FURFURAL PRODUCTION FROM MICROALGAE

BY

JOHN ENEMONA OGUCHE

DEPARTMENT OF CHEMICAL ENGINEERING
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2018

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BY

John Enemona OGUCHE, **B.ENG. (CHEMICAL) (ABU, ZARIA) 2014.**

(P15EGCE8052)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIERMENTS FOR THE AWARD
OF MASTER OF SCIENCE IN CHEMICAL ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING
FACULTY OF ENGINEERING
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2018

Declaration

I hereby declare that the work in this dissertation entitled 'FURFURAL PRODUCTION FROM MICROALGAE has been carried out by me in the Department of Chemical Engineering. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

OGUCHE John Enemona	_____	_____
Name of Student	Signature	Date

Certification

This dissertation entitled 'FURFURAL PRODUCTION FROM MICROALGAE' by John Enemona OGUCHE meets the regulations governing the award of the degree of Master of Science (Chemical Engineering) of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Dr. A.O. Ameh		
Chairman Supervisory Committee	Signature	Date
Dr. Y. Tanimu		
Member Supervisory Committee	Signature	Date
Prof. B.J. El-Yakubu		
Head of Department	Signature	Date
Prof. S. Z. Abubakar		
Dean, School of Postgraduate Studies	Signature	Date

Dedication

This dissertation is dedicated to God Almighty for seen me throughout the M.Sc. programme, to the memory of my late parent Mr. Apeh Job Oguche and Mrs. MaryOmo-ojo Apeh and to my siblings: Sunday, Ojochenemi, Samuel and Regina.

Acknowledgments

I wish to express my profound gratitude to God Almighty for His wisdom, knowledge to carry out this dissertation, in HIM I LIVE, TRUST, MOVE and have my BEING.

My sincere and enormous appreciation goes to my Supervisors: Dr.O.A. Ameh and Dr.Y.Tanimu, whose without them the successful completion of this research work would not have been possible and for their constant contributions, honesty, tolerances, guidance, simplicity and patience towards the success of this research work. Their doors are always open to me for advice, directions,corrections and prompt response to me whenever I ran to them.

I would also like to thank the staff of Chemical Engineering Department, Ahmadu Bello University,Zaria for their series of contributions throughout the various phases of this research defenses. My appreciation also goes to the Department of Botany, for allowing me to carry out the first phase of this research work in their laboratory, Multiuser General Laboratory, NARICT, IAR and ABU Water Work.

I sincerely appreciate my brother Inspector Sunday Apeh and his family. To my gentle brother, Samuel Apeh and his family, my sisters; Mrs. Ojochenemi Lawrence, who raised me up as a mother and sis. Regina Oguche, indeed they are all wonderful and so I appreciate their efforts towards the success of this study. Their immense sacrifices and contributions cannot be over emphasized in my life.

My enormous gratitude goes to my major supervisor and his wife for their immense contribution to my life, financially, physically and spiritually. Their contributions in my life know no bounds. I really want to appreciate them for taking me as a son and allowing me to grow under their shoulder. I specially want to thank Mrs. Phoebe Ameh for her advice, encouragement and taking care of me as a son. Their lives are worthy of emulation and their contribution in my life cannot be over emphasized. May God almighty reward all their labour over my life in Jesus name (Amen).

To the Beautiful Bride Christian Fellowship (BBCF), Shalom! I really appreciate her prayers, encouragement, love and spiritual guidance. Thanks to the fellowship for re-shaping and imparting my life, putting me to the path of life and for imparting me to know the truth and revelation amidst confusion and bondages of denominationalism in this end time! I appreciate the fellowship for lighting and illuminating my understanding to see and know the truth and come out of denominationalism. I sincerely want to appreciate my pastor A.O Ameh. May Almighty God renew His revelation and anointing in his life and lift him up for the earth to hear his voice in Jesus name (Amen). To every member of BBCF: Bros; Jonathan, Micah, David, Salem, Timothy, Nathan, Ezekieletc and Sis Phoebe, Lovet and Elliora,you are all wonderful. God blesses and keeps us safe in HIM.

Finally to the memory of my late parents. I appreciate their advice, quest and love for education. Their memories remain in my mind forever. To my big aunty, Esther Ugwuanyi, Isaac Ugwuanyi , Ogechi and Mama O.g I say thanks for their sacrifice and supports and everyone who contributed towards the success of this programme. To my friends; TEN-TEN, Chioma and Serah I say thanks and to all my colleagues: P15EGCE…. you are all wonderful and a big Thank you all. Remain blessed and SHALOM!!!.

Abstract

This research focused on the production of furfural from microalgae biomass and optimizing the process parameters using Response Surface Methodology (RSM). Furfural production was carried out by hydrolysis of Pentosans to Pentoses where Pentosans in hemicelluloses is hydrolyzed with dilute mineral acids and NaCl as a promoter at relatively low temperature to pentoses and subsequent dehydration of pentoses to furfural in a single stage process. Central Composite Design (CCD) was employed to determine the effect of process parameters: reaction temperature (39.43 ^{o}C-165^{o}C), reaction time (30- 90 min), and concentration of 1M H_2SO_4 (35 mL/g of microalgae biomass used -70 mL/g of microalgae biomass used) on the yield of furfural. The regression analysis showed good fit of the experimental data to the second-order polynomial (Quadratic model) with coefficient of determination (R^2) value of 0.9807 and model *F*-value of 56.54. The model was found to be significant as its Predicted R^2 of 0.8771 was in close agreement with the Adjusted R^2 of 0.9634. Sodium chloride concentration have significant effect on furfural yield; furfural yield increased with increase in sodium chloride concentration up to about 1.0g/g of biomass, however, it prolongs reaction time and subsequent increase in sodium chloride concentration led to decrease in furfural yield after 1.0 (g / g of biomass used) concentration of the salt and make furfural become very pink instead of been colorless. Furfural yield of 73.10% was obtained at optimum conditions of reaction temperature of 140^{o}C, H_2SO_4 concentration of 35.02 mL/g of microalgae biomass used and reaction time of 65.3 min.Validation experiments conducted at the optimum conditions gave an experimental value of 73.10% which was in close agreement with the predicted value of 69.36%.The polymerization of by- products were observed to be accelerated as the process variables were varied therefore; optimization of these process conditions was helpful to facilitate furfural production and to obtain high yield of 73.10% which was recorded.FTIR result confirm the presence of carbonyl and aldehyde functional groups at wave length of 1674.27cm^{-1}and 2924.18cm^{-1} respectively, while GC-MS analysis of the sample confirms the presence of furfural of relative percent with respect to the column area of 50.86% with the GC-MS Spectrum showing a molecular ions peak at mass to charge (m/z) ratio of 96 g/mol which corellate to a molecular formular of furfural of $C_5H_4O_2$.

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Abbreviations

GHG	Green House Gases
GW	Global Warming
RSM	Response Surface Methodology
CCD	Central Composite Design
DOE	Design of Experiment
FTIR	Fourier Transform Infrared Spectroscopy
GCMS	Gas Chromatogram Mass Spectroscopy
THF	Tetrahydrofuran
MeTHF	Methyltetrahydrofuran
HMF	5-Hydroxymethylfurfural

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The need to replace fossil-based chemicals with renewable alternatives has led to an increased interest in the production of platform chemicals originating from lignocellulosic biomass (Sweyger *et al.*, 2016). Although, the search for an efficient process to convert lignocellulosic biomass into platform chemicals is an important challenge, thus it requires process intensification. Lignocellulosic biomass is any organic matter that is available in a renewable origin which includes energy crops, agricultural residues, aquatic plants, wood and wood residues as well as other waste materials (Maity, 2015). Algae-based fuels and other algae derived products have recently received growing interest for energy, food, and pharmaceutical industries (Chisti, 2007). Most algae are capable of producing energy-rich oils, and other species not rich in oil have also been isolated (Rodolfi *et al.*, 2009). It has been established by Jones and Mayfield (2012) that microalgae offer several advantages over terrestrial plants as a source of transportation biofuels, including high growth rates, high lipid content, the ability to grow large cultures on non-agricultural land, and the ability to rapidly improve on strains genetically and produce co-products. Chisti (2007) has opined that despite these promising characteristics, the economic viability of algae-based biofuels is still uncertain. Recent estimates place a barrel of algae-based oil at US $450–$2300, compared with US $80–110 for crude oil in 2012 (Chisti,2007; Alabi *et al.*,2009). Efforts to lower the cost of algae oil production are currently focused on nutrient sourcing and usage, harvesting, strain isolation, production management, fuel extraction, co-product development, and

residual biomass utilization for production of important economic products such as proteins, furfural and glucose (Hannon *et al*., 2010). Other options of utilization include anaerobic digestion of biomass to produce methane (Zamalloa *et al.*, 2011), pyrolysis of dry biomass to produce bio-oil (Becker, 2007) or use as a fertilizer (Mulbry *et al.*, 2005). Zeitsch (2000) reported that in theory, any material containing a large amount of the pentose (five carbons) sugars arabinose and xylose can serve as a raw material for furfural production. Hence, in this research microalgal is suitable for this purpose. Microalgae are microscopic unicellular organisms capable of converting solar energy to chemical energy via photosynthesis (Posten *et al.*, 2008). They contain numerous bioactive compounds that can be harnessed for commercial use. The potential of microalgal photosynthesis for the production of valuable compounds or for energetic use is widely recognized due to their more efficient utilization of sunlight energy as compared with higher plants. Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for health, food and feed additives, cosmetics and for energy production (furfural) (Indira and Biswajit, 2012).Due to their abundance and their pentosans content,microalgae biomass have long been considered as an alternatives energy source (Spolaore *et al*., 2006). Factors such as climate change, population growth, diminishing oil reserve and sustainability issues in the fishing industry have led to resurgence of interest in microalgae as a source of biomass, biofuels,fish meal replacement and food (Becker, 2007;Hu *et al*., 2008;Huntington,2009; Stephen*et al*., 2010 and Day *et al*.,2012).Algae are important non-vascular plants that have many research applications, including high species diversity, biofuel sources, and adsorption of heavy metals and, following

processing, are used as ingredients in health supplements, food and so on (Chen, 2013).The use of algal biomass as feedstock for the food, cosmetics, nutraceutical, pharmaceutical, bio fertilizer, and biofuel industry are of great interest and is an actively investigated field of research (Fernando *et al.*, 2016). The concept of utilizing algae in biorefineries is a promising and economically viable alternative for the production of bio product, such as furfural. It is crucial during this period that institutionalframeworks (i.e. policies) to promote development, commercialization andstimulate the evolution of the algal biomass industries as a source of renewable fuels,high value chemical, are very important. Also the potential of microalgal photosynthesis for the production of valuable compounds or for energetic use is widely recognized due to their more efficient utilization of sunlight energy as compared with higher plants (Spolaore *et al.*, 2006).Furfural is a colorless liquid with almond odour which turn dark brown when expose to air. Furfural is a promising platform chemical for the production of fuels and high value-added chemicals in industries; it serves as feedstock for the production of a wide range of value added products and chemicals such as furfuryl alcohol. (Zeitsch, 2000). Furfural, a chemical similar to 5-hydroxymethylfurfural, is one of the furan derivatives produced from the hemicellulosic fraction of lignocellulosic, which is considered a promising commodity bio-based chemical because of the possibility of its use in the production of several products such as antacids, paints, fuel additives and fertilizers (Machado *et al*., 2016). As one of the major constituents of the algae biomass, furfural (up to 50%) are expected to play an important role in algae biorefinery (Barbosa *et al*., 2014) as it has several applications such as reactive solvent, good wetting agent, unique chemical feedstock for other furan derivatives, precursor for wide range of

chemical synthesis, Purification technology for C_4 and C_5 hydrocarbons and refining of lubricating oils as well as decolorizing agent.Furfural is a chemical that is produced from pentosans, the 5-carbon sugars available in biomass hemicellulose. Most furfural production plants utilize merely hemicellulose for furfural, and the residue is burnt or used as a fertilizer after neutralization (Hoydonckx *et al.,* 2007). Bozell and Petersen (2010) demonstrated that furfural has been recognized as one of the top chemicals from biorefinery carbohydrates. In their work, catalysts, reaction temperature, reaction media and selectivity of dehydration were mentioned as obstacles to improving furfural production, as well as the technology required for the production of derivatives. On industrial scale**,** furfural production from pure sugars would not be cost effective (Cai *et al*., 2014). Thus, the starting material for furfural production would include a mixture of sugars, hemicellulose, lignin, organic acids and other minor components originating from biomass after extraction of biodiesel (Marcotullio, 2014).

Shafeeq *et al* (2015) reported that furfural yield increases rapidly with increase in the concentration of acid up to 15%, but concluded that when acid concentration is increased to 20%, the yield slightly decreases and gives reasons that at higher concentration of acid, it becomes very difficult to isolate furfural produced in liquid phase by the standard method of laboratory separation i.e. solvent extraction using ether.They also stated that the factors upon which the yield of furfural depends are:concentration interms of acid/solid (volume to weight Ratio), temperature, and time of digestion and pressure. However, in their work, they were only able to investigate only the effects of acids concentration from 5%-20% on furfural yield. Thus, in this research, effects of concentration in terms of acid/solid (volume to weight Ratio), temperature, and time on

furfural yield will be investigated and the need to use other extracting solvents such as, sulphuric acid and chloroform to investigate this effect on furfural yield. All economic studies forecast that, in the coming years, there will be a worldwide increase in biofuel demand (Chisti, 2007).This is caused by the increasing depletion of fossil fuel, global climate change due to consumption of fossil fuel by the ever growing world population. Thus making algae a promising source of fuel and feedstock for production of platform chemical such as furfural. This organism contains many types of valuable ingredients, grow very rapidly, and can be cultivated and harvested in relatively simple ways.It is important to note that the autotrophic nature of algae makes them a huge biotechnologically valuable for the production of furfural. Also, its less expensive in production, does not required a large land to grow and can be harvested as many times as possible compared to other sources such as agricultural feedstocks.

1.2 Problem Statement

While much attention has been given to researches on the uses of lignocellulosic materials (from agricultural wastes) in the production of furfural, the uses of microalgae for furfural production have not been widely reported. Consequently there is underutilization of microalgae biomass which remains as waste after extraction of biodiesel.Furthermore, previous researchers have reported low yield of furfural from agricultural feedstock. However, optimization of process parameters such as reaction temperature, acid concentration and reaction time to obtain high yield of furfural have not been carried out.

1.3 Research Justification

The justifications for this research are as follows:

i. It is a means of converting waste to wealth by utilizing the residual biomass that remains after production of biodiesel
ii. Low cost of production and sustainable sources since its obtained from local raw material (algae) which is renewable
iii. Microalgae can be raised in wastewater and on land unsuitable for food crops
iv. Economic viability of biomass as sources of energy hinges upon high-value co-products such as furfural
v. Microalgae have high growth rates compared to agricultural cropsas sources of biomass.
vi. Cheap and abundant source of feedstock
vii. It enhances biotechnology development in Nigeria by providing experimental data for design of bioreactors.
viii. It helpsto address problem of energy security, and environmental pollution by producing abundance of biomass

1.4 Aim and Objectives

The aim of this research is to study the effect of process conditions on the yield of furfural from microalgae biomass

The specific objectives are:

i. To evaluate the effects of reaction temperature, concentration of acids (catalyst) and reaction time as well as sodium chloride concentration on the yield of furfural
ii. To optimize furfural yield using RSM (CCD) methodology
iii. To characterize furfural produced using GC-MS and FTIR.

1.5 Research Scope

The scope of this work is limited to:

i. Production of furfural from microalgae
ii. Evaluating the effect of process conditions such as temperature, acid concentration, sodium chloride concentration and reaction time on furfural yield from microalgae biomass
iii. Determination of the optimum process conditions for furfural production from microalgae.
iv. Characterisation of furfural using GC-MS, FTIR.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Overview

Algae-based fuels and other algae derived products have recently received growing interest from the energy, food, and pharmaceutical industries (Chisti, 2007). As such, research to improve alternative and renewable energy sources is of great importance. More so, the use of clean energies which can be a solution for shortage in petroleum supply(Machado *et al.*, 2016). In recent years, an increasing effort has been devoted to findpaths to utilize biomass as feedstock for the production of organic chemicals because ofits abundance, renewability and worldwide distribution (Martinand Ignacio, 2013). Theincrease in atmospheric CO_2, resulting from burning of fossil fuel (Fernando *et al.*, 2016) releases large amountsof CO_2, a gas that contributes to global warming and ocean acidification (Fernando *et al.*, 2016). Algae cancapture this CO_2 to produce large amounts of biomass since they are generated fromnatural cycles; they do not cause alterations in the planet thermal balance, and areprofitable (Jones and Mayfield, 2012). A great interest in the utilization of agriculture andforestry residues to produce renewable fuels, such as ethanol, has been observed hencebioethanol is one of potentially viable alternative energy source but despite the hugeamount of ethanol production already existent, its production from alternative sources canbe interesting due to an interesting byproduct (furfural) which can be produced alongwith ethanol (Cai *et al.*,2013).

The need for research in algae is imperative because of its emergence as one of the most promising long-term, sustainable sources of biomass and oils for fuel, food, feed, and

other co-products due their large number, cheap, and abundance and equally distributed everyone. Algae offer several advantages over agricultural (terrestrial) plants such as; a source of transportation biofuels, including faster growth rates, high lipid content, the ability to grow large cultures on non-agricultural land, and the ability to rapidly improve strains and produce co-products but it is important to note that the autotrophic nature of algae makes them a huge biotechnological value for the production of furfural (Machado *et al.*, 2016).On the world markets, the cost of fossil fuel has increased as the demand for energy and petrochemical products increases. According to the Energy Information Administration 2011 Report (Energy Information Administration, 2011), world energy consumption will increase by 53 percent (from 505 quadrillion Btu in 2008 to 770 quadrillion Btu in 2035). However, our societies heavily rely on fossil resources. About 82% of the world's energy needs are currently covered by fossil resources (Soetaert and Vandamme, 2009). Severe economic problem can occur as a consequence of unexpected disruption in oil supply. In addition, environmental problems such as greenhouse gas emissions, air pollution and acid rain by using fossil energy are still at issue (Chang, 2012).

2.2 Algae

Algae are a heterogeneous group of organisms. They are aquatic or live in damp habitats on land. Cell size can vary from 1 µm up to tenths of meters and the complexity from a rather simple spherical cell to a highly differentiated plant (Jonathan *et al.,* 2012). There are two classifications of algae: macroalgae and microalgae. Macroalgae are the large multi-cellular algae often seen growing in ponds. These larger algae can grow in a variety of ways. Microalgae are microscopic plant-like organisms that normally grow in

suspension within a body of water and are capable of converting solar energy to chemical energy (Spolaore *et al.,*2006). Algal biomass contains three main components: carbohydrates, proteins, and lipids / natural oils (Tingting, 2014). Most microalgae have a cell wall made up of cellulose with the exception of diatoms which have a silica cell wall (Becker, 2007).They contain numerous bioactive compounds that can be harnessed for commercial use (Spolaore *et al.,* 2006). Thus, Hemicellulose conversion to furfural has a high-potential to be unlocked (Machado *et al.*, 2016).

2.2.1 Use of microalgae

Some of the uses of microalgae are discussed in this section.

2.2.1.1 Microalgae biodiesel and chemicals

Microalgae biodiesel is one technology in the biodiesel field and a lot of researchers use microalgae to produce chemicals, oils and polysaccharides (Munro *et al*., 1999). With photosynthesis; microalgae absorb light to produce carbohydrates, lipids and proteins. However, its efficiency is much better than crops plants, and the capacity of producing oil is also better than the crops plants, (Chisti, 2007). The land usage is also an important issue, comparing to microalgae oil extraction, and large cultivation area needs more crop oil. It's also used as feedstock production for production of valuable chemical (Machado *et al.*, 2016).

2.2.1.2 Bio-products from microalgae

Cultivated algae serve many other purposes, including cosmetics, animal feed, bioplastic production, dyes and colorant production, chemical feedstock production, medicine and pharmaceutical ingredients (Spolaore *et al.,* 2006).

2.2.1.3 Pollution controland water treatment

Algae are used in wastewater treatment plants. Algae are used to treat polluted water by using the extra nutrients in the polluted water as food. Special water treatment plants use algae to clean the water by inserting the algae into polluted water (Spolaore *et al.*, 2006).The algae absorb the extra nutrients to grow and the algae releases oxygen into the environment (Liu *et al.*,2006.). They act as a "biofilter", this is like a sieve to filter out the nutrients from the polluted water and release clean water. With concern over global warming, new methods for the thorough and efficient capture of CO_2 are being sought out. The carbon dioxide that a carbon-fuel burning plant produces can feed into open or closed algae systems, fixing the CO_2 and accelerating algae growth. Algae cultivation is under study for uranium/plutonium sequestration and purifying fertilizer runoff (Cai *et al.,* 2013). *Chlorella,* particularly a transgenic strain which carries an extra mercury reductasegene, has been studied as an agent for environmental remediation due to its ability to reduce Hg^{2+} to the less toxic elemental mercury (Indrian and Biswajit, 2012).

2.2.1.4. Uses in pharmaceuticals and in medicine

Microalgae are rich sources of novel and biologically active primary and secondary metabolites. These metabolites may be potential bioactive compounds of interest in the

pharmaceutical industry (Rania and Hala, 2008). Microalgae contain numerous bioactive compounds that can be harnessed for commercial use. They have emerged as important sources of proteins and value added compounds with pharmaceutical and nutritional importance. The microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture. In medicine**,** Algae is used as one of important medical source due to its antioxidant, anticancer, antiviral properties (Spolaore *et al.,* 2006). Microalgae can also be used to treat medical problems. Brown microalgae have been used for colds. Brown microalgae have many vitamins such as A, B, C, E and K. Macroalgae contains a mineral called "Iodine" which can be made into tablets (Chaput *et al.,* 2012). Brown macroalgae such as *Laminaria digitata* contains a substance called "alginate" and this can be used in medicine for making bandages. Maerl is a red macroalgae that is processed and used for its nutrients. Maerl contains calcium and magnesium. It is sucked up by a pipe from the bottom of the sea. Maerl can also be used as a nutritional supplement for people who need extra calcium (Indrian and Biswajit, 2012).

2.2.2 Production of microalgae

A number of algae production technologies are currently under development, from open ponds and closed Photobioreactors, from fermentation tanks to hybrid systems, to some that combine these various methods, hence there is no one single way to grow algae at commercial scale, and this versatility is one of algae's strengths (Indrian and Biswajit, 2012). However, in most cases, the approach taken is designed solely to maximize algae growth for production of fuel, chemicals or other industrial products. How exactly these systems operate is often a function of where they are located and the desired end-product.

2.2.3 Principles of microalgae culturing

To be able to isolate algae from its natural environment one has to mimic both its chemical and physical habitat. The basic problem in establishing algal cultures is the design of the media (Algal cell culture) (Andrew *et al.*, 2008). Natural water is very dilute but at the same instances a very complex media. This is why purified offshore water (or artificial seawater) is used as the basis for marine alga culturing media. To do this, precisely defined quantities of major nutrients (i.e. nitrogen, phosphorus, and silica), minor nutrients (i.e. copper, zinc, cobalt, manganese, molybdenum, iron, selenium) and vitamins (i.e. B12, thiamin and biotin) could be added (Ethier *et al.*, 2011). Light intensity, light quality and day length are parameters that can have profound effects on algal growth (Carvalho *et al.*, 2011). In general, cultured algae are adapted to rather low light intensities and the temperature range is quite broad. Beside the physiological parameters, many algae also need specific biological parameters to be able to grow. These parameters are often completely unknown, which creates problems. Many marine algal species can only be cultivated for some generations in natural, untreated seawater. The exclusion of some of the accompanying species is usually possible (i.e. predators), but often the algae of interest cease to grow after exclusion of all accompanying species (i.e. small flagellates and bacteria). If this is due to the algal species need for prey organisms or if they live in a mutualistic kind of mood, still remains to be solved (Carvalho *et al.*, 2011). Mixed cultures create problems when studying algae. It should therefore be pointed out that, at the moment, there are very limited numbers of algal species that are used in biogenetic engineering studies, since these studies nearly always require one to grow the algal species without other organisms present in the culture (i.e.

axenic cultures). It should also be mentioned that several species of macroalgae are commercially and scientifically cultivated (He *et al.*, 2012). Algae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. The terminologies used to describe the type of algal culture include:

2.2.3.1 Indoor/Outdoor

Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods (Hu, 2004).

2.2.3.2 Open/Closed

Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc. (Hu, 2004).

2.2.3.3 Axenic (sterile)/ Xenic

Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. The latter makes it impractical for commercial operations (Tingting, 2014).

2.2.3.4 Batch culture

In limited volume (batch) cultures, resources are finite. When the resources present in the culture medium are abundant, growth occurs according to a sigmoid curve, but once the resources have been utilized by the cells, the cultures die unless supplied with new medium (Hu, 2004). In practice this is done by sub culturing, i.e. transferring a small

volume of existing culture to a large volume of fresh culture medium at regular intervals. The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested (Tingting, 2014). Although often considered as the most reliable method, batch culture is not necessarily the most efficient method. Batch cultures are harvested just prior to the initiation of the stationary phase and must thus always be maintained for a substantial period of time past the maximum specific growth rate. Also, the quality of the harvested cells may be less predictable than that in continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase) (Weston *et al.*, 2013). Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture. Batch cultures also require a lot of labor to harvest, clean, sterilize, refill, and inoculate the containers.

2.2.3.5 Continuous culture

In continuous cultures, resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practice, a volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed (Ethier *et al.*,

2011). The continuous culture method, (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished, (Tarlan and Vidadi, 2001).

Turbid stat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system. While Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant (Hallmann, 2007).

2.2.3.6 Semi-continuous culture

The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment (Hu, 2004).The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size. Table 2.1 summarizes the major advantages and disadvantages of the various algal culture techniques.

Table 2.1. Advantages and disadvantages of various algal culture techniques

Culture type	Advantages	Disadvantages
Indoors	A high degree of control (predictable)	Expensive
Outdoors	Cheaper	Little control (less predictable)
Closed	Contamination less likely	Expensive
Open		Contamination more likely
Axenic	Predictable, less prone to crashes	Expensive, difficult
Non-axenic	Cheaper, less difficult	More prone to crashes
Continuous	Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods	Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high
Semi-continuous	Easier, somewhat efficient	Sporadic quality, less reliable
Batch	Easiest, most reliable	Least efficient, quality may be inconsistent

Source: (Hu, 2004).

2.2.4 Advantages of algae over other agricultural crops

Some of the advantages of microalgae for furfural production over agricultural feedstocks are:

i. Algae can be harvested every day instead of just once or twice a year.

ii. Algae can be raised on land unsuitable for food crops.

iii. Algae can use water not suitable for irrigation (salt and brackish water)

iv. Algae require less land to produce an equivalent amount of furfural, proteins and glucose.

v. For every one ton of biomass produced, 1.9 tons of CO_2 are consumed using microalgae as feedstock.

vi. Significant quantities of algal biomass and algal residual products such as proteins, glucose will become available as algae production increases.

vii. Residual algal solids remaining after oil extraction are a valuable source of furfural, protein and glucose (Machado *et al.*, 2016).

2.3 Hemicellulose

Hemicellulose is the second most abundant polymer containing about 20–60 % of lignocellulose biomass (Ferrentino, 2007). It is not chemically homogeneous like cellulose (Figure 2.1).

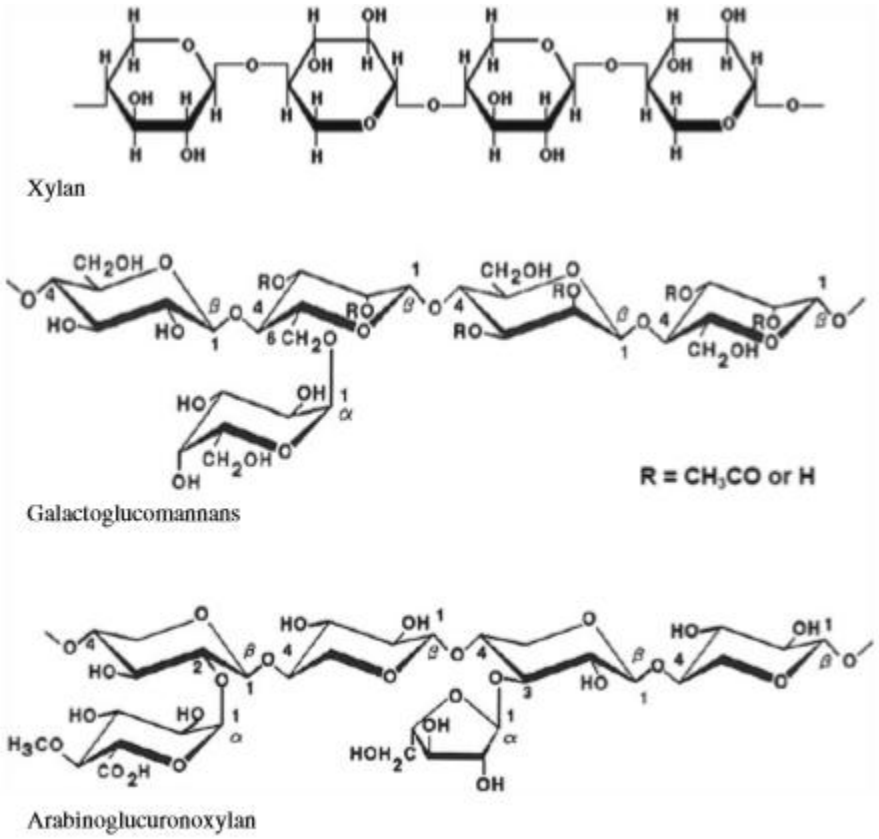


Figure2.1: Structure of hemicelluloses (Bozell and Petersen, 2010).

Hemicellulose has branches with short lateral chains consisting of different types of sugars. These monosaccharaides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (4-O-methylglucuronic, D-glucuronic, and D-galactouronic acids). The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by beta-(1, 4)-glycosidic bonds and occasionally beta-(1,3)-glycosidic bonds. Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. Hemicelluloses have lower molecular weight compared to cellulose and branches with short lateral chains that are easily hydrolysed (Saha, 2003). Hemicelluloses are found to differ in composition mainly in third generation biofuels (Algae) and in agricultural biomass like straw and grasses,

hemicelluloses are composed mainly of xylan while softwood hemicelluloses contain mainly glucomannan (Figure 2.1). In many plants, xylans are heteropolysaccharides with backbone chains of 1, 4- linked beta-D-xylopyranose units. In addition to xylose, xylan may contain arabinose, glucuronic acid, or its 4-O-methyl ether, acetic acid, ferulic and p-coumaric acids. Xylan can be extracted easily in an acid or alkaline environment while extraction of glucomannan requires stronger alkaline environment (Balaban and Ucar 1999; Fengel and Wegener, 1984). Among the key components of lignocellulosics, hemicelluloses are the most thermo-chemically sensitive (Hendricks and Zeeman 2009; Levan *et al.*, 1990). Nevertheless, severity parameters should be carefully optimized to avoid the formation of hemicellulose degradation products such as furoic acids and hydroxymethyl furfurals (Jeon *et al.*, 2016). Pentosan composition of differents types of feedstocks that can be used for furfural production is presented in Table 2.2. It is clearly notice that microalgae have higher pentosan content compared to agricultural crops; it also require less land area, used land unsuitable for agriculture,grow faster and can be harvested daily.

Table 2.2: Pentosan content of various feedstocks that could be employed for furfural production

Feedstocks	Pentosan content (%)
Corn cobs	25
Rice husk	16
Sugarcane baggase	25
Cotton seed hull	28
Microalgae	32
Wheat straw	24
Maple wood	16
Peanuts shell	3
Barley straw	24
Almond husk	30
Paper residue	13
Soy straw	12
Rice straw	25
Softwood (pine)	21

Source: (Ashutosh *et al.*, 2017)

The choice of acid catalyst to be used is also shown in Table 2.3 and it has been reported by Zhiping *et al.* (2015) that H_2SO_4 has high percentage of pentose conversion and highest furfural yield. Also H_2SO_4 has destructive ability to remove lignin from the feedstock and dilute quickly compared to other catalyst.

Table 2.3: Conversion of pentose into furfural using various catalysts

Catalyst	Pentose conversion (%)	Furfural yield (%)
No catalyst	4.20	2.00
HZSM-5	35.00	22.10
H_2SO_4	98.00	73.00
HCl	100.00	70.00
$AlCl_3$-$6H_2O$	100.00	58.60
PTSA-H_2O	100.00	58.50
Amberlyst-15	85.40	61.20

Source: (Zhiping *et al*., 2015)

2.4 Furfural

Furfural is an organic compound produced from a variety of agricultural byproducts, including corncobs, oat, wheatbran, and sawdust. The name *furfural* comes from the Latin word *furfur*, meaning ‘‘bran’’, referring to its usual source. Aside from ethanol, acetic acid and sugar it is one of the oldest renewable chemicals (Peters, 1936). Chemically, furfural is an aldehyde of furan. It is a colorless oily liquid with the odor of almonds, which quickly darkens when exposed to air. Its IUPAC name is Furan-2-carbaldehyde while other names furfural, furan-2-carboxaldehyde, fural, furfuraldehyde, 2-furaldehyde, pyromucic aldehyde. Furfural is a promising platform chemical for the production of fuels and high value-added chemicals in industries; it serves as feedstock for the production of a wide range of value added products and chemicals such as furfuryl alcohol (Zeitsch, 2000).

Furfural, a chemical similar to 5-hydroxymethylfurfural, is one of the furan derivatives produced from the hemicellulosic fraction of lignocellulosic, which is considered a promising commodity bio-based chemical because of the possibility of its use in the production of several products such as antacids, paints, fuel additives and fertilizers and many others that are normally produced from non-renewable resources (Machado *et al*., 2016).Figure 2.2 shows the chemical structure of furfural and furan compound.

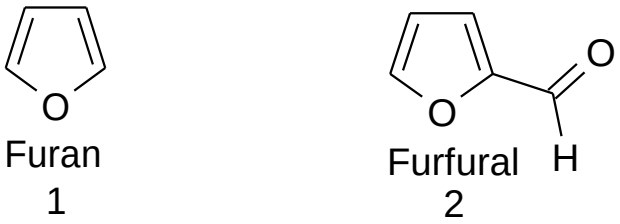


Figure 2.2: Furfural structure

Furfural or furan-2-carbaldehyde is a compound easily produced from xylose or directly from the hemicellulosic biomass dehydration. Due to its composition, rich in D-xylose, xylan should also become a good model for the direct conversion into furfural before starting the experiments with xylose-rich feedstock. Furfural is now recognized as a top-value bio-based chemical and expresses good potential as a selective solvent for diesel fuels, resins and lubricating oils. It could be also employed as precursor in the chemical industry for the preparation of various solvents such as tetrahydrofuran (THF), methyltetrahydrofuran (MeTHF), but also for the transformation into furfuryl alcohol, a well-known commercial available monomer. Its industrial production is currently carried out by using strong mineral acid such as HCl (Frederic, 2016) orH_2SO_4 (Shafeeq *et al.*, 2015) in batch or continuous reactions against pentosan containing lignocellulosic biomass.

2.4.1 Brief history of furfural

Furfural was first isolated in 1821 and published in 1832 by the GermanchemistJohann Wolfgang Döbereiner, who produced a small sample as a byproduct of formic acid synthesis. (Stenhouse, 1843).At the time, formic acid was formed by the distillation of dead ants, and Döbereiner's ant bodies probably contained some plant matter. In 1840, the Scottish chemist John Stenhouse found that the same chemical could be produced by distilling a wide variety of crop materials, including corn, oats, bran, and sawdust, with aqueous sulfuric acid; he also determined an empirical formula of ($C_5H_4O_2$) (Stenhouse, 1843)George Fownes named this oil furfurol in 1845 (from *furfur* - bran, and *oleum*) (George, 1845). This name persisted prominently in the literature until 1901 when the German chemist Carl Harries deduced furfural's structure (Brownlee and Miner, 1948). Furfural remained a relatively obscure chemical until 1922, (Peters, 1936).when the Quaker Oats Company began mass-production from oat hul (Brownlee and Miner, 1948). Today, furfural is still produced from agricultural byproducts like sugarcane bagasse and corn cobs but there is little yield of the product from these sources (Shafeeq *et al.*, 2015). The main countries producing furfural today are the Dominican Republic, South Africa and China (Werpy and Peterson, 2004). A number of plants have the polysaccharide hemicellulose which is a polymer of sugar along with five carbon atoms. On reaction with sulfuric acid, this hemicellulose hydrolysis to produce xylose. In the presence of same operating environment of heat and acid, xylose along with other five carbon sugars dehydrate and release three water molecules to produce furfural. Furfural also known as (2-furfural aldehydes, furfural, 2-Furan Carboxaldehyde, Furfuraldehyde, fural) is the principal member of five membered heterocyclic compounds called ―furans which are

characterized by an unsaturated ring of four carbon atom and one oxygen atom. Its chemical formula is OC_4H_3CHO. Furfural behavior in chemical reactions is similar to other aldehydes and aromatics. This shows its diminished aromaticity with regards to benzene. Furfural can be easily hydrogenated to the corresponding tetrahydrofuran derivatives (Zeitsch, 2000).

2.4.2 Market demand of furfural

According to Transparency market research analysis, (TMRA, 2016),Global furfural demand was 3000 kilo tons in 2016. The Strong shift towards the development of bio-based chemicals on account of volatile petrochemical prices and growing environmental concerns is expected to remain a key driver for the growth of global furfural market (TMRA, 2016) A strong push for sustainable chemistry on a political and regulatory level across key regions is expected to play a critical role in furfural market development. Furfural has gained acceptance as a substitute for petrochemicals as a building block for polymers and solid elastomers. In addition, furfural derivatives such as 5-hydroxymethylfurfural (HMF), furan-2, 5-dicarboxylic acid (FDCA) and polytetrahydrofuran (poly-THF) have also been gaining importance as potential secondary fuel sources (Daniel *et al.*, 2012). This trend is expected to drive further furfural demand over the next six years (TMRA, 2016).Production technologies involved in furfural production have witnessed very little advancement in the past. Many manufacturers produce furfural at less than 50% yield through Quaker Oats technology (Daniel *et al.*, 2012). Advancements in production processes are necessary to improve yield and reduce production costs. Increasing research and development activities are

being conducted by various companies and research institutes to improve the production process.

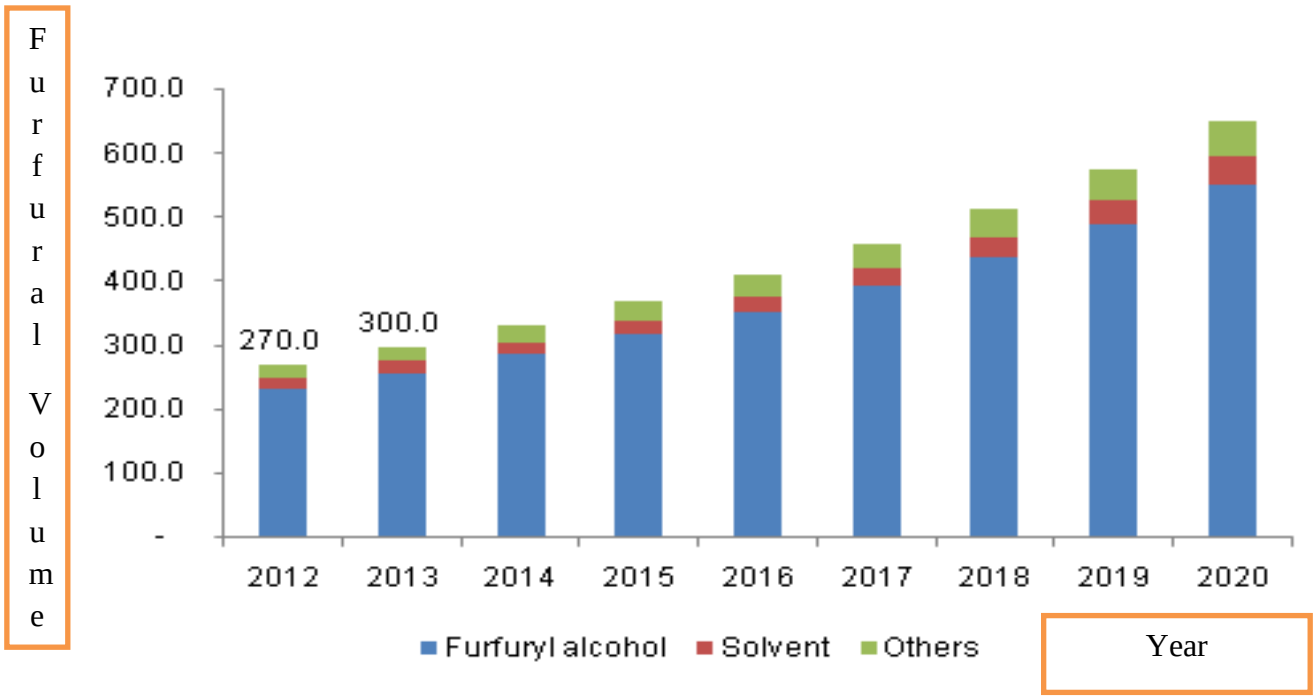


Figure 2.3:Global furfural market volume by application, 2012-2020 (GVR, 2015).

Furfuryl alcohol emerged as the largest application segment and accounted for over 85% of overall furfural market in 2013 (FMI, 2017). Furfuryl alcohol is primarily used as a binder in foundries. Growing demand for metals such as steel, magnesium and copper on account of rapid industrialization in emerging economies of Asia Pacific and Latin America is expected to drive and fuel the demand for furfuryl alcohol Furan derivatives find application in polymers, textiles, and construction materials. Novel applications in timber treatment, soil conditioning and biofuels have also emerged over a couple of years (Daniel *et al.*, 2012). Furfural has also been gaining prominence as a sustainable blend-stock in fuels such as diesel. High oxygen content in it compared to conventional fuel additives improves combustion and reduces smoke emissions in diesel engines, consequently lowering diesel costs and dependence on crude oil (Daniel *et al.*, 2012).

2.4.3 Applications of furfural

The various uses and application of furfural are:

2.4.3.1 Refining of lubricating oils and decolorizing agent

Furfural has several large scale applications due to its solubility characteristics and its easy of recuperation by steam distillation. Furfural has the unique property to dissolve aromatics and other unsaturated olefins. Today all major oil companies use furfural as selective solvent in the refining of lubricating oils (Machado *et al.*, 2016). The first refining unit was built by a subsidiary of Texaco, the Indian Refining Company. This technology leads to production of high quality motor oil with improved temperature-viscosity properties. Aromatics, polar components, mercaptans are removed from petroleum by means of furfural extraction (Jeon *et al.*, 2016). Furfural can also be used as decolorizing agent to refine crude. Furfural is used in recovery of lubricants from cracked crude solvent extraction (Daniel *et al.*, 2012).

2.4.3.2 Purification technology for C_4 and C_5 hydrocarbons

During the Second World War the purification technology of butadiene was developed in the United States for the manufacture of synthetic rubber. By extractive distillation with furfural butadiene or isoprene can be separated from other C_4 and C_5 hydrocarbons, respectively (Cai *et al.*, 2014).

2.4.3.3 Reactive solvent and good wetting agent

Furfural is unusually effective as a solvent for phenolic resins. In the manufacture of abrasive wheels, brake linings and refractory products for the steel industry furfural is known as a reactive solvent and excellent wetting agent (Jeon *et al.*, 2016).

2.4.3.4 Unique chemical feedstock for other furan derivatives

Furfural is as chemical feedstock that can be used for the production of other derivatives chemicals such as furfuryl alcohol and other 5-membered oxygen-containing heterocycles, like furan, methylfuran, acetylfuran, furfurylamine and furoic acid (Watson and Connors, 2008).

2.4.3.5 Precursor for wide range of chemical syntheses

Furfural can be used as precursor for wide range of chemical syntheses e.g. tetrahydrofuran. Furfural has unique properties and host of opportunities are available in drug and specialty chemical manufacture, replacement of phenol in foundry resins, and specialized polymers are some possibilities. For example, di-furfural is stronger than other high strength polymers (Stephens *et al.*, 2010).

It is use as high value flavor compound and used as a binder in catalyst for the new pebble bed reactors. Also used as boiler and floor grouting, adhesives used in foundry cores and moulds (Machado *et al.*, 2016). Industrially, furfural is used to produces polyamide, and polyurethane as well as nematodes control agents, fungicides and adhesives. Furfural is also used in the manufacturing of different types of thermosetting copolymer resins.

2.4.4 Physical properties of furfural

The physical property of furfural is shown in Table 2.4

Table 2.4:Physical properties of furfural

Properties	Value
Molecular weight	96g/mole
Molecular formular	$C_5 H_4 O_2$
Boiling point	161.85 ^{0}C
Melting/freezing point	-36.15°C
Relative density	1.16 g/ml
Vapor density	3.31
Critical temperature	396.9°C
Flash point	59.85°C
Auto-ignition temperature	315.41°C
Flammable limit	Upper 19.3%
Color	Colorless
Physical state	Liquid
Odour	Almond-like

Source: (Yan, 2014).

2.4.5 Chemical properties of furfural

Chemically, furfural participates in the same kinds of reactions as other aldehydes and other aromatic compounds. Indicating its diminished aromaticity relative to benzene, furfural is readily hydrogenated to the corresponding tetrahydrofuran derivatives (Sitthisa *et al.*, 2011). When heated in the presence of acids, furfural irreversibly solidifies into a hard thermosetting resin. Furfural has two important functional groups, an aldehyde

(CHO) and a conjugated system (C=C-C=C), it is a versatile compound for many applications (Tingting, 2014). Furfural aldehyde group can undergo different types of reactions such as acetylation, acylation, aldol and Knoevenagel condensation, and reduction to alcohols, reductive amination to amines, decarboxylation, oxidation to carboxylic acids and Grignard reactions (Daniel *et al.*, 2012). The furan ring system (C=C-C=C) can undergo alkylation, hydrogenation, oxidation, halogenation, open ring reactions and nitration (Win, 2005). The main advantage of furfural is that it is made from renewable resources. Furfural (from pentosans), along with ethanol (from cellulose) and phenol (from lignin) are the main commercial or semi commercial outputs from the chemical processing of wood. Yan (2014) reported that furfural has been produced for 75 years or more, so it is not new, although new and more efficient processing technologies as well as new feedstocks are emerging that could create new product possibilities for a chemical industry that is increasingly concerned about its dependence on non-renewable feedstock and energy inputs. It may also be possible to integrate furfural with other chemical and ethanol plants. As it is with many biomass transformations, Furfural investment would be likely to have impact at a local or regional level where raw materials can be sourced at low cost, either at processing plants or direct from farms in, for example, an agro forestry biochemical structure (Corporation and Services DOI, 2006).Furfural is an important renewable, non-petroleum based, chemical feedstock. Hydrogenation of furfural provides Furfuryl Alcohol (FA), which is a useful chemical intermediate and which may be further hydrogenated to tetrahydrofurfuryl alcohol (THFA) (Werpy and Peterson, 2004).THFA is used as a non-hazardous solvent in agricultural formulations and as an adjuvant to help herbicides penetrate the leaf

structure. Furfural is used to make other furan chemicals, such as furoic acid, via oxidation, (Harrison and Moyle, 1963) and furan itself via palladium catalyzed vapor phase decarbonylation. Furfural is also an important chemical solvent. (Zamalloa *et al.*, 2011) Among other uses of furfural includes;Refining of lubricating oils and decolorizing agent, Unique chemical feedstock for other furan derivatives, Purification technology for C_4 and C_5 hydrocarbons, Reactive solvent and good wetting agent. The main furfural derivatives and its applications are shown in Table 2.5

Table 2.5:Furfural derivatives and its applications

Derivatives	Production	Utilization
Furfuryl alcohol	Furfural catalytic hydrogenation	Production of resins and tetrahydrofurfuryl alcohol; intermediate in fragrances production, lysine and vitamin C
Furan	Furfural catalytic decarbonylation	Production of tetrahydrofuran and acetyl furan
2-methylfuran	Furfural and 5-methyl-furfural Decarbonylation	Solvent and monomer
Tetrahydrofuran	Furan hydrogenation	Industrial solvent, polymers fabrication, adhesives, pharmaceuticals products
Furfurylamine	Furfural reductive amination	Production of substances with pharmacological and pesticide Activities
Furoic acid	Furfural oxidation	Synthesis of medication and perfumes
Levulinic acid	Acid hydrolysis of furfuryl alcohol, hydrolysis of acetyl succinate ester	Production of succinic acid and δ-aminolevulinic acid
Succinic acid	Alcoholic fermentation	Food additives and synthesis of pharmaceuticals products

Source: (Taylor *et al.*, 2016)

2.4.6 Furfural production

Many plant materials contain the polysaccharidehemicellulose, a polymer of sugars containing five carbon atoms each (Zamalloa *et al.*, 2011).When heated with sulphuric acid, hemicellulose undergoes hydrolysis to yield these sugars, principally xylose(Hoydonckx *et al.*, 2007).Under the same conditions of heat and acid, xylose and other five carbon sugars undergo dehydration, losing three water molecules to become furfural (Win, 2005). The conversion of pentosans to furfural takes place in two steps:

1. Hydrolysis of Pentosans to Pentoses: Pentosans in hemicelluloses is hydrolyzed with dilute mineral acids at relatively low temperature to pentosans liquor. A major 75% of these pentosans is readily hydrolysable to pentoses.

2.Conversion of Pentoses to Furfural: The formation of furfural from pentoses is an ordinary process of dehydration with the elimination of three molecules of water. Furfural has rather high order of stability towards heat in the absence of catalyst and oxygen. The initial decomposition temperature has been found to be 565 °C for a contact period of 20 seconds. In commercial terms, the most important intermediary derived from furfural is (FA). This Furfural alcohol primarily used to make furan resins, but it may also be converted into Tetra Hydro Furfuryl Alcohol (THFA) (Daniel *et al.*, 2012). The factors upon which the yield of furfural depends are Acid/Solid (volume to weight Ratio), Temperature, Time of Digestion and Pressure (Shafeeq *et al.*, 2015). Pretreatments such as dilute acid at elevated temperature are effective for the hydrolysis of pentose polymers in hemicellulose and also increase the access of enzymes to cellulose fibers (Mansilla *et al.*, 1998).

Xylans, one of the types of hemicelluloses present in abundance in Lignocellulosics, are composed mainly by pentoses and generally are the major constituents of hemicelluloses in grasses and woods. Lately, studies focusing in the conversion of xylans to bioenergy, chemicals and biomaterials have received a lot of attention in the context of biorefineries (Marcotullio, 2014). Among the products which can be obtained from pentoses, there is furfural, which is a promising alternative, since it is a versatile compound that can be used in the synthesis of several important chemicals, such as furan and furfuryl alcohol, and it is vastly used in several applications in refining oil, plastics production, pharmaceutical and agrochemical industries, (Wang *et al*., 2015). Nowadays there is not a synthetic route for furfural production, so it is exclusively produced from acid hydrolysis and dehydration of pentose, mainly xylose, this way it is possible to produce it from hemicelluloses as shown in Figure 2.4 and Figure 2.5 respectively.

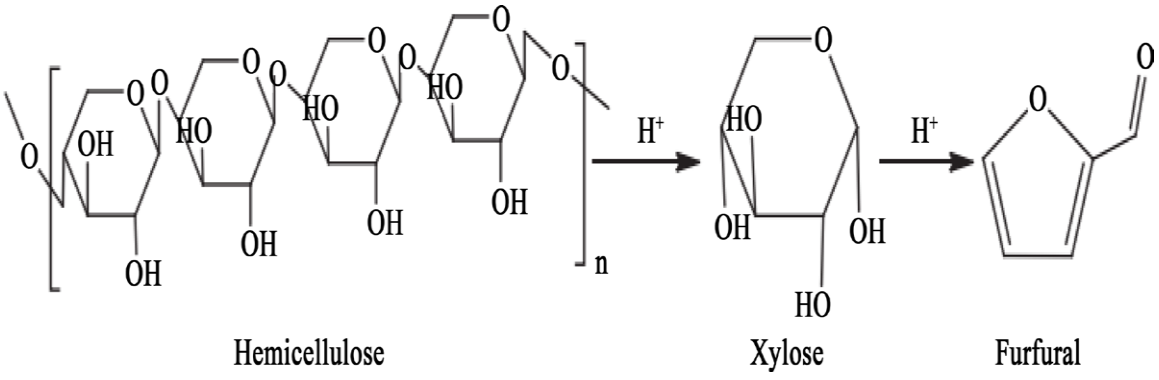


Figure 2.4: Furfural production from hemicelluloses (Yan, 2014)

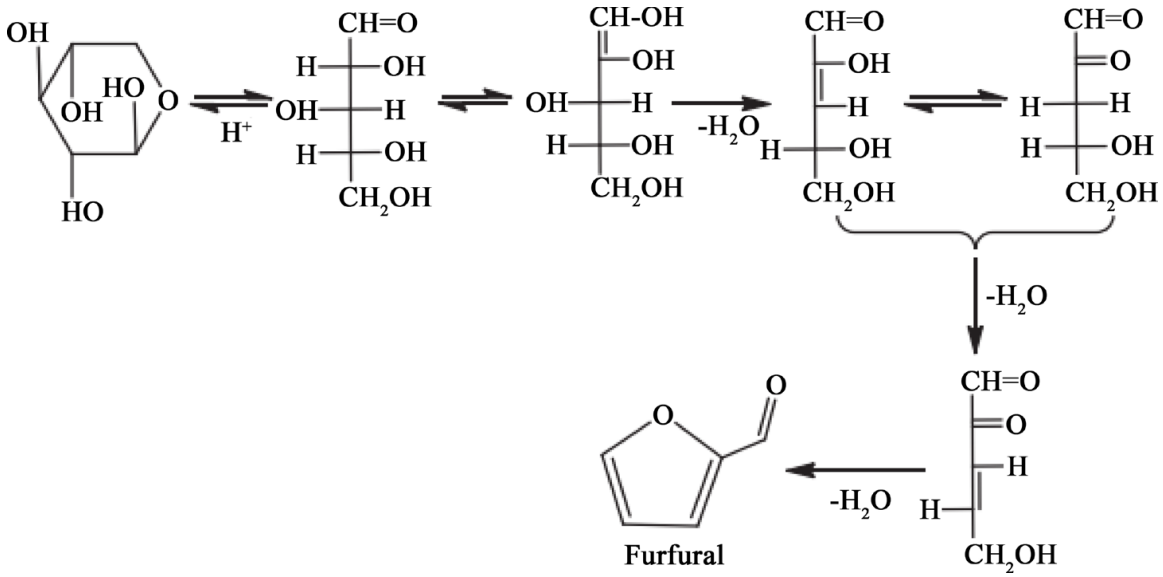


Figure 2.5: Dehydration of pentoses for furfural production (Yan, 2014)

There are two processes for furfural production, one that happens in an only step and the other which happens in a two-step process. In the one-step process, depolymerization of pentosans in xylose by acids and dehydration to furfural occurs simultaneously. In the two-step process, dissolution and depolymerization of pentosans occur in mild condition, followed by dehydration of xylose to furfural (Barbosa *et al.*, 2014). Hemicelluloses hydrolysis stage occurs in four steps as shown in Figure 2.6.

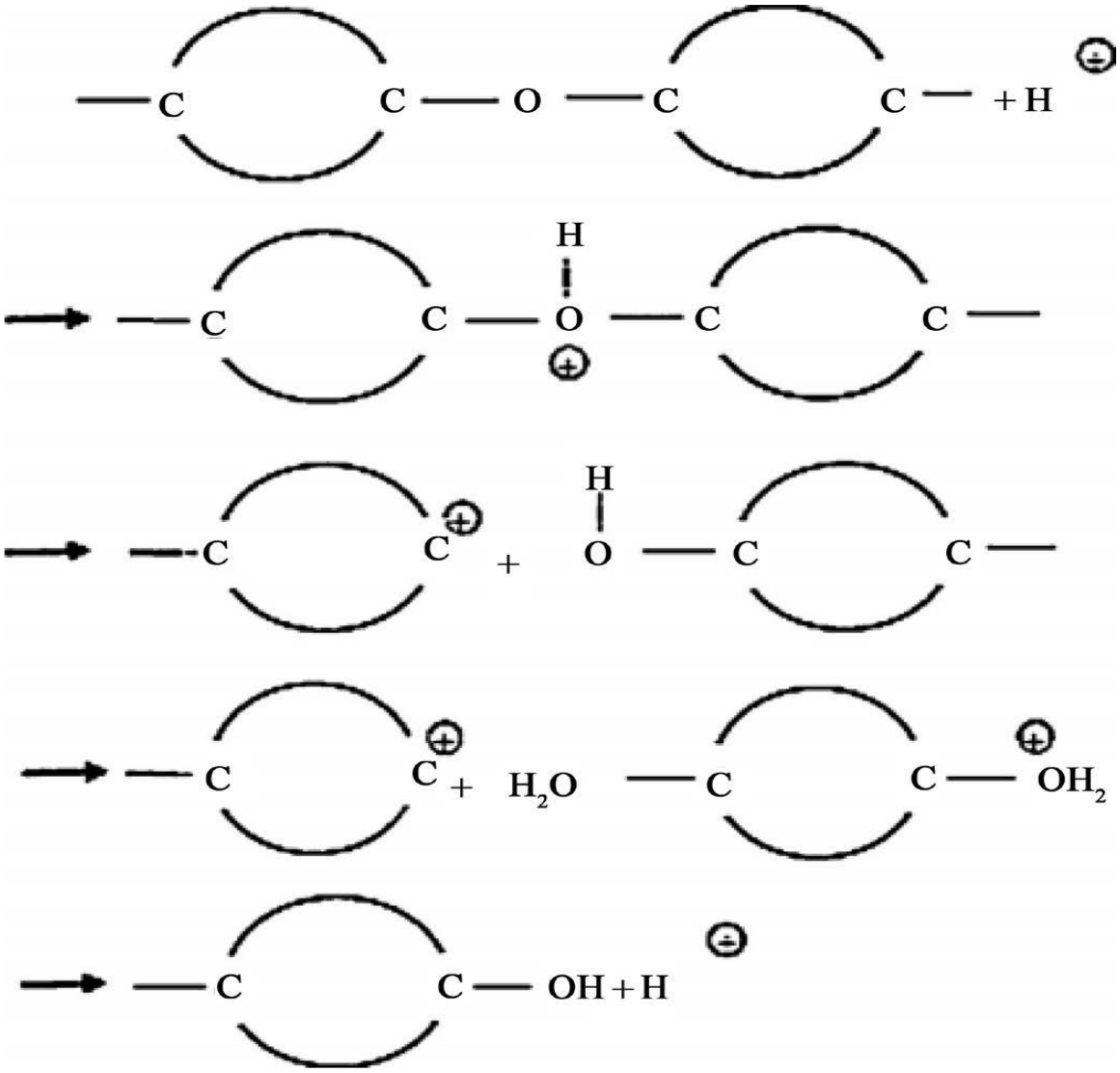


Figure 2. 6: Steps of hemicelluloses hydrolysis to xylose (Machado *et al,* 2016)

The four steps involves in hemicelluloses hydrolysis to xylose are;

i. Protonation of oxygen bond of the ether leading to the formation of trivalent oxygen.

ii. Cleavage of the oxygen bond forms a carbocation in one side and a hydroxyl group in the other side.

iii. Reaction of carbocation with water.

iv. H+ liberation and formation of hydroxyl group.

On the other hand, cyclodehydration for furfural formation occurs via two subsequent eliminations. In the first step, when H+ attacks a non-bonding electron pair of hydroxyl oxygen bound to a carbon atom, a trivalent oxygen atom positively charged is created. Since oxygen is more electronegative, the charge passes to the neighbor carbon causing the cleavage of the carbon- oxygen bond, and leading to the liberation of a water molecule. In this situation, two electron from C-X bonds are attracted to form a double bond C=C. During this step, the ring is opened and the freed hydrogen seeks another non-bound pair of electrons. In the same way as in the first elimination, another water molecule is liberated and another double bond C=C is established. In the last elimination type 1,4, oxygen trivalent ion do not create another C=C bond, but closes the open ring due to the fact that atoms which participate in double bonds form planar structures characterized by bond angles of 120˚, leading to furfural formation. Finally, liberation of H+ ends the process, (Machado *et al.,* 2016). Furfural is commercially produced in batch or continuous processes using mineral acids as catalysts, even though this process presents drawbacks as corrosion, difficult product and catalysts recuperation from reaction mixture and risks to health and the environment(Marcotullio, 2014).

In Cai *et al* (2013), integrated furfural production as a renewable fuel and chemical platform from lignocellulosic biomass, they reported that Furfural is a natural precursor to furan-based chemicals and has the potential to become a major renewable platform chemical for the production of biochemical and biofuels. However, they also establish that current industrial furfural production relies on relatively old and inefficient strategies that have hindered its capacity, and low production yields have strongly diminished its competitiveness with petroleum-based alternatives in the global market. They critically

analyzed past and current progress to enhance furfural production from lignocellulosic biomass. First, important chemical and fuel products derived from the catalytic conversion of furfural are outlined. They conclude that furfural is a promising renewable platform chemical for production of biochemicals and biofuels from low cost and abundant lignocellulosic biomass.

2.4.7 Acid hydrolysis of pentosans

Acid hydrolysis is a process in which a protic acid is used to catalyze the cleavage of a chemical bond via a nucleophilic substitution reaction, with the addition of the elements of water (H_2O). It can be defined as an acid catalyzed nucleophilic acyl substitution reaction.

Pentosan + water → pentose

$$C_5H_8O_4 + H_2O \rightarrow C_5H_{10}O_5 \quad \text{.....2.1}$$

The deconstruction of lignocellulosic biomass via hydrolysis is readily catalyzed by strong acids, which speed up the protonation of the oxygen bridges connecting the sugar monomers. Mineral acids (HCl, H_2SO_4, and HNO_3) are effective catalysts for depolymerization of hemicellulose and cellulose, as they have the necessary strength to break down the crystalline structure of the cellulose fibers (Daniel *et al.*, 2012). Acid catalyzed hydrolysis has been conducted in industrial scale for a long time. In 1922, The Quaker Oat Company developed the first commercial process for production of furfural from oat hulls in Cedar Rapids, Iowa, using liquid acid catalysts (Brownlee *et al.*, 1948). The first production of furfural was achieved by passing superheated steam through biomass (Brownlee *et al.*, 1948). However, at high enough temperatures, sulfuric acid is not necessary since the acetic acid released during the depolymerization of hemicellulose

is able to catalyze the dehydration reaction and produce furfural (Shafeeq *et al.*, 2015). The acid catalyzed hydrolysis of polysaccharides comprises the steps listed above for hemicelluloses hydrolysis to xylose. The sequence is repeated until the polysaccharide is fully converted into the corresponding sugars. However, during this process the lignin fraction cannot be dissolved and remains unreacted. Therefore, further refining is necessary. The catalytic effect of the acid is highly dependent on the proton transfer efficiency of the catalyst, rather than the proton concentration in the system. In fact, it has been observed that even negligible hydrogen ion concentrations may have a catalytic effect (Zeitsch, 2000). In this context, the hydrogen ion concentration is highly dependent on the temperature. It has been observed that the dielectric constant of water, responsible for the dissociation of the acids, decreases with temperature, which strongly diminishes the dissociation of the acid (Zeitsch, 2000).

2.4.8 Dehydration of pentose (sugars)

Dehydration of pentose takes place as shown in the reaction:

Pentose → furfural + water

$C_5H_{10}O_5 \rightarrow C_5H_4O_2 + 3H_2O$ ……...2.2

Dehydration of sugars: The formation of furfural and hydroxymethylfurfural can be accomplished by triple dehydration of xylose (pentose) and glucose (hexose), respectively, in the presence of an acid catalyst (Figure 4). Pentoses and hexoses are mostly present in the ring form, since the open-chain aldehydes only amount to less than 1 wt. %, (Daniel *et al.,* 2012). Isomerization of glucose to fructose is desirable because the latter is more effective for production of short title 9 HMF. For example, Sn-

containing zeolites have been found to be very active and selective towards fructose (31 wt. %), with small production of mannose (9 wt. %) at mild temperatures (110–140 °C), (Daniel *et al.*, 2012). As illustrated in Figure 2.7, furfural is produce along with other byproducts such as hydroxymethylfurfural, Humins due to side reaction and conversion of lignin and cellulose components of the biomass.

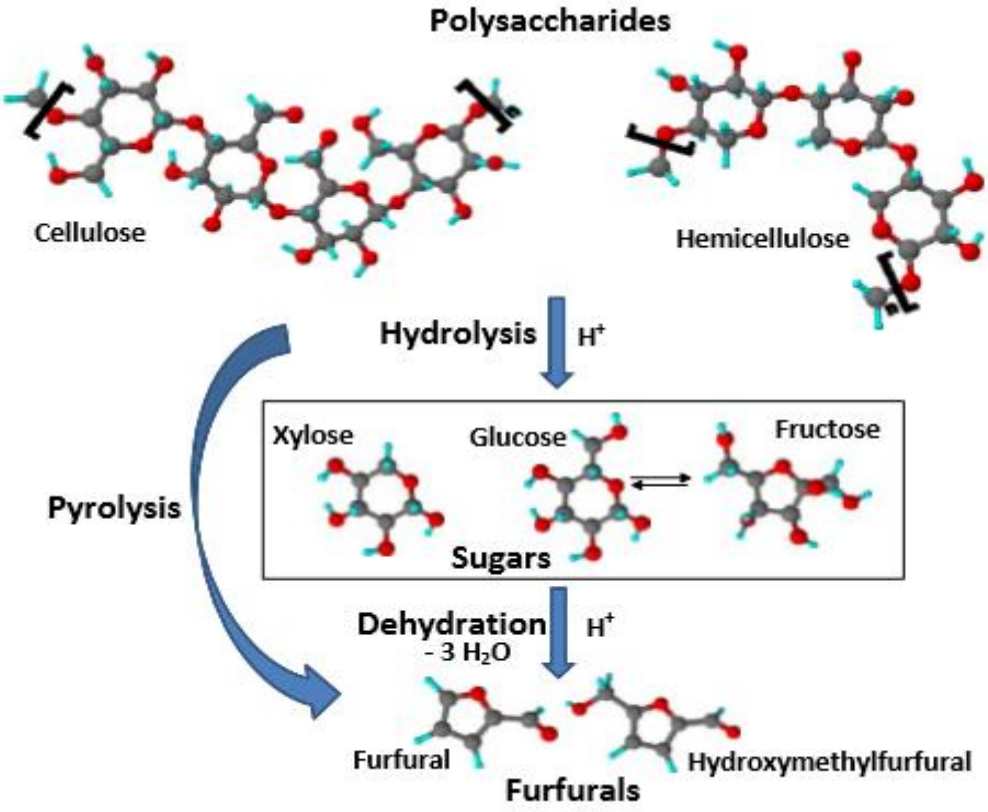


Figure 2.7: Production of furfurals from lignocellulosic biomass (Daniel *et al.*, 2012).

2.4.9 Furfural formation and degredation chemistry

The search for the dominating reaction mechanism is one the key aspect in understanding the furfural formation phenomena and forming an appropriate kinetic model (Daniel *et*

al., 2012).In-depth knowledge helps to make suitable process changes as a results of which side and loss reactions are minimized and yield enhancements achieved. The focus is on xylose dehydration into furfural and furfural degredation under the same conditions. The hydrolysis of biomass to hemicellulosic sugars happens at higher rate than xylose degredation and depends on the biomass used (Borrega *et al*., 2011; Guerra-Rodriquez *et al*., 2012; Rafiqul and Sakinah, 2012; Dussan *et al*., 2013).Thus the hydrolysis mechanism and kinetics have not been discussed. However when biomass are used, a wide product spectrum is identified including compounds originating from both sugars and ligin (Du *et al*., 2010).These compounds most probably have some interactions with furfural, sugars or catalyst affecting furfural yield.

2.4.7.1 Mechanism of furfural production

Classical route of furfural formation initiates from acyclic xylose (1) or related ketoses (3-xylulose) and proceed via acyclic intermediates (2,4 and 5) as shown in Figure2.7 (Feather *et al*., 1972). Recently,this mechanism was used as a pilar in which β-elimination (Hurd and Isenhour,1932) and chromiun catalysed 1,2 hydride shift (Binder *et al*.,2010) mechanism were group (Dannon *et al*.,2014b).This grouped mechanism is represented in Figure 2.7.The reaction steps catalysed by hallide ions or metal cations are identified in Figure2.7.These compounds are known to enhance furfural formation.Another mechanism proposed by Antal *et al* (1991) start from xylopyranose and proceeds via cyclic intermediates. Accoording to them,no clear evidence has been found that the keto form of 3-deoxyglucose (5) exist in the reaction media which is inconsistent with the fact that enolisation and de-enolisation are happening in the particular reaction conditions i.e lyxose is formed from xylose

therefore Antal *et al.*,(1991) settled on mechanism presented in Figure 2.8 where 2,5-anhydroxylose is the main intermediate reacting to furfural.At the same time acyclic xylose reacts through glyceraldehyde to pyruvalde.

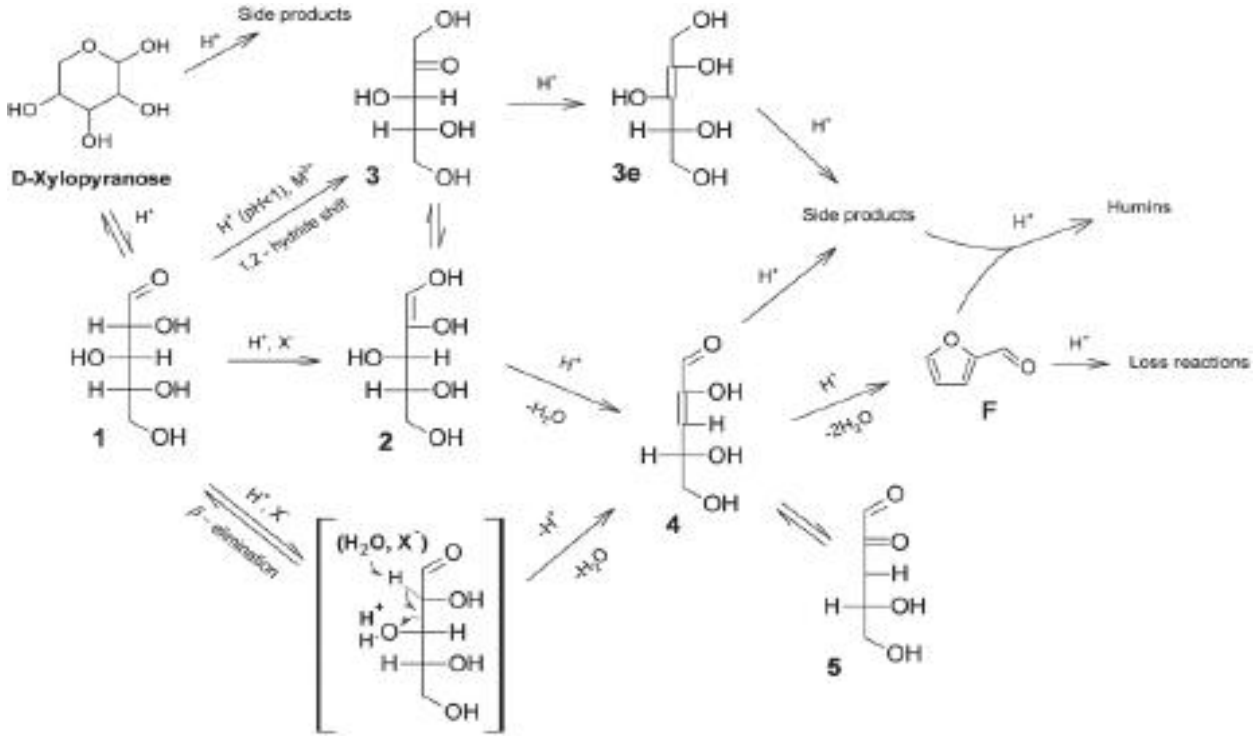


Figure 2.8: Mechanism for furfural formation via acyclic intermediates.X denotes halide ions and M^{3+} metal cations (Antal *et al.*, 1991)

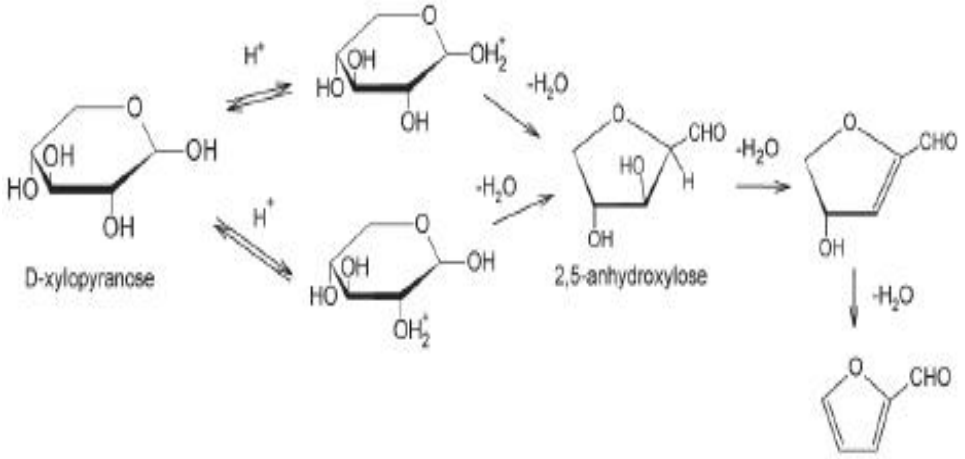


Figure 2.9: Mechanism for furfural formation via cyclic intermediates (Antal *et al.*, 1991)

Generally,the xylose dehydration reaction starts with protonation of –OH groups or the ring –O-. Importantly,Qian *et al* (2005b) and Rasmussen *et al* (2014)highlighted that the reaction conditions change the solvent watersurr oundings, and, consequently, affect the site of the protonation. In addition, solubility and mixing may also have an effect on the course of onthe reaction. Therefore, experimental observations in the actual conditions are neede

As a conclusion, several mechanisms leading to furfural seem to coexist, and one mechanism can be favoured over others by choosing a suitable solvent/catalyst system and reaction conditions.This will have an effect not only furfural yield but also on the formation of other degredation products which could have economic value in the future.

2.5 Design of Experiment

Design of Experiment (DOE) is a statistical method of systematically planning and conducting scientific studies that investigate independent experimental variables of interest altogether at a time in order to determine their effects on a given response. It is more efficient and reliable than the traditional methods of changing "one factor at a time" in order to observe the factors impact on a given response (Montgomery and Rupp, 2005). The designs of experiment help the experimenter to identify factors that affect the response. Once the important factors have been identified, the next step is to determine the settings for these factors that result in the optimum value of the response. The optimum value of the response may either be a maximum value, a minimum value or

even kept at constant (particular range) depending upon the product or process in question. For example, if the response in an experiment is the yield from a chemical process, then the objective might be to find the settings of the factors affecting the yield so that the yield is maximized. On the other hand, if the response in an experiment is the number of defects, then the goal would be to find the factor settings that minimize the number of defects. Methodologies that help the experimenter reach the goal of optimum response are referred to as response surface methods (Raissi and Eslami, 2009). These methods are exclusively used to examine the "surface," or the relationship between the response and the factors affecting the response. Regression models are used for the analysis of the response, as the focus now is on the nature of the relationship between the response and the factors, rather than identification of the important factors (Montgomery *et al*., 1994).

2.5.1 Response surface methodology

Response Surface Methodology (RSM) is an up to date approach for constructing approximation models based on either physical experiments, computer experiments (simulation) and experimented observation (Montgomery *et al*., 1994). Response Surface Methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The method was introduced by George E.P.Box and K. B. Wilson in 1951. The main idea of RSM is to use a sequence of design experiments to obtain an optimal response. Box and Wilson suggest using a second-degree polynomial model to do this. It is a collection of mathematical and statistical techniques for empirical model building (Raissi and Eslami, 2009).Statistical approaches such as RSM can be

employed to maximize the production of a special substance by optimization of operational factors. In contrast to conventional methods of keeping one factor at a time, the interaction among process variables can be determined by statistical techniques (Asadi *et al*., 2017).

2.5.2 Central composite design

Central Composite Designs (CCD) are two level full factorial 2^K or fractional factorial $2k^{-f}$ designs augmented by a number of center points and other chosen runs. These designs are such that they allow the estimation of all the regression parameters required to fit a second order model to a given response (Asadi *et al*., 2017). The simplest of the central composite designs can be used to fit a second order model to a response with two factors. The design consists of a 2^2full factorial design augmented by a few runs at the center point. Aaxial points or star points represent runs where all but one of the factors is set at their mid-levels. The number of axial points in a central composite design having 2k factors is 2^k. The distance of the axial points from the center point is denoted by α and is always specified in terms of coded values. CCD has advantages of Rotatability compared to Box-Behnken, 3-level factorials methods of RSM (Raissi and Eslami, 2009).A central composite design is said to be rotatable if the variance of any predicted value of the response, for any level of the factors depends only on the distance of the point from the center of the design, regardless of the direction. In other words, a rotatable central composite design provides constant variance of the estimated response corresponding to all new observation points that are at the same distance from the center point of the design in terms of the coded variables. It is the property of rotating points of

the design about the center of the factor space. i.e. moments of the distribution of the design points are constant. Another good property of CCD is uniformity which helps to control the number of center points is uniform precision (or Uniformity) (Montgomery and Rupp, 2005).

2.5.3 Advantages of central composite design methodology

Central composite design has several advantages over other methods of response surface methodology such as Box-Behnken, Tauguchi methods and it includes the following:

1. It is rotatable

2. CCD designs provide high quality predictions over the entire design space

3. Central composite designs are much more flexible with respect to the issue of two way interactions. They are comprised of a standard 2*k factorial, center points, and axial points. Because their core is a 2*k factorial you have the option of running a full factorial at the center or, if you don't desire information on some or all of the two way interactions you can run the core as a fractional factorial design. Thus, if all of the two way interactions are not of interest, the CCD will be more efficient than the Box-Behnken.

4. The axial (sometimes called star points) are outside of the bounds of the design space box defined by the factorial part of the design. They are chosen to produce "rotatability". i.e. the predicted response is capable of being estimated with equal variance regardless of the direction from the center of the design space. Thereby the axial points will correspond to your design space minimum and maximum and the min/max points of the

factorial will be something greater or less than the overall minimum and maximum (Raissi and Eslami, 2009)

2.5.4 Optimization using RSM; desirability function approach

The desirability function was developed by Harrington in 1965 to simultaneously optimize the multiple responses but was later modified by Derringer and Suich in 1980 to improve its practicality (Raissi and Eslami, 2009).The desirability usually lays between 0 and 1 and it respresents the closeness of a response to its ideal value. However, if a response falls within unacceptable intervals, the desirability is zero (0) which is not a desirable but if a response falls within an ideal intervals (a response reaches its ideal value), the desirability is one (1) which is most desirable. Meanwhile when a response falls within the tolerance intervals but not at its ideal interval (when a response its fails to reach its ideal value) then the desirability lies between 0 and 1.Finally, the more closely the response approaches the ideal intervals or its ideal values, the closer the desirability is to 1 (Asadi *et al*., 2017).

2.6 Characterization of Furfural

All the important characterization techniques used to confirm furfural chemicals are: Gas chromatography–mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy.

2.6.1 Fourier-transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and in some

cases, inorganic materials (Griffiths and Hasseth, 2007). The FTIR Analysis method uses infrared light to scan test samples and observe chemical properties. The first step is to collect background spectra to subtract from the test spectra to ensure the actual sample is all that is analyzed. Next, the sample is analyzed by fully-computerized Fourier Transform Infrared Spectroscopy system which generates the absorbance spectra showing the unique chemical bonds of the sample material. This profile is in the form of an absorption spectrum which shows peaks representing components in higher concentration. Absorbance peaks on the spectrum indicate functional groups such as alkanes, ketones, acid chlorides etc (Griffiths and Hasseth, 2007). Different types of bonds, and thus different functional groups, absorb infrared radiation of different wavelengths. Although the analysis is performed in absorbance, it can be converted to transmittance, since they are simply the inversions of each other. The analytical spectrum is then compared in a reference library program with cataloged spectra to identify components or to find a "best match" for unknown material using the cataloged spectra for known materials. FTIR is a technique used to obtain an infraredspectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time (Griffiths and Hasseth, 2007).

2.6.2Gas chromatography–mass spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample (David *et al.*, 2011). Applications of GC-MS

include drug detection, fire investigation,environmentalanalysis, explosives investigation and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification (Raja *et al*., 2011). Like liquid chromatography – mass spectrometry, it allows analysis and detection even of tiny amounts of substance. GC-MS has been regarded as a 'gold standard' for forensic substance identification because it is used to perform a 100% specific test, which positively identifies the presence of a particular substance (David *et al*., 2011).

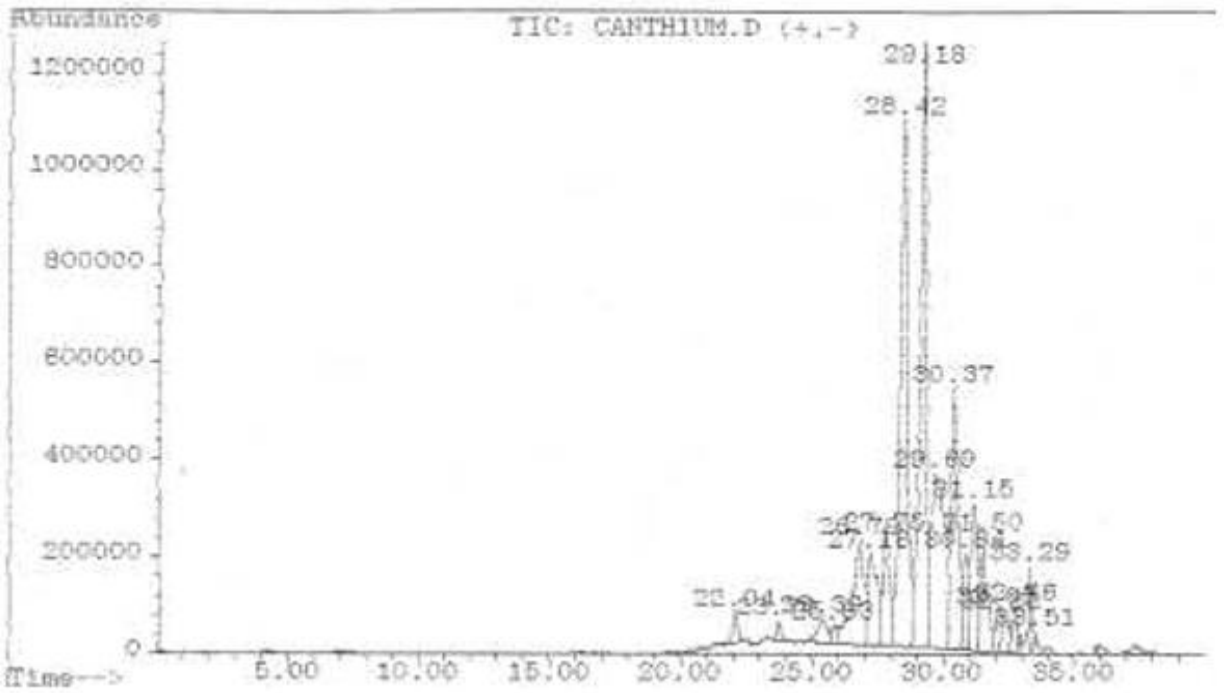


Figure 2.10: GC-MS chromatogram of ethanolic leaf extract of canthium dicoccum (Raja *et al.*, 2011).

The equipment used for gas chromatography generally consists of an injection port at one end of a metal column packed with substrate material and a detector at the other end of the column. A carrier gas propels the sample down the column. The technician uses flow meters and pressure gauges to maintain a constant gas flow. A gas that does not react with the sample or column is essential for reliable results. For this reason, carrier gases are usually argon, helium, hydrogen, nitrogen, or hydrogen (Frederic, 2012).Many analysts use helium because it does not react. Hydrogen usually is a good carrier gas but it may react and convert the sample into another substance. The ultimate choice for a carrier gas may depend on the type of detector used. To ensure proper separation, the sample must enter the column in a discreet, compact packet (Frederic, 2012). Normally the sample is injected into the injection port with a hypodermic needle and syringe capable of measuring the specimen amount. The needle is stuck into a replaceable neoprene or silicone rubber septum that covers the injection port. The injection port is maintained at a temperature at which the sample vaporizes immediately (Raja *et al.*, 2011). Ideally, the sample spreads evenly along the cross section of the column, forming a plug. The column is a metal tube, often packed with a sand-like material to promote maximum separation. The GC instrument uses a detector to measure the different compounds as they emerge from the column. Among the available detectors are the argon ionization detector, flame ionization detector, flame emission detector, cross section detector, thermal conductivity detector, and the electron capture detector. Choosing the proper detector depends upon the use (Frederic, 2012). Some considerations are that the flame detectors destroy the sample, the thermal conductivity detector is universally sensitive, and the argon ionization detector requires argon as a carrier gas. The spectral

output is usually stored electronically and displayed on a monitor. The amount of time that a compound is retained in the GC column is known as the retention time. However, retention time is not a reliable factor to determine the identity of a compound. If two samples do not have equal retention times, those samples are not the same substance (Frederic, 2012). However, identical retention times for two samples only indicate a possibility that the samples are the same substance. Potentially thousands of chemicals may have the same retention time, peak shape, and detector response. MS analysis requires a pure gaseous sample. The sample inlet is maintained at a high temperature; up to 400°C (752°F), to ensure that the sample stays a gas (Frederic, 2012). Next the specimen enters the ionization chamber. A beam of electrons is accelerated with a high voltage. The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons. Each fragment is charged and travels to the accelerator as an individual particle. In the acceleration chamber the charged particle's velocity increases due to the influence of an accelerating voltage. For one value of voltage only one mass accelerates sufficiently to reach the detector. The accelerating voltage varies to cover a range of masses so that all fragments reach the detector (Frederic, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter contains all the lists of materials, equipment with their various sources, manufacturer as well as detailed methodology followed during the laboratory work. The methodology for this research is splitted into three phases. The first phases of the experiment focused on feedstock (dried microalgae biomass) synthesis. The second phases of the experiment dwell on (DOE) using version 6.0 of design expert to design the experiment prior to laboratory work while the last phase of the experiment was aimed at optimizing the factors affecting furfural yield

3.1Materials

A list of materials used in this research is shows in Table 3.1

Table 3.1 List of materials used in the research.

S/NO	Materials	Quality/sources	Manufacturer
1	Alum	Analytical grade	Sigma Aldrich
2	Dried microalgae biomass	ABU Water Work	-
3	H_2SO_4	Analytical grade	Sigma Aldrich
4	Distilled water	Analytical grade	Chemical Eng.Dept. ABU
5	Filter Paper	Analytical grade	Sigma Aldrich
6	Waste water	ABU waste water oxidation pond	Not applicable
7	Funnels	Analytical grade	Pyrex
8	Rubber tanks	Pyrex	Sigma Aldrich
9	Sodium Chloride	Analytical grade	Sigma Aldrich
10	Chloroform	Analytical grade	Sigma Aldrich

The microalgae were collected from waste water pond at Ahmadu Bello University (ABU), Zaria and identified at Botany Department ABU Zaria while all reagents used in

this research work were of analytical grade and obtained from sigma Aldrich through their distributor in Nigeria.

Some of the equipment that was used during this research work are presented in Table 3.2

Table 3.2: **Equipment/Apparatus used during the research work**

S/NO	APPARATUS/ EQUIPMENT	MANUFACTURER/MODEL
1	Digital weighing balance	AEAdam/PW184
2	Beaker	Pyrex
3	Vacuum Pump	GallenKamp PA2435
4	Oven	GallenKamp PA2345
5	GCMS Machine	Shimadzu/GCMS-QP2010 Plus
6	FTIR Machine	Shimadzu FTIR-8400S
7	Quick fit Thermometer	Pyrex
8	Separating funnel	Pyrex
9	Condenser	Pyrex 24/29
10	Heating mantle	GallenKamp PA2243
11	Water bath	GallenKamp PA2342
12	Rotary evaporator	Shimadzu PA43320
13	Sample bottles	Pyrex
14	3-Neck Round bottom flask	Pyrex
15	Retort stand	GallenKamp PA2243

3.2 Methodology

The methodology for this research was splitted into three phases. The first phases of the experiment focused on feedstock (dried microalgae biomass) synthesis where wet microalgae biomass was collected from ABU Zaria waste water oxidation pond after coagulation using alum, then the microalgae was then filtered using vacuum filter and dried in oven at about 80 ^{O}C to obtained dried microalgae biomass and finely grounded and sieved to 1000 μm mesh size. The prepared samples were analyzed for dried moisture content, ash contents, lignin, oil content, and cellulose and hemicellulose contents after the oven drying at Institute for Agricultural Research (IAR/ABU), Zaria while preliminary study was done to investigate the use of microalgae to produce furfural and effect of sodium chloride concentration on furfural yield was investigated.

The second phases of the experiment dwell on design of the experiment using version 6.0 of (DOE).Here the experiment was designedusing a rotatable CCD a RSM by setting the lower and upper limit for the three factors (reaction temperature, reaction time and acid concentration in terms of solid to acid ratio). The DOE varies the three various factors considered at different conditions and this was taken to the laboratory to carry out the production of furfural at these various conditions while the various effect of these factors were observed and evaluated.

The last phase of the experiment was aimed at optimizing the factors affecting furfural yield where the DOE predicted ten (8) optimum solutions which was later validated in the laboratory and subsequently the optimum conditions was obtained from validated

(verified) experiment based on their desirability followed by final Characterisation of furfural produced.

3.3 Physicochemical Characterization of dried Microalgae Biomass

Physicochemical characteristics such as dried moisture content, ash contents, lignin, oil content, and cellulose and hemicellulose contents were carried out using Association of Official Analytical Chemist (AOAC, 2006) methodology at Institute for Agricultural Research (IAR/ABU), Zaria.



Plate 1: Typical microalgae biomass obtained from ABU sewage oxidation pond

3.3.1 Dried matter (DM)

Everything in feed other than water is known as the dried matter (AOAC,2006).It is determined by heating the dried microalgae biomass sample in an oven at about 70^0C for 8-12 hours until all water was evaporated and no further loss of weight was observed

while the percentage of the sample that is not water is referred to as the dried matter of the sample. The difference between the moisture % and 100 gives the dried matter

3.3.2 Ash content

Ash is considered to be the mineral content of the feed sample. It is determined by burning a dry sample in a very hot oven (500-600^{0}C) to burn off all the organic matter. Ash does not give detail information on the levels of each of the minerals. Thus more mineral analysis is required to assay the various mineral constituents of the sample. The ash content is calculated using the relation (AOAC, 2006)

$$\%Ash = \frac{weig\,ht\ of\ ash}{weig\,ht\ of\ sample} \times 100 \quad \ldots\ldots 3.1$$

3.3.3 Cellulose content

The cellulose content is determined using Crampton and Maynard method (AOAC, 2006). The dried microalgae biomass is digested with acetic and nitric acid which dissolves all other constituents in the sample such as protein, disacharides and fatty acids etc while the mineral and the cellulose are unaffected. The residue is made acidic and alcohol free by washing. The alcohol removes water while benzene removed pigments and fats from the sample. The residue is finally dried and ashed. The cellulose content is determined by measuring the difference between the dried weight before ashing and weight after ashing. It is determined using this relation

$$Cellulose\% = \frac{weig\,ht\ of\ crucibles\ + abestos\ + material\ \ befor\ \ ashing - weig\,ht\ of\ crucible\ \ and\ content\ \ after\ \ ashing}{weig\,ht\ of\ material\ \ on\ dry\ \ matter\ \ basis} \times 100 \quad \ldots\ldots 3.2$$

3.3.4 Hemicellulose content

The hemicellulose content is determined by boiling the extracted cells wall residue with about 8 % of sulphuric acid for one hour in order to hydrolyse the hemicellulose without dissolving cellulose, lignin and insoluble ash.The crucible is heated, fitterd and rinsed with hot water to remove all acid and it was then dried in an oven for 100^{o}C,cooled in a desiccator and weighed. The residue was ashed and re-weighed.

$$\text{Hemicellulose\%} = \frac{weig\,ht\ of\ cell\ wall\ residue - weig\,ht\ of\ extracted\ \ cell\ wall\ residue}{weig\,ht\ of\ sample\ \ on\ dry\ matter\ \ basis} \times 100 \ldots\ldots 3.3$$

(AOAC, 2006).

3.3.5 Lignin content

The lignin is determined by placing in an oven an extracted residue from the sample after adding 2% of pepsin I N/10 HCl, 40 % of formaldehyde, 72% sulphuric and little quantity of chloroform and acetic acid. The sample is digested for 12hours at 40^{0}C. The Non-digestion residue is recovered, filtered and washed with hot water, hot alcohol and hot benzene and hot ether. Conc. sulphuric acids was added and stir vigorously then chloroform and acetic acid was added and boiled gently and a clear solution and lignin after rinse with water to drains off the colorless. The residue was dried and ashed (AOAC, 2006).

$$\text{Lignin\%} = \frac{weig\,ht\ of\ residue\ \ before\ \ ashing - weig\,ht\ of\ residue\ \ after\ \ ashing}{weig\,ht\ of\ sample\ \ on\ dry\ matter\ \ basis} \times 100 \ldots\ldots\ldots\ldots 3.4$$

3.4 Central Composite Design of Experiment for Furfural Production

Design expert software version 6.0 already installed on a DELL laptop running window 10 operating system was used. The option ‘’new design was clicked to open a new experiment for furfural production process. The response surface methodology menu on the left hand corner was clicked and CCD method was selected.Three (3) factors having specified lower and upper levels in Table 3.3 was formulated while the response for the experiment was designated to be furfural yield (%). A total of 20 experiments were obtained; 14 non center points and 6 center points. The developed matrix is shown in Table 3.4.

Table 3.3: Design summary of process factor for response surface methodology using a rotatable CCD

S/NO	Process factors	Units	Low level(-)	High level (+)
1	Reaction Temperature	^{0}C	65	140
2	Reaction Time	Min.	30	90
3	Acid Concentration	ml/g of samples	35	70

Block =1 Alpha = 1.68, chloroform concentration fixed at 40ml and sodium chloride concentration fixed at 1.0g/g of microalgae biomass used per run.

Table 3.4:Design of furfural production experiment

Std	Run	Factor 1 A:Temperature (^{0}C)	Factor 2 B:Concentration (ml/g)	Factor 3 Time (Min)
17	1	102.50	52.50	60.00
13	2	102.50	52.50	9.55
3	3	65.00	70.00	30.00
1	4	65.00	35.00	30.00
8	5	140.00	70.00	90.00
10	6	165.57	52.50	60.00
6	7	140.00	35.00	90.00
4	8	140.00	70.00	30.00
7	9	65.00	70.00	90.00
19	10	102.50	52.50	60.00
2	11	140.00	35.00	30.00
18	12	102.50	52.50	60.00
5	13	65.00	35.00	90.00
9	14	39.43	52.50	60.00
16	15	102.50	52.50	60.00
15	16	102.50	52.50	60.00
14	17	102.50	52.50	110.45
20	18	102.50	52.50	60.00
12	19	102.50	81.93	60.00
11	20	102.50	23.07	60.00

The design matrix in Table 3.4 was strictly followed during the laboratory work where each of the experiment was run and each run was repeated twice and average yield of furfural was recorded for each of the run which is presented in chapter four under results and discussion.

3.5 Laboratory Production of Furfural

Microalgae were collected from waste water pond at Ahmadu Bello University (ABU, Zaria) and were identified at botany department ABU, Zaria.The microalgae were then coagulated using about 60g of alum in about twenty-five liters of waste water. The microalgae was then filtered using vacuum filter and dried in oven at about 80 ^{0}C and finely grounded and sieved to 1000 μm mesh size.The prepared samples were analyzed

for dried moisture content, ash contents, lignin, oil content, cellulose and hemicellulose contents after the oven drying at Institute for Agricultural Research (IAR/ABU), Zaria. Furfural production was carried out using a batch reactor and a distillation system, while the acid hydrolysis of the microalgae samples took place in the batch reactor. Aqueous sulphuric acid (various amount) of 1M concentration, uniform quantity 1.0 g of sodium chloride per g of microalgae sample, was introduced into a 500 ml 3-necked round bottom flask, fixedto a Vigreux column and a condenser and the reaction mixture was heated. The effluent from the batch reactor experienced rapid distillation at 105^{o}C, then the aqueous distillate mixture was collected over 40 ml chloroform in a separating funnel and two distinct layers were formed which are, the furfural-chloroform layer as well as the aqueous layer (Ameh *et al.*, 2016). After distillation for various process factors of reaction time, temperature and acid concentration, the furfural-chloroform layer was decanted and then poured into a conical flask. The decanted furfural-chloroform mixture was subjected to a rotary evaporation at a temperature of 65^{o}C for 10 min where all the chloroform was obsereved to be evaporated leaving the furfural as the products. This procedure was repeated for various reaction temperatures, concentration and reaction time.

The experimental set up during the research is shown in plates II and plate III.



Plate II: Experimental set up (distillation and batch reactor system) for production of furfural from microalgae



Plate III: Experimental set up (rotary evaporation system) for production of furfural from microalgae

3.6 Optimization of Furfural Yield from Microalgae

The optimization features of the design expert software was used based on furfural yield obtained per experiment conducted using design matrix of Table 3.4 for the purpose of

optimising the objective function (furfural yield) and the constraints. The optimisation goal and constraint is summarize in Table 3.5

Table 3.5:Design expert optimisation (Goal and constraints) summary for furfural yield from microalgae using a rotatable CCD

S/NO	Constraint(Process factors)	Goal	Lower	Upper
1	A:Temperature (^{0}C)	Is in Range	39.43	165
2	B:Acid Concentration ml/g	Is in Range	35	70
3	Reaction Time (min)	Is in Range	30	90
4	Furfural yield (%)	Maximize	7.87	100

Profitability of furfural (High furfural yield at optimum process conditions) was the objectives function upon which an optimal solution was selected among the numerous (8) solutions predicted by the software.A predicted furfural yield was obtained based on computed process parameters for furfural yield and was validated (verified) in the laboratory experiment.The verification experiment was repeated twice and average of furfural yield was recorded as the actual yield.The difference between the predicted and validated (actual) yield of furfural was recorded and discussed in chapter four.

The block diagram of the methodology for the research is shown in Figure 3.1. It shows a step by step process taking throughout during the research .

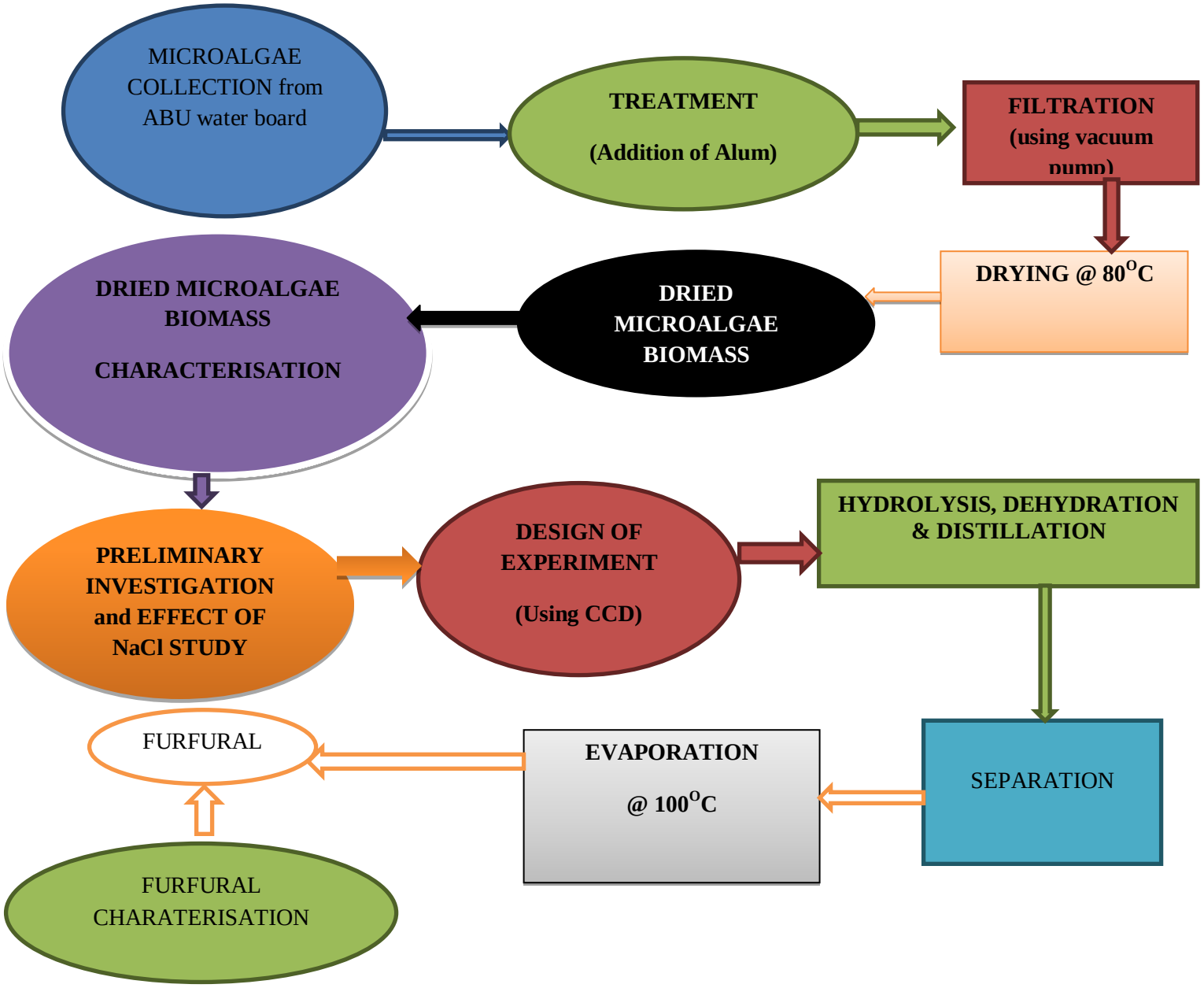


Figure 3.1: Methodology for furfural production from microalgae

3.7 Characterizationof Microalgae Synthesized

The microalgae collected from waste water pond at Ahmadu Bello University (ABU), Zaria were identified at botany department ABU, Zaria. The species of microalgal from

the waste pond are identifiedand it was further characterized and analyzed for dried moisture content, ash contents, lignin, oil content (proximate analysis) and cellulose and hemicellulose contents after the oven drying at Institute for Agricultural Research (IAR/ABU), Zaria.The analysis was done using AOAC, 2006. The result of physicochemical characterization is presented in Chapter Four.

3.8 Characterization of Furfural Produced

The furfural produced was characterized using FTIR and GCMS aside physicochemical characterization. Also the commercial furfural was obtained and characterized using both FTIR and GCMS and the results of both produced and commercial furfural bought was compared and discussed in Chapter Four.

3.8.1 Fourier-transform infrared spectroscopy (FTIR) characterization of furfural produced

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and in some cases, inorganic materials. Fourier Transform Infrared Spectroscopy analysis was used to identified the aldehyde functional group present in the furfural produced where about 1 g of the produced furfural was placed in between the glass sample holder and charged into the FTIR spectrophotometer for spectra analysis of all the functional group presents in the sample and the absorbance of the FTIR spectrum was also obtained. Also the above procedure was used to characterize commercial furfural bought.

3.8.2 Gas chromatogram-mass spectroscopy (GC-MS) characterization of furfural produced

Shimadzu GCMS-QP2010 Plus machine was used for the chemical analysis of both furfural produced and commercial furfural bought. Little quantity of the sample was charged into the column operated at about 60 ^{0}C while the injection mode was splinted at about 200 ^{0}C. Nitrogen was used as the mobile-phase gas. The difference in the chemical properties between molecules in the mixture and their relative affinity for the stationary phase of the column promote separation of the molecules as the samples travel down the length of the column. The molecule are retained by the column and then come off from the column at different times (retention time) and this allows the mass spectrometer to capture, ionize, accelerate, deflect and detect the ionizable molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragment and detecting these fragments using their mass to charge ratio (m/z). The various chemical components presents was identified by the detector.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter discuss all results obtain during this research work.

4.1 Microalgae Biomass Characterization

The identified species of microalgae from the waste oxidation pond ABU, Zaria includes members of the chlorophyta (green algae) such as *Closterium* sp, *Scenedesmus Opoliensis*, *Cosmarium* spp, *Scenedesmus Obliquus* ,*Scenedesmus bijuga*, *Staurastrum* spp and members of the cyanobacteria (blue green algae) such as, *Oscillatoria* spp, *Spirulina* sp and *Merismopedia* sp. and it was further characterized and analyzed for dried moisture content, ash contents, lignin, oil content, and cellulose and hemicellulose contents after the oven drying at Institute for Agricultural Research (IAR/ABU), Zaria.The analysis was done using AOAC (2006) methodology. The result of physicochemical characterization (proximate and fibre analyses) of the microalgae is presented in Table 4.1.

Table 4.1: Composition of some physicochemical properties of microalgae feedstock used (proximate analysis)

Parameter	Value
Moisture content (M.C)	2.99
Total dried matter (DM)	97.01
Ash Content	47.55
Crude protein content (C.P)	20.50
Oil content	0.00
Crude Fiber content (C.F)	2.36
Net protein fiber content (NFE)	26.60
Lignin content	16.43
Cellulose content	29.44
Hemicellulose	54.14

The microalgae biomass used had high percentage content of hemicellulose (54.13%) this makes it suitable as a good feedstock for furfural production. Also the moisture (2.99%) content is low signifying that the microalgae biomass has total dried matter (97.01%). This is higher than agricultural feedstock (sugarcane bagasse 32 %) as reported by Ameh *et al* (2016); rice husk (19 %) reported by Ambalkar and Talib (2012); maize hemicellulose (32 and 30 %) as reported by Wenjuan *et al* (2016) and Shafeeq *et al* (2015) respectively. Thus microalgae should give higher percentage of furfural yield because of the higher hemicellulose content. Also the percentage oil content of the microalgae is 0.00% which indicates that a high percentage of biomass produced can be converted to furfural. The proximate analysis sum to 100% (Cellulose 29.44% + Hemicellulose 54.13% + lignin 16.43%) while the Ash Content (47.55%), Crude Protein C.P (20.50%), Crude Fibre C.F(2.36%) and Net Protein Fibre NFP (26.60%) equal to the total dried matter (97.01%). The dried matter plus moisture content sum to 100%.Athough the high ash content recorded mighty be as a result of quantity of alum added to the microalgae biomass for coagulation and also high yield of furfural is obtained due to the nature of hydrolysis (acid hydrolysis) that was carry out thereby leading to the breaking of lignin ,organic acids and other minor components originating from biomass into furfural.(Marcotullio,2014).

4.2Preliminary Investigation and the Effect of Sodium Chloride on the Yield of Furfural

Preliminary investigation was done to measure values such as colour, physical state, boiling point and density. The density (1.17 g/ml) and boiling point (160.48 ^{0}C) which were within the range of reported literature value by Ameh *et al.* (2016) and Yan *et al*.

(2014) of 1.16 g/ml. However, the variations in boiling point (161.85 ^{0}C) and density (1.16 g/ml) could also be attributed to difference in the origin and type of feedstock used to that reported in literatures. The effect of sodium chloride concentration in the distilling medium was studied while the rate of furfural yield with respect to salt concentration was also investigated by varrying the concentration of sodium chloride at a constant temperature of 140^{o}C and reaction time of 60min in 40 ml of 1M concentration of sulfuric acid.The concentration of sodium chloride is defined as the ratio of mass of sodium chloride used to the ratio of microalgae biomass used. Initially, as sodium chloride concentration increasefrom 0.33- 1.0 g / g gram of microalgae biomass used, it lead to an increased in furfural yield from 25% to 60.3%. This is becauseit is evident from the experimental results (Figure 4.1) that, with increasing sodium chloride salt concentration, xylose reacts significantly faster to furfural, while no significant effect is observed on the side reactions, resulting in a remarkable selectivity improvement. This can be due that the mechanism of furfural formationfrompentoses is considered to proceed via the acyclic form of the pentoses, through a first 1,2-enediol formation and subsequent three dehydration steps yielding furfural (Ahmad *et al.*, 1995).The presence of Cl $^{-}$favors the formation of the 1, 2-enediol, with respect to both the aldoseandketoseform of the sugar, byastabilizingeffect, thusfavoringthesubsequentdehydration reactionsin presence of an acid which correspond to the observation of Marcotullio (2014).Thus NaCl is confirmed Fulmer*et al.* (1936) to affect formation of xylose in acidic solutions.However furfural yield decrease from 60.3% to 16% as the concentration of sodium chloride increased from (1.0g to 2.0g) per g of microalgae biomass used due to excess of chloride ions relative to hydrogen ions

thereby leading to undesirable reactions hence result to formation of other products such as 5-methyfurfural, furoic acids and humins. In Figure 4.1, the highest yield of furfural(60.3%) was observed at 1.0 g of sodium chloride concentration, per gram of microalgae biomass used and it's because at this concentration of sodium chloride,it accelerates the formation of furfural before decomposition to other products that could have taken place which is desirable hence the highest yield of furfural was observed at this concentration of sodium chloride. As the concentration of sodium chloride increases (Figure 4.1) from 1.0 g to 2.0 g per gram of microalgae biomass used,it was observed that, no further increase in furfural yield was observed with increase in concentration of sodium chloride because as the concentration of sodium chloride increases, it was observed that it led to excessive rise in temperature which results to an increased in the rate of furfural degredation, polymerisation and decomposition of biomass to other by-products which is an indicative of decrease in furfural yield observed. Also as the concentrations of sodium chloride increases from 1.0 g – 2.0 g per gram of microalgae biomass, furfural yield decreases from 60.3% to 19%.This is xylose is consumed at a rate that exceeds the rate of furfural formation suggesting loses of xylose due to self-condensations. In addition as the concentration of sodium chloride increases, furfural distillate becomes very light pink instead of being colorless, this may be attributed to polymerisation of furfural to other products as a result of rise in temperature. It was also observe that an increase in Sodium chloride led to increase in the formation of bubbles and caused entrainments in the distillation reactor as a result of rise in temperature hence sodium chloride concentration have significant effect on furfural yield; Sodium chloride salt is shown to enhance the reaction of xylose to furfural in aqueous acidic solution as

Cl− ionspromotetheformationofthe1,2-enediolfromthe acyclic form of the aldose, which undergoes subsequent acid catalyzed dehydration reactions to furfural. However, excess of the saltsled to excess of chloride ions relative to hydrogen ions which can led to undesirable reaction caused by accumulated 1,2-enediol .This results does not agree with Shafeeq *et al*. (2015) who concluded that sodium chloride concentration does not have effect on furfural yield. Although, in their work they did not vary the concentrations of sodium chloride but used a constant concentration of 20 g/ per g of the biomass used. The observable trend of initially increase in sodium chloride with corresponding increase in furfural yield then later decreases as concentration of the salt increases is in agreement with the reported of Enslow *et al*.,2015. Other literature works by Ameh *et al. (*2016),Shaukat *et al. (*2012) andWenjuan *et al.* (2016) did not investigate the effect of sodium chloride concentration on furfural hence this results (effects of sodium chloride concentration) show one of the most important factor affecting furfural yield.

Figure 4.1 shows the effect of sodium chloride concentration on furfural yield.

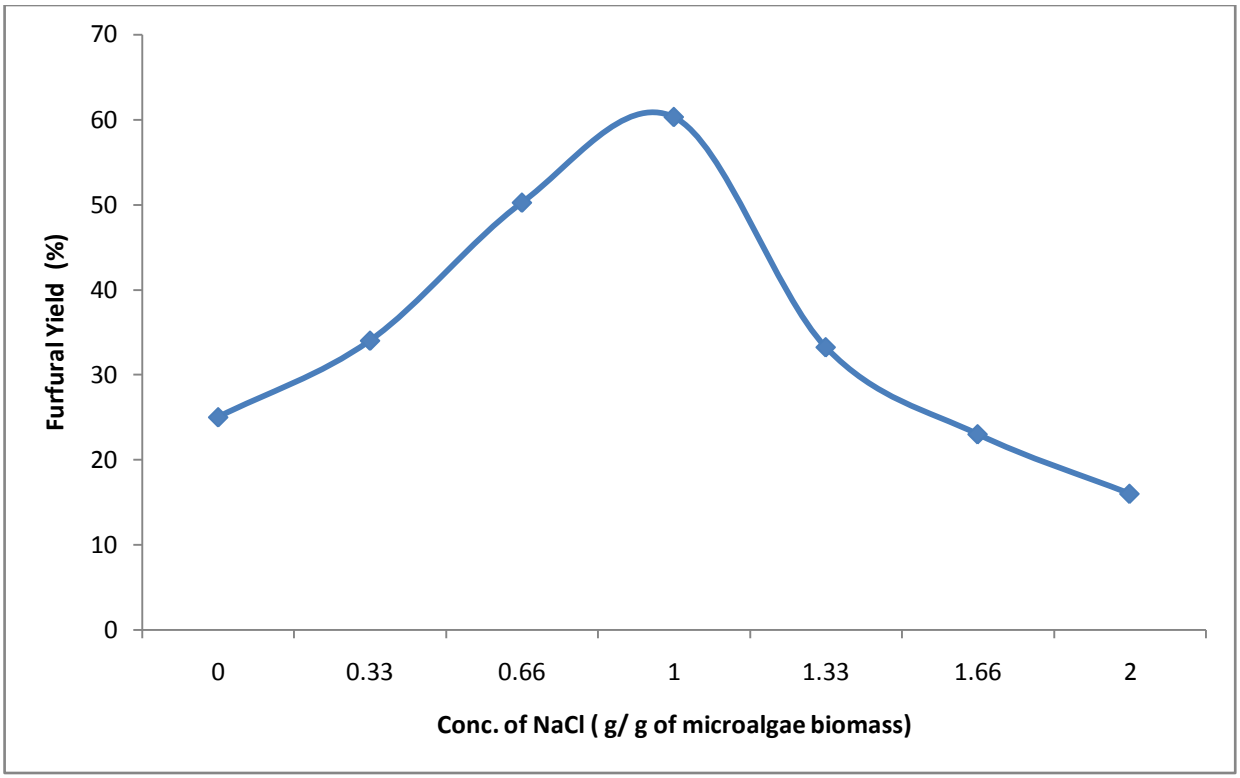


Figure 4.1: Effect of sodium chloride on furfural yield

4.3 Response Surface Modeling of Furfural Production from Microalgae Biomass

The effect of furfural production factors on the performance of twenty Experimental runs used in production of furfural was studied using CCD through acids hydrolysis and dehydration in a batch reactor system.

Furfural yield was used as the response and basis to evaluate the effects of various process parameters studied. Furfural yield is defined as the ratio of the mass of furfural produced to the mass of microalgae biomass used, and it was obtained gravimetrically. The yield of furfural was calculated according to Equation 4.1

$$\text{Furfural (\%)} = \frac{\text{mass of furfural produced (g)}}{\text{mass of microalgae biomass (g)}} \times 100 \quad \text{.....(4.1)}$$

The yields of furfural from the various runs as shown in Table 4.2

Table 4.2: Experimental (CCD) design and results (conversion of microalgae to furfural)

Experimental Run	A	B	C	Response (FURFURAL YIELD) (%)
1	102.50	52.50	60.00	55.07
2	65.00	70.00	30.00	27.67
3	39.43	52.50	60.00	20.00
4	140.00	35.00	30.00	60.05
5	102.50	52.50	60.00	54.55
6	105.50	52.50	60.00	54.71
7	102.50	52.50	60.00	56.00
8	65.00	70.00	90.00	51.00
9	102.50	52.50	110.45	45.00
10	102.50	52.50	60.00	60.00
11	102.50	52.50	9.55	15.67
12	140.00	70.00	90.00	58.00
13	65.00	35.00	90.00	43.00
14	65.00	35.00	30.00	7.87
15	165.57	52.50	60.00	73.33
16	140.00	35.00	90.00	64.20
17	102.50	81.93	60.00	57.40
18	140.00	70.00	30.00	67.10
19	102.50	52.50	60.00	54.82
20	102.50	23.07	60.00	49.00

Key; A= **Temperature (^oC), B= Concentration of H_2SO_4 (mL/g) C= Reaction Time (min)**

Table 4.2 shows the design matrix of the (CCD) for furfural production from microalgae and their corresponding responses obtained after performing the experiments at various process parameters.

4.3.1 Statistical analysis of CCD design for furfural production from microalgae

The experimental design had a total of 14 different combinations and 6 repeated (center point) for furfural production from microalgae. Statistical analysis was done on the input response (furfural yield) to select the type of model that fit best as presented in Table 4 .3

Table 4.3:Model statistical summary of CCD for furfural yield

Source	Std dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	Precision	Adequate Precision	Lack Of Fit Value
Linear	10.49	0.7009	0.6448	0.4818	3000.84	11.090	0.0005
Quadratic	3.37	0.9807	0.9634	0.8771	723.07	29.437	0.0714
2FI	9.15	0.8151	0.7298	0.6799	1882.80	12.240	0.0008

As shown in Table 4.3, three different models were suggested by the Design of experiment (DOE) and from Table 4.3, it can be observed that quadratic model came out the best model hence it was selected for the design and optimization of furfural production from microalgae as the quadratic model has the highest R-Squared value, a close value between the R-Squared 0.9807 and Adj R-Squared 0.9634 as well as the Predicted R-Squared 0.8771 compared to linear and 2 factorial models.

4.3.2 Analysis of variance (ANOVA) for furfural production

Table 4.4, shows the ANOVA of the selected quadratic model fitting the furural production using regression coefficient. A full quadratic model for predicting furfural yield was obtained.The major indicators demonstrating the significance and adequacy of this model include p-value (Prob>F) of 0.0001 which is less than 0.05 hence the model is significant at confidence level of 95 % while the lack of fit is insignifficant thus confirming the adeuacy of the quadratic model selected which is in agreement with Montgomery *et al.* (1994). Another significance indicator of a good model is the model F-value (Fisher variation Ratio) which was found to be 56.54.Also from Table 4.4, it can be seen that, the significant model terms are: A, B, C, A^2, C^2, AB, AC, and BC.

However, this does not mean the model term B^2isnot important in this study but it shows that, the model terms A, B, C, A^2, C^2, AB, AC,and BC contributed more in the yield of furfural as compared toB^2model term. It also mean that temperature and time are highly important than acid concentrations, thus the results obtained showed that the model is good.

Table 4.4:Analysis of variance (ANOVA) for response surface quadratic model for furfural production from microalgae

FULL QUADRATIC MODEL					**MODEL ACCURACY**	
Terms	**Mean Square**	**F-value**	**P-value**	**Remarks**	**Correlation Coefficients**	**Values**
Model	640.89	56.54	< 0.0001	Significant	R-squared	0.9807
A	3213.79	283.51	<0.0001	Significant	Adj.R-squared	0.9634
B	133.99	11.82	0.0064	Significant	Pred.R-squared	0.8771
C	774.37	68.32	<0.0001	Significant	Adeq.Precision	29.437
A^2	73.26	6.40	0.0292	Significant		
B^2	0.044	0.003921	0.9513	Insignificant		
C^2	928.90	81.95	<0.0001	Significant		
AB	90.79	8.01	0.0178	Significant		
AC	502.60	44.34	<0.0001	Significant		
BC	78.44	6.92	0.0251	Significant		
Residual	11.33	-	-	-		
Lack of fit	18.29	4.17	0.0714	Insignificant		
Pure error	4.38	-	-	-		

Where, A= Temperature (^oC), B= Concentration of 1M H_2SO_4 (mL/g) and C= time (min).

It can be observed that the quadratic model have high R^2 value of 0.9807. The difference (1.73 %) between R-Squared 0.9807 and Adj R-Squared 0.9634 is less than 2 % hence confirms that qudratic model fitted best which is a desirable property of a good model.Also the lack of fit value is not significant prior to relative error and exceeded the 5 % for the which is one of the major createria for a model to fit. Likewise the quadratic

model has the highest adequate precission of 29.437 compared to other models indicating a good signal to noise ratio since a ratio greater than 4 is desired and can be used to navigate the design space thus, the quadratic model can adequately predict furfural yield based on investigated process parameters affecting its production.

4.3.3 The quadratic model equation selected for furfural yield from microalgae.

The model equation is

$$\text{Yied} = +55.71 + 15.34\text{A} + 3.13\text{B} + 7.53\text{C} - 2.25\text{A}^2 + 0.056\text{B}^2 - 8.03\text{C}^2 - 3.37\text{AB} - 7.93\text{AC} - 3.13\text{BC} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.2$$

Equation 4.2 shows the yield of furfural from microalgae where the linear terms A= reaction temperature (^{o}C), B= Concentration of of 1 M H_2SO_4 Catalyst (ml/g) And C= reaction time (min). A, B and C represent the linaer terms , A^2,B^2 and C^2 denote the quadratic terms while AB, AC, and BC are the products terms. Equation 4.2 can be used to predict the yield of furfural at different conditions of the parameters prior to laboratory.The model equation can also be used to determine the effects of the various parameters considered.

4.3.4Normal distribution

The normal distribution plot shows if the residual follow a normal distribution i.e if the response follows a straight line with little possibility of scattering.Thus, from Figure 4.1 shown that, the data follow a normal distribution for the percentage of furfural yield since its followed a straight line with little scattering which is also evident in plot of predicted versus actual yield of furfural as presented in Figure 4.2.

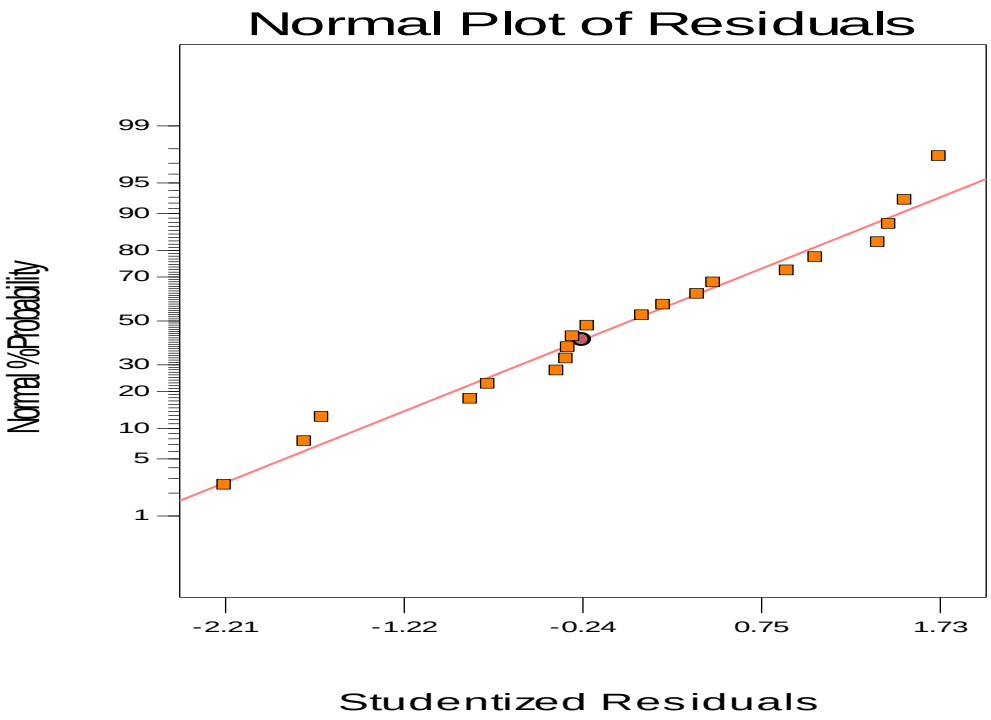


Figure 4.2: Normal probability plot of studentised residuals for percentage of furfural yield

4.3.5 Predicted and actual yield relationship

Figure 4.3 shows the relationships between the predicted and actual responses. The Figure presented the design expert parity plot of the predicted furfural yield against their respective actual responses for the evaluation and optimization of process parameters of furfural from microalgae. As shown in Figure 4.3, the data points were well distributed close to the regression line, which suggested an excellent relationship between the predicted and experimental (actual) values of the response. That there was no outlier among the data point suggests inappropriate underlying assumptions of this analysis. The approximate distribution of data points along a straight line in both Figures 4.3 showed a good correlation between the actual (experimental) and predicted values. The data points as shown in the Figure 4.3 are well distributed close to the regression which revealed that,

the model developed is highly significant and adequate to represent the actual relationship between input variables and output response.

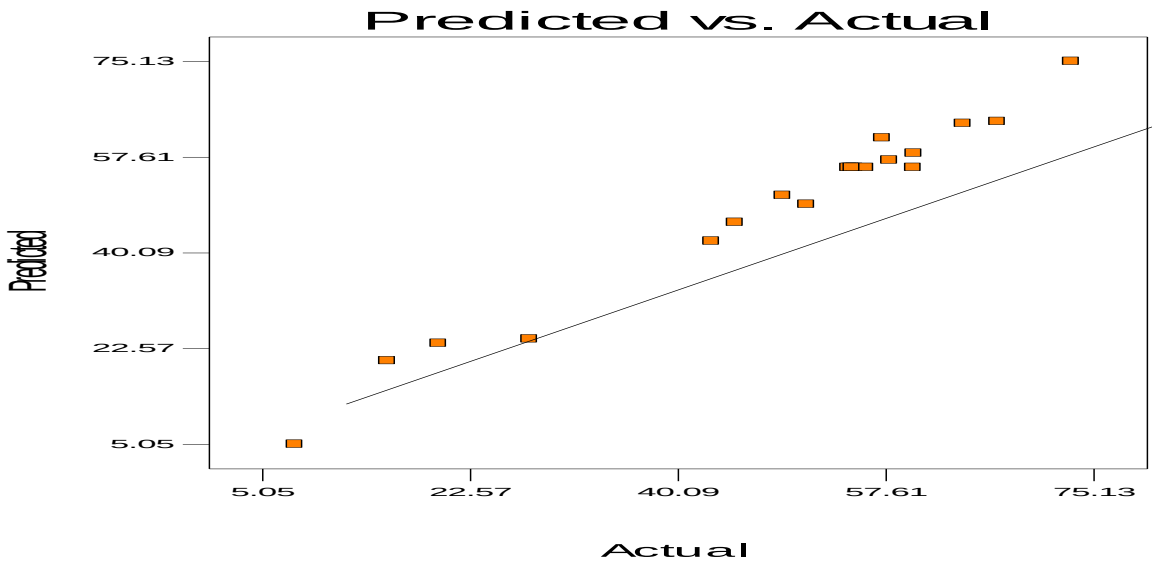


Figure 4.3: Correlation between Actual (experimental) and predicted yield of furfural production from microalgae

4.3.6 Main effects of investigated furfural production factors on furfural yield

This sections discussed the individual effects of the factor considered.The individual effects of temperature,reaction time and acids concentration plots are presented in the appendix A-1

4.3.6.1 A-Reaction Temperature:

The effect of temperature on furfural production was investigated using furfural yield as the response.The range of temperature considered and design by DOE were between 39.43 ^{o}C to 165^{o}C. As shown in Appendix A. It was observed that, furfural yield increased with increased in temperature, this because conversion of xylose to furfural required high activation energy, However, excess high temperature leads to occurrences of side reaction which is evident in the GC-MS analysis.This is in agreement with the result of Wenjuan*et al* (2016) who recorded 61.06% yield of furfural from corncob.

However, at higher temperature, it was observed that furfural degredation increases, this trend may be due that at high temperature, furfural cannot be recovered from the reactor due to its degradation, because, as temperature increases, furfural degradation rate also increase which agrees with the result of Wang *et al* (2015).

4.3.6.2 B-Acids Concentration

Also concentration of 1M H_2SO_4 (ml/g) interms of acid to solid ratio was successfully investigated using furfural yield as a response. The concentration of H_2SO_4 (ml/g) was varied between 35 ml/g to 70 ml/g as shown in the appendix A. The effect of acid concentration on furfural yield was observed and at high concentration, furfural degradation is accelerated rapidly thereby leading to loss of furfural yield. It was noticed also that initially acid concentration increased with increase in furfural yield but subsequent increase in acid concentration does not increase furfural yield,this may occur because at high concentration, it's difficult to separate furfural produced in liquid phase using standard laboratory separation methods, this result is in accordance with that of Shafeeq *et al* (2015).He established that the factors upon which furfural yield depends are Acid/solid volume ratio, temperature and reaction time hence it can be said that furfural yield remained constant despite increase in acid concentration in that acids to biomass ratio has reached its optimum and furfural yield may not increase despite increase in acids concentrations.

4.3.6.3 C-Reaction Time

The time range considered in this research ranges from 30 – 90 mins while furfural yield was maintained as the response.The effect of time on furfural yield is presented in

Appendix A. Although increase in furfural yield was observed with increase in reaction time from 30 -90 mins. This observation is in harmony with the result of Shaukat *et al* (2012) who also reported that increase in percentage yield of furfural increase with time. After certain time, It was observed that increase in time leads to decrease in furfural yield, this could probably be that greater extent of pentosans in the microalgae must have been be already converted to furfural suggesting that furfural undergoes secondary reaction in the presence of an acid after a long period of reactiontime,hence, there is formation of side reaction as reaction continues thereby leading to furfural degradation and reduction in yield, this also correspond to the result of Ayse *et al* (2013).

4.3.7 Interaction effects of investigated factors on furfural yield

The 3D-Surface diagram of significant model term interactions among the variables varied and the response yield are represented by a 3D- surface plot.The 3D response surface plots generally illustrate the effects of the independent variables and their interactive effects on the responses. The 3D plots shown in Figures 4.5, 4.6 and 4.7 respectively illustrate an interactive effect of temperature and concentration, temperature and reaction time as well as concentration and reaction time with their corresponding furfuralyield respectively.

4.3.7.1 Pertubation plot

Pertubation plot shows an interactive effects of all the factors considerd in an investigation.Pertubation plot lumped all the factors considerd and their synergy with respect to the objective fuction (response of the model) in this case furfural yield.

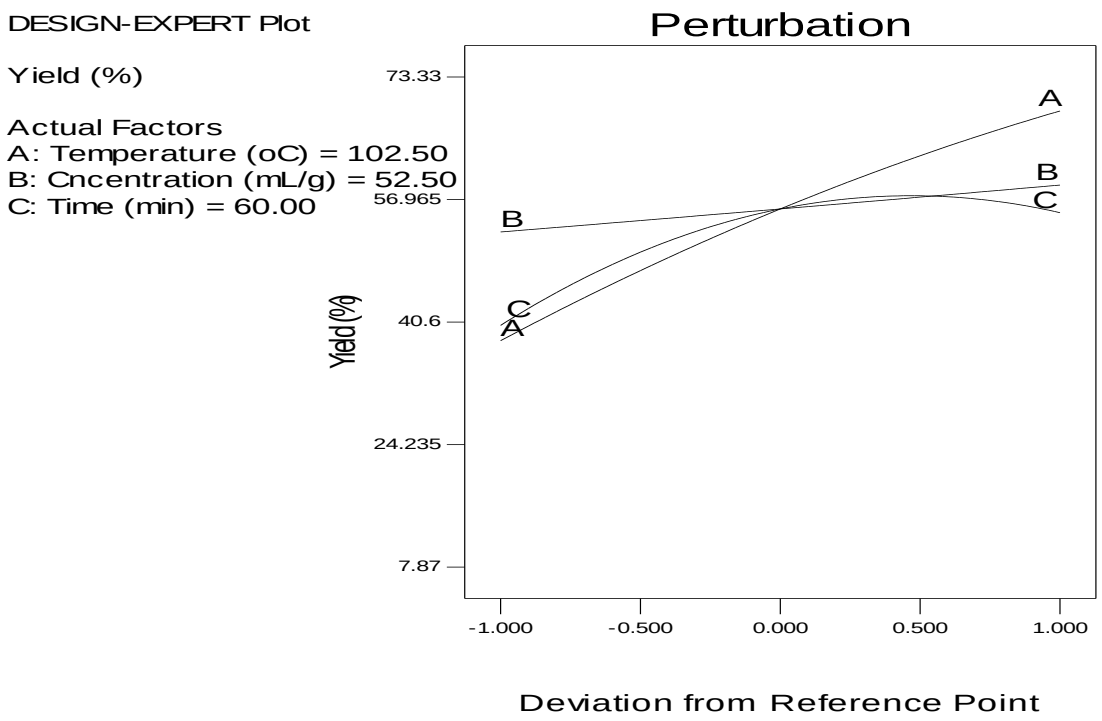


Figure 4.4: Perturbation plots of the main effects for furfural yield

Although the P-values for the main effects showed that, all the factors are significant, equally perturbation plot can show the comparative effects of all the investigated independent variables on furfural yield. From Figure 4.4, temperature (A) and reaction time (C) shows a consistent lumped curvature indicating that both have more effects on furfural yield compared to acid concentrations (B) whose effect is almost constant on furfural yield. Within the range of this experimental design, it is evident that the effects of the three lumped factors will seize to be significant beyond 60% yield of furfural. Meanwhile, increase in reaction temperature will favor the yield and possibly increase furfural yield up to about 73% while increase in reaction time decrease furfural yield. From this observation, it can be said that reaction temperature have highest effects on

furfural yield however, a synergy among these three factors contributed immensely to the yield of furfural as well.

4.3.7.2 Interaction between A-Temperature and B-Acids concentration (AB)

The effects of temperature and acid concentration on furfural yield for furfural production from microalgae is depicted in Figure 4.5. As the temperature and concentration of acid vary (either increasing or decreasing), the yield of furfural changes. As shown in the same Figure 4.4 for instance, at a temperature of 140 ^{0}C (x-axis) and acid concentration of 70 mL/g (z-axis), the corresponding furfural yield on y-axis is 69.08%.Also, the interactive effects temperature and concentration is illustrated by the contour plot (Appendix A), it was observed that as temperature increased from 65 ^{0}C to 121.15 ^{0}C, furfural yield also increased from about 37.9% to 62.85% while increase in concentration of acids from 35 ml/g to about 70ml/g shows insignificants increased in furfural yield.

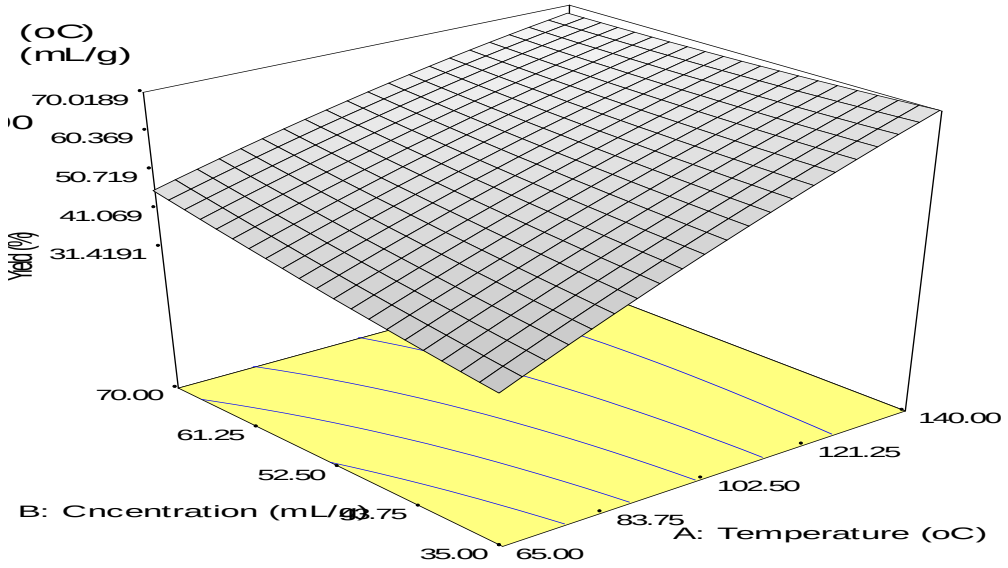


Figure 4.5: 3D Plot of furfural yield against concentration versus temperature.

4.3.7.3 Interaction between A-Temperature and C-Time (AC)

Also the effects of temperature and reaction time on furfural yield for furfural production from microalgae are depicted in Figure 4.6. As the temperature and reaction time vary (either increasing or decreasing), the yield of furfural changes. As shown in the same Figure 4.4 for instance, at a temperature of 140 ^{0}C (x-axis) and reaction time of 90 minutes (z-axis), the corresponding furfural yield on y-axis is 60.43%. Thus, several plots for other runs of varying parameters against furfural yield in the same graph resulted to the 3D surface plots as shown in Figure 4.6. Also, the variations in furfural yield showed that the interactions between the variable parameters are significant, as evidenced from the elliptical nature of the plot. This observation shows that reaction time and temperature interaction have more effects on furfural yield compared to temperature and acid concentrations. The significant interaction of time and temperature is illustrated by the contour plot in appendix A, it was noticed that as both time and temperature increases, there was a significant corresponding increase in furfural yield which was also indicated by the elliptical nature of the plot hence time and temperature shows a very significant interaction.

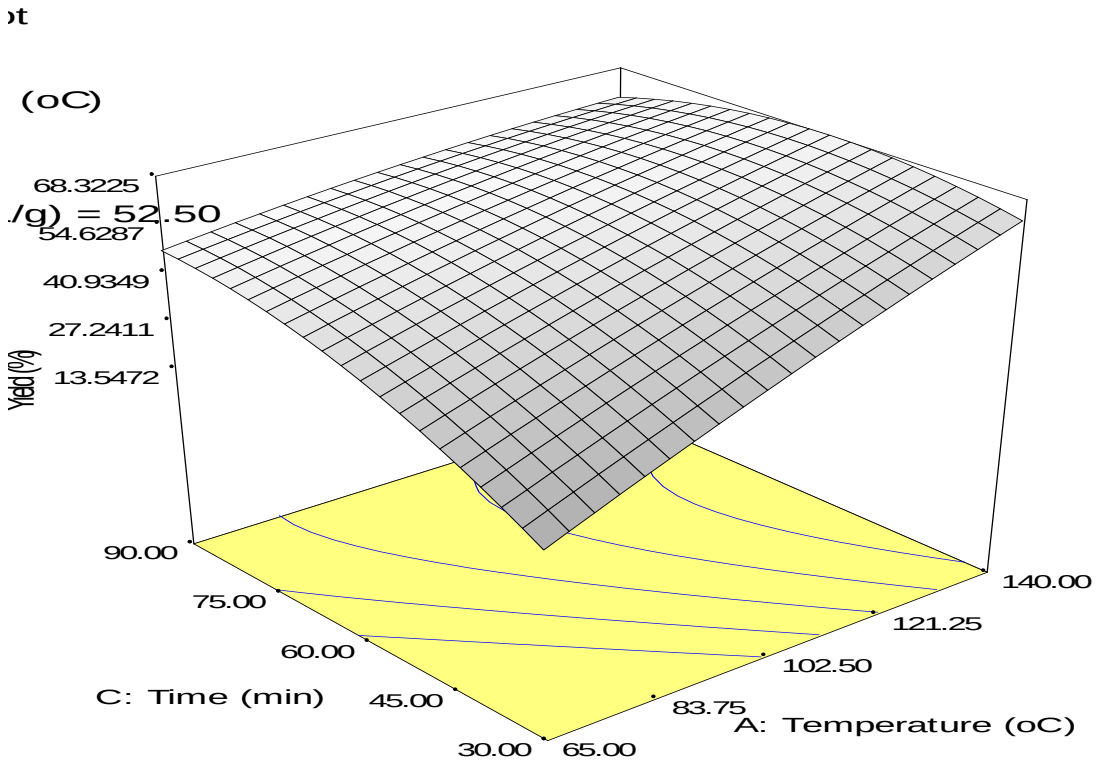


Figure 4.6:3D Plot of furfural yield against reaction time versus temperature.

4.3.7.4 Interaction between B-Acids concentration and C-Time (BC)

The effects of acid concentration and reaction time on furfural yield for furfural production from microalgae is depicted in Figure 4.7. As the acid concentration and reaction time vary (either increasing or decreasing), the yield of furfural changes. As shown in the same Figure 4.6 for instance, at acid concentration of 70 mL/g (x-axis) and reaction time of 90 minutes (z-axis), the corresponding furfural yield on y-axis is 59.45.43%. Thus, several plots for other runs of varying parameters against furfural yield in the same graph resulted to the 3D surface plots as shown in Figure 4.6. Also, the variations in furfural yield showed that the interactions between the variable parameters are significant, as evidenced from the elliptical nature of the contour plot. This resultswas

an improvement of Shafeeq*et al*. (2015), who concluded that furfural yield does not increase beyond 20 ml/g of acids concentration thus, furfural yield increase up to about 35ml/g of acids concentrations.

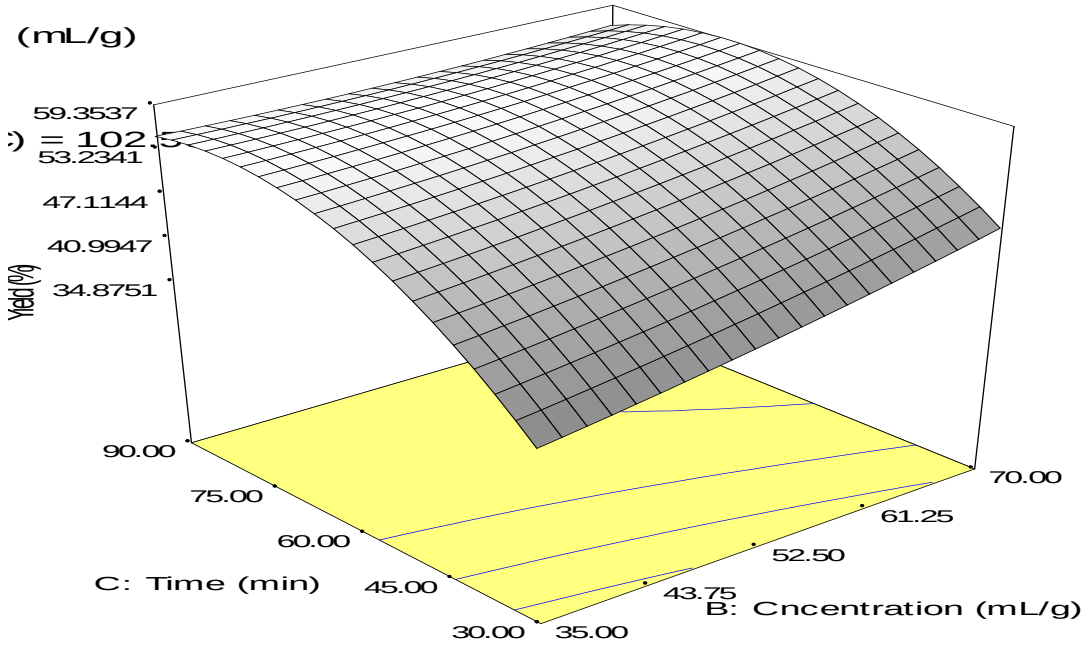


Figure 4.7: 3D Plot of furfural yield against reaction time versus concentration.

4.4Optimization of Furfural Yield

High yield of furfural was desired hence the goal of optimization of this study was to find optimal conditions among independent (process) variables that maximize the desirability function of furfural yield. It was evident from section 4.3.6.3 that minimum reaction time is required since prolong reaction time leads to furfural degradation and side reaction and increased cost of production. Therefore, the goal for reaction time was set to be between range of two points hence optimum range was set within the range whose furfural yield have been predicted as shown in Figure 4.5. Likewise, the observations during the

experimental work as evident in the discussion from section 4.3.6.1 to 4.3.6.3 and illustrated in Figures 4.5 to 4.7 guided the optimization decision such that all factors were set in a range to reduce overall production cost as shown in Table 4.5

Table 4.5:Desirability specifications for process parameters and response

S/NO	Constraint(Process factors)	Goal	Lower	Upper
1	A:Temperature (^{0}C)	Is in Range	39.43	165
2	B:Acid Concentration ml/g	Is in Range	35	70
3	Reaction Time (min)	Is in Range	30	90
4	Furfural yield (%)	Maximize	7.87	73.33

After the above specifications were set based on the observation from production process and the interactive effects of the various factors considered, a set of Eight (8) optimal solutions were generated and five (5) solutions out of the eight generated were selected and validated to see how well the model predicts the magnitude of the response. The results of validated and predicted optimized solutions are presented in Table 4.6

Table 4.6: Optimization result (validation experiment of optimized solution)

S/NO	Temperature (^{o}C)	Concentration of 1M H_2SO_4 (mL/g)	Time (min)	Predicted yield	Experimental (Actual)Yield	Desirability
1	140	35.00	65.10	69.32	73.10	0.939
2	140	38.41	63.12	69.15	66.45	0.936
3	140	65.00	55.13	68.87	66.00	0.932
4	140	49.52	61.07	68.83	65.56	0.931
5	140	70.00	53.86	68.99	65.61	0.934

The results of the validated experiment shown in Table 4.6 i.e the predicted and actual value of optimization solutions are in closed range suggesting that the quadratic model is adequate and successfully predicted the yield of furfural from microalgae. The deviation

(2.2%) from the predicted and validated experiments could be attributed to experimental error (pure error) or lack of fit error.

4.5 Furfural Characterisation

Important charaterisation techniques were carried out and the results are presented and discussed as follows:

4.5.1 Physicochemical properties of furfural produced

The physicochemical properties of furfural produced is presented in Table 4.7

Table 4.7: **Physicochemical properties of furfural produced**

Properties	**Present work**	**Literature value (Yan *et al* .,2014)**
Color	Colorless	Colorless
Density	1.17	1.16
Boiling point	160.48 ^{o}C	161.85 ^{o}C
Odour	Almond-like	Almond –like
Physical state	Liquid	Liquid
Molecular weight	95.0g/mole	96g/mole
Chemical formular	$C_5H_4O_2$	$C_5H_4O_2$

Some other measured values such as density were within the range of reported literature value by Ameh *et al* (2016) and Yan *et al* (2014) of 1.16 g/ml. However, the variations in some could also be attributed to difference in the origin and type of feedstock used to that reported in literatures.

4.5.2 Qualitative chemical analysis of furfural using fourier transform infrared spectroscopy (FTIR)

Qualitative Chemical analysis of furfural using Fourier Transform Infrared Spectroscopy (FTIR) was done on furfural produced to determine the functional group present and both the commercial and furfural produced was qualitatively characterized and compared.

Figure 4.8 shows the FTIR result, of furfural produced and that of commercial. Furfural produce was characterized using FTIR and Figure 4.8 shows Fourier Transform Infrared Spectroscopy Analysis of furfural produce and Commercial Furfural as both exhibit similar trends.

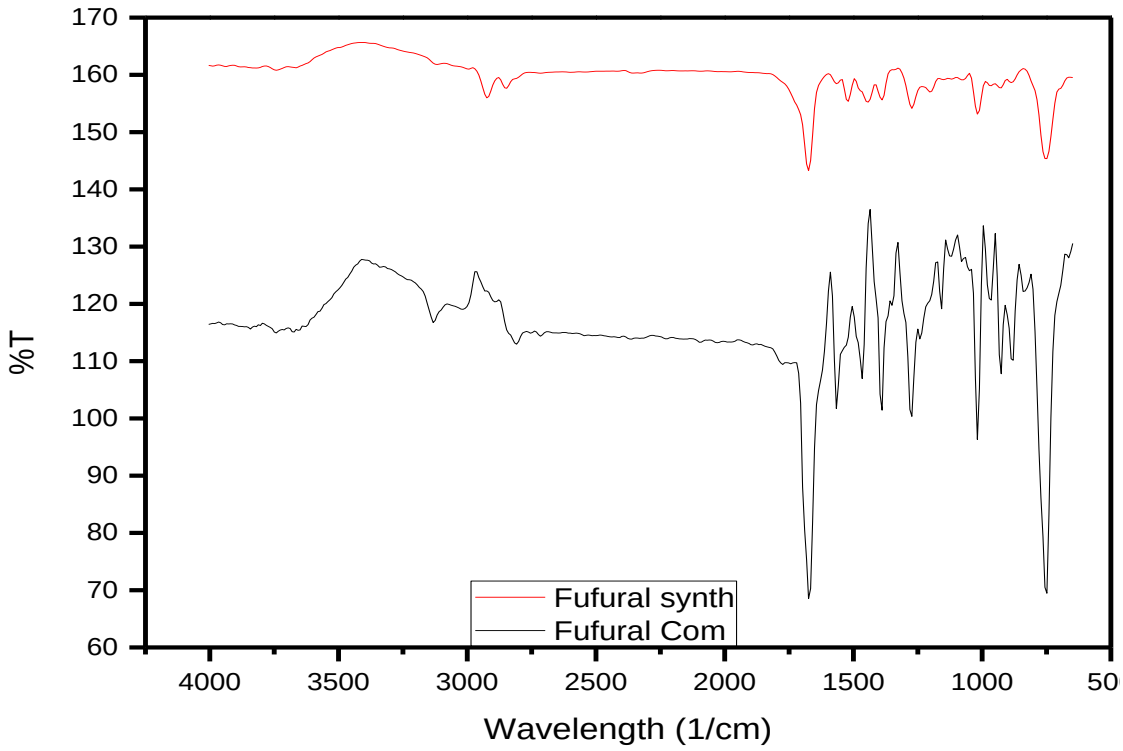


Figure 4.8: Fourier transform infrared spectroscopy analysis of furfural produced and that of commercial furfural

Table 4.8 indicates the FTIR Functional groups presents in both the commercial and furfural produced.

Table 4.8: FTIR functional groups presents in both the commercial and furfural produced

Wave number (cm^{-1})	**Functional groups**	**Class of Compound**
1685 – 1674.27	**C=O stretch**	Conjugated Carbonyl group
1685 – 1710	C=C-CH=OC=O stretch	Aldehydes
753 – 770	C-H stretch	Aromatics
1566.25 – 1388.79	C-C stretch	Aromatics
1018.45 – 1273.06	C-O stretch	Alcohols

As shown in Table 4.8, theFourier Transform Infrared Spectrum exhibited a strong absorption at 1674 cm^{-1} indicating a significant functional, the presence of conjugated carbonyl C=O group. The presence of an aldehyde was further proven by two peaks attained at 3019.55 cm^{-1} and 2924.16 cm^{-1}.These absorption shows a moderate intense stretching of aldehydic C=H as shown in Figure 4.8. Also from Figure 4.8, no broad peaks were observed at the area of 3400 cm^{-1} - 2500 cm^{-1}which belong to OH group, this confirms the absence of carboxylic group.If the sample belong to ester group,when a double bond is adjacent to a strong C=C peak would have be obsereved in 1674.27 cm^{-1} to 1685 cm^{-1} region. The strong peaks indicated from 1566.25 cm^{-1} to 1388.79 cm^{-1} are inactive stretcing of C=C from aromatic ring while the aromatic bending out of plane peaks was observed from 964.44 cm^{-1} to 849.5 cm^{-1}.Two strong peaks at 1018.45 $cm^{-1}$and

1273.06 cm^{-1} indicates C-O stretching vibration. The Infrared Spectrum of furfural produce was compared to that of commercial in Figure 4.8 and both followed a similar peaks trend. The FTIR peak shows a similar trends with that of Ong and Sashikala (2007) when they produced furfural from rice husk.

4.5.3Quantitative chemical analysis of furfural using GC-MS

Qualitative Chemical analysis of furfural using GC-MS was done on the furfural produced to determine the quantity (percentage composition) furfural present in the sample produces.

The Gas Chromatogram of furfural produced (Figure 4.9) was compared with that of commercial furfural in Figure 4.10 and both followed a similar pattern.

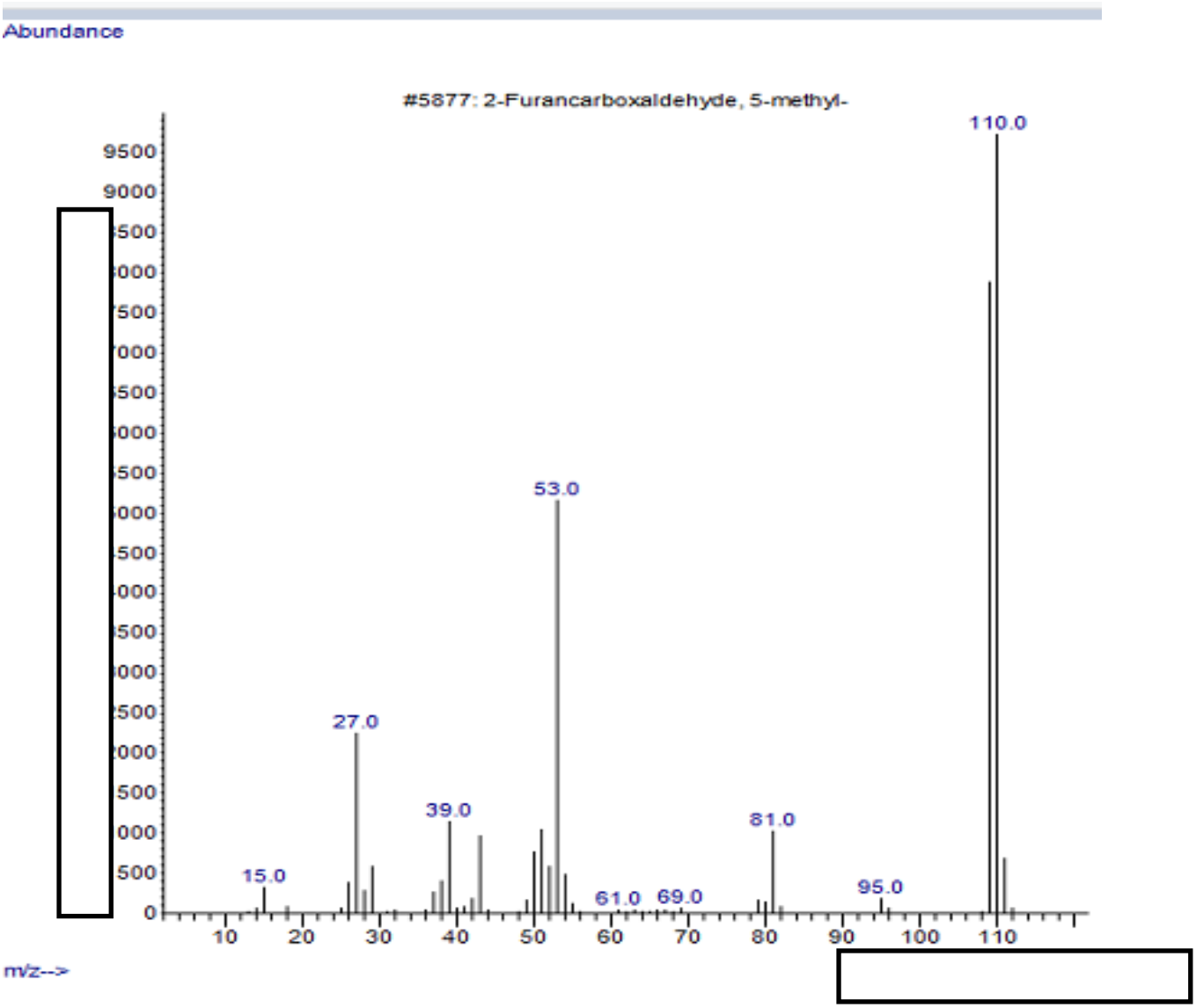


Figure 4.9: Gas Chromatogram-Peak Furfural Produced from Microalgae Biomass

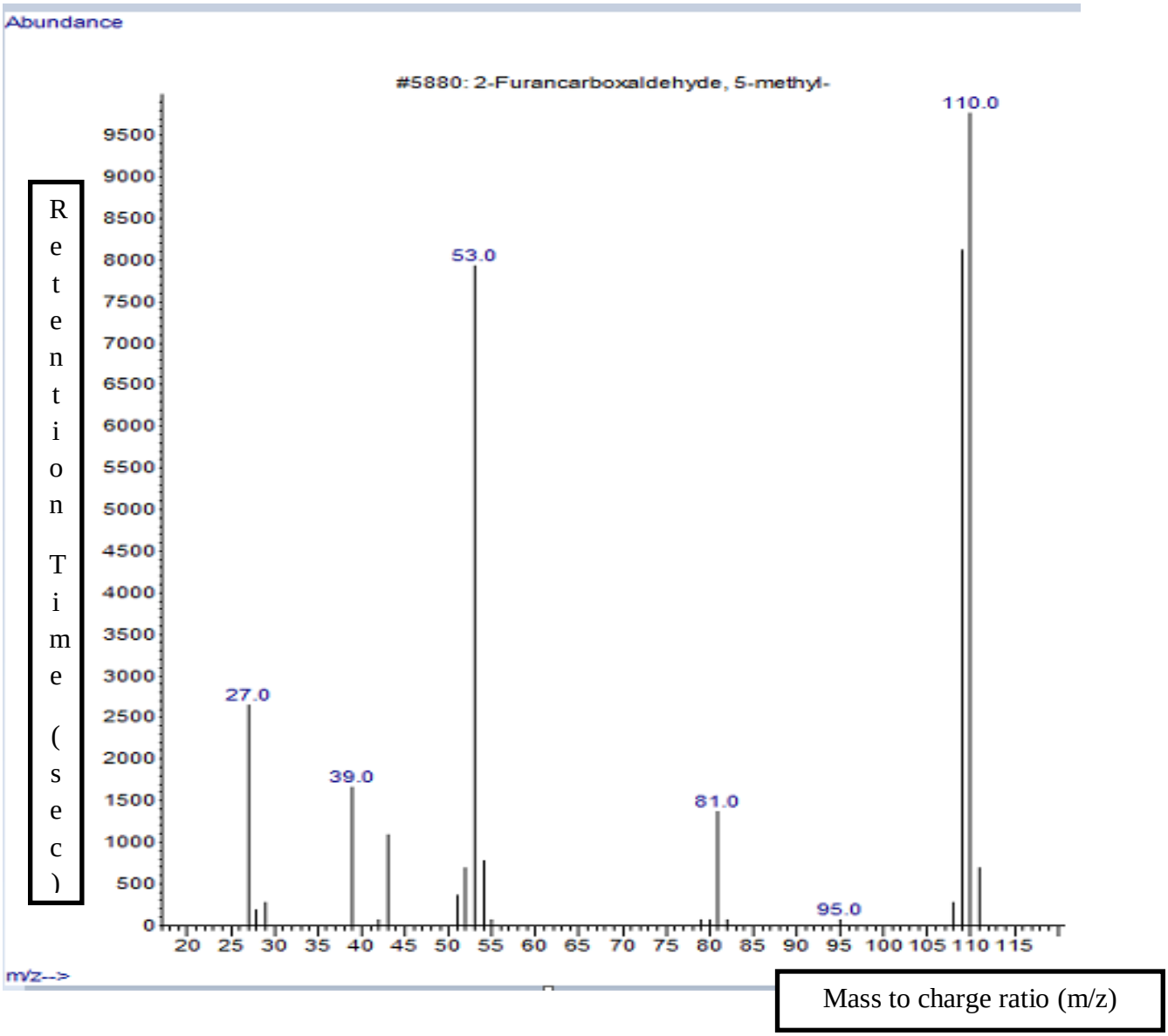


Figure 4.10: Gas chromatogram -peak of commercial furfural

Table 4.9 and 4.10 shows the (quantitative analysis) various constituent presents in furfural produce and commercial furfural respectively.

Table 4.9:Quantitative analysis (various constituents present) of furfural produced

CONSTITUENT (S)	% COMPOSITION	FORMULAR	MOLAR MASS
Thiospenecarboxylic	0.50	$C_5H_4O_2S$	128
Pentenal	0.41	$C_5H_{10}O$	86
2-Furcarboxaldehyde	50.86	$C_5H_4O_2$	96
2-Furanethanol	4.16	$C_5H_8O_2$	112
Cyclopentane	0.16	C_5H_{10}	70
Biphenyl	0.07	$C_{12}H_{10}$	154
Carbonic acids	0.32	CH_2O_3	62
P-xylene	5.38	C_8H_{10}	106
Pyridine	0.43	C_5H_5N	79.8
Benzenealdehyde	0.07	C_4H_5CHO	106.12
5-Hydroxylmethylfurfural	26.74	$C_6H_4O_2$	108
Aminopyrindine	4.16	$C_4H_5N_3$	95
Heptanonitrile	0.20	$C_7H_{13}N$	111
Benzeneacettlaldehyde	0.61	$C_9H_{10}O$	134
2-Furanone	0.50	$C_4H_4O_2$	84
2-Furanylvinylamine	0.11	C_6H_7NO	109.12
5-Methylfuranone	5.32	$C_5H_6O_2$	98.10

X=94.03%

Where X is the percentage of furfural compound and Non furfural compound =5.97%

Table 4.10: Quantitative analysis (various constituents present) of commercial furfural

CONSTITUENT (S)	% COMPOSITION	FORMULAR	MOLAR MASS
Thiospenecarboxylic	5.32	$C_5H_4O_2S$	128
Pentenal	0.41	$C_5H_{10}O$	86
2-Furcarboxaldehyde	7.87	$C_5H_4O_2$	96
2-Furanethanol	20.07	$C_5H_8O_2$	112
Cyclopentane	0.08	C_5H_{10}	70
Azulene	0.14	$C_{10}H_8$	106
Carbonic acids	0.20	CH_2O_3	62
P-xylene	9.68	C_8H_{10}	106
Methoxylacetic	0.23	$C_3H_6O_3$	90
Tricosene	0.21	$C_{23}H_{46}$	322
5-Hydroxylmethylfurfural	55.37	$C_6H_4O_2$	108
Aziridine	0.08	C_2H_5N	43
Trifluroacetic acid	0.26	$C_2H_2F_2$	62
Formaldehyde	0.08	CH_2O	30

X=83.39%

Where X is the percentage of furfural compound and non-furfural compound =16.61%

Results obtained from Gas Chromatogram (Figure 4.9 and Figure 4.10) shows that furfural has a retention time of 7.834sec with a relative's abundance of 100%. The gas chromatogram spectrum shows molecular ions peaks at mass to charge (m/z) ratio of 96 which corellate to a molecular formular of furfural of $C_5H_4O_2$. However, a peak at m/z of 95.0 (Figure 4.9) was obtained due to loss of hydrogen to form a carbonium ion.An electron was given away by the aldehyde carbon to hydrogen to form this fragmentation

since more stable cation can be formed due to formation of hydrocarbon pattern,peak at m/z of 110 becomes dominant. The gas chromatogram of furfural produced was compared with that of commercial furfural in Figure 4.10 and both followed a similar pattern.Table 4.9 and 4.10 shows the various constituent presents in furfural produce, furfural has higher percentage of 50.86% in furfural produce while 7.87 % in the commercial furfural. The low percentage of furfural recorded in the commercial may be due to adulterations and having stayed for long period. Also the limitation to calibrate the GCMS machine used for specifically furfural could affect the quantification of furfural present in both produced and commercial samples as the percentage recorded in Table 4.9 and Table 4.10 are relative with respect to the area of the column of chromatogram.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

A numbers of conclusions have been drawn from this research work while a few recommendations are made.

5.1Conclusion

Based on the results presented and discussed in chapter four, the following conclusions are made;

1. From the crude fibre analysis carried out on microalgae, it was observed that microalgae feedstock had higher hemicellulose contents (54.13%) compared to agricultural feedstocks such as corn cob (30%), rice husk (19%) and sugarcane bagasse (32%) as reported by Chen *et al*, 2015 hence it is a suitable raw material for production of furfural. Furfural yield increases with increased in temperature from 65°C (39%) to 140°C, (70%). Initially acid concentration increased with increase in furfural yield very slightly from10 ml/g to 30 ml/g and furfural yield almost remained constant after 35 ml/g concentration of the acid as an increase in furfural yield was observed with increase in reaction time from 30 min (46.56 %) to 65 min (73.33 %). However, elongated period of reaction time beyond 70 min lead to formation of side reaction and reduction in furfural yield to43 %.
2. Furfural production was successfully optimized using (CCD) RSM methodology and the optimal conditions for furfural productions from microalgae were determined to be: 140 °C, concentration of sulphuric acid (acids to solid ratio) 35.06ml/g and reaction time of 65.32 min as well as 1.0 g of sodium chloride per

gram of microalgae biomass used with furfural yield of 73.10%.

3. FTIRResult confirms the presence of carbonyl and aldehyde functional groups at wave length of 1674.27cm-1 and 2924.18cm $^{-1}$ respectively while GC-MS results confirms the presences of furfural (50.86%) with a molar mass of 96 g/mol at peak of mass to charge ratio of 96 and relative percentage of 94.03% of furfural compound. Both FTIR and GCMS of furfural produced and commercial followed a similar pattern.

5.2 Recommendations

1. Since microalgae produce high yield of furfural, a pilot plant study should be carried to enhance indigenous technology and mass production.
2. Downstream processing should be done to separate furfural from other components such 5-Hydroxylmethylfurfural, 2-Furanethanol etc.
3. library data for HPLC should be provided in order to carry out HPLC analysis to help fully determine the structure of furfural
4. Other acids catalyst such as HCl and Phosphoric acids should be used to produce furfural to investigate their effects on furfural yield
5. Effect of sodium chloride study should be incorporated into the response surface methodology (RSM) to evaluate its interaction with other factors such as temperature, time.
6. Further research should be done on the kinetic model of the reaction to establish the design parameters for the plant needed for the production of furfural

7. Nigerian government should investment in field of research for the utilization of our abundance resources, considering the large amount of lignocellulosic materials we have in the country which are virtually regarded as wastes.

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APPENDICES

APPENDIX A

Appendix A-1 Design Expert Plots And Results

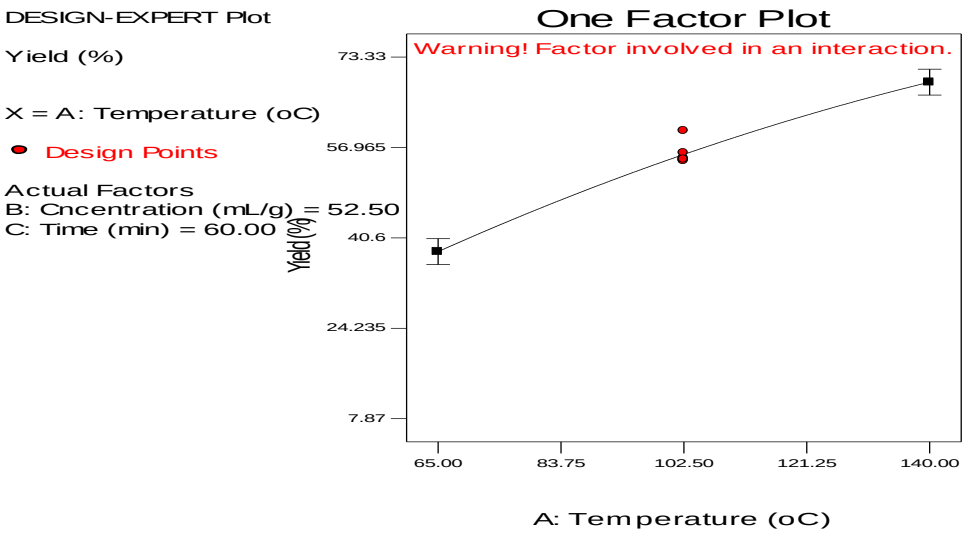


Figure A-1: One factor plot: temperature vs. yield

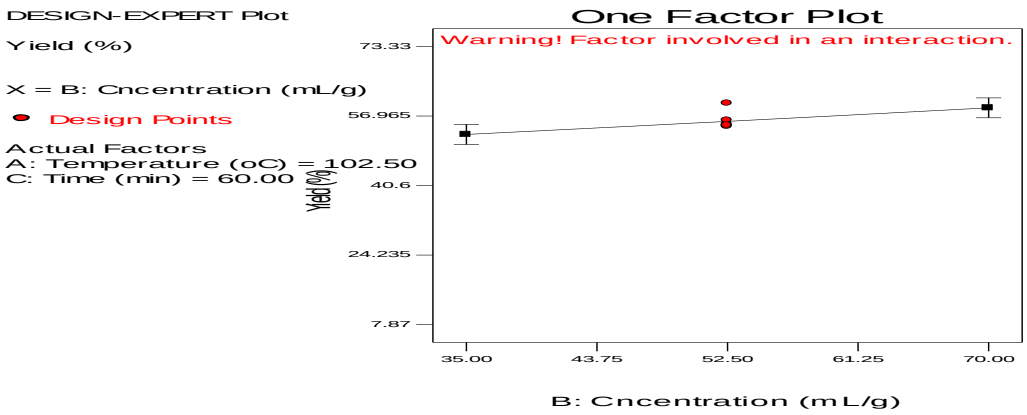


Figure A-2: One factor plot: Concentration vs. Yield

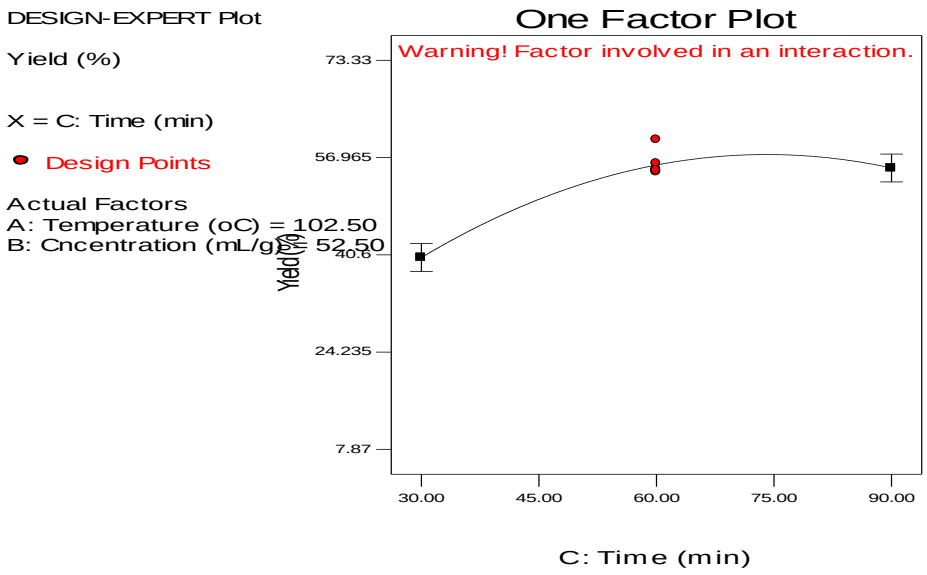


Figure A-3: One factor plot: time vs. yield

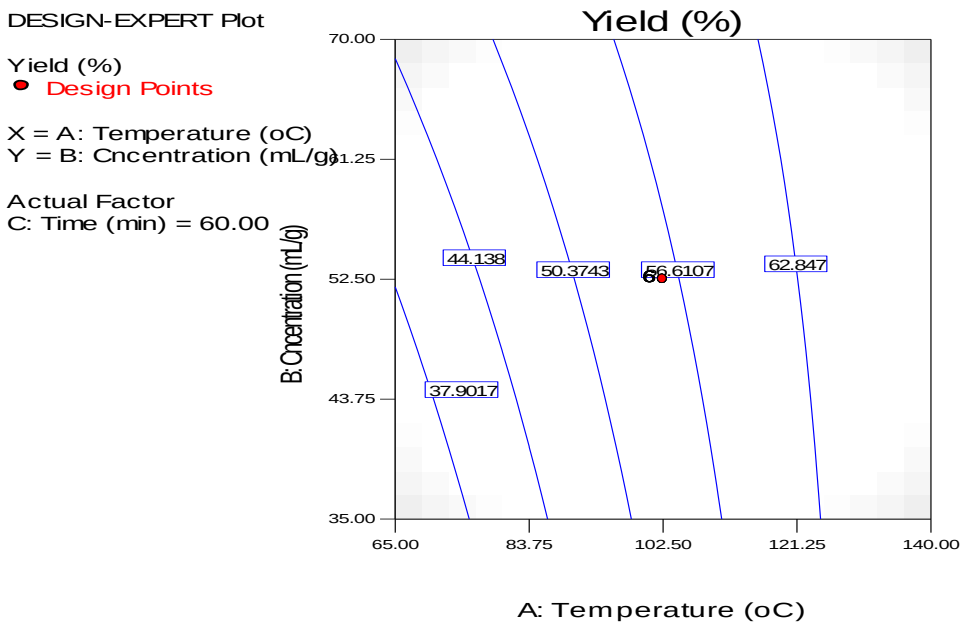


Figure A-4: Contour plot of temperature and concentration interaction

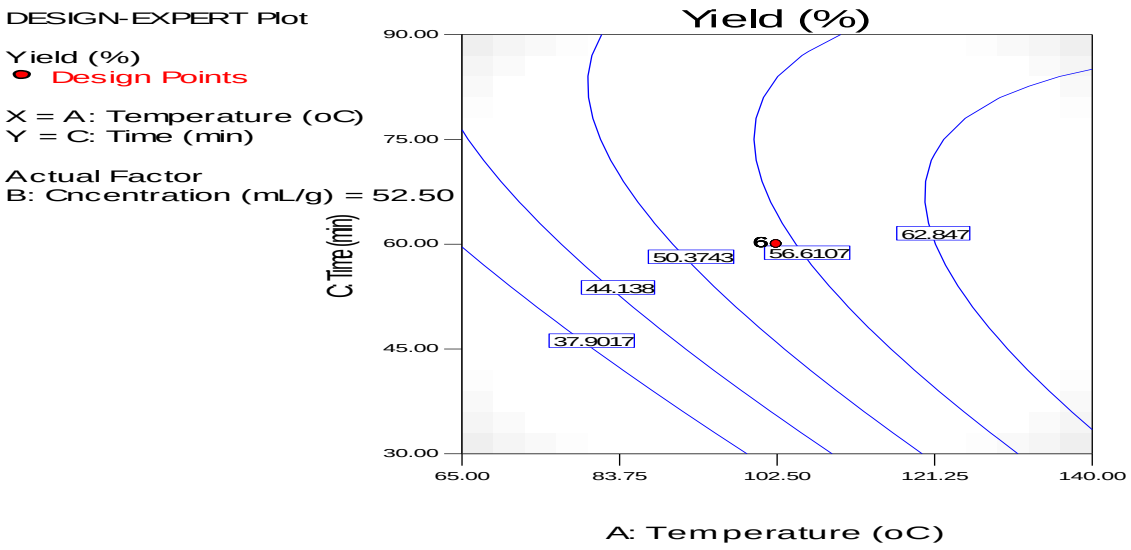


Figure A-5: Contour plot of Temperature and time interaction

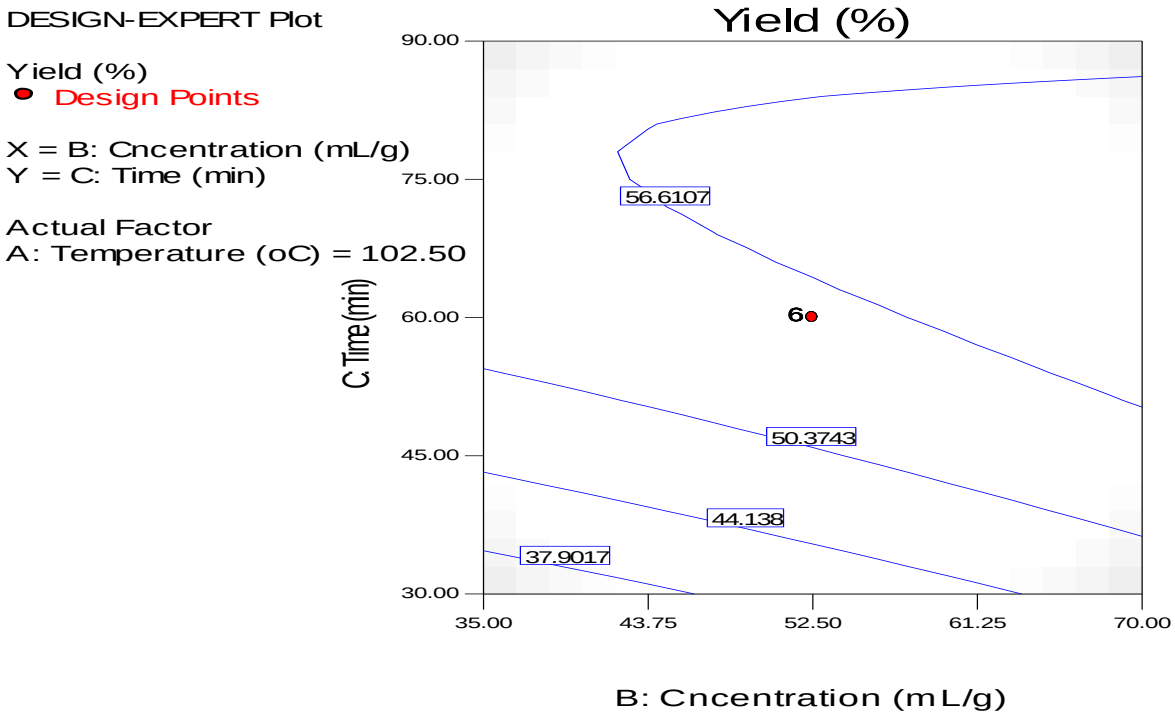


Figure A-6: Contour plot of concentration and time interaction

Table A.1 Central composite design matrix of furfural yield with their respective predicted and actual yield

Exp'tal Run	A	B	C	Experimental (FURFURAL YIELD) (%)	Predicted furfural yield
1	102.50	52.50	60.00	55.07	55.71
2	65.00	70.00	30.00	27.67	24.31
3	39.43	52.50	60.00	20.00	23.53
4	140.00	35.00	30.00	60.05	58.32
5	102.50	52.50	60.00	54.55	55.71
6	105.50	52.50	60.00	54.71	55.71
7	102.50	52.50	60.00	56.00	55.71
8	65.00	70.00	90.00	51.00	48.96
9	102.50	52.50	110.45	45.00	45.66
10	102.50	52.50	60.00	60.00	55.71
11	102.50	52.50	9.55	15.67	20.30
12	140.00	70.00	90.00	58.00	57.05
13	65.00	35.00	90.00	43.00	42.22
14	65.00	35.00	30.00	7.87	5.05
15	165.57	52.50	60.00	73.33	75.13
16	140.00	35.00	90.00	64.20	63.79
17	102.50	81.93	60.00	57.40	61.13
18	140.00	70.00	30.00	67.10	64.11
19	102.50	52.50	60.00	54.82	55.71
20	102.50	23.07	60.00	49.00	50.60

Key; A= **Temperature (^{o}C), B= Concentration of H_2SO_4 (mL/g) C= Reaction Time (min).**

Table A-2 Correlation coefficient data for furfural production from microalgae

Std. Dev	3.37	R-Squared	0.9807
Mean	48.72	Adj R-Squared	0.9634
C.V.	6.9	Pred R-Squared	0.8771
PRESS	723.07	Adeq Precision	29.437

APPENDIX B

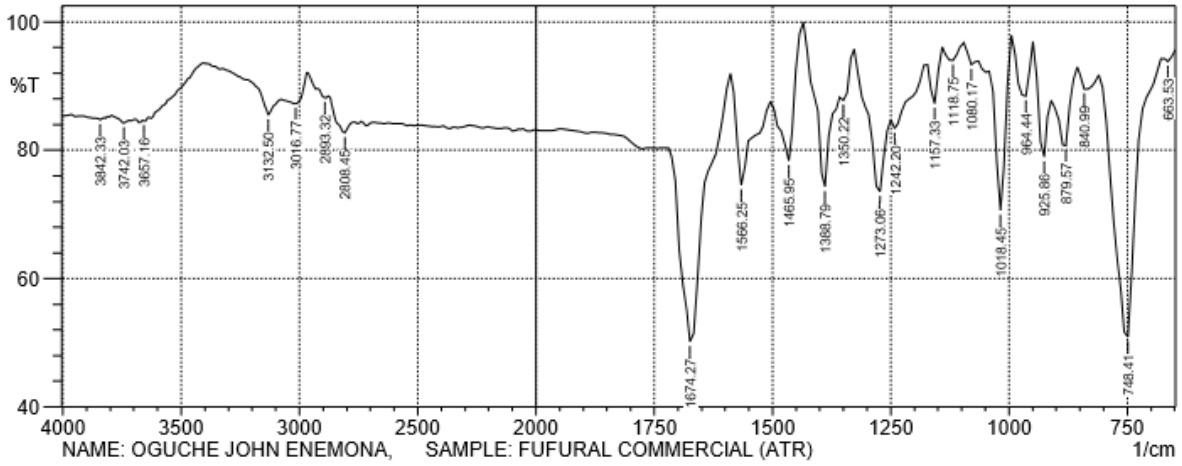
CHARATERIZATION RESULT

FTIR ANALYSIS RESULT

NATIONAL REASEARCH INSTITUTE FOR CHEMICAL TECHNOLOGY, ZARIA

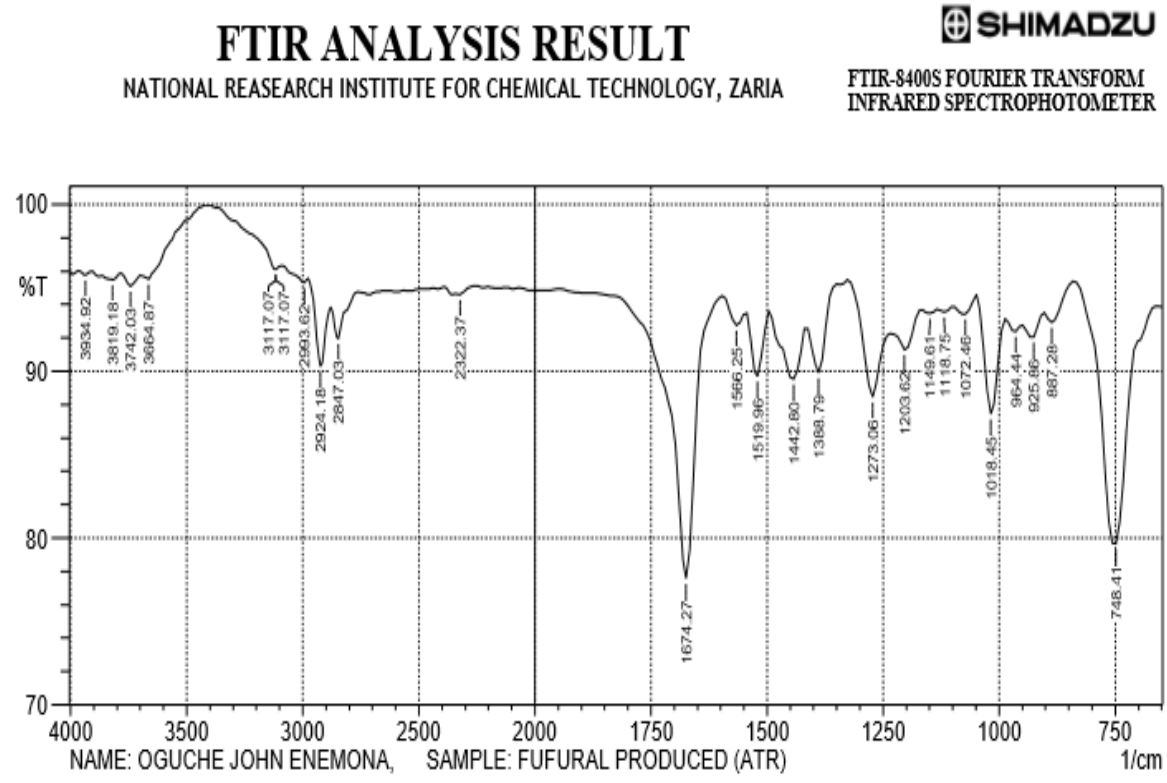
SHIMADZU

FTIR-8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	663.53	93.813	1.157	678.97	648.1	0.772	0.08
2	748.41	50.898	42.033	810.13	678.97	16.383	12.247
3	840.99	89.521	3.042	856.42	810.13	1.984	0.385
4	879.57	80.708	10.026	910.43	856.42	3.591	1.203
5	925.86	78.973	12.438	949.01	910.43	2.589	1.231
6	964.44	88.408	8.848	995.3	949.01	1.678	1.153
7	1018.45	70.573	24.562	1041.6	995.3	3.745	2.734
8	1080.17	93.327	1.983	1095.6	1064.74	0.775	0.13
9	1118.75	94.009	2.419	1141.9	1095.6	1.017	0.286
10	1157.33	87.296	7.401	1172.76	1141.9	1.27	0.538
11	1242.2	83.43	2.133	1249.91	1172.76	4.314	0.368
12	1273.06	73.531	14.497	1327.07	1249.91	6.208	2.705
13	1350.22	87.74	2.439	1357.93	1327.07	1.303	0.182
14	1388.79	74.318	18.669	1435.09	1357.93	5.152	3.07
15	1465.95	78.358	16.126	1504.53	1435.09	4.426	2.429
16	1566.25	74.529	16.26	1589.4	1504.53	7.051	3.07
17	1674.27	50.211	34.701	1728.28	1589.4	20.699	11.605
18	2808.45	82.765	3.082	2877.89	2769.87	7.852	0.996
19	2893.32	88.184	0.648	2924.18	2877.89	2.417	0.078
20	3016.77	87.227	3.05	3078.49	2970.48	5.964	1.001
21	3132.5	85.531	3.439	3325.39	3078.49	11.583	0.622
22	3657.16	84.54	0.263	3664.87	3641.73	1.673	0.024
23	3742.03	84.197	0.69	3796.04	3718.88	5.534	0.106
24	3842.33	84.704	0.33	3857.76	3826.9	2.194	0.021

Figure B.1 FTIR RESULTS OF COMMERCIAL FURF

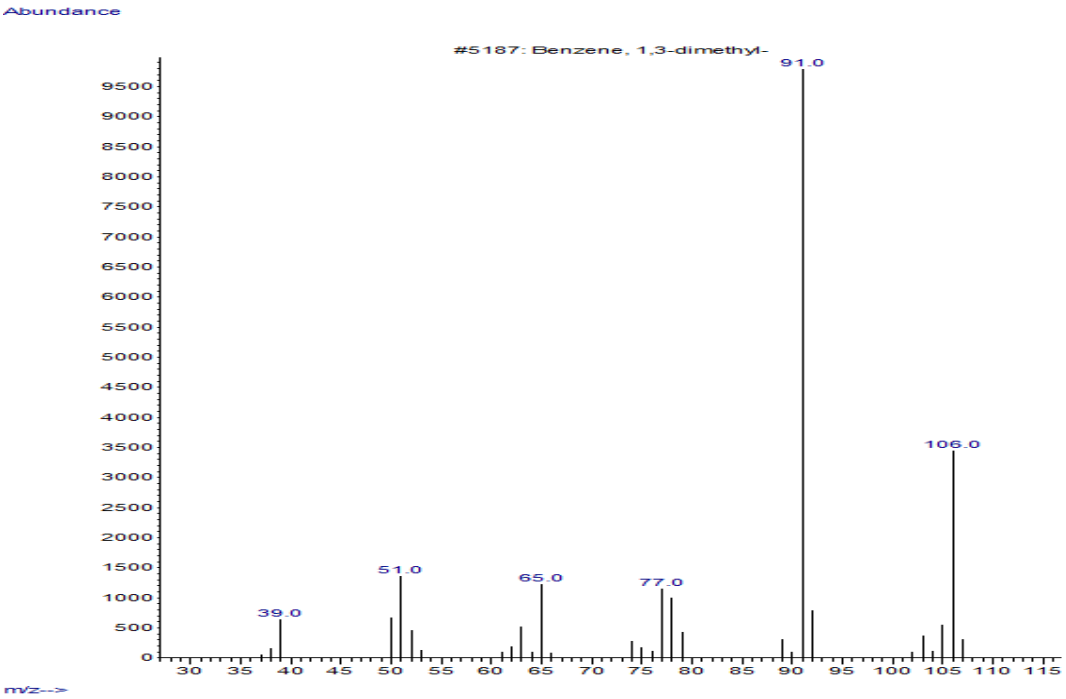


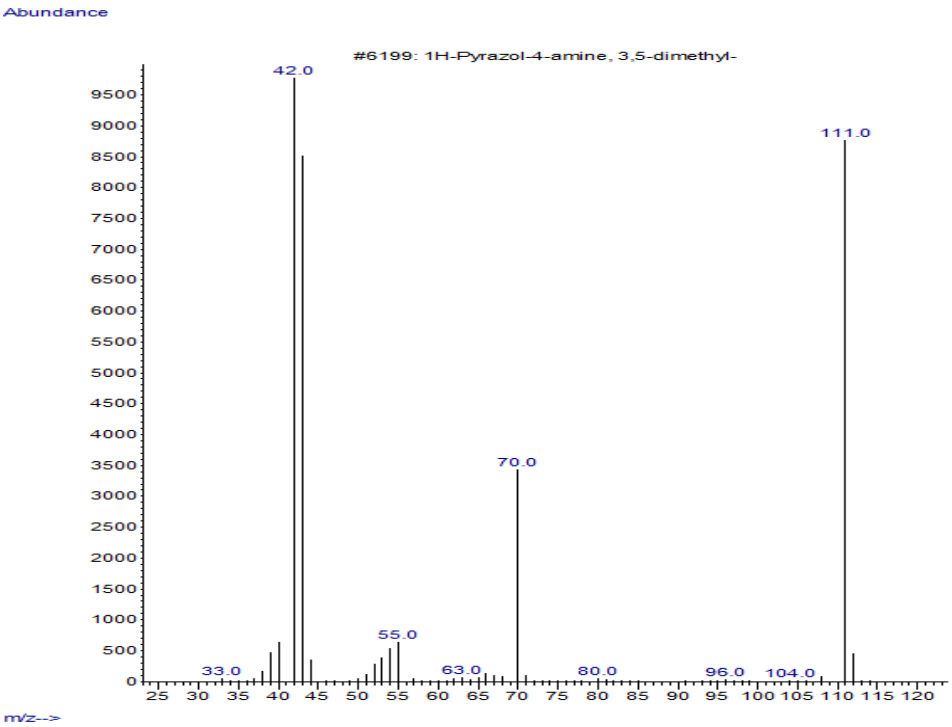
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	748.41	79.679	14.931	840.99	663.53	8.907	4.661
2	887.28	92.948	1.05	902.72	840.99	1.676	0.148
3	925.86	92.035	1.109	949.01	902.72	1.547	0.119
4	964.44	92.402	0.508	987.59	949.01	1.274	0.049
5	1018.45	87.443	6.417	1049.31	987.59	2.609	0.91
6	1072.46	93.44	0.821	1103.32	1049.31	1.509	0.109
7	1118.75	93.56	0.209	1134.18	1103.32	0.878	0.016
8	1149.61	93.462	0.222	1165.04	1134.18	0.893	0.018
9	1203.62	91.31	1.577	1234.48	1165.04	2.42	0.219
10	1273.06	88.461	5.145	1327.07	1234.48	3.45	0.901
11	1388.79	89.93	3.81	1419.66	1350.22	2.403	0.497
12	1442.8	89.542	3.342	1496.81	1419.66	3.083	0.686
13	1519.96	89.686	3.867	1543.1	1496.81	1.807	0.467
14	1566.25	92.757	1.164	1597.11	1543.1	1.588	0.135
15	1674.27	77.583	16.934	1828.58	1597.11	10.827	5.159
16	2322.37	94.612	0.123	2337.8	2252.93	1.938	0
17	2847.03	91.956	2.123	2877.89	2762.16	3.378	0.423
18	2924.18	90.285	4.346	2978.19	2877.89	3.234	0.859
19	2993.62	95.296	0.371	3086.21	2978.19	2.035	0.091
20	3117.07	96.113	0.597	3363.97	3086.21	2.372	-0.002
21	3117.07	96.113	0.597	3363.97	3086.21	2.372	-0.002
22	3664.87	95.536	0.643	3695.73	3402.54	2.641	-0.156
23	3742.03	95.099	0.7	3780.6	3695.73	1.716	0.132
24	3819.18	95.491	0.299	3865.48	3780.6	1.65	0.063
25	3934.92	95.768	0.233	3965.78	3911.77	0.984	0.028

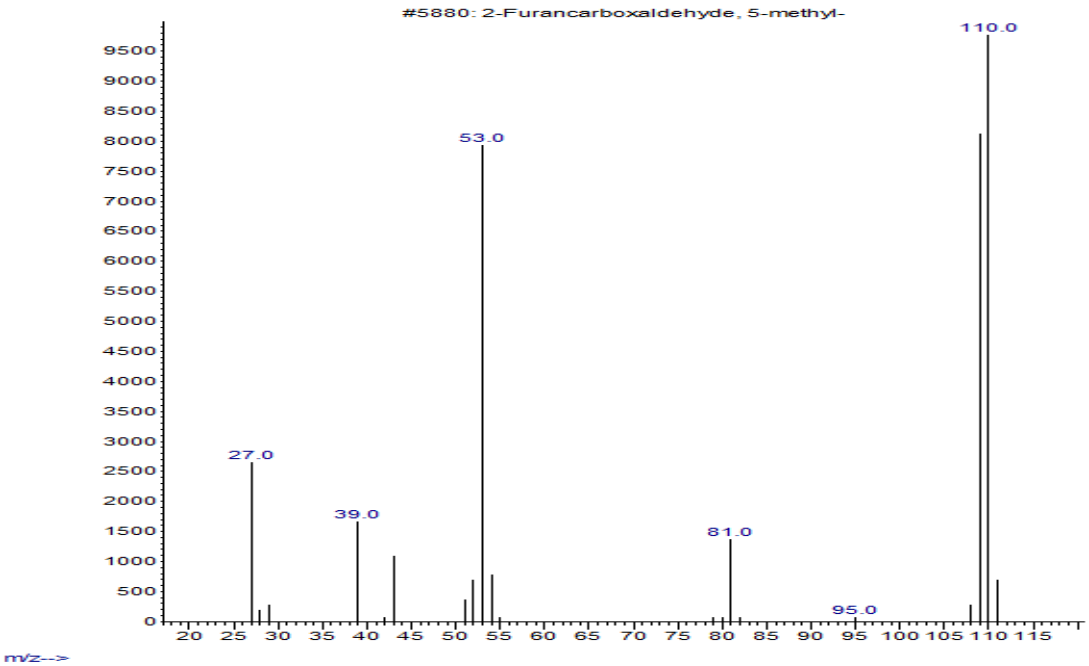
Figure B.2: FTIR results of furfural produced

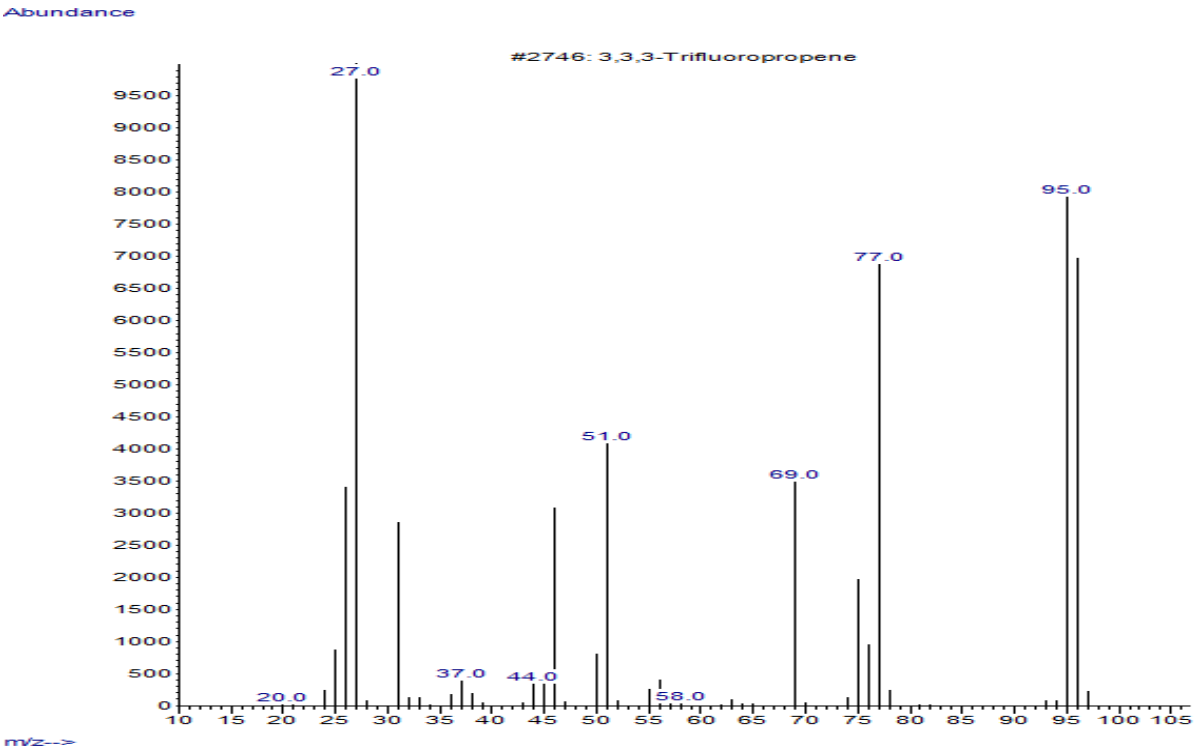
APPENDIX C

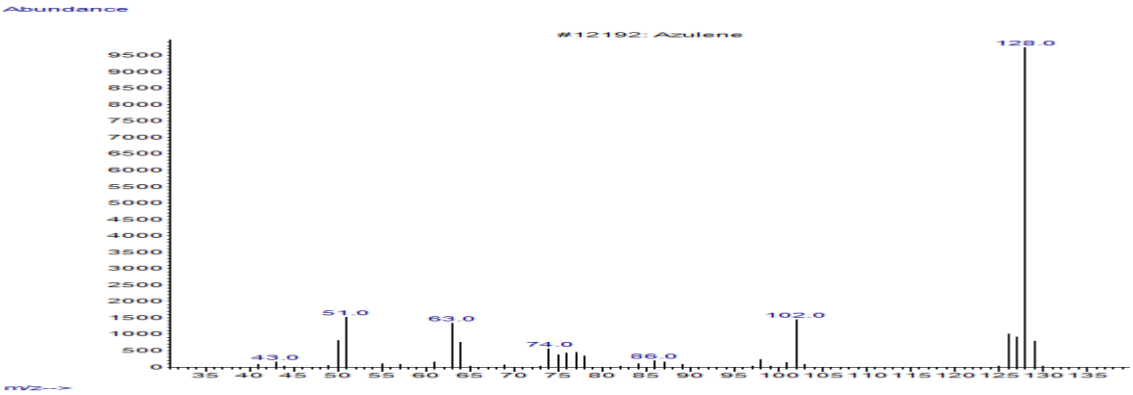
Appendix C-1 MASS SPETRA OF FURFURAL PRODUCED GCMS PEAKS

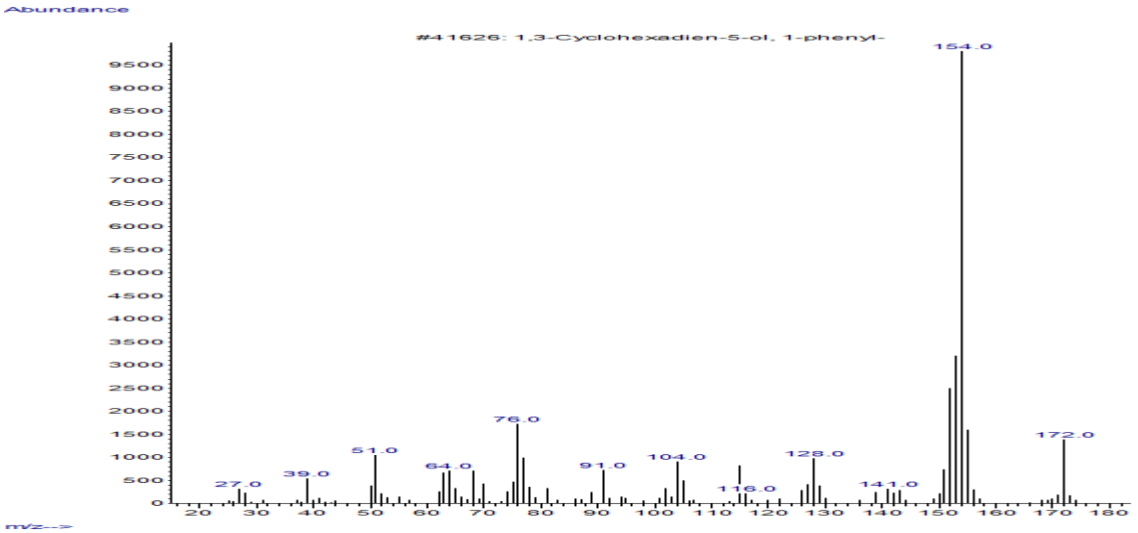


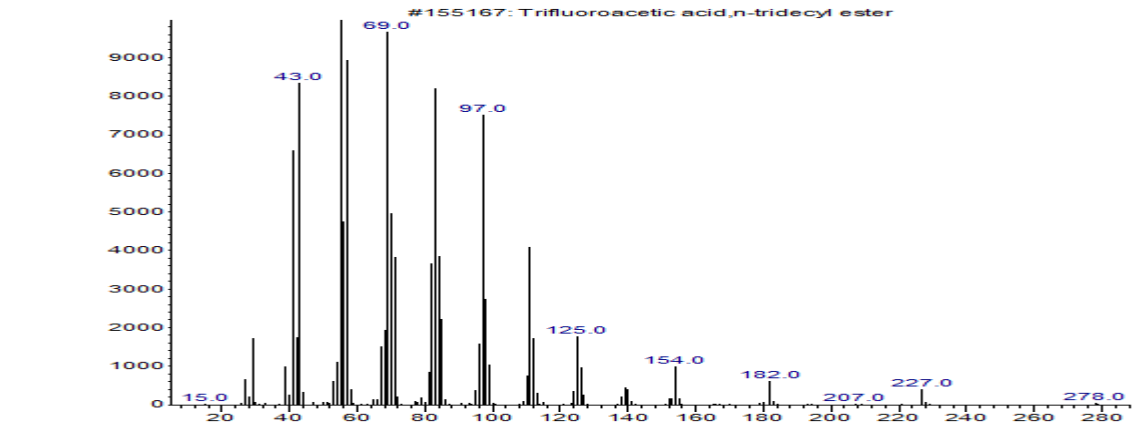

Abundance
#6199: 1H-Pyrazol-4-amine, 3,5-dimethyl-
42.0
111.0
70.0
55.0
33.0
63.0
80.0
96.0
104.0
m/z-->

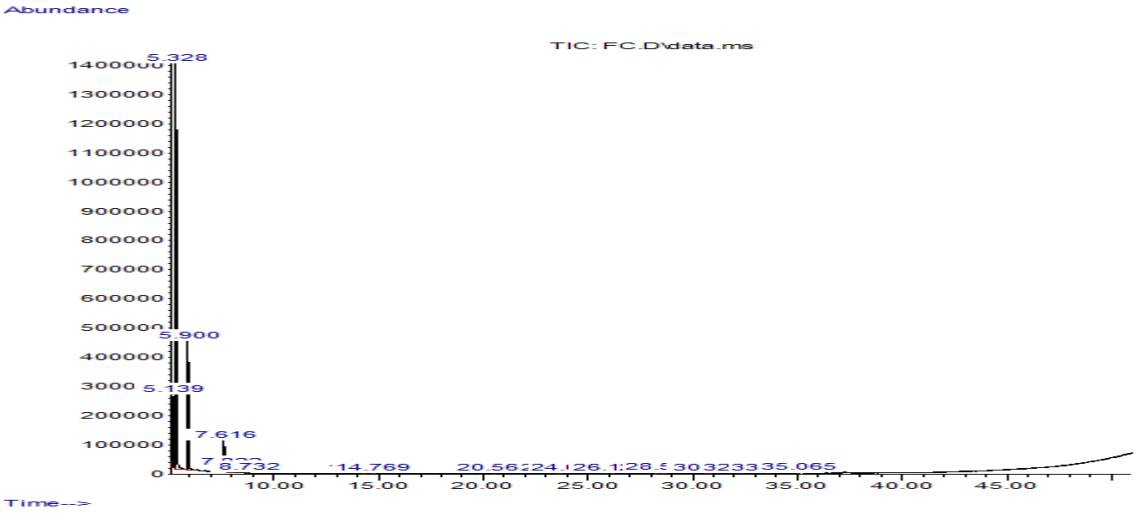

Abundance
#5880: 2-Furancarboxaldehyde, 5-methyl-
110.0
53.0
27.0
39.0
81.0
95.0
m/z-->

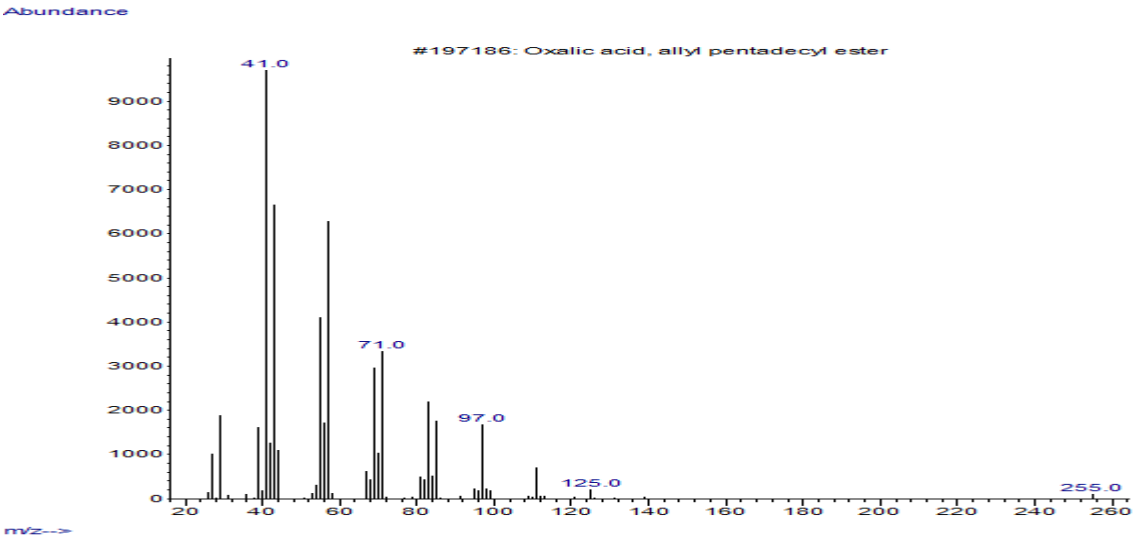

Abundance
#2746: 3,3,3-Trifluoropropene
27.0
95.0
77.0
51.0
69.0
37.0
44.0
20.0
58.0
m/z-->

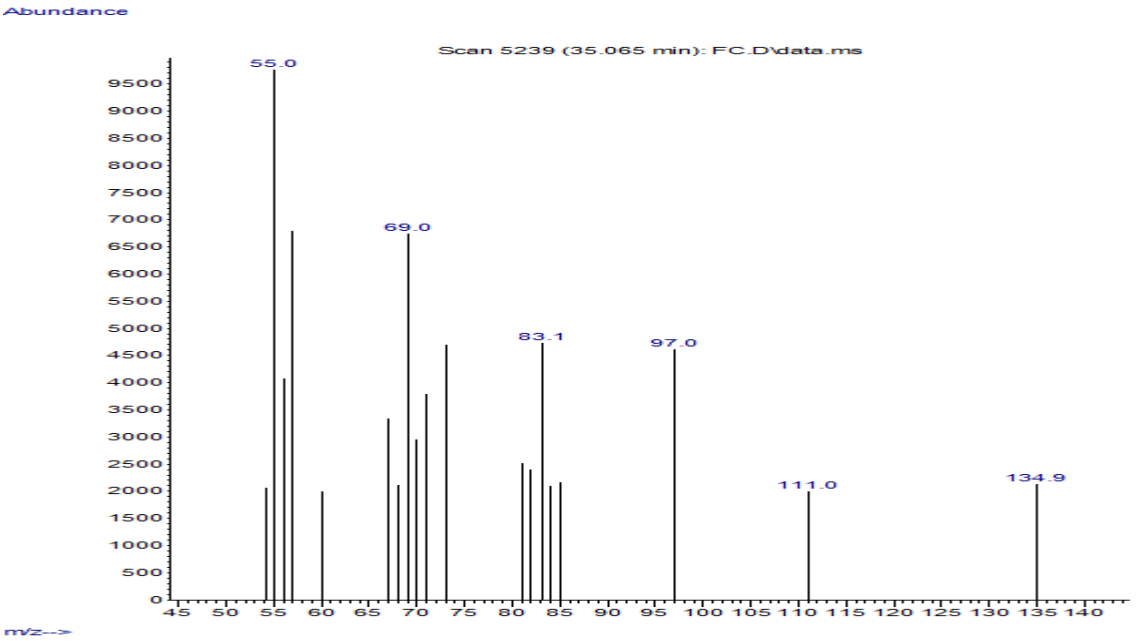

Abundance
#12192: Azulene
128.0
51.0
63.0
102.0
74.0
43.0
86.0
m/z-->

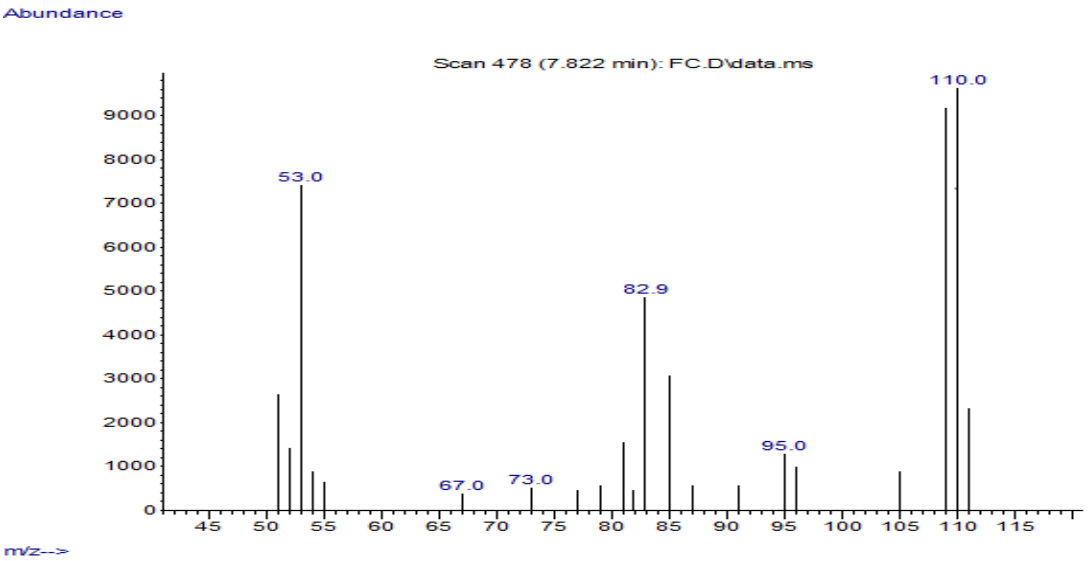

Abundance
#41626: 1,3-Cyclohexadien-5-ol, 1-phenyl-
154.0
172.0
76.0
51.0
39.0
27.0
64.0
91.0
104.0
128.0
116.0
141.0
m/z-->

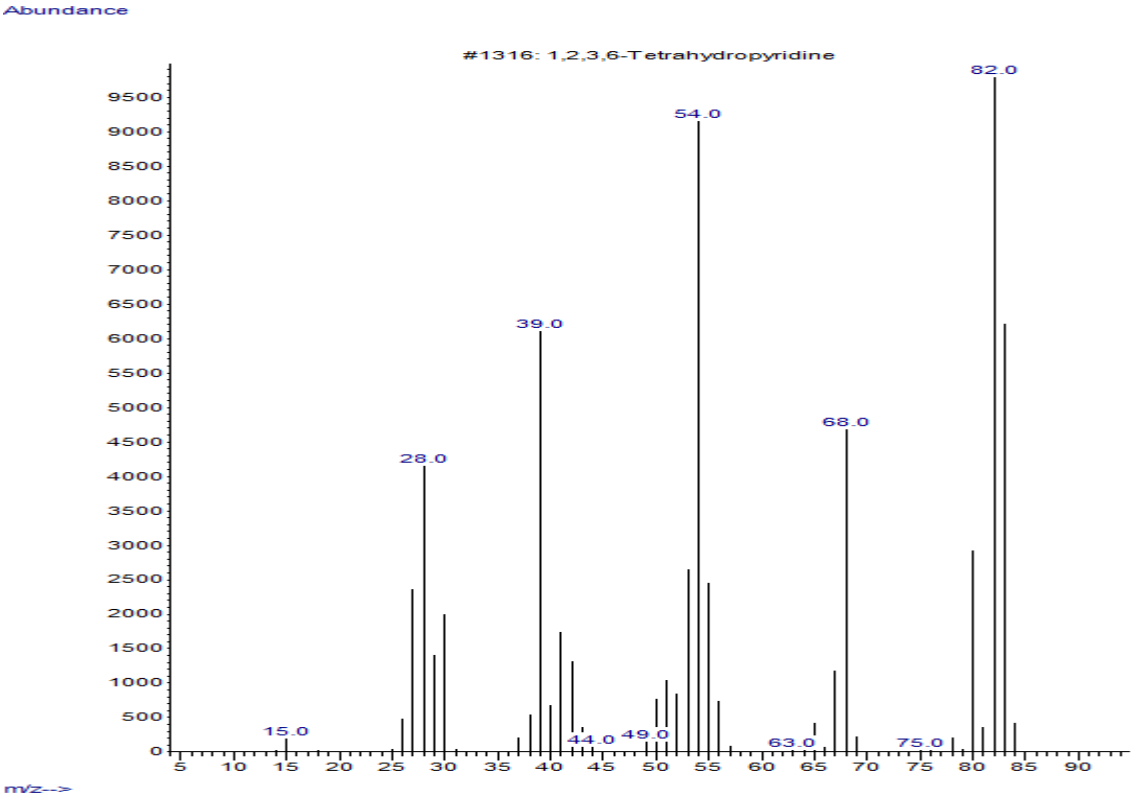

Abundance
#155167: Trifluoroacetic acid,n-tridecyl ester
69.0
43.0
97.0
125.0
154.0
182.0
227.0
207.0
278.0
15.0
m/z-->

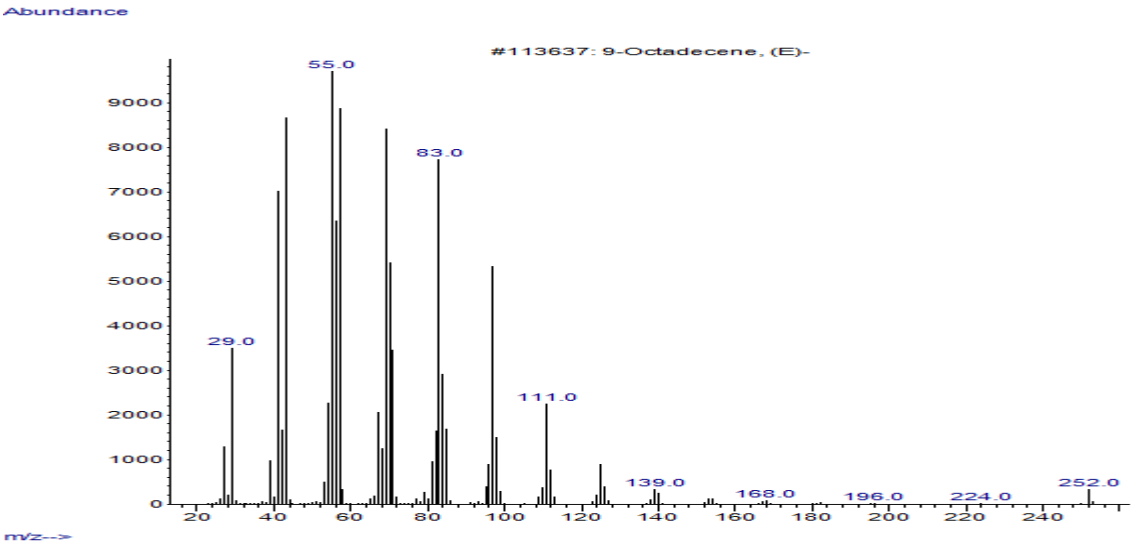

Abundance
TIC: FC.D\data.ms
5.328
5.900
5.139
7.616
8.732
14.769
20.562
24.126
28.530
32.323
35.065
Time-->

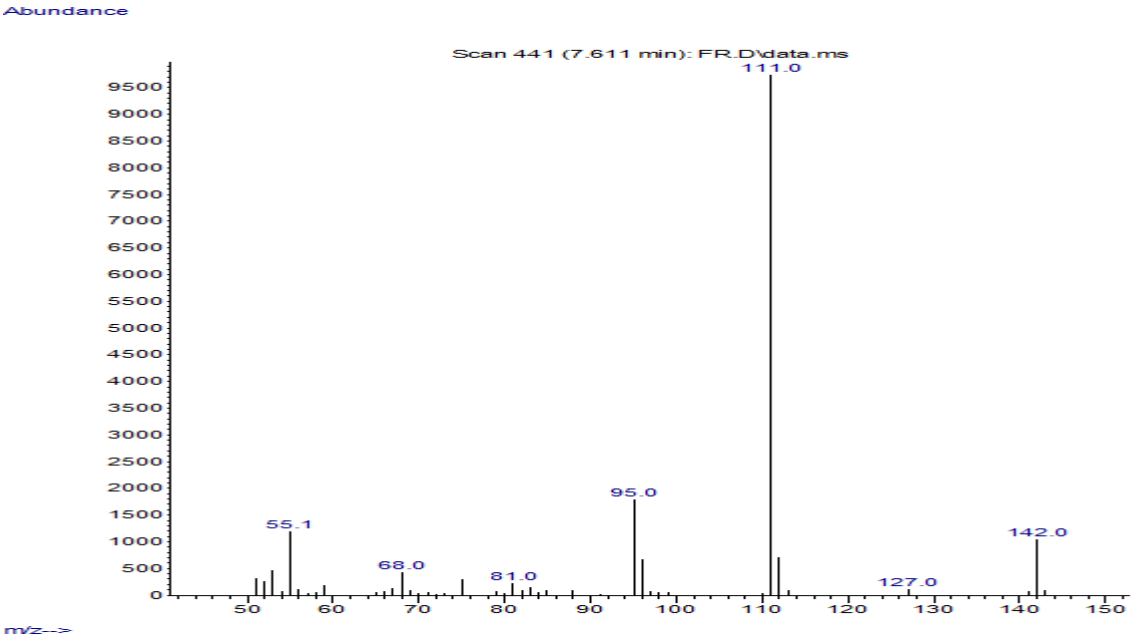

Abundance
#197186: Oxalic acid, allyl pentadecyl ester
41.0
71.0
97.0
125.0
255.0
m/z-->


Abundance
Scan 5239 (35.065 min): FC.D\data.ms
55.0
69.0
83.1
97.0
111.0
134.9
m/z-->


Abundance
Scan 478 (7.822 min): FC.D\data.ms
110.0
53.0
82.9
95.0
67.0
73.0
m/z-->


Abundance
#1316: 1,2,3,6-Tetrahydropyridine
82.0
54.0
39.0
68.0
28.0
15.0
44.0
49.0
63.0
75.0
m/z-->


Abundance
#113637: 9-Octadecene, (E)-
55.0
83.0
29.0
111.0
139.0
168.0
196.0
224.0
252.0
m/z-->


Abundance
Scan 441 (7.611 min): FR.D\data.ms
111.0
95.0
55.1
142.0
68.0
81.0
127.0
m/z-->

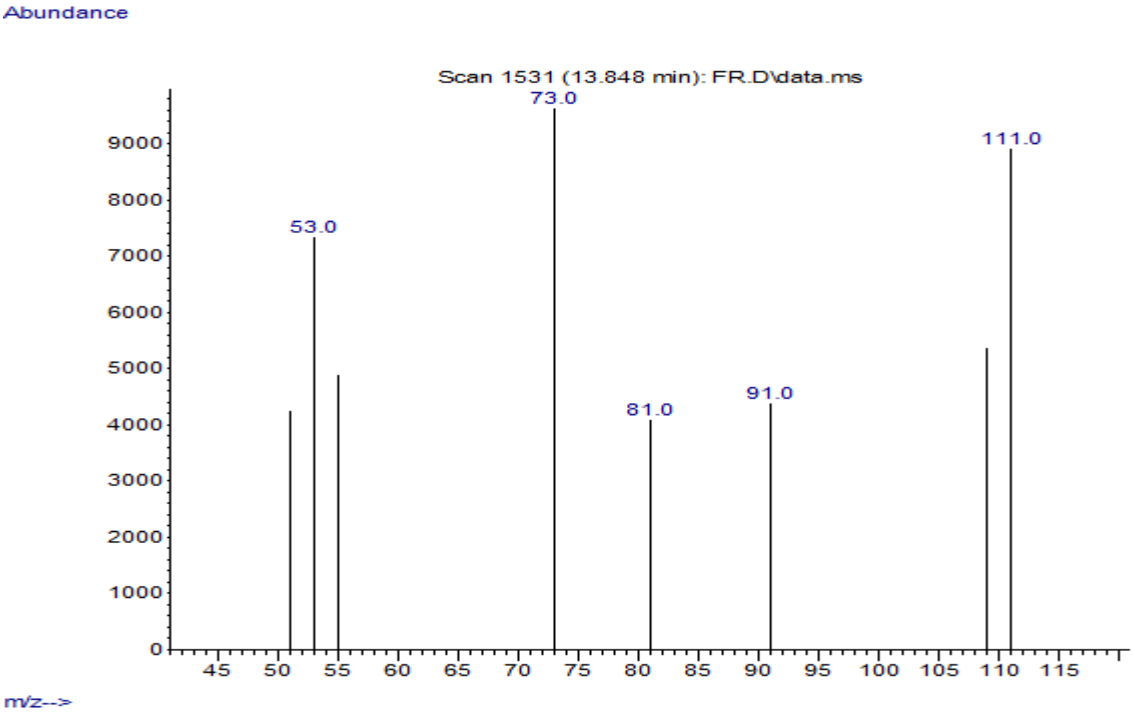




Plate A-1: Experimental set up during the research work



Plate A-II: Experimental set up and laboratory exercise for rotary evaporator during the research work.



Plate A-III: Mr. Oguche J.E carrying out laboratory exercise for this research.