



Saccharification of *Acacia auriculiformis* Benth. biomass for the production of glucose using phosphoric acid pretreatment

Nitesh K. Mund*, Nihar R. Nayak

Regional Plant Resource Centre, Nayapalli, Bhubaneswar- 751015, Odisha, India.

Received: December 14, 2016; Accepted: December 29, 2016

Abstract: *Acacia auriculiformis* is an exotic plant species luxuriantly grown in different parts of India. Because of its drought tolerance potential and wood quality, it is widely grown as timber crops in some part of the country. In this study we have addressed the possible use of *A.auriculiformis* for production of glucose, an intermediate for the production of ethanol. Chemical composition of this species was carried out and it was found that this species contains about 34 % glucan. Using Cellulose –Solvent and Organic-solvent based Lignocellulose Fractionation pretreatment the biomass of the species was hydrolyzed at two different dosages of cellulase. It was found that even at low cellulase loadings [1 Filter Paper Unit (FPU)] about 10 International Units (IU) of β -glucosidase about 88 % glucan conversion took place. This study suggests the use of this species as a biofuel feedstock for next generation fuels.

Key words: *A. auriculiformis*; enzymatic saccharification; pretreatment

Introduction

Energy is one of the most important features for socio-economic status and development of a country. Its production and consumption level exhibits not only the economic status but also the standard of living and per capita income of a country. Since last few decades fossil fuel has been dominating over all other types of energy resources particularly in transportation sector because of its easy availability and cost effectiveness. However, the limited source of fossil fuel has put the world fuel scenario in such a position that the price of crude fossil oil is fluctuating so rapidly that the cost crude oil has increased up to \$ 140 per barrel. Rise in the price of fossil fuel has put many countries into a serious problem worldwide particularly to the developing countries and the countries with limited or no fossil fuel reserve of its own like India. So far as the Indian fuel context is concerned, it has been depending on the foreign countries (mostly Arab countries) to meet its energy needs due to lack of enough fuel reserve of its own. Moreover, fossil fuel generates greenhouse gases declining the quality of the atmosphere. Viewing such a critical situation of increasing price, limited resource and non-renewability of fossil fuels, the concept of biofuels strikes the mind (Fairley, 2011; Carroll and Somerville, 2009; Tilman et al., 2012; and Graham-Row, 2011). Biofuels are derived from renewable plant products providing a platform to think of the sustainable development (Regalbutto, 2009). In future, these fuels may play a key role as being the main substitute of the present-day fuels in order to support the transportation sector of India. Production of biofuels started with the fermentation of sugar from corn and sugarcane to ethanol and is found to be successful. Later the technology moved to extract sugars from cellulose of

woody biomass (including crop and forest residues and wood chips) enzymatically and convert them to ethanol. However, ethanol has several disadvantages and proved not to be substitute for gasoline. Productions of ethanol from corn starch and sugarcane sugar compete with food. Conversion of lignocellulosic feedstocks to ethanol is a complicated process and thus becoming more expensive than corn based ethanol. Improvement in this technology has taken place that converts sugars into hydrocarbon with the help of some of the genetically altered organisms. Dissolved sugars can also be converted into hydrocarbons through routes that resemble petroleum processing more than fermentation. Crystallinity of the biomass due to the presence of extensive hydrogen bonds in cellulose microfibrils, which hinders the cellulase enzymes to digest the substrate (Karp *et al.*, 2015). In addition to this, presence of lignin makes the lignocellulosic biomass more recalcitrance. Hence, a pretreatment is needed either to remove the lignin from the biomass or to reduce the crystallinity. Different kinds of pretreatment processes have been developed in recent times with higher efficiency towards removing lignin. Alkali pretreatment using NaOH, dilute acid pretreatment using H₂SO₄ are thoroughly established technologies being used industrially. However, both of these pretreatments are not efficient enough to release the glucose from cellulose substrates at lower cellulase enzyme loadings. Herein, we have addressed recently developed COSLIF pretreatment which uses phosphoric acid as a cellulose solvent and alcohol as an organic solvent which efficiently deconstruct the biomass.

*Corresponding Author:

Mr. Nitesh K. Mund,

Research scholar,

Regional Plant Resource Centre,

Nayapalli, Bhubaneswar- 751015,

Odisha, India.

E-mail: mund.nitesh@gmail.com



This study was carried out to standardize the saccharification process of the *A. auriculiformis* in a laboratory scale, one of the important steps for the bioethanol production. Biomass of the species of the mature plants was collected and compositions were analyzed. The biomass was pretreated with COSLIF and the glucose released percentages after 72 h were calculated. *A. auriculiformis* is a tree of 8- 20 m in height belonging to the family Mimosaceae. It has been planted for wood production and erosion control in many tropical areas of the world. This species is fast growing; nitrogen fixing and can grow very well on marginal soils. No major pest and disease problem has been detected and it shows good tolerance to moderate dry seasons. It can also withstand water logging stress. The wood is heavy, hard, and durable and has a high calorific value. It is an ideal fuelwood used in China, India and throughout Asia. Charcoal is not too heavy and glows well with no smoke or sparks. In our estimation of wood density, it shows 849.9 kg/m³. *A. auriculiformis* is native to Australia, Papua New Guinea and Indonesia. It has been introduced in India due to its above-mentioned characters. With the support of the National Bank for Agriculture and Rural Development this plant has been planted in Tamil Nadu, Kerala, West Bengal, Andhra Pradesh, Karnataka and many other parts of India for rehabilitation of waste lands of India. No major research work has been reported on this species except few medicinal properties.

Materials and Methods

The biomass of *A. auriculiformis* was collected from Regional Plant Resource Centre, Bhubaneswar, India and used for all the experiments. Biomass was air dried and milled using a laboratory cutting mill (Wiley Online). Ground biomass was sieved to obtain particle size between 40-60 mesh for compositional analysis and pretreatment studies. The chemicals used HiMedia (India). Cellulase from *Trichoderma reesei* and glucosidase from *Aspergillus niger* were procured from Sigma (C2730 and 49291 respectively). Cellulase enzyme had an activity 64 FPU ml⁻¹, while the β -glucosidase activity of glucosidase enzyme was 683 IU g⁻¹.

Analysis of biomass composition

Moisture content (%) air dried biomass was estimated by incubating biomass in a hot air oven maintained at 105°C until the constant weight was achieved. Both moisture content and % total solid were calculated using the equation described elsewhere (sluiter *et al.*, 2008^a). Glucan, lignins, ash and extractive contents were measured using Standard National Renewable Energy Laboratory (NREL) protocols. (sluiter *et al.*, 2008^b) About 300 mg biomass digested with 72% H₂SO₄ at 30°C in a water bath for 1 h followed by a secondary digestion with 4% H₂SO₄ (by adding 84 g DI water) at 121°C for 60 min in an autoclave (Nat Steel, India, liquid setting). Acid soluble lignin (ASL) and glucan were estimated from the hydrolysate after neutralization. Glucose was analyzed by using glucose assay kit (Cayman, 10009582). Amount of

extractive in biomass was estimated by using 95 % ethanol in a soxhlet apparatus with a reflux time 24 h and 4-5 siphon cycles per hour (sluiter *et al.*, 2005^a). For the determination of ash, oven dried biomass of particle size of 1 mm were kept in a crucible and burnt in a muffle furnace at 575 °C for 4 h (sluiter *et al.*, 2005^b)

COSLIF Pretreatment

For COSLIF pretreatment, 1.05 g biomass of particle size 40-60 mesh was digested at a fixed percentage of H₃PO₄(85%) in 50 ml plastic centrifuge tubes at 50°C for 45 min in water bath (zhang *et al.*, 2007 and Zhu *et al.*, 2009) 20 ml of 95 % (v/v) pre-chilled ethanol was added to the tube and vortexed vigorously. After the addition of ethanol, Separation of liquid was carried out in a swing bucket centrifuge at 4000 rpm at room temperature for 10 min two times. In the second phase 40 ml ethanol was used. To remove any trace of ethanol, washing with addition of DI-water was carried out for two additional times (each time with 40 ml). Pretreated biomass pellets were neutralized by addition of 2 M Na₂CO₃.

Enzymatic saccharification of pretreated samples

For enzymatic hydrolysis, neutralized pellets were dissolved in 35 ml 50 mM sodium citrate buffer (pH 4.8) supplemented with 0.1% (w/v) sodium azide. Hydrolysate were prewarmed at 50°C and two different dosages of cellulase (1 FPU and 5 FPU) along with 10 IU β -glucosidase were added. Enzymatic hydrolysis studies were carried out in 125 ml plastic bottles in a rotary shaker set at 250 RPM and 50°C. Hydrolysis after different time intervals (2h, 4h, 6h, 12h, 24h, 48h and 72h) was evaluated by taking 1 ml of well mixed samples and centrifuging them at 13,500 rpm for 5 min. From the supernatant, glucose content was estimated and glucose released percentages were calculated by using the formula

Glucose released (%) =

[Glucose (mg) in hydrolysate X 0.9 / glucan (mg) in untreated sample] X 100

FE-SEM Analysis

Both untreated and COSLIF treated biomass were examined under the field emission scanning electron microscope (Carl Zeiss Sigma VP FE-SEM). The samples coated with gold particles and imaged under vacuum condition at accelerating voltage of 2 kV.

Results

Compositional analysis of biomass

From the results of compositional analysis, it was found that *A.auriculiformis* contained 33.98 ± 0.825 % glucan, 25.62 ± 1.2% acid insoluble lignin 7.2 % ash and 14.2 % extractives.

Enzymatic hydrolysis of pretreated biomass

COSLIF pretreated samples digested with two different dosages of cellulase (1 FPU and 5 FPU) along with a fixed β -glucosidase dosage (10 IU). Progress in enzymatic hydrolysis shown in Fig.1. When the biomass

was digested with 5 FPU cellulase initial glucose release was faster and 54.12% glucose got released after 1 h of digestion. After 6 h of digestion 80.3 % glucan got converted and the eventual glucose release percentage was 94.14 % after 72h. In contrast, pretreated biomass when hydrolysed with 1 FPU cellulase initial glucose release was lower and it was only 57.25% after 6 h, however, upon the completion of hydrolysis after 72 h higher fraction of glucan got converted into glucose (87.53%). Lower dosage of cellulase though resulted in less glucan digestibilities during initial hours, yet appreciable amount of glucose release was noticed after 72h.

Scanning electron micrographs of the raw and pretreated samples have been shown in the Fig. 2. The untreated biomass observed under the scanning electron microscope (Fig.2 a) comprises intact cells with fibrillar structures. COSLIF pretreated feedstock (Fig.2b) shows a clear destruction of cell structure in which no intact cell or fibrillar structure is visible. This result suggests that the COSLIF pretreatment with H_3PO_4 destructed the intact or crystalline structure of cell wall which resulted in the increase in surface area for cellulase enzyme to act. Similar effects have been reported from other biomass like switch grass (Rollin *et al.*, 2010) and industrial hemp stalks (Moxley *et al.*, 2008).

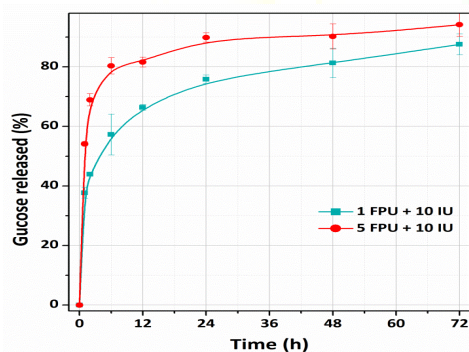


Figure 1: Glucan conversion (%) profiles of COSLIF (85 % H_3PO_4 , 50°C, 45 min) pretreated biomass at different time intervals of enzymatic hydrolysis with the use of 1 FPU and 5 FPU cellulase along with 10 IU β -glucosidase

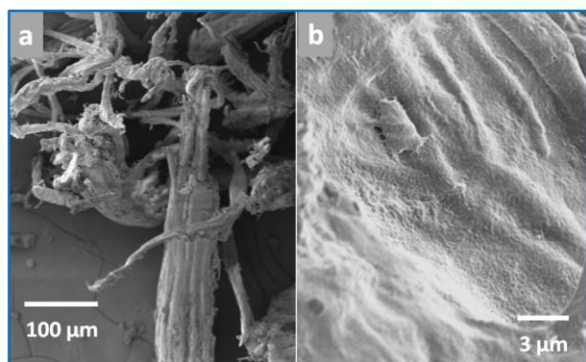


Figure 2: Scanning electron microscopic images of different biomass
a. Untreated biomass,
b. COSLIF (85 % H_3PO_4 , 50°C, 45 min) pretreated biomass.

Discussion

Higher glucan digestibilities even at lower cellulase suggests that COSLIF could make the amorphous by deconstruction of internal bonds which increases the cellulose accessibility, hence, lower dosage of cellulase can even digest higher fraction of cellulose. These findings could be correlated to the electron micrographs obtained from both pretreated and untreated biomass. In COSLIF pretreatment recovery of the cellulose solvent and organic solvent is one of the important steps. Fractional loss of any of the pretreating candidates could have a negative impact on the process. Ethanol, herein, used a organic solvent which solubilizes the pretreated acid without dilution. As the boiling point of the water and ethanol are different, recovery of ethanol could be carried out using distillation process (Zhang *et al.*, 2007).

Conclusion

Characteristics of *A. auriculiformis* provide a scope to be used as a feedstock for next generation fuels. Drought tolerance capacity of the species makes it a suitable candidate to be brought under cultivation in rain fed areas. COSLIF pretreatment used in the study shows the efficiency of the glucan conversion procedure even at the use of extremely lower dosage of cellulase, which could be further used in industrial scales.

References

- Karp EM, Resch MG, Donohoe BS, Ciesielski PN, O'Brien MH, Nill JE, Mittal A, Bidy MJ, Beckham GT, Alkaline Pretreatment of Switchgrass, ACS Sustainable Chem Eng, 2015,3:1479–1491
- Rollin JA, Zhu Z, Sathitsuksanoh N, Zhang YHP, increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia, Biotechnol Bioeng, 2010,108, 22–30
- Moxley G, Zhu Z, Zhang YHP, Efficient sugar release by the cellulose solvent-based lignocelluloses fractionation technology and enzymatic cellulose hydrolysis, J Agric Food Chem, 2008, 56, 7885–7890
- Zhang YHP, Ding SY, Mielenz JR, Cui JB, Elander RT, Laser M, Himmel ME, McMillan JR, Lynd LR, Fractionating recalcitrant lignocellulose at modest reaction conditions, biotechnol Bioeng, 2007, 97, 214–223.
- Zhu Z, Sathitsuksanoh N, Vinzant T, Schell DJ, McMillan JD, Zhang YHP, Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: enzymatic hydrolysis, supramolecular structure and substrate accessibility, Biotechnol Bioeng, 2009, 103, 715–724.
- Carroll A, Somerville C, Cellulosic biofuel, Annu Rev, Plant Biol, 2008, 60: 165-82.
- Fairley P, Next generation biofuels, Nature, 2011, 474: S2-S5.

8. Graham-Rowe D, Beyond food versus fuel, Nature,2011, 474: S6-S7.
9. Tilman D, Socolow R, Foley JA, Hill J, Larson E, Lynd L, Pacala S, Reilly J, Searchinger T, Somerville C, Williams R, Beneficial biofuels—the food, energy, and environment trilemma, Science, 2012, 325: 270-271.
10. Regalbuto JR, Cellulosic biofuels—got Gasoline? Science, 2009, 325: 822-824.
11. Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Wolfe J, Determination of total solids in biomass and total dissolved solids in liquid process samples, Technical report NREL/TP-510-42623 prepared for National Renewable Energy Laboratory: Golden, CO, 2008^a.
12. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D, Determination of structural carbohydrates and lignin in biomass, Technical report NREL/TP-510-42618 prepared for National Renewable Energy Laboratory, Golden, CO, 2008^b.
13. Sluiter A, Hames B, Ruiz R, Scarlata R, Sluiter J, Templeton D, Crocker D, Determination of extractives in biomass, Technical report NREL/TP-510-42619 prepared for National Renewable Energy Laboratory: Golden, CO, 2005^a.
14. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D, Determination of ash in biomass, Technical report NREL/TP-510-42622 prepared for National Renewable Energy Laboratory: Golden, CO, 2005^b.

Cite this article as:

Nitesh K. Mund and Nihar R. Nayak. Saccharification of *Acacia auriculiformis* Benth. biomass for the production of glucose using phosphoric acid pretreatment. *Annals of Plant Sciences* 6.01 (2017): 1512-1515.

DOI: <http://dx.doi.org/10.21746/aps.2017.01.005>

Source of support: Nil.

Conflict of interest: Nil