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Optimization of Microwave-Assisted Extraction for Enhancing Reducing Sugar of Water Hyacinth pretreatment before Organic Fertilizer Production at Klong Yong Community in Phutthamonthon, Nakhon Pathom, Thailand

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Abstract

Water hyacinth is an aquatic plant that has emerged as a major invasive weed and high reproduction rate at Soi Bon canal in Klong Yong, Phutthamonthon, Thailand as a lignocellulosic plant material, it can be made into an organic fertilizer at 60-90 days. Microwave-assisted extraction was to investigate the effect of different solvents concentration, various sizes of material and the optimization model for hydrolyzed cellulose and hemicellulose from lignin structure disruption of water hyacinth. The result showed that calcium hydroxide solvent was the best solvent for total reducing sugar extraction from water hyacinth with size as 3-5 cm. Box-Behnken design was conducted for microwave-assisted pretreatment at 450 watts with using three parameters; the solid to liquid ratios as 1:10, 1:15, and 1:20 with volume of liquid at 30 ml, extraction times of 20, 30, and 40 minutes, and calcium hydroxide solvent at various concentrations as 0.1, 0.55, and 1 %wt. The optimum conditions of total reducing sugar from water hyacinth solution were 54 mg/g at the solid to liquid ratio as 1:10, concentration of calcium hydroxide at 0.55 %wt, and 30 minutes of extraction time. Microwave-assisted pretreatment was an alternative of organic fertilizer production at shorter times for fermentation.

Keywords: Water Hyacinth; Total Reducing Sugar; Lignocellulosic; Microwave-Assisted Extraction; Box-Behnken

Introduction

The water hyacinth (*Eichhornia crassipes*) is an aquatic plant that originated from South America [1,2]. Water hyacinth lives in tropical and sub-tropical regions such as in Indonesia and Thailand. In Thailand, water hyacinth can be found in lakes, storages, and rivers. Water hyacinth has characterizes such as rapid growth rates, extensive dispersal capabilities, large and rapid reproductive output and broad environmental tolerance [2]. These makes water hyacinth become water pollution that cause major problem in the area, such as block water flow in the river, reduce oxygen content in the water that makes reduction of fishes in the water, increase sedimentation, and also can provoke health problem [2-4]. Current solution, especially in Thailand, for this problem is dispose water hyacinth manually someplace else. This solution is temporary solution and requires high cost.

Despite of disadvantages, as one of the biomass material, water hyacinth has many advantages. Water hyacinth can be made into organic fertilizer [5], craft [1], bio adsorbent [6], bioethanol [7], animal feed [8], and Biogas [9] also can be produced from water hyacinth. Those benefits of water hyacinth can have good impacts to the community. For example is water hyacinth craft that can be done by local community and can increase income of the community. But this has limitation due to it can only use small amount of water hyacinth. The other benefit is water hyacinth organic fertilizer. This organic fertilizer also can be done by local community and also can increase community income and most important is can reduce dependence on the use of chemical fertilizers. Many researchers had studied organic fertilizer from water hyacinth. Muoma John [10] and Polpraset, et al. [5] had done research of making water hyacinth organic fertilizer. In addition, the use of chemical fertilizers continuously raises environmental and health problem and also can reduce soil quality so that it can reduce crop productivity. On the other hand, organic fertilizer can improve fertility of the soil and improve the production of the crop such as improve paddy production in Indonesia.

Water hyacinth is one of the lignocellulosic material as it has lignin, cellulose, and hemicellulose and these are the primary building block of plant cell material. Table 1 shows the components of each material in water hyacinth from many researchers.

Lignin	Cellulose	Hemicellulose	References
9.27	19.5	33.4	[11]
7	31	22	[12]
7.8	17.8	43.4	[13]

Table 1: Components of lignocellulosic material in water hyacinth [11].

To extract cellulose and hemicellulose, and to degrade lignin, water hyacinth must be pretreatment. Among of these materials, lignin is the most difficult material to be degraded due to lignin is rigid structure compare to cellulose and hemicellulose. Lignin appears in forms of crystalline and amorphous. The efficiency of pretreatment can be evaluated through the material dissolved, increase of anaerobic biodegradation and operational costs. In lignocellulosic biomass, the effect of heat occurs at a temperature of 150-180°C where hemicellulose and lignin first began to dissolve [14].

Many researchers had done many experiments to extract cellulose and hemicellulose and degrade lignin more efficient, such as using mechanical, thermal, acid hydrolysis, alkaline hydrolysis, or combination of these methods. One of the pretreatment is microwave pretreatment. This pretreatment is using radiation of electromagnetic energy that is converted into heat energy. This pretreatment has many advantages, such as can penetrates directly into material, heat transfer is rapid and uniform, energy efficient due to it takes short time for the process and no temperature gradient. In conventional heating, the heat transfer from heat source to material through convection, conduction, and radiation processes. This will make heat is not uniform and it has temperature gradient. This process also requires longer time compare to microwave treatment.

Microwave irradiation can alter the structure of cellulosic biomass, including increasing the specific surface area, decreasing the polymerization and crystalline cellulose, the hydrolysis of hemicellulose and lignin depolymerization [15]. Thermal pretreatment with microwaves can destroy complex structure of lignocellulosic material. Eskicioglu, et al. [16] compared the pretreatment using microwave and conventional heating on waste activated sludge; both pretreatment methods equally provided an easy route to dissolve, but with microwave heating, the production of methane increased (± 16 % after 15 days at mesophilic digestion conditions and temperature 96°C). Budiyono, et al. [17] had studied the effects of microwave pretreatment of fresh water hyacinth for biogas production and the optimum condition for this was at power 560 Watt for 7 minutes and for this condition produce 75.12 mililiter biogas per gram of total solids. Ethaib, et al. [18] evaluated the effect of acid and alkali during microwave pretreatment on dragon fruit foliage which gave result of monomeric sugar was found 15.56 mg/g at 800 W and 0.1 N NaOH at 5 minutes of treatment time. Therefore, microwave pretreatment can be used as an approach over conventional heating for pretreatment of biomass in biogas production. Optimization of bioethanol production using water hyacinth had been studied by Zhang, et al. [19] using microwave pretreatment at power 150 W, H2SO4 concentration at 1 wt% for 20 minutes treatment time and bioethanol produced was found 1.291 g/L as the optimum result. Zhu, et al. [20] reported that microwave pretreatment on rice straw was an effective pretreatment method for increasing the rate of hydrolysis. The effect of microwave pretreatment on biogas production from waste activated sludge [21-24], dairy manure [25], kitchen waste [26], food residues [27], municipal solid waste [28,29] and comparison of the use of microwave and ultrasonic in palm oil mill effluent [30] had been studied.

For optimization of the process, Box-Behnken design method was being used for this experiment. Yang, et al. [7] had studied the optimization using Box-Behnken design for maximizing biofuel content of water hyacinth using microwave pretreatment power at 1110W, 3.5 min treatment time. Yang, et al. [7] using Box-Benhken parameters are

Microwave power, amount of absorbent, and treatment time. Previous researchers have studied production of water hyacinth organic fertilizer using subcritical pre- treatment and convensional method (without pretreatment) and the result was the fertilizer can be produced in 10 days (subcritical method) and 30days-58days (conventional method) [5,10] Also, previous researchers had studied the effect of microwave pretreatment on lignocellulosic biomass using strong base (NaOH) and strong acid (H2SO4) [14,31]. However, there is no researcher investigating the influence of the microwave pretreatment on water hyacinth for organic fertilizer production using calcium hydroxide solvent. The objective of this research was to study the effect of microwave pretreatment at various microwave levels of power, solvent concentration and times on improving the digestion of water hyacinth.

Material and Methods

Material

The water hyacinth was collected from Soi Bon canal in Klong Yong, Phutthamonthon, in Nakhon Pathom Province, Thailand. The water hyacinth was then sun dried for 14 days and chopping at 3-5 cm long.

- **Microwave-assisted pretreatment**

Microwave-assisted pretreatment were performed using Anton Paar Multiwave PRO which rotor type is Rotor 8N, and pressure vessel NXF100. The pretreatment was conducted at power 450 W, various solid to ratio content, various treatment time, and various solvent concentration. For this experiment were using calcium hydroxides. %Brix was conducted using digital refractometer.

- **Box-Behnken Design**

Optimization for this experiment were using Box-Behnken design. Factors and level for Box- Behnken design as below Table 2.

Variables	Codes	Ranges and level		
		-1	0	1
Ratio	x1	1.10	1.15	1.20
Concentration	x2	0.1 %wt	0.55 %wt	1 %wt
Time	x3	20 min	30 min	40 min

Table 2: Variables and codes for BBD.

- **Reducing sugar analysis**

The reducing sugar analysis was performed using 3.5 - dinitrosalicylic acid (DNS) [32]. The reagent is a solution formed by the following compounds: 3,5-Dinitrosalicylic acid which acts as an oxidant, Sodium-potassium tartrate was used in which to prevents the dissolution of oxygen in the reagent and sodium hydroxide to provide the medium required for the redox reaction to occur. The analysis was performed using UV VIS Spectrophotometer at wave length 540 nm [33] (Figure 1a,b).

Result and Discussion

Effect of microwave pretreatment on water hyacinth. From Figure 1 showing that microwave pretreatment making water hyacinth darker and softer also making chemical composition of water hyacinth also has certain change. From Zhao, et al. [34], the efficiency of removal of volatile solids, hemicellulose, cellulose, and lignin before and after pretreatment was 13.6%, 29.5%, 5%, and 5.9%. From Table 3 below experimental temperature range from 175˚C to 222˚C and pressure was 20 bar. From experimental temperature and pressure, water in microwave pretreatment process was in sub-critical condition. In sub-critical condition, polarity of water decreases with increasing temperature and water becomes more non-polar and a good solvent for organic compounds. And as temperature increases, water density decreases which leads to increase in diffusivity and hence to increase in degradation of cellulose, hemicellulose and lignin in the tight biomass matrix [34]. Microwave heating heats polar substances. Water is a strongly polar substance, whereas water molecules absorb microwave irradiation, generate heat, and rapidly vaporize during microwave irradiation. The rapid vibration of the water molecules leads to a rapid increase of the osmotic pressure, which disrupts the cell wall of water hyacinth. This process will change structural composition and appearance of water hyacinth.

Figure 1: a. Before pretreatment.

Figure 1: b. After pretreatment.

- **Box-Behnken Design**

From Table 3 below, experimental value obtained of %brix for Box-Behnken design range from 1.8-5.4 %brix.

No	x1 = Solid to solvent ratio	x2 =Concentrati on	x3 = Time	Y = Yield (%Brix)	Temp (^{0}C)
1	-1 (1:10)	-1 (0.1%wt)	0 (30)	3.4	196
2	1 (1:20)	-1 (0.1%wt)	0 (30)	1.9	184
3	-1 (1:10)	1 (1 %wt)	0 (30)	4.3	200
4	1 (1:20)	1 (1 %wt)	0 (30)	1.8	198
5	-1 (1:10)	0 (0.55%wt)	-1 (20)	5.4	196
6	1 (1:20)	0 (0.55%wt)	-1 (20)	2	175
7	-1 (1:10)	0 (0.55%wt)	1 (40)	3.7	222
8	1 (1:20)	0 (0.55%wt)	1 (40)	2.2	211
9	0 (1:15)	-1 (0.1%wt)	-1 (20)	2.2	184
10	0 (1:15)	1 (1 %wt)	-1 (20)	2.4	187
11	0 (1:15)	-1 (0.1%wt)	1 (40)	2.6	209
12	0 (1:15)	1 (1 %wt)	1 (40)	2.5	212
13	0 (1:15)	0 (0.55%wt)	0 (30)	5.2	209
14	0 (1:15)	0 (0.55%wt)	0 (30)	5.3	210
15	0 (1:15)	0 (0.55%wt)	0 (30)	5.3	209

Table 3: Box-Behnken design.

By using Microsoft Excel® 2013, regression model from applies regression analysis as shown in Eq.1 below

$$Y=5.27-1.11X1+0.11X2-0.13X3-0.76X_1^2-1.66X_2^2-1.88X_3^2-0.25X1.X2+0.48X1.X3-0.08X2.X3$$

Where Y is Experimental yield, X_1 is solid to solvent ratio, X_2 is solvent concentration (calcium hydroxide) and X_3 is experimental time. From regression analysis, the value of Multiple R, R Square, and Adjusted R Square is 0.987, 0.975, and 0.931 confirming the Regression model is well fit to the actual data. The 3D-response surface graphs were plotted and shown in Figures 2-4 that illustrate the interaction effect of variables on %brix.

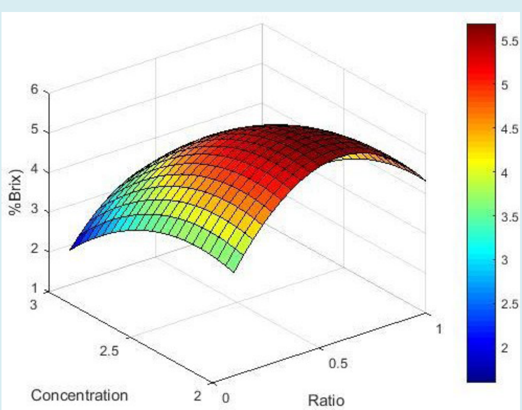

Figure 2: The 3D-graph showing the effect of concentration of solvent and solid to solvent ratio at 30 minutes treatment time.

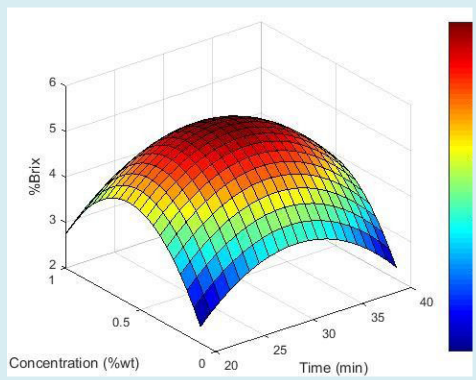

Figure 3: The 3D-graph showing the effect of concentration of solvent and treatment time at solid to solvent ratio.

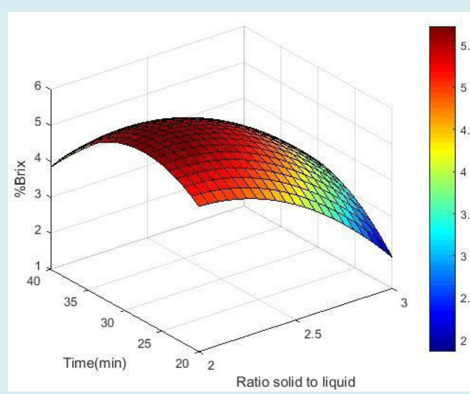

Figure 4: The 3D-graph showing the effect of concentration of solvent and treatment time at solid to solvent ratio 1:15.

The optimum condition for microwave pretreatment using calcium hydroxide solvent was observed at the solid to liquid ratio as 1:10, concentration of calcium hydroxide at 0.55 %wt, and 30 minutes of extraction time, and predicted %brix was 5.74. From Figure 3, higher solvent concentration and higher treatment time at certain solid to solvent ratio, the yield becoming lower. Also at low solvent concentration and shorter treatment time, the yield also lower. This is means that solvent concentration and treatment time was important impact during hydrolysis.

- **Reducing sugar analysis**

The method demonstrated linearity range from 0.15-1.4 mg/ml with linearity curve as Figure 5 below

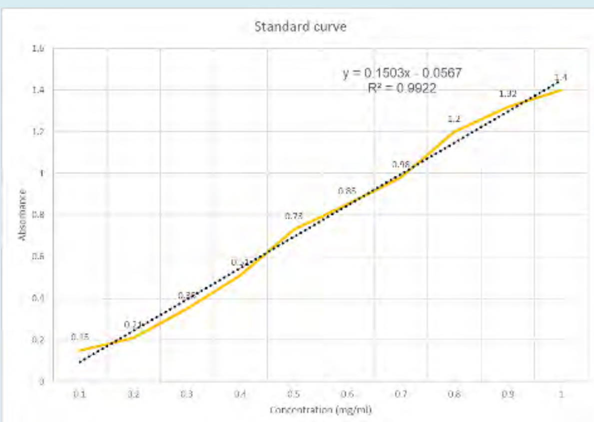

Figure 5: Standard curve.

From the Figure 5, R^2 is 0.9922 and the calibration curve is represented as y = 0.1503x - 0.0567. The amount of total reducing sugars released from the substrate equated to the effectiveness of the pretreatment method. Experimental amount of total reducing sugar of water hyacinth before and after microwave pretreatment was 27 mg/g WH and 54 mg/g WH. The amount of reducing sugar after microwave pretreatment was obtained from optimum condition.

As Rezania, et al. [35] obtained for reducing sugar from water hyacinth was 25+1.5 mg/g of WH for untreated and 95+3.1 mg/g for microwave-alkali treatment. Similarly, Harun, et al. [36] obtained 24.7 mg sugar/g dry matter of sugars in untreated WH. Also in a study 99 mg/g yield of total reducing sugar from untreated WH was produced during hydrolysis [37-43].

Conclusion

Water hyacinth can be utilized into many organic materials, such as organic fertilizer, bioethanol, and many more. Water hyacinth can be hydrolyzed using many pretreatment methods and many solvent, one of the method is microwave method using calcium hydroxide solvent. Water hyacinth was hydrolyzed using different solvent concentration (calcium hydroxide), different treatment time, and various solid to solvent ratio. And the optimum condition obtained at solid to liquid ratio as 1:10, concentration of calcium hydroxide at 0.55 %wt, and 30 minutes of extraction time with total reducing sugar was 54 mg/g WH.

Microwave pretreatment using calcium hydroxide as solvent can be one of the method to hydrolyzed lignocellulosic material from water hyacinth.

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