PRODUCTION OF BIOETHANOL FROM WASTE PAPER

Project Report

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BONAFIDE CERTIFICATE

This is to certify that the project report titled "**PRODUCTION OF BIOETHANOL FROM WASTE PAPER**" is the bonafide work of **MARY TANIA CHRISTOPHER (SHR15BT034)** during her VIIIth semester, in the partial fulfilment of the requirements of the APJ Abdul Kalam Technological University for the award of B.Tech degree in Biotechnology Engineering, under our supervision.

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COURSE OBJECTIVES

1	To apply engineering knowledge in practical problem solving.
2	To foster innovation in design of products, processes or systems.

3	To develop creative thinking in finding viable solutions to engineering problems.

COURSE OUTCOMES

CO1	Think innovatively on the development of components, products, processes or technologies in the engineering field.
CO2	Apply knowledge gained in solving real life engineering problems.
CO3	Develop a sustainable, ethical and eco friendly system which aids mankind.

PROJECT OBJECTIVES

1	To find an alternate way to minimise the waste paper load on municipal waste
2	Better understanding of biomass transfer.
3	Optimise a low cost and efficient process that gives an improved product yield.

PROJECT OUTCOMES

PR1	To identify a problem and to think innovatively on the development of components, products, processes or technologies in the engineering field
PR2	To apply the gained knowledge in solving real life engineering problems

MAPPING OF COs WITH POs

	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12
C01	-	-	3	3	-	3	3	3	-	-	-	-
C02	-	-	3	3	-	-	-	-	-	-	-	-
C03	-	-	3	3	3	3	3	3	3	3	3	3

MAPPING OF COs WITH PSO's

	CO1	CO2	CO3
PSO1	2	-	3
PSO2	-	-	-
PSO3	2	3	-

MAPPING OF CO's WITH PR's

	CO1	CO2	CO3
PR1	2	1	-
PR2	-	-	1

#1-Weak correlation, 2-Moderate correlation, 3- Substantial correlation

ABSTRACT

Paper, which is one of the largest constituents of municipal solid waste, has become a severe problem for disposal in developed and developing countries due to the shrinking landfill capacity. Newspaper, which is a cellulosic feedstock, is emerging as an attractive option for the production of bio-ethanol because of lower feedstock costs, higher potential for fossil fuel displacement and reduction in greenhouse gas emissions. The main objective of the current project is to minimise the newspaper load on municipal solid waste by efficiently utilising the waste newspaper for the production of bio-ethanol. The possibility of using waste paper as a cheap feedstock for ethanol production arose from the well-publicised concern about rising landfill costs resulting from shrinking landfill capacity. Bio-ethanol provides a higher degree of energy security nationally, is sustainable, cost effective and environment friendly usages. Biofuels reduce the dependency on conventional fossil fuels, as a vast population of India uses fossil fuels in their daily routine.

In this project, the effect of various pretreatments on hydrolysis of waste paper was analysed. The waste paper which is rich in cellulose is then converted to sugars and the subsequent production of bio-ethanol through fermentation was also studied. The influence of different parameters on bioethanol production from waste paper has been studied and optimised. The focus of this project is to improve the yields achieved through the pretreatment and subsequent ethanol production from waste paper so that it could be a potential feedstock for the production of bioethanol in an industrial scale.

Key words: Acid hydrolysis, Fermentation, Bioethanol

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CHAPTER 1

INTRODUCTION

The advent of the electronic age has influenced the use of paper among people greatly. People have started to consider going paperless. But still there seems to be a long way to go to become completely independent of paper. The environmental impact of paper has led to changes in industry and behaviour at both business and personal levels. With the use of modern technology such as the printing press and the highly mechanised harvesting of wood, disposable paper became a relatively cheap commodity, which led to a high level of consumption and waste. The rise in global environmental issues such as air and water pollution, climate change, overflowing landfills and clear cutting have all led to increased government regulations. There is now a trend towards sustainability in the pulp and paper industry as it moves to reduce clear cutting, water use, greenhouse gas emissions, fossil fuel consumption and clean up its impacts on local water supplies and air pollution.Worldwide, the pulp and paper industry is the fifth largest consumer of energy, accounting for four percent of all the world's energy use. The pulp and paper industry uses more water to produce a ton of product than any other industry.[1] There are a lot of varieties of papers produced and each and every one of them requires different raw materials, energy input, water consumption etc. Differences among the grades and types of paper are determined by several factors: the type of fibre used; the preparation of the pulp, either by mechanical (groundwood) or chemical (primarily sulfite, soda, or sulfate) methods, or by a combination of the two; by the addition of other materials to the pulp, among the most common being bleach or colouring and sizing, the latter to retard penetration by ink; by conditions under which the sheet is formed, including its weight; and by the physical or chemical treatments applied to the finished sheet.

Although wood has become the major source of fibre for papermaking, rag fibres are still used for paper of maximum strength, durability, and permanence. Recycled waste paper (including newsprint) and paperboard are also important sources. Other fibres used include straw, bagasse (residue from crushed sugarcane), esparto, bamboo, flax, hemp, jute, and kenaf. Some paper, particularly specialty items, is made from synthetic fibres.[2]

From newspapers to paper wrappings, paper seems to be present everywhere and most of them end up in the landfills which suggests the staggering amount of paper wastes that are produced everywhere. There was a time when paper was a rare and precious commodity. Now it fills our planet. It was initially invented as a tool for communication, but today, paper is used more for packaging, decorating and so on. Deforestation is one of the main environmental problems we are facing in these times. It is also a process that utilises a large amount of water. The recycling of paper or its use in some other form will result in the reduction of the wastage of energy as well as resources.

The paper waste that is coming as a major waste product in the municipal solid wastes accounts for a huge amount of the total wastes generated and are contributing greatly to landfills. The data from the U.S. Environmental Protection Agency (EPA) shows how much greater the amount of paper is present as a waste.

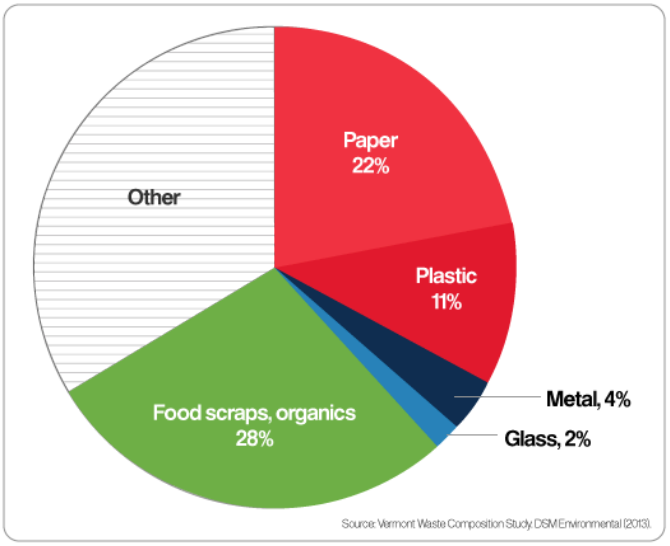


Figure 1: Pie chart of average person's daily trash

Considering the biology of paper, it can be seen as a cellulose fibre web along with some bleaches. An additional component, i.e. ink will be seen on printed paper. Cellulose, the major component with which paper is made, is a polysaccharide. It is an organic compound with the formula $(C_6H_{10}O_5)_n$ which consists of a linear chain of several hundred to many thousands of β linked D-glucose units. Glucose is an excellent

substrate for undergoing fermentation. The generation of individual glucose molecules can be done by making the cellulose undergo hydrolysis. Hydrolysis can be achieved in a number of ways like Concentrated Acid Hydrolysis, Dilute Acid Hydrolysis, Enzymatic Hydrolysis. The so produced glucose from hydrolysis can be utilised by various fermentative organisms like *Saccharomyces cerevisiae* to form bioethanol which is a major biofuel. The bioconversion of glucose to ethyl alcohol (ethanol) is performed by the process of alcoholic fermentation. Ethanol fermentation, also called alcoholic fermentation, is a biological process which converts sugars such as glucose, fructose and sucrose into cellular energy, producing ethanol and carbon dioxide as by-products. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered as an anaerobic process. In this process the energy is derived from biomass.

Bioethanol has been added as an oxygenate to gasoline. Oxygenates are usually employed as gasoline additives to reduce carbon monoxide and soot that is created during the burning of the fuel. Various concentrations of bioethanol gasoline mixtures have been permitted in different countries. In India, the ratios of blend seen are E5 (5% Ethanol+ 95% Gasoline) to E20 (20% Ethanol+ 80% Gasoline).[3] The bioethanol formed from the fermentative procedures when applied to petroleum blending will lead to the efficient utilisation of the fermentation product of waste paper. Thus the production of bioethanol from waste paper can be seen as a method to decrease the presence of paper as a waste product and in turn convert it into something of great commercial value using the various biological processes.

CHAPTER 2
LITERATURE REVIEW

The conversion of waste paper to bioethanol can be performed only after following a series of steps. Each of these steps require the application of different kinds of chemicals at different concentrations and for various time intervals. The steps followed can be broadly given as drinking, acid hydrolysis and alcohol fermentation that finally give the product as ethanol or ethyl acetate (CH_3CH_2OH). Each of these steps have their role in forming the product needed for the next stage. The application of living organisms as such occurs in the last stage wherein the product formed is mostly intracellular and it needs to be isolated using purification methods. Pretreatment methods such as carbon dioxide explosion , steam explosion, chemical pretreatment, biological pretreatment with bacteria, ozonolysis, and liquid hot water etc. have been evaluated by various authors. Acids, e.g., phosphoric acid, have further more proved effective in the fractionation of waste or recycled newspaper,enhancing sugar release.[4] Studies on simultaneous saccharification and fermentation (SSF) of cardboard, waste newsprint, copier paper, and office paper have been reported, and several studies have been published on using separate hydrolysis and fermentation for bioethanol production from waste paper.

2.1 De-inking

The process of removal of printing ink from the waste paper is known as de-inking.This is an important step in the production of bioethanol from waste paper as the ink components can hamper the fermentation process. The de-inking process mainly involves the detachment of ink from the surface of recovered newsprint and magazines (the ink removal process) and the floating away of that ink using foam (the ink collection process). A de-inking agent has two primary functions: reducing the surface tension between the pulp fibre and ink during the ink removal process to facilitate the detachment of ink and foaming a froth that floats the ink off during the ink collection process, thereby separating the ink from the pulp slurry.[5]

De-inking experiments using enzymes achieved generally better results in comparison with those in which the enzymes were previously deactivated. Despite the positive effect of activated enzymes as compared to deactivated, the application of enzymes appeared disadvantageous compared with the conventional de-inking in terms of specks surface of the deinked paper sheets. Apart from the type of enzyme preparation itself, another critical factor affecting the effectiveness of the enzymatic treatment was the addition of nitric acid used to achieve acidic conditions for the optimum enzyme function. Further studies have to be performed on enzyme preparations functioning under alkaline conditions while the effect of enzyme amounts to the de-inking and the subsequent impacts on mechanical strength of produced papers also needs to be investigated.

2.2 Acid Hydrolysis

Acid hydrolysis is done to break down cellulose into glucose units, which can be easily used as a substrate by the microorganisms for fermentation. Dilute-acid hydrolysis is the most commonly applied method among the chemical hydrolysis methods for the production of ethanol from biomass. It is a method that can be used either as a pretreatment preceding enzymatic hydrolysis, or as the actual method of hydrolyzing lignocellulose to the sugars. Different types of reactors such as batch, plug flow, percolation,countercurrent, and shrinking-bed reactors for either pretreatment or hydrolysis of lignocellulosic materials by the dilute acid processes have been applied so far. The dilute-acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis. One of the main advantages of dilute acid hydrolysis is achieving high xylan to xylose conversion yields, which is necessary to achieve favourable overall process economics in ethanol production from lignocellulose., An organic of aqueous–organic solvent mixture with addition of an inorganic acid catalyst (H_2SO_4 or HCl) is used to break the internal lignin and hemicellulose bonds.[6] In the acid hydrolysis, one uses a strong acid such as sulfuric acid (H_2SO_4) or the hydrochloric acid (HCl), for the disruption of chemical bonds. This process is performed at relatively high temperatures, approximately 120°C and 1 atm pressure.[7] The yield from dilute acid hydrolysis of the lignocellulosic content at moderate temperature and atmospheric

pressure is small and it increases gradually with increase in the concentration of acid used for hydrolysis until a critical point is reached.

2.3 Ethanol fermentation

Alcoholic fermentation is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing ethanol and carbon dioxide as by-products. Fermentation can be performed as a batch, fed-batch, or continuous process. The most suitable choice will depend on the kinetic properties of the microorganism in addition to aspects of process economics. Immobilisation and recirculation of cells are ways of increasing the cell mass concentration in the fermenter which leads to a higher productivity. The higher the productivity, the smaller the fermenter required and therefore, the capital cost is usually lower. As lignocellulosic hydrolysates contain several monosaccharides, co-cultuting two microorganisms has been suggested since one microorganism cannot ferment all substrates optimally. It has been claimed that for a fermentation with *S. cerevisiae*, continuous operation is more resistant to contamination due to the high ethanol concentration and the low substrate concentration in the fermentation broth.[8] In the fermentation of pentose-tich substrates, the situation is somewhat different because a higher fermentation pH is required regardless of whether recombinant bacteria, pentose-fermenting yeasts or S. cerevisiae in combination with xylose isomerase are used.[9] Additionally, the final ethanol concentration will be lower than in a *S. cerevisiae* fermentation due to the limited ethanol tolerance of the other microorganisms. Fermentation was carried out using commercially available yeast, *Saccharomyces cerevisiae*. The pH of hydrolysed broth was adjusted to 4.6 and an inoculum of active yeast (in log phase) was added to the hydrolysed broth. The fermentation was carried out at 360℃ until maximum sugars were converted into bioethanol. The reducing sugar utilisation during fermentation was analysed by DNS method.[10] It may be noted that the fermentation process would be economical if both the pentose and hexose sugars in the hydrolysate are converted to bioethanol. Saccharomyces Cerevisiae can ferment only hexose sugars into ethanol. In one of the studies on fermentation using Saccharomyces cerevisiae for various

substrates namely, groundnut hull, and rice husk, the yields of 0.142g per gram of groundnut oil and 0.108g per gram of rice husk were reported.[11]

2.4 Bioethanol

Biofuels are liquid or gaseous fuels that are produced from biodegradable fractions of products, remains from agricultural production and forestry, as well as biodegradable fractions of industrial and municipal wastes.

Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid. It is a biodegradable, less toxic fuel and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol.Ethanol has a Gross Calorific Value (GCV) of 29.7 MJ/kg.[12] By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions.

Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from fuel or energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, waste straw, willow trees, sawdust, reed canary grass, cord grasses,and sorghum plants. There is also ongoing research and development into the use of municipal solid wastes to produce ethanol fuel.

Bioethanol has a number of benefits when compared to conventional fuels. Firstly, it is produced from a renewable resource (such as crops). There is therefore little or no net carbon dioxide added to the atmosphere, making bioethanol an environmentally beneficial energy source. The road transport network contributes a great deal to the release of greenhouse gas emissions into the atmosphere, and with the use of bioethanol, emission rates can be drastically reduced. It is also biodegradable, and less toxic than fossil fuels. In addition, blending bioethanol with petrol compensates for the diminishing oil supplies across the globe thereby ensuring higher fuel security and avoiding foreign reliance for fuel supply between countries. The rural community will also benefit from the increased demand to grow the necessary crops required for producing bioethanol. It also reduces the emission of carbon monoxide produced by old

vehicle engines and thus improves air quality. Another key benefit of bioethanol is the ease of integrating it with the existing road transport fuel system – bioethanol can be easily blended with conventional fuels (up to 15%) without any need for engine modifications.

The determination or detection of the produced bioethanol can be done by going for various detection techniques of alcohol. It can be done by studying the various characteristics of the produced distilled solution and comparing it with the standard values. Some characteristics that can be chosen are specific gravity, pH, chemical reactions of alcohol etc. Among the reactions, esterification is a major one. The Esters can be produced by reacting alcohol with carboxylic acids in the presence of an acid catalyst. The catalyst used is usually concentrated sulphuric acid. Dry hydrogen chloride gas is used in some cases, but these tend to involve aromatic esters (ones containing a benzene ring). The development of esters can be easily detected when there is development of a fruity smell. And this can be used as a detection reaction for the presence of alcohol.[13] The comparison of properties like specific gravity, pH etc can be done by the aid of various instruments like alcoholmeter, pH meter etc. An alcoholmeter is a hydrometer that indicates the alcoholic strength of liquids which are essentially a mixture of alcohol and water. Another important character is the flashpoint and fire point. These denote the ability of vapours of a solution to combust. Flashpoint denotes the minimum temperature at which contact with an ignition source like spark would lead to the vapour to catch fire for instance. Fire Point on the other hand is the minimum temperature needed to sustain the fire caught by vapour for a minimum of 5 seconds even after the source of ignition is removed. These parameters can be found out by the use of specialised equipment called Pensky Martens flash point Apparatus.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

- Test tubes
- Petri dishes
- Inoculation loop
- Conical flasks
- Measuring cylinder
- Glass rods
- Standard flask
- Pipettes
- Beakers
- Cuvettes
- Sieves
- Hydrometer

3.2 CHEMICAL REAGENTS

- Hydrogen Peroxide
- Sodium hydroxide
- Sulphuric Acid
- Iodine
- DNS Reagent
- Sodium Silicate
- SDS
- Potato Dextrose Agar
- Agar agar
- Sodium potassium tartrate

3.3 INSTRUMENTS

- Laminar airflow chamber
- Hot Air Oven
- Spectrophotometer
- Electric Blender
- Simple Distillation Unit
- Flash point Apparatus
- Shake flask incubator
- Centrifuge
- Refrigerator
- Colorimeter
- pH meter

3.4 METHODS

The procedures followed for the various phases of the project are mentioned below:

3.4.1 Collection of Waste Paper

- Waste papers were collected from the premises of Sahrdaya College, Thrissur. The materials were collected in polythene bags and kept in the laboratory where the experiments were performed.
- 800g of collected waste papers were shredded into small pieces, washed with warm water, pulverised in electric blender to form fluffy wool like substrate and dried in oven at 65℃ for 24 hours.
- The waste paper obtained was separated into two categories i.e de-inked paper and untreated paper to find out which has more release of glucose.

3.4.2 De-inking

De-inking is the process of removing printing ink from paper fibres.

- This was achieved by treating 800g of pulverised paper with a de-inking solution.
- Components of the de-inking solution[14] (1000 ml) are as follows:

Sl.no	Component	Composition (grams)
1.	NaOH	21
2.	Na_2SiO_3	42
3.	SDS	42
4	H_2O_2	21

Table 1 : Composition of the de-inking solution

The reaction mixture was kept for 2 days for de-inking. After 2 days, the reaction mixture was taken to wash with water. 5 washes were done in order to completely remove the ink and the detergent content of the mixture. Samples after each wash were

checked for their absorbance at 520 nm. The wavelength was fixed as 520 nm after doing standardisation.

Standardisation of wavelength: To find out the wavelength at which the sample gave maximum absorbance, the standardisation of wavelength was done using a colorimeter. The same sample with maximum colour was checked for the absorbance at the range between 440 nm to 670 nm.

3.4.3 Acid Hydrolysis

Acid hydrolysis is done in order to break down the cellulose into glucose units, which can be easily used as a substrate by the microorganisms for fermentation.

Time (min)	**Glucose Concentration (mg/mL)**	
	Dilute Acid Hydrolysis	**Concentrated Acid Hydrolysis**
30	0	0
60	0.25	0.68
90	0.78	1.43
120	0.99	3.36
150	0.83	2.03
180	0.64	1.54

Table 2: Acid concentration at changing time intervals

3.4.3.1 Time

The influence of time on the hydrolysis process was determined by keeping the sample for hydrolysis in varying range of 30-180 min.After hydrolysis,the samples were

filtered and centrifuged to find out the hydrolysate product and checked for it's glucose content.

3.4.3.2 Temperature

The influence of temperature was determined in the range of 25-110℃.After hydrolysis the samples were filtered and centrifuged to obtain the hydrolysate product.

Test Number	Reaction Volume (mL)	Time (Min)	Temperature (℃)	Glucose Conc. (mg/mL)
1	200	120	25	0
2	200	120	40	0.25
3	200	120	60	0.79
4	200	120	90	1.64
5	200	120	110	2.36

Table 3: Effect on temperature on glucose concentration

3.4.3.3 Process

A comparison was done for the hydrolysis capacity of both concentrated as well as dilute sulphuric acid and its glucose content was studied.

- **Conc acid Hydrolysis**

5 grams of paper was soaked in different amounts of sulphuric acid (5% weight). The acid used was 10% by volume.

- **Dilute Acid Hydrolysis**

5 grams of paper was soaked in different amounts of sulphuric acid (5% weight). The

acid used was 98% by volume.

After hydrolysis, the samples were filtered and centrifuged to obtain the hydrolysate product. After hydrolysis, the substrate was washed in running tap water and excess water was removed. Again the substrate was dried in hot air oven at 65℃ for 24 hours. The result of the hydrolysis reaction can be found out by checking for the amount of glucose released after each stage.

Sugar Analysis using DNS assay

1. Add 3 ml of DNS reagent to 3 ml of glucose sample in a lightly capped test tube. (To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used.)
2. Heat the mixture at 90º C for 5-15 minutes to develop the red-brown colour.
3. Add 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution to stabilise the colour.
4. After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm.

3.4.4 Ethanol fermentation

The process which converts sugars such as glucose into cellular energy, producing ethanol and carbon dioxide as by-products. Alcohol fermentation is to be done using two types of organisms. The normal yeast - *Saccharomyces cerevisiae* is normally used.

- Weigh 2.4g of Potato dextrose broth in 100 ml distilled water. The mixture was sterilised for 20 minutes.
- After sterilising, the mixture was cooled down and using laminar airflow chamber the microorganism culture of *Saccharomyces cerevisiae* small quantity was added into the conical flask containing the mixture.
- The mixture was kept in the shaker at 120 rpm for 48 hours.
- After that the sub cultured conical flask was stored in a refrigerator at 4℃ for further use. It was subcultured at proper intervals.
- The cultured yeast was then allowed to grow on the treated and hydrolysed waste paper as substrate for fermentation.

3.4.5 Purification

After fermentation there are various by-products that are found inside the flask. So that various purification techniques are adopted in order to obtain the desired product. Distillation is commonly used in the biorefinery processing of bioethanol production. This adds a significant energy load on the production process.

3.4.5.1 Distillation

For distillation purposes, the supernatant from the fermentation process was poured in the round bottom flasks by setting at 70 ℃. The sample was distilled twice to improve the quality of the obtained bioethanol.

Figure 2: Simple Distillation

3.4.6 Identification Tests for Ethanol

1. Density : The total distilled sample was taken in a measuring cylinder and its weight was found out. The volume was also noted to find out the density.
2. Specific Gravity : The distilled sample was taken in a measuring cylinder. The hydrometer which is used for measuring the specific gravity of the solution was put into the measuring cylinder and the readings were noted.

3. pH : The sample was also tested for its pH using a pH meter by dipping the glass electrode in the solution and taking the reading after a stable value was obtained.

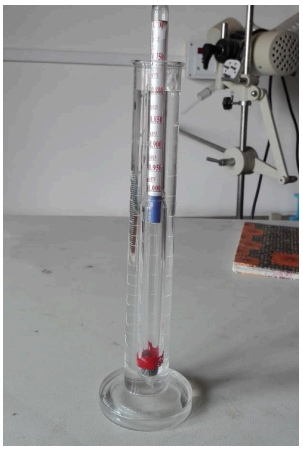


Figure 3 : Hydrometer

4. Combustion test : About 5 mL ethanol was transferred to a large test tube.This test tube was held with a test tube holder and heated until the liquid was boiling. The open end of the test tube was held to the flame and the ethanol vapours were ignited.
5. The triiodomethane (iodoform) test[15] : It can be used to identify the presence of a $CH_3CH(OH)$ group in alcohols. About 5 mL of ethanol was transferred to a clean and dry test tube.To this 25 drops of iodine solution was added and enough sodium hydroxide solution was added afterwards to remove the colour of the iodine. The solution was mixed gently for a few minutes along with gentle heating to observe any colour change.
6. The chemical confirmation test was also performed by carrying out an esterification reaction with the sample and a carboxylic acid. The carboxylic acid used was acetic acid and the catalyst acid used was concentrated sulphuric acid.Acetic acid and ethanol samples were warmed together in the presence of a few drops of concentrated sulphuric acid in order to observe the smell of the esters formed.
7. The flash point as well as the fire point was found out using a Pensky Martin Open cup apparatus. The distilled sample is filled in the cup upto the filling mark. All accessories like the thermometer are suitably fixed. The sample is then heated. The

test flame is then lit and adjusted. The flashpoint and the firepoint are noted by checking the formation and the sustenance of the flame.

8. Determine bioethanol yield

   Bioethanol produced was analysed by High Performance Liquid Chromatography (HPLC).20 µL of the sample was injected into HPLC system to determine the bioethanol yield.The HPLC analysis parameters were determined using the following conditions: column C18 RP (53 x 7mm); injector temperature was 30℃.20 µL of the sample was injected into the HPLC system. The mobile phase was phosphoric acid and the flow rate was 1.5mL/min; and detection was set at a wavelength of 210 nm.

CHAPTER 4

RESULTS

The following results were observed for each of the steps performed.

Figure 4: Shredded waste paper before oven drying.

Figure 5: Shredded waste paper after oven drying

4.1 Deinking Results

The deinking process was performed using the given medium obtained from literature. The paper sludge solution with maximum colour was selected for performing the

standardisation of wavelength to be selected for measuring each of the values. The different ranges of wavelengths in the colorimeter were tested to find out the wavelength that gave maximum absorbance value.

Wavelength (nm)	Absorbance (OD)
440	0.33
470	0.46
510	0.65
520	1.47
540	1.01
570	0.81
600	0.46
670	0.31

Table 4: Standardisation of Wavelength

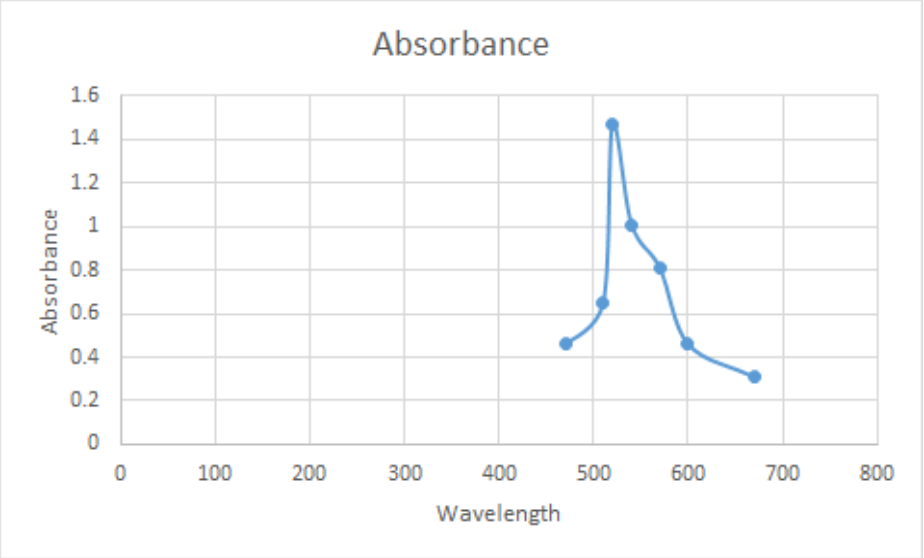


Figure 6: Standardisation of wavelength to find absorption maxima

The absorption maxima was found to be at 520 nm.

The following observations were made at 520 nm using a spectrophotometer

WASH	ABSORBANCE AT 520 nm
I	1.37
II	0.69
III	0.37
IV	0.22

Table 5: Spectrophotometer readings of paper after various washes

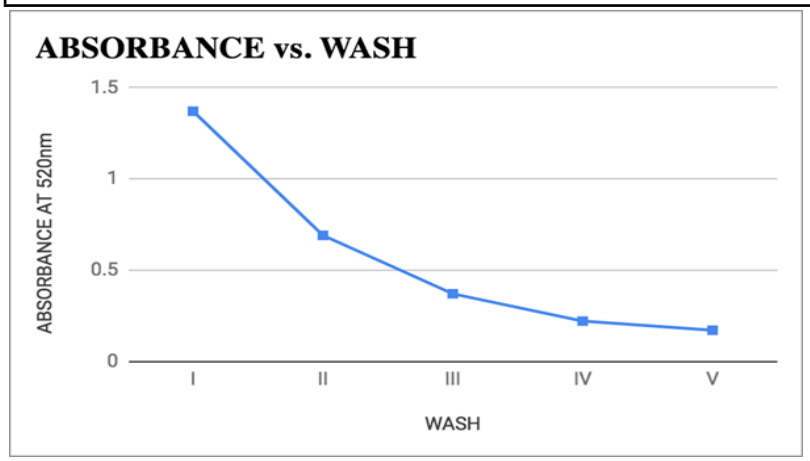


Figure 7: Graph of Absorbance vs Wash number

Figure 8: De-inked paper

4.2 Acid Hydrolysis

Various parameters were checked to find an optimum condition for glucose release,

which are as follows

4.2.1 Time

The influence of time on the process was determined in the rage of 30-180 min. After hydrolysis, the samples were filtered and centrifuged to obtain the hydrolysate product and checked for its glucose content.

Test Number	Reaction Volume (mL)	Time (min)	Glucose Conc (mg/mL)
1	100	30	0.23
2	100	60	1.67
3	100	90	3.26
4	100	120	4.69
5	100	150	2.17
6	100	180	1.86

Table 6: Effect of Hydrolysis time on Glucose Concentration for 100mL

After performing hydrolysis at various acid concentrations, the samples were tested by the Millers DNS Method to find out which one has the maximum glucose release.

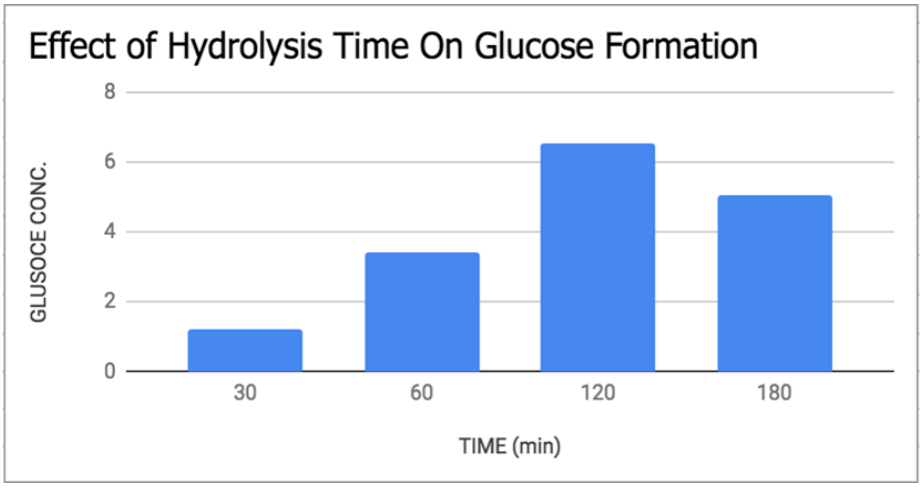


Figure 9: Effect of Hydrolysis time on glucose formation

4.2.2 Temperature

The influence of temperature on the process was determined in the rage of 25 - 110 ℃. After hydrolysis, the samples were filtered and centrifuged to obtain the hydrolysate product and checked for its glucose content.

Test Number	Reaction Volume (mL)	Time (min)	Temperature (℃)	Glucose Conc (mg/mL)
1	200	120	25	0
2	200	120	40	0.25
3	200	120	60	0.79
4	200	120	90	1.64
5	200	120	110	2.36

Table 7: Glucose released at different temperatures

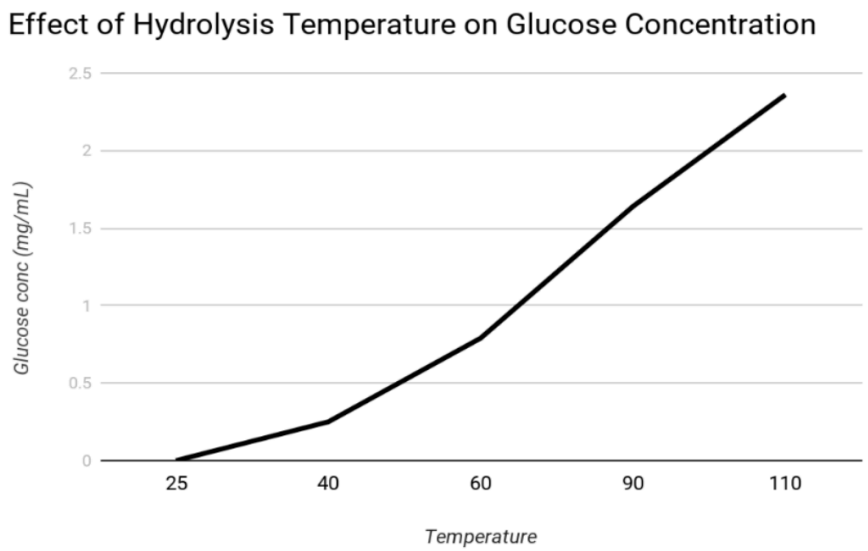


Figure 10: Effect of hydrolysis temperature on glucose concentration

4.2.3 Process

The amount of glucose released from the untreated paper in two different hydrolysis process is as shown :

Time (min)	**Glucose released in Dilute Acid Hydrolysis (mg/mL)**	**Glucose released in Concentrated Acid Hydrolysis (mg/mL)**
30	0	0
60	0.25	0.68
90	0.78	1.43
120	0.99	3.36
150	0.83	2.03
180	0.64	1.54

Table 8: Glucose released at different concentrations of acids

The amount of glucose released from the de-inked paper in two different hydrolysis process is as shown :

Time (min)	**Dilute Acid Hydrolysis (mg/mL)**	**Concentrated Acid Hydrolysis (mg/mL)**
30	0	0.23
60	0.36	1.67
90	1.49	3.26
120	2.07	4.69
150	1.87	2.17
180	1.05	1.86

Table 9: Glucose released at different concentrations of acids

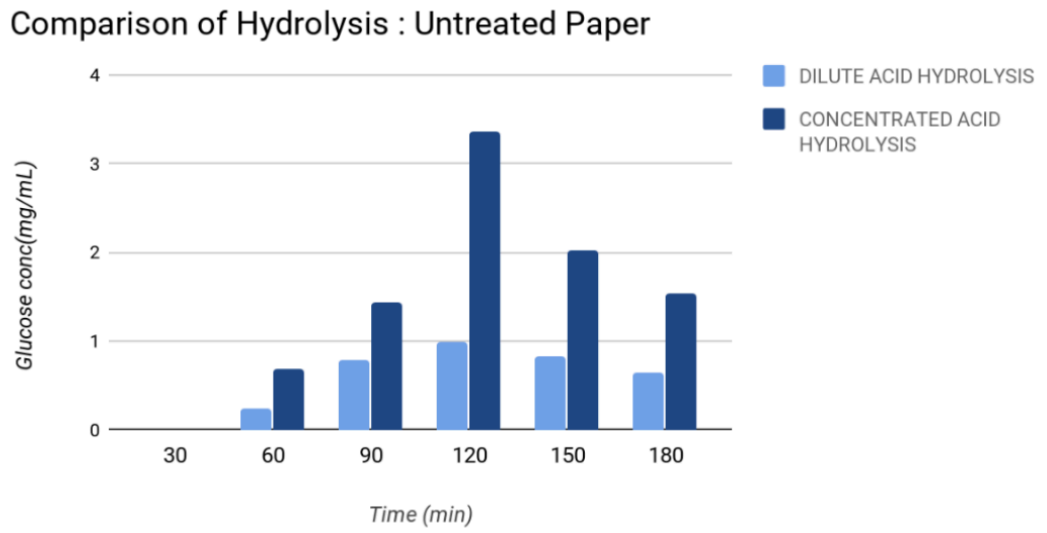


Figure 11: Comparison of hydrolysis in untreated paper

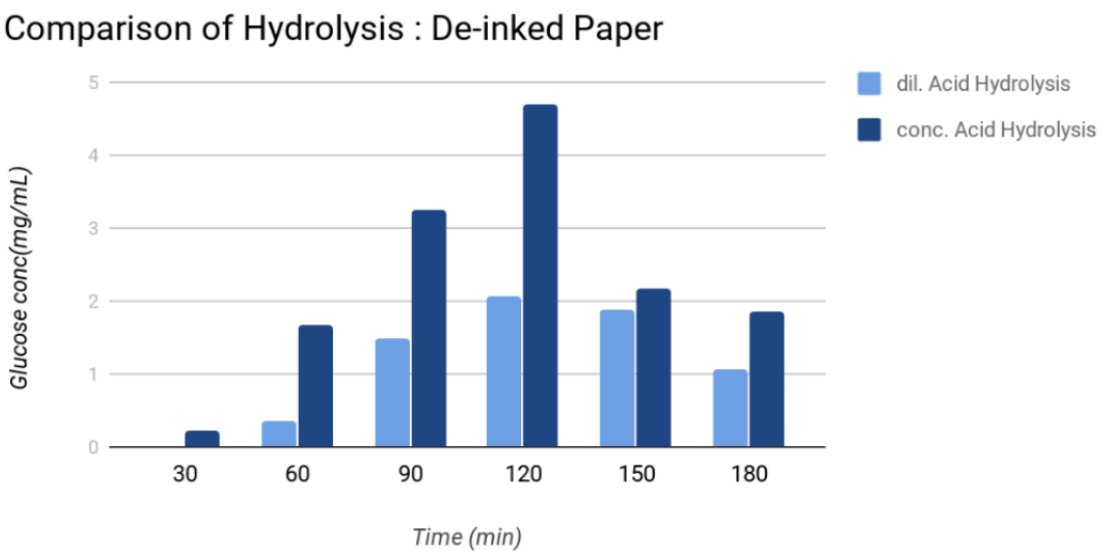


Figure 12: Comparison of hydrolysis in de-inked paper

4.3 Fermentation

The fermentation broth was taken to measure the turbidity and cell density of the culture after an incubation period of 4 days.Growth of yeast cultures was monitored by using a spectrophotometer to measure the cell suspension optical density (or

absorbance) at 600nm. It was found to be 2.34 OD which indicated an ambient growth condition of the yeast.

4.4 Bioethanol Characterisation

After distillation process, the resulting sample showed the following characteristics :

4.4.1 Density

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol[16]
0.814 g/mL	0.802 g/mL	0.7892 g/mL

4.4.2 Specific Gravity

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol
0.81	0.8	0.79

4.4.3 pH

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol
7.31	7.25	7.33

4.4.4 Combustion Test

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol[17]
Flame that is mostly blue with yellow accents,which was quite hard to see.	Flame that is mostly blue with yellow accents,which was quite hard to see.	Ethanol burns with a pale blue flame with no smoke.

4.4.5 The triiodomethane (iodoform) test

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol

Pale yellow precipitate	Pale yellow precipitate	Pale yellow precipitate formation, along with a faint disinfectant smell

4.4.6 Esterification Reaction

Bioethanol obtained from Untreated Paper + Carboxylic acid	Bioethanol obtained from De-inked Paper + Carboxylic acid	Ethanol + Carboxylic acid
Fruity smell	Fruity smell	Development of fruity smell due to ester formation.

4.4.7 Flashpoint

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper	Ethanol (40-50 %)
33 °C	27 °C	24-26 °C

4.4.8 Calibration curve

In this study a calibration curve was drawn to determine total ethanol concentration in water from ethanol samples. A linear graph of standardisation of ethanol was drawn using 95% ethanol as a standard. The standard was prepared at different concentrations.The amount of ethanol added to each vial is shown in the Table 10.

Concentration (%)	Volume of Ethanol Standard (mL)	Volume of Mobile Phase (mL)	Peak Area
25	0.5	1.5	5229
50	1.0	1.0	15330
75	1.5	0.5	28520
100	2.0	0	31494

Table10 : Standard Calculation

The calibration equation of the ethanol standard was determined to be y = 367.94x-2853 (R^2 = 0.9515) where y is the peak area of ethanol and x is the concentration of ethanol. The Ethanol standard curve is as shown.

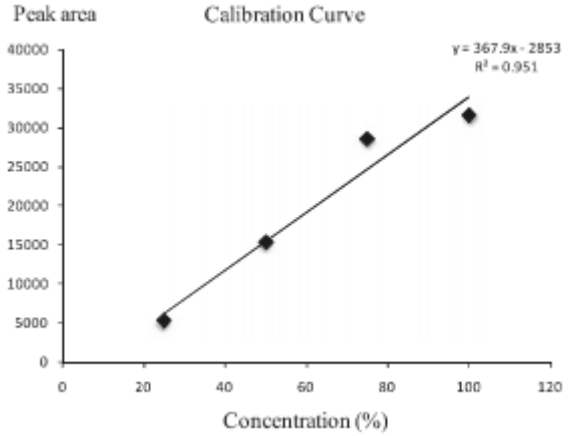


Figure 13 : Ethanol Standard Curve

4.4.9 Yield

Bioethanol obtained from Untreated Paper	Bioethanol obtained from De-inked Paper
41.3%	58.26%

CHAPTER 5

CONCLUSIONS

The present work deals with the studies on production of bioethanol from waste paper which is one of the largest constituents of municipal solid waste. Experiments were carried for pretreatment of the substrate, hydrolysis and fermentation of the hydrolysate. The de-inking procedure was performed using the medium composition obtained through literature. It had SDS and hydrogen peroxide as its main contents which played a major role in removing the ink and thereby decreasing the absorbance of the samples after each wash. The next step was to hydrolyse the cellulose to give out glucose monomers that could then be utilised by bacteria for alcohol fermentation. Various concentrations of hydrochloric acid were allowed to contact the deinked paper for varying contact times. The optimised condition for the pretreatment using acid hydrolysis was found to be 5% concentration of H_2SO_4 at 110ºC and 120 minutes of contact time. The maximum amount of cellulose recovered under these optimum parameters was almost about 45%. Next, the yeast *Saccharomyces cerevisiae* was allowed to ferment the reducing sugar into bioethanol. Waste papers are rich in cellulose content which can be effectively used for the production of bioethanol up to a certain level of purity. Production of biofuels from waste paper shows that S*accharomyces cerevisiae* are effective for producing best quality ethanol.

The conversion process employed for waste papers (which are basically made up of cellulose) was achieved by hydrolysis, where the complex cellulose structure was broken down into simple fermentable sugar. The amount of fermentable sugar that was obtained from the hydrolysis of the wastepaper was obtained to be 40%, with only slight variation from the standard. The waste paper substrate (basically made up of starch), was converted into simple fermentable sugar by microbial process i.e.,S*accharomyces cerevisiae.* The hydrolysis of waste paper substrate yielded 45%. In conclusion, more treatments like enzymatic conversion need to be examined to check for the production of a higher bioethanol yield from waste paper.

CHAPTER 6
ECONOMIC CONSIDERATIONS

The growing demand for energy, increase in oil prices and the environmental problems as a result of the use of fossil fuels has become a great challenge facing the world. Therefore, we are compelled to move towards sustainable alternative sources for energy, keeping in mind the overall economic scenario.

Waste paper has a potential to be used as an excellent alternative feedstock for fermentable sugars production due to its high cellulose content (50-60%), relative abundance and low cost. Utilisation of waste paper as feedstock for the production of other value added products is considered as a much valuable and an alternative route for waste management and reduced raw material cost.

The consideration of economic aspects in the production of bioethanol from waste paper takes place when going for setting up of a plant that can perform the complete steps mentioned in this work. It includes various variable costs like : cost of collection as well as transport of waste paper, cost of raw material contents used in deinking process, cost of acid and alkali utilised in the hydrolysis step as well as medium supplements used in fermentation stage. Along with these other fixed costs like labour, maintenance, insurance and so on also needs considerations.

In order to determine the degree of competitiveness for the bioethanol industry, it is important to compare the derived bioethanol cost with petroleum prices (premium unleaded petrol). Bioethanol has less energy content (21.2 MJ per L) than petrol (31.2 MJ per L) meaning that 1 litre of bioethanol replaces around 0.68 litre of petrol. The minimum selling price of bioethanol at pump consists of the MESP (Minimum Ethanol Selling Price), transportation and distribution costs as well as tax. With the exception of magazine paper, the overall pump prices of bioethanol produced from waste papers are close to that of petrol. It has been shown that Bioethanol from cardboard, office paper with and without dilute acid pretreatment, and newspaper using Cellic Ctec 1, a non-commercial enzyme complex (cellulolytic enzyme cocktail which is also used for enzymatic hydrolysis) are economically competitive with petrol, demonstrating a great economic feasibility of bioethanol produced from waste papers.

However, Minimum Ethanol Selling Prices/cost (petrol equivalent) of all waste papers-derived bioethanol is higher than the petrol production cost. That means the economically competitiveness of bioethanol to petrol is because of the lower fuel tax when compared to that of petrol.[18]

Even though the process of deinking has increased bioethanol yield, it has also substantially increased the overall cost for the small scale operation. The scale up of the process might help in decreasing the total cost for running the operation. The processes performed in normal paper without deinking has resulted in an economically sound result with sufficient yield.

CHAPTER 7
FUTURE PROSPECTS

- Utilise new species other than the conventional yeast for fermentation

  Many literatures suggest that not only the conventional yeast but also many other species like *Pseudomonas aeruginosa* - LV strain, genetically modified *Escherichia coli* etc. Many lactic acid bacteria, enterobacteria and clostridia form considerable amounts of ethanol as a reduced end product to maintain their redox balance. Also the organisms in the *Zymomonas* species are able to form alcohol as their main product by utilising the Entner–Doudoroff pathway. The application of these organisms for alcohol fermentation can be studied.
- Check the efficiency of other forms of hydrolysis like enzymatic hydrolysis

  The present study is done using the process of acid hydrolysis to release the glucose monomers from cellulose. The same procedure can be performed using the application of enzymes like cellulases that have been found to have more efficiency. The amount of glucose released and the concentration of enzymes used can be directly related after studying the activity of the enzyme and the glucose quantification using various assays like DNS assay.
- Scale up the entire process for treating kilograms of waste paper.

  The present study was done using weights of paper less than 1 kilogram. The process efficiency and the amount of alcohol production needs to be studied and quantified when the amount of paper used as substrate is in the range of 2 to 5 kilograms.
- The design of a bioreactor assembly that will perform the steps mentioned in the project using the optimised parameters and yield bioethanol in an economic scale.

REFERENCES

1. Paper, Printing & The Environment. Earth Greetings Co., Glandore, South Australia.[online] Available online at : http://www.earthgreetings.com.au/htm%20pages/environment/paper&environment.html[Accessed Date 29.09.2018].
2. R.B. Nair, P.R. Lennartsson, M.J. Taherzadeh , Bioethanol Production From Agricultural and Municipal Wastes (2016)
3. 2006 IPCC Guidelines for National Greenhouse Gas Inventories,Volume 2 : Energy
4. Edem Cudjoe Bensah,Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations,International Journal of Chemical Engineering,Volume 2013, Article ID 719607
5. Hiromichi,Deinking agent for waste paper,Ecomaterials, Proceedings of the Symposium K: Environment Conscious Materials of the 3rd IUMRS International Conference on Advanced Materials, Sunshine City, Ikebukuro, Tokyo, Japan, August 31–September 4, 1993
6. Bishnu. J, MeghRaj. B, Dinint. S, Jarin. J, Rajani. M and Lakshmaiah. S., 2011. Lignocellulosic ethanol production: Current practices and recent developments. Biotechnology and Molecular Biology Review, Vol 6(8), pp.172-182.
7. A. Shahbazi, B. Zhang,Dilute and concentrated acid hydrolysis of lignocellulosic biomass, in Bioalcohol Production, 2010, Pages 143-158
8. Bibi Zainab and Aslam Fakhra.Production of Ethanol by fermentation process by using Yeast Saccharomyces cerevisiae. International Research Journal of Environment Sciences, Vol.3(7) , 2014, pp.24-32.
9. Fuad Salem Eshaq et. al. Spirogyra biomass a renewable source for bioethanol Production | International Journal of Engineering Science and Technology Vol. 2(12), 2010, 7045-7054
10. G. L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., 1959, 31, 426–428
11. Shruti A. Byadgi,Production of Bioethanol from Waste Newspaper,Procedia Environmental Sciences 35 (2016) 555 – 562

12. Engineering ToolBox, (2003). *Fuels - Higher and Lower Calorific Values*. [online] Available at : https://www.engineeringtoolbox.com/fuels-higher-calorific-values-d_169.html [Accessed Date 29.10.2018].
13. *Esterification, Jim Clark,2003 [online] Available at :* https://www.chemguide.co.uk/organicprops/alcohols/esterification.html [Accessed Date 20.02.2019].
14. Ossi Laitinen, Components Removal in Flotation Deinking :Article · January 2008 *in* Progress in Paper Recycling 17(4):15-22 · January 2008
15. The Triiodomethane (Iodoform) Reaction(online) Available at : http://chemwiki.ucdavis.edu/Organic_Chemistry/Alcohols/Reactivity_of_Alcohols/The_Triiodomethane_(Iodoform)_Reaction [Accessed Date 15.02.2019].
16. Engineering ToolBox, (2018). *Ethanol - Density and Specific Weight*. [online] Available at: https://www.engineeringtoolbox.com/ethanol-ethyl-alcohol-density-specific-weight-temperature-pressure-d_2028.html [Accessed Date 14.03.2019].
17. American Herbal Products Association. August 2017. Ethanol Identity Test Methods. AHPA: Silver Spring, MD.21 CFR 111.75(a)(2)
18. Lei Wang, Mahdi Sharifzadeh, Richard Templer and Richard J. Murphy, Technology performance and economic feasibility of bioethanol production from various waste papers,Energy & Environmental Science, 2012; Energy Environ. Sci., 2012,5, 5717

APPENDIX

Kerala Veterinary and Animal Sciences University

CENTRAL INSTRUMENT LABORATORY
Santosh Nagar, Mannuthy, Thrissur, Kerala 680651, India
+91 487 237 0451

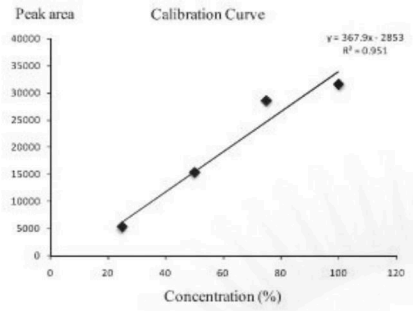
TEST REPORT

Test Report Number : CIL/19/R1043
Name of Manufacture : Mary Tania Christopher
Customer Address : Thejus Hostel, Sahrdaya College Of Engineering,Kodakara,Thrissur
Manufacturing License Number : Not Mentioned
Test Requisition Number & Date : LWA723 15/03/19
Date of Sample Received : 15/04/19
Sample Analysis Date : 17/04/19
Sample Name : Bioethanol
Sample ID : R1043
Batch No : Not Mentioned
Batch Size : Not Mentioned
Date of Manufacturing : Not Mentioned
Date of Expiry : Not Mentioned
Quantity of Sample Received : 100 mL
Sample Drawn By : The Party
Sample Condition : Received in Good Condition

Chromatographic conditions
Column : C18 RP (53 x 7mm);
Mobile Phase : Phosphoric acid
Flow rate : 1.5 mL per min
Column : C18 Symmetry (4.6 x 150mm, 5m, Make:Xterra)
Detector wavelength : 210 nm
Injection volume : 20µL
Injector temperature was 30°C
Run time : 10 min

Sl.no	Parameters	Result	Specification	Test Method
1	Ethanol Concentration	32.59 %	-	CIL/LWA/HPLC-02
2	Ethanol Concentration	45.97 %	-	CIL/LWA/HPLC-02

Standard Curve



Authorised Signature

NOTE : The test results relate only to the sample tested.The report shall not be reproduced except in full, without the written approval of the laboratory.