ACIDIC PRETREATMENT AND ENZYMATIC HYDROLYSIS OF WASTE PAPER FOR RELEASE OF SUGAR

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Waste paper- Recycling.

Biofuel.

بسم الله الرحمن الرحيم

"In the name of ALLAH The Most Gracious and The Most Beneficial"

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Dated: ______________

FINAL APPROVAL

It is certified that we have read the final thesis entitled **"Acidic Pretreatment and Enzymatic Hydrolysis of Waste Paper for Release of Sugar"** submitted by **Mr. Asghar Hayat** and it is our Judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS Degree in Biotechnology.

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(MSBT)

This humble effort is

Dedicated

To

My beloved

Family

&

Teachers

Who inspired me for higher ideals of Life

DECLARATION

I hereby solemnly declare that the work **"Acidic Pretreatment and Enzymatic Hydrolysis of Waste Paper for Release of Sugar"** presented in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis bas been previously presented for any other degree.

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Asghar Hayat

CONTENTS

ABBREVIATIONS

DM	Dry Matter
EU	European Union
FTIR	Fourier Transform Infrared Spectroscopy (FTIR) Analysis
IEA	International Energy Agency
ISO	International Standards Organization
LCC	Lignin Carbohydrate Complex
LPG	Liquid Petroleum Gas
PCRET	Pakistan Council of Renewable Energy Technologies
PEPA	Pakistan Environmental Protection Agency
PKR	Pakistani Rupees
SSF	Simultaneous Saccharification and Fermentation
TGA	Thermo Gravimetric Analysis
TOE	Tons in Oil Equivalent
UNDP	United Nation Development Program
USA	United State of America
US DOE	United States Department of Energy

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ABSTRACT

Fossils fuel is reducing day by day throughout the world and biofuel is alternative source of energy. One of cheapest source of biofuel is wastepaper. The present study is carried out to hydrolyze wastepaper (office paper, newspaper and tissue paper) with different conc. of dilute sulfuric acid (i.e. 0.5%, 0.75% and 2%) using various temperature (90°C, 100°C and 110°C) at different retention times (15, 30 and 45 Minutes). Hydrolyzed wastepaper was then treated with enzymes cellulase and β-glucosidase for the release of sugars. The obtained sugars were analyzed by HPLC coupled with RI detector. In office paper cellulose, hemicellulose and lignin were estimated 40%, 32.5% and 22.5% respectively. In newspaper cellulose, hemicellulose and lignin were estimated 46.5%, 30.5% and 22.5% while, in tissue paper cellulose, hemicellulose and lignin were estimated 62%, 22% and 15.5% respectively. Optimum conditions for sugar contents of 50% in office paper, 65% in newspaper and tissue paper were observed in 2% concentration of acid on 110°C temperature for 30 minutes. During enzymatic hydrolysis of acidic pretreated substrate were hydrolyzed with enzymes cellulase and β-glucosidase with different time period (12, 24, 36, 48, 60 and 72 hours) and obtained 5.5 mg/ml, 7.5 mg/ml, 9.5 mg/ml, 12.2 mg/ml, 19.6 mg/ml and 18.3 mg/ml respectively of glucose from office paper, 6.5 mg/ml, 10.6 mg/ml, 16.4 mg/ml, 22.6 mg/ml, 20.8 mg/ml and 20.4 mg/ml respectively of glucose were found from newspaper and 7.2 mg/ml, 12 mg/ml, 16.3 mg/ml, 21.5 mg/ml, 23.4 mg/ml and 21.5mg/ml respectively of glucose recorded from tissue paper. In enzymatic hydrolysis the optimum conditions for more sugar releasing from the office paper was in 60 hours while for newspaper was in 48 hours and for tissue paper was in 60 hours were recorded. Through enzymatic hydrolyzed resulted that sugar (glucose and xylose) were recorded. Analysis of sugar yield from wastepaper through HPLC showed that there was little bit high amount of glucose. This research of acid pretreatment and enzymatic hydrolysis was an economical method to improve glucose conversion from wastepaper.

INTRODUCTION

INTRODUCTION

Fossil fuel is economically source of energy today in world. But due to increasing in human population the need of fossils fuel is very increased. There are many different methods are available to calculate current and undiscovered reservoirs of fossils fuel in the world and draw conclusion that the turn down of fossils fuel production in the world. The economic complexity and human behavior is reducing one type of energy resource by the development of technology. It is estimated in 50 years that the use of non-fossils fuel is less than one quarter of fossil fuels (York, 2012). It is expected that annual crude oil production will reduce from the 25 billion barrels to just about 5 billion barrels in 2050. So there is a need to explore other sources of energy (Campbell and Laherrere1998).

Energy is required for performing all forms of activities around the world. Today human life depends on energy due to its ability to perform work for the progress and sustainability of society. It is present in our food, it also run our vehicles and provides light throughout the globe. Energy Information Administration (EIA) have estimated that in 2005 the production of fossils fuel reached over 72 million barrels per day in the whole world and it is increasing day by day and reached over to 75 million barrels per day. The climate also changes by the increasing of world fossil fuels production in the world and the depletion of fossils fuel is the main challenge for future. The prices of fossils fuel are rapidly increasing from 1998 to date, the analysis of prices from 1998 to date describe the big variations between supply and increased demand. Due to this scenario the prices fluctuate at a very high rate due change in rate of demand and supply (Hook and Tang 2013).

Energy is an important part of human society today throughout the world and plays an important role in the development in every sector of life including economy and industrial sector. This result in increasing the quality of life and makes life more progress oriented. From a decade of years, people and governments which want to solve environmental problems and energy safety have guide to the encouragement of renewable energy sources. Biomass is the one of the big alternate of fossils fuel which can play important role in a more varied and sustainable energy blind (Ramachandra *et al.*, 2004).

At one side there are concerns regarding crude oil production, and on other side there is growing challenge regarding global supply to meet market demand. Since 2005 the production of fossils fuel has not increased by the time to meet its requirement. Before 2005 the production of fossil fuel is improved with rising demand but the supply has been less or more stable over the eight years to date (Murray *at al.*, 2012).

World needs more energy than it has today. The world is continuously being affected by energy crisis due to high energy demand. Reliance on the maximum use of petroleum product and high rate of growing population of the world must be reduced to a level where it can be controlled and does not affect the economy and society (Vaclav, 2010). Extreme use of crude oil is not only diminishing resources, but it is also slowly increasing the level of carbon dioxide in atmosphere which is responsible for global warming. Whereas natural changes occur in local and universal climates, this is the time for scientific communities and governments to sit together and discus climatic changes which occur due to activities harmful for environment. It is thus required to design quick and thoughtful procedures to reduce harmful impacts. The levels of greenhouse gases are gradually increasing and it is high from past eight hundred thousand years. If these levels of carbon dioxide are not controlled, big changes occur in global climate which effect on human normal life and their financial system. The International Energy Agency (IEA) has drawn few very important points; implementation of these points can help to control the rising greenhouse emissions (Laurent *et al.*, 2012).

United States Department of Energy (USDOE) has published that there is a need to speed up the research on conversion of biomass to energy which will help biofuels becoming more useful and cost effective. New Jersey OF United State annually produces 7.4 million tons of biomass which could deliver up to 5.2 million megawatt hours of power electricity and 250 million gallons of gasoline that could be used for transportation fuel consumption (Brennan-Tonetta *et al.*, 2014). A big source of biomass is the lingo-cellulosic biomass because it is mostly well matched for energy function due its presence everywhere, low price and environment friendly. Most of the energy production circles depend upon cellulosic biomass because it has near-zero greenhouse emissions (Demirbas, 2009).

U.S department of energy in 2013 have reported that the demand of energy is increasing continuously and worldwide pressure is created to produce energy by an alternative sources like biomass which is environment friendly. EU has the target of producing 20% of the world energy from renewable sources till to 2020 and according to National Renewable Energy Action Plan (NREAPs) more than third of the EU electricity consumption will come from renewable energy sources and the share of renewable energy sources in transport is projected to 111.2% in 2020. Wood biomass is harvested from forest contribute directly in residential and commercial use (Lacoa *et al.*, 2014).
Average biomass utilization in houses is about 2325 kg of firewood or 1480 kg of dung or 1160 kg of crop annually. The HESS evaluated that the total wood stock is almost 210 million tons among yearly production of 22.7 million tons. Likewise annually crop residue (principally cotton sticks) were present about 12-15 million tons (Anwer, 2001).

Total Amount of Renewable Energy Resources was 21.6%, 18.7% and 19.75 in different years 1973, 2004 and 2010 respectively (Ozturk *et al.*, 2013). Lignocellulosic material in plant cells gets energy from sun light during photosynthesis and contains different organic molecules likes carbon and hydrogen (Alvira *at al.*, 2010). The production of bio-fuel from lingo-cellulosic material is the second generation phenomena. In this process the lignocellulosic material that has six carbon sugars is used for the production of bio-ethanol (Mosier *et al.*, 2005). The lignocellulosic biomass consisted of three components, lignin, hemicellulose and cellulose which are associated with each other and forms a compact structure. The lignin is outermost part of biomass consisting of phenolic material that provides resistance from microbial and chemical attacks (Zhang *et al.*, 2010).

Energy which is renewable is the more fruitful to meet the sustainable development. Increasing use of fossil fuel throughout the world is depleting the reservoir consequently adding to the pollution and cause climate changes; it is a big threat for globe. There are large alternates known as renewable energy; are still undergoing commercial development. But some new technologies were developed which includes Brazilian sugarcane ethanol is a world energy product which is fully competitive with motor gasoline and good for replication in many countries (Goldemberg, 2007).

The plants materials that are made up by the combination of carbon, hydrogen, oxygen and other elements are known as biomass. The biomass is synthesized in sunlight in the presence of CO_2 and H_2O to form glucose which is then utilized by plant to form its structure. Biomass is structurally a chemical bond energy store that is gain from sunlight by breaking such bonds through oxidation process energy can be obtained from biomass (McKendry, 2002). Biomass is the big source of energy and having vast availability. Still now six biomass resources are identified for production of energy like: energy crop on surplus crop land, forest residue degraded land, agriculture residues, organic wastes and animals wastes (Hoogwijk *et al.,* 2003). Biomass can be utilized for the production of many types of energies including thermal energy, biofuels like bio ethanol, biogas, and biodiesel. These products are useful for combustions in different energy consuming components like boilers, fuel cells and combustion engines. Different methods and techniques can be worked on to gain energy from biomass (Sriram and Shahidehpour 2005).

Plant biomass is complex structure of polysaccharide that is consisting of cellulose, lignin and hemicelluloses. The most abundant form of polysaccharide on the earth is cellulose which is linear form of glucose. By enzymatic and chemical process cellulose component of plant is converted into simple sugar compound and then ethanol by fermentation (McKendry, 2002). Biomass like corn stove and sugarcane bagasse household feedstock these lignocellulosic biomass has ability to produce sizeable amount of bio-fuel and bio based material. Conversion of lignocellulosic material to alcohol basically consists of four basic steps like pretreatment, enzymatic hydrolysis, fermentation and alcohol purification. During the process of bio-fuel preparation process, first need to address the quick elimination of lignin and hemicelluloses by the step of pretreatment. In recent time it has been confirmed that the dilute acid pre-hydrolysis could achieve better results in low time and get better cellulose hydrolysis. But pretreatment working surrounding must be customized to the particular chemical and structural composition of the biomass (Eggeman, 2005).

Lignocellulosic material like municipal wastes, woody crops, agricultural waste and woodland residues present in large amount throughout the world in different weather condition, this biomass can be made low priced for the production of bio-fuel. These biomass are plentiful and viable in price, cellulosic material can offer unique resource which are using for producing organic product. Lignicellulosic materials give new road for production of bio fuel and other organic compounds which could be very effective in those areas of globe where there is a deficiency of fossils fuels. Biomass consists of carbohydrates in the form of cellulose, hemicellulose or lignin therefore in production of bio fuel and other organic compounds. The global production of biofuel has dramatically increased in recent years from 18.2 billion letters to 60.6 billion letters from 2000 to 2007 by the use of cheap substrate (Saini *et al.,* 2014).

Waste paper is made up of cellulose, hemicellulose and lignin (wood). These materials are found in agricultural waste, domestic waste and industrial waste. One of the sources of bio mass is waste paper. Which is not only cheap also readily available throughout country. Waste paper is in different form newspaper, office paper and tissue papers. The cellulosic material can be hydrolyzed via chemical or enzyme and fermented to produce biofuels (Saha, 2002). Lignocellulosic material has been identified as big source of sugar and then fermented to biomaterial and fuel. From last 80 years many method have been expanded for the conversion of lignocellulosic material to biofuel. Now we consider the natural struggle of plant cell wall to microbes and enzymatic degradation, collectively called "Biomass recalcitrance (Michael *et al.,* 2007).

It is estimated that the world has produced about 87 gigalitres of biofuel in 2008 which is equal to the consumed fuel in Germany that year. These biofuels were produced from plants, raising concerns regarding energy and CO_2 effects and competition between use of land for production of fuel, animals feed, food, ecosystem and fiber. Remaining technologies implementation is most effective to convert the lignocellulosic material to biofuels. However there has been less discussion of what type of plants can be useful for bioenergy (Somerville *et al.,* 2010).

Basically paper is made up of plants raw material like cellulose fibers and wood. Recycled papers, and agricultural residues contain 60% of the cellulose fibers. In developing countries it is obtained from non-wood raw materials such as bagasse, cereal straw, bamboo, reeds and esparto grass (Gullichsen and Fogelholm 2000).Paper has different kind of varieties like newspaper, office or white paper and packing paper. After working the papers is thrown everywhere which make environment polluted, waste the biomass and increase the cost for demand of new paper. If there is awareness in society for reuse of waste paper it will beneficial for environment and economy. The recycling of newspaper can save environment and also will reduce the cost of paper and will provide another feedstock for the production of biofuels. Very large amount of waste paper is destroyed and cannot be returned for use in the future. Awareness of people must be raised to the level that people must think of this waste as a feedstock for renewable energy resource. The problem in this issue is that the requirements for the production of biofuel from paper are that the temperature needs to be controlled and enzymes used must give sufficient yield (Guerfal *et al.*, 2014).

Daily mostly papers are discarded in the form of waste from offices, houses and different stores which bad affect the environment and pollute water systems. Waste paper is lignocellulosic material it is used as solid fuel and can give 16MJ/Kg of energy. Bio fuel is an alternative fuel which can be prepared from a different kind of waste material in which paper product is one of the big sources. The method discussed here is known as simultaneous Saccharification and Fermentation (SSF). It utilizes cellulase enzyme to break down the cellulose and yeast to ferment the resulting glucose. The ethanol produced can be used in blended form with gasoline as well as in neat form. Ethanol burns to give out less harmful gasses into the environment, it is renewable and clean energy (Gavrilescu, 2008).Hydrolysis of the cellulosic material in fermentable sugar is the first move for the manufacture of bio fuel. Pretreatment of cellulosic biomass is complicated then the hydrolysis of polysaccharides likes starch. The hemicelluloses are hydrolyzed to five carbon sugar which is hard to ferment. The cellulosic hydrolyzed into six carbon sugar which is very easily fermented. There are two methods for hydrolyze cellulose chemically, biologically and enzymatically (Sun and Cheng 2002).

Chemical method uses strong acids whereas enzymatic process utilizes variety of microorganisms. Different substances are used for the hydrolysis of cellulose such as Sulfuric acid, Hydrochloric acid, heterogeneous solid acids, sulfonated carbonaceous based acids, polymer based acids and magnetic solid acids (Huang and Fu 2013). The funguses involved in the conversion of biomass into energy are brown fungus and white fungus which produce enzymes that degrade the cellulose and gain energy from it. Brown rots are microorganisms that are used for treatment of waste paper that attack on cellulose. However white and soft rots attack lignin and hemicellulose. The cellulase enzyme that is produced by fungus such as (*Trichoderma, Penicillium, Aspergillus, Schizophyllum and chrysosporium*) and bacterias (*Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Clostridium, Cellulomonas and Streptomyces*). Enzymatic hydrolysis process is low in cost as compare to other methods. The products of the hydrolysis are usually reducing sugars including glucose (Sun and Cheng 2002).

Pakistan is a developing country which has approximately 200 million populations, it is estimated that it produces more or less thousand tons of waste material on daily bases. Waste material contains every kind of waste like disposable plastics, rubber, wasted foods, slaughter house wastes and several other types of biomass. Due to underdeveloped or nonexistent recycling industries and weak management system, the collection of recyclable waste material is very poor because the recycled waste material like newspaper, bottles, tin cans, bread, books and magazines are passing through different hand from sweepers to street pickers to small dealers and to recycling industries. Step by step passing of waste to industries can check the cost of waste materials and but no one is interested for establishment of recycling industries in small cities. Pakistan Environmental Protection Agency (PEPA) has reported in 2005 that 1.896 kg/house/day to 4.29 kg/house/day produces waste in major cities. The collection rate of solid waste by respective municipality's ranges from 51% to 69% of the total waste and uncollected waste i.e. 31% to 49% remains on street, corners, open spaces and vacant plots; polluting the environment (Mahar *et al.*, 2007).

Seeing the demographical position, agricultural activities and meteorological sequences, there are different renewable assets are technically feasible and have big opportunities in Pakistan, which comprises solar, water, wind and wastes (solid wastes, chicken farms, forestry waste, paper waste, agriculture waste, furniture and factory waste, hospital waste and animals farms). Pakistan can be helped from these alternates' sources of energy. Pakistan was producing maximum energy from aquatic resources but now this tendency is decreasing. But due many issues in dams and other problems hydal generation is moving to thermal power plants. These plants drive on fuel like oil (petrol, diesel, etc.) and gas per unit cost is very high. Enormous quantity of oil is being wasted due to import which is not sufficient assets in Pakistan. Available assets of oil and gas are contributing only 5 and 48.8% in total energy generation and are estimated to reduce in about 16 years (Mahmood, 2014).

Use of oil in thermal plants has two main drawbacks, first environmental pollution by release of carbon dioxide, carbon monoxide and nitrogen and use of fossils fuel. In the present situation show that there will be need a dependable solution to achieve the energy requirements of this country is to exploit the use of renewable energy resources. These resources in the form of biomass are available selected area. It is estimated that the available biomass is about average of 129 t/d of biomass (Dodic *et al.*, 2010).

Pakistan is expected to turn as energy passageway for the area as it grips important strategic location by neighboring with India, China, Arabian Sea, Afghanistan and Iran. Due to this geographic position of Pakistan will have to struggle for energy self-reliance. The available energy is in smaller amount to fulfill the requirement. Day by day energy need are increased on gas, oil, liquid petroleum gas (LPG) and electricity has been reported. Pakistan economic survey has reported the annual cumulative growth rates of material are as under Gas 3.7%, Oil 5.7%, LPG 14.3%, Coal 7.5%, Hydal electricity 0.6%, Nuclear electricity 1.7%, Imported electricity 25.5%. It was reported that the total used of energy in 2009-2010 was -.64% and it is reduced upto-3.09% per capita available energy in Tons in oil equivalent (TOE). It is suggested that the available resources will have to 29.06% of its energy deficit in 2021-2022. European Union has enacted that the members of EU should be producing almost 22.1% of their energy from renewable resources to meet with the pledge of producing energy from good alternative sources (Nayyar *et al.*, 2014).

Pakistan by following same code of conduct may fulfill its energy needs and satisfy the role of being an environment friendly nation. Keeping in view the economical importance of lignocellulosic biomass the present study was carried out with the following objectives:

1. Optimization of dilute sulfuric acid pretreatment condition from waste paper.
2. Enzymatic hydrolysis of waste paper with cellulase.
3. Production of fermentable sugars under different temperature and reaction time
4. Analysis of various sugar by using High Performance Liquid Chromatography

REVIEW OF LETRATURE

REVIEW OF LITERATURE

Tengborg *et al.,* (2001) have evaluated a research to enzymatically hydrolyzed the softwood of plants for the production of bioethanol due to presence of high contents hexoses. Complete conversion of cellulose to glucose of softwood by enzymatic hydrolysis is not sufficient. The great importance in overall economy is improvement of cellulose conversion. It has been investigated in study that spurce influence on the conversion of cellulose. By the addition of β-glucosidase increased the cellulose conversion by enzymatic process as compare to cellulose. The addition of large amount of enzyme increases the conversion of spurce into glucose when all the step of hydrolysis was followed. It conversion process is greatly influence by temperature, pH and time at dry matter (DM) content. At optimum temperature, 4.9 pH. 144 h and 5% DM the conversion of cellulose was 69.2%. When the DM of substrate was decrease from 5% to 2% the conversion of cellulose reached to 79.7%.

McKendry (2002) in part1 paper described the production of energy from biomass. Biomass is the most common source of energy in developing countries like Asian, African and Latin American. To replace the fossils energy source much attention is needed to identify the biomass that are high energy sources. The main source of biomass is mostly plants residues (rice and wheat straw, sugar cane bags and unwanted grasses), animals manure and paper wastes. The production of energy from biomass has great impact on global warming.

McKendry (2002) in part 2 paper discus the conversion technologies for energy production from biomass. Increasing environmental pollutions have led to call for the use of renewable energy sources. Plant biomass is the potential source of energy. The energy gain from biomass is usually used as electricity and engine combustion. Using of biomass as energy reduce the pollution level as well increase the resources of energy.

Perez *et al.,* (2002) have analyzed the biodegradation of biomass. Cellulose, lignin and lignocelluloses are the major sources of biomass. The process of degradation is completed by using microorganisms that are producing enzyme. Using enzyme and microorganisms' for the degradation of biomass is environment friendly technology. This research study describes advancement in treatment of lignocellulose for biofuel production. Such biotechnological methods are applied on paper and pulp manufacturers.

Heller *et al.,* (2004) describe the production of energy from willow biomass their environmental benefits of production electricity. Willow is the important source of electrical energy in USA. The biofuel gained from biomass has positive impact on environment as compared to fossils fuel. It is conform by Heller *et al* conducting a research that 10% biomass gives 8.9% energy, decrees 7-10% potential global warming, reduced 9.5% emission of SO_2 and also expected to reduction of NO_2. It is estimated that willow biomass provide gasification and direct fire generating facilities and avoid pollution as compare to other energy sources.

Park *et al.,* (2004) have conducted a research to investigate the lactic acid production in office waste paper with help of enzymatic hydrolysate. He has been used the culture of *Rhizpuse oryzae* for 4 days which contain 7 xylose, 82 glucose and 34 cellobiose per gram per liter. The lactic acid yield production glucose medium was only 16.3g/l/d. The production lactic acid yield from office waste paper was very low which comprised the ratio of 1:2:7 or 2:1:7 of xylose: cellobiose: glucose. The lactic acid yield production was similar to that glucose production. This indicates that xylose inhibited the production rate that derived from hemicelluloses and yield may be inhibited by some compound that derived from paper pulp.

Park and Okuda, (2004) have evaluated enzyme to hydrolyzed waste paper. They have used three kind of enzyme Meicelase, Cellulosin T2 and Acremonium cellulase. Enzymes were loaded from 1 to 10% for the conversion of waste papers to investigate the sugar. The conversion of waste was increased by increasing enzyme load. If the load enzyme is increases to 10% acremoniumcellulase reached to 79% conversion of waste paper which 17% than Meiclase and 13% than cellulosin T2. The conversion is calculated by using formula $x=kE^{m}t^{(aE+b)}$ m,a and k used as constant and E indicate the start concentration.

Wen *et al.*, 2004 have conducted a research on animal manure that are the major source of lignocellulose. In such a research they have hydrolyze the manure into fermentable sugar. When the animal manure were treated with 3% sulfuric acid for one hour under the temperature of 110 °C hemicelluloses was degraded into arabinose, xylose and galactose. The treated materials were treated with enzyme to hydrolyze cellulose. It is found that by reducing the particle size to 590- μm result in high glucose yield and by addition of 2% tween-80 result in 20% glucose yield. Such process achieved 11.32 g/100 glucose yields of which is equal to 40% conversion

Wu and Cheng (2005) have analyzed commercial cellulase gain from *Trichoderma reesei* in his laboratory. Waste paper was hydrolyzed with vertically hanging immobilized cellulase. The major hydrolysis products were glucose, xylose and cellobiose. The diameter of cellulase pellet was 4.190±0.291mm. the UV irradiation deactivate the activity of immobilized cellulase. The activity of immobilized cellulase is restoring by washing with distilled water. The main advantage of the vertically hanging cellulase is that it can be reuse and recycle. The overall application of immobilized cellulase for waste paper hydrolysis issuccessful.

Cara *et al.*, (2008) have investigated biomass of olive tree for the production of sugar with help of dilute sulphuric acid. The acid was in different concentration 0.25 w/w, 0.6% w/w, 1.0% w/w and 1.4% w/w. With help of 1% sulphuric acid and 83% hemicelluloses sugar from raw material at 170 °C. But in case enzyme hydrolysis yield was 76.5% with 1.45 acids at 210 °C. It was noted that the recovery of sugar was very poor in prehydrolytic. The generated fermentable sugar by pretreatment and enzyme hydrolysis were taking into account and sugar was calculated. The research conform that from 100gram biomass of olive release 36.3 g sugar by treating to 1% sulphuric acid at 180 °C.

Mirza *et al.*, (2008) have described the biomass energy utilization in Pakistan. Energy plays a vital role in the development of any country. Biomass is a traditional that has been used by mankind for thousands of years so there are different kind of biomass which is also in use now days like agricultural residues, animal manure and firewood. Biomass meets every day energy requirement of low income and rural house hold in Pakistan. It has been estimated by the scientist that the average using of biomass/house/annum in Pakistan contain 1160 kg agricultural residues, 1430 kg of animal manure and 2325 kg firewood. It is also mentioned in study that PCRET has installed 60,000 energy-conserving biogas plants use domestically in rural area. Research on biodiesel production from biomass is also under process. Electricity is also generated from sugarcane bagasse. Some effort is needed for government to financially support scientists and institutions that are showing entrusts for the production energy from biomass as well recycling municipal waste.

Perez *et al.*, (2008) have mention in his paper that wheat straw is big source of bioethanol. They have design liquid hot water method for pretreatment of wheat straw for the production of ethanol. The solid residue of sugar from hemicelluloses was obtained by filtration when they were enzymatically treated. The result of conducted research shows that the obtained yield of hemicelluloses derived sugar was 43.6% in a liquid solution for 40 min under 188 °C and enzymatically treated yield was 79.8%. The yield gaining response was variable under different temperature and time. At 184 °C and 24min the yield was 71.2% but at 214 °C and 2.7 min yield reached to 90.6%. it show that decreasing both time and temperature result in low yield but increasing the temperature and reducing the time duration give the highest recovery of fermentable sugar.

Sanchez and Cardona in (2008) have conducted a research in which they have deals with biotechnological process for the production of ethanol from different raw materials like (sugar cane, starchy materials and lignocelluloses biomass). The complexity of biomass is described by different stages that are involved in the conversion of lignocellulose. The fermentation process of three groups for feedstock is discus for the production of ethanol fuel. Some conclusions are considered in current research study for the conversion of feedstock into ethanol fuel under pretreatment and biological process.

Demirbaş (2009) analyzed research for the production of bioethanol from cellulosic materials and their use in motor as fuels. Ethanol is basically alcohol a liquid biofuel that is produce by the fermentation of sugar, cellulose and starch. The cellulose biomass is resource of urban wastes, forest and agricultural materials. Different kinds of acids, enzymes, microorganisms and conversion technologies are used to break down the hemicelluloses into sugar and others biofuels products. The cellulose and hemicellulose portion of biomass is hydrolyzed by enzyme and acid into sugar. The sugar is fermented into bioethanol which are used as motor or engine fuels.

Binder and Raines (2010) have reported in his that plant biomass is an abundant source of energy. They have also reported a high yielding chemical process to hydrolyzed pant biomass into monosaccharide sugar. The gradual addition of water into the chloride ion liquid process result in to production of 90% glucose from cellulose and 80% sugar from corn stover. Yield from ionic liquid is recovered by ion-exclusion chromatography. This is a simple process which does not require edible plant and cellulase.

Gupta and Lee (2010) have investigated biomass degradation by sodium hydroxide and ammonia to enhance the digestibility of enzyme. The effect of pretreatment was increase by H_2O_2 supply with alkaline. At high temperature H_2O_2 was unstable but they were supply in step wise from low to high so this was found effective. During alkaline free treatment solubilized hemicelluloses was found in a liquid that are exist as lignin carbohydrate complex (LCC). The formation of LCC protects hemicelluloses sugar from degradation. The formation of lignin is precipitated and analyses TGA and FTIR. The data gain from TGA indicate that NaOH lignin has uniform structure and high O/C ratio then ammonia lignin and FTIR data indicate that NaOH lignin has lower aroma content but higher guaiacyl stricter than ammonia lignin.

Lee *et al.,* (2010) have conducted a research to enhance the digestibility of enzyme against waste paper. The pretreatment was conducted on different concentration of sulphuric acid. The optimum temperature they have noted 2% sulphuric acid, 150 °C for 15 minutes which have approximately 75% remove lignin and hemicelluloses to intact the cellulose. Then 94% enzyme digestibility was gained the dissolution yield was strongly related to enzymatic digestibility.

Laopaiboon *et al.*, (2010) have acidically hydrolysis of sugarcane bagasse for lactic acid production. They have used two types of acids hydrochloric acid and sulphuric acid at different condition. The concentration of acid was from (0.5 to 1% v/v), temperature was (90-120 °C) and reaction time was from (1-5 h). Under the condition of 0.5% of HCl at 100 °C for 5 h the maximum enzymatic efficiency was 10.85 which contain the component xylose, 22.59; arabinose, 1.29; glucose, 1.50; furfural, 1.19 and acetic acid, 0.15 g/l. The yield of lactic acid was increase by the addition of *Lactococcus lactis* in hydrolysis. The final products of fermentation process were ethanol, 5.24, formic acid, 6.04, lactic acid, 10.85 and acetic acid, 7.87.

Amjid *et al.,* (2011) have conducted a research study on biogas the renewable energy source for Pakistan. They have mention that developing countries are also in energy crisis. Pakistan annually spend 7 billion dollar for the import of fossils fuels to fill the demand of energy so renewable energy production from biomass are the best option for the substitution of conventional energy. Pakistan has a great opportunity for production of biogas it has 159 domestic buffalo and cattle which produces 652 million kg manure per day if that is use for the generation of energy it produce 16.3 million m^3 biogas/day and approximately produce 21 billion tons of bio fertilizers annually. The domestic biogas use in Pakistan were started in 1959 now it has gained a lot of achievement and government have stated biogas support program in 2000 which have installed 1200 biogas plants and it is expected to install 10000 plants within next five years. Only $10m^3$ size of biogas plant save 92062 PKR annually. The demand of biogas is increasing steadily because are low cast and can be installed at low price.

Chaudry and Qazi (2011) have analyzed the energy challenges for Pakistan. They have mentioned in study the depletion of fuel sources due increase in population and development of large industries. It is essential to save the energy reservoir by using renewable energy source like solar energy, biomass and wind energy. It is mention in study that the South Asian countries rural areas household follows the traditional uses of energy as fuel like animal dung, firewood, gobar gas and crop residues while the transition of energy uses are only seen in urban and semi urban areas and such changes are very slow in rural area. In Pakistan majority of the rural household follow the traditional methods of energy uses and most of the potential are wasted due to lake of scientific methods. It is needed to develop modern bio-energy technologies for the conversion of biomass in to energy to overcome the energy need of Pakistan. We are trying to recall the attention of people for the current issues and challenges related to energy crises and also to give awareness for the utilization of biomass as energy sources.

Harun and Qanquas (2011) perform a research on renewahle energy biomass to produce Ethanol. In a present study they have examine the enzymatic hydrolysis of *Chloroccum* specie with cellulase obtained from *Trichoderma reesei* ATCC 26921. Kinetic hydrolysis was fitted with Michaelis–Menten's model. The obtained glucose yield was 64.25% w/w under the temperature of 40 °C . The kinetic study production of glucose and cellubiose were double as compare to fast glucose and cellubiose production.

Anwar *et al.*, (2012) have analyzed that biomass is an abundant source of biofuel that are produce by using *Fusarium oxyporum* and *Sacchromyces cervesiae*. They have use rice which is cheaper source for the production of biofuel. The conversion of biomass into bioethanol requires acid treatment at 1st step. Inhibitory compound are reduce by using Response surface methodology during sulphuric acid treatment. The biomass is treated with 1.5% dilute sulphuric acid under 100 °C for 30 minutes. During hydrolysis 16.52mg/Ml glucose are obtained by using 1mL enzyme at 50 °C for 72 hours. Glucose is then converted into bioethanol by using *Sacchromyces cervesiae* and *Fusarium oxysporum*.

Dubey *et al.,* (2012) have evaluated acid pretreatment of waste paper for the production of bioethanol. The presence of 70.12 % holocellulose makes waste paper as source of renewable energy. It was found that waste paper contain 16.33 % lignin, 7.42% pentosan, 61.5% cellulose and 8 moisture. For the waste paper pretreatment condition of dilute acid were put vary of solid/ liquid ratio 1:8 to 1:14, the reaction time was set from 1 to 6 hours and the temperature of autoclave were set for 120 ^{o}c. waste paper acidic hydrolysis through yeast *Pichia stipitis* at optimum temperature resulted in ethanol production 3.73 ± 0.16 g/l.

Nakazawa *et al.,* (2012) have developed a recombinant strain of *Trichoderma reesei* X3AB1 for the pretreatment of cellulosic biomass. The developed strain express β-glucosidase 1 that is highly specific under the control promoter *xyn3*. B-glucosidase1 produce from *Trichoderma reesei* has 25 fold higher activities against cellulose then parent strain. The Xylanase activity was fined 30% lower due absence of xyn3. When X3AB1 was grown on 0.5% xylan and 1% Avicel it produces 3.3 and 2.3 fold more β-xylosidase and Xylanase respectively. The supplement from X3AB1 saccharified NaOH-pretreated rice straw at low dose of enzymes. Its indicate that developed strain is good for biomass conversion.

Wang *et al.*, (2012) have conducted a research to hydrolyzed waste paper for the production of biofuel. The waste paper like (office paper, magazine and newspaper) they have used for their research was oven dry. They have found 10% efficiency enzymatic conversion of wet newspaper and solid loading hydrolysis found 15% using two enzymes. The research shows that office paper and cardboard are found more suitable for bioethanol production than newspaper and office paper. The experimental data describe the parameter of glucan and xylan hydrolysis. This model provides design required for commercial production of bioethanol from waste.

Das *et al.,* (2013) have conducted a research to produce enzyme cellulase and xylanase from *Aspergillus fumigates* ABK9 and for production surface methodology was employed to optimize mixed substrate solid state fermentation. The enzyme production were influence by different parameters like substrate amount (10.0 to 10.5g), pH (6.1 to 6.2), fermentation time (86–88 h), and substrate ratio (wheat bran: rice straw) (1.1). Under these conditions endoglucanase, β-glucosidase, FPase (filter paper degrading activity) and xylanase activities of 826.2, 255.16, 102.5 and 1130.4 U/g, respectively were obtained. The enzyme was obtained from cocktail extracted to increase the fermentation process of waste paper brightness till to 82.8% ISO.

Guerfal *et al* (2014) have reported in his research that biomass is an energy sources which reduce the use of petroleum bass energy. They have investigated newspaper and office waste paper through enzymatic hydrolysis to produce fermentable sugar. The enzyme were obtain from two microbes *Trichoderma reesei* Rut-C30 and *Aspergillus niger* F83. Under hydrolysis condition the sacchrification of newspaper was 67% and office paper was 92%. The release sugar from waste paper was then converted into ethanol by help of *Saccharomyces cerevisiae.*

MATERIAL & METHOD

MATERIAL AND METHODS

This study was conducted at Biotechnology Laboratory in PMAS Arid Agricultural University Rawalpindi and at department of Bioinformatics and Biotechnology, International Islamic University Islamabad.

3.1 Sample

Three types of papers were used for this study like used confidential office papers which was completely demolished by using different machine and then burnt, old newspapers and used tissue papers, these tissue papers were used by employees of different offices. These papers have been used in experiments were collected from different private offices of Islamabad (Figure 3.1 (a-c)).

3.2 Sample collection

The sample waste paper (office paper, newspaper and tissue paper) were collected from local areas of district Islamabad, Pakistan.

3.3 Sample Preparation

After collection the sample (office paper, newspaper and tissue papers) was shredded into small pieces by using machine (AS1225CD AURORA) shown in the Figure 3.2. Further substrate was chopped into small pieces, dried in sunlight and packed in polyethene bag before taken into laboratory. The preparation of the samples was carried out according to methodology described by Laboratory Analytical Procedure (Haines *et al.*, 2004).

3.4 Moisture Content Determination

Three washed and dried large petri dishes were taken and weighted with electric balance and weights of dishes were noted. 20 grams of each sample (office paper, newspaper and tissue paper) were collected in dishes and again weighted with electric balance and sample were air tightly packed and dried by using oven for an overnight (12 hours) on 110 ^{o}C and weighted again with the help of electrical balance (Lin *et al.*, 2010).

Figure 3.2 Shredding machine:

3.5 Estimation of Cellulose

Estimation of cellulose was made by the method of Gopal and Ranjhan, 1980. In this method one gram (W_1) of sample (office paper) was taken into round bottom flask and added 30 ml of 80% acetic acid. The sample with acetic acid mixed with 2 ml of concentrated nitric acid and refluxed for 20 minutes. After this the sample was filtered and residues were dried by using oven at 105°C for a night and again weighted the sample (W_2). The sample was than heated and converted in ash by using muffle furnace at 550°C for 6 hours and weighted again (W_3). The cellulose content (%) was calculated by the following formula (Gopal and Ranjhan 1980). Similarly processes were done for newspaper and tissue paper. Amount of cellulose content (%) was estimated (Figure 3.3).

$$\text{Cellulose contents (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

3.6 Estimation of Lignin

Estimation of lignin was carried out by the method as describe by Milagres (1994). In this method 1gm of dried sample (office paper) (W_1) was taken in round bottom flask and 70 ml of 1.25 % of H_2SO_4 were added and refluxed the mixture for 2 hours. After that the solution was filtrated and separates the residue and added into 30 ml of 70% of H_2SO_4 and stirred for 120 minutes with the help of magnetic stirrer. After stirring excesses amount of water was added to dilute the solution. The sample was filtered and residues were dried by using oven for an overnight and weighted again with the help of balance (W_2). By applying formula given below, the lignin content was calculated (Milagres, 1994). Same processes of estimation of lignin were done in newspaper and tissue paper.

$$\text{Lignin contents (\%)} = \frac{W_2}{W_1} \times 100$$

3.7 Estimation of Hemicellulose

Estimation of hemicellulose was carried out by simple calculation (Figure 3.3).

$$\text{Cellulose} + \text{Lignin} - 100 = \text{Hemicellulose}$$

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Figure 3.1(a) Office paper

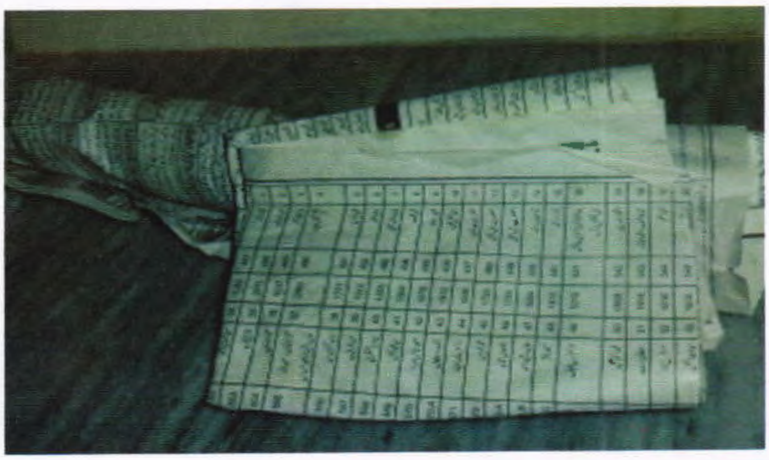

Figure 3.1 (b) Newspaper

Figure 3.1 (c) Tissue paper

Figure 3.2 Shredding machine:

3.5 Estimation of Cellulose

Estimation of cellulose was made by the method of Gopal and Ranjhan, 1980. In this method one gram (W_1) of sample (office paper) was taken into round bottom flask and added 30 ml of 80% acetic acid. The sample with acetic acid mixed with 2 ml of concentrated nitric acid and refluxed for 20 minutes. After this the sample was filtered and residues were dried by using oven at 105°C for a night and again weighted the sample (W_2). The sample was than heated and converted in ash by using muffle furnace at 550°C for 6 hours and weighted again (W_3). The cellulose content (%) was calculated by the following formula (Gopal and Ranjhan 1980). Similarly processes were done for newspaper and tissue paper. Amount of cellulose content (%) was estimated (Figure 3.3).

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$$\text{Lignin contents (\%)} = \frac{W_2}{W_1} \times 100$$

3.7 Estimation of Hemicellulose

Estimation of hemicellulose was carried out by simple calculation (Figure 3.3).

$$\text{Cellulose} + \text{Lignin} - 100 = \text{Hemicellulose}$$

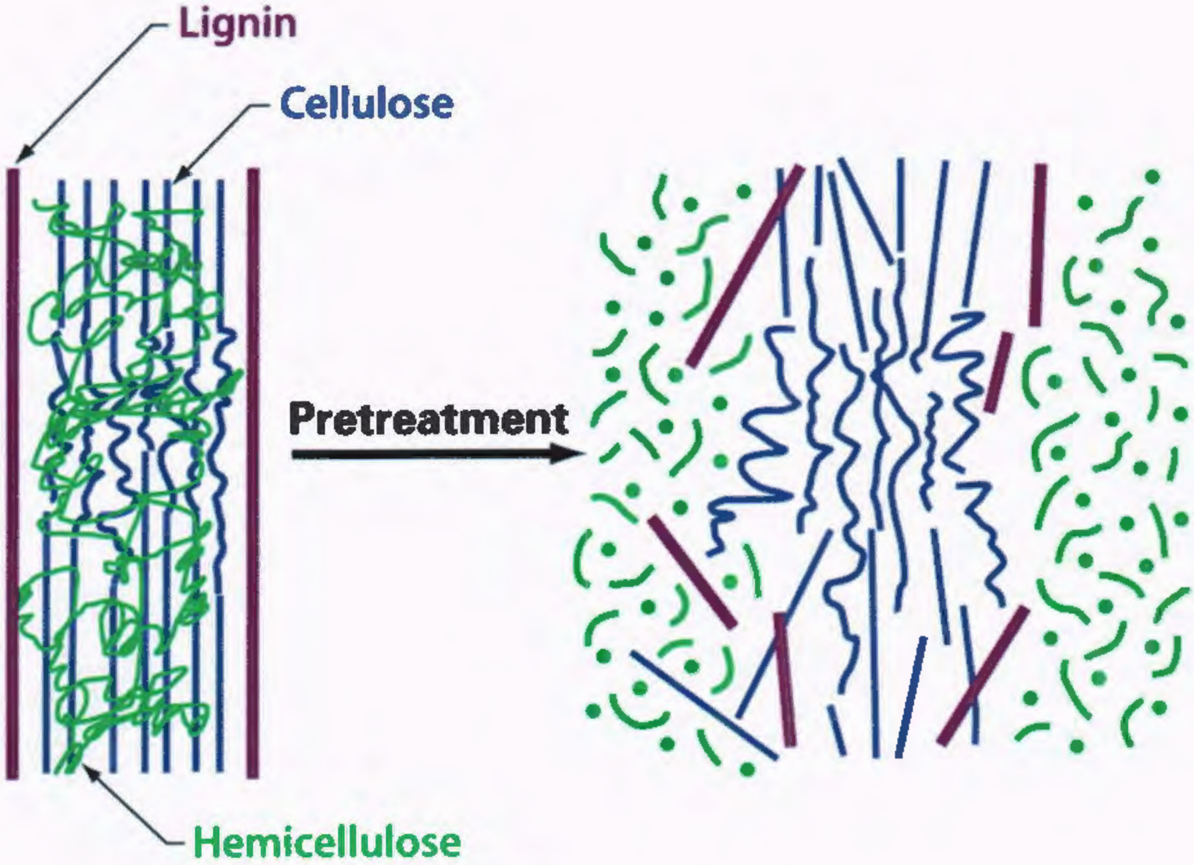

Figure 3.3 Diagrammatically presentation of polymers of lignocellulosic contents (Mosier *at al.*, 2005)

3.8 Dilute Acid Pretreatment

The dry biomass of (e. i. office paper, newspaper and tissue paper) were treated with dilute sulfuric acid with final concentrations of 0.5, 0.75 and 2 % (w/w) at 90°C, 100°C & 110°C with residence times of 15, 30 and 45 minutes were used in order to know the best conditions which was given maximum fermentable sugars. The pretreated biomass was adjusted to pH 5.0 with 10 M NaOH before enzymatic Saccharification (Saha, 2005).

3.9 Enzymatic Saccharification

After dilute acid pretreatment, enzymatic hydrolysis at the solid loading of 5% dry mass (DM, w/w) of each sample was performed using cellulases and β-glucosidase for maximum of 72 hours in a water bath separately. Sodium citrate buffer (50mM, pH 4.8) is used in the mixture to maintain the pH at 4.8 (Salam *et al.*, 2013).

3.10 Detoxification with Ca(OH)2

Detoxification of pretreated hydrolyzate of sample (office paper, newspaper and tissue paper) was carried out in a 250 ml flask in an incubated water bath shaker with Ca(OH)2. Involves increasing the pH of the hydrolyzates to 9, 10, 11 or 12, keeping up to 90 min at different temperatures of 30, 45 and 60 degrees C, followed by readjustment of the pH to 5 (Purwadi *et al.*, 2004).

3.11 Estimation of Total Sugar

After converting the sample into small pieces, investigated the sugar contents by the following process. Total sugar contents of the filtrate were measured. 1 ml of each filtrate was taken and 5 ml of concentrated H_2SO_4 were added. Each sample was left for 20 min at room temperature after proper mixing and then optical density (OD) was measured by spectrophotometer (Figure 3.4) at 470 nm against blank (Dubois *et al.*, 1956)

3.12 Analytical Procedure

The total reducing sugars from three different types of sample (office paper, newspaper and tissue paper) were determined separately by DNS (Dinitrosalicylic acid) method using glucose as the standard and High Performance Liquid Chromatography (HPLC) (Figure 3.5) quantification of released sugar was analyzed by HPLC RI. The analysis of sugar was carried out on temperature 35°C and rate of flow was 1 ml min^{-1} with 70%

acetonitrile and 25% of water, mixture as a mobile phase. Samples were centrifuged at 4000 rpm for 10 minutes. Residue were filtered and diluted many time before inserted in HPLC (Herrera *et al*, 2003).

3.13 Statistical Analysis

The data obtained was statistical analyzed with Analysis of Variance (ANOVA) by using MS Excel software.

Figure 3.4 Spectrophotometer:

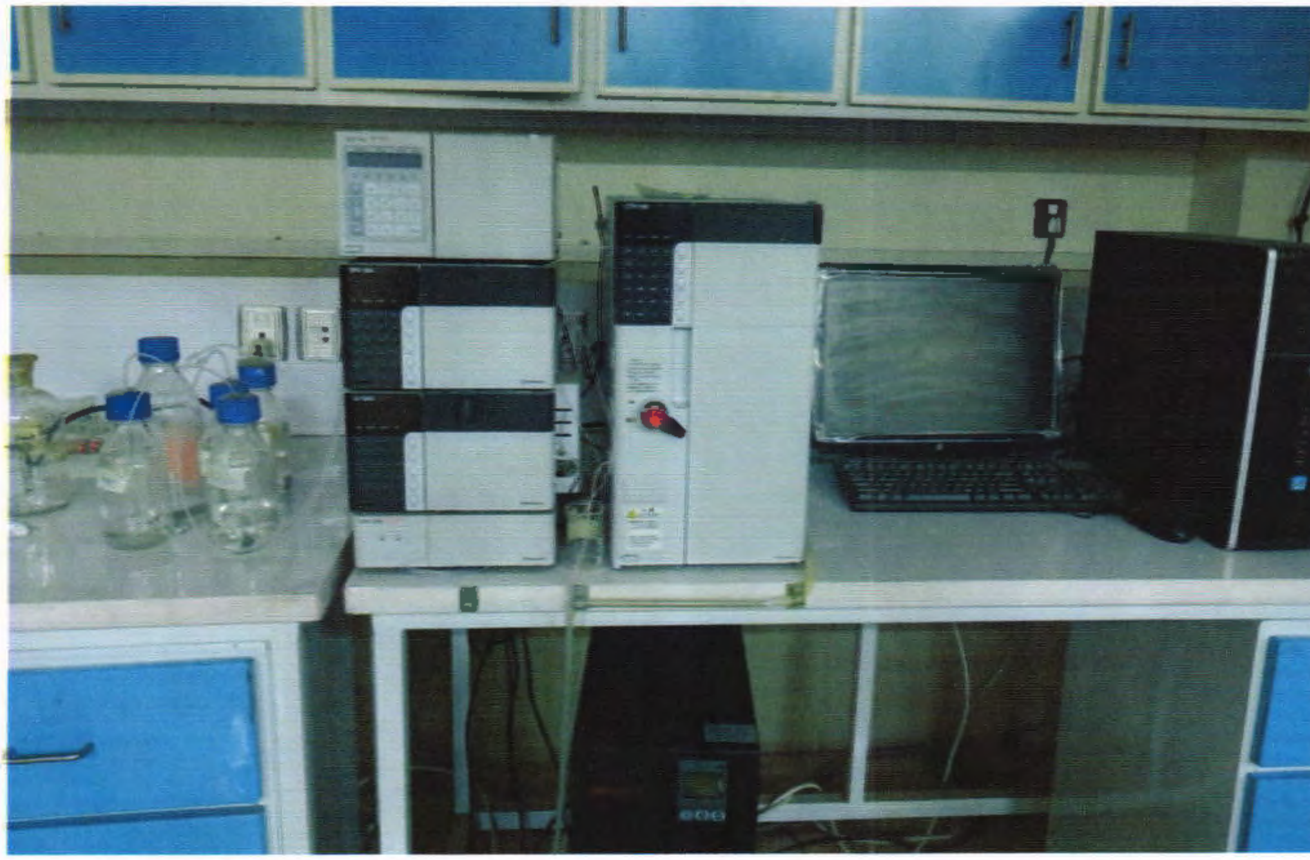

Figure 3.4 High Performances Liquid Chromatography (HPLC) instrument for sugar analysis:

RESULTS

RESULT

Waste paper comes from different sources in a country, for example from residential area, commercial area, institutions and municipal service. Waste paper has diverse effects on the environment which is collectively known as paper pollution. Discarded papers are a big part landfill sites.

4.1 Substrate Evaluation

To explore the best source for sugar lignocellulosic contents were estimated. Result revealed that in office paper cellulose 40%, hemicellulose 32.5% and lignin 22.5% were assessed. In newspaper cellulose 46.5%, hemicellulose 30.5% and lignin 22.5% were observed while in tissue paper cellulose 62%, hemicellulose 22% and lignin 15.5% were found (Table 4.1). The suitable substrate for Maximum cellulosic contents were found is tissue paper.

Table 4.1: Presence of cellulose, hemicellulose and lignin contents in substrates

Samples	Cellulose %	Hemicellulose %	Lignin %
Office paper	40± 2.5	32.5 ± 2.5	22.5 ± 2.5
Newspaper	45 ± 1.5	30.5 ± 5	25 ± 5
Tissue paper	60 ± 2	25 ± 5	15 ± 5

*Data presented in mean ± SD for three different samples.

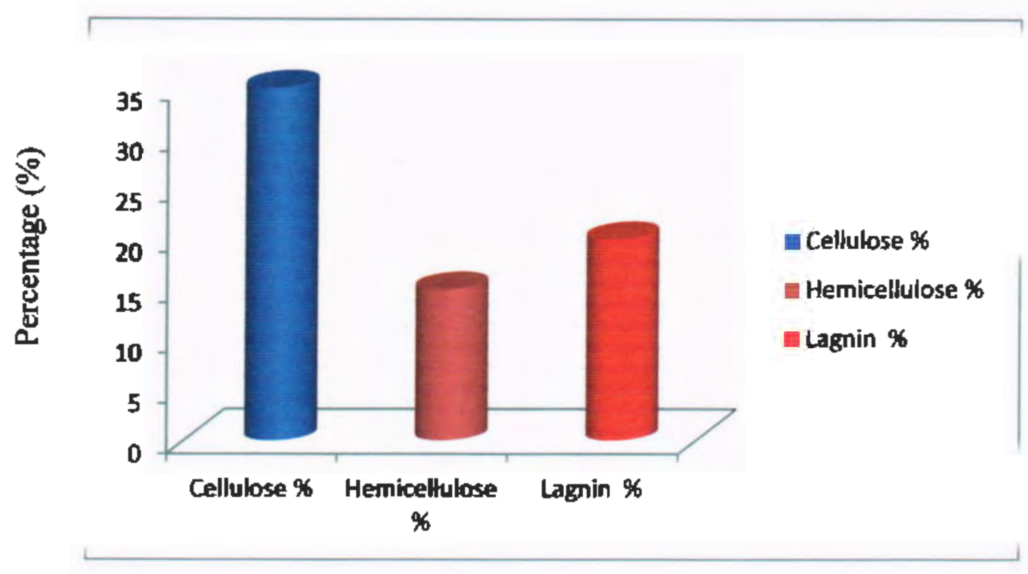

Figure 4.1: Presence of cellulose, hemicellulose and lignin contents in office paper

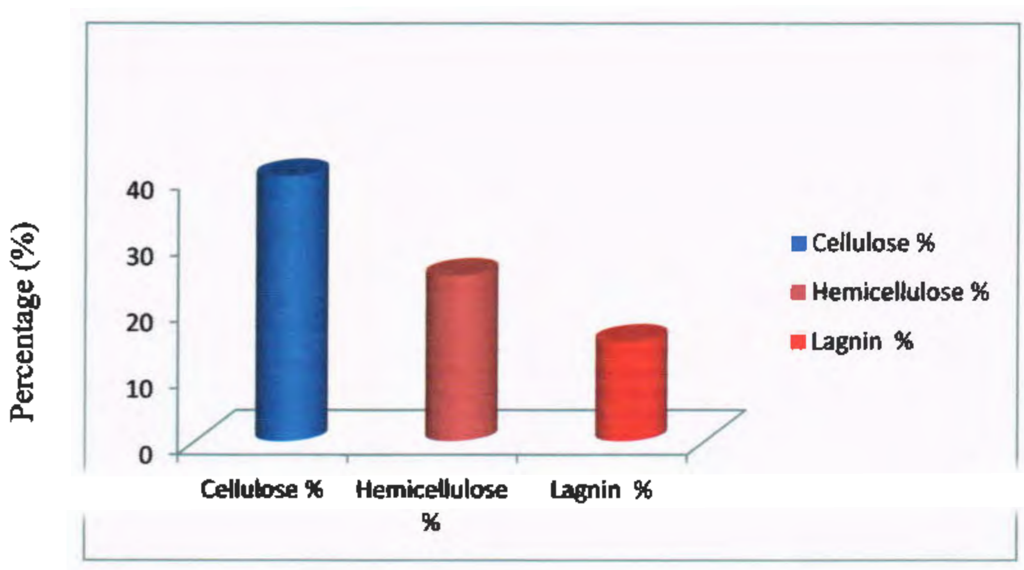

Figure 4.2: Presence of cellulose, hemicellulose and lignin contents in newspaper

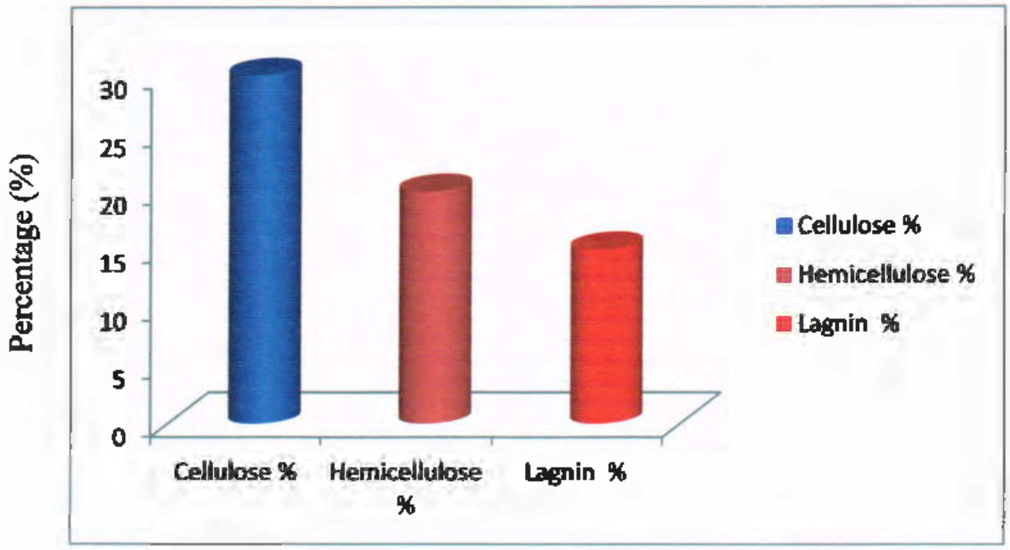

Figure 4.3: Presence of cellulose, hemicellulose and lignin contents in tissue paper

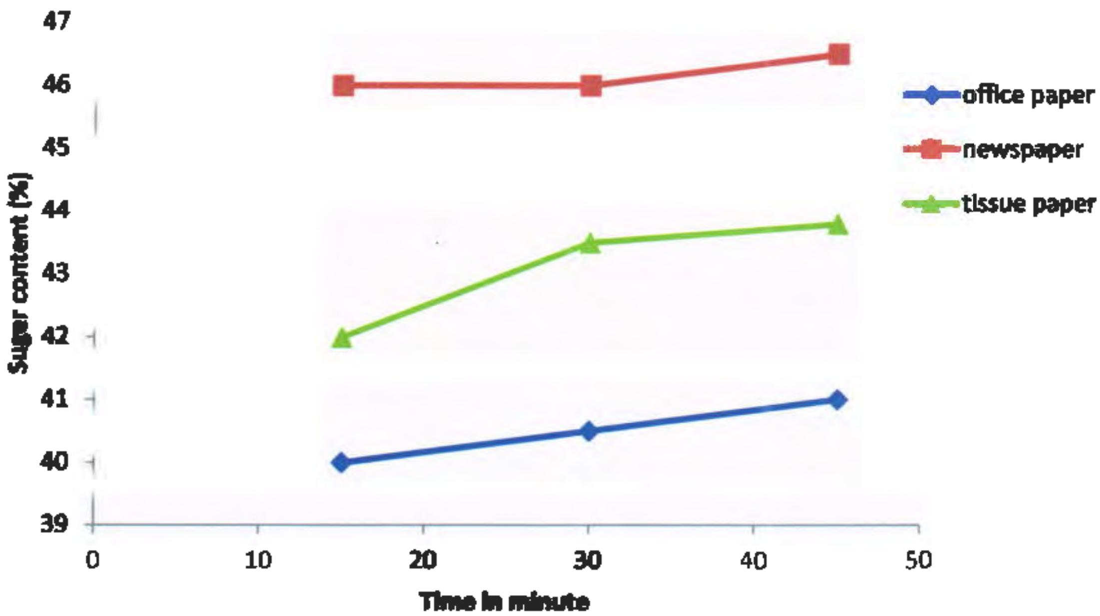

Figure 4.4 (a): A proportional of sugar contents at 0.5% dilute sulfuric acid at 90°C temperatures

4.2 Optimization of acidic dilute sulfuric acid pretreatment

The substrates (office paper, newspaper and tissue paper) were treated with different concentration of sulfuric acid 0.5%, 0.75% and 2% at various temperatures 90°C, 100°C & 110°C and with various interval of times 15, 30 and 45 minutes. Our study recorded the highest concentration of sugar (48.8%) with 0.5% concentration of sulfuric acid treatment from newspaper, and lowest sugar concentration (45.2%) was recorded for the office paper on same temperature and time interval (Figure 4.4 a, 4.4 b and 4.4 c).

While the highest concentration of sugar contents (56%) was found in substrate (newspaper) and lowest sugar concentration (47.5%) was observed from the office paper at 0.75% concentration of dilute sulfuric acid (Figure 4.5 a, 4.5 b and 4.5 c). The highest concentration of sugar contents (65%) was recorded in substrate (newspaper) at 2% concentration of sulfuric acid. And lowest sugar concentration (50%) was recorded for the office paper (Figure 4.6 a, Figure 4.6 b and Figure 4.6 c).

During optimization of dilute sulfuric acid it was found that the optimum condition were about 2% sulfuric acid, 100°C and 30 minutes for removal of 65% of sugar contents from newspaper and tissue paper but removal of sugar contents from office paper was 50%.

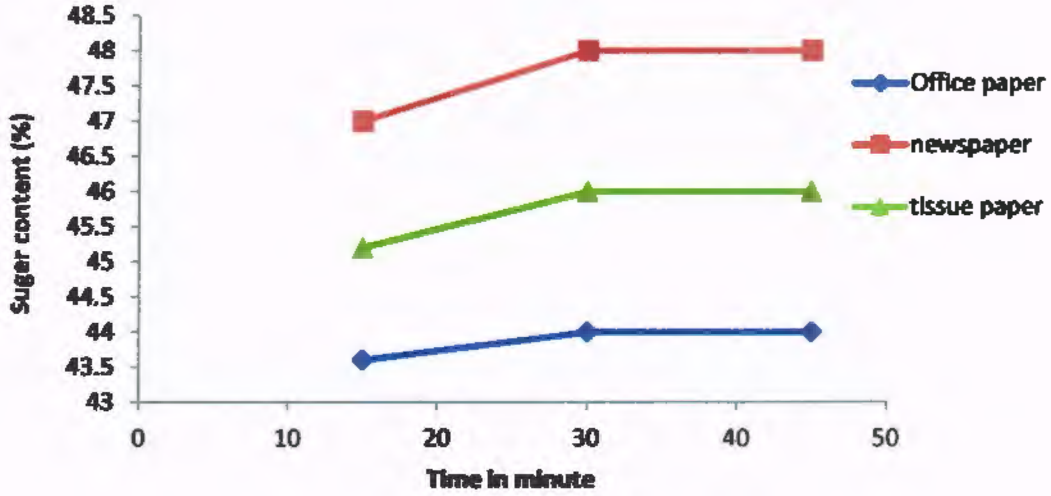

Figure 4.4 (b): A proportional of sugar contents at 0.5% dilute sulfuric acid at 100°C temperatures

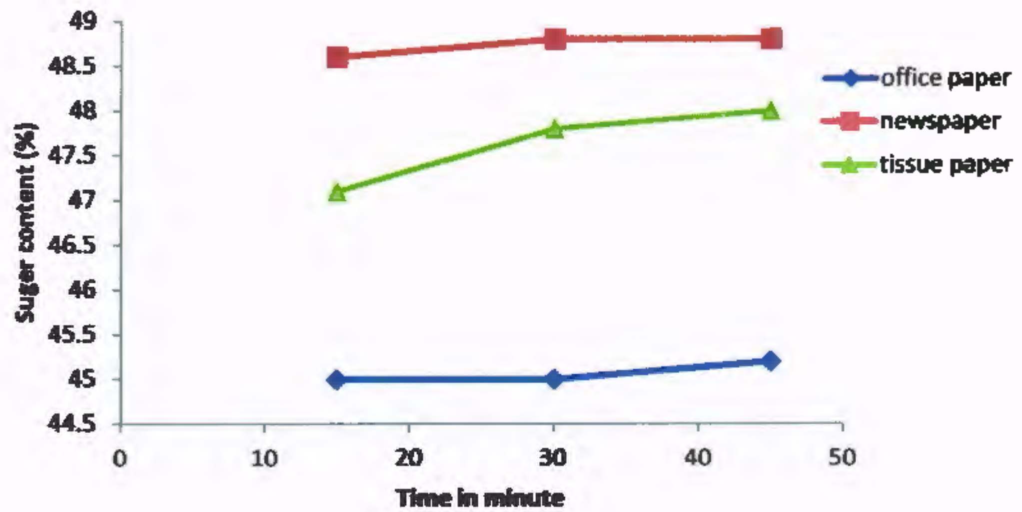

Figure 4.4 (c): A proportional of sugar contents at 0.5% dilute sulfuric acid at 110°C temperatures

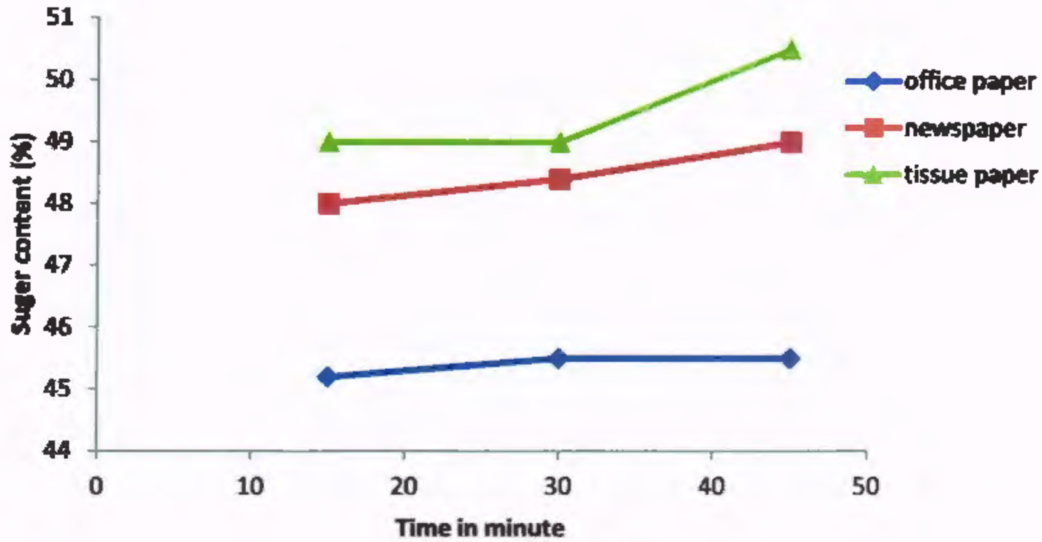

Figure 4.5 (a): A proportional of sugar contents at 0.75% dilute sulfuric acid at 90^0C temperatures

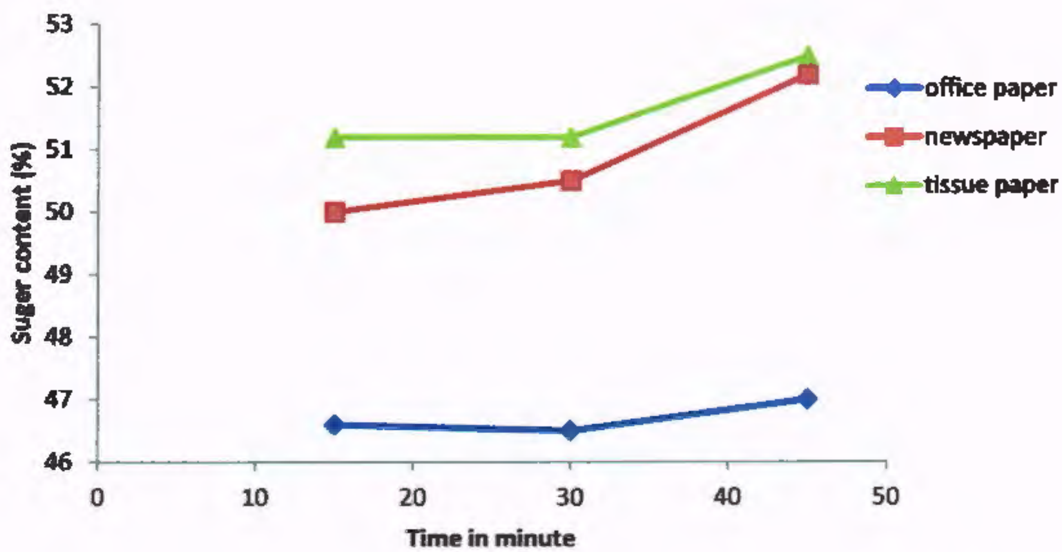

Figure 4.5 (b): A proportional of sugar contents at 0.75% dilute sulfuric acid at 100^0C temperatures

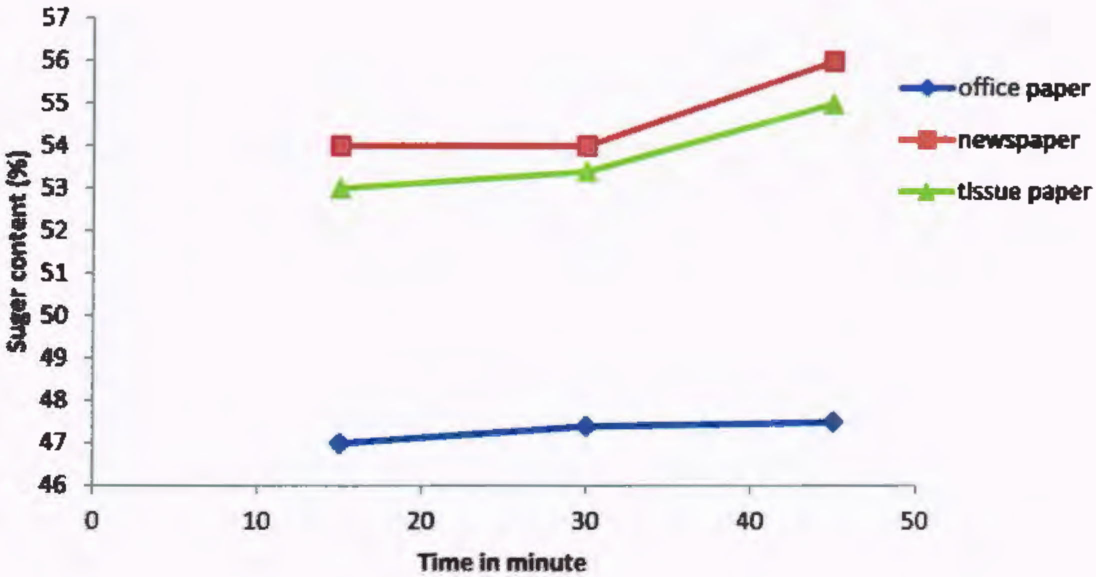

Figure 4.5 (c): A proportional of sugar contents at 0.75% dilute sulfuric acid at 110□C temperatures

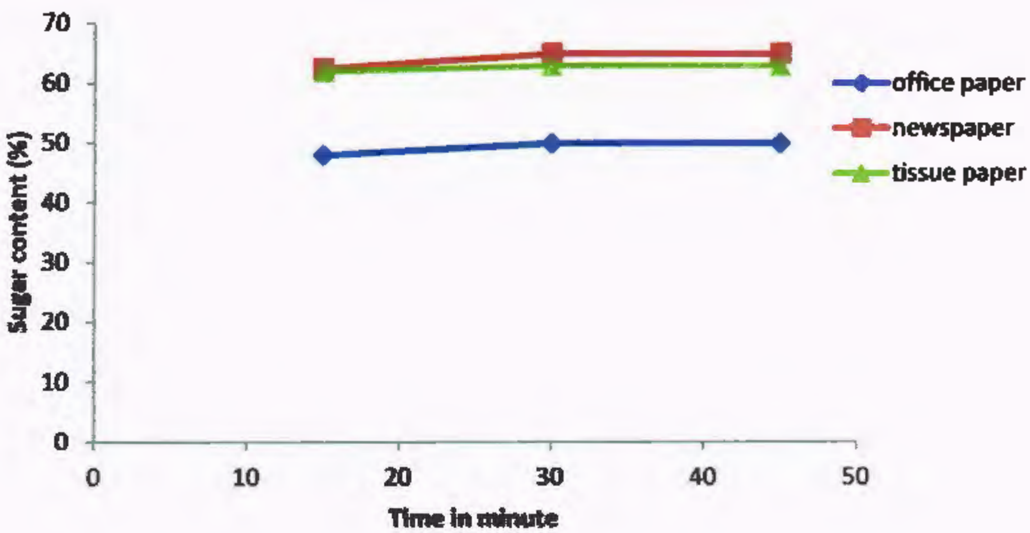

Figure 4.6 (a): A proportional of sugar contents at 2% dilute sulfuric acid at 90^0C temperatures

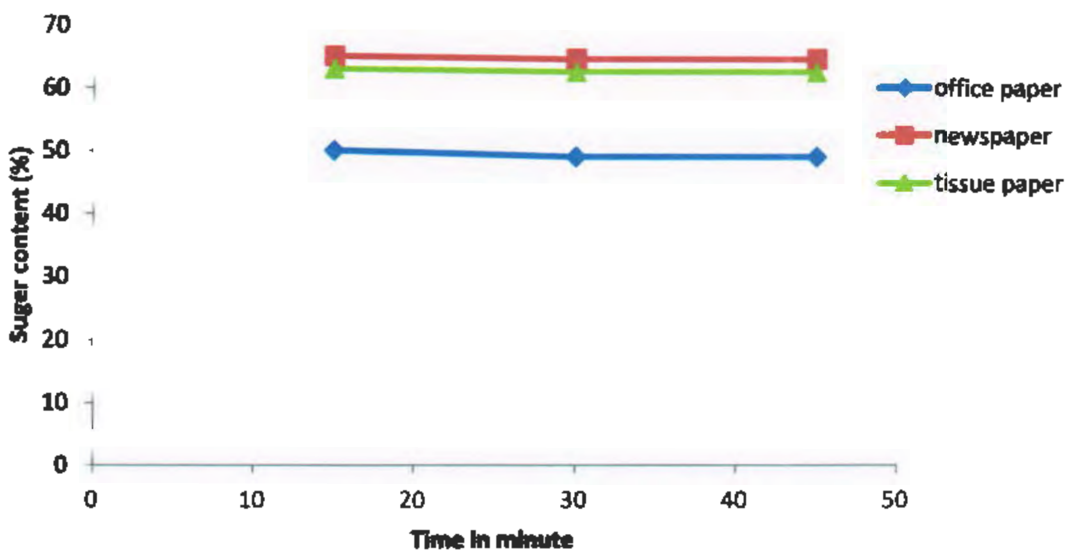

Figure 4.6 (b): A proportional of sugar contents at 2% dilute sulfuric acid at 100^0C temperatures

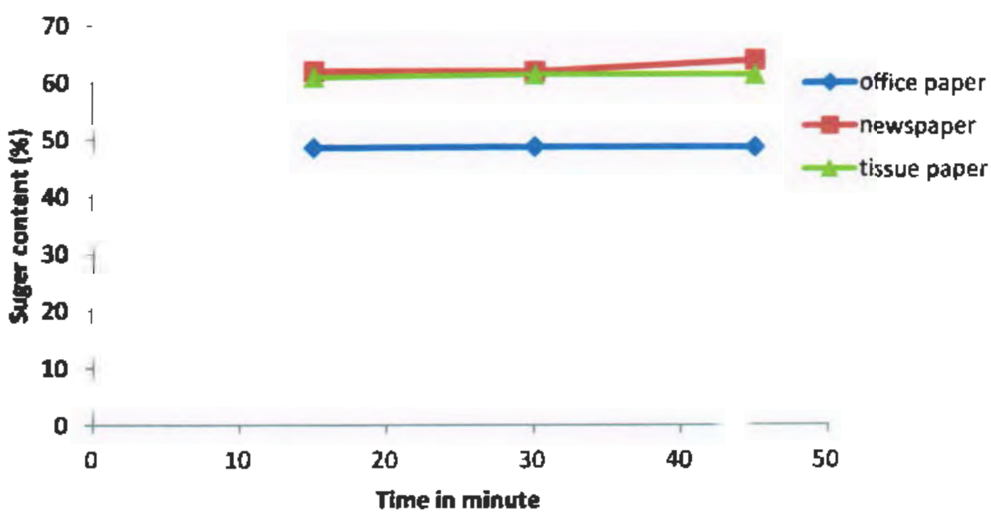

Figure 4.6 (c): A proportional of sugar contents at 2% dilute sulfuric acid at 110^0C temperatures

4.3 Enzymatic hydrolysis

During present study after acidic pretreatment the enzymatic saccharification of substrate (office paper) was carried out for 12, 24, 36, 48, 60 and 72 hours. The cellulase and β-glucosidase with enzyme activity of 12 FPU/g and 23 U/g were used. 5% (w/v) substrate (office paper) was taken and hydrolyzed with enzymes on given time and released sugar 5.5 mg/ml, 7.5 mg/ml, 9.5 mg/ml, 12.2 mg/ml, 19.6 mg/ml and 18.3 mg/ml respectively were observed (Table 4.2).

Same process of enzymatic saccharification was carried out on other substrate (newspaper) the same amounts of enzymes were applied on various time periods (T1, T2, T3. T4, T5 & T6) and resulted sugar 6.5 mg/ml, 10.6 mg/ml, 16.4 mg/ml, 22.6 mg/ml, 20.8 mg/ml and 20.4 mg/ml respectively were recorded (Table 4.3).

Similarly the process of enzymatic saccharification was carried out on other substrate (tissue paper) the same amounts of enzyme were used. Same concentration of substrate (tissue paper) was hydrolyzed with enzymes on different time periods (T1, T2, T3. T4, T5 & T6) and sugar 7.2 mg/ml, 12 mg/ml, 16.3 mg/ml, 21.5 mg/ml, 23.4 mg/ml and 21.5mg/ml respectively were observed (Table 4.4). Optimum conditions for more sugar releasing from the office paper was 60 hours (T5) while for newspaper was 48 hours (T4) and for tissue paper was 60 hours (T5).

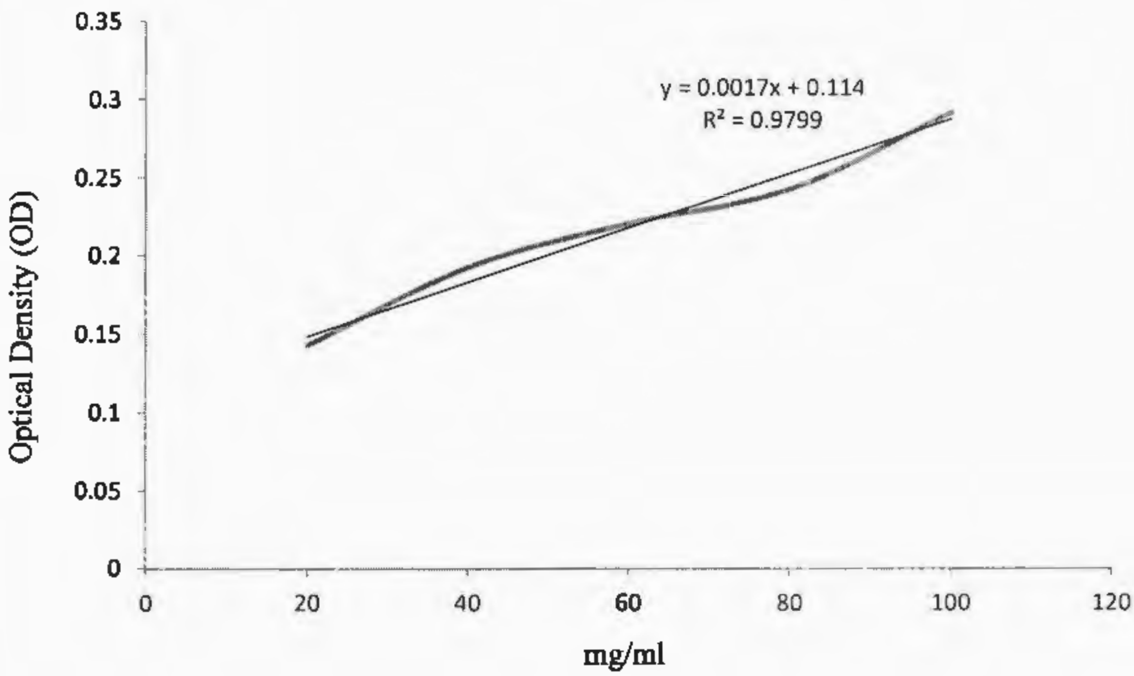

Figure 4.7: Standard curve for the estimation of the sugar by Spectophotometer

Table 4.2: Enzymes and substrate (office paper) loading and glucose production

Time	Enzymes Concentration.		Substrate	Glucose
	Cellulase	β-glucosidase	% (w/v)	mg/ml
T1	12 FPU/g	23U/g	5	5.5 ± 0.5
T2	12 FPU/g	23U/g	5	7.5 ± 0.3
T3	12 FPU/g	23U/g	5	9.5 ± 0.5
T4	12 FPU/g	23U/g	5	12.2 ± 0.2
T5	12 FPU/g	23U/g	5	19.6 ± 0.2
T6	12 FPU/g	23U/g	5	18.3 ± 0.3

*Data presented in mean ± SD for glucose released from office paper.

Table 4.3: Enzymes and substrate (newspaper) loading and glucose production

Time	Enzymes Concentration		Substrate	Glucose
	Cellulase	β-glucosidase	% (w/v)	mg/ml
T1	12 FPU/g	23U/g	5	6.5 ± 0.3
T2	12 FPU/g	23U/g	5	10.6 ± 0.2
T3	12 FPU/g	23U/g	5	16.4 ± 0.3
T4	12 FPU/g	23U/g	5	22.6 ± 0.2
T5	12 FPU/g	23U/g	5	20.8 ± 0.4
T6	12 FPU/g	23U/g	5	20.4 ± 0.2

*Data presented in mean ± SD for glucose released from newspaper.

Table 4.4: Enzymes and substrate (tissue paper) loading and glucose production

Time	Enzymes Concentration		Substrate	Glucose
	Cellulase	β- glucosidase	% (w/v)	mg/ml
T1	12 FPU/g	23U/g	5	7.2 ± 0.5
T2	12 FPU/g	23U/g	5	12 ± 0.5
T3	12 FPU/g	23U/g	5	16.3 ± 0.3
T4	12 FPU/g	23U/g	5	21.5 ± 0.5
T5	12 FPU/g	23U/g	5	23.4 ± 0.2
T6	12 FPU/g	23U/g	5	21.5 ± 0.5

*Data presented in mean ± SD for glucose released from tissue paper.

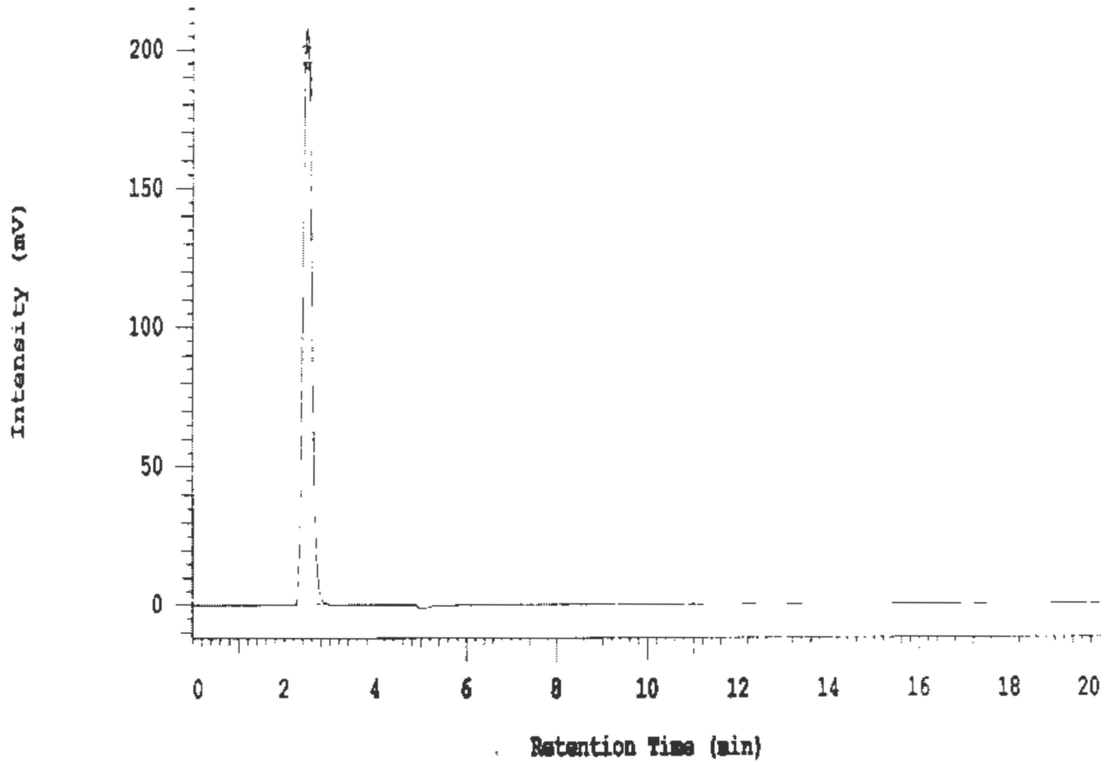

Figure 4.8 HPLC Glucose Standard

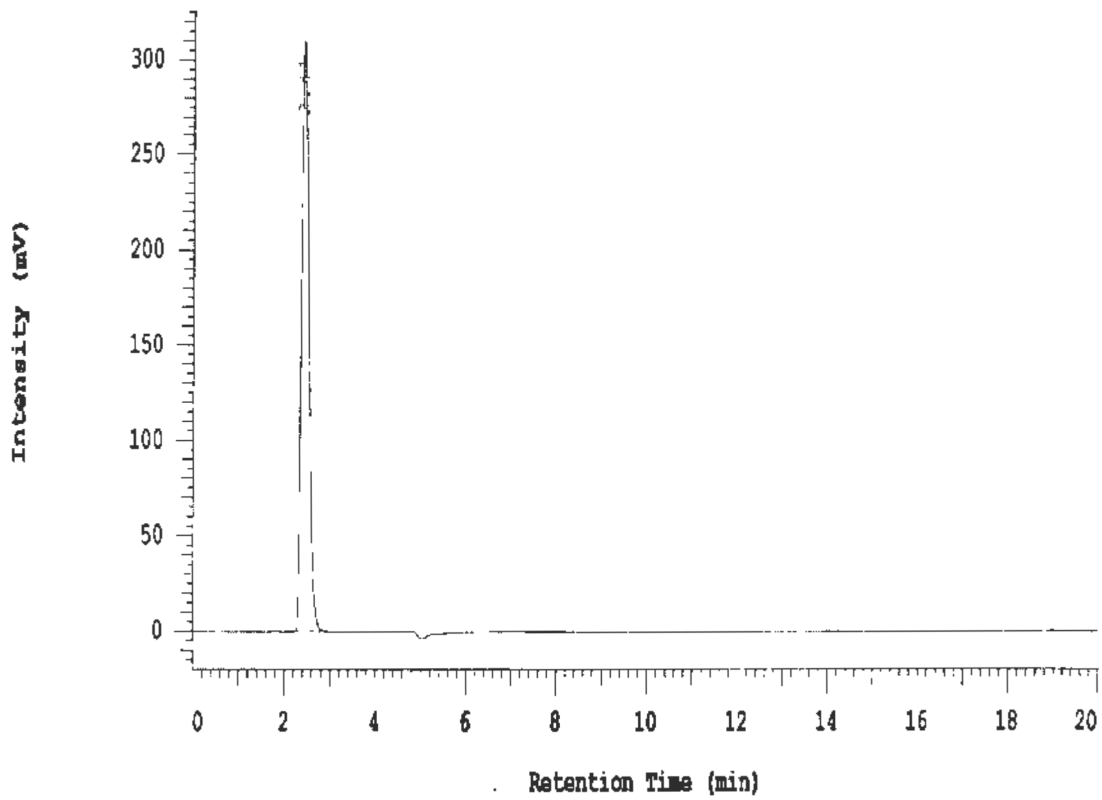

Figure 4.9 HPLC Xylose Standard

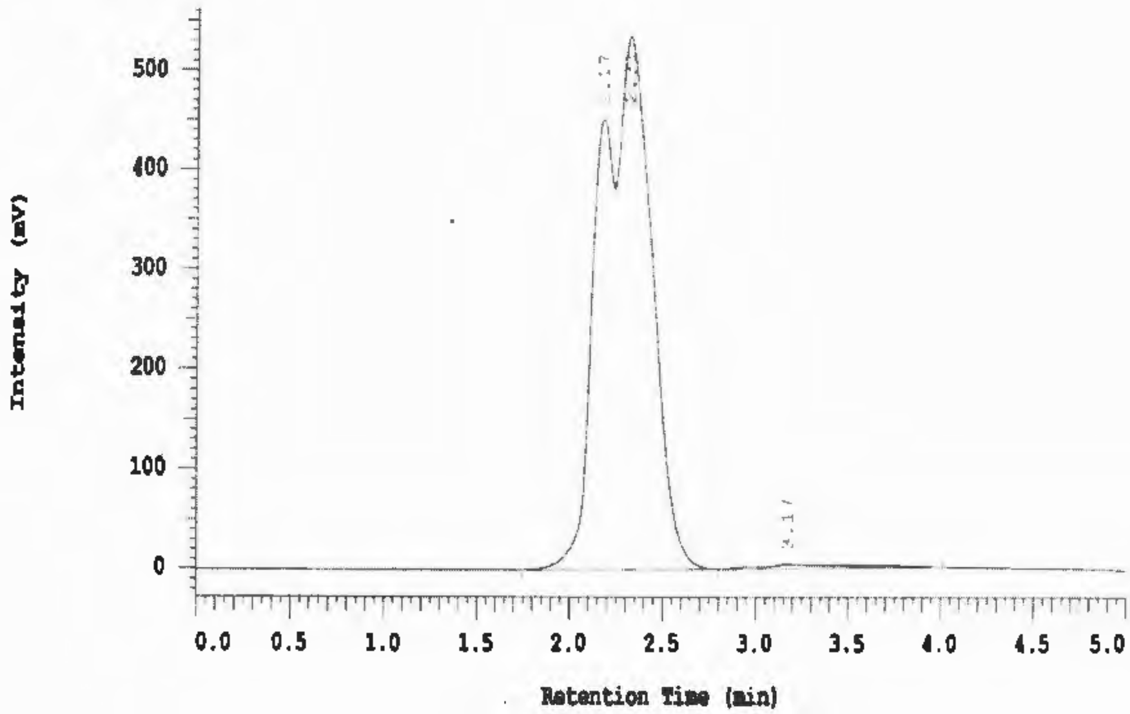

Figure 4.10 HPLC Glucose and Xylose Peaks

4.4 Analysis of glucose through HPLC

Through enzymatic hydrolyzed resulted that sugar (glucose and xylose) were recorded. Analysis of sugar yield from wastepaper through HPLC showed that there was little bit high amount of glucose 22 at 2.8 retention time with 229898.52 peak area in substrate (tissue paper). While in newspaper recorded low concentration of glucose 20 as compared to tissue paper on same retention time. Similarly in office paper the concentration of glucose was 18 on 2.5 retention time (Table 4.8).

Table 4.5 Analysis of glucose through HPLC

Sample	Retention time (minutes)	Peak area	Glucose mg/ml
Office paper	2.5	228924.12	18
Newspaper	2.8	229832.28	20
Tissue paper	2.8	229898.52	22

DISCUSSION

DISCUSSION

Fossil fuel is economically source of energy today in world. But due to increasing in human population the need of fossils fuel is very increased. It is estimated in 50 years that the use of bio fuel is less than one quarter of fossil fuels (York 2012).The prices of fossils fuel are rapidly increasing day by day and analyses of prices describe the big variations between supply and increased demand (Hook and Tang 2013). U.S department of energy in 2013 have reported that the demand of energy is increasing continuously and worldwide pressure is created to produce energy by an alternative sources like biomass which is environment friendly (Lacoa *et al.,* 2014). The plants materials that are made up by the combination of carbon, hydrogen, oxygen and other elements are known as biomass. Still now six biomass resources are identified for production of energy like: energy crop on surplus cropland, forest residue degraded land, agriculture residues, organic wastes and animals wastes (Hoogwijk *et al.,* 2003).

Waste paper is made up of cellulose, hemicellulose and lignin. These materials are found in agricultural waste, domestic waste and industrial waste. One of the sources of biomass is waste paper. Which is not only cheap also readily available throughout country. Waste paper is in different form newspaper, office paper and tissue papers. The cellulosic material can be hydrolyzed via chemical or enzyme and fermented to produce biofuels (Saha, 2002).

The research has been conducted on waste paper to hydrolyze by dilute sulfuric acid pretreatment. It was found that waste paper like (newspaper, tissue paper and office paper) contain different lignocellulosic material (cellulose, lignin and hemicellulose) in different percentage. It is resulted that the cellulose is found in highest amount 62% in tissue paper and 46.5% in newspaper and in range of 40% in office papers, in case of lignin 15 to 22% were found in all subjected waste paper while hemicellulose were found in highest amount 32% in newspaper, 25 % in tissue paper and lowest amount of 17% in office paper. The results are in accordance with Kim *et al,.* (2012) who conducted a research on physical and chemical characteristics of products from the torrefaction of yellow paper. He also reported that yellow waste paper contains cellulose 37 % and hemicellulose 26%. According to Foyle *et al.,* (2007) Compositional analysis of lignocellulosic material waste paper and straw. They have reported that waste paper contains37% cellulose, 28% hemicellulose and 21% lignin. Jung *et al.,* (2013) Optimization of concentrated acid hydrolysis of waste paper using surface methodology. They have reported that the waste paper (newspaper) contains 57% cellulose, 8.4% hemicellulose and 17.2% lignin and office paper contained 59.2% cellulose and 9.0% hemicellulose and 17.5% ash content. Lee *et al.,* (2000) Dilute-acid hydrolysis of lignocellulosic biomass. They reported that waste paper consist of 16-22% of lignin and 60-75% of cellulose. Deves and Hunche (1998) Composition and Microbial degradeability in the soil of farmyard manure from ecologically-managed farms they reported tissue paper consist of 60-70 cellulose, 10-20% hemicellulose and 5-10 lignin which conform our finding about sugar contents.

During optimization of dilute sulfuric acid it was found that the optimum condition were about 2% sulfuric acid, 110°C and 30 minutes for removal of 65% of sugar contents from newspaper and tissue paper but removal of sugar contents from office paper was 50%. The results are in accordance with Lee *et al.,* (2010) Pretreatment of waste newspaper using ethylene glycol for bioethenol production. They have reported that the optimum condition were about 2% sulfuric acid, 150°C and 15 minutes for removal of 60% hemicellulose and 75% lignin from newspaper. Jung *et al.,* (2013) Optimization of concentrated acid hydrolysis of waste paper using surface methodology. They have reported that the waste paper (office paper) the optimum concentrated acid hydrolysis conditions were acid concentration of 70.8%, loading sulfuric acid of 3.2 ml and a reaction time of 3.6 Hours. Dubey *et al.,* (2012) Bioethenol production from waste paper. They have reported the optimum condition for acid hydrolysis of waste paper were 0.50 N sulfuric acid at 120°C for 2 hours.

In enzymatic hydrolysis of acidic pretreatment substrate the current study was found that the Concentration of substrate (office paper, newspaper and tissue paper) 5% (w/v) was taken and hydrolyzed with enzymes on six different time periods (12, 24, 36, 48, 60 and 72 hours) and obtained 5.5 mg/ml, 7.5 mg/ml, 9.5 mg/ml, 12.2 mg/ml, 19.6 mg/ml and 18.3 mg/ml respectively of glucose from office paper, 6.5 mg/ml, 10.6 mg/ml, 16.4

mg/ml, 22.6 mg/ml, 20.8 mg/ml and 20.4 mg/ml respectively of glucose were found from newspaper and 7.2 mg/ml, 12 mg/ml, 16.3 mg/ml, 21.5 mg/ml, 23.4 mg/ml and 21.5mg/ml respectively of glucose recorded from tissue paper. The results are in accordance with Elliston *et al.,* (2013) High concentration of cellulosic ethanol achieved by fed batch semi simultaneous saccharification of waste paper. They have reported that the use of 16 FPU/g cellulase, and 30 U/g β-glycosidase on different time periods (H1 & H2) on 5% substrate (w/v) and resulted sugar concentration of 7.5 mg/mL and 14.4 mg/mL respectively. Kuhad *et al.,* (2010) Fed batch enzymatic saccharification of newspaper. Reported that the use of enzyme β-glycosidase (60 Ug-1), exoglucanase (20 U g-1) and xylanase (80 U g-1) maximum releasing of 14.64 g L-1 sugar. Hilloise (2016) Improvement in enzymatic hydrolysis of waste paper with chemical pretreatment and enzyme loading. He reported that the use of enzyme 5.2, 7.8 and 10.4 FPU/g on different hydrolysis time 24, 48 and 72 h on substrate *(2.25, 3.00 and 3.75 g). Maximum glucose concentration was 23 g/l.

CONCLUSION

CONCLUSION

In present study the wastepaper was used for the release of sugar. It seems achievable method for energy conservation and waste minimization. Dilute acidic pretreatment of office paper cellulose, hemicellulose and lignin were estimated 40%, 32.5% and 22.5% respectively. Similarly in newspaper cellulose, hemicellulose and lignin were estimated 46.5%, 30.5% and 22.5% while, in tissue paper cellulose, hemicellulose and lignin were estimated 62%, 22% and 15.5% respectively. The experimental assessments were well within the estimated value of the model. By operating the pretreatment at optimum conditions and enzymatic hydrolysis, these avoids using the longest possible time and highest sulfuric acid loading, subsequently reduces the overall production cost. The research is needed to make this process economically feasible. Ultimately the optimal hydrolysis conditions and enzymatic hydrolysis for the glucose recovery at the laboratory scale may potentially be scaled up to the production level. This research of acid pretreatment and enzymatic hydrolysis was an economical method to improve glucose conversion from waste paper.

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