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Formulating a fully converged biorefining chain with zero wastewater generation by recycling stillage liquid to dry acid pretreatment operation

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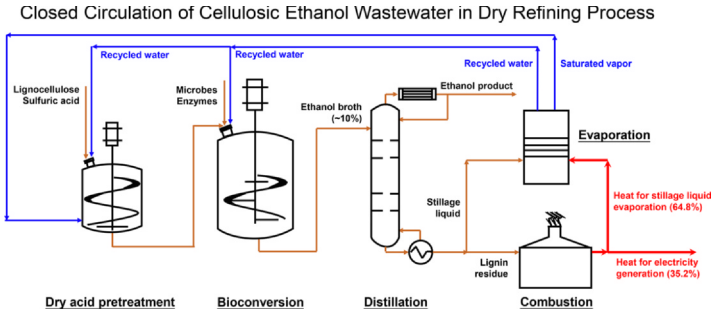
GRAPHICAL ABSTRACT



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ABSTRACT

Huge wastewater generation is the major challenge of biorefinery technology for production of cellulosic ethanol. This study designed and verified a method for completely recycling of wastewater stream (the stillage liquid from the beer column) in cellulosic ethanol production by dry biorefining processing. When the stillage liquid was directly recycled to dry acid pretreatment operation, ethanol production gradually reduced after two recycles primarily because the inorganic compounds accumulated by around 139%. To ultimately solve this technical barrier, the stillage liquid was evaporated and condensed into distillated water, then recycled to the pretreatment for complete dry biorefining process. This strategy supported a stable cellulosic ethanol production, and the overall mass and heat balance confirmed that only 65% of the lignin residue consumption was used for wastewater evaporation with 35% surplus for electricity generation. This study provided a fully converged biorefining process with a closed-loop wastewater recycling.

1. Introduction

One of the major challenges for cellulosic ethanol production is its high wastewater generation (Alvira et al., 2010; Hu et al., 2017). Biorefinery process generally requires high water consumption and consequently generates large quantity of wastewater. For the most widely applied dilute acid pretreatment (~30% solids loading), 16.6 ton wastewater is generated with the production of per ton ethanol (Humbird et al., 2010; 2011). Ammonia fiber expansion (AFEX) (Uppugundla et al., 2014; Kim and Dale, 2015), deacetylation and mechanical refining (DMR) (Chen et al., 2015; 2016), and steam explosion (SE) (Liu and Chen, 2016) even generate greater wastewater at 20.7, 115.2, and 115.7 ton per ton ethanol, respectively (Liu and Bao, 2017a). Such wastewater generation in cellulosic ethanol production is

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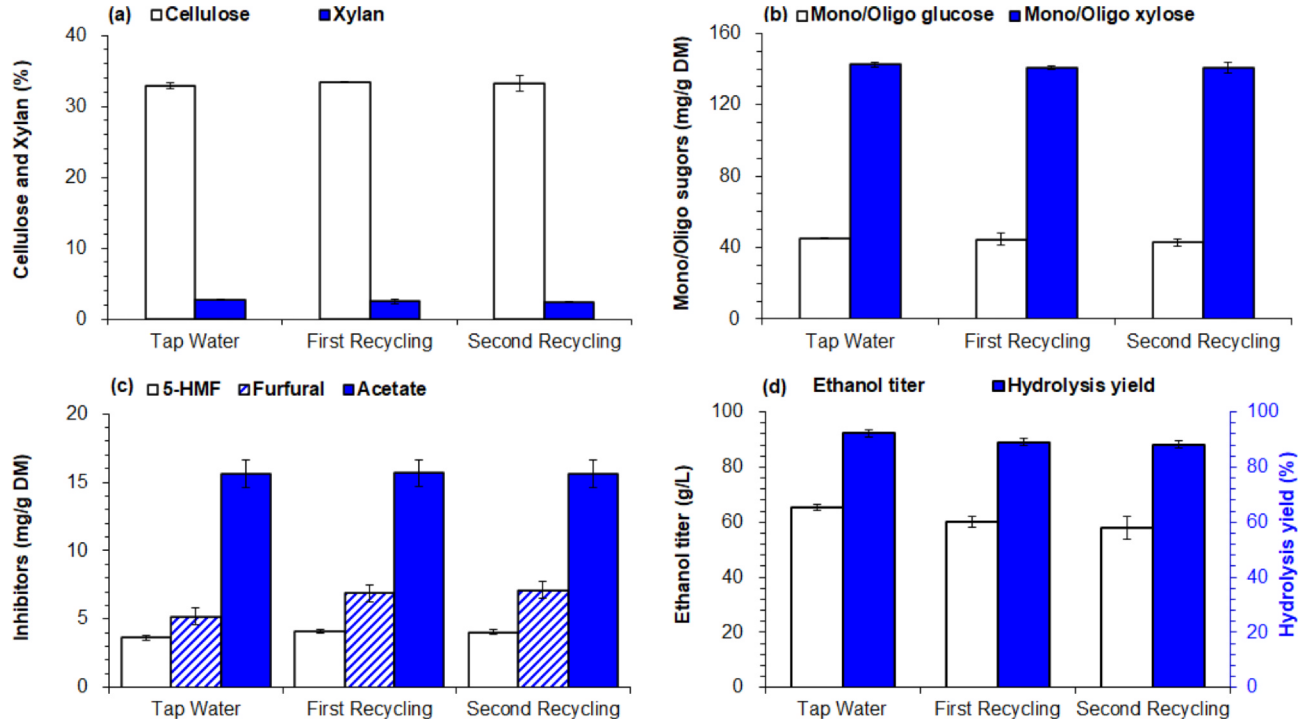

Fig. 1. Evaluation of direct recycling stillage liquid to pretreatment operation with the feedstock of raw wheat straw. (a) Cellulose and xylan content. (b) Mono and oligo-glucose, mono and oligo-xylose content. (c) Inhibitors content. (d) Enzymatic hydrolysis yield and final ethanol titer in SSCF. Tap water, pretreatment using tap water; First recycling, pretreatment using the first recycled stillage liquid; Second recycling, pretreatment using the second recycled stillage liquid.

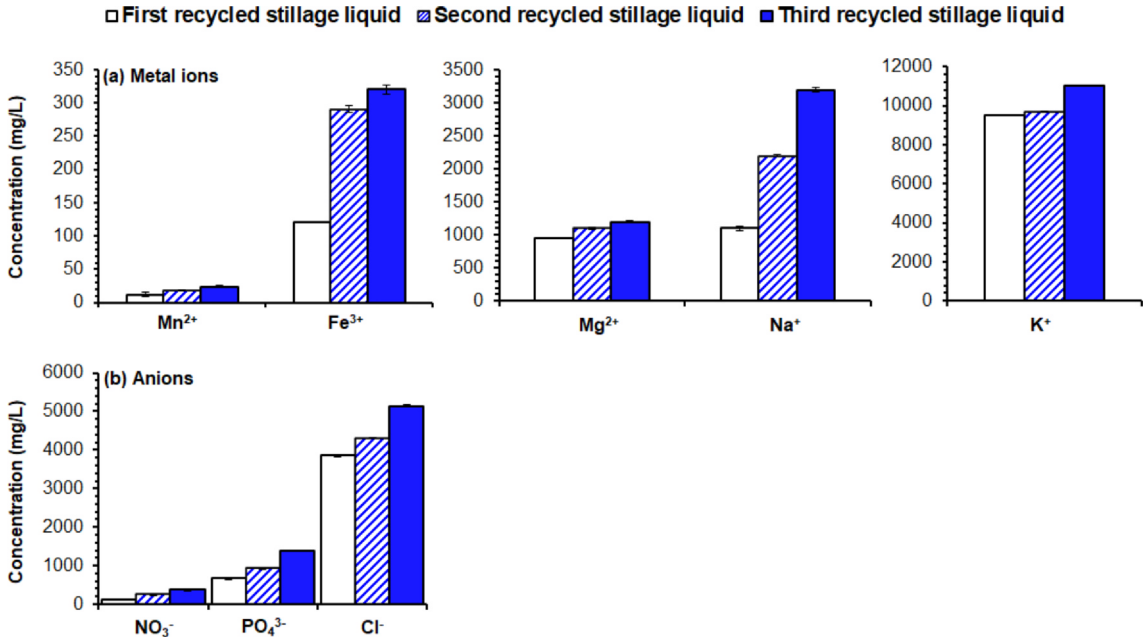

Fig. 2. The concentration of main ions in the recycled wastewater. (a) Metal ions concentration. (b) Anions concentration.

far higher than that in corn ethanol production (8.3 ton per ton ethanol) (Liu and Bao, 2017a). Meanwhile, the high COD of cellulosic ethanol wastewater leads to an extra-ordinary expense to meet the discharge standard by regular approaches such as anaerobic digestion (Tian et al., 2013; Hu et al., 2017), aerobic biochemical (Wang et al., 2017a; Hu et al., 2017), electrochemical oxidation/ozone oxidation (Hu et al., 2017). The high salinity of cellulosic ethanol wastewater further increased the expense in desalination by expensive reverse osmosis and electrodialysis/evaporative crystallization (Luiz et al., 2015). Obviously, the massive generation of such wastewater almost completely eliminated the feasibility of commercial cellulosic ethanol production.

As an alternative approach for regular wastewater treatment, recycling of the wastewater to the fermentation operation has been reported (Puengprasert et al., 2020; Wang et al., 2014, 2017b; Yang et al., 2016; Zhang et al., 2009). However, the harmful inhibitors, salts and metabolites in the wastewater are frequently lethal to cell growth and metabolism of fermentation strains. In this study, a new strategy was designed and verified by recycling the stillage wastewater to the pretreatment operation of dry biorefining process. In this dry biorefining process, the wastewater generation has already been cut to only 8.8 ton

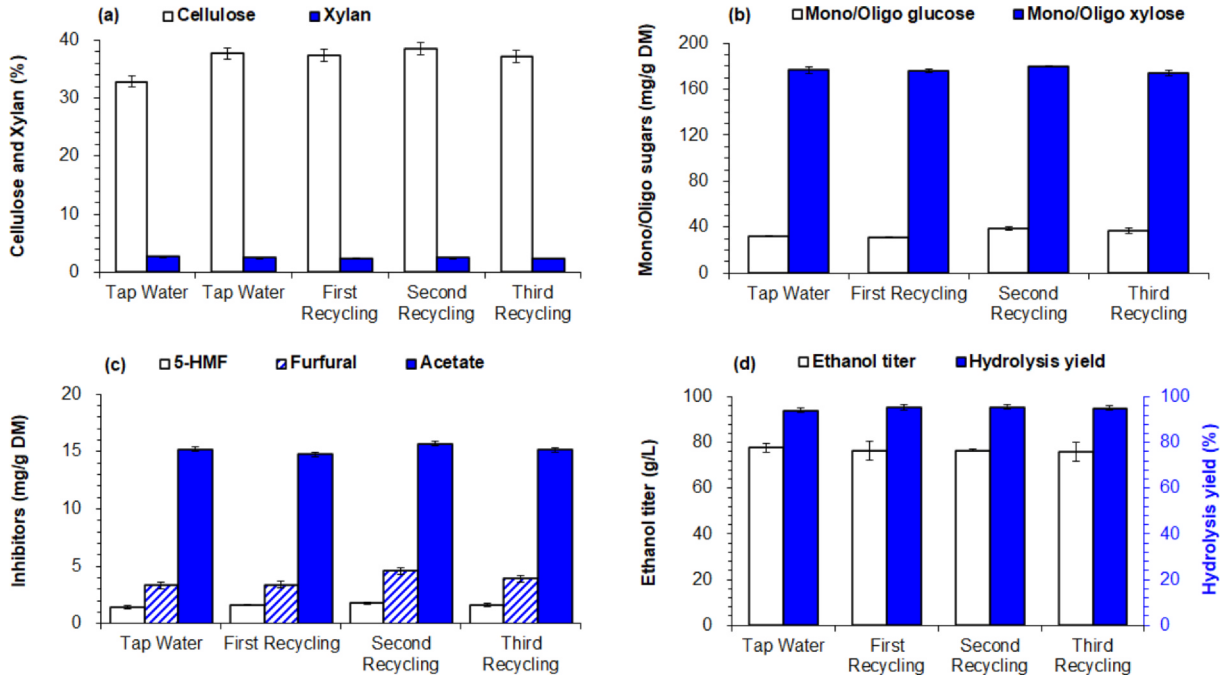

Fig. 3. Evaluation of recycling treated stillage liquid to pretreatment operation using de-ashed wheat straw. (a) Cellulose and xylan content. (b) Mono and oligo-glucose, mono and oligo-xylose content. (c) Inhibitors content. (d) Enzymatic hydrolysis yield and final ethanol titer in SSCF. Tap water, pretreatment using tap water; First recycling, pretreatment using the first recycled treated stillage liquid; Second recycling, pretreatment using the second recycled treated stillage liquid; Third recycling, pretreatment using the third recycled treated stillage liquid.

Table 1
Ions concentration during the recycling of the treated stillage liquid in dry biorefining process.

Water type	K^+ (mg/L)	Na^+	Mg^{2+}	Fe^{3+}	Mn^{2+}	Cl^-	PO_4^{3-}	NO_3^-
First recycled stillage liquid	3300	343	980	163	15	290	852	355
Second recycled stillage liquid	3400	337	980	168	14	305	858	374
Third recycled stillage liquid	3469	348	976	162	17	301	874	380
Fourth recycled stillage liquid	3389	342	978	164	16	298	861	369
Treated stillage liquid	1	2	1	0	0	2	0	1
Distilled water	1	5	1	0	0	7	7	2

per ton ethanol (close to corn ethanol wastewater generation at 8.3 ton per ton ethanol). No wastewater was generated in the dry acid pretreatment and biodetoxification operations, and the only wastewater outlet was the stillage stream of the first distillation column (beer column) (Liu and Bao, 2017a; Liu et al., 2018). In the direct recycling scenario, the organic matters (inhibitors and metabolites) in the wastewater were basically degraded at harsh pretreatment operation, and the degradation products were thoroughly converted to non-toxic compounds or ultimately to CO_2 and water in the subsequent biodetoxification step using unique biodetoxification microbes. The remaining problem in this scenario was that the accumulation of soluble salts in the wastewater gradually inhibited the fermenting microbes. Considering the surplus heat generation from lignin residue combustion, the evaporation of the wastewater was proposed in dry biorefining process. The condensed water was recycled to the pretreatment, which perfectly supported the cellulosic ethanol production process. The mass balance and energy balance were finally calculated to verify the feasibility of evaporation strategy. This study gives an important option for cellulosic ethanol wastewater treatment operation in biorefinery processing.

2. Materials and methods

2.1. Feedstock, enzymes, and reagents

Wheat straw was harvested from Jining, Shandong, China in the summer of 2018, then air-dried and milled using a hammer crusher to pass through the 10 mm (diameter) apertures. The raw wheat straw contained 35.3% of cellulose, 23.2% of hemicellulose, 16.4% of lignin, and 9.5% of ash based on weight percentage assayed according to NREL protocol (Sluiter et al., 2012). De-ash operation of the raw wheat straw was conducted according to He et al. (2014a); (2014b;).

Commercial cellulase Cellic CTec 2.0 was purchased from Novozymes (China), Beijing, China with the filter paper activity of 203.2 FPU/mL, cellobiase activity of 4,900 CBU/mL and protein content of 87.3 mg/mL assayed in our previous study (Han et al., 2018; 2019a; 2019b). Yeast extract was purchased from Oxoid Co., Basingstoke, UK. Other analytical grade chemicals were purchased from Lingfeng Chemical Reagent Co., China.

2.2. Strains and media

The biodetoxification strain *Amorphotheca resinae* ZN1 was isolated in our previous study (Zhang et al., 2010) and stored in China General Microorganisms Collection Center (CGMCC), Beijing, China as CGMCC

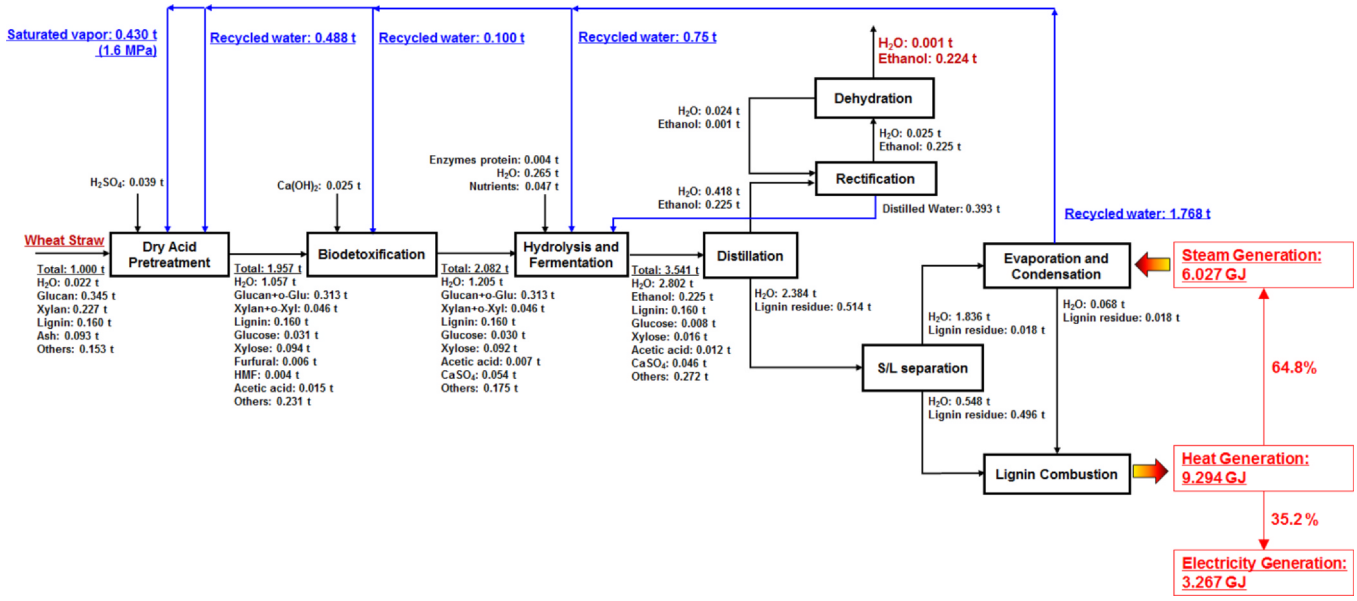

Fig. 4. Mass balance and energy balance of dry biorefining chain. The stillage liquid was evaporated to generate saturated vapor and condensed water. The saturated vapor was used for dry acid pretreatment, and the condensed water was recycled to dry acid pretreatment unit, biodetoxification unit, and enzymatic hydrolysis and fermentation unit, to form an enclosed circulation of cellulosic ethanol wastewater in dry biorefining chain. Lignin residue was used as the combustion fuel for evaporation, and the boiler efficiency here was calculated as 80%.

7452. *A. resinae* ZN1 was maintained on potato-dextrose-agar (PDA) slant prepared by boiling 200 g of potatoes in 1 L of deionized water for 30 min with the addition of 20 g of glucose and 15 g of agar.

The ethanol fermenting strain *Saccharomyces cerevisiae* XH7 was cultured in YPD medium used for seeds culture contained 20 g/L of glucose, 20 g/L of peptone, and 10 g/L of yeast extract (Li et al., 2016).

2.3. Dry biorefining procedure

Wheat straw was pretreated using dry acid pretreatment method (Han and Bao, 2018; He et al., 2014a; Liu et al., 2018). Briefly, wheat straw and sulfuric acid solution at the solid/liquid ratio of 2:1 (w/w) were together fed into the 20 L reactor and maintained at 175 °C for 5 min under mild agitation. The solids content after pretreatment was around 45% (w/w, dry base) and no wastewater was generated. The hydrolysis efficiency of the pretreated wheat straw was assayed according to the NREL LAP-009 protocol (Brown and Torget, 1996). Briefly, 2.5% (w/w) of the pretreated feedstock solids was hydrolyzed using 26 mg cellulase protein/g cellulose (20 FPU/g DM) at pH 4.8, 50 °C for 72 h.

The pretreated wheat straw was biodetoxified to remove the inhibitors (Zhang et al., 2010). Briefly, the pretreated feedstock was neutralized using 20% (w/w) $Ca(OH)_2$ to pH 5–6 and then disk milled. *A. resinae* ZN1 was inoculated at 10% (w/w) of the solid feedstock and maintained in a 15 L bioreactor for 48–72 h at 28 °C and 0.8 vvm of aeration. No wastewater steams were generated from the biodetoxification operation.

Simultaneous saccharification and co-fermentation (SSCF) was carried out using the pretreated and biodetoxified wheat straw in a 5 L helical agitated bioreactor (Han and Bao, 2018; Liu et al., 2018). After 12 h prehydrolysis at 50 °C, the xylose-utilizing strain *S. cerevisiae* XH7 was inoculated into the slurry at 10% (v/v) ratio after a short adaption, and the SSCF was performed at 30 °C, 200 rpm, pH 5.5 for 72 h. The short adaption before inoculation was carried out using the pretreated corn stover as the carbon source (Liu et al., 2018).

All the experiments in biorefining process were performed in duplicate, and the data shown here was the average of two parallel experiments.

2.4. Wastewater recycling operation

The fermentation broth was distillated in a glass distillation column (inner diameter of 40 mm) filling with stainless theta ring packings (3 mm in diameter). The distillate contained ~ 35% ethanol (w/w) and the stillage was filtrated by a press filter with a fabric cloth to obtain the wastewater and the high lignin containing solids residue (Liu and Bao, 2017b). In Strategy One, the wastewater was directly recycled to pretreatment operation to replace tap water. In Strategy Two, the wastewater was evaporated to obtain the condensed water using the glass distillation column and this treated wastewater was subsequently recycled to pretreatment operation.

2.5. Analysis of sugars, ethanol, inhibitors and ions

Cellulose and hemicellulose contents were assayed using a two-step sulfuric acid hydrolysis method (Sluiter et al., 2012). Oligomers of glucan and xylan were measured using a one-step sulfuric acid hydrolysis method (Sluiter et al., 2008). Glucose, xylose, ethanol, acetic acid, furfural and 5-hydroxymethylfurfural (HMF) were assayed by HPLC (LC-20AD pump, RID-10A detector, Shimadzu, Japan) with Bio-Rad Aminex HPX-87H column (Bio-Rad, Hercules, USA) at 65 °C and 0.6 mL/min of 5 mM H_2SO_4 solution. The ions contents were measured by Inductively Coupled Plasma Atomic Emission Spectrometry and Ion Chromatography according to Han and Bao (2018).

2.6. Analysis of higher heating value and chemical oxygen demand

Lignin residue cake with the thickness of around 4 mm and moisture of 46% (w/w) was dried at 105 °C until constant weight. The higher heating value (HHV) of lignin residue was analyzed by a calorimeter (MMC 274 multi-module, Netzsch Co., Germany) according to the China National Standard Method GB/T213-2003 (Determination of Calorific Value of Coal).

Chemical oxygen demand (COD) of the wastewater was analyzed according to the China National Environmental Protection Standard Method HJ/T399-2007 (Water Quality Series, Determination of COD). Briefly, the oxidation was performed in a digester (Hach DRB200, Hach Inc, USA) at 165 °C for 15 min, and the absorbance was measured at

600 nm by a spectrophotometer (Hach DR5000, Hach Inc, USA). Potassium dichromate was used as oxidizing agent and potassium biphthalate was used as control.

3. Results and discussion

3.1. Direct recycling of stillage liquid to pretreatment operation

The only wastewater stream generated from the dry biorefining process is the stillage stream of the first distillation column (beer column) (Liu et al., 2018). The stillage stream is filtrated in the consequent solid–liquid separation operation into one solid stream (lignin residue as combustion fuel) and one liquid stream, stillage liquid, as the wastewater outlet of cellulosic ethanol production. The stillage liquid is approximately 8–9 metric tons per ton of fuel ethanol production by dry biorefining process, which is already close to the wastewater generation of advanced corn ethanol production technology (Liu and Bao, 2017a; 2017b). The purpose of this study is to find a practical way to completely recycle the stillage liquid to reduce the burden of wastewater treatment.

As the first trial of recycling, the stillage liquid was directly recycled to pretreatment operation as the solvent of sulfuric acid catalyst solution preparation, followed by the complete dry biorefining steps including biodetoxification, simultaneous saccharification and co-fermentation for ethanol production, distillation, and solid–liquid separation. After two recycling cycles, the properties of pretreated wheat straw using the recycled stillage liquid and tap water were very close in the contents of cellulose, residual xylan, mono- and oligo-sugars, and acetic acid, while the contents of furfural and HMF were slightly increased (Fig. 1). The high COD concentration (84,600 and 97,800 mg/L) in the two rounds of stillage liquid recycling did not lead to the strong negative impact on pretreatment efficiency, partially because the dominant organic matters in the wastewater were soluble lignin derivatives and they were easily condensed during pretreatment operation at high temperature and low pH (Takada et al., 2019). The slightly higher inhibitors were efficiently converted to CO_2 and non-toxic compounds by the unique biodetoxification method using *A. resinae* ZN1. The enzymatic hydrolysis yield slightly declined from 92.4%, 89.0% to 88.1%, and the ethanol concentration also gradually reduced from 65.3, 60.3 to 57.8 g/L using tap water and the two rounds of stillage liquid in pretreatment operation (Fig. 1**d**).

Fig. 2 shows that the inorganic compounds (salts) accumulated during the direct recycling of stillage liquid, although partial soluble ions were adsorbed onto lignin residues. The accumulating salts concentration on ethanol fermentability was tested and the results confirmed that high salts concentration decreased the ethanol yield, which was in agreement with the previous studies on cellulase activity (Yu and Chen, 2010) and yeast viability (Casey et al., 2013; Zaky et al., 2018). Since the accumulation of salts is essentially inevitable in the direct recycling of wastewater, cellulosic ethanol production is less possible to maintain the similar efficiency after several rounds of wastewater recycling. A more effective approach of wastewater recycling is needed to stabilize the fermentation efficiency.

3.2. An alternative approach of wastewater recycling by evaporation operation

To stop the ions accumulation tendency in wastewater recycling, a de-ash step was tried to remove partial ash of the raw wheat straw feedstock before pretreatment. The ethanol production was consequently increased due to the increased cellulose and xylose content in the saccharification and fermentation step (Fig. 3). However, the metal ions and anions were still accumulating with the wastewater recycling (Table 1) and the de-ash operation only had a limited effect at the initial recycling rounds.

Considering the very high COD and salinity in the stillage wastewater, the regular treatment is complex and expensive on the removals of soluble organic compounds, ammonium nitrogen and inorganic salts (Hu et al., 2017; Luiz et al., 2015; Tian et al., 2013). Evaporation of the stillage liquid seems to be an ultimate solution for eliminating the ions accumulation from cellulosic ethanol production. Generally, the complete evaporation of wastewater is not an optimal solution because of the high heating energy requirement. However, biorefinery process might be an exception because the large amount of high heating-value lignin residue is obtained after the whole biorefining process to be used as combustion fuel for evaporation (Aden et al., 2002). Here we tested the evaporation concept by recycling the condensed water of evaporated stillage liquid to the pretreatment unit.

The results show that after three rounds of recycling, the pretreated wheat straw was essentially the same as the original pretreated one in chemical composition, enzymatic hydrolysis and ethanol fermentability (Fig. 3). The ions content in each batch of wastewater (Table 1) and solids residue were also unchanged. Almost all the ions were removed and the ions content of treated wastewater was even lower than that of distilled water (Table 1). The stable pretreatment efficiency as indicated by the enzymatic hydrolysis yield (94.1%, 95.4%, 95.5% and 95.1%) and the ethanol titer (77.2, 76.2, 76.5 and 76.8 g/L) suggests the success of an enclosed circulation of cellulosic ethanol wastewater by evaporation strategy.

3.3. Feasibility of wastewater evaporation strategy in a closed circulation of cellulosic ethanol production

To evaluate the feasibility of the wastewater evaporation strategy for recycling, the mass balance and the energy balance of the dry biorefining process were calculated (Fig. 4). The mass balance shows that one metric ton of wheat straw could be used to produce 0.225 ton of ethanol with the generation of 1.854 ton of wastewater and 0.514 ton of lignin residue. Approximately 65% of the lignin residue (heat value of 18.1 GJ/t) was used as combustion fuel to evaporate the stillage liquid to generate the saturate vapor (1.6 MPa) and the condensed water for pretreatment, detoxification and SSCF. The results suggest that the lignin residue is sufficient as combustion fuel to evaporate the wastewater without outside energy input in the present process, in which 65% of lignin is used for vaporization of stillage liquid and the rest 35% still goes to electricity generation. If it combines with direct recycling for several times, the energy consumption will be further reduced and more lignin residue is preserved for electricity generation. The evaporation strategy completely eliminated the wastewater generation and realized the closed-loop operation of biorefining chain. It could elevate the feasibility of cellulosic ethanol production by simplifying the wastewater treatment unit from cellulosic ethanol plant, which is primarily built in the rural area without advanced infrastructure and wastewater treatment system (Liu and Bao, 2019a; Zhang et al., 2016). Furthermore, the ash of lignin combustion could be used as phosphate and potash fertilizers for selling (Liu and Bao, 2019b).

However, this evaporation recycling strategy has its limitations in actual applications. When the wastewater generation of a specific biorefinery technology is 50% greater than that in the present dry biorefining technology, the residual 35% of lignin will be completely consumed without surplus for electricity generation. The further increase in wastewater generation will require considerable outside heating energy input for evaporation as in the cases of dilute acid pretreatment (Humbird et al., 2010; 2011), ammonia fiber explosion (AFEX) (Uppugundla et al., 2014; Kim and Dale, 2015), deacetylation and mechanical refining (DMR) (Chen et al., 2015; 2016), and steam explosion (SE) (Liu and Chen, 2016). In such a scenario, the convergence and enclosure of the biorefining process will be broken down. In other word, the present evaporation recycling strategy only works for the minimum wastewater generating processes such as our dry biorefining process (Liu et al., 2018; Liu and Bao, 2017a).

Several technical problems are still existed including (i) Salts in the

lignin residues may prevent the full combustion of lignin and lead to corrosion of reactors (Fang and Jia, 2012; Niu et al., 2016; Nunes et al., 2016); (ii) High COD in the wastewater may hinder the salts crystallization (Bian et al., 2020; Ge et al., 2019) and increase the burden of combustion (Liu and Bao, 2019b); (iii) Very large evaporator is required and the fouling in the evaporator might be a practical problem (Gurram and Menkhaus, 2013; Demirskyy et al., 2016); (iv) Volatile compounds may be present in the steam and a gas treatment device should be designed to absorb the volatile compounds as in the case of pulping industry (Chagger et al., 1998; Zhu et al., 1999). These problems should be carefully considered before the practical applications.

4. Conclusions

Direct recycling of the wastewater to pretreatment unit led to a high but gradually reduced ethanol concentration primarily due to the accumulation of salts. The wastewater could be treated by evaporation and subsequently recycled to the pretreatment unit, leading to a stable cellulosic ethanol concentration in biorefinery. The combustion of lignin residue could satisfy the requirement of evaporation of cellulosic ethanol wastewater generated from our dry biorefining technology, which successfully realized the closed-loop operation of the biorefinery plant without wastewater discharge. This study provided a reasonable and effective solution of wastewater treatment in cellulosic ethanol biorefinery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.124077.

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