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Evaluation energy efficiency of bioconversion knot rejects to ethanol in comparison to other thermochemically pretreated biomass

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HIGHLIGHTS

- Energy efficiency of bioconversion sulfite pulp mill rejects and thermochemically pretreated biomass.
- Effects of disk refining on bioconversion of sulfite pulp mill rejects.
- Rejects show superior energy efficiency over pretreated biomass.

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ABSTRACT

Rejects from sulfite pulp mill that otherwise would be disposed of by incineration were converted to ethanol by a combined physical–biological process that was comprised of physical refining and simultaneous saccharification and fermentation (SSF). The energy efficiency was evaluated with comparison to thermochemically pretreated biomass, such as those pretreated by dilute acid (DA) and sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL). It was observed that the structure deconstruction of rejects by physical refining was indispensable to effective bioconversion but more energy intensive than that of thermochemically pretreated biomass. Fortunately, the energy consumption was compensated by the reduced enzyme dosage and the elevated ethanol yield. Furthermore, adjustment of disk-plates gap led to reduction in energy consumption with negligible influence on ethanol yield. In this context, energy efficiency up to 717.7% was achieved for rejects, much higher than that of SPORL sample (283.7%) and DA sample (152.8%).

1. Introduction

Cellulosic ethanol represents the best sustainable, secure, and renewable alternative to fossil fuels, and now becomes the focus of worldwide research and investment (Badger, 2002). Cellulosic ethanol is produced from lignocellulose, a structural material that comprises much of the mass of plants, such as corn stover, switchgrass, and woodchips (Sun and Cheng, 2002; Zaldivar et al., 2001). Production of cellulosic ethanol has the advantage of abundant and diverse raw materials compared to ethanol processes that use corn and cane, but requires a greater amount of physicochemical pretreatments to make the sugar monomers available to the microorganisms that are typically used to produce ethanol by fermentation(Alvira et al., 2010). Furthermore, the pretreatment of lignocellulose feedstocks, such as the commonly used acid hydrolysis, steam explosion, and ammonia fiber expansion, are rather energy intensive due to the required extreme conditions such as high temperature and high pressure, making it a major bottleneck hindering economic bioconversion (Cardona Alzate and Sánchez Toro, 2006; Hamelinck et al., 2005; Zhu and Pan, 2010). In this context, efforts had been contributed to biological pretreatment to replace or complement thermochemical methods for biofuel production with the purpose of process energy reduction (Lemée et al., 2012; Salvachúa et al., 2011). Biological pretreatments was verified a promising technique and has advantages in biofuel production, but at the same time it was recognized very slow and requires careful control of growth conditions, thus less attractive commercially (Cheng and Timilsina, 2011; Zheng et al., 2009). Considering the intensive energy consumption of thermochemical pretreatments and time consuming biological pretreat-

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ments, more and more attentions are paid to the utilization of cellulosic wastes, which have been already physicochemically treated in processes of target product (Ballesteros et al., 2001; Duff and Murray, 1996; Li et al., 2007; Taherzadeh and Karimi, 2008; Zhang et al., 2010). It is believed such conversion from the wastes to clean renewable fuel can not only promote the energy efficiency of bioconversion, but also bring about profound benefits to society, environment and economy. This will definitely mitigate the dependence on fossil energy, lower greenhouse gases emission, and create a new industry of jobs and economic growth.

In fact, bioconversion of cellulosic waste to ethanol had been widely studied, especially the knot rejects (KR) from sulfite pulp mill. Knot rejects that are generated in screening process after digestion of woodchips consist mostly of botanical knots and insufficiently cooked woodchips. Knot rejects are conventionally disposed of by combustion as a method to reduce landfill (Monte et al., 2009). But the calorific value of rejects is low due to poor dewatering efficiency. Rather than combustion, knot rejects are now considered as suitable feedstock for cost-competitive biofuel production because the sulfite cooking that is usually carried out between pH 1.5 and 5 using bisulfite and sulfurous acid at 130–160 °C for 4–14 h renders extra thermochemical pretreatment unnecessary (Biermann, 1993). Furthermore, the utilization of knot rejects for biofuel production will make pulp mills a bio-refining system with multitude products, which was considered to be profitable by the increased revenues from biofuel and the cost cut on wastes disposal. It is for this reason that intensive research had been conducted in this field. Helle et al. (2007) have reported the addition of reject knots from the pulp line to spent sulfite liquor could significantly increase the sugar content in fermentation broth, and therefore fortify the fermentation of spent liquor. A similar research conducted by Lai (2010) showed the bioconversion of sludge, which was made up of pulp fines and rejects, was greatly enhanced when combined with sulfite spent liquor, yielding 50% ethanol conversion. Recently, Menind et al. (2012) reported that pulp rejects could be effectively used for bioethanol production with an optimized ethanol yield of 78.9 g kg^{-1}. Although the bioconversion of rejects to ethanol had been actively explored as mentioned above, the economics are a little concerned. The study of Helle et al. (2007) revealed that the high capital cost of hydrolysis tank and the cellulase enzyme required for effective hydrolysis were the major economic bottlenecks that hindered the bioconversion of knot rejects. In that context, Zhang et al. (2010) focused on the development of economic scheme for bioconversion. It was observed that the enzyme cost was reduced when enzyme recycling was performed. However, the enzyme recycling though membrane separation was economically infeasible due to the rapid and recurrent membrane fouling. Because the knot rejects from pulp mill consist mostly of botanical knots and insufficiently cooked woodchips that have extremely strong structure which impedes the access of enzyme to cellulose fiber, knot rejects are recalcitrant to enzymatic hydrolysis and this is why a great quantity of enzyme was required for effective bioconversion. For this reason, further study is needed to develop an approach that is capable of overcoming the recalcitrance of knot rejects by fibrillating knot rejects and boosting the accessible area of cellulose fiber exposing to enzyme. In addition, energy consumption evaluation will also be needed to demonstrate conclusively that the bioconversion of knot rejects by the developed approach is much more energy efficient than bioconversion of lignocellulosic biomass from conventional thermochemical pretreatments.

The goal of the present study was to evaluate the economic viability of the bioconversion of knot rejects. Especially, physical refining was proposed to enhance digestibility of knot rejects for superior energy efficiency over woody biomass that was conventionally pretreated by thermochemical methods.

2. Experimental

2.1. Materials

Knot rejects used in this study were friendly offered by a sulfite pulp mill in US with production capacity of 300 ton per day (tpd) pulp from mixture of hardwood and softwood. Up to 15–30 tpd of rejects were collected from the continuous digester. The rejects were botanical knots that were insufficiently cooked with size ranged from 6 mm to 38 mm. This material of rejects is rich in carbohydrate as shown in Table 1 but currently being fed to a wood waste boiler for energy recovery in the mill, and mostly as a method to reduce land-filling. The rejects were received from the mill as it were and stored in a freezer at a temperature of approximately −16 °C until used. The chemical compositions of rejects were listed in Table 1.

Fresh aspen (*Populus tremuloides*) wood logs were obtained from northern Wisconsin, USA and debarked and chipped at the Forest Products Laboratory, Madison, Wisconsin. The size of the wood chips used for the study ranged from 6 to 38 mm in two dimensions with a thickness variation from 1 to 5 mm. The chips were stored in a freezer at a temperature of approximately −16 °C until used.

Celluclast 1.5 L and Novozyme 188 (β-glucosidase) were generously provided by Novozymes North America (Franklinton, NC). Sodium acetate, sulfuric acid, and sodium bisulfite were used as received from Sigma–Aldrich (St. Louis, MO). All other chemicals, including culture media ingredients, were received from Fisher Scientific (Hanover Park, IL). All chemicals were all ACS reagent grade.

2.2. Microorganism and culture

Saccharomyces. cerevisiae FPL-450 (ATCC® Number 9080) was grown at 30 °C for 2 days on YPD-agar plates containing 10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose, and 20 g L^{-1} agar. A colony from the plate was then transferred by loop to a liquid YP medium supplemented with 30 g L^{-1} glucose in a flask. The *S. cerevisiae* FPL-450 seed was grown overnight at 30 °C with agitation at 90 rpm on a shaking bed until the biomass concentration reached 2 g L^{-1} as monitored by optical density at 600 nm measurements (Agilent 8453, UV–visible spectroscopy system, Agilent Technologies, Santa Clara, CA).

2.3. Pretreatment and substrate production

Aspen wood chips were directly pretreated by dilute sulfuric acid (DA) and sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL). A laboratory wood pulping digester of capacity of 23 L was used to conduct pretreatment as described in our previous studies (Wang et al., 2011; Zhu et al., 2009). All pretreatments were conducted using 150 g wood chips in oven-dried (od) weight in a 1-L reactor with a liquor to wood ratio of 3:1 at 170 °C for 20 min. DA pretreatment was conducted at sulfuric acid concentration 0.2% (v/v) or 1.10% (w/w) on od wood (DA-A_2B_0). Two SPORL pretreatments were conducted, sulfuric acid concentration 0.2% (v/v) and sodium bisulfite 1.5% (w/w) on od wood (SPORL-$A_2B_{1.5}$), and sulfuric acid concentration 0.2% (v/v) and sodium bisulfite 3% (w/w) on od wood (SPORL-A_2B_3). The pretreated wood chips were then subjected to physical refining under atmospheric conditions after the separation of the solids from the pretreatment hydrolysate for solid substrate generation. The refiner was equipped with plates of pattern D2-B505 (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH). The disk-plates gap was set at 40‰ inch. Water was added during physical refining, which resulted in substrate slurry with solid consistency of approxi-

Table 1
Chemical composition of wood chips and substrates as well as solid yield.

Sample label	Klason lignin	Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan	Pretreatment yield [a]	Washing yield [a]	Solid substrate yield [a]
Aspen wood	20.8	0.29	0.33	0.20	43.78	16.40	1.57	100	100	100
Substrates										
Knot rejects (KR)	20.1	nd [b]	nd	nd	68.77	4.68	1.64		91.7	91.7
SPORL-A_2B_3	24.8	0.91	nd	nd	68.78	2.27	0.20	81.2	79.7	64.7
SPORL-$A_2B_{1.5}$	26.4	0.85	nd	nd	68.22	2.06	0.29	78.6	78.9	62.0
DA-A_2B_0	29.6	0.84	nd	nd	64.70	2.80	0.10	84.8	78.4	66.5

[a] Defined as percent of starting materials recovered as insoluble solids (w/w). Specifically, pretreatment yield was calculated by dividing the amount of the pretreated wood chips by the amount of original wood chips; washing yield was the percent of pretreated wood recovered as substrate after physical refining and washing; solid substrate yield is calculated by multiplying pretreatment yield by washing yield.
[b] Not detectable.

mately 10%. The energy consumption of physical refining was recorded (Table 2) as described elsewhere (Zhu et al., 2010). The size-reduced solid was directly dewatered by pressing using a canvas bag to a solid content of approximately 30%, without a separate washing step. The yield of solid substrate in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. The chemical compositions of original wood and pretreated substrates, as well as solid yield were listed in Table 1.

Similarly, knot rejects were fed directly into the disk refiner for structure deconstruction. Different from the pretreated wood chips, three plate-gap sets were applied to knot reject to produce substrates with different fiber sizes. Substrates obtained at plate-gap of 15‰ inch, 40‰ inch, and 80‰ inch were abbreviated as KR-15, KR-40, and KR-80, respectively. The solid substrate yield and energy consumption of physical refining were listed in Table 1 and Table 2, respectively.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted using commercial enzymes at 2% substrate solid loading (w/v) in 50-mL of sodium acetate buffer (pH 4.8, concentration 50 mM) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 50 °C and 200 rpm. An enzyme mixture of Celluclast 1.5 L cellulase and Novozyme 188 β-glucosidase was used for enzymatic hydrolysis. Cellulase and β-glucosidase loadings were 10 FPU/g glucan and 15 CBU/glucan, respectively, if not otherwise specified. Hydrolysate was sampled periodically for glucose concentration. Each data of glucose titer (Table 2) is the average of three replicates analyses of the same sample.

2.5. Quasi-simultaneous enzymatic saccharification and fermentation (SSF)

Quasi-SSF were carried out in 250 mL Erlenmeyer flasks using a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 35 °C and 90 rpm with 10% (w/v) solid substrate. The enzyme loadings were the same as for enzymatic hydrolysis described above, namely cellulase 10.0 FPU g^{-1} glucan and β-glucosidase 15 CBU g^{-1} glucan. The solid substrate was first liquefied at 50 °C and 200 rpm for 120 min on the shaker incubator before adding the yeast *S. cerevisiae* FPL-450. Initial cell concentration for all SSF experiments was 0.4 mg dry cell g^{-1} substrate. No nutrients were added for all fermentation experiments. Samples of the fermentation broth were taken every 24 h and centrifuged at 10,000 rpm for 5 min and were stored at −4 °C until analyzed for ethanol concentration. Reported results of ethanol titer (Table 2) are the average of three replicates with an average relative standard deviation of about 4%.

2.6. Analytical methods

The chemical compositions of the original wood chips, pretreated biomass, and knot rejects were analyzed by the Analytical and Microscopy Laboratory of the Forest Products Laboratory using improved high-performance anion exchange chromatography with pulsed amperometric detection (Davis, 1998). Solid materials were Wiley milled (model #2, Arthur Thomas Co, Philadelphia, PA) using

Table 2
Details about physical refining, enzymatic hydrolysis and SSF of substrates from knot rejects and pretreated wood chips.

Sample labels [a]		KR-15	KR-40	KR-80	SPORL-A_2B_3	SPORL-$A_2B_{1.5}$	DA-A_2B_0
Physical refining							
Energy consumption (GJ ton^{-1}) [b]		1.479	1.285	1.006	0.079	0.169	0.343
Enzymatic hydrolysis							
Glucose titer (g L^{-1})	24 h	11.36	11.19	9.98	5.74	5.40	5.08
	48 h	12.82	12.74	11.68	7.45	6.41	5.85
	72 h	13.88	13.39	12.66	8.22	7.00	6.40
Glucan conversion@72 h%		90.79	87.6	82.81	53.77	46.17	44.48
Simultaneous enzymatic saccharification and fermentation (SSF)							
Ethanol titer (g L^{-1})	24 h	16.72	13.44	10.16	11.52	6.62	6.52
	48 h	25.46	24.5	23.59	16.31	13.90	13.72
	72 h	28.84	28.19	27.53	22.33	15.75	14.81
	96 h	30.72	29.44	28.17	27.13	20.95	17.62
	120 h	32.04	31.10	30.21	29.68	23.25	21.22
Ethanol yield@120 h%		82.78	80.46	78.15	76.76	60.63	58.36

[a] KR-15, KR-K40, and KR-80 stand for substrates obtained from physical refining at 15‰ inch, 40‰ inch, and 80‰ inch of disk plate gap, respectively. SPORL-A_2B_3, SPORL-$A_2B_{1.5}$ and DA-A_2B_0 were substrates that were obtained by physical refining at 40‰ inch of disk plate gap.
[b] GJ stands for gigajoule. The unit of energy consumption is GJ ton^{-1} knot rejects for KR-15, KR-40 and KR-80, and GJ ton^{-1} pretreated wood chips for SPORL-A_2B_3, SPORL-$A_2B_{1.5}$ and DA-A_2B_0.

a 20-mesh outlet screen (1 mm). The samples were hydrolyzed using sulfuric acid in two stages. The hydrolysis conditions were an acid concentration of 72% (v/v) at 30 °C and 3.6% (v/v) at 120 °C for the first and second stages, respectively. The hydrolysis duration time was 1 h for both stages. Monosaccharides in hydrolysate were then determined using a Dionex (Sunnyvale, CA) HPLC system (ICS-3000) equipped with an AS3500 autosampler, a GP40 gradient pump, an anion exchange column (Dionex, CarboPac PA1) and an ED40 electrochemical detector(Pan et al., 2005). Deionized water was used as eluent at a flow rate of 1.0 mL/min. Aliquots (20 μl) were injected after passing through a 0.45 μm nylon syringe filter (Chromatographic Specialties, Brockville, Canada). Optimization of baseline stability and detector sensitivity was achieved by postcolumn addition of 0.2 M NaOH. The column was reconditioned using 1 M NaOH after each analysis. Monosaccharides were quantified with reference to standards using the same analytical procedure. Klason lignin content of pulps was measured gravimetrically after washing and drying the solid residue from the acid hydrolysis according to the TAPPI T-222 standard method.

Ethanol analysis in the cellulosic substrate fermentation broth was carried out using a gas chromatograph (GC, model 7890, Agilent Technologies, Palo Alto, CA) through direct sample injection using an external standard for calibration. The sample was centrifuged and the supernatant was filtered before injection to the GC column. The GC is equipped with a flame ionization detector and Agilent DB Wax column of 30 m with an ID 0.32 mm. A universal guard column was used to reduce column contamination. All analyses were carried out in duplicate at a minimum. The average data were reported. The standard deviations were calculated as a measurements error. For fast analysis, glucose in the enzymatic hydrolysate was measured in duplicate using a commercial glucose analyzer (YSI 2700S, YSI, Yellow Springs, OH).

2.7. Definitions and calculations

Glucan conversion was defined as the percentage of glucan in the substrate converted to glucose enzymatically, and was applied to evaluate the performance of enzymatic hydrolysis. Ethanol yield through SSF was defined as the percentage of substrate glucan fermented to ethanol. To evaluate the bioconversion performance of feedstocks, a term of energy efficiency, η_{Energy}, is defined as a percentage of the net energy output to the total energy consumption for bioconversion, i.e.,

$$\eta_{Energy} = \frac{E_{output} - E_{input}}{E_{input}} = \frac{E_{ethanol} - (E_{wood\_chipping} + E_{thermal\_pretreatment} + E_{physical\_refining})}{E_{wood\_chipping} + E_{thermal\_pretreatment} + E_{physical\_refining}} \quad (1)$$

where E_{output} denotes energy output from ethanol, which is calculated by multiplying ethanol production by calorific value of ethanol (Gross Calorific Value of ethanol is 29.7 GJ/ton). E_{input} denotes total energy input for bioconversion, including wood log chipping energy $E_{wood\_chipping}$, thermal pretreatment energy $E_{thermal\_pretreatment}$ and physical refining energy $E_{physical\_refining}$. $E_{wood\_chipping}$ was estimated to be 0.18 GJ ton^{-1} wood according to the study of Klugman et al. (2007). $E_{thermal\_pretreatment}$ was based on thermodynamic calculations of enthalpy of the pulp suspension at 25% solids consistency (liquid to wood ratio = 3/1) at the pretreatment temperature of 170 °C, i.e., 1.25 GJ ton^{-1} wood, with consideration of 50% thermal energy recovery. $E_{physical\_refining}$ was calculated based on data record of power during physical refining.

3. Results and discussion

3.1. Energy consumption of physical refining for knot rejects and thermochemically pretreated woody biomass

Rejects obtained from sulfite pulp mill mainly consist of wood knots and insufficiently cooked wood chips. The strong structure makes it difficult to be accessed by enzyme although knot rejects had been thermochemically treated in digester for pulp production. Therefore, structure deconstruction that results in separated fibers and even external fibrillation is crucially needed to enhance the digestibility of substrate by boosting the accessible area to enzyme molecules. Structure deconstruction of knot rejects was performed by physical refining at different disk-plates gaps and the corresponding energy consumption was listed in Table 2. Generally, refining of knot rejects was observed extremely energy intensive compared to thermochemically pretreated biomass. For the same plate-gap of 40‰ inch, the energy consumption of knot rejects (KR-40, 1.285 GJ ton^{-1}) was as high as four to fifteen times of energy consumption of pretreated biomass dependent on the pretreatment method applied (DA-A_2B_0, 0.343 GJ ton^{-1}; SPORL-A_2B_3 0.079 GJ ton^{-1}). Although nearly a third of energy consumption can be cut for knot rejects by widening plate-gap from 15‰ inch (KR-15, 1.479 GJ ton^{-1}) to 80‰ inch (KR-80, 1.006 GJ ton^{-1}), the refining energy consumption of knot rejects was still much higher than pretreated biomass.

3.2. Enhanced enzymatic digestibility by physical refining

The structure deconstruction of knot rejects by physical refining was an energy intensive process as mentioned above. In this context, knot rejects were directly subjected to enzymatic hydrolysis without any treatment to see whether the energy consuming process of physical refining was indispensable to bioconversion. For a comprehensive understanding, the enzymatic hydrolysis under various conditions was conducted to both unrefined rejects and refined rejects (Fig. 1). It was observed that no matter enzymatic hydrolysis was conducted at low substrate consistency of 2% (Fig. 1a) or at medium substrate consistency of 10% (Fig. 1b), the glucan conversion of unrefined rejects was far below the glucan conversion of the refined rejects, even with a higher enzyme dosage. This can be explained by the production of separated and fragmented fibers that were resulted from the structure deconstruction of knot rejects by shear force of physical refining. Furthermore, the results in Fig. 1 also reflected the economic potential of physical refining because of the reduced enzyme dosage and elevated glucan conversion. Again, this was ascribed to the significant increase of accessible surface of substrate to cellulase as a result of fibrillation.

3.3. Comparison of enzymatic digestibility among refined knot rejects, DA substrates, and SPORL substrates

Following physical refining is the step of enzymatic hydrolysis in which the glucan in substrate was converted to fermentable sugar. The glucan conversion was plotted against time as shown in Fig. 2. Overall, the glucan conversion of DA substrate, SPORL substrate, and refined rejects were in ascending order. Specifically, at the same plate-gap of physical refining, the glucan conversion@72 h of the refined rejects (KR-40, 87.60%) was as much as 60% higher than SPORL substrates (SPORL-A_2B_3, 53.77%), and almost twice the glucan conversion of DA substrates (DA-A_2B_0, 44.48%). The reducing of disk-plates gap led to significant improvement in enzymatic digestibility. Up to 8% gain in glucan conversion@72 h was achieved by plate-gap adjustment from 80‰ inch

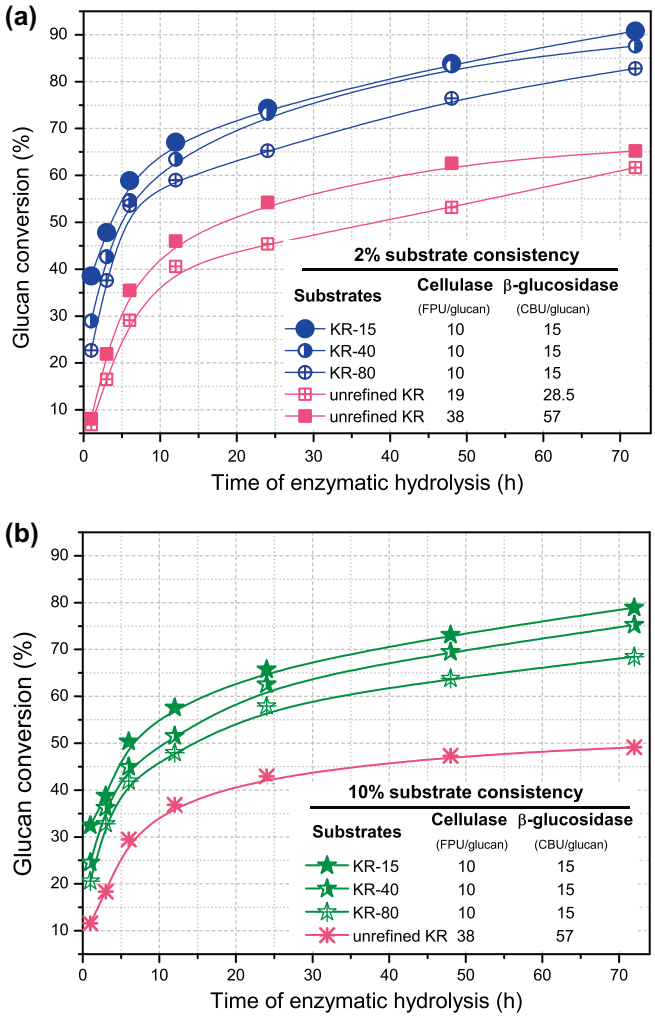

Fig. 1. Enzymatic hydrolysis of refined rejects and unrefined rejects at various conditions, (a) enzymatic hydrolysis was conducted at 2% (w/v) substrate consistency and (b) enzymatic hydrolysis was conducted at 10% (w/v) substrate consistency.

of KR-80 to 40‰ inch of KR-40 (Table 2) due to the increased accessible area to cellulase as a result of further fiberization of knot rejects.

3.4. Fermentation of refined knot rejects, SPORL substrate, and DA substrate

Given the variation of glucan content in different substrates, instead of the terminal ethanol concentration in fermentation broth, the ethanol yield, defined as the percentage of substrate glucan fermented to ethanol, was applied to evaluate the potential of bioconversion of refined rejects and thermochemically pretreated substrates. From Fig. 3, KR-15 showed highest terminal ethanol yield of 82.78% over other substrates especially thermochemically pretreated substrates. However, SPORL-A_2B_3 showed a terminal ethanol yield of 76.76% that was as high as 78.15% of KR-80, while SPORL-$A_2B_{1.5}$ with a lower bisulfite dosage showed a much lower terminal ethanol yield of 60.63%, but was still slightly higher than 58.36% of DA-A_2B_0. This observation suggested the importance of bisulfite in removal of recalcitrance of biomass. The ethanol yields of KR-15, KR-40 and KR-80 showed the same order of rank as glucan conversion as discussed above. In addition, the difference of ethanol yields among KR-15, KR-40 and KR-80 diminished as enzymatic hydrolysis proceeded, which could be interpreted by the nature of heterogeneous reaction.

3.5. Preliminary evaluation on energy efficiency of bioconversion

Energy efficiency was used to evaluate the performance of bioconversion routes. The energy efficiency is dependent on ethanol production and energy consumption which consists of wood chipping energy, thermal pretreatment energy and physical refining energy as expressed in Eq. (1). The results in Table 3 indicated the refined rejects was an excellent substrate for bioconversion because the ethanol production was much higher than that of SPORL and DA samples. However, the physical refining of rejects consumed much more energy than SPORL and DA samples due to the hard structure of rejects. Fortunately, the intensive energy consumption was compensated by the remarkable ethanol production, led to energy efficiency up to 717.7% for KR-40. The decrease in disk-plates gap from 80‰ inch to 15‰ inch resulted in 6% promotion in ethanol production (from 351.0 L/ton to 372.4 L/ton). Mean-

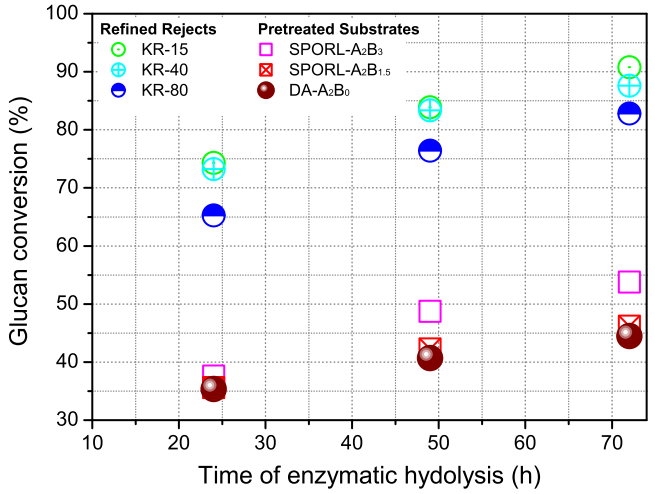

Fig. 2. Correlations between glucan conversion and enzyamtic hydrolysis time for all substrates investigated. Enzymatic hydrolysis was conducted at 2% substrate solid loading (w/v) with cellulase 7.5 FPU g^{-1} glucan and β-glucosidase 11.25CBU g^{-1} glucan for the SPORL and DA pretreated substrates.

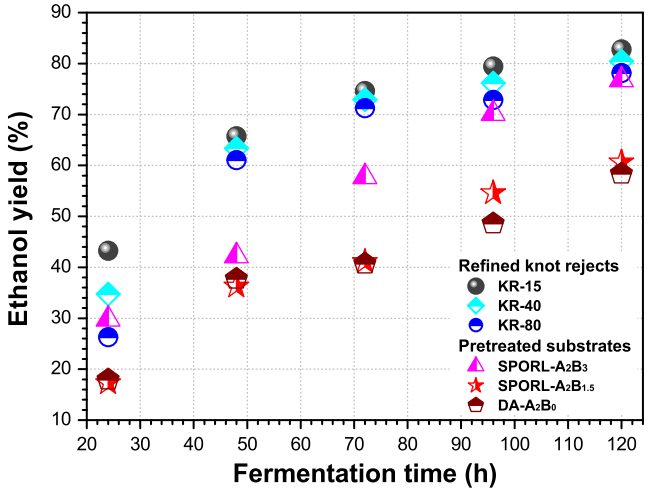

Fig. 3. Time-dependent ethanol yield of refined knot rejects, SPORL substrate, and DA substrate, SSF was conducted at 10% (w/v) substrate consistency with cellulase 10.0 FPU g^{-1} glucan, β-glucosidase 15CBU g^{-1} glucan, and initial cell concentration of *S. cerevisiae* 0.4 mg dry cell g^{-1} substrate.

Table 3
Energy consumption, ethanol production and energy efficiencies of KR and SPORL/DA pretreated substrates.

Sample label	$E_{wood\_chipping}$ & $E_{thermal\_pretreatment}$ [a] (GJ ton^{-1}) [b]	$E_{physical\_refining}$ [a] (GJ ton^{-1}) [b]	SSF ethanol production (L ton^{-1}) [b]	$E_{ethanol}$ [a] (GJ ton^{-1}) [b]	η_{Energy} (%)
KR-15	0	1.479	372.4	8.726	490.0
KR-40	0	1.285	361.7	8.476	559.6
KR-80	0	1.006	351.0	8.226	717.7
SPORL-A_2B_3	1.43[a]	0.056	243.4	5.703	283.7
SPORL-$A_2B_{1.5}$	1.43	0.105	182.7	4.281	178.9
DA-A_2B_0	1.43	0.228	178.9	4.192	152.8

[a] $E_{wood\_chipping}$, $E_{thermal\_pretreatment}$, $E_{physical\_refining}$ and $E_{ethanol}$ were used for determination of η_{Energy} as expressed in Eq. (1).
[b] The unit ton refers to weight of knot rejects for KR-15, KR-40 and KR-80, and weight of wood chips for SPORL-A_2B_3, SPORL-$A_2B_{1.5}$ and DA-A_2B_0.

while, energy consumption of physical refining increased by nearly a half (from 1.006 GJ ton^{-1} to 1.479 GJ ton^{-1}). Therefore, the trade-off between ethanol production and energy consumption should be seriously considered for the evaluation of feedstocks and process. For thermochemically pretreated samples, the lowest energy efficiency 152.8% of DA-A_2B_0 was resulted from the highest energy consumption and lowest ethanol production, while for refined rejects, the highest energy efficiency 717.7% of KR-80 was the result of trade-off between ethanol yield and energy consumption.

4. Conclusions

The present study demonstrated the great economic potential of knot rejects in ethanol production. Although the bioconversion of knot rejects avoided the operations of wood chipping and thermochemical pretreatment, but the physical refining, an indispensable process for enzymatic digestibility promotion, was observed more energy intensive than SPORL or DA pretreated biomass. Fortunately, the physical refining of rejects led to remarkable ethanol yield, which overcompensated the energy consumption and resulted in superior energy efficiency over SPORL or DA samples.

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