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ORIGINAL ARTICLE

Microwave Accelerated Dilute Acid Hydrolysis of *Pongamia* Pressed Oil Cake for Release of Fermentable Sugars

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ABSTRACT
Bioethanol is produced from various sugar sources but the economic viability of such process mainly depends on the cost of the feedstock, processing methods and its availability. Some of the current processing methods are not only energy intensive; they generate fermentation inhibitors, thus hampering the economic viability of the overall process. This paper describes the use of microwave energy along with acid hydrolysis to produce fermentable sugars from low-cost feedstock viz. de-oiled cake of Pongamia pinnata. The process variables for digestion of starchy and lignocellulosic content of oil cake to produce reducing sugars were screened and optimized. The volume of reaction, solids concentration, sulfuric acid concentration and exposure time were significant in increasing sugar yield. The presence of un-extracted lipid in the biomass had no significant role in the hydrolytic reaction. Further, the fermentation inhibitors (furfural and HMF) were much lower in the case of the microwave-treated process. Sugar yield of 193.4±14.2 g total sugar per kg oil cake and 154.9 ± 8.8 g reducing sugar per kg oil cake under optimized conditions are reported.
Keywords; *Biomass to biofuels, Microwave, Acid hydrolysis, Pongamia oil cake, Fermentable sugars, Response surface methodology.*

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INTRODUCTION

The term "Biofuel" is attributed to fuel that is derived from any organic material or biomass. Ethanol is one of the most trusted alternate fuel for the future that reduce GHG emissions and being produced on a commercial scale worldwide [1]. Production of bioethanol differs considerably with the raw material involved. But, some of the major stages in the process remain the same. The process may take place in different conditions such as temperature, pressure, microorganisms, presence of oxygen and several other influencing parameters. But, in general, these stages include hydrolysis (chemical, physical and/or enzymatic), fermentation and distillation [2, 3]. The schematic illustration of the ethanol production from pressed oil cake or any biomass is shown in Figure 1.

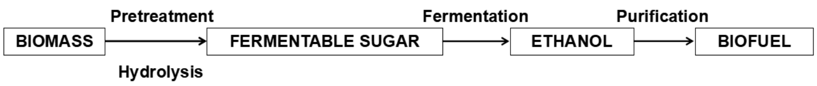

Figure 1: Schematic illustration of the ethanol production from biomass.

The efficient application of cellulose is limited by the presence of strong hydrogen bonds between the molecules in the polymer [3, 4]. Prior to conversion of lignocellulosic biomass to fuel, a pretreatment step is necessary to hydrolyze the polymers, namely cellulose and hemicellulose into their monomeric compounds. A pretreatment method uses techniques such as physical treatment, chemical treatment, physico-chemical treatment, biological treatment and combination of these [2, 5–7]. This pretreatment alters the structure and strength of cellulosic biomass and makes the cellulose more accessible for further modification. Then, the cellulose and hemicellulose can be hydrolysed into its constituent sugars using acids or enzymes [5, 8]. One of the commonly used methods is combined acid treatment along with steam explosion [9, 10]. This process is simpler t han other methods and consumes lesser energy. But, it co-produces sugar degradation products such as furfural and hydroxymethyl furfural (HMF). Hemicelluloses undergoes dehydration with formation of 2-furaldehyde (furfural) and the hexoses are dehydrated to 5-hydroxymethyl-2-furaldehyde (HMF) [9, 11]. These degradation products when present in fermentation medium act as inhibitors for ethanol production [12–14].

India being the world's third largest emitter of GHG, aims to increase its biofuel economy to $15.6 billion by 2020 [15]. To meet the required quantity of ethanol production as per revised Indian Biofuel policy, there is a need to tackle several associated challenges such as, identification and utilization of under-utilized biomass, process challenges and purification technology. The biodiesel industries in India depend upon the oil from non-edible sources and co-produce pressed oil cake in large quantity [16, 17]. This de-oiled cake is rich in carbohydrates and is a potential biomass to produce bioethanol. The major challenge is the process of converting the complex carbohydrates to fermentable sugars, upon which the microbes or enzymes can act to produce ethanol [11, 18].*Pongamia pinnata* is one of the widely used non-edible sources to produce biodiesel in India [16, 18]. Due to this the de-oiled cake, a by-product of biodiesel industry, is available in plenty. Doshi and Srivastava [19] reported glucose yield of 66 mg reducing sugars per gram of *Pongamia* seed cake using two step pretreatment method. In the first step, residues were treated with 0.5% sulfuric acid at a temperature of 121 ^{0}C for 90 min. In the second step residues were treated with 5% sulfuric acid for 70 hours at 50 ^{0}C. Higher yield of glucose from de-oiled *Pongamia* seed cake was reported by Radhakumari et al. [20]. Here, they used a single step process with 7.5% sulfuric acid and digested the biomass for 60 minutes using an autoclave; obtained yield of 0.245g of glucose per g of seed cake. Apart from sulfuric acid, other acids like hydrochloric acid, acetic acid has been used for the digestion of biomass. Hydrochloric acid (2% w/w) treated *Pongamia* de-oiled cake at 100 ^{0}C for 60 minutes yielded 0.132 g of glucose per g of seed cake [21].

Microwave radiation accelerates chemical processes through the intrinsic heat generation because of extensive molecular collisions, vibration of polar molecules and ionic movement [22]. The performance of a microwave process is mainly attributed to the dielectric properties of the lignocellulosic material and the surrounding medium. Microwave has been used for pretreatment of biomass for release of fermentable sugars [23–25].

In the current study, microwave radiation is used as heating medium to facilitate acid hydrolysis of *Pongamia pinnata* pressed oil cake. The present work reports the use of microwave energy for acid hydrolysis of pressed *Pongamia* oil cake to release fermentable sugars with lower energy requirement. Further, this method produces lower quantity of fermentation inhibitors, thus favouring alcoholic fermentation of the product mixture.

MATERIAL AND METHODS

Biomass and chemicals: The biomass, de-oiled cake of *Pongamia pinnata*, used for acid hydrolysis experiments was procured from a biodiesel production centre which produced biodiesel from *Pongamia* oil. The de-oiled cake was the residue obtained through expulsion of oil from its seed using mechanical process with a helical screw extruder. The fresh cakes were procured and finely powdered using a mixer-grinder. The composition of the biomass was determined as per NREL methods [26]. The lipid was quantified using gravimetric analysis following Soxhlet extraction using chloroform – methanol solvent system. The powder was dried overnight at 60 ^{0}C and stored in airtight container until its use for further experiments. All the chemicals used were of reagent grade and used without any further modifications.

Microwave assisted acid digestion process: For the hydrolysis experiments, a microwave oven with 5 fixed power levels (Onida, Power Solo 17D) was used. The power level when used beyond 40% results in evaporation of the liquid fraction and the sample dries off very quickly, hence, a fixed microwave exposure power of 280 W (40% of maximum output power 700 W) was used for all experiments. Hydrolysates obtained through sulfuric acid catalyzed hydrolysis are more suitable for further fermentation process [10]. Hence sulfuric acid was selected as catalyst for hydrolysis. Six grams of powdered biomass was taken in a flat bottom Erlenmeyer flask to which, 3 %(v/v) sulfuric acid was added to obtain 6 %(w/v) solids concentration. The sample was exposed to microwave radiation at 280 W for 5 minutes, cooled and centrifuged, and the supernatant solution was analyzed for its composition.

Quantification methods: The supernatant solution obtained after acid digestion process was neutralized using a few drops of 5 N aqueous NaOH solution, filtered using Whatman No.1 filter and then the filtrate was analyzed for total sugar, reducing sugar, inhibitors furfural and 5-hydroxy methyl furfural (HMF). Spectrophotometer based anthrone method for total sugars [27] and DNS method for reducing sugars [28] was used for estimation. Glucose was used as standard. Inhibitors furfural was estimated using ethanol-HCl–aniline method [29] and HMF by White's method [30].

Factorial design experiments
Analysis of variables that affects the release of fermentable sugars and inhibitors was assessed by performing a series of statistically designed experiments. A 'resolution IV' 2 level fractional factorial experimental design was employed. Since the microwave heating differs from other methods of heating, some predetermined theoretical analysis was performed to choose the factors. Certain aspects, such as volumetric heating, mixing of contents within the reaction vessel in microwave oven, surface area available for catalysis, absorption of microwave in presence of entrainer molecule, presence of lipid in the biomass were considered along with conventional factors. Based on this, the variables chosen for experiment were volume of reaction mixture, solids concentration, sulfuric acid concentration, time of exposure, aeration condition, particle size, pre-treatment with hexane and glycerol concentration. Here, glycerol was chosen as an entrainer molecule which is one of the major by-product of biodiesel industry. About 16 experiments were conducted with orthogonally designed combination of factors at 2 levels (high and low). The factors and their levels used are listed in Table 1. Experiments were conducted in random order.

Table 1: Parameter levels for fractional factorial design.

Parameter	Factor code	Unit	L (Low)	H (High)
Volume of reaction mixture	A	mL	50	100
Solids concentration	B	%(w/v)	2	6
Sulfuric acid concentration	C	%(v/v)	0	3
Time of exposure	D	min	2	5
Aeration condition	E	(LPH)	No (0)	Yes (1.5)
Particle size	F	µm	< 300	420 - 600
Pre-treatment with Hexane	G		No	Yes
Glycerol concentration	H	%(v/v)	0	3

Acid hydrolysis using conventional heating: An autoclave based pressurized steam at 15 psig (or 2 bar) is commonly used conventional method of hydrolysis of biomass to yield fermentable sugars [31]. The biomass was treated under similar conditions of reaction composition under microwave and pressurized steam. Solids concentration of 2 % & 6 %(w/v) treated with 0 % & 3 % (v/v) sulfuric acid was used in both methods. The heating time was chosen as 2 min and 5 min for microwave, 15 min and 30 min for conventional method of heating. Experiments were conducted in triplicate. Further, statistical analysis was performed to ascertain the difference between the two methods to produce reducing sugars, furfural and HMF.

Optimization of microwave assisted hydrolysis of pressed Pongamia oil cake: The significant factors identified through fractional factorial design were optimized using 4 factors 5 level central composite rotatable experimental design (CCRD). The experimental sets were designed using Minitab v15. The factors and their levels are given in Table 2. The reaction volume was varied from 50 ml to 150 ml based on the observation post fractional factorial experiments. Experiments were conducted in random order to minimize the biasing effect by unexplained variables. The concentration of total sugars and reducing sugars were measured as response variables.

Table 2:Experimental combinations for response surface optimization of microwave assisted acid hydrolysis of pressed *Pongamia* oil cake.

Variable	Unit	Coded Levels				
		-2	-1	0	1	2
Volume	ml	50	75	100	125	150
Solids Conc.	%(w/v)	2	3	4	5	6
H_2SO_4 Conc.	%(v/v)	0.00	0.75	1.50	2.25	3.00
Time	min	2	3	4	5	6

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \qquad \text{(Eq. 1)}$$

A quadratic model (Eq. 1) was fit to the response variables using Minitab v15 where y is the response (sugar concentration); xi and xj are coded independent variables and β0, βi, βii and βij are intercept, linear, quadratic and interaction constant coefficients, respectively. The statistical analysis and multi-response optimization was carried out to maximize the sugar concentrations. The optimal conditions were validated by performing experiments in triplicate at optimal settings and compared with predicted sugar concentrations.

RESULTS AND DISCUSSION

Biomass characterization: The composition of pressed oil cake of *Pongamia pinnata* was determined using NREL procedures. The percentage composition of powdered biomass is given in Table 3. The biomass has about 60% of sugar which can be hydrolysed for use in sugar based fermentation process. The lipid present in the biomass depends upon the extent to which oil has been expelled during oil extraction process. The presence of lipid may affect the sugar hydrolysis reaction through adverse reactions. To test the effect of lipids on hydrolysis, extraction of lipids prior to microwave experiment was considered as one of the parameter.

Table 3: Composition of pressed oil cake of *Pongamia pinnata*.

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

Acid hydrolysis facilitated by microwave

Fractional factorial design: Fractional factorial experiments were carried out to screen the significant parameters that affect the acid hydrolysis process for conversion of complex carbohydrates to fermentable sugars using microwave radiation. The parameters were used at 2 levels and statistically designed experiments were carried out in randomized order. After centrifugation, the supernatant was estimated for sugar concentration (total sugar and reducing sugar) and fermentation inhibitors concentration (furfural and HMF). The concentrations of these products of microwave assisted acid digestion are given in Table 4.

Table 4: FFD experimental combinations and hydrolysate concentration.

Run #	Factors[a]								TS (g/L)	RS (g/L)	Furfural (mg/L)	HMF (mg/L)
	A	B	C	D	E	F	G	H				
1	H	H	H	H	H	H	H	H	20.94	7.63	38.9	17.7
2	H	H	H	H	L	H	H	L	5.32	2.26	9.5	20.2
3	H	H	L	L	H	L	L	H	4.68	2.46	12.2	0.0
4	H	H	L	L	L	L	L	L	13.77	6.14	18.6	58.8
5	H	L	H	H	H	H	L	L	2.47	0.51	6.8	0.0
6	H	L	H	H	L	L	H	H	1.96	0.68	10.8	0.0
7	H	L	L	L	L	H	L	H	2.07	1.69	21.6	4.0
8	H	L	L	L	H	L	H	L	2.36	1.94	1.7	0.0
9	L	H	H	H	H	H	L	L	10.25	5.01	8.1	46.8
10	L	H	H	H	H	L	H	L	12.31	2.35	56.8	6.1
11	L	H	L	L	L	H	L	H	10.44	2.23	13.5	0.0
12	L	H	L	L	L	L	H	H	15.90	6.60	23.5	0.0
13	L	L	H	H	H	L	L	H	7.32	3.88	3.2	154.6
14	L	L	H	H	L	H	H	L	9.60	3.94	9.4	15.9
15	L	L	L	L	H	H	H	H	2.31	0.81	8.1	42.9
16	L	L	L	L	L	L	L	L	3.59	0.66	4.0	24.0

[a] A = Volume; B = Solids concentration; C = Sulfuric acid concentration; D = Time of exposure; E = Aeration; F = Particle size; G = Extraction with Hexane; H = Glycerol concentration; TS = Total sugar; RS = Reducing sugar; L = Low; H=High.

Statistical Analysis: The variance in data were analyzed to obtain high sugar concentration. Although it is desirous to have low furfural & HMF concentration, the ANOVA showed, there is no significant effect of process parameters. Moreover, the concentration of furfural and HMF were very low. Response curve was modelled for total sugar, reducing sugar and inhibitor (HMF + Furfural) concentration using a linear fit model. ANOVA was used to ascertain the reduced fit model at 95% confidence level and t-test was used to screen the significant parameters. The ANOVA of the linear fit model equations is given in Table 5 for reducing sugars and total sugars. The F test shows that linear model is statistically significant (Total sugar: $F_{8,7}$=6.47, p<0.05; Reducing sugar: $F_{8,7}$=8.19, p<0.05) and 7 degrees of freedom for error provides stronger evidence for the built model. None of the parameters were significant in production of inhibitors even at 80% confidence level.

Table 5: ANOVA for fractional factorial design[a].

Source	Total Sugar					Reducing Sugar				
	DF	SS	MS	*F*	*p*	DF	SS	MS	*F*	*p*
Main Effects	8	439.9	54.99	6.47	0.012	8	69.08	8.64	8.19	0.006
Residual Error	7	59.5	8.50			7	7.38	1.06		
Total	15	499.4				15	76.46			
R^2	88.08%					90.34%				

[a] DF = Degrees of Freedom, SS = Sum of Squares error, MS = Mean Sum of squares error.

The statistically significant parameters were identified using t-test (95% confidence level). The parameters having statistically significant effect on synthesis of total sugars (Figure 2a) were, sulfuric acid concentration, solids concentration and time of exposure. Even though time of exposure in case of reducing sugars (Figure 2b) was not significant at 95% level, it was significant at 81% level (or α = 0.19). To accommodate the effects for both total sugar and reducing sugar, time of exposure could be considered to have significant effect. In case of fermentation inhibitors (furfural and HMF) production, none of the parameters have shown significant effect. These inhibitors are known to inhibit ethanol fermenting strains at above 1000 mg/L concentrations [13, 32]. The concentration of inhibitors in all experimental combinations were much lower than biological significance level with a maximum concentration of 160 mg/L.

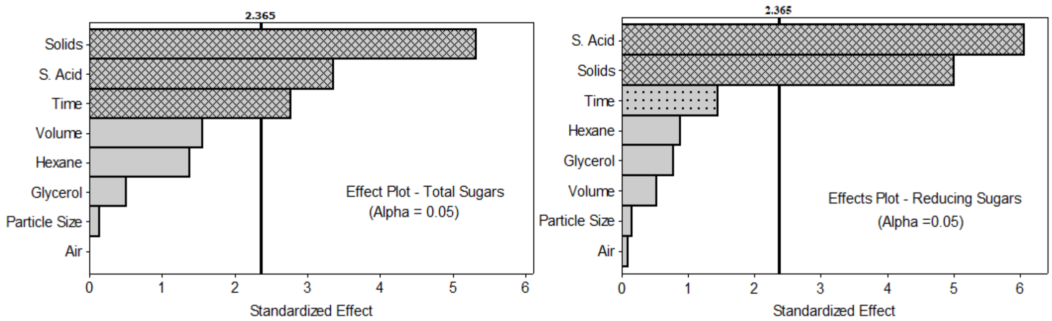

Figure 2: Standardized effects plot for total sugars (a) and reducing sugars (b).

Analysis of parametric effect: The main effects of parameters chosen for experiments deduced from linear fit model are reported in Table 6. Presence of air increases the chance of structural deformation in case of lignocellulosic digestions [33]. In addition, aeration was expected to aid in mixing of reaction components. But aeration increased the chances of oxidative degradation of sugars in to inhibitors. Particle size below 1 mm size was found to have no contribution towards improvement in sugars concentration. The analysis showed that there was 49% chance that particles of size lower than 300μm tends to increase inhibitors concentration. Also, Ballesteros et al. [34] had shown that particle size of 5 mm – 10 mm tend to yield more soluble sugars. Perhaps, the smaller particles have a great chance to undergo digestion quickly due to easy access to acid and microwave radiation.

The oil cake was pre-treated with hexane to remove lipids. Agitated cold extraction using hexane (1:10 w/v ratio) at room temperature for 48 hours was carried out to remove lipids. This method could remove 65% of lipid, leaving about 6% lipid in the oil cake un-extracted. Thus, the hexane treated and untreated biomass had lipid composition of 6% and 16% respectively. The statistical analysis showed, the presence of lipid did not have any significant effect on the release of sugars under microwave irradiation. But there was 50% chance that hexane treated biomass tend to produce lower amount of sugar degradation products. Since the concentration of furfural and HMF were quite lower than the biological significance limit, solvent extraction to eliminate lipids may not be necessary. Further, estimation of lipids in the hydrolysate using sulpho-phospho-vanillin method [35] showed very minute quantities of total lipids.

Table 6: Evaluation of parametric effects on *Pongamia* cake hydrolysis.

Term	Total Sugar		Reducing Sugars		Fermentation Inhibitors	
	% Effect	p-value	% Effect	p-value	% Effect	p-value
Aeration	1.6	0.999	-0.01	0.927	81.8	0.326
Particle Size	-2.5	0.901	2.4	0.882	-53.3	0.513
Hexane treatment	14.6	0.210	26.0	0.406	-55.2	0.499
Reaction Volume	-8.7	0.163	-29.3	0.614	-94.5	0.262
Solids concentration	82.7*	0.001	100.0*	0.002	11.3	0.888
Sulfuric acid concentration	100.0*	0.012	63.2*	0.001	100.0	0.237
Glycerol	12.7	0.626	9.6	0.466	31.1	0.700
Time of exposure	23.8*	0.028	52.2	0.192	88.7	0.290

* Significant at 95% level

Microwave heating is known to be a volumetric and molecular level heating process [36]. The volume of reaction showed no statistically significant effect on release of sugars from complex carbohydrates. But, there was about 74% chance of producing lower amounts of HMF and furfural if larger reaction volumes are used. In this case, volume was varied from 50 ml (low) to 100 ml (high). It is quite possible that heat released during microwave heating dissipated quickly in lower volumes and favored the inhibitors production. To ascertain this fact, a few experiments were carried out at 150 ml reaction volume (data not reported). When larger volume was used, furfural and HMF produced at very low concentration. It indicated that higher reaction volume helped in sinking the heat into diluent rather than accelerating the sugar degradation process.

Even though, the solids concentration alone did not show much impact on the formation of furfurals and HMF, presence of higher amount of sulfuric acid showed a stronger effect. Presence of higher amounts of acid increased the combined severity factor of the process. Thus, increasing chances of enhanced degradation reaction of monomeric sugars to furfurals and other low molecular weight products [9].Glycerol, an entrainer molecule and a green solvent, was added to improve the efficiency of microwave absorption. But glycerol showed insignificant impact on production of sugar and fermentation inhibitors. Increase in exposure time also increased the chance of microwave contact with reaction mixture. This in turn increased the rate of reaction. In the case of hydrolysis of carbohydrates to sugars, the effect was low but exposure time played a dominant role during the formation sugar degradation products. Thus, exposure time was considered as one of the major factor to achieve the goal of maximizing sugar concentration along with reduced degradation reaction. To increase sugar concentration and reduce degradation products, the important variables that need to be considered are reaction volume, solids concentration, sulfuric acid concentration and microwave exposure time. In addition, particle size in the range of 600-1000μm could be a suitable choice. Aeration, hexane treatment and addition of glycerol are not necessary to achieve the aforementioned objectives.

Comparison with conventional heating method

A set of experiments were conducted to find the effect of microwave on the hydrolysis of biomass and its synergistic effect with sulfuric acid pretreatment, where, 2% & 6% (w/v) solids concentration with 0 and 3% (v/v) sulfuric acid final concentration were used. Absence of sulfuric acid acts as a control and brings out the effect of microwave alone on hydrolysis reaction. Similar experiments were conducted using an autoclave where, pressurized steam was used at 15 psig or 2 bar (absolute). The yield of reducing sugars and fermentation inhibitors (furfural and HMF) in both methods are represented in Figure 3 and Figure 4 respectively. Microwave - sulfuric acid hydrolysis process resulted in 7.12±0.16 g/L reducing sugar concentration in 5 min amounting to a yield of 118.7 ± 2.7g reducing sugar per kg of oil cake. A higher yield of 194.1 ± 3.1g reducing sugar per kg of oil cake was obtained in 5 min of microwave exposure but the reducing sugar concentration in hydrolysate was as low as 3.92 ± 0.12g/L. In all these cases, the furfural and HMF were very low in comparison with conventional method.

Presence of sulfuric acid increased the release of fermentable sugars and microwave exposure increased the rate of reaction, thereby reducing the time of reaction. The amount of sugar released with microwave alone is quite higher in comparison with conventional heating method without acid. In addition, microwave exposure produced lower amounts of inhibitors in comparison with the conventional heating method. This signifies the synergistic effect of microwave with sulfuric acid on hydrolysis of biomass to release higher amounts of sugars in short time along with lower amounts of fermentation inhibitors.

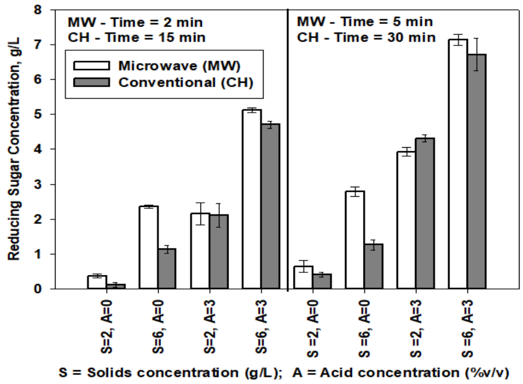

Figure 3: Reducing sugar concentration in microwave and conventional methods.

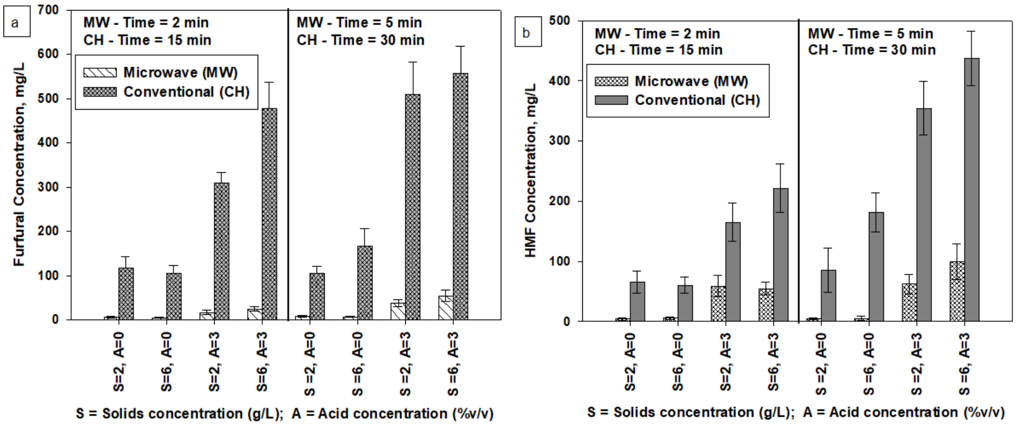

Figure 4: Concentration of fermentation inhibitors released in microwave and conventional methods of processing: a) Furfural concentration; b) HMF concentration.

Optimization of process parameters using CCRD

The factors that significantly influence the microwave mediated acid hydrolysis of pressed oil cake of *Pongamia pinnata* were optimized using response surface methodology or CCRD. The factors sulfuric acid concentration, solids concentration, microwave exposure time and reaction volume were optimized. The experimental combinations and their responses (concentration of total sugar and reducing sugar) are reported in Table 7. The responses were modeled to quadratic equation (Eq. 1) and ANOVA was performed. The significant linear, quadratic and interaction terms were retained to obtain reduced fit model. The reduced fit models for total sugar (TS)concentration (Eq. 2.) and reducing sugar (RS) concentration (Eq. 3.) were used for to determine the optimal condition.

Insignificant *lack of fit* test [TS: (p=0.406) > 0.05; RS: (p=0.098) > 0.05] in ANOVA table for reduced fit models (Table 8) indicated that the predicted models fit well with the experimental data. These model equations were optimized with a goal to maximize the sugar yields using multi-response optimization. The predicted optimal condition was as follows: 6 % (w/w) solids concentration treated with 2.74 %(v/v) sulfuric acid concentration in a reaction volume of 75 ml for 5.5 min under microwave power of 280 W. The particle size of solids used was in the range of 600 – 1000 μm with mean particle size 825 μm. Under the optimal condition, the predicted concentrations were 12.91 ± 1.47 g/L total sugar and 8.66 ± 0.58 g/L reducing sugar. Experiments conducted to validate the predicted models yielded 11.60 ± 0.85 g/L total sugar and 9.29 ± 0.53 g/L reducing sugar. Difference between predicted and experimental results was insignificant as determined by Student's t test. Hence, under optimal conditions, yield of 193.4 ± 14.2 g-TS per kg-oil cake and 154.9 ± 8.8 g-RS per kg-oil cake are reported. The response curves plotted at optimal condition indicated that there was significant amount of quadratic effect shown by factors on total sugar production (Figure 5a). However, the interaction effects were more significant in case of reducing sugar production (Figure 5b).

$$TS = 10.086 + 0.731 A + 1.152 B + 0.504 C + 1.536 D - 0.906 A*A - 0.701 B*B - 0.541 D*D + 0.923 B*C \quad (Eq. 2)$$

$$RS = 4.473 - 0.2891 A + 0.7157 B + 0.2277 C + 0.7004 D + 0.2502 D*D - 0.427 A*B + 0.405 A*C - 0.776 A*D - 0.350 C*D \quad (Eq. 3)$$

Table 7: Experimental runs for response surface optimization of microwave assisted hydrolysis of *Pongamia* oil cake.

Run No.	Factors				Response (g/L)	
	A: Volume	B: Solids Conc.	C: H_2SO_4 Conc.	D: Time	TotalSugar	Reducing Sugar
1	-1	-1	-1	-1	4.34	2.64
2	+1	-1	-1	-1	6.49	3.45
3	-1	+1	-1	-1	4.87	4.42
4	+1	+1	-1	-1	6.92	4.08
5	-1	-1	+1	-1	3.51	2.21
6	+1	-1	+1	-1	7.06	4.76
7	-1	+1	+1	-1	9.25	5.13
8	+1	+1	+1	-1	9.34	6.04
9	-1	-1	-1	+1	8.81	6.12
10	+1	-1	-1	+1	10.56	3.91
11	-1	+1	-1	+1	10.18	8.12
12	+1	+1	-1	+1	10.65	4.34
13	-1	-1	+1	+1	5.76	4.23
14	+1	-1	+1	+1	9.33	4.21
15	-1	+1	+1	+1	11.83	7.23
16	+1	+1	+1	+1	12.44	4.76
17	-2	0	0	0	5.09	4.85
18	+2	0	0	0	6.75	3.66
19	0	-2	0	0	4.73	2.89
20	0	+2	0	0	8.75	5.19
21	0	0	-2	0	7.18	4.05
22	0	0	+2	0	10.38	6.03
23	0	0	0	-2	5.12	3.81
24	0	0	0	+2	9.65	7.12
25	0	0	0	0	11.18	4.68
26	0	0	0	0	10.98	4.12
27	0	0	0	0	11.77	5.23
28	0	0	0	0	10.06	3.89
29	0	0	0	0	8.59	4.15
30	0	0	0	0	9.48	4.89

Table 8: ANOVA table for reduced fit models of total sugar and reducing sugar obtained through response surface modeling

	Total Sugar#					Reducing Sugar#				
Source	DF	SS	MS	*F*	*p*	DF	SS	MS	*F*	*p*
Model terms	8	157.625	19.703	14.73	<0.001	9	46.253	5.139	22.34	<0.001
Linear	4	107.387	26.847	20.07	<0.001	4	27.316	6.829	29.69	<0.001
Quadratic	3	36.613	12.204	9.12	<0.001	1	1.803	1.803	7.84	0.011
Interaction	1	13.625	13.625	10.18	0.004	4	17.134	4.284	18.62	<0.001
Error	21	28.095	1.338			20	4.600	0.230		
Lack of fit	16	21.024	1.314	0.93	0.590	15	3.245	0.216	0.80	0.660
Pure error	5	7.070	1.414			5	1.356	0.271		
Total	29	185.719				29	50.854			
R^2	84.9%					90.9%				

#DF = Degrees of Freedom, SS = Sum of Squares error, MS = Mean Sum of squares error

The reducing sugar yield in microwave assisted process is comparatively in line with reported sugar yields that used conventional method but has significantly brought down the reaction time by many folds [18–21]. The enhancement of rate of reaction by microwave radiation is mainly attributed to its interaction at molecular level vibrations [22, 36]. Consequently, this interaction brings down the activation energy necessary for rate limiting steps, thereby increasing rate of the reaction. The only current disadvantage of this method is the problems associated with scaling it up to industrial level [9].

Due to its inherent processing speed, the continuous microwave system shows higher productivity [36]. Perhaps, the use of continuous or semi-batch microwave processing system may prove advantageous.

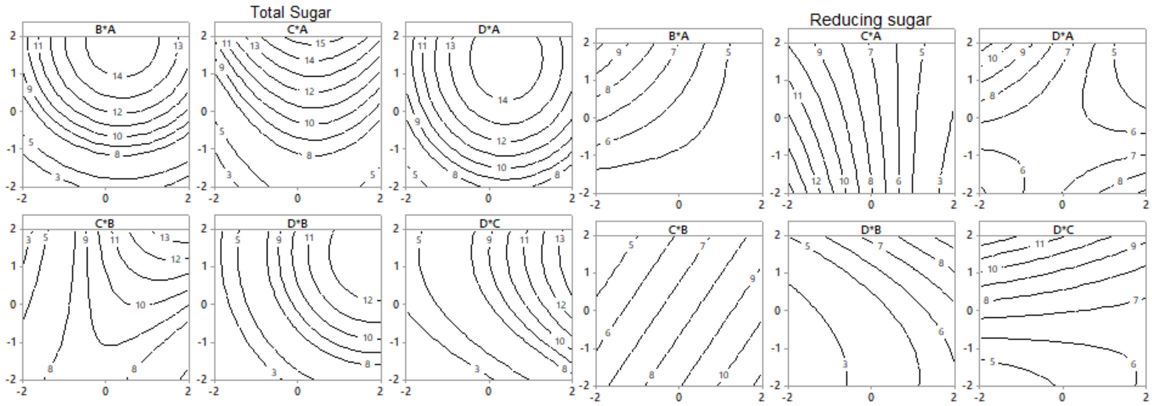

Figure 5: Contour plots under optimal condition for release of total sugar (a), reducingsugar (b).

CONCLUSION

A statistical experimental design was used to identify or screen the parameters that enhance microwave assisted acid hydrolysis of biomass to fermentable sugars. Sulfuric acid concentration, solids concentration, and microwave exposure time were found to be statistically significant in releasing higher amounts of fermentable sugars. Higher reaction volume and particle size in the range 600 – 1000 µm are suitable choice to achieve the desired goal. The fermentation inhibitors furfural and HMF that co-produced in the hydrolysis reaction were minimal and below the biologically effective limits. Further, microwave along with sulfuric acid not only increases the rate of reaction, thereby reducing reaction time but also helped in decreasing further degradation of sugars to furfural and HMF. Hydrolysis of pressed oil cake of *Pongamia* with microwave heating was optimized. Under optimal conditions total sugars yield of 193.4 ± 14.2 g per kg oil cake and reducing sugars yield of 154.9 ± 8.8 g per kg oil cake were obtained.

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