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Production of Ethanol by fermentation process by using Yeast *Saccharomyces cerevisae*

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Abstract

The world is facing a serious energy crisis all over the world due to development in industrial and transportation sectors and population explosion as well. So there is a need to find out the alternative techniques. Ethanol is a renewable energy resource derived from the domestic resources like the waste tissue paper, corn, wheat straw, algae, food and newspaper waste mixture and also from paper waste. Present study deals with the Production of Ethanol from the waste tissue paper by the simultaneous saccharification. Waste tissue paper samples were collected from different restaurants. Sampling strategy was the composite sampling. Ethanol was prepared from tissue paper waste and paper waste with different ratios of sulfuric acid. The variation in the quality of ethanol is due to the raw material used. Quality assessment parameters were also measured. All the results of quality parameters were within the range of pure ethanol. Quality assessment parameters showed that ethanol produced meet the ASTM quality standards indicating that used/waste tissue paper can be used as a substitute to the traditional energy fuels as well as protect the environment.

Keywords: Energy crisis, fermentation, ASTM quality standards, simultaneous saccharification, yeast (Saccharomyces cerevisae).

Introduction

Now a day energy crisis are seeing everywhere around the world which then leads to increasing electric energy's desire as compared to its supply which is very less. Any immense bottle neck or increase in the energy resources supply to a country's economy is called as energy crisis[1]. This can pass on to the scarcity of electricity or additionally oil and other non renewable resources. It is the need of the time that we should focus to inspect new energy resources because for about 40 years our present oil reserves will last at most and before then will decline significantly[2]. This may b cause some fear by realizing that our present energy resources are declining and leads to devastating results for the global life quality and global economy as well[3].

Biofuel is one of the best alternative fuels in order to trounce the energy crisis. It is a type of fuel which derives its energy from the carbon fixation biologically. The fuels included in biofuels are derived from the solid biomass, conversion of biomass, various biogases and liquid fuels as well[4]. Biofuels are driven by the factors like need for increasing energy security and hikes and gaining the scientific and public attention. Ethanol can be made by any organic material which contains sugar, starch or cellulose[5]. According to the new technologies ethanol is producing from the cellulose in the woody fibers from the crops, grasses and trees residues. Ethanol is an environmental friendly fuel. It is biodegradable and water soluble as well. In comparison with gasoline, if a fuel spill occurs, its effects are less severe environmentally[6]. Ethanol can cause lower emissions of CO (carbon monoxide) when using as the fuel additive because ethanol contains oxygen. As compared to the conventional gasoline the blends of E10 resulted in 12-25 % less emissions of carbon monoxide. In the areas that are failed to meet the air quality standards of Environmental protection agency for CO, E10 is used there widely[7].

Biofuel can be produced by means of different sources like waste chicken feathers, cellulosic biomass, food and organic waste. Other types of fuels can be produced by mixed paper waste that is separated from the municipal solid waste. Cash crops can also be utilized to produce bioethanol like Jatropha curcus, cotton, maize, and corn and wheat etc[8]. The biofuel besides this can also be produced from the microbes and plants. The plants or microbes generated the fats or carbohydrates which are advanced to produced biofuel. Some other sources of bioethanol production are algae and waste cooking oil[9]. Cellulose is the major content of all the crops. It can be renovated into glucose and ethanol can be produced after the process of fermentation[10]. The ethanol fuel produced from the cellulose promise very less environmental emissions of GHGs because cellulosic fibers are the universal and biggest part of the cell wall of plant. It has been stated by the IEA (International Energy Agency) that ethanol fuel produced from the cellulose will show the much bigger contribution than in the past[11]. Every year Pakistan produced molasses around 2 million tons in which at the ostensible rate of 35 dollars per ton, 45 million tons of molasses were exported last year earning only 47 million dollars[12]. According to some industrial source if the molasses are converted to ethanol (ethyl glycol) then more foreign exchange can be gained[13]. Recovery of ethanol on an average from one ton of the molasses is approximately at 240 liters – 270 liters which also include its dependency on the type of molasses used. Pakistan can become the self sufficient in fuel production because it has the tendency to produce at least 500 million liters of biofuel (ethanol) and hence eliminate the debit of trade[14]. The present study was intended to focus on reevaluation of waste tissue papers after converting it into ethanol fuel which is very important economically.

Material and Methods

Experimental performance: All the experimental work was done in the Environmental science research lab at LCWU, Lahore. The present study deals with the study of producing bioethanol from the waste tissue papers by the process of fermentation. Samples were collected from the three different restaurants located in Lahore. Regular visits done for the sampling purposes and regular laboratory work were also done. Samples were collected manually according to the sampling plan in the plastic bags. Every time three samples were collected from the three restaurants to make one composite sample. Hence, total 48 random samples were collected to make 16 composite samples. Each sample contains 5kg of the tissue paper waste. Samples were taken in the clean plastic bags. Plastic bags were capped properly and preserved at the room temperature.

Drying and weighing of tissue papers: The drying of tissue paper waste was done at first. The tissue papers were placed in an oven at 100^0C for about 40 minutes until all the moisture content was removed from the tissue papers. After drying the tissue paper waste was weighed. For this purpose the weighing balance was used. Seven different samples were prepared in which five samples with 100 g tissue paper waste. To produce ethanol from the paper waste, one sample was prepared with 100 g of paper and the other one was prepared with 50g tissue papers and 50 g papers. For this purpose the weighing balance was turned on. The samples in a tray were placed on it and the reading of desired weight was noted in grams on weighing balance.

Soaking in H_2SO_4 and hydrolysis of the samples: Next the tissue papers were soaked in H_2SO_4 (5 % by weight of H_2SO_4). The H_2SO_4 was taken in the different variations i.e. 300ml, 400ml, 500ml, 600ml, 700ml, 500ml, 500ml for the tissue papers, papers and mixture of both (50g tissue papers and 50g papers) respectively. Afterwards the samples were placed in the autoclave for the hydrolysis. Acid hydrolysis was done due to its economic importance. The autoclave was maintained at 120^0C for about 3 hours.

Filtration of the samples: The filtration of all the samples was done in the filtration assembly. The filtration was done twice in order to get the pure filtrate without any residue. The second time, filtration was done by the help of filter paper. Different quantity of the filtrate can be obtained from the different samples.

Neutralization of the samples filtrates: After filtration all the samples were neutralized by adding potassium hydroxide solution (KOH solution). The KOH solution was prepared by adding 40 g of potassium hydroxide in 100mill liter of distilled water. The potassium hydroxide solution was added according to the different concentration i.e. 50ml, 75ml, 100ml, 125ml, 150ml, 40ml and 25ml in all the samples respectively.

Fermentation: The fermentation of all the samples was done at the room temperature. The fermentation can be done by adding Yeast (*Sacchromyces cerevisiae)* in each sample. The Yeast can be added in the different concentration i.e. 2.5g, 5g, 10g, 15g, 20g, 10g and 10g respectively. The samples were placed at 30^0C for the fermentation for about 24hours.

Distillation: After fermentation all the samples were ready for the distillation. The distillation was done in the distillation assembly for about 8hours. The distillation can be held twice in order to optimize the production of bioethanol in the final product. In order to get the large quantity of bioethanol, another sample was prepared with the different concentrations of all things in it. In that sample 700g of tissue papers were soaked in 3500ml H_2SO_4 (5% by weight of H_2SO_4). The amount of 400ml of KOH solution (180g KOH dissolved in 300ml distilled water) was added in order to neutralize and the amount of Yeast added was 66g for fermentation and after fermentation the sample was left for the distillation (twice).

Table 1
The comparison of pH value of the prepared ethanol with the standard ethanol after double distillation

Comparison of the pH value of the prepared ethanol samples with standard ethanol					
Sr. No.	No. of Samples	1	2	3	Mean ± SD
1	100g tissue paper + 700ml H2SO4	6.9	7.0	6.8	6.9 ± 0.1
2	50g tissue paper + 50g paper	7.6	7.5	7.7	7.6 ± 0.1

Table 2
The comparison of percentage of alcohol of the prepared ethanol with the standard ethanol after double distillation

Comparison of the percentage of alcohol in prepared ethanol samples with standard ethanol					
Sr. No.	**No. of Samples**	**1**	**2**	**3**	**Mean ± SD**
1	100g tissue paper + 700ml H2SO4	85	85.1	84.9	85 ± 0.1
2	50g tissue paper + 50g paper	89.9	90.1	90	90 ± 0.1

Table 3
The comparison of the viscosity value of the prepared ethanol with the standard ethanol after double distillation

Comparison of the viscosity value of the prepared ethanol samples with standard ethanol					
Sr. no.	No. of Samples	1	2	3	Mean ± SD
1	100g tissue paper + 700ml H2SO4	0.0011	0.0013	0.0012	0.0013 ± 1E-04
2	50g tissue paper + 50g paper	0.0013	0.0011	0.0012	0.0012 ± 1E-04

Table 4
The comparison of density of the prepared ethanol with the standard ethanol after double distillation

Comparison of the density value of the prepared ethanol samples with standard ethanol					
Sr. no.	**No. of Samples**	**1**	**2**	**3**	**Mean ± SD**
1	100g tissue paper + 700ml H2SO4	0.791	0.790	0.792	0.791 ± 0.001
2	50g tissue paper + 50g paper	0.783	0.785	0.784	0.783 ± 0.001

Table 5
The comparison of value of specific gravity of the prepared ethanol with the standard ethanol after double distillation

Comparison of the specific gravity value of the prepared ethanol samples with standard ethanol					
Sr. No.	**No. of Samples**	**1**	**2**	**3**	**Mean ± SD**
1	100g tissue paper + 700ml H2SO4	0.799	0.798	0.797	0.799 ± 0.001
2	50g tissue paper + 50g paper	0.788	0.787	0.789	0.788 ± 0.001

Table 6
The comparison of the boiling point of the prepared ethanol with the standard ethanol after double distillation

Comparison of the boiling point value of the prepared ethanol samples with standard ethanol					
Sr. No.	**No. of Samples**	**1**	**2**	**3**	**Mean ± SD**
1	100g tissue paper + 700ml H2SO4	79	80	78	79 ± 1
2	50g tissue paper + 50g paper	78	79	80	79 ± 1

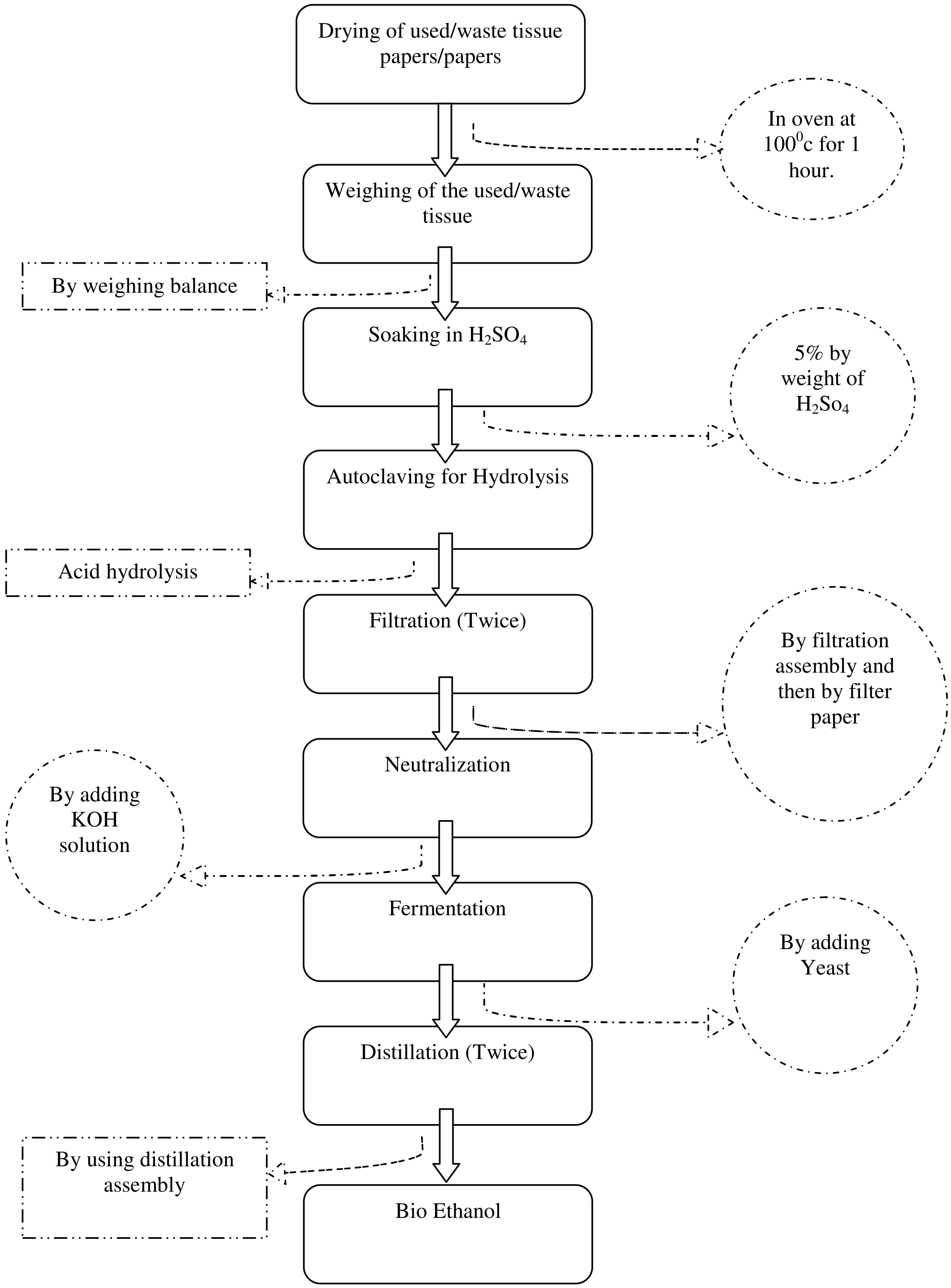

Figure-1
Process Flowchart (Production of Bioethanol from tissue paper waste)

Results and Discussion

The study was conducted for the production of bioethanol from the waste tissue papers through the fermentation process by using *Sacchromyces cerevisiae*. Figure-2 shows the value of pH of the prepared samples and their comparison with the standard ethanol after double distillation. Two samples were left for the double distillation i.e. sample 5 and sample 7. The pH of the sample 7 has more close enough value of pH with the pure ethanol i.e. 7.6± 0.1 while the sample 5 has the pH value of 6.9 ± 0.1

Figure-3 shows the comparison of percentage of the sample of the prepared ethanol sample with the pure ethanol after double distillation. According to the figure the sample 7 has 90± 0.1 of alcohol while sample 5 has 85 ± 0.1 of alcohol in it. So the sample 7 has more close enough value of percentage of alcohol to the standard alcohol i.e. 95%.

Figure-4 shows the value of viscosity in Pa of the sample and its comparison with the standard ethanol after double distillation. The sample 5 has the viscosity value of 0.0013± 1E-04 while the sample 7 has the viscosity value of 0.0011 ± 1E-04. The sample 5 has more close viscosity value to the standard ethanol so it is more pure.

Figure-5 shows the comparison of value of density in g/cm^3 of the samples with the standard ethanol sample after double distillation. The sample 5 has value of density 0.791 ± 0.001whilethe sample 7 has the density value of 0.783 ± 0.001. According to the figure the sample 7 has more close value of density to the standard ethanol than the sample 5.

Figure-6 shows the value of the specific gravity in g/cm^3 of the samples after double distillation and their comparison with the standard ethanol. The sample 5 has the specific gravity of 0.799 ± 0.001 while the sample 7 has the value of specific gravity of 0.788 ± 0.001.So the sample 7 has more close enough value of the specific gravity to the standard ethanol as compared to sample 5.

Figure-7 shows the boiling point in ^{o}C of the prepared ethanol samples and their comparison with the standard ethanol. According to the figure the sample 5 has the boiling point of 79 ± 1 while the sample 7 has also the boiling point of 79 ± 1. So both the samples has boiling point close enough to the boiling point of the standard ethanol.

The ratio of the substance's density compared to the reference substance's density is called as specific gravity of a substance[15]. Specific gravity of all the ethanol samples produced from the tissue paper waste was determined by using a specific gravity bottle[16]. The value of specific gravity ranged from 0.788g/cm^3 to 0.799 g/cm^3. According to ASTM standard the value of specific gravity for the ethanol is 0.785 g/cm^3. The value of specific gravity of the prepared ethanol is within the range of ASTM standard. The slight deviations from the standard value are due to the presence of some impurities[17].

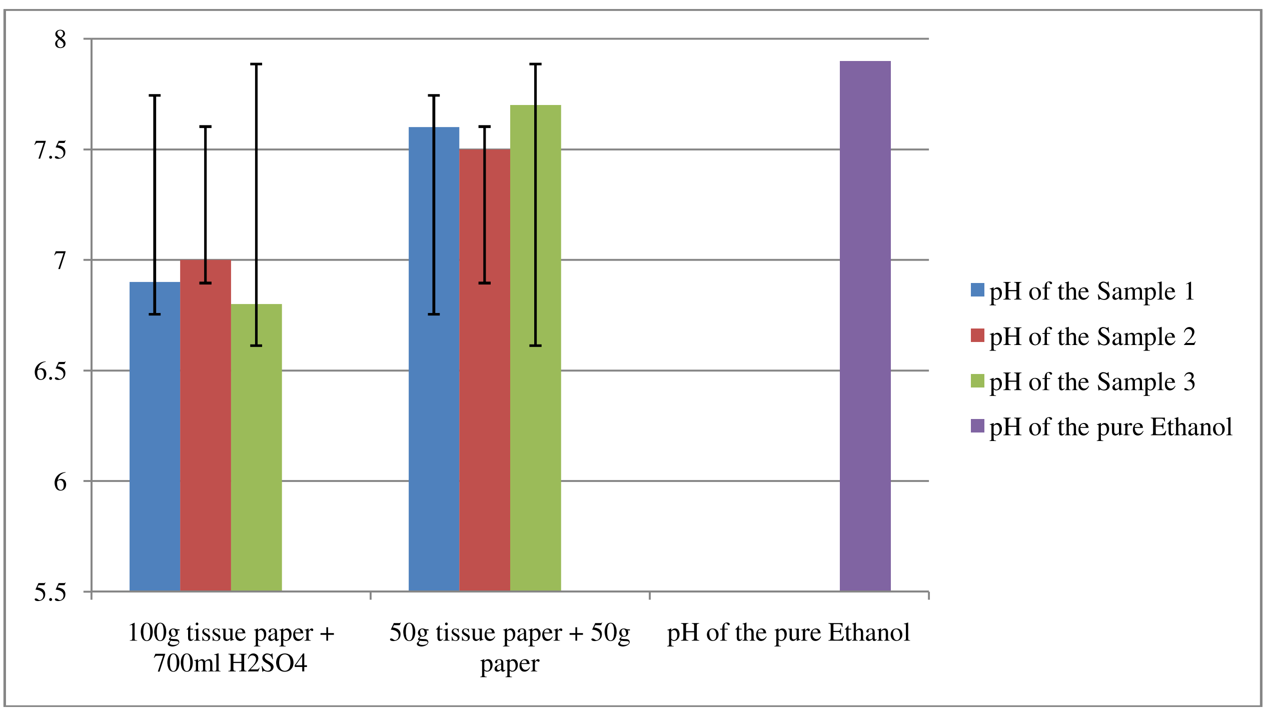

Figure-2
Graph showing the comparison of pH value of the prepared ethanol with the standard ethanol after double distillation

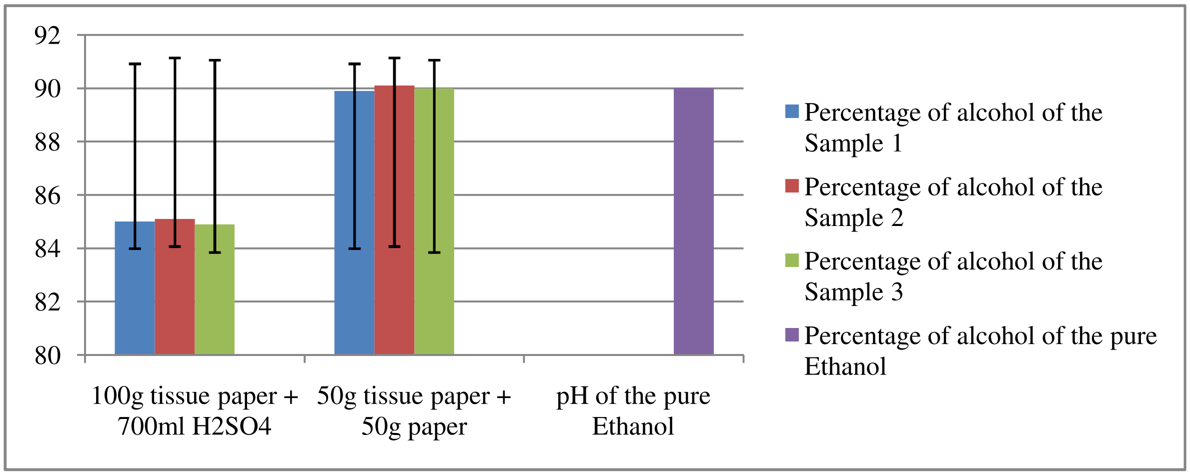

Figure-3
Graph showing the comparison of percentage of alcohol of the prepared ethanol with the standard ethanol after double distillation

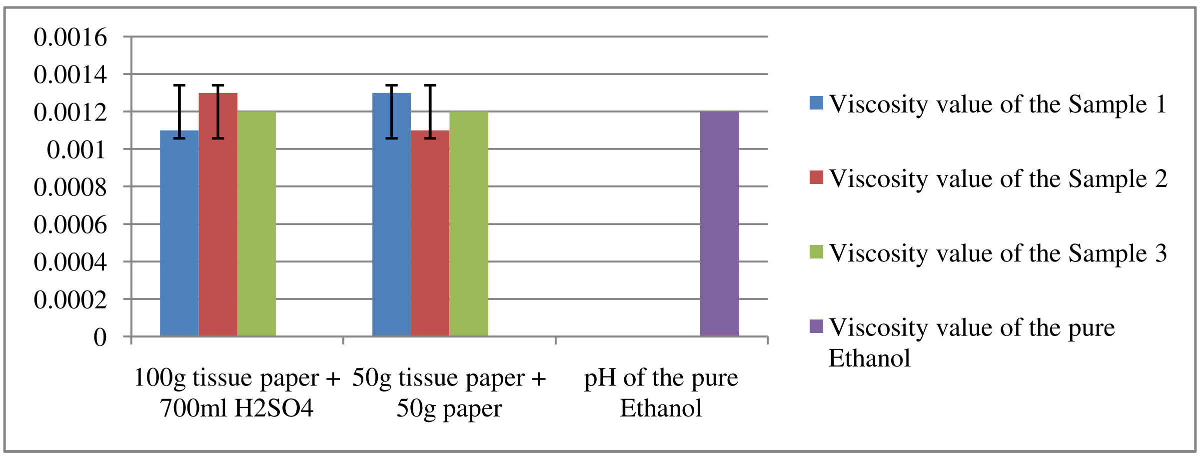

Figure-4
Graph showing the comparison of the viscosity value of the prepared ethanol with the standard ethanol after double distillation

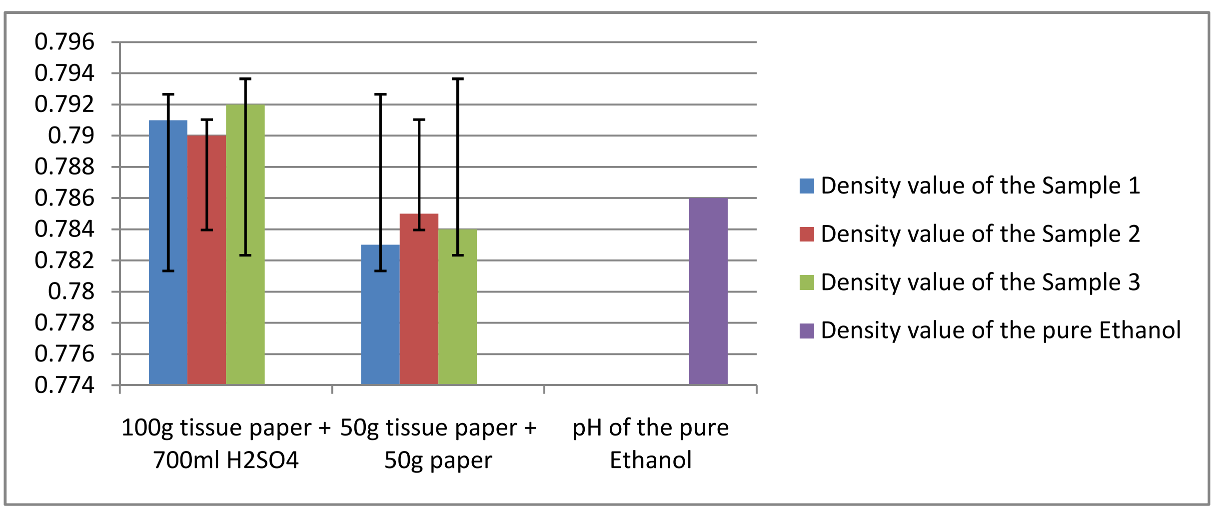

Figure-5
Graph showing the comparison of density of the prepared ethanol with the standard ethanol after double distillation

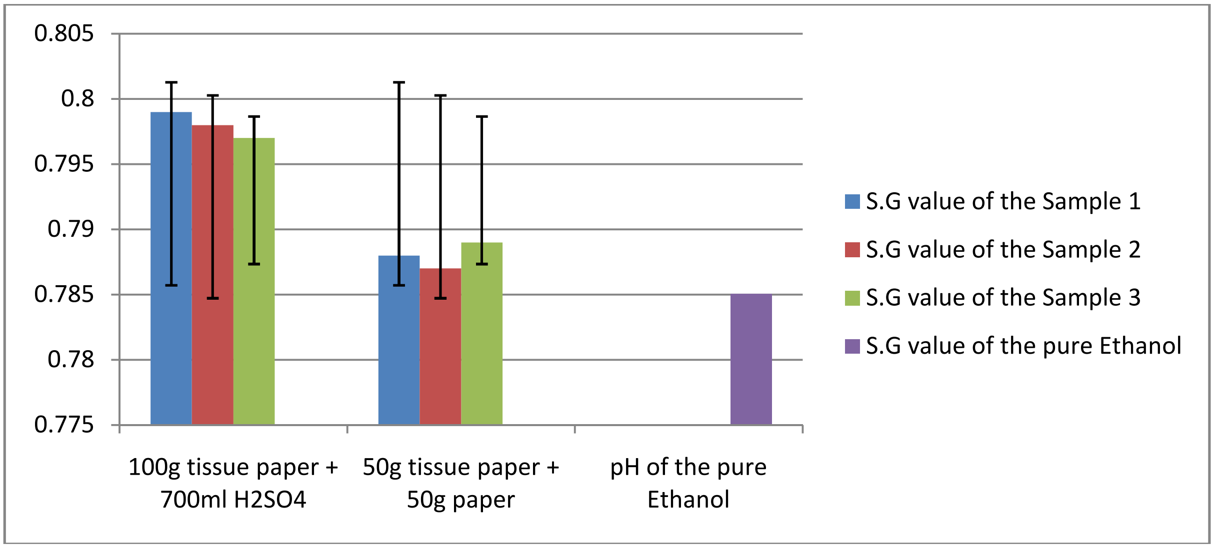

Figure-6
Graph showing the comparison of value of specific gravity of the prepared ethanol with the standard ethanol after double distillation

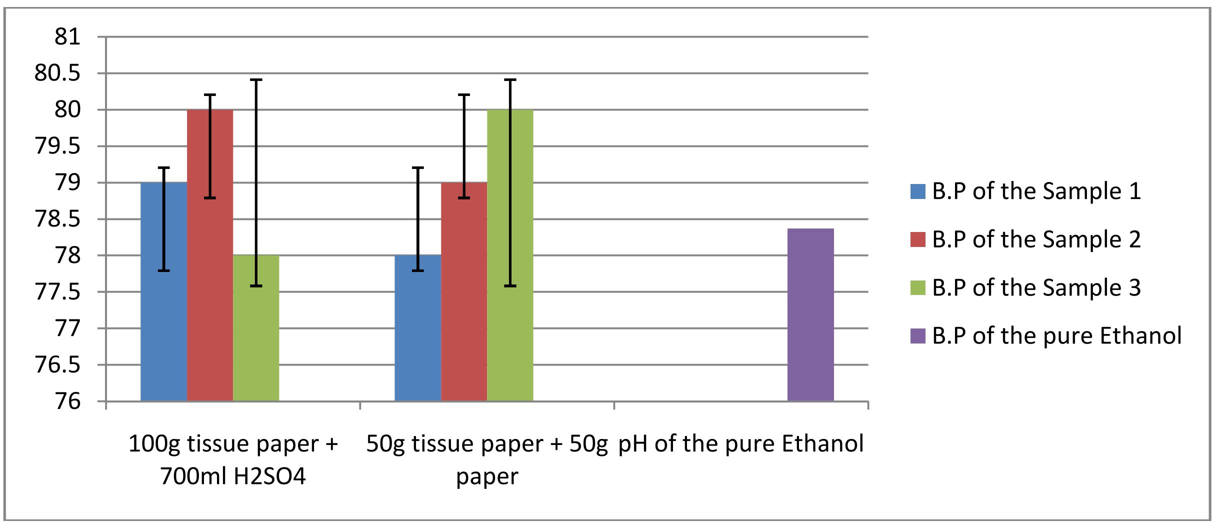

Figure-7
Graph showing the comparison of the boiling point of the prepared ethanol with the standard ethanol after double distillation

The density of a material is defined as the mass of the material per unit its volume. The density (g/cm^3) of the ethanol produced from the tissue paper was determined which is the weight of the substance compared to water at a constant specified temperature (usually at 4^{o}C). The density of the sample was measured with the hydrometer. Density actually reflects the properties of the chemical compounds that make up ethanol[18]. The value of the density was ranged from 0.791 g/cm^3 to 0.783 g/cm^3. According to ASTM standards, the value of density for the ethanol is 0.786 g/cm$^{3.}$ These values of the prepared ethanol meet the density value of the pure ethanol. Bioethanol demonstrate temperature dependent behavior that is qualitatively similar to the biofuels[19].

pH can be defined as which is the concentration of hydrogen ions in the sample. pH values for the ethanol produced from the waste tissue paper were measured for all the ethanol samples with the help of pH meter which is the concentration of hydrogen ions in the ethanol. The value ranged from 6.9-7.6[20]. The highest value of pH was of sample 7 i.e. 7.6. According to ASTM standards, pH of the ethanol should be 6.5-7.9. Slight variations in the pH readings from the standard values were happening because of the presence of impurities, water content that may be present after the double distillation[21].

Boiling point can be defined as the temperature at which a substance changes in to vapor state and liquid's vapor pressure become equal to the surrounding's vapor pressure. In order to check the quality of ethanol, boiling point of all ethanol samples were determined. The boiling point of the both the samples was 79°C. According to the study of Pasquini & Bueno in 2007 the boiling point of the pure ethanol was 78.3°C. So the boiling point of both samples is close to the boiling point of pure ethanol[22].

Kinematic viscosity can be defined as the measure of fluid resistance to its plodding deformation by the tensile stress or shear stress. It corresponds to the informal conception of thickness for the liquids. Kinematic viscosity (pa) for the all ethanol samples produced from the waste tissue paper was measured with the help of Viscometer. It is a measurement of the fluid thickness or resistance to flow under the gravity. The value ranged from 0.0011pa to 0.0013pa. The viscosity value for the ethanol was 0.0012pa according to the ASTM standards. So both the samples after double distillation have the viscosity value close enough to the ASTM standards[22]. The value of Kinematic viscosity is deviated because of the reduction in the intermolecular forces among the molecules of the original species i.e. dipolar interactions in the esters and hydrogen bonding in the alcohols. It is also due to the more difficult accommodation of the alcohol and esters molecules[23].

The quality of producing ethanol is affected due to the water droplets and impurities during the production which can be handled by comparing with ASTM standards. Now the government should pay attention towards the new technological innovations to overcome the global fuel crisis and the production of bioethanol fuel from environmentally friendly and waste stocks like a tissue paper waste[24].

Conclusion

The world energy crisis is increasing day by day due to the increase in population and our more dependency on the resources which are non renewable and less on the renewable resources. Our main dependency for the energy needs is on the non renewable resources like as oil, coal and gas for over 80%. Due to which there is a need to find the alternative biofuels such as biodiesel and bioethanol in order to cope with the energy crisis. Present study concluded that dependence on the petroleum reserves can be minimized by introducing alternative methods of ethanol production from tissue paper and paper waste. The ethanol production from the tissue paper waste can reduce the disposal load on the landfills and more over dependency on the non renewable resources. According to the results the ethanol can be available at the cheap rates which make it more valuable alternative biofuel than other petro diesel fuels. The emission produced by the bioethanol is 90% less than other traditional fuels. Being an environmental friendly fuel it can reduce global warming, emission of greenhouse gases and VOCs. The production of bioethanol will support sustainable development of non renewable resources. There is an urgent to support and commercialize the production of biofuels in order to get rid of the energy crisis and to strengthen the economy of the country.

Future Scope: Bioethanol is a renewable energy resource which has many advantages. It has been derived from the domestic resource and hence reduces the dependency on the fossil fuels. Besides this it is also non toxic and biodegradable. It can also reduce the emissions of harmful gases to over 84-90%. Further detailed study should be conducted on the vehicular emissions using Bioethanol as a fuel as it produce less emissions of CO_2, NO_x , VOCs and other traditional fuels. Commercialization of bioethanol will stable the fluctuation in the fuel prices as well as increases the employment opportunities.

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