ORIGINAL RESEARCH PAPER

STUDY OF THE PREPARATION OF SUGAR FROM HIGH-LIGNIN LIGNOCELLULOSE APPLYING SUBCRITICAL WATER AND ENZYMATIC HYDROLYSIS: SYNTHESIS AND CONSUMABLE COST EVALUATION

Hanny F. Sangian, Dian Ranggina, Galih M. Ginting, Ageng A. Purba, Setiyo Gunawan, Arief Widjaja[*]

Sepuluh Nopember Institute of Technology, Department of Chemical Engineering, 60111, Surabaya, Indonesia

*Corresponding author: arief_w@chem-eng.its.ac.id

Abstract: This study concern sugars hydrolyzed from the high-lignin coconut coir dust using moderate subcritical water (SCW) hydrolysis at pressures 20-40 bar for 1 h and to evaluate the consumable costs driver generated. The SCW method produced two products, sugar liquid and solid (SCW-treated substrate). The solid was proceeded to prepare the sugar *via* enzymatic hydrolysis using pure cellulase. Yield of sugar hydrolyzed from lignocellulose by SCW technique was 0.25 gram sugar/gram cellulose +hemicellulose, or 0.09-gram sugar/gram lignocellulose at 160 °C and 40 bar. While, the maximum yield of sugar liberated enzymatically from SCW-treated solid was 0.35-gram sugar/gram cellulose+hemicellulose, or 0.13-gram sugar/gram SCW-treated solid. It was found that carbon dioxide gas was the highest cost driving in SCW hydrolysis.

Keywords: *cellulose, cellulase, coconut coir dust, hemicellulose, lignin, reducing sugar*

13

INTRODUCTION

The global warming caused by emission of greenhouse gases, especially carbon dioxide gas from burning fossil fuels, received attention from scientists concerning sustainability of life on the planet. Increasing of global temperatures on the Earth's surface was due to infrared wave trapped in the atmosphere and it transfers into heat. Global warming has resulted an economic losses caused by flood, famine and a prolonged summer crop failure. Economic losses are estimated increase every year and reach 600 billion dollars per year in the next 15 years [1].

Accumulation of carbon dioxide on Earth's atmosphere is seen as the driving force of global warming [2]. Fossil fuels, oil, coal and gas, are still the main energy sources nowadays. The world's reserves of fossil energies will be exhausted in the coming years. The new formula used to calculate the time fossil energies will run out employing the Klass model. It was calculated that petroleum, coal and gas, would finish 35, 107 and 37 years to go [3].

To overcome the scarcity of energy is to look for new and renewable energy. Energy sources, which are abundant in the earth's surface, are lignocellulosic materials. Lignocelluloses were biologically synthesized through photosynthesis at production rate of one hundred Giga tons per year [4]. Lignocelluloses are enzymatically hydrolyzed into sugars and then fermented to biofuels. Lignocellulose contained in plant composed of cellulose, hemicellulose and lignin. Indonesia is a tropical country with a variety of plants and abundant agricultural wastes, which are source of lignocellulose, such as coconut coir dust.

Pretreatment on lignocellulose, including lignin degradation with conventional techniques have been managed to modify crystal structure of cellulose in order to increase digestibility of enzymatic hydrolysis. Ionic liquid (IL) has been applied on lignocellulosic pretreatments since many advantages such as low vapor pressure, high thermal stability, easy to dissolve many compounds, and easy to be recycled [5 - 6]. The low lignin lignocelluloses, sugarcane bagasse and cotton waste, were hydrolyzed directly to sugars without delignification [7 - 9]. However, these methods have weaknesses such as the use of large amounts of acid and alkaline, difficult to recycle due to the similarity of the boiling points of the acid and water. Ionic liquid is still expensive render it is difficult to apply in large scale.

On the other hand, the subcritical water (SCW) has several advantages, the technology is environmentally friendly, water as the reactant acts as a catalyst and its operation is easy to handle. An SCW method has been applied on pure cellulose and lignocellulose to change the structure from high-to low crystallinity called amorphous structure. Furthermore, SCW could dissolve directly reducing sugars in water in the subcritical state [10 - 13]. However, SCW equipment must be made of anti-corrosion material (alloy) that is resistant to high temperature and pressure and requires a special feed pump. Furthermore, SCW method is conducted in an extreme condition so that SCW method is still expensive [14].

This work was aimed to convert the high lignin lignocellulose, coconut coir dust to reducing sugars by SCW treatment as well as to analyze the consumable cost driver generated from SCW hydrolysis. The procedure of sugar preparation using SCW technique follows: reducing the size of substrate by mechanical technique, treating biomass by SCW method, separating the liquid sugar and solid and hydrolyzing

enzymatically of SCW-treated solid. The reducing sugars liberated via SCW, enzymatic hydrolysis were measured by DNS technique, and the structure of substrate was analyzed by using XRD. Finally, economic evaluation of consumable cost driver was conducted to analyze the feasibility of SCW method on sugar manufacture.

MATERIALS AND METHODS

Material preparation and SCW design

Prior to hydrolysis using subcritical water treatment, coconut coir dust was dried under sunlight for days to reduce the water content and then milled until the size at range of 70-120 mesh. Since the size was not uniform, particles were screened using screener *(Retsch GmBh Rheinische Strade 36 4278, Haan*, Germany) and obtained around 100 mesh.
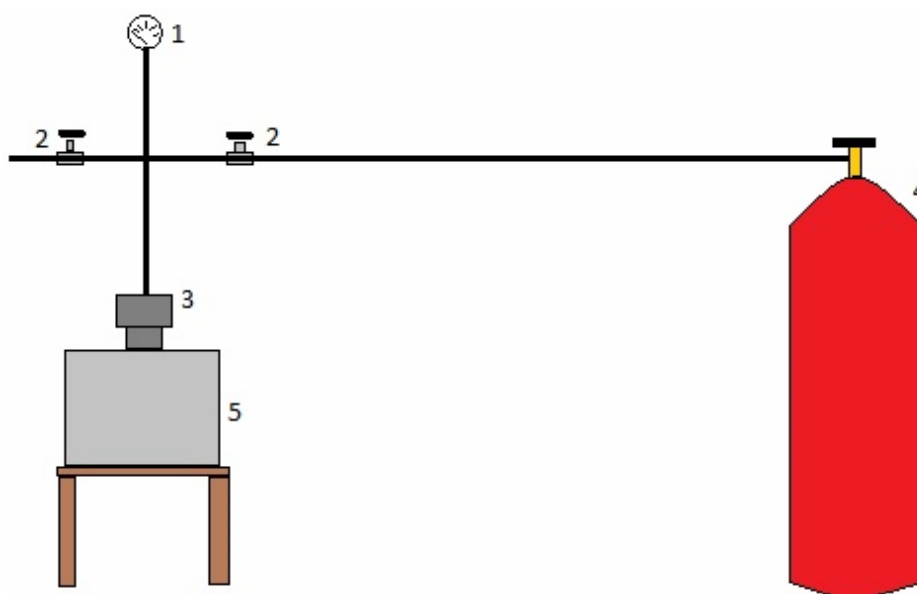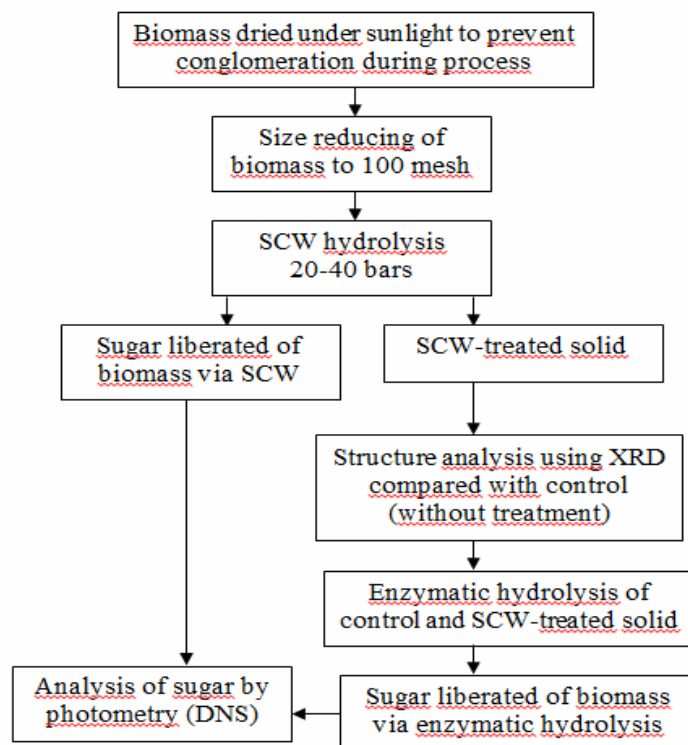

Figure 1. *Design of SCW reactor used in this study*

Figure 1 shows the design of an SCW reactor adapted from work as previously published [10]. The system consists of (1) pressure meter measures the pressure generated inside reactor due to buildup of gases, carbon dioxide and water. (2) Swagelok-valve regulates, directs or controls the flow of fluids, carbon dioxide and water by opening and closing. (3) Five-hundred milliliter non-magnetic SS reactor. (4) Carbon dioxide container to pressurize the reactor, and (5) heater generates heat to increase the temperature inside reactor. The temperature was controlled by PID instrument with reading signal transmitted by thermocouple immersed in liquid. The reactor used was pressurized by carbon dioxide with purity was at 99.9 %. Chemical compositions of substrate were analyzed using gravity meter, which was as previously proposed by Rathin Datta [15].

Procedure of experiment

The procedure of the preparation of sugar hydrolyzed of biomass using SCW and enzymatic hydrolysis is shown in Figure 2. Four grams biomass, which has been dried and reduced its size to 100 mesh, was mixed with 75 mL distilled water inside reactor and the temperature was increased until subcritical water condition was attained.

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┌─────────────────────────────────┐
│ Biomass dried under sunlight to  │
│ prevent conglomeration during    │
│ process                          │
└─────────────────────────────────┘
              │
┌─────────────────────────────────┐
│ Size reducing of biomass to      │
│ 100 mesh                         │
└─────────────────────────────────┘
              │
┌─────────────────────────────────┐
│ SCW hydrolysis 20-40 bars        │
└─────────────────────────────────┘
      │                    │
┌──────────────┐   ┌──────────────┐
│ Sugar        │   │ SCW-treated  │
│ liberated of │   │ solid        │
│ biomass via  │   └──────────────┘
│ SCW          │          │
└──────────────┘   ┌──────────────────────┐
      │            │ Structure analysis   │
      │            │ using XRD compared   │
      │            │ with control         │
      │            │ (without treatment)  │
      │            └──────────────────────┘
      │                    │
      │            ┌──────────────────────┐
      │            │ Enzymatic hydrolysis │
      │            │ of control and       │
      │            │ SCW-treated solid    │
      │            └──────────────────────┘
      │                    │
┌──────────────┐   ┌──────────────────────┐
│ Analysis of  │◄──│ Sugar liberated of   │
│ sugar by     │   │ biomass via          │
│ photometry   │   │ enzymatic hydrolysis │
│ (DNS)        │   └──────────────────────┘
└──────────────┘
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Figure 2. Procedural diagram of the preparation of fermentable sugars hydrolyzed from high lignin biomass using SCW and enzymatic hydrolysis

Temperatures were varied at 110, 120, 130, 140, 150, 160 and 170 °C and pressures were controlled at 20, 30 and 40 bar for one-hour hydrolysis. After hydrolysis, the temperature was decreased using ice water bath until 30 °C. After pretreatment, products liquid sugar and solid were separated using screener paper Whatman No. 2 (GF/B 25 mm) *via* vacuum flask. The SCW-treated solid was dried using oven at 60 °C for 48 h and proceeded to enzymatic hydrolysis. The XRD was applied to analyze the crystalline structure of SCW-treated solid and control.

Enzymatic hydrolysis

The SCW-treated solid, which was not converted into sugars on subcritical water treatment, was subjected to enzymatic hydrolysis using pure cellulase from *A. niger* (Sigma-Aldrich, St. Louis, MO, USA) at 60 °C for 48 h. Enzymatic hydrolysis of SCW treated-solid and sugar analysis followed procedure described in literature [11, 16]. Sodium acetate buffer (*p*H 3, 30 mL, 0.1 M) was poured into treated-substrate inside Erlenmeyer reactor. The mixture was incubated in an oil bath controlled at 60 °C and

slowly stirred using stirrer bar for 48 h. When the pure cellulase added, enzymatic hydrolysis started to release sugar. To measure amount of sugar released by enzymatic reaction at any time, 0.2 mL samples were periodically moved from flask and dissolved with 1.8 mL distilled water and 3 mL DNS solution and then amount of sugar was analyzed using spectrophotometer (CECIL 1001, Cambridge, United Kingdom). The amount of sugar released from SCW and enzymatic hydrolysis was measured by performing spectrophotometer setting a wavelength of 540 nm. Prior to analysis, the mixture was isolated in the tube and put in boiling water for ten minutes to stop the reaction. The sample tube was moved to an ice-water bath and then was rotated in centrifuge equipment (Hermle Labortechnik GmbH-Z 326 K, Wehingen Germany) to settle the solid and pulp. The analysis was conducted in duplicate.

RESULTS AND DISCUSSION

Chemical compositions of substrates

Table 1 describes the chemical compositions vs pressure and temperature of cellulose and hemicellulose contained inside substrates, or without pretreatment, which were calculated by employing Chesson method as previously proposed work [15].

Table 1. *Chemical composition of substrate, control and SCW-treated solid*

T [ºC]	P [bar]	Cellulose [%]	Hemicellulose [%]	Lignin [%]
Control		**16.90**	**20.05**	**51.30**
110	20	24.71	12.71	50.72
120	20	23.71	11.38	52.38
130	20	24.42	9.44	55.18
130	30	22.79	11.88	51.38
150	30	22.25	6.15	55.92
170	30	23.42	5.74	65.75
140	40	20.91	11.29	54.25
160	40	24.46	3.37	56.56

The trends of chemical contents of biomass treated by SCW in various temperatures and pressures are shown in table above that the first three points are control, without pretreatment. Chemical compositions of control were 16.90 % cellulose, 20.05 % hemicellulose and 51.30 % lignin, which differed slightly with other reports, especially cellulose, hemicellulose and lignin [17, 18]. The origin, species and ripeness of raw materials may affect to biomass composition [19]. After SCW treatment, cellulose lignin contents increased, while hemicellulose significantly decreased whose results have not previously reported.

It also shows the effect of pressure and temperatrure on chemical compositions of lignocellulose. Generally, the cellulose composition did not change significantly as temperature and pressure increased in SCW condition. Increasing temperature at *P*

constant resulted in the decrease of hemicellulose content. It was found that hemicellulose content also decreased as pressure increased. It showed that hemicellulose could be degraded effectively into sugars in SCW conditions since it behaves an amorphous structure, meanwhile cellulose is crystalline structure [20].

In this study, coconut coir dust ±4 g was treated by SCW to produce reducing sugar and the solid. The substrate which was not converted into sugar, was procedeed enzymatic hydrolysis. Other data shows that the increase of pressure and temperature resulted in decreasing of lignins-their connections were brokendown and lignins were dissolved in water under high pressure and temperature.

Structural Analysis of substrates

An XRD method was performed to know the structure of substrates, control and SCW-treated substrate. Quantity of crystalline index (CrI) is one of ways of XRD technique to understand the lattice arrangement inside material. For original cellulose called cellulose I displays vivid peak at 22-23°, which related to (002) plane.
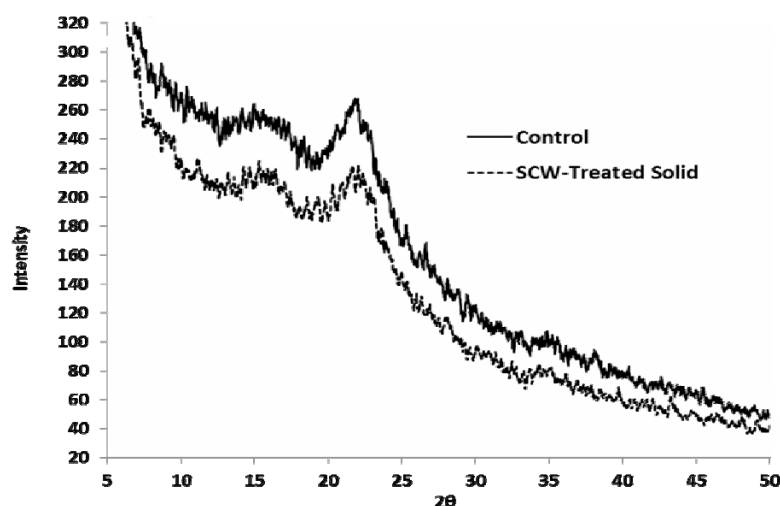
Figure 3. *An XRD pattern of substrate, control (without pretreatment) and SCW-treated solid at 160 ^{o}C, 40 bar for 1 h pretreatment of present study*

Table 2. *Crystalline index of solids, before and after SCW treatment*

Solids	I_{002}	I_{AM}	Cristalline index (CrI)
Before treatment	298	184	0.56
After SCW treatment	216	158	0.48

To measure the crystalline index (CrI) of cellulose from the X ray diffraction spectra (XRD), was calculated from the height division the intensity of the crystalline peak (I_{002} - I_{AM}) and total intensity (I_{002}) after subtraction of blank signal measured without sample. Cellulose I is original cellulose, whose properties are high crystallinity and clear peaks at the angles (16, 22 and 35°) as previously published study [21].

After pretreatment with SCW, peaks (002) and (101) decreased significantly, relating to the crystalline alteration of the solid from cellulose I to cellulose II whose property was an amorphous structure and could improve of enzymatic hydrolysis. Figure 3 shows the

XRD patterns of SCW-treated substrate (160 °C, 40 bar) and non-pretreatment substrate. The value of I_{002} shows the crystallinity of cellulose and is measured at $2\theta = 23^o$, meanwhile, I_{am} describes the amorphous quantity calculated at $2\theta = 18^o$. When the substrate was treated by SCW at 160 ^{o}C, 40 bar, the CrI declined significantly to 0.48 compared to control (without pretreatment) recorded at 0.56. The CrI decrease correlated to the change of cellulose surface. It means that SCW, including subcritical carbon dioxide (SCC) declined the crystalline index of cellulose as previously reported works [22, 23].

Water in subcritical condition shows some important properties such as, low viscosity, low electrical constant and high solubility of organics material. Those properties cause cellulose to interact electrically with water ions, H^+ and OH^- in order that glicosidic bonds connecting monomers are broken down to produce sugar and lower chain polysaccharide. In this situation, subcritical water acted as a medium, or solvent to improve reaction rate. The properties of water, dielectrical constant, solubility and acidity as well as ionization constant are suitable with cellulose so biomass was dissolved as previously reviewed by other authors [24].

Effect of pressure and temperature on sugar released by SCW-hydrolysis

Sugar released by SCW-hydrolysis was measured using DNS (*Dinitrosalicylic acid*) method. Table 3 describes the effect of temperature and pressure on sugar liberated when SCW technique employed for 1 h hydrolysis.

As shown in Table 3, coconut coir dust liberated in average 3.00 g·L^{-1} reducing sugar at 20 bar, at three temperatures, 110, 120 and 130 °C. If pressure added to 30 bar, sugar dissolved into water increased significantly at 4.22 g·L^{-1} with temperatures varied at 130-170 °C.

Table 3. *Amount and yield of sugar converted from coconut coir dust for 1 h hydrolysis using SCW method*

P bar	T [^{o}C]	Sugar concentration [g·L^{-1}]	Vol [mL]	Mass [g]	Yield	
					Yield (1) [g.sugar/g.solid]	Yield (2) [g.sugar/cell.+hemi.]
20	110	2.47	75	0.19	0.05	0.12
20	120	3.01	75	0.23	0.06	0.15
20	130	3.46	75	0.26	0.06	0.18
30	130	4.01	75	0.30	0.07	0.20
30	150	4.22	75	0.32	0.08	0.21
30	170	4.44	75	0.33	0.08	0.22
40	140	4.22	75	0.32	0.08	0.21
40	160	4.96	75	0.37	0.09	0.25

The increase pressure 10 to 40 bar, the amount of sugar broken down from cellulose and hemicellulose chains was in average 4.60 g·L^{-1}, increase 9 % from previous data recorded. The highest sugar obtained from substrate treated by SCW was 4.96 g·L^{-1} at
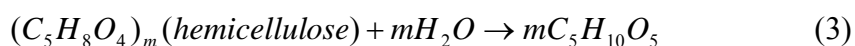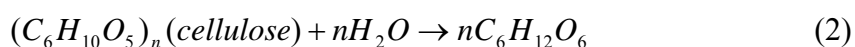
temperature 160 °C and pressure 40 bar, which increased around 100 percent compared with initial temperature and pressure, 110 °C and 20 bar observed at 2.47 g·L^{-1}.

The amount of sugar increased as pressure and temperature increased as revealed in Table 3. Some monomers in cellulose and hemicellulose were hydrolyzed into sugars by water acting as solvent, catalyst and reactant [14]. When water attains subcritical conditions, the H-bound weakens so that water under ways dissociation as follows:

$$2H_2O \rightarrow H_3O^+ + HO^- \tag{1}$$

The ionization constant increases as pressure and temperature turns up [24]. The biomass was dissolved into water in SCW-cellulose and hemicellulose was hydrolyzed by water ions so sugars dominated by glucose and xylose were liberated as follows:

$$(C_6H_{10}O_5)_n (cellulose) + nH_2O \rightarrow nC_6H_{12}O_6 \tag{2}$$

$$(C_5H_8O_4)_m (hemicellulose) + mH_2O \rightarrow mC_5H_{10}O_5 \tag{3}$$

To know the percentage of biomass hydrolyzed to reducing sugar is expressed by yield of sugar. There are two yields, the first is defined as mass of sugar obtained (in g) per mass of dried coconut coir dust input inside reactor (in g). The weight of sugar was calculated by employing the relation of amount of sugar (g·L^{-1}) multiplied by suspension volume. The second definition is mass of sugar divided cellulose+hemicellulose.

As shown in Table 3, the yield of sugar released at 20 bar (110 °C) was 0.05 g.sugar/g. solid, or 0.12 g.sugar/g.cellulose+hemicellulose, which was the smallest yield obtained. If the pressure increased by 10 bar to 30 bar at 130 °C, biomass released monomers, 0.20 g.sugar/g.celulosa+hemicellulose, which rose 67 percent. At pressure 40 bar, 160 °C, the sugar recovered of biomass was 0.09 g sugar/g solid, or 0.25 g.sugar/g.cellulose+hemicellulose that sugar increase was 108 percent from the first conditions, 20 bar, 110 °C, resulted at 0.12 g.sugar/g. cellulose+hemicellulose.

The data show that the yield of sugar released from SCW-hydrolysis was competitive as compared to that treated by conventional methods. The most important advantage of SCW hydrolysis, which is not owned by conventional methods, mostly was zero-wastewater.

Other authors reported that sugar yield of SCW-hydrolysis of whole coconut husk was 0.12 g.sugar/g.dried solid at 259 °C, 200 bar and for 30 min [17]. The results were higher than that to the present work since the previous study used whole coconut coir and employed high pressure and temperature. The difference of biomass type and origin may influence to the chemical composition as previously published work [19]. To degrade more cellulose, the temperature should be increased to near or up critical point of water and carbon dioxide. The total sugar released from SCW treatment declined as temperature and pressure decreased [25, 26].

Consumable cost driver on SCW hydrolysis

Some authors reported that manufacture of sugar hydrolyzed from biomass using SCW and supercritical methods were not feasible for industrial scale [14]. The present study was also conducted to analyze the consumables cost driver on moderate SCW method

whose pressures and temperatures were only 20-40 bar and 110-160 °C, respectively using high-lignin lignocellulose-coconut coir dust, which was abundant in Indonesia.

Manufacture of sugar hydrolyzed from lignocelluloses using SCW technique produces no waste. From this point of view this method is much easier to handle than that conventional technique, such as acid, alkaline and ionic liquid. The chemical methods use consumables such as water, raw material, acid, electricity, enzymes, base, nitrogen gas, ionic liquid and ethanol. The chemical pretreatments in wash step employs very much water to neutralize chemicals from substrates. For example, expenditure of electricity on ionic liquid technique generates a high cost since it follows some steps, physical and chemical pretreatments including wash steps and enzymatic hydrolysis [27, 28].

To find the mass of carbondioxide needed in SCW hydrolysis, it was necessary to calculate the compressibility factor (Z) of gas using relation as previously described study [29, 30]:

$$Z = PV/nRT = Z^0 + \omega Z^1 \tag{4}$$

$$Z^0 = 1 + B^0 P_r/T_r \tag{5}$$

$$Z^1 = B^1 P_r/T_r \tag{6}$$

$$B^0 = 0.083 - 0.422/T_r^{1.6} \tag{7}$$

$$B^1 = 0.139 - 0.172/T_r^{4.2} \tag{8}$$

By inserted equations 5-8 into initial equation, Z, so equation (4) becomes

$$Z = 1 + 0.083 - 0.422/T_r^{1.6} P_r/T_r + \omega [0.139 - 0.172/T_r^{4.2} P_r/T_r] \tag{9}$$

where ω is acentric factor defined as $\omega = -1.0 - \log (P_r^{sat})_{Tr=0.47}$; T_r is reduced temperature, T/T_c; P_r is reduced pressure, P/P_c; Tc and Pc are critical temperature (K) and pressure (bar). Quantity P_r^{sat} is the reduced vapor pressure (bar). Table 4 shows the compressibility factor and gas needed in subcritical water treatment. By assuming carbon dioxide dominated inside reactor, at 40 bar and 160°C (433K), $T_r = 433/304 = 1.42$, $P_r = 40/73.8 = 0.54$, acentric factor $\omega = 0.224$, so the compressibility factor of carbon dioxide is 1.04 as shown in Table 4. Carbon dioxide gas employed was 23.47 g per 4 g biomass, or 5.87 g/g biomass.

Table 4. *Compressibility factor and mass of carbon dioxide needed in SCW hydrolysis with acentric factor ω = 0.224*

T [ºC]	T [K]	P [bar]	T_r	P_r	Z	n [mol]	m [g]	g CO_2/ g solid
110	383	20	1.40	0.27	1.06	0.29	12.99	3.25
120	393	20	1.44	0.27	1.06	0.28	12.62	3.16
130	403	20	1.47	0.27	1.07	0.27	12.28	3.07
130	403	30	1.47	0.41	1.05	0.43	18.79	4.70
150	423	30	1.55	0.41	1.06	0.40	17.77	4.44
170	443	30	1.62	0.41	1.06	0.38	16.86	4.22
140	413	40	1.51	0.54	1.03	0.56	24.83	6.21
160	433	40	1.58	0.54	1.04	0.53	23.47	5.87

On the other hand, the SCW hydrolysis only uses a less consumable materials namely water, electricity and raw material. Electricity did not dominate the overall expenditures of all consumable materials on SCW technique since hydrolysis took below 1 h. In this work, four grams lignocellulose was added into 75 mL fresh water inside SCW reactor. Electricity price was about US$ 0.11 per kilowatt-hour in East Java Indonesia, while, the water employed was US$ 0.0002 per kilogram.

Table 5. *The cost per kg coconut coir dust and percentage of consumable cost in the present work using SCW hydrolysis in Lab scale at 40 bar and 160 °C*

No	Expenditure	Price [US$/kg]	Consumables per biomass [g/g]	Cost/kg biomass [US$/kg]	Percentage of cost drivers [percent]
1	Water	0.0002	18.75	0.00375	0.59
2	Raw material	0.02	1	0.02	3.15
3	Electricity	-	-	0.024	3.79
4	Carbon dioxide	0.1	5.87	0.59	92.47
Total				0.63	100

Table 5 shows the cost per kilogram biomass and percentage of consumable costs of SCW hydrolysis using high-lignin lignocellulose, coconut coir dust at 40 bar and 160 °C. It surprisingly found the expenditure of electricity was extremely low recorded at US$ 0.024 per kilogram biomass using SCW hydrolysis. The electrical energy consumed per kilogram biomass, which was transfer to heat by SCW hydrolysis was multiplication of heat capacity of materials involved and the change of temperature, $Q = C\Delta T$ [31]. The low expense was due to minimum usage of electricity taken only 1h compared to that conventional technique consuming electricity more than 48 h [5 - 9].

The SCW hydrolysis was much more efficient in electricity usage than those conventional techniques (ionic liquid, acid and alkaline methods). Two critical points discovered in SCW hydrolysis were the low energy usage and the less water employing since hydrolysis was conducted shortly as well as there was no wash steps.

In this study, SCW almost did not produce wastewater so it was environmentally friendly. However, the SCW equipment needs expensive components such as valves, reactor, carbon dioxide-pressurized flask and pipes that all parts were of high quality to ensure savety. Furthermore, SCW hydrolysis generated an unexpected cost, i.e; carbon dioxide cost mostly dominated of overall costs that were 92.47 % (at 40 bar and 160 °C).

This study suggests SCW method needs a breakthrough to reduce the cost especially on pressurized gas employed. It is necessary to consider the usage of gas from air despite it is low in purity. Of course, if higher P &T employed, the cost of consumable gas used goes up-the product price will rise unprecedented.

The cost of manufacture per kilogram sugar was defined as division of cost per kilogram biomass and yield (1) shown in Table 6. As shown in that the cost decreased as the pressure and temperature increased since the yield went up significantly. This work has not obtained the optimum value of yield of sugar for pressure and temperature was set at 40 bar, 160 °C as maximum conditions. The cost of preparation for yield 0.09 g/g was US$ 7 per kilogram sugar that pressure and temperature were 40 bar and 160 °C compared to that US$ 7.75/kg sugar of previous conditions. These data showed

the cost of preparation at 40 bar and 160 °C declined about 9.70 % from initial conditions, 20 bar and 110 °C, recorded at US$ 7.40/kg sugar and decreased about 29 percent from that without pretreatment noted at US$ 9.82/kg sugar. It was well understood the cost was generated from the work done by electrical energy to compress the gas into liquid phase when it was prepared.

Table 6. *The cost per kilogram sugar obtained versus to temperature, or pressure on SCW method*

P [bar]	T [K]	Yield (1)	Cost/kg biomass [US$/kg]	cost/ kg.sugar [US$/kg]
20	110	0.05	0.37	7.40
20	120	0.06	0.36	6.00
20	130	0.06	0.35	5.83
30	130	0.07	0.51	7.28
30	150	0.08	0.49	6.12
30	170	0.08	0.47	5.87
40	140	0.08	0.62	7.75
40	160	0.09	0.63	7.00

Enzymatic Hydrolysis of SCW-treated solid

After SCW pretreatment, solids, which were not converted into sugar, were proceded to enzymatic hydrolysis.
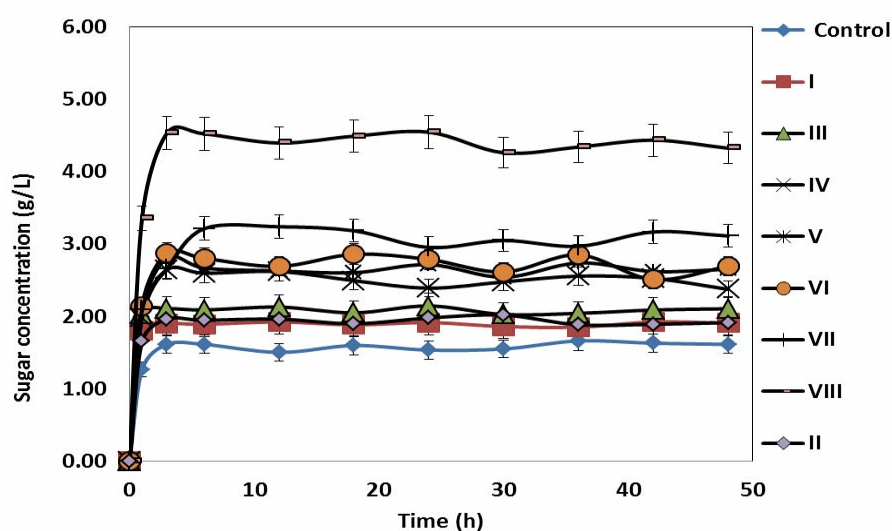
Figure 7. *Time course of the effect of SCW-pretreatment on enzymatic hydrolysis of coconut coir dust using cellulase for 48 h and at 60 °C.*
Variables: Control (without treatment), I (20 bar, 110 °C), II (20 bar, 120 °C), III (20 bar, 130 °C), IV (30 bar, 130 °C), V (30 bar, 150 °C), VI (30 bar, 170 °C), VII (40 bar, 140 °C), VIII (40 bar, 160 °C) for 1 h SCW-treated method

Figure 7 shows time course of the effect of SCW-pretreatment on enzymatic hydrolysis of using cellulase for 48 h and at 60 °C. The amount of sugar released enzymatically of SCW-treated solid increased as pressure and temperature added. Sugar concentration obtained from solid treated at 20 bar and 110 °C was 1.91 $g \cdot L^{-1}$, which rose 18 percent from control recorded at 1.62 $g \cdot L^{-1}$. When temperature added 10 °C to 120 °C with pressure maintained at 20 bar, solid liberated 1.92 $g \cdot L^{-1}$ sugar, increased slightly from previous variable. The sugar concentration was 2.11 $g \cdot L^{-1}$ for biomass treated by subcritical water at 130 °C and the pressure was controlled at 20 bar.

When pressure was increased to 30 bar at 130 °C, 2.38 $g \cdot L^{-1}$ sugar was released from solid, which was less than that liberated from substrate treated at 30 bar, 150 °C obtained of 2.65 $g \cdot L^{-1}$. At 140 °C, carbon dioxide was increased its capacity entering reactor via valve to 40 bar, 140 °C, sugars escaped from polysaccharide chains were 3.11 $g \cdot L^{-1}$, rose very significant about 99 percent from that obtained of non-pretreatment. The highest sugar hydrolyzed enzymatically from SCW treated solid (40 bar, 160 °C) was 4.32 $g \cdot L^{-1}$ that the increase was 167 % compared with control as previously described. These results were indicative that the amount of sugar hydrolyzed enzymatically could be significantly affected by SCW conditions, pressure and temperature as well as influenced by time of hydrolysis [22].

When SCW properties performed, a part of lignin removed from biomass and the structure of cellulose transformed to amorphous crystal called as cellulose II that improved the digestibility of enzymatic hydrolysis [11].

The last study was to analyze the yield of sugar obtained from SCW-treated solid using an enzymatic hydrolysis. The mass of sugar (g) obtained was divided by dried solid-the first yield, meanwhile the second yield divided by mass of theoretical total reducing sugar (TRS) in also g unit. The weight of TRS was derived by multiplying the amount of sugar (in $g \cdot L^{-1}$) and the volume of suspension (mL). The T TRS (theoretical total reducing sugars) resulted from the chemical contents (cellulose+hemicellulose) of control (0.169 plus 0.200 multiplied by the weight of solid).

Table 7. Yield of sugar hydrolyzed enzymatically from coconut coir dust treated by SCW method

P [bar]	T [K]	Yield (1)	Yield (2)
20	110	0.06	0.16
20	120	0.06	0.16
20	130	0.06	0.17
30	130	0.07	0.19
30	150	0.08	0.21
30	170	0.08	0.22
40	140	0.09	0.25
40	160	0.13	0.35

Table 7 shows the yield of sugar liberated enzymatically from coconut coir dust treated by SCW method. In this investigation, the highest yield recorded was of 0.13 g.sugar/g.dried solid, or 0.35 g.sugar/g.(cellulose+hemicellulose) from the substrate that was pretreated with SCW at 40 bar, 160 °C. The increase was more than 160 percent from non-pretreatment substrate noted 0.05 g.sugar/g.dried solid, or 0.13 g.sugar/g.(cellulose+hemicellulose).

The enzymatic yields (in g.TRS/g.cellulose+hemicellulose) of solids treated by SCW for control and various variables were 0.16, 0.17, 0.19, 0.21, 0.22, 0.25 and 0.35, respectively as shown in Figure 7. Therefore, the SCW-treated substrate conditioned at 160 °C and 40 bar was the most easily hydrolyzed by cellulase compared to other substrates. This result related to the structure of treated substrate, which has transformed into an amorphous structure as previously described [32].

Generally, the yields of sugar released enzymatically from SCW-treated solids were higher than that from the non-pretreatment solid. After pretreatment, the surface area of the substrate was opened due to delignification and the decrease of the crystalline index, CrI. SCW pretreatment can modify the lattice structure of the cellulose so the enzyme easily attacks the glicosidic connections to produce sugar, or other lower chain polysaccharides. In the pretreated solids, the molecules inside the cellulose moved apart from each other, resulting in an amorphous structure. In SCW conditions, the water dissolved some lignin so that cellulose was exposed providing an open way of enzymes to easily access to the surface of cellulose and hemicellulose enabling their hydrolyzing to reducing sugars [11 - 13].

CONCLUSIONS

Reducing sugar has been successfully prepared from coconut coir dust using SCW and enzymatic methods for 1 h treatment. The lignocellulose treated with SCW produced solid and liquid containing sugar. Based on XRD investigation, the crystalline index decreased significantly indicating a change to amorphous structure that easily attacked by enzymes for enzymatic hydrolysis. The consumable cost per kilogram sugar used in SCW-hydrolysis was lower than that control, without pretreatment. The sugar liberated enzymatically of solid treated by SCW increased extremely to about 160 % compared with that without pretreatment.

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