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Quantitative Estimation of Bioethanol Produced From Lignocellulosic & Household Wastes

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Abstract: **Bio ethanol is a liquid bio fuel which can be produced from several different biomass feed stocks and conversion technologies. Ligno-cellulosic waste materials such as fruit waste, agricultural waste, biodiesel waste glycerol and bagasse can be used to produce bio ethanol. Bio ethanol is identical to ethanol from other sources but has the advantage that the raw materials required to produce bio ethanol are highly abundant, diverse, and renewable. Bio ethanol produced from ligno-cellulosic materials, shows energetic, economic and environmental advantages in comparison to bio ethanol from starch or molasses. Ligno-cellulosic waste materials can be processed to rupture cell walls and liberate the sugars needed to produce ethanol. Cellulose molecules are similar to starch and contain long chains of glucose molecules. However, their structural configuration and the encapsulation by lignin make cellulosic materials comparatively more difficult to hydrolyze than starchy materials. Cellulose requires hydrolysis to liberate the cellulose and hemicelluloses from the lignin encapsulation and access its crystalline structure. This can be performed by chemical and enzymatic hydrolysis of ligno-cellulosic material. The extracted sugar molecules from the ligno-cellulosic material need to be converted into ethanol by submerged batch fermentation. Quantitative estimation of bio ethanol produced was carried out by biochemical method and by using a gas chromatograph.**

Keywords: **Molasses, Lignin, Cellulose, Ethanol, Glycerol, Gas chromatography.**

I. INTRODUCTION

An advantage of ethanol (CH_3CH_2OH *or* C_2H_6O) is that it has a higher octane rating than ethanol-free gasoline available at roadside gas stations, which allows an increase of an engine's compression ratio for increased thermal efficiency. In high-altitude locations, some Ethanol also called *ethyl alcohol , pure alcohol, grain alcohol, or drinking alcohol*, is a *volatile, flammable, colorless liquid*. Bio ethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Current plant design does not provide for converting the lignin portion of plant raw materials to fuel components by fermentation.

Ethanol fuel is the most common bio fuel worldwide, particularly in Brazil. Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch from which alcoholic beverages such as whiskey, can be made . The ethanol production methods used are enzyme digestion , fermentation of the sugars, distillation and drying. The distillation process requires significant energy input for heat (often unsustainable natural gas fossil fuel, but cellulosic biomass such as bagasse, the waste left after sugar cane is pressed to extract its juice, can also be used more sustainably).

Ethanol is also used to fuel bio ethanol fireplaces. As they do not require a chimney and are *"flue less"*, bioethanol fires are extremely useful for newly built homes and apartments without a flue. The downsides to these fireplaces is that their heat output is slightly less than electric heat or gas fires, and precautions must be taken to avoid carbon monoxide poisoning.

Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. The word *alcohol* derives from Arabic *al-kuhul*, which denotes a fine powder of antimony used as an eye makeup. *Alcohol* originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage.

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide.

In the production of beverages, such as whiskey and brandy, the impurities supply the flavor. Starches from potatoes, corn, wheat, and other plants can also be used in the production of ethanol by fermentation. However, the starches must first be broken down into simple sugars. An enzyme released by germinating barley, diastase, converts starches into sugars. Thus , the germination of barley, called malting, is the first step in brewing beer from starchy plants, such as corn and wheat.

The ethanol produced by fermentation ranges in concentration from a few percent up to about 14 percent. About 14 percent, ethanol destroys the zymase enzyme and fermentation stops. Ethanol is normally concentrated by distillation of aqueous solutions, but the composition of the vapor from aqueous ethanol is 96 percent ethanol and 4 percent water. Therefore, pure ethanol cannot be obtained by distillation. Commercial ethanol contains 95 percent by volume of ethanol and 5 percent of water. Dehydrating agents can be used to remove the remaining water and produce absolute ethanol.

Balat et al(2008) highlighted the production of ethanol (bio ethanol) from biomass to reduce both consumption of crude oil and environmental pollution. The study revealed that bio ethanol is appropriate for the mixed fuel in the gasoline engine because of its high octane number, and its low ketene number and high heat of vaporization impede self-ignition in the diesel engine. The study also implied the disadvantages of bio ethanol which include its lower energy density than gasoline, its corrosiveness, low flame luminosity, lower vapor pressure (making cold starts difficult), miscibility with water, and toxicity to ecosystems.

II. MATERIALS AND METHODS

2.1 Prepration of PDA culture media

Material Required:- Potato, dextrose, sugar, agar, peptone, beef extract, beaker, measuring cylinder, funnel, filter paper, muslin cloth, mortar and pestle, heater, autoclave, laminar air flow, chamber, test tubes, petriplates, al foils, cotton rolls etc, knife, etc.

2.1.1 Procedure-PDA media Preparation

Take potatoes peel off and weigh 50g.Cut it into small pieces with a knife and boil it in a beaker containing water for 20-30 min, fill the pieces give a whitish appearance or easily penetrated by a glass rod. Decant the excess supernatant Mash the potatoes and filter the sol through a muslin cloth. Take the potato extract in a beaker and add 5g of dextrose and 5g of agar. Make up the vol. to 250ml by adding distilled water to it. Boil the flask a little to dissolve the ingredients .Cotton plug it and cover it with a foil. Autoclave the flask along with petri plates at 121˚C for 20 min at 15 Psi pressure.

2.1.2 Preparation of PDA plates

Take the entire material in a laminar air flow cabinet which is previously sterilized for 30 min in u.v. light.When temp cools down a little pour about 15-20 ml of medium aseptically into the bottom half of Petriplates. Place the plates on a horizontal base and after 20-30 min, it solidifies to form PDA plates.

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Inoculate the different source on PDA plates:-Taking the PDA plates and inoculate the soil sample as a pour plate method. With a different PDA plate curd pour on the it. Incubate these plates under the BOD incubator for 48 hours for its growth. After that recognized the colonies with the help of gram staining.

2.2 Collection of the raw material

Collect the lignocelluloses waste material from different sources like house-hold wastes, agricultural wastes, fruit waste, and industrial wastes. E.g.- baggase, pea *waste*,, orange peels, molasses.

2.3 Hydrolysis of ligno cellulosic material

The ligno-cellulosic waste was collected and dried overnight. The dried waste was powdered and was hydrolysed using chemical and enzymatic method. Chemical hydrolysis was carried out using dilute H_2SO_4.Dilute sulphuric acid solutions of 0.3M and 0.5M were prepared and 5 grams of powdered ligno-cellulosic waste was added in each acid solution. The acid solutions were kept for 24 hours for hydrolysis. The acid solutions were then kept at high temperature of 121^oC and high pressure of 15 psi for 1 hour.

2.3.1 Enzymatic Hydrolysis

Another basic method of hydrolysis is enzymatic hydrolysis. Enzymatic are naturally occurring plant proteins that cause chemical reactions to occur. The most commonly used micro-organism to produce ethanol is *Saccharomyces cerevisiae* is a species of yeast. It is perhaps the most useful yeast, having been important to wine making, baking and brewing since ancient times.

Enzymatic hydrolysis was done using enzyme amylase. The enzyme solution was prepared at a concentration of 1 mg/ml. 5 grams of lingo-cellulosic waste was added in 20 ml enzyme solution and volume was made up to 100 ml using distilled water. The solution containing lingo-cellulosic waste and enzyme was kept overnight for hydrolysis.

2.4 Calorimetric determination of glucose by dinitro salysilic acid (DNS) method

3,5-Dinitrosalicylic acid is an aromatic compound which reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, that absorbs light strongly at 540 nm. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions.

2.4.1 Standard Maltose solution

Weigh 20 mg of maltose and transfer it into a volumetric flask dissolve and make up the volume 10 ml with distill water

2.4.2 DNS Reagent

1g of DNS dissolved, 200 mg of phenol and 50 mg of sodium sulphide in 100 ml of 1% (w/v) NaOH. Store this reagent in refrigerator.

2.4.3 Pottasium-sodium tartrate tetrahydrate (40% w/v)

Standard Maltose solution:-

Component	Volume
Maltose	20mg
Distill water	10ml

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DNS Reagent:-

Components	Volume
DNS	0.5 g
Phenol crystal	100 mg
Sodium sulphite	25 mg
NaOH	0.5 g
Distill water	50 ml

Procedure:-

1 ml of sugar solution and 2 ml of DNS was added and vortexed. The tubes were heated in water bath for 5 min and then 1 ml of 40% K-Na tartarate was added. Tubes were cooled at room temp and 6 ml distill water added. Absorbance was measured at 540 nm using spectrophotometer.

2.5 Fermentation

Anaerobic batch fermentation of 100 ml broth media consisting of and hydrolysed lingo-cellulosic waste was carried out in order to convert the released sugars into ethanol, the conversion process being accomplished by the enzymes released by *Saccharomyces cerevisiae*. The pH of the solution was brought to 5 -6 by adding required amount of NaOH to accommodate yeast growth. The fermentation media was prepared by addition of yeast extract, urea and dextrose in the following amount per 100 ml of fermentation media.

Yeast extract = 0.2 grams

Urea = 1 gram

Dextrose = 15 grams

The fermentation media containing hydrolysed material was completely sterilized by autoclaving (120°C, 15 psi pressure and 60 mins) before inoculating the yeast.

2.6 Quantitative Estimation Of Bio-Ethanol

Samples were regularly collected from each of the four fermentation broths for three days for quantitative estimation of ethanol. Quantitative estimation was done by both biochemical method and by using a gas chromatograph.

2.6.1 Biochemical Method

Quantitative estimation of ethanol using biochemical method was done by potassium dichromate method. Most of the chemical oxidation methods are based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid with the formation of acetic acid. This reaction is popular because potassium dichromate is easily available in high purity and the solution is indefinitely stable in air. The theoretical reaction stoichiometry is shown below:

2Cr2O7-- + 3C2H5OH + 16H+ -----> 4Cr+++ + 3CH3COOH + 11H2O

2.6.1.1 Acid dichromate solution: 125 ml of water was added to a 500 ml conical flask. Then 325 ml of concentrated sulphuric acid was carefully added. The flask was cooled under cold water tap and 34 grams of potassium dichromate was added. Dilute to 500 ml with distilled water.

2.6.1.2 2 M Sodium Hydroxide Solution: Add 40 grams of NaOH in 1000 ml of distilled water.

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Procedure:-

10-50 microlitres of absolute alcohol was taken in different test tubes and the volume was made up to 500 microliters by adding distilled water in each test tube.30 microlitres of test sample was taken and the volume was made up to 500 microliters by adding distilled water in test tube.1 ml of potassium dichromate reagent was added in each test tube. Then 2 ml of sodium hydroxide solution was added in each test tube. The test tubes were incubated at 50^0C for 30 minutes. The absorbance was measured at 600 nm by using a spectrophotometer.

2.7 Gas Chromatography

Gas chromatography- specifically gas-liquid chromatography-involves a sampling being vaporized and injected on to the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. It exploits differences in the partition coefficients between a stationary liquid phase and a mobile gas phase of the volatilized analyzed they are carried through the column by the mobile gas phase .the temperature of the column is raised to 50-300 C volatilization.

III. RESULTS

The various lingo cellulosic waste materials have complex carbohydrate in them which cannot be directly converted to ethanol by yeast cells. Thus different Hydrolysis methods with acid, autoclave and enzyme were used to break down the complex sugars into simple monosaccharide units. The total sugar content of the hydrolyzed sugar was measured by DNS method.

Table: 1: DNS Test Observation & Graph (Standard)

S.NO	STANDARD MALTOSE	CONCENTRATION OF MALTOSE (mg/ml)	D.W (ml)	DNS (ml)	POTASSIUM SODIUM TATRATE (ml)	D.W (ml)	O.D
1	BLANK	0	1	2	1	6	0
2	0.1	0.2	0.9	2	1	6	0.16
3	0.2	0.4	0.8	2	1	6	0.32
4	0.3	0.6	0.7	2	1	6	0.48
5	0.4	0.8	0.6	2	1	6	0.62
6	0.5	1.0	0.5	2	1	6	0.78
7	0.6	1.2	0.4	2	1	6	0.94
8	0.7	1.4	0.3	2	1	6	1.09
9	0.8	1.6	0.2	2	1	6	1.24
10	0.9	1.8	0.1	2	1	6	1.39
11	1.0	2.0	0	2	1	6	1.55

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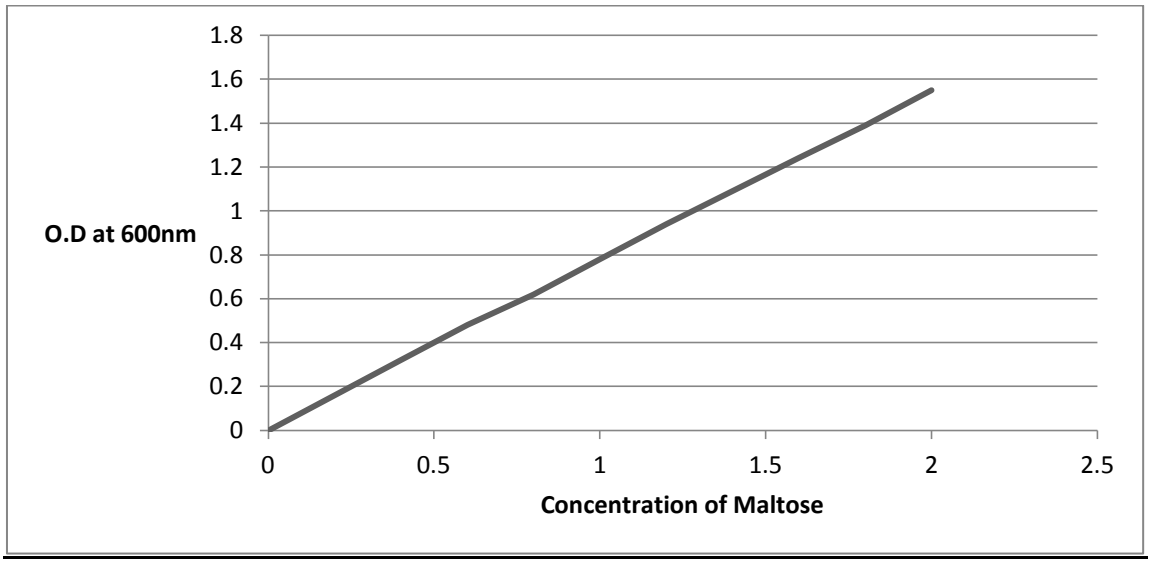

Fig: 1: DNS Test Observation & Graph of standard

Table: 2: Observation of Sugar concentration of 0.3 M hydrolyzed.

S.NO	SAMPLE	O.D Before autoclave	O.D After autoclave	Concentration of sugar (mg/ml) after Autoclave
1	BLANK	0.00	0.00	
2	Bagasse	1.22	1.54	2.05
3	Pea waste	0.26	0.38	0.49
4	Orange Pulp	1.00	1.02	1.16
5	Molasses	1.49	1.69	3.16

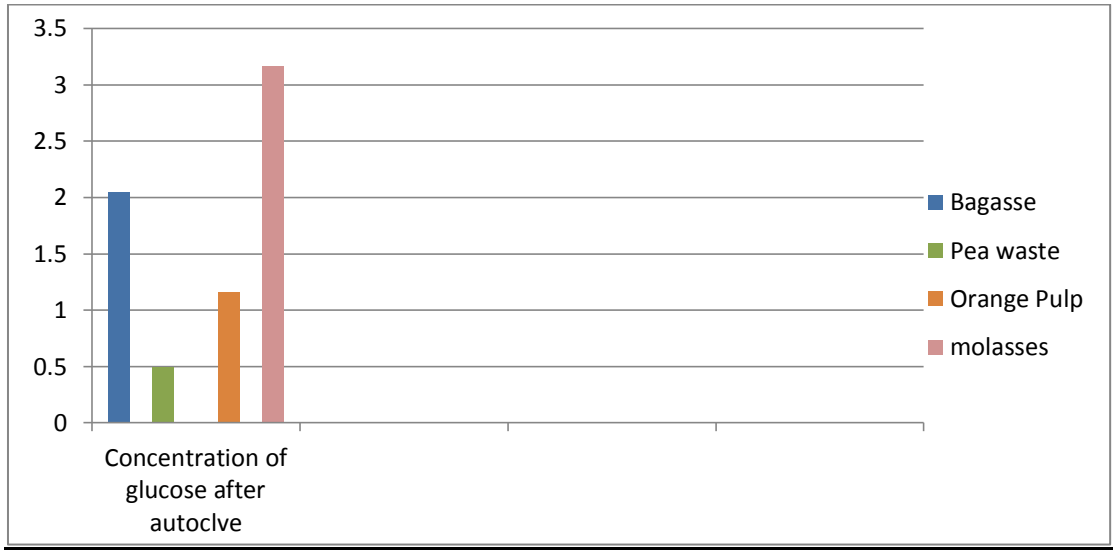

Fig: 2: Concentration of sugar in 0.3 M hydrolyzed compound

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Table: 3: For 0.5 M hydrolyzed compound Sugar concentration.

S.NO	SAMPLE	O.D Before autoclave	O.D After autoclave	Concentration of sugar (mg/ml) after Autoclave
1	BLANK	0.00	0.00	
2	Bagasse	1.42	1.70	2.45
3	Molasses	1.89	1.99	3.56

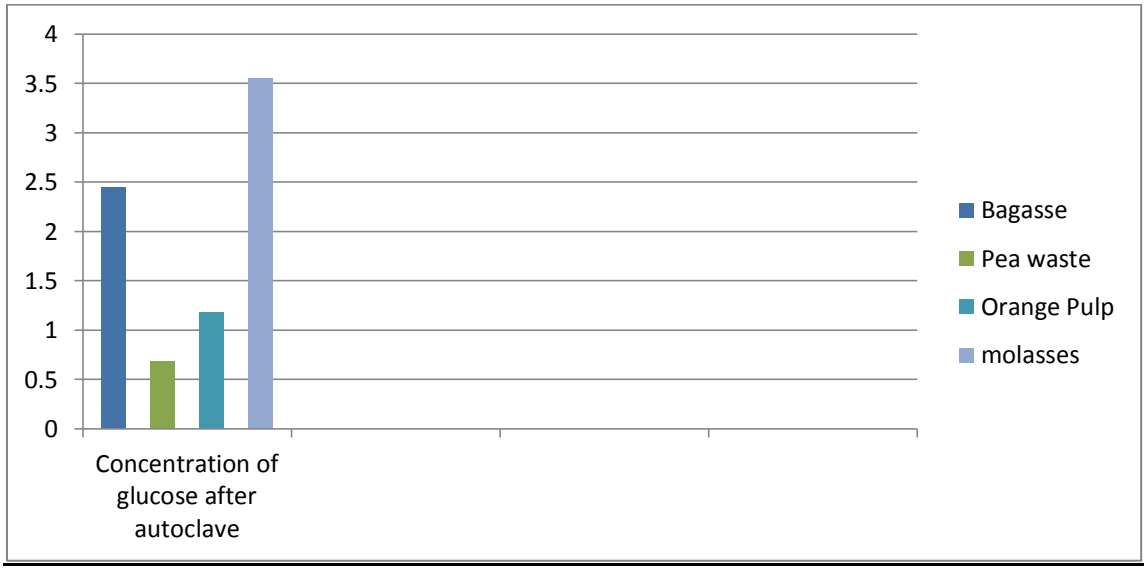

Fig.:3 Graphical representation of concentration of Glucose of 0.5 M hydrolyzed compound DNS result.

Table:4 For Enzyme hydrolyzed compound Sugar concentration.

S.NO	STANDARD MALTOSE	O.D Before autoclave	O.D After autoclave	Concentration of sugar (mg/ml) after Autoclave
1	BLANK	0.00	0.00	
2	Bagasse	1.03	1.45	1.87
3	Pea waste	1.45	1.51	1.94
4	Orange Pulp	0.95	1.12	1.44
5	Molasses	1.68	1.84	2.37

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Fig: 4: Graphical representation of Enzyme hydrolyzed compound sugar concentration in various samples

Table: 5: Absorbance of standard ethanol

Potassium Dichromate (Standard):

Sample Concentration	Amount of sample(Absolute alcohal)	Distilled Water (µl)	$Kr_2Cr_2O_7$ +D.W+H_2SO_4 (ml)	2M NaOH (ml)	Absorbance at 600 nm
10%	50	450	2	1	0.35
20%	100	400	2	1	0.38
30%	150	350	2	1	0.41
40%	200	300	2	1	0.45
50%	250	250	2	1	0.50
60%	300	200	2	1	0.54
70%	350	150	2	1	0.57
80%	400	100	2	1	0.61
90%	450	50	2	1	0.66
100%	500	00	2	1	0.72

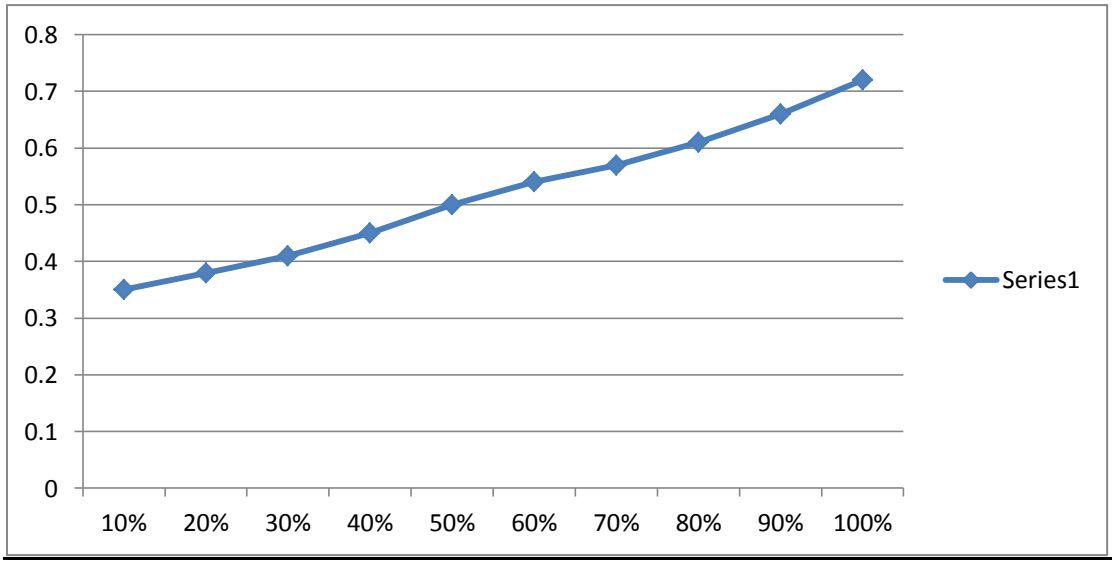

Fig.5 Graphical presentation of Standard alcohol by potassium dichromate test

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Table: 6: Dichromate test of Sample hydrolyzed by 0.3 M Sulphuric acid-

S.No.	SAMPLE NAME	AMOUNT OF PRODUCED ETHANOL IN % age
1	Bagasse	63 %
2	Potato	60%
3	Pea	58%
4	Molasses	95%
5	Orange Peel	58%
6	Orange Pulp	63%

Table: 7: Potassium dichromate test of ethanol produced in different samples hydrolyzed using 0.3 M Sulphuric acid

Sl. no.	Sample Name	OD at 600 nm				Ethanol
		Day 1	Day 2	Day 3	Day 4	
1	Bagasse	0.52	0.6	0.45	0.29	0.6
2	Potato	0.4	0.58	0.42	0.31	0.58
3	Pea	0.52	0.56	0.42	0.26	0.56
4	Molasses	0.46	0.9	0.67	0.49	0.9
5	Orange peel	0.46	0.56	0.45	0.4	0.56
6	Orange pulp	0.44	0.53	0.46	0.31	0.53

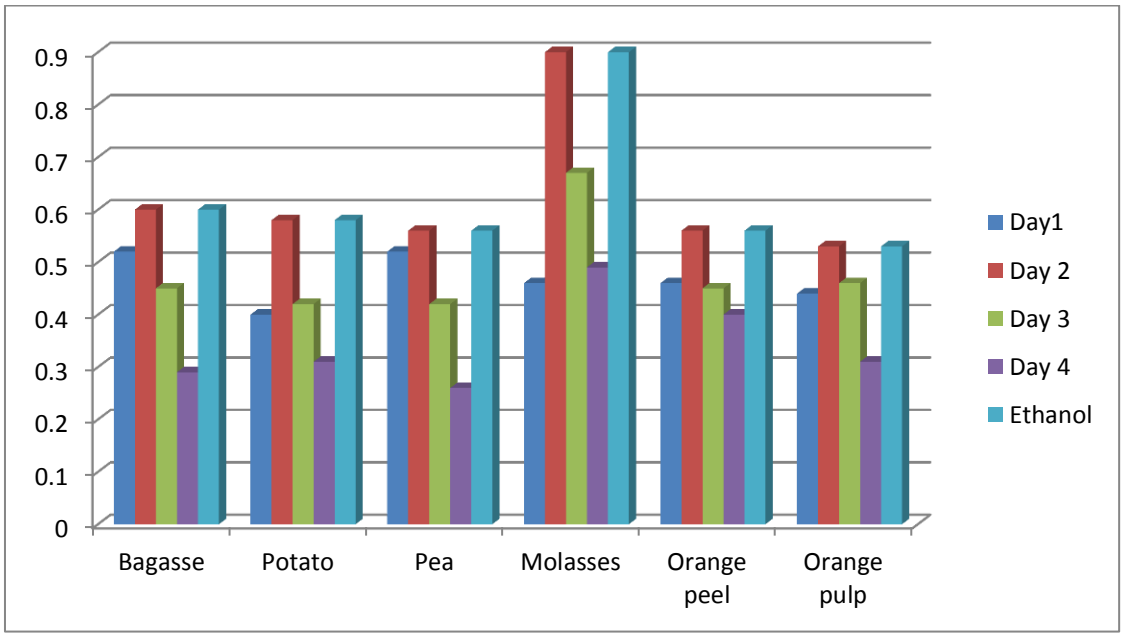

Fig: 6 : Dichromate test of Samples Hydrolysed by Enzyme

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Table: 8: Potassium dichromate test of ethanol produced in different samples hydrolyzed using Enzyme on 2 nd day.

S.No.	SAMPLE NAME	AMOUNT OF PRODUCED ETHANOL IN % age
1	Bagasse	42%
3	Pea waste	24%
4	Molasses	85%
5	Orange Peel	56%
6	Orange Pulp	53%

Table: 9: Estimation of the amount of ethanol produced by using Potassium Dichromate test of 0.5M acid hydrolyzed samples.

Sample	Potassium dichromate (ml)	2M NaOH (ml)	Amount of sample (µl)	D/W (µl)	O.D. at 600nm			
					First Day	Second day	Third day	Fourth day
Agricultural Wastes (Mixture of all lignocellulosic & household waste)	1	2	250	250	0.74	**0.80**	0.65	0.40

SAMPLE NAME	AMOUNT OF PRODUCED ETHANOL IN % age
Agricultural Wastes	**80%**

Table: 10, 11, 12& 13. Estimation of ethanol in various samples using Gas Chromatography Method

				Ethanol Concentration (gm/l)		
Sl no	Sample	Hydrolysis	R.T (Peak Obtnd.)	Day 1	Day 2	Day 3
1	Bagasse	0.3 M H2SO4	2.1	10.78	13.17	11.69
2		0.5 M H2SO4	2.2	16.14	25.3	23.66
3		Amylase Enzyme	2.15	19.14	21.16	20.54

Table:10

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Sl no	Sample	Hydrolysis	R.T (Peak Obtnd.)	Day 1	Day 2	Day 3
1	Pea waste	0.3 M H2SO4	2.15	17.13	18.42	11.69
2		0.5 M H2SO4	2.31	19.6	13.17	20.1
3		Amylase Enzyme	2.2	13.56	20.54	15.43

Table 11

Sl no	Sample	Hydrolysis	R.T (Peak Obtnd.)	Day 1	Day 2	Day 3
1	Orange Pulp	0.3 M H2SO4	2.15	2.29	10.12	11.69
2		0.5 M H2SO4	2.01	10.78	21.68	12.33
3		Amylase Enzyme	2.2	4.3	11.5	9.85

Table 12

Sl no	Sample	Hydrolisis	R.T (Peak Obtnd.)	Day 1	Day 2	Day 3
1	Molasses	0.3 M H2SO4	2.16	20.73	21.71	20.89
2		0.5 M H2SO4	2.42	28.59	30.25	28.9
3		Amylase Enzyme	2.15	21.5	23.25	22.31

Table: 13

Table: 14: Estimation of the amount of ethanol produced by using a gas chromatograph in Bagasse.

S. No.	SAMPLE	HYDROLYSIS	R.T.(PEAK OBTAINED)	DAY 1 ETHANOL COCN (gm/l)	DAY 2 ETHANOL COCN (gm/l)	DAY 3 ETHANOL COCN (gm/l)
1.	Bagasse	0.3 M H_2SO_4	2.10	10.78	13.17	11.69
2.	Bagasse	0.5 M H_2SO_4	2.20	16.14	25.30	23.66
3.	Bagasse	Amylase Enzyme	2.15	19.14	21.16	20.54

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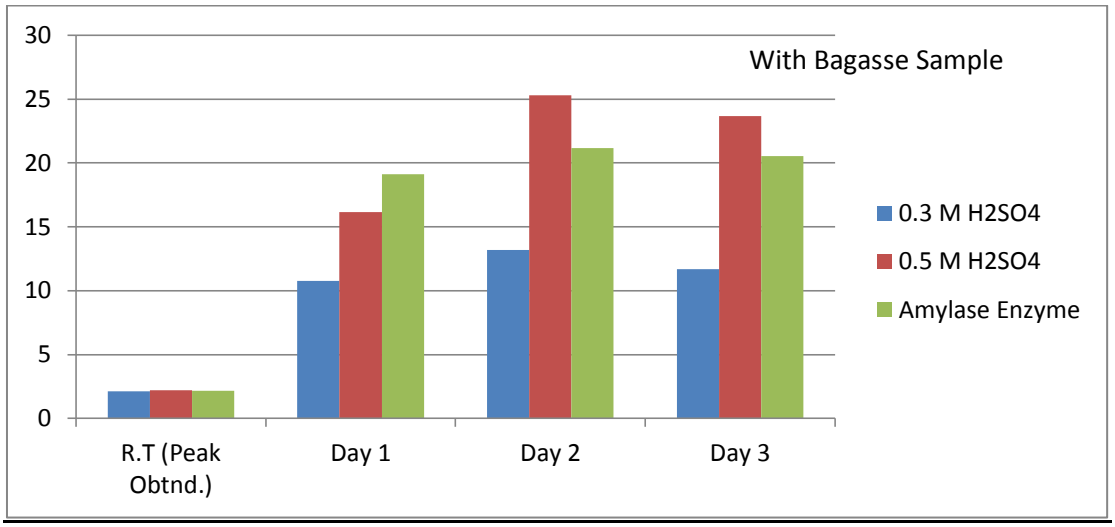

Fig; 7: Graph showing ethanol production in bagasse by Gas chromatography

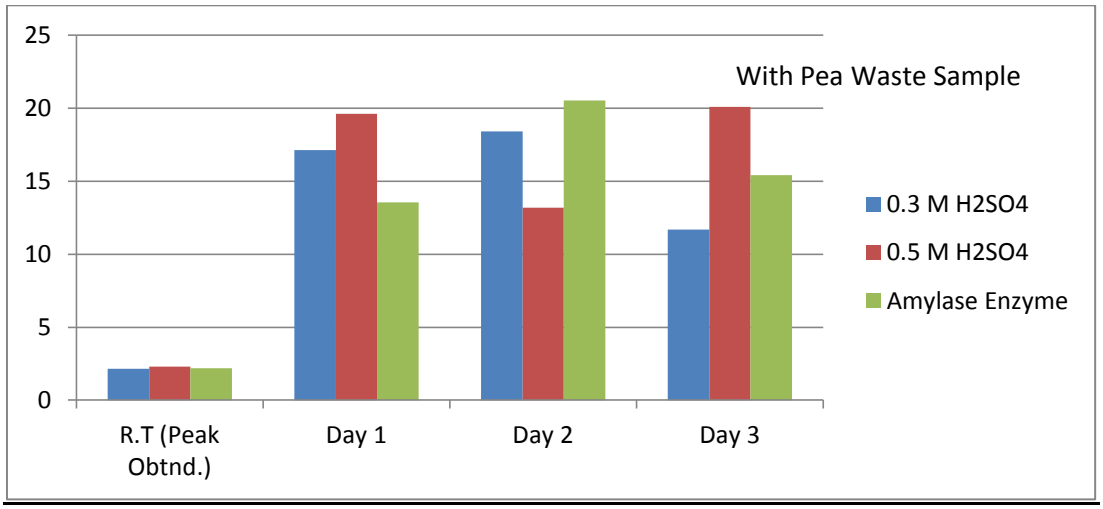

Fig:8: Graph showing ethanol production in pea waste by gas chromatography

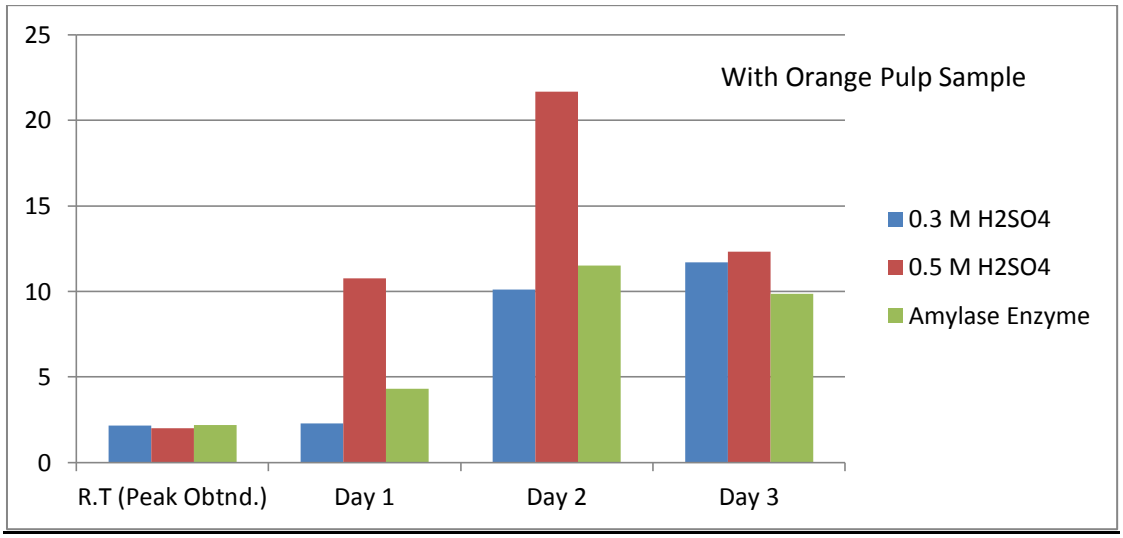

Fig: 9: Graph showing ethanol production in orange pulp by gas chromatography

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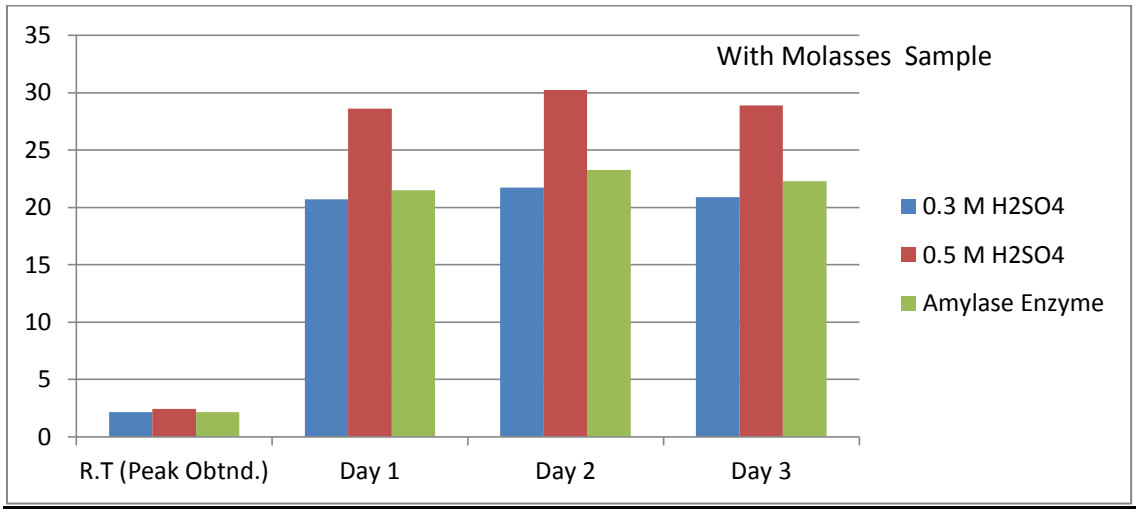

Figure: 10: Graph showing ethanol production in molasses by gas chromatography

Table: 15: Estimation of the amount of ethanol produced by gas chromatograph in Pea.

S. No.	SAMPLE	HYDROLYSIS	R.T. (PEAK OBTAINED)	DAY 1 ETHANOL COCN (gm/l)	DAY 2 ETHANOL COCN (gm/l)	DAY 3 ETHANOL COCN (gm/l)
1.	Pea waste	0.3 M H_2SO_4	2.15	17.13	18.42	14.69
2.	Pea waste	0.5 M H_2SO_4	2.31	19.6	13.17	20.10
3.	Pea waste	Amylase Enzyme	2.20	13.56	20.54	15.43

Table : 16: Estimation of the amount of ethanol produced by gas chromatograph in orange Pulp.

S. No.	SAMPLE	HYDROLYSIS	R.T. (PEAK OBTAINED)	DAY 1 ETHANOL COCN (gm/l)	DAY 2 ETHANOL COCN (gm/l)	DAY 3 ETHANOL COCN (gm/l)
1.	Orange pulp	0.3 M H_2SO_4	2.16	2.29	10.12	11.69
2.	Orange pulp	0.5 M H_2SO_4	2.01	10.78	21.68	12.33
3.	Orange pulp	Amylase Enzyme	2.20	4.30	11.50	9.85

Table: 17: stimation of the amount of ethanol produced by gas chromatograph in Molasses

S. No.	SAMPLE	HYDROLYSIS	R.T. (PEAK OBTAINED)	DAY 1 ETHANOL COCN (gm/l)	DAY 2 ETHANOL COCN (gm/l)	DAY 3 ETHANOL COCN (gm/l)
1.	Molasses	0.3 M H_2SO_4	2.16	20.73	21.71	20.89
2.	Molasses	0.5 M H_2SO_4	2.42	28.59	30.25	28.90
3.	Molasses	Amylase Enzyme	2.15	21.50	23.25	22.31

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Table: 18 Estimation of the amount of ethanol produced in Small scale Fermenter. (Mixture of all lingo cellulosic and household waste) by using a gas chromatograph in Agricultural Wastes

S. No.	Sample	Hydrolysis	R.T (Peak Obtained)	Area obtained on 2nd day peak	Day 1 Ethanol Conc. (gm/l)	Day 2 Ethanol Conc. (gm/l)	Day 3 Ethanol Conc. (gm/l)
1	Agricultural Wastes	0.5 M H_2SO_4	2.40	6681463	19.49	21.16	18.99

Table: 19: Estimation of amount of ethanol in agricultural wastes after distillation

S. No.	Sample	R.T (Peak Obtained)	Area obtained on 2nd day peak	Day 1 Ethanol Conc. (gm/l)
1	Agricultural Wastes	2.32	17612669	30.49

IV. DISCUSSION

The chemical and enzymatic hydrolysis of different ligno-cellulosic waste to convert cellulose into reducing sugars had shown positive results. The maximum amount of sugar was produced from hydrolyzed bagasse which is a waste product of sugar cane industry. Agriculture waste also produced sufficient amount of sugar. The released sugars were fermented for 2 days to produce ethanol and estimation of ethanol was done after every 24 hours. Ethanol estimation was done from preliminary potassium dichromate method and by using a gas chromatograph after bioethanol was successfully produced from different ligno-cellulosic wastes.

Gas chromatography has advantages over potassium dichromate method as it is rapid, sensitive and accurate. Potassium dichromate method is preliminary method for quantification of ethanol. More amount of ethanol was estimated by potassium dichromate method than by using a gas chromatograph as the chances of error are more in potassium dichromate method. Thus, amount of ethanol estimated by using gas chromatograph was considered more appropriate.

The maximum amount of ethanol estimated using a gas chromatograph was 30.25 grams/litre which was produced from Molasses on second day which was used as standard. Bagasse produced 25.30 grams/liter of ethanol on second day when hydrolyzed using 0.5 M of sulphuric acid Pea waste, Orange pulp which was household waste produced ethanol was about 13.17 gm/L, 21.68 gm/L respectively when hydrolyzed by 0.5 M H2SO4 And Agriculture waste (mixture of all household & lignocellulosic waste) in small scale 3 liter fermentor fermentation was done and produced ethanol of amount 21.16 gm/L on second day and after purification(distillation) production was 30.49 gm/L.

This recovery of ethanol is comparable to other studies done by different scientist on different raw materials. Thus, agricultural wastes can be economically used for ethanol production.

V. CONCLUSION

It can be concluded that ligno-cellulosic waste materials such as agriculture waste, sugarcane waste, fruit waste and biodiesel waste have the capability to undergo acid and enzymatic hydrolysis and fermentation to produce bioethanol. Ligno-cellulosic wastes are rich in cellulose thus can be used to produce cellulosic ethanol. Ligno-cellulosic wastes consist of high amount of glucose which can be converted to bioethanol. Use of ethanol as a fuel can reduce greenhouse gas emission thus reduces air pollution. Fuel ethanol reduces the dependency on the fossil fuels by reducing the use of petroleum for automobile transporation.

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