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Catalysed-microwave based Pretreatment of Lignocellulosic Biomass of *Camelina Sativa* L. for Bio-Fuel Production

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ABSTRACT

Lignocellulosic biomasses are promising alternative resource for bio-fuel production. But due to the recalcitrant nature of lignin and hemicellulose, necessitates an efficient pre-treatment process to improve the yield of reducing sugars and maximising the enzymatic hydrolysis efficiency. Catalysed-microwave pre-treatment may be a good alternative as compared to other methods since it can reduce the time and improve the enzymatic activity during hydrolysis. The aim of this study was to evaluate the efficiency of the catalysed-microwave based pre-treatment of lignocellulosic biomass of *Camelina sativa* straw (CSS) to overcome the recalcitrant nature of cellulosic biomass. The microwave-alkaline (2 % NaOH) pre-treatment of CSS at 250 W for 10 min yields maximum (~422 mg/g) total soluble sugars (TSS) production during hydrolysis. Likewise, the maximum glucose content (~294 mg/g) was measured in 2 % alkaline-microwave pre-treatment for 10 min at RT. However, slight increase in lignin degradation was observed with the increase in alkaline hydroxide concentration and microwave irradiation exposure time. The maximum degradation in lignin content (~83 %) was measured in 3 % alkaline-microwave pre-treatment for 20 min at RT. Our results suggest that the microwave-alkaline pre-treatment approach may be employed for comprehensive utilisation of CSS biomass of *Camelina sativa* L. cv. Calena (EC643910) for bio-fuel production.

Keywords: Bio-fuel; *Camelina sativa* straw; Catalysed-microwave pre-treatment; Hydrolysis lignocellulosic biomass; Fossil fuels

NOMENCLATURE

ANOVA	Analysis of variance
CSS	*Camelina sativa* straw
DMRT	Duncans multiple range test
DNS	Dinitrosalicylic acid reagents
LSD	Least significant difference
RT	Reaction time
SDW	Sterile distilled water
TSS	Total soluble sugars

1. INTRODUCTION

The extreme consumption of fossil fuels and its declining resources have reinforced the search for the development and identify of new renewable fuel alternatives from biological source known as Bio-fuel[1]. It is expected that by the end ~2040 all the stocks of fossil fuels will be depleted and we have to depend on biofuel for energy requirements[2]. Therefore, in the current scenario a tremendous amount of research on the development of technologies for efficient lignocellulosic biomass (mainly consists of three bio-polymers, cellulose, hemicelluloses, and lignin) conversion to biofuels (bio-ethanol) has proven the importance of the second-generation bio-fuel technology as alternative to fossil fuel[3,4]. Lignocellulosic feedstocks such as solid waste, wood, agricultural and forest residues are inexpensive, renewable, and abundant source of bio-ethanol production and can also contributes in the reduction of green house gas emission of fossil fuels[5]. Although, the conversion of lignocellulosic biomass to bio-fuel is a promising technology but it has various limitations and challenges, which includes handling and transport of lignocellulosic biomass, and its efficient pre-treatment methods for total delignification. Therefore, the use of efficient pre-treatment methods can improve the efficiency of the whole process of enzymatic saccharification for increasing yield of fermentable sugars[6].

Pre-treatment is an important step for efficient conversion of lignocellulosic biomass to bio-fuels that affects the methodology and efficiency of the subsequent saccharification process. The main goal of pre-treatment is to reduce the recalcitrant nature of lignocellulosic biomass and increasing the biomass surface area, as well as reducing cellulose crystallinity[7,8]. Since the recalcitrant nature of lignocellulosic biomass severely impedes the yield of fermentable sugars during hydrolysis. Pre-treatment process makes cellulose more available to hydrolytic enzymes for further conversion of carbohydrate polymers into fermentable sugars. A number of pre-treatment methods have been developed such as physical, chemical, physico-chemical, biological processes or combinations thereof[8]. Most of these methods have their own advantages and limitations and also influence with the nature of biomass undertaken. Mostly these methods suffer from relatively low sugar yields, severe reaction conditions,

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large capital investments, time taking and high cost with great investment risks[9].

The microwave irradiation based pre-treatment method has been widely used now these days because of its easy operation and high heating efficiency[10]. The main advantages of this method are uniform and selective processing of lignocellulosic biomass with precise control and thereby reduction of overall energy requirements[11]. Also, the microwave irradiation causes rapid and volumetric heating since the heat is generated internally through direct contact between the electromagnetic waves and components of the lignocellulosic biomass. Thus, it is a promising pre-treatment process since it utilises both thermal and specific (non-thermal) effects generated by microwave irradiation in an aqueous environments[12]. These effects can cause fragmentation and swelling, leading to degradation of lignin and hemicellulose in biomass[13]. Microwave irradiation studies have proven the improvement in total reducing sugars production as compared to untreated biomass[13,14].

The aim of the this study was to evaluate the efficiency of the catalysed-microwave based pre-treatment of lignocellulosic biomass of *Camelina sativa* straw (CSS) to overcome the recalcitrant nature of cellulosic biomass and to enhance fermentable sugar production during hydrolysis for the production of bio-ethanol or other value added products. This efficient catalysed-microwave based pre-treatment approach may be employed for comprehensive utilisation of lignocellulosic biomass of *Camelina sativa* L. cv. Calena (EC643910).

2. MATERIAL AND METHOD

The *Camelina sativa* cv. Calina (EC643910) plants were cultivated at open fields at Defence Institute of Bio-Energy Research, Haldwani. The mature (~3 months old) *Camelina sativa* straw (CSS) were harvested (initial moisture content ~5 %) and washed with sterile distilled water to expel all undesirable matter followed by oven drying at 80 °C for 24 h to obtained constant dry weight. Dried CSS sample was ground with the help of milling machine to obtained optimum particle size (~1-2 mm). These milled CSS samples were stored in sealed plastic bags at room temperature until used for catalysed-microwave assisted pre-treatments experiments. All chemicals (analytical grade) used in the present study were procured from Sigma-Aldrich, USA.

2.1 Catalysed-microwave Assisted Pre-treatment

Grounded CSS (~1-2 mm) samples (10.0 g) were initially treated with different concentration solutions of sodium hydroxide (1 to 3%; w/v), dilute sulphuric acid (1 and 2%; w/v) and sterile distilled water in a 500 ml beaker, separately. After that, the microwave assisted pre-treatment of lignocellulosic biomass of CSS was done at 121 °C by using Kenstar multi grill convection microwave oven (wavelength of microwave: 12.2 cm; Power Supply: 230 V, AC 50 Hz) at 250 W at ambient temperature (25 °C). After catalysed microwave assisted pretreatment, mixtures were filtered through filter paper (Wattman filter paper 00) to separate solid residue and liquid (filtrate). The filtrate was used for all further biochemical analysis.

2.2 Estimation of Total Soluble Sugar

The TSS was estimated using anthrone method[15]. The CSS extract (1 mL) was mixed with freshly prepared anthrone (3 mL) and the tubes was subsequently heated at 100 °C for 10 min. The reaction was terminated by placing the tubes on ice for 5 min. After that, the absorbance (Labomed Inc., UV-VIS spectrophotometer, USA) was measured at 620 nm, and TSS content (lg g-1 FW) was estimated from a standard curve of D-glucose (Sigma-Aldrich, USA).

2.3 Glucose Estimation

The glucose content was measured by using dinitrosalicylic acid reagents (DNS) method of Miller[16]. The DNS reagent (3 mL) was added to CSS extract (3 mL) sample in a test tube. The reaction mixture was heated at 90 °C for 15 min to develop the red-brown color. After that, added 1 ml of a 40 per cent potassium sodium tartrate (Rochelle salt) solution for stabilising the color of the reaction mixture. The reaction mixture was cooled to room temperature. The absorbance was measured at 575 nm by using spectrophotometer (Labomed Inc., UV-VIS spectrophotometer, USA).

2.4 Estimation of Lignin Content

The lignin content was estimated according to Stange and McDonald[17]. The CSS extract (1 mL) sample was added to cold neutral detergent solution (10 mL) in a refluxing flask. Added decahydronaphthalene (2 mL) and sodium sulphate (0.5 g) into the refluxing flask and reaction mixture was heated to boil and reflux for 60 min. The reaction mixture was filtered through sintered glass crucible (G-2) by suction and wash with warm water followed by two acetone washings. After that the residue was transferred to a crucible and subsequently dried at 100 °C for 8 h. The lignin content was weighed after cooling of crucible in a desiccator.

2.5 Statistical Analysis

All biochemical estimations were carried out in triplicates. The CropStat (7.2.2007.2 module) software was used for statistical analysis (ANOVA) for all experiments. The treatments and controls means values were compared by least significant difference (LSD) test at a significance level of $P \leq 0.05$. Duncans multiple range test (DMRT) was also performed to check the significance of the differences between mean values.

3. RESULTS AND DISCUSSION

Camelina sativa L. cv. Calena (EC643910) is an important short-duration (mature in 80–90 days) non-edible oilseed bio-fuel crop that grows well on relatively saline soils and adapted to cool and semi-arid regions of Eastern Europe and southwest Asia[18,19]. In India, it is a newly introduced for its potential commercialisation as bio-fuel crop. DIBER-DRDO, Haldwani is the pioneer agency in India to introduce this crop as alternate bio-fuel crop and also standardize its agro-technology in different climacteric zone of India for harnessing its potential as high quality renewable bio-fuel[20,21]. The cellulosic biomass of Camelina is an important resource of bio-fuel production. In order to efficient utilisation of its cellulosic biomass (straw) an attempt has been made in present study to develop an efficient and eco-friendly pre-treatment process for conversion of CSS into the fermentable sugar that can be used further to make bio-fuel (Bio-ethanol).

3.1 Effect of Catalysed-microwave based Pre-treatment of CSS on Total Soluble Sugars

For this purpose, we used dilute alkali (NaOH; 1 % and 2%; w/v) and acid (H_2SO_4; 1 % and 2%; w/v) catalysed micro-wave irradiation method due to their high heating efficacy, lower energy consumption, and easy operation. Our results showed that the microwave-alkaline (2% NaOH) pre-treatment of lignocellulosic biomass of CSS at 250 W for 10 min may overcome the recalcitrant nature of cellulosic biomass and to enhance total soluble sugars (TSS) production during hydrolysis for the production of bio-ethanol or other value added products. The maximum total soluble sugar yield (~422 mg/g) was obtained at pre-treatment of CSS at 250 W for 10 min (Fig. 1). The alkali hydroxide treatment showed significant (P<0.05) catalytic performance as compared to dilute sulphuric acid and sterile distilled water (SDW) in converting hemicellulose into total soluble sugars under microwave irradiations.

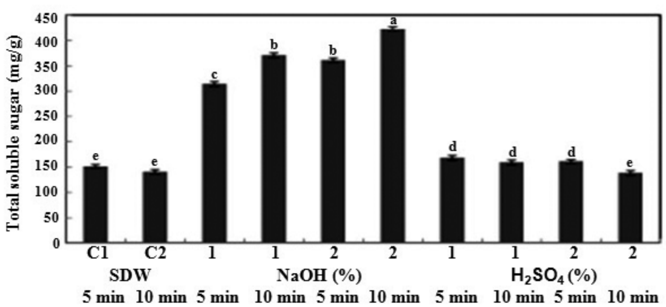

Figure 1. Effect of catalysed-microwave based pre-treatment [NaOH (1 % and 2%); H_2SO_4 (1 % and 2%); and sterile distilled water (at 250 W for 5 and 10 min] of lignocellulosic biomass of *Camelina sativa* straw on total soluble sugars yield. Bar shown means (n = 3) ± SE are statistically significant (P≤0.05) according to least significant difference test. Different letters used in each column indicate significant differences at *P*≤0.05, according to Duncans multiple range test.

The microwave-dilute sulphuric acid pre-treatment of CSS causes degradation of the solid skeleton of the lignocellulosic materials that enhance the porosity of biomass and makes it easy to proceed in further hydrolysis reactions[22]. However, the microwave-alkaline pre-treatment method (generally all the hydroxide of S-block elements), actively transforms the structure of lignin, partial decrystalisation of cellulose and partial solvation of hemicellulose[23]. Therefore, a microwave-alkaline method was preferred for further study to optimize its concentration and time for pre-treatment of CSS biomass. The effectiveness of microwave-alkali pre-treatment method on various cellulosic biomass *viz*. corn, switch grass, bagasse, wheat, rice straw, hardwood, and softwood have been also showed by various workers[8,13,24-25].

3.2 Effect of Microwave-alkaline Pre-treatment of CSS on Glucose Yield

To standardise the concentration and time of alkali hydroxides (NaOH) used for efficient conversion of CSS biomass into simple sugars, the glucose content were measured in various microwave-alkaline (1 to 3%) pre-treatment combinations at different time interval (5 to 20 min). The maximum glucose content (~294 mg/g) was measured in 2% alkaline-microwave pre-treatment for 10 min at RT combination (Fig. 2). Our results showed that glucose yield significantly (P<0.05) increased with increasing alkali concentration up to 2% NaOH concentration, after that it significantly decreased in further increase in NaOH concentration (3%). Also the exposure time of microwave irradiation is important since the glucose yield was maximum at 10 min, after that it decreased significantly (P<0.05) with the increase of exposure time (Fig. 2). Ethiab[14], *et al*. observed the maximum glucose content by using alkali as a catalyst during pre-treatment of lignocellulosic biomass of dragon fruit foliage.

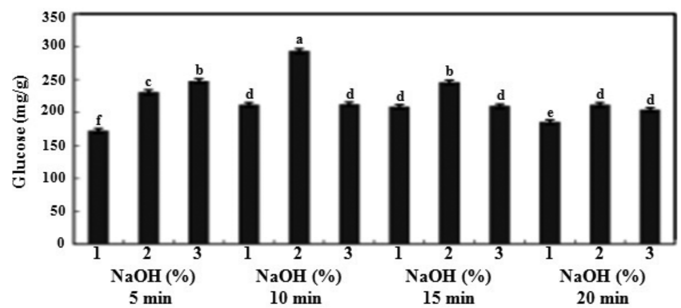

Figure 2. Estimation of glucose due to microwave-alkaline (1 to 3% NaOH) pre-treatment of lignocellulosic biomass of *Camelina sativa* straw at 250 W for 5 to 20 min. Bar shown means (n = 3) ± SE are statistically significant (P≤0.05) according to least significant difference test. Different letters used in each column indicate significant differences at *P*≤0.05, according to Duncans multiple range test.

3.3 Lignin Degradation Caused by Microwave-alkaline Pre-treatment of CSS

Lignin degradation is an important step since it releases other lignocellulosic components (Cellulose and hemicellulose) of cell wall for further hydrolysis into simple sugars. Therefore, the delignification processes can enhance the rate and extent of enzymatic hydrolysis during pre-treatment methods[26]. In this effort, the percentage of lignin degradation was measured in different microwave-alkaline (1 to 3%) pre-treatment combinations at different time interval (5 to 20 min). In pre-treatment experiments, slight increase in lignin degradation was observed with the increase in alkaline hydroxide concentration and microwave irradiation exposure time. In some case this increase was not significant (P<0.05). The maximum degradation in lignin content (~83%) was measured in microwave-alkaline (3% NaOH) pre-treatment for 20 min at RT combination (Fig. 3). Similarly, Moony[27], *et al*.showed significant degradation of lignin content during microwave- alkaline pre-treatment at a particular temperature and catalyst concentration. Nomanbhay[12], *et al*. also reported

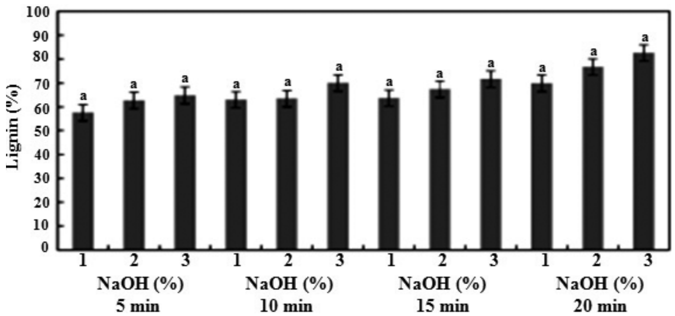

Figure 3. Lignin reduction caused by microwave-alkaline (1 to 3% NaOH) pre-treatment of lignocellulosic biomass of *Camelina sativa* straw at 250 W for 5 to 20 min. Bar shown means (n = 3) ± SE are statistically significant (P≤0.05) according to least significant difference test. Different letters used in each column indicate significant differences at *P*≤0.05, according to Duncans multiple range test.

~74 % lignin degradation due to microwave-alkaline pre-treatment condition (3 % NaOH at 180 W for 12 min) in EFB of Oil Palm.

4. CONCLUSIONS

Catalysed-microwave pretreatment method is an efficient technology for the conversion of lignocellulosic biomass of *Camelina sativa* into simple sugars that may be utilised for the production of bio-fuel (bio-ethanol). Our results showed that the microwave-alkaline (2 % NaOH) pre-treatment of lignocellulosic biomass of CSS at 250 W for 10 min yields maximum total soluble sugars (TSS) and glucose content ~422 mg/g and ~294 mg/g, respectively, during hydrolysis at RT. However, slight increase in lignin degradation was observed with the increase in alkaline hydroxide concentration and microwave irradiation exposure time. Also, the maximum degradation in lignin content (~83 %) was measured in microwave-alkaline (3 % NaOH) pre-treatment for 20 min at RT. Our results suggest that the efficient microwave-alkaline based pre-treatment approach may be employed for comprehensive utilisation of CSS biomass of *Camelina sativa* L. cv. Calena (EC643910).

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Contribution in the current study, he has contributed in critical revision and proofreading work of this MS.