Synthesis And Production Of Bio-Ethanol From Waste Paper

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Abstract
Paper, which is one of the largest constituent of Municipal solid waste, has become a severe problem for disposal in developed and developing countries due to the shrinking landfill capacity. It is very important and challenging task in managing the solid waste. Newspaper, which is a cellulosic feed stock, is emerging as an attractive option for the production of bio-ethanol because of lower feedstock costs, higher potential for fossil fuel displacement and also there will be reduction in greenhouse gas emission as compared to production of ethanol from corn.. In the present study, paper, which is one of the largest constituent of municipal solid waste, has become a severe problem for disposal in developed and developing countries due to the shrinking landfill capacity. It is very important and challenging task in managing the solid waste. There will be reduction in greenhouse gas emission as compared to production of ethanol from corn. The main objective of this project is to minimize the newspaper load on municipal solid waste by efficiently utilizing the waste newspaper in the production of bio-ethanol. Experimental studies had carried out to optimize the pre-treatment process for increasing the efficiency of acidic hydrolysis and enzymatic hydrolysis, the efficient conversion of cellulosic carbohydrates to hexoses and monomers cellulose degrading microorganisms and to convert the sugars released to Ethanol by using Fermentation process. Pretreatment, hydrolysis and fermentation are the steps involved in the production of Bioethanol. In the pre-treatment process, the Lignin, Hemicellulose and Cellulose are separated to enhance the hydrolysis process. The optimized condition for the pre-treatment was found to be 5% concentration of H_2SO_4 at 121ºC and 45 minutes. During enzymatic hydrolysis treatment, spores of Aspergillus species were used. There was a sharp increase in reducing sugars in enzymatic hydrolysis compared to acid hydrolysis of 557.8 to 592.1 mg/ml. Similarly, production of bioethanol is enhanced in enzymatic hydrolysis paper hydrolysate when compared to acid hydrolysis of 1.8 to 2.12 % in 20 gm paper hydrolysate and the yield was estimated using specific gravity method. This study revealed the possibility of producing bioethanol from locally available waste paper using cheap, simple and adaptable technology and using better yeast strains for fermentation and production.

Keywords: *Paper waste, Bio-ethanol, Fermentation, Characterization, Saccharomyces Cerevisiae*

Introduction

Increase in the population over the last century lead to the increase of the energy consumption world wide. To meet the increased energy demand crude oil has been used as the major resource. The global oil production would decline to 5 billion barrels from 25 billion barrels approximately. Due to this unavoidable depletion of the world petroleum resources in the coming years the worldwide interested aroused in seeking an alternative non-petroleum based energy source, (Zhi Sheng Zu et al.). One of the best alternative fuels in order to beat severely the energy crises is from Biofuel. From biologically carbon fixation the energy is derived from Biomass. The various factors like need for increasing energy security and hikes and gaining the scientific and public attention the biomass are driven. The main contents of ethanol are sugar, starch or cellulose. The Bioethanol is one of the environment friendly fuels, the effects on environment is less because the Ethanol contains oxygen With comparison to the conventional gasoline the blends of E10 resulted in 12-25% less emission of carbon monoxide, [23]. The sugarcane and corn are the first generation bio-fuels. Due to vast increase in the ethanol production using these crops they cause immoderate pressure on the global food supply. The second generation biofuels can be produced by means of different sources like waste chicken feathers, cellulosic biomass food and organic waste. The cellulosic biomass, such as agricultural residue and industrial waste are the most abundant and cheap source of renewable energy in the world. The second generation biofuels may also include the fuels produced from mixed paper waste which is separated from the municipal solid waste, cash crops Jatropha, Hong, Cotton, Maize etc. can be utilized to produce bioethanol. The third generation biofuels can be

produced from micro-organisms mainly Algae. The fourth generation biofuels produced from vegetable oil, biodiesel.

Ethanol obtained from cellulose is an environmental friendly fuel. It is bio-degradable and water soluble and produces lower emissions of carbon monoxide [3]. The hydrolysis can be carried out by enzymes or acids [4]. The main disadvantages of enzymatic hydrolysis are that it requires a pretreatment of the raw material to improve the enzymatic digestibility [5] and longer retention time [6]. Acid hydrolysis may be carried out with diluted or concentrated acid. The advantages of diluted acid hydrolysis are that it attacks polysaccharides, being easier to hydrolyze than cellulose and acid losses are not important [7]. The present research is based on bioethanol production from waste office paper. This feedstock results to be attractive for biofuel production due to it's available. Despite of recycling efforts have been strengthened in the last years, the recycling rate is about a 65%, since the quality of the paper decreases with the recycling process [8]. Then, waste office paper could be a suitable raw material for obtaining bioethanol.

The need for energy is increasing continuously, because of rapid increase in industrialization and vehicles. The sources of this energy are petroleum, natural gas, and coal, hydro and nuclear. The increasing the concern of fuel as well as the escalating social and industrial awareness lead to exploration for clean renewable fuels. Therefore, bioethanol produced from renewable energy sources such sugary and starchy materials, fruit wastes are believed to be one of these options, and it is concurrently being practiced in waste management. Thus, bioethanol has become important in recent times as the world to find an alternative energy as suitable substitute for fossil fuel and to reduce greenhouse gas emissions. Apart from being alternative energy, bioethanol has been shown to be less polluter compared to nitrous oxide, carbon monoxide and Sulphur oxide. It is considered to be non-toxic and biodegradable, as well as obtainable from renewable source. Those papaya and banana peels and seed have several characteristics that make them potential feed stocks for bioethanol production. They have high cellulose and hemicellulose contents can be readily hydrolyzed in to fermentable sugar. In terms of chemical composition, ethanol is common molecule in biological systems, being the end product of metabolism (fermentation). Bioethanol can be produced from biomass by hydrolysis and sugar fermentation process [2, 18].

The study mainly involves in the production, purification and characterization of bio-ethanol from waste paper. In the process of ethanol production there will be newspapers from locally available wastes was chosen, due to less availability of other wastes and production of bio ethanol by using biochemical Conversion Process is eco- friendly and non- toxic to environment.

II. Study Area

The study area of the research is in Arba-Minch town which is separately located in southern Ethiopia, in the Gamo zone of the southern nations, nationality, and people region.

III. Equipment

Plastic bags: - to collect and transport samples to the laboratory.

Knife: - for cutting the fruit wastes in to pieces. Digital ovens: - to dry the sample.

Crushers: - to crush the dried sample.

Balances: - to weigh samples and yeast.

Digital pH meter: - to measure the pH of the hydrolytes before fermentation.

Thermometer: - to control temperature of the sample under experiment (fermentation and distillation) isothermally at the set point.

Vessels: - to hold samples and additives for hydrolysis, fermentation and distillation experiments. Graduated cylinders of different volumes: - for volume measurement. Autoclave: - for sterilization and hydrolysis.Beaker: - for density measurement.

Fermentation and distillation set ups: - to ferment and distill respectively

IV. Chemicals

Sulfuric Acid (H_2SO_4) 5 M: - used as a pretreatment and hydrolysis of waste paper.

Sodium Hydroxide (NaOH): - used to adjust the pH of soluble cellulose and hemicelluloses before fermentation).

Mg NO_3: -to prepare yeast activation

$K_2Cr_2O_7$: -at ethanol estimation to provide color.Yeast (Saccharomyces cerevisiae): -to degrade glucose to carbon dioxide &ethanol

V. Preparation of Substrate

Easily available waste newspaper was collected from Arba Minch town. The waste newspaper dried in a hot air oven at 106 ºC for 6 hours and waste newspaper wash chopped into pieces. The dire mass of sample was ground to a maximum size ranging 1-2 mm approx. Further sieve by passing through mesh sieve of size 0.35 mm and 0.95 mm and store for the experiments.

VI. Acidic Hydrolysis

The different weight of dried and chopped sample (20, 15, and 5) gm. of waste newspaperwas mixed with 200 ml of 5% of concentrate d H_2SO_4 in a conical flask. The acid hydrolysis reaction was catalysed at 121 ºC at15 lb pressure for 15 min of t reatment time. After that, the hydrolysate was cooled do

wn to room temperature and carefully filtered asepticall y using Whattman filter paper no.1 to remove the unhyd rolysed material andsolid residues. The filtrate was coll ected and the total content of ethanol and reducing suga r was estimated before fermentation

VII. Detoxification of acid hydrolysis

The detoxification of hemicelluloses acid hydrolysis by boiling and over liming to pH 10 with solid Na (OH) and sulphite synergize better ferment ability of the hydrolyzate. Boiling assists to reduce volatile compounds, such as furfural and phenols and over-liming with Na (OH) remove the concentration of other acid compounds. Increase of pH up to 10 helps in precipitation of heavy metals. The furfurals are transforming into furfural acid and condense with other components of pre-hydrolyzate (Stricklank et al., 1985). Finally the pH had been maintained to 5.2 suitable for the growth of *Saccharomyces cereviceae*. After detoxification and before detoxification 2ml of sample were taken for reducing sugar estimation.

VIII. Enzymatic hydrolysis

To convert cellulose into simple monomeric carbohydrate used Aspergillus grown on subordinate dextrose agar plate. The inoculum was transferred into prepared each sample for enzymatic hydrolysis after the identification of Aspergillus was done by using microscope. The Aspergillus inoculated sample was kept at the room temperature for 4 days in an incubator shaker at 100 RPM at 37ºC for continuous shaking.

IX. Inoculum preparation

Aspergillus was inoculated from tomato mold by striking method. Aspergillus was grown on petriplates containing Sabourdenet dextrose agar and grown in incubator and kept for 72 hour. The morphology of the Aspergillus was identified by using microscope. Cell from single well isolated colony were inoculated in conical flask that containing prepared sample for each enzymatic and acidic hydrolysis. The Aspergillus inoculated sample was kept at the room temperature and kept for 4 days in an incubator shaker at 100 RPM at 37ºC for continuous shaking. The inoculum was mixed in 1ml of distilled water before mixing in to the liquefy prepared samples to help the growth of the inoculum properly.

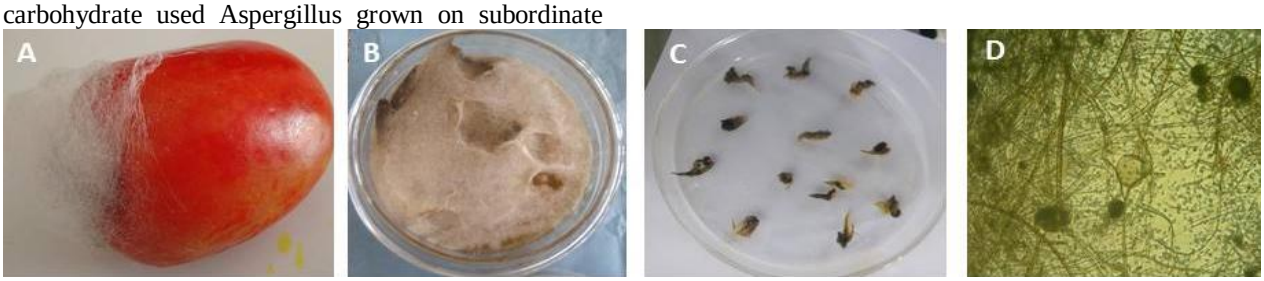

Figure 3: Isolation of Aspergillus species (A) Mold on Tomato (B) Petri plate culture of Aspergillus indicating black colour (C) Equal size of loop full of inoculum (D) Microscopic examination of Aspergillus fruiting body with spores.

X. Estimation of reducing sugar

Liquefied slurry was transferred into test tube and reducing sugars were estimated by DNSA reagent (Dinitrosalicylic acid). DNSA reagent were prepared by dissolving 0.5g of dinitrosalicylic acid, in 10ml of 2N NaOH in one beaker and dissolve 15gm of sodium Potassium Tartrate in another beaker and separately stir until dissolved after that both of the separated sample were mixed in to 250 beaker and heat on hot plate and mixed to homogenized. Distilled water were added to the volume 50ml and kept in 4ºC refrigerator and prepared all sample for

reducing sugar estimation. Then DNSA reagent were mixed with the prepared sample .After addition of DNSA reagent to the standard solution and test solution as shown in Table 1 below, whole reaction mixture was incubated in boiling water bath at 90ºC for 15 min until red--brown color followed to stabilize color of reaction mixture. Subsequently observance was measured at 510 nm. The whole set of experiment was performed in triplicate such as for enzymatic, acidic (before detoxification and after detoxification) for each sample and also for each standard. The glucose estimation of concentration was 1mg/1ml subsequently observance were measured at 510 nm. (Kumar.et al.,2013)

Table 1: Estimation of reducing sugars using glucose as standard.

Volume of glucose	Concentration of Glucose	Distilled H2O	DNSA reagent	Distilled H2O	OD at 540nm
0.3ml	0 mg	1ml	1ml	3ml	0.000
0.3ml	0.2 mg	0.8ml	1ml	3ml	0.114
0.3ml	0.4 mg	0.6ml	1ml	3ml	0.289
0.3ml	0.6 mg	0.4ml	1ml	3ml	0.490
0.3ml	0.8 mg	0.2ml	1ml	3ml	0.685
0.3ml	1.0 mg	0.2ml	1ml	3ml	0.788

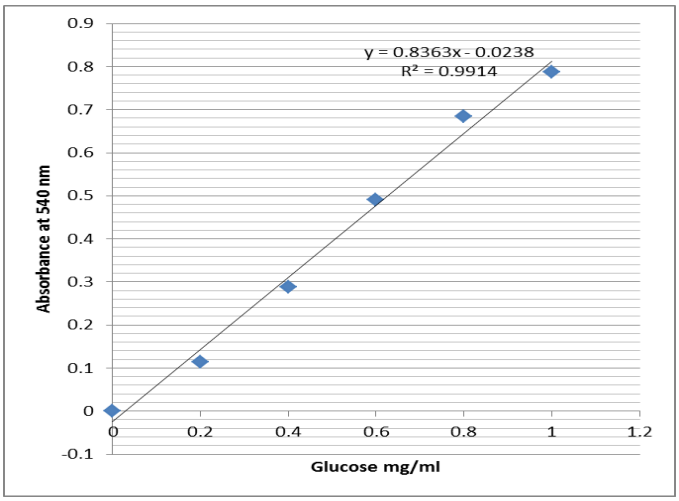

Figure 4: Standard regression curve for reducing sugar where glucose is used as standard.

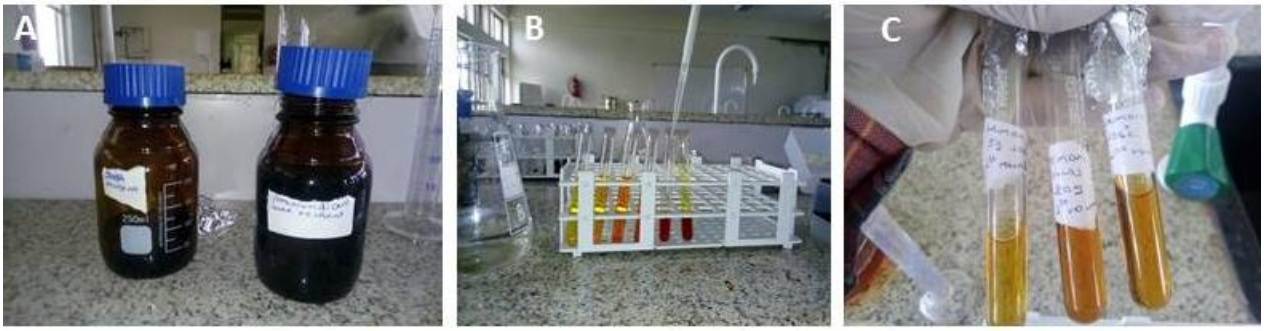

Figure 5: (A) DNSA reagent (B) Standard curve samples of glucose (C) Testing samples of paper of 5, 10 and 20 gm paper biomass hydrolysate.

XI. Yeast preparation

Commercial yeasts are found in preserved form which needs to be activated using 32 ml distilled water; 20g of commercial Baker's yeast *Saccharomyces cerevisiae* (instant premium) was added in a 250 ml conical flask. The prepared yeast used for each acidic and enzymatic hydrolyzed sample. Then the neatly prepared conical flasks containing yeast were shacked and properly covered with aluminum foil. The conical flask was then a shaking for few min and keep for 30 min.

XII. Fermentation

The fermentation process was carried out in shaker incubator, at 30 °C, with stirring at 180 rpm, for a 72h. The prepared hydrolyisates were adjusted to pH of 4.5-5.2 which is optimum for *Saccharomyces cervisiae* using 2N HCL solution. Before conducting fermentation, preparation of media for the yeast is a must. In order to prepare the media, the favorable condition for yeast growth must be established to supply the required amount of nutrients.

XIII. Procedure for Fermentation

To perform the experiment, the sample was conditioned to temperature of 30^0C before fermentation step is started. This is the temperature at which all fermentation experiments are carried out. Then the pH was checked before adding the cultured media and it was maintained by 2M of NaOH. The pH 4.5-5.2 was optimum for yeast culture. The cultured media of 6.25 ml was used for each of acidic hydrolysate sample, and enzymatic hydrolysate samples the were mixed in Erlenmeyer flask. For every 2hrs the fermentation mixture was shaked with manually. At the end the mixture was placed at a temperature of 30^0C for 72 hours. After 72 hours of fermentation, the samples were taken out and distilled for purification of crude bioethanol

Figure 6: pH adjustment

Figure 7: Fermentation samples

XIV. Distillation

Distillation was the final step in the production of ethanol from waste paper. The aim of this step of the experiment was to purify the alcohol. Distillation is the method used to separate two liquid based on the difference of their boiling points. In this experiment simple distillation set up was used at a temperature of 78^0C for 4 hours on water bath. Water bath was used in order to control the temperature at set point. The fermented product is separated by distillation into alcohol and stillage (Sheorain, 2000).

Figure 8: Simple Distillation setup

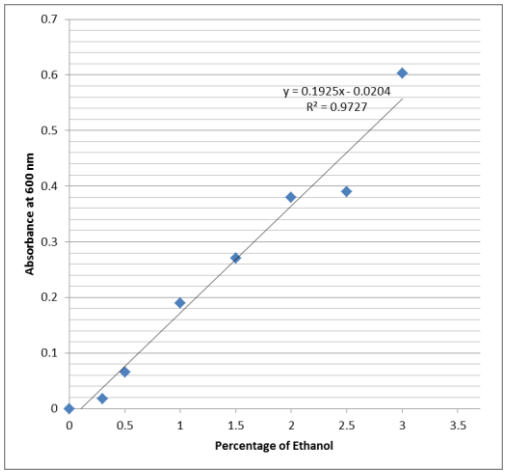

Figure 9: Standard regression curve for percentage of ethanol were absolute ethanol is used as standard

XV. Ethanol Estimation

Ethanol estimation was done spectrum photometric wielding Potassium dichromate method which requires Acid Dichromate Solution (7.5 gm Potassium dichromate in 250 ml 5 M H2SO4) and 10 mg/ml Ethanol stock solution (Caputi et al., 1968). In 300 µl of fermented sample, 3 ml of acid dichromate solution was add and left for 30 min

in room temperature. Oxidation of ethanol were changed the color from yellow to green. Absorbance will be measure at 590 nm against standard graph.

Table 2: Estimation of percentage of ethanol using absolute ethanol as standard.

Standard % Ethanol /ml	Volume of ethanol (ml)	Distilled Water (ml)	Potassium dichromate reagent (ml)	Absorbace at 600 nm
0	0	1	3	0
0.3	0.3	0.7	3	0.0173
0.5	0.3	0.7	3	0.0658
1	0.3	0.7	3	0.19
1.5	0.3	0.7	3	0.27
2	0.3	0.7	3	0.38
2.5	0.3	0.7	3	0.39
3	0.3	0.7	3	0.603

XVI. Experimental analysis

A. Identification of Bioethanol

About 5 ml of distilled fermented wash/bio-ethanol samples was taken in test tube and 1 percent potassium dichromate solution and 5drops of H_2SO_4 was added the distilled ethanol sample add on it. The color of the sample solution was observed [20].

B. Determination of Moisture Content

First, the empty dish was weighed. Then accurately weight of waste newspaper was into an empty dish. The sample were dried in an oven at 105^0C; weighing each 2 h until constant weight was obtained and finally the weight was taken and compared with the initially recorded weight. The percentage weight in the waste newspaper was calculated using the formula.

$$\text{Moisture \%} = \frac{(W1-W2)}{W2} \times 100$$

Where: W_1 = Original weight of the sample before drying

W_2 = Weight of the sample after drying.

C. Determination of density of ethanol

The density of ethanol was determined by using a density formul.25ml sample was poured in 25gm of beaker. Mass of beaker containing sample of bio-ethanol was measured by using electrical beam balance. Mass of bio-ethanol sample was known by subtracting mass of beaker from mass of beaker and sample. Density was calculated by the following formula

Density=mass of sample/volume of sample

D. Determination of Specific Gravity

Use density bottle to determine the density of the oil. Weigh a clean and dry bottle of 25ml capacity (W_0), and then fill with the Ethanol insert stopper and reweigh to give (W_2). Substitute the ethanol with water after washing and drying the bottle and weigh to give (W_1). The expression for specific gravity (Sp.gr) is given by: Specific gravity of ethanol $= \frac{[W2-W0]}{[W1-W0]}$ = Mass of the substance / Mass of an equal volume of water .Where

W0= Mass of empty bottle

W1= Mass of water with bottle

W2= Mass of ethanol with bottle

E. Determination of Viscosity

Clean and dry viscometer was selected with a flow time above 200 seconds for the fluid to be tested. Filter the sample through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The sample was Charged to the viscosity meter with the sample by inverting the tube's thinner arm into the liquid sample and drawn up suction force to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometers was placed into a holder and insert it to a constant temperature bath set at 29°C and allow approximately 10 minutes for the sample to come to the bath temperature at 29°C. Apply the suction force then to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flows freely from the upper timing mark to the lower timing mark will be recorded. The value was calculated by the following equation.

Viscosity= number of drop sample*density of water*viscosity of water

Number of drop of water*density of ethanol

F. Determination of pH Value

Around 25 ml of the sample was poured into a clean dry 50 ml beaker and 13ml of hot distilled water was

added to the sample in the beaker and stirred slowly. It was then cooled in a cold-water bath to 25°C. The pH electrode was standardized with buffer solution and the electrode immersed into the sample and the pH value was read and recorded.

Replications and Statistical Analysis

All experiments were repeated three times on different days. The average values ±SE are presented for few observations only. Statistical analysis of the data was done using the software Sigma plot (version 12.0) and Microsoft excel 2010.

RESULT AND DISCUSSION

More recently, due to scarcity of natural resources like petrochemical products and natural gas, many scientists have been trying to develop new renewable energy resources. As it is mentioned earlier in study that nowadays bioethanol is used as alternative to petrochemical, due to this reason many researchers have tried to find out various sources for production of bioethanol from different cell biomass including plant lignocellulose material is one of the resources for bioethanol. Several techniques have been used for conversion of biomass into ethanol like acid treatment of biomass before fermentation and enzymatic hydrolysis etc. In this present study, three potent weeds were selected from Abaya campus, Arba Minch University, which contains rich amount of carbohydrate of total biomass on dry weight basis.

The conversion of lignocellulose to pentose and hexose monomers was successfully achieved through biochemically of heat acid hydrolysis and enzymatic hydrolysis flowed by autoclaving at 121 ^{0}C, 15 lb pressure for 15 min for every treatment time. Figure 10 illustrates that the increase of waste paper biomass from 5 gm to 20 gms, there was a marked increase the reducing sugars. Similarly, quantity of reducing sugars were around 4 fold increase was obtained after conversion of acid hydrolysate to detoxification of acid by maintaining the pH to alkaline in waste paper biomass hydrolysate were 196. to 756.1 mg/ml for *Euphorbia*, 449.1 to 557.8 mg/ml for *Parthenium* and 192.9 to 557.8 mg/ml for 20 gm of waste paper biomass respectively (Fig. 10 and Table 4).

Table 3: Estimation of reducing sugars before and after detoxification

Biomass	Absorbance at 540 nm for Acid Hydrolysis	Glucose mg/ml	Absorbance at 540 nm after detoxification of hydrolysate	Glucose mg/ml
Paper 5 gm	0.032	56.140351	0.151	264.9123
Paper 10 gm	0.072	126.31579	0.24	421.0526
Paper 20 gm	0.11	192.98246	0.318	557.8947

These values are slightly different from those reported in the earlier literature (Mathewson, 1980) reported higher values of fermentable sugars respectively from papers and food substrates. The slight departure from literature for the old newspapers might be as a result of reaction residence time, concentration of acid used, pre-hydrolysis of the substrate and size reduction. In the case of maize substrate, the variation might be traced to the ratio of the malt to substrate used for conversion. Since the process is microbial, the actual ratio of malt to substrate can be attained after several attempts.

Reducing sugars were estimated for all three samples shows that there was a marked increase sugars was (178.9 mg/ml, 205.2 mg/ml and 592.1 mg/ml) for 5, 10 and 20 gms of waste paper biomass in enzymatic hydrolysate with inoculum *Aspergillus* spores (Table 4 and Fig. 11). Similar results were obtained through enzymatic hydrolysis of corncob from cellulosic hydrolysate were investigated by Chen *et al.*, 2007 after corncob was pretreated by 1% H2So4 at 1080C for 3 h, cellulosic residue was hydrolyzed by cellulose from *Trichoderma reesei*.

Table 4: Reducing sugar with absorbance at 540nm for enzymatic hydrolysis

Biomass	Absorbance at 540 nm for Enzymatic Hydrolysis	Glucose mg/ml
Paper 5 gm	0.136	178.9474
Paper 10 gm	0.156	205.2632
Paper 20 gm	0.45	592.1053

The production of bioethanol using Baker's yeast *Saccharomyces cerevisiae* during acid hydrolysis is higher in enzymatic hydrolysis when compared to acid hydrolysis of 0.9 to 1.8 %, and 1.01 to 2.12 % from 5 gms to 20 gms waste paper biomass hydrolysate for 72 hr of fermentation respectively (Fig. 12). Similarly, according to Narayanan *et al.*, 2013, in saccharification stage pretreated water hyacinth was added to the growth media and inoculated with saccharification micro-organisms *Trichoderma reesei* and *Aspergillus niger*, the optimum saccharification period for both micro-organisms was found to be 72 hours.

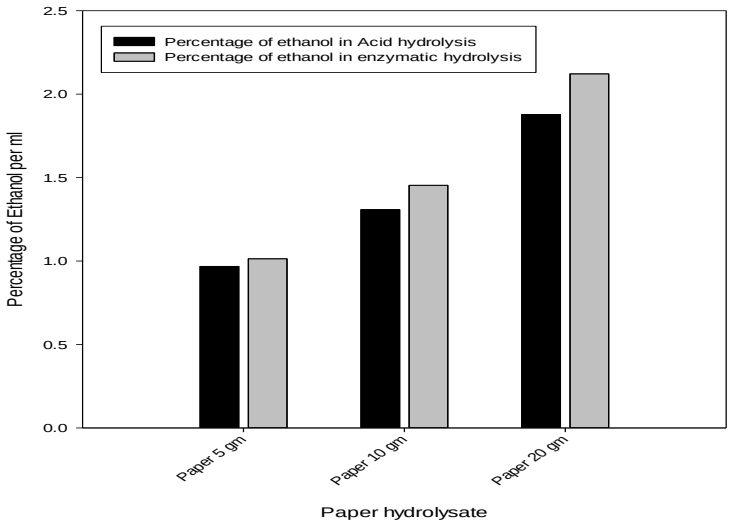

Fig. 12: Percentage of ethanol after fermentation in acid hydrolysis and enzymatic hydrolysis of three different quantity of samples of waste paper biomass.

The standard density value of bio-ethanol at 25°C is 0.78522g/cm^3 and density our sample is 0.783g/cm^3 it approaches to the standard value of ethanol, but it is not reach the standard due to systematic error of handling the measuring material. The boiling point of standard ethanol is 78.24°C, but the boiling point of our sample is 78 C° and it approaches to the standard value. The viscosity of the standard ethanol at 25°c is 1.074 Pas. However, viscosity of our product is 1.069 which is for some extent it is far from the standard due to errors like, observing time correctly when ethanol flow from upper mark to reach lower labeled mark, lack of ability to use the instrument ethanol is soluble to water [miscible with water]. The specific gravity of our product is 0.805 it is less by 0.0046 from standard specific gravity of ethanol may show some uncertainty due to systematic or instrumental error.

Conclusions and Recommendations

Conclusions

Production of conventional fuels are depleting unprecedentedly by profuse consumption in human civilization since few decades after industrial revolution. On the other hand, perpetuating depletion of forestry and exponential increase of pollutions has astronomical effects on global warming. These factors necessitate to find some effective alternative to

conventional fuels. Biofuel plays a crucial role as alternative source of conventional fuel in ameliorating the balancing of global warming.

Bioethanol production from waste materials like plant and agricultural wastes and field crops and animal wastes including paper wastes from different sources was successful. The best conditions for acid hydrolysis of 5g, 10g and 20g of paper waste raw material were incubated in 200 ml of sulfuric acid and 120 min of reaction time in an autoclave at 121 ºC is best way to get good amount of reducing sugars. After fermentation with *Saccharomyces cerevisiae*, there was a good amount of bioethanol in enzymatic hydrolysis rather than acid hydrolysis method. Finally, some properties were measured according the standards and the obtained bioethanol conforms to the set limit. Based on the obtained results, it can be concluded that waste paper different sources is a suitable raw material for bioethanol production.

Recommendations:

- Further, purification of ethanol through advanced methods may give better production and yield.

- Addition of synthetic enzymes in fermentation production medium may help the further production and yield in the large scale synthesis.
- We performed the fermentation production in batch culture, but in future, if we use continuous fermentation production method, we may get more production and yield at the industrial level.

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