Waste Biomass Valor (2014) 5:963–971
DOI 10.1007/s12649-014-9305-8

ORIGINAL PAPER

Factors Affecting Bioethanol Production from Lignocellulosic Biomass (*Calliandra calothyrsus*)

S. Y. Adaganti · V. S. Yaliwal · B. M. Kulkarni · G. P. Desai · N. R. Banapurmath

Received: 30 August 2013 / Accepted: 7 April 2014 / Published online: 27 April 2014

Abstract India is in need of renewable fuels for transportation and power generation applications. Bio-ethanol, a second generation fuel is considered as one of the most important promising alternative fuel for both petrol and diesel engine applications. The molasses feedstock is the main source for ethanol production in India, but it is hardly sufficient to meet the current growing demand. Lignocellulosic biomass is an alternative, renewable and sustainable feedstock to meet the demand of ethanol. In the present study, experiments were carried out with the main objective of bio-ethanol production from *Calliandra calothyrsus* shrub, a potential lignocellulosic raw material for cellulose-to-bioethanol process. In view of this, *C. calothyrsus* biomass was pretreated with hydrothermal explosion using hot water, a method prior to hydrolysis process to produce fermentable sugars. Based on the experimental results, 2.67 and 1.72 g/L glucose was obtained with H_2SO_4 and HCl acid hydrolysis respectively for pretreated biomass. Also the present research work involves experimental investigations of bioethanol production from *C. calothyrsus* using batch fermentation. The results revealed that pH 4.5, temperature 30 °C and incubation period of 72 h were found to be favorable for producing maximum bioethanol yield. Further, study on hydrolysis was extended using enzyme, that resulted in 16.5 and 10.25 g/L glucose with and without pretreatment respectively.

Keywords *Calliandra calothyrsus* · Bioethanol · Biodiesel · *Saccharomyces cerevisiae*

S. Y. Adaganti (✉)
Chemical Engineering Department, SDM College of Engineering and Technology, Dharwad, Karnataka, India
e-mail: shivanand@sify.com

V. S. Yaliwal (✉)
Mechanical Engineering Department, SDM College of Engineering and Technology, Dharwad, Karnataka, India
e-mail: vsyaliwal2000@rediffmail.com

B. M. Kulkarni · G. P. Desai
Chemical Engineering Department, Babuji Institute of Engineering and Technology, Davangere, Karnataka, India

N. R. Banapurmath
Mechanical Engineering Department, BVB College of Engineering and Technology, Hubli, Karnataka, India

Introduction

In the present energy scenario, demand for energy is on a continual increase. Environmental concerns and depletion of fossil fuel reserves have led to the extensive search for alternate fuels [1, 2]. For a sustainable development through biomass present a very promising alternative fuel since biofuels derived from biomass have numerous advantages compared to fossil fuels [3–10]. Bioethanol production from conventional feed stocks like sugarcane and starch rich feed stocks such as corn, potato etc., has been reported in the literatures as first generation process. However, they have social and economical barriers [11, 12]. Therefore, second generation processes to produce bioethanol are gaining momentum. The second generation processes will use lignocellulosic biomass (corn stover, sugarcane bagasse, straws, stalks and switch grass) for this purpose and biosphere clearly has sufficient supplies of lignocellulosic materials. Bio-ethanol from lignocellulosic biomass is one of the important alternatives being considered due to the easy adaptability of this fuel to existing engines with higher octane rating [13, 14]. The production of ethanol from lignocellulosic biomass has been reported.

These sources have widespread abundance and the cost of their procurement is relatively cheap [15–21].

Lignocellulosic biomass contains 35–50 % cellulose, 25–30 % hemicelluloses and 20–25 % lignin [11, 22]. The various processes involved during the conversion of lignocellulose biomass to ethanol are: pretreatment, acid or enzymatic hydrolysis, fermentation and separation [20]. The task of hydrolyzing lignocellulose to fermentable monosaccharides is still technically problematic because the digestibility of cellulose is hindered by many physico-chemical, structural and compositional factors. Owing to these structural characteristics, pretreatment is an essential step for obtaining potentially fermentable sugars in the hydrolysis (saccharification) step. The aim of the pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose for enhancing enzymes accessibility to the cellulose during hydrolysis step [23–27].

Current pretreatment research is focused on identifying, developing, evaluating, and demonstrating promising approaches that primarily support hydrolysis of the pretreated biomass. Several investigators have reported various pretreatment methods such as physical (mechanical), physico-chemical, chemical and biological pretreatment methods using wide variety of feed stocks [20, 27, 28]. One of the most promising pretreatment method appears to be physico-chemical pretreatment i.e., hydrothermal pretreatment. Several researchers have investigated different hydrothermal pretreatment methods like steam explosion, ammonia fiber explosion, CO_2 explosion and liquid hot water process for bioconversion of lignocellulosic biomass to ethanol [21, 25, 29–35]. In the present work, to enhance the effectiveness of pretreatment, hydrothermal explosion method has been studied where in liquid hot water and steam explosion methods were combined. It is the most attractive pretreatment method for lignocellulosic materials due to non use of chemicals and efficient biomass disruption characteristics.

In this context, the present work focus on the hydrothermal explosion pretreatment method for bioethanol production using *Calliandra calothyrsus* shrub, a potential lignocellulosic biomass for cellulose-to-bioethanol process which can be an alternative to starch or sugar-containing feedstock. Further, various factors affecting bioethanol yield is presented subsequently.

Status of Bioethanol Fuels in India

India has 16 % of the world's population and the fourth largest petroleum consumer but has only 0.5 % of the oil and gas resources of the world. And the energy needs of the country are met from 80 % of oil imports [36]. In the year 2003, Planning Commission, Government of India has reported bio-ethanol and biodiesel as fuels for the nation and proposed blending in a gasoline and diesel up to 10–20 % by 2011–2012. The current share of biofuels in the consumption of transportation fuels is extremely low and is confined mainly to 5 % blending of ethanol in gasoline even though government had made it mandatory in 11 states and three union territories of the nation. India has set a target to reduce 50 % crude oil import by 2020 and 75 % by 2025 and eventually achieve self-sufficiency and energy independence by 2030.

According to the survey, it is observed that around 330 distilleries in India produce ethanol which is predominantly produced from molasses with an installed annual capacity of 4 billion liters, 60 % is mainly consumed by the beverage industry, 25 % share going into chemical industry and remaining 15 % share for blending with fossil fuel, which is not sufficient to meet the energy demand [36]. About 1.5 billion liters of ethanol is required for 10 % blending to meet the current demand and projected to be 2.2 billion liters in 2017 [36]. Currently, the government does not allow the use of imported ethanol for the Ethanol Blending Program and the focus is completely on indigenously produced ethanol. To overcome these challenges, second generation ethanol produced from lignocellulosic biomass is an alternative to ethanol derived from molasses. Since, India is land of agriculture producing nearly 500 million tons of biomass per year and hence producing ethanol from biomass is promising renewable source [37].

Characterization of Biomass and Bioethanol

In the present study, bio-ethanol is derived from *C. calothyrsus,* a lignocellulosic biomass. *C. calothyrsus* was collected locally from the Western region of Karnataka (India). It is widely distributed in humid/sub humid regions of central and South America and Mexico, It is also found in Africa abundantly and widely used as fodder and fuel wood. It is popularly known as red *Calliandra,* one of the promising lignocellulosic feed stock for bioethanol production due to its high cellulose content. *C. calothyrsus* is a small, perennial, thornless multi-stemmed shrub which grows 2–12 m in height with a trunk diameter of 25–40 cm; it belongs to the family of *mimosoideae*. It grows in all soil types and sandy clays. The cellulose of red *Calliandra* can be harnessed for ethanol production. The leaves and twigs were removed and stems were cut into useable sizes, dried and stored for studies. Samples were ground in a grinder. Figure 1a and b shows the *C. calothyrsus* plant, and stem of *C. calothyrsus* shrub. Figure 1(c) shows sample of raw material.

The raw material was oven dried at 105 °C until constant weight and stored in a sealed plastic bag at ambient

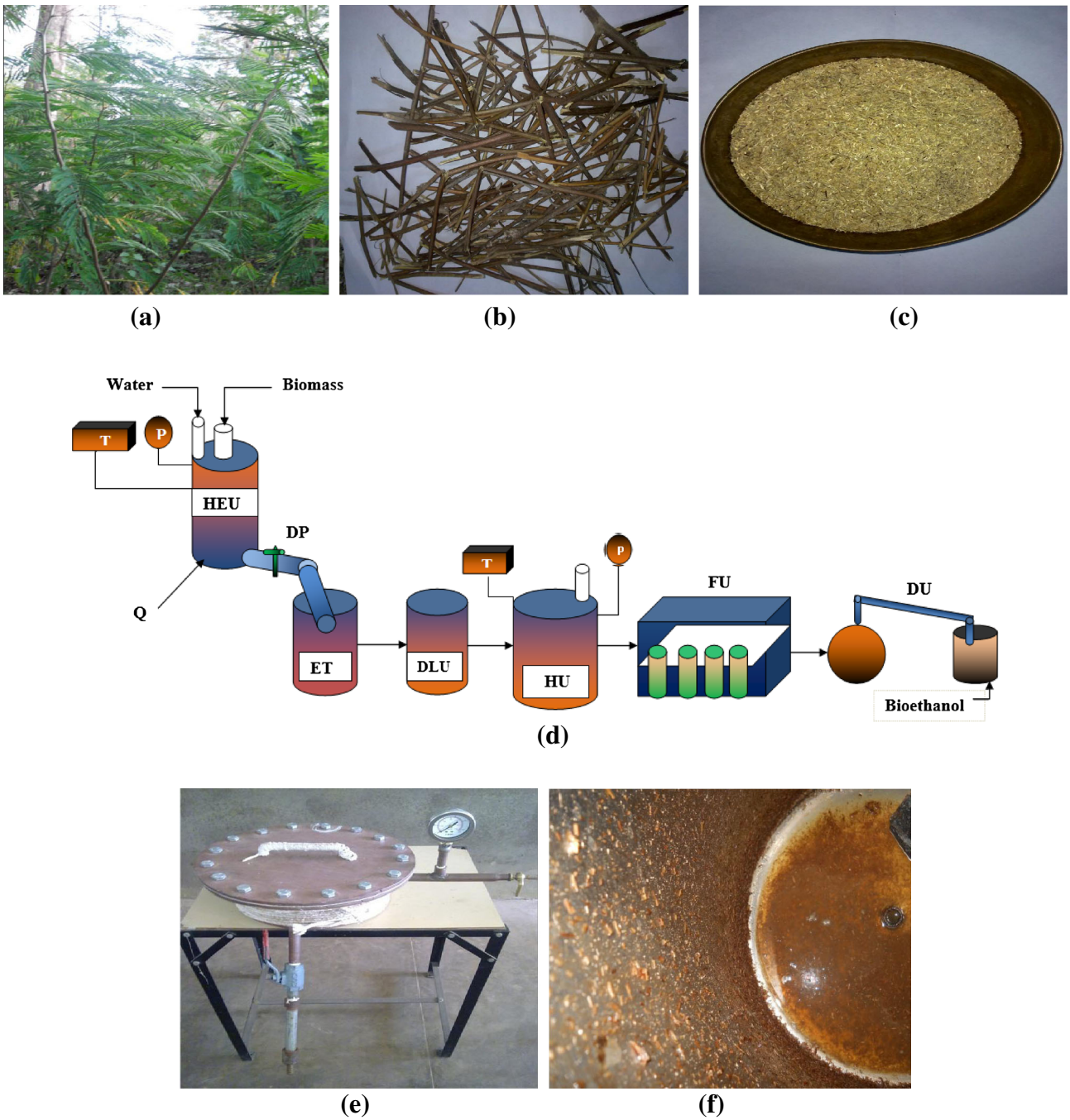
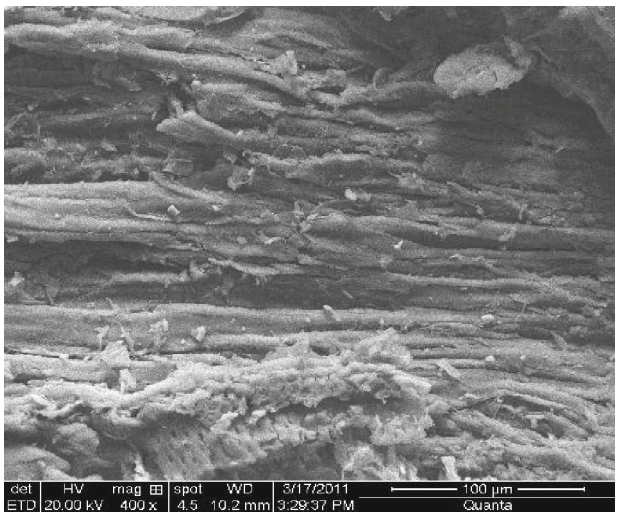
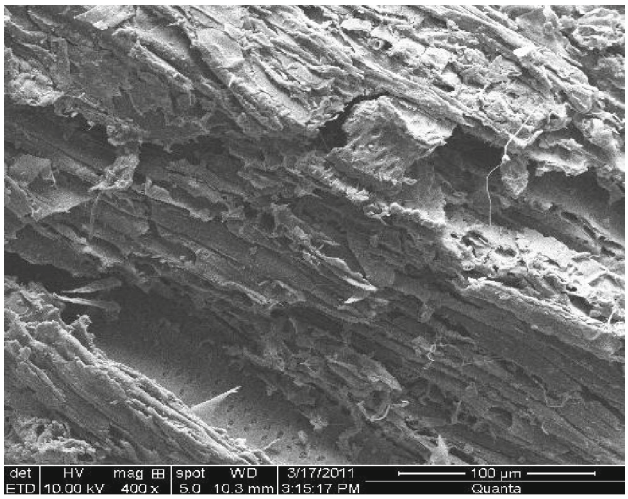


Fig. 1 **a** *Calliandra calothyrsus* shrub. **b** Stem of *Calliandra calothyrsus*. **c** Raw material. **d** Line diagram of bioethanol production, *T* temperature sensor, *P* pressure sensor, *HEU* hydrothermal explosion unit, *ET* explosion tank, *DLU* delignification unit, *HU* hydrolysis unit, *FU* fermentation unit (rotary incubator), *DU* distillation unit, *Q* heat supply. **e** Hydrothermal explosion unit. **f** Hydrothermal explosion of *Calliandra calothyrsus*

temperature for chemical analysis. Composition of the raw material and properties of bioethanol were determined at our college laboratory and are presented in Tables 1 and 2. Cellulose and hemicellulose were measured according to the procedures reported in the literature [38]. Klason lignin, ethanol–benzene solubles and ash content was determined by using TAPPI Standard Test Methods T 222 om-06, T 204 cm-07, T 413 om-06 respectively. Due to minor significance of other fractions present in the material, they were not determined.



(a)



(b)

Fig. 2 SEM images of *Calliandra calothyrsus.* **a** Before hydrothermal explosion. **b** After hydrothermal explosion

Production of Bioethanol from Lignocellulosic Biomass

In the present work, bioethanol was produced from *C. calothyrsus* biomass using hydrothermal explosion pretreatment followed by acid hydrolysis, fermentation and separation. Figure 1d shows line diagram of bioethanol production.

Pretreatment

The dried stem of *C. calothyrsus* was reduced to the size of 3 mm and stored before pretreatment. In this study, hydrothermal explosion treatments were carried out without chemicals in pilot scale equipment with a 5-L reactor (with a maximal operating pressure of 12 bars) provided with a quick-opening ball valve. Raw material and water (1:50 w/v) was taken in hydrothermal explosion unit and the ball valve was closed. The vessel was heated and the temperature is maintained at 152 °C for 45 min. After the desired time, the ball valve was opened, which caused a rapid explosive decompression and disintegration of biomass material.

During the hydrothermal pretreatment, biomass extractives were washed with hot water, hemicellulose is solubilized completely and partial hydrolysis of lignin. At the end of treatment, the solid residue was recovered by filtration and washed with distilled water to remove the water-soluble fraction. Morphological characteristic of the biomass before and after hydrothermal explosion is discussed in the subsequent paragraph (Fig. 2a, b).

Table 1 Composition of *C. calothyrsus* used for bioethanol production

Raw material	*Calliandra calothyrsus* stem (%)
Cellulose	40–45
Hemicellulose	16–20
Lignin	25–30
Ash	3–4
Extractives	16–18

Table 2 Properties of bioethanol

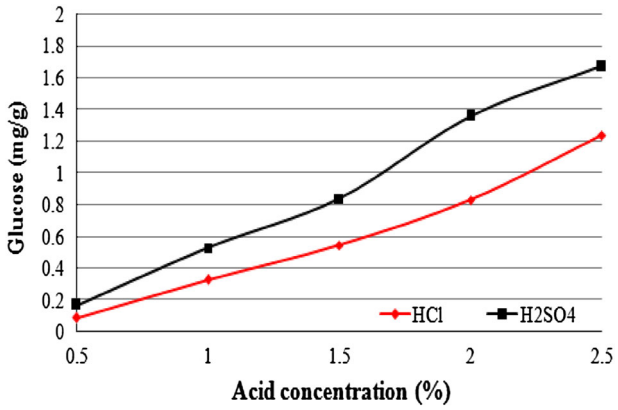
Property	Ethanol from molasses	Bioethanol from *Calliandra calothyrsus*	Test method
Density (kg/m^3)	781	790	ASTM D1298
Kinematic viscosity (cst) @ 40 °C	1.24	1.0016	ASTM D445
Flash point °C	14	12	ASTM D93
Calorific value (kJ/kg)	21.05 (kJ/L)	21.25 (kJ/L)	ASTM 2382

Delignification

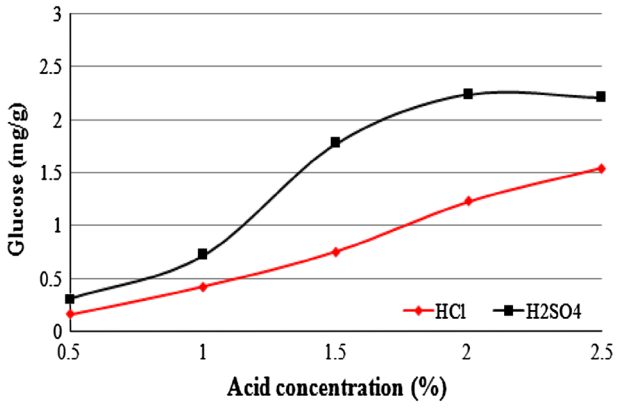
Delignification of *C. calothyrsus* was performed using 10 % NaOH for 2 h at 70 °C. The biomass is thoroughly washed with distilled water until it was colorless and neutral, and stored in a sealed bag before hydrolysis [26].

Acid Hydrolysis

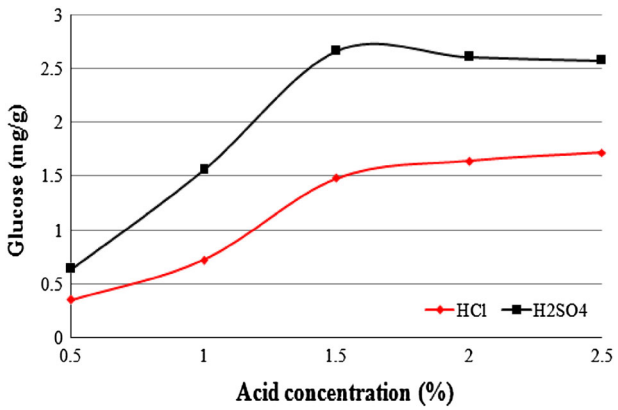
Batch experiments were conducted to study the effect of parameters on hydrolysis reaction using HCl and H_2SO_4



(a)

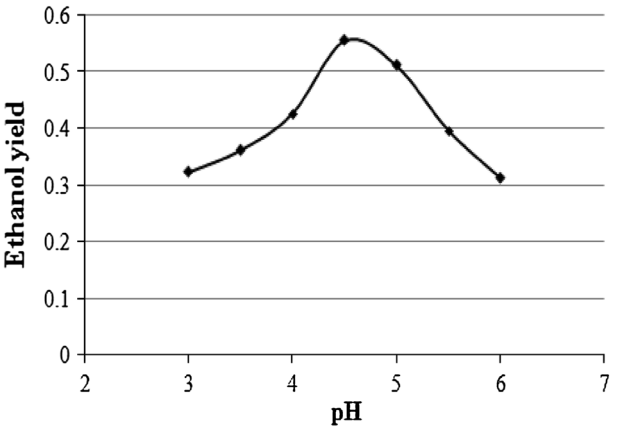


(b)

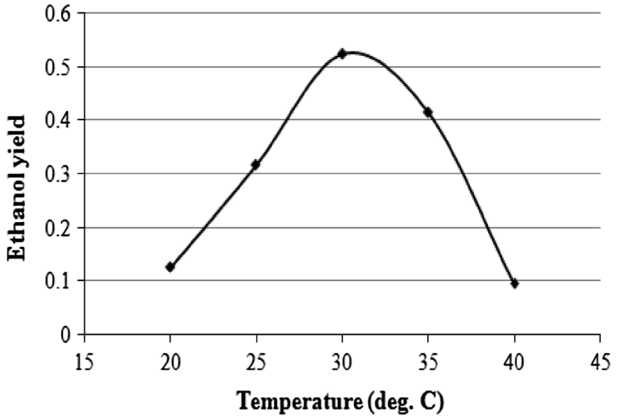


(c)

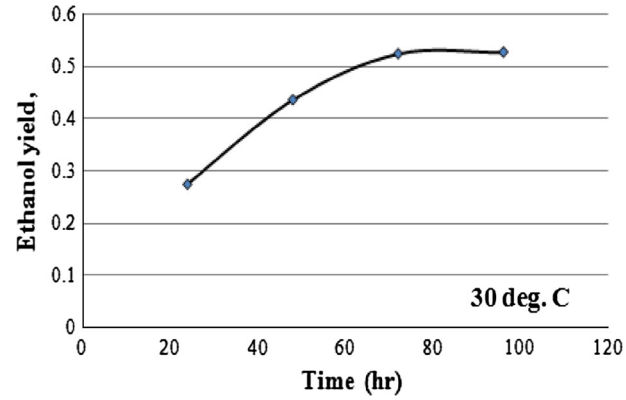
Fig. 3 Glucose yield at varied acid concentration. **a** Glucose yield at varied acid concentration for 60 min. **b** Glucose yield at varied acid concentration for 90 min. **c** Glucose yield at varied acid concentration for 120 min



(a)



(b)



(c)

Fig. 4 **a** Ethanol production during pH variations. **b** Ethanol production from temperature variation. **c** Ethanol production for different time interval

acids to catalyze the hydrolysis of cellulose. In this study, the amount of dry feedstock solid to liquid loading of 1:5 was treated in an autoclave at constant temperature of 120 °C with different residence times (60, 90 and 120 min) and at different acid concentrations (0.5, 1.0, 1.5, 2.0, 2.5 % w/w). Once the selected temperature and time was reached, allowing a few minutes for the temperature to drop below 40 °C, solid and liquid fractions were separated by filtration and later neutralized by adding calcium carbonate. The liquid fraction was analyzed for the

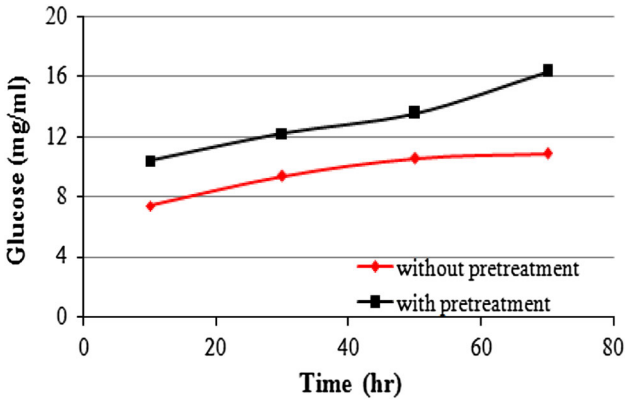


Fig. 5 Glucose produced with and without pretreatment

monomeric sugar contents by using 3,5-dinitrosalicylic acid (DNS) method with UV/Visible spectrophotometer (UV-1700, Shimadzu).

Fermentation

Ethanol production generally requires the fermentation of the glucose (hydrolyzate) using yeast (*Saccharomyces cerevisiae*). Within the experiments, several physical conditions regarding bioethanol fermentation were investigated. Specifically, reaction temperature, incubation time and pH were studied to determine their effect on the fermentation of cellulosic biomass into ethanol. For each physical condition, three runs were conducted over a period of approximately 2 weeks. Within the three runs, each individual run tested the physical condition at a varying degree, e.g. varied incubation time, reaction temperature and pH within the reaction flask.

For fermentation, the inoculation media for yeast cultivation was prepared in a 2 L volumetric flask with distilled, de-ionized water. Glucose 20 g/L, Peptone 10 g/L and yeast extract 20 g/L are added to the water to create the inoculation media. The solution was placed into an autoclave for sterilization (45 min at 121 °C) to prevent any microorganisms other than yeast from growing. The yeast was decanted from the inoculation media via a centrifuge. The nutrient media was prepared by adding 7.5 g ammonium sulphate ($(NH_4)_2SO_4$), 3.5 g potassium hydrogen phosphate ($KHPO_4$), 0.75 g magnesium sulphate ($MgSO_4$) and 1 g calcium chloride ($CaCl_2{\cdot}2H_2O$) to 500 ml of distilled water and dissolving completely. The media was autoclaved for 30 min at a temperature of 120 °C and a pressure of 103.45 kN/m^2 (15 psi).

The fermentation was carried out in 250-ml Erlenmeyer flask with 100 ml of hydrolyzate which was inoculated with 5 % yeast. The batch fermentation was operated at 30 °C for 3 days in the rotary incubator at a speed of 200 rpm under static condition. The samples were collected at regular time intervals to determine ethanol production. The broth was centrifuged to separate the supernatant, which was dried and later distilled to obtain bioethanol.

Enzyme Hydrolysis

An investigation was made to study the impact of enzyme hydrolysis on *C. calothyrsus* biomass to produce glucose. Enzymatic hydrolysis was carried for both pretreated and untreated biomass at 50 °C and pH of 4.8, which is milder than conventional acid hydrolysis. Commercially available cellulose enzyme (ONOZUKA R-10) was used to break cellulose into glucose. The activity of enzyme was found to be 15 FPU/ml, and it was used throughout the experimentation. It was carried out by adding 0.05 M sodium citrate buffer to maintain 4.8 pH and 50 °C in a rotary shaker with agitation speed of 140 rpm for 70 h. Samples were then taken for glucose analysis at 10, 30, 50 and 70 h during enzymatic hydrolysis.

Experimental Set Up

Pretreatment was performed in a laboratory-scale hydrothermal unit provided with temperature and pressure gauges. Figure 1e shows hydrothermal explosion unit of 5 liter capacity with 8 mm thick. Figure 1f shows hydrothermally exploded sample of *C. calothyrsus* biomass.

Results and Discussions

Scanning Electronic Microscopy

Scanning Electronic Micrographs of the sample was obtained to verify material structural changes caused by the hydrothermal explosion. The SEM image Fig. 2a of untreated sample indicated compact and regular surface structure of fiber bundles in a biomass without pores. After pretreatment, considerable damage to the fibers could be observed clearly in Fig. 2b. The surface is uneven and the fibers are separated, causing a significant increase in surface area. Scanning electronic microscopy (SEM) of the untreated and pretreated residue was carried out with Hitachi S-4800 (Japan) instrument at 15 kV.

Optimization of Glucose Yield Using Acid Hydrolysis

Acid hydrolysis was conducted for pretreated biomass with different reaction conditions. For the study, different process variables viz. acid concentration, type of acid and reaction time were considered to achieve maximum glucose yield. Finally, each variable was evaluated to determine the favorable conditions for maximum yield of glucose.

Batch experiments were conducted using 2 M H_2SO_4 and 2 M HCl as the reaction catalyst at reaction temperature of 120 °C. Hydrolysis of biomass was conducted at various concentrations of acid ranging from 0.5 to 2.5 % in steps of 0.5 % and at reaction time of 60, 90 and 120 min. Figure 3a, b and c show the variation of conversion with different acid concentrations. Results revealed that the high concentration of acid enhances reaction rate while improving the sugar concentration as acid catalyze the hydrolysis of cellulose. The catalyst activity was proportional to H^+ concentration. Formation of more hydrogen ions in the aqueous solution resulted in higher cellulosic hydrolysis process [39]. Therefore, H_2SO_4 resulted in higher glucose yield compared to HCl. It could be attributed to increased reaction rate due to higher breaking of glycosidic bonds in the cellulose. Hence it causes high conversion of cellulose fraction into glucose. The results obtained were in good agreement with published literatures [33].

From Fig. 3b, it is observed that, degradation of glucose leads to furfurals under severe conditions i.e. with 2.5 % H_2SO_4 acid concentration and 90 min reaction time [26]. Figure 3c shows decreased conversion for 2 % HCl acid and 120 min reaction time. However, at constant reaction temperature of 120 °C, reaction time 120 min, with 1.5 % H_2SO_4 and 2.5 % HCl glucose, yield was found to be 2.67 and 1.72 g/L respectively as it is evident from Fig. 3c. It was observed that the yield did not increase beyond 1.67 g/L with 1.5 % HCl acid concentration and 120 min reaction time. Results indicated lower yield with higher concentration of H_2SO_4 and longer reaction time. It may be due to degradation of sugar to hydroxyl methyl furfurals. Based on the results obtained it can be concluded that, the acid concentration was found to have more dominating effect than reaction time during hydrolysis. Higher yield may also be due to higher temperature, as it provides greater energy to break linkages of fibers in a biomass. Similar trends were observed with published literature [40].

Beside the orderly crystalline structure in the cellulose molecules, the presence of lignin fraction in the cellulose materials is also responsible in defending the cellulose from hydrolysis [41].

Fermentation

Variation of bioethanol yield with respect to pH, temperature and time interval (Fig. 4) has been discussed in the subsequent paragraph.

Effect of pH on Fermentation

Figure 4a shows the effect of pH on the yeast's ability to convert glucose into ethanol. In the present work, Baker's yeast was used as it has an internal pH of about 5.0 and slightly acidic condition favors the reproduction and growth of yeast. Hence as the pH increases, the reaction yield decreases. The experimentation concludes that a slightly acidic pH around 4.5, favors yeast fermentation. However, at 6 pH reaction yield is nearly 20 % lower than the 4.5 pH, suggesting that neutral or basic pH's will greatly inhibit overall yeast health [42]. Finally, it is observed that lower pH levels ensure that the yeast function well under minimal internal stress. Hence, it can ferment glucose into bioethanol more efficiently. Around 4.5 pH, 72 h of incubation period provides maximum sugar conversion (52 %). Fermentation pH of the medium is an important parameter affecting the growth and product formation [43].

Effect of Temperature on Fermentation

Figure 4b represents the effect of temperature on bioethanol yield during fermentation stage. The temperature variation experiment investigated range of temperatures affecting the production of ethanol using yeast. Incubation temperature for maximum ethanol yield of 52 % was found to be 30 °C. It could be due to the fact that the yeast was under minimal stress and lower inhabitation from bioethanol obtained. The yeast cells at 30 °C are structurally sound and are capable of healthy and efficient reproduction. High temperature results in considerably higher thermal stress on the yeast as it reproduces and hence slows reproduction rate of yeast to consume the available glucose substrate for ethanol production. Hence, it is found that lower temperature favors bioethanol fermentation.

Effect of Time on Fermentation

Figure 4c shows ethanol yield at different time interval for 4.5 pH and reaction temperature of 30 °C. During initial stages of fermentation, yeast adapts themselves to growth conditions so there was no glucose to ethanol conversion. Later ethanol yield increased exponentially till 72 h. After 72 h of fermentation, the growth rate was found to slow down due to glucose depletion and the ethanol was used as a carbon source by the yeast for its growth after the 72 h [44]. The time taken for maximum sugar conversion was 72 h, 4.5 pH and incubation temperature of 30 °C.

Optimization of Glucose Yield Using Enzymatic Hydrolysis

Enzymatic hydrolysis yields are shown in Fig. 5 as a function of time for pretreated (hydrothermal explosion) and untreated biomass. Experiments on enzymatic hydrolysis showed remarkable changes in the glucose yield compared to acid hydrolysis (Fig. 3a, b, c). Pretreated biomass showed positive

effect on saccharification of *C. calothyrsus* cellulose fraction compared to untreated biomass. It is observed that enzymatic hydrolysis for pretreated biomass resulted in 16.5 g/L compared to 10.25 g/L of glucose for untreated biomass. This was contributed by solubilisation of hemicellulose fraction, reduced cellulose crystallinity and more surface area accessibility (lignin removal) during pretreatment favoring enzyme accessibility to cellulose [30, 45]. Pretreated biomass followed by enzymatic hydrolysis is a very effective method compared to acid hydrolysis of pretreated biomass for ethanol production from *C. calothyrsus* shrub.

Equation (1) shows the disintegration process of the cellulose molecules by water into glucose.

$$(C_6H_{12}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \quad (1)$$

Conclusions

The following conclusions were made from the study.

In recent years, bioethanol has become more attractive biofuel for engine application and the fact that it is made from renewable resources. Investigators/researchers have investigated the combustion in both SI and CI engine using ethanol as single and dual fuel operation (blended fuel with diesel and biodiesels as a fuel). In this context, an attempt has been made to study the bioethanol production to meet the current demand. The overall observation based on the study is that, acid concentration, pH, and enzyme play an important role in production process and associated reactions. Some important conclusions are summarized below.

1. The current study offered an opportunity in exploring the potential of using a locally available *C. calothyrsus* shrub rich in cellulose, as an alternative biomass for production of bioethanol due to its low price and renewable property.
2. SEM images illustrated changes in the structure of *C. calothyrsus* biomass making feedstock more porous and loose, indicating higher specific surface using hydrothermal explosion pretreatment.
3. Glucose yield of 2.67 g/L was observed during H_2SO_4 acid hydrolysis at 120 °C reaction temperature, 120 min reaction time and 1.5 % H_2SO_4 concentration. Similarly for 2.5 % HCl concentration glucose yield was found to be 1.72 g/L.
4. Based on the experimental results of this study, it is concluded that the maximum ethanol yield (52 %) from *C. calothyrsus* using *S. cerevisiae* can be achieved by maintaining temperature of 30 °C, pH 4.5 and incubation period of 72 h.
5. Enzymatic hydrolysis of pretreated biomass was 16.5 g/L glucose, and for untreated biomass yield was 10.25 g/L. Results showed enzymatic hydrolysis provides better glucose yield than acid hydrolysis.
6. Pretreated biomass is a very effective method in preparing the substrate as it enhances accessibility of cellulose and showed positive effect on saccharification of *C. calothyrsus* cellulose fraction.

On the whole, it seems that bioethanol is a promising alternative and renewable fuel. Investment cost reduction and technology advancements in renewable energy helps to enhance the usage of renewable and alternative fuels for engine applications. Internal combustion engines fueled by bioethanol and its blends with technological advancements are convenient and economically viable and can serve as a future option for engine applications.

References

1. Wyman, C.E.: What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnol. **25**, 153–157 (2007)
2. Lynd, L.R., Laser, Mark S., Bransby, David, Dale, B.E., Davison, B., Hamilton, R., Himmel, M., Keller, M., McMillan, J.D., Sheehan, J., Wyman, C.E.: Nat. Biotechnol. **26**(2), 169–172 (2008)
3. Park, S.H., Yoon, S.H., Suh, H.K., Lee, C.S.: Effect of the temperature variation on properties of biodiesel and biodiesel- ethanol blend fuels. Oil Gas Sci. Technol. Rev. IFP **63**, 737–745 (2008)
4. Xingcai, L., Zhen, H., Wugao, Z., Degang, L.: The influence of ethanol additives on the performance and combustion characteristics of diesel engines. Combust. Sci. Technol. **176**, 1309–1329 (2004)
5. Chen, H., Shuai, S., Wang, J.: Study on combustion characteristics and PM emission of diesel engines using ester-ethanol-diesel blended fuels. Proc. Combust. Inst **31**, 2981–2989 (2007)
6. Li, De-gang, Huang Zhen, Lu, Xingcai, Yang Jian-guang: Physicochemical properties of ethanol diesel blend fuels on performance and emission of diesel engine. Renewable Energy **30**(6), 967–976 (2005)
7. Hansen, A.C., Zhang, Q., Lyne, P.W.L.: Ethanol-diesel fuel blends-a review. Bioresour. Technol. **96**, 277–285 (2005)
8. Corkwell, K.C., Jackson, M.M., Daly, D.T.: Review of exhaust emissions of compression ignition engines operating on E diesel fuel blends, SAE International Paper no.: 2003-01-3283 (2003)
9. Banapurmath, N.R., Tewari, P.G., Yaliwal, V.S.: Fuel efficiency—improving fuel efficiency of compression ignition engines fuelled with vegetable oil, Nova Science Publishers, Inc., ISBN: 978-1-61122-194-7: 2–36 (2010)
10. Yaliwal, V.S., Nataraja, K. M., Banapurmath, N. R., Tewari P.G.: Honge oil methyl ester and producer gas-fuelled dual-fuel engine operated with varying compression ratios. International Journal of Sustainable Engineering, Article in press. doi:10.1080/19397038.2013.837108 (2013)
11. Mabee, W. E., Saddler, J. N.: Ethanol from Lignocellulosics: Policy options to support bioethanol production, IEA Task 39, Forest Products Biotechnology, University of British Columbia. Report T39-P2, 1–16 (2005)
12. Mitchell, D.: A note of rising food prices, Policy research working paper 4682. Development Prospects Group, The World Bank, Washington D.C., USA, 1–21 (2008)

13. Wheals, A.E., Bassoc, L.C., Alves, D.M.G., Amorimd, H.V.: Fuel ethanol after 25 years. Trends Biotechnol. **17**(12), 482–487 (1999)
14. Grad, P.: Biofuelling Brazil—an overview of the bioethanol success story in Brazil. Biofuels **7**(3), 56–59 (2006)
15. Dien, B.S., Li, X.L., Iten, L.B., Jordan, D.B., Nichols, N.N., Bryan, O., Cotta, M.A.: Enzymatic saccharification of hot-water pretreated corn fiber for production of mono-saccharides. Enzyme Microb. Technol. **39**(5), 1137–1144 (2006)
16. Laser, M., Schulman, D., Allen, S.G., Lichwa, J., Antal, M.J., Lynd, L.R.: A comparison of liquid hot water and steam pretreatments of sugar cane bagase for conversion to ethanol. Bioresour. Technol. **81**, 33–44 (2002)
17. Xu, J., Thomsen, M.H., Thomsen, A.B.: Pretreatment on corn stover with low concentration of formic acid. J. Microbiol. Biotechnol. **19**(8), 845–850 (2009)
18. Muralidharan, M., Mathew, P., Thariyan Sumit Roy, Subrahmanyam, J. P., Subbarao P. M. V.: Use of Pongamia Biodiesel in CI Engines for Rural Application. 3rd International Conference on Automotive and Fuel Technology, Society of Automotive Engineer Paper No.: 2004-28-0030, 1–8 (2004)
19. Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploger, A., Schmidt, A.S.: Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnol. Bioenergy **49**(5), 568–577 (1996)
20. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M.: Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. **96**(6), 673–686 (2005)
21. Perez, J.A., Ballesteros, I., Ballesteros, M., Sáez, F., Negro, M.J., Manzanares, P.: Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. Fuel **87**, 3640–3647 (2008)
22. Nigam, P.S., Gupta, N., Anthwal, A.: Pre-treatment of agro-industrial residues. In: Nigam, P.S., Pandey, A. (eds.) Biotechnology foragro-industrial residues utilization, 1st edn, pp. 13–33. Springer publications, Netherlands (2009)
23. Zhang, Y.H.: Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. Ind. Microbiol. Biotechnol. **35**, 367–375 (2008)
24. Hendriks, A.T.W.M., Zeeman, G.: Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. **100**(1), 10–18 (2009)
25. Kumar, R., Singh, S., Singh, O.V.: Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. Ind. Microbiol. Biotechnol. **35**, 377–391 (2008)
26. Taherzadeh, M.J., Karimi, K.: Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci. **9**, 1621–1651 (2008)
27. Yang, B., Wyman, C.E.: Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod. Biorefin. **2**(1), 26–40 (2008)
28. Sun, F.B., Cheng, H.Z.: Evaluation of enzymatic hydrolysis of wheat straw pretreated by atmospheric glycerol autocatalysis. J. Chem. Technol. Biotechnol. **82**, 1039–1044 (2007)
29. Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B.: Biomass pretreatment: fundamentals toward application. Biotechnol. Adv. **29**, 675–685 (2011)
30. Laureano-Perez, L., Teymouri, F., Alizadeh, H., Dale, B.E.: Understanding factors that limit enzymatic hydrolysis of biomass. Appl. Biochem. Biotechnol. **121**(124), 1081–1099 (2005)
31. Balan, V., Bals, B., Chundawat, S.P., Marshall, D., Dale, B.E.: Lignocellulosic biomass pretreatment using AFEX. Methods Mol. Biol. **581**, 61–77 (2009). doi:10.1007/978-1-60761-214-8_5
32. Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K.: Bioethanol production from agricultural wastes: an overview. Renewable Energy **37**(1), 19–27 (2012)
33. Mosier, N.S., Ladisch, C.M., Ladich, M.R.: Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. **6**(79), 610–618 (2002)
34. Kim, Y., Mosier, N.S., Ladisch, M.R.: Enzymatic digestion of liquid hot water pretreated hybrid poplar. Biotechnol. Prog. **25**(2), 340–348 (2009)
35. Yu, G., Yano, S., Inoue, H., Inoue, S., Endo, T., Sawayama, S.: Pretreatment of rice straw by a hot-compressed water process for enzymatic hydrolysis. Appl. Biochem. Biotechnol. **160**(2), 539–551 (2010)
36. Sukumaran, R.K., Surender, V.J., Sindhu, R., Binod, P., Janu, K.U., Sajna, K.V., Rajasree, K.P., Pandey, A.: Lignocellulosic ethanol in India-prospects, challenges and feedstock availability. Bioresour. Technol. **101**, 4826–4833 (2010)
37. Ravindranath, N.H., Somashekar, H.I., Nagaraja, M.S., Sudha, P., Sangeetha, G., Bhattacharya, S.C., Abdul Salam, P.: Assessment of sustainable nonplantation biomass resources potential for energy in India. Biomass Bioenergy **29**(3), 178–190 (2005)
38. Goering, H. K., Van Soest, P.J.: Forage fiber analysis (apparatus, reagents, procedures and some applications). USDA Agricultural Handbook No.: 379 (1970)
39. Romero, I., Ruiz, E., Castro, E., Maya, M.: Acid hydrolysis of olive tree biomass. Chem. Eng. Res. Design **88**, 633–671 (2010)
40. Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N.: The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb. Technol. **24**, 151–159 (1999)
41. Lenihan, P., Orozco, A., O'nill, E., Ahmad, M.N.M., Rooney, D.W., Walker, G.M.: Dilute acid hydrolysis of lignocellulosic biomass. J. Chem. Eng. **156**, 395–410 (2010)
42. Reed, G.: Production of fermentation alcohol as a fuel source. In: Prescott & Dunn's Industrial Microbiology, fifth ed. Reprinted by CBS Publishers, New Delhi, 835–860 (2002)
43. Pramanik, K.: Parametric studies on batch alcohol fermentation using saccharomyces yeast extracted from toddy. J. Chin. Inst. Chem. Eng. **34**(4), 487–492 (2003)
44. Olaniran, A.O., Maharaj, Y.R., Pillay, B.: Effects of fermentation temperature on the composition of beer volatile compounds, organoleptic quality and spent yeast density. Electron. J. Biotechnol. **14**(2), 5 (2011)
45. Kabel, M.A., Bos, G., Zeevalking, J., Voragen, A.G.J., Schols, H.A.: Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. Bioresour. Technol. **98**, 2034–2042 (2007)