An- Najah National University

Faculty of Graduate Studies

Enzymatic Hydrolysis of Olive Industry Solid Waste into Glucose, the Precursor of Bioethanol

By

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This Thesis was defended successfully on 16/9/2015 and approved by:

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Dedication

The candle that melts to illuminate my paths

The flower that wilt for a fragrance smell

The tender which overflows with no limits

The symbol that embodies the struggle and immortality

To my beloved parents

The blood may bind us

But the love unites us surly

To my brother and sisters

Someone I'm proud to be related to

Support, encourage me all the time

To my life partner

Those who've been fortunate with them

Who have stood with me thru good and bad times

To my close friends

Acknowledgement

Initially, praise and thanks to Almighty Allah for his blessings and assisting towards achieving my project with the best image.

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الإقرار

انا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

Enzymatic Hydrolysis Of Olive Industry Solid Waste Into Glucose, The Precursor Of Bioethanol

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The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Signature: **التوقيع :**

Date: **التاريخ:** ١٦/ ٩/ ٢٠١٥

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List of abbreviations

Symbol	Abbreviation
OISW	Olive industry solid waste
OILW	Olive industry liquid waste
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CO2	Carbon dioxide
US	United state
DOE	Department of energy
PC	Plant cellulose
BC	Bacteria cellulose
pH	Power of hydrogen
OD weight	On dry weight

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Abstract

Jeft is a solid by-product generated during olive oil production. As a lignocellulose, jeft material is consist of cellulose, hemicellulose, lignin and other extractives. Previous research work successfully extracted about 40-45% cellulose from jeft raw materials. Jeft is considered as a biomass sources for the production of renewable energy. Our study investigated the process of hydrolyzing jeft into its monomers, glucose through the a two-step process that involves activation by pre-treatment of olive pulp with water, NaOH, $Ca(OH)_2$ and acetic acid. The activation step was followed by an enzymatic hydrolysis of cellulose to glucose.

A combination of two enzymes cellulase and β-glucosidase were used in the hydrolysis. The aim of this step was to convert glucose to ethanol which is used as fuel source. The highest yield of 85.02% glucose was obtained using enzyme concentration cellulose of 15.0 mg/g jeft and β-glucosidase 20.0 mg/g jeft at 45°C with 10% NaOH pre-treated jeft sample for 8 hr at pH = 4.8.

Whereas a pure cellulose produces about 95.30% glucose which is considered the highest yield obtained in our project in the same condition listed above.

Chapter One

Introduction

1.1 Background

The olive oil industry represents one of the most economically important agro-food sectors in the Mediterranean and Middle Eastern regions including Palestine. For example, according to the Palestinian Ministry of Agriculture, the West Bank and Jordan produce approximately 135 thousand metric tons of olive fruits every year [1].

In general, olive mill waste consists of about 44% of olive industry solid wastes (OISW = pomace, also locally known as Jeft)) and 56% of olive industry liquid waste (OILW). These wastes are acidic, have extremely high biological oxygen demand (BOD) and chemical oxygen demand (COD) values [2]. The waste materials pose a challenge in waste management to the olive mills and a concern to environmentalists, for it presents a serious disposal problem [2]. The size and magnitude of the olive production worldwide means that huge amounts of unexploited agronomic wastes are generated [2], thus posing acute environmental problems in the region.

In addition, olive industry loses economic value by disposing the OISW effluent or selling it for a low price to other industries. In many countries, it is usually burned or left to rot, thus releasing more CO_2 into the atmosphere that participate to the global warming. On the other hand, the OILW tends to be disposed off via the sewage system or unguided release above ground, which has negative implications on wild life as well as on surface and

underground water quality. Consequently, olive industry waste is a major problem facing by industrialist in view of increasing environmental standards day by day. In addition, olive industry loses economic value by disposing the solid wastes or selling them for a low price.

The challenge is to utilize and convert this waste materials into value added material. One of the most useful materials that could be obtained from OISW is ethanol. Ethanol is a valuable material with an enormous number of applications. The most useful among these is automotive fuel. The production of ethanol for fuel applications is becoming increasingly important in the world to decrease the relying on the un-renewable fossil fuels. Additionally, there is a growing international concern about the threat of global climate change. Policy makers across the globe are seeking the most effective methods for reducing the buildup of greenhouse gases that may cause global climate change. Their main goal is to promote a cleaner environment and reduce dependence on imported petroleum products. Projects in the area of converting feedstock to bio-ethanol are funded all over the world. For instance, The US Department of Energy (DOE) is promoting the development of ethanol from lignocellulosic feedstock as an alternative to conventional petroleum-based transportation fuels. DOE funds both fundamental and applied research in this area [3].

The production of bio-ethanol from OISW has not been fully explored. None of the published work in this filed has resulted in the development of commercial process for production of bio-ethanol from Jeft [4-6]. With

this work we are hoping to develop an efficient technology for converting Jeft into ethanol that could be commercialized at low cost.

Preliminary results showed that OISW could be a valuable source of pure glucose that could be feasibly used to produce ethanol. The preliminary results were obtained by subjecting cellulose obtained in pure form to acid hydrolysis.

With this technology, Palestine will have a new access to fuel, fine chemicals and another important source of income. Economically and environmentally speaking, we could predict that this new source of bio-ethanol will be especially important for the Palestinian economy and the global environment .

1.2 Jeft as Biomass

Biomass is produced from the reaction between CO_2 and H_2O using sunlight as the energy source, this process produce O_2. The primary products formed are hexoses sugar which polymerizes to form the main plants component cellulose. Glucose also polymerizes with pentose sugars to the second major components of plants hemicellulose. The third component is lignin. It is a three-dimensional cross-linked polymer composed of substituted phenols, it gives strength to plants together with cellulose and hemicellulose. Lipids, starch, and terpenes are intervention in the composition of plants biomass [7].

Biomass is a term that includes organic materials which is considered a renewable source of energy. These matters are taken from plants (wood plants), animals and organic wastes [8].

The use of biomass as a source of energy play a vital role in contributing to reduce dependence on non-renewable energy sources. As a result it led to alleviate global warming [9].

The major component of biomass is lignocellulose which consists of cellulose, hemicellulose and lignin that are strongly linked together. The proportion of biomass ingredients vary according to the type of source, but in general it consist of four main components [9]:

1- Cellulose
2- Hemicellulose
3- Lignin
4- Other extractives

1.2.1 Cellulose

Cellulose is considered as abundant renewable biological resource of energy with low-cost. The production of bio-products and bioenergy from low cost renewable lignocellulosic materials have benefits to the local economy and environment [10].

Cellulose is the most important structure in plants cell wall skeletal components, and is considered as inexhaustible organic polysaccharide raw material that consist of repeated connection of D-glucose units as appeared in Fig 1.1. This polymers is characterized as linear, stiff-chain homopolymer in its structure and many interesting properties such as its hydrophilicity, chirality and biodegradability [11].

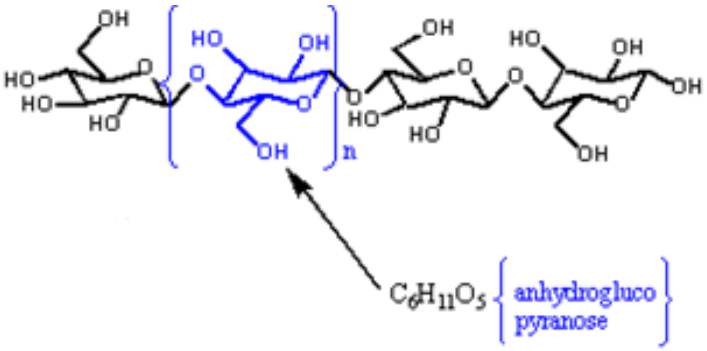


Figure 1.1: chemical structure of cellulose [12].

Cellulose polymer which is water insoluble was found as a main structure of plants and natural fibers defined as plant cellulose (PC). Also it is synthesized by some types of bacteria and defined as (BC). These two types are chemically identical but different in physical properties [13].

Cellulose is a large molecule considered as a major constituent of lignocellulose biomass representing about 45% of dry weight of wood in plants. This molecule is composed of D-glucose subunits which are linked together by β-(1,4)-glycosidic bond between the equatorial OH group at C4 of the first glucose unit and at the C1of the next glucose subunit to form cellobiose molecules. Cellobioses are linked together to form long chains called elemental fibrils which is connected together by H-bonds and van der waals forces and group together to constitute cellulose polymers as appeared in Fig 1.2 [14].

Cellulose chains can arrange in two ways:

1- Crystalline cellulose: an organized conformation to produce.
2- Amorphous cellulose: Non-organized conformation. This type of cellulose is more susceptible to enzymatic hydrolysis [14].

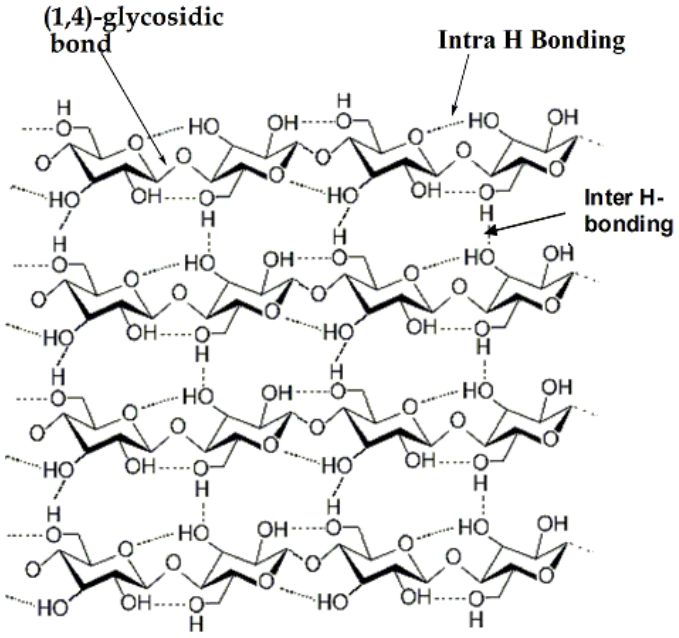


Figure 1.2: 3-D Structure of Cellulose Shows Intra- and Inter-H Bonding [15].

1.2.1.1 Cellulose Application:

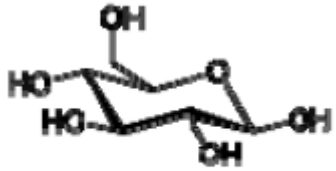
Cellulose is a raw material used for multiple industrial applications. Its directly used in a conventional filed as paper and cardboard production. Cellulose is not used in conventional field only, but also it can be chemically modified to produce cellulose derivatives. These derivatives are used in versatile industrial sectors, such as coatings, laminations, absorbents and optical films materials. Additionally, these derivatives can be used also in pharmaceutical industries, food and cosmetics products. In 2003 about 3.2 million tons of cellulose was used in the production of regenerated fibers and films and cellulose derivatives [16].

1.2.2 Hemicellulose

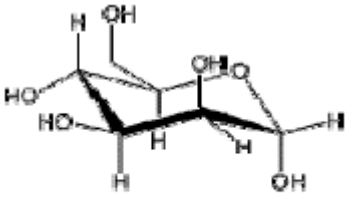
The second component of lignocellulose biomass is hemicellulose. It is a polysaccharide polymer consist of five carbon sugars include xylose and

arabinose and six carbon sugars involve galactose, glucose, and mannose as appeared in **Fig 1.3**. It's found inside plant cell wall in addition to cellulose and lignin and bind to cellulose through pectin to form cross-linked fibers. The ratio of these components is different according to the lignocellulose material, the proportion is ranging from 26-43% [17].

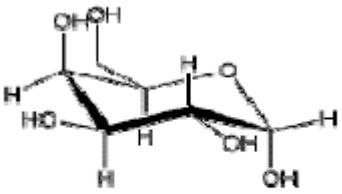
Compared to cellulose, hemicellulose has lower molecular weight and consists of shorter chains. It consist of about 500-3000 sugar units with branched structure. However, cellulose is unbranched polymer contain about 7,000-15,000 glucose. In contrast to cellulose, hemicellulose is easy to hydrolyze due to its random, amorphous structure as a result little strength. While cellulose as mentioned before is crystalline and amorphous. The crystalline reign is strong and resist the mild hydrolysis process [17].



D- glucose



D- mannose



D- galactose

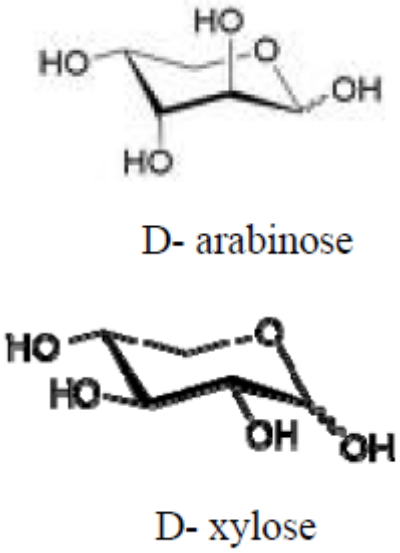


Figure 1.3: Monomers of Hemicelluloses [15]

Hemicellulose can be hydrolyzed by chemicals or enzymes to produce sugar monomers which then subjected to fermentation to produce biofuels. In chemical hydrolysis, the process is carried out by using diluted acid, concentrated acid, or alkali. While enzymatic hydrolysis require a complex of hydrolytic enzymes. This complex consists of endo-1, 4-β-xylanase, β-D-xylosidase, acetyl xylan esterase, α-glucuronidase and arabinose [18].

1.2.3 Lignin

The third component of lignocellulose biomass is Lignin. It is a complex polymer of phenyl propane molecules linked together in three dimension shape as appeared in Fig 1.4 [18]. Lignin is an aromatic and hydrophobic large macromolecules with molecular mass over 10,000 atomic mass unit resides in the wood and cell walls of plants so as to fill the gaps that exist between cellulose hemicellulose and other components, therefore it provides the support for the cell wall and the plant as a whole [19].

The phenyl propane units are linked together by two types of linkages:

1- Condensed linkages (e.g., 5–5 and β-1 linkages)
2- Ether linkages (e.g., ß -O-4 and α -O-4), while the ether linkages are the dominant linkages [12].

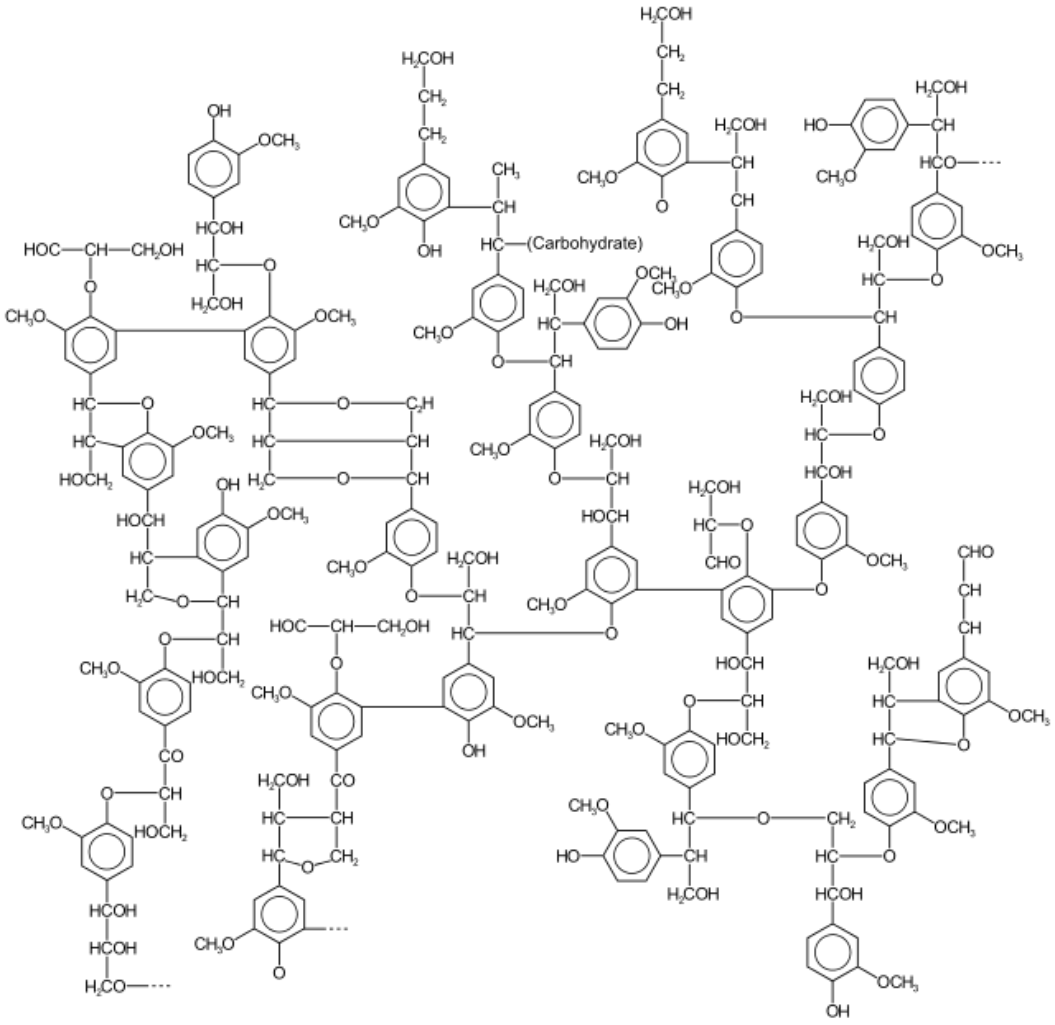


Figure 1.4: Chemical structure of lignin

1.3 Bioethanol production:

Biorefineries is a term describes the process for production of chemicals, materials and fuels from biomass. Petroleum and the fossil fuels are limited, environmentally unfriendly, and unsustainable energy sources in the world. So the industrial scientists have tended to look for alternative

safe resources of energy from raw materials such as biomass of plants which is sustainable and economic [20].

1.3.1 Bioethanol as value added product:

Petroleum and other fossil fuels resources releases CO_2 during the consumption process as a by-product which is considered as a major cause of global warming issue, so the production of bioethanol from natural source is cleaner burning than fossil fuels [21].

Cellulose is considered as a major source of renewable energy of raw materials. Therefore, the benefits of cellulosic wastes is important to produce energy. Cellulase enzymes play a role in the hydrolysis of cellulose [22].

1.3.2 Conversion of jeft biomass to bioethanol:

1.3.2.1 Pretreatment process

Lignocellulose biomass should be exposed to pretreatment process as a first step during ethanol production. This step is important so as to decrease the crystalline degree of cellulose, decrease its particle size, increase the surface area, and separate cellulose, hemicellulose and lignin from each other. Therefore change the structure of biomass and produce high yield of glucose [23]. Pretreatment methods include physical, chemical, and thermal or a combination of the three.

1.3.2.2 Processes of cellulose hydrolysis

Cellulose can be hydrolyzed into glucose sugars by two methods [18]:

1- Chemical hydrolysis by using acids or bases.

2- Biological hydrolysis by using enzymes.

In chemical hydrolysis, the lignocellulose biomass is treated with acids (concentrated or diluted) or alkali (such as NaOH) at specific temperature and pH for a period of time so as to produce glucose sugar monomers.

In enzymatic hydrolysis, enzymes have high molecular weight, specific and biological effective catalysts consists of proteins that increase the rate of reaction [18].

Table (1.1) shows Some differences between enzymatic and chemical hydrolysis [18]:

Factor	Enzymatic hydrolysis	Chemical hydrolysis
Temperature	low temperature (40 – 50 °C)	high temperature
pH	Mild (4 – 5)	Low pH (acidic hydrolysis) or high pH (basic hydrolysis)
Yield	High (about 100%)	low yield, high % of byproducts
Effect on environment	Environmental friendly	Generates Fumes and side products
Equipment	Any equipment	Special equipment it causes sever corrosion

1.3.2.2.1 Cellulase enzymes:

Cellulases are the enzymes that produced by fungi, bacteria, protozoans, plants, and animals. The catalytic effect include hydrolyze β-1,4 linkages in cellulose chains. The complete hydrolysis process of cellulose requires a combination of three types of celluases include: 1- endoglucanases, 2- exoglucanases, 3- β-glucosidase [10].

Endoglucanases is responsible for cleaving internal β-1,4-glycosidic bond, while exoglucanases play a role in release of cellobiose from reducing and non-reducing ends of cellulose. β-glucosidase have a vital role in the cellulolytic process by hydrolyze cellobiose molecules to produce glucose monomers and reduces cellobiose inhibition of endoglucanase and exoglucanase enzymes [22].

1.4 Aims of the project

In this project, the main component of the jeft which is cellulose will be hydrolyzed into it its repeat unit glucose, the precursor for bioethanol.

The primary objective of this work is to define the optimum enzymetic conditions for conversion of olive waste (Jeft) or cellulose extracted from Jeft into monomeric sugar glucose. In this project the following process for conversion of Jeft or cellulose to glucose will be evaluated. The process involves several treatments that together form a unique reaction network. These reactions could be summarized as follows:

1.4.1 Activation

Activation will be performed by subjecting jeft to various treatments which include: water, sodium hydroxide, calcium hydroxide, and acetic acid. The treatment will be conducted under various conditions of temperature and time

1.4.1.1 Hydrolysis

In this process, acids or enzymes or a combination of both are used to catalyze the conversion complex polysaccharides in Jeft to simple sugar.

After the pretreatment process, Jeft will be subjected to hydrolysis by one on the following two processes acid (dilute and concentrated) or enzymatic.

a) Acid Hydrolysis

Was conducted previously as described by reference [15].

b) Enzyme Hydrolysis

In this process biological hydrolysis of cellulose will be carried out using a combination of two enzymes. Enzyme treatment is expected to have the potential to improve conversion efficiencies and production economics.

For enzymes to work efficiently, they must obtain access to the molecules to be hydrolyzed. This requires some kind of pretreatment process (activation) to remove hemicelluloses and break down the cellulose or removal of the lignin to expose the cellulose and hemicelluloses molecules to enzyme.

After the process is evaluated conditions for conversion of Jeft into monomeric sugar glucose will be optimized to obtain maximum yield at low cost.

A continuation of this work will be:

1.5 Scale up the bench process into a large scale process.

1.6 Determine the feasibility study of the developed process.

1.7 Develop a single step process for converting Jeft into ethanol.

Chapter Two

Experimental Part

2.1 General experimental

All reagents were purchased from Aldrich Chemical Company, and used without any more purification unless otherwise specified. Kraft pulping and prehydrolysis treatment were performed using a high Parr Peactor model: Buchiglasuste , bmd 300. Jeft was obtained from an olive factory from Tulkarm city in Palestine and stored in a freezer at about -5 ^{o}C to 0 ^{o}C. Cellulose extracted from jeft and jeft samples were converted into glucose using the following methods:

1. Pre-hydrolysis treatment stage.
2. Enzymatic hydrolysis.

Glucose analysis was performed using a calibrated blood glucose meter with brand name ACCU-CHEK® performa Nano. It it's a trademark of Roche Company, made in USA.

2.1.1 Analysis of the Hydrolysis Product

2.1.1.1 Calibration of Glucose meter by preparation of calibration curve

The calibration curve was prepared according to the following procedure: a series of standard solutions of glucose ranging from concentrations 300.0 mg/dl to 700 mg/dl were prepared. Then analyzed by blood glucose meter to determine their glucose concentration . Results are summarized in the following table.

Table 2.1: Analysis of samples of glucose with known concentrations

Standard solution mg/dl	Measurement mg/dl
300	17
400	27
500	34
600	43
700	49

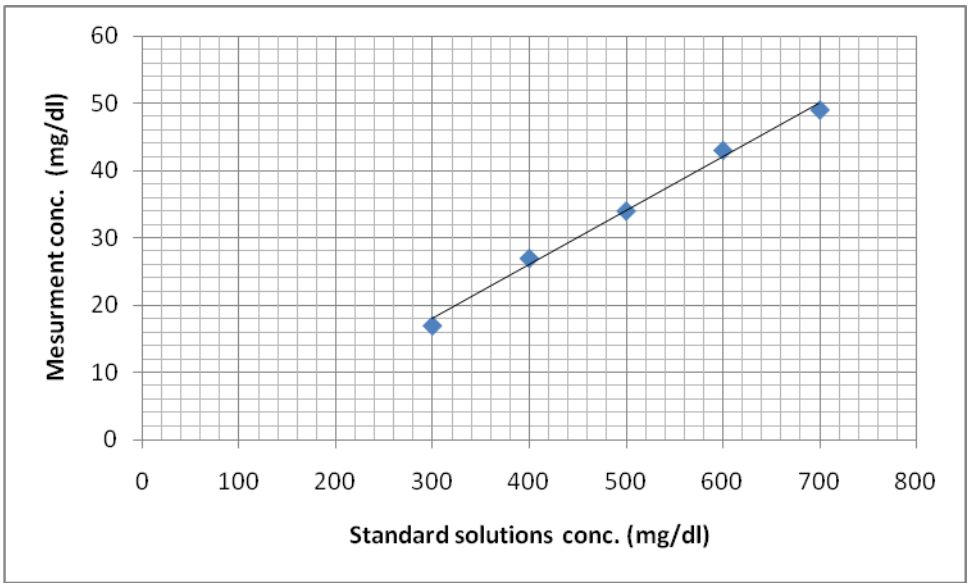


Figure 2.1: Calibration curve for determining glucose concentration.

Calibration curve was drawn by plotting the measurements against actual glucose concentration.

2.2 Jeft hydrolysis into sugar glucose

2.2.1 Pre-hydrolysis treatment with water

To a 100.0 g of jeft (OD wt. 80.0 g) in a reactor vessel (1L) was added to a 100.0 mL of water (Jeft to water ratio is 1:1). The reactor was sealed, pressurized to 50 psi using N_2 and heated, the temperature was raised to 160 ^{o}C in about 30 min and maintained at this temperature for 1 hr. The mixture was then cooled to room temperature filtered by suction filtration,

washed with distilled water (3 x 200 ml) and stored in a plastic bag in the refrigerator for future use.

This experiment was performed twice and repeated using Jeft to water ratios of 1: 2.5 and 1: 4 shown in Table 2.2

Table 2.2: Preparing different ratios of jeft:water (1:2.5 and 1:4) with 100g jeft

Jeft weight (g)	OD weight of jeft	Weight of water	Jeft to water ratio
100	80	250	1 to 2.5
100	80	400	1 to 4

2.2.2 Enzymatic hydrolysis of water-treated Jeft:

Enzymatic Hydrolysis was performed using the following enzymes:

1. Cellulase enzyme .
2. β-glucosidase enzyme .

An acetate buffer solution with a pH of 4.8 solution was prepared by dissolving 2 g sodium acetate salt in 100 ml distilled water and adding acetic acid solution until we reach pH = 4.8 measuring by pH meter.

A sample of water treated Jeft (10.0 g) was suspended in a 100 mL buffer solution in a 250 mL a glass bottle. The two enzymes cellulse and β-glucosidase were then added to the mixture. The concentration of enzymes calculated based on the dry weight of the jeft samples.

The bottle was caped and clamped to a shaker and placed in a water bath at about 45 °C. After 2 , 4 , 6 and 8 hr, a few milliliters were taken from the bottle, placed in a test tube, centrifuged to remove the suspension and the glucose content was measured using a blood glucose meter using pre-prepared calibration curve.

2.2.2.1 Study the effect of reaction time on rate of hydrolysis

2.2.2.1.1 Hydrolysis with enzyme concentration cellulase (3.0 mg/g jeft) and β-glucosidase (4.0 mg/g jeft)

Table 2.3: Dry weights of water treated jeft samples used in this experiment

Water Pre-treated Jeft Sample	Dry weight in 10.0 g sample (g)
jeft : water = 1:1	4.252 g
jeft : water = 1:2.5	4.333 g
jeft : water = 1:4	4.872 g

Results from this experiment are summarized in Tables 2.4, 2.5, 2.6.

Table 2.4: Results for Hydrolysis of water pre-treated Jeft (1: 1) using enzymes concentrations (Cellulase = 3.0 mg/g jeft and β glucosidase = 4.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	Low	-
4	Low	-
6	22	4.21 % *
8	24	4.59 %

*** % conversion = [glucose conc.(mg/dl)/10]*dry wt (g)*0.45**

Glucose conc. = 22 mg/dl solution.

Dry weight = 4.252 g.

0.45 : perrcent of cellulose extracted from jeft.

% conversion = [22/10]*4.252*0.45

= 4.21%.

2.2.2.2 Study the effect of amount of water used for Jeft activation on rate of hydrolysis.

Table 2.5: Results for Hydrolysis of water-treated Jeft (1 : 2.5) using enzyme concentration (Cellulase = 3.0 mg/g jeft and β glucosidase = 4.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	Low	-
4	Low	-
6	22	4.29 %
8	25	4.87 %

Table 2.6: Results for Hydrolysis of water treated Jeft (1:4) Using Enzyme concentration (Cellulase = 3.0 mg/g jeft and β glucosidase = 4.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	Low	-
4	23	5.04 %
6	24	5.26 %
8	27	5.92 %

2.2.2.3 study the effect of enzyme concentration on rate of hydrolysis

2.2.2.1.2 Hydrolysis with enzyme concentration cellulase (5.0 mg/g jeft) and β-glucosidase (6.0 mg/g jeft)

The previous hydrolysis procedure in page 21-22 was followed except that, in this experiment, hydrolysis was performed at different concentrations of cellulase (5.0 mg/g jeft) and β glucosidase = (6.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time

ranging from 2 hr to 8 hr. Results of these experiments are summarized in Tables 2.7, 2.8, 2.9.

Table 2.7: Results for Hydrolysis of water treated Jeft (1:1) using enzyme concentration (Cellulase = 5.0 mg/g jeft and β glucosidase = 6.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	30	5.74 %
4	34	6.51 %
6	37	7.08 %
8	34	6.51 %

Table 2.8: Results for Hydrolysis of water treated Jeft (1:2.5) Using Enzyme concentration (Cellulase = 5.0 mg/g jeft and β glucosidase = 6.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	33	6.43 %
4	36	7.02 %
6	40	7.80 %
8	40	7.80 %

Table 2.9: Results for Hydrolysis of water treated Jeft (1:4) using enzyme concentration (Cellulase = 5.0 mg/g jeft and β glucosidase = 6.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	34	7.45 %
4	40	8.77 %
6	42	9.20 %
8	41	8.99 %

2.2.2.1.3 Hydrolysis with enzyme concentration cellulase (7.0 mg/g jeft) and β-glucosidase (9.0 mg/g jeft):

The previous hydrolysis procedure in page 23-24 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (7.0 mg/g jeft) and β glucosidase = (9.0 mg/g jeft). The hydrolysis was performed as described before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.10, 2.11, 2.12.

Table 2.10: Results for Hydrolysis of water treated Jeft (1:1) Using enzymes concentrations (Cellulase = 7.0 mg/g Jeft and β glucosidase = 9.0 mg/g Jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	36	6.89 %
4	41	7.84 %
6	41	7.84 %
8	45	8.61 %

Table 2.11: Results for Hydrolysis of water treated Jeft (1:2.5) using enzyme concentrations (Cellulase = 7.0 mg/g Jeft and β glucosidase = 9.0 mg/g Jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	36	7.02 %
4	42	8.19 %
6	45	8.77 %
8	47	9.16 %

Table 2.12: Results for Hydrolysis of water treated Jeft (1:4) using enzyme concentration (Cellulase = 7.0 mg/g jeft and β glucosidase 9.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	37	8.11 %
4	43	9.43 %
6	45	9.87 %
8	49	10.74 %

2.2.2.1.4 Hydrolysis with enzyme concentration cellulase (9.0 mg/g jeft) and β-glucosidase (12.0 mg/g jeft):

The previous hydrolysis procedure in page 25-26 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (9.0 mg/g jeft) and β glucosidase = (12.0 mg/g jeft). The hydrolysis was performed as described before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.13, 2.14, 2.15.

Table 2.13: Results for Hydrolysis of water treated Jeft (1:1) using enzyme concentration (Cellulase = 9.0 mg/g jeft and β glucosidase = 12.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	60	11.48 %
4	70	13.39 %
6	78	14.92 %
8	82	15.69 %

Table 2.14: Results for Hydrolysis of water treated Jeft (1: 2.5) using enzyme concentration (Cellulase = 9.0 mg/g jeft , β glucosidase = 12.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	63	12.28 %
4	76	14.82 %
6	81	15.80 %
8	89	17.35 %

Table 2.15: Results for Hydrolysis of water treated Jeft (1: 4) using enzyme concentration (Cellulase = 9.0 mg/g jeft and β glucosidase = 12.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	64	14.03 %
4	78	17.10 %
6	88	19.29 %
8	93	20.39 %

2.2.2.1.5 Hydrolysis with enzyme concentration cellulase (11.0 mg/g jeft) and β-glucosidase (14.0 mg/g jeft):

The above hydrolysis procedure was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (11.0 mg/g jeft) and β glucosidase = (14.0 mg/g jeft). The hydrolysis was performed as described before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.16, 2.17, 2.18.

Table 2.16: Results for Hydrolysis of water treated Jeft (1:1) Using Enzyme concentration (Cellulase = 11.0 mg/g jeft and β glucosidase = 14.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	92	17.60 %
4	108	20.66 %
6	116	22.19 %
8	128	24.50 %

Table 2.17: Results for Hydrolysis of water treated Jeft (1:2.5) using enzyme concentration (Cellulase = 11.0 mg/g jeft and β glucosidase = 14.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	93	18.13 %
4	110	21.45 %
6	120	23.40 %
8	132	25.74 %

Table 2.18: Results for Hydrolysis of water-treated Jeft (1:1) using enzyme concentration (Cellulase = 11.0 mg/g jeft and β glucosidase = 14.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	98	21.49 %
4	114	24.99 %
6	125	27.41 %
8	133	29.16 %

2.2.2.1.6 Hydrolysis with enzyme concentration cellulase (13.0 mg/g jeft) and β-glucosidase (17.0 mg/g jeft):

The previous hydrolysis procedure in page 29-30 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (13.0 mg/g jeft) and β glucosidase = (17.0 mg/g jeft). The hydrolysis was performed as described before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.19, 2.20, 2.21.

Table 2.19: Results for Hydrolysis of water treated Jeft (1:1) using enzyme concentration (Cellulase = 13.0 mg/g jeft and β glucosidase = 17.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	124	23.73 %
4	139	26.60 %
6	156	29.85 %
8	169	32.34 %

Table 2.20: Results for Hydrolysis of water treated jeft (1:2.5) Using Enzyme concentration (Cellulase = 13.0 mg/g jeft and β glucosidase = 17.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	126	24.57 %
4	144	28.08 %
6	159	31.00 %
8	171	33.34 %

Table 2.21: Results for Hydrolysis of water treated Jeft (1:1) Using Enzyme concentration (Cellulase = 13.0 mg/g jeft and β glucosidase = 17.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	126	27.62 %
4	147	32.23 %
6	160	35.08 %
8	181	39.68 %

2.2.2.1.7 Hydrolysis with enzyme concentration cellulase (15.0 mg/g jeft) and β-glucosidase (20.0 mg/g jeft):

The previous hydrolysis procedure in page 31-32 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr

Results from this experiment are summarized in Tables 2.22, 2.23, 2.24.

Table 2.22: Results for Hydrolysis of water treated jeft (1:1) using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	264	50.51 %
4	289	55.30 %
6	301	57.59 %
8	314	60.08 %

Table 2.23: Results for Hydrolysis of water treated Jeft (1:2.5) using enzyme concentration (Cellulase = 15.0 mg/g jeft, β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	264	51.48 %
4	301	58.69 %
6	314	61.26 %
8	314	61.26 %

Table 2.24: Results for Hydrolysis of water treated Jeft (1:4) using enzyme concentration (cellulase = 15.0 mg/g jeft, β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	276	60.51 %
4	301	65.99 %
6	314	68.84 %
8	326	71.47 %

2.2.2.1.8 Hydrolysis with enzyme concentration cellulase (17.0mg/g jeft) and β-glucosidase (26.0mg/g jeft):

The previous hydrolysis procedure in page 33-34 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (17.0 mg/g jeft) and β glucosidase = (26.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.25, 2.26, 2.27.

Table 2.25: Results for Hydrolysis of water treated Jeft (1:4) using enzyme concentration (cellulase = 17.0 mg/g jeft, β glucosidase = 26.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	260	49.75 %
4	283	54.15 %
6	295	56.46 %
8	301	57.59 %

Table 2.26: Results for Hydrolysis of water treated jeft (1:4) using enzyme concentration (cellulase = 17.0 mg/g jeft, β glucosidase = 26.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	260	50.70 %
4	297	57.91 %
6	315	61.42 %
8	310	60.45 %

Table 2.27: Results for Hydrolysis of water treated Jeft (1:4) using enzyme concentration (cellulase = 17.0 mg/g jeft, β glucosidase = 26.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	271	59.41 %
4	302	66.21 %
6	308	67.53 %
8	321	70.38 %

2.2.3 Pre-hydrolysis treatment with an aqueous solution of sodium hydroxide

To a 100.0 g of jeft (OD wt. 80.0 g) in a reactor vessel (1L) was added a 400.0 mL of an aqueous solution of sodium hydroxide with various concentrations (1%, 2.5%, 5%, 7.5%, and 10.0%). The reactor was sealed, pressurized to 50 psi using N_2 and heated, the temperature was raised to 160 ^oC in about 30 min and maintained at this temperature for 1 hr. The mixture was then cooled to room temperature filtered by suction filtration, washed with distilled water until the pH is neutral.

2.2.4 Enzymatic hydrolysis of sodium hydroxide-treated Jeft:

The enzymatic hydrolysis was performed as previously described before using the enzymes cellulase and β-glucosidase.

A sample of water treated Jeft (10.0 g) was suspended in a 100 mL buffer solution in a 250 mL a glass bottle. The two enzymes cellulse and β-glucosidase were then added to the mixture. The concentration of enzymes were calculated based on the dry weight of the jeft samples. The bottle was caped and clamped to a shaker and placed in a water bath at about 45 ^oC. After 2, 4, 6 and 8 hr a few milliliters were taken from the bottle, placed in a test tube, centrifuged to remove the suspension and the glucose content was measured using a blood glucose meter using pre-prepared calibration curve.

Table 2.28: Dry weights of jeft samples treated with NaOH solutions

NaOH Pre-treated Jeft Sample	Dry weight in 10.0 g sample (g)
1.0 %	4.12 g
2.5 %	4.08 g
5.0 %	3.72 g
7.5 %	3.73 g
10 %	3.81 g

2.2.4.1 Hydrolysis with enzyme concentration cellulase (15.0 mg/g jeft) and β-glucosidase (20.0 mg/g jeft):

The previous hydrolysis procedure in page 37 was followed except that, in this experiment, hydrolysis was performed at different concentration of cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.29, 2.30, 2.31, 2.32, 2.33.

Table 2.29: Results for Hydrolysis of 1% NaOH treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	289	53.58 %
4	351	65.08 %
6	363	67.30 %
8	363	67.30 %

Table 2.30: Results for Hydrolysis of 2.5% NaOH treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	301	55.26 %
4	363	66.65 %
6	376	69.03 %
8	388	71.24 %

Table 2.31: Results for Hydrolysis of 5% NaOH treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	376	62.94 %
4	450	75.33 %
6	487	81.52 %
8	487	81.52 %

Table 2.32: Results for Hydrolysis of 7.5% NaOH treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	388	65.13 %
4	435	73.01 %
6	450	75.53 %
8	500	83.92 %

Table 2.33: Results for Hydrolysis of 10% NaOH treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	388	64.43 %
4	450	74.72 %
6	475	78.87 %
8	512	85.02 %

2.2.5 Pre-hydrolysis treatment with an aqueous solution of calcium hydroxide

To a 100.0 g of jeft (OD wt. 80.0 g) in a reactor vessel (1L) was added a 400.0 mL of an aqueous solution of calcium hydroxide with various concentrations (1%, 2.5%, 5%, 7.5%, and 10.0%). The reactor was sealed, pressurized to 50 psi using N_2 and heated, the temperature was raised to 160 ^{o}C in about 30 min and maintained at this temperature for 1 hr. The mixture was then cooled to room temperature filtered by suction filtration, washed with distilled water until the pH is neutral.

2.2.6 Enzymatic hydrolysis of calcium hydroxide-treated Jeft:

The enzymatic hydrolysis of jeft treated with an aqeous solution of calcium hydroxide was performed as before using the f enzymes cellulase and β-glucosidase.

A sample of water treated Jeft (10.0 g) was suspended in a 100 mL buffer solution in a 250 mL a glass bottle. The two enzymes cellulse and β-glucosidase were then added to the mixture. The concentration of enzymes were calculated based on the dry weight of the jeft samples. The bottle was caped and clamped to a shaker and placed in a water bath at about 45 ^{o}C. After 2, 4, 6 and 8 hr a few milliliter were taken from the bottle, placed in a test tube, centrifuged to remove the suspension and the glucose content was measured using a blood glucose meter using pre-prepared calibration curve.

Table 2.34: Dry weights of $Ca(OH)_2$ treated jeft samples:

Concentration of sodium hydroxide used	Dry weight (g)
1 %	4.23 g
2.5 %	4.39 g
5 %	5.53 g
7.5 %	4.35 g
10 %	4.51 g

2.2.6.1 Hydrolysis with enzyme concentration cellulase (15.0 mg/g jeft) and β-glucosidase (20.0 mg/g jeft):

The previous hydrolysis procedure in page 41 was followed using the amount of enzyme: cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.35, 2.36, 2.37, 2.38, 2.39.

Table 2.35: Results for hydrolysis of 1% $Ca(OH)_2$ treated jeft with enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	125	23.79 %
4	150	28.55 %
6	167	31.79 %
8	186	35.41 %

Table 2.36: Results for hydrolysis of 2.5% $Ca(OH)_2$ treated jeft with enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	129	25.48 %
4	163	32.20 %
6	183	36.15 %
8	192	37.93 %

Table 2.37: Results for hydrolysis of 5% $Ca(OH)_2$ treated jeft with enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	132	32.85 %
4	165	41.06 %
6	189	47.03 %
8	196	48.77 %

Table 2.38: Results for hydrolysis of 7.5% $Ca(OH)_2$ treated jeft with enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	252	49.33 %
4	289	56.57 %
6	301	58.92 %
8	363	71.06 %

Table 2.39: Results for hydrolysis of 10.0% $Ca(OH)_2$ treated jeft with enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	252	51.14 %
4	326	66.16 %
6	357	72.45 %
8	363	75.09 %

2.2.7 Pre-hydrolysis treatment with an aqueous solution of acetic acid

To a 100.0 g of jeft (OD wt. 80.0 g) in a reactor vessel (1L) was added a 400.0 mL of an aqueous solution of acetic acid with various concentrations (1%, 2.5%, 5%, 7.5%, and 10.0%). The reactor was sealed, pressurized to 50 psi using N_2 and heated, the temperature was raised to 160 ^oC in about 30 min and maintained at this temperature for 1 hr. The mixture was then cooled to room temperature filtered by suction filtration, washed with distilled water until the pH was neutral.

2.2.8 Enzymatic hydrolysis of acetic acid pre-treated Jeft:

The enzymatic hydrolysis of jeft treated with an aqeous solution of aceti acid was performed as before using the enzymes cellulase and β-glucosidase.

A sample of water treated Jeft (10.0 g) was suspended in a 100 mL buffer solution in a 250 mL a glass bottle. The two enzymes cellulse and β-glucosidase were then added to the mixture. The concentration of enzymes calculated based on the dry weight of the jeft samples. The bottle was

caped and clamped to a shaker and placed in a water bath at about 45 ^{o}C. After 2, 4, 6 and 8 hr a few milliliter were taken from the bottle, placed in a test tube, centrifuged to remove the suspension and the glucose content was measured using a blood glucose meter using pre-prepared calibration curve.

Table 2.40: Dry weights of acetic acid treated jeft samples:

Concentration of acetic acid used	Dry weight of jeft samples (g)
1 %	5.03 g
2.5 %	3.92 g
5 %	4.22 g
7.5 %	3.56 g
10 %	4.40 g

2.2.8.1 Hydrolysis with enzyme concentration cellulase (15.0 mg/g jeft) and β-glucosidase (20.0 mg/g jeft):

The previous hydrolysis procedure in page 46 was followed using the amount of enzyme: cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr. Results from this experiment are summarized in Tables 2.41, 2.42, 2.43, 2.44, 2.45.

Table 2.41: Results for hydrolysis of 1% acetic acid treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	135	30.55 %
4	161	36.44 %
6	178	40.29 %
8	189	42.78 %

Table 2.42: Results for hydrolysis of 2.5% acetic acid treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	134	23.64 %
4	152	26.81 %
6	171	30.16 %
8	184	32.46 %

Table 2.43: Results for hydrolysis of 5.0% acetic acid treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	118	22.41 %
4	155	29.43 %
6	171	32.47 %
8	182	34.56 %

Table 2.44: Results for hydrolysis of 7.5% acetic acid treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	127	20.35 %
4	165	26.43 %
6	186	29.89 %
8	196	31.40 %

Table 2.45: Results for hydrolysis of 10% acetic acid treated Jeft using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion (%)
2	123	24.35 %
4	141	27.92 %
6	159	31.48 %
8	167	33.07 %

2.3 Kraft pulping of jeft:

2.3.1 Pre-treatment of jeft for kraft pulping

To a 100.0 g of jeft (OD wt. 80.0 g) in a reactor vessel (1L) was added a 100.0 mL of water (Jeft to water ratio is 1:1). The reactor was sealed, pressurized to 50 psi using N_2 and heated, the temperature was raised to 160 ^{o}C in about 30 min and maintained at this temperature for 1 hr. The mixture was then cooled to room temperature filtered by suction filtration, washed with distilled water (3 x 200 ml) and stored in a plastic bag in the refrigerator for future use.

2.3.2 Jeft pulping

The pretreated jeft was returned to the reactor vessel and to it was added a solution of sodium hydroxide (25.9 g) and sodium sulfide (13.02 g) in 300 mL water (this solution has sulfidity of 25.2% and effective alkaline 13%). The vessel was then attached to the high barr reactor, sealed and pressurized to 50 psi with nitrogen. It was then heated by an outer jacket containing electrical wires to 160 °C in about 30 min. The vessel contents

were mixed with a mixer that is rotating in the reactor vessel via a motor. The temperature was measured with a thermometric sensor installed inside the reactor. The pulp that resulted eventually was washed with plenty of tap water and filtered by suction filtration. a small sample from the product (2.0 g) was dried in an oven at 100 ^{o}C for 4 hr and used to determine oven dried weight (OD wt.).

% of dry weight = (dry weight / wet weight) * 100

Table 2.46: Dry weight of Kraft pulping samples :

Sample	Dry weight of 10g (g)
Kraft pulping	3.20 g

2.3.3 Hydrolysis of jeft pulp into glucose

The previous hydrolysis procedure in page 52 was followed using the amount of enzyme: cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as before at 45 °C and for periods of time ranging from 2 hr to 8 hr.

Table 2.47: Results for Hydrolysis of jeft_pulp sample using enzyme concentration (Cellulase = 15.0 mg/g pulp and β glucosidase = 20.0 mg/g pulp).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion to glucose (%)
2	313	45.07 %
4	413	59.47 %
6	462	66.53 %
8	512	73.73 %

2.3.4 Bleaching of pulp extracted from jeft:

The pulp product obtained from the previous step was bleached using the following process.

2.3.4.1 Bleaching sequence:

Bleaching process reported in reference [24] and reference [12] was adopted to obtain lignin free jeft pulp (pure cellulose).

Table 2.48: Dry weight of Cellulose samples :-

Sample	Dry weight (g)
Pure cellulose obtained from jeft	9.67 g

2.3.4.2 Hydrolysis of jeft bleached pulp into glucose

The previous hydrolysis procedure in page 54 was followed using the amount of enzyme: cellulase (15.0 mg/g jeft) and β glucosidase = (20.0 mg/g jeft). The hydrolysis was performed as described before at 45 °C and for periods of time ranging from 2 hr to 8 hr.

Table 2.49: Results for hydrolysis of cellulose sample using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	301	65.49 %
4	363	79.00 %
6	413	89.86 %
8	438	95.30 %

2.4 Hydrolysis of sodium hydroxide pre-treated jeft with multidoses of enzyme

Enzymatic Hydrolysis was performed as descibed before using the f enzymes: Cellulase enzyme and β-glucosidase enzyme .

A 100 mL buffer acetate solution with a pH of 4.8 was added to a 10.0 g sample of sodium hydroxide treated Jeft (see section 2.2.3) in a 250 mL a glass bottle. The two enzymes cellulse and β-glucosidase were then added to the mixture. The concentration of enzymes was calculated based on the dry weight of the jeft samples. The bottle was caped and clamped to a shaker and placed in a water bath at about 45 ^{o}C. After about 90 min the hydrolysis reaction was stopped by filtering the mixture. The filtrate was analyzed for glucose contents and the solid mass was returned to the glass bottle and new buffer solution and enzymes were added. The process was repeated several time as shown in table 2.48.

Table 2.50: Results for hydrolysis of 10% NaOH treated Jeft after removing the buffer solution 90 min using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (min)	Concentration of Glucose (mg/dl)	Conversion %
90	289	49.55 %
180	41	7.03 %
270	Zero	_
360	Zero	_

2.5 Hydrolysis of sodium hydroxide pre-treated jeft under a pressure of air

a 100.0 g of jeft pretreated with 10% NaOH (OD wt. 80.0 g) in a reactor vessel (1L) was added to a 400.0 mL of buffer solution (pH 4.8) followed with the two enzymes. The reactor was sealed, pressurized to 10 psi with air and mixed at low speed for the time shown in table 2.49. The product was filtered and analyzed for glucose.

Table 2.51: Results for Hydrolysis of 10% NaOH treated jeft under air pressure using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft) .

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	451	77.32 %
4	451	77.32 %
6	531	91.04 %
8	511	87.61 %

2.6 Hydrolysis of water pre-treated jeft under a pressure of air

a 100.0 g of jeft pretreated with water(OD wt. 80.0 g) using jeft to water ratio (1:4) in a reactor vessel (1L) was added to a 400.0 mL of buffer solution (pH 4.8) followed with the two enzymes. The reactor was sealed, pressurized to 10 psi with air and mixed at low speed for the time shown in table 2.49. The product was filtered and analyzed for glucose.

Table 2.52: Results for Hydrolysis of water treated jeft with ratio (1:4) under air pressure using enzyme concentration (Cellulase = 15.0 mg/g jeft and β glucosidase = 20.0 mg/g jeft).

Hydrolysis Time (hr)	Concentration of Glucose (mg/dl)	Conversion %
2	227	49.77 %
4	252	55.25 %
6	264	57.88 %
8	252	55.25 %

Chapter Three

Results and Discussion

Cellulose hydrolysis means depolymerization of cellulose polymer into its glucose monomers. Cellulose hydrolysis can be performed using several methods among those that were mentioned in chapter one are:

1. Acid hydrolysis.
2. Enzymatic hydrolysis.

The acid hydrolysis was done previously at our laboratory and results were published in a MS thesis [15]. This thesis summarizes methods and results of cellulose Jeft hydrolysis using enzyme.

Cellulose consists of polymeric chains that associate with each other through H-bonding. It consists of two regions amorphous and crystalline. The amorphous region is the open region of the cellulose, reagents can access it and interacts with the cellulose repeat units. However the crystalline region is completely closed and mild reagents cannot access it. Therefore, complete hydrolysis of cellulose chain into its monomeric anhydroglucose repeat unit required an activation stage. In this stage special reagents are used to open the crystalline structure and make accessible for reagents. In this work several activating reagent were tried among these hot water, different bases (NaOH, $Ca(OH)_2$) , and acid. In this work the enzymatic hydrolysis stage was proceeded by an activation stage. In this thesis, the effect of different activating agents on cellulose hydrolysis was also evaluated.

It was reported in the literature that the 45 ^{o}C is the optimum temperature to the highest enzyme activities and highest life time. So the hydrolysis in all reactions was performed at 45°C and controlled in a bath with a thermostat. The hydrolysis was performed on pure of cellulose extracted form jeft using method published in a MS thesis [15] and on Jeft raw material, results obtained from both material were compared.

In experimental work, the effect of enzyme concentration on rate of hydrolysis was evlauated, which involved testing several concentrations of enzymes ranging from 3.0 mg cellulase/g jeft to 4.0 mg β-glucosidase/g jeft were used. The hydrolysis time on rate of cellulose hydrolysis was also examined. The hydrolysis time used in all experiments ranged from 2-8 hr. The hydrolysis was performed in a buffer solution as stated in the experimental part. The effect of concentraton of cellulose in the buffer solution on the rate of hydrolysis was also studied. Our main goal from the testing all these variable is determine the conditions that produce the highest concentration of glucose.

3.1 Analysis of the Hydrolysis Product

The hydrolysis product was analyzed using blood glucose meter as shown in the experimental part. The concentration of produced glucose from cellulose hydrolysis was calculated from the calibration curve shown in Fig 3.1

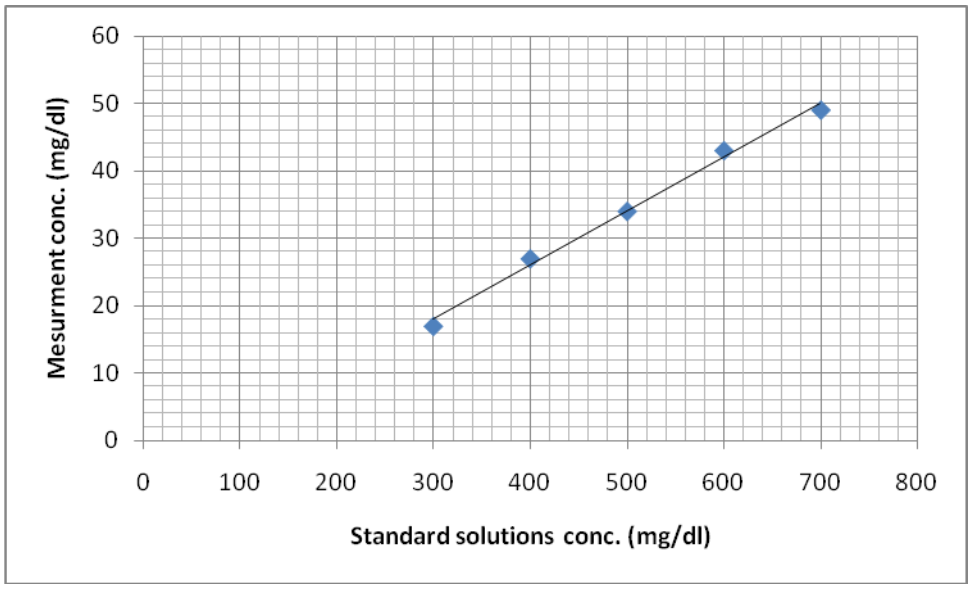


Figure 3.1: Calibration curve for determining glucose concentration.

in standard samples with variuos and known concentrations. Detailed procedure for the generation of the calibration curve is shown in the experimental part **Chapter 2**.

3.1.1 Hydrolysis of Jeft pre-activated with hot water:

3.1.1.1 Effect of reaction time on rate of hydrolysis

The sample of jeft was first activated with hot water in a 1:1 ratio then subjectd to enzymetic hydrolysis time of 6.0 hr and 8.0 hr, concentration of enzymes used were 3.0 mg/g cellulase and 4.0 mg/g β-glucosidase shown in Fig 3.2.

Table 2.4 shows the highest concentration of glucose was determined to be 22.0 mg/dL, 24.0 mg/dL for a hydrolysis time of 6 hr and 8 hr, respectively. Hydrolysis rate was very low, so higher concentrations of these enzymes were tried as shown in Fig 3.2.

However, these results indicate that there is a direct proportionality between glucose concentration and treatment time.

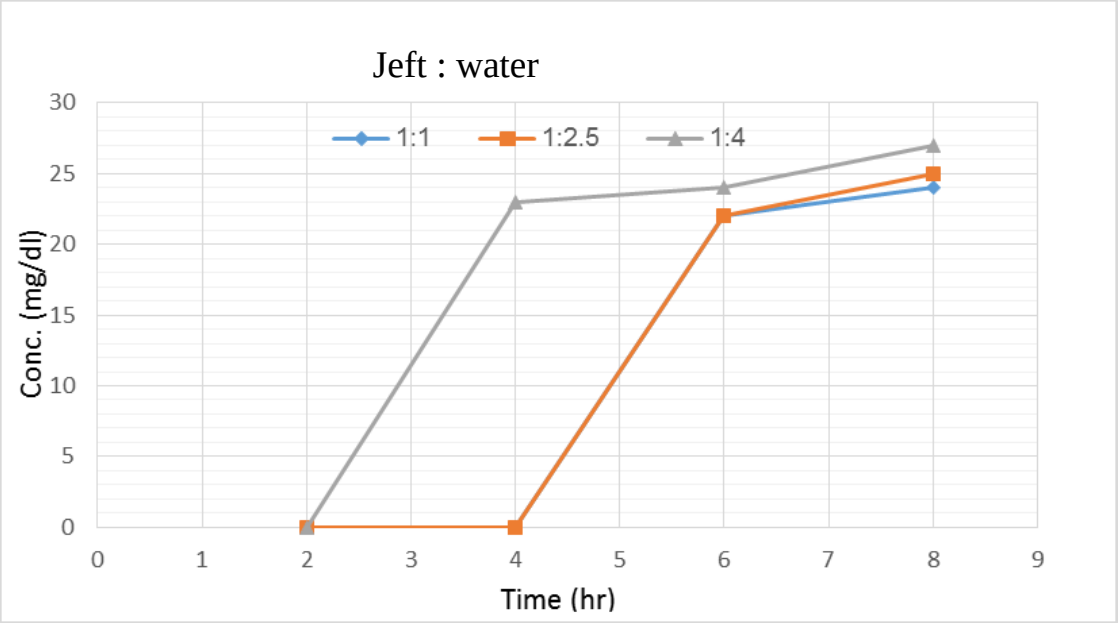


Figure 3.2: Results for Hydrolysis of Jeft as a function of time.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)].

The percent conversion of cellulose to glucose was calculated using the following equation:

% conversion = [concentration of glucose(mg/dl)/10] * dry weight (g) * 0.45

Where 0.45 is a percent of cellulose extracted from jeft [12]

3.1.1.2 Effect of amount of water used for Jeft activation on rate of hydrolysis.

The effect of increasing the proportion of water to jeft during the activation stage on rate of hydrolysis was also studied. The jeft activation with water was perfromed for 2 hr at a ratio of Jeft to water 1:2.5 and 1:4.

Concentration of enzymes used were 3.0 mg/g cellulase and 4.0 mg/g β-glucosidase. The results are shown Table 2.5 and Table 2.6.

The realtionship between aamount of water to jeft on rate of hydrolysis is shown in Figure 3.3. As shown in Figure 3.3 the best results was obtained using Jeft to water in 1:1 ratio. So, increasing amount of water in the activation step satge was not nessecasry.

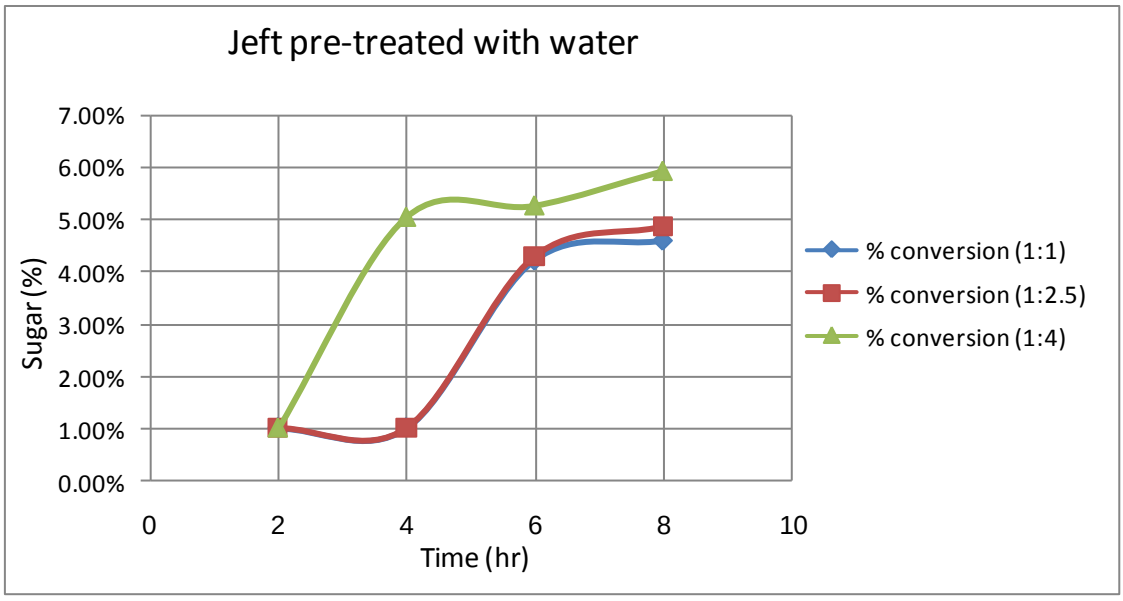


Figure 3.3: the percent conversion of jeft pretreated with water at three different water to jeft ratio

3.1.1.3 Effect of enzyme concentration on rate of hydrolysis

The effect of increasing the concentration of enzymes on rate of hydrolysis was then studied. So, the second step hydrolysis was performed using 5mg/g cellulase and 6 mg/g β-glucosidase. The results are illustrated in Table 2.7, Table 2.8 , Table 29 respectively for various proportion of jeft to water as previously arranged.

The concentration of cellulase and β-glucosidase enzymes were increased sequentially until the optimum and best concentration of enzymes so as so produce high yeild of glucose was obtained.

The results are featured in tabels from Table 2.10 to Table 2.27.

Contrary to chemical hydrolysis, the enzymatic hydrolysis doesn't convert glucose to other chemicals such as furfural and other by-products. As a result, the concentration of glucose was increased to an optimum level [18].

The best result was obtained using enzymes concentration by proportion of cellulase enzyme with 15mg/g jeft and β-glucosidase 20mg/ g jeft, and at a treatment time for 8 and jeft to water proportion 1:4. Theses results are shown in Fig 3.4 and the relation between time and conversion percent is shown in Fig 3.5.

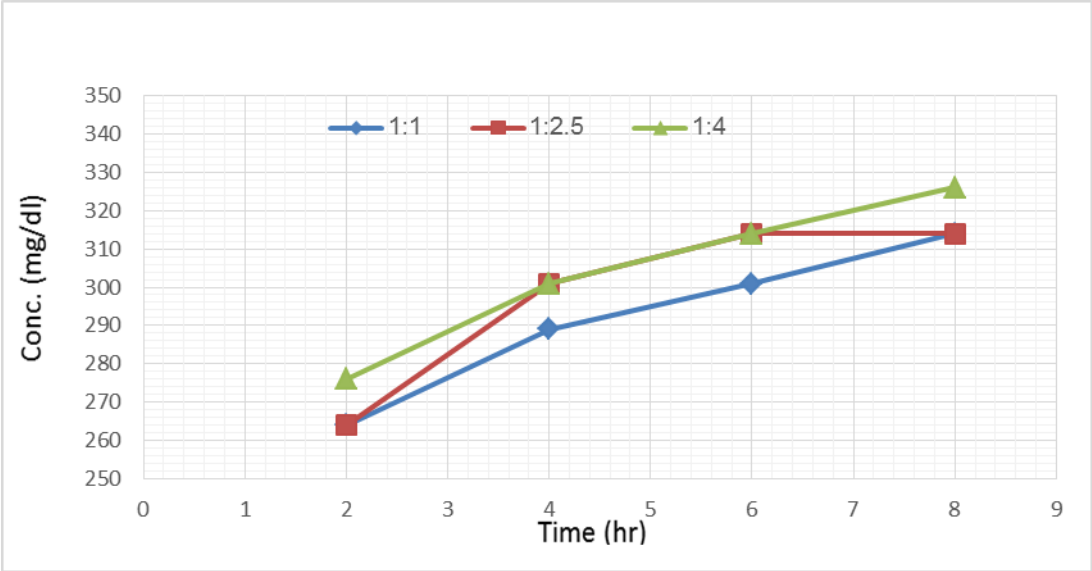


Figure 3.4: Results for Hydrolysis of Jeft using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)].

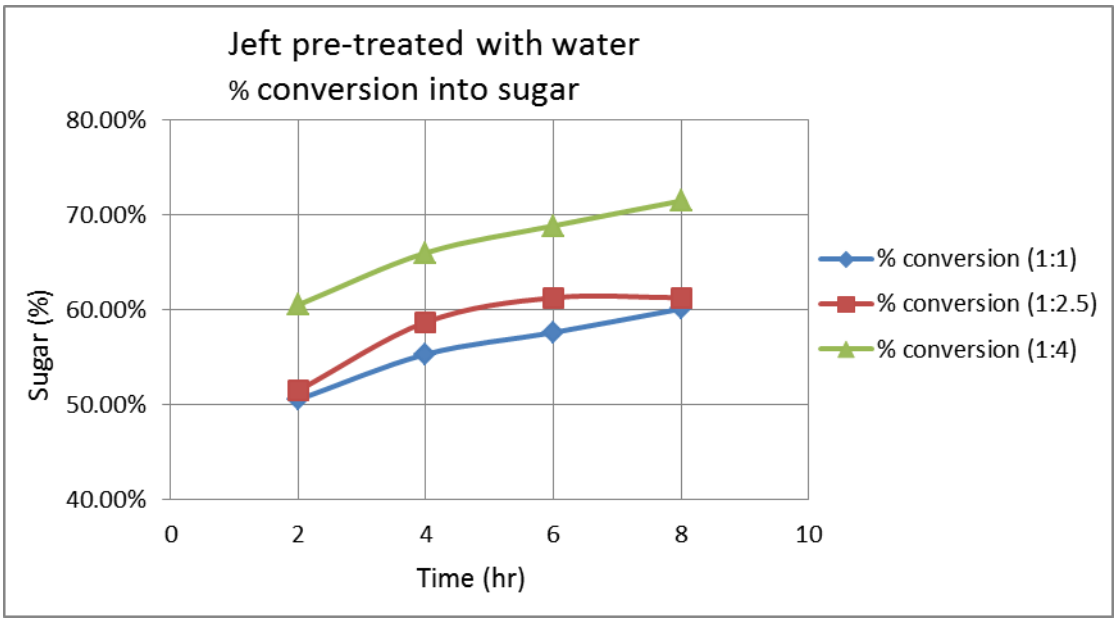


Figure 3.5: the percent conversion of jeft pretreated with water at three different water to jeft ration for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft , β glucosidase = 20.0 mg/g jeft .

3.1.2 Hydrolysis of Sodium Hydroxide-treated jeft samples:

Five solution of NaOH with different concentration ; 1%, 2.5%, 5%, 7.5%, 10% were prepared. The treatment was performed in buffer solution of 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at temperature 45 °C for 2, 4, 6 and 8 hr period. The results are summerized in **Tables 2.29, 2.30, 2.31, 2.32** and **2.33** in **chapter 2**. These results are shown in **Fig 3.6** which indicates that concentraion of glucose increased by increasing the treatment time and percent of NaOH in solution from 363 mg/dl in 1% NaOH solution to 512 mg/dl in 10% NaOH solution; both of them treated after 8 hr.

Also, the relationship between the treatment time (hr) and the conversion percent is illustrated in **Fig 3.7.**

The highest concentration of glucose was obtained by using 10% sodium hydroxide solution equal 512 mg/dl at 45°C for 8 hr.

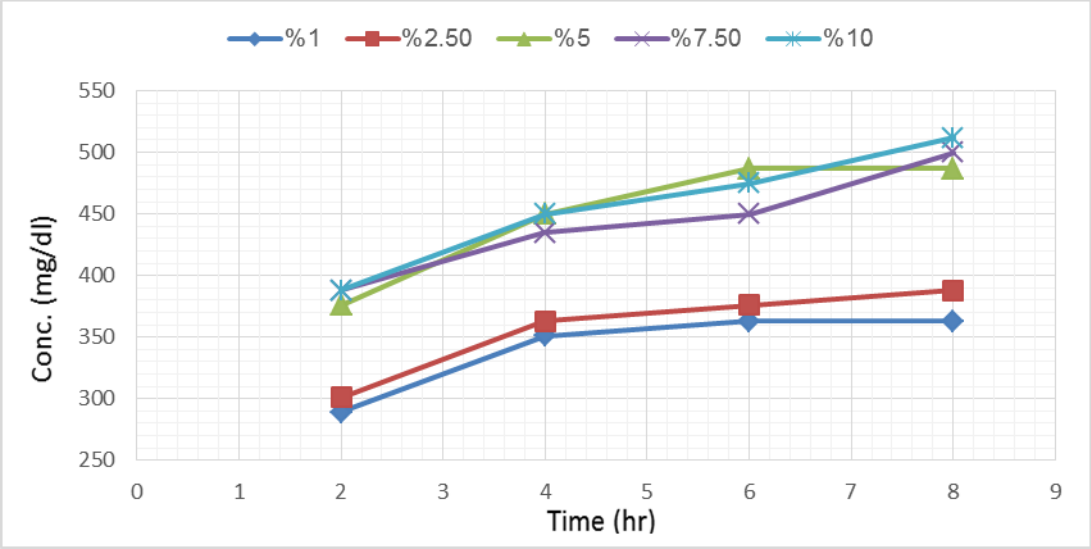


Figure 3.6: Results for hydrolysis of NaOH-treated Jeft using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at different percents . [**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)].

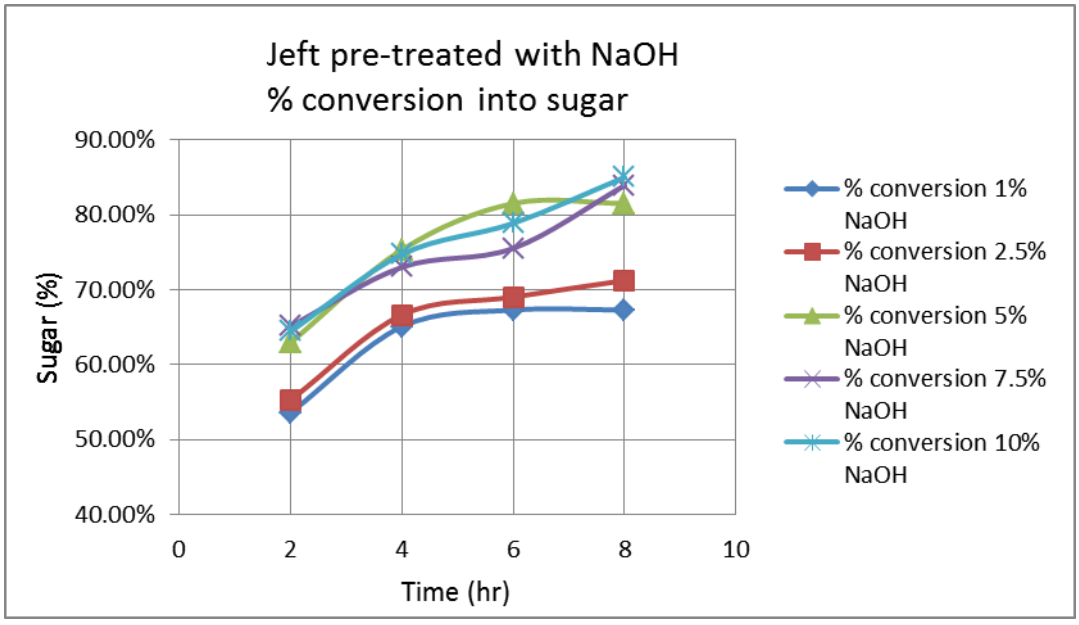


Figure 3.7: the percent conversion of jeft pretreated with NaOH at five different NaOH percents for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft , β glucosidase = 20.0 mg/g jeft .

3.1.3 Hydrolysis of calcium Hydroxide-treated jeft samples:

Five solution of $Ca(OH)_2$ with different concentration ; 1%, 2.5%, 5%, 7.5%, 10% were prepared. The treatment was performed in buffer solution of 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at temperature 45 °C for 2, 4, 6 and 8 hr period. The data were reported in **Tables 2.35, 2.36, 2.37, 2.38** and **2.39** in **chapter 2**. These results are graphed in **Fig 3.8,** which indicates that concentraion of glucose increased by increasing the treatment time and percent of $Ca(OH)_2$ in solution from 186 mg/dl in 1% $Ca(OH)_2$ solution to 363 mg/dl in 10% $Ca(OH)_2$ solution; both of them treated after 8 hr.

The highest concentration of glucose was resulted by using 10% calcium hydroxide solution equal 363 mg/dl at 45°C for 8 hr.

Also, the relationship between the treatment time (hr) and the conversion percent is plotted in **Fig 3.9.**

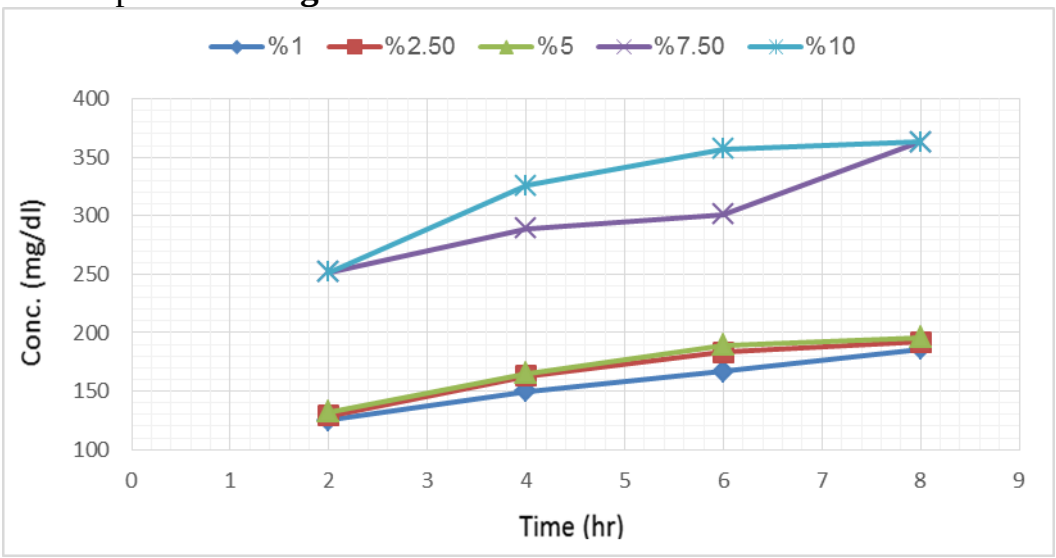


Figure 3.8: Results for hydrolysis of $Ca(OH)_2$-treated Jeft using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at different percents.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)] .

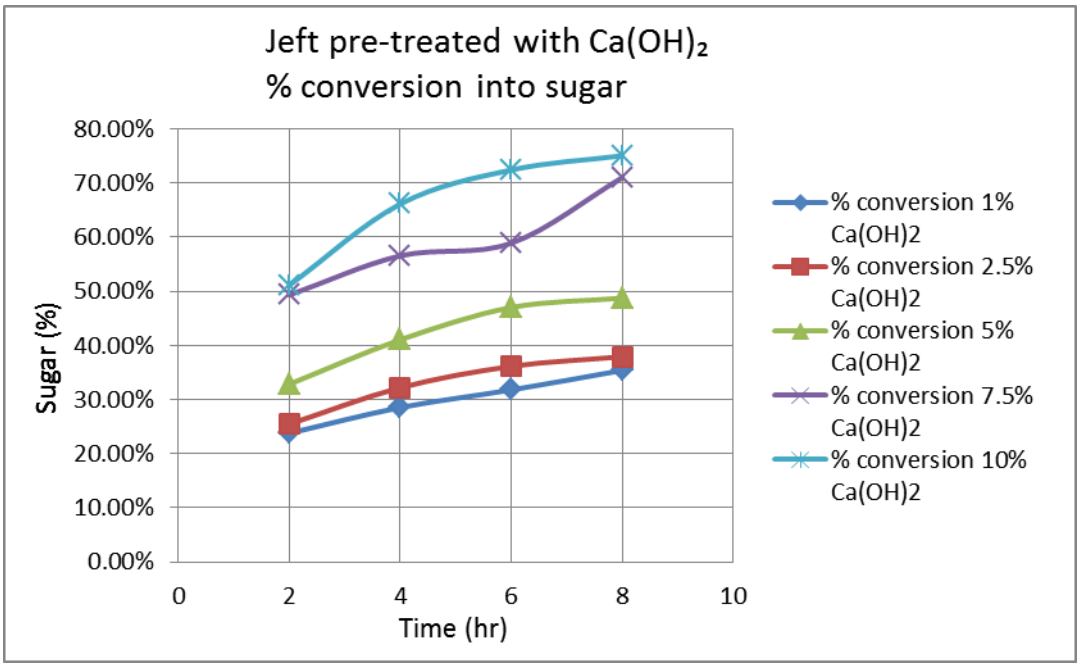


Figure 3.9: the percent conversion of jeft pretreated with $Ca(OH)_2$ at five different $Ca(OH)_2$ percents for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft , β glucosidase = 20.0 mg/g jeft.

3.1.4 Hydrolysis of acetic acid-treated jeft samples:

Five solution of acetic acid with different concentration ; 1%, 2.5%, 5%, 7.5%, 10% were prepared. The treatment was performed in buffer solution of 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at temperature 45 °C for 2, 4, 6 and 8 hr period. The data was reported in Tables 2.41, 2.42, 2.43, 2.44 and 2.45 in chapter 2. These results are shown in Fig 3.10, which indicates that concentraion of glucose increased by increasing the treatment time and percent of acetic acid in solution from 189 mg/dl in 1% acetic acid solution to 196 mg/dl in 7.5% acetic acid solution; both of them treated after 8 hr.

Fig 3.11 represent the relationship between the treatment time (hr) and the percent of glucose conversion.

The highest concentration of glucose was obtained by using 10% acetic acid solution equal 196 mg/dl at 45°C for 8 hr.

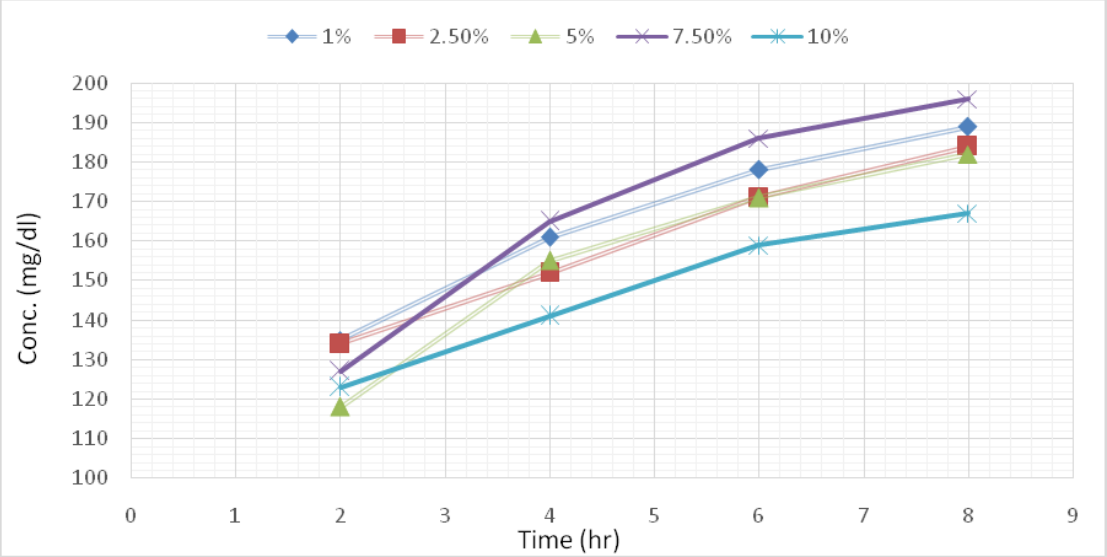


Figure 3.10: Results for hydrolysis of acetic acid-treated Jeft using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at different percents.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)] .

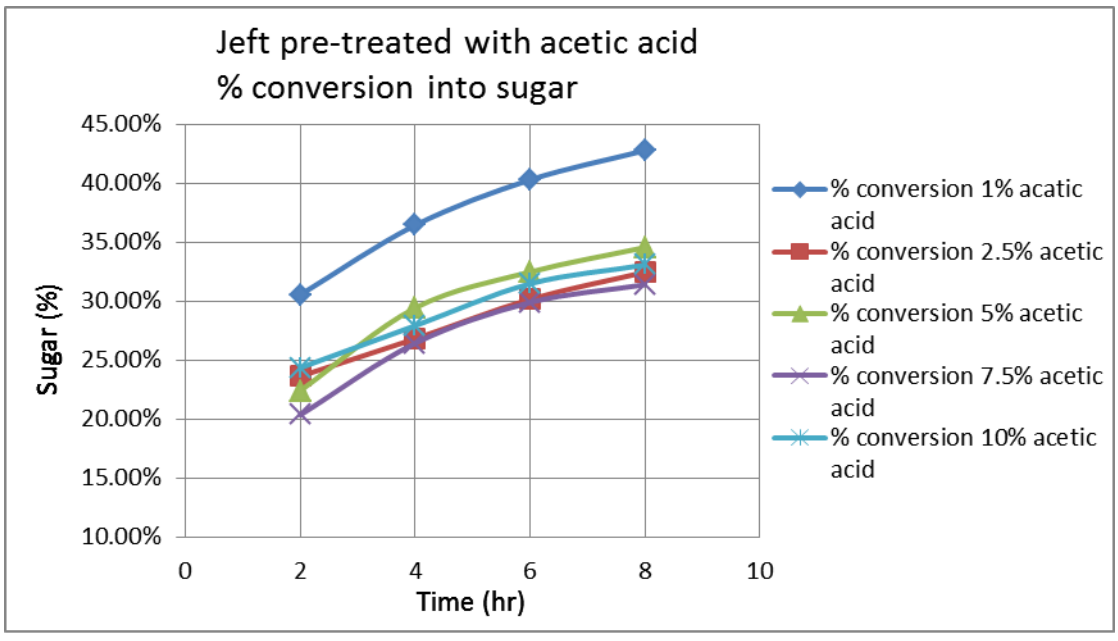


Figure 3.11: the percent conversion of jeft pretreated with acetic acid at five different acetic acid percents for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft , β glucosidase = 20.0 mg/g jeft.

3.1.5 Hydrolysis of jeft pulp and extracted cellulose samples:

A jeft sample was exposed to kraft pulping and cellulose extracts were prepared. The treatment was performed in buffer solution of 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at temperature 45 °C for 2, 4, 6 and 8 hr period. The results are graphed in Tables 2.47and 2.49 in chapter 2. These results are shown in Fig 3.12 which indicates that concentraion of glucose in extracted cellulose was lower than kraft pulping sample by increasing the treatment time because it has lower dry weight than pulp. But as a final result, the cellulose has higher percent conversion to glucose than pulp as illustrated in Fig 3.13.

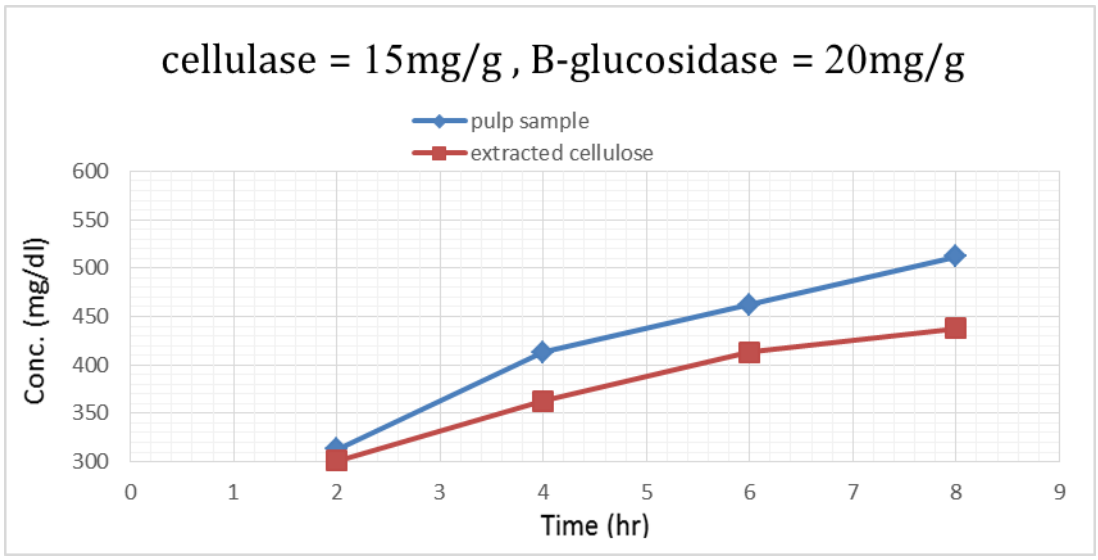


Figure 3.12: Results for hydrolysis of kraft pulping jeft sample and extracted cellulose using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)].

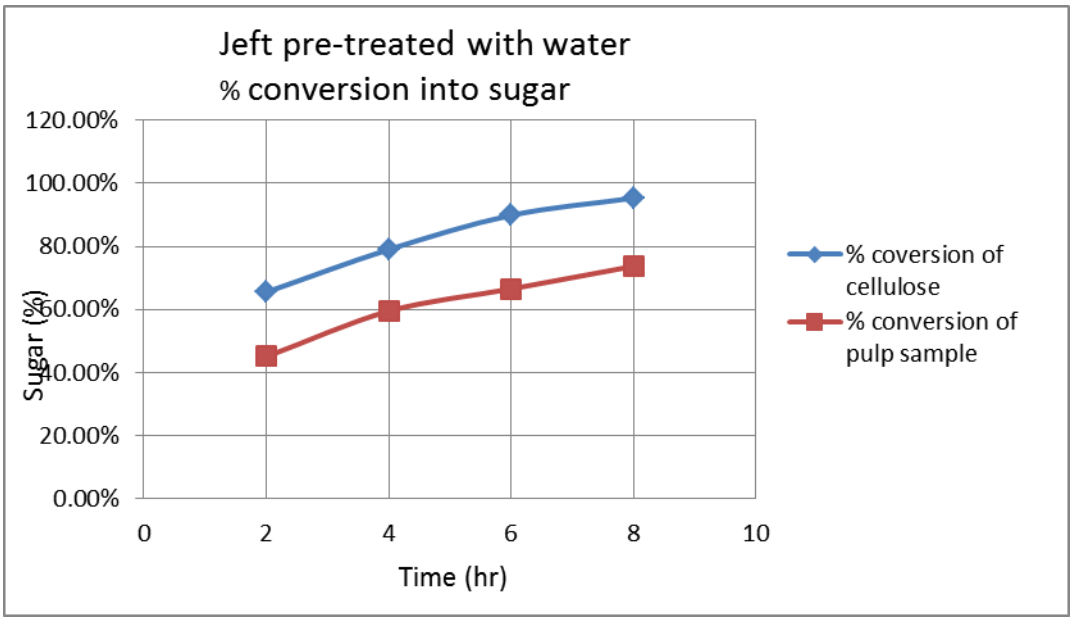


Figure 3.13: the percent conversion of cellulose and kraft pulp samples for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft, β glucosidase = 20.0 mg/g jeft.

3.1.6 Hydrolysis of sodium hydroxide pre-treated and water pre-treated jeft under a pressure of air:

A samples of 10% NaOH pre-treated and 1:4 water pre-treated were prepared and hydrolyzed in a reactor vessel under air pressure at 45°C . The treatment was performed in buffer solution of 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft at temperature for 2, 4, 6 and 8 hr period. The results are summerized in Tables 2.51and 2.52 in chapter 2. These results are shown in Fig 3.14 which indicates that concentraion of glucose in 10% NaOH treated jeft under air pressure sample is more than water treated jeft with ratio (1:4) under air pressure sample. Also the percent conversion of glucose is increased proportional with treatment time as show in Fig 3.15.

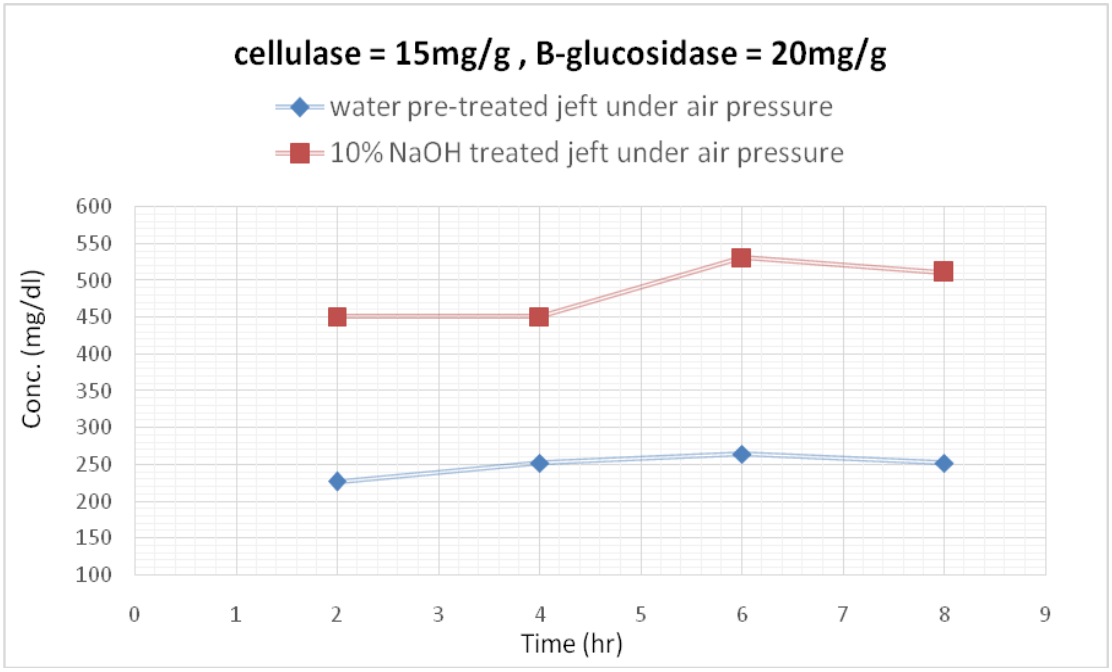


Figure 3.14: Results for hydrolysis of reactor treated sample under air pressure with 10% NaOH and water treated jeft with ratio (1:4) using 15mg cellulase/g jeft and 20mg β-glucosidase/g jeft.

[**conc.** : glucose concentration (mg/dl), **Time** : Treatment time (hr)] .

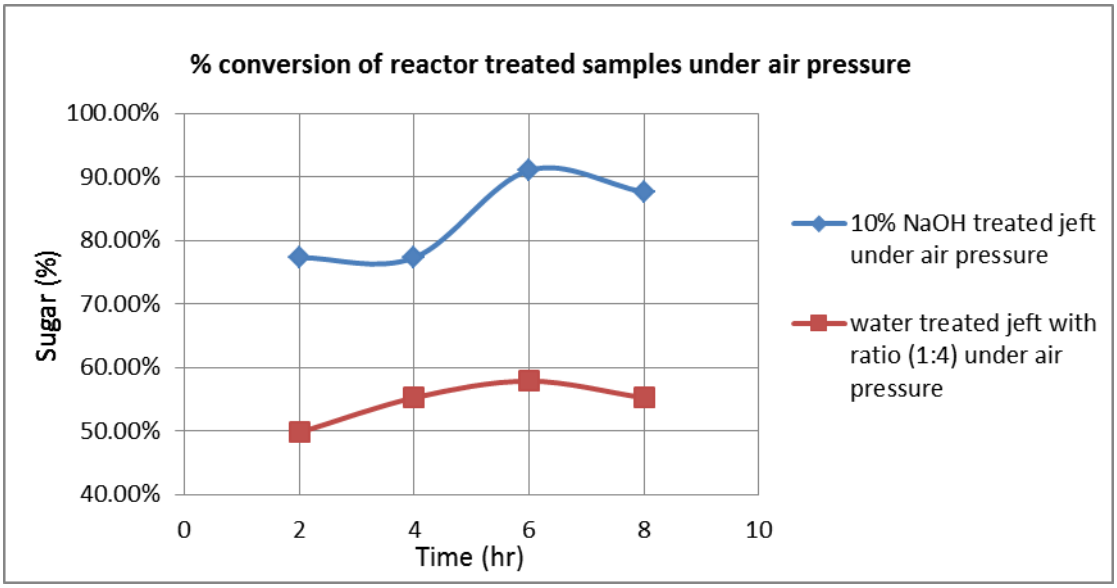


Figure 3.15: the percent conversion of reactor treated sample under air pressure with 10% NaOH and water treated jeft with ratio (1:4) for 2.0 hr using at enzyme dose of Cellulase = 15.0 mg/g jeft, β glucosidase = 20.0 mg/g jeft.

Conclusion

Jeft which is represent olive industry solid waste is considered as attractive material for researchers to convert this waste material into useful products such as fuels. Jeft consists of four main components: cellulose, hemicellulose, lignin and other extractives.

Cellulose represent about 40-45% from the olive pulp as we pointed previously.

The main objective of our research is to determine the optimum condition for the conversion cellulose material to glucose sugar by specific enzymes so as to produce bioethanol later on.

The parameters optimized are: jeft to water ratio, treatment time, enzyme concentrations, temperature and pH reaction media are evaluated.

In this work, several processes involving several treatments for conversion of cellulose to glucose were done and the optimal conditions were determined.

During the process, several conditions were proposed and optimal parameters were determined.

As a result, the best conditions are:

1- enzyme concentration cellulase 15.0 mg/g jeft and β-glucosidase 20.0 mg/g jeft.
2- Temperature 45°C.
3- Time 8 hr.
4- pH media 4.8.
5- best sample is 10% NaOH pre – treated jeft.

The best result we have obtained is 85.02% glucose percent form jeft raw material and 95.30% from pure cellulose at these condition.

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جامعة النجاح الوطنية
كلية الدراسات العليا

استخدام الانزيمات في تحليل المواد الصلبة (جفت الزيتون) وتحويله الى سكر الجلوكوز، المؤدي الى تكون الايثانول الحيوي

اعداد
إسراء جمال محمد داغر

اشراف
أ.د. شحدة جودة
د. عثمان حامد

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس, فلسطين
2015

استخدام الانزيمات في تحليل المواد الصلبة (جفت الزيتون) وتحويله ال سكر الجلوكوز، المؤدي إلى تكون الايثانول الحيوي

اعداد

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اشراف

أ.د. شحدة جودة

د. عثمان حامد

الملخص

الجفت هو عبارة عن المادة الصلبة الناتجة عن عملية عصر الزيتون والذي يتكون بشكل اساسي من السيليلوز, شبه السيليلوز واللجنين بالاضافة الى مكونات جانبية اخرى.

اشارت الابحاث السابقة الى ان الباحثون نجحوا في استخراج السيليوز بنسبة حوالي 40-45% من مادة الجفت الخام.

تعتبر مادة الجفت مصدرا حيويا لانتاج الطاقة المتجددة عن طريق تحليل مادة الجفت الى مكوناتها الاساسية وهي سكريات احادية بسيطة بخطوتين وهما:

1- معالجة وتنشيط مادة الجفت وتفكيكها بواسطة الماء, هيدروكسيد الصوديوم, هيدروكسيد الكالسيوم وحمض الخليك لتسهيل عملية دخول الانزيمات في الخطوة التالية.

2- الخطوة الثانية تتضمن اختراق الانزيمات مادة السيليلوز وتحللها الى سكريات احادية بسيطة.

تتكون هذه الانزيمات من انزيمين وهما سيليوليز وبيتا- جلوكوزايديز وهما المسؤولان عن تحليل مادة السليلوز الى سكريات. الهدف من عملية تحول السيليلوز الى سكر هو تحويل السكريات الاحادية الى مادة الايثانول الحيوي والذي يعتبر مصدر متجدد للطاقة بواسطة عملية التخمر.

من خلال هذا البحث توصلنا الى ان اعلى نسبة سكر حصلنا عليها من الجفت الخام هي 85.02% وذلك من خلال الظروف التالية:

1- تركيز انزيم السيليوليز = 15ملغم/غم جفت و تركيز انزيم ال بيتا- جلوكوزايديز = 20ملغم/غم جفت.

2- درجة الحرارة المثلى 45 درجة مئوية.

3- الوقت 8 ساعات.

4- درجة حموضة الوسط المحيط 4.8 .

في حين اظهرت الدراسة ان تحلل مادة السيليوز والمستخلصة حققت اعلى نسبة سكر والتي وصلت الى 95.30% في نفس الظروف المذكورة اعلاه.