Optimization and Utilization of Wastepaper for Bio-energy Production

Solomon Hailu[1], Solomon Kahsay G. Mariam[1], Tesfay Berhe[1, 2]

[1]Department of Biological and Chemical Engineering, Mekelle Institute of Technology, Mekelle University, Tigray, Ethiopia
[2]Department of Chemical Engineering, Kombolcha Institute of Technology, Wollo University, Amhara, Ethiopia

Email address:
tesadi@gmail.com (T. Berhe)

Abstract: Bioenergy future depends on an increased share of renewable energy, especially in developing countries. Bioconversion of lignocellulosic based biomass to ethanol is significantly hindered by the structural and chemical complexity of biomass, which makes these materials a challenge to be used as feed stocks for cellulosic ethanol production. Bioethanol is one of the most important alternative renewable energy sources that substitute the fossil fuels. Wastepaper has a content of cellulose and hemicelluloses, which make it suitable as fermentation substrate when hydrolyzed. The objective of this work is ethanol production from wastepaper by fermentation process. Eight laboratory experiments were conducted to produce bioethanol from wastepaper. By using Design Expert 7 software, it was formulated the dilute acid hydrolysis step to investigate the effects of hydrolysis parameters on yield of ethanol and optimum condition. All the three hydrolysis parameters were significant variables for the yield of ethanol. The optimum combinations of the three factors chosen for optimum ethanol yield 10.86 ml/50 g sample were 92.59°C hydrolysis temperature, 30 minutes hydrolysis time and 1%v/v acid concentration.

Keywords: Bioethanol, Distillation, Fermentation, Hydrolysis, Wastepaper

1. Introduction

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Crude oil has been the major resource to meet the increased energy demand (Campbell et al, 1998). As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the greenhouse gas emission. At the same time, increasing waste generation linked to rising population and living standards is a worldwide challenge to waste management systems. World energy consumption is predicted to increase by 50% to 2030 according to the United States Energy Information Agency (EIA, 2009). The combustion of fossil fuel is responsible for 73% of the CO_2 emission. In this scenario, renewable sources might serve as an alternative. The alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available. Numerous potential alternative fuels have been proposed, including bioethanol, biodiesel, methanol, hydrogen, boron, natural gas, liquefied petroleum gas (LPG), Fischer–Tropsch fuel, p-series, electricity, and solar fuels. Biofuels which include bioethanol, vegetable oils, biodiesel, biogas, biosynthetic gas (bio-syngas), bio-oil, bio-char, Fischer–Tropsch liquids, and biohydrogen offer many advantages over petroleum-based fuels (Demirbas, 2008). These advantages include biofuels are easily available from common biomass sources, they represent a CO_2-cycle in combustion, biofuels have a considerable environmentally friendly potential, there are many benefits to the environment, economy and consumers in using biofuels, and they are biodegradable and contribute to sustainability (Ngunzi, 2014).

Bioethanol have the major applications in automobile, beverage, pharmaceuticals industry etc. due to economically as well as ecofriendly (Galbe and Zacchi, 2007). Bioethanol is appropriate for the mixed fuel in the gasoline engine due to its high octane number, due to its low cetane number and high heat of vaporization impede self-ignition in the diesel engine. Absolute ethanol and 95% ethanol are themselves act as good solvents, somewhat less polar than water and used in perfumes, paints and tinctures. Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% (Cardona and

Sanchez, 2007).

Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars. Currently, the technology for lignocellulosic ethanol production relies mainly on pretreatment, chemical or enzymatic hydrolysis, fermentation and product separation or distillation (Balat, 2010). An appropriate pretreatment strategy is essential for the efficient enzyme hydrolysis of lignocellulosic biomass as lignin hinders the saccharification process (Gaur, 2006). Efficient saccharification of cellulose is of great importance from a viewpoint of the disposal of cellulosic wastes and the utilization of cellulose as a renewable resource in relation to the preventing of a greenhouse effect due to carbon dioxide.

Various raw materials like sugarcane juice and molasses, sugar beet, beet molasses, sweet sorghum (Stokes, 2005) and starchy materials like sweet potato, corn cobs and hulls, cellulosic materials like cocoa, pineapples and sugarcane waste coffee husk and milk/cheese/whey using lactose hydrolyzing fermenting strains has available in literature. The yield of bioethanol from corn and sugar are high and the techniques are mature. However, it increases the risk of causing global food shortage (MoFED, 2010). Therefore, another alternative material has been used which contain lignocellulosic such as weed, grass, saw dust, municipal solid waste, woody biomass, and paper mill waste.

Wastepaper consists of a considerable share of municipal and industrial waste even though recycling efforts have been strengthened in recent years by legal provisions like the packaging directive (Forum for Agricultural Research in Africa, 2008). However, the recycling rate of wastepaper is low and the recycled wastepaper has a low grade paper product because of shorted fiber length. Since the shortening of paper fibers decreases the quality of paper, the maximum ratio of paper-to-paper recycling is said to be 65% (Ikeda et al., 2006). This means that a certain fraction of paper would always be sent to disposal. Still, wastepaper is considered as one of the prospective and renewable biomass materials to produce bioethanol.

The enzymatic hydrolysis of wastepaper is desirable from the standpoint of green and clean processing, although the process presents such challenges as a slow reaction rate and low process efficiency, mainly due to the high crystallinity of cellulose, the presence of some lignin, low specific surface area of the material, and the complexity of celluloses as multicomponent enzyme systems, etc (Hahn-Hagerdal et al., 2006).

Moreover, this alternative outlet for waste papers could help reduce pressure on other waste management options (i.e. recycling, incineration and landfill) from the increasing waste generation due to rising population and ultimately we create aesthetically very attractive city (Halidini et al, 2014). Therefore, the aim of this work is to investigate the possibility of using and transforming different wastepaper to ethanol by fermentation using S. Cereviciae thereby contributing towards alternative energy supply as well as creating an employment opportunity. Also to study the optimum hydrolysis of different waste papers with diluted sulfuric acid.

2. Material and Methods

2.1. Materials

Wastepaper: The wastepaper was collected from the campus area of Mekelle University, Ethiopia. The wastepaper was then dried in oven at 60°C for 48 hours). The dried paper was placed in a mortar crusher and the maximum particle sizes of 3 mm. The sample of larger particle size than 3 mm was cut over and over again until all particle size became 3 mm. The cut material was kept at low temperature until the next stage of experiment.

Chemical: Sulfuric acid (98% H_2SO_4), sodium hydroxide (NaOH) solution, dry instant yeast (*saccharomyces cerevisiae*), yeast extracts (agar), urea, dextrose sugar, ($MgSO_4.7H_2O$), distilled water, and potassium dichromate were used during the experiment.

Equipment: Scissor, oven, balance, digital pH meter, shaking incubator, centrifuge, flasks of different volumes, graduated cylinders of different volumes, autoclave, alcoholmeter, shaker, rotary evaporator, computer, aluminum foil, and cotton were used during the experiment.

2.2. Methods

Steam Pretreatment: The purpose of pretreatment is to remove lignin, reduce cellulose crystallinity, and increases the porosity of the materials (Prasad, 2003). Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

Procedure in Steam Pretreatment: First distilled water was prepared and then 50 g of the cut sample was soaked in a distilled water of 500 mL in conical flasks for 24 hours. The conical flasks were capped with the help of aluminum foil. Then the lignocellulosic biomass was rapidly heated at 121°C by high-pressure steam without addition of any chemicals in autoclave. The biomass/steam mixture was held for 15 minutes to promote hemicellulose hydrolysis, and terminated by an explosive decompression. After finishing the given pretreatment time and temperature the sample in autoclave was allowed to cool and the soluble portion was separated from the non-soluble portion. The non-soluble portion was hydrolyzed in the next steps and the soluble solution was placed in another conical flask.

Dilute Acid Hydrolysis: The carbohydrate polymers in lignocellulosic materials need to be converted to simple sugars before fermentation, through a process called hydrolysis. Various methods for the hydrolysis of lignocellulosic materials have recently been described. The most commonly applied methods can be classified in two groups: chemical hydrolysis and enzymatic hydrolysis (Silverstein, 2004). Even though there are many types of hydrolysis types, dilute acid hydrolysis is an easy and productive process and the amount

of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. This process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing.

The three-parameter and two-level ($2^3 = 8$) factorial design was applied to hydrolysis step of the experimentation. Three experiments were carried out in series for each sample: hydrolysis, fermentation, and distillation. Because of the series nature of the three experiments, it was not simple to assess the contribution of change in parametric values of each experiment on the final result (Sun and Cheng, 2002). For that reason the factorial design was applied only to the hydrolysis experiment and parameters were changed at two levels to see their directional effects on the response parameter (ethanol yield). The parametric values for the rest of the experiments were kept constant for the experiment to be sharply justified. Therefore, the optimum values of main variables in hydrolysis process (time, acid concentration, and temperature) which give high ethanol yield were set. Table 1 shows the hydrolysis parameters and their respective maximum and minimum.

Table 1. Minimum and maximum value of parameters.

S.No	Experiments	Minimum	Maximum
1	Hydrolysis temperature (°C)	80	100
2	Hydrolysis time(minutes)	30	60
3	Acid concentration(% by volume of distilled water)	1	5

The tabulated numeric representation of the factorial design of this research is shown in Table 2. For each one the hydrolysis parameters (acid concentration, hydrolysis time and hydrolysis temperature) there are four low and four high levels in the design shown in the table (Table 2). 50 g of ground wastepaper was used for each experiment and the factors for hydrolysis were time (30 to 60 minutes), hydrolysis temperature (80 to 100°C), and acid concentration (1 to 5%).

Table 2. Numeric values of parameters in hydrolysis according to factorial design.

Sample Number	Acid concentration (% by volume)	Hydrolysis time(min)	Hydrolysis temperature(°C)	Coded representation of parameters
1	1	30	80	- - -
2	5	30	80	+ - -
3	1	60	80	- + -
4	5	60	80	+ + -
5	1	30	100	- - +
6	5	30	100	+ - +
7	1	60	100	- + +
8	5	60	100	+ + +

Procedures in Dilute Acid Hydrolysis: Sulfuric acid (by volume to water) was diluted to 1% and 5% concentration. The 50 g ground wastepaper of 10% Weight per Volume to the prepared dilute acid solution (1 to 5%) was then added into the glass vessel. Then the prepared sample was hydrolyzed in autoclave with the vessels unlidded between 80 and 100°C for 30 to 60 minutes. Centrifugation and then filtration was used to separate the solid particles from the liquid in the hydrolyzate (remove the non-fermentable lignin portion). The diluted hydrolyzed samples were conditioned to temperature of 30°C before fermentation step was started. This was the temperature at which all fermentation experiments were carried out.

Fermentation: The supernatant from dilute acid hydrolysis of lignocelluloses can contain both six-carbon (hexoses) and five-carbon (pentoses) sugars (if both cellulose and hemicellulose are hydrolyzed). Depending on the lignocelluloses source, the hydrolysate typically consists of glucose, xylose, arabinose, galactose, mannose, fucose, and rhamnose. Microorganisms can be used to ferment all lignocellulose-derived sugars to bioethanol.

Microorganisms: S. cereviciae was purchased from local market and it was used in all experiments throughout this work. Media which is favorable for the yeast was prepared.

Procedures in Media Preparation: First, 100 ml media containing 10 gm Sugar (Dextrose), 0.2 gm yeast extract, 1.0gm urea and 1.0g $MgSO_4.7H_2O$ was prepared. The 100 ml media was sterilized in autoclave. Next to the 100 ml media, 0.5 gm of yeast, *Saccharomyces cerevisiae* was added in a 250 ml conical flask and then properly covered with aluminum foil. The conical flasks were then placed in a shaking incubator for 24 hours at a temperature of 30°C and 200 rpm.

Adjustment of pH: Before addition of any microorganism to the diluted hydrolyzed sample, pH of these samples had to be adjusted. Otherwise the microorganism will die in hyper acidic or basic state. A pH of around 5.0 - 5.5 was maintained. The hydrolyzed samples were primarily checked for pH using a digital pH meter. The pH then was adjusted to 5.0 - 5.5. When the pH went below 5.0 - 5.5, sodium hydroxide solution was added drop wise to the flask with constant stirring until the pH reaches to a range of 5.0 - 5.5. When the pH went beyond 5.0 - 5.5, concentrated sulfuric acid was added drop wise to maintain the pH in the range.

Procedures of the Experiment: Fermentation was taken place in shaking incubator. The shaking incubator was set at 30°C and the prepared samples were dipped into the water-filled-beaker until a set temperature and temperature of the water in the beaker became equal. The yeast, *Saccharomyces Cerevisiae* culture was added with the proportion of 1:10 to the hydrolyzed sample. The vessel was lidded with a piece of ginned cotton covered with aluminum foil. Fermentation was let take place. After 72 hours of fermentation, the sample was taken out and distilled. The

parameters of fermentation i.e. incubation time, yeast concentration (yeast proportion) and fermentation temperature was set to be at 72 hours, 10% and 30°C respectively.

Distillation: A distillation system was used to separate the bioethanol from water in the liquid mixture. All distillation experiments were carried out at a temperature of 85°C and a distillation time of 3 hours by rotary evaporator.

Identification of Bioethanol: About 5 ml fermented sample was taken and pinch of potassium dichromate and a few drops of H_2SO_4 were added. Color change from pink to green indicated the presence of bioethanol.

Determination of Ethanol Concentration: The ethanol concentration of the samples collected every 3 hours interval by rotary evaporator of fermented solution was measured by alcoholmeter. An alcoholmeter is a hydrometer which is used for determining the alcoholic strength of liquids. It only measures the density of the fluid. Alcohol meters have scales marked with volume percent of potential alcohol, based on a pre-calculated specific gravity.

Procedure: Each sample was poured in to a graduated cylinder leaving enough space for the alcoholmeter to disperse the liquid. Then the alcoholmeter was dipped in to the sample and spinned to dislodge any bubbles that may have been in the liquid, and could potentially cause the alcoholmeter to float slightly higher, resulting in a low reading. Finally a reading was taken from the mark where the liquid level crosses the stem of the alcoholmeter. This was the percentage alcohol by volume of the sample.

Data Analysis: Design expert® 7 software was used to determine the effect of three hydrolysis parameters (time, temperature, and acid concentration) on ethanol yield, and to optimize these hydrolysis parameters. The response variable was ethanol yield. Significance of the result was set from analysis of variance (ANOVA).

3. Result and Discussion

Effect of Temperature and Time:

Figure 1(a) & (b) show effect of temperature and time on yield of ethanol when acid concentration was at the center point. Ethanol yield increased with increasing hydrolysis temperature when hydrolysis time was at low level. Similarly, ethanol yield increased with increasing hydrolysis time when hydrolysis temperature was at low level. This is because at low temperature and time cellulose might not be converted to fermentable sugars and at high temperature and time the fermentable sugars might be converted to non-fermentable molecules. Hence both temperature and time have interaction effect, in addition to main effect for the yield of ethanol production.

Figure 1(c) shows the contour plot graph showing predicted response of ethanol yield as a function of hydrolysis time and hydrolysis temperature. Ethanol yield increased as hydrolysis time increases at lower level temperature and it decrease when the hydrolysis time and temperature became higher and higher.

The response surface figure 1 (d), obtained from hydrolysis temperature and time shows that ethanol yield increased with increasing time when hydrolysis temperature was at low level and with increasing hydrolysis temperature when time was at low level.
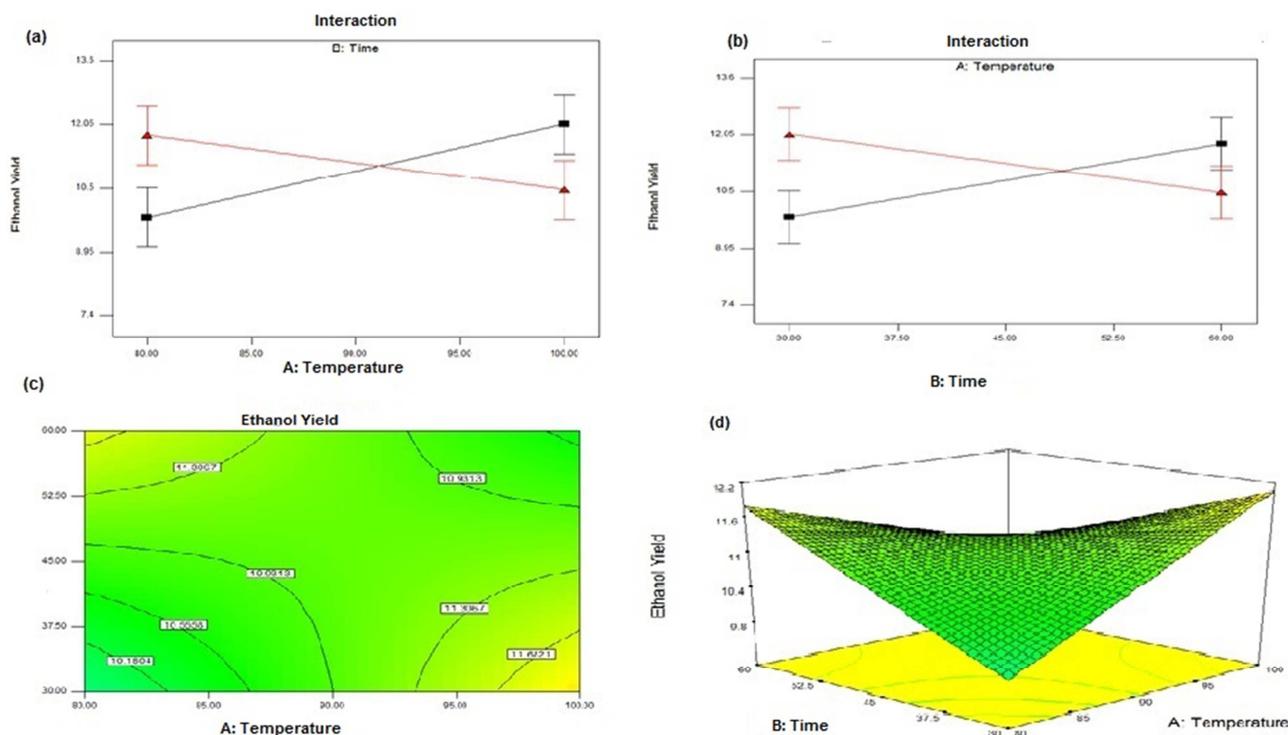

Figure 1. *Effect of temperature and time on yield of ethanol when acid concentration was at the center point (a, b, c & d) (a) The effects of temperature and time (fixed) on the yield of ethanol, when the concentration was at the center point (b) The effects of temperature(fixed) and time on the yield of ethanol, when the concentration was at the center point (c) Contour plots of the effects of time and temperature on the yield of ethanol (d) Response surfaces plot of the effects of temperature and time on the yield ethanol.*

Effect of Acid concentration and time:

Figure 2 (a) & (b) show effect of acid concentration and time on yield of ethanol when hydrolysis temperature was at the center point. Ethanol yield increased with increasing acid concentration when hydrolysis time was at low level. Similarly, ethanol yield increased with increasing hydrolysis time when acid concentration was at low level. This is because at low concentration and time cellulose might not be converted to fermentable sugars and at high concentration and time the fermentable sugars might be converted to non fermentable molecules. Hence both time and acid concentration have interaction effect, in addition to main effect for the yield of ethanol production.

Figure 2 (c) shows the contour plot graph showing predicted response of ethanol yield as a function of hydrolysis time and acid concentration. Ethanol yield increased as hydrolysis time increases at lower level acid concentration and it decrease when the hydrolysis time and acid concentration became higher and higher.

The response surface figure 2 (d), obtained from hydrolysis time and acid concentration shows as hydrolysis time increases at lower level of acid concentration and as increase level of acid concentration and lower level of time gives a positive effect on the yield of ethanol.
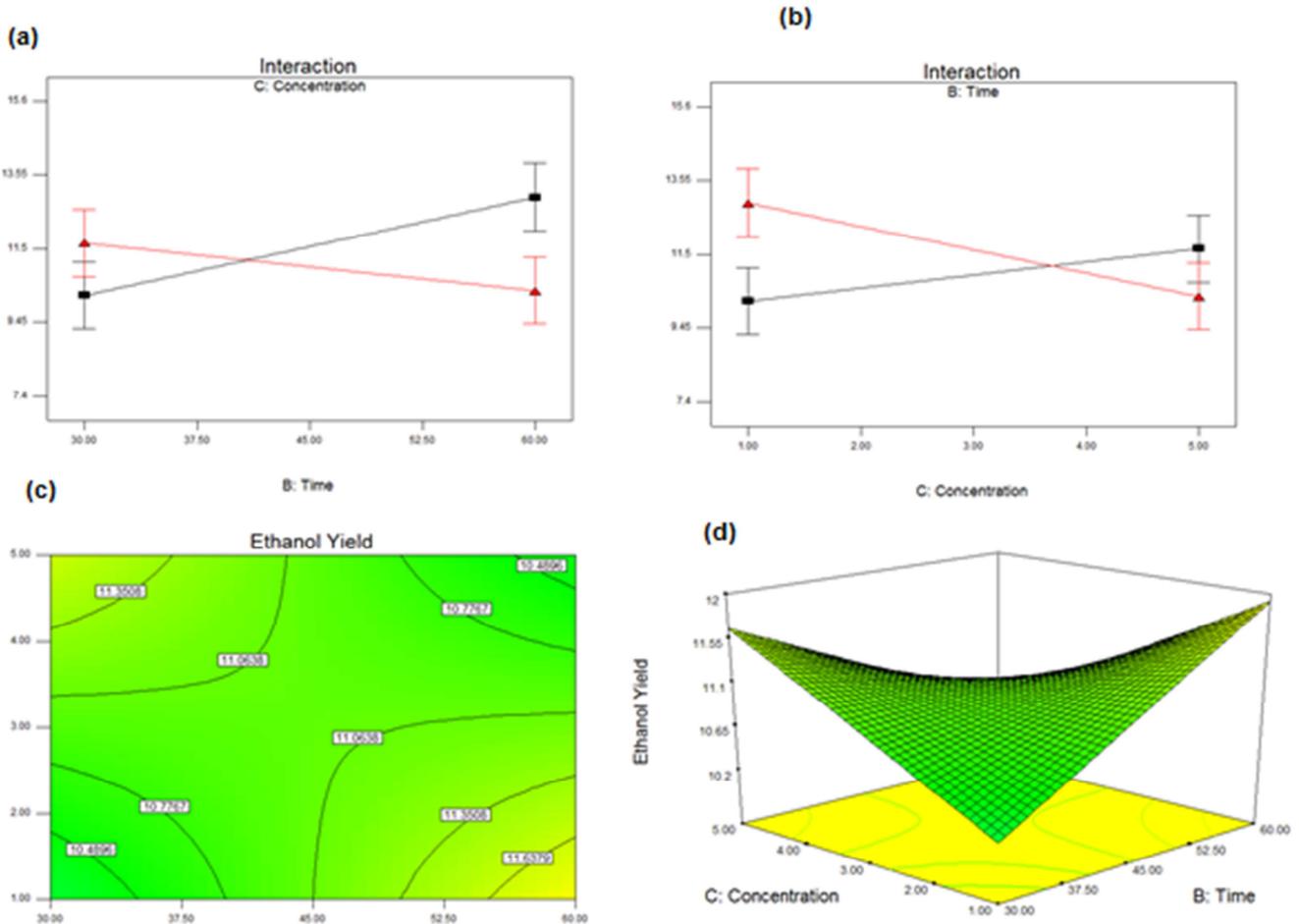

Figure 2. Effect of acid concentration and time on the yield of ethanol when temperature was at the center point. (a)The effects of time and acid concentration (fixed) on the yield of ethanol, (b) The effects of time (fixed) and acid concentration on the yield of ethanol, (c) Contour plots of the effects of acid concentration and time on ethanol yield, (d) Response surface plots of the effects of acid concentration and time on ethanol yield.

Effect of temperature and acid concentration

Figure 3 (a) & (b) show effect of acid concentration and temperature on yield of ethanol when hydrolysis time was at the center point. Ethanol yield increased with increasing acid concentration when hydrolysis temperature was at low level and with increasing hydrolysis temperature when acid concentration was at low level. At lower temperature and acid concentration the cellulose might not be hydrolyzed to fermentable sugars and at higher acid concentration and time the cellulose might be converted to non fermentable molecules.

Hence both temperature and acid concentration have interaction effect, in addition to main effect for the yield of ethanol production.

Figure 3 (c) shows contour plot graph showing predicted response of ethanol yield as a function of hydrolysis temperature and acid concentration. Yield of ethanol increase with increasing acid concentration at low level of hydrolysis temperature and with increasing hydrolysis temperature at low level of acid concentration.

The response surface figure 3 (d), obtained from hydrolysis

temperature and acid concentration shows ethanol yield increased with increasing acid concentration when hydrolysis temperature was at low level and with increasing hydrolysis

temperature when acid concentration was at low level. This was consistent with the study on ethanol production from mango and banana peel reported by Taye in 2009.
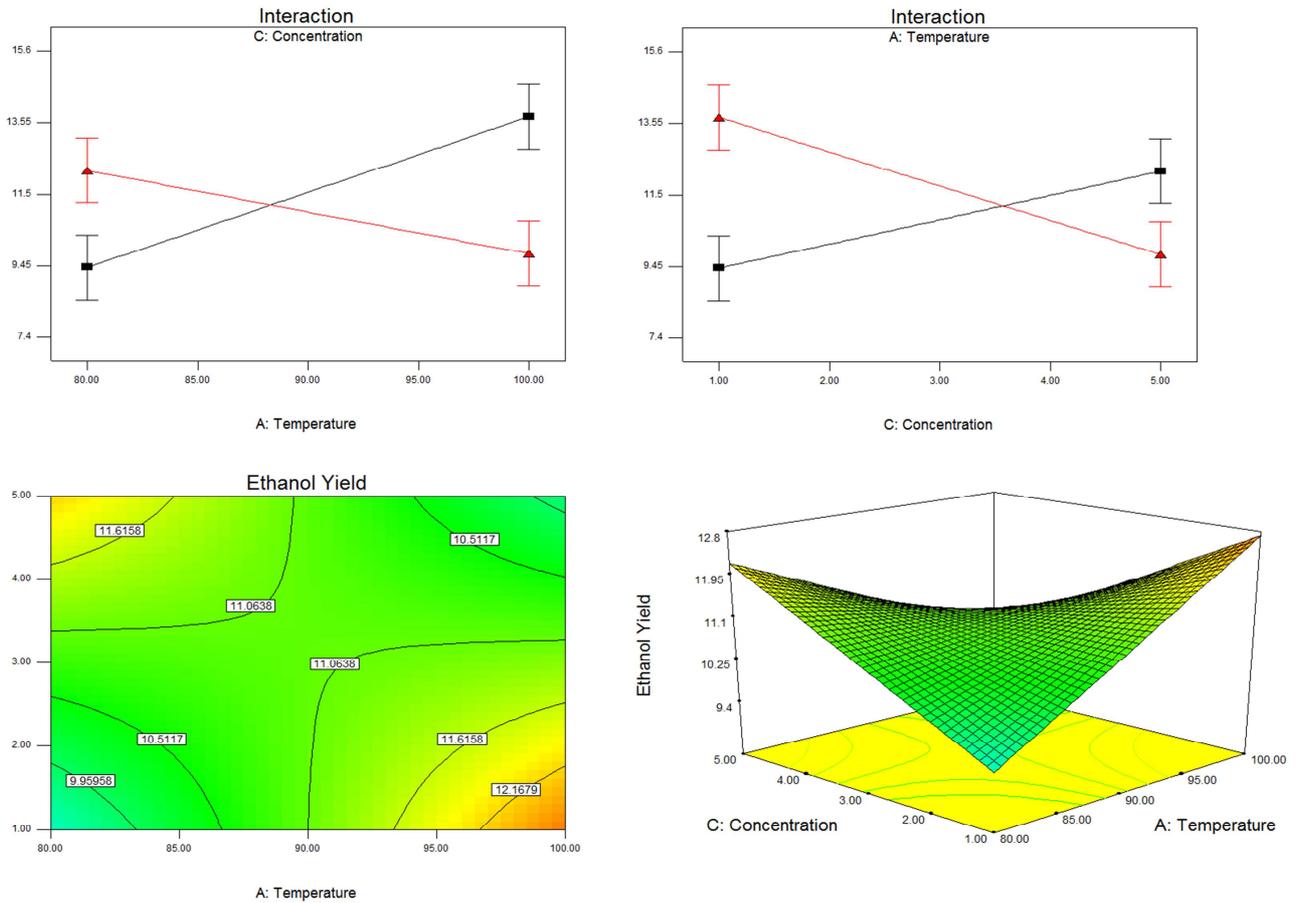

Figure 3. *Effect of temperature and acid concentration on the yield of ethanol when time was at the center point, (a) The effects of temperature and acid concentration (fixed) on the yield of ethanol, (b) The effects of temperature (fixed) and acid concentration on the yield of ethanol, (c) Contour plots of the effects acid concentration and temperature and (d) Response surface plots of the effects acid concentration and temperature.*

Therefore, if it is required to increase ethanol yield with increasing acid concentration, low level of hydrolysis temperature is required. Or else, increased temperature while keeping acid concentration at low level depending on whether economic optimum or technical optimum is the choice or the combination. Technically, optimum operations do not necessarily provide economically optimum performances. Variables such as environment, socio-economic situations and accessibility of relevant technologies must be evaluated to systematically combine the two optimums. From environmental point of view, pollution-free or closed-loop systems are preferred to environmental unfriendly and open end processes. Taking in to consideration the economy of production, using as low quantity of chemicals as possible is beneficial for environment in that treatment and disposal costs are significant portions of production costs. Therefore, using as minimum acid as possible is advisable if possible. Increasing hydrolysis temperature and reducing acid concentration could be economically feasible and environmentally beneficial as heat for steam generation could be derived from non-fermentable lignin-rich fraction of the

waste without the threat of any polluting emissions.

When the above results were compiled to one, high hydrolysis time and high hydrolysis temperature would yield maximum ethanol yield at low acid concentration. This conclusion was consistent with the actual data at 1% acid concentration, 60 minutes hydrolysis time and 100°C hydrolysis temperatures. The maximum ethanol yield found was 13.515 at (1% v, 60minutes, 100°C) of acid concentration, hydrolysis time and hydrolysis temperature respectively.

Table 3. *Optimization criteria for optimum yield of ethanol.*

Parameter	Purpose	Minimum Value	Maximum value
Acid concentration (%)	Minimize	1	5
Temperature (°C)	Minimize	80	100
Time (min)	Minimize	30	60
Ethanol Yield (mL/50g sample)	Maximize	7.42	13.515

Optimization of Hydrolysis Parameters: Design expert® 7 software was used for optimization of hydrolysis parameters. The optimization of hydrolysis criteria for ethanol production from wastepaper using dilute acid treatment are summarized

in Table 3.

Design expert® 7 software calculates 15 optimum possible solutions for ethanol production using different hydrolysis parameters as shown in Table 3. The optimum combinations of the three factors chosen for optimum ethanol yield (10.8538) were 92.5°C (hydrolysis temperature), 30 minutes (hydrolysis time) and 1%v acid concentration. This was global optimum combination of the factors. The local optimization usually requires hundreds or perhaps thousands of experiments. The

gross choice between these two factors values do not purely rely on the technical success of the experiment. It also had to consider the economic variables of ethanol production and also whether the production is pilot scale or large scale. The Figure 4, Figure 5 and Figure 6 show contours plot and response surfaces plot generated by Design expert® 7 software for the optimum combinations of the three factors chosen respectively.
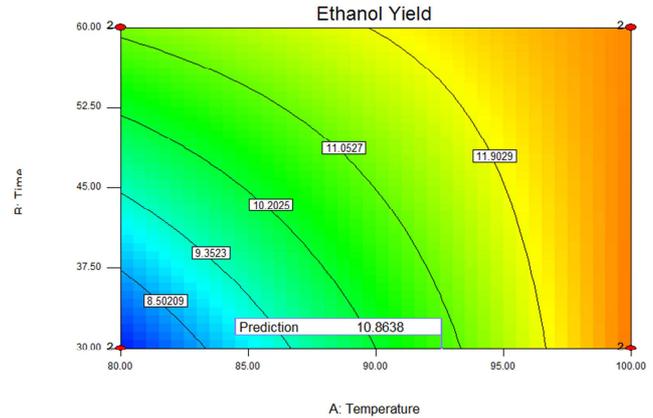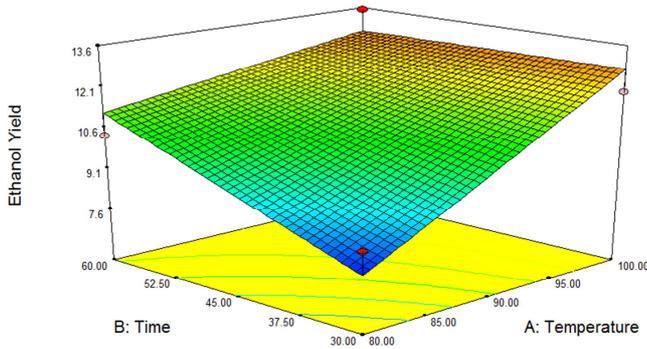

Figure 4. Optimization of time and temperature (a) response surface method, (b) counter plot.
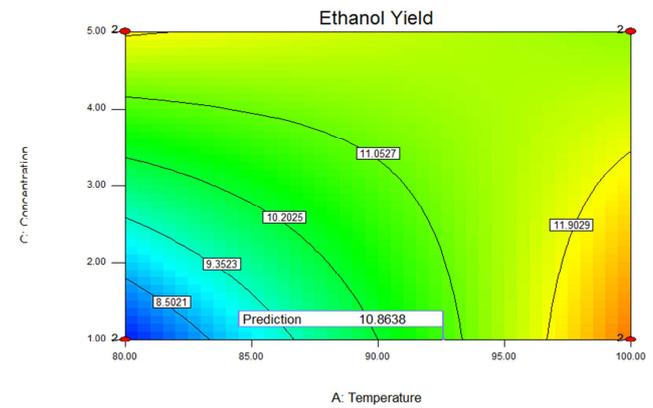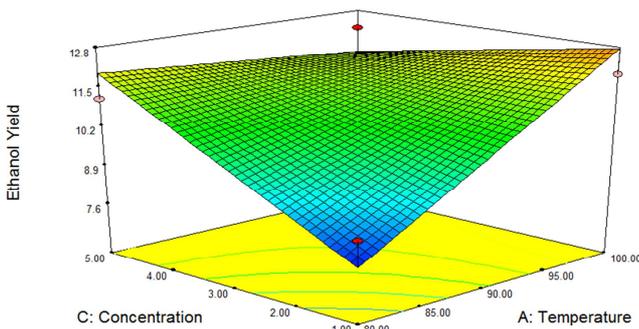

Figure 5. Optimization of concentration and temperature (a) response surface method, (b) counter plot.
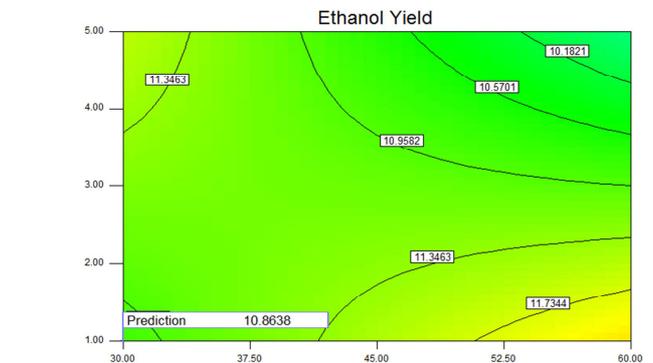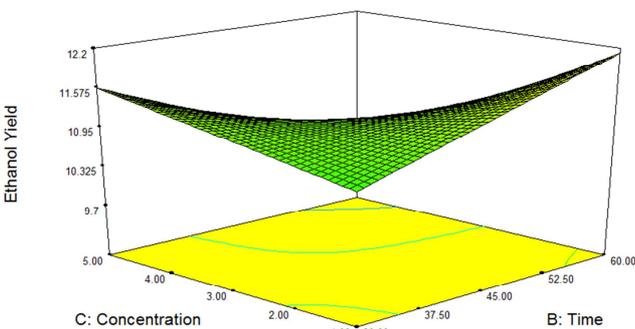
Figure 6. Optimization of concentration and time (a) response surface method, (b) counter plot.

To see the consistence between the theoretical ethanol yield at theoretical combination of parametric values and the actual result at that point, an experiment with hydrolysis acidic concentration, temperature and time were conducted at the optimized conditions. The actual result of ethanol yield at theoretical combination (10.45) was slightly lower than what was expected (10.86).

4. Conclusion

It is concluding that wastepaper is promising lignocellulosic feedstock for bioethanol production. One of the most important factors in the acid treatment of lignocellulose is the determination of optimal conditions required to provide the maximum yield of fermentable sugars and the least amount of inhibitors. All the three hydrolysis parameters were significant variables for the yield of ethanol. Yield of ethanol decreases at very high and low hydrolysis temperature, hydrolysis time and acid concentration. The optimum combinations of the three factors chosen for optimum ethanol yield (10.86 ml/50 g sample) were 92.59°C (hydrolysis temperature), 30 minutes (hydrolysis time) and 1% v acid concentration. Ethanol production from wastepaper is doubtlessly an attractive business from economic and environmental point of view.

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