Bioresource Technology Reports 30 (2025) 102152

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Lactic acid fermentation of sugars produced by fast pyrolysis of cellulose and effects of by-products on fermentation

Yasuko Maruichi, Takashi Nomura, Eiji Minami, Haruo Kawamoto *

Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Keywords:
Cellulose saccharification
Fast pyrolysis
Levoglucosan
Lactic acid fermentation
Fermentation inhibitors
Glycolaldehyde

ABSTRACT

Saccharification of cellulose is a crucial step in the conversion of biomass into bio-based chemicals and fuels. Among various saccharification methods, fast pyrolysis of cellulose was conducted, resulting in the production of bio-oil containing levoglucosan as the major component, along with various by-products such as glycolaldehyde, glyoxal, methylglyoxal, furfural, 5-hydroxymethylfurfural, acetic acid, formic acid, and formaldehyde. The levoglucosan in the bio-oil was hydrolyzed into glucose and used as a sugar solution for lactic acid fermentation. However, the presence and concentrations of these by-products significantly affected the fermentation process. The inhibitory effects of the by-products were ranked in the order of aldehydes > organic acids > furans, as these compounds interfere with the microbial activity necessary for efficient lactic acid production. To address this issue, an ion-exchange resin containing amino groups was utilized to purify the sugar solution by selectively removing the aldehyde-containing compounds, which comprise the majority of inhibitory substances. This purification enabled successful lactic acid fermentation, even at sugar concentrations that were previously inhibitory. These findings highlight the potential of bio-oil derived from cellulose pyrolysis as a sustainable feedstock for bioprocessing, provided that effective purification strategies are implemented.

1. Introduction

To address the challenges of global warming and fossil resource depletion, it is essential to replace fossil fuels with renewable resources. Cellulose, a major component of wood and other lignocellulosic biomass, can be converted to glucose, which can then be fermented to produce ethanol, lactic acid, acetic acid, and other valuable chemicals. These chemicals are used in the wide range of applications, including pharmaceuticals, surfactants, and bioplastics. Among bioplastics, polylactic acid (PLA) is the most widely produced due to its environmentally friendly properties and compostability. In 2023, the global production capacity of PLA was approximately 0.7 million tons (European bioplastics, 2023). PLA is synthesized from lactic acid, which is typically derived from edible resources such as starch (e.g., corn) and sugar (e.g., sugarcane). To produce 1 kg of PLA, approximately 1.28 kg of corn is required (Benavides et al., 2020). However, as the demand for bioplastics continues to grow, the corresponding need for raw materials is also increasing. Meeting this demand solely through edible sugar sources will become increasingly difficult due to competition with food production (Colwill et al., 2012). Therefore, utilizing cellulosic biomass as a non-edible and sustainable alternative is a promising strategy to overcome these limitations.

For the production of lactic acid from cellulose, saccharification is a key step. Following saccharification, lactic acid and other chemicals can be produced via fermentation, as in conventional methods. The main approaches to cellulose saccharification are acid hydrolysis and enzymatic saccharification. Acid hydrolysis is relatively simple but requires harsh conditions, such as the use of concentrated sulfuric acid, which leads to the issues such as waste acid generation and equipment corrosion (Rinaldi and Schuth, 2009). In contrast, enzymatic saccharification operates under mild conditions but necessitates chemical or physical pretreatment and has a long reaction time (Canilha et al., 2012). Moreover, achieving high-concentration sugar solutions remains a major challenge for both methods. Consequently, the industrial-scale application of cellulose saccharification remains limited, highlighting the need for more efficient and practical saccharification technologies.

To address this issue, we focused on a pyrolysis-based approach as an alternative method for saccharification. Pyrolysis is a thermochemical

Abbreviations: LG, levoglucosan; AGF, 1,6-anhydro-β-D-glucofuranose; GA, glycolaldehyde; GO, glyoxal; MeGO, methylglyoxal; AcOH, acetic acid; FcOH, formic acid; FF, furfural; 5-HMF, 5-hydroxymethylfurfural; Falde, formaldehyde.

* Corresponding author.
E-mail address: Kawamoto.haruo.2m@kyoto-u.ac.jp (H. Kawamoto).

https://doi.org/10.1016/j.biteb.2025.102152
Received 30 March 2025; Received in revised form 1 May 2025; Accepted 13 May 2025
Available online 18 May 2025

process carried out in the absence of oxygen. One advantage of pyrolysis-based saccharification is its ability to produce highly concentrated sugar solutions, as the process takes place under dry conditions. During pyrolysis, cellulose is decomposed into char, gases, and a liquid product known as bio-oil (Jahirul et al., 2012). The primary component of bio-oil derived from cellulose pyrolysis is levoglucosan (LG, 1,6-anhydro-β-D-glucopyranose) (Shafizadeh et al., 1979), which has attracted significant attention due to its potential to be converted into fermentable glucose and other high-value chemicals (Junior et al., 2020). Fast pyrolysis, characterized by rapid heating rates, is widely recognized for enhancing the yields of both bio-oil and LG (Liu et al., 2014). However, pyrolysis also generates low-molecular-weight by-products, such as glycolaldehyde, furfural, 5-hydroxymethylfurfural, and acetic acid, which can negatively affect fermentation process and the production of lactic acid and other chemicals (Shen et al., 2015).

Numerous studies have investigated the ethanol fermentability of sugar solutions obtained from the pyrolysis of lignocellulosic biomass (Bennett et al., 2009; Islam et al., 2015, 2018; Lian et al., 2010; Wang et al., 2012). Wang et al. (2012) investigated ethanol fermentation using sugar solutions derived from the fast pyrolysis of loblolly pine particles. They found that when more than 10 % of bio-oil hydrolysate was added to YPD medium (1 % yeast extract, 2 % peptone and 2 % glucose), the by-products present in the bio-oil completely inhibited ethanol fermentation. Lian et al. (2010) studied the effects of by-products on ethanol fermentation and reported that carboxylic acids and phenols exhibited the highest toxicity, while furans and alkanes were mildly toxic. They also demonstrated that ethanol fermentation could proceed successfully after detoxification, achieving 94.6 % of the theoretical yield. Therefore, a detoxification process is essential for enabling ethanol fermentation using sugar solutions derived from bio-oil. Various detoxification methods have been proposed, including solvent extraction, adsorption using absorbents such as activated carbon and ion-exchange resins, and neutralization (Canilha et al., 2012; Islam et al., 2015). For ethanol fermentation, the inhibitory effects of bio-oil-derived by-products have been well characterized, and it has been confirmed that fermentation is feasible following detoxification.

However, there are few reports on the lactic acid fermentability of sugar solutions obtained through the pyrolysis of lignocellulosic biomass. In the case of lactic acid fermentation of sugar solution obtained by enzymatic saccharification of lignocellulose, by-products such as furans, organic acids, and phenols generated during pre-treatment are known to inhibit the fermentation process (Abdel-Rahman et al., 2021; Abdel-Rahman and Sonomoto, 2016; Ajala et al., 2020; Cubas-Cano et al., 2018; van der Pol et al., 2014; Yankov, 2022). Similar inhibitory effects may occur with sugar solutions derived from pyrolysis, but it remains unclear which specific by-products in bio-oil inhibit lactic acid fermentation and to what extent they affect the process.

In this study, the lactic acid fermentability of sugar solutions derived from the fast pyrolysis of cellulose was investigated. Bio-oil containing levoglucosan and various by-products was produced through a fast pyrolysis of cellulose. The by-products present in the bio-oil were identified using ^{1}H NMR spectroscopy. Levoglucosan in the bio-oil was hydrolyzed into glucose, which was subsequently utilized for lactic acid fermentation. The inhibitory effects of several by-products in the bio-oil, including glycolaldehyde, glyoxal, methylglyoxal, furfural, 5-hydroxymethylfurfural, acetic acid and formic acid, on lactic acid fermentation were also investigated. Furthermore, the pyrolysis products were purified using an ion-exchange resin containing amino groups, and the purified sugar solutions were successfully applied to lactic acid fermentation.

2. Material and methods

2.1. Materials

Cellulose powder (Avicel PH-101, Asahi Kasei, Co., Ltd., Tokyo, Japan) was used for pyrolysis. The solid acid catalyst Amberlyst 15JWET was obtained from Organo Co. (Tokyo, Japan). Methanol, yeast extract, NaCl, K_2HPO_4, glucose, dimethyl sulfoxide-d_6 (DMSO-d_6), maleic acid, hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), $CaCO_3$, glycolaldehyde, glyoxal, methylglyoxal, furfural, 5-hydroxymethylfurfural, acetic acid and formic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Hypolypepton, and hypolypepton S were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). All reagents and materials were used without further purification.

2.2. Fast pyrolysis of cellulose

Fast pyrolysis was conducted using an IR image furnace (RHL-E45N, ADVANCE RIKO, Kanagawa, Japan) flowing the method of Nomura et al. (2021). A quartz tube (internal diameter: 30 mm, length: 495 mm) was placed inside a cylindrical furnace. A quartz boat (length: 35 mm, width: 10 mm, height: 6 mm, AS ONE Corporation, Osaka, Japan) containing 300 mg of cellulose, or a ceramic boat (length: 88 mm, width: 13 mm, height: 10 mm, AS ONE Corporation, Osaka, Japan) containing 930 mg of cellulose, was placed on a stainless steel mesh positioned at the center of the reactor. A 10 L Tedlar® sampling bag containing 30 mL of methanol was attached to the outlet of the reactor tube to collect volatile products. Prior to pyrolysis, the air inside the reactor system was purged with nitrogen. The fast pyrolysis conditions included a nitrogen flow rate of 5 L/min, an IR output of 1.0 kW, and irradiation continued until mist production ceased. The irradiation time was set to 30 s for the 300 mg sample and 60 s for the 930 mg sample. Pyrolysis was performed 1–3 times under identical conditions, and the condensates in the sample bag and on the reactor walls were extracted using 200 mL of methanol.

Accurate temperature measuring during pyrolysis is challenging, as the thermocouple itself may be heated by infrared radiation. However, it is known that fast pyrolysis of cellulose occurs within the range of 400–450 °C, which is higher than the temperature associated with slow pyrolysis (around 360 °C) (Shoji et al., 2014). In our experiments, the occurrence of fast pyrolysis was also confirmed by the formation of melt char, which is characteristic of fast pyrolysis conditions.

2.3. Purification of bio-oil products from the fast pyrolysis of cellulose using ion-exchange resin

A styrene-based polyamine ion-exchange resin (DIAION™ WA21J, Mitsubishi Chemicals Co. Ltd., Tokyo Japan) was used in the purification process. Prior to use, the resin was pretreated by passing 180 mL of 4 % NaOH through a column packed with 60 mL of resin at a flow rate of 1.6 mL/min. The NaOH was then removed by rinsing the resin with pure water until the pH of effluent reached 7. The products obtained from the fast pyrolysis of cellulose were purified by passing the solution through the pretreated column at a flow rate of 1.2 mL/min. The amino groups in the ion-exchange resin reacted with the carbonyl carbon of aldehydes, forming Schiff base through subsequent dehydration. This process enabled the adsorption and removal of glycolaldehyde, glyoxal, methylglyoxal, furfural, 5-hydroxymethylfurfural from the pyrolysis products. After the purification, the column was washed with distilled water until a total volume of 100 mL had passed through. The purified products were recovered as a yellow syrup by evaporating the water using a rotary evaporator.

2.4. Hydrolysis of pyrolysis products before and after purification

LG and 1,6-anhydro-β-D-glucofuranose (AGF) were hydrolyzed to glucose following the method described by Nomura et al. (2024). The pyrolysis products, both before and after purification, were dissolved in 3 mL of distilled water. Hydrolysis was carried out by adding 2 mL of the solid acid catalyst Amberlyst 15JWET and a stirrer bar to the solution, followed by microwave heating at 120 °C for 30 min using a microwave synthesis system (Discover SP, CEM, Matthews, NC, USA).

2.5. Lactic acid fermentation

The microorganism used for lactic acid fermentation was *Lactococcus lactis* subsp. *lactis* (NBRC100933), which was cultured at 30 °C for 3 days in the following medium: 3.0 g/L yeast extract, 5.0 g/L NaCl, 2.5 g/L K_2HPO_4, 2.5 g/L glucose, 17.0 g/L hypolypepton, and 3.0 g/L hypolypepton S. Calcium carbonate ($CaCO_3$) was added as a neutralizing agent at 50 wt% relative to sugar source. Each component of the medium was autoclaved at 121 °C for 20 min prior to use.

For lactic acid fermentation, four types of sugar solution were used as sugar resources: (i) a model solution (pure glucose), (ii) a solution obtained by pyrolysis (before purification), (iii) a solution obtained by pyrolysis (after purification), and (iv) pure glucose supplemented with inhibitors. The glucose concentrations were adjusted to approximately 10 or 20 g/L. A total of 4.5 mL of sugar solution and 0.5 mL of preculture medium were added to a vial, and fermentation was conducted at 30 °C with stirring. All fermentation experiments were performed in triplicate, and average values are reported.

2.6. Characterization

2.6.1. Proton nuclear magnetic resonance (^{1}H NMR)

Proton (^{1}H)-nuclear magnetic resonance (NMR) analyses were performed on a Bruker AC-400 spectrometer (400 MHz, Bruker, MA, USA) at 25 °C to identify and quantify the products. For the analysis of pyrolysis products dissolved in methanol, 10 mL of the solution was taken, and the methanol was evaporated. The resulting syrup was dissolved in 0.7 mL of DMSO-d_6 containing maleic acid as an internal standard and 5.0 mg of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) for the in situ derivatization of aldehydes into their corresponding oximes. NMR data were processed using Bruker TopSpin 4.0.8 software. Subsequently, signal assignments were made based on literature data (Fukutome et al., 2015), and quantification was performed by comparing the peak area of a specific proton in the target product with that of the internal standard, maleic acid.

2.6.2. High-performance liquid chromatography (HPLC)

The concentration of glucose and lactic acid were analyzed by high performance liquid chromatography (HPLC) system (LC-20AD; Shimadzu, Kyoto, Japan) equipped with a refractive index (RI) detector and an HPX-87H column (300 mm × 7.8 mm, Bio-Rad Laboratories, Inc., Hercules, CA, USA) maintained at 45 °C. The column was eluted with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. A 0.2 mL sample of the fermented solution was collected using a syringe. Microorganisms and sediments in the fermented solution were removed by passing the sample through a cartridge with a 0.45 μm filter (Sigma-Aldrich, St. Louis, MO, USA), followed by centrifugation.

3. Results and discussion

3.1. Products from fast pyrolysis of cellulose

Fig. 1 shows the ^{1}H NMR spectra of the products obtained from the fast-pyrolysis of cellulose. As previously reported, signals assigned to levoglucosan (LG), 1,6-anhydro-β-D-glucofuranose (AGF), glycolaldehyde (GA), glyoxal (GO), methylglyoxal (MeGO), furfural (FF), 5-hydroxymethylfurfural (5-HMF), acetic acid (AcOH), formic acid (FcOH), and formaldehyde (Falde) were identified. Their yields (wt%, based on the initial cellulose) are shown in Fig. 2. The total yield of these products was 58.50 wt%, with the remaining portion consisting of char, gases and unidentified products.

LG, the major product, was quantified from the proton signal of C1—H (around 5.1 ppm), with a yield of 51.60 wt%. This value is consistent with previous reports (Kwon et al., 2007; Nomura et al., 2021;

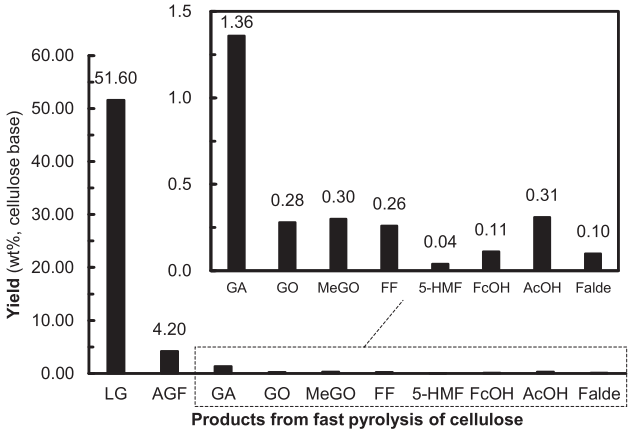

Fig. 2. The yields of products obtained from the fast pyrolysis of cellulose, quantified by ^{1}H NMR analysis.

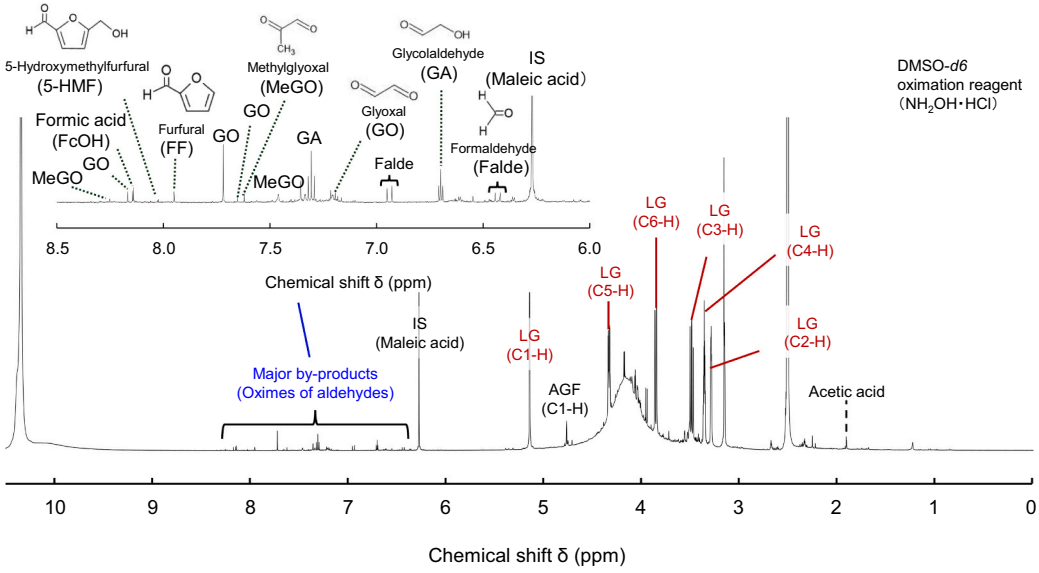

Fig. 1. The ^{1}H NMR spectra of the products obtained from the fast pyrolysis of cellulose.

Shafizadeh et al., 1979). A proton signal corresponding to C1—H of AGF, which can also be hydrolyzed to glucose like LG, was detected around 4.8 ppm, with a yield of 4.20 wt%. Regarding the by-products, the methyl proton signal of AcOH was observed around 1.9 ppm, and multiple oxime H—C=N(OH) signals were detected in the 6.0 to 8.5 ppm range. In this region, GA, GO, MeGO, FF, 5-HMF, FcOH, and Fald were identified. The total yield of by-products was 2.76 wt%, with GA being the most abundant by-product, at 1.36 wt%.

LG and AGF can be hydrolyzed into glucose and subsequently used for fermentation, whereas the other by-products cannot be converted into glucose and are therefore considered non-fermentable. To assess whether such a small quantity of by-products could inhibit lactic acid fermentation, fermentation experiments were conducted using bio-oil derived from the fast pyrolysis of cellulose.

3.2. Fermentability of sugar solutions from fast pyrolysis of cellulose

The sugar solution obtained by hydrolyzing bio-oil—converting LG and AGF into glucose—was used for lactic acid fermentation. The concentrations of each component in the sugar solutions used for fermentation are shown in Table 1. According to HPLC analysis, LG and AGF were not detected in the sugar solutions, indicating that they were completely converted into glucose during hydrolysis. Sugar solutions were prepared with glucose concentrations of approximately 10 g/L and 20 g/L, using either a model solution or a solution obtained from the fast pyrolysis of cellulose (prior to purification). It should be noted that higher glucose concentration in sugar solutions obtained from the fast pyrolysis of cellulose followed by hydrolysis are associated with increased concentrations of by-products. Therefore, when using sugar solutions obtained through fast pyrolysis for lactic acid fermentation, a glucose concentration of 20 g/L is expected to result in more significant inhibition than 10 g/L due to the higher concentration of by-products that inhibit lactic acid fermentation (Peng et al., 2013; van der Pol et al., 2016; Zhang et al., 2016).

The results of lactic acid fermentation using sugar solutions obtained from cellulose are shown in Fig. 3 and compared with those of the model sugar solution. When fermentation was conducted at a sugar concentration of approximately 10 g/L, the model sugar solution was completely consumed within 10 h, accompanied by a corresponding increase in lactic acid concentration (Fig. 3 (a), circle plot). In contrast, when a sugar solution derived from cellulose was used, glucose was fully consumed within 20 h, and lactic acid production was observed (Fig. 3 (a), square plot). These results indicate that lactic acid fermentation proceeded even with the sugar solution obtained from the pyrolysis of cellulose, although a longer time was required. This delay, compared to the model sugar solution, is likely attributable to the inhibitory effects of by-products other than LG and AGF, as has been reported in previously studies on ethanol fermentation (Jayakody et al., 2011; Lian et al., 2010; Sjulander and Kikas, 2020; Wang et al., 2012).

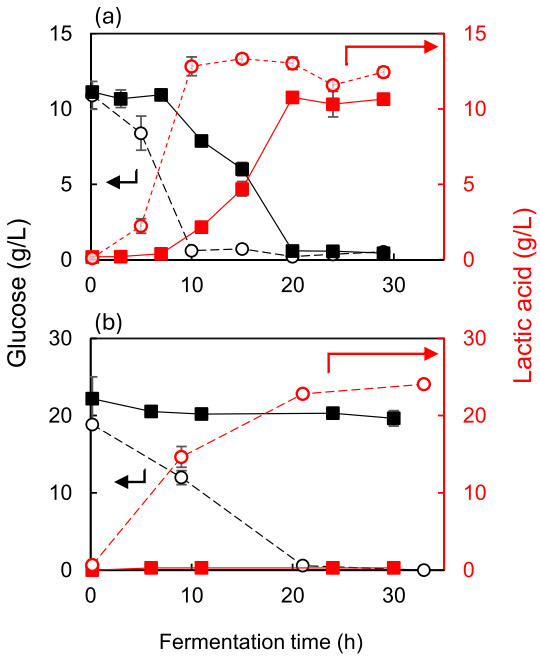

Fig. 3. Results of lactic acid fermentation using a model glucose solution and a sugar solution obtained from the fast pyrolysis of cellulose, followed by hydrolysis, at glucose concentrations of approximately 10 g/L (a) and 20 g/L (b). Black line: glucose, red line: lactic acid, ○: model sugar solution; ■: sugar solution obtained from the fast pyrolysis of cellulose, followed by hydrolysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

When the glucose concentration was increased to approximately 20 g/L, the model sugar solution (18.8 g/L) was completely consumed within 21 h, resulting in lactic acid production (Fig. 3 (b), circle plot). In contrast, the sugar solution obtained from cellulose (22.2 g/L) showed no glucose consumption even after 30 h, and no lactic acid production was observed (Fig. 3 (b), square plot). These results suggest that at a glucose concentration of 10 g/L, pyrolysis by-products merely delayed lactic acid fermentation, whereas at 20 g/L, the elevated concentration of these by-products was sufficient to completely inhibit fermentation.

3.3. Effects of by-products as inhibitors on lactic acid fermentation

To better understand the inhibitors of lactic acid fermentation, by-products generated from cellulose pyrolysis—GA, GO, MeGO, FF, 5-HMF, FcOH and AcOH—were added to a 20 g/L pure glucose solution

Table 1
Concentration of each product in the sugar solution used for lactic acid fermentation.

	Sugar solution products (g/L)	Abbr.	10 (g/L)		20 (g/L)	
			model sugar solution	sugar solution obtained from the fast pyrolysis of cellulose	model sugar solution	sugar solution obtained from the fast pyrolysis of cellulose
Sugar*	Glucose	–	10.9 ± 0.9	11.1 ± 0.3	18.8 ± 0.3	22.2 ± 2.8
Aldehyde**	glycolaldehyde	GA	–	0.40	–	0.80
	glyoxal	GO	–	0.08	–	0.16
	methylglyoxal	MeGO	–	0.10	–	0.20
	formaldehyde	Falde	–	0.03	–	0.06
Organic acids**	acetic acid	AcOH	–	0.09	–	0.18
	formic acid	FcOH	–	0.03	–	0.06
Furans**	furfural	FF	–	0.08	–	0.16
	5-hydroxymethyl furfural	5-HMF	–	0.01	–	0.02

* Measured by HPLC.
** Measured by ^{1}H NMR and calculated.

at concentration ranging from 0.1 to 5.0 g/L. Lactic acid production after 24 h of fermentation was then compared (Fig. 4). These by-products were categorized into three groups: aldehydes (GA, GO, and MeGO), furans (FF and 5-HMF), and organic acids (FcOH and AcOH). Although FF and 5-HMF possess aldehyde groups, they were classified as furans in this study due to their furan ring structures.

The results of lactic acid fermentation using sugar solutions containing by-products are shown in Fig. 4. The inhibitory effects followed the order: aldehydes > organic acids > furans. When 0.5 g/L of aldehydes was added to the sugar solution, lactic acid production was almost completely suppressed (Fig. 4(a)). Among the by-products present in the 20 g/L sugar solution derived from pyrolysis products—where lactic acid fermentation was not occur—the concentration of aldehydes GA, GO, and MeGO were 0.8 g/L, 0.16 g/L, and 0.20 g/L, respectively (Table 1). At concentrations of 0.16 g/L GO and 0.20 g/L MeGO, only minor inhibitory effects on fermentation were observed (Fig. 4(a)). However, the concentration of GA in the sugar solution obtained from fast pyrolysis of cellulose was 0.8 g/L, exceeding the 0.5 g/L threshold, indicated to completely inhibit lactic acid fermentation. These findings indicate that the inhibition of lactic acid fermentation in sugar solutions derived from fast pyrolysis of cellulose was primarily caused by GA. GA is known to inhibit yeast growth and ethanol production due to its toxicity, which stems from covalent binding to proteins, leading to cellular damages (Jayakody et al., 2011, 2017). Regarding lactic acid fermentation, Li et al. (2015) reported that lignin-derived aromatic aldehydes such as pyrogallol aldehyde, syringaldehyde, *o*-phthalaldehyde, and 4-hydroxybenzaldehyde inhibited cell growth and lactic acid fermentation by *Lactobacillus delbrueckii*, likely due to the reaction between their carbonyl groups with amino groups in proteins. Therefore, GA is believed to affect lactic acid bacteria through a similar mechanism.

When organic acids were present in the sugar solution, lactic acid was still produced; however, the production rates gradually declined as the concentration of organic acids increased, indicating moderate inhibitory effects (Fig. 4(b)). Undissociated acid molecules, which are liposoluble, can pass through cell membranes and acidify the intracellular environment. Microorganisms expend energy to maintain intracellular pH homeostasis, thereby inhibiting their growth and fermentation (Sjulander and Kikas, 2020; Zhang et al., 2016).

In the case of furans, sugar solutions containing less than 1.0 g/L of furans produced nearly the same amount of lactic acid as the model glucose solution. However, when the concentration of furans exceeded 1.0 g/L, lactic acid production decreased, suggesting that small amounts of furans do not strongly inhibit fermentation (Fig. 4(c)). Furans are known to inhibit microbial enzymes such as alcohol dehydrogenase and other glycolytic enzymes, leading to reduced intracellular ATP production, which is essential for energy transport (Sjulander and Kikas, 2020). Since ATP is critical for cellular activity, reduced ATP levels result in fermentation inhibition. In addition, furans induce the formation of reactive oxygen species (ROS), which damage cellular components such as mitochondria, vacuole membranes, the actin cytoskeleton, and nuclear chromatin (Sjulander and Kikas, 2020).

As reported by Zhang et al. (2016), mixtures of alkali-pretreated corn cob (ACC) or acid-catalyzed steam-exploded corn stover (ASCS) hydrolysate strongly inhibited lactic acid production, even when the concentrations of individual inhibitors were below their respective toxic thresholds. In the present study, organic acids and furans in the sugar solution obtained from the fast pyrolysis of cellulose were present at low concentrations and did not exhibit significant toxicity when assessed individually. However, the additive or synergistic effects of multiple inhibitors should not be overlooked (Jönsson and Martín, 2016). Further research on the combined impact of various inhibitors on lactic acid fermentation by *Lactococcus lactis* subsp. *lactis* is essential for understanding their synergistic effects.

3.4. Purification by ion-exchange resin

To remove inhibitory compounds and enable fermentation, bio-oil was purified using an ion-exchange resin containing amino groups, which selectively react with aldehydes and ketones to form stable Schiff bases. Fig. 5 shows the optical images and ^{1}H NMR spectra of the cellulose pyrolysis products before and after purification. Table 2 summarizes the yields of LG, AGF and other by-products before and after purification. Prior to purification, the liquid appeared brown; however, after purification, it became light yellow and more transparent. In the ^{1}H NMR spectra before purification, strong signals corresponding to aldehyde oximes were detected in the 6.0–8.5 ppm region. After purification, almost no signals were observed in this region, confirming that aldehydes, furans and formic acids were effectively removed by the ion-exchange resin. Since 77 % of the LG remained after purification, this method demonstrates high selectivity for removing inhibitory by-products while preserving the target compound.

Lactic acid fermentation was performed using a purified sugar solution derived from cellulose pyrolysis and a model glucose solution (Fig. 6). After purification, lactic acid production from the cellulose-derived sugar solution at a concentration of 18.4 g/L—previously unable to support fermentation—became comparable to that of the model glucose solution. These results indicate that the removal of by-products is essential for enabling lactic acid fermentation using sugar solutions derived from cellulose pyrolysis.

4. Conclusion

Bio-oil containing LG as the major component was obtained through the fast pyrolysis of cellulose. In addition to LG, the bio-oil contained various by-products, including glycolaldehyde, glyoxal, methylglyoxal, furfural, 5-hydroxymethylfurfural, acetic acid, formic acid, and

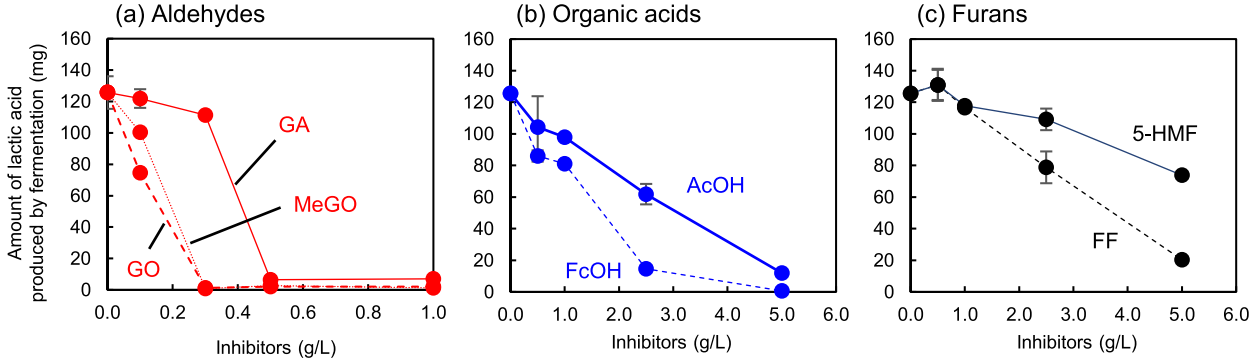

Fig. 4. Effects of by-products obtained from the fast pyrolysis of cellulose on lactic acid fermentation: (a) aldehydes, (b) organic acids, and (c) furans. Each by-product was added to a 20 g/L glucose solution at concentrations ranging from 0.1 to 5.0 g/L, and the amount of lactic acid produced after 24 h of fermentation was compared.

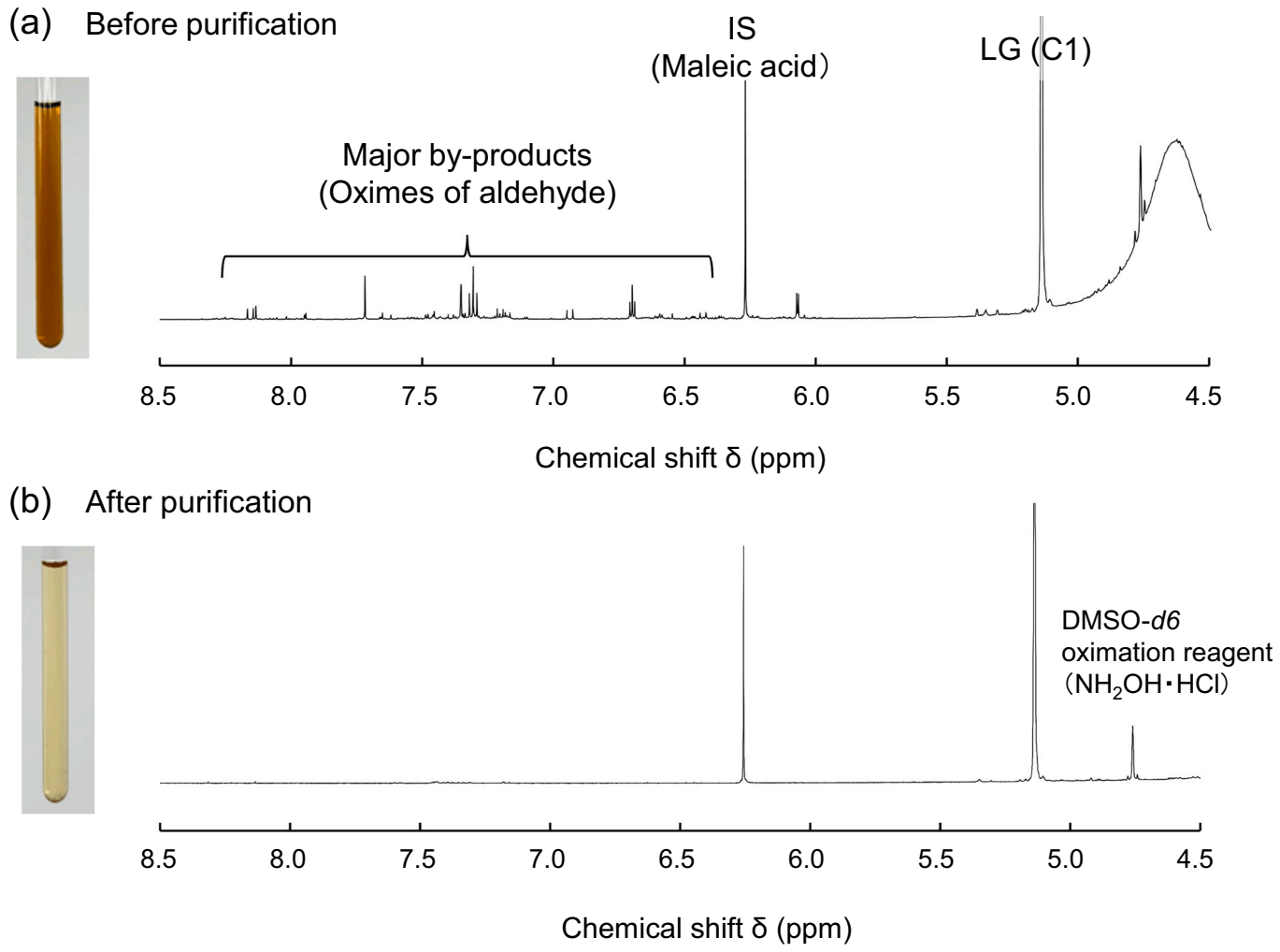

Fig. 5. ^{1}H NMR spectra of the products from the fast pyrolysis of cellulose: (a) before purification with ion-exchange resin and (b) after purification.

Table 2
The yields of pyrolysis products before and after purification quantified by ^{1}H NMR analysis.

Product		LG	AGF	GA	GO	MeGO	FF	5-HMF	FcOH	AcOH	Falde
Yield (wt%, cellulose base)	Before purification	46.70	3.80	1.37	0.36	0.19	0.02	0.12	0.06	0.10	0.04
	After purification	35.90	6.40	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01

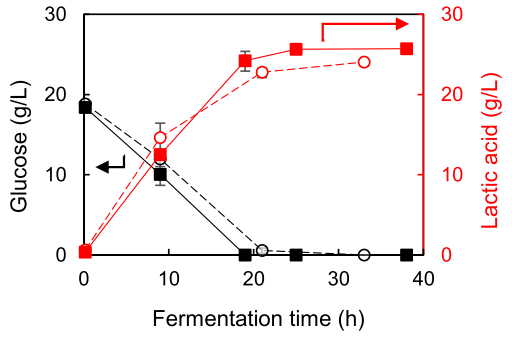

Fig. 6. The results of lactic acid fermentation of model glucose solution and purified pyrolysis sugar solution. Black line: glucose, red line: lactic acid, ○: model sugar solution; ■: purified sugar solution obtained from the fast pyrolysis of cellulose, followed by hydrolysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

formaldehyde. LG in the bio-oil was hydrolyzed into glucose and subsequently used as a sugar solution for lactic acid fermentation. However, lactic acid fermentation was inhibited by the presence of by-products at high sugar solution concentrations. The inhibitory effects followed the order: aldehydes > organic acids > furans. These inhibitory compounds were successfully removed using an ion-exchange resin containing amino groups. Following purification, lactic acid fermentation proceeded even at sugar concentrations that had previously resulted in complete inhibition. This study highlights the critical importance of removing inhibitors to enable effective utilization of pyrolyzed sugar solutions for bioproduction.

CRediT authorship contribution statement

Yasuko Maruichi: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Takashi Nomura:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Eiji Minami:** Writing – review & editing, Supervision. **Haruo Kawamoto:** Writing –

review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding sources

This work was supported by the JST Mirai Program, Japan (Grant no. JPMJMI20E3) and by the Japan Society for the Promotion of Science (JSPS) KAKENHI program (Grant nos. JP19H03019 and JP20J12367).

Declaration of competing interest

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

Acknowledgements

We would like to express our deepest gratitude to Dr. Taku Omura for his helpful advice during the writing of this manuscript.

Data availability

Data will be made available on request.

References

Abdel-Rahman, M.A., Sonomoto, K., 2016. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. J. Biotechnol. 236, 176–192. https://doi.org/10.1016/J.JBIOTEC.2016.08.008.

Abdel-Rahman, M.A., Hassan, S.E.D., Fouda, A., Radwan, A.A., Barghoth, M.G., Desouky, S.G., 2021. Evaluating the effect of lignocellulose-derived microbial inhibitors on the growth and lactic acid production by *Bacillus coagulans* Azu-10. Fermentation 7, 17. https://doi.org/10.3390/FERMENTATION7010017.

Ajala, E.O., Olonade, Y.O., Ajala, M.A., Akinpelu, G.S., 2020. Lactic acid production from lignocellulose – a review of major challenges and selected solutions. ChemBioEng Rev. 7, 38–49. https://doi.org/10.1002/CBEN.201900018.

Benavides, P.T., Lee, U., Zarè-Mehrjerdi, O., 2020. Life cycle greenhouse gas emissions and energy use of polylactic acid, bio-derived polyethylene, and fossil-derived polyethylene. J. Clean. Prod. 277, 1–26. https://doi.org/10.1016/j.jclepro.2020.124010.

Bennett, N.M., Helle, S.S., Duff, S.J.B., 2009. Extraction and hydrolysis of levoglucosan from pyrolysis oil. Bioresour. Technol. 100, 6059–6063. https://doi.org/10.1016/j.biortech.2009.06.067.

Canilha, L., Chandel, A.K., Dos Suzane Santos Milessi, T., Antunes, F.A.F., Da Luiz Costa Freitas, W., Das Graças Almeida Felipe, M., Da Silva, S.S., 2012. Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. J. Biomed. Biotechnol. 2012, 989572. https://doi.org/10.1155/2012/989572.

Colwill, J.A., Wright, E.I., Rahimifard, S., Clegg, A.J., 2012. Bio-plastics in the context of competing demands on agricultural land in 2050. Int. J. Sustain. Eng. 5, 3–16. https://doi.org/10.1080/19397038.2011.602439.

Cubas-Cano, E., González-Fernández, C., Ballesteros, M., Tomás-Pejó, E., 2018. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. Biofuels Bioprod. Biorefin. 12, 290–303. https://doi.org/10.1002/BBB.

European bioplastics, 2023. Bioplastic market development update 2023. https://www.european-bioplastics.org/bioplastics-market-development-update-2023-2/ (accessed 31 January 2025).

Fukutome, A., Kawamoto, H., Saka, S., 2015. Processes forming gas, tar, and coke in cellulose gasification from gas-phase reactions of levoglucosan as intermediate. ChemSusChem 8, 2240–2249. https://doi.org/10.1002/cssc.201500275.

Islam, Z.U., Zhisheng, Y., Hassan, E.B., Dongdong, C., Hongxun, Z., 2015. Microbial conversion of pyrolytic products to biofuels: a novel and sustainable approach toward second-generation biofuels. J. Ind. Microbiol. Biotechnol. 42, 1557–1579. https://doi.org/10.1007/s10295-015-1687-5.

Islam, Z.U., Klykov, S.P., Yu, Z., Chang, D., Hassan, E.B., Zhang, H., 2018. Fermentation of detoxified acid-hydrolyzed pyrolytic anhydrosugars into bioethanol with saccharomyces cerevisiae. Appl. Biochem. Microbiol. 54, 58–70. https://doi.org/10.1134/S0003683818010143.

Jahirul, M.I., Rasul, M.G., Chowdhury, A.A., Ashwath, N., 2012. Biofuels production through biomass pyrolysis - a technological review. Energies 5, 4952–5001. https://doi.org/10.3390/en5124952.

Jayakody, L.N., Hayashi, N., Kitagaki, H., 2011. Identification of glycolaldehyde as the key inhibitor of bioethanol fermentation by yeast and genome-wide analysis of its toxicity. Biotechnol. Lett. 33, 285–292. https://doi.org/10.1007/s10529-010-0437-z.

Jayakody, L.N., Ferdouse, J., Hayashi, N., Kitagaki, H., 2017. Identification and detoxification of glycolaldehyde, an unattended bioethanol fermentation inhibitor. Crit. Rev. Biotechnol. 37, 177–189. https://doi.org/10.3109/07388551.2015.1128877.

Jönsson, L.J., Martín, C., 2016. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour. Technol. 199, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009.

Junior, I.I., Nascimento, M.A. do, Souza, R.O.M.A. de, Dufour, A., Wojcieszak, R., 2020. Levoglucosan: a promising platform molecule? Green Chem. 22 (18), 5859–5880. https://doi.org/10.1039/D0GC01490G.

Kwon, G.J., Kim, D.Y., Kimura, S., Kuga, S., 2007. Rapid-cooling, continuous-feed pyrolyzer for biomass processing. Preparation of levoglucosan from cellulose and starch. J. Anal. Appl. Pyrolysis 80, 1–5. https://doi.org/10.1016/j.jaap.2006.12.012.

Li, J., Zhu, C., Tu, M., Han, P., Wu, Y., 2015. Effect of carbonyl inhibitors and their H_2O_2 detoxification on lactic acid fermentation. Appl. Biochem. Biotechnol. 175, 3657–3672. https://doi.org/10.1007/S12010-015-1533-2/FIGURES/6.

Lian, J., Chen, S., Zhou, S., Wang, Z., O'Fallon, J., Li, C.Z., Garcia-Perez, M., 2010. Separation, hydrolysis and fermentation of pyrolytic sugars to produce ethanol and lipids. Bioresour. Technol. 101, 9688–9699. https://doi.org/10.1016/j.biortech.2010.07.071.

Liu, C., Wang, H., Karim, A.M., Sun, J., Wang, Y., 2014. Catalytic fast pyrolysis of lignocellulosic biomass. Chem. Soc. Rev. 43, 7594–7623. https://doi.org/10.1039/c3cs60414d.

Nomura, T., Mizuno, H., Minami, E., Kawamoto, H., 2021. Fast pyrolysis of cellulose by infrared heating. Energies 14, 1842. https://doi.org/10.3390/EN14071842.

Nomura, T., Minami, E., Kawamoto, H., 2024. Microwave effect in hydrolysis of levoglucosan with a solid acid catalyst for pyrolysis-based cellulose saccharification. ChemistryOpen 13, e202300311. https://doi.org/10.1002/open.202300311.

Peng, L., Wang, L., Che, C., Yang, G., Yu, B., Ma, Y., 2013. Bacillus sp. strain P38: an efficient producer of l-lactate from cellulosic hydrolysate, with high tolerance for 2-furfural. Bioresour. Technol. 149, 169–176. https://doi.org/10.1016/j.biortech.2013.09.047.

van der Pol, E.C., Bakker, R.R., Baets, P., Eggink, G., 2014. By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio) chemicals and fuels. Appl. Microbiol. Biotechnol. 98, 9579–9593. https://doi.org/10.1007/S00253-014-6158-9. /TABLES/3.

van der Pol, E.C., Vaessen, E., Weusthuis, R.A., Eggink, G., 2016. Identifying inhibitory effects of lignocellulosic by-products on growth of lactic acid producing micro-organisms using a rapid small-scale screening method. Bioresour. Technol. 209, 297–304. https://doi.org/10.1016/j.biortech.2016.03.037.

Rinaldi, R., Schuth, F., 2009. Acid hydrolysis of cellulose as the entry point into biorefinary schemes. ChemSusChem 2, 1096–1107.

Shafizadeh, F., Furneaux, R.H., Cochran, T.G., Scholl, J.P., Sakai, Y., 1979. Production of levoglucosan and glucose from pyrolysis of cellulosic materials. J. Appl. Polym. Sci. 23, 3525–3539. https://doi.org/10.1002/APP.1979.070231209.

Shen, D., Jin, W., Hu, J., Xiao, R., Luo, K., 2015. An overview on fast pyrolysis of the main constituents in lignocellulosic biomass to valued-added chemicals: Structures, pathways and interactions. Renew. Sust. Energ. Rev. 51, 761–774. https://doi.org/10.1016/j.rser.2015.06.054.

Shoji, T., Kawamoto, H., Saka, S., 2014. Boiling point of levoglucosan and devolatilization temperatures in cellulose pyrolysis measured at different heating area temperatures. J. Anal. Appl. Pyrolysis 109, 185–195. https://doi.org/10.1016/j.jaap.2014.06.014.

Sjulander, N., Kikas, T., 2020. Origin, impact and control of lignocellulosic inhibitors in bioethanol production—a review. Energies 13, 4751. https://doi.org/10.3390/en13184751.

Wang, H., Livingston, D., Srinivasan, R., Li, Q., Steele, P., Yu, F., 2012. Detoxification and fermentation of pyrolytic sugar for ethanol production. Appl. Biochem. Biotechnol. 168, 1568–1583. https://doi.org/10.1007/s12010-012-9879-1.

Yankov, D., 2022. Fermentative lactic acid production from lignocellulosic feedstocks: from source to purified product. Front. Chem. 10, 823005. https://doi.org/10.3389/FCHEM.2022.823005/XML/NLM.

Zhang, L., Li, X., Yong, Q., Shang-Tian, Ouyang, J., Yu, S., 2016. Impacts of lignocellulose-derived inhibitors on l-lactic acid fermentation by Rhizopus oryzae. Bioresour. Technol. 203, 173–180.