Acid Based Hydrolysis Pretreatment of Sugarcane Leaves To Manufacture Ethanol

Varsha N.Yadav[1], Prof.Rajendra S. Raut[2]
[1] Shivajirao. S. Jondhale college of Engineering , Dombivli Thane
[2]AlSSMS college of engineering, Pune

Abstract- *The use of ethanol is increasing throughout the world for number of reasons. It is mainly used as potential alternative for fuels to fulfill the need of country for economic development. The production of ethanol in our country does not satisfy the need from industrial sector. So large amount of ethanol we have to import from foreign country. Hence to avoid this dependency we have to increase the production of ethanol in our country. Ethanol is mainly produced from sugarcane molasses and grains. As sugarcane is water intensive crop, its production is depending upon whether conditions. As a weather condition fluctuates it affects the production and cost. And we can not increase the production of sugarcane beyond certain limit. At the same time these raw materials were mainly used as food resources. Hence unintentionally it creates the diversion of food resources, prices increases which results to food v/s fuel crisis. Hence to overcome the situation this is the today's need to develop the bio ethanol from such materials which does not used as food resources. Lignocellulosic material is found as feasible raw material for the development of bio ethanol. But its commercialization is highly limited because of its high cost processing technologies and enzymes. Hence the production of bio ethanol from lignocellulosic raw material being challenge in the future. Lignocellulosic biomass is made up of cellulose, hemicelluloses and lignin. The desired cellulose content is generally bounded by hemicelluloses and then lignin. The lignin is necessary to separate to increase the accessibility of cellulose by carrying out the pretreatments. The numbers of pretreatment methods are available but not the single method is resulted as best option because as the raw material gets change the effective pretreatment process also changes. These processes are still under research to increase the economy and efficiency. This document presents why sugarcane leaves as raw material, general procedure to carry out the pretreatment, DNSA method to find the content of reducing sugar, acid based hydrolysis processes at optimized condition.*

Keywords– Sugarcane Leaves, Lignocellulose, Hydrolysis, Bio-ethanol

I. INTRODUCTION

In recent few years high fuel prices and environmental concern leads the development of bio fuels. These bio fuels are used as potential alternative for the economic development of the country. Ethanol is manufactured from molasses of sugarcane and grains in the first generation of bio fuels. The raw materials were mainly used as food resources only. But same time government also provide subsidies , tax break so the land that was previously used for crops now being used to grow crops for bio fuels. Hence unintentionally it creates the diversion of food resources, prices increases which results to food v/s fuel crisis. As sugarcane is water intensive crop, its production is depending upon whether conditions. As a weather condition fluctuates it affects the production and cost. And we can not increase the production of sugarcane beyond certain limit. Hence to overcome the situation this is the today's need to develop the bio ethanol from such materials which does not used as food resources. Lignocellulosic material is found as feasible raw material for the development of bio ethanol. But its commercialization is highly limited because of its high cost processing technologies and enzymes. Hence the production of bio ethanol from lignocellulosic raw material being challenge in the future. Lignocellulosic biomass is made up of cellulose, hemicelluloses and lignin. The desired cellulose content is generally bounded by hemicelluloses and then lignin. The lignin is necessary to separate to increase the accessibility of cellulose by carrying out the pretreatments. The numbers of pretreatment methods are available but not the single method is resulted as best option because as the raw material gets change the effective pretreatment process also changes. These processes are still under research to increase the economy and efficiency. Currently, the entire bio-ethanol requirement for blending mandates has to come from molasses, a byproduct of sugarcane. The availability of molasses depends on cane and sugar production that are cyclical in nature. Lower molasses availability will put pressure on molasses prices which in-turn affects ethanol production from molasses. Molasses prices in the last decade have fluctuated substantially. Additionally, ethanol produced has many other alternative uses such as potable alcohol, and in chemical and pharmaceutical industries. During a normal year,

cane converted into sugar generates enough molasses to produce alcohol that can meet the needs of both the potable and chemical sectors (30-40% each) and another 20-30% surplus alcohol is available for conversion into ethanol for blending. The amount of residues from sugar cane harvesting depends on many factors such as: harvesting system, topping height, cane variety, age of crop, climate, soil and others. Therefore, with the purpose of excluding the effects of harvesting conditions, experiments were carried out to determine de amount of trash (dry leaves, green leaves and tops) available in sugar cane field before harvesting.

II. MATERIALS AND METHODS

Estimation of reducing sugar by dinitrosalicylic acid (DNSA Method)

Principle:

DNSA Method is used for estimating concentration of reducing sugar. This method is invented by G. Miller in 1959.Reducing sugar is a basic solution of aldehyde or ketone and having tendency to reduce many of the reagents. The aldehyde group of glucose converts 3,5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid, which is the reduced form of DNS. The formation of 3-amino-5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength 540 nm. The absorbance measured using a spectrophotometer is directly proportional to the amount of reducing sugar.

Materials:

1. Sodium potassium tartrate: Dissolve 45 gm. of sodium potassium tartrate in 75 ml of H_2O.
2. 3,5-DNS solution: Dissolve 1.5 gm of DNS reagent in 30 mL of 2 M/liter NaOH.
3. 2 molar NaOH: 80 gms of NaOH dissolved in 1 liter of water.
4. DNS reagent: Prepare fresh by mixing the reagents (1) and (2) make up the volume to 150 ml with water.
5. Standard sugar sodium:

a. Stock standard sugar sodium: 250 mg of glucose in water and make up the volume to 100 ml.
b. Working standard sodium: Take 10 mL from this stock solution and make up the volume to 100 ml.

Procedure:

1. Take clean and dry test tube
2. Pipette out standard solution in the range of 0 to 2 ml
3. Make up the final volume in all the tubes to 2 mL with distilled water concentrations ranging from 0 to 750 mg.
4. Add 1 mL DNS reagent to all the test tubes and mix well and capped it (To avoid the loss of liquid due to evaporation)
5. Keep the test tube in a boiling water bath for 10 minute.
6. Take the tubes and cool to room temperature. Read extinction at 540 nm against the blank.
7. Prepare standard curves of the sugars provided and use them to estimate the concentration of the unknowns provided.

General procedure for pre-treatment:

- Allow the sugar cane leaves to dry it in shade drying,
- Cut it into small pieces,
- Grind it till getting very small particle size,
- Sieve it up to the required size by using sieve shaker.
- Weight as per the requirement by using weighing balance,
- Prepare solution as required to perform the experiment
- Filter the solution after caring out the experiment
- Perform the analysis by using UV-spectrometer at 540 nm to find the glucose concentration in ppm.

1. Conc. H2SO4 pre-treatment

(H2SO4 Concentration 72%, temp. 35 deg C. time 60 min).

Procedure-

1. Select the raw material sugar cane leaves due to the high contents of cellulose & hemicelluloses,
2. Allow to dry it in shade drying,
3. cut it into small pieces,
4. Grind it till getting very small particle size,
5. Sieve it up to the size of 1 mm. 1.5mm & 2 mm. by using sieve shaker.
6. Weight as per the requirement by using weighing balance,
7. Prepare 72% H2SO4 solution (i.e. 978.26ml H2O+21.74ml H2SO4
8. Allow to deep sugarcane pieces into the solution.
9. Optimize the parameter (i.e. residence time, particle size & Concentration by performing the expt.)
10. Filter the solution after caring out the experiment
11. Perform the analysis by using UV-spectrometer to find the glucose concentration in ppm.

Dilute acid hydrolysis:

(H2SO4 Concentration up to 4%, temp. 121degC , Time 30 min.)
Procedure-

1. Select the raw material sugar cane leaves due to the high contents of cellulose & hemicelluloses,
2. Allow to dry it in shade drying,
3. cut it into small pieces,
4. Grind it till getting very small particle size,
5. Sieve it up to the size of 1.5mm by using sieve shaker.
6. Weight as per the requirement by using weighing balance,
7. Prepare 3%, 4%, 5% H2SO4 solution
8. Allow to deep sugarcane pieces into the solution.
9. Optimize the parameter (i.e. residence time, temperature & Concentration by performing the expt.
10. Filter the solution after caring out the experiment
11. Perform the analysis by using UV-spectrometer to find the glucose concentration in ppm.

III. RESULTS AND DISCUSSION

Std. Curve preparation for determination of total reducing sugar by DNSA method.

Table 1. DNSA method curve preparation

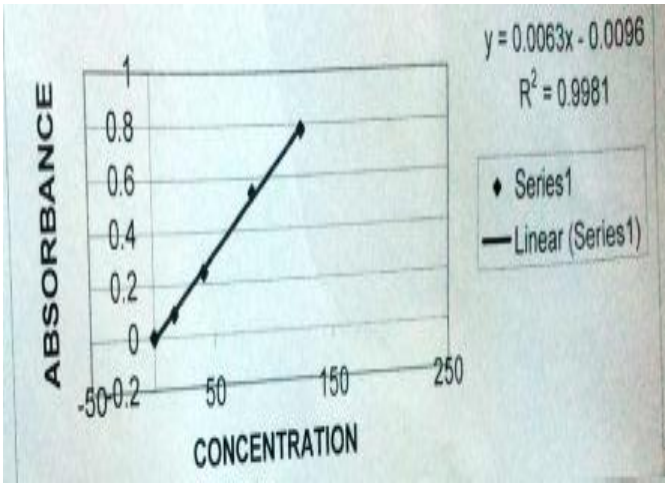
Sr No.	Stock	D / W	DNS	Boling water bath	Cooling	Absorbance
	Amt.(ml.)	(ml.)	(ml.)	(Minutes)	Room temp.	@ 540nm
1	0	2	1	10	RT	0
2	0.2	1.8	1	10	RT	0.0867
3	0.5	1.5	1	10	RT	0.2433
4	1	1	1	10	RT	0.539
5	1.5	0.5	1	10	RT	0.776

To calculate concentration,

Table 2. Calculation

No.	Stock amt(ml.)	Concentration
1	0	0=0
2	0.2	(0.2*250)/3=16.7
3	0.5	(0.5*250)/3=41.7
4	1	(1*250)/3=83.3
5	1.5	(1.5*250)/3=125

Calibration curve:



Graph 1 .Calibration curve for DNSA method

Y=0.0063 X -0.0096

This linear equation can be used to find out the unknown concentration of sample on the basis of absorbance value.

1. Conc. H2SO4 pre treatment

(H2SO4 Concentration 72%, temp. 35 deg C. time 60 min).

Table 3. Expt. No :1

No.	Particle size	Concentration	Residence time	Temp.	Absorbance @540nm
	mm.	Gm.	Min.	degC	
1	1.5	10	60	30	
2	1.5	10	60	35	
3	1.5	10	60	40	

Result: Sugar cane leaves having particle size 1.5 mm. Are not sustaining with 72% H2SO4 solution, Sample is black in colour having very fine suspended particles which cannot be separated even after vacuum filtration & centrifugation at 2000 rpm. So it cannot be analyzed by UV.

Table 4 .Expt. no: 2

Sr No.	Particle size	Loading	Residence time	Temp	Absorbance @ 540 nm
	(mm)	(gm)	(min)	(deg C)	
1	>2	10	60	30	
2	>2	10	60	35	
3	>2	10	60	40	

The particle size has increased than 2 mm , the sample is clear but black in colour which can not be analyzed by DNSA method @ 540 nm using UV spectroscopy.

Fehling's solution test :

- Fehling's solution is a chemical reagent used to differentiate between water soluble carbohydrate and ketone functional groups and as a test for reducing sugars
- The test was developed by German chemist Hermann von Fehling in 1849

Materials :

Two solutions are required:

Fehling's " A " : 7 gram CuSO4. H2O dissolved in distilled water containing 2 drops of dilute sulfuric acid.
Fehling's " B " : 35 gram of potassium tartrate and 12 gram of NaOH in 100 ml of distilled water.

Procedure :

- For the test :
- Mix 15 ml of solution "A" with 15 ml of solution "B"
- Add 2 ml of this mixture to an empty test tube
- Add 3 drops of the compound to be tested to the tube
- Place the tube in a water bath at 60 deg C

Result:

- Even after the addition of fehling's solution to the sample still sample is black in colour , hence it can not be analysed by Fehling's solution test.

Dilute acid hydrolysis:

(H2SO4 Concentration up to 4%, temp.121degC , Time 30 min.)

Table 5.Run-1

No.	Loading	Particle size	Concentration	Temperature	Time	Absorbance	Concentration
	Gm.	mm.		degC	Min.	@540 nm	Mg/lit
1	10	1.5	3%	150	30	0.364	59.3
2	10	1.5	4%	150	30	0.474	76.76
3	10	1.5	5%	150	30	0.328	53.59

Result: Optimized concentration is 4%.

Table 6.Run-2

By maintaining constant 4% sulfuric acid concentration, now will vary the temperature.

No.	Loading	Particle size	Concentration	Temperature	Time	Absorbance	Concentration
	Gm.	mm.		degC	Min.	@540 nm	Mg/lit
1	10	1.5	4%	120	30	0.0571	10.58
2	10	1.5	4%	150	30	0.474	76.76
3	10	1.5	4%	180	30	0.448	72.73

Result : Optimized temp. is 150deg C.

Table 7.Run-3

Now maintaining the concentration and temperature constant throught and vary thetime.

No.	Loading	Particle size	Concentration	Temperature	Time	Absorbance	Concentration
	Gm.	mm.		degC	Min.	@540 nm	Mg/lit
1	10	1.5	4%	150	20	0.1241	21.22
2	10	1.5	4%	150	30	0.474	76.76
3	10	1.5	4%	150	40	0.343	55.96

Result : Optimized time is 30 min.

Hence, Optimized parameters of dilute acid hydrolysis with sugar cane leaves as raw material are concentration 4%, temperature 150 deg C, time 30 in giving maximum content of reducing sugar 76.76 mg/lit.

IV. CONCLUSION

To meet the targeted blending requirements alternative feed stocks will have to play a more important role to fill the current and future gap between demand and supply of bio ethanol. Sugar cane residue is one such alternative feedstock that can be used. Production of ethanol from sugar cane residue has mainly consist of two steps The conversion of cellulose to glucose and the conversion of glucose to ethanol. In the conversion of cellulose to glucose pretreatment plays a vital role to break the structure, and improve the yield.It is not possible to extract the content of reducing sugar

from sugar cane leaves by using concentrated acid pretreatment method because the presence of 72 % concentrated sulphuric acid. Sugarcane leaves are not sustaining with the highly concentrated acid.To extract the reducing sugar from sugar cane leaves dilute acid pretreatment method at optimized condition is the best option. With 4 % concentration of sulfuric acid at 150 degree C for 30 min is found to be most effective pretreatment with maximum reducing sugar 76.76 mg/ lit.

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REFRENCES

[1] J.Y. Zhu and X.J.pan ,(2009), " Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, Bioresource Technology 101(2010) ,4492-4496.

[2] Parveenkumar, Diene M.Barrett,MichaelJ.Delwiche,and Pieter Stroeve,(2009)," Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and bio fuel production",Industrial and Engineering Chemistry Research

[3] EdgardGnansounou , and ArnauadDauriat,(2005), " Ethanol fuel from biomass: A review ,Journal of Scientific & Industrial Research Vol 64 , 809-821

[4] Mohammad Taherzadeh and KeikhosroKarimi ,(2007) "Enzyme based hydrolysis processes for ethanol from lignocellulosic materials: A review", Bio Resources 2(3),707-729

[5] Mohammad Taherzadeh and KeikhosroKarimi ,(2007) "Acid based hydrolysis processes for ethanol from lignocellulosic materials : A review" Bio Resources 2(3) , 472-499

[6] P.C.Badger,(2002) "Ethanol from cellulose : A general review", Trends in new crops and new uses, 17-21

[7] Valery B. Agbor , NazimCicek , Richard Sparling , Alex Berlin , David B. Levin,(2011),"Biomass pretreatment : Fundamentals towards application", Biotechnology Advances 29,675-685

[8] Wennan Zhang , (2010)" Automotive fuels from biomass via gasification", Fuel Processing Technology 91 , 866-876