STUDIES ON BIOETHANOL PRODUCTION FROM CASSAVA STEM AND SAWDUST

A PROJECT REPORT

Submitted by

ARUN PANDIAN C
(Reg. No.1120203001)

in partial fulfillment for the requirement of award of the degree

of

M.TECH (BIOTECHNOLOGY)

FACULTY OF TECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE 641 049
(An Autonomous Institution Affiliated to Anna University, Chennai)

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BONAFIDE CERTIFICATE

Certified that this project work entitled **STUDIES ON BIOETHANOL PRODUCTION FROM CASSAVA STEM AND SAWDUST** is the bonafide work of **Mr. ARUN PANDIAN, C (Reg.No. 1120203001)** who carried out the research under my supervision. Certified further that to the best of my knowledge, the work reported herein does not from part of any other thesis or dissertation, on the basis of which, a degree or award was conferred on an earlier occasion on this or any other students.

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ABSTRACT

Cassava stem and Sawdust, wastes generated from sago and timber based industries, are utilized for the bioethanol production. Response surface methodology (RSM) based Box-Behnken design (BBD) was employed to optimize the weak acid and enzymatic hydrolysis for cassava stem and sawdust. In weak acid hydrolysis, three factors considered for this study were oxalic acid concentration (OAC) (0.5 – 1 %), time (10 – 30 min) and reaction temperature solid to liquid ratio (0.05 – 0.20 g/mL) at constant quantity of cassava stem (4.5 g) and temperature 121°C, 15 psi pressure. For enzymatic hydrolysis of sawdust, three factors considered for this study were acetate buffer volume (4-6 mL), incubation temperature (40-50°C) and incubation time (20-60 min) at constant quantity of sawdust (0.5 g) and cellulase enzyme (250 µl). For cassava stem, the maximum total reducing sugars (TRS) concentration produced by weak acid hydrolysis was 2.325 mg/mL at optimum conditions of 1.47 % w/v of OAC, and 29 min. Then the cassava stem hydrolysate was fermented for overliming-detoxified and non-detoxified samples. It was found that maximum ethanol concentration was 1.592 % (v/v) and 1.243 % (v/v) for *Kluyveromyces marxianus* with *Fusarium oxysporum* and *K. marxianus* with *Zymomonas mobilis* for detoxified hydrolysates. Also maximum ethanol concentration was 1.526 % (v/v) and 1.194 % (v/v) for *K. marxianus* with *F. oxysporum* and *K. marxianus* with *Z. mobilis* for non detoxified hydrolysates. For sawdust, the maximum TRS concentration produced by enzymatic hydrolysis was 1.40 mg/mL at optimum conditions of 4.17 mL buffer, 49°C and 21 min. The sawdust hydrolysate was fermented for overliming-detoxified by *Klebsiella oxytoca* and it was found that maximum ethanol concentration was 0.051% v/v.

Keywords: Cassava stem, Sawdust, Weak acid hydrolysis, enzymatic hydrolysis, Fermentation, Ethanol

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LIST OF ABBREVIATIONS

GWe	Gigawatt – electric
GHG	Green house gases
LUC	Land-use change
DP	Degree of polymerization
FFVs	Flexible fuel vehicles
UV	Ultraviolet
DNS	Dinitro salicylic
TRS	Total reducing sugar
MSDS	Material safety data sheet
BBD	Box – Behnken design
OAC	Oxalic acid concentration
SLR	Solid to liquid ratio
CI	Confidence interval
VIF	Variable inflation factor
ANOVA	Analysis of variance
SEM	Scanning electron microscope
FBR	Fluidized bed reactor
MFR	Mixed Flow Reactor
RSM	Response surface methodology

CHAPTER 1
INTRODUCTION

1.1 OVERVIEW

Today, ethanol is used as a wide range of purposes, from producing medicine and synthesizing chemical products to fuelling our heaters, lamps and vehicles. Some of the oldest internal combustion engines actually ran on ethanol, something which makes ethanol history closely intertwined with car history. Today, ethanol vehicles are more popular than ever before. But the history of the intricate relationship between ethanol and man goes much further back than the history of our modern, vehicle filled society.

The fermentation of sugar into ethanol is one of the earliest organic reactions that man learned to carry out and the history of man-made ethanol is very long. Ethanol is a powerful psychoactive substance and ethanol history is filled with accounts detailing its use as a recreational drug. Dried ethanol residue have been found on 9000 year old pottery in China which indicates that Neolithic people in this part of the world may have consumed alcoholic beverages

Beer and wine will normally not develop alcohol content over roughly 15% alcohol by volume, since a higher concentration makes it impossible for most yeast to reproduce. Eventually, humans found out that a higher ethanol concentration could be obtained through distillation. Distillation is a process where a mixture is separated into various components based on their individual volatility. Fermented solutions have been distilled since ancient times in order to produce distilled beverages with high ethanol content.

In cold parts of Central Asia, freeze distillation was discovered and the earliest evidence of it being used dates back to the early middle age. It is known that alcohol was distilled in *Schola Medica Salernitana* in southern Italy during the 12th century, and that fractional distillation was invented by Tadeo Alderotti in the 13th century.

The year 1796 is significant for ethanol history because this is when Johann Tobias Lowitz obtained pure ethanol by filtering distilled ethanol through activated charcoal. Antoine Lavoisier was able to ascertain that ethanol consists of hydrogen, oxygen and carbon, but it wasn't until the early 19th century that the chemical formula was determined by Nicolas-Théodore de Saussure. During the mid 1800s, ethanol became one of the first structural formulas to be determined – another vital step in the history of ethanol. (ethanolhistory.com)

1.1.1 CHALLENGES AND OPPORTUNITIES IN BIOETHANOL PRODUCTION

The strong emphasis on biofuels in the 21st century is attributed primarily to four interrelated factors: (1) increasing atmospheric concentrations of CO_2 and other greenhouse gases (GHGs), (2) risks of abrupt climate change, (3) rapidly increasing global energy demand, and (4) food insecurity affecting more than 1 billion people or about 15% of the global population. Production of biofuels can also improve the rural economy by creation of new jobs. Biomass – based electricity schemes provide more than 9 GWe of generation capacity worldwide (Mirza et al.,, 2008).

1.1.2 ECONOMICAL AND ENVIRONMENTAL IMPACTS ON BIOETHANOL PRODUCTION

In 1925, Henry Ford predicted that the fuel of the future is going to come from every bit of vegetable matter that can be converted. Today Henry Ford's futuristic vision significance can be easily understood. In the present scenario, the importance of alternative fuel source has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for the safe and better environment, with an inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy. The burning fossil fuel at the current rate is likely to create an environmental crisis globally. Use of fossil fuel generates carbon dioxide, methane and a significant quantity of nitrous oxide. Most of these harmful gases are formed due to incomplete combustion of fossil fuel; since ethanol contains 35% oxygen that may result in a more complete combustion of fuel and thus reduces tailpipe emissions.

Biomass utilization into ethanol production offer environmental benefits in terms of non-renewable energy consumption and global warming impact. All environment flows were examined from the product life cycle, its production and extraction from raw materials through intermediate conversion process, transportation, distribution and use. Dilute acid process was found better than the enzyme process in terms of greenhouse gas potential, natural resource depletion, acidification potential and eutrophication potential. (Chandel et al.,, 2007)

1.1.3 PAST, CURRENT AND FUTURE LAND USE IN BIOETHANOL PRODUCTION

Production of biofuels requires cultivation of the biofuel crops, processing, and transportation, leading to greenhouse gas emissions (GHG). Presently, most biofuels are produced based on conventional food and feed crops. Technologies for the

conversion of lignocellulosic feedstocks, such as perennial grasses or short rotation woody crops, have yet to become commercially available. However, the rapidly growing demand for biofuels may require new cropland which is made available through the conversion of native ecosystems such as peat lands, forests, grasslands, fallow lands, and marginal crop lands. Expansion of biofuel crop cultivation leads to direct and also in many instances indirect land-use change (LUC). Globally arable land (i.e. land that is planted to temporary crops or is temporarily fallow) accounts for 28% of the total agricultural area of 4967 Mha. Permanent pasture accounts for 68% of agricultural area, the most dominant land use supporting very low intensity cattle production on a large share of the land claimed.

Expansion of biofuel crops are likely to come from the conversion of permanent pasture area or forests. Pasture is often marginal cropland because of low precipitation or rocky, infertile soils. However, demand for crops is large enough to drive the conversion of pasture, even if it is marginal cropland. The increasing pressure on permanent pasture will mainly come from the expansion of arable land (largely crop land particularly in developing countries). Arable land is projected by, FAO 2008, to increase by 6% and 12% in 2015 and 2030 respectively compared to 1999 area of 956 Mha. (Ravindranath et al.,, 2009)

1.1.4 KEY BARRIERS OF BIOETHANOL PRODUCTION

Using lignocellulosic residues as a substrate of bioethanol production is a promising technology to depreciate the dependency of conventional fuels. Although the availability of residues are abundant, the technology to produce bioethanol is limited due to certain barriers. Some of the key barriers of bioethanol production are discussed below

- Factors like lignin content, crystallinity of cellulose and particle size, limit the digestibility of the hemicellulose and cellulose present in the lignocellulosic biomass
- Hydrolysis process is an important step in bioethanol production. But due to complex nature of lignocellulosic residues it is not easy to digest by any enzymes or acids, there are some factors which limits the hydrolysis process are degree of polymerization (DP), moisture content, available surface area and lignin content
- The pore size of the substrate in relation to the size of the enzymes also a barrier in the enzymatic hydrolysis of lignocellulosic biomass
- The recalcitrance of lignocellulose is one of the major barriers to the economical production of bioethanol. Typically, both physical and chemical pretreatments have been used. Physical pretreatment refers to the reduction of physical size of biomass feedstock to increase enzyme-accessible surface areas and decrease the crystallinity of cellulose. Chemical pretreatment refers to the process of using chemicals to remove or modify key chemical components that protect cellulose in biomass, mainly hemicellulose and lignin. Lack of low-cost and high-activity cellulose hydrolytic enzymes is another barrier to cellulosic bioethanol production (Hendriks and Zeeman, 2009)

1.1.5 BIOETHANOL AND VEHICLE COMPATIBILITY

All petrol vehicles are able to operate on petrol and ethanol blends up to 10 percent. But due to energy crisis, the demand of petrol goes higher in recent years. For this reason Flexible Fuel Vehicles (FFVs) are developed to reduce the dependant on petrol. FFVs are engineered to run on blends of gasoline and ethanol in any percentage up to 85 percent. E85 is 85 percent ethanol and 15 percent gasoline. (alternativefuels.about.com, 2013)

Due to higher alcohol content in E85 (85 % ethanol), it can deteriorate the non-synthetic, rubber parts in fuel systems which is considered to be a caustic compound. Therefore, these systems require synthetic gaskets and seals. In addition, some metals can be damaged by high levels of alcohol and vehicles with these systems often need to have stainless steel or another composite metal that will not be damaged by the alcohol.

Along with alcohol's caustic nature, it also has different burn characteristics than petrol. To compensate, the engine management systems are designed with additional specific sensors to measure the alcohol concentration and signal the on-board computer to adjust parameters such as ignition time, fuel flow rate and air – fuel ratio. (http://alternativefuels.about.com/od/generalmaintenance/a/ffvmaint.htm). The E-85 fuelled vehicle is better vehicle than the gasoline fuelled car by balancing of all the 3E"s the energy, environmental and economic aspects. E-85 fuelled FFV (fossil fuelled vehicle) is about 15% higher efficient when compared to gasoline fuelled car. It also lowers the pollutant emission viz. particulate matter, CO_2, CO, NO_x emission than gasoline fuelled car. (Chandel et al.,, 2007)

1.2 MOTIVATION

Since 1987, it is been a steady increase in crude oil price in the world. The main reasons for the drastic change in the price are increase in population, over consumption and energy. Fossil fuel is almost devastated by our actions and it cannot be recovered but rather we could be versatile. For this reason, production of fuels from biological source is a sole promising technology for future global sustainment.

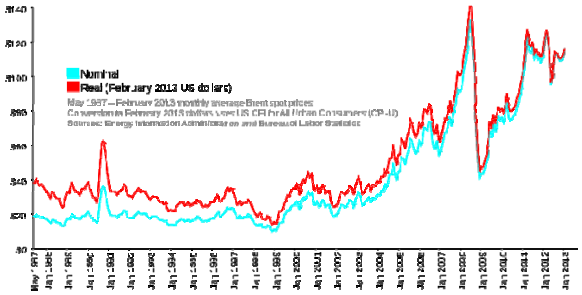


Fig. 1.1 Crude oil price changes from May 1987 to Jan 2013

In the above graph it is clear that, in over the decades the crude oil price is increased very often and it will continue in further year. This is the right time to be versatile and adopting better technology to fulfil the global requirement. And it is required by the highly populated countries like India to meet its requirement and decrease its demand.

1.3 OBJECTIVES

- To characterize the cassava stem and sawdust
- To develop an effective pretreatment technology that does not require expensive chemicals and high pressure equipments
- To optimize pretreatment and hydrolysis process for the residues using response surface methodology
- To optimize co-fermentation process for the residues

CHAPTER 2
REVIEW OF LITERATURE

2.1 GENERAL

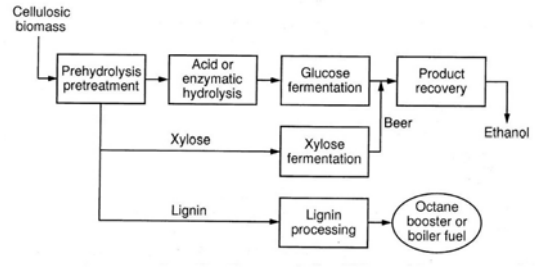


Fig 2.1 Overall process flow diagram for production of ethanol from lignocellulosic material

Lignocellulosic biomass has been put forward as a feasible alternative due to its abundance in nature and the large quantities generated as waste from agricultural and industrial activities. Lignocellulosic biomass is primarily composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are the main substrates used for ethanol production, but lignin is composed of aromatic lignols that need to be separated and removed before enzymatic hydrolysis. Today, expensive pretreatments are the main reason for unsuccessful implementation of complex lignocellulosic biomasses as a starting material for ethanol production

Production of ethanol from lignocellulosic biomass contains three major processes, including pretreatment, hydrolysis, and fermentation. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub microscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. Hydrolysis refers to the processes that convert the polysaccharides into monomeric sugars. The fermentable sugars obtained from hydrolysis process could be fermented into ethanol by ethanol producing microorganisms, which can be either naturally occurred or genetically modified.

2.2 PRETREATMENT METHODS

Table 2.1 Pretreatment methods for various substrates

S.No.	Substrate	Pretreatment Method	Reference
1	Switch grass and Sugarcane Bagasse	Steam Explosion	Ewanick and Bura (2011)
2	Animal manure	Acid treatment	Wen et al., (2003)
3	Cassava Starch	Sodium acetate buffer	Ruiz et al., (2011)
4	Cassava pulp	Drying and Sieved	Rattanachomsri et al., (2009)
5	Rice straw	Acid pretreatment	Kim et al., (2010)
6	Photoperiod sensitive (PS) sorghum	Acid treatment	Xu et al., (2011)
7	Norway spruce	Pressurized hot water	Leppa¨nen et al., (2010)
8	Algae	Drying	Vardon et al., (2012)
9	Sunflower stalks	Milling	Martinez et al., (2012)
10	Rice straw	Alkali pretreatment	Watanabe et al., (2012)
11	Wheat straw	Alkali pretreatment	Singh and Bishnoi (2013)
12	Corn, wheat bran and pine sawdust	Alkali pretreatment	Giordano et al., (2011)
13	Rice straw	Alkali pretreatment	Singh and Bishnoi (2012)
14	Corn stover	Alkali pretreatment	Zhu et al., (2010)
15	Rapeseed straw	Wet and Dry process	Wi et al., (2011)
16	*Miscanthus x giganteus*	Acid pretreatment	Haverty et al., (2012)
17	Sugarcane Bagasse	Acid pretreatment	Candido et al., (2012)
18	*Miscanthus x giganteus*	Acid pretreatment	Vanderghem et al., (2012)
19	Rice straw	Acid pretreatment	Kang et al., (2011)
20	Spruce wood chips	Solvent pretreatment	Shafiei et al., (2012)
21	Cassava waste water	Gasoline treated	Aisien et al., (2010)
22	Sugarcane Bagasse	Water prehydrolysis	Silverstein et al., (2006)
23	Cotton stalks	Acid - Alkali treatment	Abril et al., (2012)
24	Corn stover	Milled, washed and dried followed by hot steam	Zhang et al., (2011)
25	Fresh-chipped spruce (*Picea abies*) free from bark	Milled and sieved followed by acid treatment	Monavaroi et al., (2011)
26	Spruce bark	Steam Explosion (SE)and Hot Water Extraction (HWE)	Kemppainen et al., (2012)
27	Maize	Mashing	Pejin et al., (2009)
28	Sorghum	Milling	Corredor et al., (2005)
29	Bagasse, corn cob, and rice straw	Acid pretreatment	Sumphanwanich et al., (2008)
30	Sugarcane Bagasse, Cassava stalks, peanut, shells, and rice hulls and rice hulls	Dilute acid prehydrolysis	Martin et al., (2007)
31	Sweet sorghum fresh stalks	Chopping	Siwarasak et al., (2011)
32	Old oil palm trunk	Chopping	Yamada et al., (2010)
33	Mango	Washing	Reddy et al., (2007)
34	Tapioca Stem var. 226 White Rose to Ethanol	Dilute acid and alkali pretreatment	Magesh et al., (2011)
35	Wheat straw	Milling, Sieving and Soaking	Olofsson et al., (2010)
36	Cassava bagasse	Milling and Hydrothermal treatment	Gaewchingduang and Pengthemkeerati (2010)
37	Cotton Gin Waste	Steam explosion	Shen and Agblevor (2008)
38	Barley straw, Hemp, Grass, Cellulose and Newspaper	Chemical pretreatment	Sigurbjornsdottir and Orlygsson (2012)
39	Sugarcane bagasse and Straw	Ball Milling (BM) and Wet Disk Milling (WDM) pretreatment	Silva et al., (2010)
40	Corncob	Acid and Alkali pretreatment	Zhang et al., (2010)
41	Waste newsprint	Defibration	Park et al., (2010)
42	X-ER	Milling	Liu et al., (2012)
43	Sorghum	Alkali pretreatment	Chen et al., (2012)
44	Water hyacinth	Alkaline–oxidative (A/O) pretreatment	Ahn et al., (2012)
45	*Kappaphycus alvarezii* (Red seaweed)	Drying	Meinita et al., (2012)
46	*Kappaphhycus alvarezii* (Seaweed)	Drying	Meinita et al., (2012)
47	Olive Cake	Grounding	Asli and Qatibi (2009)
48	Wheat Straw	Alkali pretreatment	Han et al., (2009)
49	White Rose Tapioca Stem	Crushing and Milling	Magesh et al., (2011)
50	Kinnow mandarin (*Citrus reticulata*) waste	Hydrothermal	Oberoi et al., (2010)
51	Corn stover	Alkaline peroxide	Banerjee et al., (2011)
52	Wheat straw	Lime treatment	Mass et al., (2008)
53	*Eucalyptus globulus* wood	Hydrothermal	Romani et al., (2010)
54	Hazelnut shell	Alkaline treatment	Arslan and Eken-Saracoglu (2010)
55	Corn fibre	SO_2-Catalyzed Steam	Bura et al., (2003)
56	Rice straw	Extrusion/ Extraction	Chen et al., (2011)
57	*Salix*	Steam	Sassner et al., (2006)
58	Wheat straw	Supercritical CO_2	Alinia et al., (2010)
59	*Eucalyptus globulus* wood	Acid treatment	Gütsch et al., (2012)
60	Wheat straw	Steam, Steam/Acetic	Zabihi et al., (2010)

		acid and Steam/Ethanol	
61	Rice straw	Dilute acid treatment	Karimi et al., (2006)
62	Rice straw	Ammonia and Ionic liquid	Nguyen et al., (2010)
63	Wheat and Rice straw	Fungal pretreatment	Patel et al., (2007)

2.3 HYDROLYSIS OF CELLULOSE

Table 2.2 Types of hydrolysis process

S.No.	Hydrolysis	Reference
1	Enzymatic method	Alinia et al., (2010)
2	Cellulolytic organism	Mansfield, S.D. and Meder, R. (2003)
3	Acid – catalyzed	Gütsch, J.S. et al., (2012)

2.4 DETOXIFICATION METHODS

Table 2.3 Types of detoxification methods

S.No.	Substrate	Detoxification Method	Reference
1	Corn stover	Bio-detoxification using *Aspergillus nidulans*	Yu et al., (2011)
2	Steam exploded wheat straw	Laccase detoxification	Moreno et al., (2012)
3	Lignocellulosic hydrolysate	*In situ* detoxification	Tian et al., (2009)
4	Red seaweek	Activation carbon and overliming	Meinita et al., (2012)
5	Corncob	In situ detoxification by *Clavispora* strain NRRL T-50464	Liu et al., (2011)
6	Corn stover	Activated carbon	Banerjee et al., (2011)
7	Rice straw	Overliming	Chen et al., (2011)
8	Lignocellulose	Biological method	Zhang et al., (2011)
9	Hazelnut shell	Overliming and Charcoal	Arslan et al., (2010)
10	D – xylose	*In situ* detoxification by *Saccharomyces cerevisiae* strain NRRLY-50049	Ma et al., (2012)
11	Black locust	Ammonia	Garlock et al., (2012)
12	Lignocellulose	*In situ* detoxification by *Saccharomyces cerevisiae*	Geddes et al., (2011)
13	Wheat straw	Overliming using calcium hydroxide	Saha et al., (2005)
14	Corn cub hemicellulose hydrolysate	Overliming and overliming plus activated carbon	Ge et al., (2011)
15	Grass straw	Overliming	Kumar et al., (2011)

2.5 CO-FERMENTATION PROCESS

Table 2.4 Co-fermenting organisms for various substrates

S.No.	Substrate	Co-fermenting organism	Reference
1	Wheat straw	*Saccharomyces cerevisiae*, TMB3400	Olofsson et al., (2010)
2	Hazelnut shell	*Pichia Stipitis*	Arslan, et al., (2010)
3	Corn stover	*Saccharomyces cerevisiae* 424A(LNH-ST)	Jin et al.,, (2012)

2.6 ETHANOL PRODUCTION PROCESS FROM LIGNO-CELLULOSIC MATERIAL

Ewanick and Bura, (2011) determined the effect of moisture content of Switchgrass and Sugarcane bagasse on overall ethanol yield. The overall ethanol yield after simultaneous saccharification and fermentation of hexose was 18 - 28% higher in samples that were not soaked with 3% w/w SO_2 and water.

Xu et al., (2011) investigated the growing high-starch duckweed for its conversion to bioethanol as a novel technology to supplement maize-based production. The ethanol yield of duckweed reached 6.42×10^3 kg/ha, about 50% higher than that of maize-based ethanol production, which makes duckweed a competitive starch source for fuel ethanol production.

Pramanik, (2003) dealt with the development of a *Saccharomyces cerevisiae* yeast strain from toddy and the study of important process parameters which will facilitate the fermentation of sugar to alcohol using the developed strain. A higher concentration and yield of ethanol were obtained using a yeast strain developed from toddy compared to baker's yeast *Saccharomyces cerevisiae*.

Wen et al., (2003) converted animal manure into value-added products provide a potential alternatives for treatment and disposal of such materials. The optimized hydrolysis process achieved a glucose yield of 11.32g/100g manure, which corresponded to about 40% cellulose conversion.

Antonini et al., (2005) presented here three approaches to this subject: the distribution of killer phenotype among the yeasts from the process; the selection of ethanol-producing strains with killer activity; and the discrimination (fingerprinting) of yeast strains using their differential sensitivity to killer toxins and finally concluded that the selection of yeast strains capable to express the killer activity in fermentation conditions is an interesting and useful approach, which could give them competitive advantage. Besides, the killer system may be also an effective and inexpensive tool for the yeast fingerprinting.

Razmovski and Pejin, (1996) immobilized the *Saccharomyces diastaticus* cell onto beech wood chips of different particle size and three pH values. Ethanol production and the respiration quotient (RQ) were at a maximum at a dilution rate of 0.16/h. The reactor was operated under steady-state conditions for 30 d.

Kim et al., (2010) used cost effective lignocellulosic biomass for bio-based chemical production requires the discovery of novel strains and processes. They used *Lactobacillus pentosus* JH5XP5 for the utilization of glucose and pentoses from rice straw as lignocellulosic biomass. It produced a significant amount of ethanol without acetate formation. The yields of ethanol were 2.0 to 2.5-fold higher than those of lactate when glucose, galactose or maltose was used either as a single carbon source or simultaneously with glucose.

Xu et al., (2011) studied the Photoperiod Sensitive (PS) sorghum which has the great potential for bioethanol production. Under the processed conditions the total ethanol yield is 74.5% (about 0.2 g ethanol from 1 g PS sorghum) and detailed mass balance was also provided.

Leppa¨nen et al., (2010) extracted Norway spruce saw meal with pressurized hot water at 120–240°C using a flow-through system. Partial degradation of cellulose seemed to take place only at 240°C. Of the total amount of extracted hemicelluloses, 4–22% was hydrolyzed to monosaccharides.

Xu and Tschirner, (2011) investigated the most critical steps in producing lignocellulose-based bio-ethanol through consolidated bioprocessing (CBP). They were investigated with a co-culture consisting of *Clostridium themocellum* and *Clostridium thermolacticum*. The ethanol yield observed in the coculture was higher (up to twofold) than in mono-cultures, especially in MCC fermentation. The ethanol yield (as a percentage of the theoretical maximum) observed was 75% (w/w) for MCC and 90% (w/w) for xylose.

Martinez et al., (2012) analysed the effect exerted by the phosphoric acid concentration (C_{Ao}) in sunflower-stalk hydrolysis at 95°C, considering the results in relation to the yields in D-glucose and total reducing sugars. The hydrolysates were fermented with *Hansenula polymorpha*, ATCC 34438, registering better results in the production of ethanol that of xylitol. For an initial concentration of total reducing sugars of 13.3 g/L, xylitol yield was 0.023 g/g, and ethanol yield was 0.14 g/g

Moreno et al., (2011) evaluated to detoxify the whole slurry from steam-exploded wheat straw. For it, two different strategies, laccase treatment before or after enzymatic hydrolysis, were employed and the thermotolerant yeast *Kluyveromyces marxianus* used as a fermenting organisms

Jin et al., (2011) investigated Xylose consumption by *Saccharomyces cerevisiae* 424A (LNH-ST) during Simultaneous Saccharification and Co-Fermentation (SSCF) of AFEXTM pretreated switchgrass was inhibited by unhydrolyzed solids. Low temperature (30°C) and ethanol inhibition were shown to be the factors limiting hydrolysis rate and hence productivity during SSCF

Watanabe et al., (2012) developed Repeated-batch Simultaneous Saccharification and Fermentation (SSF) of alkali-treated rice straw using immobilized yeast to produce ethanol. In repeated-batch SSF of 20% (w/w) rice straw, stable ethanol production of approx. 38 g/L and an ethanol yield of 84.7% were obtained

Singh and Bishnoi, (2012) used microwave alkali pretreated wheat straw as substrate for ethanol production from *Saccharomyces cerevisiae*. Under optimum conditions ethanol production studied at bioreactor level and obtained ethanol concentration 16.4 g/L with ethanol productivities 0.45 g/L/h obtained at pH 5.5, temperature 30°C, inoculums level 3.3% and total reducing sugar concentration 6.5%

Giordano et al., (2011) compared the classic Plackett–Burman design (PB) ANOVA analysis and a genetic algorithm (GA) approach to identify significant factors. Interestingly, some interactions were found to be significant by the GA approach and allowed to identify significant factors, that otherwise, based only in the classic PB analysis, would have not been taken into account in a further optimization step

Singh and Bishnoi, (2011) used statistical experimental designs were used for optimization of critical nutrients and process variables for ethanol production. Under optimum conditions ethanol production studied at fermenter level and ethanol concentration 19.2 g/L, ethanol productivity 0.53 g/L/h and ethanol yield to consumed sugar 0.50 g/g obtained

Sills and Gossett, (2011) used Fourier Transform Infrared, Attenuated Total Reflectance (FTIR-ATR) spectroscopy combined with partial least squares (PLS) regression accurately predicted 72 h glucose and xylose conversions (g sugars/100 g potential sugars) and yields (g sugars/100 g dry solids) from cellulase- mediated hydrolysis of alkali-pretreated lignocellulose. Glucose conversion increased with NaOH loading for all biomasses with maximum occurring at the highest pretreatment level (20g NaOH per 100 g TS)

Zhu et al., (2010) applied alkaline pretreatment to enhance biogas production from corn stover through solid-state anaerobic digestion. Different NaOH loadings (1%, 2.5%, 5.0% and 7.5% (w/w)) were tested for solid-state pretreatment of corn stover. The highest biogas yield of 372.4 L/kg VS was obtained with 5% NaOH-pretreated corn stover, which was 37.0% higher than that of the untreated corn stover. However, a higher NaOH loading of 7.5% caused faster production of volatile fatty acids during the hydrolysis

Romero et al., (2005) studied the feasibility of using phosphoric acid to hydrolyze the hemicellulosic fraction of olive tree pruning, as a step in the bioconversion process to produce ethanol. Milled olive tree pruning was submitted to hydrolysis at 90°C by phosphoric acid in a concentration range 0.3–8 N for 240 min. The hydrolysates were then fermented by *Pachysolen tannophilus*. The maximum ethanol yield (0.38 kg/kg, equivalent to 74.5% of the theoretical yield) was obtained when hydrolysing with N/2 phosphoric acid

Wi et al., (2010) found a pretreatment process that enhances enzymatic conversion of biomass to sugars. Rapeseed straw was pretreated by two processes: a wet process involving wet milling plus a popping treatment, and a dry process involving popping plus dry milling. In enzymatic hydrolysis performance, the wet process presented the best glucose yield, with a 93.1% conversion, while the dry process yielded 69.6%, and the un-pretreated process yielded <20%.

Yu et al., (2010) investigated the process of ethanol production from Steam-Exploded Corn Stover (SECS), a cellulose-degradation strain of *Aspergillus nidulans* (FLZ10), whether it could remove the inhibitors released from steam exploded pretreatment, and thereby be used for biological detoxification on *Saccharomyces cerevisiae*. An ethanol yield of 0.45 g/g on glucose was obtained in the hydrolysate biodetoxified by *Aspergillus nidulans* FLZ10.

Haverty et al., (2012) dealt with the novel approach to the performic acid pulping of biomass enables effective delignification and fractionation in a time frame not achieved heretofore. An autothermal decomposition reaction was triggered when 100 mg/L $Fe_2(SO_4)_3$ in 4.0 M NaOH was added to 5% or 7.5% H_2O_2 in aqueous formic acid containing chipped *Miscanthus* x *giganteus*. Hemicellulose removal to the liquor was 68% and 89% for the 5% and 7.5% peroxide solutions. Crystalline cellulose yields were >99% and >95% and the rate of glucose release from cellulase digestion of the pulps in 24 h was more than 20-fold that for the raw *Miscanthus*

Vanderghem et al., (2011) employed a Box–Behnken experimental design and response surface methodology to optimize the pretreatment parameters of a formic/acetic acid delignification treatment of *Miscanthus* × *giganteus* for enzymatic hydrolysis. According to the response surface analysis the optimum conditions predicted for a maximum enzymatic digestibility of the glucan (75.3%) would be obtained using a cooking time of 3 h, at 107°C and with a formic acid/acetic acid/water is 40/40/20 (%)

Kang et al., (2011) studied the pretreatment of rice straw with hypochlorite-hydrogen peroxide (Ox-B) solution. The optimal pretreatment conditions were determined via response surface methodology, and the pretreated rice straw was hydrolyzed with enzymes. The fermentation of enzymatic hydrolysates containing 8.14 g/L D-glucose and 4.49 g/L D-xylose with *Pichia stipitis* generated 3.65 g/L of ethanol with a corresponding yield of 0.37 g/g. The maximum possible ethanol conversion rate is 72.54%

Shafiei et al., (2012) used the pretreatment with three green cellulose solvents, N-Methylmorpholine-N-oxide (NMMO), 1-Ethyl-3- Methylimidazolium Acetate ([EMIM][OAc]), and 1-Butyl-3-Methylimidazolium Acetate ([BMIM][OAc]) for improvement of ethanol production. The ethanol yield from the untreated spruce chips and powder were 2.7% and 9.7% of the maximum theoretical yield, respectively, whereas pretreatment of these materials with [EMIM][OAc] improved the ethanol yield to 66.8% and 81.5%, respectively.

Aisien et al., (2010) investigated the suitability of blending ethanol produced from cassava waste water with gasoline as a source of automobile fuel. The results show that 7% ethanol blend increased the Reid vapour pressure (RVP) to 8 psi, reduced T_{50} to 57°C and driveability index (D.I) was reduced to 933.8. 10% ethanol blend

increased the R.V.P to 8.6 psi, reduced T_{50} to 67^{o}C and D.I was reduced to 970.7. Corresponding values for 20% and 30% were 8.8 psi, 68^{o}C, 979.7 and 9 psi, 72^{o}C, 926.6 respectively. A linear increase in research octane number (R.O.N) with a slope of 0.3 was observed

Silverstein et al., (2006) investigated the effectiveness of Sulphuric acid (H_2SO_4), Sodium hydroxide (NaOH), Hydrogen peroxide (H_2O_2), and Ozone pretreatments for conversion of cotton stalks to ethanol. Sulphuric acid pretreatment resulted in the highest xylan reduction (95.23% for 2% sulphuric acid, 90 min, 121°C/15 psi) but the lowest cellulose to glucose conversion during hydrolysis (23.85%). Sodium hydroxide pretreatment resulted in the highest level of delignification (65.63% for 2% NaOH, 90 min, 121°C/15 psi) and cellulose conversion (60.8%). Hydrogen peroxide pretreatment resulted in significantly lower ($p \leq 0.05$) delignification (maximum of 29.51% for 2% of H_2O_2, 30 min, 121°C/15 psi) and cellulose conversion (49.8%) than sodium hydroxide pretreatment, but had a higher ($p \leq 0.05$) cellulose conversion than sulphuric acid pretreatment. Ozone did not cause any significant changes in lignin, xylan, or glucan contents over time

Zhang et al., (2011) extensively studied two rarely noticed but important parameters of the dilute sulfuric acid pretreatment of lignocellulose biomass, the feedstock filling ratio to the pretreatment reactor and the solids/liquid pre-soaking ratio. This method was applied to various lignocellulose feedstocks successfully and provided a practical means to produce ethanol economically feasible

Monavari et al., (2010) studied the employment of metal salts in dilute-acid pretreatment the severity can be reduced due to reduced activation energy. This study reports on a dilute-acid steam pretreatment of spruce chips by addition of a small amount of Ferrous sulfate to the acid catalyst, i.e., SO_2, H_2SO_3 or H_2SO_4. The utilization of Ferrous sulfate resulted in a slightly increased overall glucose yield (from 74 to 78% of the theoretical value) in pretreatment with SO_2 and H_2SO_3

Kemppainen et al., (2012) studied the enzymatic hydrolysis and fermentation of spruce bark sugars to ethanol were studied after three different pretreatments: Steam Explosion (SE), Hot Water Extraction (HWE) at 80°C, and Sequential Hot Water Extraction and Steam Explosion (HWE + SE), and the recovery of different components was determined during the pretreatments. The best steam explosion conditions were 5 min at 190°C without acid catalyst. Ethanol was produced efficiently with the yeast *Saccharomyces cerevisiae* from the pretreated and hydrolysed materials suggesting the suitability of spruce bark to various lignocellulosic ethanol process concepts

Corredor et al., (2005) investigated the effect of decortication as a pretreatment method on ethanol production from sorghum as well as its impact on Distiller's Dry Grains with Solubles (DDGS) quality. Ethanol yield was 3.3 to 11.1% for sorghum with 10% decortication and increased 7.6 to 18.1% for sorghums with 20% decortication

Sumphanwanich et al., (2008) found that bagasse, corn cob, and rice straw agricultural wastes consist of 37, 39 and 34% cellulose and 24, 41 and 22% hemicellulose respectively, on a dry solid (w/w) basis. Pretreatment by 141 mM Sulphuric acid, bagasse waste released glucose (134 mg/g) at a higher level than that from corn cob (75 mg/g) and rice straw (8 mg/g). Ethanol conversion yield of corn cob hydrolysate was 0.45± 0.006 g/g and for bagasse hydrolysate was 0.49 ± 0.007 g/g

Martin et al., (2007) investigated the potential of dilute-acid prehydrolysis as a pretreatment method for sugarcane bagasse, rice hulls, peanut shells, and cassava stalks. The prehydrolysis was performed at 122°C during 20, 40, or 60 min using 2% H_2SO_4 at a solid-to-liquid ratio of 1:10. All prehydrolysates were readily fermentable by *Saccharomyces cerevisiae*. Sugarcane bagasse produces 73–81% of xylan, Rice hulls produce 46.9 - 61.4% of glucose, Peanut Shells and Cassava Stalks produce 43.6 and 45.1% of xylan respectively

Garrote et al., (2001) treated the *Eucalyptus* wood samples with water under selected operational conditions (autohydrolysis reaction) to obtain liquid phase containing hemicellulose-degrading products (mainly acetylated xylooligosaccharides, xylose and acetic acid). Autohydrolysis is leading to fractionation of biomass into soluble sugar oligomers for ethanol production

Siwarasak et al., (2011) investigated the use of *Trichoderma reesei* RT-P1 crude enzyme powder and of this powder with 10% v/v *Saccharomyces cerevisiae* for ethanol fermentation of sweet sorghum fresh stalks. At the optimal conditions above, ethanol concentration, productivity and yield of the Cowley cultivar (35.00 g/L, 0.18 g/L h and 0.38 g ethanol/g substrate, respectively) were higher than those of the Keller cultivar (20.46 g/L, 0.11 g/L h and 0.28 g ethanol/g substrate)

Saucedo-Luna et al., (2010) characterized the chemical composition of Bagasse of Agave Tequilana (BAT), which was further saccharified and fermented to produce ethanol. BAT was constituted by cellulose (42%), hemicellulose (20%), lignin (15%), and other (23%). Saccharification of BAT was carried out at 147°C with 2% Sulfuric acid for 15 min, yielding 25.8 g/l of fermentable sugars, corresponding to 36.1% of saccharificable material. The final optimized process generated 8.99 g ethanol/50 g of BAT, corresponding to an overall 56.75% of theoretical ethanol (w/w)

Magesh et al., (2011) studied the Simultaneous Saccharification and Fermentation (SSF) of tapioca stem var. 226 white rose to ethanol using cellulase enzyme and *Saccharomyces cerevisiae* in a fermentor. The optimum values of particle size, substrate concentration, pH and temperature were found to be 100 mesh size, 50 g/l, 5 and 35°C respectively with the maximum ethanol concentration of 13.6 g/L

Olofsson et al., (2010) recognized Simultaneous Saccharification and Co-Fermentation (SSCF) as a feasible option for ethanol production from xylose-rich lignocellulosic materials. By using both enzyme and substrate feeding, the xylose conversion in SSCF could be increased from 40% to 50% in comparison to substrate feeding only. In addition, by this design of the feeding strategy, it was possible to process a WIS content corresponding to 11% in SSCF and obtain an ethanol yield on fermentable sugars of 0.35 g/g

Gaewchingduang and Pengthemkeerati, (2010) investigated the optimal condition for hydrothermally pretreating cassava baggasses with or without acid addition. For acid hydrolysis, pretreating cassava baggasses with sulfuric acid at 120^{o}C for 60 min gave a maximum reducing sugar yield. enzymatic hydrolysis in a combination with hydrothermal pretreatment was an alternative to enhance efficiency reducing sugar production from cassava bagasse

Shen and Agblevor, (2008) investigated the hydrolytic kinetics of Cotton Gin Waste (CGW) at various initial concentrations of two enzymes, Novozymes NS50052 and Spezyme AO3117. The concentrations of reducing sugars reached 6.41 g/L and 4.93 g/L after 7 h of hydrolysis at the initial Novozymes enzyme loading of 12.3 filter

paper unit (FPU)/g substrate and Spezyme loading of 3.68 FPU/g substrate, respectively. The average diffusivities of Novozyme and Spezyme enzymes on the CGW were estimated to be $7.14\times10^{-17}m^2/s$ and $5.58\times10^{-17}m^2/s$, respectively

Sigurbjornsdottir and Orlygsson, (2011) investigated Combined Biohydrogen and Bioethanol (CHE) production from monosugars, polymeric carbohydrates and hydrolysates made from various lignocellulosic biomasses by strain AK54, a saccharolytic, thermophilic ethanol and hydrogen producing bacterium isolated from a hot spring in Iceland. The highest hydrogen was also produced from cellulose hydrolysates or 6.7 mol-H_2/g TS pretreated with alkali (12.2 mol-H_2/g glucose equivalents) but of the lignocellulosic biomass, highest yields were from grass pretreated with base (4.9 mol-H_2/g TS)

Silva et al., (2010) compared the effectiveness of Ball Milling (BM) and Wet Disk Milling (WDM) on treating sugarcane bagasse and straw. Glucose and xylose hydrolysis yields at optimum conditions for BM-treated bagasse and straw were 78.7%, 72.1%, 77.6% and 56.8% respectively. Maximum glucose and xylose yields for bagasse and straw using WDM were 49.3%, 36.7%, 68.0% and 44.9% respectively. Bagasse and straw BM hydrolysates were fermented by *Saccharomyces cerevisiae* strains. Ethanol yields from total fermentable sugars using a C6-fermenting strain reached 89.8% and 91.8% for bagasse and straw hydrolysates, respectively, and 82% and 78% when using a C6/C5 fermenting strain

Zhang et al., (2009) obtained high concentration of ethanol from cellulose, corn cob was pretreated with acid and alkali to remove non-cellulose components, and then subjected to Simultaneous Saccharification and Fermentation (SSF). An ethanol concentration as high as 69.2 g/L was achieved with 19% Dry Matter (DM) using batch SSF, resulting in an 81.2% overall ethanol yield. A fed-batch process using a high solid concentration was also investigated. Fresh substrate was pretreated with dilute Sulfuric acid–Sodium hydroxide, and then added at different amounts during the first 24 h, to yield a final dry matter content of 25% (w/v). SSF conditions with cellulose loading of 22.8 FPU/g glucan, dry yeast (*Saccharomyces cerevisiae*) loading of 5 g/L and substrate supplementation every 4 h yielded the highest ethanol concentration of 84.7 g/L after 96 h. This corresponded to a 79% overall ethanol yield

Park et al., (2009) evaluated the thermotolerant ethanol-fermenting yeast, *Saccharomyces cerevisiae* KNU5377, isolated from sludge of a local industrial complex stream in Korea, for its capability for lignocellulosic ethanol production from waste newsprint in high temperature. The maximum production of 8.4% (v/v) ethanol was obtained when 250 g (w/v)/L of dry-defibrated waste newspaper was used for ethanol production by SSF

Liu et al., (2011) reports a new yeast strain of *Clavispora* NRRL Y-50464 that is able to utilize cellobiose as sole source of carbon and produce sufficient native Beta-glucosidase enzyme activity for cellulosic ethanol production using SSF. Ethanol production of 23 g/L was obtained using 25% solids loading at 37°C by SSF without addition of exogenous Beta-glucosidase

Chen et al., (2012) investigated the efficiency of a batch microwave-assisted ammonia heating system as pretreatment for sweet sorghum bagasse and its effect on porosity, chemical composition, particle size, enzymatic hydrolysis and fermentation into ethanol evaluated. The best glucose yields and ethanol yields (from glucose only) among all different pretreatment conditions averaged 42/100 g dry biomass and 21/100 g dry biomass, respectively with 1–2 mm sorghum bagasse pretreated at 130°C for 1 h

McIntosh et al., (2012) empirically determined conditions for optimal pretreatment of eucalypt (*Eucalyptus dunnii*) and spotted gum (*Corymbia citriodora*) forestry thinning residues for bioethanol production using a 3^3 factorial design. *Saccharomyces cerevisiae* efficiently fermented crude *E. dunnii* hydrolysate within 30 h, yielding 18 g/L ethanol, representing glucose to ethanol conversion rate of 0.475 g/g (92%)

Ahn et al., (2011) optimized Alkaline–Oxidative (A/O) pretreatment and enzymatic saccharification for bioethanol fermentation from water hyacinth by *Saccharomyces cerevisiae*. The yield of ethanol in batch fermentation was 0.35 g ethanol/g biomass. Continuous fermentation was carried out at a dilution rate of 0.11 (per h) and the ethanol productivity was 0.77 [g/(l h)]

Meinita et al., (2011) investigated the effect of fermentation inhibitors to the *Kappaphycus alvarezii* (red seaweed) hydrolysate on cell growth and ethanol fermentation. Detoxification by activated charcoal strongly improved the fermentability of dilute acid hydrolysate in the production of bioethanol from *K. alvarezii* with *Saccharomyces cerevisiae*. The optimal detoxifying conditions were found to be below an activated charcoal concentration of 5%

Meinita et al., (2011) examined the hydrolysis of marine algal biomass *Kappaphhycus alvarezii* using two different acid catalysts. The optimal conditions for hydrolysis were achieved at a Sulfuric acid concentration, temperature and reaction time of 0.2 M, 130°C and 15 min, respectively

Asli and Qatibi, (2009) investigated the inexpensive production of sugars from lignocellulose is an essential step for the use of biomass to produce fuel ethanol. The lignocellulosic component of the olive cake was dilute-acid pretreated at a 13.5% olive-cake loading with 1.75% (w/v) Sulfuric acid and heating at 160°C for 10 min. Soluble sugars resulting from the pretreatment process were fermented using *E. coli* FBR5. 8.1 g of ethanol/L was obtained from hydrolysates containing 18.1 g of soluble sugars

Han et al., (2009) examined the effectiveness of ammonia percolation pretreatment of wheat straw for ethanol production. The experiments were performed at treatment temperature of 50-170°C and residence time of 10-150 min. The pretreated wheat straw was hydrolyzed by a cellulase complex (NS50013) and β-glucosidase (NS50010) at 45°C. After saccharification, *Saccharomyces cerevisiae* was added for fermentation. The ethanol concentration reached 24.15 g/L in 24 h

Collares et al., (2011) focused the evaluation of enzymatic hydrolysis of starch from cassava using pectinase, α-amylase and amyloglucosidase. The amyloglucosidase showed to be significant in the process since after its addition to the reaction media was verified an increasing of 30-50% in the amount of total reducing sugar released. At optimized condition the maximum productivity obtained was 22.9 g/(L·h)

Kumoro et al., (2008) studied the hydrolysis of powdered fibrous sago waste by Sulphuric acid and Glucoamylase. The optimum condition for acid hydrolysis was found to be at 90°C using 1.5 M Sulphuric acid concentration and reaction time of 120 min, yielding 0.6234 g glucose/g waste. The optimum condition for enzymatic hydrolysis using glucoamylase was found to be at enzyme concentration of 6 AGU/ml and reaction time of 30 min, yielding 0.5646 g glucose/g waste

Tian et al., (2009) demonstrated that the yeast strains Y1, Y4 and Y7 have high conversion efficiencies for sugars and high abilities to tolerate or metabolize

inhibitors in dilute-acid lignocellulosic hydrolysates. Strains Y1 and Y4 completely consumed the glucose within 24 h in dilute-acid lignocellulosic hydrolysate during *in situ* detoxification, and the maximum ethanol yields reached 0.49 g and 0.45 g ethanol/g glucose, equivalent to maximum theoretical values of 96% and 88.2%, respectively

Magesh et al., (2011) studied the direct fermentation of 226 white rose tapioca stem to ethanol by *Fusarium oxysporum* in a batch reactor. The optimum process conditions were then obtained using response surface methodology. The quadratic model indicated that substrate concentration of 33g/l, pH 5.5 and a temperature of 30.13^{o}C were found to be optimum for maximum ethanol concentration of 8.64g/L

Baskar et al., (2008) study the effect of starch concentration, temperature, time and enzyme concentration were studied and optimized for hydrolysis of cassava *(Manihot esculenta)* starch powder (of mesh 80/120) into glucose syrup by immobilized (using Polyacrylamide gel) α-amylase using central composite design. The optimum value of starch concentration temperature, time and enzyme concentration were found to be 4.5% (w/v), 45^{o}C, 150 min, and 1% (w/v) enzyme. The maximum glucose yield at optimum condition was 5.17 mg/mL

Oberoi et al., (2010) used the dried, ground, and hydrothermally pretreated Kinnow mandarin (*Citrus reticulata*) waste to produce ethanol via Simultaneous Saccharification and Fermentation (SSF). Central composite design was used to optimize cellulase and pectinase concentrations, temperature, and time for SSF. The validation experiment using 6 FPU g/ds cellulase and 60 IU g/ds pectinase at 37°C for 12 h in a laboratory batch fermenter resulted in ethanol concentration and productivity of 42 g/L and 3.50 g/L/h, respectively

Banerjee et al., (2011) studied the feasibility of scaling-up the AHP process and integrating it with enzymatic hydrolysis and fermentation. Corn stover (1 kg) was subjected to AHP pretreatment, hydrolyzed enzymatically, and the resulting sugars fermented to ethanol. During fermentation using a glucose- and xylose-utilizing strain of *Saccharomyces cerevisiae*, all of the Glucose and 67% of the Xylose were consumed in 120 h. The final ethanol titre was 13.7 g/L

Maas et al., (2008) describes an integrated pilot-scale process where lime-treated wheat straw with a high dry-matter content (around 35% by weight) is converted to ethanol via simultaneous saccharification and fermentation by commercial hydrolytic enzymes and bakers' yeast (*Saccharomyces cerevisiae*). Based on the achieved experimental values, 16.7 kg of pretreated wheat straw could be converted to 1.7 kg of ethanol, 1.1 kg of methane, 4.1 kg of carbon dioxide, around 3.4 kg of compost and 6.6 kg of lignin-rich residue

Romaní et al., (2010) pretreated *Eucalyptus globulus* wood samples in aqueous media under non-isothermal conditions to reach maximal temperatures (TMAX) in the range 195–250°C. The overall glucose yield decreased for substrates pretreated at TMAX above 220°C owing to cellulose losses. Using substrates pretreated at TMAX is 220°C, up to 94% of polysaccharides were recovered in the hydrolysis media as mono- or oligo-saccharides

Arslan and Eken-Saracoglu, (2010) investigated the use of hazelnut shell as a renewable and low cost lignocellulosic material for bioethanol production for the first time. Fermentation of hazelnut shell hydrolysate by *Pichia stipitis* were evaluated with shaking flasks experiments. Hazelnut shells hydrolysis with 0.7 M H_2SO_4 yielded 49 g/l total reducing sugars. Under the best assayed conditions, ethanol concentration of 16.79 g/l was reached from a hazelnut shell hydrolysate containing initial 50 g total reducing sugar per L after partial synthetic xylose supplementation

Chen et al., (2011) used a combination of a twin-screw extrusion and an acid-catalyzed hot water extraction process performed at a bench-scale to prepare high monomeric xylose hydrolysate for cellulosic production. The optimal condition for the extrusion step was determined to be 40 rpm with 3% H_2SO_4 at 120°C; the optimal condition for the extraction step was determined to be 130°C for 20 min. After the pretreatment at the optimal condition, 83.7% of the xylan was converted to monomeric xylose, and the concentration reached levels of 53.7 g/L. Finally, after the subsequent enzymatic hydrolysis, an 80% yield of the total saccharification was obtained

Sassner et al., (2005) performed Simultaneous Saccharification and Fermentation (SSF) on the slurries resulting from steam pretreatment of non-, SO_2- and H_2SO_4-impregnated *Salix* chips. At a water-insoluble solid concentration of 9%, 32 g/L ethanol was obtained after 78 h of SSF, using 3.3 g/L baker's yeast (*Saccharomyces cerevisiae*) cultivated on the pretreatment liquid. This corresponds to an overall ethanol yield of 76% of the theoretical, based on the glucan and mannan content in the raw material

Alinia et al., (2010) experimental apparatus has been designed to investigate the effect of pretreatment of dry and wet wheat straw using supercritical carbon dioxide alone and the combination of supercritical CO_2+ steam by varying the temperature (160-200°C) and the residence time (10, 30, 60 or 70 min). The pretreatment of dry wheat straw by steam explosion and supercritical CO_2 at steam temperature and retention time of 200°C and 15 min and supercritical CO_2 conditions of 12 MPa, 190°C and 60 min, resulted in the best overall yield for sugar (234.6 g/kg)

Gütsch et al., (2012) Three different acids (Acetic, Oxalic and Sulfuric acid) were tested for their catalytic activity during the pretreatment of *Eucalyptus globulus* wood comparatively to autohydrolysis in order to extract valuable products prior to kraft pulping and to reduce lignin precipitation in the pretreatment step. At wood yields below 80% glucose dissolution is enhanced using acid catalysts, thus reduced yields are expected during subsequent kraft cooking. Lignin in the hydrolyzate originates from acid-soluble lignin in wood. The amount of insoluble lignin is drastically reduced using strong acid catalysts

Zabihi et al., (2010) found that additives which increase the efficiency of the sugar production. The pretreatment of wheat straw by steam explosion soaked with acetic acid or ethanol prior to the pretreatment was investigated by varying the temperature (180–225) °C and the retention time (3–60 min). The pretreatment of wheat straw by steam explosion at 210°C and 10 min, by steam/acetic acid at 220°C and 8 min and by steam/ethanol at 220°C and 5 min resulted in the best overall yield of reducing sugar which was found to be 177.3 g/kg on dry solids (DS) basis, 244.1 g/kg DS, and 264.3 g/kg DS, respectively

Karimi et al., (2006) investigated and compared the ethanol production from rice straw by Simultaneous Saccharification and Fermentation (SSF) with *Mucor indicus*, *Rhizopus oryzae*, and *Saccharomyces cerevisiae* with pure cellulose. The SSF experiments were carried out aerobically and anaerobically at 38°C, 50 g/L dry matter (DM) solid substrate concentration and 15 or 30 filter paper unit (FPU)/g DM of a commercial cellulase. *R. oryzae* had the best ethanol yield as 74% from rice straw followed by *M. indicus* with an overall yield of 68% with 15 FPU/g DM of cellulase. Glycerol was the main by-product of the SSF by *M. indicus* and *S. cerevisiae* with yields 117 and 90 mg/g of equivalent glucose in the pretreated straw,

respectively, while *R. oryzae* produced lactic acid as the major by-product with yield 60 mg/g glucose equivalent in pretreated rice straw under anaerobic conditions

CHAPTER 3
MATERIALS AND METHODS

3.1 MATERIALS

Cassava stem and Sawdust was collected from local Sago and Timber industry in Coimbatore and Salem district. Cassava stem was dried and milled by using Jaw crusher (Almech Enterprise, Coimbatore). It was sieved by using sieve shaker (Lawrence & Mayo) to get desired sizes. Then the samples were dried and stored in an air-tight container at 4°C. UV-Visible spectrophotometer of Shimadzu (Model: UV-1800) was used for measuring the absorbance of solutions. A trial version of Design Expert 8.0.1 was used for Box-Behnken Design experiments.

3.2 METHODS

3.2.1 CHARACTERIZATION OF SUBSTRATES

Table 3.1 Characterization of substrates

S.No.	Parameter	Method	Reference
1	Total sugars	Dubois method	Dubois et al., 1956
2	Starch	Anthrone method	Kim et al., 1992
3	Cellulose	Goering method	Goering et al., 1975
4	Hemi cellulose		
5	Lignin		
6	Total protein	Lowry's method	Lowry et al., 1951
7	Crude fibre	Digestion	Maynard et al., 1970
8	Fat	Soxhlet method	Soxhlet, 1879
9	Total nitrogen	Pellet and Young	Pellet and Young, 1980
		method	
10	Moisture	Oven	International standard: ISO 1741, 2010
11	Total solids	Oven	International standard: ISO 1741, 2010
12	Total reducing sugars	DNS assay	Miller, 1959
13	Ash	Furnace	International standard: ISO 3593, 2010
14	pH	pH metry	Black and Allen, 1973
15	FTIR	KBR method	Michell, 1989
16	SEM	Microscopy	-

3.2.2 PRETREATMENT BY WEAK ACID HYDROLYSIS

Pretreatment was done to Cassava stem to break down the complex structure of lignocellulosic material to cellulose, hemi-cellulose and lignin. Selection of acids was done based on its K_a value. Acid strength increases with increase in K_a value, the following table shows that the acids with respective K_a value

Table 3.2 Acids and its K_a value

S.No.	Acid	K_a
1	$HClO_4$	10^{10}
2	HCl	1.3×10^{6}
3	HNO_3	2.4×10^{1}
4	H_2O_2	2.5×10^{-12}
5	H_2O	1.82×10^{-16}
6	H_2SO_4	1.0×10^{3}
7	H_3PO_4	7.5×10^{-3}
8	$(COOH)_2$	5.6×10^{-2}
9	HCOOH	1.8×10^{-4}
10	CH_3COOH	1.8×10^{-5}

In order to determine the maximum sugar removal, samples were treated with these acids. DNS assay was performed to estimate the TRS content present in the sample with xylose as a standard. HClO4, H_2O_2, $(COOH)_2$ and H_2O showed a maximum TRS value and it was selected for further screening. Material Safety Data Sheet (MSDS) provided the hazardous level of acids and finally oxalic acid was selected with minimum hazardous level. Box – Behnken Design (BBD) was performed to optimize the reaction condition for Cassava stem by using oxalic acid.

3.2.3 ENZYMATIC HYDROLYSIS OF CELLULOSE

Enzymatic hydrolysis was done by using Cellulase from *Trichoderma reesei*, ATCC 26921, procured Sigma – Aldrich. Sawdust having high cellulose content was hydrolysed using cellulase. The enzymatic hydrolysis was performed at different volumes of acetate buffer with pH 5.0 (4, 5 and 6 mL), temperature (40, 45 and 50°C) and time (20, 40 and 60 min). BBD based on 3 factor-3 level was performed to optimize TRS production. Cellulase was loaded at the rate of 250μL/g sawdust to all the tubes. After the enzymatic hydrolysis, samples were centrifuged at 5,000 rpm for 10 min and TRS concentration was determined with glucose as a standard.

3.2.4 DETOXIFICATION USING $Ca(OH)_2$

Before the detoxification process, hydrolysates from weak acid hydrolysis and enzymatic hydrolysis were pooled. Detoxification was done by using overliming

treatment by increasing the pH of hydrolysates 9 to 10 by gradual addition of $Ca(OH)_2$ (0.16 g/100 mL water). The hydrolysate was placed over the stirrer plate and heated to 50°C. $Ca(OH)_2$ was added gradually and mixed by using stirrer until it reaches pH 9 to 10. The hydrolysate was maintained at 50°C for 30 min without stirring. Then the mixture of hydrolysate and $Ca(OH)_2$ was filtered and the recovered solids ($CaSO_4$) were discarded. The overlimed filtrate pH was adjusted to 6.5 using 0.1N hydrochloric acid for further analysis. (Mohamad, N.L. et al.,, 2011)

3.2.5 GROWTH CURVE STUDIES

Fusarium oxysporum, *Klebsiella oxytoca*, *Zymomonas mobilis* and *Kluyveromyces marxianus* were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. 100 mL of the respective medium was prepared and autoclaved at 121°C, 15 psi for 15 min. For fungi, 2% w/v (on wet basis) of mycelia was inoculated to autoclaved medium and placed in shaker at 100 rpm. For bacteria, 1% v/v of respective overnight culture was inoculated to medium and placed in shaker at 100 rpm. Initial absorbance of the cultures was noted using visible spectrophotometer at 600 nm with 2 mL of sterile medium as a reference. Absorbance of *Klebsiella oxytoca* and *Zymomonas mobilis* was recorded at 1 h time interval and for *Fusarium oxysporum* and *Kluyveromyces marxianus* at 3 h time interval. Growth curve was plotted till the organisms reached their stationary phase.

3.2.6 CO-FERMENTATION

Fermentation was carried out in 250 mL Erlenmeyer flask containing 100 mL of both detoxified and non-detoxified hydrolysate. Combination of *Kluyveromyces marxianus* with *Zymomonas mobilis* and *Kluyveromyces marxianus* with *Fusarium oxysporum* (5 mL each) was added to detoxified and non-detoxified Cassava stem hydrolysate. The mixture was kept in shaker at 100 rpm and ethanol concentration was determined at regular interval of 24, 36 and 48 h. 10 mL of *Klebsiella oxytoca* was added to detoxified sawdust hydrolysate and it was kept in shaker at 100 rpm and ethanol concentration was determined at regular interval of 24, 36 and 48 h.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF CASSAVA STEM AND SAWDUST

Table 4.1 Characterization of Cassava stem and Sawdust

S.No.	Parameter	Cassava stem (%)	Sawdust (%)
1	Total sugars	3.57	1.31%
2	Starch	0.98	0.31
3	Cellulose	37	51
4	Hemicellulose	29	8
5	Lignin	9.1	24. 7
6	Total protein	10.36	9.41
7	Crude fibre	29	56
8	Fat	ND	ND
9	Moisture	7	5
10	Total solids	93	95
11	Total reducing sugars	2.55	1.15
12	Ash	7	2.3
13	pH	9.28	6.12

The industrial residues like Cassava stem and Sawdust were selected to evaluate their potential to ethanol production. The characterization of selected residues was carried out using standard methods outlined in the literature. It was found that Cassava stem and Sawdust are lignocellulosic materials.

4.2 OPTIMIZATION OF PRETREATMENT BY WEAK ACID HYDROLYSIS FOR CASSAVA STEM BY RESPONSE SURFACE METHODOLOGY

Selection of acids was done based on its Ka value and respective TRS concentration was tabulated below.

Table 4.2 Screening of acids based on its Ka value

S.No.	Acid	Ka value	A_{540}	
			Cassava stem	Sawdust
1	HCl	1.3×10^{6}	0.230	0.011
2	HNO_3	2.4×10^{1}	0.208	0.038
3	H_2O_2	2.5×10^{-12}	1.413	0.209
4	H_2O	1.82×10^{-16}	0.501	1.105
5	H_2SO_4	1.0×10^{3}	0.114	0.010
6	H_3PO_4	7.5×10^{-3}	0.190	0.211
7	$(COOH)_2$	5.6×10^{-2}	1.202	1.037
8	HCOOH	1.8×10^{-4}	0.215	0.026
9	CH_3COOH	1.8×10^{-5}	0.209	0.380
10	$HClO_4$	10^{10}	1.861	0.778

$HClO_4$, H_2O_2, $(COOH)_2$ and H_2O were selected for further screening. Next step screening was done by comparing the hazardous, fire and reactivity level of the above acids (Material Safety Data Sheet)

Table 4.3 Screening of acids based on its hazardous, fire and reactivity level

S.No.	Acid	Hazardous level (blue)	Fire level (Red)	Reactivity level (Orange)
1	$HClO_4$	3	0	3
2	H_2O_2	2	0	1
3	$(COOH)_2$	3	1	0
4	H_2O	0	0	0

Table 4.4 Hazardous, fire and reactivity level value and its consequences

S.No.	Level	Blue (Health and Hazard)	Red (Flammability)	Red (Reactivity)
1	0	No risk	No burn	No reaction
2	1	Irritation	Burn occurs only after preheating	Normal under fire condition

3	2	Temporary injury	Burn occurs after heating slightly	Unstable under high temperature/pressure
4	3	Major injury	Fire at normal temperature	May form explosive mixtures with H_2O
5	4	Life threatening/ permanent damage	Flammable gases/very volatile	Readily capable of explosive water reaction

Based on the Material Safety Data Sheet (MSDS) data listed above, oxalic acid shows low risks of hazardous, fire and reactivity nature and selected for further analysis.

Table 4.5 Independent variables and levels used for Box – Behnken design for Cassava stem

Variables	Symbol	Unit	Levels		
			-1	0	1
Oxalic acid concentration	A	% w/v	1	2	3
Time	B	Min	20	40	60
Solid to liquid ratio	C	g/mL	0.05	0.125	0.20

Table 4.6 Box-Behnken design matrix employed for three independent variables for cassava stem

S. No.	OAC (% w/v) (A)	Time (min) (B)	SLR (g/mL) (C)	TRS (mg/mL)
1	1.5	20	0.05	0.457
2	1.5	30	0.125	1.956
3	0.5	30	0.125	1.030
4	1	30	0.05	1.156
5	1.5	10	0.125	1.192
6	1	10	0.20	1.509
7	0.5	20	0.05	1.025
8	1	20	0.125	1.217
9	0.5	20	0.20	0.784
10	1	20	0.125	1.217
11	1.5	20	0.20	2.325
12	1	10	0.05	0.573
13	0.5	10	0.125	1.101
14	1	30	0.20	1.401
15	1.5	20	0.05	0.457

In order to optimize the BBD experimental design, three-level-three-factors BBD was adopted, required 15 experiments included three centre points. The parameters, which were selected for the study of TRS concentration and their respective levels, were as follows: oxalic acid concentration (0.5 - 1.5 % w/v), time (10 – 30 min) and slurry to liquid ratio (0.05 – 0.20 g/mL) (Table 4.5). All 15 of the designed experiments were conducted, and the results were analyzed via multiple regression. The coefficients of a full model were evaluated via regression analysis and tested for significance. A coefficient of determination (R^2=0.9901) showed that the model was significant in predicting TRS concentration. Finally, the best fitting model was determined via regression. This showed that three linear coefficients (A, B, C), three quadratic coefficients (A^2, B^2, C^2), and three cross-product coefficients (AB, AC, BC) were significant (Table 4.6).

Table 4.7 Analysis of variance for selected Box – Behnken design for Cassava stem

Source	Sum of Squares	df	Mean Square	F – Value	p-value Prob > F
Model	3.056795	6	0.509466	33.98863	< 0.0001
A-Oxalic acid concentration	0.495013	1	0.495013	33.02439	0.0007
B-Time	0.170528	1	0.170528	11.37665	0.0119
C-Solid to liquid ratio	0.985608	1	0.985608	65.7541	< 0.0001
AB	0.174306	1	0.174306	11.62871	0.0113
AC	1.11197	1	1.11197	74.18426	< 0.0001
BC	0.11937	1	0.11937	7.963697	0.0257
A^2	0.009768	1	0.009768	0.844735	0.4101
B^2	0.015792	1	0.015792	1.3657	0.3074
C^2	0.061827	1	0.061827	5.346723	0.0818
Residual	0.104925	7	0.014989		
Lack of Fit	0.104925	6	0.017488		
Pure Error	0	1	0		
Cor. Total	3.16172	13			

Table 4.8 Regression coefficient and significance of response quadratic model for cassava stem

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.210214	1	0.032721	1.132841	1.287587	
A-Oxalic acid concentration	0.24875	1	0.043286	0.146395	0.351105	1
B-Time	0.146	1	0.043286	0.043645	0.248355	1
C-Solid to liquid ratio	0.351	1	0.043286	0.248645	0.453355	1
AB	0.20875	1	0.061215	0.063999	0.353501	1
AC	0.52725	1	0.061215	0.382499	0.672001	1
BC	-0.17275	1	0.061215	-0.3175	-0.028	1
A^2	0.05525	1	0.060113	-0.11165	0.222151	1
B^2	0.07025	1	0.060113	-0.09665	0.237151	1
C^2	-0.139	1	0.060113	-0.3059	0.027901	1

The analysis of variance (ANOVA) for the response surface quadratic model is provided in Table 4.7. The coefficients of the response surface quadratic model were also evaluated. A *p*-value showed that all of the linear coefficients were more highly significant than their quadratic and cross-product term. However, in order to minimize error, all of the coefficients were considered in the design. According to the ANOVA analysis of factors, it was noted that, lack of fit is not significant. This indicates that the model does indeed represent the actual relationships of reaction parameters, which are well within the selected ranges (Table 4.7).

The final estimative response model equation (based on the actual value) by which the TRS concentration was estimated was as follows:

Equation for Cassava stem

[TRS] = +2.38247-2.99308*A-0.026179*B+0.34112*C+0.050800*A*B+ 17.12667*A*C-0.27967*B*C+0.22100*A^2+7.02503E-004*B^2- 24.71107*C^2

Table 4.9 Validation of the model for Cassava stem

S. No.	OAC (% w/v)	Time (min)	SLR (g/mL)	TRS (mg/mL)		Mean	Standard deviation	Coefficient of variation
				Exp	Pre			
1.	1.47	29	0.04	0.702	1.005	0.931	0.134	14.4%
2.	1.47	29	0.04	1.045				
3.	1.47	29	0.04	0.975				
4.	1.47	29	0.04	0.940				
5.	1.47	29	0.04	0.995				

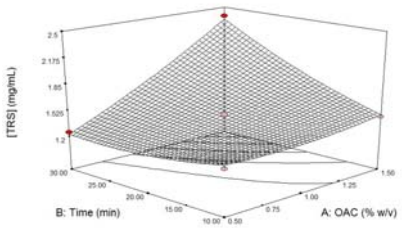


Fig 4.1 Response surface plots representing the effect of oxalic acid concentration (OAC), time, and their TRS concentration on bioethanol production

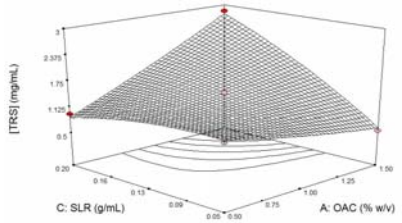


Fig 4.2 Response surface plots representing the effect of oxalic acid concentration (OAC), solid to liquid ratio (SLR), and their TRS concentration on bioethanol production

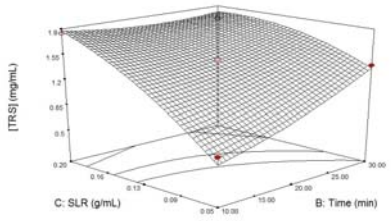


Fig 4.3 Response surface plots representing the effect of time, solid to liquid ratio (SLR), and their TRS concentration on bioethanol production

Fig 4.1 represents the effects of different oxalic acid concentration (OAC) and time on TRS concentration at constant temperature of 121°C and 15 psi pressure. An increase in oxalic acid concentration has no significant effect in the change of TRS concentration. But increase in hydrolysis time has significant change in TRS concentration. This shows that, hydrolysis time placed a predominant role in the increase of TRS concentration.

Fig 4.2 represents the effects of different oxalic acid concentration and slurry to liquid ratio (SLR) on TRS production at constant temperature of 121°C and 15 psi pressure. Increase in both SLR and OAC have no significant change in the TRS concentration.

Fig 4.3 represents the effects of different time and SLR on TRS concentration at constant temperature of 121°C and 15 psi pressure. From the figure it is clear that both time and SLR have significant increment in TRS concentration. It is concluded that, the appropriate optimum conditions for the maximum TRS concentration was found to be 1.47 % w/v of OAC and 29 min of time.

4.3 OPTIMIZATION OF ENZYMATIC HYDROLYSIS FOR SAWDUST BY USING RESPONSE SURFACE METHODOLOGY

The lignocellulosic nature of sawdust was hydrolysed by using cellulase. RSM-BBD was employed to optimize the production of TRS from sawdust. Fifteen experiments were performed by using BBD with buffer volume, temperature and time as factors. Table 4.10 shows the BBD matrix employed for three independent variables with TRS concentration.

Table 4.10 Independent variables and levels used for Box – Behnken design for sawdust

Variables	Symbol	Unit	Levels		
			-1	0	1
Buffer volume	A	mL	4	5	6
Temperature	B	°C	40	45	50
Time	C	min	20	40	60

Table 4.11 Box-Behnken design matrix employed for three independent variables for sawdust

S. No	Buffer volume (mL) (A)	Temp (°C) (B)	Time (min) (C)	TRS (mg/mL)
1	5	45	40	1.11
2	4	45	20	1.37
3	6	45	60	1.26
4	4	50	40	1.40
5	5	45	40	1.08
6	4	40	40	1.23
7	5	45	40	1.06
8	5	50	60	1.36
9	6	50	40	1.13
10	6	45	20	1.03
11	6	40	40	1.21
12	5	50	20	1.21
13	4	45	60	1.30
14	5	40	60	1.40
15	5	40	20	1.09

Table 4.12 Analysis of variance for selected Box – Behnken design for Sawdust

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.115165	9	0.012796	8.596914	0.0145
A-Buffer	0.029646	1	0.029646	19.91745	0.0066
B-Temp	0.002113	1	0.002113	1.419262	0.2870
C-Time	0.024753	1	0.024753	16.63014	0.0096
A×B	0.008742	1	0.008742	5.873392	0.0599
A×C	0.011881	1	0.011881	7.982129	0.0369
B×C	0.003192	1	0.003192	2.144681	0.2029
A^2	0.008156	1	0.008156	5.479732	0.0663
B^2	0.016699	1	0.016699	11.21885	0.0203
C^2	0.015124	1	0.015124	10.1607	0.0243
Residual	0.007442	5	0.001488		
Lack of Fit	0.00656	3	0.002187	4.958617	0.1724
Pure Error	0.000882	2	0.000441		

Cor. Total	0.122607	14			

Table 4.13 Regression coefficient and significance of response quadratic model for sawdust

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.78	1	0.022274	0.722742	0.837258	
A-Buffer	-0.06088	1	0.01364	-0.09594	-0.02581	1
B-Temp	0.01625	1	0.01364	-0.01881	0.051313	1
C-Time	0.055625	1	0.01364	0.020562	0.090688	1
AB	-0.04675	1	0.01929	-0.09634	0.002837	1
AC	0.0545	1	0.01929	0.004913	0.104087	1
BC	-0.02825	1	0.01929	-0.07784	0.021337	1
A^2	0.047	1	0.020078	-0.00461	0.098612	1.01
B^2	0.06725	1	0.020078	0.015638	0.118862	1.01
C^2	0.064	1	0.020078	0.012388	0.115612	1.01

The regression coefficients and significance levels are given in Table 4.11. The developed model was verified by performing trials under optimum conditions. The result of the experiments at optimum conditions was presented in terms of predicted and experimental values. A coefficient of determination (R^2=0.9393) showed that the model was significant in predicting TRS concentration.

The analysis of variance (ANOVA) for the response surface quadratic model is provided in Table 4.11. A *p* – value showed that all of the linear coefficients were more highly significant than their quadratic and cross product terms. However, in order to minimize error, all of the coefficients were considered in the design. According to the ANOVA analysis of factors, lack of fit was not significant and the model was significant. It indicates that the model does indeed represent the actual relationships of reaction parameters, which are well within the selected ranges (Table 4.11).

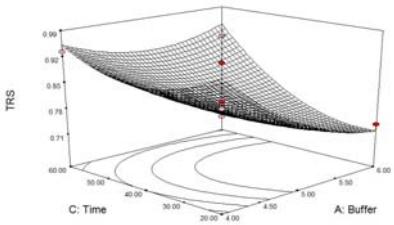


Fig 4.5 Response surface plots representing the effect of Buffer, Time and their TRS concentration on bioethanol production

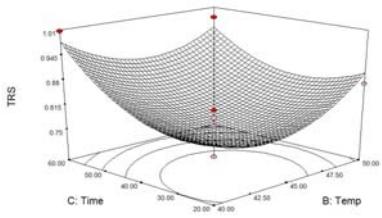


Fig 4.6 Response surface plots representing the effect of temperature, time and their TRS concentration on bioethanol production

Figs. 4.4 – 4.6 show the isoresponse surface plots for the optimization conditions of TRS production. From the plots, it was easy and convenient to understand the interactions between two parameters and also to locate the optimum levels.

The final estimated response model equation, based on actual values, for the production of TRS as follows:

$$[TRS]=+5.638-0.219*A-0.181*B-0.0109*C-9.35E-3*A*B+2.725E-3*A*C-2.825E-4*B*C+0.047*A^2+2.69E3*B^2+1.6E-4*C^2$$

Table 4.14 Validation of the model for Sawdust

S. No.	Buffer volume (mL)	Temp (°C)	Time (min)	TRS (mg/mL)		Mean	Standard deviation	Coefficient of variation
				Exp	Pre			
1.	7	1.39	0.19	1.30				
2.	7	1.39	0.19	0.979				
3.	7	1.39	0.19	1.009	0.993	1.057	0.137	12.96%
4.	7	1.39	0.19	0.988				
5.	7	1.39	0.19	1.007				

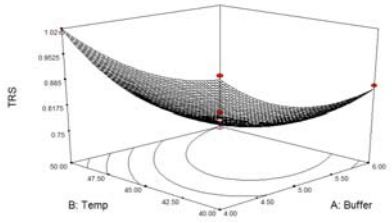


Fig 4.4 Response surface plots representing the effect of buffer, temperature and their TRS concentration on bioethanol production

The effect of temperature and buffer on TRS production is shown in fig. 4.4. TRS production increased with the increase of temperature, increase in the buffer volume has no significant change in the TRS concentration. In fig. 4.3 TRS production increased with decrease in the buffer volume and increase in hydrolysis time increased the TRS formation. Finally, the effect of temperature and time on TRS production is shown in fig. 4.6. TRS production was increased by both increase in time and temperature. It is found that the isoresponse surface between temperature and time showed good interaction. Therefore, optimum TRS production could be obtained at low buffer volume, high temperature and long time.

4.4 GROWTH CURVE STUDIES

Growth curve studies for *Klebsiella oxytoca* revealed that its log phase started at 3rd hour and ended at 8th hour (Fig 4.7). Thus mid log phase was found to be at 6th hour approximately.

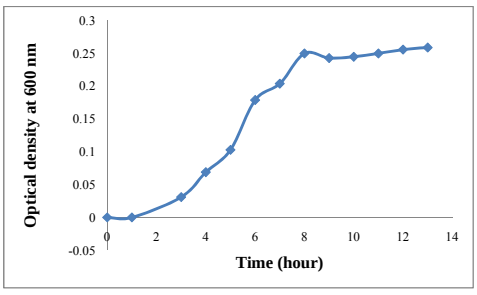


Fig 4.7 Growth curve for *Klebsiella oxytoca*

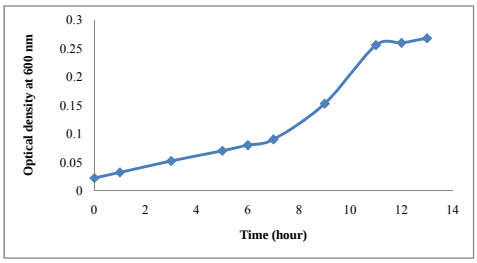


Fig 4.8 Growth curve for *Kluyveromyces marxianus*

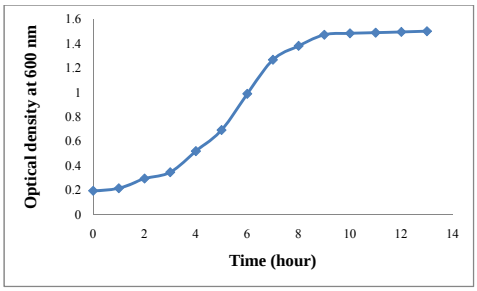


Fig 4.9 Growth curve for *Zymomonas mobilis*

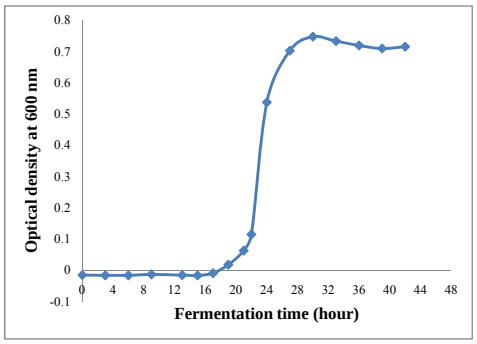


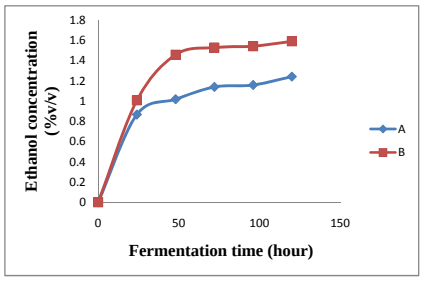
Fig 4.10 Growth curve for *Fusarium oxysporum*

Growth curve studies for *Kluyveromyces marxianus* revealed that its log phase started at immediately after inoculation and ended at 11th hour (Fig 4.8). Thus mid log phase was found to be at 6th hour approximately.

Growth curve studies for *Zymomonas mobilis* revealed that its log phase started at 3rd hour and ended at 9th hour (Fig 4.10). Thus mid log phase was found to be at 6th hour approximately.

Growth curve studies for *Fusarium oxysporum* revealed that its log phase started at 17th hour and ended at 32nd hour (Fig 4.10). Thus mid log phase was found to be at 24th hour approximately.

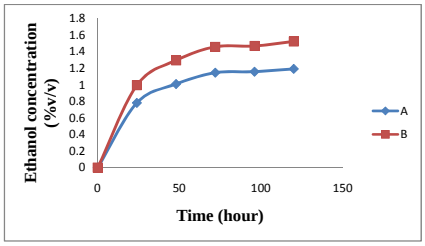
4.5 CO-FERMENTATION



A – *Kluyveromyces marxianus* and *Zymomonas mobilis*

B - *Kluyveromyces marxianus* and *Fusarium oxysporum*

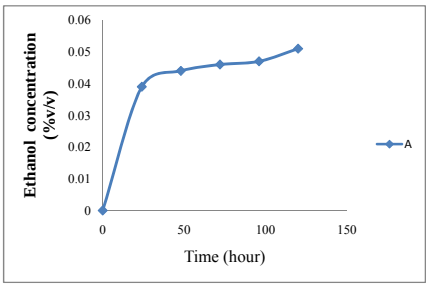
Fig 4.11 Effect of detoxified cassava stem hydrolysate on ethanol concentration by KM+ZM and KM+FO



A – *Kluyveromyces marxianus* and *Zymomonas mobilis*

B - *Kluyveromyces marxianus* and *Fusarium oxysporum*

Fig 4.12 Effect of non - detoxified cassava stem hydrolysate on ethanol concentration by KM+ZM and KM+FO



A – *Klebsiella oxytoca*

Fig 4.13 Effect of detoxified sawdust hydrolysate on ethanol concentration by *Klebsiella oxytoca*

Fig. 4.11 shows the effect of fermentation time on conversion of TRS to ethanol with detoxification. During 5 days of fermentation time, nearly all fermentable sugars were converted to bioethanol. It was evident from fig. 4.11 that maximum ethanol concentration was 1.592 %v/v and 1.243 %v/v for *Kluyveromyces marxianus* with *Fusarium oxysporum* and *Kluyveromyces marxianus* with *Zymomonas mobilis* respectively.

In non – detoxified cassava stem hydrolysate, the maximum utilization of total sugars and non-reducing sugars for ethanol production by co-fermenting *Kluyveromyces marxianus* with *Fusarium oxysporum* and *Kluyveromyces marxianus* with *Zymomonas mobilis* were recorded as 1.526 %v/v and 1.194 %v/v respectively (fig 4.12).

Fig. 4.13 shows the effect of fermentation time on conversion of TRS to ethanol with detoxified sawdust. *Klebsiella oxytoca* was inoculated with the hydrolysate for the effective conversion of bioethanol. During 5 days of fermentation time, nearly all fermentable sugars were converted to bioethanol. It was evident from fig. 4.13 shows that maximum ethanol concentration was 0.051 %v/v.

4.6 SCANNING ELECTRON MICROSCOPE (SEM) IMAGE OF CASSAVA STEM BEFORE AND AFTER PRETREATMENT

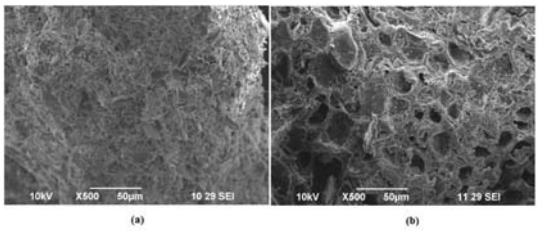


Fig 4.14 Scanning electron microscope (SEM) image: (a) Cassava stem before pretreatment, (b) Cassava stem after pretreatment

Fig 4.14 (a & b) shows the scanning electron microscope (SEM) image of native cassava stem, i.e. cassava stem before pretreated with acid and after pretreated cassava stem and it is very clear that the disruption of the cell wall occurred due to the action of oxalic acid concentration of 1.47 % with temperature 121°C, 15 psi pressure for 29 min. Pores present in the fig 4.14 (b) revealed the release of hemicellulose from the complex structure of lignocellulosic material.

4.7 SCANNING ELECTRON MICROSCOPE SEM IMAGE OF SAWDUST BEFORE AND AFTER HYDROLYSIS

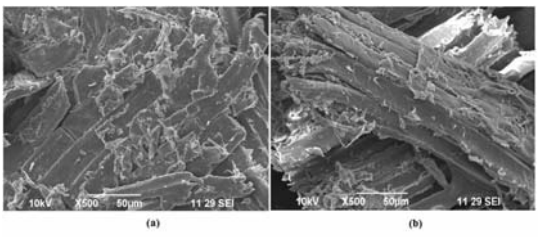


Fig 4.15 Scanning electron microscope (SEM) image: (a) Sawdust before hydrolysis, (b) Sawdust after hydrolysis

Fig 4.15 (a) shows the scanning electron microscope (SEM) image of raw sawdust, i.e. sawdust before hydrolysis. Fig 4.15 (b) shows the SEM image of after hydrolyzed with cellulase and it is very clear that the disruption of the cell wall occurred due to the action of cellulase loaded at the concentration of 250 µL with acetate buffer volume of 4.17 mL at 49°C for 20 min.

CHAPTER 5
DESIGN OF FLUIDIZED BED REACTOR FOR CONTINUOUS ETHANOL PRODUCTION

5.1 DESIGN OF FLUIDIZED BED REACTOR (FBR)

Performance equation of ideal Mixed Flow Reactor (MFR) is given by

$$\frac{V}{F_{A0}} = \frac{X_A}{-r_A} \quad (5.1)$$

Where,

V is the volume of reactor

F_{A0} is the feed rate = $V_0 C_{A0}$

V_0 is the volumetric flow rate

C_{A0} is the initial concentration of substrate

X_A is the fractional conversion

$-r_A$ is the rate of the reaction

Since the rate of reaction in FBR depends on mass of catalyst, equation (5.1) should be rewritten in terms of mass of the catalyst. Therefore equation (5.1) becomes,

$$\frac{V}{V_0 C_{A0}} = \frac{X_A}{-r_A} \quad (5.2)$$

Density of catalyst, $\rho_c = \frac{\text{Mass of catalyst } M_C}{\text{Volume of catalyst } V_C}$

$$\rho_c = \frac{M_C}{V}$$

$$V = \frac{M_C}{\rho_C} \quad (5.3)$$

Assuming that volume of catalyst is equal to volume of reactor and by substituting equation (3) in equation (2) we get,

$$\frac{M_C}{\rho_C V_0 C_{A0}} = \frac{X_A}{-r_A} \quad (5.4)$$

The rate of enzymatic reaction is given by Michaelis – Menten equation,

$$-r_s = \frac{V_{max}[S]}{K_m+[S]} = \frac{V_{max}[C_S]}{K_m+[C_S]} \quad (5.5)$$

Expressing equation (5.4) in terms of substrate S instead of reactant A, equation (5.4) becomes,

$$\frac{M_C}{\rho_C V_0 C_{S0}} = \frac{X_S}{-r_S} \quad (5.6)$$

By substituting equation (5.5) in (5.6) we get

$$\frac{M_C}{\rho_C V_0 C_{S0}} = \frac{X_S}{\frac{V_{max} C_S}{V_m+C_S}}$$

$$\frac{M_C}{\rho_C V_0 C_{S0}} = \frac{(K_m+C_S)X_S}{V_{max} C_S} \quad (5.7)$$

$$M_C = \frac{\rho_C V_0 (C_{S0} - C_S)}{V_{max} C_S}$$

The above equation is performance equation of fluidized bed reactor.

CHAPTER 6
CONCLUSION

In this study the effectiveness of pretreatment and enzymatic hydrolysis of Cassava stem and Sawdust for the bioethanol production was investigated. Response Surface Methodology (RSM) based Box – Behnken Design (BBD) was employed for the optimization of weak acid hydrolysis and enzymatic hydrolysis process of Cassava stem and Sawdust respectively. The overall data presented here increase the understanding of the effect of optimization for the enhancement of bioethanol yield. The yield of mixed cultures *K. marxianus* and *Z. mobilis* (1.243% v/v), *K. marxianus* and *F. oxysporum* (1.592% v/v) were obtained during the 5th day of fermentation period for detoxified Cassava stem hydrolysate. Also, yield of mixed cultures *K. marxianus* and *Z. mobilis* (1.194 %v/v), *K. marxianus* and *F. oxysporum* (1.526% v/v) were obtained during the 5th day of fermentation period for non – detoxified Cassava stem hydrolysate. For Sawdust, *K. oxytoca* yielded a maximum ethanol yield of 0.051% v/v during 5th day of fermentation. Finally it is evident that, the mixed culture of *K. marxianus* and *F. oxysporum* produced a maximum ethanol yield from detoxified and non – detoxified Cassava stem. Thus co – fermentation method used in this study was shown to be potential to other lignocellulosic biomass with high efficiency of pretreated and hydrolysed effect.

APPENDICES

APPENDIX 1

A1.1 Composition of growth media

The composition of growth media employed for 5 different microbes are provided in the tables below.

Table A1.1 Composition of Potato Sucrose medium employed for *Fusarium oxysporum*

Components	Quantity
Potatoes (scrubbed and diced)	200 g
Sucrose	20 g
Agar	20 g
Distilled water	1 L

Table A1.2 Composition of the medium employed for *Kluyveromyces marxianus*

Components	Quantity
Malt extract	3 g
Yeast extract	3 g
Peptone	5 g
Glucose	10 g
Distilled water	1 L

Table A1.3 Composition of the medium employed for *Zymomonas mobilis*

Components	Quantity
Yeast extract	11.11 g
Distilled water	1 L
20% Glucose	111.11 mL
Magnesium chloride	11.11 mL
Ammonium sulphate	11.11 mL
Pottasium dihydrogen phosphate	11.11 mL

Table A1.4 Composition of the medium employed for *Klebsiella oxytoca*

Components	Quantity
Beef extract	1 g
Yeast extract	2 g
Peptone	5 g
Sodium chloride	5 g
Distilled water	1 L

APPENDIX 2

A2.1 Composition of Sodium-acetate buffer

Stock Solution:

Solution A: 0.2M of acetic acid (11.5ml in 1000 ml)

Solution B: 0.2M of sodium acetate (16.4g in 1000ml)

"x" ml of solution A and "y" ml of solution B is diluted to 100ml with distilled water

x	y	pH
41.0	9.0	4.0
36.8	13.2	4.2
30.5	19.5	4.4
25.5	24.5	4.6
20.0	30.0	4.8
14.8	35.2	5.0
10.5	39.5	5.2
8.8	41.2	5.4
4.8	45.2	5.6
87.7	12.3	6
68.5	31.5	6.5
39	61	7
16	84	7.5
5.3	94.7	8

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