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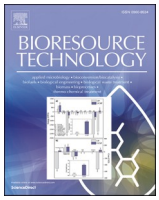


Short Communication

Recycling of waste calcium carbonate in lignocellulosic biorefining chain for chiral lactic acid production

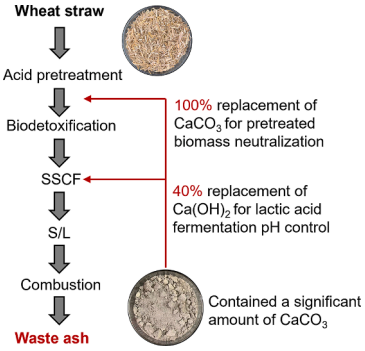
Bin Zhang [a], Qingmei Han [a], Qi Liu [a], Ya Wang [b], Jie Bao [a,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China
[b] School of Chemistry and Chemical Engineering, Shihezi University, Beisi Road, Shihezi, Xinjiang 800032, China

HIGHLIGHTS

- The waste combustion ash from dry biorefinery contained high amount of $CaCO_3$.
- The waste ash was efficiently recycled as neutralizer in biorefining chain.
- No negative effects when 100% of $CaCO_3$ and 40% of $Ca(OH)_2$ was replaced by ash.
- The cellulosic L-lactic acid titer reached 102.4 ± 3.6 g/L with waste ash recycling.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the major end-products of lignocellulosic biorefining chain is the solid residues containing various compounds. The present approach to solid residues treatment is combustion for generation of heat and electricity. This study investigated the potential for recycling of the combustion ash from the solid residues after lignocellulosic dry biorefining process. A range of characterizations showed that the combustion ash contained a high amount of calcium carbonate. By recycling the ash as the neutralizer in biorefining process, the waste calcium carbonate in the ash was efficiently utilized for pretreated biomass neutralization and can replace 40 % of calcium hydroxide for lactic acid production. The chiral L-lactic acid titer reached 102.4 ± 3.6 g/L from 20 % (w/w) solids loading of wheat straw. Three feasible strategies of ash recycling for the investigated biorefinery concept were further proposed base on the rigorous calcium mass calculation, which can efficiently reduce the consumption of neutralizers.

1. Introduction

Circular bioeconomy utilizes sustainable biomass as an integral component to generate bioproducts in a biorefinery (Ubando et al., 2020). Driven by the potential benefits including reduced emissions of greenhouse gases, decreased dependence on fossils for energy generation, maximal utilization of natural resources, low toxic generation and maximum security to food resources (Qin et al., 2021), the biorefinery of

* Corresponding author.
E-mail address: jbao@ecust.edu.cn (J. Bao).

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second-generation lignocellulosic feedstock gains more and more attention to promote circular bioeconomy.

Lignocellulosic biorefinery should maximize the utilization of biomass and minimize the wastes and emissions associated with the conversion of bioproducts (Velidandi et al., 2023). But most of the current lignocellulosic biorefineries encounter difficulties in realizing the concept of circular bioeconomy, where generate a large amount of wastewater and toxic compounds leading to the significant stagnation in sustainable operation and commercialization (Almuth & Rachel, 2018). The recently developed dry biorefinery technology has significantly reduced the freshwater input and wastewater emission by operating pretreatment at high solids loading (~70 %, w/w) and solid state biodetoxification. (Zhang et al., 2021). However, the efficient utilization of solid residues after the enzymatic hydrolysis, fermentation, and solid/liquid separation remains a major concern. Solid residues contain complicated components including lignin residues, insoluble salts (both organics and inorganics), cellulase enzymes, original plant proteins, fermentation microbial cell mass, metabolites, etc. The most feasible way of solid residues utilization is combustion to generate heat and electricity for biorefining process use and sell the surplus electricity to the grid (Humbird et al., 2011; Liu and Bao, 2017a). The combustion ash is therefore generated in large quantities.

The resource utilization of the combustion ash generated in biorefining process has not yet been sufficiently consolidated. In the report by National Renewable Energy Laboratory (NREL) (Humbird et al., 2011), the combustion ash was landfilled along with the calcium sulfate. Liu and Bao (2019) suggested that the combustion ash containing high amount of phosphorous and potassium elements should be recycled to farmland for reduction of chemical fertilizer usage. However, disposing of this ash directly into the environment can be extremely impactful. Owing to the presence of residual carbon and high silica content, it can generate harmful contaminants, such as soil acidification, accumulation of ash in river beds, as well as changes in soil and water characteristics (Moraes et al., 2014). Many studies explored the recycling potentials of fly ash generated from biomass combustion power plant for use as adsorbent, synthesis of zeolite, cements, building materials, etc. (Cabrera et al., 2015; Zacco et al., 2014). A novel application of fly ash had been proposed as a substitute for alkali in Waelze process (Huang et al., 2017), resulting in less environmental impact compared to the practices in landfill, cement, and brick. In dry biorefining process, the acid catalyst in pretreatment should be neutralized to the pH close to cellulase enzyme for hydrolysis. In the consequent fermentations, specifically in case of the target products are acidic compounds such as chiral lactic acid, these acids should also be neutralized to the pH to match the requirement of fermentation strain growth and metabolism. For choosing neutralizer used in biorefining process, calcium hydroxide or calcium carbonate is preferred because the calcium salts are generally insoluble or only slightly soluble in fermentation broth, thus the removal of these calcium salts in the form of insoluble solids is easily conducted without the negative effect of highly concentrated salts on the downstream wastewater treatment.

In this study, the combustion ash generated from lignocellulosic dry biorefining process of chiral lactic acid production was found to contain the high-level calcium carbonate with the potential as neutralizer on neutralizing the pretreatment acid catalyst, and controlling lactic acid fermentation pH. A significant amount of calcium carbonate or calcium hydroxide could be saved by recycling the combustion ash as neutralizer. This study complements a new approach to waste treatments based on the concept of circular economy for lignocellulosic biorefinery.

2. Materials and methods

2.1. Feedstock

Raw wheat straw was harvested from Nanyang city, Henan province, China, in spring 2021. The main compositions of raw wheat straw are 34.3 % (w/w) of cellulose, 19.3 % (w/w) of xylan, 22.1 % (w/w) of lignin, and 8.4 % (w/w) of ash based on dry matter, which was determined by two-step acid hydrolysis method (Sluiter et al., 2012).

2.2. Strains and medium

The biodetoxification strain was *Peacilomyces variotii* FN89 (CGMCC 17665) was cultured and preserved on PDA plate (Zhang et al., 2021). The chiral L-lactic acid producing strain was engineered *Pediococcus acidilactici* ZY271 (CGMCC 13611) and cultured in the simplified MRS medium (He et al., 2022).

2.3. Lignocellulosic dry biorefining process

Wheat straw was pretreated at ~ 70 % (w/w) of solids loading, 175 ℃ for 5 min with the oxalic acid dosage of 4.0 % (w/w) in a 20 L reactor equipped with a helical impeller (Zhang et al., 2021). The saturated water steam (1.6 MPa, 201 °C) was produced from a steam generator machine (HX-36D, Huazheng Boiler Co., Shanghai, China). The pretreated wheat straw was neutralized to ~ 5.0 according to the Base pH Approaching method (Han & Bao, 2018). The solid state biodetoxification of pretreated wheat straw was conducted at 1 vvm for 36 h in a 15 L bioreactor (Baoxing Biotech Co., Shanghai, China) (Zhang et al., 2021). The simultaneous saccharification and co-fermentation (SSCF) method was adopted for cellulosic chiral L-lactic acid production at 20 % (w/w) solids loading (He et al., 2022).

2.4. Ash preparation

The fermentation broth was centrifugated at 8,000 rpm for 10 min to collect the solid residues. The solid residues were washed at least three times at ~ 10 % (w/w) solids loading. The washed solid residues were air dried until the moisture content below 10 % (w/w). The solid residues were pulverized and combusted in muffle furnace (SX2-2.5–10, Boxun Industrial Co., LTD, Shanghai, China). The preheated was conducted at 75 ℃ for 30 min, and then the temperature was increased to 575 ℃ and maintained for at least 4 h.

2.5. Characterizations of the ash

The surface morphology of ash was characterized using scanning electron microscope (SEM, S-3400 N, Hitachi Co., Japan) equipped with energy dispersive X-ray spectrometer (EDS, 133 eV/Falion 60S, EDAX Inc., USA). The ash mixed with dried KBr powder was analyzed by Fourier transform infrared spectrometry (FTIR, Nicolet 6700, Nicolet Co., USA). The mineral phase compositions of ash were analyzed using X-ray diffraction (XRD, Dmax 2550VB, Rigaku Co., Japan). The acid neutralization capacity of the ash was characterized by titration using 0.1 M of standard hydrochloric acid solution in the magnetically stirred suspension containing 1 g/L (dry weight) of ash.

2.6. Analytical methods

The calcium content was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, 725 ICP-OES, Agilent Co., USA) (Han & Bao, 2018). Sugars, inhibitors, and lactic acid were determined by Shimadzu HPLC system equipped with Bio-rad Aminex HPX-87H column and RID-10A detector (He et al., 2022).

3. Results and discussion

3.1. Combustion ash generation in general dry biorefining process for lactic acid production

The process flowsheet of overall dry biorefining was shown in Fig. S1 (see supplementary material), and the mass balance was calculated

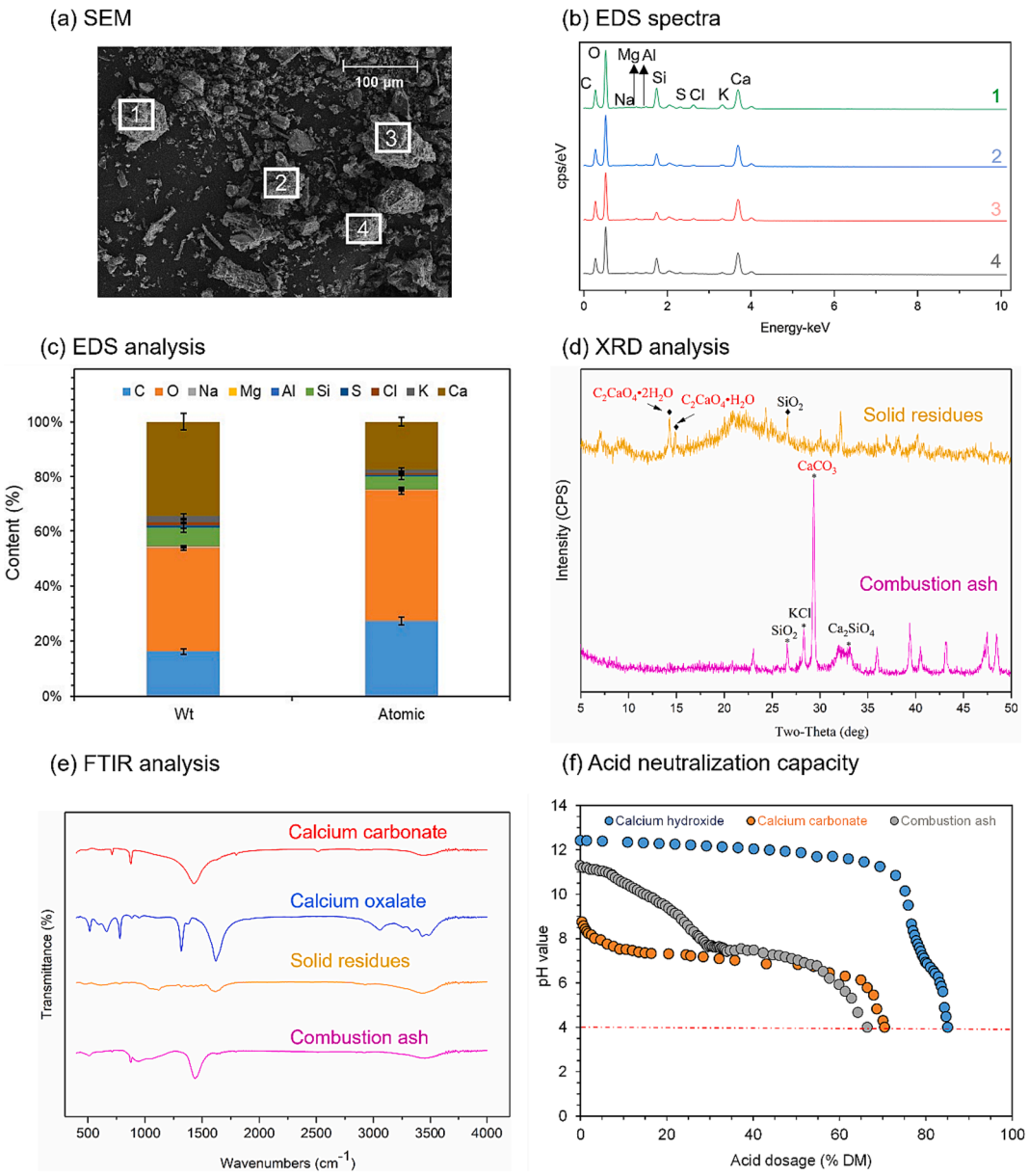

Fig. 1. Characterizations of combustion ash after dry biorefinery processing. (a) SEM; (b) EDS spectra; (c) EDS analysis. The regions marked by numbers in SEM photo were analyzed by EDS. The EDS was conducted by line scanning and analyzed according to k linear energy, Wt (%) indicates the elemental mass percentage. Atomic (%) indicates the atomic number percentage. (d) XRD analysis; (e) FTIR analysis; (f) Acid neutralization capacity. The acid neutralization capacity of ash, $CaCO_3$ or $Ca(OH)_2$ was defined as the dosage of 0.1 M standard HCl solution required to neutralize 1 g/L suspension of ash, $CaCO_3$ or $Ca(OH)_2$ to pH 4.0.

starting from 1,000 kg (dry weight) of wheat straw. The ash in raw wheat straw was derived from the dust and the plant structural ash (Han & Bao, 2018), which is different from the final combustion ash. The acid pretreated wheat straw was neutralized by adding calcium carbonate. Biodetoxification in this study refers to a complete and ultimate biodegradation of the inhibitors accumulated in the pretreated wheat straw feedstock by a unique microorganism *P. variotii* FN89. The process was conducted in the form of solid state fermentation, and the major inhibitors including furfural, 5-hydroxymethylfurfural (HMF), and acetic acid were removed. (Zhang et al., 2021). The biodetoxified biomass was used for high-purity chiral L-lactic acid production by SSCF using 20 % (w/w) calcium hydroxide slurry as neutralizer (Fig. 1c). After the operation of solid/liquid separation of the fermentation broth, the solid residues were collected and combusted for combined heat and power generation (Liu and Bao, 2017b). Approximately 97.0 kg of combustion ash was generated for processing one ton of raw wheat straw.

3.2. Characterization of the ash from solid residues combustion

The ash from the combustion of solid residues in cellulosic lactic acid biorefining process was characterized with a view to exploiting its potential for recovery of resources. The results of SEM/EDS showed that the ash contained 37.7 ± 0.8 % (w/w) oxygen, 34.4 ± 3.0 % (w/w) calcium, 16.2 ± 1.0 % (w/w) carbon, 6.4 ± 1.5 % (w/w) silicon, and other elements (Fig. 1a-1c). Due to the de-ashing operation in the pre-handling step before pretreatment (He et al., 2014), the silicon content in the ash was relatively lower than in the ash from the combustion of raw biomass or municipal solid waste (Tharajak & Sanpo, 2019). On the other hand, the combustion ash produced from cellulosic lactic acid biorefining process contained more calcium. The insoluble calcium salt precipitates generated by adding the calcium-based neutralizers in biorefining process were mixed with the solid residues and ultimately enriched in the ash after combustion, leading to the elevated content of

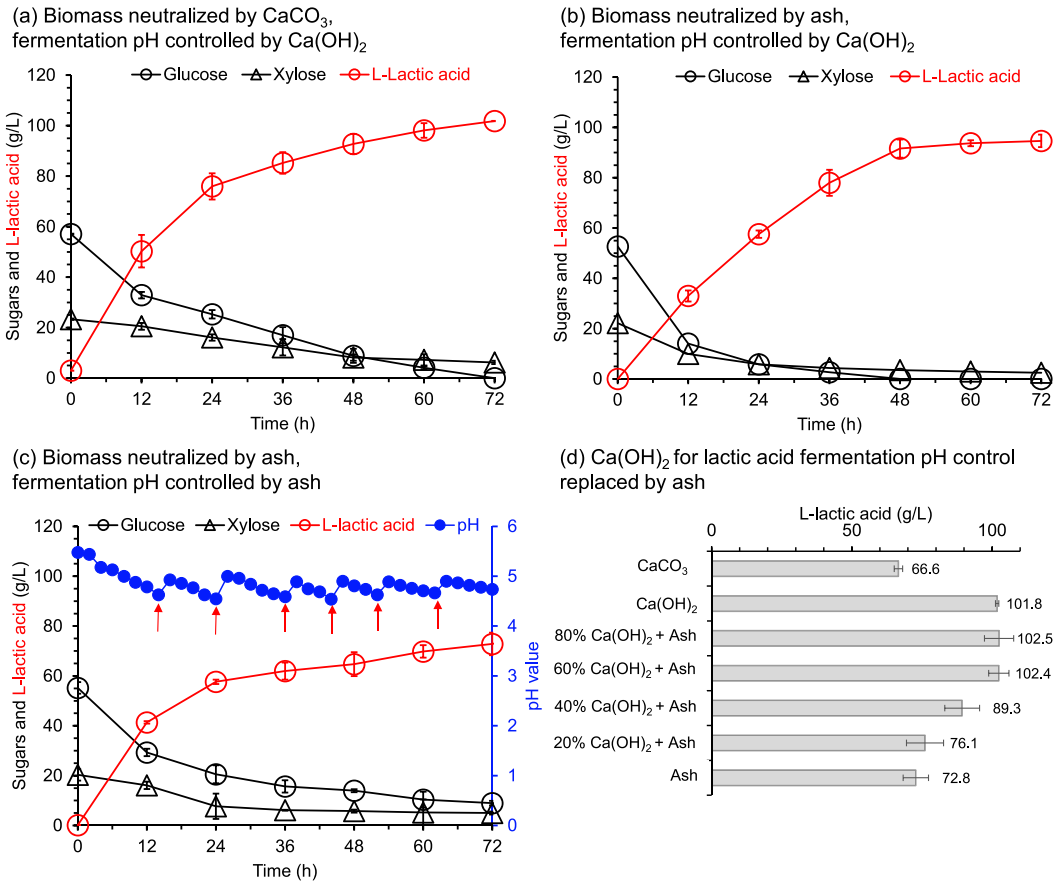

Fig. 2. L-lactic acid fermentation from acid pretreated and biodetoxified wheat straw. (a) Neutralized by $CaCO_3$ and pH controlled by $Ca(OH)_2$. (b) Neutralized by the combustion ash and pH controlled by $Ca(OH)_2$. (c) Neutralized by combustion ash and pH controlled by combustion ash. Red arrows in (c), combustion ash was added periodically. (d) Cellulosic L-lactic acid fermentation with pH controlled by $CaCO_3$, combustion ash, $Ca(OH)_2$, or combined combustion ash and $Ca(OH)_2$. The acid pretreated wheat straw was neutralized by the combustion ash. The fermentation pH was controlled by the one-time addition of 60 g/L $CaCO_3$, batch addition of the combustion ash, or automatic addition of the slurry of $Ca(OH)_2$ and the combustion ash. Fermentation conditions: 20 % (w/w) solids loading, 4 mg protein/g DM, 42 ℃, 200 rpm.

calcium in the ash.

The solid residues and combustion ash were further analyzed by XRD and FTIR (Fig. 1d and 1e). The result of XRD showed that the solid residues contained calcium oxalate component, which was derived from the neutralization of pretreatment catalyst of oxalic acid (Fig. 1d). The combustion ash contained a significant amount of calcium carbonate component, while the components of potassium chloride and calcium silicate were also identified, the component of calcium oxalate was not detected. The FTIR spectrum of combustion ash showed the bonds in 1427 cm^{-1} and 875 cm^{-1}, which were consistent with standard calcium carbonate (Fig. 1e) (Pinzari et al., 2010). The standard calcium oxalate showed the peaks in 516 cm^{-1}, 781 cm^{-1}, 1317 cm^{-1}, 1619 cm^{-1} (Pinzari et al., 2010). The FTIR spectrum of calcium oxalate was not obvious owing to its low content in solid residues. The aromatic ring of the lignin in 1600 cm^{-1} may have been overlapped in the underlying FTIR absorbance of calcium oxalate (Bui et al., 2015). The integrated results of XRD and FTIR indicated that there was a transformation of insoluble calcium oxalate in solid residues to calcium carbonate in the ash during the combustion.

The acid neutralization capacity of the ash was characterized and compared with that of the pure calcium carbonate or calcium hydroxide. The well mixed suspension at the concentration of 1 g/L (dry weight) of insoluble ash, calcium carbonate, or calcium hydroxide was titrated using 0.1 M of the standard hydrochloric acid solution, respectively (Fig. 1f). The initial pH of the suspension of calcium carbonate and calcium hydroxide were 8.5 and 12.5, while the pH of ash suspension was 11.5 due to the presence of trace amounts of soluble alkaline components, such as sodium hydroxide, basic magnesium chloride, and basic calcium chloride in the ash (Wang et al., 2021). For the titration of the three suspensions to pH value of 4.0, the acid dosage was 66.4 %, 70.4 %, and 85.0 % for the suspensions of ash, calcium carbonate, and calcium hydroxide, respectively. The combustion ash had a similar acid neutralization capacity compared to calcium carbonate.

3.3. *Recycle combustion ash as neutralizer in biorefining process*

The combustion ash has the potential to be used as a neutralizer equivalent to calcium carbonate in biorefining process (Fig. 1). The oxalic acid pretreated wheat straw was neutralized to the pH value of ~ 4.5 by adding the ash, then biodetoxified and used for L-lactic acid fermentation. The L-lactic acid titer reached 94.6 ± 2.5 g/L at 72 h, which is similar to that using standard calcium carbonate as neutralizer (101.8 ± 0.1 g/L at 72 h) (Fig. 2ab).

The combustion ash was further used for pH control during cellulosic lactic acid fermentation (Fig. 2c). However, the one-time addition of the ash before the fermentation led to a dramatic increase in the pH value of the hydrolysate (above 10.0) owing to the presence of trace amounts of soluble alkaline components (Fig. 1f). Therefore, the batch addition was used to neutralize the produced lactic acid during the fermentation. Briefly, 1 % (w/v) of the ash was added when the pH value of the hydrolysate decreased to the suboptimal pH (4.5) for lactic acid fermentation (Fig. 2c, red arrows). The pH values of the whole fermentation

process were controlled between 4.5 and 5.5, and the final L-lactic acid titer reached 72.8 ± 4.5 g/L at 72 h (Fig. 2c).

The cellulosic lactic acid titer using the combustion ash as neutralizer was similar to that using calcium carbonate, but lower than that using 20 % (w/w) calcium hydroxide slurry (Fig. 2d). The ash was then used to replace calcium hydroxide in gradients for neutralization in lactic acid fermentation (Fig. 2d). The results showed there was almost no negative effect on fermentation efficiency when 20 % (w/w) or 40 % (w/w) of calcium hydroxide was replaced by the ash. The fermentation efficiency decreased with the further increasing proportions of the ash in slurry. In short, the combustion ash can replace 100 % of the calcium carbonate for neutralizing the acid pretreated biomass, as well as up to 40 % (w/w) of calcium hydroxide for controlling the fermentation pH value with no negative effects on cellulosic lactic acid fermentation efficiency in lignocellulosic dry biorefining process.

3.4. Calcium mass balance

The calcium contents in raw wheat straw, solid residues, and ash were practically measured, and the mass balance of dry biorefining process based on calcium was conducted from 1,000 kg of dry wheat straw (Fig. S2) (see supplementary material). The pretreated wheat straw was neutralized by adding 44.5 kg of calcium carbonate. During L-lactic acid production, 20 % (w/w) of calcium hydroxide slurry was automatically added to control the fermentation pH at 5.5, and a total of 178.6 kg of calcium hydroxide was used.

According to the experimental results, the practical neutralization capacity of 1 g of the ash was equal to 0.83 g of calcium carbonate or 0.37 g of calcium hydroxide. Three feasible strategies for ash recycling in biorefining process were therefore proposed based on the rigorous calcium mass calculations. In scenario 1, one batch produced ash was recycled to replace 100 % of calcium carbonate for pretreated biomass neutralization and 9.2 % of calcium hydroxide for lactic acid fermentation. In scenario 2, one batch produced ash was recycled to replace 20.4 % of calcium hydroxide for lactic acid fermentation only. In scenario 3, two and a half batches produced ash was recycled to replace 100 % of calcium carbonate and up to 40 % of calcium hydroxide used in fermentation according to the experimental results (Fig. 2d). Compared to the biorefining process without ash recycling, these proposed strategies can efficiently reduce the consumption of neutralizers and the emissions of waste solids.

4. Conclusion

The waste combustion ash generated from lignocellulosic dry biorefinery was fully characterized and recycled as a neutralizer for acid pretreated wheat straw and lactic acid fermentation in biorefining process. No adverse effects were observed when 100 % of calcium carbonate and 40 % of calcium hydroxide was replace by the ash. The feasible strategies for ash recycling were proposed based on the rigorous calcium mass balance. The further investigation would focus on assessing the impact of ash recycling on economic and ecological aspects in the overall biorefining process.

CRediT authorship contribution statement

Bin Zhang: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization. **Qingmei Han:** Writing – original draft, Methodology, Investigation. **Qi Liu:** Writing – original draft, Methodology, Investigation. **Ya Wang:** Writing – original draft, Methodology, Investigation. **Jie Bao:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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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.2024.130303.

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