



Enhancing the enzymatic digestibility of oil palm biomass using supercritical carbon dioxide-based pretreatment towards biorefinery application

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ABSTRACT

In realizing the vast potential of lignocellulosic biomass residues generated by oil palm industry under the regime of 'one-size-fits-all' biorefinery concept, supercritical carbon dioxide (SC-CO₂) technology offers great promise. Herein, we demonstrated a new eco-friendly pretreatment of oil palm trunk biomass based on SC-CO₂ under controlled conditions of pressure (25, 35 and 45 MPa), temperature (80, 100 and 120 °C) and moisture content (0, 20, 40 and 60 %), and investigated their effect on enzymatic hydrolysis yields of the pretreated fractions. During pretreatment, biomass sample reacted with hot water in SC-CO₂ which led to high diffusivities, promoting its disruption and xylan solubilisation as supported by various characterization techniques. This study illustrates that hydrolysis yields are influenced by process parameters, particularly the moisture content played a crucial role in the biomass disruption. The SC-CO₂ pretreatment resulted in enhanced digestibility for all the pretreated fractions compared to the untreated biomass.

1. Introduction

Over the years, trend towards environmental sustainability has augmented the interest of researchers in identifying simple and eco-friendly processing technologies for lignocellulosic biomass in the second generation biorefinery (Luterbacher et al., 2017; Sankaran et al., 2020). Numerous novel strategies have been developed by researchers for the processing of lignocellulosic biomass aimed to ensure environmental sustainability and economic viability of a biorefinery (Soltanian et al., 2020). In this regard, supercritical fluids have gained a great deal of scientific attention worldwide (Toscan et al., 2017; Putrino et al., 2020). In principle, supercritical fluid refers to any substance that is above its critical conditions (temperature and pressure) and exhibits unique properties of liquids in terms of density and gases in terms of compressibility. The supercritical carbon dioxide (SC-CO₂) is essentially abundant, non-toxic, non-flammable, very volatile, cheap, recyclable and exhibits easy-reaching critical point (31.1 °C and 7.36 MPa) (Serna et al., 2016). The use of SC-CO₂ technology for biomass pretreatment involves in-situ formation of carbonic acid that promotes the removal of

xylan from lignocellulosic biomass and subsequent drastic release of pressure creates a physical "explosion effect". This results in a highly disrupted biomass heterostructure counteracting biomass recalcitrance with a positive effect on the bioconversion yields due to improved enzyme accessibility (Zhao et al., 2019).

The oil palm industry generates a vast amount of wide-ranging lignocellulosic biomass residues, accounting for over 90 % of the total biomass, from 5.85 million ha of oil palm plantation on annual basis in Malaysia (MPOB, 2019). However, a large proportion of this biomass is left at the plantation sites (Onoja et al., 2018). The traditional biomass waste recycling that includes open field disposal (Uemura et al., 2013), composting (Krishnan et al., 2017) and mulching (Moradi et al., 2015) are not considered sustainable solutions (Bruckman, 2016). Earlier, our study on the physicochemical properties of important agricultural residues available in Malaysia revealed that oil palm trunk (OPT) could be an ideal biorefinery feedstock because of its high cellulose and low lignin contents, whilst, despite these benefits it is mostly left unharvested in the plantation area (Sohni et al., 2018). Numerous pretreatment strategies have been put forward to date employing acid (Chin

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et al., 2011; Khamtib et al., 2011; Noparat et al., 2015), aqueous ammonia (Jung et al., 2011; Maryanto et al., 2018), sodium hydroxide (Lai and Idris, 2016; Prawitwong et al., 2012), sulfite (Noparat et al., 2017), hydrothermal (Eom et al., 2015), steam explosion (Punsuvon, 2013), organosolv (Rattanaporn et al., 2018), and lime (Sithikitpanya et al., 2018) to enhance bioconversion yields of OPT. However, biomass processing in most cases being chemical and energy intensive is not sufficiently eco-friendly, together with intricacies pertaining to equipment deterioration and recycling of chemicals, hence it is not an economically beneficial option for industrial gains as well (Mohtar et al., 2015; Serna et al., 2016; Watkins et al., 2015). In contrast, SC-CO₂ processing is a low-environmental impact technology because temporary acidification because of carbonic acid exists as long as the system is kept pressurized and after system depressurisation, pH of the residual solution rises again (Toscan et al., 2017). Other beneficial features include low carbon dioxide cost, no generation of toxic compounds, low-temperature condition as well as ability to handle large amount of solids (Soltanian et al., 2020).

The SC-CO₂ technology has been the subject of scientific investigation, for instance numerous studies on the extraction of waxes and lipid components (Attard et al., 2015; Kawahito et al., 2008; Ortega et al., 2017), and fractionation of bio-oils (Chan et al., 2020; Cheng et al., 2016; Montesantos et al., 2019) from the lignocellulosic biomass have been reported. The implementation of SC-CO₂ technology as an alternative for conventional biomass pretreatment strategy is limited to cellulose (Kim and Hong, 2001), wheat straw (Alinia et al., 2010), guayule biomass (Islam et al., 2018; Moharreri et al., 2017; Srinivasan and Ju, 2010), sugarcane bagasse (Benazzi et al., 2013a; Phan and Tan, 2014; Silveira et al., 2015; Toscan et al., 2017), rice husk (Serna et al., 2016), soybean hulls (Islam et al., 2017), corn stover (Huisheng et al., 2013) and switch-grass (Luterbacher et al., 2010), among others. It is important to mention that the selection of optimal experimental conditions depends upon physicochemical properties of biomass feedstock (Huisheng et al., 2013). Liu et al. studied a high-pressure CO₂-based pretreatment of selected lignocellulosic biomasses, and identified temperature and moisture content as two vital parameters for achieving maximum reducing sugar yields (Liu et al., 2014). However, the use of high temperature simultaneously accelerates dissolution and degradation of cellulose. This would not only reduce the amount of regenerated cellulose but it also consumes a lot of energy (Goshadrou and Lefsrud, 2017). On the other hand, moisture content of the biomass is known to have a positive effect during SC-CO₂ pretreatment because the binding of cellulose and hemicellulose with lignin becomes softened, whilst the pressurized biphasic system of CO₂-H₂O mixture forms carbonic acid in-situ, promoting slight hydrolysis of the biomass. This, as a result, enhances the enzymatic digestibility of pretreated biomass compared to untreated fraction (Islam et al., 2017; Moharreri et al., 2017). Earlier, Zheng et al. conducted SC-CO₂ pretreatment of cellulose using low temperature range (35–80 °C) without moisture control (Zheng et al., 1998). A number of studies have revealed that moisture content plays a crucial role in SC-CO₂ pretreatment (Kim and Hong, 2001; Serna et al., 2016; Srinivasan and Ju, 2010). According to (Zhao et al., 2019), the actual role of moisture content in SC-CO₂ processing has not been completely established till date. Although their study mainly focused on low temperature SC-CO₂ pretreatment of biomasses (50–80 °C, 17.5–25.0 MPa) with moisture content fixed at 75 % for extended time (12–60 h) but a mechanism to highlight the role of moisture was also proposed. Very recently, Putrino et al. SC-CO₂ pretreated green coconut shell (20 MPa, 70 °C and 1 h) to enhance hydrolysis of its enzymatic cellulose and recommended further studies on the effect of moisture during biomass processing (Putrino et al., 2020).

Although SC-CO₂ technology has been investigated for the extraction and processing of palm oil (Birtigh et al., 1995; ; Markom et al., 2001; Davarnejad et al., 2008), but its usage as a pretreatment method for oil palm biomass residues is, however, limited and still at infancy stage. Thus SC-CO₂-based processing of oil palm biomass deserves scientific

attention in order to understand the real possibilities of SC-CO₂ technology in the oil palm biomass-based biorefinery. To the best of our knowledge, no work on SC-CO₂ pretreatment of OPT biomass has been reported to date. Therefore, the aim of present work was to systematically perform SC-CO₂-based processing of OPT under different process conditions; pressure (25 MPa, 35 MPa, 45 MPa), temperature (80 °C, 100 °C, 120 °C) with varying moisture content (0 %, 20 %, 40 % and 60 %). To assess whether SC-CO₂ pretreatment was effective or not, enzymatic hydrolysis reactions were run to compare the digestibility of SC-CO₂ treated fractions with the untreated biomass. This study would improve our understanding on the effect of SC-CO₂ process conditions on enzymatic digestibility of oil palm biomass. Also, it provides useful insights about the influence of moisture content and how structural and compositional variations occurs in the lignocellulosic biomass as a function of process conditions. We advocate that the implementation of SC-CO₂-based clean technology for biomass pretreatment under mild experimental conditions is deemed to be vital for the environmental sustainability and economic viability of oil palm biomass-based biorefineries.

2. Material and methods

2.1. Materials

For present work, calcium carbonate, citric acid, sodium hydroxide, sodium azide, sodium chlorite, hydrochloric acid, sulphuric acid, toluene and ethyl alcohol of analytical grade were purchased from Sigma-Aldrich. Sodium potassium tartrate tetrahydrate was purchased from Merck and 3,5-dinitrosalicylic acid (DNS) was obtained from Fischer Scientific. For compositional analysis, D-cellobiose, and sugar standards D-(+)-glucose, D-(+)-xylose and L-(+)-arabinose were purchased from Sigma-Aldrich. The commercial enzyme cocktail used for the hydrolysis (Cellic CTec2) was provided by Novozyme and its activity was determined as per National Renewable Energy Laboratory (NREL) method (Adney and Baker, 1996). The OPT biomass samples were collected from Selangor, Malaysia, and were processed into uniform material suitable for compositional analysis and chemical processing as provided in Fig. S11 (see Supporting Information).

2.2. SC-CO₂-based processing of OPT

A supercritical CO₂ extraction unit (Separex) at School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM) with high pressure pretreatment reactor of 5 L capacity made of stainless steel with two sealing screw-caps having dimensions as follows; height: 29 cm, OD: 14 cm, ID: 12 cm was used. Fig. 1 shows the process flow diagram for high pressure pretreatment experiment. About 100 g biomass was introduced into high pressure reactor after the desired temperature was reached and reactor pressure was held at the desired level for the selected time duration as provided in Table 1. The temperature was kept 80 °C in most of the runs because elevated temperatures could lead to the formation of potential fermentation inhibitors (Benazzi et al., 2013a). The presence of moisture is crucial in SC-CO₂ processing of biomass as reported elsewhere (Alinia et al., 2010; Srinivasan and Ju, 2010). Thus, experiments were run with moisture content held at 0 % (no addition of water) as well as 20 %, 40 % and 60 % adjusted by spraying a calculated amount of water over the biomass (Serna et al., 2016). The effect of pressure was studied at 25 MPa, 35 MPa and 45 MPa based on earlier reported work (Gao et al., 2010; King et al., 2012; Zhao et al., 2019). In a typical procedure, CO₂ pumped from the supply cylinder was first cooled to -7 °C and then directed towards preheated reactor charged with sample. In this work, holding time of 60 min was based on earlier reported works (Alinia et al., 2010; King et al., 2012; Phan and Tan, 2014). It ranged from the time when desired pressure was reached to the release of pressure from the reactor.

Finally, the reactor was instantaneously depressurized to create an

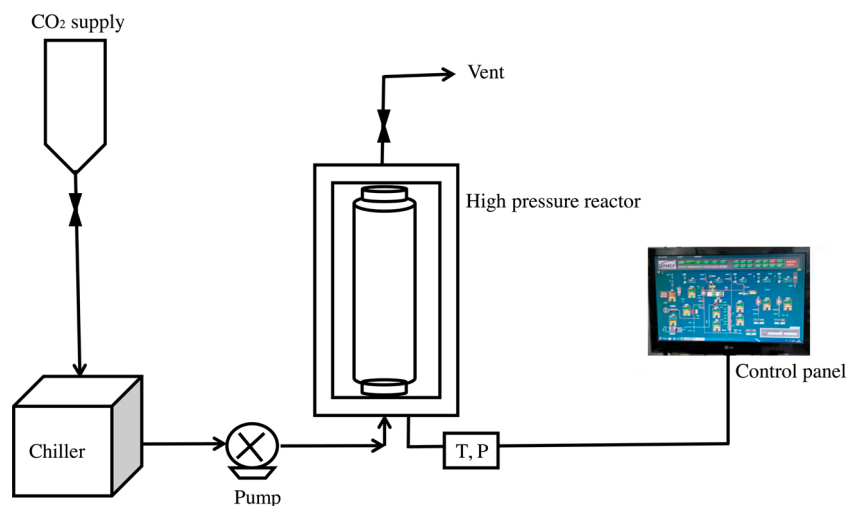


Fig. 1. Schematic diagram of SC-CO₂ reactor.

Table 1

Selected supercritical carbon dioxide (SC-CO₂)-based pretreatment conditions and sample codes of oil palm trunk (OPT).

Assay No.	OPT fraction	Moisture (%)	Temperature (°C)	Pressure (MPa)
1	OPT Control	–	–	–
2	SC-OPT ^{dry} , 80°C, 25MPa	0	80	25
3	SC-OPT ^{20%} , 80°C, 25MPa	20	80	25
4	SC-OPT ^{40%} , 80°C, 25MPa	40	80	25
5	SC-OPT ^{60%} , 80°C, 25MPa	60	80	25
6	SC-OPT ^{60%} , 80°C, 35MPa	60	80	35
7	SC-OPT ^{60%} , 80°C, 45MPa	60	80	45
8	SC-OPT ^{60%} , 100°C, 35MPa	60	100	35
9	SC-OPT ^{60%} , 120°C, 35MPa	60	120	35

explosion effect. The pressure was released at constant rate by opening a micro metering valve which took approximately 3 min. The reactor was opened up and biomass sample was collected followed by extensive washing with water. The pretreated biomass was later transferred to a clean plastic bag which was stored at 4 °C prior to further analysis. A fraction of pretreated solid sample was placed on an aluminium foil as thin layer followed by overnight drying in the oven at 40 °C before the compositional analysis.

2.3. Compositional analysis of biomass to determine structural carbohydrates and lignin

The effect of SC-CO₂-based processing on relative quantity of structural carbohydrates and lignin in the raw and SC-CO₂ treated OPT samples was assessed. The tested biomass fraction was initially converted into a uniform material suitable for compositional analysis according to NREL/TP-510-42620 (Hames et al., 2008). The total moisture and extractive contents in the OPT biomass were determined according to NREL/TP-510-42621 (Sluiter et al., 2008a) and NREL/TP-510-42619 (Sluiter et al., 2008b), respectively. The structural carbohydrates and lignin content in the biomass fractions were determined as per NREL/TP-510-42618 using standard NREL protocol (Sluiter et al., 2012). In a typical procedure, 3 mL of 72 % w/w H₂SO₄ was added to the

pressure tube containing extractive free OPT biomass (0.3 g) followed by incubation in the water bath (30 °C) for 60 min. Afterwards, the acid was diluted to a 4 % concentration by adding deionized water followed by autoclave for one hour at 121 °C. Acid soluble lignin (ASL) was quantified via UV-spectrophotometry at 320 nm with absorptivity of 30 Lg⁻¹ cm⁻¹. Whilst, acid insoluble lignin (AIL) collected on the filtering crucibles (porosity # 4) by vacuum filtration of hydrolysis solution was dried at 105 °C for 4 h to measure AIL content in the biomass. The filtrate was analyzed for cellobiose, glucose, xylose and arabinose obtained after two step acid hydrolysis of extractives free OPT using high performance liquid chromatography (HPLC) (Waters, USA) equipped with a Sugar-Pak™ 1, 6.5 × 300 mm column (Waters, USA) and refractive index (RI) detector. Ultrapure water with conductivity of 18.2 MΩ filtered through 0.4 μm nylon filter and degassed using multifrequency ultrasonic cleaning unit, TI-H-10 (100–120 V oder 230 V) was used as a mobile phase with the flow rate of 0.5 mL/min. The glucan, xylan and arabinan components of biomass composition were calculated using Eqs. 1–3, respectively.

$$\text{Glucan (\%)} = \frac{\text{Glucose recovered from acid hydrolysis (mg)} \times 0.9}{\text{Wt. of OPT (mg)}} \times 100 \quad (1)$$

$$\text{Xylan (\%)} = \frac{\text{Xylose recovered from acid hydrolysis (mg)} \times 0.88}{\text{Wt. of OPT (mg)}} \times 100 \quad (2)$$

$$\text{Arabinan (\%)} = \frac{\text{Arabinose recovered from acid hydrolysis (mg)} \times 0.88}{\text{Wt. of OPT (mg)}} \times 100 \quad (3)$$

2.4. Enzymatic hydrolysis

The efficacy of SC-CO₂-based processing was evaluated by performing enzymatic hydrolysis of native biomass and pretreated OPT fractions using Cellic CTec2 (Novozyme). The enzymatic hydrolysis of biomass based on NREL standard method was conducted in duplicate on a shaking incubator (IKA KS 4000i Control Orbital Shaker) at 50 °C (Selig et al., 2008). A predetermined quantity of solid substrate adjusted for the presence of moisture was loaded into the vial followed by addition of 1 mL sodium azide solution (20 mg/mL) as an antimicrobial agent. Later, 0.05 M citrate buffer solution (pH = 4.8) was added to make final volume equal to 100 mL after accounting for the enzyme dosage (30, 60, 70 and 80 FPU/g glucan) to be added. The resulting

suspension was placed in the shaking incubator with rotation speed of 150 rpm at 50 °C for 60 min. Finally, Cellic CTec2 (Novozyme) was loaded into each vial. Aliquots were taken at specific intervals, and were chilled straightaway on the ice bath. Collected portions were centrifuged at 7000g for 10 min, filtered and stored at -20 °C. To quantify the released glucose during hydrolysis, hydrolysate liquid samples were thawed and filtered through a 0.2 µm filter before HPLC analysis. The cellulose conversion (%) was calculated using Eq. 4.

$$\text{Cellulose conversion (\%)} = \frac{\text{Glucose released (g)} \times 0.9}{\text{Glucan added (g)}} \times 100 \quad (4)$$

2.5. Examination of surface transformations

The morphology of OPT biomass material was examined by means of scanning electron microscopy (SEM). For SEM imaging, biomass sample was fitted onto aluminium stubs via carbon double-sided tape and then was coated with gold using a sputter coating machine (Leica EM SCD005, Czech Republic). The surface of biomass was later examined using SEM, Quanta FEG 450, FEI, The Netherlands.

2.6. Functional groups analysis

The presence of various functional groups in untreated and SC-CO₂ pretreated fractions was established using Thermo Fisher Scientific (Nicolet iS50) Fourier transform infrared (FTIR) spectrometer by using

KBr method.

2.7. Crystallinity studies using X-ray diffraction (XRD)

To assess the crystallinity of biomass samples, XRD measurements were performed on ARL™ X'TRA Powder Diffractometer set at 40 kV and 50 Ma using monochromatic Cu Kα radiation ($\lambda = 1.5406 \text{ \AA}$) in the angular range of $2\theta = 5\text{--}80^\circ$ with a scan rate of $2^\circ 2\theta/\text{min}$. The crystallinity index (CrI) was measured from the diffractogram using Eq. 5.

$$\text{CrI (\%)} = \frac{A_c}{A_c + A_a} \times 100 \quad (5)$$

Where CrI (%) refer to the crystallinity percentage, A_c shows area under crystalline peaks and A_a is the area under amorphous region in XRD pattern.

3. Results and discussion

3.1. Influence of SC-CO₂ process conditions on biomass composition

The compositional analysis was carried out to compare the effects of process conditions on glucan, xylan and lignin contents of biomass fractions. Summarized in Fig. 2, raw biomass contained $44.43 \pm 0.14 \%$ glucan, $22.31 \pm 0.07 \%$ xylan, $2.93 \pm 0.20 \%$ arabinan, $26.20 \pm 0.063 \%$ AIL and $1.20 \pm 0.046 \%$ ASL. We first investigated the effect of moisture (0 %, 20 %, 40 % and 60 %) and it was found that the quantity of xylan

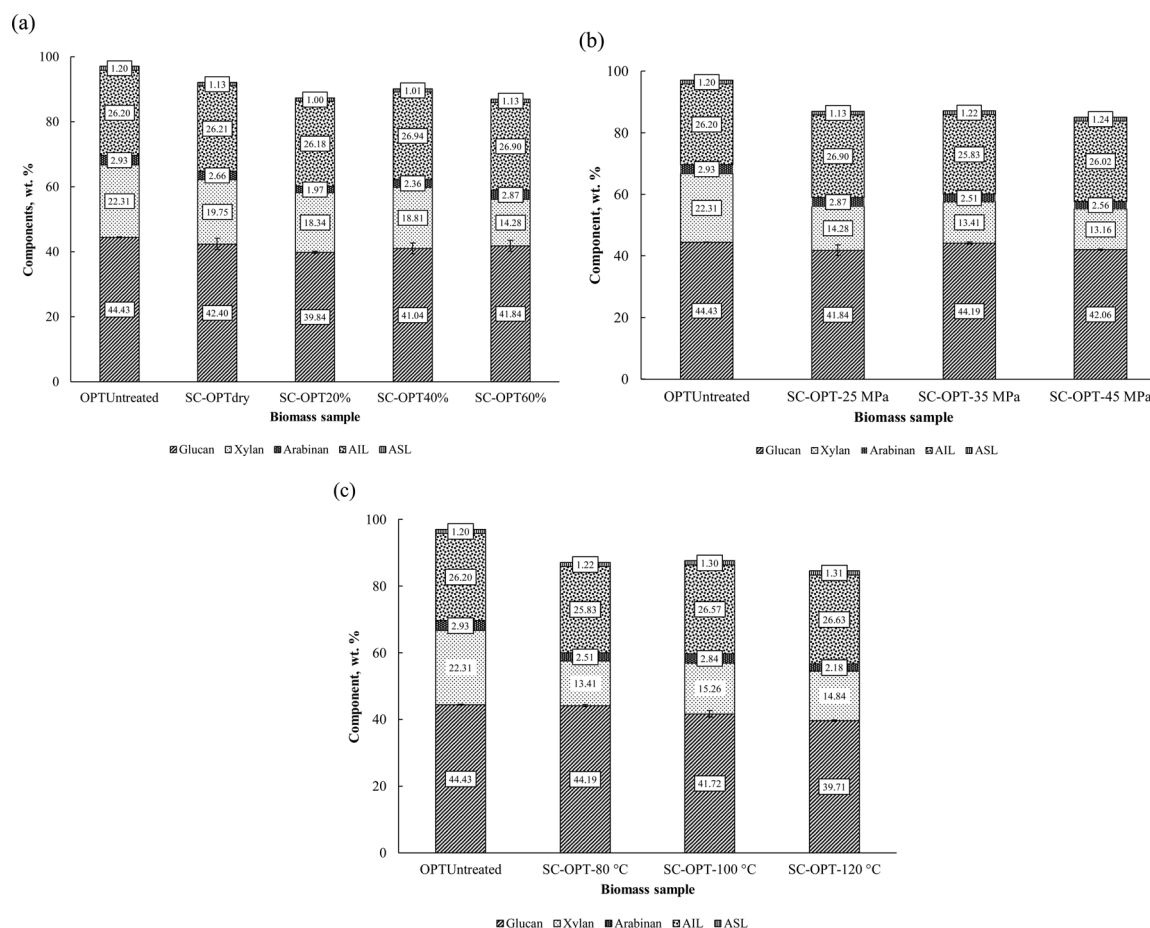


Fig. 2. Composition of oil palm trunk (OPT) fractions showing the effect of supercritical carbon dioxide (SC-CO₂)-based processing with varying (a) moisture content (0 %, 20 %, 40 %, 60 %) executed at 25 MPa and 80 °C, (b) pressure (25 MPa, 35 MPa, 45 MPa) with 60 % moisture content at 80 °C and (c) temperature (80 °C, 100 °C, 120 °C) at fixed pressure of 35 MPa with 60 % moisture content.

Glucan, xylan, acid soluble lignin (ASL) and acid insoluble lignin (AIL) contents in the biomass are provided. All experiments were done in replicate, and mean values are reported.

decreased from 22.31 % in case of untreated biomass to 14.28 % in the biomass fraction processed under SC-CO₂ (25 MPa) at 80 °C with 60 % moisture as shown in Fig. 2a. Most importantly, it was noticed that increasing the moisture content simultaneously accelerated xylan solubilisation. This can be ascribed to the pressurized biphasic system of CO₂-H₂O mixture which forms carbonic acid in-situ, thereby promoting xylan hydrolysis (Islam et al., 2017; Moharreri et al., 2017). Biomass pretreated under SC-CO₂ (25 MPa) at 80 °C with no moisture underwent very little xylan solubilisation (19.75 %), compared to the biomass processed with 20 % (18.34), 40 % (18.81 %) and 60 % (14.28 %) moisture content. The analysis of biomass composition pretreated as a function of increasing pressure revealed that xylan contents in pretreated fractions were 14.28 % (25 MPa), 13.41 % (35 MPa) and 13.16 % (45 MPa), compared to 22.31 % in the control (untreated OPT biomass). In the next phase, SC-CO₂ pretreatment as a function of temperature revealed very close values of xylan content for all the pretreated biomass fractions, i.e. 13.41 %, 15.26 % and 14.84 % when executed at 80 °C, 100 °C, and 120 °C, respectively. It appears that the effect of temperature on xylan content of biomass was less pronounced during SC-CO₂ pretreatment. This might be due to a decrease in the solubility and dissolution of CO₂ in the medium at a high temperature (Benazzi et al., 2013b). The glucan content in pretreated biomass fractions were 41.84 %, 41.72 % and 39.71 % when processed at 80 °C, 100 °C and 120 °C, respectively, with fixed moisture (60 %) at 35 MPa, compared to the control (44.43 %). This result shows that the decomposition of polymer is enhanced at higher temperature (Benazzi et al., 2013a). The lignin content remained roughly close, i.e. 26 % for both untreated as well as pretreated biomass materials under given experimental conditions, very similar with the earlier reports on rice straw (Gao et al., 2010) and green coconut fiber (Putrino et al., 2020) conversion. From the data compiled in Fig. 2, it can be inferred that the material loss mainly resulted from solubilisation of xylan backbone of lignocellulosic biomass (Islam et al., 2017; Moharreri et al., 2017) and was more noticeable in case of OPT processed with 60 % moisture at 80 °C under elevated pressure. It appears that the selected pretreatment conditions were severe enough to dissolve most of the xylan part of OPT, leaving a solid residue with relatively higher glucan and lignin content.

3.2. Enzymatic saccharification

The results of enzymatic hydrolysis carried out at enzyme and substrate loading of 30 FPU/g and 3 g, respectively, as a function of time are shown in Fig. 3. Total sugar conversion is mainly determined by glucose conversion because xylan content for all the pretreated biomass was not as much of glucan. In case of untreated OPT biomass, it was found that cellulose conversion was only 12.31 %. In contrast, SC-CO₂ pretreatments resulted in enhanced digestibility for all the pretreated fractions. Other researchers also evaluated SC-CO₂ pretreatment of lignocellulosic residues and obtained improved sugar yield after enzymatic hydrolysis of pretreated fractions. In one study on rice straw, SC-CO₂ pretreatment (10–30 MPa, 40–110 °C for 15–45 min) brought about significant enhancement in glucose recovery (32.4 %) as compared to untreated biomass (27.7 %) (Gao et al., 2010). (Narayananaswamy et al., 2011) observed that the pretreatment of corn stover with 75 % moisture content at 24 MPa and 150 °C for 1 h had glucose yield of 30 % compared with 12 % from untreated corn stover. In present study, enzymatic digestibility of the biomass progressively increased with moisture content showing peak value (27.58 %) when pretreated at P = 25 MPa; T = 80 °C with 60 % moisture level. This most likely is associated with the hydrolysis of hemicellulose fraction of biomass due to in-situ formation of carbonic acid in CO₂/H₂O biphasic system at elevated pressure (Toscan et al., 2017). Alinia and team also verified that the yield of reducing sugar from hydrolysis of wheat straw was higher in case of wet biomass (Alinia et al., 2010). In present work, the pretreatment of biomass at P = 35 MPa; T = 80 °C for 60 min with 60 % moisture content greatly facilitated enzymatic digestibility. However, a

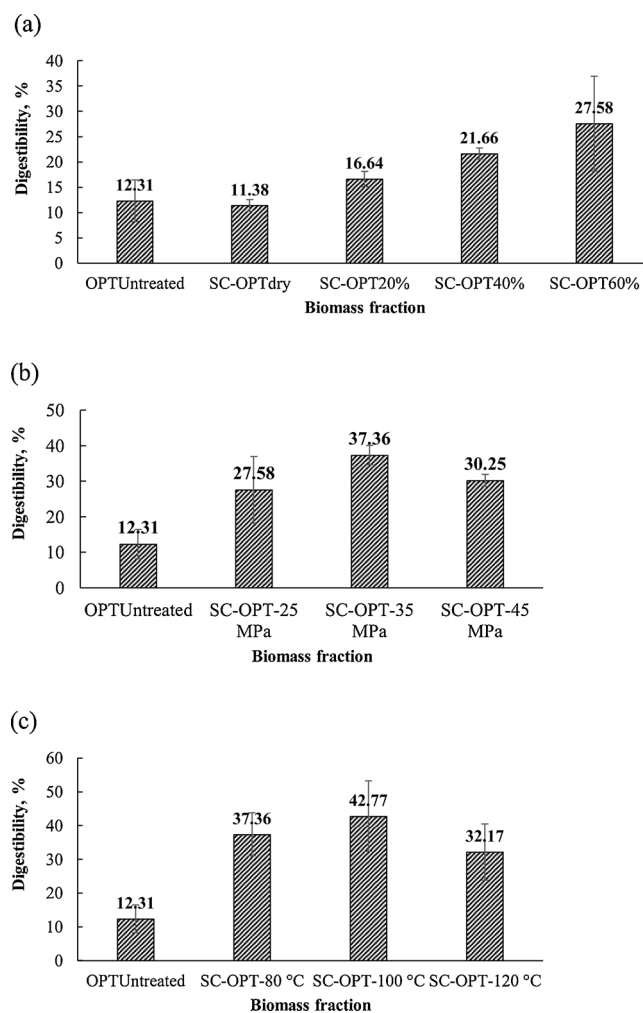


Fig. 3. Enzymatic digestibilities of untreated oil palm trunk (OPT) and supercritical carbon dioxide (SC-CO₂) treated oil palm trunk (SC-OPT) fractions showing the effect of varying (a) moisture (0 %, 20 %, 40 % and 60 % at P = 25 MPa; T = 80 °C), (b) pressure (25 MPa, 35 MPa and 45 MPa at T = 80 °C with 60 % moisture) and (c) temperature (80 °C, 100 °C and 120 °C at P = 35 MPa with 60 % moisture). Enzymatic hydrolysis was carried out at enzyme and substrate loading of 30 FPU/g glucan and 3 g, respectively for 72 h. Error bars refer to the standard deviation for replicate measurements.

decrease in the hydrolysis yield was found at 45 MPa as shown in Fig. 3b, most likely due to the solubility changes of water. The amount of water in bulk SC-CO₂ being more than that confined inside biomass structure had an adverse effect on biomass swelling function with weakened explosion effect thereafter. This negative effect related to pressure was also reported by other researchers (Kim and Hong, 2001; Zhao et al., 2019). The hydrolysis yield was 42.77 % for biomass processed at 100 °C under SC-CO₂ (35 MPa) with 60 % moisture, compared to the untreated biomass (12.31 %). However, further increasing the pretreatment temperature (120 °C) did not greatly increase the hydrolysis yield (32.17 %) (Fig. 3c). This might be because of polymer degradation at temperatures greater than 100 °C (Zhao et al., 2019) as well as due to low solubility and dissolution of CO₂ in the medium at a constant pressure (Zhao et al., 2019). Table 2 summarizes the results of enzymatic hydrolysis reactions of biomass fraction processed at P = 35 MPa; T = 80 °C with 60 % moisture at different enzyme loadings, FPU/g of glucan. The best cellulose conversion of 61.14 ± 7.56 % was achieved for SC-CO₂ treated OPT relative to 37.09 ± 1.01 % in case of untreated OPT at an enzyme loading of 80 FPU/g of glucan.

Table 2

Enzymatic digestibilities of supercritical carbon dioxide (SC-CO₂) treated oil palm trunk (SC-OPT) processed at P = 35 MPa; T = 80 °C with 60 % moisture using different enzyme loadings, FPU/g of glucan, for 72 h compared to untreated oil palm trunk (OPT).

Dosage (FPU/g of glucan)	Untreated OPT (%)	SC-OPT (%)
60	25.95 ± 6.36	56.10 ± 3.93
70	28.91 ± 5.35	58.06 ± 2.33
80	37.09 ± 1.01	61.14 ± 7.56

3.3. Surface modification of biomass after SC-CO₂-based pretreatment

The accessibility of enzymes towards cellulosic fibers is one of the critical factors that could affect hydrolysis process (Kumar et al., 2009). The role of moisture content was evaluated in more detail using SEM because of its crucial role in SC-CO₂ pretreatment. As can be seen in Fig. 4a, SEM micrograph of untreated OPT revealed intact morphology, a smooth and compact structure without any disruption, cracks or fragments. On the other hand, SC-CO₂ pretreatment significantly altered biomass morphology with substantial roughness, holes and several fragments were seen over the surface of biomass material (Fig. 4b–d). It was also observed that biomass disruption varied as a function of moisture content. Examining the SEM images of biomass pretreated without added moisture (Fig. 4b and Fig. SI2) showed physically damaged surface with pores distributed all over its surface. However, as clearly shown in Fig. 4c–d, damage to the tissues was more pronounced in case of biomass pretreated with 60 % moisture as compared to the other fractions. This was likely due to the greater penetration of gas molecules (due to biomass swelling) as well as greater dissolution of xylan backbone (due to carbonic acid) (Moharreri et al., 2017). It appears that moisture content promoted biomass swelling that in turn facilitated the subsequent drastic release of pressure, hence caused a physical “explosion effect”. This might be the reason why enhanced

bioconversions were achieved in case of wet biomass in agreement with the previous discussion. The microstructure of OPT biomass pretreated at 45 MPa (SC-OPT⁶⁰ %, 45MPa, 80 °C) resulted in less physical damage (Fig. SI3a), as compared to SC-CO₂ processing carried out at 100 °C (Fig. SI3b). The decrease in OPT biomass rigidity at elevated temperature might have helped in promoting CO₂ uptake, hence surface disruption was more extensive and clearly noticeable in the biomass pretreated at 100 °C (Kumar et al., 2010).

3.4. Functional groups analysis

The FTIR spectra (Fig. 5) of OPT fractions subjected to SC-CO₂ pretreatment with varying moisture content were recorded and compared

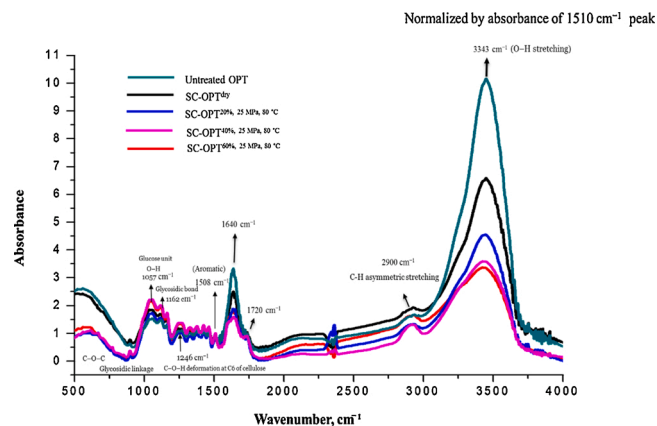


Fig. 5. FTIR spectra of untreated oil palm trunk (OPT^{raw}) and treated OPT fractions processed under SC-CO₂ conditions of 25 MPa at 80 °C.

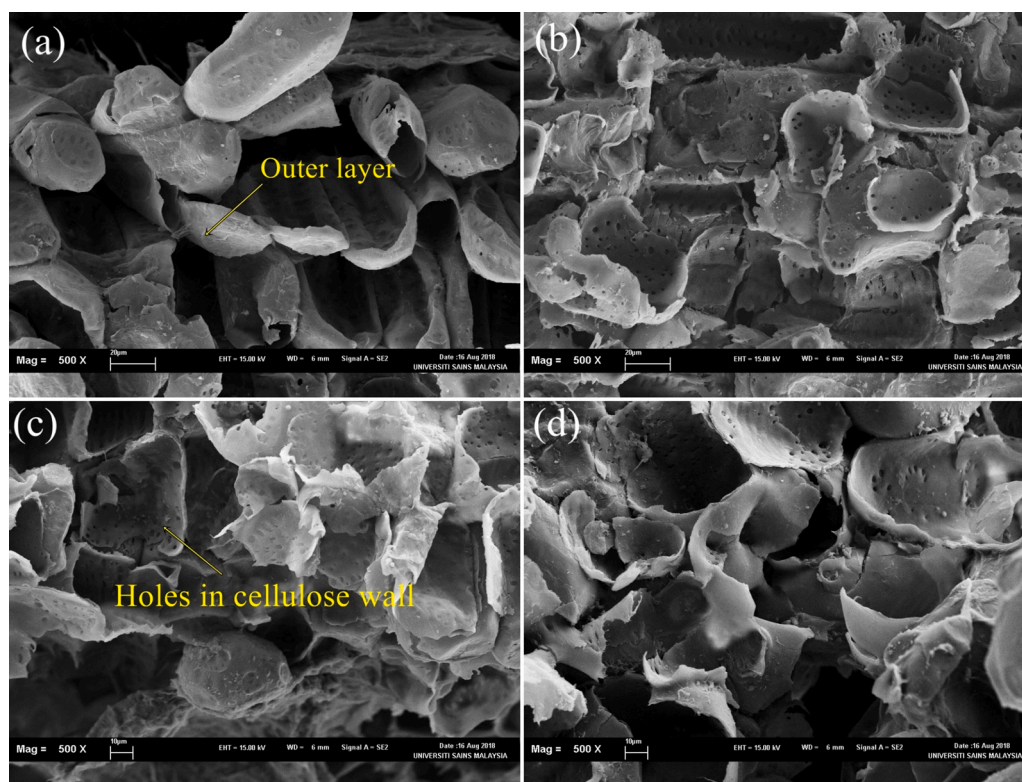


Fig. 4. SEM micrographs of (a) untreated oil palm trunk (OPT), (b) supercritical carbon dioxide (SC-CO₂) treated oil palm trunk (SC-OPT) fractions processed at 25 MPa and 80 °C without moisture (SC-OPT^{dry}, 25MPa, 80 °C), (c) SC-OPT pretreated at 25 MPa and 80 °C with 40 % moisture (SC-OPT⁴⁰ %, 25MPa, 80 °C) and (d) SC-OPT pretreated at 25 MPa and 80 °C with 60 % moisture (SC-OPT⁶⁰ %, 25MPa, 80 °C).

to that of raw biomass to further substantiate the results. The outcomes of functional group analysis were consistent with the data shown in Fig. 2. The absorption band 1515 cm^{-1} was chosen as an internal standard to determine relative absorbance. The peak assignments for both raw and SC- CO_2 pretreated OPT samples showed some variations in terms of relative absorption intensities of certain peaks as evident from Fig. 5, implying some changes in the distribution of cellulose and hemicellulose in the pretreated biomass. As can be seen in Fig. 5, a reasonably strong unstructured IR band ranging from 3500 to 3100 cm^{-1} because of superposition of stretching of abundant alcoholic and phenolic hydroxyl groups in cellulose, hemicellulose and lignin decreased in intensity after SC- CO_2 processing. This might be due to the degradation of bonds between carbohydrate and lignin and, in addition to the breakdown of H-bonds between cellulosic chains (Adel et al., 2011). Band positioned at 2900 cm^{-1} due to C–H stretch associated with cellulose was relatively more prominent in pretreated OPT as compared to the untreated biomass. A strong IR absorption at 1640 cm^{-1} in the untreated OPT can be ascribed to absorbed water due to strong affinity of hemicellulose for water which progressively became weaker in the pretreated OPT fractions (Chen et al., 2011). The absorption bands at 1300 – 1600 cm^{-1} related to lignin were more intense in pretreated biomass compared to raw OPT showing that SC- CO_2 pretreatment had essentially no effect on the solubilisation of lignin at selected conditions (Lee et al., 2017). A characteristic absorption band for lignin observed at 1508 cm^{-1} was more prominent in case of pretreated OPT (Li and Kiran, 1988). The linkage C=O between lignin and hemicellulose at 1720 cm^{-1} was also less prominent in pretreated OPT. The absorbance at multiple positions that were not noticeable in raw OPT became more prominent in the pretreated biomass, for instance absorption at 897 , 1057 , 1115 , 1162 and 1377 cm^{-1} can be identified for cellulose, whilst these peaks were not prominent in case of raw OPT, indicating an increase in the relative quantity of cellulose. This might be due to the removal of hemicellulose which led to an overall enhancement effect on the characteristics of cellulose in the biomass (Chen et al., 2012; Xue et al., 2012). The presence of moisture during SC- CO_2 treatment has been reported to enhance hydrolysis of hemicellulose fraction of biomass due to in-situ formation of carbonic acid. This acid being unstable will decompose and produce hydronium ions which, in turn, reduce the pH of reaction mixture, causing the hydrolysis of hemicellulose in $\text{CO}_2/\text{H}_2\text{O}$ biphasic system at elevated pressure (Toscan et al., 2017).

3.5. XRD analysis

The XRD profiles of raw and pretreated OPT are compiled in Fig. 6. Using these diffractograms, CrI values were calculated using Eq. 5 and the results showed that untreated OPT had a low crystallinity value (43.9 %) which might be due to the presence of xylan and lignin components. The increase in CrI value after SC- CO_2 processing showed the presence of crystalline cellulose in the OPT. The maximum value of CrI was found in case of biomass pretreated with 60 % moisture (51.4 %) relative to the fraction pretreated without moisture (47.1 %) despite identical process conditions (25 MPa, $T = 80^\circ\text{C}$). This can be attributed to the break-down of amorphous cellulose and xylan dissolution under acidic conditions. These results are consistent with the data shown in Fig. 2. Similar findings regarding variation in CrI value were reported by (Santos et al., 2011), (Srinivasan and Ju, 2012), (Serna et al., 2016) and (Zhao et al., 2019), where an overall improvement in the crystallinity was attained after SC- CO_2 pretreatment process. For the biomass pretreated at 35 MPa and 100°C with 60 % moisture, a slight decrease in crystallinity was observed relative to the untreated OPT as reported elsewhere where SC- CO_2 processing had a little or no effect on crystallinity (Yin et al., 2014) (Liu et al., 2014). It should however be kept in mind that crystallinity is not the only factor that could affect enzymatic hydrolysis yields from lignocellulosic biomass (Narayanaswamy et al., 2011), nevertheless, this aspect needs further investigation.

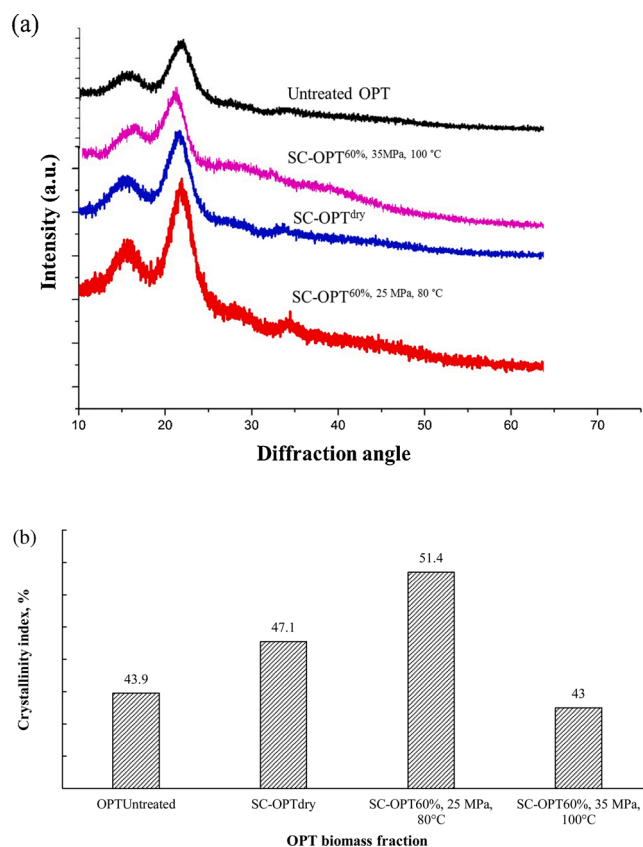


Fig. 6. The XRD profiles (a) and corresponding CrI values (b) for supercritical carbon dioxide (SC- CO_2) treated oil palm trunk (SC-OPT) biomass fractions at different process parameters compared to untreated OPT.

4. Conclusions

We have successfully demonstrated a facile and green pretreatment approach based on SC- CO_2 for oil palm trunk biomass that enhances its enzymatic hydrolysis yields for biorefinery application. The data suggested that SC- CO_2 process conditions had a profound impact on structure, composition and enzymatic hydrolysis of biomass material. This study revealed that the moisture content played a crucial role during SC- CO_2 pretreatment. As a result, enhanced bioconversion was achieved most likely due to the carbonic acid formation and better explosion effects during pretreatment. It is advocated that the SC- CO_2 -based method is very promising as compared to conventional pretreatment strategies because it avoids the use of toxic chemicals or excess water and offers possibility of solvent recycling, hence bids sustainability benefits. Overall rationale is that the SC- CO_2 -based technology is an eco-friendly alternative approach for processing different kinds of lignocellulosic biomass residues generated by oil palm industry in a single processing unit under virtually same conditions and equipment, and its integration in the oil palm industry would provide a new impetus towards the establishment of next generation oil palm biomass-based biorefineries.

CRediT authorship contribution statement

Saima Sohni: Investigation, Writing - original draft. **Rokiah Hashim:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing. **Hafiz Nidaullah:** Investigation, Writing - review & editing. **Othman Sulaiman:** Supervision, Writing - review & editing. **Leh Cheu Peng:** Writing - review & editing. **Junidah Lamaming:** Writing - review & editing. **Takamitsu Arai:** Conceptualization, Funding acquisition, Writing - review & editing. **Akihiko Kosugi:**

Conceptualization, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2020.112923>.

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