EFFECT OF pH AND SUBSTRATE CONCENTRATION ON BIO-ETHANOL PRODUCTION FROM POTATO PEEL WASTE USING *Saccharomyces cerevisiae*

M. Sc. THESIS

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MAY, 2015

HARAMAYA UNIVERSITY, HARAMAYA

Effect of pH and Substrate Concentration on Ethanol Production from Potato Peel Waste Using Saccharomyces cerevisiae

A Thesis Submitted to the College of Natural and Computational Sciences, Department of Biology, School of Graduate Studies

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

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MAY, 2015

HARAMAYA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by **Alemayehu Gashaw** entitled: **EFFECT OF pH AND SUBSTRATE CONCENTRATION ON BIO ETHANOL PRODUCTION FROM POTATO PEEL WASTE USING S*accharomyces cerevisiae***. We recommended that it be submitted as fulfilling the thesis requirement.

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ACKNOWLEDGMENTS

First and foremost, I would like to thank God for giving me the strength and persistence to complete this study. I would also like to thank many people whose advice and support were valuable throughout the pursuit of this thesis.

I want to express my sincere gratitude and heartfelt thanks and appreciation to my major advisor Dr. Meseret Chimdessa Egigu for his valuable advice, support and comments in my research work and for his insight and guidance starting from proposal development to completion of my research thesis. I also acknowledge my co-advisor Dr. Manikandan Muthsuwamy for his valuable guidance, constructive comments and support starting from my research proposal development to completion of my thesis.

My special acknowledgement goes to my mother, W/ro Beletu Gashaw Mengesha for her financial support and helpful advice throughout my research work. She has worked hard to keep me accomplish my goals. I am also thankful to my aunt w/ro Ateda Gashaw Mengesha, my uncle Ato Lakew Gashaw Mengesha, Ato Mamush Gashaw Mengesha and my best friend Obsa Gizaw and W/ro Almaz Tesaw for financial support and helpful advice for my thesis.

I would also like to express my sincere thank to Metahara sugar factory managers for allowing me use their laboratory and all Metahara sugar factory laboratory technicians for their support. I am also thankful to Metahara Sugar Factor Preparatory School and Hospital for their support to use some laboratory equipment.

I am also thankful to all my staff members and management at Kersa preparatory school for their helpful advice and support to use some paper and print for my research thesis.

ABBREVIATIONS AND ACRONOMYS

CRD	Completely Randomize Design
DNS	Di Nitro Salicylic acid
E85	Ethanol85
FFCO	Fineli Food Composition
FFVs	Flexible Fuel Vehicles
HMF	Hydroxyl Methyl Furfural
LSD	Least significant difference
MTBE	Methyl tertiary butyl ether
RAO	Regional Accounting Office
RFA	Renewable Fuel Association
SPSS	Statistical Package for Social Studies
SSF	Simultaneous Saccharification and fermentation
VD	Volume of Distillate
WB	Weight Bottle
WEB	Weight of Empty Bottle

TABLE OF CONTENTS

Continue

List of table

LIST OF FIGURE

Effect of pH and Substrate Concentration on Bio-Ethanol Production from Potato Peel Waste Using *Saccharomyces cerevisiae*

ABSTRACT

Fermentation is a metabolic process of microorganisms to obtain energy by breaking down organic compound. *Potato peel waste was fermented with different concentration substrate (1gm, 2.5gm, 5gm, 10gm, 15gm, 20gm and 25gm) with 1% yeast and pH4.5 in different fermentation period (4th day, 8th day, 12th and 16th days) with the aim of optimizing the substrate concentration for ethanol production. Among the different substrate concentration 10 gram of substrate produced more quantity of ethanol on 4th day of fermentation. Accordingly, 10 gram substrate considered as optimum for ethanol production and subjected to acid pre-treatment. After the acid pre-treatment, ethanol production was boosted as compared with untreated 10 gram substrate. The maximum ethanol production for 10g untreated substrate was only 5.06%, whereas that of acid-pretreated was 7.46%. The reducing sugars contents decreased gradually as the fermentation period increased. Moreover, on 4th day of fermentation period, the highest reducing sugar concentration (29.33mg/ml) was obtained from acid pretreated potato peel waste of 10gram substrate, whereas the same substrate concentration showed 26.08mg/ml in untreated substrate. The cell biomass was higher in acid pre-treated substrate (31.2mg/ml) than untreated ones (26.6mg/ml). Moreover, the acid pretreated substrates showed the less lignin content than the untreated substrate, whereas highest removal of lignin percentage (7.4%) was observed in 10gram acid pretreated substrate compared with untreated substrate. The production of ethanol was increased with increasing the level of pH up to 4.5 after this suddenly declined rate of ethanol production. The maximum ethanol production (7.46%) was observed at pH 4.5 in acid pre-treated. The cumulative bio ethanol production was observed in different fermentation periods for optimum substrate concentration in acid pre-treated and untreated substrate. The maximum ethanol production was observed on 4th day of fermentation in acid pre-treated substrate. If the fermentation period increases, the rate ethanol production also decreased in treated and untreated substrate (10gram). The study revealed that it is possible to produce bio-ethanol from potato peel waste. Therefore, it is recommended to conduct pilot studies to maximize ethanol yield.*

***Key word**: batch fermentation, cell biomass, untreated substrate and acid pretreated substrate*

INTRODUCTION

Ethanol can be produced by fermentation from different kinds of raw materials. The raw materials are classified into three categories of agricultural raw materials: simple sugars, starch and cellulose. Increased ethanol concentration of microbial fermentation has been examined as a strategy to reduce energy cost in downstream distillation and waste treatment. Some sugars can be converted directly to ethanol, whereas starch and cellulose must first be hydrolyzed to sugar before conversion to ethanol. Most of the polymeric raw materials are available at prices lower than refined sugars. Consequently, each country may preferably develop ethanol production based on the available raw material in that country (Philppidis *et al*., 1993).

The species of *Saccharomyces cerevisiae* is a traditional ethanol producer, yet it is sensitive to high concentration of ethanol. Ethanol diffuses freely across biological membrane in yeast cell allowing equalization of ethanol concentration between intracellular and extracellular pools. As a result, the increased ethanol concentration in a medium inhibits cell growth, damage cells viability and reduce ethanol yield. The microorganisms used to carry out the fermentation process are just as important as the substrate, and they have also been the target of much research. *Saccharomyces cerevisiae*, also known as baker's yeast, is the most widely used fermentation microbe because of the baking and brewing industries. This is due to the fact that some species adopt different metabolic path ways by having special genes or special enzymes such as invertase genes and invertase enzymes, respectively for conversion of sugars to ethanol or other species (Fregonesi *et al*., 2007).

Currently, potatoes are alternative feed stocks for bio ethanol production. Potatoes are starchy crops, which do not require complex pretreatment. Although it is a high value crop, a significant amount of potato (e.g. 18% in the potato chip industry) is lost during processing because of poor quality (Fadel, 2000). Therefore, waste from potato industry could be the carbon source for bio ethanol production as it is cheaper compared to other feed stocks which are considered as available food sources. Byproducts of potato industry are currently, utilized as animal feed, however, ethanol production could be an alternative for the industry to utilize the waste.

Ethanol is produced from byproducts of French fry processing, whereas the stillage (unfermented solids) of ethanol production is used for cattle feed (Mann *et al*., 2002). Furthermore, *S. cerevisiae* and other microorganisms typically used in first generation bio ethanol processes are not able to convert all the sugars which constitute lignocellulosic biomass (Bothast *et al.*, 1999), therefore compromising the efficiency of the process.

In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure. The cellulose and the hemi cellulose portions are broken down (hydrolyzed) by enzymes or dilute acids into sucrose sugar that is then fermented into ethanol. The lignin which is also present in the biomass is normally used as a fuel for the ethanol production plants boilers. The world ethanol production has reached about 51,000 million liters (RFA, 2007), being the USA and Brazil the first producers and India stands fourth among the top fuel ethanol producers. Since it is estimated that the fossil fuels will be running out by the next few decades, attention has currently been dedicated to the conversion of biomass into fuel ethanol. Main feed stocks for bio-ethanol production are sugarcane (in Brazil) and corn grains (in USA), while many other agricultural raw materials are also used worldwide. Among the three major types of raw materials, the production of ethanol from sugary and starchy materials are easier as compared to lingo-cellulosic materials since it requires additional technical challenges such as pretreatment (Petersson *et al*., 2007).

Researchers have shown that the optimum temperature for ethanol production through microbial activity is 30^0C. Though at slightly higher temperature microbial growth rate and ethanol yield may be adversely affected, some strains of *Saccharomyces cerevisiae* have also been capable of growing and fermenting sugar at about 40-45^0C under batch fermentation (Amutha and Paramasamy, 2001). Similarly, the substrate concentration and pH level, which influences the survival of yeast, will limit the production of ethanol (Amuth and Paramasamy, 2001).The bio ethanol concentration gradually increases along with the increase in pH and reaches a maximum percentage of bio ethanol concentration when pH is equal to 4 and later it starts declining due to the lesser activity of yeast. De Vasconcelos et al. (1998) and Nigam (1999) are also observed the maximum ethanol productivity at pH=4.2 to 4.5.

Furthermore the use of high technology and complicated instrumentation methods with high operating costs may in turn limit their commercialization and industrial application in the developing countries (Isarankura et al., 2007).

Research efforts are focused to design and improve a process, which would produce sustainable transportation fuel using low cost feed stocks. Many agricultural raw materials rich in fermentable carbohydrates were tested worldwide for bioconversion from sugar to ethanol, but the cost of carbohydrate raw materials has become a limiting factor for large scale production by the industries employing fermentation processes. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feed stocks such as lingo-cellulosic biomass and agro-food wastes, are being considered to make bio-ethanol competitive in the open market (Campo*et.al* ., 2006). The production of ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganism is the only possible way to meet the great demand for ethanol in the present situation of energy crisis (Pramanik and RAO, 2005).

Ethanol is an important chemical product with emerging potential as bio fuel to replace the fossil fuel. An eco-friendly bio ethanol is one of the alternative fuels that can be used unmodified petrol engines with current fuelling infrastructure and it is easily applicable in present combustion engine as mixing with gasoline (Hansen *et al*., 1985). Ethanol produced by microbial fermentation of biomass is used primarily as a substitute for gasoline, usually blended, although it can also be used on its own.

Minor applications commonly include industrial use or rocket fuel. In developing countries it is also commonly used to replace kerosene for cooking and illumination. Besides being a renewable energy source, bio ethanol has other advantages over fossil fuels: it emits less CO_2 when burned (Brown *et al.*, 1998), which in addition is compensated by CO_2 uptake from the biomass; it does not emit toxic gases (such as CO and nitrate oxides) or particles when burned; less energy is required to produce ethanol than the equivalent amount of gasoline; it is rapidly biodegraded in the environment; it has a higher octane rating than gasoline (Bailey, 1996).

Ethanol is a clean-burning, renewable, domestically produced product made from fermented agricultural products. Ethanol contains oxygen, which provides a cleaner and more efficient burn of the fuel. When used in vehicles, ethanol reduces carbon dioxide, a major contributor to global warming. Although burning ethanol still releases carbon dioxide during production and combustion, it is recycled by the crops that produce ethanol. This creates a cycle in which greenhouse gases are used instead of being emitted into the environment (Akpan *et al*., 2004).

This study is therefore designed to investigate the impacts of varying levels of pH and substrate on ethanol production from potato peel waste after acid pre-treatment with the following general and specific objectives.

General objective

- To investigate bio ethanol production from potato bio-waste using baker's yeast as fermentative agent.

Specific objectives

- To identify the optimum substrate concentration for bio ethanol production
- To evaluate the effect of different pH levels on bio ethanol production from potato peel waste fermentation
- To examine the effect of acid pre-treatment substrate on bio ethanol production

2. LITERATURE REVIEW

2.1 Bio ethanol as a source of energy

Ethanol, which is also called ethyl alcohol, is colorless compound with a chemical formula of C2H5OH (Kaur and Kocher, 2002). It is one of the bio energy sources with high efficiency and low negative impact on which is potato environment. Various raw materials have been used as carbon source for ethanol production, one of peel. Bio fuel as an alternative source of energy may be the proper solution to this problem. Among the commercially available bio fuel for special attention deserves dehydrated ethyl alcohol derived from plant products in the course of ethanol fermentation, so called bio ethanol. The use of ethanol as an alternative energy source creates the need for possibility of using cheap raw material, new technological solution and effective fermenting microorganisms (Aldiguier *et al*., 2004, Karim *et al*., 2006, Krishnan *et al*., 2000, Kupczyk and Eilski, 2002, Linde *et al*., 2007).

Development of technology for the production of bio ethanol based on agro industry waste material reduce the cost of ethanol production and help to eliminate these waste from dried trade. Measurable economic and environmental aspects of such proceedings are also significant. Results of previous studies (Ohgren*et al*., 2006) indicate that the production of ethanol can be used as in a wide range of waste products of food industry. These include potato peel waste (Arapoglou *et al*., 2010).

Ethanol can be used to power motor vehicle by mixing with gasoline (Balat, 2006; Hill *et al*., 2006). Many car manufactures are now producing flexible fuel vehicle (FFV's), which can safely run on any combination of bio ethanol and gasoline up to 110% bio ethanol (Miller, 1959). Increasing numbers of light trucks are sold as flexible fuel vehicles, capable of burning a variety of fuel, including mixes of gasoline and bio ethanol and other alternative fuels. Vehicles that can run on pure bio ethanol are rare and require special engineering to function, which is why fuels for FFVs usually contain at least some amount of gasoline. One commonly used bio ethanol blend is called E85, and it contains 15% gasoline and 85% bio ethanol. Producers add this small amount of gasoline to the ethanol to make the vehicle start better in cold weather. E85 is generally priced at about the same level as gasoline (Chen *et al*., 2007).

Ethanol has been valuable industrial solvent, germicide, antifreeze and is a major feedstock for a number of chemical derivatives, polymers, esters etc. (Maiorella *et al*., 1984). The shortage of crude oil coupled with environmental problems associated with its use has led to extensive research for alternative energy sources. Ethanol, in spite of its lower heating value may become a partial replacement of fossil fuels, particularly for automotive use. It is a natural fuel, which burns cleaner than petrol and causes less environmental problems. It is a readily available liquid that can be produced and utilized with existing technologies (Aslam, 1999). The most economic way of manufacturing ethanol from variety of renewable sources involves fermentation by yeasts (Reed, 2001). Molasses is the most important worldwide raw material for ethanol fermentation (Maiorella *et al*., 1984). It provides a cheap source of fermentable sugars and contains some formative nutrients as well (Aslam, 1999). Approximately 70% of the current production process for ethanol is through a batch-wise fermentation, which disadvantages it because of high capital costs, labour intensity and difficult process and product quality control. An obvious method to avoid or minimize these difficulties is to use a continuous production process; to achieve this it is necessary to have a good knowledge of the chemical transformation reaction and their kinetics. The traditional method of starch hydrolysis to glucose (or fermentable sugars) is highly energy intensive as it involves the units operations of liquefaction by sulfuric acid. The manufacture of ethanol or other feed stock from starchy material therefore is not economically feasible in comparison with directly fermentable sugars such as glucose. Starch is the major dietary source of carbohydrate and abundant storage polysaccharides in plants, occurring as granules of size 1µm to 100µm (Phillips and Willians, 2000). It is composed of a mixture of two kinds of polyglucan, namely amylose and amylopectin. Amylopectin is highly branched polymer, consisting of short α-1-4, obligo polymers linked by largest molecules in nature .Amylose is a linear component mostly comprised α-1, 4-linkage, with average degree of polymerization up to 6,000 and molecular mass of 105 to 106 g/mol. Depending on the botanical source, the amylose content varies from 0 to 70% (Viswannathan, 1999). The most important origins of starch are maize, potato and wheat.

2.2 Saccharomyces cerevisiae as Bio ethanol Producer from potato waste

Ethanol production is among the oldest technology and produced commercially by fermentation of potato peel waste or other material with sugar content. The fermentation process involves the conversion of sugars to alcohol and carbondioxde by yeast Saccharomyces cerevisiae. The principal biological agents of fermentation are yeast belonging to the genus S.cerevisiae which can catalyze alcoholic fermentation to ethanol production. *Saccharomyces cerevisiae* meet their energy demand by converting the carbon sources to by products such as carbon dioxide, lactic acid and ethanol. Ethanol is one of the end products of fermentation, which can be performed by *Saccharomyces cerevisiae*. Fermentation is an energy generation process with no electron transport mechanisms (Shuler and Kargi, 2008). Fermentation is carried out in anaerobic environment, but *Saccharomyces cerevisiae* needs small amount of oxygen to synthesis fatty acid and sterol (Sanchez and Cadona, 2008).

One of the biological wastes that can serve as substrate for bio ethanol production is potato waste. The mashed potato flakes contain 73% of carbohydrate (FFCO, 2008). It also contains minerals such as sodium, potassium, magnesium, calcium, phosphorous, zink, selenium, and iodine. Based on the composition of potato flakes, potato mash could be a feasible feed stock for micro organisms to produce bio ethanol. Fadel (2000) and Liimatainnen *et al*. (2004) showed that potato waste can be a carbon source for yeast during alcohol fermentation from the waste of potato chip industry and different potato cultivations, respectively. Yamadal *et al*. (2009) also reported that bio ethanol can be produced from the byproduct of potato processing plants.

2.3. Potato peel waste used as substrate

Agricultural activity and food industry generate considerable quantities of wastes which are rich in an organic matter and could constitute new materials for value added products. The peel waste holds the range of carbohydrate polymers; makes it idyllic as source of energy through anaerobic digestion (Talebnia, 2008).

Potato peel waste is one such product which is abundant and very low utility and low price. The utilization of industrial solid potato waste for bio ethanol production could reduce the pollution and bring down the ethanol production. These advantages are withdrawal the interest of researcher around the world to investigate its potential for bio ethanol production. The ideal

source for bio ethanol production should be cost-competitive and rich in carbon content. Besides using low cost materials, many other technologies have also been carried out to make ethanol production efficient and cost-competitive such as employing strain with high ethanol production ability (Nakamura *et al*., 1997; Kannanet *al*., 1998; Kiranet *al*., 1999).

The production of bio ethanol also depend up on strains of yeast such as Saccharomyces cerevisiae, suitable substrate and methods employed for bio ethanol production greatly increase the efficiency bio ethanol production. For bio ethanol production, potatoes are cheap substrate because it is rich starch and require less processing than other grains. After suitable processing, good quality of ethanol can be produced from potato peel waste which can be used for both fuel as well potable purposes. Recently, due to shortage in natural resource of petrochemical and natural gas, researchers have focused to produce new renewable energy source. To achieve this goal, scientists take interest on the production of bio ethanol using biomass and agricultural waste. Potato possesses great amount of starch that could be hydrolyzed in to sugar and easily fermented to produce bio ethanol and found suitable to be used as alternative energy source (Chandel et al., 2007

2.4. Hydrolysis of potato peel waste

Hydrolysis is the process of breaking down of potato peel waste amylopectin and amylose linkages into fermentable sugar, and is needed before the fermentation of starch material. Hydrolysis is carried out at high temperature (90^0c-110^0c). At low temperature, hydrolysis of starch is possible and can contribute to energy savings (Sanchez and Cardona, 2008). To convert starch of potato peel waste into fermentable sugars, either acid hydrolysis or enzyme addition should be done. Both hydrolysis methods have disadvantage and advantage. The limitations of acid hydrolysis include the byproduct inhibition on the growth of yeast (such as 5-hydroxy methyl furfural (5-HMF)), neutralization before fermentation, and expensive constructional material (Tasic *et al*., 2009). On other hand, high prices of enzyme play a crucial role when feasibility is concerned for enzyme hydrolysis. Enzyme hydrolysis is chosen despite the high cost of enzyme and initially investment (Tasic *et al*., 2009) because of high conversion yield of glucose.

2.4.1 Acid pretreatment substrate

Acid pretreatment is one the oldest factor and the most commonly used method. Both concentrated and diluted acids are applied in this method. Concentrated acids disturb the hydrogen bonds in crystalline cellulose and convert crystalline cellulose into amorphous cellulose. The advantage of applying concentrated acid pretreatment is that it is not specific to bio mass type. Concentrated acid such as H_2SO_4 have been used to treat lignocellulosic material. Although they are powerful agent for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactor that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Siver and Zacchi, 1995). Diluted acid hydrolysis has been successfully developed for pretreatment for lignocellulosic material. Diluted sulphuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian *et al*., 1997).

In addition, mild temperature condition and high monosaccharide yield (90%) are the characteristic method appealing for decades (Stavrinides et *al.,* 2010). The issues with concentrated acid especially at higher temperature (200^0c-500^0c) dead to the formation of furfural or hydroxyl methyl furfural, which reduce the sugar, yield (Demibras, 2006). The other drawback for concentrated acid pretreatment is high corrosion of the equipment and high acid recycling cost (Alvirap *et al*., 2010).Facing these issues, diluted acid pretreatment become more favorable in the bio fuel industry. There are typically two types of diluted acid pretreatment such as high solid loading (10-40%) at low temperature (less than 160^0c) in batch pretreatment and low solid loading (5-10%) at high temperature (more than 160^0c) in continuous flow pretreatment (Kumar *et al* ., 2009). Besides reactions with cellulose, diluted acid pretreatment also cause hemicelluloses dissociation. This water soluble sugar monomers and oligomer released from cell wall and enzyme digestibility are both increased. Although diluted acid is not able to remove lignin researcher suggested that the lignin in biomass was modified (Yang and Wyman, 2008). As a result the diluted acid pretreatment flexibility in choosing feed stock. Biomass with lower lignin content is preferred. Due to the mild condition, extension of time or increase of temperature is required (Stavrinides *et al*., 2010).

Environment toxicity and equipment corrosion leads to the limitation of applying this pretreatment (Hinman *et al*,. 1992). Furthermore, the byproducts of this pretreatment process, including fulfural (from pentose), 5- hydroxyl methyl fulfural (from hexose), and acetic acid and

metal ions are all considered to be inhibiting the subsequent fermentation. These inhibitors are known to decrease the growth rate of microorganism's process (Palmquist and Habn-Hagerdal, 2000). Cleaning processes like stream stripping are necessary prior to fermentation (Mes-Hartree and Saddler, 1983).

2.4.2 Biological hydrolysis

Biological technologies are also been used for hydrolysis process. There are two technologies being developed for hydrolysis such as enzymatic conversion and direct microbial conversion (Pessoa et *al*,. 1997).Yeasts are facultative anaerobes and can grow with or without oxygen. In the presence of oxygen, they convert sugars to CO_2, energy and biomass. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate by-products such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production. The main carbon and energy source for most yeast is glucose supplied from starch, which is converted to the glycoltic pathway to pyruvate and by the Krebs cycle to anabolites and energy in the form of ATP. Yeasts are classified according to their modes of further energy production from pyruvate to respiration and fermentation. These processes are regulated by environmental factors, mainly glucose and oxygen concentrations. In respiration, pyruvate is decarboxylated in the mitochondrion to acetyl-CoA, which is completely oxidized in the citric acid cycle to CO_2, energy and intermediates to promote yeast growth. In anaerobic conditions, glucose is slowly utilized to produce the energy required just to keep the yeast cell alive. This process is called fermentation, in which the sugars are not completely oxidized to CO_2 and ethanol *(*Bekatorou *et al.,* 2006 and Scragg, 1991*).*

Starch is very important and abundant natural solid substrate. Many microorganisms are capable to hydrolyze starch, but generally its efficient hydrolysis requires previous gelatinization. Many microorganisms can hydrolyze starch, especially fungi which are then suitable for Simultaneous Saccharification and fermentation (SSF) application involving starchy substrate. Microorganisms generally prefer gelatinized starch but large quantity of energy is required for gelatinization so it would be attractive to use organisms growing well on raw (ungelatinized) starch. Biological routes are built around using enzymes to break down cellulose (cellulase) and hemicelluloses (hemicellulase) to sugar. These sugars are then fermented to ethanol or other products which are recovered and purified to meet market requirements (Wyman *et al.,* 2005).

Biological conversion promises low cost because it has the potential to achieve nearly theoretical yields and the modern tools of biotechnological can improve key process steps. Cellulosic biomass must be pretreated to realize high yields vital to commercial success in biological conversion (Moseir *et al*., 2005).

2.5 Factors that affect yeast cells growth for bio ethanol production

During ethanol fermentation, yeast cells are negatively affected by various stress factors. These stress factors include extreme temperatures, pH, nutrient deficiency, contamination, cell metabolic conditions such as high sugar content tolerance, ethanol tolerance etc. Potential environmental stresses that affect *S.cerevisiae* occur during alcoholic fermentation (Bai *et al*., 2008). Many of them act synergistically to affect yeast cell more severely than any single stress, leading to reduced yeast viability as well as lower ethanol yield. Stress can cause structural changes and metabolic changes in an organisms acting as expression activator for genes involved in the synthesis of specific compounds that protect cell (Banat *et al*., 1998).*Saccharomyces cerevisiae* is an important microorganism in bio-industry and its tolerance to temperature and ethanol concentration is one of the main characteristics used for deciding whether it can be used as a bio-fermentation resource (Osho, 2005). Thus, in the industrial ethanol production, there are many important factors which should be considered such as ethanol or sugar tolerance of strains, and enzymatic activities for good operation (Furukava *et al*., 2004). One of the problems associated with fermentation of sugar is the high temperatures (35-45°C) and high ethanol concentration (over 20%). Tolerance to high temperatures and ethanol concentrations are important factors of microorganisms for increasing efficiency at industrial scale.

2.5.1Temperature

The fermentation efficiency of *S. cerevisiae* at high temperatures is very low due to increased fluidity in membranes to which the yeast responds by changing its Stress or environmental stimuli can cause structural changes and/or metabolic changes in an organism acting as expression activator for genes involved in the synthesis of specific compounds that protect the organism (Lieckefeld *et al*., 1993). The factors triggering the expression of this type of genes can be biotic or abiotic. Biotic factors induce changes in the gene expression of the guest, giving rise to the synthesis of specific compounds that generate resistance to the strange organism. Abiotic stresses can be temperature, osmotic stress, anaerobic conditions, heavy metals, growth

regulators, ultraviolet or gamma radiation, metabolic repressors, and pH (Brosnan *et al*., 2000). Stress due to temperature has been the most studied abiotic factor, where both heat and cold induce the synthesis or storing of a group of proteins that increase stress resistance (Steensma and Linde, 2001). Some successful attempts to adapt yeasts to high temperatures have been described, *S. cerevisiae* yeasts, capable of fermenting at 40 and 45°C have been obtained using progressive cultures (De Barros *et al.*, 1998).

Excess amount of ethanol has been reported to cause mitochondrial DNA damage and degrades bio membranes in yeast cells (Swiecilo *et al*., 2000). Ethanol can dissolve fatty acid constituents of the cell membranes; disrupt cytoplasm membrane rigidity (Osho, 2005). Many reports have accented a relationship between the fatty acid compositions of lipid membranes and ethanol stress tolerance (You *et al*., 2003) which stop mitochondrial bio molecules translocation and proton motive force (Ekunsanmi and Odunfa, 1990) and finally cause cell death. According to these phenomena, resistant strains to ethanol have many mechanisms to overcome ethanol perils. Invertase enzyme activity propriety in the yeast strain is very important. Invertase is one of the important extracellular enzymes in *Saccharomyces* that is responsible for converting sucrose to its subunits, glucose and fructose (Sengupta *et al*., 2000).

Temperature is one the most important factors that affect ethanol production by yeast cell using potato peel waste as carbon source. The fermentation process always accompanied with evolution of heat that raises the temperature of fermenter (Jones *et a*l., 1994). Ethanol fermentation at high temperature is a key requirement for effective ethanol production in tropical country where the average day time temperatures are usually high throughout the year. The advantages of rapid fermentation at high temperatures are not only a reduction in the cooling costs but also decreased risk of contamination.

To achieve high temperature fermentation, it is necessary to use an effective yeast strain that can tolerate high temperatures. Some strains of *Saccharomyces cerevisiae* are able to grow and produce ethanol at 42-44^{0}c (Sree *et al.,* 2000; Edgardo *et al*., 2008). Further increasing in temperature and pH reduces the percentage of ethanol production and it is mainly due to denaturation of yeast cell, while an excellent temperature for maximum productivity occurs at 320C for maximum strain. It is therefore, necessary to select temperature the optimum temperature at the yeast strain can ferment sugar (Yah et al., 2010).

2.5.2. Effect of pH on Ethanol production

The pH of the bio reactor affects the enzymes inside the cells and changes the rates of reaction. Yeast cells have evolved so that they can thrive in more acid environments than many competing organisms. As the yeast cells consume their nitrogen source, hydrogen ions are released and decreasing the pH solution. Buffers are often used to maintain the solution within the desired range. The pH of solution has several effects on the structure and activity of enzymes and hence depolarization. Enzymes are molecules containing a large number of acids and basic groups, mainly situated on their surface. The changes on these groups vary, according to their acid dissociation constants, with the pH of the solution. Thus, pH affects the reactivity of the catalytically active group (Nester et al., 2001).

2.6. Fermentation process

Fermentation is a metabolic process of microorganisms to obtain energy by breaking down organic compound. While microorganisms derive their energy, some by products are lactic acid, butanol, carbon dioxide, ethanol and cellulose. In ethanol fermentation, derivation of energy from sugar by either yeast or bacteria, produce carbon dioxide and ethanol. Because yeasts produce their energy without need for oxygen, ethanol fermentation methods are other important aspects of ethanol fermentation. Batch fermentation has been applied in the ethanol industry.
Traditionally, lignocellulosic material can be the first converted to fermentable sugars consisting of mainly glucose, followed by fermentation to ethanol by using special yeasts. This method called separate hydrolysis and fermentation (SHF) (Kang *et al*,. 1992). For the SHF method, yeast is used for ethanol and CO_2 productions in the absence of oxygen. The main production ethanol has been distilled from solutions containing yeast cells and biomass residues to reach a higher concentration around 96%. The residues from distillation are used for live stock feed due to high protein content. This traditional yeast fermentation can be used as a standard method to show the presence of the pretreatment effects on hydrolysis process.

Batch fermentation is carried out in cultured vessels with an initial amount of medium, and fermentation is performed after sterilization of media and adjustment of pH by either acid or alkali. Batch process is the simplest method of fermentation (of hydrolysis), with the simplest control system, in comparison to other modes of operation. After inoculating the medium, nothing is added in the batch fermentation, except possibly base for pH control. The medium has

essentially the same composition as the hydrolyzed with the exception of possible additions of minerals or other supplemental nutrients (Taherzadesh and Karim, 2007). .

After inoculation of yeast, production of ethanol takes place by controlling temperature, pH, agitation and aeration depending on the characteristics of cultured microorganisms. Because no medium addition occurs, the growth of microorganism follows four main phases of the growth curve which include the lag phase, log phase, stationary phase and decline phase. The growth of microorganism is slow in the lag phase, because this is an adaptation time for cells to a new environment and cells may have new metabolic path ways or synthesis enzymes (Shuler and Kargi, 2002). Stationary phase refers to the period where microbial growth rate equal to death rate. In this phase, cells are still able to produce secondary product (Shuler and Kargi, 2002). The last period of batch fermentation is the phase in which death rate is higher than growth rate due to lack of nutrients or accumulation of inhibitor primary or secondary metabolite.

2.7. Current Bio ethanol production process

Ethanol has been produced by anaerobic yeast fermentation of simple sugars since early recorded history. This fermentation used the natural yeast found on fruits and the sugars of these fruit produce ethanol. A current practice utilizes enzymes and acid to efficiently hydrolysis grains or tuber starch to glucose for fermentation to ethanol. The fixed amount of ethanol is produced, along with other feed products and CO_2, and has almost no process flexibility. Some solids are removed initially remaining only the starch slurry fed to ethanol production step. This process has the capability to produce various end product and considerable higher process flexibility, compared to the dry milling (Fernado et al., 2006).

3. MATERIALS AND METHODS

3.1. Description of study area

The experiment was conducted in the ethanol production laboratory at Metahara sugar factory. Metahara sugar factory is located 200km the south east of the capital city, Addis Ababa and located in Oromia Regional State, East Shoa Zone, Fentale Woreda and 8kms away from Metahara town. The average maximum and minimum air temperature of the study area is 35^0C and 21^0C respectively and the soil temperature at depth of 0.05m and 0.1m is 33^0C and 31^0C respectively. The soils of the area include sandy loam, loam, silt and clay loam where silt dominates the larger portion of the area (Belayneh, 2005).

3.2 Substrate and yeast samples preparation

The substrate to be used for ethanol production was potato peel waste. Potato peel waste was obtained from House hold. The collected potato peel was allowed to dry in an oven (65^0C for 48hr). One kilogram of potato peel sample was pulverized with a blender, packed in plastic container and stored in the refrigerator until analysis to prevent the sample from contamination. The 1% baker's yeast (*S. cerevisiae*) was prepared by using distilled water. From this 10 ml was used as inoculums (Dhopeshwarker *et al.*, 2001).

3.3 Preparation of nutrient solution

Nutrient supplements that enhance the growth of yeast was prepared by adding 0.1 g KH_2PO_4, 0.5 g $CaCl_2$, 0.05 g $MgSO_4$, 0.1 g Na_2SO_4 and 0.1 g $(NH_4)_2SO_4$ per litter (Abouzeid and Reddy , 1986).

3.4. Fermentation experiment

Fermentation experiment was designed in a CRD factorial and three replications for each treatment and control. There were seven treatments to be carried out. Fermentation (Experiment) was carried out using the samples (potato peel) by using baker's yeasts. To evaluate the impact of substrate concentration and select the optimum one, different amount of substrates (1gm, 2.5gm, 5gm, 10gm, 15gm, 20gm and 25gm) were considered and separately mixed with equal volume of

distilled water and 100 ml nutrient solution in 250 ml conical flasks. Substrate acid pretreatment was carried out in 500ml conical flasks by using dilute sulphuric acid hydrolysis to enhance the rate of conversion of glucose into bio-ethanol by the action of baker's yeast (*S. cerevisiae*).

The acid concentration during hydrolysis leads to a corresponding increase in the concentration of hydrogen ions which in turn increase the rate of the hydrolysis reactions and consequently the rate at which the Glycosidic bonds was broken to be increased into fermentable sugar (Kumar et al., 2009; Mosier et al. 2005).

For this, dilute H_2SO_4 (10ml) (1.25%) was mixed with substrate to be treated and the mixture was shaken & heated up to 100^oC for 15 minutes. Sodium hydroxide was added to adjust the pH to 4.5 (Cheng, 2002). The effect of pretreatment on fermentation was compared with fermentation on untreated substrates.

To evaluate the impact of pH on the optimum substrate concentration, the pH of the solution was adjusted to get different levels (2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5) by adding sulfuric acid or NaOH. In all cases 1% of inoculums were added. The production of ethanol was estimated at 4 days interval (i.e., on 4^{th}, 8^{th}, 12^{th}, and 16^{th} day).

3. 5 Parameters determined

Besides ethanol, reducing sugar and cell density were the parameters measured for the optimum substrate concentration. For this, first, samples ($30cm^3$) were taken on 4^{th}, 8^{th}, 12^{th}, and 16^{th} days after the start of fermentation and centrifuged at 400 rpm for 30 minutes to remove the cell suspensions. The supernatant fluid was filtered through Whatman No.1 filter paper and the filtrate was used to determine the reducing sugar and ethanol concentration (Amadi *et al.*, 2004).

3.5.1 Quantitative Analysis of Reducing Sugar

Reducing sugar like glucose concentration was determined by using 3, 5-dinitrosalicylic acid (DNS) reagent as described by Miller (1959). A sample (0.05ml) was taken from the filtrate, and mixed with Citrate buffer (pH=6.5) (0.35ml) and DNS (0.6ml), and then the sample was boiled for 5 minutes immediately to stop the reaction. The absorbance was measured at 540nm by using spectrophotometer (Bennet, 1971).

3.5.2 Estimation of Bio-ethanol Production

The amount of bio-ethanol produced from potato peel was analyzed from the filtrate. For this, the filtrate was distilled at 78^{o}C by using rotary evaporator. The quantitative analysis was carried out by determining the densities of the distillates as follows (Amadi *et al.*, 2004).

$$\text{Density} = \frac{(WB+D)-WEB}{DV}$$

WB = Weight of the Bottle

WEB = Weight of Empty Bottle

VD = Volume of Distillate

3.5.3. Cell Density (Biomass)

Cell density was measured at the interval of 4th day, 8th day, 12th day, and 16th day by using spectrophotometer (Humas Think HS 3300, Korea) at 600 nm absorbance (Summer et al., 2004).

3.6 Lignin Measurement

The lignin content in the optimum substrate concentration (treated and untreated) samples was hydrolyzed by using equal volume of 1.25% H_2SO_4 for two hours. The residues were filtered and washed with distilled water for ten minutes to neutralized H_2SO_4 and then oven dried at 105^{o}C for 10 hours for a constant weight. The amount of lignin percentage was expressed by using the following formula (Krisztina *et al.,* 2009).

$$\%\text{Lignin} = \frac{Lignin\ Weight}{Biomass} \text{x} 100$$

3. 7. Data analysis

The data obtained from laboratory experiment was analyzed and summarized into tables and graphs by using Microsoft office excel spreadsheet and Statistical Package for Social Studies (SPSS) version 17.Duncan's multiple range tests and LSD (least significant difference) test were used to identify significant differences among treatment means. P values < 0.05 were considered significant in all cases.

4. Results and Discussion

4.1 Effect of substrate (untreated) concentration on Bio ethanol production

Ethanol production was observed in all substrate concentration levels on fourth days of fermentation (Fig. 1). Many researchers (Ruchi *et al*., 2011; Nyachaka *et al*., 2013) reported the start of ethanol production on fourth days of fermentation. Though all substrate concentrations resulted in ethanol production starting from the fourth day of fermentation, 10 g substrate was found to yield high amount of ethanol throughout the incubation period. Therefore as 10 g substrate is yielding the maximum amount of ethanol, it can be considered as the optimum concentration. Moreover, it was observed that ethanol production decreased as time for fermentation increased, suggesting that less amount of substrate remained to be ethanol.

Table-1. Effect of substrate concentration (untreated) on ethanol production

Substrate conc.(gm)	Potato Peel Waste Ethanol Production (%)			
	Untreated Substrate			
	4^{th} day	8^{th} day	12^{th} day	16^{th} day
1	2.35 ± 0.09^{Ga}	2.13 ± 0.05^{Gb}	1.97 ± 0.09^{Gc}	1.55 ± 0.06^{Gd}
2.5	2.71 ± 0.03^{Fa}	2.41 ± 0.03^{Fb}	2.24 ± 0.05^{Fc}	1.96 ± 0.08^{Fd}
5	3.91 ± 0.06^{Ca}	3.61 ± 0.05^{Ea}	3.36 ± 06^{Cc}	2.65 ± 0.12^{Cd}
10	5.06 ± 0.00^{Aa}	4.40 ± 0.09^{Ab}	3.58 ± 0.09^{Ac}	2.52 ± 0.1^{Ad}
15	4.45 ± 0.06^{Ba}	4.16 ± 0.08^{Bb}	3.57 ± 0.11^{Bc}	2.23 ± 0.01^{Bd}
20	3.59 ± 0.02^{Da}	2.98 ± 0.06^{Db}	2.30 ± 0.06^{Dc}	2.07 ± 0.07^{Dd}
25	2.83 ± 0.05^{Ea}	2.42 ± 0.06^{Eb}	2.17 ± 0.08^{Ec}	1.78 ± 0.01^{Ed}

(Means ± SD), n = 3 n = number of experimental replicates, SD = standard deviation

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between the treatments.

Once the 10g was identified as an optimum concentration, it was subjected to acid pre-treatment to check if ethanol production can be boosted as compared to the same concentration of

untreated substrate. The result showed that acid pretreatment significantly increased ethanol production on the fourth day of fermentation (Fig. 1).

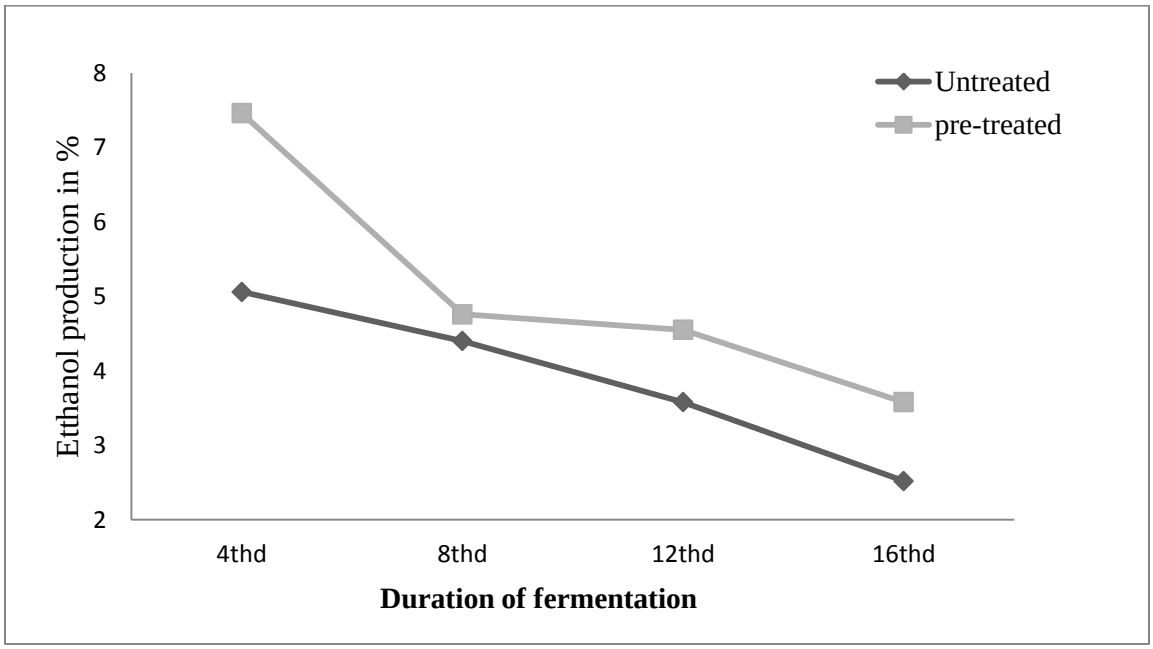

Figure 1Comparison of untreated and acid pre-treated optimum substrate concentration (10gm) on ethanol production

The study revealed that ethanol productions were significantly different at 5% level of significance between acid pre-treated and untreated substrates. For example, the maximum ethanol production for 10g untreated substrate was only 5.06%, whereas that of acid-pretreated was 7.46%. This may due to the fact that acid pre-treatment facilitates degradation of cellulosic material to be converted into ethanol. Chandel *et al.* (2007) previously reported that starch in potato will be hydrolyzed in to sugar during acid pre-treatment and easily fermented to produce bio ethanol. Moreover, Sharma *et al*, (2007) reported that acid pretreatment makes the structure of the substrate less complex, and it becomes more accessible to the enzyme, and hence, more reducing sugars are released.

4.1.1 Reducing sugar

The reducing sugar concentrations were analyzed for optimum substrate concentration (10 gram) in acid pretreated and untreated substrate. The reducing sugars contents decreased gradually as the fermentation period increased. Moreover, on 4th day of fermentation period, the highest reducing sugar concentration (29.33mg/ml) was obtained from acid pretreated potato peel waste of 10gram substrate, whereas the same substrate concentration showed 26.08mg/ml in untreated substrate (Fig.3). After 4th day, the concentrations of reducing sugar were decreased in treated and untreated substrate. The trend of reducing sugar content was in line with ethanol production, suggesting that acid-pre-treatment facilitated conversion of complex carbohydrates into simple sugars that would eventually be converted into ethanol.

Current result showed the concentration of residual sugars go down consistently during the first 96 hrs (4th day) of fermentation. This was due to rapid increase in ethanol concentration, at the same time 4th day the maximum concentration of ethanol was achieved; showing the consumed sugar was converted to ethanol. The current study is similar with that of Swine *et al*. (2007) who reported that maximum ethanol concentration was obtained at 4th day with high concentration of reducing sugar in submerged shake-flask fermentation of Mahula flowers with high reducing sugar concentration. The correlation coefficient determinations of reducing sugar for acid treated and untreated substrates were 0.933 and 0.950 respectively.

This indicated that the correlation of reducing sugar was found to be significant at the 0.01 level.

Table 2 Reducing sugar concentration (mg/ml) in potato peel waste at different fermentation period

Substrate (gm)	Reducing Sugar Concentration at Different Fermentation Period							
	Acid Pretreated substrate				Untreated substrate			
	4th day	8th day	12th day	16th day	4th day	8th day	12thday	16th day
1	2.30 ± 0.18^{Ga}	0.99 ± 0.06^{Gb}	0.57 ± 0.06^{Gc}	0.049 ± 0.00^{Gd}	1.53 ± 0.02^{Ga}	0.80 ± 0.0^{Gb}	0.47 ± 0.02^{Gc}	0.023 ± 0.02^{Gd}
2.5	5.16 ± 0.1^{Fa}	1.86 ± 0.03^{Fb}	1.18 ± 0.08^{Fc}	1.03 ± 0.05^{Fd}	4.04 ± 0.04^{Fa}	1.47 ± 0.09^{Fb}	0.89 ± 0.04^{Fc}	0.67 ± 0.05^{Fd}
5	10.73 ± 0.39^{Ca}	5.91 ± 0.07^{Cb}	2.05 ± 0.15^{Cc}	1.45 ± 0.35^{Cd}	8.15 ± 0.03^{Ca}	3.17 ± 0.04^{Cb}	1.62 ± 0.03^{Cc}	0.82 ± 0.04^{Cd}
10	29.33 ± 0.47^{Aa}	7.31 ± 0.34^{Ab}	2.86 ± 0.11^{Ac}	1.89 ± 0.15^{Ad}	26.08 ± 0.3^{Aa}	5.38 ± 0.04^{Ab}	2.71 ± 0.04^{Ac}	1.50 ± 0.07^{Ad}
15	19.33 ± 0.57^{Ba}	6.53 ± 0.02^{Bb}	2.81 ± 0.17^{Bc}	1.53 ± 0.06^{Bd}	15.88 ± 0.0^{Ba}	4.26 ± 0.03^{Bb}	2.17 ± 0.03^{Bc}	1.11 ± 0.02^{Bd}
20	8.73 ± 0.25^{Da}	2.79 ± 0.08^{Db}	1.66 ± 0.21^{Dc}	1.35 ± 0.13^{Dd}	6.57 ± 0.03^{Da}	1.78 ± 0.01^{Db}	1.18 ± 0.03^{Dc}	0.73 ± 0.03^{Dd}
25	7.07 ± 0.14^{Ea}	1.41 ± 0.16^{Eb}	1.33 ± 0.22^{Ec}	1.12 ± 0.03^{Ed}	4.83 ± 0.02^{Ea}	1.59 ± 0.03^{Eb}	0.98 ± 0.03^{Ec}	0.56 ± 0.02^{Ed}

(Means ±SD), n = 3 n = number of experimental replicates, SD = standard deviation

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between the treatments

4.1.2 Cell Biomass

The cell biomass was also observed for optimum substrate concentration (10 gram) and it was indicated that yeast biomass was increased at 4^{th} day in both untreated and pretreated substrates. However, the amount is higher in acid pre-treated substrate (31.2mg/ml) than untreated ones (26.6mg/ml), and this corresponded with the highest ethanol production. However, after 4^{th} day of fermentation the cell biomass concentration found to be decreased. This may be due to high alcohol content and decrease in fermentable sugar. A rapid bioconversion of sugars to ethanol during the initial stages could also be observed in the entire sample from the increased cell mass of yeast and also from the decreasing trend in the amount of residual sugars in the fermentation medium. The increasing ethanol production with increasing cell biomass indicated that the amount of yeast influenced ethanol production (Akin-Osanaiye *et al.*, 2005).

Table 3 Cell density (mg/ml) in potato peel waste at different fermentation period

substrate (gm)	Cell density at Different Fermentation Period							
	Acid Pretreated substrate				Untreated substrate			
	4th day	**8th day**	**12th day**	**16th day**	**4th day**	**8th day**	**12th day**	**16th day**
1	15.5±0.5^{Ga}	13.5±0.2^{Gb}	6.9±0.19^{Gc}	6.1±0.02^{Gd}	11.3±0.42^{Ga}	6.3±0.13^{Gb}	4.4±0.36^{Gc}	2.6±0.19^{Gd}
2.5	19.2±0.8^{Fa}	14.6±0.35^{Fb}	11.4±0.03^{Fc}	10.0±0.03^{Fd}	14.4±0.1^{Fa}	10.7±0.53^{Fb}	6.7±0.4^{Fc}	3.9±0.35^{Fd}
5	24.8±0.26^{Ca}	22.4±0.18^{Cb}	14.0±0.05^{Cc}	11.9±0.08^{Cd}	23.5±0.12^{Ca}	15.1±0.25^{Cb}	14.4±0.24^{Cc}	6.4±0.36^{Cd}
10	31.2±0.8^{Aa}	22.8±0.09^{Ab}	17.2±0.01^{Ac}	13.1±0.06^{Ad}	26.6±0.02^{Aa}	19.4±0.08^{Ab}	15.9±0.17^{Ac}	12.2±0.67^{Ad}
15	26.4±0.4^{Ba}	21.4±0.11^{Bb}	13.9±0.02^{Bc}	12.6±0.03^{Bd}	24.3±0.42^{Ba}	16.3±0.56^{Bb}	14.8±0.12^{Bc}	9.3±0.22^{Bd}
20	21.7±0.8^{Da}	15.4±0.04^{Db}	9.6±0.05^{Dc}	11.2±0.05^{Dd}	18.2±0.15^{Da}	13.3±0.04^{Db}	9.7±0.2^{Dc}	5.4±0.26^{Dd}
25	20.3±0.29^{Ea}	14.7±0.05^{Eb}	12.0±0.01^{Ec}	11.0±0.03^{Ed}	15.4±0.3^{Ea}	13.9±0.11^{Eb}	8.2±0.43^{Ec}	3.6±0.1^{Ed}

(Means ±SD), n = 3 n = number of experimental replicates, SD = standard deviation

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between the treatments

4.1.3 Effect of acid pretreatment on lignin content in optimum substrate (10 gram) concentration of untreated and acid pre-treated potato peel waste substrate

The effect of acid pretreatment on lignin content was observed for optimum substrate concentration. Significant difference was observed between the acid pretreated and untreated substrates in lignin content. The acid pretreated substrates showed the less lignin content than the untreated substrate, whereas highest removal of lignin percentage (7.4%) was observed in 10gram acid pretreated substrate compared with untreated substrate (14.3%) (Fig.5).This may be one of the reasons why 10gram acid pretreated substrate produced more ethanol when compared with untreated substrate. It has been stated that during the acid pretreatment the structure of lignin is disrupted, thus making the carbohydrates more accessible to enzymes (Yang and Wyman, 2004). With this chemical pretreatment, mainly employing chemical agents such as acids and alkalis can enhance hydrolysis and improve glucose recovery from cellulose because of the removal of hemicelluloses or lignin (Mosier *et al*., 2005). Moreover, diluted acid hydrolysis has been successfully developed for pretreatment for lignocellulosic material. Diluted sulphuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian *et al*., 1997).

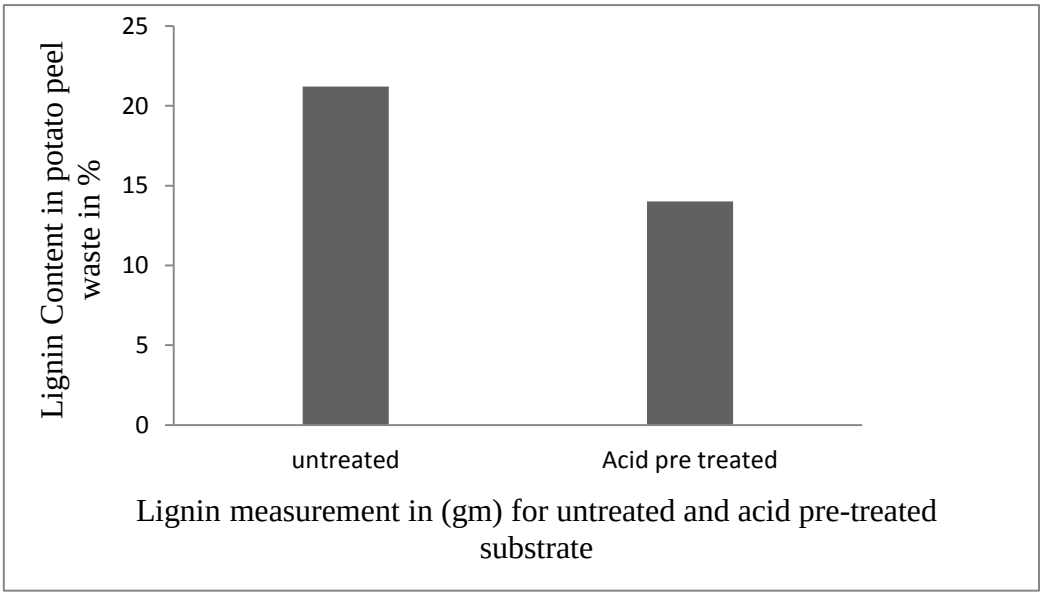

Figure 2. Effect of acid pretreatment on lignin content in optimum substrate (10 gram) concentration of untreated and acid pre-treated potato peel waste

4.2 Effect of pH on ethanol production in optimum substrate concentration (10gram) in untreated and acid-pre-treated potato peel waste

The effect of pH on bio ethanol production was also observed for optimum substrate concentration (10gram) in acid pre-treated and untreated substrate (Fig. 6). The results showed that production of ethanol depended on level of pH. In this experiment, production of ethanol was increased with increasing the level of pH up to 4.5 after this suddenly declined. In line with this, during ethanol fermentation, yeast cells are negatively affected by various stress factors. These stress factors include extreme temperatures, pH, nutrient deficiency, contamination, cell metabolic conditions such as high sugar content tolerance, ethanol tolerance etc. Potential environmental stresses that affect *Saccharomyces cerevisiae* occur during alcoholic fermentation (Bai *et al*., 2008).

The maximum ethanol production (7.46%) was observed at pH 4.5 in acid pre-treated. This result was in accordance with that of Krishna *et al*. (2001) who showed that maximum ethanol yield from wheat straw at pH 4.5. Roukas (1994) studied the effect of pH on ethanol production from corn pod by *S. cerevisiae* and found that the maximum ethanol concentration, ethanol yield, and fermentation efficiency were obtained at pH 4.5. In addition to this, Cheng(2002), Vasconcelos *et al*. (1998) and Nigam (1999) have also observed the maximum ethanol productivity at pH of 4.2 to 4.5.

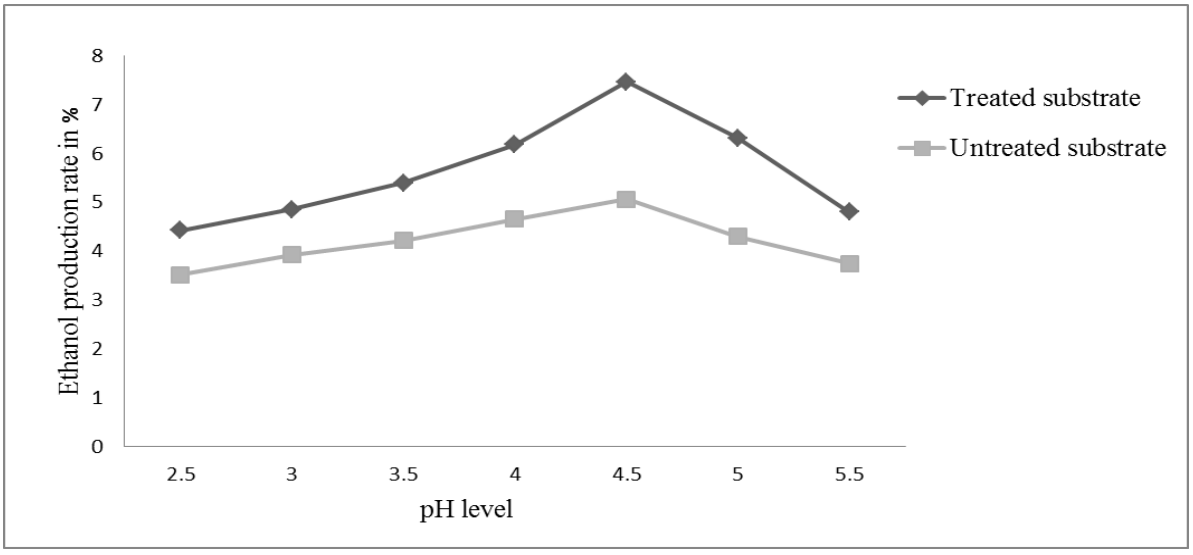

Figure3. Effect of pH on bio-ethanol production in potato peel waste acid pretreated and untreated substrate in 10gram at 4th day fermentation

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4.3. Cumulative ethanol production from treated and untreated substrate of potato peel waste in optimum (10 gram) substrate concentration

The cumulative bio ethanol production was observed in different fermentation periods for optimum substrate concentration in acid pre-treated and untreated substrate. The maximum ethanol productions were observed at 4th day of fermentation in acid pre-treated substrate. If the fermentation period increases the rate ethanol production also decreased in treated and untreated substrate.

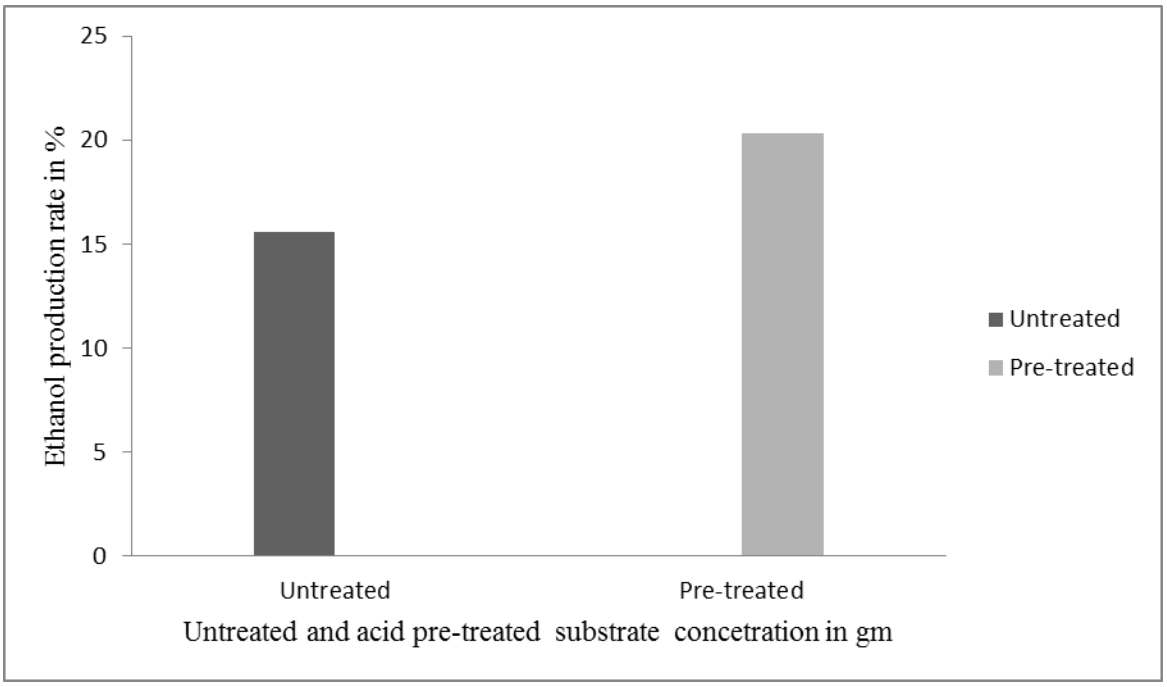

Figure -4: Cumulative ethanol production in acid pre-treated and untreated substrate.

Finally overall cumulative ethanol production was observed for optimum substrate concentration. It indicated that acid pre-treated substrate produce more ethanol than untreated substrate. In line with this, Fadel (2000) and Liimatainnen et al. (2004) showed that potato waste can be a carbon source for yeast during alcohol fermentation from the waste of potato chip industry and different potato cultivations, respectively. Yamadal et al. (2009) also reported that bio ethanol can be produced from the waste of potato processing industry.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

With the aim of ethanol production from potato peel waste, a series of experiments were carried out with different substrate concentration (1gm, 2.5gm, 5gm, 10gm, 15gm, 20gm and 25gm) using batch digester up to 16th day. The fermentation was carried out to obtain optimum substrate concentration for maximum ethanol production. Though all substrate concentrations resulted in ethanol production starting from the fourth day of fermentation, 10 g substrate was found to yield high amount of ethanol throughout the incubation period. Therefore as 10 g substrate is yielding the maximum amount of ethanol, it can be considered as the optimum concentration. Moreover, it was observed that ethanol production decreased as time for fermentation increased

The reducing sugar concentrations were analyzed for optimum substrate concentration (10 gram) in acid pretreated and untreated substrate. The reducing sugars decreased gradually as the fermentation period increased. Moreover, on 4th day of fermentation period, the highest reducing sugar concentration (29.33mg/ml) was obtained from acid pretreated potato peel waste of 10gram substrate. The cell biomass was also observed for optimum substrate concentration (10 gram) and it was indicated that yeast biomass was increased at 4th day. The comparison between acid pretreated and untreated substrate, acid pretreated showed the highest cell biomass concentration.

The acid pretreated substrates showed the less lignin content than the untreated substrate. Whereas, highest removal of lignin percentage (7.4%) was observed in 10gram acid pretreated substrate compared with untreated substrate. The productions of ethanol depended up on level of pH. In this experiment, ethanol production was increased with increasing the level of pH up to 4.5 after this suddenly decline the production rate of ethanol.

5.2. Conclusion

From the experiment, it was proved that the bio-ethanol yield could be produced from potato peel waste. Bio-ethanol production from treated and untreated substrates was statistically significant at $p<0.05$. Among the different substrate concentration 10 gram substrate showed the highest percentage of ethanol production. The substrates were treated with different pH, the maximum ethanol production was observed with pH4.5. Therefore, substrate and pH concentration are directly proportional with ethanol production. Comparatively the reducing sugar utilization was more in acid pretreated substrates than untreated ones. This may be due to substrate limitation and decrease in cell biomass (yeast). The study also identified that substrate with high initial concentration had high lignin content and pretreatment is not only used in releasing reducing sugars, but also reduces the amount of lignin content in the substrates. Therefore, it can be concluded that maximum ethanol productions were achieved with 10gram potato peel acid pretreated substrate with pH4.

5.3 Recommendations

- The study revealed that it is possible to produce bio-ethanol from potato peel waste. Therefore, it is recommended to conduct pilot studies to maximize ethanol yield.
- Further work is again necessary to look at the effect of inhibitor on bio-ethanol production as a result of pretreatment.
- It is also recommended to check the bio-ethanol quality of potato peel waste by Gas chromatography too.
- Further study is very important to describe how absolute bio-ethanol can be produced from potato peel wastes by using rotary evaporator, because it is difficult to make pure ethanol since there are other chemicals that can evaporate below the boiling point of ethanol (78^0C).

6. REFERENCES

Abouzeid, M., and C. Reddy. 1986. Direct fermentation of potato starch ethanol produced by coculture of Aspergilla's Niger and Saccharomyces cerevisiae. *Applied and environmental micro biology* 52(5): 1055-1059.

Adarsh, R.Asha, D.L., Balaji, R.R. 2010. "Production of bio ethanol from pectobacterium carotovorum induced soft rotten potatoes". *African Journal of Microbiology Research; 4:1340-1342.*

Akin-Osaniye BC, Nzelibe HC, Agbaji AS. 2005. Production of ethanol from carica papaya (paw paw) agro waste effect of Saccharification and different treatments on the ethanol yield. *Afr.S. Biotechnology.,* 4(7):657-659.

Akpan I, Uraih N, Obuekuwe and Kenebomah M. 2004.Physicochemical characteristics of Mahihot esculenta plant. *Acta Biotechnological*, 18(1):39-45.

Aldiguier A, S., Alfenores. Cameleyre X., Goma G., Uribelarrea J.L., Guillouet E.S., Molina-Jouve C. 2004. Synergistic temperature and ethanol effect on *Saccharomyces cerevisiae* dynamic behavior in ethanol bio fuel production. *Bio process Biocyst.Eng.* 26, 217-222.

Alvirap, Tomas-PejoE, Ballesteros M, Negro MJ. 2010. Pretreatment technologies for efficient bio ethanol production process on enzymatic hydrolysis: *A review bio resource Technology*, 101(23):4851-61.

Amadi, E. N. Agomuo, And C.O .Ibegbulan, *research methods in Biochemistry* Supreme Publishers, Owerri, Nigeria, 2004. Pp. 93-99. And Technology news Clippings, pp 1-3, and utilization of Bio ethanol fuel, *Applied Energy*. Pp. 2273-2282.

Amutha, R. and G. Paramasamy. 2001. Production of ethanol from liquefied cassava starch *and Methanol*. *US Government Printing Office*; Washington, DC.

Arapoglou D., Varzakas Th., Vlyssides A., Israilides C., 2010. Ethanol production potato peel waste (PPW): *Waste Management* 30(10):1898-902.

Aslam, M. 1999. Ethanol Production for motor Vehicles and Chemical Feedback. *Pakistan Academy of Science, Islamabad*, pp. 3-37.

Ayudhya,C.,Tantimongcolwat,T.,Kongpanpee,T.,Prabkate,P.,&Prachayasittikul,V.2007. Appropriate Technology For The Bioconversion Of Water Hyacinth To Liquid Ethanol: *Future Prospects For Community Strengthening And Sustainable Development*.6:167 176.

Bai, F.W., Anderson, W.A., Moo-young, M. 2008. Ethanol fermentation technologies from sugar and starch feed stocks, *Biotechnology Advances*, 26, 89-105.

Bailey, B. K. 1996. Performance of ethanol as a transportation fuel. Paghes 37-60 in C. E. Wyman, Editor. Handbook on bio ethanol: production and utilization. Taylor and Francis, Bristol, Pennsylvania, USA.

Balat. H. 2006. Investigations of bio ethanol production from potato peel waste. *Applied energy;* 86, 2273-2282.

Banat, I., Nigam, P., singh, D., Marchant, R., Mc Hale, A. 1998. Review: ethanol production at elevated temperatures and alcohols concentrations: Part 1-yeasts in general. *World J Microbial Biotechnology*, 14, and 809-12

Bekatorou, A., Psarianos.C, andKoutins, A. 2006. Production of food grade yeasts. *Food Technology Biotechnology*, 44:407-415

Belayneh, M. Z. 2005. Environmental and socioeconomic impact of irrigation development at Ambibar irrigation project in Ethiopia. M. Sc.Thesis, Arba Minch University, Ethiopia.

Bennett, C. 1971. Spectrophotometric Acid Dichromate Method for the Determination of Ethyl Alcohol .*Am .J. Med. Technol*., 37: 217-220. *BioCycle* 43(12): 38

Bothast, R. J., Nichols, N. N. &Dien, B. S. 1999. Fermentations with new recombinant organisms. *Biotechnology program*, v.15, p. 867-75.

Brosnan MP, Donnelly D, James TC, Bond U. 2000. The stress response is repressed during fermentation in brewery strains of yeast. *J. Appl. Microbial*., 88: 746-755.

Brown, M. A., Levine, M. D., Romm, J. P., Rosenfeld, A. H. &Koomey, J.G. 1998. Engineering and Economic Studies of Energy Technology to Reduce Greenhouse Gas Emision: Opportunities and Changelles. *Annual Review of Energy and the Environment*, 23, 287-385.

Camacho, Ruiz, L, Perez-Guerra N, Perez R .2003. Factors affecting the growth of *S. cerevisiae* in batch culture and in solid sate fermentation. *Ejeafche*, 2(5): 531-541.

Campo, Id., Alegria,I. ,Zazpe, M., Echeverria, M., & Echeverria, I. 2006. Diluted Acid Hydrolysis used to Pretreatment agro-Food wastes for Bio-ethanol Production. *Asian Journal of Experimental Biological Science,* 42: 214- 221.

Chandel AK, Rudravaram, R, Narasu ML, Raov, Ravindra P. 2004. Economics and environmental impact of bio ethanol production technologies: an apparatus. *Bio technology Mal. Bio.Rev.*2 (1): 14-32.

Chandel AK., Rudravaram R, Narasu ML, Rao V, Ravindra P. 2007. Economics and environmental impacts of bio ethanol production technologies: an appraisal. *Bio technology and Molecular Biology Rev.,* 2(1):014-032.

Cheng, C.K., Hajar H. Hani, Ku Syahidah Ku Ismail, 2007. Production of Bio ethanol obtained from oil Palm Emptyfruit Bunch*ICoSM*: 69-72.

De Barros LM, Sodden A, Martens AL, Henschke PA, Langridge, P .1998. Differentiation and species identification of yeast using PCR. *Int. J. Syst. Bacterial*., 48: 279-286.

De Vasconcelos, J.N., Lopes, C.E., and de Franca, F.P. 1998. Yeast immobilization on cane stalk for fermentation. *International sugar J.*100 {1190}, 73-75

Demibras A. 2006. Furfural production from fruit shells by acid catalyzed hydrolysis. Energy source, part A: *Recovery, utilization, and environmental effects*; 28(2):157-165.

Dhopeshwarker, R., Javadav, U., R., D' Souza, L .Jayaprakash, D. 2001. Continuous ethanol production by fermentation of waste banana peels using flocculating yeast. *Indian journal of chemical Technology* 40:B25

Edgard. A, Carolina P, Manunuel R, Juanita F, BaezaJ .2008. Selection of thermo tolerant yeast strain Saccharomyces Cerevisiae for bio ethanol production. *Enzyme microbiology Technol* 43:120-123 crossRef.

Ekunsanmi TJ, Odunfa SA .1990. Ethanol tolerance, sugar tolerance and invertase activities of some yeast strains isolated from steep water of fermenting cassava tubers. *J. Appl. Bacterial.,* 69: 672-675.

Esteghlalian A, Hahimoto A .G, Fenske JJ, Penner MH .1997. Modeling and optimization of the dilute sulphuric acid pretreatment of corn stove, polar and switch grass. *Bioresource Technol.* 59:129-136.

Fadel, M. 2000. Alcohol production obtained from potato industry starchy waste. *Egyptian Journal of microbiology* 35 (3):273-287.

Fernado S, AVikaris, Chandrapal C, Murali N. 2006. Bio refinery: current status, challenges and Future direction. *Energy and Fuel* 20, 1727-1737.

FFC, 2008.Fineli Food Composition Data base, mashed potato powder composition.Available at: http/www. Finely fi / food.phpFoodid=206 and Lang=en Accessed 25 September. Francis. *Food Technology Biotechnology* 44:407-415.

Fregonesi, A.J. Moran-pawo. I. Joekes, J.AugustR.Rodrigue, E.Tonella, K.Althoff. 2007. Continuous Fermentation of sugar cane syrup using immobilized yeast cells. *Bioengineer*, 97: 48-52.

Furukava K, Kitano H, Mizoguchi H, Hara S. 2004. Effect of cellular inositol content carried out for ethanol tolerance of *S. cerevisiae* in sake brewing J. *Bioscience Bioeng.* 98: 107-113.

Hansen, A. C., Q. Zhang, W. Peter, L.Lyne. 1985. Ethanol disedfuel blend a review, *Bioresource Technol.* 96: 277-285.

Hill, J. Nelson, E.Tilman, d.polasky, S. and Tiffancy, D. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol bio fuels. *Proceedings of the national Academy of science, USA*, 103, 11206-11210.

Hinman. ND, Schell.DJ. Riley J, Bergeron, PW Walter PJ. P.1992. Preliminary estimate of the cost of ethanol production for SSF technology. *Applied biochemistry*.34-35(1):639-49.

Isarankura-Na-Ayudhya, C.Tantimongcolwat, T., kongpapee, prabkate, P.Prachayasittikul, V.2007. Appropriate technology for the bio conversion of water hyacinth (Eichhornia crassipes) to liquid ethanol: future prospects for community strengthening and sustainable development. *EXCLI Journal G,* 167-176.

J. Cheng and Y. Sun.2002." hydrolysis of lignocellulosic material for ethanol production: a review. *Bio resource Technology*, vol-83 no.1, pp.1-11.

Jones, A.M., K.C.Thomass, and W.M. Ingledew.1994. Ethanolic fermentation black strap molasses and sugar cane juice using high temperature gravity technology: *Journal of agricultural and food chemistry,* 42(5): p. 1242-1246.

Kannan T.R., G.Sangiliyandi, P. Gunase Karan. 1998. Improved ethanol production from sucrose by a mutant of Zymomonas mobilis lacking sucrases in immobilized cell fermentation. *Enzyme Microb Technol.,* 22: 179-184.

Karakashew D, Thomsen AB. 2007. Angelidakil.Anaerobic biotechnological approaches for production of liquid energy carriers from biomass. *Biotechnology letter*, 29(7):1005-12.

Karim K, Emtiazi G., Taherzadeh M.J. 2006. Ethanol produced from dilute acid pretreatment rice straw by simultaneous Saccharification and fermentation with mucorindicus, Rhizopusoryzzae, and Saccharomyces cerevisiae: *Enzyme microbial Techn*. 40, 138-144.

Kaur, M and P, Kocher. 2002. Ethanol production from molasses and sugar cane juice by an adapted strain of S.cerevisiae. *Ind.J.Microbial*. 42: 255-257.

Krishna AS, Reddy, J. T and ChowdaryGV.2001. Simultaneously Saccharification of lignocellulosic wastes into ethanol using thermo tolerant yeast. *Bioresource. Tchnolo*.177, 193-196.

Krishnan M.S., Taylor F., Davision B.H., Nghiem N.P. 2000. Economic analysis of fuel ethanol and production from corn starch using fluidized-bed- bioreactors. *Bio resource Technology*, 75: 99-105.

Krisztina, K, M.Stefano, George and Z.Guido. 2009. Enzymatic Hydrolysis of Steam Pretreated Lingo-cellulosic Materials with *Trichoderma Atroviride* Enzymes Produced In-House. *Bioresource Technol*ogy, 100:1350-1357.

Kumar D, Barrett DM, Delluiche MJ, Stroeve P.2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production: *Industrial Engineering chemistry Research*, 48(8); 3713-29.

Kupczyk A., Eikilski A. 2002. Bio ethanol szansadlapolskiegorolnictawa.*WiesJutra*, 5(46), 13-25.

Lieckefeldt E, Meyer W, Boner T. 1993. Rapid identification and differentiation of yeasts by DNA and PCR finger printing J. Basic. *Microbiology*, 33: 413-426.

Liimatainnen, H, Kuokkanen T. and Kaariainen J.2004. Development of bio ethanol production obtained from waste potatoes, waste minimization and Resource use optimization. *Conference, June 10thUniversity of Oulu, Finland, and pp*.123-129.

Linde M., Galben M., Zacchi G. 2007. Simultaneous Saccharification and fermentation of steam pretreated barley straw at low enzyme loadings and low yeast concentration; *Enzyme microb. Technol*. 40, 1100-1107.

Maiorella B.L. Blanch HW and Wilke C.R. 1984. Feed component inhibition in Ethanolic fermentation by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, 24, 1155-1166.

Mann, P., S. Siewert**,** J. Totter. 2002. Fermenting potato peels and chips into ethanol. *BioCycle,* 43: 38-40.Direct Link |

Mes-Hartree M, SaddlerJN.1983. The nature of inhibitory materials present in pretreated lignocellulosic substrates which inhibits the enzymatic hydrolysis cellulose. *Biotechnology letters*; 5(8):531-6.*Microbiol.*, 33: 413-426.

Miller, G. 1959. Use of dinitrosalicylic acid used as reagent for determination of reducing sugar; *Analyt. Chem.*, 31:426-428.

Mosier N, Wyman CE, Dale B, Elander R, Lee YY, Holtzapple M .2005. Feature of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technol*. 96(6):673-86.

Nakamura, Y., F. Kobaya, M. Ohnaga, T. Sawada. 1997. Alcohol fermentation of starch by genetic recombinant yeast has gluco-amylase activity. *Biotechnology Bio engineering,* 53:21-25.

Nigam, J.N. 1999. Continuous ethanol production obtained from simple pineapple cannery waste. *J. of Bio ethanol,* 72, 197-202.

Nyachaka C.J. Yawal D. S. Pam G. 2013. Production and performance Evaluation of Bio ethanol fuel from Groundnuts shell waste. *American Journal of engineering Research (AJER) e-ISS*: 2320-0847 P-ISSN: 2320-0936 volume-02, ISSW-12, pp-303-312 www.ajer.org.

Ohgren K., Rudolf A., Galbe M., Zacchi G. 2006. Fuel ethanol produced from pretreated corn Stover using SSF at higher dry matter content. *Biomass Bio energy*. 30, 863-869

Osho A. 2005. Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. *Afr. J. Biotechnology.*, 4: 660-662.

PalmquistE, Habn-HagerdasB.2000. Fermentation of lignocellulosichydrolysisate. Inhibitor and mechanisms of inhibition. Bioresource Technol. 74(1):25-33.

Pessoa JR, Manciha IM, Satos.1997. Acid hydrolysis of hemicelluloses from sugar cane bagasse. Brazilian journal of chemical engineering.14 (3).

Petersson, A., Thomsen, M. H., Hauggaard -Nielsen, H. & Thomsen, A.B. 2007. Potential Bio-ethanol and Biogas Production Using Lingo-cellulosic Biomass from Winter Rye, oilseed Rape and Faba Bean. 31: 812 - 819. Pp14.

Phillips, GO, Williams PA. 2000. Hand book of Hydrocolloids, CRC. *Press, M,* 472pp.

Philppidis GP, Smith TK and Wyman CE. 1993. Biotechnology Bio Engineering. 4(9):846-853

Pramanik, K., & RAO, De. 2005. Kinetic Study On Ethanol Fermentation of Grape Waste Using Saccharomyces Cerevisiae Yeast Isolated From Toddy. 85: 53 -58.

Reed G. 2001. Production of fermentation alcohol used as a fuel source. *The AVI Publishing company, Inc. USA*. 2003.

Renewable Fuel Association (RFA). 2007. Industry statistics available at: http:// www.ethanolrfa.org /industry/statistics/.

Roukas, T. 1994. Solid-state fermentation of carob pods for ethanol production. *Appl.Microbiol Biotechnology*. 41:296-301.

Ruchi Mutreja, Debasish Da**s**, Dinesh Goyal, Arun Goyal .2011. "Bioconversion of agricultural waste to ethanol by SSF using recombinant cellulase from *Clostridium thermocellum*". *Enzyme Research*, Vol 2011, 10 pages. doi:10.4061/2011/340279.

Sengupta S, Jana ML, Sengupta D, Naskar AK. 2000. A note on the estimation of microbial glycosidase activities by dinitrosalicylic acid reagent. Appl.Microbiol. Biotechnology. 53: 732-735.

Sharma-shivappa, R.R, R.R, R.R., Boyette, M.D., and Osborne, J. 2007. A comparision of chemical pretreatment methods for improving Saccharification of cotton stalks. *Bioresource, Technol*., 98,300-11.

Shuler, M.L. and Kargi, F. (2002). Bioprocess Engineering: Basic Concepts, 2nd edition, Prentice Hall, Upper Saddle River, New Jersey

Sivers, M.V., Zacchi, G. 1995. ATechno-economic comparision of three processes for the production of ethanol from pine. Bioresource. Technol.51, 43-52.

Sree NK, Sridharm, Suresh K, Banat IM, VenkateswarRao L .2000. Isolation of thermo tolerant, osmotolerant,occculating Saccharomyces Cerevisiae for ethanol production. *Bioresource Technology*, 72:43-46 crossRef.

Stavrinides A.J., Phipps D. A., Al-Shamma'a A. 2010. Current and developing lignocellulosic pretreatment methods for bio ethanol production. *Bean (Built Environment & Natural Environment) 5th Annual Conference. Liverpool, UK.* -233 p.

Steensma, H.Y, TerLinde JJ .2001. Plasmids with the Crerecombinase and the dominant Nat marker, suitable for use in phototrophic strains of *S. cerevisiae* and *Kluyveromyces lactis*. *Yeast*, 18: 469-472.

Summer, J.B. and G.F.Somers. 2004. Laboratory Experiments in Biological Chemistry. Academic Press, Inc., New York

Sun Y, Cheng J. 2002. Hydrolysis of lignocellulose materials for ethanol production: A Review. *Bioresource Technology*, 83(1); 1-11.

Swiecilo A, Krawiec Z, Wawryn J, Bartosz G .2000. Effect of stress on the life span of the yeast *S. cerevisiae*. *Acta Biochim Polonica,* 47: 355-364.

Swine, M.R., S.Kar, A.K.Sahoo, R.C.Ray. 2007. Ethanol fermentation of mahula (Madhuca latifolial.) flowers using free and immobilized yeast Saccharomyces cerevisiae. *MicrobiologyResearch,* 162:93-98.

Taherzadesh and Karimi.2007. Bioresource, 2(4):707-738. Acid- based hydrolysis process for Ethanol from lignocellulosic cmaterials: A: *A review. Bio resource;* 2:472-499.

Talebnia F. 2007. Ethanol production from cellulosic biomass by encapsulated *Saccharomyces cerevisiae*. PH.D thesis: Chalmers University Technol. Gothenburg (Sweden).

Tasic, M. B., B.V. Konstanntinovic, M.L. Lazic, V.B. Veljkovic. 2009. The acid hydrolysis of potato tuber mass in bio ethanol production. *Biochemical engineering journal,* 43:208-211.

Viswannathan A. 1999. Effect of degree octenyl succinate starch in the emulsification activity on different oil phase; *Journal of envymironmental polymer degradation*, 7:191-196.

Wymann CE, Dale BE, Elander RT, Holtzapple M, LadischMR, Lee YY. 2005a. Coordinated development of leading biomass pretreatment technologies. *Bio resourTechnol,* 96:1959-66.

Yah, C. S., S.E. Iyuke, E.I.Unuabonah, O.Pillay, C. Vishanta and S.M.Tessa. 2010. Temperature optimization for bio ethanol production from corn cabs using mixed yeast strain. *Online Journal of biological science*, 10(2): p.103.

Yamada S., N. Shinomiya, K. Ohbo, M. Sekikawa, y. Oda. 2009. Enzymatic hydrolysis and ethanol fermentation of by products from potato processing plant. *Food science and Technology Research*, 15 (6): 653-658.

Yang, B, Wyman, C. 2008. Pretreatment: The key to unlocking low cost cellulosic ethanol. *Bio fuels, Bio products and Bio refining*, 2(1):26-40.

You, K.M, Rosenfild CL, Knipple DC .2003. Ethanol tolerance in the *S.cerevisiae* is dependent on cellular oleic acid content. *Appl. EnvironmentMicrobiolology*, 69: 1499-1503.