Scientific Journal of Impact Factor(SJIF): 3.134

e-ISSN(O): 2348-4470
p-ISSN(P): 2348-6406

International Journal of Advance Engineering and Research Development

Volume 2,Issue 5, May -2015

Optimization of Pretreatment and Hydrolysis process in the production of Bioethanol

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Abstract : *Paper, which is one of the largest constituent of Municipal solid waste, has become a severe problem in developed and developing countries due to the shrinking land fill capacity. It is very important and challenging task in managing the solid waste. The Newspaper which is a cellulosic material is the potential source of Ethanol. The main objectives of the current project is, to minimize the newspaper load on municipal solid waste by efficiently utilizing the waste news paper in the production of Bioethanol, to optimize the pretreatment process for increasing the efficiency of bacterial hydrolysis also in the efficient conversion of Cellulose to sugars from cellulose degrading micro organisms. In this paper efforts are made to optimize the pretreatment process which helped to separate Lignin, Hemicelluloses and Cellulose and enhance the hydrolysis process. The optimized condition for the pretreatment was found to be 1.1% concentration of H_2SO_4 at 121ºC and 60 minutes. The bacteria Cytophaga was used for hydrolysis process, which helped in converting the cellulose to sugars and was analyzed using HPLC (High Performance Liquid Chromatography.*

I. INTRODUCTON

With increases in petroleum prices have sparked renewed interest in alternative fuels, especially renewable fuels such as ethanol. Ethanol is an excellent light duty vehicle (LDV) fuel that can be used in conventional vehicles in blends of up to 10% ethanol and 90% gasoline (E10), while flexible fuel vehicles, of which there are more than five million in the United States today, can run on mixtures containing as high as 85% ethanol blended with 15% gasoline (E85). Municipal solid waste (MSW) is emerging as an attractive option because of developments in conversion technology, lower feedstock costs, and higher potential for fossil fuel displacement and reduction in greenhouse gas (GHG) emissions compared to corn-ethanol. Paper waste recovered from Municipal Solid Waste (MSW) is an especially appealing feedstock for ethanol, because cellulosic materials such as paper, from about 40- 50% of the dry weight of a typical MSW stream. Mainly the municipal solid waste consists of the components like Food waste, Wood, leaf, Garden or Yard trimmings, Rubber, Leather, Textiles, metals(Ferrous and Non ferrous), Glass and large amount of Paper and Paper boards. Mainly the composition of paper in municipal solid waste is upto 35 to 40% by weight.

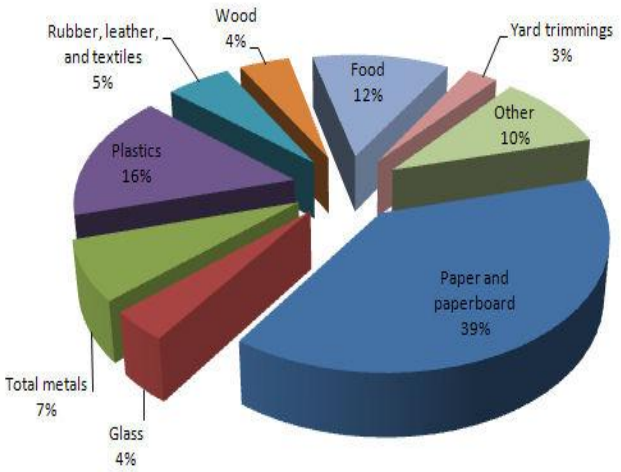

Figure 1: composition of different components in municipal solid waste.

The possibility of using wastepaper as a cheap feedstock for ethanol production arises from the well publicized concern about rising landfill costs resulting from the shrinking landfill capacity. Since wastepaper is the single largest material category in the Municipal Solid Waste (MSW) stream, it is the main target of the efforts to reduce the MSW burden. In India the wastepaper constitutes about 35-40% of total quantity of recyclables of dry waste in urban areas. The main composition of news paper are Celluose, Hemicellulose and Lignin. Cellulose which is about 30-50%, hemicelluloses 15-35% and lignin which consists upto 10-20%. Cellulose and hemicelluloses make up approximately 70% of the entire biomass of the entire biomass.

II.MATERIAL AND METHODOLOGY

2.1 Physical treatment

News paper is collected and it is made into smaller pieces and oven dried for few hours at 60ºC to constant weight and stored in a sealed plastic bag at room temperature. As the dilute acid treatment is economical and it is less corrosive, simple technique is required, effective in enhancing the hydrolysis process, it is selected for the pretreatment process.

2.2 Estimation of different parameters in raw substrate.
Estimation of cellulose
Procedure: Three milliliters of acetic/nitric reagent was added to one gram of the powdered News paper taken in test tube and mixed thoroughly in a cyclo mixer. The sample was placed in water bath and heated at 100°C, cooled and centrifuged for 20 minutes at 10000 RPM. The supernatant was discarded and the residue washed with distilled water. Ten milliters of 67% sulphuric acid was added and allowed to stand for an hour. One millilitre of the solution was diluted with 100 ml of distilled water. To 1 ml of the diluted sample, 10 ml of anthrone reagent was added and mixed well. The sample was then heated in boiling water bath for 10 minutes and cooled to room temperature. Absorbance was measured at 630 nm. One hundred (100) milligram of cellulose solution was prepared in a test tube as standard and the procedure above was repeated for the standards.

Moisture content in substrate
The moisture content is determined by taking the known amount of the sample and it is kept in oven for 24 hours at 60°C and the final weight is noted. Percentage Moisture content is calculated using the formula:

Moisture content=(Initial weight-final weight)/ * 100

Ash content of the substrate
To determine the ash content, the known amount of substrate is kept in the oven for few hours with the temperature of 660°C. The final weight is obtained.

Dilute acid pretreatment

The sulphuric acid concentrations 0.5,1,2,3,4% (w/v) were prepared. Solid to liquid ratio of 1:50 (w/v) are added in the conical flask. The treatment was performed at 121ºC for the different residence time of 30min, 60min, and 90minutes. After heating for the particular residence time it is centrifuged for 15min with 100 rpm and supernatant is discarded and residue is washed with distilled water two to three times to neutralize the acid, 10ml of 67% sulphuric acid is added to the residue and it is incubated for 1hour. After 1 hour 1ml of the sample is collected and it is diluted to 100ml. From the diluted solution, 1ml is taken and 10ml of Anthrone reagent is added and it is mixed well, and heated in boiling water bath for 10min, then the samples are cooled and the color is noted at 630nm using Spectrophotometer. Further the concentrations and the residence time is varied for optimization of the pretreatment.

Bacterial hydrolysis of substrate

The pretreated samples are taken and they adjusted with the pH 7 for the better Enzymatic activities, then the residue is oven dried till the constant and it is sterilized and taken for hydrolysis process.

The organism Cytophaga (bacteria) is purchased from NCR Pune. Cytophaga was used as a novel organism for cellulose hydrolysis. Inoculum is prepared for Cytophaga medium.

50ml of the 24 hours grown inoculum broth was added to the oven dried pretreated substrate in the conical flask in a laminar air flow and are placed in rotary shaker at 100rpm at 37ºC. Further at regular intervals the hydrolysate are collected and analysed for conversion of cellulose to sugars i.e reducing sugars, the reducing sugars is analyzed usinghigh Performance Liquid Chromatography. Hydrolysate samples were collected until the constant value is obtained for reducing sugar. The maximum sugars released during the period of hydrolysis of pretreated substrates were selected for fermentation process.

III.RESULTS AND DISCUSSION

3.1 Cellulose content

The cellulose content in the news paper is determined using Anthrone method. It was found that the cellulose content present in the news paper is about 47%.

3.2 Moisture content in substrate
The moisture content is determined by oven drying method and the moisture was found to be 4.4% in the substrate.

3.3 Ash content of the substrate

To determine the ash content the known amount of substrate is kept in the oven for few hour with the temperature of 660°C. The final weight is obtained. The ash content present in the sample is 5.9%.

3.4 Pretreatment

The study performed using different pretreatment parameters of acid concentrations and reaction time in order to optimize the pretreatment conditions. The unknown absorbance is obtained from the standard graphs. The graph of glucose released Vs sulphuric acid concentration is plotted as shown below.

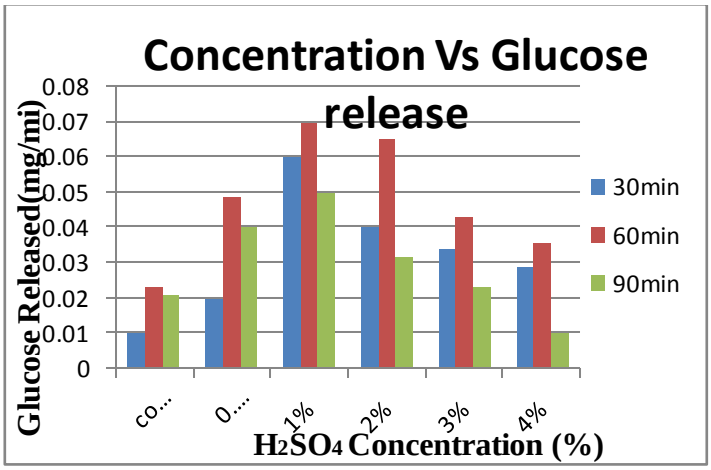

Figure 2: The graph of glucose released Vs concentration

From the results of above graph we can say that the maximum concentration is between 1% to 2% concentrations of H_2SO_4. And from the above graph the residence time is observed to be maximum at 60minutes.

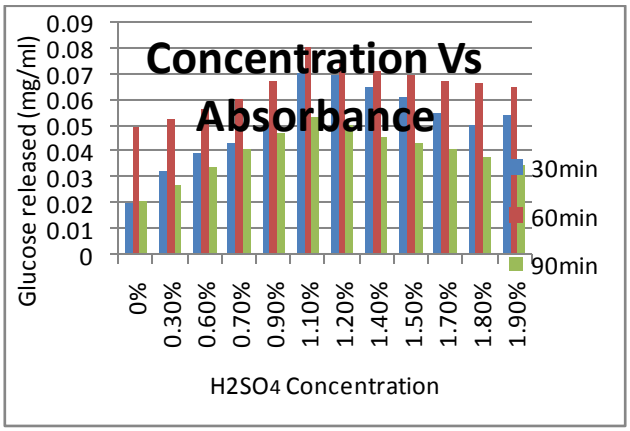

Figure 3: The graph showing glucose released Vs the different conc. of sulphuric ascid

In the above graph the time is varied and the concentrations are varied according to the results of the 1st graph. The maximum glucose release is obtained at 1.1% concentration and with the residence time of 60minutes.

Hence the optimized condition for the pretreatment from the above results are 1.1% concentration of H_2SO_4 and the residence time of 60minutes.

3.5 Hydrolysis process

The hydrolysis is carried out using both isolated and pure culture organisms. Maximum cellulose released during pretretment optimization was selected for Hydrolysis. The pretreated substrate pH was adjusted to 7.0 before hydrolysis and oven dried till constant weight at 60°C. At regular intervals the glucose released is observed using High Performance Liquid Chromatography (HPLC). Taking the areas from the graph obtained by different hours and comparing the results with standard sugars the concentration of sugars released is obtained.

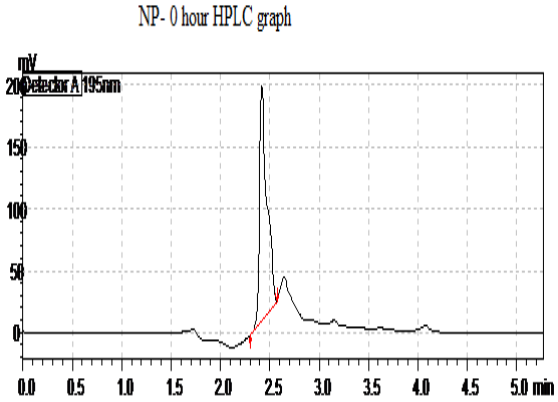

Figure 4: Shows the 0 hour reading of Hydrolysis process using HPLC

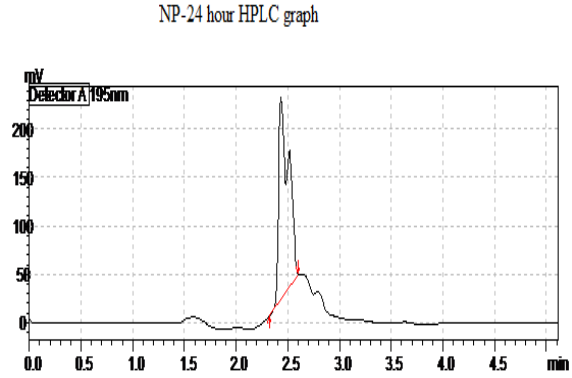

Figure 5: Shows the 24 hour reading of Hydrolysis process using HPLC

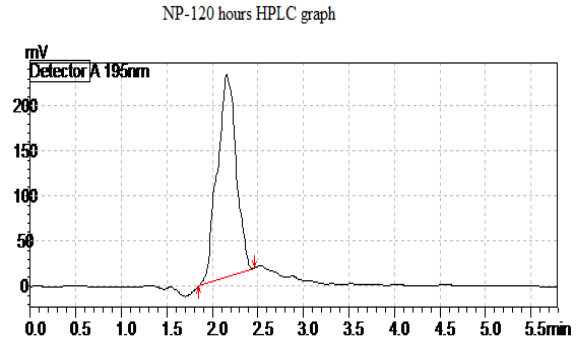

Figure 6: Shows the120 hour reading of Hydrolysis process using HPLC

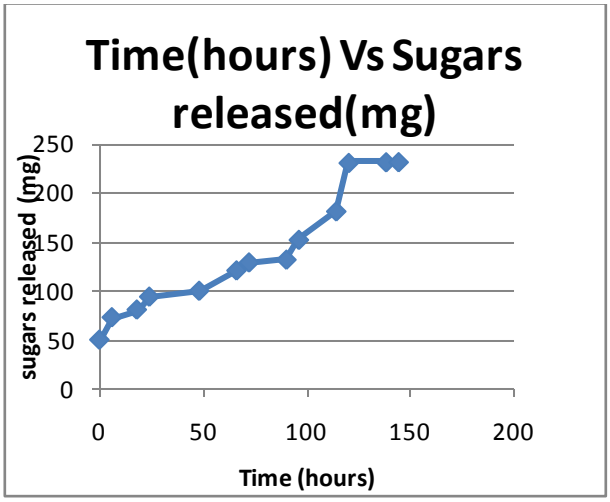

Figure7: The graph shows the variation of sugar released w.r.to time.

The HPLC reading is taken at every regular intervals, 0, 24, 48……until there is constant value of reducing sugars is noted. Taking the standard HPLC reading of glucose and comparing it with the readings obtained, the concentrations is determined From the above graph, we can observe that the sugars released increases with respect to 50.12mg of sugars per gram of substrate, and the sugars released at 144 hours was found to be 232.12mg per gram of the substrate. Hence 144 hours with the concentration of 232.12mg is the optimized condition for the Hydrolysis process.

IV. CONCLUSION

News paper is taken as the substrate for bioethanol production in order to reduce the load on municipal waste. The cellulose present in the news paper is obtained to be 47%. The highest conversion percentage resulting from newspaper to glucose resulting from the bacterial hydrolysis was achieved using the 1.1% H_2SO_4 for the period of 60minutes with a temperature of 121°C. The pretreatment proved to be effective in enhancing the conversion of waste cellulose material to glucose. Using HPLC the sugars released was analysed. At 144 hours the hydrolysis process was found to effective in releasing the sugars. Hence, at 144 hours with the concentration of 232.12mg per gram of substrate is the optimized condition for the hydrolysis process and this was taken for fermentation process.

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