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FULL LENGTH ARTICLE

Modeling of microwave assisted coconut leaf acid hydrolysis pre-treatment process for maximum release of reducing sugars

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ABSTRACT

Conversion of lignocellulosic material into reducing sugars will be the economical way of developing media for fermentation. Maximum carbon source should be present in the media in order to felicitate the growth of microorganism. In the current investigation attempt is made to develop the model for microwave assisted hydrolysis. Significant parameters responsible for maximum release of reducing sugars have been screened (Concentration of HCl, weight of coconut leaf, time and power of irradiation) using one factor at a time (OFAT) method. Further response surface methodology (RSM) is adopted to identify the optimum condition. Model equation is developed based on RSM revealed the optimum conditions of 10.58% v/v of HCl concentration, 15.12 g/100ml of coconut leaf with predicted concentration of 16.6g/L of reducing sugars.

INTRODUCTION

The increasing population of world and the growing per capita energy demand boost the interest in producing energy from various renewable resources to replace fossil fuel. Lignocellulosic materials are one of the most abundant and cost effective raw materials that can be used for production of energy [1]. Coconut palm are abundantly available in the coastal areas. Agricultural residues can be used as a biomass for the production of bioethanol. *Cocos nucifera*, the coconut palm, is a monocot perennial member of the Arecaceae (palm family), cultivated in tropical areas worldwide for its fruit and fiber. Coconut palms are medium-sized, solitary herbaceous plants. Although treelike in form, their trunks are composed not of wood, but of fibrous, stout, overlapping stems, and may grow to 25 m tall (80 feet), topped by a crown of pinnately compound leaves up to 4 meters (15 feet) long[2]. The leaf of this plant which is an agricultural residue can be used as a biomass for the production of bioethanol. A number of pretreatment methods have been developed for improving hydrolysis of lignocellulosic biomaterials. Most microwave pretreatment is generally carried out at elevated temperature (>160°C). Some previous studies have shown that application of microwave irradiation pretreatment may significantly increase the conversion of starch materials to glucose [3,4]. Combination microwave treatment with either acid or alkali or combined acid/alkali might be an alternative for pre-treatment of lignocellulosic materials has been recently explored [5]. Microwaves (MW) are electromagnetic waves spanning a frequency range from 300 (3×108 cycles/s) to 300 GHz (3×1011 cycles/s), with most industrial and household microwave systems operating at either 900 or 2.45 GHz [6]. Microwaves interact with polar molecules and ions in a material, and result in both thermal and non-thermal effects that drive physical, chemical or biological reactions [7]. Microwave irradiation has been commonly used in chemistry because it has high heating efficiency and can, in some cases, increase reaction rate and reduce reaction time. There are many examples of application of microwave irradiation in the field of organic synthesis [8], chemical catalysis [9], and solid state reactions [10]. A number of industries have benefited from the distinction between microwave heating and conventional heating such as in food processing field. Especially, energy conversion of microwave irradiation lead to volumetrically heat generation with the target material rather than through the surface of the material, as is the case with conventional heating [11]. Microwave irradiation can affect in a positive way in biomass digestion. In the current investigation MW pretreatment was carried out to release reducing sugars from coconut palm leaf. One factor at a time (OFAT) method was adopted to screen the effective parameters.

METHODS

Processing of Coconut Leaf

The dried coconut leaves were cut into small pieces and sun dried to remove the moisture content. After sun drying leaf was kept in hot air oven at 90°C for complete removal of moisture. After drying, the leaves are then powdered using mixer. The powder is sieved using mesh number 10. The powder is stored in refrigerator until the further use.

Optimization of pre-treatment of Coconut Leaf (CL)

Pre-treatment of Coconut leaf was carried out by acid hydrolysis with hydrochloric acid.

Screening of significant parameters and their levels by OFAT

The conventional OFAT approach was used to select the significant physical parameters and the initial test range of the two variables i.e. HCl concentration (X1 = % v/v), and Coconut palm powder concentration (X2 =%w/v) for the pre-treatment processes. The effect of the parameters on the pre-treatment process was checked varying one parameter at a time and keeping the other parameters and process conditions constant (Table 1).Power of irradiation is fixed at 300watts and time of irradiation at 8 min, since the further increase in these parameters exhibited evaporation of sample. All the experiments were performed in duplicates. The experiments were carried out in 250 ml conical flasks containing 100 ml of acid solution of appropriate strength, with appropriate quantity of Coconut palm powder (Table 1). The Flasks were heated in microwave for eight minutes and the estimation of reducing sugars released was carried out by dinitrosalicylic acid (DNSA) method. The parameter levels at which maximum reducing sugars were extracted, were chosen as the centre point values to enhance the pre-treatment process by Rapid Surface Methodology(RSM).

Table 1: Selected parameters and their levels for OFAT studies of Coconut Leaf Powder pre-treatment.

Parameter	Notation	Test Range
HCl concentration(% v/v)	X1	2 - 15
Weight of Coconut leaf (g/100ml)	X2	3-14

Central Composite Design

Two experimental factors: HCl concentration (X1, %v/v) and weight of coconut leaf (X2, g/100ml) were selected for RSM optimization. These factors showed significant effect on the pre-treatment process during OFAT studies and their levels were optimized for maximum extraction of reducing sugars from coconut leaf using central composite design (CCD) for acid hydrolysis. The concentration of reducing sugars extracted on pre-treatment was determined as the output (Y).CCD was designed with two factors and five levels (Tables 2), and it consisted of 12 experimental runs (Tables 3). The levels of other non significant factors were kept constant at the tested centre values during the OFAT studies. These optimization experiments were designed by STATISTICA software [12]. The data of pre-treatment studies obtained was subjected to analysis by using analysis of variance (ANOVA) using the same software. A second order polynomial model was utilised to obtain the mathematical response between the response and the independent variables

$$Y = \beta o + \sum \beta i Xi + \sum \beta ii Xi^2 + \sum_{i \neq j} \beta ij Xi Xj$$

Where *Y* is the dependent response, the *βo, βi, βii,βij* are estimates of polynomial coefficients and *Xi, Xj* represent independent variables.

Table 2: Independent parameters and their coded levels for pre-treatment of Coconut leaf by acid hydrolysis optimized by CCD.

Factors	Notations	Levels				
		-α	-1	0	1	+α
HCl Concentration(%v/v)	X1	9.17	10	12	14	14.83
Weight of Coconut leaf (g/100ml)	X2	10.88	11	13	14	15.12

Table 3:CCD for two variables and experimental results with respect to pre-treatment of DOSC by acid hydrolysis.

Run No.	X1	X2	Y_{ex}
1	10.00	11.00	10.87
2	10.00	14.00	14.48
3	1400	11.00	13.74
4	14.00	14.00	7.43
5	9.17	13.00	13.86
6	14.83	13.00	6.23
7	12.00	10.88	13.36
8	12.00	15.12	14.24
9	12.00	13.00	14.84
10	12.00	13.00	14.84
11	12.00	13.00	14.84
12	12.00	13.00	14.84

X1:HCl Conc.(%v/v),X2:Weight of coconut leaf(g/100ml)
Y_{ex}(mg/ml): Concentration of RRS from Coconut leaf after pre-treatment with HCl

The optimization experiments were carried out in 250 ml conical flasks containing 100 ml of acid solution as per Tables 4. The time and power of irradiation was maintained at 8 mins and 300W (from OFAT studies). The estimation of released reducing sugars was carried out by DNSA method [13].

Estimation of released reducing sugars by DNSA method

The samples subjected to acid hydrolysis were neutralised to pH 7.0 by treating with sodium hydroxide solution and sulphuric acid solution. The expected products of hydrolysis are glucose, sucrose, galactose, cellobiose. All of these saccharides are reducing sugars. Therefore, the concentration of released reducing sugars (RRS) was then estimated by the colorimetric method using the UV Visible spectrophotometer at 540 nm with 3, 5- DNSA reagent with glucose as standard [13].

RESULTS AND DISCUSSION

Optimisation of pre-treatment of Dry Coconut leaf

Selection of significant parameters and their levels by OFAT

The significant physical parameters and the initial test range of four factors (Table 1) in the pre-treatment process were obtained by OFAT. When acid concentration was varied keeping the other factors constant (X2=5%w/v; X3=8mins; X4=300W),the reducing sugar concentration increased with increased acid concentration upto 12%.Similarly when dry coconut leaf concentration was varied keeping other factors constant (X1=12%v/v;X3=8mins;X4=300W),it was observed that the reducing sugar concentration had increased with an increase in coconut leaf concentration upto 13g.On the basis of these results obtained, levels of factors were determined and subjected to optimisation by CCD. The insignificant independent parameters, i.e. Time (X3) and power of irradiation (X4) were maintained at the centre of their levels (8mins and 300W respectively).

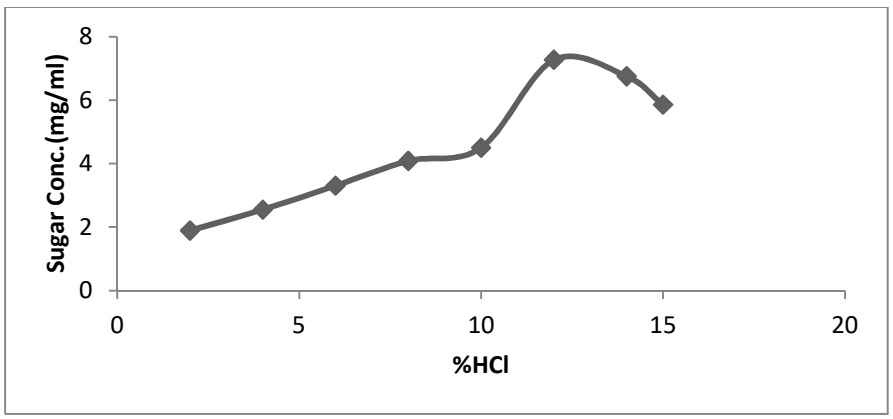

Fig 1:Release of reducing sugar on acid hydrolysis of coconut leaf at different HCl concentrations.

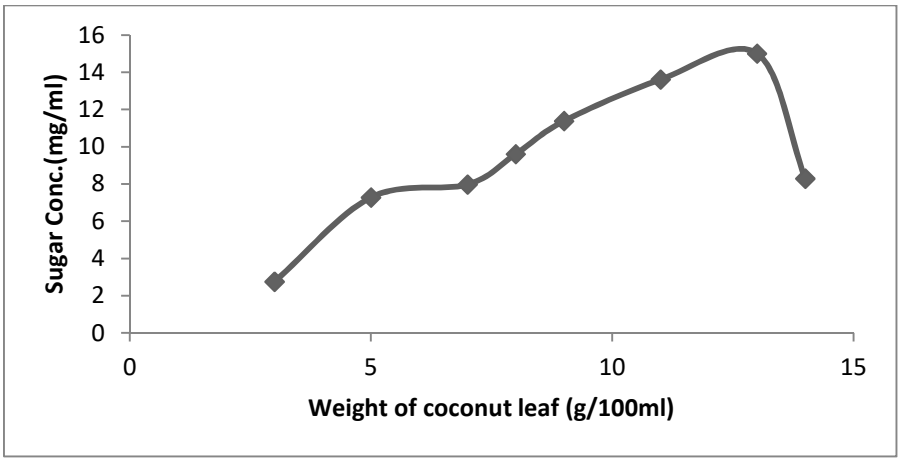

Fig 2:Release of reducing sugar on acid hydrolysis of coconut leaf at different Concentration intervals of coconut leaf.

Optimisation of parameters for release of reducing sugars by acid hydrolysis by CCD

The influence of HCl Concentration (X1) and Coconut leaf concentration (X2) on release of reducing sugars after acid hydrolysis of Coconut leaf was determined by CCD as indicated in Table 4. This table also present the observed values for RRS concentration by acid hydrolysis at different combinations of the independent parameters.

Tables 3 indicate the ANOVA table for release of reducing sugars on acid hydrolysis of coconut leaf respectively. Table 3 shows that the linear effects of both the independent variables and the interaction between HCl concentration and Coconut leaf concentration showed highly significant effect on the reducing sugar concentration released by
acid hydrolysis of Coconut leaf (Y).

Table 3: ANOVA table for release of reducing sugar on acid hydrolysis of Coconut leaf as affected by HCl conc. and Coconut leaf concentration. $R^2 = 0.9505$; $p<0.05$ is significant

	F	p-value
X1(L)	49.26	0.000
X2(L)	0.55	0.485
X1(Q)	46.01	0.001
X2(Q)	1.08	0.340
X1 by X2	35.04	0.001

The response surface graph for reducing sugars as a function of %HCl concentration and weight of coconut leaf is depicted in Figure 3. It was observed that release of reducing sugars increased with increase of weight of coconut leaf at a particular HCl concentration.

The regression equations for release of reducing sugars by acid hydrolysis of Coconut leaf, as a function of the two independent variables (X1 and X2) and their linear and quadratic interactions, is represented by the following:

Ypr=-218.7+24.74*X1+14.30*X2-0.6074*X1*X1-0.155*X2*X2-0.870*X1*X2

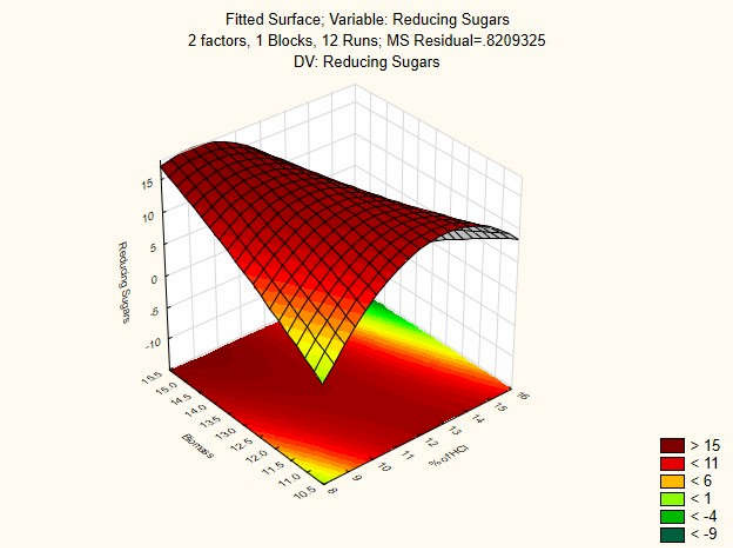

Fig 3:Three dimensional plot showing effect of HCl Concentration,Coconut leaf concentration on acid hydrolysis of coconut leaf.

The optimized levels of variables (X1 and X2) for the maximum release of reducing sugars by both the hydrolysis were determined by desirability profiles (Figure 4).On the basis of desirability plots the optimized factors for obtaining maximum reducing sugars were 10.58% v/v of HCl concentration at 15.12 g/100ml of coconut leaf. The desirability function to get the maximum release of reducing sugars was plotted by the least square method. The level of variable giving the highest desirability (1.0) was selected as the optimum level. Predicted reducing sugar concentration at optimized condition is 16.6g/L.

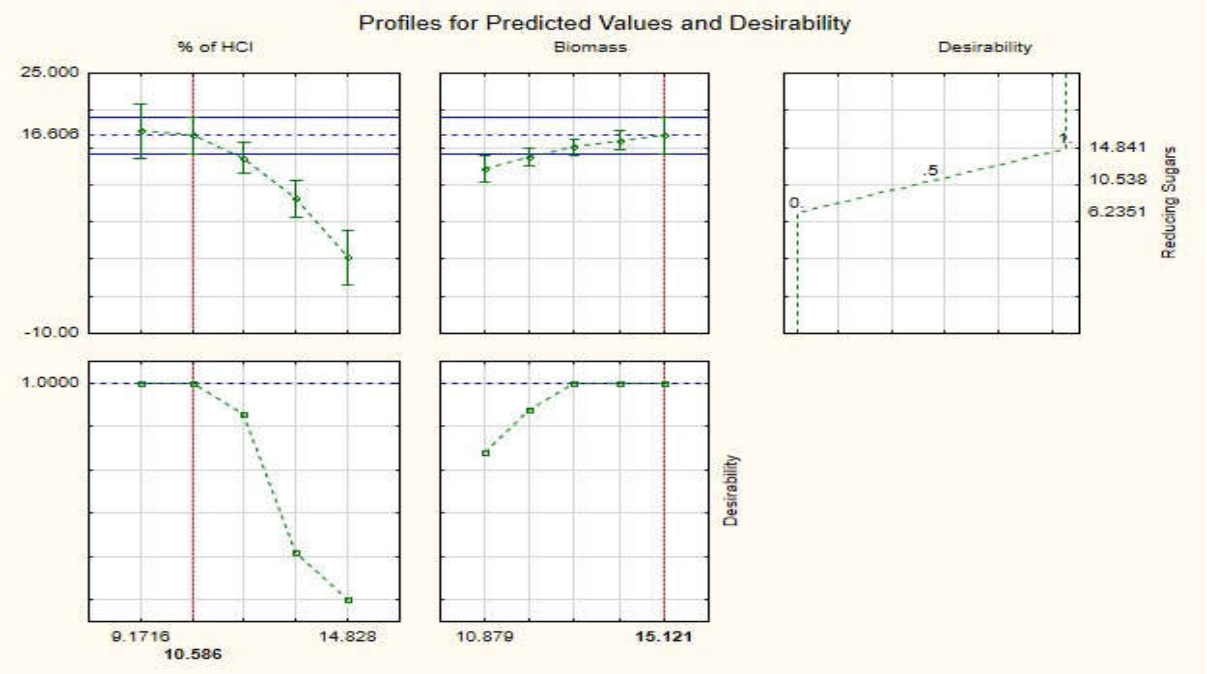

Fig 4: Profiles for desirability levels of factors i.e. X1(HCl Conc.%v/v) and X2 (Weight of coconut leaf g/100ml) for maximum release of reducing sugars on acid hydrolysis of Coconut leaf.

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REFERENCES

1. Romani A., Garrote G., Parajo J.G, (2012) Bioethanol production from autohydrolyzed Eucalyptus globules by simultaneous saccharification and fermentation operating at high solids loading, Fuel, 94, 305 –312.
2. P.K. Thampan. 1981. Handbook on Coconut Palm. Oxford & IBH Publishing Co.

3. S. Zhu, Y. Wu, Z. Yu, Q. Chen, G. Wu, F. Yu, C. Wang and S. Jin,(2006) Microwave Assisted Alkali Pre-treatment of Wheat Straw and Its Enzymatic Hydrolysis, *Process Biochemistry*, 94(3), 437-442.
4. T. Palav and K. Seetharaman, (2007) Impact of Microwave Heating on the Physico-Chemical Properties of a Starch- Water Model System, *Carbohydrate Polymers*, 67(4) 596-604.
5. P. Binod, K. Satyanagalakshimi, R. Sindhu, K. Usha Janu, R. K. Sukumaran and A. Pandey, (2012) Short Duration Mi-crowave Assisted Pretreatment Enhances the Enzymatic Saccharification and Fermentable Sugar Yield from Sug-arcane Bagasse, *Renewable Energy* 37(12) 109-116.
6. Shi, J.; Pu, Y.Q.; Yang, B.; Ragauskas, A.J.; and Wyman, C.E. (2011). Comparison of microwaves to fluidized sand baths for heating tubular reactors for hydrothermal and dilute acid batch pretreatment of corn stover. *Journal of Bioresource Technology*, 102(10), 5952-5961.
7. Sridar, V. (1998). Microwave radiation as a catalyst for chemical reactions. *Journal of Current Science,* 74, 446-450.
8. Majetich, G.; and Hicks, R. (1995). The use of microwave heating to promote organic reactions. *Journal of Microwave Power Electromagnetic Energy,* 30 (1), 27-45.
9. Wan, J.; Tse, M.; Husby, H.; and Depew, M. (1990). High-power pulsed microwave catalytic processes: decomposition of methane. *Journal of Microwave Power Electromagnetic Energy,* 25(1), 32-38.
10. Caddick, S. (1995). Microwave assisted organic reactions. *Journal of Tetrahedron,* 51 (38), 10403–10432.
11. Farag, S.; Sobhy, A.; Akyel, C.; Doucet, J.; and Chaouki, J. (2012). Temperature profile prediction within selected materials heated by microwaves at 2.45GHz. *Journal of Applied Thermal Engineering*, 36,360- 369.
12. Statsoft (1999) Statistics for Windows, Statsoft Inc., Tulsa, USA.
13. Miller G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 1959, 31(3), 426– 428

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