

Cellulosic Conversion to Bioethanol from Pongamia Pod – A Biodiesel Industry Waste

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ABSTRACT:- Lignocellulosic biomass is the most abundant, sustainable and a promising source of renewable energy, present on the earth. This study investigates the potential of Pongamia pod, a waste residue left after biodiesel production from Pongamia Pinnata, as a source of bioethanol. Pretreatment optimization of finely ground samples by dilute sulphuric acid concentration of 1.0% and steam heating for 60 minutes released maximum cellulose of 297mg g⁻¹ of substrate (54% cellulose). The pretreated substrate was hydrolysed by cellulose degrading bacteria, isolated from the soil samples collected from Western Ghats of Belgaum region. Comparison of cellulose degradation capacity between isolated bacteria and pure culture was carried out. The analysis of hydrolysed product shows that maximum cellulose degradation by the isolated bacteria released 169mg of reducing sugars per gram of substrate, as compared to 162mg by pure culture. The sugars released during hydrolysis were converted into ethanol by fermentation by using yeast *Saccharomyces cerevisiae*. Fermentation result showed that bioethanol production of 7.1% v/v (isolated bacteria) and 6.6% v/v (pure culture) in the fermented broth thus indicating Pongamia pod as a potential source for bioethanol production.

Keywords:- Cellulosic ethanol, Cellulose degrading bacteria, Lignocellulose, Pongamia pod, Pretreatment.

I. INTRODUCTION

The demand for energy has increased steadily in the past few years due to rapid growth in population along with heavily industrialization of many countries. This energy demand at present is mainly met by fossil fuels. The use of fossil fuel has brought about negative climate change due to pollution, caused by release of unburnt petroleum products, by various sources like industries and vehicles. Due to this, there is a growing concerns regarding energy security as it is difficult to meet the fast growing demand for petroleum products, and also the global warming due to climate change in recent years. Keeping these in view, there is necessary felt to reduce the burden of using petroleum resources and to protect the degrading environment. Use of renewable sources like lignocellulosic waste for bioethanol production is one such attempt in addressing these issues. The use of renewable cellulosic wastes for the production of bioethanol has attracted researchers, as the feedstocks are renewable, cheaply available and do not create food versus fuel crisis. Lignocellulosic material is composed of cellulose, hemicellulose and lignin. Cellulose is the major component of the lignocellulosic material consisting of 40-60% dry weight, hemicellulose is the second major component consisting of 25-35% on dry basis, while the lignin is the third major component consisting of 20-35% on dry basis [1].

II. MATERIALS AND METHODS

2.1 Collection of substrate

Pongamia pod is used as a substrate for the production of bioethanol. *Pongamia* pod is collected from the Bagalkot District Biofuel Information and Demonstration Centre (BIDC-STEP), Bagalkot. The substrates were collected in a dust free and fungus free state. The substrate were dried in sunlight and were crushed by mechanical crusher to a fine powder and was screened by mesh of size 1mm.

2.2 Chemical Analysis of the substrate

Composition of the substrate and its properties were analyzed before pretreatment. The cellulose content and total carbohydrate in the substrate was estimated by anthrone method [2], Moisture content and ash content of the substrate were also performed [3].

2.3 Collection of Soil samples

Soil samples were collected from western ghats of Belgaum region, as it possess wide biodiversity of microorganisms. Soil samples were collected at a depth of 10-15 cm from top soil cover [4]. A total of ten soil samples were collected and stored at a temperature of 4°C before use to cease microbial activity.

2.4 Isolation of cellulose degrading bacteria

Isolation of cellulose degrading bacteria was done by using soil samples from western ghats. Pour plate technique on a basal media was used for initial isolation of bacteria from grown colonies. Basal media had the following media composition 2.5 % NaNO₃, 0.2 % MgSO₄, 2.0% KH₂PO₄, 0.2 % NaCl, 0.1 % CaCL₂.6H₂O, pH 6.8-7.0 [5]. Individual colonies were selected to further confirm the cellulolytic potential of the selected bacteria. This confirmation was done by inoculating the selected individual bacteria colony onto cellulose Congo red dye. The composition of cellulose Congo red dye is 0.5 % KH₂PO₄, 0.25 % MgSO₄, 2.0% Cellulose, 2.0 % Gelatin, 15.0 % Agar, and 0.2 % Congo red, pH 6.8-7.0 [5]. The growing bacterial colony having the largest clear zoning was confirmed to have highest cellulose degrading capacity [5]. This bacteria was selected for hydrolysis of the pretreated substrate.

2.5 Optimization of pretreatment process

The pre-treatment optimization for the substrate was carried out by using different combination of dilute sulphuric acid ranging from 0% to 6% and heating period of 30, 60 and 90 minutes at 121°C and 15lb pressure. 1gm of substrate was added with 10ml of dilute sulphuric acid (1:10). Cellulose release during this optimization process was analysed by anthrone method [2]. The substrate releasing maximum cellulose during optimum conditions during pretreatment were selected for hydrolysis process.

2.6 Hydrolysis of the pretreated substrate

Maximum cellulose released during the pretreatment were hydrolysed by the isolated cellulose degrading bacteria. The pretreated substrate were washed with distilled water several times to neutralise the acid concentration. The substrate was oven dried till constant weight and the pH was adjusted to 7.0. Pure culture *Cytophaga hutchisonni* (CH) (NCIM 2338) was procured from National Collection of Industrial Microorganisms (NCIM), Pune. Comparison study between isolated cellulose degrading bacteria and the pure culture, CH was performed. A 24hr grown inoculum of isolated cellulose degrading bacteria and pure culture, CH were added to the pretreated substrate. Reducing sugars release during substrate hydrolysis were analysed by Dinitrosalicylic Acid (DNS) method every 24hr from zero hour, for both the organisms [2]. Maximum sugars released during this period were further taken for fermentation to produce bioethanol.

2.7 Fermentation of hydrolysed broth

Fermentation is 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 36°C until maximum sugars are converted into bioethanol. The reducing sugar utilization during fermentation was analysed by DNS method [2] and the bioethanol production was analysed by using specific gravity method [6].

Calculation for specific gravity:

$$\text{Specific Gravity} = \frac{W2 - W1}{W3 - W1}$$

Where,

W1 = empty weight of specific gravity bottle

W2 = Weight of sample + specific gravity bottle

W3 = Weight of distilled water + specific gravity bottle

III. RESULTS AND DISCUSSION

3.1 Chemical analysis of the substrate

The chemical analysis of the substrate showed that the substrate consisted of 55% cellulose, Ash content of about 2.6% and moisture content of about 6%.

3.2 Isolation of Cellulose degrading bacteria

Total ten soil samples were inoculated in Petri plates, around 80 to 90 colonies were observed on each plate. The isolation of cellulose degrading bacteria was carried out on the basis of clear zone formation around the growing bacterial colonies, developed on the cellulose Congo red agar medium. Eight colonies per plate were selected randomly to grow on cellulose Congo red media. Larger the diameter of clear zone, higher the

cellulose degradation or hydrolysis capacity To select effective cellulose degrading bacteria, Petri plate was divided into four parts with single colony of bacteria was streaked onto one of the four quadrants (Figure 4.4). Clear zone is measured and the clear zone measuring more than one centimetre is selected for hydrolysis of substrate (Table 4.2). Total 26 effective cellulose degrading bacteria were selected and deposited in the Department of Biotechnology. In the present studies one very effective bacteria, WG3 was selected.

Table 1: Isolation and screening of cellulose degrading bacteria.

Sl.no	Soil sample	Number of colonies on BSM	Number of isolates selected from BSM to grow on Congo red media	Number of isolates showing maximum clear zone(>1cm)
1	WG1	89	8	3
2	WG2	75	8	3
3	WG3	91	8	4
4	WG4	78	8	2
5	WG5	65	8	2
6	WG6	89	8	2
7	WG7	81	8	3
8	WG8	82	8	2
9	WG9	86	8	3
10	WG10	83	8	2



Figure 1: Clear zone formation around the growing bacterial colonies indicating Cellulose degradation/hydrolysis.

3.3 Optimization of pretreatment of the substrate

Pretreatment optimization of substrate by various concentrations of dilute sulphuric acid (0% to 6%) at varying heating period (30, 60 and 90 minutes). Of the pretreated substrates dilute sulphuric acid concentration of 1.0% at 60 min of heating at 121°C was found to release maximum cellulose of 297mg per gram of substrate. This resulted in 55% of cellulose recovery from the substrate.

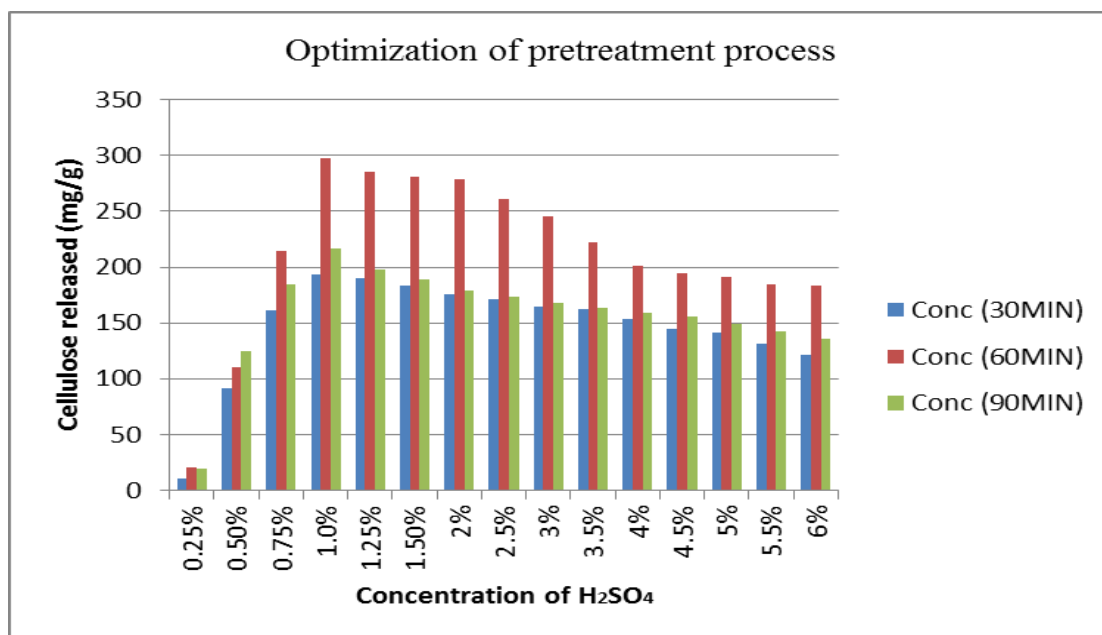


Figure 2 : Pretreatment optimization of substrate.

3.4 Hydrolysis of the pretreated substrate:

Hydrolysis is carried out to convert the cellulose obtained from pretreated substrate to fermentable sugars at 7 pH. Hydrolysis was carried out using isolated bacteria, WG3 and pure culture, CH. The maximum sugars released during the period of hydrolysis were selected for fermentation process.

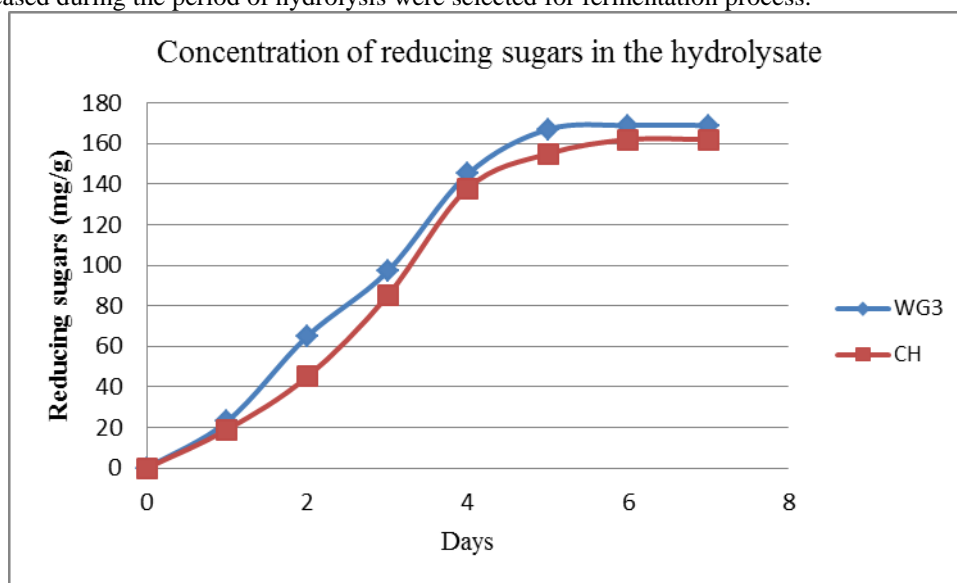


Figure 3: Hydrolysis of pretreated substrate.

Maximum cellulose degradation was observed in the isolated organism, WG3 as compared to the pure culture *Cytophaga hutchisonni*. The isolated organism released 169 mg of reducing sugars per gram of substrate as compared to the 162 mg from pure culture *Cytophaga hutchisonni*. Conversion was observed within 24h of inoculation. On 5th day maximum conversion is recorded (Figure 4.6). In both the cultures, growth was recorded constant after 5th day.

3.5 Fermentation of the hydrolysed broth

Fermentation of sugars released during hydrolysis of substrates was carried out by yeast *Saccharomyces cerevisiae* at pH 4.6 and 34°C temperature, to convert into bioethanol. Fresh inoculum of yeast (5% v/v) was added to the hydrolysed broth. Fermentation was carried out for six days with fermented samples being collected every twenty four hours for analysis of reducing sugar by DNS method for substrate utilization. Determination of bioethanol produced was carried out by specific gravity method.

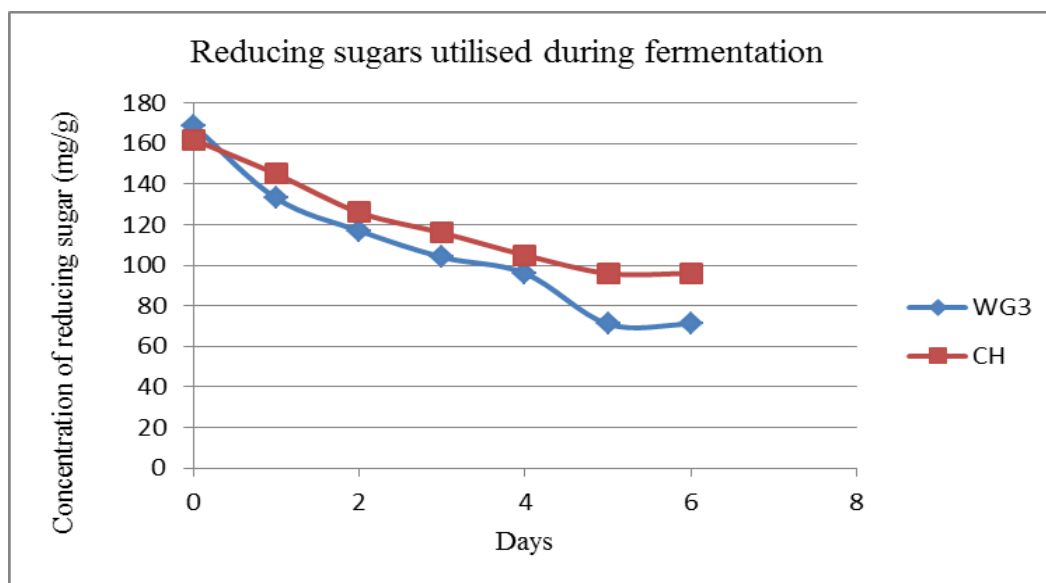


Figure 4: Utilization of reducing sugars during fermentation.

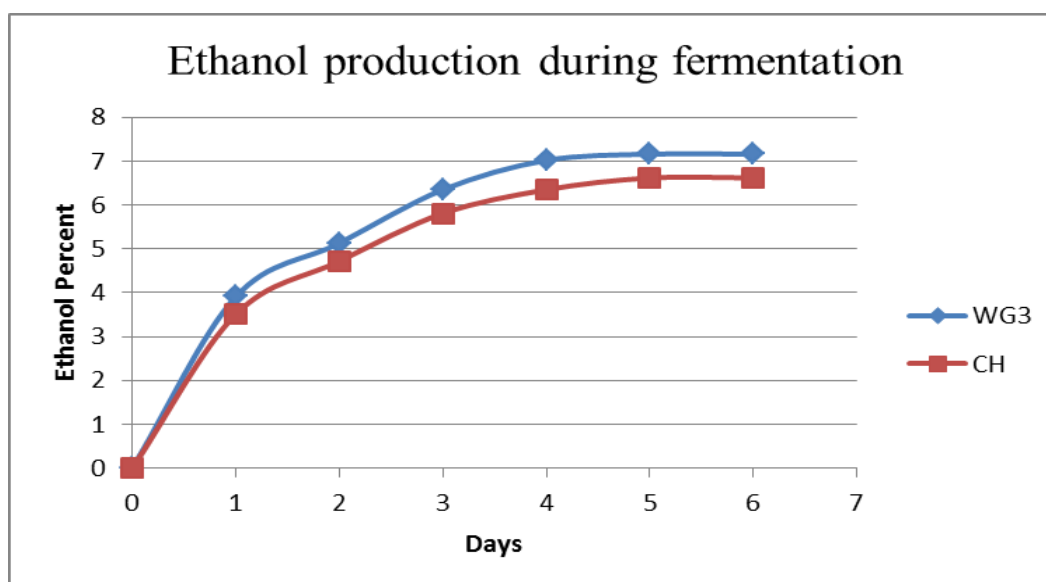


Figure 5: Percent of bioethanol produced.

Ethanol production was determined by specific gravity method. The maximum percent of bioethanol produced from *Pongamia* pod during the present studies was 7.1 % v/v (from isolated organism, WG3) and 6.6 % v/v (from pure culture, CH).

IV. CONCLUSION

In the present study, cellulosic waste such as *Pongamia* pod is a one of the good raw material for cellulosic bioethanol production. In order to separate the cellulose, hemicellulose and lignin, dilute sulphuric acid of concentration 1% and a heating period of 60 min at a temperature of 121°C was found to be most effective. The isolated cellulose degrading bacteria, WG3 converted maximum amount of cellulose into sugars as compared to pure culture, *Cytophaga hutchisonni*. The sugars released during hydrolysis were converted into ethanol by *Saccharomyces cerevisiae* during fermentation. Based on these results, *Pongamia* pod can be used as a potential feedstock for bioethanol production. The biological hydrolysis of cellulose by isolated bacteria instead of costly cellulase enzymes or by acid hydrolysis is more economical and environment friendly.

ACKNOWLEDGMENT

The author would like to thank Dr. Bharati S. Meti for her constant support and guidance during project work. The author also acknowledges TEQIP-II for funding the project and making the project successful.

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