Research Article

# Effect of Pre-treatment Method on Biomethanation of Jatropha De-Oiled Cake

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**Accepted on:** 05-05-2016; **Finalized on:** 30-06-2016.

**ABSTRACT**

Lignocelluloses are often a major or sometimes the sole components of different waste streams from various industries, forestry, agriculture and municipalities. Hydrolysis of these materials is the first step for either digestion to biogas (methane) or fermentation to ethanol. However, enzymatic hydrolysis of lignocelluloses with no pretreatment is usually not so effective because of high stability of the materials to enzymatic or bacterial attacks. The reason for improved rate of hydrolysis by removal of lignin might be related to a better surface accessibility for enzymes by increasing the population of pores after removing of lignin. We used different methods of pretreatment for jatropha deoiled cake, which then was used for its conversion to biogas. Effective parameters in pretreatment of lignocelluloses, such as protection by lignin are described here. Several pretreatment methods were used to study their effects on delignification and anaerobic digestion of biomass was seen. We have tried different pretreatments method for delignification of jatropha deoiled cake and were found that acid hydrolysis with 50% conc. sulphuric acid yield more acid soluble lignin as compared to 72% conc. sulphuric, resulting in comparatively economical method for pretreatment. Further, alkaline hydrolysis can also be claimed as a good method for delignification of jatropha deoiled cake. However, the methane content in biogas obtained was maximum with acid treated biomass as compared to alkaline treated biomass.

**Keywords:** lignocelluloses, Pretreatment, Delignification, enzymatic hydrolysis.

## INTRODUCTION

Production of waste materials is an undeniable part of human society. The wastes are produced by different sectors including industries, forestry, agriculture and municipalities and hospitals. The production of waste and the "throw-away philosophy" results in several environmental problems such as global warming, acid rain, health issues and safety hazards. Un-utilization of waste or non proper disposal of waste prevent unsustainable development of nation as much of lignocelluloses biomass waste contain the energy resource in term of cellulose which can be converted into other source of energy by different technique such as anaerobic digestion, fermentation and pyrolysis. Govt of India promotes different schemes to manage solid waste generation by 3R'S that is reduce, reuse and recycle in which most of solid waste are anaerobically degraded to produce biogas which after purification of methane gas is utilized for power generation, fuel for vehicle, and cooking gas. The Reuse of biomass waste for energy in industry lead to sustainable development of nation by minimizing/reduced use of non renewable resources of energy[1].

Present gap between energy demand and supply can be reduced by proper utilization of waste for energy /products. As per study we have come to know that 25 kg of cow dung produce $Im^3$ of biogas which further produce 0.98kw power. Cow dung biomass 750 million tons is available annually in India which can be anaerobically digested in bioreactor to produce biogas and provide alternate to different energy resources such as LPG, Coal, Inorganic fertilizer, CNG., etc. Biogas is energy source that is used as car fuel, or for production of heat or electricity in different countries[2]. Biogas production from biomass is an old and almost established process. Forestry and agriculture residues are by nature heterogeneous in composition. Sugars, starches, lipids and proteins present in it are among the materials easily degradable by microorganisms, while some other fractions, such as lignocelluloses and keratin are more difficult to degrade[4], but for this lignocelluloses material should be accessible to the enzymes for biodegradation. Lignocelluloses are composed of cellulose, hemicelluloses, lignin, extractives and several inorganic materials. Cellulose is a linear syndiotactic (alternating spatial arrangement of the side chains) homopolymer composed of D-anhydroglucopyranose units which are linked together by *β*-(I→4)-glycosidic bonds. Taking the dimer cellobiose as the basic unit, cellulose can be considered as an isotactic polymer of cellobiose. The cellulose chains arranged into microfibrils which are stabilized by hydrogen bonds[5]. These fibrils are attached to each other by hemicelluloses and amorphous polymers of different sugars as well as other polymers such as pectin and covered by lignin. Hemi cellulose has a lower molecular weight than cellulose and is composed of mainly pentose (like xylose and arabinose) and hexoses (like mannose, glucose and galactose). It also has considerable side chain branching consisting of hydrolysable polymers. Lignin is a very complex molecule constructed of phenyl propane units linked in a three dimensional structure which is

particularly difficult to biodegrade. Lignin is the most recalcitrant component of the plant cell wall and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation. Generally, softwoods contain more lignin than hardwoods and most of the agriculture residues. Lignin is one of the drawbacks of using lignocelluloses biomass in anaerobic digestion, as it makes lignocelluloses resistant to chemical and biological degradation. The structure of these naturally occurring cellulose fibrils is mostly crystalline in nature and highly resistant to attack by enzymes (limited accessibility of cellulose chains). Cellulose is more susceptible to enzymatic degradation in its noncrystalline form. The presence of lignin also decreases enzymatic hydrolysis, as it does not allow enzymes to act on cellulose.[6] If enzymatic hydrolysis of biomass is to proceed in typical processes, the crystalline structure of cellulose needs to be disrupted, accessible area increased, and the lignin and hemicelluloses separated from the cellulose before treatment with enzymes. The main goal of pretreatment is to overcome this recalcitrance. The cellulose and hemicelluloses are cemented together by lignin. Lignin is responsible for integrity, structural rigidity and prevention of swelling of lignocelluloses. Thus, lignin content and distribution constitute the most recognized factor which is responsible for recalcitrance of lignocellulosic materials to enzymatic degradation by limiting the enzyme accessibility; therefore the delignification processes can improve the rate and extent of enzymatic hydrolysis from the matrix polymers, and to make it more accessible for enzymatic hydrolysis. The reason for improved rate of hydrolysis by removal of lignin might be related to a better surface accessibility for enzymes by increasing the population of pores after removing of lignin. Jatropha deoiled cake cannot be used for any purpose as its toxic in nature in raw form. Jatropha deoiled cake can be anaerobically converted to biogas.

The present work deal with improvement of methane gas from lignocelluloses biomass jatropha deoiled cake by different physical and chemical pretreatment method which remove lignin and decreases crystalline nature of cellulose and treated biomass an aerobically digested in laboratory bioreactor to produce biogas.

We used different methods of pretreatment (Acid, Alkaline and Thermal) for biomass, which then was used for its conversion to biogas.

## MATERIALS AND METHODS

All the chemicals used (conc. sulphuric acid, sodium hydroxide and distilled water) were from Rankem and the standard lignin used was from Merck. Standard biogas sample were from Centurion scientific.

### Preparation of Standard Lignin Solution

Standard lignin samples of 1 and 10 ppm were prepared by dissolving weighed quantity of pure lignin (Rankem) in appropriate volume of distilled water.

Substrate: Jatropha de-oiled cake was prepared by grinding jatropha seed in press machine.

### Experimental Method

#### *Pretreatment of Jatropha De Oiled Cake (JDOC)*

### Production of Biogas from Pre-Treated JDOC

### Analysis of Biogas Produced

### Pretreatment of Jatropha De Oiled Cake (JDOC)

Jatropha biomass pretreatment was done by acid, alkaline and thermal methods.

#### *Acid Treatment of Jatropha Biomass*

Jatropha biomass was treated with 72% $H_2SO_4$ for 30 minutes at 121°C and 15 psi pressure[7]. The solution was filtered and the filtrate was studied under UV-Visible spectrophotometer.

The residue was treated similarly for the second crop of the filtrate, which further was studied similarly under UV-Vis. Similar procedure was repeated 4 times. Similar studies were done using variable concentrations of sulfuric acid for different time period under different temperatures. All the set ups were completed as per the first one up to similar level of successive steps.

#### *Thermal Hydrolysis of Jatropha Deoiled Cake*

Jatropha de oiled cake was treated in presence of water for period of 2 hr in autoclave at 121°C and 15 psi.

The reaction mixture was filtered and absorbance of filtrate was noted at 205nm. Same procedure was repeated three times with residue from first, second and third stage. Similar treatments were done at different time periods.

#### *Thermal Followed by Alkaline Hydrolysis of Jatropha Deoiled Cake*

Jatropha de oiled cake was treated in presence of water for period of 2 hr in autoclave at 121°C and 15 psi. The reaction mixture was filtered and absorbance of filtrate was noted at 205nm.

Same procedure was repeated three times with residue from first, second and third stage. Residue from third stage was treated in presence of alkaline environment in autoclave for period of 2hr at 121°C and 15 psi and the absorbance of filtrate was noted.

### Production of Biogas from Pre-Treated JDOC

**Table 1:** Reaction Conditions

| | |
|---|---|
| RPM | 200 |
| Working volume | 600 ml |
| Total volume | 1 l |
| Temperature | 37°C |
| pH | 6.5 |
| HRT | 36 hrs |
| Reactor type | Batch |

Jatropha biomass after treatment with acid, alkali, thermal were subjected to bio-reactor separately in form of slurry of conc. 10% with culture of microorganism responsible for biogas generation, such as methanogenic, acetogenic bacteria. Gas was collected with biogas balloon via gas nozzle of digester.

**Figure1:** Batch type Stirrer Tank Bioreactor

**Analysis of Biogas Produced**

Sample of gas collected from biogas balloon of digesters was injected into GC (Nucon -5700) sampler injector with injection syringe. System Specifications are given below:

- Injection volume - 100µl
- Mobile phase - Argon
- Column Make – Stainless Steel
- Column ID - (HEYSEP. Q)
- Run time - 10 minute
- Column length - 2mtr

## RESULTS AND DISCUSSION

**For Delignification**

The absorbance of lignin standard is shown in Table-2.

**Table 2:** OD of Standard lignin solution

| Concentration | Standard lignin absorbance (205nm) |
|---|---|
| 10ppm | 3.365 |
| 1ppm | 0.849 |

**Acid Pretreatment of Jatropha Deoiled Cake**

The observations obtained from acid treatment of jatropha de-oiled cake are summarized in Table-3.

**Table 3:** Absorbance of Acid Treated Biomass

| Name of Experiment | Absorbance of 10 ppm solution | Time of Autoclave |
|---|---|---|
| 72% of sulphuric acid Stage 1 | 1.309 | 30 mins |
| 72% of sulphuric acid Stage 2 | 0.54 | 30 mins |
| 72% of sulphuric acid Stage 3 | 0.529 | 30 mins |
| 72% of sulphuric acid | 2.419 | 1 hour |
| 65% of sulphuric acid | 2.69 | 1 hour |
| 60% of sulphuric acid | 2.692 | 1 hour |
| 50% of sulphuric acid | 3.088 | 1 hour |
| 4% of sulphuric acid | 1.82 | 1 hour |

**Table 4:** Absorbance of Soluble lignin after Thermal Treatment

| Name of Experiment | Absorbance of 10 ppm solution | Time of Autoclave |
|---|---|---|
| Stage 1 | 2.231 | 2 hours |
| Stage 2 | 1.631 | 2 hours |
| Stage 3 | 0.769 | 2 hours |
| Sample+ 100ml of distilled water, then kept in autoclave at 121 °C and 15 psi for 1hr | 2.014 | 1 hour |

**Table 5:** Absorbance of Soluble lignin after Thermal and Alkaline Treatment

| Name of Experiment | Absorbance of 10 ppm solution | Time of Autoclave |
|---|---|---|
| Thermal hydrolysis Stage 1 | 2.231 | 2 hours |
| Thermal hydrolysis Stage 2 | 1.631 | 2 hours |
| Thermal hydrolysis Stage 3 | 0.769 | 2 hours |
| Alkaline hydrolysis | 2.53 | 2 hours |

(Table 3) Above observations indicate that acid hydrolysis after third stage treatment has now no longer effect on delignification.

Further, 50% of conc. sulphuric acid has great impact on delignification of jatropha deoiled cake as compare to using 72% sulphuric acid.

**Thermal Hydrolysis of Jatropha Deoiled Cake**

The observations obtained from thermal treatment of jatropha de-oiled cake are summarized in Table-4.

**Thermal Followed by Alkaline Hydrolysis of Jatropha Deoiled Cake**

The observations obtained from thermal treatment of jatropha de-oiled cake are summarized in Table-5.

Results of alkaline treatment show that however after third stage aqueous medium was no longer beneficial for the removal of lignin, but more lignin was reported to be extracted out in the presence of 2% NaOH.

**G.C Analysis of Gas**

Chromatograph obtain with standard biogas sample is shown in Fig-2.

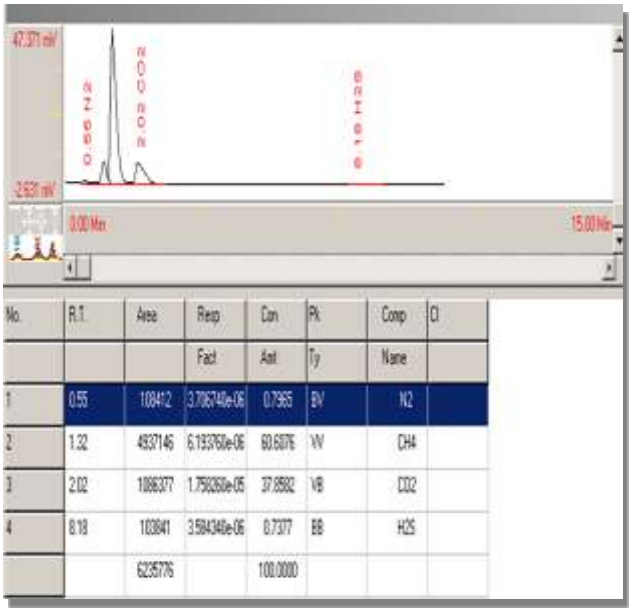


**Figure 2:** Chromatograph of Standard Biogas Sample

Above figure indicates the components of standard bio gas sample at different retention times.

Concentration of each component gas in biogas is shown in table.

This set was run to check the suitability of the system.

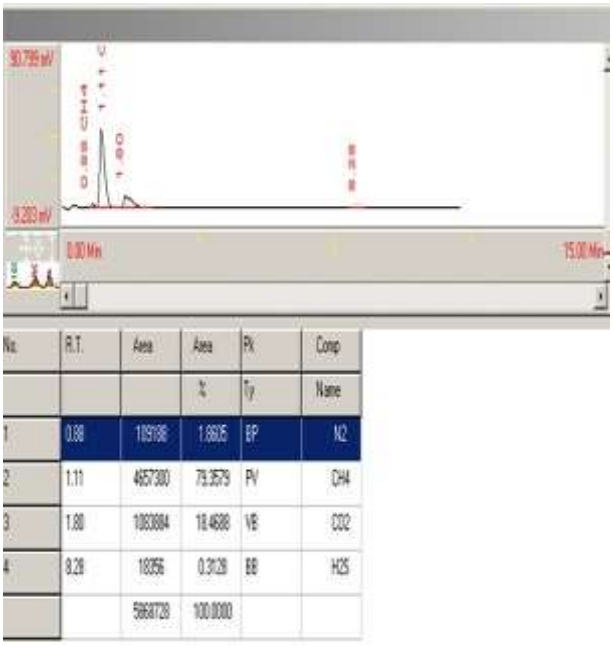
**Chromatograph of gas obtain after anaerobic digestion of acid treated (50%) biomass shown in Fig-3.**

Below observation indicates that gas produced after the anaerobic digestion of acid treated (50%) biomass yields 85.72% methane.

| No. | R.T. | Area | Resp Fact | Con Amt | Pk Ty | Comp Name | Cl |
|---|---|---|---|---|---|---|---|
| 1 | 0.72 | 406365 | 3.706740e-06 | 5.2263 | BV | N2 | |
| 2 | 1.25 | 3989081 | 6.193760e-06 | 85.7263 | VB | CH4 | |
| 3 | 1.57 | 108569 | 1.758260e-05 | 6.6233 | TTT | CO2 | |
| 4 | 8.11 | 194520 | 3.584340e-06 | 2.4241 | BB | H2S | |
| | | 4698935 | | 100.0000 | | | |

**Figure 3:** Chromatograph of Acid Treated Biomass

**Chromatograph of gas obtained after anaerobic digestion of thermal treated biomass shown in Fig-4.**



| No. | R.T. | Area | Area % | Pk Ty | Comp Name |
|---|---|---|---|---|---|
| 1 | 0.88 | 109186 | 1.6805 | BP | N2 |
| 2 | 1.11 | 4657380 | 79.3579 | PV | CH4 |
| 3 | 1.88 | 1083884 | 18.4688 | VB | CO2 |
| 4 | 8.28 | 18056 | 0.3128 | BB | H2S |
| | | 5868729 | 100.0000 | | |

**Figure 4:** Chromatograph of Thermal Pretreated Biomass

Above data indicates that anaerobic digestion of thermal treated biomass leads to methane content of 79.36% in total volume of biogas.

**Chromatograph of gas obtains after anaerobic digestion of alkaline treated biomass shown in Fig-5.**

Below chromatograph indicates that biogas generated after alkali treatments of jatropha deoiled cake has 81.12% of methane and 8.7% of carbon dioxide in total volume of biogas.

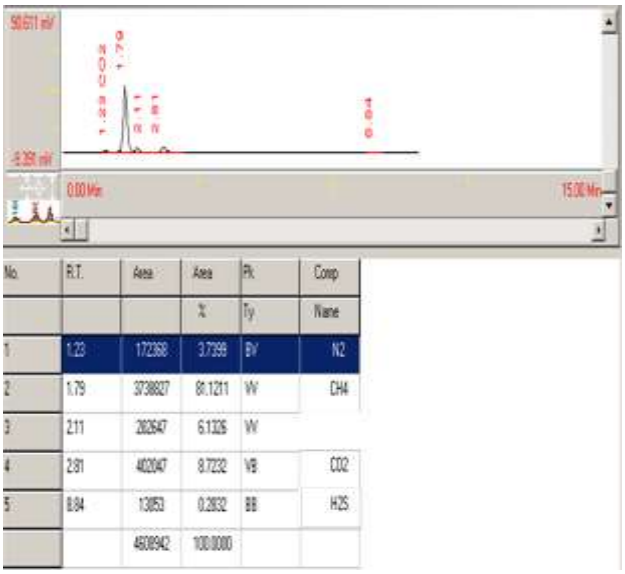



**Figure 5:** Chromatograph of Alkali Treated Biomass

## CONCLUSION

We have tried different pretreatment methods for delignification of jatropha deoiled cake and found that acid hydrolysis with 50% conc. sulphuric acid yields more acid soluble lignin as compared to 72% conc. sulphuric, resulting in comparatively economical method for pretreatment. Further, alkaline hydrolysis can also be claimed as a good method for delignification of jatropha deoiled cake. However, the methane content in biogas obtained was maximum after acid treated biomass as compared to alkaline treated biomass.

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**Source of Support:** Nil, **Conflict of Interest:** None.