ADDIS ABABA UNIVERSITY

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SCHOOL OF CHEMICAL AND BIO ENGINEERING

SYNTHESIS AND CHARACTERIZATION OF FURFURAL FROM MICROALGAE

A thesis submitted to Addis Ababa Institute of Technology, School of Chemical and Bio Engineering in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering (Process Engineering)

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This is to certify that the thesis prepared by Yeshidagna Nigussie, entitled: ***"Synthesis and characterization of furfural from microalgae"*** and submitted in partial fulfillment of the requirements for the degree of Masters of Science in Chemical Engineering (Process Engineering) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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I certify that this research work titled "**Synthesis and characterization of furfural from microalgae**" is my own work. The work has not been presented elsewhere for assessment. Where material has been used from other sources it has been properly acknowledged / referred.

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ABSTRACT

This research has mainly focused on the synthesis of furfural from microalgae biomass and optimizing the process parameters using Response Surface Methodology (RSM). The raw material microalgae were characterized for proximate and biochemical compositions and the HPLC analysis of the acid hydrolyzed sample of algae shows sufficient amount of pentose's, particularly xylose with concentration of 12.89g/100mL, which indicates its abundance for furfural synthesis. Furfural production was carried out by hydrolysis of algal biomass with dilute Sulfuric acid. NaCl has been used as pentosane conversion enhancer and subsequent dehydration of pentoses to furfural occurred in the process at relatively low temperature. Response surface methodology, Central Composite experimental design, was applied to investigate and find optimum furfural synthesis parameters. Reaction temperature (110-160 ℃), Acid concentration (0.1M-1.5M) and Time (30-90 min) has been selected as the major study parameters. Furfural yield has been examined as experimental response. A total of 20 experiments were conducted, besides the proximate and compositional analysis of the raw material. The important synthesis parameters which affect the yield of furfural were investigated using the analysis of variance (ANOVA). Reaction temperature was found to be the most significant parameter which has a significant effect on the response. 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 0.9973 and model F-value of 409.63. The model was found to be significant as its Predicted R^2 of 0.9822 was in close agreement with the Adjusted R^2 of 0.9949. The individual and interaction effect of the selected parameters on the yield of furfural was studied and optimum conditions were established. A temperature of 150 ℃, an Acid concentration of 1.5 M and reaction time of 45 min were found to be the optimum conditions and the validation experiment resulted in optimum furfural yield of 46.37% with a 2.6% deviation from the optimum value determined by the design expert software. FTIR result confirm the presence of conjugated carbonyl groups at a band intensity of 1670 cm-1 and aldehyde functional groups at wave length of 2816 cm^{-1} and 2850 cm^{-1}. Also the HPLC analysis of the product confirms the presence of furfural. Generally, the results found in this study showed that viability of microalgae biomass as an alternative option for the production of furfural.

Keywords: Dehydration, Furfural, Hydrolysis, Microalgae, Pentose, Xylose

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CCD	Central Composite Design
DOE	Design of Experiment
FA	Furfuryl Alcohol
FC	Fixed Carbon
FTIR	Fourier Transform Infrared Spectroscopy
HMF	5-Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
LAP	Laboratory Analytical Procedures
MF	Methylfuran
MTHF	Methyltetrahydrofuran
NREL	National Renewable Energy Laboratory
RSM	Response Surface Methodology
THF	Tetrahydrofuran
THFA	Tetrahydrofurfuryl alcohol

1. Introduction

1.1 Background of the study

In recent years, the production of bio-based chemicals produced from inexhaustible renewable biomass resources has gained worldwide attention due to the environmental pollutions and problems associated with the exploitation of fossil resources. Renewable biomass, in bio-based economy principle, is considered as the primary essential feedstock. Compared to fossil resources, biomass is over-functionalized with hydroxyl groups and hence has a high oxygen content. Understanding the way to convert these molecules into high-value and high-quality add chemicals is important as they can substitute their fossil-based equivalents. These bio-based chemicals and materials are synthesized from feedstocks rich in carbohydrates and starch. (Sweygers et al., 2020). Biomass, a renewable non-fossil carbon energy source, is regarded as an ideal alternative to traditional fossil resources because it is environmental-friendly and abundant. In recent decades, great interests have been devoted to produce bio-fuels and bio-chemicals using non-edible lignocellulosic and algal biomass, which is abundant in agricultural residues and waste streams. The utilization of lignocellulosic and algal biomass avoids the food-versus-fuel debate and can potentially significantly reduce CO_2 emissions. It is one of the most promising options for the green and sustainable production of fuels and chemicals. Lignocellulosic and algal biomass has drawn a lot of attention because of their high carbohydrate and lipid content. Lignocellulosic biomass is any organic and natural matter that's available in a renewable origin which incorporates energy crops, agricultural residues, aquatic plants, wood and wood residues as well as different waste materials.(Maity, 2015) Lignocellulosic biomass mainly contains cellulose, hemicellulose and lignin, which comprise 40-50%, 25-35% and 15-20% of lignocellulose, respectively. The main approaches for the conversion of lignocellulosic biomass are gasification, pyrolysis and hydrolysis.(Li et.al., 2016). The acid-catalyzed hydrolysis is a more complicated process that deconstructs lignocellulose into a series of C5-C6 sugars, which can be further rearranged with partial removal of their oxygen atoms to form various renewable platform compounds such as furfural, 5-hydroxymethylfurfural (HMF), lactic acid and levulinic acid.

Furfural, a chemical similar to 5-hydroxymethylfurfural, is one of the furan subordinate derivatives made out of the hemicellulosic fraction of lignocellulosic, is a promising renewable platform

compound derived from lignocellulosic biomass, which can be further converted to bio-chemicals and bio-fuels. Furfural has been chosen as one of the top 30 biomass-derived platform compounds by the U.S. Department of Energy on the basis of several indicators such as the raw material, estimated processing cost, technical complexity and market potentials. Furfural is an organic compound with the formula C_4H_3OCHO. It is a colorless liquid, although industrial and commercial samples are often reddish brown. It comprises of an aldehyde group attached to the 2-position of furan. It is a product of the dehydration of pentose sugars, as occur in a variety of agricultural byproducts, including oats, corncobs, wheat bran, and sawdust. The name furfural originates from the Latin word furfur, meaning bran, pertaining to its usual source. Beside from ethanol, acetic acid and sugar it is one of the oldest known renewable chemicals.

Furfural is produced from biomass that contain pentosans, which are aldose sugars, composed of small rings formed from short five-member chains, that constitute a class of complex carbohydrates, found in cellulose of numerous woody plants such as corn cobs, bagasse, rice straw and oat hulls etc.(Win, 2005). Furfural is a clear, colorless or reddish-brown liquid with a characteristic 'almond-benzaldehyde' odor. The molecular formula is $C_5H_4O_2$. Its synonyms are: 2-furancarboxaldehyde, furaldehyde, 2-furanaldehyde, 2-furfuraldehyde, fural, furfurol. When exposed to sunlight in the presence of oxygen auto-oxidation occurs and the colorless furfural darkens to a dark red/brown color (Brenkem Consultants Asia, 2011.)

In theory, any material containing pentosans can be utilized for the production of furfural. Technically furfural is produced by acid hydrolysis of the pentosan contained in biomass. Low cost lignocellulosic biomass that can be found as residue or waste material from various segments, like corn stover, bagasse, verge cuttings, food waste and sawdust, is targeted primarily. However, when the bio-based economy gains traction on a world scale, the vast demand of feedstock would force the cultivation of dedicated crops for bio-fuel and bio-based chemicals production, which should be able to grow on polluted, infertile and non-arable land to limit (or even avoid) the competition with food and feed crops.(Sweygers et al., 2020)

Despite the very fact that the preferred feedstock for furfural production are agricultural residues and farming deposits due to their homogeneity and regular availability in large quantities from food processing plants, Furfural can be solely produced from algal biomass by dehydrating

pentose. Algae-based products and fuels have recently gained growing interest for energy, food, and pharmaceutical industries.(Chisti, 2008) Most algae are equipped for 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 & Mayfield, (2012) that microalgae offer several advantages over terrestrial plants as a source of transportation biofuels and bio chemicals, including high growth rates, high carbohydrate and lipid content, the potential and flexibility to grow large cultures on non-agricultural land, and the ability to rapidly improve on strains genetically and produce co-products.

Algae are very important non-vascular plants with high species diversity having many research applications, including biofuel sources, adsorption of heavy metals, used as ingredients in health supplements, food and so on.(FAO, 2020.) The utilization 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 area of research.(Fernando et.al., 2006) The idea of using algae in biorefineries is a promising and financially feasible option for the production of bioproduct, such as biodiesel and furfural. It is crucial during this period that institutional frameworks (i.e. policies) to promote development, commercialization and stimulate the evolution of the algal biomass industries as a source of renewable fuels and 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 vascular plants.(Spolaore et.al., 2006)

1.2 Statement of the problem

Mankind is now faced with the challenges of the expanding demand for fuels and chemicals driven by worldwide population growth, diminishing fossil resources, energy and chemical products guarantee due to political and health issues, and environmental problems caused by CO_2 emissions and the resulting global warming. Different alternative forms of renewable energy resources have been explored to develop sustainable processes that eliminate problems associated with conventional petrochemical based products. Biomasses such as corn, sugarcane and soy, or waste materials, such as forestry, agricultural and industrial wastes, have already been used to produce biofuels or biochemical (Kim & Dale, 2004) However, most of these resources require water and utilize land to grow. This may lead to deforestation and compete directly with food production.

Aquatic biomass comprises a diverse group of organisms ranging from macroalgae to microalgae. Currently, algae are receiving increasing interest as source of high added value products, such as biochemicals and biopharmaceutics and biofuels such as biogas or bioethanol (D'Este, 2017).

Beside the rising concerns regarding environmental effects the great demand for furfural in the global market is another key driving factor for production of furfural. The global furfural market demand was 300 kilotons in 2013. It is projected to expand at a rate of 11.9% from 2014 to 2020. 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 (Anonymous, 2016).

The chemical industry sector in Ethiopia is though, at an infant stage and needs to be developed to the level demanded to support the rapidly growing economy of the country. Many chemical inputs and chemical products are still imported with a huge sum of foreign currency from abroad for a national manufacturing processes and direct consumption. Although, there is no particular data regarding the demand of furfural in Ethiopia, the growth of major end-use industries such as chemicals, pharmaceuticals, and flavoring intermediates is expected to increase the demand of furfural, which is a versatile chemical platform, for the emerging chemical industries in the country.

Apart from these effects, recent researches regarding furfural production has been focused on utilization of lignocellulosic materials (from agricultural biomass and food processing plant wastes), the use of microalgae for furfural production have not been broadly reported on the open literature. Consequently, there is underutilization of microalgae biomass for the production of furfural. Moreover, most of the previous researchers have focused on searching novel process and green catalyst by using reagent grade xylose and xylan instead of raw biomass for simplification of analysis. However, optimization of process parameters such as reaction temperature, acid concentration and reaction time to obtain high yield of furfural from the raw biomass have not been widely carried out. Therefore, in this study the optimization of process parameters from the raw biomass has been incorporated. Generally, this study proposed the use of underutilized non edible raw material that is microalgae for the production of highly demanded and versatile biochemical, Furfural, in efficient way.

1.3 Research justification

Any material containing any amount of pentose (five carbon) sugars arabinose and xylose can serve as a raw material for furfural production (Zeitsch, 2000). Microalgae are microscopic photosynthetic organisms which has a similar photosynthetic mechanism to land-based plants. There is also cell wall composition similarity to vascular plants, which makes them suitable for production of biochemical including furfural. The cell walls of microalgae's consisted of carbohydrates, lipids, proteins and other constitutes. Monosaccharide analysis of isolated cell walls of microalgae's revealed the presence of sugars like glucose**,** xylose and galactose etc. Harnessing this monosaccharide sugars effectively is useful in producing bio based chemicals. When it comes to the potential to produce fuel, no feedstock can match algae in terms of quantity or diversity(Gouveia, 2011).

Algae's also offers several benefits. The main advantages of microalgae are (Gouveia, 2011); Chisti, 2008; Rodolfi et al., 2009)

- A higher photon conversion efficiency (approximately 3–8% against 0.5% for terrestrial plants), which represents higher biomass yields per hectare) and grow at high rates (e.g. 1–3 doublings/day)
- Ability to grow in a liquid medium, with better handling, and can utilize salt and waste water streams (saline/brackish water/coastal seawater), thereby reducing freshwater use
- It utilizes nitrogen and phosphorous from a variety of wastewater sources (e.g. agricultural run-off, concentrated animal feed operations and industrial and municipal wastewaters) providing the additional benefit of wastewater bioremediation
- It uses marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands) and thereby does not compete with arable land for food production
- Production is not seasonal and can be harvested batch-wise nearly all-year-round
- Cultures can be induced to produce a high concentration of feedstock (oil, starch, biomass)
- The conversion of light to chemical energy can be responsible for a wide range of fuel synthesis: protons and electrons (for biohydrogen), sugars and starch (for bioethanol and

others), oils (for biodiesel) and biomass (for biomethane), via biochemical, thermochemical, chemical and direct combustion processes

- They produce value-added co-products or by-products (e.g. proteins, polysaccharides, pigments, biopolymers, animal feed, fertilizers…).
- Many species of algae can be genetically induced to produce high concentrations of a chosen, valuable compound, such as proteins, carbohydrates, lipids and pigments depending up on growth conditions.
- Small amount or Lack of lignin enables easier thermo chemical and bio chemical conversion to fuels and chemicals when compared to woody biomass.

1.4 Objectives

1.4.1 General objective

The general objective of this study was to synthesize and characterize furfural from microalgae.

1.4.2 Specific objectives

The specific objectives are to:

- Identify the species of the microalgae biomass.
- Characterize the algal biomass for production of furfural.
- Synthesize furfural from microalgae biomass.
- Investigate the individual and interaction effect of reaction temperature, concentration of acid and reaction time in the yield of furfural using response surface methodology.
- Determine the optimum operating parameters using response surface methodology.
- Characterize the physicochemical properties of furfural produced.

1.5 Scope of the study

This research was an experimental study to show the possibility of synthesizing furfural using microalgae as a raw material. The thesis work generally covers microalgae characterization, production of furfural using sulfuric acid as catalyst and product characterization. Standard procedures and test methods are used throughout the process.

1.6 Significance of the study

The study has great significance in terms of assuring the production of an alternative form of top platform chemical which has a versatile application in plastics, pharmaceutical, agrochemical and other industries. The study has a significance in contributing to the existing knowledge by introducing underutilized biomass for production of furfural. The algal biomass that can be used for the production of furfural is a nonedible feedstock that can be cultivated in wastewater ponds and unsuitable lands for food crops and can be harvested within few days. This study is a means of converting underutilized biomass to wealth by utilizing cheap raw material and easy process within cost effective methods. So the study supports the development of sustainable utilization of renewable materials for production of valuable products and also the study used to promote the usage of microalgae for synthesis of bio based product.

2. Literature Review

2.1 Algae: - General overview

Algae are a highly diverse group of organisms with important functions in aquatic habitats. They are defined as photoautotrophic organisms with unicellular reproductive structures, chlorophyll *a* and a thallus that is not differentiated into roots, stems and leaves. As per to various taxonomic classifications, the number of algal divisions ranges from 4 to 13, with as many as 24 classes, and about 30,000 species. The term "algae" includes macroalgae, or seaweeds, and microalgae. Although there is no recognized definition for microalgae, the term generally refers to those algae that are too small to be seen clearly with the naked eye, with a size ranging from 1 to 50 μm.

The word 'microalgae' in applied phycology refers to Oxygenic photosynthetic bacteria (i.e. cyanobacteria) and microscopic algae. The cell structure consists of a distinction between microalgae and prokaryotic bacteria. Eukaryote species have a genuine membrane-bound nucleus, which has a real membrane-bound nucleus. The bulk of the genome is spread over a collection of chromosomes and the nucleolus. On the contrary, prokaryotic Cyanobacteria lack a membrane-bound nucleus, mitochondria and any other membrane-bound organelles (Ashok et.al., 2015).

2.2 Structure of algae

2.2.1 Cell wall

The cell wall of eukaryotic microalgae is generally comprised of a microfibrillar layer of cellulose, which may be surrounded by an amorphous layer. A laminated polysaccharide cover may be present in the outside of the outer amorphous layer, The cell wall is concealed by the Golgi apparatus and its composition can be more or less complex, containing: 25-35% cellulose, 15-30% hemicellulose, 35% pectin and 5-10% glycoproteins (Wang, 1995). The cell wall has a significant role in harsh environmental conditions and is a key determinant of algal resistance to bacterial lysis (Cole, 2003). Some species lack cell wall, while others like *Staurastrum sp.* contain a lignin-like substance that account for nearly 6% of the cell wall weight and increases with the age of the culture (Cole, 2003). Algaenan and sporopollenin are two polymers often associated with the cell wall resistance. The cell wall composition of various microalgae is summarized in Table 2.1.

2.2.2 Cell composition

The comparative amount of carbohydrates, proteins and lipids that microalgae contain when they are grown under optimal conditions is similar for different species. The cell composition of a single species can, however, vary depending on the growth factors, substantially. Chlorella sp., for instance, *Botryococcus braunii* and *Dunaliella salina* have the following biochemical composition: 20-40% carbohydrate, 30-50% proteins and 8-15% lipids under suitable environmental conditions.(Hu et al., 2008) However, under unsuitable environmental conditions these species can accumulate up to 80% fatty acids, 80% hydrocarbons and 40% glycerol. The dynamic cell composition and cellular metabolism of microalgae's is influenced by nutrient concentration, salinity and environmental conditions like light and temperature. Lipids accumulation in many microalgae species such as such as *Chlorella sp*. and *Nannochloropsis sp* is enhanced by nitrogen starvation strategy (Hu et al., 2008). The main storage product (e.g. lipids and sugars) for different microalgae classes is reported in Table 2.1.

2.3 Classification of algae

Traditionally, the classification of microalgae has been by their color given by dominant pigments (green, red, blue or golden-brown algae). Nowadays, microalgae classification schemes are based on cell wall constituents, kind of pigments, and chemical nature of storage products. The occurrence of flagellate cells, the structure of flagella, the scheme and path of nuclear and cell division, the presence of endoplasmic reticulum envelope around the chloroplast and the possible connection between the endoplasmic reticulum and nuclear membrane are considered as an additional criterion for the classification. (Lee, 1989.) separated algal divisions into four groups according to the number of additional membranes around the chloroplast envelope.

Table 2.1 Classes of Eukaryotic Microalgae and their main characteristics (Hu et al., 2008)

Classes	Genus	Storage product	Cell wall	Number of membranes	Habit
Rhodophyta	*Gracilaria* *Gelidium* *Chondrus*	Starch	Cellulose Galactan Xilan $CaCO_3$	2	Freshwater Seawater Blackish water
Chlorophyta	*Chlorella* *Scenedesmus* *Spirogyra* *Ulva* *Haematococcus* *Botrycoccus* *Braunii*	Sugars Starch Fructan	Cellulose Mannan Proteins $CaCO_3$(some species naked)	2	Freshwater Seawater Blackish water Soil
Dinophyta (Pyrrophyceae)	*Gymnodinium* *Ceratium* *Alexandrium*	Starch Glycan Lipids	Absent or cellulose	3	Freshwater Seawater Blackish water
Chrysophyta	*Dinobryon* *Surirella*	Chrysolaminarin Lipids	Often absent or Cellulose Silica $CaCO_3$	4	Mainly fresh water Seawater Blackish water Soil
Bacillariophyta	*Bacillaria*	Chrysolaminarin Lipids	Silica	4	Freshwater Seawater Blackish water Soil
Eustigmatophyta	*Euglena* *Phacus* *Nannochloropsis*	Paramylon Lipids Sugars Eicosapentaenoic acid	Absent or Polysaccharide	4	Freshwater Seawater Blackish water Soil

The first group includes prokaryotic algae. The other groups are classified with respect to the evolution of the chloroplast, and include eukaryotic algae. The second group (which includes Rhodophyta and Chlorophyta) has the chloroplast surrounded by only two chloroplast membranes; while the third and fourth groups have the chloroplast surrounded by one (Dinophyta) or two (*Chrysophyta*, *Bacillariophyta*, *Eustigmatophyta* and *Phaeophyta*) additional endoplasmic reticulum membranes, respectively.

2.4 Applications of algae

The interest in microalgae lies in their potential utilization to produce biomass for food, feed, fine chemicals and biofuels. In recent years, algae are mainly generated in the human food and animal feed industries on a large scale in order to get high-value ingredients, like omega-3 and carotenoids, for cosmetics and pharmaceutical goods. Nevertheless, new attempts have been made in the last 10 years to do researches on areas of biofuels from algae. While there was disbelief and criticism about the revival of algal biofuels research, the continuous increase in global demand for oil, and environmental concerns associated with fossil fuels have turned attention back to the topic. The first study of algae as a substrate was for the production of biofuels in the 1950s. However, in the 1970s, during the oil crisis, there was great effort to promote this new source of energy has been intensively prompted. The key countries which supported research on algal biofuels were United States and Japan. In the last decade, great advances in the cultivation of microalgae in raceway ponds and biodiesel processing was carried out (Wiley et.al., 2011).

2.4.1 Use as Bio-fertilizer

Microalgae are used as bio-fertilizers and soil conditioners in agricultural field. Many cyanobacteria are capable of fixing atmospheric nitrogen and are used as bio-fertilizers in an efficient way. Cyanobacteria play a significant role in of soil fertility maintenance and build-up, thus increasing plant growth and yield as a natural bio-fertilizes. In plant cultivation, the agricultural value of cyanobacteria is directly related to their ability to fix nitrogen and other beneficial effects on plants and soil. Nitrogen is the second limiting factor next to water for plant growth and the deficiency of this element is met by fertilizers. With the use of Blue green algae (BGA), in addition to the increase in yield and saving of fertilizer nitrogen, the soil physico-chemical properties also improved. Gradual accumulation of residual soil nitrogen and carbon, soil

pH change, and electrical conductivity were observed. The consistency and quality of the grain has increased in terms of protein content. Blue green algae belonging to the *Nostoc, Anabaena, Tolypothrix* and *Aulosira* genera fix atmospheric nitrogen and are used as paddy crop inoculants under both upland and low land crop growth conditions.

2.4.2 Use in pharmaceuticals

Algal species are a rich source of new and biologically active metabolites. These metabolites may be bioactive potential metabolites, which have enormous contribution in the pharmaceutical industry. The presence of bioactive substances in microalgae is due to the inhibitory interaction between the co-occurring producers and competitors in the aquatic habitat. Microalgae contain a variety of bioactive compounds that can be harnessed for commercial applications. They are a great source of valued add compounds and proteins with nutritional importance for pharmaceutical industries. As a natural source of bioactive molecules, microalgae have a considerable attraction, because they have the potential in culture to provide bioactive compounds, which are difficult to produce by chemical synthesis. Antibacterial activity in vitro against gram positive and gram negative bacteria has been proved by using cell extracts from different unicellular algae. (e.g. *Chlorella vulgaris*). It's also been reported that a wide range of in vitro active antifungal activities are obtained from extracts of green algae, diatoms and dinoflagellates. Microalgae, for instance *Ochromonas sp., Prymnesium parvum* and variety of algae produce toxins which will have significant pharmaceutical applications. (Borowitzka, 1995).

2.4.3 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. With photosynthesis; microalgae absorb light to produce carbohydrates, lipids and proteins. Its efficiency is much better than crops plants, and the capacity of producing oil is also better than the crops plants (Chisti, 2008). 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.

2.4.4 Pollution control and water treatment

Algae are used in wastewater treatment plants. They 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. 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 CO2 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., 2014). 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.

2.5 Cultivation of microalgae

The cultivation of microalgae is one of the most important aspects of algal biofuels and products. Numerous types of algal cultivation systems are in practice. However, most of them are mainly based either on open ponds or raceways and closed bioreactor systems.

2.5.1 Open pond culture systems

Open pond systems are the first and most common oldest system for mass cultivation of micro algae. The open pond is usually between 1 and 100 cm deep, from about one acre to several acres in size. Major pond systems are circular ponds, shallow big ponds, and raceway ponds. Borowitzka suggested that these systems are depending upon types of algal species, climatic conditions, and the cost of lands and water. The most popular type is the paddle-wheel raceway pond, because its shape resembles a race track and the liquid is circulated around the pond by a paddle wheel (Chisti, 2008). Raceway ponds are utilized mostly for algal cultivation and wastewater treatment. The major advantage of open ponds is that they are easier to construct and operate than closed cultivation systems. However, major drawbacks include high land requirements, poor light utilization by the cells, contamination issues, diffusion of CO_2 to the atmosphere, and water loss

due to evaporation. Due to such drawbacks, closed photobioreactors are preferred for cultivation of microalgae over the open pond.

2.5.2 Closed photobioreactors

Closed photo bioreactors (PBR) are very flexible structures and that can be placed both indoors and outdoors with synthetic light and natural light, respectively. these structures have triumph over the major issues related to open-pond cultures. mainly photoautotrophic algae are cultivated in open structures, whereas closed cultivation structures are used for each photoautotrophic and heterotrophic cultivations. The closed photo bioreactors are tubular transparent vessels of various shape and sizes. The most famous of these systems are tubular PBR, helical PBR, airlift PBR, and flat panel PBR (flat plate). however, because of more advantages, tubular PBR is widely used in this discipline and those can be run both vertically or horizontally. It has some of clean transparent tubes, composed of either glass or plastic measuring 10 cm or less in diameter, which allows in for sufficient mild penetration. furthermore, algal biomass is avoided from settling by using maintaining highly turbulent go with the flow within the reactor with either a mechanical pump or an airlift pump (Chisti, 2008).

In algae cultivations, the value of the nutrient media is one of the fundamental hurdles for economic biomass production. therefore, efforts are being made to substitute the more expensive nutrient media with relatively less expensive nutrient assets. amongst diverse alternatives in recent times the usage of diverse sorts of wastewater is in practice which serves dual advantages of biomass production in conjunction with waste water remedies.

2.5.3 Culture using deep sea water

Deep-sea water (DSW) utilization has additionally received massive interest from beyond few decades due to its hugely to be had quantity and capability for recycling energy. DSW incorporates traces of various elements and nutrients that could be stimulating the production of precise components/metabolites within the microalgae (Chen et.al., 2011). it is reported that the oil-rich microalga *Chlorella sorokiniana* CY1 cultured in 50% DSW in BG-11 medium to decide its boom and oil manufacturing and this subculture technique achieved a comparatively better biomass (2.4 g/L) alongside a better oil yield of 176.6 mg/L/day. numerous other research additionally validated

that with minor changes of composition or the addition of a comparatively smaller amount of vitamins, DSW will be used for excessive biomass yield of diverse marine microalgae species.

2.6 Hemicellulose

Hemicelluloses are a group of polysaccharides present in the primary and secondary cell walls of all terrestrial and fresh water plants, and algae. When extractives (compounds which are soluble in cold water or in neutral organic solvents) and lignin are removed from wood, it yields a fibrous product termed holocellulose, which represents the sum total of cellulose and other polysaccharides. The later one is the hemicelluloses (polyoses). Hemicelluloses, highly branched heterogeneous polysaccharides, are the second most abundant natural polysaccharide after cellulose. Hemicelluloses rank second to cellulose in abundance in wood and cereal straws, comprising roughly one-fourth to one-third of most plant materials, and this amount will vary according to the particular plant species. They are composed of a wide variety of sugars such as, pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose, and rhamnose), and sugar acids (glucuronic acid, 4-O-methyl glucuronic acid, and galacturonic acid) (Sjostrom, 1993). The principal component of hemicelluloses in softwood is glucomannan, while in hardwood and herbaceous plants such as grasses and straw is xylan, which contains xylose and uronic acid (Fengel & Wegener, 1989). Hemicelluloses are linked to cellulose via intermolecular hydrogen bonding and van der Waals forces, and to lignin via cinnamate acid ester linkages, and to other hemicelluloses through covalent and hydrogen bonds (Sjostrom, 1993). The degree of polymerization in hemicellulose is lower than cellulose, having an average of about 100–200 and the molecules are highly branched (Rowell, 2013). The branching nature in hemicellulose arises beyond the linkage that exist the β -(1-4) glyocsidic bond there are also β-(1-2), β-(1-3) and β-(1-6), which varies depending on the hemicellulose components (Fengel & Wegener, 1989) The uronic acids in the hemicellulose components structure are always found in branching side. Due to the combination of several sugar units and its amorphous structure, the hemicellulose is more soluble in water and therefore easily degraded by dilute acid or base than cellulose. They are often regarded as the most thermo-chemically sensitive components among the key constitutions of lignocellulosic biomass. In the lignocellulosic materials, the cellulose and lignin are intimately linked, because hemicellulose acts as matrix of the cell wall component (Fengel & Wegener,

1989). The amount of hemicelluloses in lignocellulosic materials can range from 20 to 40% of the dry biomass weight depending on the type of species (Rowell, 2013).

The hemicelluloses are potentially very useful. Studies on utilization of hemicelluloses from biomass have demonstrated to be a potential fermentation feedstock in production of ethanol, acetone, butanol, and xylitol. The current uses of xylan on an industrial scale involve their conversion to xylose, xylitol and furfural. For instance, xylitol is produced by hydrolysis of xylan, crystallization of xylose, and hydrogenation. This has been tested in a variety of food products. In addition, some important applications for xylans have been discovered. These include uses in chiral separations, cholesterol depressant, table disintegrant and dietary fibre. Evidently, hemicellulosic biopolymers have a very wide variety of direct food and non-food applications. In particular, some hemicelluloses from higher plants and herbs represent a potential source of pharmacologically active polysaccharides. Glucuronic acid-containing (acidic) xylans isolated from annual plant residues such as bamboo leaves, corn stalks, wheat straw as well as hardwood have been reported to inhibit markedly the growth of sarcoma-180 and other tumors, probably due to the indirect stimulation of the non-specific immunological host defense. Arabino-(glucurono) xylans isolated from Echinacea purpurea, Eupatorium perfoliatum have been reported to have immune stimulating effects (Paul Gatenholm, 2003). Similarly, 4-0- methylglucuronoxylan from Chamormlla recutita and the acetic, highly branched heteroxylan from plantago species have been found to have anti-inflammatory activity.

2.6.1 Structure

Like most polysaccharides from plant origin hemicelluloses display a large poly diversity and poly molecularity. This corresponds to their being present in a variety of plant species and to their distribution in several types of tissues and cells. All straw hemicelluloses are characterized by a β l,4-linked-Dxylopyranosyl main chain which carries a variable number of neutral or uremic monosaccharide substituents. For example, the hemicellulosic fraction, isolated with 0.5 M NaOH at 37°C for 2 h from wheat straw, was confirmed to be a β (1-4) xylan with side chains consisting of L-arabinofuranosyl and Dxylopyranosyl groups attached in position 3, and D glucopyranosyluronic acid or 4-O-Methyl-D-glucopyranosyluronic acid group attached at position 2. For every 15 D-xylopyranosyl residues in the main chain, there was one L - arabinofiiranosyl

group. For 19 such D-xylopyranosyl residues, there was one D-xylopyranosyl group, and for -26 such D-xylopyranosyl residues, there was one uronic acid unit (Fig. 2.1) (Sun et.al., 1996 : Sun, Sun, & Tomkinson, 2003).

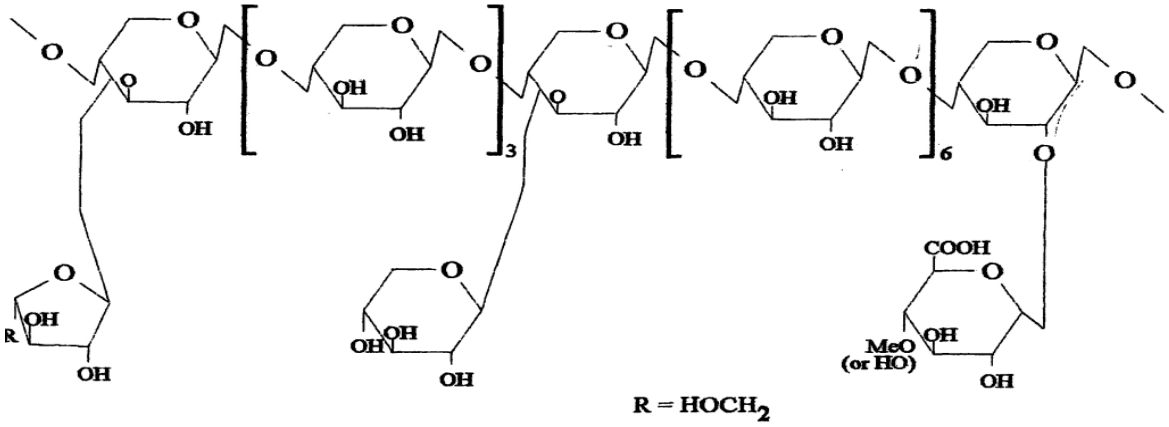

Figure 2-1 A structure of hemicelluloses (wheat straw) (Paul Gatenholm, 2003)

2.6.2 Isolation of hemicellulose

Hemicelluloses are relatively easily hydrolyzed by acids to their monomeric components consisting of D-glucose, D-mannose, D-xylose, L-arabinose, and small amounts of L-rhamnose (Sjostrom, 1993) In fact, hemicelluloses Isolation requires hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by an extraction into aqueous media. However, the liberation of the xylan component from the cell wall is hindered by the presence of lignin network as well as ester and ether lignin-hemicellulose linkages. In addition, hydrogen bonds between individual polysaccharide cell wall components may impede isolation of the hemicellulosic component. For quantitative isolation of hemicelluloses, the material must first be pre-extracted, preferably with ethanol-toluene, in order to eliminate all lipophilic and hydrophilic non-structural components. In the second step, the material is delignified and then the obtained holocellulose is extracted with alkali. Delignification is usually performed with chlorine, chlorine dioxide or peroxyacetic acid (Paul Gatenholm, 2003). There is no completely satisfactory method for preparation of holocellulose. Some loss of the hydroxycinnamic acid appendix and degradation of the hemicelluloses are obviously inevitable. In order to avoid saponification of ester linkages, the holocellulose is extracted in succession with dimethylsulphoxide and water. As the yield obtained

using this procedure seldom exceeds 50%, most commercial hemicelluloses are usually exited with alkali. In the case of the product obtained, except for the fact that acetyl, feruloyl-, and p-coumaroyl appendices have been saponified, is quite similar to the native polysaccharide. Nowadays, the hazardous and expensive $NaClO_2$-dehgnification step is replaced aqueous alcohol treatment procedure, which produce polysaccharide rich in xylan fractions contaminated with lignin and degradation products of the cell wall components. However, the yield of the prepared hemicelluloses was lower in comparison to those obtained from partially delignified raw materials. Another potential method for isolating of hemicelluloses has been discovered by Gabrielii et al., in which the raw material was extracted by an alkali followed by hydrogen peroxide treatment and ultrafiltration. Spray drying were used to recover the hemicelluloses. Another possible technique in the industrial separation of polymers from cereal straw and wood samples is the isolation of hemicelluloses by steam explosion. (Glasser et.al., 2000). The use of an extruder-type twin-screw reactor makes the extraction more feasible. The extractability of hemicelluloses from annual plants is easier than that of wood hemicelluloses due to the lower amounts and different structure of lignin. It can be affected by the alkali type and isolation conditions and improved by a multistep mechanical-chemical treatment.

Ultrasound-assisted extraction is another method of hemicellulose extraction. In the absence of ultrasound-assisted extraction, the hemicellulose obtained by of ultrasound-assisted extraction appears to be more linear and less acidic than hemicellulose extracted by alkali. In addition, hemicellulose preparation obtained by ultrasound-assisted extraction lower content of associative lignin content, but higher molecular weight and slightly higher thermal stability than hemicelluloses isolated by alkali without ultrasonic irradiation. seemed more linear and less acidic than that of the hemicelluloses extracted by alkali in the absence of ultrasonic irradiation.

2.7 Furfural

Furfural (Figure 2.2) is a heterocyclic aldehyde, produced by the dehydration of xylose, a monosaccharide often found in large quantities in the hemicellulose fraction of biomass. 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 has a broad spectrum of industrial applications, such as the production of plastics, pharmaceuticals, agro-chemical products, and non-petroleum-derived chemicals (Mamman et al., 2008). Furfural is not produced from petroleum or fossil fuels, therefore current production methods from biomass do not displace production from petroleum. However, recent research has reported a furfural-to-distillate fuel pathway that could potentially make furfural derivatives a drop in replacement for petroleum-derived distillate fuels (jet and diesel).

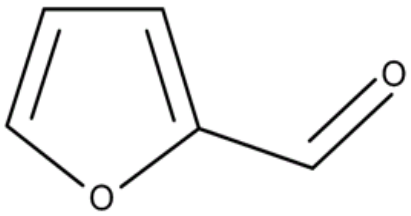

Figure 2-2 The chemical structure of furfural ($C_5H_4O_2$) ("Furfural CAS 98-01-1 n.d")

2.7.1 Applications of furfural

Of the many actual and potential applications of furfural, the treatment is limited to a few fields where furfural is used as it is. It is merely noted that 65 percent of all furfural produced is converted to furfuryl alcohol (Zeitsch, 2000). The applications of furfural as it is and cases where furfural is employed as an input material for the synthesis of other chemicals are discussed in this here under.

2.7.1.1 Furfural as an extractant

The application of furfural as an extractant relies on a phenomenon called "intermolecular conjugation". This means that when molecules with conjugated double bonds like furfural come in contact with other molecules containing double bonds, they form an enlarged conjugated covalent bond system, and this enlargement liberates energy analogous to intramolecular bond formation. Consequently, furfural hooks on to molecules containing double bonds, but "ignores" molecules without double bonds. For this reason, furfural is used

(a) to remove aromatics from lubricating oils to improve the viscosity/temperature relationship, (b) to remove aromatics from diesel fuels to improve the ignition characteristics, and (c) to obtain

unsaturated compounds (compounds with double bonds) from vegetable oils such as soybean oil to make "drying oils" suitable for paints and varnishes as only the double bond compounds are capable of "drying" by oxidation with air to form cross-linked polymers. In the applications (a) and (b), the desired product (freed of aromatics) is the raffinate, while in application (c) the desired product (rich in unsaturated compounds) is obtained from the extract. Of course, in liquid/liquid extraction, the extractant must form two liquid phases with the feed stock. With lubricating oil, diesel fuel, and soybean oil, furfural is capable of satisfying this condition as its oxygen atoms make it a more polar compound.(Zeitsch, 2000)

2.7.1.2 Furfural as a fungicide

Furfural is an exceptionally powerful fungicide. It was found that while even excessive concentrations of formaldehyde (10-15 %) do not prevent the growth of the mold penicillium, one of the most common fungus, as little as 0.5 % furfural is sufficient to totally prevent the growth of mold even under conditions otherwise most favorable. It was once found that furfural is particularly effective in inhibiting the growth of wheat smut (*Tilletis foetens*). This fungus is destroyed when the wheat is soaked for 3 hours in an 0.05 % aqueous solutuion of furfural, whereas with a formaldehyde solution of the same strength a period of 12 hours of soaking is necessary to kill the smut. Most note worthily, as far as seeds are concerned, it was discovered that their treatment with furfural does not diminish their growth and germination power to any significant extent, whereas treatment with the same concentration of formaldehyde proved massively toxic. Wheat can be soaked for 6 hours in an 0.5 % aqueous solution of furfural with a reduction of only 4 % of the germination power of the seeds, whereas the same time of soaking in 0.5 % aqueous formaldehyde destroys the germination power completely. From the treatment of seeds, the application of furfural as a fungicide was extended to growing plants and to wood.(Zeitsch, 2000)

2.7.1.3 Furfural as a nematocide

Plant-parasitic nematodes, also called eelworms, are small cylindrical organisms usually between 0.5 and 3.0 mm long. Nematodes considered agricultural pests include root-knot nematodes which cause serious rankling on the roots of many crops. Infected plants grow poorly and shrivel promptly in warm weather. Worldwide, plant-parasitic nematodes cause an estimated agricultural loss of 60 billion U.S. dollars per annum. (Zeitsch, 2000).

As indicated in various publications, furfural and other aromatic compounds such as benzaldehyde and thymol were found to be very effective nematode control agents. Although they do not kill nematodes, they change the microflora of the soil to a degree that the nematode population diminishes to zero. It was found that these "indirectly acting nematocides" stimulate a rapid development of bacteria antagonistic to nematodes, so that these agents can be said to breed microorganisms capable of killing nematodes in a very biological fashion.

In contrast with "directly acting nematocides" (nematocides working by toxicity), furfural has the following advantages:

(1) For equivalent effect, it costs considerably less.

(2) It is nontoxic for humans. Furfural is present in fruit juices, beer, and bread.

(3) It is safely and easily applicable (as a saturated aqueous solution, 7.9 ml per 100 ml of water at 20 °C).

(4) It is nonsystemic, which means that it is not taken up by the plant to be protected, so that it can be applied till harvest time. This is prohibited for other nematocides.

Furfural is not only nontoxic for humans but it is also harmless for the environment. By contrast, methyl bromide, a widely used nematocide having a boiling point of 4.5 °C is a threat for the ozone layer, and with this being so, according to a directive of the united nations, it had phased out by the year 2002. Considering these all advantages, the application of furfural as a nematocide has the potential of turning into the best outlet for furfural, requiring the construction of many more furfural plants to satisfy the agricultural interest.

2.7.1.4 Furfural as transportation fuel, gasoline additive, lubricant and resin

The aldol condensation of furfural and acetone followed by hydrogenation results in the production of high yields of liquid alkanes which are utilized as transportation fuels (Huber, 2006). Furfural is used as an agent for decolorizing crude wood rosin. It is widely used as a solvent in petroleum refining, lubricants and specialist adhesives. Furfural can be converted by hydrogenation to 2-methylfuran and 2-methyltetrahydrofuran which are used as gasoline additives. Furfural as well as furfuryl alcohol can be used individually or in combination with phenol, acetone or urea to make

resins in the production and manufacture of casting molds, automotive brake linings, abrasive wheels, refectory products of the steel industry, fiberglass and some aircraft components.

2.7.1.5. Furfural in the synthesis of pharmaceuticals, chemicals and biopolymers

Furfural is an essential portion of pharmaceutical building blocks. It is used in the manufacture of furan, an intermediate in the synthesis of pharmaceuticals, agricultural and fine chemicals as well as stabilizers. Furfural, made from biomass has been utilized to feature the synthesis options of petrochemical derived polymers (polyesters) which are used as drop-in alternative to manufacture bio-renewable plastics because of their similarity with their petrochemical equivalents. Bio-renewable polyethylene furanoate is an alternate and green plastic, used for soft drink bottles. Bioplastics are also used extensively for consumer electronics, automotive accessories, packaging, catering products and toys. (Zeitsch, 2000)

Furfural is also used inside the formation of spandex, a synthetic fiber (polyurethane-polyurea copolymer), known for its exceptional elasticity. However, it's strong, but less durable than natural latex, which is its major non-synthetic competitor. Furfural is an efficient lignin crosslinking agent that can replace formaldehyde in timber glues. It's far utilized in formulations for rigid and flexible polyurethanes, which can be used in automotive packaging and furniture industries for use as foams, coatings, adhesives, sealants, etc.

2.7.1.6. Furfural as a jet fuel blendstock

Furfural and its derivatives have the potential to make jet fuel range alkenes and to serve as gasoline blendstock. Furfuryl alcohol was one amongst its components that can be utilized as hypergolic starter fluids that ignite liquid rocket fuels spontaneously in space shuttles. These starter fluids also ignite without oxygen (i.e. in space).(Zeitsch, 2000)

2.7.1.7. Furfural in books

The papers in books comprises hemicelluloses which are the sources for synthesis of furfural. Furfural is one of the many chemicals that contribute to the aroma of books. The gradual degradation of the complex mixture of volatile chemicals within paper, used in the manufacture of books, produces the aroma of books which is associated with furfural.

2.7.1.8. Furfural residues as soil enhancers and organic fertilizers

Furfural residues are rich in carbon, contain valuable nutrients and are acidic (with pH = 2). (Cai et al., 2014) reported that furfural residues can be used to improve properties such as the pH (reduced alkalinity), water permeability, bulk density, compactness, and retention ability of soil. (Xin et.al., 2008) also recently proposed a production method for a soil fertilizer, consisting of a mixture of solid residues from seaweed and furfural processing units. This multifunctional organic fertilizer, with an extensive application range is easy to prepare at a low integrated cost and has also shown fantastic outcomes on crop yields.

2.7.2 Furfural to value-added products

Most furfural is catalytically hydrogenated to value-added products, several of which are described in (Yan et.al., 2014) and summarized in Table 2.2. Production of jet fuel from furfural has been explored by Yan. Furfural produced from the hemicellulose fraction of red maple wood was converted to a mixture of linear and branched alkanes with carbon numbers ranging from C7 to C31 in a three-step process. First furfural is condensed with acetone under basic conditions to produce a conjugated C13 compound, furfural-acetone-furfural (FAF). Second, FAF is hydrogenated over a noble metal catalyst to improve thermal stability. Third, the hydrogenated product is processed with hydrogen co feed over bifunctional catalysts to eliminate the remaining oxygen and isomerize the product to linear and branched alkanes.

Table 2.2 Chemicals and Products Derived from Furfural

End Use/Application	Notes
Furfuryl alcohol (FA)	It is estimated that about 62% (Yan et al. 2014) of furfural produced each year is converted to furfuryl alcohol, most of which is used for the production of foundry resins. The anti-corrosion properties of FA make it useful in the manufacture of furan fiber-reinforced plastics for piping. FA can also be converted to 1-pentanol, which is a solvent for coating DVDs (Yan et al. 2014).
Tetrahydrofurfuryl alcohol (THFA)	THFA is a "green" solvent used in agricultural applications, printing inks, and industrial and electronics cleaners. It can also be converted to 1,5-pentanediol, which is used as a plasticizer (Yan et al. 2014).
2-methylfuran (MF)	MF is used as a feedstock in the production of antimalarial drugs such as chloroquine. Also used to produce methylfurfural, nitrogen and sulfur heterocycles, and functionally substituted aliphatic compounds (Yan et al. 2014).
2-methyltetrahydrofuran (MTHF)	MTHF is a specialty solvent, used mainly as a substitute for tetrahydrofuran, due to its inverse solubility with water (solubility decreases with increasing temperature). MTHF can also be used in the manufacture of lithium electrodes (Yan et al. 2014) and as a component in alternative fuels, as it has an octane number of 87 (Huber, Iborra, & Corma, 2006a).
Furan	Furan often occurs as a side reaction during hydrogenation of furfural, but can be targeted through catalytic decarboxylation of furfural at high temperatures (Yan et al. 2014). It is used as a solvent as well as in the synthesis of furan-based compounds. It is converted to tetrahydrofuran by hydrogenation. Nitro-substituted furan derivatives are used as biocides or fungicides. Sulfur-substituted furan derivatives are used as flavoring agents.
Tetrahydrofuran (THF)	Derived from furan via hydrogenation, THF is used as a solvent for many chromatography applications and as an ingredient in adhesives, PVC cements, vinyl films, and cellophane (Yan et al. 2014)
Cyclopentanone	A chemical intermediate, cyclopentanone is used in the production of pharmaceuticals, insecticides, and rubber chemicals (Yan et al. 2014).
Cyclopentanol	Cyclopentanol is used as a perfume and pharmaceutical solvent and as an intermediate chemical for dyes and pharmaceuticals (Yan et al. 2014).
Maleic Anhydride	The biggest single use of maleic anhydride is in the manufacture of unsaturated polyester resins, which are used in fiber-reinforced plastics and materials used in the boating, automotive, and construction industries. It is also used to make alkyl resins, which are used in paints and coatings.

2.7.3 Chemistry of furfural formation

Furfural is universally made from agricultural raw materials rich in pentosan. By aqueous acid catalysis, the pentosan is hydrolyzed to pentose, and this pentose is dehydrated to furfural in a unified process. The conversion of hemicellulose to furfural takes place through two steps. The first step is the hydrolysis of polysaccharide into simple sugar where xylan is broken down into the monomer xylose. Xylose is then converted to furfural in the second step through dehydration by removal of three molecules of water. (Zeitsch, 2000 ; Shafeeq et al., 2015).

2.7.3.1 Mechanism for the Hydrolysis of Pentosan

Pentosan consists predominantly of rings linked by oxygen bridges (ether bridges). As shown in Fig. 2.3, protonation of oxygen link is the first step of hydrolysis of pentosan and carbon-oxygen bond cleavage follows resulting in the formation of the hydroxyl group on one side oxygen bridge and carbonium ion is formed during the reaction on the other side. The addition of water molecule onto carbocation forms H_2O^+ group and then H_2O^+ group liberates hydrogen ion leaving a hydroxyl group behind. These steps of hydrolysis continue until the all oxygen bridge disappears.

2.7.3.2 The Mechanism for the Dehydration of Pentose

Various mechanisms for the transformation of xylose to furfural have been proposed but, most studies focus on the cyclodehydration mechanism removing three molecules of water using heterogeneous catalysts. Figure 2.3 shows the mechanism for removal of three molecules of water from xylose to form furfural. First, protonation of the free electron pair on hydroxyl group results in trivalent positively charged oxygen and the positive charge moves to the adjacent carbon atom to arrange itself to more stable conformation before C–O bond breaks and a water molecule is released. Then, the two electrons from the neighboring C–O bond are attracted to the C–C bond. This causes the formation of the double bond and the C–O bond breaks liberate another hydrogen ion. This hydrogen ion then attacks electron pair on the oxygen of another hydroxyl group to liberate another water molecule. The attacked site splits to another carbocation and water molecule. The resulting hydrogen ion migrates and attacks another non-bonded pair of electrons on another hydroxyl groups and other two water molecules are released upon dissociation. In the end, ring formation takes place by 1,4-elimination [Zeitsch 2000].

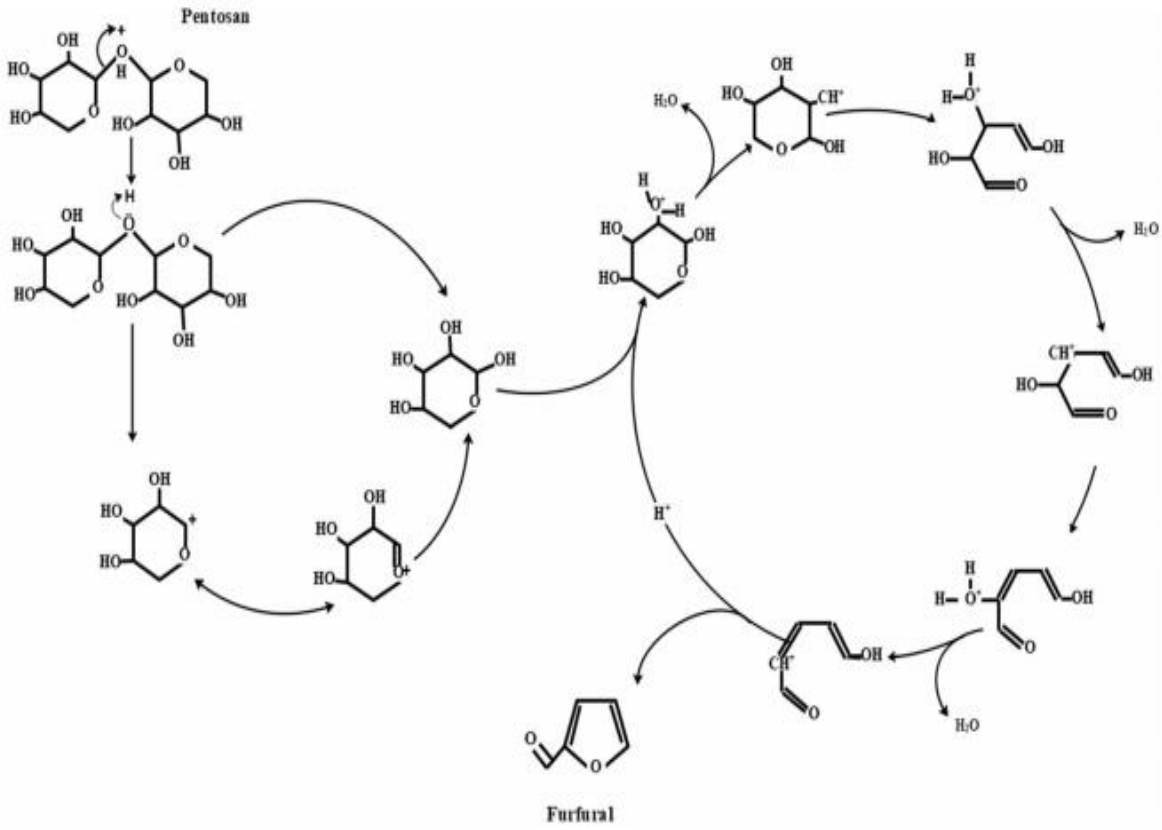

Figure 2-3 The mechanism for the hydrolysis of pentosan and the dehydration of pentose

2.7.4 Catalysts for the production of furfural

2.7.4.1 Homogeneous Catalysts

In last decades, comprehensive studies on the conversion of xylose into furfural have been performed instead of raw biomass for simplification of analysis. Homogeneous catalysts, mineral acids and metal chlorides have been investigated for furfural production. Among the mineral acids, sulfuric acid and hydrochloric acid are the most widely used catalysts for the commercial production of furfural. (Yemiş & Mazza, 2011) investigated and compared the catalytic activities of different acids including sulfuric acid, nitric acid, phosphoric acid, hydrochloric acid, acetic acid and formic acid targeting at the yield and conversion of furfural. 65% of furfural from xylose using hydrochloric acid and 84.8% furfural yield using $AlCl_3$ from xylan is recorded by Zhang et.al and Liu et.al (Zhang et.al., 2013 ; Liu, et.al., 2018).

Agirrezabal et.al investigated the effect of sodium chloride on the dehydration rate of xylose to furfural and achieved 76% furfural yield at 200 °C in a reaction time of 8 min (Agirrezabal et.al., 2013). In general, studies show that mineral acids resulted in high yield of furfural. However, mineral acids are extremely corrosive to the equipment, recovery of the catalysts from the reaction mixtures is difficult, and we cannot reuse the catalysts. Various attempts have been carried by researchers to mitigate the chemical pollution occurring because of the use of acid homogeneous catalysts.

2.7.4.2 Heterogeneous Catalysts

Solid acid catalysts could be regenerated and reused in catalytic processes with comparable efficiencies. Sulfonic acid functionalized mesoporous SBA-15 materials, modified zeolites such as HUSY, H-Beta zeolite, H-mordenite, and MCM-41 are among heterogeneous solid catalysts that were developed for furfural production to solve the problems associated with the use of homogeneous catalysts. Arenesulfonic SBA-15 catalysts were prepared via co-condensation for the dehydration for *d*-xylose to furfural (Agirrezabal et.al., 2014). These selective and hydrothermally stable catalysts incorporate high arenesulfonic-site and posse hexagonal pore arrangement. The activities of sulfonic acid functionalized mesoporous SBA-15 materials were studied with emphasis on the stability of the catalyst. At 160 °C with 99% selectivity, they obtained 82% furfural yield from *d*-xylose in water/toluene. Even though the solvent (toluene) used in the study for extraction of furfural from water medium is not a green chemical, arenesulfonic functionalized SBA-15 supports aged at higher temperatures show higher stability and recyclability (Agirrezabal et al., 2014). The most studied solid catalysts are zeolites, which are thermally and chemically stable, have tunable acidities and shape (Agirrezabal et.al., 2011). Furfural yield of lower than 40% was achieved on studies using a variety of H-zeolites (e.g., HY-faujasite, H-mordenite, and H-ferrierite) in water, water/methyl isobutyl ketone (MIBK), water/toluene and dimethyl sulfoxide (DMSO) in a batch reactor at 140–170 °C (Dias, et.al., 2005). A sulfated tin ion-exchanged montmorillonite ($SO_4$2-/SnMMT) in 2 Methyltetrahydrofuran (2-MTHF)/NaCl-water showed high activity and reusability with furfural yield of 79.64% from xylose at 160 ℃ (Lin et al., 2017). Sulfonated metal oxides and ion-exchange resins were also used for dehydration of xylose to produce furfural. 40-70% yield was achieved by using ion-

exchange resins. Metal oxides, sulfonated with H_2SO_4, such as TiO_2, ZrO_2, SnO_2, and Al_2O_3 were also studied and proved that SnO_2 gives the highest xylose conversion and furfural yield. $SO_4$2-/ZrO_2–TiO_2 gave a higher yield than the selected zeolite catalysts (Zhang, et.al., 2012).

2.7.5 Physical and chemical properties of furfural

2.7.5.1 Physical Properties

The general physical properties of furfural are given in Table 2.3. Because of its unique and alluring properties, it has been broadly used as a building block for the production of fine chemicals, the sustainable production of fuel additives and value-added chemicals.

2.6.5.2 Chemical Properties

Chemically, as other aldehydes and other aromatic compounds furfural participates in the same kinds of reactions. Indicating its diminished aromaticity relative to benzene, furfural is instantly hydrogenated to the corresponding tetrahydrofuran derivatives. When heated within the presence of acids, furfural irreversibly solidifies into a hard and tough thermosetting resin. Furfural has two important functional groups, an aldehyde (CHO) and a conjugated system (C=C-C=C), it is a flexible compound for several applications. Furfural aldehyde group can undergo through various types of chemical reactions like acetylation, acylation, aldol and Knoevenagel condensation, and reduction to alcohols, reductive amination to amines, decarboxylation, oxidation to carboxylic acids and Grignard reactions. (Yan et al., 2014 ; Zeitsch, 2000)

Table 2.3 Physical property of furfural (Furfural Wikipedia & ChemSpider.com)

Properties	Value
Molecular weight	96.085g/mole
Molecular formula	$C_5H_4O_2$
Boiling point	162 ℃
Melting/freezing point	-37 ℃
Density at 20 ℃	1.16 g/ml
Vapor density	3.31
Critical temperature	397 ℃
Flash point	62 ℃
Auto-ignition temperature	315 ℃
Odour	Almond-like
Physical state	Liquid
Refractive index @ 25 ℃	1.5261

2.8 Research Gap regarding furfural production

Several researchers have reported on the production of furfural from the dehydration of xylose, hemicellulose, and cellulosic biomass. For example, Yang et al (2012) reported 75% furfural yield from the reaction of xylose in water- tetrahydrofuran biphasic medium containing $AlCl_3 \cdot 6H_2O$ and NaCl under microwave heating at 140 ℃ and 55%, 38%, 56%, and 64% furfural yield from corn stover, pinewood, switchgrass, and poplar, respectively, using the same solvent system at 160 ℃. Zhang et al (2013) reported 85.3% furfural yield from maple wood at 170 ℃ in 0.1 M sulfuric acid for 50 min by employing simultaneous solvent extraction with Methyl isobutyl ketone(MIBK). Mittal et al (2017) used a biphasic reaction system to convert pentose sugars in biomass derived process-relevant hydrolyzates to furfural in yields approaching 80%. At the optimum reaction conditions of 0.05 M H_2SO_4, 170 ℃ for 20 min with MIBK as the solvent. Some of the researchers shows mechanisms for process improvements to achieve higher furfural yield using the following mechanisms. (1) by improving furfural removal efficiency using steam or an inert gas, e.g., the SupraYield process, which uses N_2 stripping; (2) by extracting furfural

using a secondary organic phase in a biphasic reaction, for example, using cyclopentyl methyl ether (CPME), o-nitrotoluene,20 tetrahydrofuran, and/or γ-valerolactone; (3) by using different homogeneous or heterogeneous solid catalysts, for example, maleic acid, formic acid, metal salts, and/or Amberlyst.

On industrial scale, the first furfural production plant was a batch process originally developed by Quaker Oats Technology in the 1920s in the United States. In this process, oat and oat cereal waste biomass was treated with acid (aqueous sulphuric or phosphoric acid) and steam at 153°C in a hydrolysis step which could convert the pentosans in the biomass to pentoses. The generated pentoses were then converted (i.e., cyclodehydrated) into furfural in a subsequent stage, and then furfural was recovered by steam stripping from solution. Another industrial scale producer is, Westpro which has modified the Quaker Oats Technology process in China (Huaxia Furfural Technology) into a continuous process. The company used corn cobs, rice hulls, flax dregs, cotton hulls, sugarcane bagasse, and wood. However, no detailed information is available with regard to this technology.

Recently, The laboratory-scale furfural production still practicing original or modified versions of the Quaker Oats Technology process. Jing and Lu produced furfural using autocatalytic system at higher temperature (>200 ℃) and higher pressure using reagent xylose. A maximum of 50% furfural (based on xylose) and formic acid were formed as the main product and by-products of this autocatalytic process (Jing & Lu, 2008). Aqueous acid catalytic system also used for production of furfural in laboratory scale. Several mineral acids are used for the production process. A temperature range of 120°C to 200°C has been investigated on this category. Furfural yield of 65.4% was obtained by Lamminpaa et.al using acid catalytic system. (Lamminpää, et.al., 2015). Furfural also has been produced using solid catalysts in bi-phasic system and aqueous system. In by phasic system The aqueous phase usually consists of water or a water-dimethyl sulphoxide (DMSO) mixture and an acid (HCl or H_2SO_4) is used for production of furfural at a temperature range of 110-200 ℃ giving 90% of furfural yield (Xing, et.al., 2011). Dias et.al (2005) reported 50% furfural yield using Microporous and mesoporous niobium silicates (Na,H-AM-11 and Nb-MCM-41), water-toluene solvent, 140-180 ℃.

To sum up, the production of furfural from agricultural biomass and reagent grade xylose using homogeneous and heterogeneous catalysts has been explored by several researchers. Lignocellulosic biomass from agricultural residues and reagent grad xylose was the raw materials for production of furfural. Several process parameters that affect the yield of furfural also has been investigated by the scholars. Temperature, acid concentration and time were the most important factors that alters the yield of furfural. Strong mineral acids, beside their disadvantages, are the most used and preferred catalysts in the synthesis of furfural. On this study the possibility of producing furfural from unutilized biomass i.e. microalgae have been examined using dilute sulfuric acid and the improvement of high percentage yield through optimization of process variables were executed. The most important parameters that affect the yield of furfural has been investigated. A simple production process was followed and the auxiliary chemicals that are used for the production process were easily accessible.

3. Materials and Methods

This chapter contains materials, equipment, experimental methods and experimental locations. The National Renewable Energy Laboratory (NREL) and Association of Official Analytical Chemist (AOAC) methods of testing were used for Laboratory analytical procedures. The methodology of this research work involves characterization of the feedstock (microalgae biomass), synthesis and characterization of furfural, analysis of experimental results using design expert software and optimization of experimental factors that affect the furfural yield.

3.1 Raw material collection and preparation

The Microalgae biomass (Fig. 3.1) was collected from a river found in Debre Birhan city, which is located in the northern direction of Addis Ababa, Ethiopia. A quantity (> 1 kg) of algal biomass were harvested in early January 2020. The algal biomass was washed with river water, tap water and distilled water to remove dirt and foreign materials. The fresh water algae sample was identified for algal species using standard method at the laboratory of Biology department in Addis Ababa university. The samples that were used for further thesis work was dried at 60°C and finely milled to obtain a dried homogenized microalgae biomass. The residues after the milling were analyzed for Proximate (moisture content, ash content, Volatile matter and fixed carbon) analysis and Compositional (extractives, cellulose, hemicellulose and lignin) analysis.

Figure 3-1 Milled microalgae biomass

3.2. Characterization of microalgae biomass

3.2.1 Proximate analysis

The proximate analysis gives moisture content, volatile matter content, the fixed carbon content, and the ash content (the inorganic residue remaining after combustion of the sample). These parameters were determined according to method of Association of Official Analytical Chemists(AOAC), European standard procedure and NREL laboratory analytical procedures.

- **Moisture Content**

The moisture content of the algal biomass was determined according to the published NREL Laboratory Analytical Procedures (LAP) (Wychen & Laurens, 2015). The procedure is described in the Annex.

- **Ash Content**

Similarly the Ash content of the algal biomass was determined according to the published NREL Laboratory Analytical Procedures (LAP) (Wychen & Laurens, 2015). The procedure is described in the Annex.

- **Volatile Matter Content**

The total volatile component present in the algae is determined using according to the procedures described in European Standard EN15148-2009 ("Solid biofuels - Determination of the content of volatile matter"). (British Standard Institution, 2010). The procedure is described in the Annex.

- **Fixed Carbon Content**

This is the residue left after the moisture, volatile and ash is given up. It is deduced by subtracting from 100, the percentage of moisture, volatile matter and ash content. The fixed carbon content (FC) is given as

FC = 100 – (%moisture + %volatile matter + % ash) …… *Equation 3-1*

3.2.2 Compositional analysis

The compositional analysis of the microalgae biomass involves Extractives, cellulose, hemicellulose and lignin content determination of the biomass.

❖ Solvent extractives

The solvent extractives of the algal biomass were determined according to the published NREL Laboratory Analytical Procedures (LAP). (Sluiter, 2008). The detail is described in the annex.

❖ Cellulose content determination

The Kurschner Hoffner method was used to determine the cellulose content of the algal biomass, which consists of treating 5 g of extractives-free samples with 125 ml of alcoholic nitric acid solutions under reflux during four cycles of 1 h. After each cycle, the exhausted alcoholic nitric acid solution was removed and a fresh volume is added. The alcoholic nitric acid solution consisted in mixing one volume of 65% (w/w) solution of nitric acid with four volumes of 96% purity ethanol; at the end of the four cycles, the cellulose was washed, dried and weighed (Ouensanga, 1989)

$$\%\text{Cellulose}=\frac{\text{weight of extractive free sample} - \text{weight of dried sample}}{\text{weight of extractive free sample}} * 100 \quad \text{..... } \textit{Equation 3-2}$$

❖ Hemicellulose content determination

The hemicellulose content of the microalgae was determined by the laboratory analytical procedures (LAPs) “Determination of structural carbohydrates and lignin in biomass” refined by NREL researchers. (Sluiter, 2008)

❖ Lignin content determination

The lignin content of the microalgae was also determined by the laboratory analytical procedures (LAPs) “Determination of structural carbohydrates and lignin in biomass” refined by NREL researchers. (Sluiter, 2008)

3.3 Analysis of algae hydrolyzate

The Microalgae hydrolysates were prepared using a dilute sulfuric acid pretreatment procedure. 10 g of algal biomass were pretreated in aqueous sulfuric acid at a concentration of 1.5M. The mixture then reacted for 1 h in a 250 mL Erlenmeyer flask at 120 ℃ in an autoclave. Particulates were removed by filtration and the filtrate were analyzed for monosaccharide sugar content. The Concentration of the monosaccharides sugar of the hydrolyzate was determined using HPLC. The

analysis of hydrolyzate sample were carried out in the laboratory of "*Ethiopian Conformity Assessment Enterprise*". Acidic hydrolysis was used to decompose hemicellulose of the algae into its monosaccharides like xylose, arabinose, glucose, etc. The monosaccharide standards and acid hydrolysis samples were performed with Agilent 1260 infinity II HPLC instrument equipped with a Refractive index detector(RID). The fused capillaries have 5 micrometers I.D. and a length of 150 mm. A solution consisted of water and Acetonitrile were used in the experiment.

3.4 Synthesis of furfural

The experimental runs for furfural production were done using a batch reactor, an autoclave and a distillation system. The experiments were conducted in four steps involving pretreatment of algal biomass, acid hydrolysis, separation, and furfural analysis. The acid hydrolysis of the microalgae samples took place in an autoclave and 1000 ml round bottom flask in which the dried algal biomass was hydrolyzed with aqueous Sulphuric acid of concentration ranging from 0.1M -1.5M. Uniform quantity of sodium chloride salt (1.0 g/ per g of microalgae sample) was introduced into each sample flask during the reaction process. The round bottom flask was connected with a Vigreux column and water condenser in which the distillate was collected in a receiver. The reaction was carried out at temperature variation between 110 ℃ and 160 ℃ for 30 - 90 min. The aqueous distillate mixture from the distillation column was set to flow into a separatory funnel contained with chloroform. Two layers were formed with the aqueous layer at the top and the chloroform-furfural containing layer at the bottom of the flask. The chloroform-furfural layer was subjected to the rotary evaporator at a temperature of 60 ℃ to remove the chloroform, and a clear liquid product (P-l) remained. Then this Product P-l was analyzed by FTIR and HPLC and was determined as furfural. These procedures were repeated for different reaction temperature, acid concentration and reaction time. (Barbosa et.al., 2014)

3.5 Determination of furfural yield

The colorimetric determination of furfural was carried out by concentrated hydrochloric acid-aniline method. According to Hui et.al (2019) furfural can react with aniline with the aid of concentrated HCl to form a dye, which shows characteristic ultraviolet absorption at 518 nm. The extracted solution (1 mL) and 1 mL chromogenic agent (aniline) were taken into a 25 ml volumetric flask. After that, distilled water was added to the scale line of 10 ml. Then the mixture

was allowed to stand for 15 min at room temperature in dark. Care was taken to protect the flask from sunlight. Next, the solution was charged into a cuvette for UV test. Finally, according to the standard calibration curve, the concentration of furfural was determined. The absorbance of the color was measured at 518nm using UVD-3200 Spectro UV-Vis Double beam PC 8 Scanning auto cell Uv-vis spectrophotometer. The concentration of furfural in the extracted sample was determined using the standard curve.

A standard calibration curve was obtained by plotting the UV-Vis absorbance of a series of standard furfural solutions against their corresponding concentrations. The standard calibration curve is given in Appendix A4. Then the concentration of furfural of the reaction system can be determined according to its UV-Vis absorbance. Therefore, the yield of furfural can be estimated from the following equation:

$$\text{Furfural yield} = \frac{\text{mass of produced furfural}}{\text{Mass of the starting algae Biomass}} \times 100\% \quad \text{……..} \textit{ Equation 3-3}$$

3.6 Product characterization

3.6.1 Validation of product with Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectrum was recorded by passing a beam of infrared light through the sample. The functional group analysis of the furfural containing product was carried out using Fourier-Transformed Infrared (FTIR) spectroscopy. The FTIR spectra were recorded on spectrum FT/IR-6600 type A equipped with KBr beam splitter. A regular scanning range of 400-4000 cm^{-1} was used at a spectral resolution of 4 cm^{-1}. All the spectra were recorded and processed using essential FTIR software.

3.6.2 Furfural analysis using HPLC

Agilent 1100 Series HPLC with UV detector was used to analyze and quantify furfural in the liquid product. A C8 Agilent Zorbax SB HPLC column with a length of 150 mm and the inner diameter of 4.6 mm is used for quantification. Standards were prepared with deionized HPLC grade water. The ultraviolet detection was at Wavelength of 285 nm.

3.7 Central composite experimental design

The experiments were designed according to the Central Composite design method with the selected three important Furfural synthesis parameters: Temperature , acid concentration and time. The required response of the experiment, furfural yield, was optimized after studying the influences of these independent parameters and their interaction effects. Experimental data analysis was carried out using Design- Expert 11.0.0 software. According to the central composite design a total of 20 experiments were obtained containing 14 non center points and 6 center points.

Table 3.1 Experimental levels of selected variables for Central Composite Design

Abbreviation	Variables	Units	Levels	
			Low level(-)	High level (+)
A	Temperature	℃	110	160
B	Acid Concentration	M	0.1	1.5
C	Time	min	30	90

The complete experimental design matrix of the CCD for the factorial design of furfural synthesis is shown in table 3.4. The order in which the runs were made was randomized to minimize systematic errors. The design matrix in Table 3.4 was entirely followed during the experimental work of each run.

3.8 Optimization of process variables

The optimization features of the design expert program were used for optimizing objective function (furfural yield) and constraints based on the furfural yield obtained per experiment performed using the design matrix of table 3.4. The optimization objective and constraints are discussed in chapter 4. The desirability function approach was applied to determine the optimum combination of Temperature, Acid concentration and time for the production of Furfural with respect to its yield. The assumptions were to develop a product which would have maximum yield. High yield of furfural at optimum process conditions was the objectives function upon which an optimal solution was chosen among the various solutions predicted by the software

Table 3.2 Central Composite Design matrix for furfural synthesis

	Factor 1	Factor 2	Factor 3
Run	A: Temperature (°C)	B: Concentration (M)	C: Time (min)
1	135	0.8	110.45
2	135	0.8	60
3	110	0.1	90
4	135	1.97	60
5	135	0.8	60
6	135	0.8	60
7	135	0.8	9.55
8	135	0.8	60
9	110	1.5	90
10	160	0.1	90
11	135	0.8	60
12	92.95	0.8	60
13	135	0.8	60
14	160	1.5	90
15	177.04	0.8	60
16	110	1.5	30
17	110	0.1	30
18	160	1.5	30
19	135	0.1	60
20	160	0.1	30

4. Results and Discussions

4.1. Identification of microalgae species

The microalgae species were identified using inverted Microscope at 400x resolution as per the standard laboratory reference guide. The identified species of microalgae as shown in figure 4.1 are mainly dominated by the species of *Mougeotia scalaris* (>98%), and a few amount *of Spirogyra varians* and *Zygnema aplanosporum* were found in the identified algal samples.

The dominant species of this mixed algae sample is *Mougeotia scalaris. Mougeotia scalaris* is genus of conjugating filamentous algae of the order *Zygnematales*, phylum *Charophyta*. It is widespread in freshwater habitats worldwide (John et.al., 2011). From the river where the algal samples were collected, the species was presented in the water body forming massive algal blooms. These species are belongs to the family of *Zygnemataceae*. Within this family the three most occurring genera of microalgae are —*Mougeotia*, *Spirogyra*, and *Zygnema* (Hall & McCourt, 2015). The microalgae species that are identified by the microscope are from the same family.

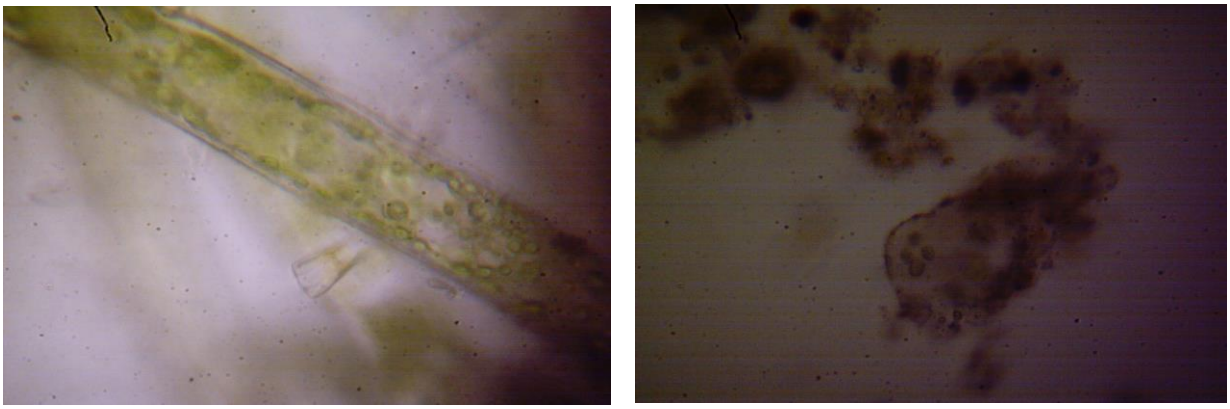

Figure 4-1 Microscopic photos of Species of Microalgae identified for furfural synthesis

Mougeotia cell wall composition has homologies with vascular plant cell walls. *Mougeotia* is rich in non-cellulosic carbohydrates and cellulose. The whole cell walls of *Mougotia* consisted of carbohydrate, acidic carbohydrates, lipid protein and others. Eight neutral sugars were present in the *Mougeotia* cell wall with glucose and xylose most abundant. Arabinose, Fucose and rhamnos are another monosaccharide that are found in the algal species (Hotchkiss, et.al., 1989). The

abundance and presence of pentose monosaccharides (Xylose and Arabinose) in this particular species of microalgae ensures its suitability for the production of furfural.

4.2. Proximate and compositional analysis of the microalgae biomass

Proximate analysis was carried out to determine % moisture content, % Ash content, % volatile matter content and % fixed carbon content of the algal biomass. The compositional analysis was carried out to determine the extractives, cellulose, hemicellulose and lignin compositions of the algae. Table 4.2 shows the proximate and compositional analysis of the algal biomass.

- **Moisture Content**

The moisture content of the dried algal biomass was determined gravimetrically according to NREL method. The moisture content was expressed on a dry weight basis. In triplicate analysis of the moisture content the following data were obtained. Moisture content determination equation written in the annex was used to calculate the moisture content.

Table 4.1 . Average moisture content of algal biomass

Run	Sample weights (g)		Moisture content (% w/w) of algal biomass	Mean ± SD
	W_1(g)	W_2(g)		
1	7.000	6.545	6.5	6.53 ±0.286
2	7.000	6.517	6.9	
3	7.000	6.566	6.2	

The moisture content of the algal biomass for the three samples were 6.5%, 6.9% and 6.2% and the average moisture content was obtained as 6.53% w/w. The moisture content of 6.53% is low and comparable to lignocellulosic and agricultural feedstock's. This indicates that air dried algal biomass requires small energy and time to remove moisture for further processes. The moisture content value of these particular algal species is closer to the value of other algal species obtained by (Laurens et al., 2012) which was 4.07 and (Ameh et.al., 2008) which was 4.16.

- **Ash, Volatile matter and Fixed Carbon**

The ash, volatile matter and fixed carbon content of the algal biomass is shown in table 4.2. Ash is the inorganic residue remaining after the water and organic matter are eliminated by heating within the presence of oxidizing agents. In short it is the measure of the amount of minerals which are not destroyed by heating. This provides a measure of the total amount of minerals within the biomass, such as Ca, Na, K and Cl. The volatile matter of biomass is the condensable vapor and permanent gases (exclusive of water vapor) released from the biomass when it is heated. Fixed carbon is the solid combustible residue that remains after biomass is heated and the volatile matter is expelled.

Table 4.2 Proximate analysis and biochemical composition of microalgae biomass

Parameters	**Values**
Moisture Content	6.53 %
Ash Content	7.92 %
Volatile Matter	74.23 %
Fixed Carbon	11.32 %
Extractives	26.24 %
Cellulose Content	39.33 %
Hemicellulose Content	29.68 %
Lignin Content	4.51 %

The algal biomass constitutes an adequate amount of hemicellulose comparable to that found in Corn Cobs (30-32 %), Rice Husk (16-18%), Bagasse (25-27%) and Cotton Husk (27%) commonly used for furfural production.(Shafeeq et al., 2015) The utilization of algal biomass for synthesis of furfural requires the biomass fractionation into cellulose, hemicelluloses and lignin. This step involves pretreatment of the biomass where a considerable part of hemicelluloses is solubilized. It is significant that the algal biomass contained low lignin and enough amount of hemicellulose to serve as a best raw material for furfural synthesis. The composition of the algal biomass is

comparatively in harmony with the studies reported by several researchers. (Ververis et al., 2007; Estevez et.al., 2008 ; Martone et al., 2009).

The relatively low content of lignin presented in the microalgae has a great effect in the synthesis of furfural. Lamminpää et.al (2015) studied the effect of the presence of lignin along with the hemicellulose on the yield of furfural and confirmed that it has a negative effect on both the conversion of xylose and the yield of furfural. Therefore, the decrease in the yield of furfural with negative effects associated with the presence of lignin will be reduced for synthesis of furfural from microalgae.

4.2.1 Analysis of hydrolyzate

The monosaccharide sugar content of the acid hydrolyzate of the algal samples were determined with HPLC and the results are presented in the table and figures here under. (Detail results are presented in Appendex A2)

Table 4.3 Acid hydrolysate of the solubilized hemicellulose fraction measured as monosaccharides

S/N	Monosaccharide sugars tested	Test result
1	Xylose, g/100ml	12.89
2	Mannose, g/100ml	<0.5
3	Arabinose, g/100ml	<0.5
4	Glucose, g/100ml	32.2
5	Galactose, g/100ml	<0.5

4.2.2 Hydrolysis efficiency

The efficiency of hydrolysis process was determined as the percentage of hemicellulose removed from the microalgae and transformed into xylose. Considering the maximum yield of furfural determined in this study the hydrolysis efficiency is calculated. The calculation assumed complete hydrolysis of hemicellulose and complete xylose to furfural conversion. The formula used was defined in equation 4.1.

$$\text{Hydrolysis Efficiency (\%)} = \frac{\text{Pentose concentration after hydrolysis}}{\textit{hemicellulose concentration before hydrolysis}} \quad \text{--------- } \textit{Equation 4.1}$$

According to the results of equation 4.1 the efficiency of the hydrolysis reaction with dilute sulfuric acid is determined as 47%. A good efficiency of hydrolysis increases the accessibility of pentose's to dehydration and therefore obtaining a value of polymerization degree close to one, so that they can be converted to furfural.

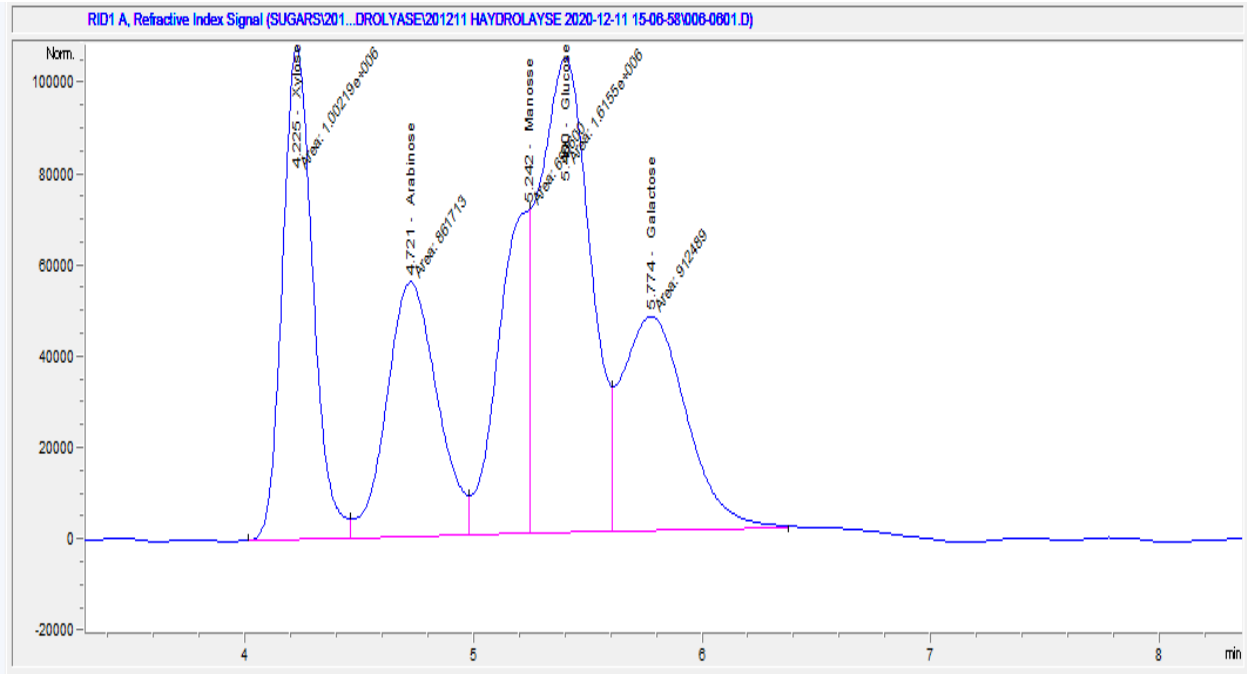

Figure 4-2 standard sugar mixture (Xylose, Arabinose, Mannose, Glucose and Galactose)

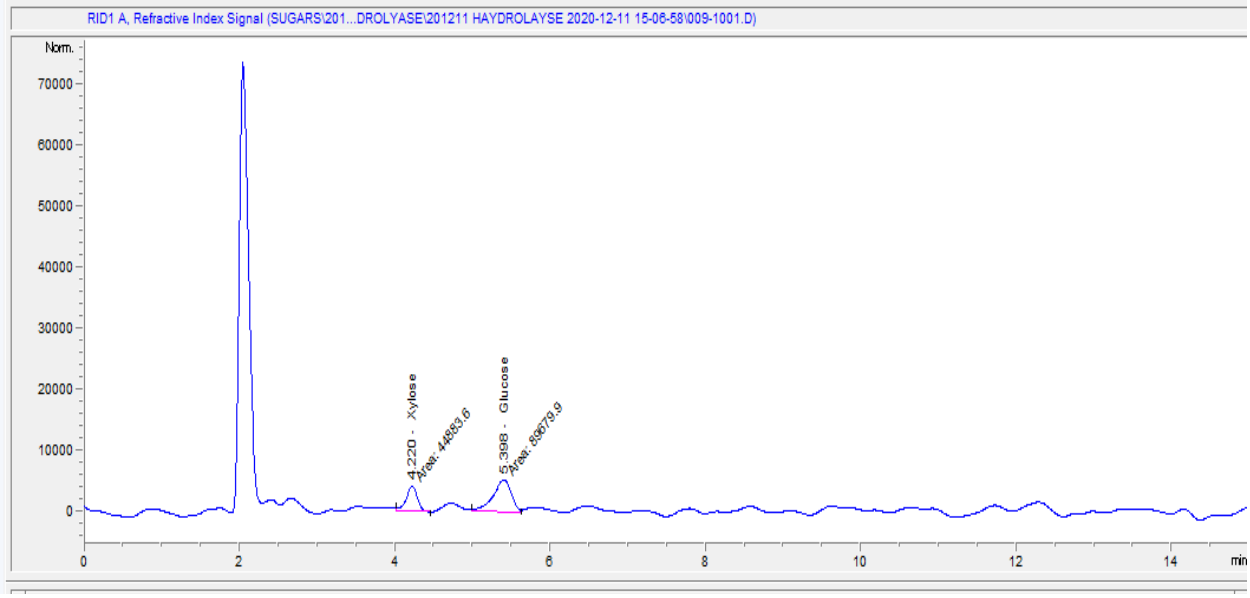

Figure 4-3 Monosaccharide sugar analysis of algae hydrolyzate

Table 4.2 and results from figure 4.2 and 4.3 shows the amount of the individual sugars. When the acid hydrolysis takes place, the hemicellulose fraction is degraded into sugars composed of mainly pentose and hexoses. Xylose and glucose was the major sugar and arabinose, mannose and galactose are the minor sugars. Xylose and arabinose, are the pentoses which are required for the synthesis of furfural. Pentose monomers under acidic condition can degrade to furfural. High Pentoses concentration is the main interest since it is the potential source for furfural production. From the hydrolysate analysis result it has been concluded that algae hemicellulose is a promising source of xylose which could be utilized in the production of furfural.

4.3 Experimental design analysis of furfural production from microalgae

Central Composite design (CCD) of Response surface methodology (RSM) of Design-Expert 11.0.0 software was used for statistical analysis and to determine the optimal conditions of the furfural synthesis process.

A least squares method was used to derive a mathematical correlation by fitting a response surface to the determined values of furfural yield. The adequacy of the model was determined by evaluating several factors obtained from the statistical analysis.

The statistical significance of the model and model variables were determined at 95% confidence interval ($\alpha = 0.05$). The results found on the experimental runs had been put to Design-Expert software and there has been found a single model that can be possibly fit or satisfy the factors significance.

The analysis of the model indicates that it is highly significant with F-value 409.63 with the corresponding P value < 0.0001. The ANOVA for the response is shown in (Table 4.8).

The model adequacy was also investigated using R^2 which were found to be **0.9973**. These imply that the model is adequate enough to predict the response in the experimental range with 99.45% variability. The experimentally found and the predicted values were in good agreement as depicted in Table 4.10. The Pred R^2 show that the model equations for the yield of furfural give good predictions with 98.22% variability. In addition, the adj-R^2 of 99.49% were in a reasonable agreement with Pred R^2 values. The degree of precision and reliability can be explained by the low value of CV which were 2.46. Furthermore, adequacy precision for each response for furfural yield was found to be 74.9880.

The value of "Prob > F" less than 0.05 indicate model terms are significant. In addition to this, the adequacy of the model was checked by constructing different diagnostic plots which are shown in Figures 4.4, 4.5, 4.6, and 4.7.

The Analysis of statistical data with design expert software is done based on the following input information's. The build information of input factors and responses of the general CCD for furfural production from microalgae are described in table 4.4 a, b and c.

Table 4.4 Build Information summary

A. Build Information Model Summary

Study Type	Response Surface
Design Type	Central Composite
Design Model	Quadratic
Runs	20
Blocks	No Blocks

B. Build Information of Factors

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Temperature	°C	Numeric	92.96	177.04	-1 ↔ 110.00	+1 ↔ 160.00	135.00	21.20
B	Acid Concentration	M	Numeric	0.1	1.98	-1 ↔ 0.10	+1 ↔ 1.50	0.8000	0.5935
C	Time	Min	Numeric	9.55	110.45	-1 ↔ 30.00	+1 ↔ 90.00	60.00	25.43

C. Build Information of Responses

Response	Name	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Model
R1	Yield	20	Polynomial	6.89	53.13	36.44	12.94	7.71	Quadratic

Based on the Central Composite Design (CCD) result and using the relationships in Table 3.4, a total of 20 runs were performed including the central point which measures process stability and inherent variability. Furfural yield was used as the response and basis to evaluate the effects of various process parameters that are studied under the production process. Furfural yield is defined as the ratio of the mass of furfural produced to the mass of microalgae biomass used. The yield of furfural was calculated according to equation 3.3. The yield of furfural from the various experimental runs with the design matrix of the Central Composite Design (CCD) are shown in the table 4.5.

4.3.1. Statistical analysis of furfural synthesis from microalgae

The Central composite experimental design had a total of 20 experiments with 14 different combinations of experiments and 6 repeated (center point) runs. Statistical analysis was done on the interactions of the process parameters (Factors) and the response to select the type of model that fit best. Model summary statistics for furfural production is presented in table 4.6. From the table it can be seen that the difference between adjusted and predicted R^2 values is less than 0.2 in the case of the quadratic model. Therefore, the quadratic model was selected according to the suggestions by the design expert software. Therefore, this model could be appropriate for the determination of optimized conditions for synthesis of furfural.

Table 4.5 Experimental (CCD) design and results (conversion of microalgae to furfural)

	Factor 1	Factor 2	Factor 3	Response 1
Run	A: Temperature (°C)	B: Concentration (M)	C: Time (min)	Yield
1	135	0.8	110.45	32.79
2	135	0.8	60	42.81
3	110	0.1	90	30.77
4	135	1.97	60	47.80
5	135	0.8	60	41.51
6	135	0.8	60	42.1
7	135	0.8	9.54	16.9
8	135	0.8	60	41.87
9	110	1.5	90	37.80
10	160	0.1	90	45.2
11	135	0.8	60	41.67
12	92.95	0.8	60	15.8
13	135	0.8	60	41.35
14	160	1.5	90	44.8
15	177.04	0.8	60	53.13
16	110	1.5	30	18.80
17	110	0.1	30	6.89
18	160	1.5	30	50.1
19	135	0.1	60	36.8
20	160	0.1	30	43.85

The model adequacy was also investigated using R^2 which were found to be **0.9973**. These imply that the model is adequate enough to predict the response in the experimental range with 99.73% variability. The experimentally found and the predicted values were in good agreement as depicted in Table 4.10. The Pred R^2 show that the model equations for the yield of furfural give good predictions with 98.22% variability. In addition, the adj-R^2 of 99.49% were in a reasonable agreement with Pred R^2 values.

Table 4.6 Model Summary Statistics for furfural production from microalgae

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Predicted residual sum of square of the model (PRESS)	
Linear	7.33	0.7150	0.6616	0.5155	1460.39	
2-level factorial (2FI)	6.48	0.8186	0.7349	0.6531	1045.56	
Quadratic	**0.9030**	**0.9973**	**0.9949**	**0.9822**	**53.78**	**Suggested**
Cubic	1.12	0.9975	0.9921	0.5476	1363.51	Aliased

Table 4.7 Fit Summary Statistics for furfural production from microalgae

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0001	< 0.0001	0.6616	0.5155	
2FI	0.1077	< 0.0001	0.7349	0.6531	
Quadratic	**< 0.0001**	**0.0524**	**0.9949**	**0.9822**	**Suggested**
Cubic	0.9704	0.0052	0.9921	0.5476	Aliased

4.3.2. Analysis of variance (ANOVA) for furfural production

The statistical significance and the goodness of fit of the developed quadratic model, as well as the effect of individual variables and their interactions were analyzed by analysis of variance (ANOVA). The results of analysis of variance are shown in table 4.8.

The F value and P-value of the obtained quadratic model presented in table 4.8 were found to be 409.63 and <0.0001 respectively. These implies that the quadratic model is significant. As a result of that, the model can sufficiently predict the % yield of furfural based on the investigated factors affecting the production process. The p-value of the main three effects Temperature, Acid

concentration and Time i.e. A, B and C respectively were significant based on their estimated p-values (<0.05) which indicated that the model was suitable for use in this experiment and suggesting that all parameters has influence on the yield of furfural. The quadratic terms (A^2 and C^2) and the interactive effects of AB, AC and BC are significant ($p<0.05$).

The lack of fit low F-value of 4.93 and P-value of 0.0524 implied that the lack of fit of the model was not significant relative to the pure error. There is good fit between the quadratic model and the experimental % yield of furfural.

Table 4.8 ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3005.94	9	333.99	409.63	< 0.0001	Significant
A-Temperature	1702.26	1	1702.26	2087.76	< 0.0001	
B-Acid Concentration	137.33	1	137.33	168.43	< 0.0001	
C-Time	315.62	1	315.62	387.10	< 0.0001	
AB	21.42	1	21.42	26.27	0.0004	
AC	274.13	1	274.13	336.21	< 0.0001	
BC	16.62	1	16.62	20.38	0.0011	
A^2	78.23	1	78.23	95.95	< 0.0001	
B^2	2.81	1	2.81	3.45	0.0928	
C^2	473.34	1	473.34	580.54	< 0.0001	
Residual	8.15	10	0.8154			
Lack of Fit	6.78	5	1.36	4.93	0.0524	not significant
Pure Error	1.38	5	0.2750			
Cor Total	3014.09	19				

4.3.3. Development of regression model equation

A model equation is a representative equation in which it represents the whole model with a single mathematical relation that helps to maximize yield and optimize operating conditions. The software package Design-Expert 11.0.0 was used to determine the statistical significance of each experimental factor and to generate the corresponding mathematical prediction models.

The final equation in terms of coded factors are given as follows

$$\text{Yield} = +41.84 + 11.16 \times A + 3.17 \times B + 4.81 \times C - 1.64 \times A \times B - 5.85 \times A \times C - 1.44 \times B \times C - 2.33 \times A^2 + 0.4420 \times B^2 - 5.73 \times C^2$$

Where: A, B, and C are Temperature, Acid concentration and time, respectively. This model equation can be used to predict the yield of furfural at different conditions of the parameters prior to laboratorial experiments.

4.3.4. Checking of data and adequacy of model

Table 4.9 Fit Statistics

Std. Dev.	0.9030		**R^2**	0.9973
Mean	36.64		**Adjusted R^2**	0.9949
C.V. %	2.46		**Predicted R^2**	0.9822
			Adeq Precision	74.9880

The coefficient of determination reflects the variability of the dependent response variable, which is explained by its relationship with the independent process variables. In this study, the R^2 value is **0.9973**, which indicates that 99.73% of the variability in the %yield of furfural was attributed to the independent factors considered, and only 0.27% of the total variations was not explained by the developed regression model. The R^2 value is close to unity, which indicated that the experimental data obtained is linearly fits in the chosen model equation. The adjusted and predicted R^2 for this study was obtained as 0.9949 and 0.9822 respectively. The difference between the adjusted R^2 and predicted R^2 values shows a good fit between the actual and predicted % yield of furfural.

The developed model has a high degree of precision. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case the adequate precision found is high enough at 74.9880. Thus, this model can be used to navigate the design space.

The coefficient of variance (C.V.) obtained for this study was found to be low (2.46%), which indicates that the experimental data are accurate and reliable to suggest that the quadratic model is capable of optimizing the process. Smaller values of CV give better reproducibility. The coefficient of variation (CV) of less than 10 indicates that the model is reproducible.

All of the above stated statistical parameters shows the reliability of the model.

4.3.5. Diagnostic plots for the adequacy of the proposed model

- **Normality of the data**

Normality of the data was done by means of normal probability plot. The normal probability plot of the residuals for furfural yield is shown in Figure: 4-4, which reveals that the residuals are falling reasonably close to the straight line with little scattering. This means that the errors are distributed normally showing no deviation of the variance. Therefore, the normality assumption was satisfied as the residual plot approximated along a straight line.

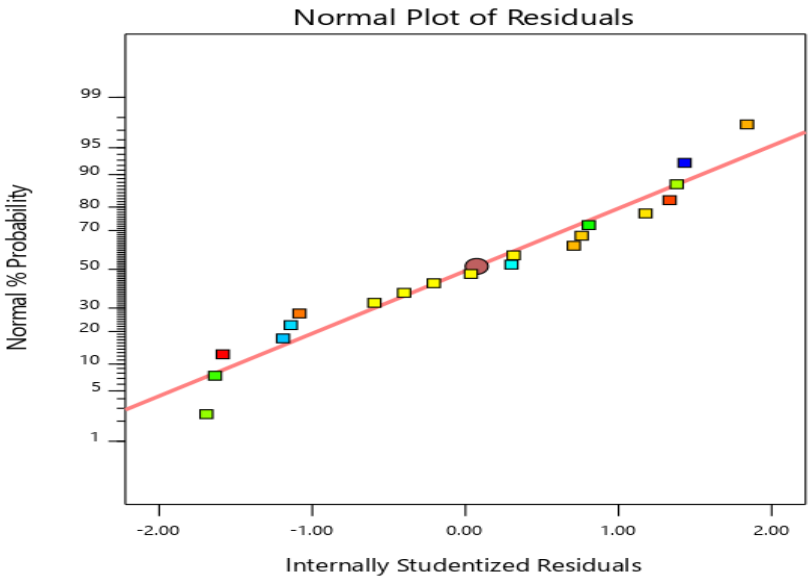

Figure 4-4 Normal probability of internally studentized residuals (Normality of the data) plot

- **Predicted vs actual yield**

Figure: 4-5 indicates the relationship between the actual and predicted values of the yield. This figure shows that the developed model is adequate. Because, the residuals have tendency to be close to the diagonal regression line suggesting a strong relationship between the actual and predicted values of the response. The predicted values obtained from the developed models were quite close to the experimental values.

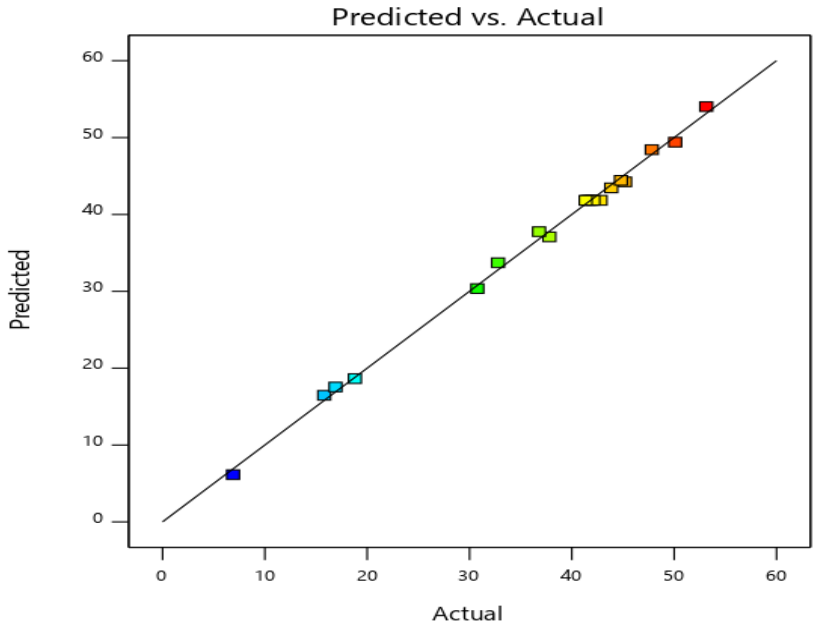

Figure 4-5 Experimental yield vs. predicted yield

- **Residuals vs Run**

Figure: 4-6 presents a plot of residuals versus experimental run order. It checks for lurking factors that may have influenced the response during the experimental run. The plot should show an arbitrary random scatter. From the plot it has been observed that all the data points lie within the limits (±3).

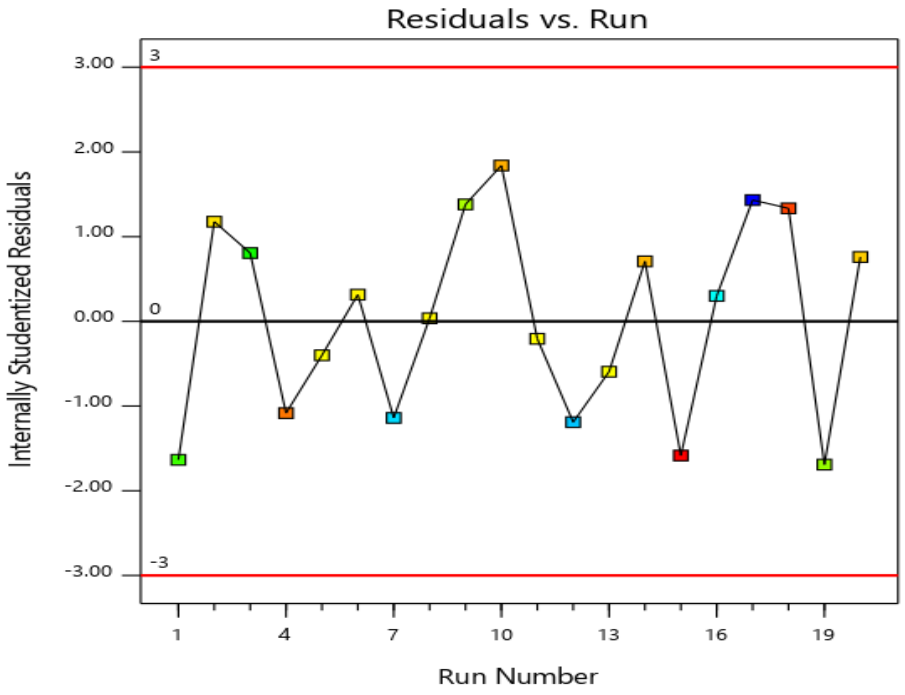

Figure 4-6 Residuals vs. experimental run number

- **Residuals vs. Predicted**

Figure: 4-7 is a plot of residuals versus the ascending predicted response. It tests the assumption of constant variance. The plot should be a random scatter (constant range of residuals across the graph). Expanding variance in this plot indicates the need for a transformation, however a constant range of residuals can be observed from fig 4.7 indicating no need for transformation. The overall impression of this plot is that the residuals distributed randomly on the plot, indicating that the variance of the original observation is constant for all values of Y.

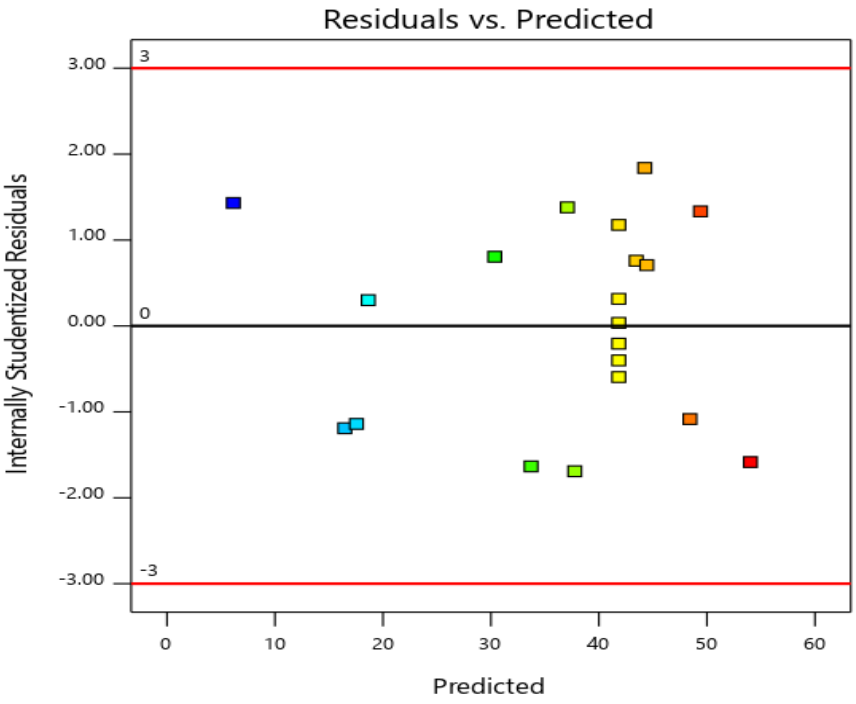

Figure 4-7 Residual versus predicted values

4.3.6. Predicted vs experimental values

According to the CCD result using Design-Expert 11.0.0 software, an experiment with hydrolysis time, temperature and sulfuric acid concentration were conducted in order to study the outcome or effect of the design. The tough correlation between the experimental and the predicted results confirmed that the response model was adequate to reflect the expected synthesis condition.

Table 4.10 Predicted and experimental values of the response of the furfural synthesis

	Actual parameters (Variables)			Yield	
Standard Order	**Temperature (℃)**	**Concentration (M)**	**Time (min)**	**Actual Value**	**Predicted Value**
1	135	0.8	110.45	32.79	33.72
2	135	0.8	60	42.81	41.84
3	110	0.1	90	30.77	30.35
4	135	1.97	60	47.81	48.42
5	135	0.8	60	41.51	41.84
6	135	0.8	60	42.10	41.84
7	135	0.8	9.54	16.90	17.55
8	135	0.8	60	41.87	41.84
9	110	1.5	90	37.80	37.08
10	160	0.1	90	45.20	44.25
11	135	0.8	60	41.68	41.84
12	92.95	0.8	60	15.80	16.47
13	135	0.8	60	41.35	41.84
14	160	1.5	90	44.80	44.43
15	177.04	0.8	60	53.13	54.03
16	110	1.5	30	18.80	18.64
17	110	0.1	30	6.89	6.15
18	160	1.5	30	50.10	49.41
19	135	0.1	60	36.80	37.76
20	160	0.1	30	43.85	43.46

4.3.7. Interaction effect of factors on yield

Interaction effects represent the combined effects of factors on the response. When an interaction effect was present, the impact of 1 factor depends on the extent of the other factor. The ability to estimate and test interaction effects between the factors involved is one of the advantage of DOE.

Response surfaces were plotted using Design-Expert version 11.0.0 software to study the effects of parameters and their interactions on yield.

The 3D-Surface diagram of significant model term interactions among the process variables and yield are illustrated by a 3D- surface plots. The 3D plots generally depict the effect of the independent variables and their interactive effects on the responses.

A. Effect of Interaction between Temperature and Acid concentration (AB) on Yield

Figure: 4.8 shows the interaction effect of Temperature and Acid concentration on the yield of furfural. As the temperature and concentration of acid varies either increasing or decreasing, the yield of furfural changes. The effect of temperature on furfural production was investigated using furfural yield as the response. The range of temperature considered and designed were between 92.95 ℃ to 177.04 ℃. The catalytic effect of sulfuric acid concentration on the dehydration of the pentose's was evaluated with the acid concentration varied from 0.1M to 1.97 M of H2SO4. The reaction temperature is the most significant factor of all thermochemical conversion processes. This is because of the conversion of xylose to furfural requires high activation energy. However, excess high temperature leads to occurrences of side reactions. This is in also studied by (O'Neil et.al., 2009) who reported the stability of furfural was low and that it degraded quickly at high temperatures. Higher temperature and higher acid concentration may favor the hydrolysis of the dehydration of pentose's to furfural. It also could accelerate many side reactions, such as condensation of furfural with intermediates, degradation of furfural, esterification of cellulose with intermediate acids, and the polymerization of furfural with lignin (Guenic et.al., 2015). As shown in the Figures at low temperatures and low acid concentrations the yield of furfural is low compared to the yield of furfural at high acid concentrations and high temperatures. For instance, at a constant time condition at a temperature of 110 ℃ and acid concentration of 0.1 M, the corresponding furfural yield was 6.89%. But when the temperature and acid concentrations rises to 160 ℃ and 1.5 M the yield of furfural also rises to 50.1%. Therefore, the interaction between temperature and Acid concentration was significantly affecting the yield.

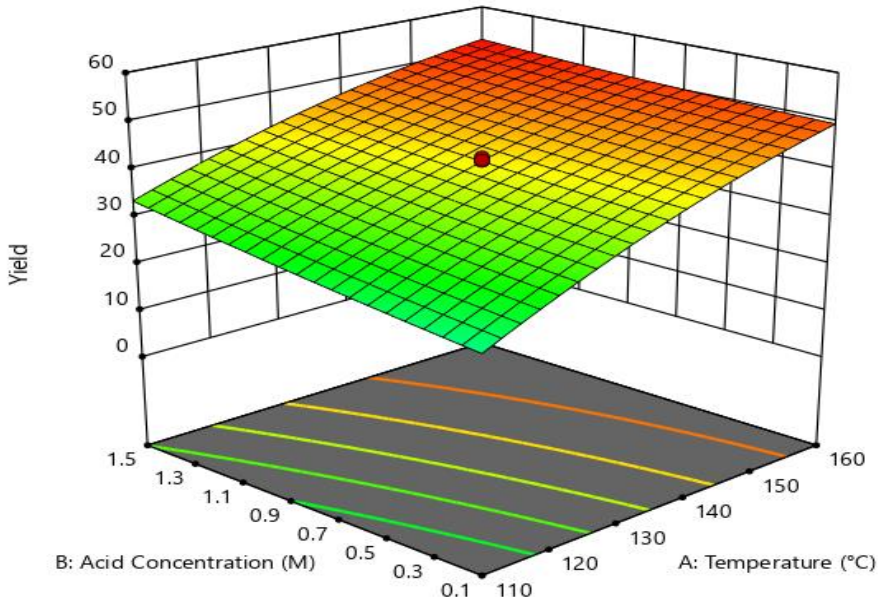

Figure 4-8 3D Plot of furfural yield against concentration versus temperature

B. Effect of Interaction between Temperature and Time (AC) on Yield

The effect of interaction between temperature and reaction time on furfural yield are depicted in Figure 4.9. It can be seen that the reaction temperature and time had a large effect on both the yield of furfural. The time range considered in this experimental work ranges from 30 – 90 min. When the reaction temperature was 110 ℃, with a 30 min reaction time, the furfural yield of 18.8 % was obtained. The furfural yield increased from 18.8% to 44.799% when conditions changed from 110 ℃ to 160 ℃ in a 90 min reaction time. As discussed before, by prolonging the reaction time and temperature it's possible to obtain high yield of furfural, but indeed long reaction time leads to furfural degradation and other side reactions, such as condensation or resinification of furfural. The reduction in furfural yield despite higher temperature and residence time can be attributed to the cross-linking and self-polymerization taking place simultaneously in the reaction which will cause the loss of furfural. Cross-polymerization reactions that occur between furfural and the intermediates of xylose-to-furfural result in the loss of furfural, while self-polymerization reactions in which case furfural reacts with itself. The consecutive condensation reactions result in undesired

by-products (Zeitsch, 2000). In general, the variations in furfural yield showed that the interaction between the temperature and time are significant, as also evidenced from the plot as it resembled to ellipse, the interaction effect of temperature and time had more effect on the yield of furfural compared to the interaction effect of temperature and acid concentration.

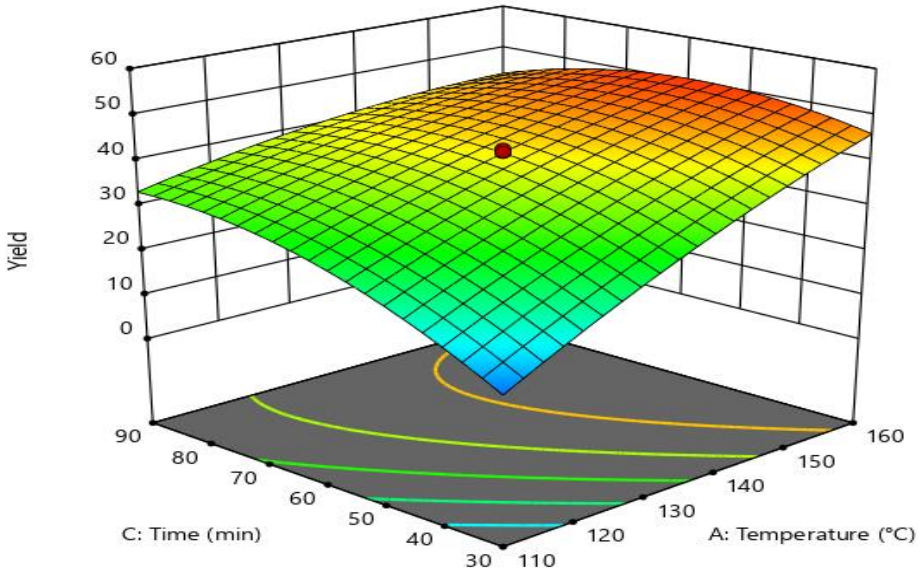

Figure 4-9 3D Plot of furfural yield against reaction time versus temperature.

C. Effect of Interaction between Acid concentration and Time (BC) on Yield

The effects of acid concentration and reaction time on yield of furfural is shown in Figure 4.13. As the acid concentration and reaction time vary either increasing or decreasing, the yield of furfural changes. From the Figure 4.10 it has been seen that at acid concentration of 0.1M and reaction time of 30 min the yield of furfural was 6.89%. As the acid concentration and time increases to 1.5M and 90 min respectively the yield of furfural was 37.8%, which shows a significant increment in the yield of furfural suggesting a strong interaction effect between the two variables.

The variations in furfural yield showed that the interactions between the variable parameters are significant, However the effect of acid concentration is not as much significant as the effect of reaction time, which is also studied by Shafeeq et.al. From this study furfural yield increases rapidly with increase in the concentration of acid up to 15%. However, when acid concentration is increased to 20%, the yield slightly decreases due to the difficulty of laboratory separation and side reaction of products. (Shafeeq et al., 2015)

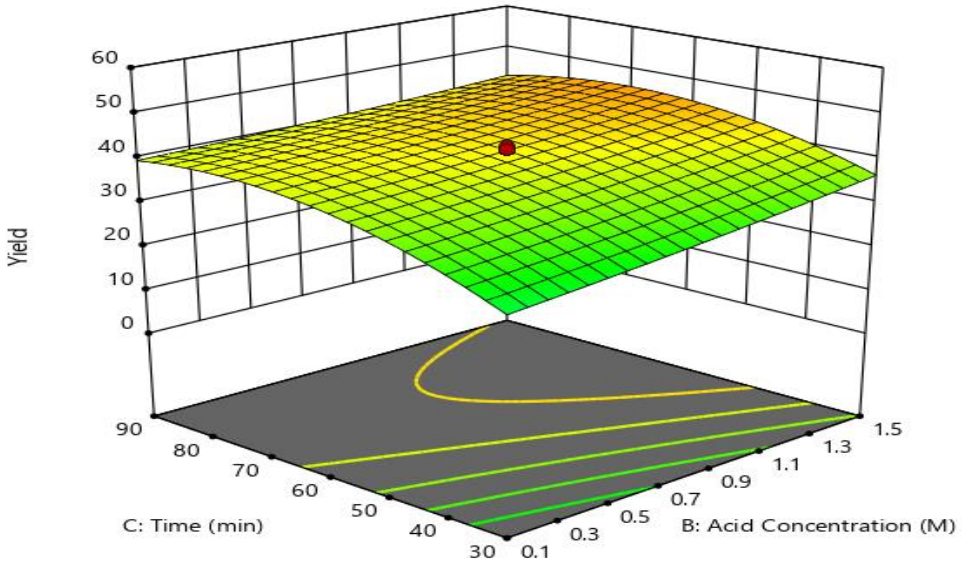

Figure 4-10 3D Plot of furfural yield against reaction time versus acid concentration.

4.3.8. Perturbation plot

The perturbation plot shows the comparison between the effect of all factors at a particular point in the design space. The response is plotted by changing only one factor over its range while holding all the other factors constant. The perturbation plot for furfural yield is shown in Figure 4.11. The yield of furfural was drawn by changing only one factor over its range while the other factors were held constant. As it has been discussed in the previous sections that the P-values for the main effects of all the factors considered in the synthesis of furfural were significant. However, perturbation plot can show the comparative effects of all the investigated independent variables on

the yield of furfural. The relatively flat line of Acid concentration shows lower effect of this factor on yield of furfural compared to the other factors in the design space. It can be seen from the perturbation plot that temperature and time had significant curvature effect. An increase in reaction temperature will favor the yield and possibly increase furfural yield up to 53.13% while increase in reaction time decrease furfural yield. From this observation, it can be said that reaction temperature has highest effects on furfural yield compared to the two factors. But it is obvious that a synergy of these three factors contributed significantly to the yield of furfural.

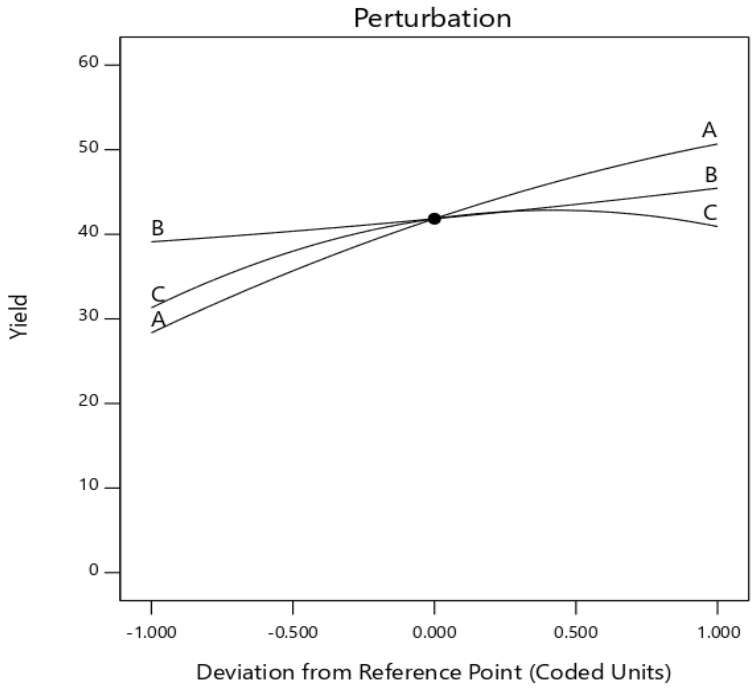

Figure 4-11 Perturbation plots of the main effects for furfural yield (A: Temperature, B: Acid Concentration and C: Time)

4.4 Optimization of process variables

The main goal of optimization of process variables for furfural production was to maximize economic benefit or increasing furfural yield by minimizing process cost. Determination of the optimum conditions for furfural yield was obtained by Design Expert 11.0.0 using numerical optimization. Multiple response method called desirability (D) function was used to maximize the desirability by finding out different combinations of independent process variables. As it has been discussed in the previous sections a prolonged reaction time will cause furfural degradation and

side reactions, while an increase in temperature causes an increment in yield of furfural along with increased energy cost. Therefore, optimizing process variables aimed to determine optimum conditions within the range of the values of the process variables by considering the constraints associated with variations of this process parameters.

Table 4.11 Constraints applied for optimization

Name	Goal	Lower Limit	Upper Limit
A:Temperature	is target = 150	92.5	177
B:Acid Concentration	is in range	0.1	1.5
C:Time	is target = 45	30	90
Yield	Maximize	6.89	53.13

The optimization feature of design expert software has found 23 best possible solutions that maximized the furfural yield. The values obtained for optimum production of furfural yield were: a temperature of 150 ℃, an Acid concentration of 1.5 M and reaction time of 45 min with 48.972% furfural yield. Desirability shows the interval between the response and its ideal value, and its value usually falls within the range 0–1. As can be seen from the optimum graph (Figure 4.12), the desirability value was 0.979, which is near to the ideal value of 1. Optimization of hydrolysis conditions is important for improving the efficiency of pentose to furfural conversion processes. The highest yield of furfural, 53.13 %, is obtained at a temperature of 177 ℃ and 60 min. This yield is obtained at expense of high energy cost and prolonged reaction time. However, the optimum yield of 48.972 is determined by the design expert software at a target temperature of 150 ℃ which can possibly ensure the requirement of optimum activation energy for the conversion of xylose to furfural. The target reaction time of 45 min also ensures the possible and sufficient residence time for conversion of xylose to furfural. The optimal yield with Possible combinations of factors that maximize the yield of furfural are listed in Appendix A5.

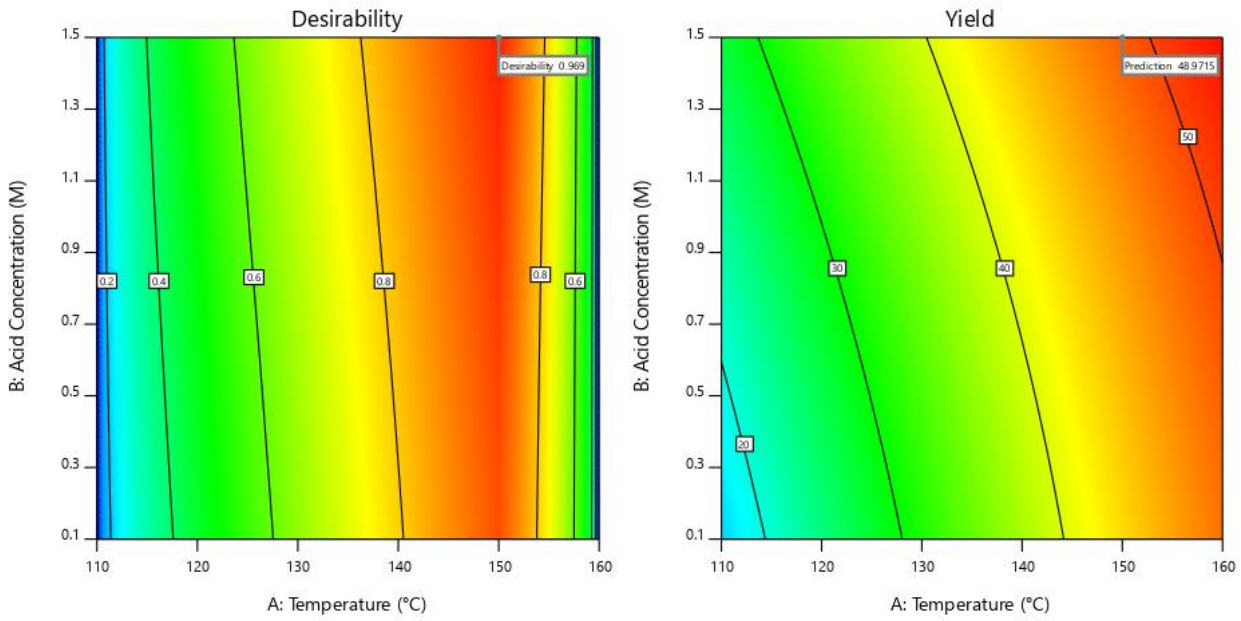

Figure 4-12 Optimized contour plot of Model Desirability

4.5 Model Validation

As determined by the CCD result using Design-Expert software, an experiment with optimized conditions of temperature 150 ℃, acid concentration 1.5 M, and time of 45 min was carried out. Furfural yield of 46.37 (average) obtained and was in good agreement with the predicted one. The results of the validated experiment show that 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 (2.6%) from the predicted and validated experiments could be attributed to experimental error (pure error) or lack of fit error.

4.6 Comparison of furfural yield

The maximum yield of furfural from microalgae in this study was 53.13 %. Ameh et al (2008) obtained 73.10% yield of furfural using a concentrated H_2SO_4 acid and various species of microalgae (*Closterium species*, *Scenedesmus Opoliensis*, *Cosmarium*, *Scenedesmus Obliquus*, *Scenedesmus bijuga Staurastrum* SPP, *Oscillatoria* SPP, *Spirulina*, *Merismopedia* and *Ankistrodesmus falcatus (Cyanobacteria)*). Jeon et.al., (2016) obtained 18.5 mol % yield of furfural from microalgae derived Alginic acid using Amberlyst-15 as a solid catalyst. (Martín & Grossmann, 2016) shows an industrial possibility of producing furfural from microalgae and

switch grass and come up with annual production capacity 9.3 MMgal/yr of furfural with a yield of 15%. In general, Although the methods and the procedures followed on this research is differ from the others, the result obtained in this study using dilute sulfuric acid shows a potential yield of furfural from microalgae.

4.7 Characterization of product

Important physicochemical characterization techniques were carried out for qualitative and quantitative determination of the product and the results are presented and discussed as follows:

4.7.1. Physical characterization of the product

The density, refractive index, color and physical appearance of the produced furfural is compared to the standard furfural as shown in the table 4.12 below. Pycnometer and refractometer are used to determine the density and refractive index of the produced product.

Table 4.12 Comparison for physical characterization (("Furfural - PubChem," 2020)*)*

Physical Property	The produced furfural	Standard furfural
Color	Reddish Brown	Appears as colorless to pale yellow or as a reddish-brown
Physical state	Liquid	Liquid
Odor	Penetrating odor	Characterizing almond like odor
Density	1.16	1.153-1.162
Refractive Index	1.519	1.52-1.529

4.7.2. FTIR spectroscopy Analysis of Furfural

FTIR analysis with a wave number range of 400 cm^{-1} to 4000 cm^{-1} was performed to detect information on the nature of the bonds and to identify different functional groups available on the furfural compound. Although the physical characterizations of the produced furfural have been done, the presence of furfural in the product can be confirmed by using FTIR Spectroscopy based on functional group similarities with pure furfural Infrared spectra. As it can be seen from Figure 2.2 and Figure 4.13, the structure of furfural is mainly characterized by the presence of aldehyde

group with C=O stretch, C=O, C=H stretch off and C=C, as well as aromatic ring furan consisting of C=C=H asymmetric stretch.

The Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of the produced furfural and its characteristic wavelength is shown in Fig. 4.13. Analysis of the spectrum of furfural is done according to the FTIR table found in the appendix A3.

The presence of a strong absorption at 1,670 cm^{-1} corresponds to the absorption of conjugated carbonyl (C=O) which occurs in conjugated aldehydes and not the ketone group. The C=O absorption is lower than the usual absorption of aldehyde due to internal hydrogen bonding which occurs in conjugated unsaturated aldehyde and conjugation lower the vibrational frequency of carbonyl compounds.

The presence of an aldehyde group and aliphatic C–H stretches was further proven by intensity bands attained at 2816 cm^{-1} and 2850 cm^{-1}. These absorption shows a medium intense stretching of aldehyde C-H (Kumar, 2006). The Strong peaks obtained at 1450 cm^{-1} and 1568 cm^{-1} shows the stretching of C=C from aromatic ring. The peaks at 1,050 cm^{-1} corresponds to the C–O stretching vibration (Ong & Sashikala, 2007). The aromatic ring and their derivatives are attributed to the C-H stretching are detected by spectral range of 880 ± 20 and 810 ± 20 which indicates the out of plane bending vibration. The presence of substituted aromatic groups is detected by the presence of peaks between 900–675 cm^{-1}.(Chan et al., 2018) In addition, there are various peaks in the liquid product which were not found in pure furfural standard. For instance, the strong and broad absorption peaks observed at a range of 1310–1390 cm^{-1} indicated the presence of phenolic groups in the sample. This indicated the diversity of the liquid products that were produced from the microalgae during the synthesis of furfural.

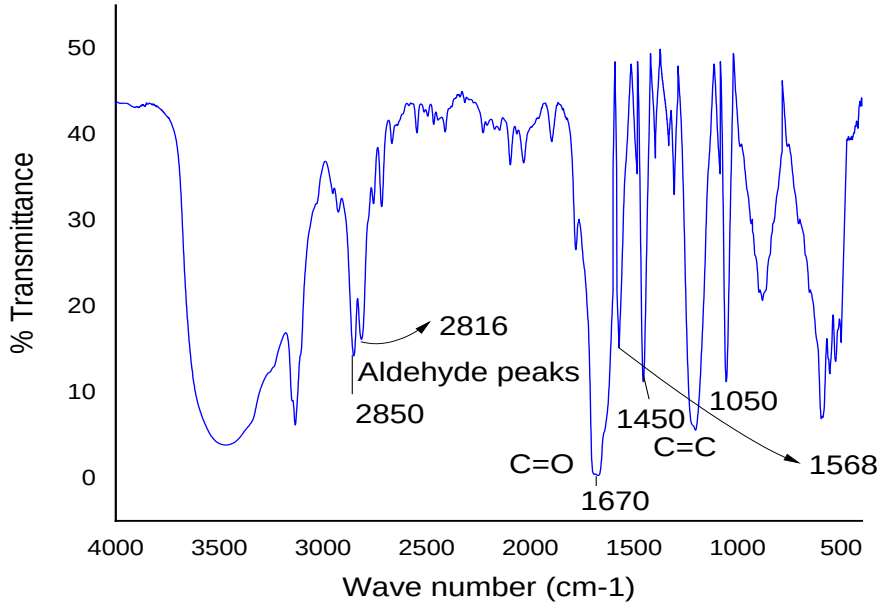

Figure 4-13 FTIR analysis of a product containing furfural

4.7.3. Furfural analysis using HPLC

Agilent 1100Series HPLC with UV detector was used to analyze and quantify furfural in the liquid product. Identification of the produced furfural using HPLC was carried out by comparing retention times(t_R), and the peak of a known standard furfural in the range of 50-300 ppm assay. The standard furfural sample is calibrated prior to the analysis of the produced furfural sample. The peak identification was based on the retention time and the ultraviolet spectrum was compared with the standard furfural. The matching between the occurrence of peaks at a retention time of the standard furfural and the component peak at the same retention time confirms the identity of the produced furfural.

Chromatogram figure 4.14 shows the peak and retention time of the standard furfural, while figure 4.15 shows the peak and the retention time of the produced furfural with respect to the standard furfural sample. The retention time is the time in which furfural took to travel through the column to the detector. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height. It is the characteristic of the furfural under the operating conditions.

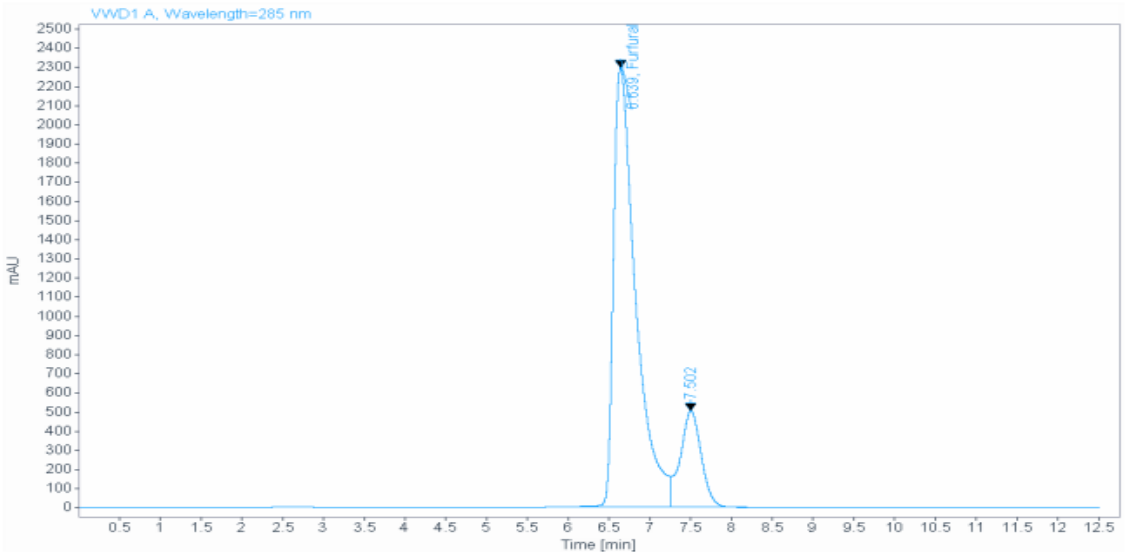

Figure 4-14 Chromatogram of standard furfural Solution

The Identification of the produced furfural were confirmed by comparing Chromatographic peak areas and the retention time of the produced furfural against the injection of a known amount of standard furfural. The average retention time of the standard furfural was 6.67 min. With this reference retention time the produced furfural is detected with a peak height of 617 and retention time of 6.67 min.

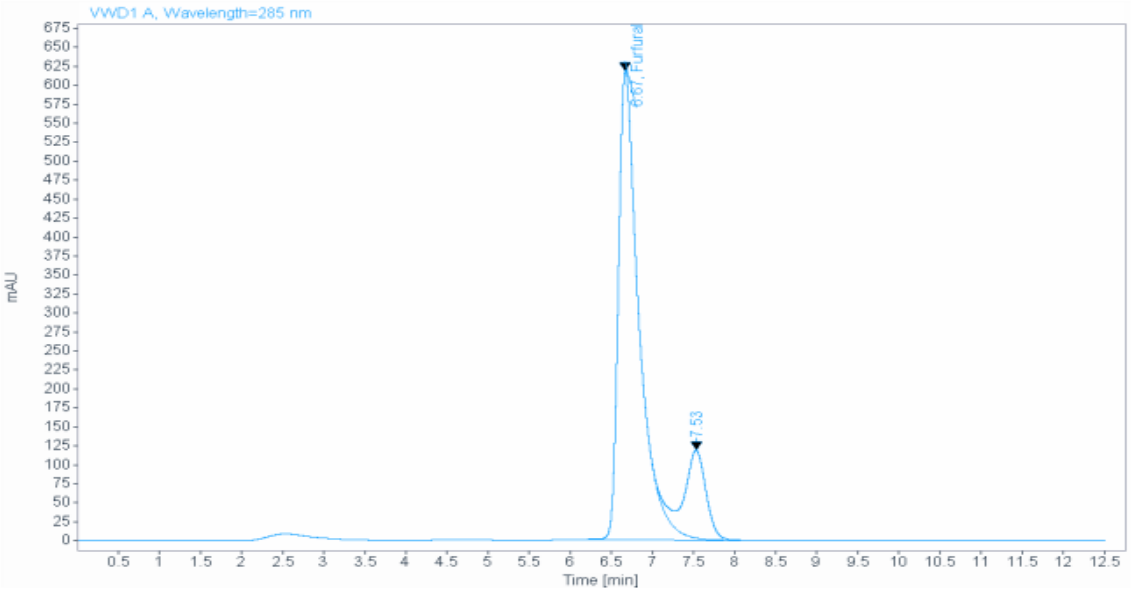

Figure 4-15 Chromatogram of produced furfural for peak and retention time identification

5. Conclusion and Recommendations

5.1 Conclusion

The objective of this thesis was to assess the possible ways for valorization of microalgae as a raw material for production of furfural. The possibility of producing furfural, a versatile platform chemical, from microalgae, which was unutilized biomass for synthesis of furfural, has been investigated throughout this study. Therefore, it has been concluded that algal biomass has a potential to be an alternative bio resource for the production of furfural.

From the compositional analysis carried out on particular microalgae species, it was observed that *mougotia scalaris* (>98%), *Spirogyra varians* and *Zygnema aplanosporum* species of microalgae feedstock from family of *Zygnemataceae* had sufficient hemicellulose content (29.68%) which is comparable to agricultural feedstock's such as Corn Cobs (30-32 %), Rice Husk (16-18%), Bagasse (25-27%) and Cotton Husk (27%) as reported by (Shafeeq et al., 2015) and hence it is a suitable alternative raw material for production of furfural. Furthermore, the hydrolysate of the algae revealed the presence of pentose sugars and it has been found a promising amount of xylose (12.89 g/100ml) which could be utilized for production of furfural

Also in this study, the furfural synthesis parameters from microalgae were investigated and optimized using the CCD RSM method. The study has shown that the experimental method can be used as an excellent tool to identify the interaction effect of the individual furfural synthesis parameters. Increasing temperature, acid concentration and residence time increases the yield of furfural; however, a temperature of 160 ℃, an Acid concentration of 1.5 M and reaction time of 54.355 min was found as optimum values.

The FT-IR analysis of the product showed that the presence of conjugated carbonyl C=O group at wave number of 1670 cm^{-1} which occurs in conjugated aldehydes. Besides the conjugated carbonyl group, Aldehyde peaks at 2816 cm^{-1} and 2850 cm^{-1} and aromatic ring with their derivatives at band intensity 880 ± 20 and 810 ± 20 conforms the presence of the desired functional groups. The produced furfural also further quantified by HPLC. The result showed that 36.38% Furfural is obtained in the final product.

5.2 Recommendations

Considering the results obtained in this research, the following recommendations are suggested for future works:

Although Sulfuric acid is the commonly used homogeneous catalyst in the production of furfural, due to its cost, effectiveness and availability, it poses an environmental problem during disposal. Since the residue from the production process is acidic, which causes a handling and disposal problem it is better either to find a suitable disposal technique or to use an alternative catalyst. Solid acid catalysts can be a good alternative to mineral acids, therefore it is recommended to study the synthesis of furfural from microalgae using heterogeneous catalysts. Also it would be far better if a green catalyst is synthesized from biomass for synthesis of furfural from microalgae.

The acid hydrolyzate analysis of the algae sample revealed the presence of sufficient hexose sugar beside the pentose's and it is recommended to study and utilize this sugars of algae for synthesis of valuable chemicals like acetone, HMF, Levulinic acid etc. which are products of hexoses.

The sole effect of sodium chloride salt on the dehydration of pentose's and yield of furfural have not been investigated in this research. It is recommended to study the effect of sodium chloride salt in the yield of furfural.

In this study it has been discovered that higher yields of furfural were achieved at higher temperatures. However, the highest temperature that was used on this research was 177 ℃, therefore it has been recommended to study the maximum temperature that yield higher furfural yield. This would be helpful in reducing the reaction time.

The Techno-economic feasibility of furfural synthesis from microalgae needs further investigation to confirm its advantages compared to recently used lignocellulosic raw materials.

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Appendices

Appendixes – A Characterization Results and Supportive Data

A1. HPLC Result of synthesized furfural

JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3.1	Page 1 of 1	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name:	Yeshidagna Nigussie	Test Report No:	051	
Contact Person:	Yeshidagna Nigussie	Report Date:	03/11/2020	
Sample Type:	Synthesized product	Test Request No:	Not Specified	
Sample Source:	Addis Ababa	Tel (Mob):	+251 910365975	
Sample Collected By:	Customer	Fax:	Not Specified	
Sample Collection Date:	Not Specified	E-mail:	Yeshidu2006@gmail.com	
Sample Receiving Date:	28/10/2020	Tested By:	LN-05	
Sample Condition:	Normal	Dates Tested:	31/10/2020	

S/N	Lab No.	Customer ID	Furfural (%)	Test Method
1	J-0275/2810/20		36.38	HPLC Method

Remark:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by

Name	Signature	Date
Henok Shiferaw		03/11/2020

Technical Signatory

Authorized by

Name	Signature	Date
Mulugeta Terefe		03/11/2020

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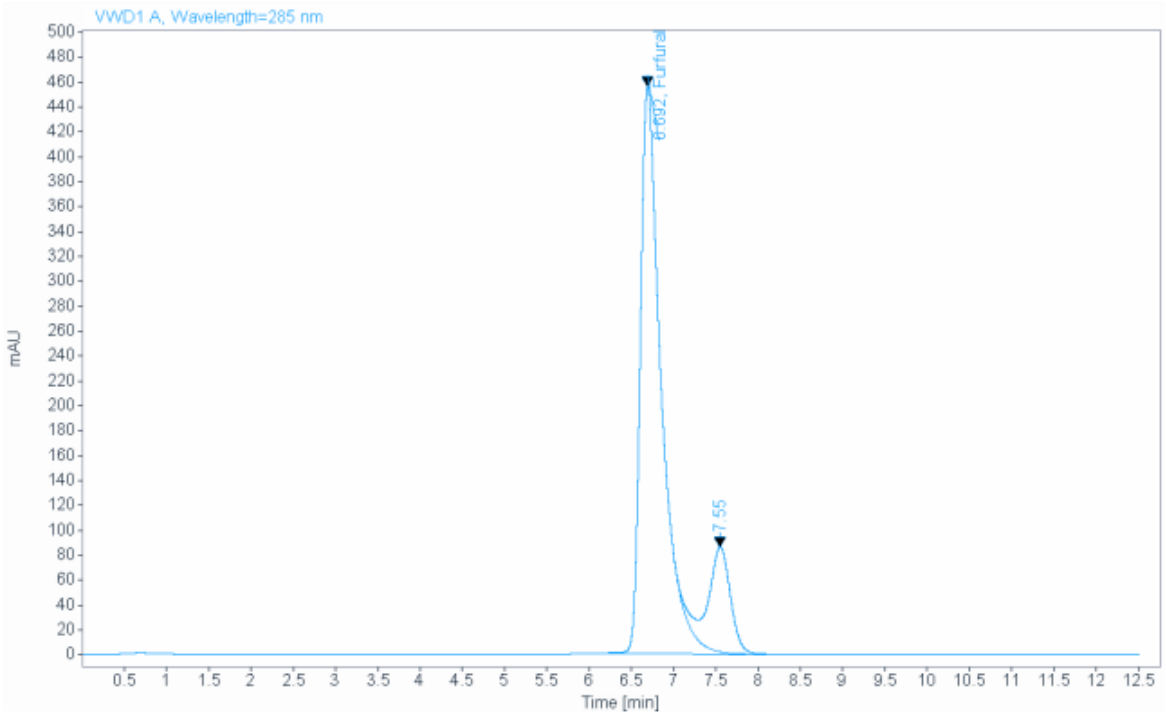
Web site: www.jijelaboglassplc.com
Physical Address: Nifas Silk Lafto Sub City, Woreda 01, Lebu, Foziana Building 6th Floor, Infront of Varnero Real Estate, Addis Ababa, Ethiopia.

HPLC Summary Report

Sequence name: Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25-11

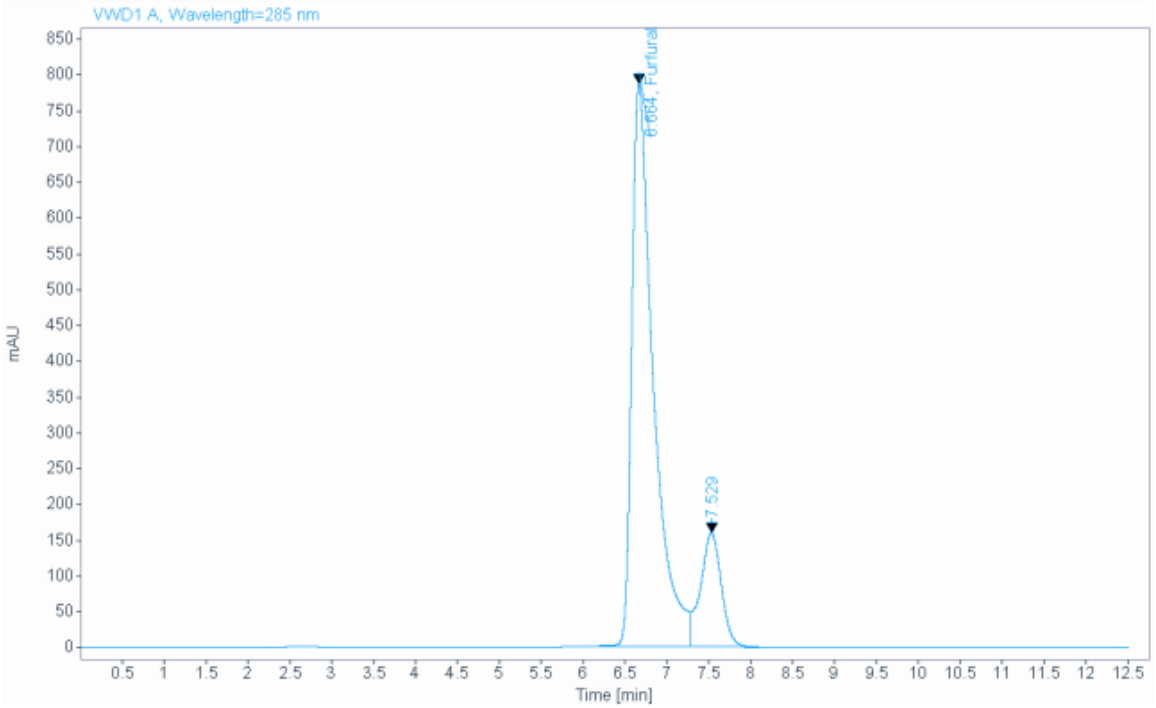
Agilent Technologies

Data file:	C:\Chem32\1\Data\furfural\Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25-11\001-0101.D		
Sample name:	50ppm Furfural		
Injection date:	10/31/2020 10:26:33 AM	**Sample type:**	Calibration
Acq. method:	Furfuran SB column.M	**Location:**	1
Analysis method:	Furfuran SB column.M	**Injection:**	1 of 1
Acq. operator:	SYSTEM	**Injection volume:**	20.000
Last changed:	10/31/2020 11:41:57 AM		



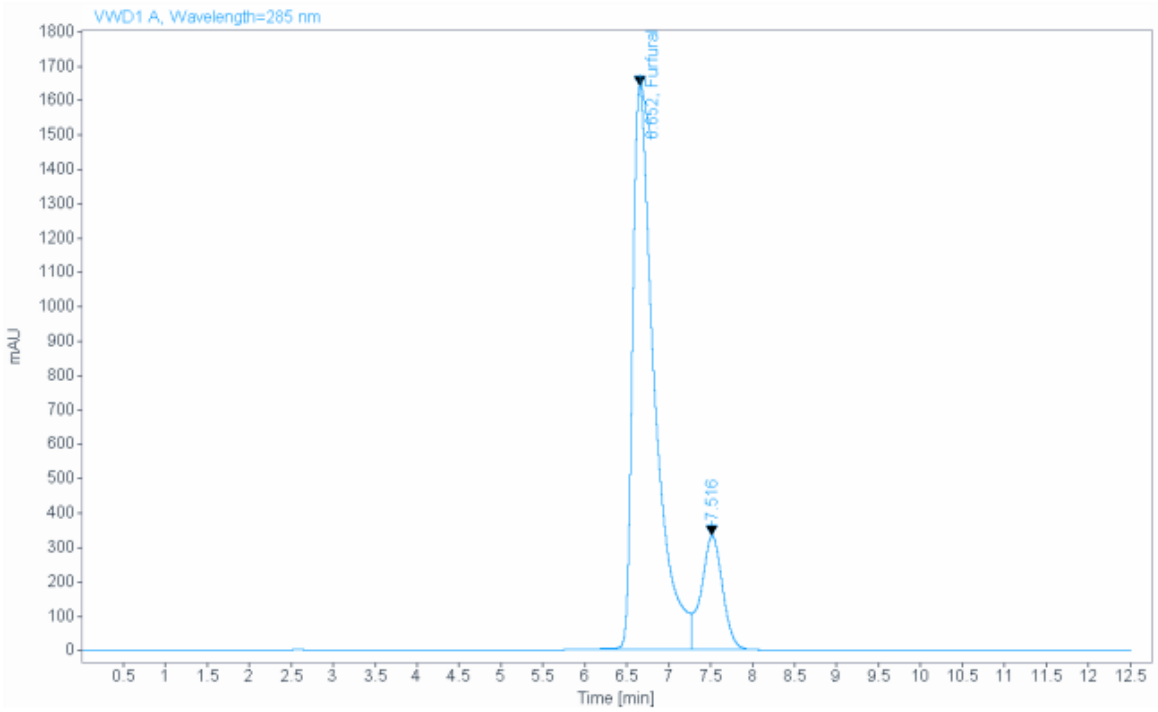
Agilent Technologies

Data file:	C:\Chem32\1\Data\furfural\Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25-11\002-0201.D		
Sample name:	100ppm Furfural		
Injection date:	10/31/2020 10:40:26 AM	**Sample type:**	Calibration
Acq. method:	Furfuran SB column.M	**Location:**	2
Analysis method:	Furfuran SB column.M	**Injection:**	1 of 1
Acq. operator:	SYSTEM	**Injection volume:**	20.000
Last changed:	10/31/2020 11:41:57 AM		



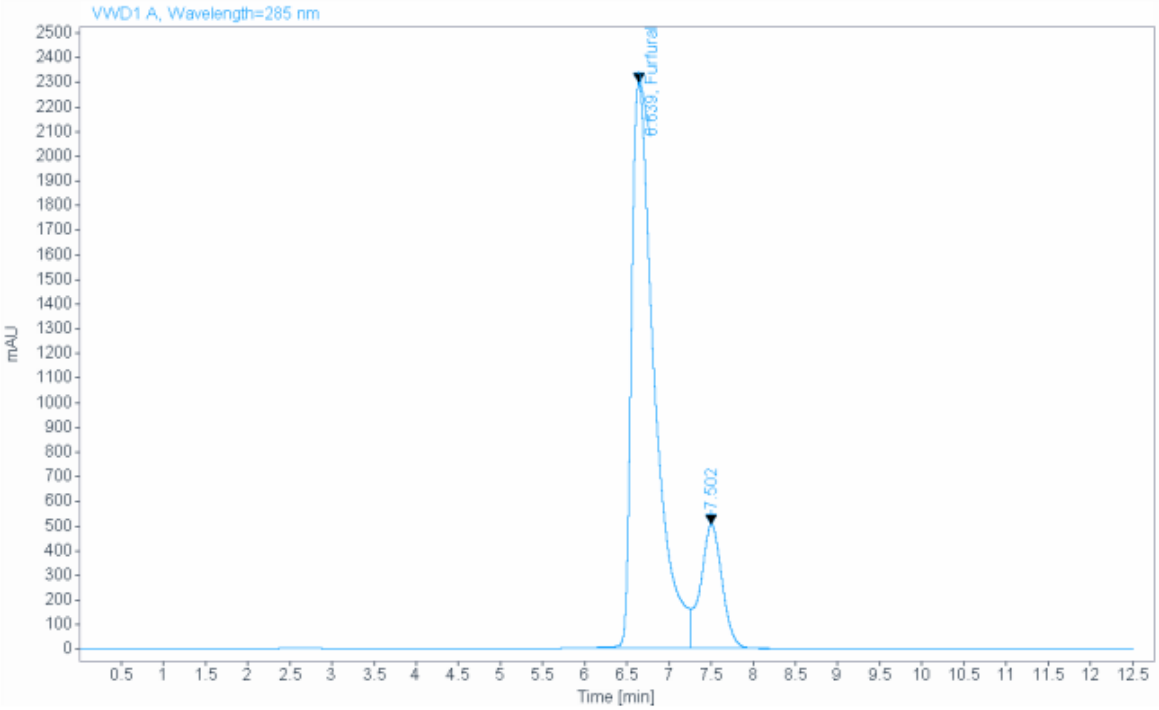
Agilent Technologies

Data file:	C:\Chem32\1\Data\furfural\Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25-11\003-0301.D		
Sample name:	200ppm Furfural		
Injection date:	10/31/2020 10:54:20 AM	**Sample type:**	Calibration
Acq. method:	Furfuran SB column.M	**Location:**	3
Analysis method:	Furfuran SB column.M	**Injection:**	1 of 1
Acq. operator:	SYSTEM	**Injection volume:**	20.000
Last changed:	10/31/2020 11:41:57 AM		



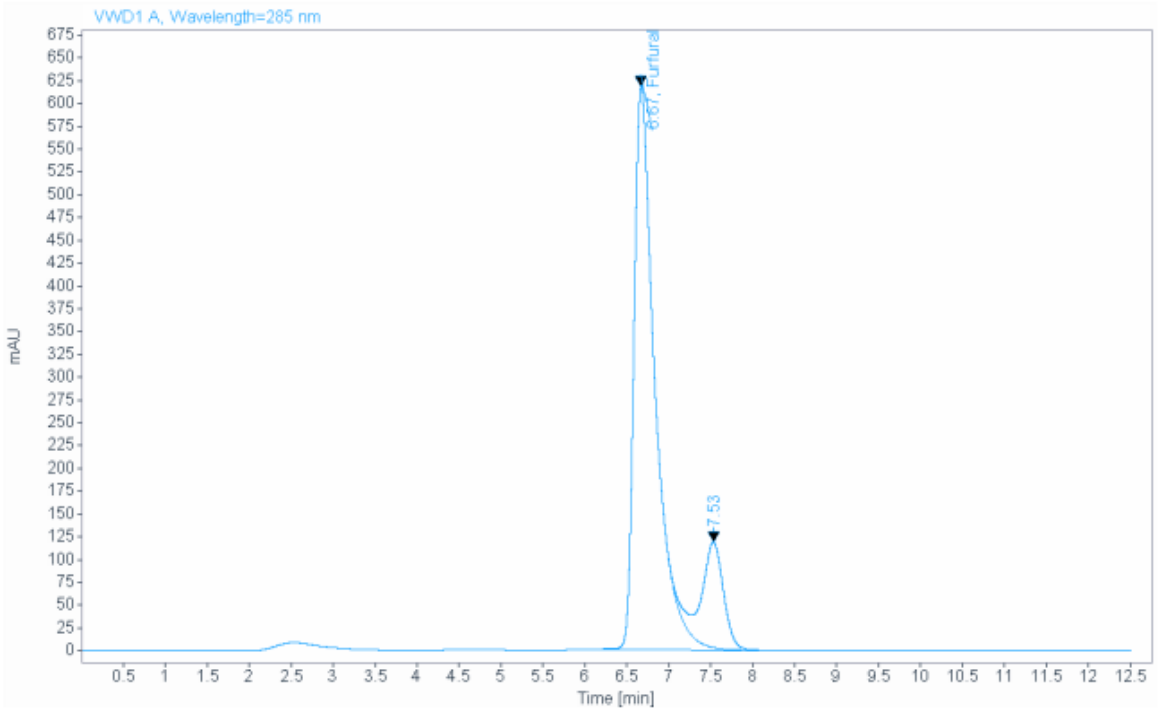
Agilent Technologies

Data file:	C:\Chem32\1\Data\furfural\Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25-11\004-0401.D		
Sample name:	300ppm Furfural		
Injection date:	10/31/2020 11:08:14 AM	**Sample type:**	Calibration
Acq. method:	Furfuran SB column.M	**Location:**	4
Analysis method:	Furfuran SB column.M	**Injection:**	1 of 1
Acq. operator:	SYSTEM	**Injection volume:**	20.000
Last changed:	10/31/2020 11:41:57 AM		



Agilent Technologies

Data file:	C:\Chem32\1\Data\furfural\Furfural J-0275-2810-20 30-10-2020 2020-10-31 10-25 -11\275.D		
Sample name:	J-0275-2810-20		
Injection date:	10/31/2020 11:22:10 AM	**Sample type:**	Sample
Acq. method:	Furfuran SB column.M	**Location:**	5
Analysis method:	Furfuran SB column.M	**Injection:**	1 of 1
Acq. operator:	SYSTEM	**Injection volume:**	20.000
Last changed:	10/31/2020 11:41:57 AM		



Agilent Technologies

Calibration sample statistics:

Compound: Furfural **Signal:** VWD1 A, Wavelength=285 nm

Line#	Location	Inj#	Type	RT [min]	Amount	Unit	Area	Height	Sample Name
1	1	1	BV R	6.692	52.27744	mg/l	7880.86963	455.36560	50ppm Furfural
2	2	1	BV	6.664	95.34592	mg/l	14078.22949	785.96863	100ppm Furfural
3	3	1	BV	6.652	203.61455	mg/l	29657.59961	1639.03638	200ppm Furfural
4	4	1	BV	6.639	298.76209	mg/l	43348.90234	2292.39795	300ppm Furfural
			Mean	6.662	162.50000		23741.4002	1293.19214	
			StdDev	0.0222	110.93008		15962.3395	832.11567	
			RSD	0.334	68.26466		67.23420	64.34587	

Agilent Technologies

Sample statistics:

Compound: Furfural **Signal:** VWD1 A, Wavelength=285 nm

Line#	Location	Inj#	Type	RT [min]	Amount	Unit	Area	Height	Sample Name
5	5	1	BV R	6.670	72.76362	mg/l	10828.73828	617.86304	J-0275-2810-20
			Mean	6.670	72.76362		10828.7382	617.86304	
			StdDev	0				0.00000	
			RSD	0.000	0.00000		0.00000	0.00000	

A2. HPLC Result of the hydrolyzate

ECAE	የኢትዮጵያ የተስማሚነት ምዘና ድርጅት Ethiopian Conformity Assessment Enterprise	Document No. TLD/F7.08-1 Copy No: - Rev No: 0
Title:	TEST REPORT ይህ የናሙና ፍተሻ ሪፖርት እንጂ ሰርተፍኬት አይደለም	Page No: 1 of 1 Effective Date: 30 Sep 19

Name and address of client:	Yeshidagna Nugussie, Addis Ababa	Test Report No:	ATR /0313/13
Tel:	+251-910-36-59-75	Test Order No:	----
Fax:	---	Reported date:	14/12/2020
E-mail:	Yeshidu2006@gmail.com	Date of sampling:	Not specified
Date sample Received:	27/11/2020	Place of sampling:	Not specified
Client Sample code:	----	Sampled and submitted by:	Client
Type of sample:	Hydrolysate	Date tested:	02-11/12/2020
Lab Designated number:	13078050	Specification/Test Method:	ES 1202:2018

S/N	Characteristics tested	Specification/ Test Method	Standard Requirements			Test result	Comment
			Min	Nom	Max		
1.	Xylose, g/100ml	CTL/SOP/M031.01				12.89	-
2.	Mannose, g/100ml	CTL/SOP/M031.01				< 0.5	-
3.	Arabinose, g/100ml	CTL/SOP/M031.01				< 0.5	-
4.	Glucose, g/100ml	CTL/SOP/M031.01				32.2	-
5.	Galactose, g/100ml	CTL/SOP/M031.01				< 0.5	-

Remark

1. This test report relates only to the specific sample product which has been tested by ECAE testing laboratory.

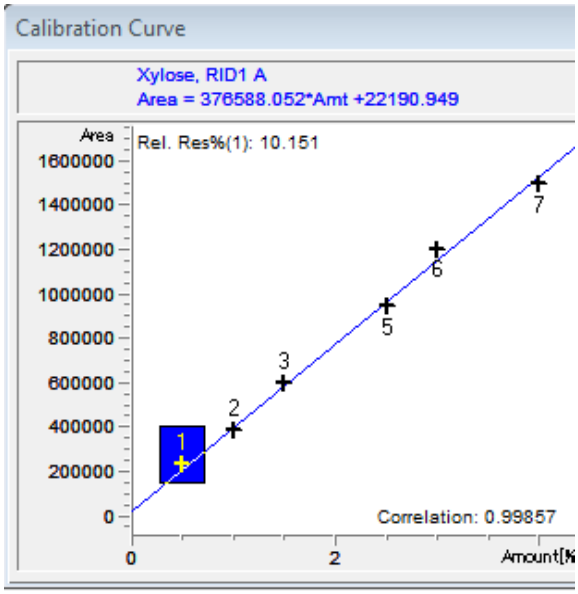
Test report authorized by, Name Metiku Dirba Position Team leader Sign. for

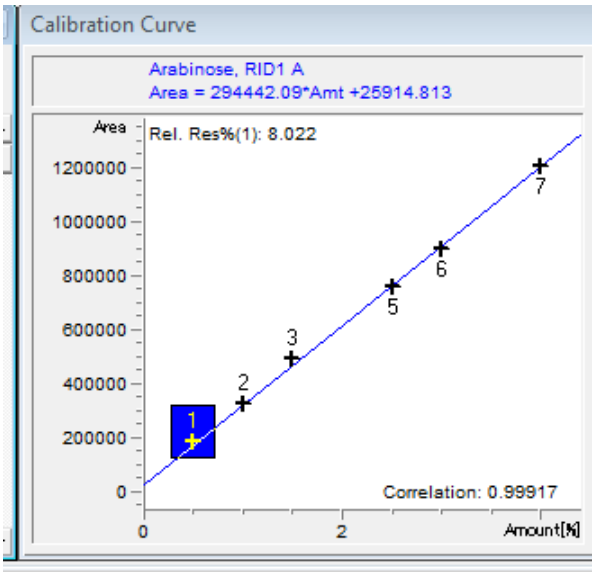
✉ 11145 ☎ 011 6 46-05-69, Fax. 011 6 45-97-20, E-mail info-cs@eca-e.com Web site: www.eca-e.com
BOLE SUBCITY, WOREDA 6, ADDIS ABABA, ETHIOPIA

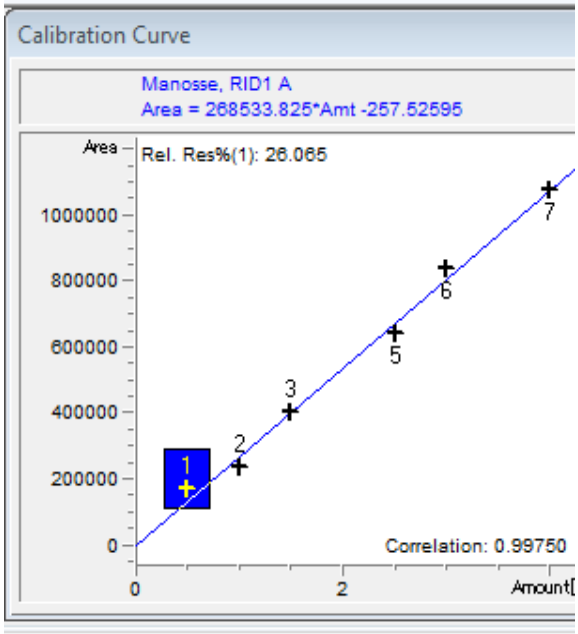
Calibration Table

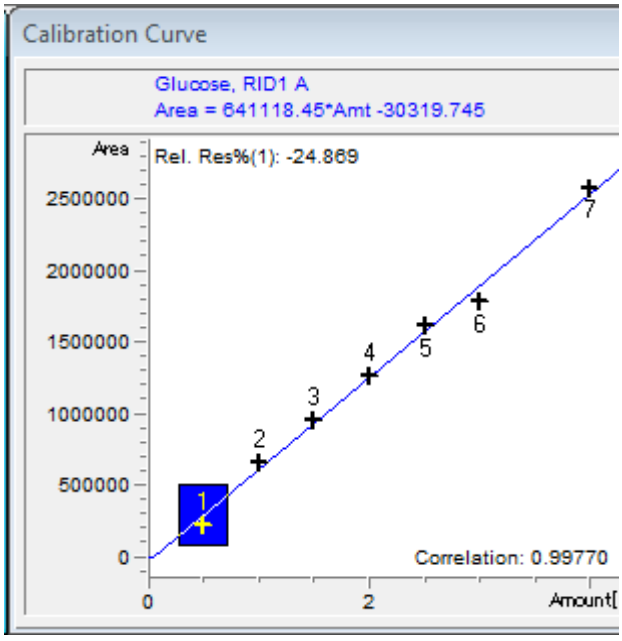
Enter | Delete | Insert... | Print | OK | Help

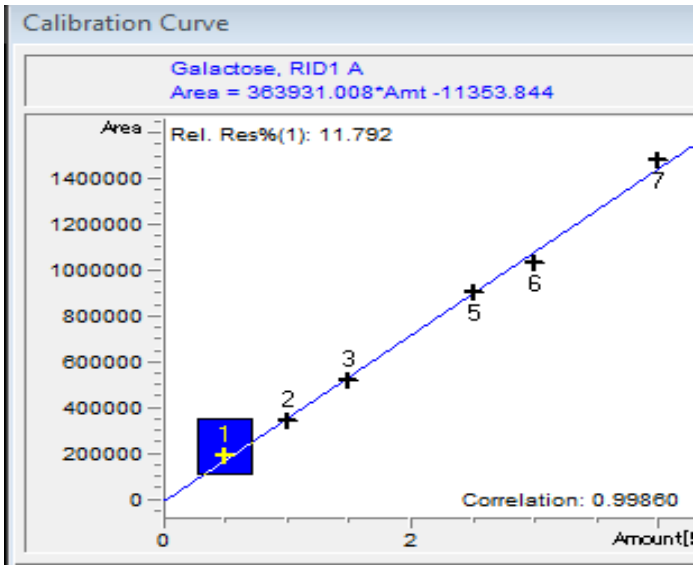
#	From	To	RT	Signal	Compound	Lvl	Amt[%]	Area	Rsp.Factor
1	4.116	4.327	4.222	RID1 A	Xylose	1	0.500	231850.000	2.1566e-6
						2	1.000	389410.000	2.5680e-6
						3	1.500	595300.000	2.5197e-6
						5	2.500	943570.000	2.6495e-6
						6	3.000	1.2036e6	2.4925e-6
						7	4.000	1.4990e6	2.6685e-6
2	4.597	4.832	4.714	RID1 A	Arabinose	1	0.500	187030.000	2.6734e-6
						2	1.000	324140.000	3.0851e-6
						3	1.500	493600.000	3.0389e-6
						5	2.500	757690.000	3.2995e-6
						6	3.000	894370.000	3.3543e-6
						7	4.000	1.2051e6	3.3192e-6
3	5.118	5.380	5.249	RID1 A	Manosse	1	0.500	168940.000	2.9596e-6
						2	1.000	233640.000	4.2801e-6
						3	1.500	401080.000	3.7399e-6
						5	2.500	639060.000	3.9120e-6
						6	3.000	837640.000	3.5815e-6
						7	4.000	1.0745e6	3.7226e-6
4	5.275	5.546	5.411	RID1 A	Glucose	1	0.500	218060.000	2.2929e-6
						2	1.000	651020.000	1.5360e-6
						3	1.500	946490.000	1.5848e-6
						4	2.000	1.2598e6	1.5876e-6
						5	2.500	1.6164e6	1.5467e-6
						6	3.000	1.7843e6	1.6813e-6
						7	4.000	2.5776e6	1.5518e-6
5	5.637	5.926	5.782	RID1 A	Galactose	1	0.500	190730.000	2.6215e-6
						2	1.000	340810.000	2.9342e-6
						3	1.500	519310.000	2.8885e-6
						5	2.500	901090.000	2.7744e-6
						6	3.000	1.0341e6	2.9012e-6
						7	4.000	1.4837e6	2.6960e-6


Calibration Curve
Xylose, RID1 A
Area = 376588.052*Amt +22190.949
Area
Rel. Res%(1): 10.151
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
1
2
3
5
6
7
Correlation: 0.99857
0
2
Amount[


Calibration Curve
Arabinose, RID1 A
Area = 294442.09*Amt +25914.813
Area
Rel. Res%(1): 8.022
1200000
1000000
800000
600000
400000
200000
0
1
2
3
5
6
7
Correlation: 0.99917
0
2
Amount[%]

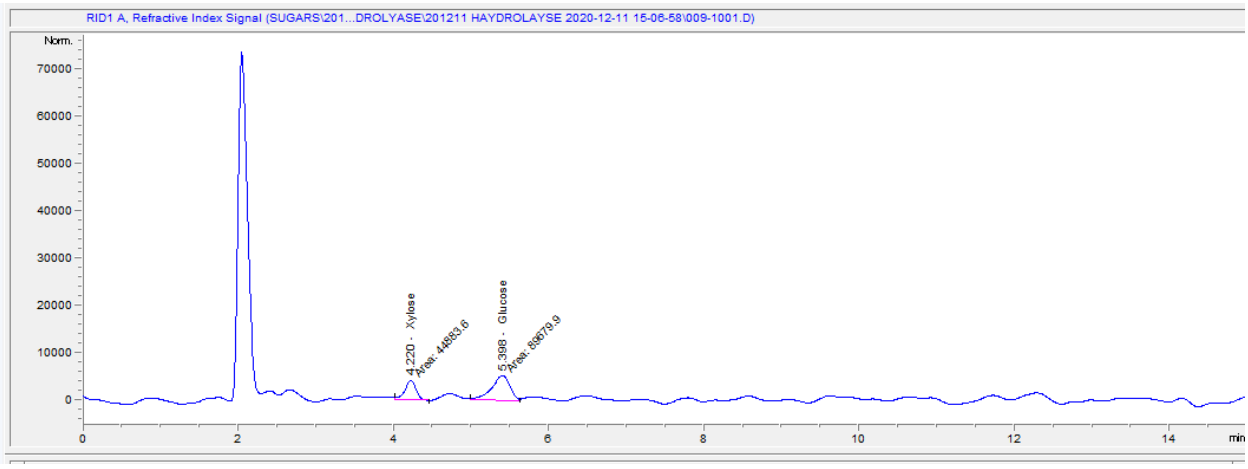

Calibration Curve
Manosse, RID1 A
Area = 268533.825*Amt -257.52595
Area
Rel. Res%(1): 26.065
1000000
800000
600000
400000
200000
0
1
2
3
5
6
7
Correlation: 0.99750
0
2
Amount[


Calibration Curve
Glucose, RID1 A
Area = 641118.45*Amt -30319.745
Area
Rel. Res%(1): -24.869
2500000
2000000
1500000
1000000
500000
0
1
2
3
4
5
6
7
Correlation: 0.99770
0
2
Amount[

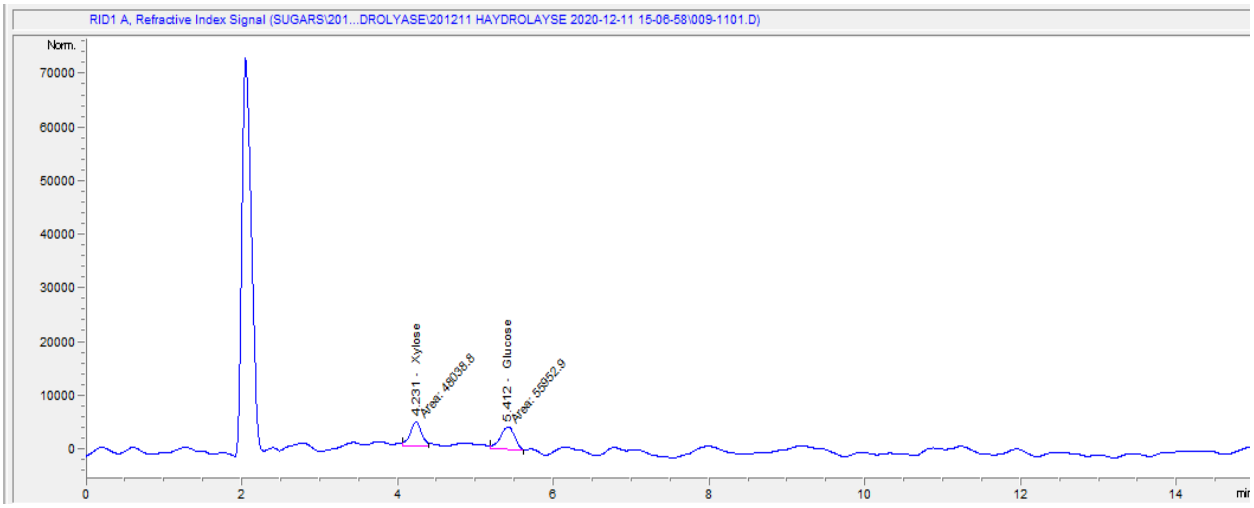


Chromatogram

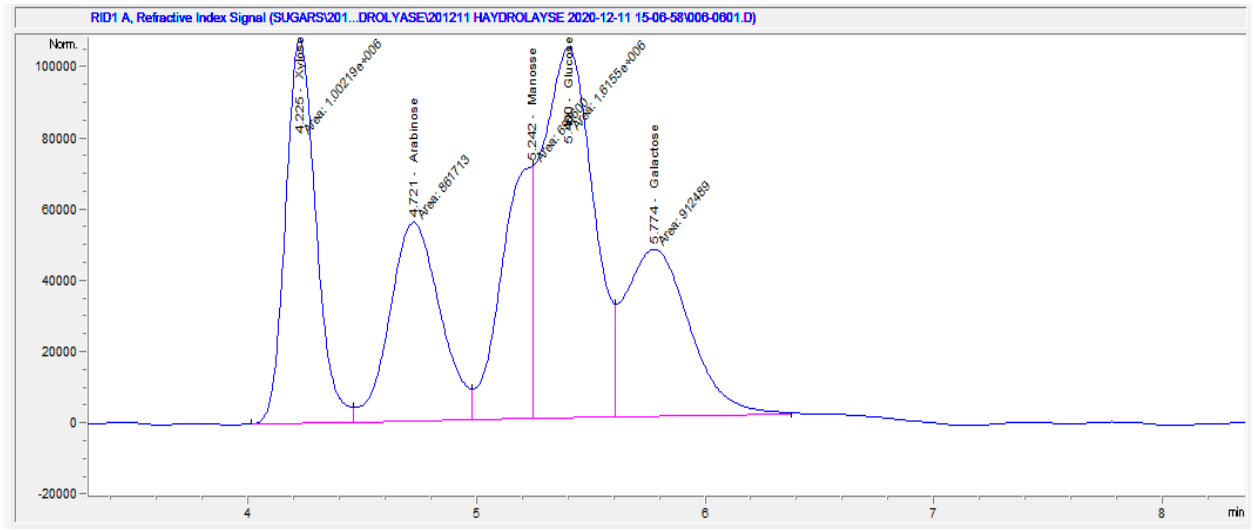
Sample A



Sample B



2.5% Sugar standard mixture (Xylose, Arabinose, Mannose, Glucose and Galactose)



A3. Characteristic IR absorption frequencies of organic functional groups

Absorption (cm^{-1})	**Appearance**	**Group**	**Compound Class**
3700-3584	medium, sharp	O-H stretching	Alcohol
3550-3200	strong, broad	O-H stretching	Alcohol
3500	Medium	N-H stretching	primary amine
3400-3300	Medium	N-H stretching	aliphatic primary amine
3350-3310	Medium	N-H stretching	secondary amine
3300-2500	strong, broad	O-H stretching	carboxylic acid
3200-2700	weak, broad	O-H stretching	Alcohol
3000-2800	strong, broad	N-H stretching	amine salt
3333-3267	strong, sharp	C-H stretching	Alkyne
3100-3000	Medium	C-H stretching	Alkene
3000-2840	Medium	C-H stretching	Alkane
2830-2695	Medium	C-H stretching	Aldehyde
2600-2550	Weak	S-H stretching	Thiol
2349	Strong	O=C=O stretching	carbon dioxide
2275-2250	strong, broad	N=C=O stretching	Isocyanate
2260-2222	Weak	CΞN stretching	Nitrile
2260-2190	Weak	CΞC stretching	Alkyne
2175-2140	Strong	S-CΞN stretching	Thiocyanate
2160-2120	Strong	N=N=N stretching	Azide
2150		C=C=O stretching	Ketene
2145-2120	Strong	N=C=N stretching	Carbodiimide
2140-2100	Weak	CΞC stretching	Alkyne
2140-1990	Strong	N=C=S stretching	Isothiocyanate
2000-1900	medium	C=C=C stretching	Allene
2000		C=C=N stretching	Ketenimine
2000-1650	Weak	C-H bending	aromatic compound
1818	Strong	C=O stretching	Anhydride
1815-1785	Strong	C=O stretching	acid halide
1800-1770	Strong	C=O stretching	conjugated acid halide
1775	Strong	C=O stretching	conjugated anhydride
1770-1780	Strong	C=O stretching	vinyl / phenyl ester
1760	Strong	C=O stretching	carboxylic acid
1750-1735	Strong	C=O stretching	Esters

1750-1735	Strong	C=O stretching	δ-lactone
1745	Strong	C=O stretching	Cyclopentanone
1740-1720	Strong	C=O stretching	Aldehyde
1730-1715	Strong	C=O stretching	α, β-unsaturated ester
1725-1705	Strong	C=O stretching	aliphatic ketone
1720-1706	Strong	C=O stretching	carboxylic acid
1710-1680	Strong	C=O stretching	conjugated acid
1710-1685	Strong	C=O stretching	conjugated aldehyde
1690	Strong	C=O stretching	primary amide
1690-1640	medium	C=N stretching	imine / oxime
1685-1666	Strong	C=O stretching	conjugated ketone
1680	Strong	C=O stretching	secondary amide
1680	Strong	C=O stretching	tertiary amide
1650	Strong	C=O stretching	δ-lactam
1678-1668	Weak	C=C stretching	Alkene
1675-1665	Weak	C=C stretching	Alkene
1675-1665	Weak	C=C stretching	Alkene
1662-1626	medium	C=C stretching	Alkene
1658-1648	medium	C=C stretching	Alkene
1650-1600	medium	C=C stretching	conjugated alkene
1650-1580	medium	N-H bending	Amine
1650-1566	medium	C=C stretching	cyclic alkene
1648-1638	Strong	C=C stretching	Alkene
1620-1610	Strong	C=C stretching	α, β-unsaturated ketone
1550-1500	Strong	N-O stretching	nitro compound
1465	medium	C-H bending	Alkane
1450	medium	C-H bending	Alkane
1390-1380	medium	C-H bending	Aldehyde

1385-1380	medium	C-H bending	Alkane
1440-1395	medium	O-H bending	carboxylic acid
1420-1330	medium	O-H bending	Alcohol
1415-1380	Strong	S=O stretching	Sulfate
1410-1380	Strong	S=O stretching	sulfonyl chloride
1400-1000	Strong	C-F stretching	fluoro compound
1390-1310	medium	O-H bending	Phenol
1372-1335	Strong	S=O stretching	Sulfonate
1370-1335	Strong	S=O stretching	Sulphonamide
1350-1342	Strong	S=O stretching	sulfonic acid
1350-1300	Strong	S=O stretching	Sulfone
1342-1266	Strong	C-N stretching	aromatic amine
1310-1250	Strong	C-O stretching	aromatic ester
1275-1200	Strong	C-O stretching	alkyl aryl ether
1250-1020	medium	C-N stretching	Amine
1225-1200	Strong	C-O stretching	vinyl ether
1210-1163	Strong	C-O stretching	Ester
1205-1124	Strong	C-O stretching	tertiary alcohol
1150-1085	Strong	C-O stretching	aliphatic ether
1124-1087	Strong	C-O stretching	secondary alcohol
1085-1050	strong	C-O stretching	primary alcohol
1070-1030	strong	S=O stretching	Sulfoxide
1050-1040	strong, broad	CO-O-CO stretching	Anhydride
995-985	strong	C=C bending	Alkene
980-960	strong	C=C bending	Alkene
895-885	strong	C=C bending	Alkene
850-550	strong	C-Cl stretching	halo compound
840-790	medium	C=C bending	Alkene
730-665	strong	C=C bending	Alkene
690-515	strong	C-Br stretching	halo compound
600-500	strong	C-I stretching	halo compound

880 ± 20	strong	C-H bending	1,2,4-trisubstituted
880 ± 20	strong	C-H bending	1,3-disubstituted
810 ± 20	strong	C-H bending	1,4-disubstituted or
780 ± 20	strong	C-H bending	1,2,3-trisubstituted
755 ± 20	strong	C-H bending	1,2-disubstituted

A4**.** Standard calibration curve for furfural.

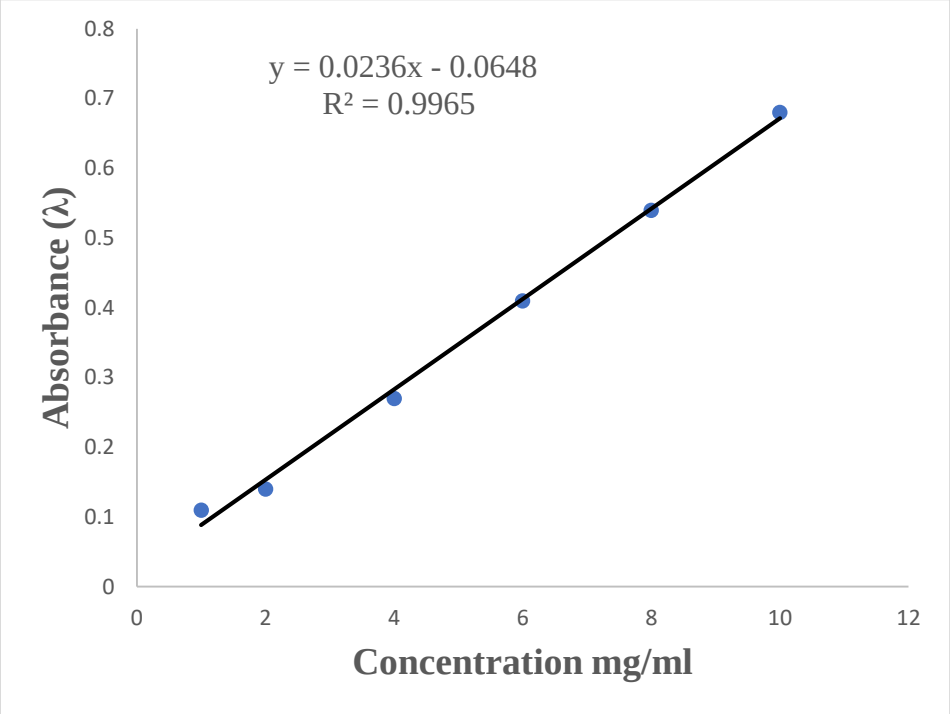


Table A5. Design Expert Desirability Solution table

Number	Temperature	Acid Concentration	Time	Yield	Desirability	
1	150.000	1.500	45.000	48.972	0.969	Selected
2	150.000	1.491	45.000	48.923	0.969	--
3	150.000	1.478	45.000	48.853	0.968	--
4	150.000	1.500	45.236	49.015	0.968	--
5	149.783	1.500	45.000	48.888	0.967	--
6	149.990	1.500	45.465	49.053	0.966	--
7	150.000	1.425	45.000	48.568	0.966	--
8	149.762	1.500	45.281	48.932	0.965	--
9	150.000	1.457	45.472	48.827	0.965	--
10	150.000	1.500	45.843	49.124	0.964	--
11	150.000	1.358	45.000	48.218	0.963	--
12	150.000	1.500	46.069	49.163	0.963	--
13	150.000	1.305	45.000	47.950	0.961	--
14	150.000	1.451	46.116	48.913	0.961	--
15	149.598	1.500	45.819	48.967	0.960	--
16	149.152	1.500	45.000	48.641	0.960	--
17	150.000	1.500	47.000	49.316	0.957	--
18	150.000	1.500	44.476	48.872	0.957	--
19	148.835	1.496	45.000	48.493	0.956	--
20	150.553	1.500	44.881	49.162	0.950	--
21	150.000	0.889	45.000	45.995	0.946	--
22	150.000	0.764	45.000	45.473	0.941	--
23	150.000	0.728	45.000	45.326	0.940	--

Appendix B: Laboratory equipment and sample photos

Blooming Charophyta green algae

Milled and dried algae

Extractives Determination

Compositional Analysis

Batch distillation

Rotary Evaporator

Furnace for Proximate Analysis

Density Determination with Pycnometer

UV Vis Spectophotometer for Absorbance measurement

Reflective Index measurement

Synthesized furfural product

Annex

❖ Moisture Content Determination

Algal biomass samples were weighed into ceramic crucibles and dried in an oven at 60 ℃ at atmospheric pressure until all water was evaporated and no further loss of weight was observed. The crucible was covered and weighed after reaching room temperature. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$Moisture\ content\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where:

W_1= Initial weight of algal biomass

W_2= weight after drying

❖ Ash Content Determination

A crucible was weighed empty, and then the algal samples were put in it. The sample and the crucible were placed in a muffle furnace for 4 hours at 600 ℃. The crucible was removed from furnace and placed in a desiccator to cool, then was reweighed. This procedure was followed by 1hr heating at 600 ℃ until sample mass varies by less than 0.3 mg from previous weighing

$$\%\ Ash = \frac{weight\ of\ ash}{weight\ of\ sample} * 100 = \frac{W_1 - W_2}{W_1} * 100$$

Where:

W_1= Original weight of the sample

W_2 = Weight of sample after ashing

❖ Volatile matter Determination

The total volatile component present in the algae is determined using a Furnace. A crucible was weighed empty, and then samples were put in it. The sample and the crucible were placed in a muffle furnace for 7 min at 900 ℃. The crucible was removed from furnace and placed in a desiccator to cool, then reweighed The amount of volatile matter in the algae was expressed in percent in the following way:

$$VM = \frac{W_1 - W_2}{W_1} * 100$$

- **Solvent extractives determination**

15g of oven dried algal biomass was loaded into a Soxhlet extraction tube using a suitable handling filter paper. 600 mL of mixture of toluene ethanol (C_7H_8 – C_2H_5OH) solvent in a ratio of 2:1 was used as solvent for extraction for 6 hours of refluxing run period. After extraction, the sample was air dried at room temperature for few minutes. Constant weight of the extracted material was achieved in an oven at 80 ℃. The % (w/w) of the extractives content was evaluated as the difference in weight between the raw extractive-laden biomass and extractive-free biomass.

$$\% \text{ Extractives} = \frac{W_1 - W_2}{W_1} * 100$$

Where;

W_1 = oven dried sample

W_2 = extractive free residue

- **Hemicellulose content determination**

Into a 1000 mL conical flask, 3 g of oven dried biomass was transferred and 450 mL of 0.5M NaOH was added. The mixture was boiled for 3.5 h. It was filtered after cooling through vacuum filtration and washed until neutral pH. The residue was dried to constant weight at 105°C in a convection oven. The difference between the sample weight before and after this treatment is the hemicelluloses content (% w/w) of dry biomass (Sluiter, 2008)

$$\% \text{ Hemicellulose} = \frac{\text{weight of Extractive free sample} - \text{weight of extracted cell wall residue}}{\text{weight of extractive free sample}} * 100$$

❖ **Lignin content determination**

In glass test tubes, 3 g of oven dried raw biomass was weighed and 10 mL of 72% H_2SO_4 was added. The sample was kept at room temperature for 2.5 h with careful shaking at 30 min intervals to allow complete hydrolysis. After the initial hydrolysis, a distilled water was added to dilute the solution into a 4% acid concentration. The second step was hydrolysis which made to occur in an autoclave for 1 h at 121 ℃. The slurry was then cooled at room temperature. Hydrolyzate was filtered using a vaccum pump with a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105 ℃ and accounting for ash by incinerating the hydrolyzed samples at 550 ℃ in a furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm. The lignin content was calculated as the summation of acid insoluble lignin and acid soluble lignin. (Sluiter, 2008).