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42**Bioresource Technology 136 (2013)** 213–221 **Contents lists available at SciVerse ScienceDirect Bioresource Technology journal homepage: www.elsevier.com/locate/biortech** Bioethanol **production**

from pretreated Melaleuca leucadendron shedding bark – Simultaneous saccharification and fermentation at high solid loading Ibrahim Nasser Ahmed a, Phuong Lan Tran Nguyen a, Lien Huong Huynh b, Suryadi Ismadji c, Yi-Hsu Ju a,⇑

6a **Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Keelung Rd., Sec. 4, Taipei 106-07, Taiwan b Department of Chemical Engineering, Can Tho University,** 3-2 Street, **Can Tho City, Viet Nam c** Department of **Chemical Engineering, Widya Mandala Surabaya Catholic University, Kalijudan 37, Surabaya 60114, Indonesia**

highlights Paper bark tree shedding bark has higher glucan component. SCW at mild temperature is efficient for pretreatment of shedding bark. High ethanol production (63.2 g/L) was achieved at 0.25 g/mL solid loading. High ethanol (43.7 g/L) and yield (91.25%) were obtained at 0.15 g/mL solid loading.

44**article info Article history: Received** 1 **January 2013 Received in revised form** 22 February **2013 Accepted 25** February **2013 Available online** 14 March **2013 Keywords:**

Paper bark tree Bioethanol Subcritical water Saccharomyces cerevisiae abstract Bioethanol production from the shedding bark of Melaleuca leucadendron (Paper-bark Tree, PBT) was studied using subcritical water (SCW) pretreatment at various severities (So). High ethanol production was attained by implementing a factorial design on three parameters (So, solid loading and enzyme load- ing) in simultaneous saccharification and fermentation (SSF) mode. Ethanol concentration of 63.2 g L 1 corresponding to ethanol yield of 80.9% were achieved from pretreated biomass (So = 2.37) at 0.25 g mL 1 solid and 16 FPU g 1 glucan enzyme loadings. Similarly at 0.15 g mL 1 solid loadings both high ethanol concentration (43.7 g L 1) and high ethanol yield (91.25%) were achieved. Regression analysis of experi- mental results shows that all process parameters had significant role on maximum ethanol production, glucose solubility, ethanol yield and ethanol volumetric productivity. SSF of SCW treated PBT biomass is economically feasible for production of bioethanol. Ó 2013 Elsevier Ltd. All rights reserved. 1. Introduction The world is facing sustaining high oil price and progressive depletion of non-renewable fuel resources, while energy consump- tion keeps growing. In addition, the intensive use of fossil fuels has led to increasing release of polluting gasses into the atmosphere, which has caused change in global climate. Increasing concerns about climate change and energy security have motivated the search for alternative energy (Valentine, 2011). The growing inter- est in gasoline substituting

43**fuels has boosted** bio **-ethanol** produc- tion **worldwide from 12 to 19.5 billion gallons in the period of 2005–2009, with the**

USA

54**and Brazil being the two largest** produc- ers **representing 54% and 34% of total production, respectively (RFA, 2010).**

51**Ethanol use in 2011 reduced tailpipe CO2-equivalent emissions by 25.3 million metric tons. That** is equivalent **to the**

⇑

19**Corresponding author. Tel.: +886 2** 27376612; **fax: +886 2** 27376644. **E-mail address:** yhju@mail **.ntust.edu.tw (Y.-H. Ju). 0960-8524/$ - see front matter** Ó 2013 **Elsevier Ltd. All rights reserved.** http://dx **.doi.**

org/10.1016/j.biortech.2013.02.097 emissions of 4 million vehicles. Moreover the most current measurement of ethanol's

41**energy balance shows a positive 1.7–2.3 score, meaning ethanol is providing twice the energy it took to**

pro- duce (RFA, 2012). Most of the ethanol produced today is from starch and sugar producing crops (RFA, 2012). The

4**use of this type of biomass has been increasingly debated due to its impact on food** supply **as well as for environmental reasons. Therefore, complex (lignocellulosic) biomass has been put forward as a feasible alternative due to its abundance in nature and the large quantities generated as waste from agricultural activities,**

its higher cellulose content and com- positional uniformity. Moreover, tree possesses a lignocellulosic

26**energy conversion factor of 16 (compared** to **1 and 8 for corn and sugarcane, respectively), and can be grown on marginal land, thereby** minimizing **encroachment on** land for growing **food**

crops (Fenning et al., 2008). Thus the conversion of

37**lignocellulosic biomass to fuel offers potential economical and environmental advantage.**

Melaleuca leucadendron (paper-bark tree, PBT) is easily recognized by its odd whitish bark, which splits and peels in many papery layers. The tree continually sheds its outer bark and ex- poses the whiter inner part. The native range of PBT

12**is along the coast of eastern Australia from Sydney northward. It is native also in New Caledonia, Papua, New Guinea, and Irian Jaya.**

PBT has high adaptability and grows well in poor soil, dry and submerged envi- ronments, swampy ground and on creek banks, and even on hill- sides if ground water remains close to the surface. In its native habitat, PBT grows to 82 ft tall and is typically found in almost pure stands or with a few associates, such as Casuarina glauca, Eucalyptus robusta, and E. tereticornis. And

12**in the continental United States,** PBT **is naturalized on a significant scale in Florida**

and plantations in Hawaii and California (Geary and Woodall, 1990). The wood is a

13**suitable timber for such uses as pulp and cabinetry; the bark** has **potential uses as an amendment to plant potting mixes and in packaging and insulation.**

The

13**entire tree can be used as a biomass fuel**

(Geiger, 1981). Its leaves and fruits are rich in essential oil (cajuput oil), and has been used as a perfume and a popular rem- edy (Guenther, 1950). The shedding bark of PBT was used as feed- stock for bioethanol production in this study.

20**Pretreatment of lignocellulose biomass is crucial for achieving effective hydrolysis of substrates** since **enzymatic hydrolysis of** na- tive **lignocelluloses produces less than 20% glucose from the** cellu- lose **fraction (Zhang and Lynd, 2004).**

Processing shortages such as

59**long residence** time, **high energy demand, high**

cost, and environ- ment pollution exist

59**in current biological, physical, chemical and physicochemical pretreatment methods**

(Shill et al., 2011). There- fore, the

53**major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still** maintain- ing a **green and energy efficient** process (Lee et al., 2009). Hot compressed water (HCW) for autohydrolysis or hydrothermal pro- cessing has attracted considerable attention. The advantages are: corrosion problems are limited, no sludge is generated, reduces the need for neutralization and conditioning chemicals since nei- ther acid nor base is added, simple to implement, capital and oper- ational costs are low, and cellulose is not significantly degraded

30**under normal operating conditions (Liu 2010;**

Mosier et al., 2005). HCW has been shown to effectively pretreat

30**lignocellulosic biomass by partially hydrolyzing the hemicelluloses and** disrupt- ing **the lignin and cellulose structures, thus increasing the surface area**

(Hendriks and Zeeman, 2009). However, HCW

35**under severe conditions may generate inhibitors for enzymatic hydrolysis and fermentation, such as vanilline, syringaldehyde, furfural and 5-** hy- droxy **methyl furfural** (HMF) (Carolina **et al.,**

2011). The

2**properties of HCW change with temperature and density**

(Kruse and Dinjus, 2007).

2**Below the critical temperature** or **at** very **high pressures** (subcritical condition) **the ionic product is up to three orders of magnitude higher than** that at **ambient conditions, which means that water is an acid/base catalyst precursor**

(Alenezi et al., 2009). Also the dielectric constant being much higher in the sub- critical than in the supercritical region of HCW, hence it favors io- nic

2**reaction. This region is used for** various **synthesis reactions but also for degradation reactions** such as **biomass liquefaction**

11**(Kruse and Dinjus, 2007). Subcritical water** (SCW), which **is defined as** HCW **at temperatures between** 100 **and** 374 °C **under high**

pres- sure, has been used for hydrolysis of lignocellulose biomass (Lu and Saka, 2010). The production of ethanol from pretreated lignocellulose mate- rial can be carried out

32**either in a** two-step **separate hydrolysis and fermentation (SHF) or in a** single stage **simultaneous** saccharifica- tion **and fermentation (SSF).**

The

9**products formed during the hydrolysis step in an SHF process, such as cellobiose and glucose, inhibit** the **cellulase** enzyme **as well as the fermenting**

microorgan- isms. However, in SSF

46**glucose produced from hydrolysis is** simul- taneously **metabolized by microorganism, thereby alleviating problems caused by product inhibition (Alfani et al., 2000).**

Moreover,

9**the SSF process has other advantages such as reduced operational costs, lower enzyme requirement and increased** pro- ductivity **(Chen et al., 2007).**

38**Conversion of lignocellulosic materials to monomeric sugars and finally** to **ethanol must be performed at low cost, while still achieving high**

yield. Hence the type of pretreatment and its after- math on the overall process are important (Mosier et al., 2005). The two important variables affecting the economic features of bio- ethanol manufacturing are solid and enzyme loadings. A threshold of economical profitable bio-ethanol production, which is 4–5 vol- ume percent in a fermentation broth (Manzanares et al., 2011), de- mands the utilization of media containing an initial solid loading of at least 0.15 g mL 1 (on dry basis). However, high solid loading cre- ates an environment in which practically no free water exists in the pretreated material which may result in limited cellulose conver- sion in enzymatic hydrolysis or in SSF, owing to mass transfer lim- itation (Romaní et al., 2012). The objective of this work was production of bioethanol, which will meet an economic profitable concentration limit, from SCW pretreated PBT shedding bark. In this study a 33 fac- torial

24**design was** implemented **to** investigated **the effects of** SCW pretreatment; solid **loading and**

enzyme loading which are be- lieved to play important roles in selected variables (ethanol con- centration, ethanol yield, volumetric yield and glucan solubilization) in SSF. 2. Methods 2.1. Raw material Shedding bark of PBT was collected from experimental farm of National Taiwan University, Da’an District, Taipei, Taiwan. The location of the farm is N 25 00059.4000; E 121 32025.100. The air dried bark was milled to pass 8 mm screen, and stored in a dessicator be- fore use. 2.2. Pretreatment

7**There are three main parts in** the **equipment** for pretreatment: **reactor, heater and control devices. The reactor** is **made** of **stainless steel** with a **total inner volume** of **about**

200 mL. It is 25 mm

34**thick and can withstand an estimated maximum operation pressure of**

100 MPa. The reactor is equipped with a thermocouple and a pres- sure gage. The

7**process was run under batch mode**

with magnetic stirring (50 rpm). For SCW pretreatment,

7**nitrogen gas (99.9%** pur- ity) **purchased from Dong-Xing Company (Taiwan) was used to maintain** constant **pressure** (20 bar) in **the reactor. Dried**

and milled bark (10 g) and deionized (DI) water (100 mL) was put in the reactor. The suspension was heated to the desired temperature (120–180 °C) and kept at that temperature for a predetermined time (15, 30 or 60 min). The subcritical condition was terminated by venting vapor in the reactor. The reactor was then cooled to room temperature and the slurry collected from the reactor was fil- tered. The filtrate was analyzed for its monomeric sugar (glucose, xylose and galactose) and inhibitors (5-hydroxymethylfurfural, furfural and phenols) contents. The collected solid was

27**washed with** DI **water and** kept **at** 4 **°C. The** extent **of**

SCW treatment can be

1**expressed in terms of severity (So), defined as the logarithm of the severity**

factor R o (Romaní et al., 2010), which was calculated using the expression: S0 ¼ log R0 ¼ log½R0 heating þ R0 coolingŠ "Z tmax TðtÞ TRef dt þ Z tF T0ðtÞ T Ref # ¼ log x ð1Þ 0 tmax x

1**dt According to this equation,** So includes **the combined effects of temperature and reaction time.**

1**In Eq. (1), tmax (min) is the time needed to achieve Tmax** (°C), **tF (min) is the time needed for the** whole **heating–cooling** period, whereas **T(t)** and T0ðtÞ **represent the temperature profiles in heating and cooling, respectively.** Calcula- tions **were made** assuming **the values reported in literature** for x **(14.75** °C) **and**

TRef (100 °C). 2.3. Chemical composition analysis Compositions of carbohydrates and lignin of SCW treated and untreated shedding barks were determined using the standard NREL method as described by Sluiter et al. (2011). In brief the sam- ple (300 mg) was treated with H2SO4 (3 mL, 72%) in a water bath (30 °C) and incubated for 1 h followed by diluting the acid to 4% by adding 84 mL DI water, autoclaved for 1 h at 121 °C. The hydro- lysis solution was vacuum filtered using filtering crucible. Acid insoluble lignin in the residue was determined after ashing in ramping furnace for 24 h. Acid soluble lignin and the major struc- tural components (glucan, xylan, and galactan) were analyzed from the hydrolysis filtrate. The liquid phase was passed through a 0.22 lm PVDF syringe filter (Testhigh), then analyzed for mono- meric glucose, xylose and galactose using a HPLC (Jasco, Japan) equipped with a Jasco 830-RI Intelligent RI detector and a Cosmosil sugar-D column (4.6 mm I.D. 250 mm). Acetonitrile: water

39**(80:20 v/v) was used as the mobile phase at a flow rate of 1.** 0 **mL min 1.** Similarly sugars in **the**

prehydrolysate (pretreatment liquor) and SSF product were analyzed using the same HPLC system. The Liquid fraction was autoclaved

56**with 4% sulfuric acid** for 1 h **at 121 °C to**

break down oligomeric sugars into monomeric ones. Sugar standards with known concentrations were also autoclaved for the same time and at the same acid concentration to calibrate hydrolysis loss factors. The total amount of oligomeric sugars in the liquid sample was then calculated as:

34**determined by gas chromatography (GC-** 14B, **Shimadzu, Japan) with a flame ionization detector.**

37**Other conditions of operation were: Nitrogen as** the **mobile phase** (30 mL **min** 1), **column**

tem- perature 40 °C, injector temperature 200 °C, and detector tempera- ture 250 °C. Injection volume was 1 lL.

50**The concentration of ethanol was calculated based on elution time and peak areas of known concentration of ethanol.**

Separations were carried out on a stabilwaxÒ – DA (fused silica, polar phase; crossbondÒ carbo- waxÒ polyethylene glycol) column. 2.4. Microorganism, medium and yeast cultivation The fermenting yeast

22**used in this study was industrial** strain Ethanol RedÒ **Saccharomyces cerevisiae.** Inoculums were prepared **by**

selecting a single colony from YPD culture plates and inoculat- ing into YPD broth medium. The media consisted of 10

23**g L 1** glu- cose, **10 g L 1 peptone, 5 g L 1 yeast extract,** 2 **g L 1 KH2PO4, and 1 g L 1 MgSO4** at **pH** 4 **.8.**

27**After incubation at 35 °C for 24 h, the**

opti- cal density (OD 660) reading of the seed culture reached between 1.5 and 2.0. 2.5. Experimental design A 33 factorial experimental design with a total of 18 experi- ments was implemented as shown in Table 4 to investigate the re- sponses (maximum ethanol concentration, Emax; maximum ethanol yield, ECmax; maximum ethanol volumetric productivity, Qp max and glucose solubility, Glus) to the three process factors at three levels (So at 1.37, 1.92 and 2.37; solid loading at 0.10 g mL 1, 0.15 g mL 1 and 0.25 g mL 1; enzyme loading at 4, 10 and 16 FPU g 1 glucan) during SSF of PBT shedding bark. The three levels of So (x1), solid loading (x2) and enzyme loading (x3) were represented by 1, 0 and 1 for low, center and high levels respectively (Table 4). Upon completion of all experiments the regression analysis of experimental data was performed in Minitab 16 software to corre- h Oligomeric sugar g L 1 i ¼ Total sugars g L in the hydrolysate corrected for degradation h 1 i h Monomeric sugar g L in the hydrolysate liquid before autoclaving 1 i ð2Þ The concentrations of 5-hydroxymethylfurfural (HMF) and fur- fural in the prehydrolysates were analyzed by HPLC (Jasco, Japan) equipped with a PU-2089 pump, a degasser, an UV-2077 detector and a Luna C-18 column (5 lm particle size, 250 4.6 mm, Phe- nomenex, USA). The column temperature was 25 °C, the mobile phase was

29**acetonitrile: water: acetic acid (11:88:1 v/v/v)** at **a flow rate of 1 mL min** 1. The **injection volume** was 20 lL **and absorption wavelength** was **276 nm. The**

same HPLC for the analysis of pheno- lic compounds was used with the mobile phase consisted of sol- vent A (water: acetic acid = 100:1, v/v) and solvent B

31**(methanol: acetonitrile: acetic acid** = 75:25 **:1, v/v/v) at a flow rate of** 1 **mL min** 1. A **gradient elution was** used **as follows: 0–2 min,**

from 0 to 5%

16**solvent B; 2 to 10 min,** from 5 **to** 25% **solvent B; 10 to 20 min,** from 25% **to** 40% **solvent B; 20 to** 30 **min,** from 40% **to** 50% **solvent B;** 30 **to** 40 **min,** from 50% **to** 100% **solvent B;** 40 **to** 45 **min,**

100% solvent B; 44 to 55 min, 100 to 5% solvent B. UV detection was performed at 280 nm. Under these conditions it allowed the simultaneous detection of hydroxybenzoic and hydroxycinnamic acids.

45**Phenolic compounds in the samples were identified by comparing their relative retention times and UV** spec- tra **with those of authentic**

standards. In the same way, the concen- trations of sugars, furfural and HMF were calculated by using calibration curves obtained from standards. Ethanol content was late the experimental data, to determine the coefficients in the model and the significance of the coefficients. The established

33**polynomial equations were used to plot**

3-D surfaces and 2-D con- tours in Minitab

33**to visualize individual and interactive effects of the process factors on the response variables within their** prede- fined **ranges.**

2.6. SSF of SCW treated solids SSF was

8**carried out in a** 250 **mL Erlenmeyer flask equipped with a bubble trap to maintain anaerobic** condition **in an orbital shaker** (150 **rpm,**

37 °C) for 120 h. The fermentation flask contained 10 mL

15**nutrient solution (containing** 10 **g L 1 peptone** and 5 **g L 1 yeast extract),**

mixed with the desired solid and enzyme loadings, 5 mL sodium citrate buffer at pH 4.8, and 10 mL inoculums. The total volume of working slurry was 100 mL. Sample (1 mL) was with- drawn from SSF medium at preset times (0, 3, 6, 12, 24, 48, 72, 96 and 120 h), centrifuged (16,000g) and analyzed for ethanol and glucose. The enzyme used in this study was ‘‘CelluclastÒ 1.5 L’’ cellulases (from Trichoderma reesei) purchased from Sigma Aldrich Co. (St. Louis, USA). Cellulase activity was determined using the Filter Paper assay. Ethanol yield (% cellulose conversion) was calculated as: Îllulose conversion ¼ 0:5½E1ðtOf½HbiŠofma½sEstŠO1H:1Š1i1Þ 100% ð3Þ

58**Where [EtOH]f: Ethanol concentration at the end of the fermentation (g L** 1) **[EtOH]o:**

5**Ethanol concentration at the beginning of fermentation (g L** 1) **which** is **zero** [Biomass]: **Dry biomass concentration at the beginning of fermentation (g L** 1 **)f: Cellulose fraction of dry biomass (g g** 1) 0.51: **Conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast 1.111: Converts cellulose to equivalent glucose**

3. Results and discussion 3.1. SCW pretreatment of PBT shedding bark In

28**biorefinery based on lignocellulosic materials, which has** sug- ars **as intermediates, it is necessary to break down the feedstock’s structure and** obtain **sugars from cellulose and hemicellulose.**

Hence pretreatment is needed to prepare the feedstock in order to improve conversion of sugars (Carolina et al., 2011). Moreover, in order to obtain

22**high ethanol concentration in fermentation, a high** cellulose **concentration in the** medium **is** required.

21**Removal of non-cellulose components by pretreatment**

is beneficial to increasing in cellulose content. HCW mainly solubilize hemicellu- lose under controlled pH; hence it allows better accessibility of cel- lulose and to avoid the formation of inhibitors (Hendriks and Zeeman, 2009). The ionic product of HCW at subcritical condition

2**is up to three orders of magnitude higher than** that at **ambient** con- ditions, **which means that** using subcritical **water** (SCW) **is**

capable of achieving the same results as employing either acidic or alkaline catalysts (Alenezi et al., 2009).

18**The hydrolysis of Japanese beech (Fagus crenata) by batch and semi-flow**

SCW at 170–290 °C demonstrated an increased

18**production of total saccharides with temperature for both batch and semi-flow hot-compressed water treatments**

(Lu and Saka, 2010). The PBT shedding bark was pre- treated with SCW at mild temperature (120–180 °C) for the pur- pose of extracting xylan and obtaining high glucan concentration in the biomass to apply for

32**simultaneous saccharification and fer- mentation.** Table 1 shows **the** lists **of the**

range of temperature and residence time and the calculated severity (So) of each pretreat- ment. The value of So ranges from 0.46 to 2.59. The yield of xylose increased with severity till it reach the peak at a So of 2.37 (Fig. 1A). However, further increase of severity (So > 2.37) resulted in a de- crease of xylose yield, presumably due to degradation. At a So of 2.25, where xylose yield is close to its peak value, significant glucan depolymerization was evident. Glucose release kept increasing with increasing So. A notable difference between solubilization of the xylan and glucan fractions was the fact that the latter did not reach its potential maximum under the study conditions (Fig. 1A), hence SCW pretreatment of PBT primarily affords xylan extraction and as a result the solid residue became rich in glucan. In pretreatment, it has been shown that cellulose degradation was more difficult than hemicellulose under the same conditions due to their different structures, but the trends of hemicellulose and cel- lulose were similar (Carolina et al., 2011). A
9

57**8 Glucose** Xylose **Concentration (g/L) 7 6 5 4 3 2 1 0** -1 **0.5**

1.0 1.5 2.0 2.5 3.0 S o B 0.040 HMF 0.035

36**Furfural Concentration (g/L) 0.** 030 **0.** 025 **0.** 020 **0.** 015 **0.** 010 **0.** 005 **0.** 000 **-0.** 005 **0.**

5 1.0 1.5 2.0 2.5 3.0 S o

27**Fig. 1.** Compositions **of sugars (A) and** inhibitors **(B)** as function **of**

severity, So, in PBT shedding bark prehydrolysate after SCW water pretreatment. During pretreatments, various inhibitors may be formed, such as phenolics, furfural and HMF. These inhibitors

49**originate from the release and subsequent degradation of** carbohydrate **and**

lig- nin.

49**Formation of these compounds is directly proportional to** pre- treatment **severity**

(Hendriks and Zeeman, 2009). In Fig. 1B, the formation of

48**furfural and HMF, the** two **major degradation** prod- ucts **of pentose** and **hexose sugars,**

is presented. Pretreatment con- ditions which increased low level accumulation of furfural (Fig. 1B) strongly correlated to conditions associated with the progression of xylose loss (Fig. 1A). In contrast, the appearance of low level of HMF does not appear to correlate to glucose loss during pretreat- ment but presumably resulted from the degradation of minor hex- ose sugars associated with the hemicelluloses fraction. In general the milder temperature (120–180 °C) of SCW pretreatment used in this study requires relatively lower energy consumption and prevents extensive xylose and glucose degradation. In addition, since no chemicals is required making SCW pretreatment an envi- ronmentally benign and economical approach. Based on the pre- treatment findings, three So values (1.81, 1.92 and 2.37), which can extract hemicellulose with very low accumulation of inhibitors and high glucan recovery, were selected for the subsequent study in this work. Table 1 SCW pretreatment conditions and the corresponding severity value for PBT shedding bark. Pretreatment No. 1 2 3 4 5 6 7 8 9 10 11 12 Temperature (°C) Time (min) Severity, So 120 120 15 30 0.46 1.37 120 140 60 15 1.81 1.63 140 140 30 60 1.92 2.23 160 160 15 30 1.97 2.19 160 180 60 15 2.44 2.18 180 30 2.37 180 60 2.59 Table 2 Compositions of SCW prehydrolysate and solid residue from PBT shedding bark (0.1 g mL 1 solid load). Composition of Prehydrolysate (g L 1) So 1.81 So 1.92 So 2.37

Xylose Glucose Galactose Olig-xylose Olig-glucose Furfural HMF 3,4 Dihydro cinnamic acid p-coumaric acid Ferulic acid Composition of solid Residue (wt.%) Xylan Glucan Lignina 0.24 3.37 NDb ND ND 0.12 0.92 4.5 ND 0.42 ND 0.0001 ND ND ND 0.0034 ND 0.0012 ND 0.00012 15 9.17 48.2 51 19 18.1 8.63 4.5 0.44 4.2 1.1 0.0064 0.001 0.01 0.008 0.008 5.4 58 16.2 a Acid soluble lignin (ASL) plus acid insoluble lignin (AIL). b Not detected. 3.2. Composition of PBT and mass balance The compositions of PBT shedding bark hydrolysis liquor (prehydrolysate) and solid residue are listed in Table 2. At low So (1.81), little xylose (1.08% of xylan) was recovered in the prehydrolysates. This does not imply poor solublization of hemi- celluloses; rather the polymer was solubilized primarily in olig- omeric forms (5% of xylan). Previous studies on hydrolysis of lignocelluloses biomass using HCW indicated that the solubilized hemicelluloses appeared mainly in oligomeric form at lower severities (Garrote et al., 1999; Lu and Saka, 2010). As So in- creased the yield of xylose also increased. A maximum of 47% of available xylan was solubilized into monomeric xylose and a total 72% of xylan in oligomeric and monomeric forms at So = 2.37 (Table 2). Hence the yield of xylose was favored at a So of 2.37, which indicates that oligomeric xylose changed into monomeric forms. At the same So (2.37) significant concentra- tion of glucose and galactose were analyzed in prehydrolysate which may resulted from dissolution of the minor hexose sugars associated with the hemicelluloses and amorphous cellulose. Moreover trace amounts phenolic compounds were identified in the prehydrolysates at So of 1.92 and 2.37. At So = 2.37, eight phenolic compounds were identified: gallic, caffeic, 3,4-dihydroxycinamic, syringic, ferulic, p-coumaric, p-hydroxybenzo- ic, and vanillic acids at trace amounts. However only 3,4- dihydroxycinamic, p-coumaric and vanillic acids were quantified (Table 2) which showed higher yields than the other phenols.

11**It has been reported that phenolic compounds exist in insoluble bound forms with lignin and carbohydrates (hemicellulose and cellulose) in** lignocellulosic biomass **cell wall**

(Hendriks and Zee- man, 2009); hence,

14**the existing bonds (ester and/or ether bonds) between these materials can be hydrolyzed by**

SCW. However further studies are needed to obtain maximum recovery of phen- olics from PBT shedding bark. The extraction of xylan and acid soluble lignin enriched glucan content in the residue solid (Table 2). Under subcritical condition water can act as acid/base catalyst and can be employed in mild hydrolysis reaction (Alenezi et al., 2009), hence this property is believed to favor xylan and lignin sol- ubilization. The major portion of acid insoluble lignin was recov- ered in the pretreated solids while the acid soluble lignin solublized in the SCW prehydrolysate as shown in the mass bal- ance (Table 3). Thus a weight loss of the residue solid after SCW pretreatment was mainly attributed to xylan and acid soluble lig- nin dissolution into prehydrolysate as monomeric/ oligomeric xy- lose, furfural, solubilized lignin and phenolics. In general, the analysis of carbohydrate and non carbohydrate composition of PBT shedding bark showed good mass balance. The calculation incorporates the assumptions of including total extractives into prehydrolysate and the balance of ash. The total mass balance was 99.6% to 95% for native and pretreated biomass from HPLC analysis (Table 3). It is obvious that xylan present in the prehydro- lysate is mainly in monomeric and oligomeric xylose forms and furfural. For total lignin mass balance the phenolic compounds re- leased and solubilized and recovered lignins were considered. A small difference in mass balance between the native and pre- treated samples confirms the

14**presence of other unknown** com- pounds **from the decomposition of** PBT **in** SCW **medium which could not be identified in this study.**

3.3. Effects of SSF parameters on glucan dissolution and ethanol production Minimizing cellulase dosage is important for cost reduction of cellulosic ethanol production. It is also important to identify the optimum solid loading. At high solid loading the glucose solubility (Glus) was delimited by the low enzyme dosage (4.0 FPU g 1) as shown in Table 4, Exp. No 1–3; and Exp. No 7–9. The Glus results clearly indicate that hydrolysis was greatly improved by using high enzyme dosage which positively affected maximum ethanol pro- duction (Emax) and ethanol yield (ECmax). Similarly high solid load- ing had a positive effect on Glus and Emax. However, it had opposite effect on ECmax (Table 4). Saccharification of SCW pretreated biomass allowed high glu- can dissolution (up to 83%) and significant differences in Glus were observed between the pretreatment severities (1.81, 1.92 and Table 3 Mass balancea in native and SCW pretreated PBT. Native So = 1.81 Solid residue Extraction liquor So = 1.92 Solid residue Extraction liquor So = 2.37 Solid residue Extraction liquor Carbohydratesb Glucan Xylan Galactan Non

56**-carbohydrates Acid insoluble lignin Acid soluble lignin Ash**

Extractives 49.7 18.4 0.42 18.6 1.2 1.62 9.7 49.15 16.1 – 18.6 1.13 1.6 – 86.6 – 1.16 0.32 – 0.11 – 9.7 11.29 47.6 9.7 – 17.5 0.63 1.4 – 76.8 1.16 8.14 0.4 – 0.74 – 9.7 20.14 41.2 5.7 – 16.7 – 1.16 – 64.8 5.75 13.2 0.32 – 1.16 – 9.7 30.13 Overall mass balance 99.6 97.9 96.94 94.9 a Composition of dry native PBT (wt.%). b Total carbohydrate in non solubilized form or solubilized monomeric, oligomeric and/or degraded products (furfural and HMF). 2.37). For instance in Table 4 Exp. No 4 affords a Glus of 25.5 g L 1 (53% of glucan), and Exp. No 10 and Exp. No 13 give a Glus of 28.5 g L 1 (58% of glucan) and 35.2 g L 1 (64% of glucan) respectively. In the same way, Exp No 5, Exp. No 11 and Exp. No 14 give a Glus of 38.4 g L 1 (53% of glucan), 48.8 g L 1 (66.4% of glucan) and 58.9 g L 1 (71.4% of glucan), respectively. Proximity in Glus be- tween the So (1.92 and 2.37) was observed when higher solid load- ings was implemented. For instance at 0.25 g mL 1 solid loading (Exp. No 6 and 9, Table 4) close glucan dissolution (54.7% and 56.9%, respectively) was observed. However, closeness in Glus did not assure comparable Emax in different experiments. This event was a result of mass transfer limitations at high solid loadings and So. As shown in Table 3, there is a clear difference in xylan and lignin recovery between the So (1.92 and 2.37) that resulted in the solubility and the viscosity difference among the slurries. On the other hand Glus from biomass at low So (1.81) was greatly reduced with increasing solid loading. For instance at 0.15 g mL 1 solid loading (Exp No 5), 53% of glucan solubilization was obtained. However at 0.25 g mL 1 solid loading (Exp No 6) only 37% of glucan solubility can be achieved. Consequently, So affected the efficiency of enzyme and yeast. In this work SSF preformed with substrates treated at So = 2.37 led to good ethanol production and yield. The experimental data given in Table 4 were used to develop a full quadratic polynomial regression model (Eqn. 4) to predict the dependent variables y (Glus Emax, ECmax and Qp max) as a function of the three process parameters: x1 (So, °C), x2 (solid loading, g mL 1) and x3 (enzyme loading, FPU g 1glucan). In Eqn.4, bo

40**is the** offset **coefficient, bi is the linear coefficient, bii is the quadratic coefficient and bij is the interaction coefficient. The**

coefficients and the corre- sponding p-values of the models are listed in Table 5. The fitted models ANOVA results (Table S1 in Supplementary material-1) showed that the R2 values were at least 0.97, which indicate the aptness of the models to explain responses. Y ¼ bo þ Xibxi þ Xibix2i þ XiXj bij xij ð4Þ 1 1 1 2 Analysis of the p-values of each term in the models was used to determine the significance levels of the three process parameters and their interactions on responses. As shown in Table 5, linear interactions of independent variables had high significant effect (p < 0.001). Moreover the combined effects of all independent vari- ables significantly contributed to dependent variables. However the square interaction of So (b11) on all dependent variables is not significant, this interaction could be removed from the equa- tions without significant effect on the accuracy of the predicted dependent variables. The equations were used to plot response surfaces and their corresponding contours to show Glus, Emax, ECmax and Qp max by different levels of the process variables with one variable fixed at center level (Supplementary material-2). Figs. S1-A and B show that there are significant interactions of So with the other process parameters on Glus. Similar interactions were ob- Table 4 Operational parameters and results in experimental design carried out to optimize the SSF of SCW treated PBT shedding bark. Exp. No Independent variablesa x1 x2 x3 Dependent variablesb S0 EL (FPU) SL (g mL 1) Glus (g/L) Emax (g/L) ECmax (g/100g) Qp max (g/L/h) 1 1.81 2 1.81 3 1.81 4 1.81 5 1.81 6 1.81 7 1.97 8 1.97 9 1.97 10 1.97 11 1.97 12 1.97 13 2.37 14 2.37 15 2.37 16 2.37 17 2.37 18 2.37 4 0.10 4 0.15 4 0.25 10 0.10 10 0.15 10 0.25 4 0.10 4 0.15 4 0.25 10 0.10 10 0.15 10 0.25 10 0.10 10 0.15 10 0.25 16 0.10 16 0.15 16 0.25 1 1 1 1 1 1 1 0 1 0 1 0 0 1 0 1 0 1 0 0 0 0 0 0 1 0 1 0 1 0 1 1 1 1 1 1 1 16.7 0 24.3 1 32.9 1 25.5 0 38.4 1 44.7 1 22.8 0 33.7 1 42 1 28.5 0 48.8 1 67 1 35.2 0 58.9 1 78.32 1 44.3 0 68.5 1 103.3 4.5 16.5 6.6 16.1 9.6 14.07 8.5 31.15 11.3 27.6 13.5 19.8 6.9 25.04 9.5 22.8 12.8 18.44 17.7 63.76 23.5 56.3 32.2 46.32 25 80.02 35.8 76.36 54.6 69.9 29.4 93.11 43.7 91.25 62.3 80.92 0.094 0.14 0.20 0.18 0.24 0.28 0.145 0.19 0.27 0.37 0.33 0.45 0.35 0.5 0.76 0.41 0.61 0.88 a So: severity, SL: solid loading (g mL 1), EL: enzyme loading (FPU/g glucan). b Glus: solublized glucose concentration (g/L), EMAX: maximum ethanol concentration (g/L), QPMAX : volumetric productivity at E MAX

36**(g/L),** ECMAX: **maximum ethanol** conversion **(g ethanol/** 100 **g** potential **ethanol).**

Table 5 Regression coefficients and statistical parameters measuring the correlation and significance of the models. Glus Emax ECmax Qp max Coefficient p-value Coefficient p-value Coefficient p-value Coefficient p-value bo b1 b2 b3 b11 b22 b33 b12 b13 b23 52.3851 11.2380 13.7366 16.9957 0.8583 -1.6183 -6.4919 3.1667 4.9623 6.4943 0.000 0.000 0.000 0.000 0.698 0.522 0.004 0.286 0.003 0.002 23.4710 14.1870 5.820 8.028 3.181 -7.544 -1.388 8.778 4.532 3.481 0.000 50.0404 0.000 24.6108 0.004 11.5537 0.000 -5.3937 0.120 0.2806 0.007 -17.7633 0.355 -0.1638 0.006 18.95 0.002 -0.098 0.021 -2.3364 0.000 0.367131 0.000 0.159475 0.000 0.096166 0.000 0.110698 0.864 0.032222 0.000 -0.074167 0.897 -0.004813 0.000 0.079167 0.915 0.058275 0.059 0.040496 0.000 0.000 0.016 0.000 0.449 0.144 0.881 0.170 0.032 0.172 served on Emax (Fig. S2), ECmax (Fig. S3) and Qp max (Fig. S4). High ethanol concentration was affected by the three process parame- ters. It can be seen from Fig. S2-A that at 0.15 g mL 1 solid loading, So P 2.3 and enzyme loading > 12.5 FPU/g glucan should be used to achieve high ethanol concentration. Fig. S2-C shows that at high PBT solid loading, high enzyme loading was required to catalyze the hydrolysis and to achieve high ethanol concentration. In gen- eral from Fig. S2-A and B that ethanol concentration higher than 40 g L 1 (economical threshold limit) can be achieved at So P 2.3, enzyme loading P 15 FPU g 1 glucan, and solid loading P 0.15 g mL 1. The interaction between solid loading and ECmax shows that solid loading had a decreasing effect on ECmax (Figs. S3-A and B), as a result of mass transfer limitation. Moreover con- sidering the regression coefficients of ECmax (Table 5), it can be seen the square interaction of solid loading (b22) had a negative values and had high significant effects ($p < 0.001$) on ECmax. 3.4. Time courses of SSF All SSFs were performed using the washed solid fraction to remove sugars solubilized during pretreatment and potential inhibitors (Fig. 1) that could affect enzymatic hydrolysis and fer- mentation. In an attempt to obtain ethanol concentration exceed- ing the economical threshold limit (4–5%, v/v), the experiments were carried out with a substrate loading of 0.15 and 0.25 g mL 1. Additionally, experiment with 0.10 g mL 1

24**was performed in order to** study **the effect of** substrate **loading**

on ethanol production by S. cerevisiae. Fig. 2 shows the time courses of ethanol and glucose concentra- tions in SSF with 0.10 g mL 1, 0.15 g mL 1 and 0.25 g mL 1 solid loadings. In general, glucose concentration increased sharply in the first 24 h of SSF depending on the solid and enzyme loadings. However, the concentration decreased as the SSF progressed which was accompanied by a rapid increase of fermentation product. In the first 12 h, the differences in ethanol concentrations were insig- nificant in all cases since yeast cells were adapting to the new envi- ronment. The delay or lag phase was more obvious and extended up to 24 h when substrate loading was 0.15 or 0.25 g mL 1. The lag phase due to the adaptation of yeast to fermentation conditions and its duration is related to solids loading (Hoyer et al., 2009). A longer lag phase in SSF performed at high solid loading of other lig- nocellulosic materials using inoculums of S. cerevisiae has been re- ported (Sassner et al., 2006). In most experiments of this work, the highest ethanol concentrations were obtained at 72 h and at high solid loadings (0.25 g mL 1 and 0.15 g mL 1). Maximum concentra- tion (63.2 g L 1) was obtained under the following conditions: pre- treatment at So = 2.37, SSF for 72 h, 0.25 g mL 1

48**solid loading and** 16 **FPU g 1** glucan **enzyme loading.**

The volumetric productivity (Qp) in the first 24 h of SSF was higher for 0.10 g mL 1 solid loading (data not shown), owing to favorable kinetics of cellulose hydroly- sis in the early stage. At higher solid loadings (0.15 g mL 1 and 0.25 g mL 1), after 24 h of SSF the slurry become less viscous hence the mass transfer limitation was reduced and Qp increased. The volumetric productivity at the highest ethanol concentration (Qp max) was analyzed (Table 4) since Qp at random time lacks practical interest (Romaní et al., 2012). The variation range determined for Qpmax was 0.094–0.88 g (L 1 h 1), and it was affected by all process parameters. All SSF carried out using So 1.81 and some of SSF implementing So 1.91 (Exp. No 7–10, Table 4) attained Emax in 48 h with ECmax values from 14.07% to 63.8%, while the rest (Exp. No 11–18, Table 4) attained maxima after 72 h of SSF, and the ECmax varied from 56.3% to 93.1%. Longer fermentation times re- sulted in higher conversion. For comparison, Table 6 lists experimental results

8**obtained in this work and** data **reported in related studies.**

At higher solid loadings (0.15 and 0.25 g mL 1), the SSF reaction matrix became A 50

52**40 Glucose (g/ L) 30 20 10 0**

30 25 20 15 10 Ethanol (g/ L) 5 0 B 70 0 20 40 60 Time (h) 80 100 120 60

52**Glucose (g/ L) 50 40 30 20 10 0**

45 40 35 30 25 20 15 Ethanol (g/ L) 10 5 0 5 C 120 0 20 40 60 80 100 120 Time (h) 70 100 Glucose (g/ L) 80 60 40 20 0 60 50 40 30 20 Ethanol (g/ L) 10 0 0 20 40 60 80 100 120 Time (h) Fig. 2. Time courses of ethanol (So 2.37, 16 FPU: (j); So 2.37, 10 FPU: (.); So 1.97, 10 FPU (d); So 1.97, 4 FPU: ( )) and glucose (So 2.37, 16 FPU: (h); So 2.37, 10 FPU: (5); So 1.97, 10 FPU: (s); So 1.97, 4 FPU: (})) concentration in SSF experiments at solid loadings: 0.10 g mL 1 (A); 0.15 g mL 1 (B); 0.25 g mL 1 (C). highly viscous with unequal distribution of slurry resulting in eth- anol yield less than the theoretical value. Pessani et al. (2011) re- ported that switchgrass treated with hydrothermolysis at 200 °C gave 22.5 g L 1 ethanol concentration and 86% ethanol yield at 0.08 g mL 1 solid loading, while at 0.12 g mL 1 solid loading etha- nol concentration

15**increased to** 32 **g L 1** but ethanol **yield decreased to**

82%. Steam exploded (205 °C) and washed corn stover at high enzyme loading (20 FPU g 1) produced an ethanol concentration of 41 g L 1 and 49 g L 1 at 0.25 g mL 1 and 0.30 g mL 1 solid load- ings, respectively with high yield (92–94%) (Lu et al., 2010). How- ever, using similar pretreatment and biomass loading (0.25 and 0.30 g mL 1), low ethanol yield (64.8% and 52.1%) and ethanol pro- duction (39

3**.3 g L 1 and** 40.6 **g L 1)**

were reported by Zhang et al. (2010) when an enzyme activity of 13.6 FPU g 1 was used. Using Table 6 Experimental data

8**obtained in this work and results reported in related studies. Raw Material Pretreatment condition** SLa **(g**

mL 1) ELb Emaxc (FPU/g) (g/L) ECmaxd Reference (%) Switchgrass Switchgrass Corn stover Corn stover Corn stover Corn stover Barley straw Barley straw Corn cobs Corn cobs Coffee residue Oil palm empty fruit bunches Olive tree pruning Eucalyptus globulus Eucalyptus globulus Eucalyptus globulus Eucalyptus grandis Paper bark tree Paper bark tree

47**Paper bark tree** Hydrothermolysis **at** 200 **°C for** 10 **min**

Hydrothermolysis at 200 °C, 10 min Steam explosion at 200

25**°C for 4 min** Steam explosion **at** 200 **°C for 4 min** Steam explosion **at** 205 **°C for** 6 **min** Steam explosion **at** 205 **°C for** 6 **min** Steam explosion **at** 210 **°C for** 5 **min** Steam explosion **at** 210 **°C for** 5 **min**

Conc. formic

22**acid hydrolysis at** 60 **°C for** 6 **h**

followed by 15% aq. ammonia delignification at 60 °C for 12 h 2 wt.% sulfuric acid at 121 °C for 45 min followed by 2 wt.% sodium hydroxide at 80 °C for 6 h. Popping pretreatment at a pressure of 1.47 MPa for 10 min 21% ammonia at 60 °C for 12 h 1% H2SO4 hydrolysis at 180 °C for 10 min Autohydrolysis at 230 °C Autohydrolysis at 230 °C Delignification with 60% ethanol 1.2% H2SO4 hydrolysis at 121 °C for 45 min Subcritical water

55**at 180 °C for 30 min** Subcritical water **at** 180 **°C for 30 min** Subcritical water **at 180 °C for** 30 **min**

0.08 58e 0.12 58e 0.30 13.6f 0.25 13.6 0.25 20 0.30 20 0.15 –g 0.10 - 0.19 30 0.25 22.8 0.10 2.23g 0.05 60 0.225 15 0.10 6.2 0.25 16 0.10 20 0.20 30 0.15 16 0.25 10 0.25 16 22.5 86 32 82 40.6 52.1 39.3 64.8 41 92 49.5 94 26 - 19.4 - 62.7 77.3 84.7 79 15.3 87.2 18.6 65.6 24.9 38 26.7 77.7 67.4 91.1 35 - 28.7 - 43.7 91.3 54.6 70 62.3 81 Pessani

10**et al., 2011** Pessani **et al., 2011** Zhang **et al.** (2010) Zhang **et al.** (2010) **Lu et al.,** 2010 **Lu et al.,** 2010 García-Aparicio **et al., 2011** García-Aparicio **et al., 2011** Zhang **et al.** (2010) Zhang **et al.** (2010) Choi **et al.,** 2012 Jung **et al.,** 2012 Manzanares **et al., 2011** Romaní **et al.** 2010 Romaní **et al., 2012** Muñoz **et al.,** 2011 Silva **et al.,**

2011 This work This work This work a SL: solid loading. b EL: enzyme loading. c Emax : maximum ethanol concentration. d ECmax: maximum ethanol yield. e 0.7 mL/g glucan of cellulase with activity 82.2 FPU/mL. f 18.3 mg protein/ g glucan with activity 0.122 FPU/mg protein. g not reported. Eucalyptus grandis pretreated with acids, Silva et al. (2011) ob- tained an ethanol concentration of 28.7 g L 1 operating under 0.20 g mL 1 solid loading, but high enzyme loading (30 FPU g 1) was required. Muñoz et al. (2011) employed organosolv-deligni- fied Eucalyptus wood to obtain substrates containing 84% cellu- lose, which was processed by SSF (0.10 g mL 1 solid and 20 FPU g 1 enzyme loadings) to reach ethanol concentration up to 35 g L 1, but some cellulose was lost in the pretreatment step. Zhang et al. (2010) reported that using a pretreated corncob first with acidic solutions and then under alkaline conditions, increas- ing the solids loading from 0.075 g mL 1 up to 0.19 g mL 1 caused a decrease of ethanol yield from 90% to 77%. Similar result was re- ported when using hardwood pretreated with liquid hot water or by acid prehydrolysis, ethanol yield was significantly reduced as solid loading was

24**increased from 0.** 09 **g** mL **1 to** 0.23 **g** mL **1**

(Man- zanares et al., 2011). Romaní et al. (2012) reported high ethanol yield (91.1%) and high ethanol concentration (67.4 g L 1) using 0.25 g mL 1 Eucalyptus globules after autohydrolysis treatment at high temperature (230 °C). This work implemented the environmentally friendly SCW pre- treatment at mild temperature (180 °C) without utilization of chemicals. High ethanol concentration (63.2 g L 1) with high ethanol yield (80.9%) was obtained at 0.25 g mL 1 solid loading. Simul- taneous high ethanol concentration (43.7 g L 1) and yield (91.25%) can be obtained at 0.15 g mL 1 solid loading.

21**These values meet the requirements for economically viable production of ethanol from** PBT lignocelluloses **on an industrial scale. 4. Conclusion The** results **of**

this study showed that Melaleuca leucadendron shedding bark has high glucan component and confirmed that SCW pretreated PBT biomass implemented in SSF mode is suitable for economically feasible production of bioethanol. Optimization of the process resulting in the identification of operational conditions (pretreatment temperature 180 °C; high solid loading 0.15– 0.25 g mL 1 and enzyme loading 16 FPU g 1 glucan) enabling simultaneously high ethanol concentration (43.7

15**g L 1** to 63 **.2 g L 1) and ethanol yield**

(91.25–80.9%).

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17**Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.02.**

097. References Alenezi, R., Leeke, G.A., Santos, R.C.D., Khan, A.R., 2009. Hydrolysis kinetics of sunflower oil under subcritical water conditions. Chem. Eng. Res. Des. 87, 867– 873. Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M., 2000. Comparison of SHF and SSF processes for the

bioconversion of steam-exploded wheat straw. J. Ind. Microbiol. Biotechnol. 25, 184–192. Carolina, C.M., Arturo, J.G., Mahmoud, E.H., 2011. A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. Process. Saf. Environ. 90, 189–202. Chen, H., Xu, J., Li, Z., 2007. Temperature cycling to improve the ethanol production with solid state simultaneous saccharification and fermentation. Appl. Biochem. Microbiol. 43, 57–60. Choi, I.S., Wi, S.G., Kim, S.B., Bae, H.J., 2012. Conversion of coffee residue waste into bioethanol with using popping pretreatment. Bioresour. Technol. 125, 132–137. Fenning, T.M., Walter, C., Gartland, K.M.A., 2008. Forest biotech and climate change. Nat. Biotechnol. 26, 615–617. García-Aparicio, M.P., Oliva, J.M., Manzanares, P., Ballesteros, M., Ballesteros, I., González, A., Negro, M.J., 2011. Second-generation ethanol production from steam exploded barley straw by Kluyveromyces marxianus CECT 10875. Fuel 90, 1624–1630. Garrote, G., Dominguez, H., Parajo, J.C., 1999. Mild autohydrolysis: and environmentally friendly technology for xylooligosaccharide production from wood. J. Chem. Technol. Biotechnol. 74, 1101–1109. Geary, T.F., Woodall, S.L., 1990. Melaleuca quinquenervia (Cav.) S. T. Blake melaleuca, in: Burns, R.M., Honkala, B.H., Silvics of North America, Vol. 2. Hardwoods, Agric. Hand b. 654, U.S. Department of Agriculture, Forest Service, Washington, DC, pp. 461–465. Geiger, R.K., 1981. Proceedings of melaleuca symposium, Fort Myers, FL, September 23–24, 1980. Florida Division of Forestry, Tallahassee, p. 140. Guenther, E., 1950. The essential oil. REK Publishing Company, Florida, Vol. IV, pp. 602–615. Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18. Hoyer, K., Galbe, M., Zacchi, G., 2009. Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content. J. Chem. Technol. Biotechnol. 84, 570–577. Jung, Y.H., Kim, I.J., Han, J.I., Choi, I.G., Kim, K.H., 2012. Aqueous ammonia pretreatment of oil palm empty fruit bunches for ethanol production. Bioresour. Technol. 102, 9806–9809. Kruse, A., Dinjus, E., 2007. Hot compressed water as reaction medium and reactant properties and synthesis reactions. J. Supercritical Fluids 39, 362–380. Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol. Bioeng. 102, 1368–1376. Liu, S., 2010. Woody biomass: Niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis. Biotechnol. Adv. 28, 563–582. Lu, Y., Wang, Y., Xu, G., Chu, J., Zhuang, Y., Zhang, S., 2010. Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. Appl. Biochem. Biotechnol. 160, 360–369. Lu, X., Saka, S., 2010. A hydrolysis of Japanese beech by batch and semi-flow water under subcritical temperatures and pressures. Biomass Bioenerg. 34, 1089– 1097. Manzanares, P., Negro, M.J., Oliva, J.M., Sáez, F., Ballesteros, I., Ballesteros, M., 2011. Different process configurations for bioethanol production from pretreated olive pruning biomass. J Chem. Technol. Biotechnol. 86, 881–887. Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreshel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005. Industrial scale-up of pH controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl. Biochem. Biotechnol. 125, 77–79. Muñoz, C., Baeza, J., Freer, J., Mendoça, R.T., 2011. Bioethanol production from tension and opposite wood of Eucalyptus globulus using organosolv pretreatment and simultaneous saccharification and fermentation. J. Ind. Microbiol. Biotechnol. 38, 1861–1866. Pessani, N.K., Atiyeh, H.K., Wilkins, M.R., Bellmer, D.D., Banat, I.M., 2011. Simultaneous saccharification and fermentation of Kanlow switchgrass by thermotolerant Kluyveromyces marxianus IMB3: The effect of enzyme loading, temperature and higher solid loadings. Bioresour. Technol. 102, 10618–10624. Renewable fuel Association, 2010. Ethanol Industry Outlook: Climate of Opportunity, Available at: http://ethanolrfa.org/page//objects/pdf/outlook/ RFAoutlook2010_fin.pdf?nocdn=1. Renewable fuel Association, 2012. Ethanol Industry Outlook: Accelerating Industry Innovation. Available at: http://ethanolrfa.3cdn.net/d4ad995ffb7ae8fbfe_ 1vm62ypzd.pdf. Romaní, A., Garrote, G., Alonso, J.L., Parajó, J.C., 2010. Bioethanol production from hydrothermally pretreated Eucalyptus globulus wood. Bioresour. Technol. 101, 8706–8712. Romaní, A., Garrote, G., Parajó, J.C., 2012. Bioethanol production from autohydrolyzed Eucalyptus globules by Simultaneous Saccharification and Fermentation operating at high solids loading. Fuel 94, 305–312. Sassner, P., Galbe, M., Zacchi, G., 2006. Bioethanol production based on simultaneous saccharification and fermentation of steam-pretreated Salix at high dry-matter content. Enzyme Microb. Technol. 39, 756–762. Shill, K., Padmanabhan, S., Xin, Q., Prausnitz, J.M., Clark, D.S., Blanch, H.W., 2011. Ionic liquid pretreatment of cellulosic biomass: Enzymatic hydrolysis and ionic liquid recycle. Biotechnol. Bioeng. 108, 511–520. Silva, N.L.C., Betancur, G.J.V., Vasquez, M.P., Gomes, E.B., Pereira, N., 2011. Ethanol production from residual wood chips of cellulose industry: acid pretreatment investigation, hemicellulosic hydrolysate fermentation, and remaining solid fraction fermentation by SSF process. Appl. Biochem. Biotechnol. 163, 928–936. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2011. Determination of structural carbohydrates and lignin in biomass, NREL. Technical, Report NREL/TP-510-42618. Valentine, S.V., 2011. Emerging symbiosis: Renewable energy and energy security. Renew. Sust. Energ. Rev. 15, 4572–4578. Zhang, J., Chu, D., Huang, J., Yu, Z., Dai, G., Bao, J., 2010. Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor. Biotechnol. Bioeng. 105, 718–728. Zhang, Y.H.P., Lynd, L.R., 2004. Review: Toward an Aggregated Understanding of Enzymatic Hydrolysis of Cellulose: Noncomplexed Cellulase Systems. Biotechnol. Bioeng. 88, 797–824. Zhang, M., Wang, F., Sua, R., Qia, W., Hea, Z., 2010. Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. Bioresour. Technol. 101, 4959–4964. Author's personal copy 214

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136 (2013) 213–221

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136 (2013) 213–221 215 216

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