

Microwave Assisted Hydrolysis of Cellulose to Release Sugars from *Pongamia* Oil Cake for its use in Bioethanol Production

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Abstract: Biomass based biofuels has been a promising alternative source of energy for the past few decades. The major hurdle is the technologies of production and challenges involved in processing time. This work describes the use microwave radiation to produce bioethanol from a byproduct of biodiesel industry viz., *Pongamia pinnata* oil seed cake. The powdered oil cake was acid treated under microwave radiation for release of reducing sugars. The kinetics of the microwave process was studied and found that microwave mediated process accelerates the acid hydrolysis of oil cake in comparison with conventional heating method. Further, the multistage process for microwave mediated acid hydrolysis was adapted to increase the overall yield. The yield did not improve beyond 3 stages. The hydrolysate obtained was fermented to produce bioethanol using *Saccharomyces cerevisiae*.

Keywords: Microwave radiation, Acid hydrolysis, Cellulosic Biomass, Biofuels, Bioethanol

Introduction

The trends in depleting fossil fuels for energy, their increasing cost and global instabilities have driven the interest to search for alternative energy sources. Biofuels, one among such renewable fuels produced from bio-based feedstock. The Indian 'National Policy on Biofuels - 2018' now allows use of several non-edible and waste materials as feedstock for production of biofuels¹. In the last two decades, several such feedstocks have been used for production of bioethanol, which is one of the biofuel. The composition of raw material determines the sequence of processes involved in bioethanol production. A schematic illustration of the ethanol production from biomass is shown in Figure 1.

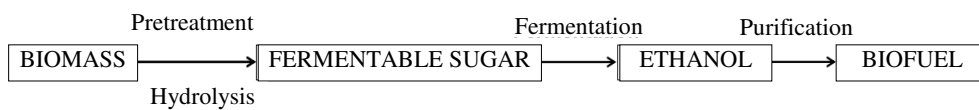


Figure 1. Scheme of biomass based bioethanol production

A pretreatment step that hydrolyzes complex sugar polymer in the biomass is essential before fermentation to produce ethanol. Several pretreatment methods such as physical treatment, chemical treatment, biological treatment, physicochemical treatment and combination processes have been reported²⁻⁴. Use of acids or enzymes alters the strength of cellulosic fibers and hydrolyze the polymer to its monomers². *Pongamia pinnata* is one of the widely used non-edible sources to produce biodiesel in India^{5,6}. Doshi and Srivastava⁷ reported reducing sugar yield of 66 mg/g of seed cake with a conventional pretreatment method by using sulfuric acid. Radhakumari *et al.*,⁸ reported a single step process using 7.5% sulfuric acid to digest the biomass using an autoclave. In 1 h of processing they obtained glucose yield of 0.245 g/g of seed cake. Apart from sulfuric acid, other acids like hydrochloric acid, acetic acid has been used for the digestion of biomass⁹. *Pongamia pinnata* oil cake has a great potential for use as feedstock for bioethanol production. But the lesser attempts have been made to improve the processing time. Improvement in processing time enhances the productivity, hence the economics of the process.

Microwave radiation accelerates chemical processes through the intrinsic heat generation because of extensive molecular collisions, vibration of polar molecules and ionic movement¹⁰. The dielectric properties of the reaction components determine the performance of a microwave process. Microwave has been successfully used for biomass pretreatment process¹¹⁻¹³. Microwave irradiation accelerates the reaction due to the thermal and non-thermal effects^{10,14}. Microwave irradiation also decreases the activation energy of the reaction. In their work on bio-diesel production, Iyyaswami *et al.*,¹⁴ have confirmed that microwave irradiation interacts with reacting molecules and brings down the activation energy required for reaction.

In this study, microwave radiation is used for hydrolysis of *Pongamia pinnata* oil cake. Kinetic studies of microwave assisted acid hydrolysis process were performed and compared with conventional heating method. The overall yield of microwave batch process was increased by subjecting the oil cake to multi stage acid hydrolysis process. After, the separation and treatment of hydrolysate, it was used for ethanolic fermentation using *Saccharomyces cerevisiae*.

Experimental

The de-oiled cake of *Pongamia pinnata* was used as biomass for acid hydrolysis experiments. It was procured from a biodiesel production unit which produced biodiesel from *Pongamia* oil. The composition of the powdered biomass was determined using NREL methods¹⁵. Soxhlet extraction with chloroform methanol solvent system was used to estimate the lipid composition in the biomass. All the chemicals used were of reagent grade and used without any further modifications.

Microwave assisted acid digestion process

A microwave oven (Onida, Power Solo 17D) with five fixed power levels was used for all the microwave mediated acid hydrolysis experiments. Since sulfuric acid is one of the most preferred inorganic catalyst for hydrolysis of carbohydrates, it was used as catalyst for all hydrolysis reactions of carbohydrates¹⁶. In a typical microwave experiment, 6 g of powdered oil cake was added to sulfuric acid solution in an Erlenmeyer flask and diluted with distilled water to make up the concentration of solids to 6% (w/v) and acid concentration to 2% (v/v). The sample was exposed to microwave radiation at 140 W for 5 min and then it was cooled and centrifuged. The supernatant solution was neutralized with a few drops of concentrated sodium hydroxide solution. Further, it was filtered using Whatman No.1 filter and then the

filtrate was analyzed for total sugar and reducing sugar concentration. Spectrophotometer based anthrone method for total sugars¹⁷ and DNS method for reducing sugars¹⁸ was used for estimation. Glucose was used as standard for both methods.

Kinetics of acid hydrolysis reaction under microwave radiation

The kinetics of acid hydrolysis reaction to produce reducing sugars from carbohydrates present in oil cake was studied by varying microwave exposure time from 1 min to 9 min. The kinetic analysis was performed for microwave output powers 140 W, 280 W and 420 W. The acid concentration in the reaction mixture was varied from 1% (v/v) to 3% (v/v). The product, reducing sugar concentration¹⁸ was estimated for reaction kinetics analysis.

Multistage microwave mediated hydrolysis of oil cake

The experimental combination giving higher reducing sugar concentration was used for multistage experiments. The kinetic analysis showed that solids at 6% (w/v) with sulfuric acid concentration of 3% (v/v) for a reaction time of 6 min gave higher reducing sugar concentration. Experiments conducted in triplicate followed the same combination for multistage experiment. The experiment scheme is shown in Figure 2. Total sugar and reducing sugar concentration was analyzed at each stage. The yield of sugars at each stage was calculated using Eq. 1 and overall yield using Eq. 2.

$$\text{Sugar yield (stage i)} = \frac{\text{Sugar concentration in stage i}}{\text{Solids concentration in stage i}} \quad (1)$$

$$\text{Overall sugar yield} = \frac{\sum (\text{Sugar concentration (g/L)} \times \text{volume of hydrolysate (L)})}{\text{Solids mass fed in stage 1 (g)}} \quad (2)$$

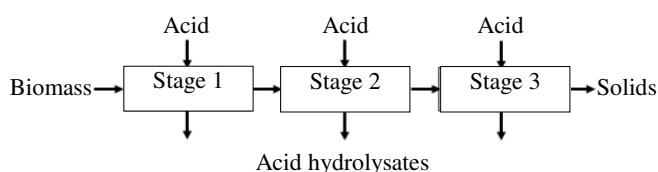


Figure 2. Scheme of multistage acid hydrolysis of oil cake

Fermentation of hydrolysates to ethanol

The acid hydrolysate obtained by microwave mediated process was used for ethanolic fermentation by using *Saccharomyces cerevisiae* MTCC 171. The reducing sugars concentration was readjusted to 6 g/L final hydrolysate broth concentration and autoclaved. No additional nutrients were added. A sub culture of *S. cerevisiae* was grown in nutrient medium for 24 h and 1 mL of this was transferred to the autoclaved hydrolysate broth. The culture flasks with hydrolysate and microbe were agitated and incubated at room temperature (25-30 °C) and 150 rpm for 24, 48 and 72 hours. The sample was distilled and ethanol was estimated using potassium dichromate method¹⁹.

Results and Discussion

Biomass composition

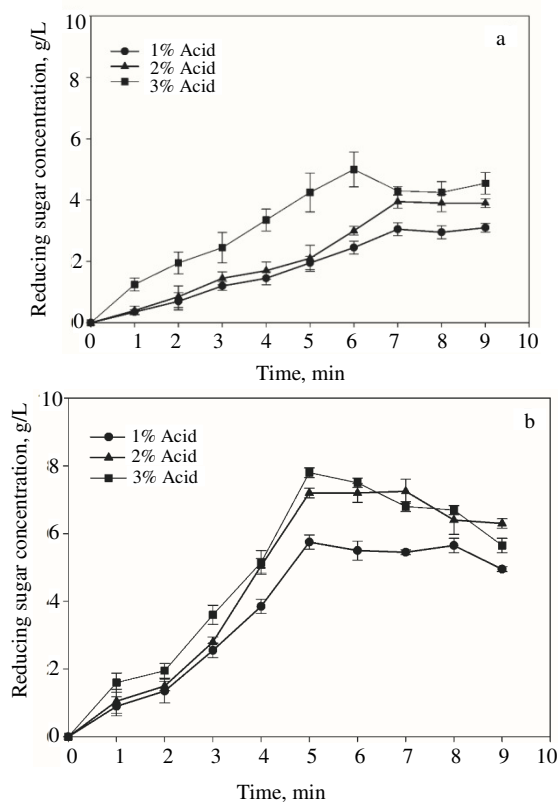
The composition of *Pongamia pinnata* oil cake was determined using NREL procedures. Table 1 represents the composition of powdered biomass. The oil cake has about 60% of the sugar that can be hydrolysed and used for ethanolic fermentation. The lipid present in the biomass depends upon the extent to which oil has been expelled during oil extraction process.

Table 1. Composition of pressed oil cake of *Pongamia pinnata*

Components	% w/w
Cellulose	27.60±1.17
Hemicellulose	31.20±0.85
Starch	1.10±0.10
Lignin	4.20±0.51
Protein	13.10±1.15
Lipid	16.45±1.13
Moisture	3.16±0.96
Ash	5.32±0.92

Kinetics of single stage acid hydrolysis facilitated by microwave

Kinetic studies of microwave facilitated acid hydrolysis of oil cake to sugars was conducted at 3 different microwave powers. The sulfuric acid concentration was also varied at these power levels. In Figure 3a. reducing sugar concentration increased with time up to 6 min at microwave power of 140 W. The concentration remained same beyond 6 min. At 280 W power, the reducing sugar concentration increased up to 5 min (Figure 3b.) and similar trend was observed with microwave power of 420 W (Figure 3c.). Highest reducing sugar concentration of 7.80 ± 0.14 g/L and total sugar concentration 10.35 ± 0.58 g/L of was obtained at 280 W and 3% (v/v) acid concentration.



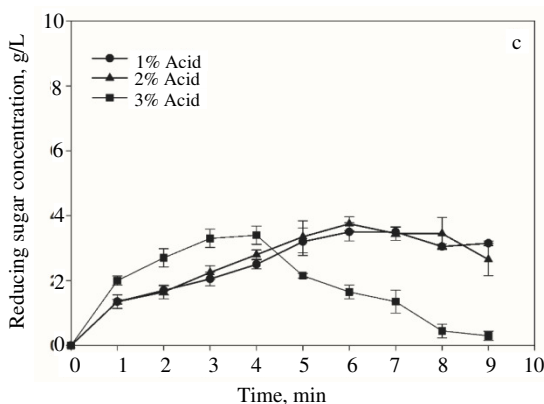


Figure 3. Kinetics of microwave facilitated acid hydrolysis of oil cake at different microwave powers: a) 140 W; b) 280 W; c) 420 W

The sugar concentration was low at 140 W and 420 W in comparison with 280 W. There could be two reasons that attribute to this behavior *viz.*, thermal effects and degradation reactions. The thermal effect is due to the microwave heating whereas degradation reaction is due to the further conversion of sugars to furfurals²⁰. At 140 W, the temperature of reaction mixture (40-60 °C) was much lower in comparison with temperature at 420 W (80-100 °C). The thermal effect caused the variation in rate of reaction by altering the rate constants of the reaction^{13,14}. Thus, it was expected that as the microwave power increased from 140 W to 420 W, reducing sugar concentration would also increase with time. In contrast, the sugar concentration decreased beyond 5 min as well as for power above 280 W. This decrease in sugar concentration could be because of further conversion of reducing sugar to its degradation product furfurals. In addition to this effect, reaction mixture was evaporated quickly at 6 min and beyond when power of 240 W and 420 W was administered, which lead to the reduction in sugar concentration. Thus, microwave power of 280 W with acid concentration 3% (v/v) and irradiation time of 5 to 6 min could be a suitable experimental combination to yield high sugar concentration.

Multistage microwave mediated hydrolysis of oil cake

Based on the kinetic analysis, biomass solids at 6% (w/v) was treated with 3% (v/v) sulfuric acid for a reaction time of 6 min at 280 W. The hydrolysate was analyzed for yield of reducing sugar concentration and total sugar concentration. The biomass solids remained after 1st stage was treated again with 3% (v/v) acid at 280 W for 6 min. This procedure was repeated for total 3 stages. The sugar concentration and yield obtained in each stage is shown in Table 2. Maximum amount of sugar was released in first stage. In stages 2 and 3 the sugar concentration is decreased possibly due to the increase in solids to acid ratio. This in turn increased the rate of undesired sugar conversion thereby increasing the concentration of furfurals.

The overall yield is a measure of removal of sugar from the biomass based on initial quantity of biomass supplied to stage 1. Since the stages were sequential, an analysis of yield progression was done. Of the sugar released in three stages, 1st stage accounted for about 60-70% of the overall yield. Use of 2nd and/or 3rd stage could improve the sugar yield but at the cost of lower sugar concentrations. To cater to the needs of ethanolic fermentation with broth sugar concentrations greater than 5 g/L, only single stage hydrolysis is recommended.

The yield of reducing sugar released is comparatively similar to that produced using conventional methods reported in literature^{6,7,9}. Due to the increased rate of reaction, reaction time is reduced by many folds.

Table 2. Sugar concentration and yield in multi stage acid hydrolysis of oil cake

Stage	Concentration, g/L		Yield, g/kg oil cake	
	Reducing sugar	Total sugar	Reducing sugar	Total sugar
1	7.71±0.34	10.02±0.52	128.4±5.6	167.0±8.7
2	3.29±0.55	5.69±0.64	51.2±9.2	117.8±12.6
3	0.51±0.18	1.42±0.35	9.3±3.3	34.7±9.4
Overall	3.84±0.08	5.71±0.21	191.8±4.1	285.5±10.3

Fermentation of single stage oil cake hydrolysate to ethanol

The acid hydrolysate produced from oil cake through single stage process was used to prepare fermentation broth to a final sugar concentration of 6 g/L. Ethanol yield obtained from *Saccharomyces cerevisiae* at different incubation time are shown in Figure 4. Ethanol titer of 2.04±0.23 g/L was produced in 72 hours using acid hydrolysate. Though, this ethanol titer is less in comparison with literature reported processes, this process can be improved by utilizing suitable high yielding strains or improving medium composition. This work demonstrates the use of acid hydrolysate obtained from microwave mediated process to produce ethanol.

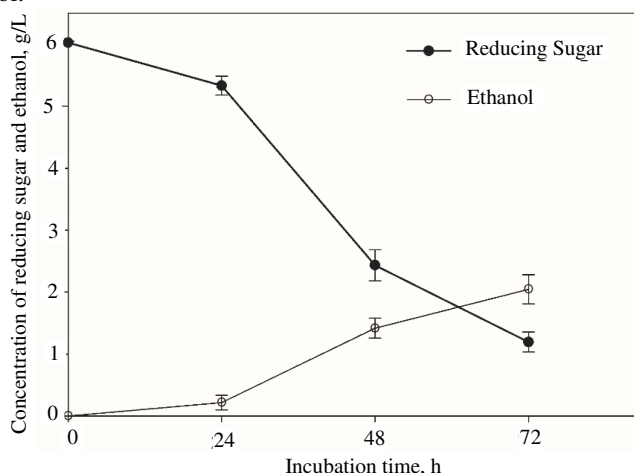


Figure 4. Ethanol production from acid hydrolysate of oil cake after incubating with *Saccharomyces cerevisiae* at different time intervals

Conclusion

Use of microwave radiation was demonstrated as a suitable heating medium for acid hydrolysis of *Pongamia pinnata* oil cake. This process released reducing sugar and use of multi stage process did not improve the sugar concentration. Hence it is suggested to use single stage process. The hydrolysate was fermented to produce ethanol using yeast. Although the ethanol produced was less, process can be improved using further using suitable improvisation techniques. This work demonstrated the use of byproduct of biodiesel industry as a suitable feedstock for bioethanol production.

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Conflict of interest

The authors declare no competing financial interest.

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