



Upcycling agricultural waste to biodegradable polyhydroxyalkanoates by combined ambient alkaline pretreatment and bacterial fermentation

Md. Anwar Hossain^a, Logan Mushill^c, Mohammad Shahinur Rahaman^a, Seth M. Mains^a, Tasia Vickers^c, Sarttrawut Tulaphol^{b,*}, Jie Dong^{c,*}, Noppadon Sathitsuksanoh^{a,d,**}

^a Department of Chemical Engineering, University of Louisville, Louisville, KY 40292, USA

^b Sustainable Polymer & Innovative Composite Materials Research Group, Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand

^c Department of Chemistry, Southern Illinois University Edwardsville, Edwardsville, IL 62026, USA

^d Department of Wood Science, University of British Columbia, Vancouver V6T 1Z4, Canada

ARTICLE INFO

Keywords:

Alkaline pretreatment
Rice straw
Hemp hurd
Polyhydroxyalkanoates
Ambient temperature
High solid loading

ABSTRACT

Medium-chain-length polyhydroxyalkanoates (mcl-PHAs), polyesters produced by bacterial fermentation of sugars and/or lipids, are potential bioplastic alternatives to petroleum-derived plastics. Lignocellulose is an abundant renewable source of sugars for bacterial fermentation. However, chemical or enzymatic release of the sugars requires a pretreatment step to dispel the rigid structure of the lignocellulose and enhance sugar access. Most pretreatments of lignocellulose involve high temperature and/or pressure, which increases processing and production costs and discourages commercialization. Here we demonstrate a chemical-biological pathway for mcl-PHAs production from rice straw and hemp hurd. We combined ambient alkaline pretreatment, enzymatic hydrolysis, and bacterial fermentation. The alkaline pretreatment reduced the particle size of pretreated solids and partially fractionated hemicellulose and lignin; these effects increased cellulose accessibility to enzymes and enabled a high sugar release (78–83% glucose yield) at a high solid loading (9 wt%). Therefore, we obtained 47 wt% and 69 wt% mcl-PHAs (with respect to gram of dry cell weight) from pretreated rice straw and hemp hurd, respectively. These findings constitute an energy-efficient pretreatment process that can be extended to other sources of lignocellulose, such as woody biomass and dedicated bioenergy crops. Moreover, mcl-PHAs from lignocellulose provide revenue to the agricultural sector, mitigate global warming from fossil fuel processing, and potentially reduce plastic pollution.

1. Introduction

A global challenge in sustainability is the transition from a fossil fuel-based economy to a bioeconomy based on renewable resources such as lignocellulose. The creation of renewable lignocellulose-derived biodegradable products provides economic, societal, and environmental benefits: (1) a reduced carbon footprint because of less reliance on fossil fuels as feedstock and energy, (2) an improved economy from adding revenue to the agricultural sector and decreasing import of fossil fuels, and (3) a biodegradable alternative for petroleum-derived plastics and lower plastic pollution that harms the environment and health (Isikgor and Becer, 2015; Pileidis and Titirici, 2016; Sheldon, 2011).

Hemp hurd (Das et al., 2020; Tulaphol et al., 2019, 2021) and rice

straw (Hossain et al., 2021; Satlewal et al., 2018) are potential renewable lignocellulose feedstocks. The estimated global production of rice straw and hemp hurd in 2019 was 670 million tons/year (Nguyen Van et al., 2019) and ~0.7–1.3 million tons/year, (Robinson, 1996) respectively. Presently, these materials are considered as agricultural waste or they are used in only low-value applications, such as garden mulch, lightweight paperboard, and acoustical ceiling. Thus, there is a vast amount of unused lignocellulose that could be upcycled into fuels and biodegradables.

To use lignocellulose, its sugars must be released efficiently for subsequent conversion into bioproducts. Because of the rigid chemical structure and low enzymatic accessibility of cellulose, a lignocellulose pretreatment step is unavoidable (Bhatia et al., 2020; Soltanian et al.,

* Corresponding authors.

** Corresponding author at: Department of Chemical Engineering, University of Louisville, Louisville, KY 40292, USA.

E-mail addresses: sarttrawut.tul@kmutt.ac.th (S. Tulaphol), jdong@siue.edu (J. Dong), n.sathitsuksanoh@louisville.edu (N. Sathitsuksanoh).

2020). Most current pretreatments of lignocellulose require high temperature and/or pressure to enhance the cellulose accessibility and release sugars (Balan et al., 2009). For example, dilute acid, (Ríos-González et al., 2021; Saha et al., 2005; Sheng et al., 2021) liquid hot water, (Jimenez-Gutierrez et al., 2021; López González and Heiermann, 2021; Pérez et al., 2008) ammonia fiber explosion/expansion (AFEX), (Bals et al., 2010; Zeng et al., 2021) sulfite (SPORL), (Zan et al., 2021; Zhu et al., 2009) and ionic liquid (Wang et al., 2017; Zhang et al., 2021) enable high sugar release from enzymatic hydrolysis, but all these lignocellulose pretreatments are conducted at the expense of high temperature (80–220 °C). High-temperature pretreatment has drawbacks: (1) high cost of energy, (Capolupo and Faraco, 2016; Yu et al., 2009) (2) generation of greenhouse gases (CO₂, CH₄) and particulates from burning coal, petroleum, and natural gas (Armaroli and Balzani, 2007; Centi and Perathoner, 2021; Fulkerson et al., 1990), and (3) degradation of sugars into fermentation inhibitors that require a detoxification step before fermentation. All of these attributes of high temperature pretreatment negatively impact the economic feasibility of using lignocellulose (Binod et al., 2012).

Pretreatment of lignocellulose with alkaline agents, such as lime and ammonia, at low temperature (<100 °C) is a promising approach to improve cellulose accessibility (Heggset et al., 2016; Zhao et al., 2008) without the economic and environmental cost of burning fuel and without sugar degradation. The low-temperature operation of alkaline pretreatments retains sugars in the pretreated solids for subsequent enzymatic hydrolysis (Chen et al., 2013). Moreover, low-temperature is a less expensive condition in terms of processing and reactor materials (Galbe and Zacchi, 2012).

Sugars released from lignocellulose can be metabolized naturally by bacterial fermentation to produce medium-chain-length polyhydroxyalkanoates (mcl-PHAs) (Ashby et al., 2001). The mcl-PHAs are biodegradable polyesters that are potential alternatives to petroleum-derived polyethylene, polystyrene, and polyethylene terephthalate (Ashby et al., 2001; Chen, 2009). In addition, mcl-PHAs are highly biocompatible, which enables biomedical applications such as implants, sutures, scaffolds, and drug delivery carriers (Hazer et al., 2012; Kim et al., 2005; Kurth et al., 2002; Rai et al., 2011). Among PHA-producing bacteria, *Pseudomonas* species produce mcl-PHAs as carbon reserves under growth-limiting conditions (i.e., nutrient-deficiency) in the presence of excess carbon (Chen, 2009). The mcl-PHAs yield from *Pseudomonas* species (*P. putida* W619, *P. putida* KT2440, and *P. fluorescens* 555) reached ~26–37 wt% (with respect to dry cell weight) from glucose as the substrate (Davis et al., 2013). Arreola-Vargas et al. (2021) used dilute acid pretreatment at 121 °C on corn stover prior to fermentation by *P. putida* into 0.25 g/L mcl-PHAs. Davis et al. (2013) compared hot water pretreatment at 120 °C and 2% NaOH pretreatment at 120 °C on ryegrass, followed by *P. putida* fermentation into 0.2 g/L mcl-PHAs. Although the alkaline pretreatment

at 120 °C was effective and the fermentation of the resulting sugar hydrolysate yielded mcl-PHAs, the high-temperature pretreatment contributes to the overall production cost of mcl-PHAs.

Here, we demonstrated the effectiveness of alkaline pretreatment of rice straw and hemp hurd at ambient temperature for production of mcl-PHAs (Scheme 1). The ambient alkaline pretreatment of rice straw and hemp hurd retained >88% glucan. Enzymatic hydrolysis of pretreated lignocellulose released >78% glucose yield. Then we assessed the compatibility of the resulting sugars for fermentation by *P. putida* into mcl-PHAs. Fermentation of resulting sugar hydrolysates by *P. putida* yielded 0.5–0.6 g mcl-PHAs/L. This alkaline pretreatment at ambient temperature/pressure to release sugars and compatibility with *P. putida* offers a simple yet energy-efficient approach for lignocellulose upcycling to mcl-PHAs for biorefineries. The knowledge gained will help in further optimizing pretreatment and fermentation conditions to maximize yields of sugars and mcl-PHAs.

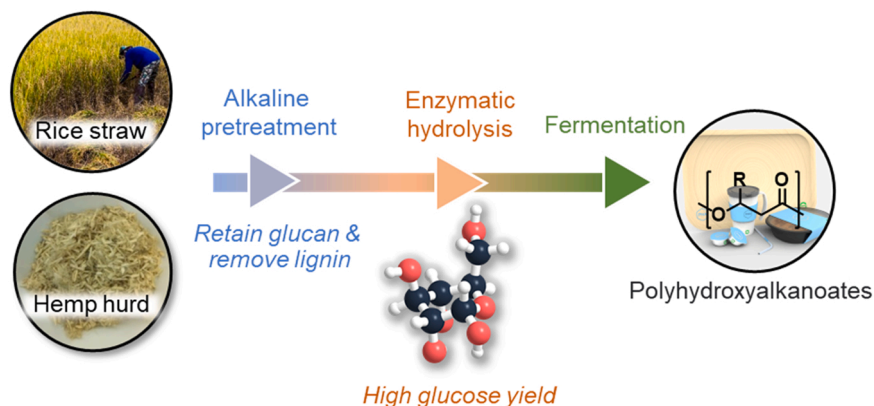
2. Material and methods

2.1. Materials

Rice straw was provided by Industrial Technology Research Institute (Taiwan). Hemp hurd was the Mammoth hemp strain and donated by Green Remedy (Louisville, KY, USA). The rice straw and hemp hurd samples were dried overnight at 80 °C, knife-milled, and sieved to the size of ~2.0–2.8 mm (7–14 mesh). The milled samples were rod shaped with an average length of ~2–5 mm. Our rice straw samples contained 30.3 wt% glucan, 15.6 wt% xylan, 0.1 wt% arabinan, 21.5 wt% Klason lignin, 2.1 wt% acid-soluble lignin, 13.2 wt% ash (~67 wt% SiO₂ in ash) and 17.2 wt% others (extractives, proteins, and wax). The composition of the hemp hurd was 31.6 wt% glucan, 9.6 wt% xylan, 1.1 wt% arabinan, 25.2 wt% Klason lignin, 3.1 wt% acid-soluble lignin, 4.9 wt% ash, 24.5 wt% others. Previous studies reported others from hemp hurd in the range of 8–26 wt% (Das et al., 2020; Stevulova et al., 2015). The amount of others in hemp hurd varied based on cultivars (Das et al., 2020) and growth conditions (Thomsen et al., 2005). Avicel PH101 was obtained from Sigma Aldrich (USA). Avicel was used as a model native cellulose, which contained both crystalline and amorphous fractions (Sathitsuksanoh et al., 2012). Regenerated amorphous cellulose (RAC) was synthesized as described elsewhere (Sathitsuksanoh et al., 2011a, 2011c). RAC was used as a model amorphous cellulose (Hossain et al., 2019). All chemicals and reagents were purchased from VWR (USA) and Sigma Aldrich (USA) as analytical grade and used as received unless otherwise noted.

2.2. Alkaline pretreatment

The alkaline pretreatment was conducted by dilute NaOH at ambient



Scheme 1. Polyhydroxyalkanoates production from rice straw and hemp hurd.

temperature. The NaOH concentration, pretreatment time, and lignocellulose loading in the range of 1–10 wt% NaOH (in water, $\rho \sim 1.0$ – 1.1 g/mL), 0.5–6.0 h, and 1–30 wt% lignocellulose loading (in dilute NaOH solution) were varied to determine the optimal pretreatment condition (see Supporting Information for detail). Briefly, lignocellulose samples were mixed with the dilute alkaline solution to the desired lignocellulose loading. For example, pretreatment at 10 wt% lignocellulose loading was conducted by adding ~ 1.0 g rice straw with 9.0 g of the dilute NaOH solution. Then, the mixture was stirred by a magnetic stir bar at 600 rpm at ambient temperature at various pretreatment durations. After pretreatment, the pretreated solid was separated by vacuum filtration using Whatman filter paper No.1. The pretreated solid was left on the filter paper and washed by DI water (~ 100 mL) until the pH of filtrate dropped to ~ 6.5 – 7.0 . The resulting samples were separated into two parts: (1) the first part was freeze-dried for compositional analysis and structural characterization, and (2) the second part was stored at 4°C and used for enzymatic hydrolysis and fermentation. Solid recovery, glucan retention, xylan removal, and lignin removal were calculated by Eqs. 1–4:

$$\text{Solid recovery (wt\%)} = \left[\frac{\text{solid}_{\text{before}} - \text{solid}_{\text{after}}}{\text{solid}_{\text{before}}} \right] \times 100 \quad (1)$$

$$\text{Glucan retention (wt\%)} = \left[\frac{\text{glucan}_{\text{before}} - \text{glucan}_{\text{after}}}{\text{glucan}_{\text{before}}} \right] \times 100 \quad (2)$$

$$\text{Xylan removal (wt\%)} = \left[\frac{\text{xylan}_{\text{before}} - \text{xylan}_{\text{after}}}{\text{xylan}_{\text{before}}} \right] \times 100 \quad (3)$$

$$\text{Lignin removal (wt\%)} = \left[\frac{\text{lignin}_{\text{before}} - \text{lignin}_{\text{after}}}{\text{lignin}_{\text{before}}} \right] \times 100 \quad (4)$$

where $\text{solid}_{\text{before}}$, $\text{glucan}_{\text{before}}$, $\text{xylan}_{\text{before}}$, and $\text{lignin}_{\text{before}}$ represent the mass of solid, glucan, xylan, and lignin before pretreatment. $\text{solid}_{\text{after}}$, $\text{glucan}_{\text{after}}$, $\text{xylan}_{\text{after}}$, and $\text{lignin}_{\text{after}}$ indicate the mass of solid, glucan, xylan, and lignin after pretreatment.

The 10 wt% solid loading, 2.5 wt% NaOH, and 6 h was found as the optimal pretreatment condition as shown in high glucan digestibility (Fig. S1–S3, see Supporting Information for detail). Therefore, this pretreatment condition was applied to hemp hurd and used to construct the mass balance.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in 50 mM phosphate buffer, pH 4.5 (6.80 g of potassium dihydrogen phosphate in 1 L water with a solid loading of 30 g glucan/L (70 g biomass/L) in 50 mL centrifuge tubes. The 30 g glucan/L solid loading was selected for a high glucose concentration to target a high titer of mcl-PHAs from subsequent fermentation. A rotary oven (Hybaid Micro-4, Hybaid limited, United Kingdom) was used to control the hydrolysis temperature. Stir bars were custom-made using a quartz coated steel rod (5 mm inner diameter x 50 mm length) to ensure uniform mixing of the solid slurry and enzymes. Hydrolysis experiments were performed in a rotary shaker at 50°C at 250 rpm. The enzyme loading was 30 mg protein/g glucan, unless otherwise noted. The Novozyme® cellulase (Ctec 2, protein concentration: 188 mg protein/mL, 0.74 FPU/mg protein) to hemicellulase (Htec 2, protein concentration: 190 mg protein/mL) ratio of 9/1 by volume was used or otherwise described. The enzyme ratio was found to be optimal to release sugars (Liu et al., 2018; Yaegashi et al., 2017). The protein concentration and cellulase activity (filter paper unit, FPU) of the enzymes were measured by the bicinchoninic acid (BCA) assay using BSA as protein standard and the filter paper assay as described elsewhere (Adney and Baker, 2008; Socha et al., 2014). The protein concentrations and cellulase activity values agreed with previous studies

(Alvira et al., 2011; Cannella et al., 2012; Zhao et al., 2015). All experiments were performed in triplicate, and data represented the mean with the standard deviation $< 9\%$. Enzymatic glucose and xylose yields were calculated by Eqs. (6–7):

$$\text{Enzymatic glucose yield (\%)} = \frac{\text{glucose released by enzymes (g)}}{\text{glucose equivalent in pretreated solid (g)}} \times 100 \quad (6)$$

$$\text{Enzymatic xylose yield (\%)} = \frac{\text{xylose released by enzymes (g)}}{\text{xylose equivalent in the pretreated solid (g)}} \times 100 \quad (7)$$

2.4. Fermentation of sugar hydrolysates from pretreated biomass

2.4.1. Bacterial strain, media, and cultivation

Pseudomonas putida KT2440 (ATCC 47054) was used in this study. *P. putida* KT2440 catabolizes glucose from the native, cyclically-operating Entner-Doudoroff (ED) pathway (Sánchez-Pascuala et al., 2019) to produce medium-chain-length polyhydroxyalkanoates (mcl-PHAs) (Scheme S1, Supporting Information). *P. putida* is a nonpathogenic, generally-regarded-as-safe, gram-negative soil bacterium that thrives in contaminated industrial pollutant sites. Therefore, *P. putida* is tolerant to inhibitors and was selected for this study (Dong et al., 2019; Horlamus et al., 2019). *P. putida* KT2440 was grown at 30°C in minimal medium: NH_4Cl 0.1 g/L (as a nitrogen source), KH_2PO_4 1.5 g/L, Na_2HPO_4 3.54 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01 g/L, ammonium ferric citrate 0.06 g/L, and trace elements (H_3BO_3 0.3 mg/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.2 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 mg/L, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.03 mg/L, $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 0.03 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.02 mg/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.01 mg/L) (Ouyang et al., 2007; Rocha et al., 2008). The limited amount of nitrogen in the minimal medium was intended to trigger the accumulation of mcl-PHAs. For carbon sources, cultures were supplemented with pure glucose (25 g/L as control) and sugar hydrolysates from pretreated biomass. The sugar hydrolysates were diluted to 25–30 g glucose/L prior to fermentation to activate the production and accumulation of mcl-PHAs, unless otherwise noted.

A single colony of *P. putida* KT2440 was first grown at 30°C in a minimal medium for ~ 12 h, as the seed culture. The seed culture (5% v/v) was then inoculated into tubes with 15 mL minimal medium for PHA production. Samples were taken periodically for optical density (OD) and sugar analysis. After 72 h, cell pellets were collected by centrifugation at 6000 rpm for 15 min, resuspended in 1 mL DI water, and centrifuged again at 10,000 rpm for 1 min to remove liquid. Cell pellets were freeze-dried for cell dry weight (DCW) and PHA analysis.

2.4.2. Extraction and quantification of medium-chain-length polyhydroxyalkanoates

Medium-chain-length polyhydroxyalkanoates (mcl-PHAs) were extracted and quantified according to Brandl et al. (1988) One milliliter of chloroform was added to 5–20 mg of lyophilized cells, followed by the addition of 0.85 mL methanol and 0.15 mL concentrated sulfuric acid. The mixture was heated for 2.5 h at 100°C . The samples were first cooled to ambient temperature, then placed on ice following addition of ~ 0.5 mL of water. The resulting slurry was centrifuged at $2000 \times g$ for 5 min, which produced a biphasic solution with chloroform on the bottom and methanol/water on top. The organic phase was removed, transferred to a new tube, and diluted to an organic phase/chloroform ratio of 1/10 or 1/20 (v/v, depending on the amount of collected cells). Samples were filtered through a $0.2\text{-}\mu\text{m}$ polyvinylidene difluoride (PVDF) syringe filter before analysis by Shimadzu QP 2020 Plus Gas chromatography (GC) equipped with mass spectrometry (MS). About 1 μL of sample was separated by GC/MS, which was equipped with a

DB-WAX column (30-m length, 0.32-mm inner diameter, 0.25- μ m film thickness; Agilent, USA). The GC oven temperature was programmed to hold at 80 °C for 5 min before increasing to 170 °C at 20 °C/min. The temperature of the injector was set to 250 °C. Ultra-high purity helium was used as the carrier gas at 5 mL/min. The MS ion source temperature was set to 200 °C with an interface temperature of 250 °C and scan from 50 to 150 *m/z*. External standards, 3-hydroxybutyrate methyl ester, 3-hydroxyhexanoate methyl ester, 3-hydroxydecanoate methyl ester, and 3-hydroxydodecanoate methyl ester, were used to confirm the presence of monomers and quantify their amounts. The yield of mcl-PHAs was calculated based on the glucose content in pretreated lignocellulose-derived hydrolysates.

2.5. Compositional analysis of biomass

The compositions of untreated and pretreated lignocellulose samples were determined by National Renewable Energy Laboratory analytical procedures (Sluiter et al., 2012). All sugar concentrations in the acid-digested solutions were analyzed by Agilent 1100 High-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID) and a diode array detector (DAD). The Aminex HPX-87H column (300 \times 7.8 mm, Bio-Rad®, Hercules, CA, USA) was used to separate sugars at 60 °C with 0.6 mL/min of 4 mM H₂SO₄ as a mobile phase. The concentrations of sugars were determined by the RID signals' peak area (HMF and furfural were determined by DAD signals at 280 nm). All sugars were calibrated against certified standards (Absolute Standards Inc., Hamden, CT, USA).

Based on the mass flow of solid and liquid streams from pretreatment and enzymatic hydrolysis, the mass balance based on 100 g of dry biomass was constructed. The solid streams were presented in the form of solid sugar polymers (glucan, xylan, arabinan, lignin). The liquid streams were presented in the form of sugar monomers (glucose, xylose, and arabinose).

2.6. Characterization of pretreated rice straw and hemp hurd

To determine the physiochemical characteristics of lignocellulose after alkaline pretreatment, the pretreated solids were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR). Untreated lignocellulose samples were used as a control.

2.6.1. Scanning electron microscopy (SEM)

SEM was performed on pretreated solids to determine the changes in morphology. The TESCAN Vega3 SEM (Warrendale, PA, USA) with an energy dispersive X-ray spectrometry (EDS) detector was used. Before performing SEM, pretreated solids were sputter-coated by an SPI-Module™ Sputter Coater (West Chester, PA, USA) using a gold target for 90 s under Argon gas.

2.6.2. X-ray diffraction (XRD)

To determine the degree of crystallinity, X-ray diffraction was performed on pretreated samples using the Bruker D8 (Billerica, MA, USA) with CuK α radiation ($\lambda = 0.15418$ Å). The scanning rate was 0.5 s/step (0.02 step increment), from 10° to 45° unless otherwise noted. The change in the degree of crystallinity of biomass was expressed in terms of the crystallinity index (CrI). The CrI value was calculated based on the Segal method (Segal et al., 1959) using the relationship between the height of the crystalline peak corresponding to (002) lattice plane (I_{002}) and the amorphous region (I_{am}), which was the minimum ($\sim 18^\circ$) between (110) and (002) lattice planes as shown in Eq. (8):

$$CrI = \frac{I_{(002)} - I_{am}}{I_{(002)}} \times 100 \quad (8)$$

2.6.3. Fourier-transform infrared (FTIR) spectroscopy

The changes in the chemical structures of pretreated lignocellulose (bond strength between sugar monomers and lignin-carbohydrates) relative to untreated samples were characterized by the JASCO 4700 FT-IR Spectrometer (Akron, OH, USA) equipped with Attenuated Total Reflection (ATR, Pike Technologies, Madison, WI, USA). The samples were scanned in the spectral range between 400 and 4000 cm^{-1} for 256 scans at 4 cm^{-1} resolution.

3. Results

First, we pretreated rice straw and hemp hurd by alkaline pretreatment at ambient condition using 10 wt% solid loading, 2.5 wt% NaOH, and 6 h. Then we hydrolyzed the pretreated lignocellulose samples to release sugars and presented the resulting sugars to *P. putida* for production of medium-chain-length polyhydroxyalkanoates (mcl-PHAs).

3.1. Mass balance of the ambient alkaline pretreatment of rice straw and hemp hurd, followed by enzymatic hydrolysis

The mass balance showed that alkaline-pretreated rice straw and hemp hurd solids retained ~ 88 – 96 wt% glucan, 56 – 87 wt% xylan, and 61 – 80 wt% lignin (Fig. 1). These high retention results suggested that alkaline treatment retained biomass components in the solids. To assess the alkaline treatment efficiency under the pre-determined optimal condition, we hydrolyzed the pretreated rice straw and hemp hurd with enzymes at a high loading of pretreated solids (~ 90 g pretreated solid/L).

To evaluate the efficiency of alkaline pretreatment, we performed the enzymatic hydrolysis using 30 g glucan/L, three times higher solid loading than that of our control. At this solid loading, initially, the pretreated lignocellulose samples acted like sponges and formed a viscous slurry for 24 h. Afterward, the slurry was thinner. Enzymatic hydrolysis of pretreated solids resulted in 78% glucose and 90% xylose yields from pretreated rice straw and 83% glucose and 83% xylose yields from pretreated hemp hurd after 72 h. As a control, we performed the enzymatic hydrolysis of untreated rice straw and hemp hurd samples at 10 g glucan/L and 30 mg protein/g glucan. We obtained < 15 wt% glucose yield after 72 h enzymatic hydrolysis of untreated biomass with 10 g glucan/L loading (~ 9 wt% rice straw and hemp hurd), which suggested resistance to enzymatic hydrolysis of untreated lignocellulose. The high enzymatic hydrolysis yields of sugars from pretreated lignocellulose suggested that the alkaline treatment at ambient condition facilitated the glucose and xylose release from rice straw and hemp hurd.

Lignin acts as a glue to bind cellulose and hemicellulose together in the plant cell wall. Hence, many researchers developed pretreatment processes to remove lignin for enhanced cellulose accessibility to enzymes and sugar release (Hossain et al., 2021; Lyu et al., 2021; Tian et al., 2020; Wang et al., 2021). On the basis of our mass balance, we obtained $> 78\%$ enzymatic hydrolysis glucose yield from biomass while retaining > 88 wt% glucan and 86 wt% xylan with 20–39 wt% lignin removal after alkaline pretreatment (Table S1). We postulated that the high enzymatic sugar yield by alkaline pretreatment at ambient temperature was a result of (1) moderate lignin removal (20–39 wt%), and (2) modified the chemical structure of lignin. Previous studies by Marcia (2009), Reinoso et al. (2018), and Yuan et al. (2021) demonstrated that a high content of para-coumaric acid (pCA) inhibited enzymatic hydrolysis. Therefore, the lignin removal by alkaline pretreatment at ambient temperature potentially removed pCA and/or modified lignin's chemical structure. As a result, the enzymes did not bind on the lignin to a great extent and resulted in more available enzymes for hydrolysis of cellulose and hemicellulose, thereby increasing enzymatic sugar release. Future studies will study the effect of ambient alkaline pretreatment on removing lignin and ferulic acid/pCA and their correlation with enzymatic hydrolysis yield.

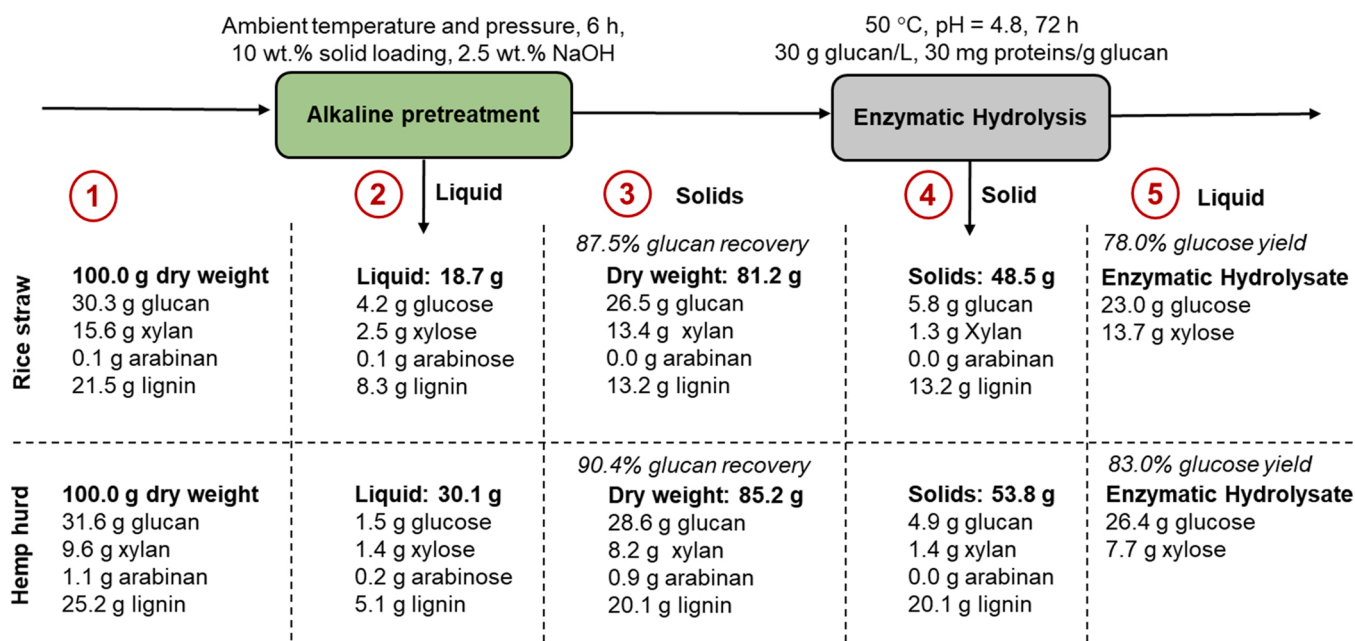


Fig. 1. Mass balance of sugar release from alkaline pretreatment of rice straw and hemp hurd followed by enzymatic hydrolysis: Pretreatment condition: ambient temperature and pressure (~25 °C and 1 atm), 10 wt% solid loading, 2.5 wt% NaOH in water for 6 h. Enzymatic hydrolysis condition: 30 g glucan/L (~92 g pretreated rice straw/L and 89 g pretreated hemp hurd/L), pH 4.8 phosphate buffer (final pH), 30 mg proteins/g glucan. The reported lignin was Klason lignin.

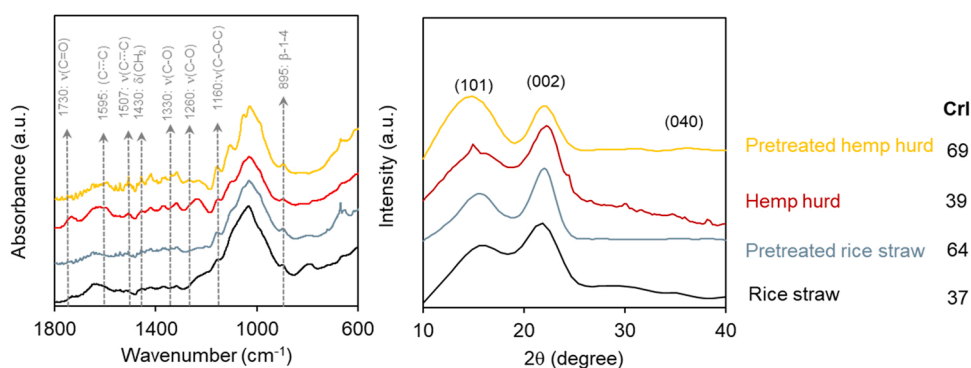


Fig. 2. XRD and FTIR spectra of rice straw and hemp hurd before and after alkaline pretreatment. CrI indicates the crystallinity index.

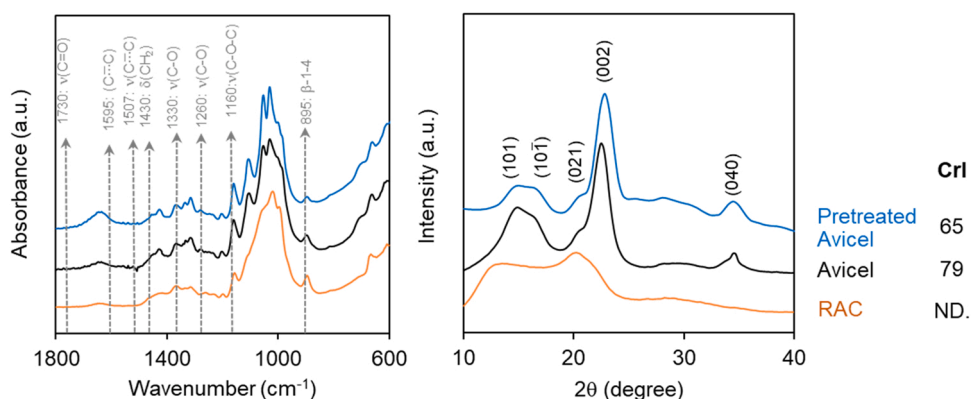


Fig. 3. XRD and FTIR spectra of Avicel before and after alkaline pretreatment. CrI indicates the crystallinity index. RAC indicates regenerated amorphous cellulose as a proxy for amorphous cellulose. ND indicates not determined.

3.2. Characterization of pretreated rice straw and hemp hurd

To identify the chemical structure responsible for the high enzymatic

sugar yields from pretreated biomass at a high glucan loading, we characterized pretreated biomass by XRD and FTIR. As a control, XRD spectra of untreated biomass showed two broad crystalline bands of

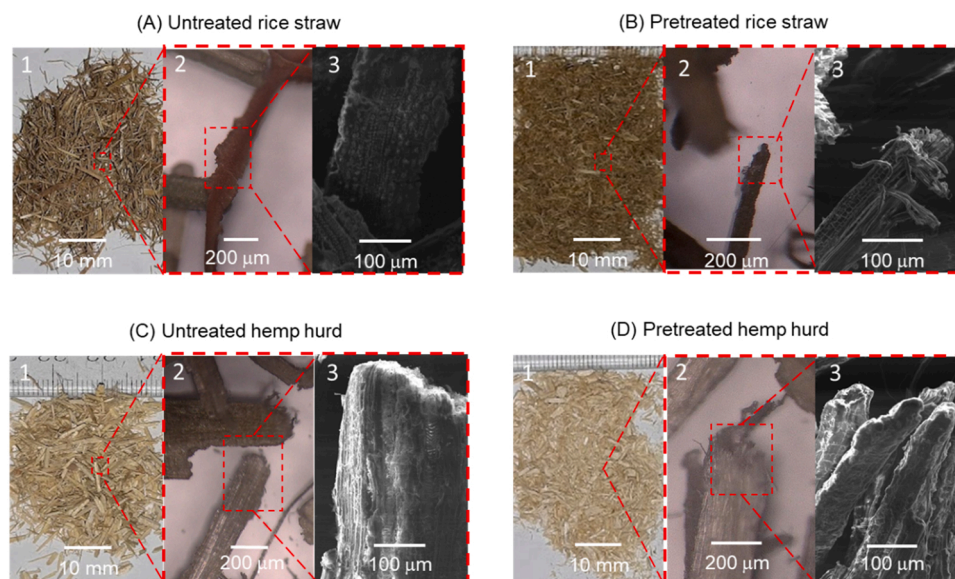


Fig. 4. Morphology of rice straw and hemp hurd before and after alkaline pretreatment. Images 1,2, and 3 were obtained by digital camera, microscope, and scanning electron microscope, respectively.

(101) and (002) crystal planes, similar to reported XRD spectra of rice straw (Kainthola et al., 2021; Li et al., 2012) and hemp hurd (Stevulova et al., 2014; Tyagi et al., 2021). After alkaline treatment, the XRD spectrum of treated biomass showed an increase in (002), which suggested an increase in the degree of crystallinity. We determined the crystallinity index of these samples based on their XRD spectra. The crystallinity index of untreated rice straw and hemp hurd was 37% and 39%, respectively. After pretreatment, the crystallinity of pretreated solids significantly increased to 64% for pretreated rice straw and 69% for pretreated hemp hurd. An increase in crystallinity index after pretreatment came from the removal of amorphous xylan and lignin (Gharpuray et al., 1983; Sathitsuksanoh et al., 2011b).

Next, we used FTIR with pretreated biomass samples to determine the changes in chemical functionality of the C-C polymer backbone and surface functional groups. As a control, FTIR spectra of untreated biomass showed functional features of β -glycosidic linkage, C-O-C stretching in cellulose and hemicellulose, C-O stretching in guaiacyl ring, C-O stretching in syringyl ring, CH_2 bending vibration from cellulose and lignin, aromatic ring stretching in lignin and ketone/aldehyde $\text{C}=\text{O}$ stretching at 895, 1160, 1260, 1330, 1430, 1507 and 1730 cm^{-1} , respectively, similar to previous reports (Lehto et al., 2018; Marchessault and Liang, 1962; Pal et al., 2016; Sills and Gossett, 2012).

Because we expected that NaOH pretreatment would decrease the cellulose crystallinity, we pretreated Avicel with alkali at the same ambient condition we used for biomass (10 wt% solid loading, 2.5 wt% NaOH, 6 h). As a control, the XRD spectrum of Avicel showed five distinct cellulose crystal planes, (101), (10 $\bar{1}$), (021), (002), and (040). The calculated CrI of Avicel was 79%, in agreement with reported values (Sathitsuksanoh et al., 2011b). After pretreatment, the CrI decreased to 65%. The 17% decrease in CrI after pretreatment suggested that alkaline pretreatment decrystallized crystalline cellulose even at ambient temperature. To examine the effect of CrI reduction on glucose yield, we performed enzymatic hydrolysis of pretreated Avicel (Fig. S4). As a control, untreated Avicel yielded 40% glucose after 72 h. The pretreated Avicel yielded 59% glucose after 72 h. These results suggested that ambient alkaline pretreatment decrystallized crystalline cellulose and improved cellulose accessibility to cellulases, which led to increased glucose yield. Overall, these characterization results suggested that partial removals of hemicellulose and lignin and decrystallizing crystalline cellulose by alkaline pretreatment increased the cellulose accessibility to enzymes and facilitated the high sugar release.

To investigate the change in morphology of the rice straw and hemp hurd induced by alkaline pretreatment, we used a microscope and SEM. As a control, untreated rice straw and hemp hurd were rod shaped with an average length of $\sim 2\text{--}5\text{ mm}$ (Fig. 4A1–2 and C1–2). In addition, they had a microfibrillar structure (Fig. 4A3 and C3). Alkaline pretreatment disrupted the fibrous bundles as evidenced by a smaller size of $< 1\text{ mm}$ (Fig. 4B1–2 and D1–2) and frayed and loose fibers at the end (Fig. 4B3 and D3). One reason for loosened and smaller fibers of pretreated rice straw was the 39% lignin removal by alkaline pretreatment (Table S1). Similarly, we observed the loose fibrous structure and smaller particles after alkaline pretreatment of hemp hurd. However, the frayed hemp hurd fibers were larger than the fibers after pretreatment of rice straw. Lignin glues the cellulose microfibrils together. Once the lignin was removed, we partially de-glued the lignocellulose structure and exposed the cellulose microfibrils to attack by alkali. Thus, we postulated that the pretreated hemp hurd samples had larger fiber sizes compared with rice straw because alkaline pretreatment of hemp hurd removed only 20% lignin compared with 40% from pretreated rice straw. Overall, the alkaline pretreatment decreased particle size of pretreated fibers and removed lignin and hemicellulose, which enhanced cellulose accessibility to enzymes and enabled a high enzymatic sugar yield (Fig. 1).

3.3. Fermentation of enzymatically derived sugars into medium-chain-length polyhydroxyalkanoates

To assess the feasibility of using biomass-derived sugars, we presented the sugar hydrolysates of pretreated rice straw and hemp hurd to *P. putida* KT2440. We monitored glucose consumption and optical density during fermentation. Nitrogen is a key element in cell growth. Without sufficient nitrogen, cells cannot produce amino acids and grow. We used a minimal medium with a limited nitrogen source to enable initial cell growth (TCA cycle, Scheme S1) and trigger mcl-PHA accumulation as a carbon source storage during the nitrogen-deficient phase (fatty acid de novo synthesis). As a control, we presented *P. putida* KT2440 with a pure glucose solution. We observed a fast glucose consumption and progressively increased optical density (Fig. 5A) indicative of cell growth. *P. putida* KT2440 grew well in sugar hydrolysates (glucose and xylose) from both rice straw and hemp hurd. The glucose consumption profiles were similar to that of pure glucose (Figs. 5B and C). The optical density for cell growth in the sugar hydrolysates was slightly lower than that in pure glucose after 72 h at a similar glucose

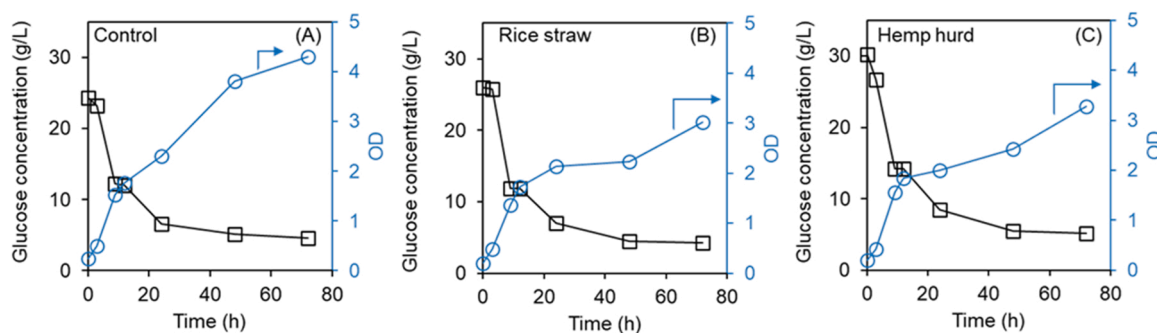


Fig. 5. Growth curves and glucose consumption of *P. putida* KT2440 in different sugar sources in 25 g/L glucose as a control (A), enzyme hydrolysates of pretreated rice straw (B) and pretreated hemp hurd (C) at ~25–30 g glucose/L.

concentration; the lower optical density suggested that pretreated lignocellulose-derived hydrolysates contained inhibitors of cell growth, such as extractives, pectin, oligosaccharides, and soluble phenolics from lignin (Arreola-Vargas et al., 2021; Guarnieri et al., 2017; Horlamus et al., 2019; Liu et al., 2019).

After 72 h fermentation, we harvested the cells, measured the yield of the dry cell weight (DCW), and determined mcl-PHA yield (Fig. 6). As a control, the DCW from growth in pure glucose was 1.67 g/L. The DCWs from both hydrolysates were ~0.89–1.00 g/L, lower than that from pure glucose. Nevertheless, cells grown in biomass-derived sugar hydrolysates accumulated 0.47 g mcl-PHAs/g DCW (0.47 g/L) from pretreated rice straw and 0.69 g mcl-PHAs/g DCW (0.61 g/L) from pretreated hemp hurd, more mcl-PHAs than cells grown on pure glucose (0.36 g mcl-PHAs/g DCW). *P. putida* KT2440 consumed glucose and xylose in hydrolysate for the cell growth. Only glucose involved with mcl-PHAs accumulation. When considering the mcl-PHAs yield per gram of glucose, the glucose control had 0.03 g mcl-PHAs/g glucose. We obtained 0.022–0.024 g mcl-PHAs/g glucose from pretreated rice straw and hemp hurd hydrolysates, respectively, which corroborated the trend of DCW.

When considered mcl-PHA yield per DCW, a glucose control provided 0.36 g mcl-PHAs/g DCW. We obtained 0.47 and 0.69 g mcl-PHAs/g DCW from hydrolysates of pretreated rice straw and hemp hurd, respectively. As suggested earlier, the lower DCW from pretreated biomass-derived sugar hydrolysates compared with that of pure glucose at similar glucose concentrations was likely due to inhibitors in the biomass-derived sugar hydrolysates. Thus, cells did not grow as well as they grew in pure glucose. In case of physiological stress, such as shortage of nitrogen and/or presence of inhibitors, *P. putida* repressed cell growth and enhanced accumulation of mcl-PHAs as shown by the

greater yield of mcl-PHAs per DCW from pretreated biomass-derived sugar hydrolysates compared with the yield from growth in pure glucose. We postulated that the lower yield of mcl-PHAs from the hydrolysate of pretreated hemp hurd compared with that from pretreated rice straw was due to the higher concentration of inhibitors that triggered the accumulation of mcl-PHAs (Liu et al., 2019).

The mcl-PHAs resulting from pure glucose growth contained four monomers, 3-hydroxy-hexanoate (C₆), 3-hydroxy-octanoate (C₈), and 3-hydroxy-decanoate (C₁₀), and 3-hydroxy-dodecanoate (C₁₂) with C₁₀ being the dominant monomer (74.5 wt%) (Fig. S4). The mcl-PHAs obtained from pretreated biomass-derived sugar hydrolysates also had four monomers. However, the C₁₂ monomers were more prevalent (15–20 wt%) compared with pure glucose growth (1.9 wt%). These results suggested that (1) *P. putida* produced mcl-PHAs under stress (presence of toxins and/or inhibitors, e.g. extractives, pectin, and oligosaccharides (Guarnieri et al., 2017; Horlamus et al., 2019), (2) the compositions of sugar hydrolysates controlled the composition of mcl-PHAs, and (3) biomass-derived sugars enabled the formation of high carbon number monomers that have enhanced mcl-PHAs properties (e.g., ductility and malleability) (Gopi et al., 2018).

4. Discussion

Efficient sugar release from lignocellulose under a mild pretreatment condition is a major challenge in creating profitable biorefineries and facilitating bioeconomy (Den et al., 2018; Islam et al., 2020; Lynd et al., 1999; Seidl and Goulart, 2016; Wyman, 1999; Yang et al., 2018; Zheng et al., 2009). We demonstrated a chemical-biological upcycling strategy for medium-chain-length polyhydroxyalkanoates (mcl-PHAs) from rice straw and hemp hurd. We combined alkaline pretreatment at ambient

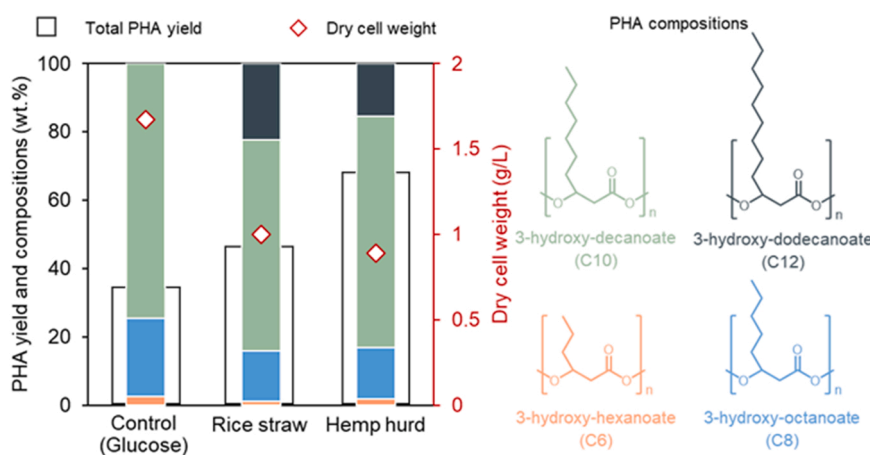


Fig. 6. Yields and compositions of medium-chain-length polyhydroxyalkanoates (mcl-PHAs) from different sugar sources. The mcl-PHA yield was calculated from mcl-PHA weight percentage in dry cell weight. See Fig. S4 for GC chromatogram and MS trace.

conditions, enzyme hydrolysis, and bacterial fermentation. The ambient alkaline pretreatment enabled enzymatic sugar release of 78–90% at a 9 wt% solid loading (~89–92 g pretreated biomass/L). The high sugar yield at a high solid loading and low-temperature operation minimized cost by eliminating the necessity of concentrating and detoxifying the sugars for downstream conversion. The resulting glucose was a direct precursor for mcl-PHAs production.

Our most significant finding was the high sugar retention in the pretreated solids and sugar release by enzymes at a high solid loading from alkaline pretreatment at ambient conditions. Pretreatment at ambient temperature and pressure retained xylan and glucan in the pretreated solid, which eliminated the need for sugar recovery from a dilute alkaline solution for further upgrading. In addition, the ambient alkaline pretreatment partially removed hemicellulose (~15 wt%) and lignin (20–39 wt%) and reduced particle size, thereby increasing cellulose accessibility to enzymes and enabling a high 78–90% sugar yield from rice straw and hemp hurd at high solid loading (90 g pretreated solid/L).

Table S2 shows selected alkaline pretreatment processes for lignocellulose. High-temperature alkaline pretreatment has been used to fractionate labile xylan into the dilute alkaline solution, (Molaverdi et al., 2021; Shi et al., 2021; Zhao et al., 2008) a procedure that requires a separation and concentration step to recover soluble xylan oligomers and xylose. Sodium hydroxide, (Chen et al., 2013; Jung et al., 2020) sodium carbonate, (Mirmohamadsadeghi et al., 2016) ammonium salts/agents, (Chundawat et al., 2020) and calcium hydroxide (lime) (Gu et al., 2015) are common alkali agents (catalysts) for alkaline pretreatment of lignocellulose. A high temperature (60–180 °C) and/or pressure (2–17 atm) is typically used to facilitate the dissolution of hemicellulose and/or lignin, enhance cellulose accessibility to enzymes, and maximize sugar release with short reaction times (Kim et al., 2016). For example, Chen et al., 2013 pretreated corn stover with NaOH at temperatures from 60° to 130 °C, solid loadings of 0.01–0.1 g NaOH/g dry biomass, for 30–120 min. Elevated temperature removed lignin and xylan, which enhanced the cellulose accessibility to enzymes, as evidenced by an increased enzymatic glucan digestibility. However, the high temperature comes with the processing and environmental costs of burning fossil fuels.

The high sugar yield at a high solid loading for enzymatic hydrolysis eliminated a sugar concentration step for subsequent fermentation because we obtained >78% sugar yield from 4 g biomass/g NaOH (alkali strength), a yield greater than reported for many studies (Heggset et al., 2016; Kim and Han, 2012; Molaverdi et al., 2021). Alkali strength is an important indicator for the alkaline pretreatment efficiency. The high enzymatic hydrolysis yield at a high alkali strength indicates the high pretreatment efficiency. Although Jung et al. (2020) and Chen et al. (2013) achieved >81% glucose yield and >69% xylose yield from pretreated corn stover and Miscanthus at >10 (w/w) alkali strength, they used temperatures greater than 121 °C. The heat supply to reach 120 °C typically comes from burning fossil fuels (natural gas), which generates CO₂ and contributes to global warming. In addition, high temperature promotes formation of degradation products (i.e., furans), common inhibitors of bacterial fermentation. As a result, the aforesaid studies required a detoxification step before fermentation. The detoxification step adds to the cost of the process.

Another significant finding was the ability to use glucose resulting from pretreated lignocellulose-derived hydrolysates to produce mcl-PHAs. We pretreated lignocellulose at ambient conditions to minimize the formation of fermentation inhibitors. However, the lignocellulose-derived hydrolysates still showed some inhibition of cell growth, as evidenced by a lower dry cell weight (DCW) compared with that of pure glucose. Surprisingly, although we obtained lower DCWs from pretreated lignocellulose-derived hydrolysates, their yields of mcl-PHAs were greater than the yield from pure glucose.

These fermentation results suggested that lignocellulose-derived hydrolysates triggered the accumulation of mcl-PHAs. We postulated

that *P. putida* responded to growth limitation (N-deficiency) (Wongsirichot et al., 2020, 2021) and physiological stress (hydrolysate derived inhibitors) (Lenz and Marchessault, 2005) by repressing the TCA cycle and triggering de novo fatty acid synthesis to produce mcl-PHAs (Fig. 7). As a result, we obtained 0.47–0.61 g mcl-PHAs/L from hydrolysates of pretreated rice straw and hemp hurd samples.

Our findings provide a new perspective on lignocellulose upcycling to mcl-PHAs. Alkaline pretreatment of lignocellulose at ambient conditions for mcl-PHAs production had not been investigated (Table 1). Current upcycling processes for mcl-PHAs use harsh pretreatment conditions (~120 °C) to break lignocellulose recalcitrance and enhance enzymatic sugar yields. For example, Ahn et al. (2015, 2016) and Sindhu et al. (2013) produced poly-3-hydroxybutyrate (PHB) from dilute acid-pretreated rice straw at 121 °C. The PHB contained 4-hydroxybutyrate monomer (C₄); the low carbon number monomers caused the PHB to be brittle because of a rigid structure and low elasticity (Mozejko-Ciesielska et al., 2019). The C₆–C₁₄ monomers of mcl-PHAs have desirable mechanical properties, e.g., elasticity and tensile strength, compared with PHB (Li et al., 2016; Mozejko-Ciesielska et al., 2019). Our results demonstrated the effectiveness of the combined ambient alkaline pretreatment, enzyme hydrolysis, and fermentation for the production of mcl-PHAs from rice straw and hemp hurd. We expect our process to extend to other agricultural wastes and organics from municipal solid waste.

Although lignocellulose upcycling to mcl-PHAs by an ambient alkaline pretreatment process is promising, the yield of mcl-PHAs needs to be improved for commercial feasibility. Future work will focus on identifying the inhibitors in hydrolysates, optimizing fermentation conditions (C/N ratio of the medium), controlling the monomer distribution of the mcl-PHAs (Arreola-Vargas et al., 2021; Ashby et al., 2001), and co-utilizing soluble lignin fractions during fermentation. In addition, *P. putida* KT2440 contains two enzymes, PP_1444 and PP_1170, that convert xylose to xylonate, yet the bacterium cannot use xylose and xylonate for the production of mcl-PHAs. Thus, we will engineer *P. putida* KT2440 to shuffle xylose and/or xylonate to central metabolism to synthesize mcl-PHAs and maximize the utilization of sugars (Bator et al., 2020). In addition, we will develop the techno-economic analysis and life-cycle analysis models of our developed ambient alkali pretreatment and determine the threshold of pretreatment temperature, pretreatment time, (Mosier et al., 2005) and alkali strength in relation to

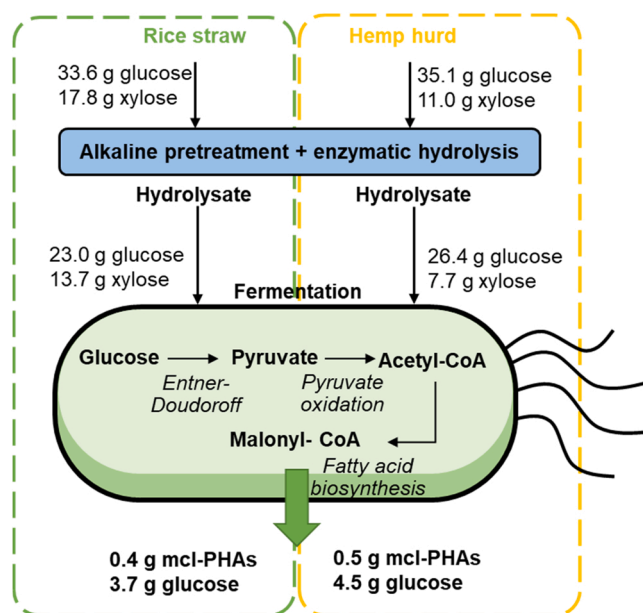


Fig. 7. Mass balance of the overall conversion of rice straw and hemp hurd into medium-chain-length polyhydroxyalkanoates (mcl-PHAs).

Table 1
Selected production of polyhydroxyalkanoates from lignocellulose.

Lignocellulose	Pretreatment	Microorganism	PHA type	PHAs titer (g/L)	References
Corn stover	1% H ₂ SO ₄ , 121 °C for 1 h	<i>Pseudomonas putida</i>	mcl-PHAs	0.25 (1.4 ^a)	(Arreola-Vargas et al., 2021)
Perennial ryegrass	2% NaOH, 120 °C for 10 min Hot water, 120 °C for 10 min	<i>Pseudomonas putida</i>	mcl-PHAs	0.2 0.2	(Davis et al., 2013)
Rice straw	6% H ₂ SO ₄ , 121 °C for 1 h	<i>Cupriavidus necator</i>	PHB	0.4	(Ahn et al., 2015)
	2% H ₂ SO ₄ , 121 °C for 1 h	<i>Cupriavidus necator</i>	P(HB-co-HV)	1.0	(Ahn et al., 2016)
	2% H ₂ SO ₄ , 121 °C for 1 h	<i>Bacillus firmus</i>	PHB	1.7	(Sindhu et al., 2013)
	2.5 wt% NaOH, ambient temperature, 6 h	<i>Pseudomonas putida</i>	mcl-PHAs	0.5	This study
Hemp	2.5 wt% NaOH, ambient temperature, 6 h	<i>Pseudomonas putida</i>	mcl-PHAs	0.6	This study

^a Supplemented sugar hydrolysates with lignin stream from alkaline pretreatment of residual solids after enzymatic hydrolysis

the environmental and processing costs for profitable biorefinery.

5. Conclusion

We upcycled hemp hurd and rice straw to biodegradable medium-chain-length polyhydroxyalkanoates (mcl-PHAs), potential replacements for petroleum-derived plastics. The ambient alkaline pretreatment process retained sugars in the pretreated solids, enhanced cellulose accessibility to enzymes, and facilitated the sugar release by enzymes at high solid loading. Moreover, the resulting lignocellulose-derived sugars were favorable for bacterial fermentation to mcl-PHAs. Production costs are the key limitation to the mcl-PHAs commercialization; our process has a relatively low cost. By showing the ability to produce high-value mcl-PHAs from lignocellulose, our study advances the bioconversion field. Moreover, the ambient alkaline pretreatment can be applied to other bacterial fermentations for other bioproducts, such as isoprenoids and methyl ketones for bio-jet and diesel fuels.

CRediT authorship contribution statement

Md. Anwar Hossain: Conceptualization, Investigation, Writing – original draft. **Logan Mushill:** Investigation. **Mohammad Shahinur Rahaman:** Investigation. **Seth Mains:** Investigation. **Tasia Vickers:** Investigation. **Sarttrawut Tulaphol:** Conceptualization, Visualization, Writing – review & editing. **Jie Dong:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Noppadon Sathitsuksanoh:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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.

Acknowledgment

A part of this material is based upon work supported by the National Science Foundation under Cooperative Agreement No. 1355438 and Internal Research Grant, Office of the Executive Vice President for Research, University of Louisville (USA). This work was performed in part at the Conn Center for Renewable Energy Research at the University of Louisville, which belongs to the National Science Foundation (USA) NNCI KY Manufacturing and Nano Integration Node, supported by ECCS-1542174. The authors would like to thank by Industrial Technology Research Institute (Taiwan) for rice straw samples and Green Remedy, Inc. (Louisville, KY, USA) for hemp hurd samples used in this study. The authors would like to thank Dr. Howard Fried for his valuable comments and suggestions on the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2022.114867](https://doi.org/10.1016/j.indcrop.2022.114867).

References

- Adney, B., Baker, J., 2008. Measurement of Cellulase Activities, Laboratory Analytical Procedure (LAP).
- Ahn, J., Jho, E.H., Nam, K., 2015. Effect of C/N ratio on polyhydroxyalkanoates (PHA) accumulation by *Cupriavidus necator* and its implication on the use of rice straw hydrolysates. *Environ. Eng. Res.* 20, 246–253.
- Ahn, J., Jho, E.H., Nam, K., 2016. Effect of acid-digested rice straw waste feeding methods on the 3HV fraction of bacterial poly (3-hydroxybutyrate-co-3-hydroxyvalerate) production. *Process Biochem.* 51, 2119–2126.
- Alvira, P., Negro, M.J., Ballesteros, M., 2011. Effect of endoxylanase and α -L-arabinofuranosidase supplementation on the enzymatic hydrolysis of steam exploded wheat straw. *Bioresour. Technol.* 102, 4552–4558.
- Armaroli, N., Balzani, V., 2007. The future of energy supply: challenges and opportunities. *Angew. Chem. Int. Ed.* 46, 52–66.
- Arreola-Vargas, J., Meng, X., Wang, Y.-Y., Ragauskas, A.J., Yuan, J.S., 2021. Enhanced medium chain length-polyhydroxyalkanoate production by co-fermentation of lignin and holocellulose hydrolysates. *Green Chem.* 23, 8226–8237.
- Ashby, R.D., Solaiman, D.K.Y., Foglia, T.A., Liu, C.-K., 2001. Glucose/lipid mixed substrates as a means of controlling the properties of medium chain length poly (hydroxyalkanoates). *Biomacromolecules* 2, 211–216.
- Balan, V., Bals, B., Chundawat, S.P.S., Marshall, D., Dale, B.E., 2009. Lignocellulosic biomass pretreatment using AFEX. In: J., M. (Ed.), *Biofuels. Methods in Molecular Biology (Methods and Protocols)*. Humana Press, Totowa, NJ, pp. 61–77.
- Bals, B., Rogers, C., Jin, M., Balan, V., Dale, B., 2010. Evaluation of ammonia fibre expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations. *Biotechnol. Biofuels* 3, 1.
- Bator, I., Wittgens, A., Rosenau, F., Tiso, T., Blank, L.M., 2020. Comparison of three xylose pathways in *Pseudomonas putida* KT2440 for the synthesis of valuable products. *Front. Bioeng. Biotechnol.* 7, 480.
- Bhatia, S.K., Jagtap, S.S., Bedekar, A.A., Bhatia, R.K., Patel, A.K., Pant, D., Rajesh Banu, J., Rao, C.V., Kim, Y.-G., Yang, Y.-H., 2020. Recent developments in pretreatment technologies on lignocellulosic biomass: effect of key parameters, technological improvements, and challenges. *Bioresour. Technol.* 300, 122724.
- Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A., 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renew. Energy* 37, 109–116.
- Brandl, H., Gross, R.A., Lenz, R.W., Fuller, R.C., 1988. *Pseudomonas oleovorans* as a source of poly (β -hydroxyalkanoates) for potential applications as biodegradable polyesters. *Appl. Environ. Microbiol.* 54, 1977–1982.
- Cannella, D., Hsieh, C.-W.C., Felby, C., Jørgensen, H., 2012. Production and effect of aldonic acids during enzymatic hydrolysis of lignocellulose at high dry matter content. *Biotechnol. Biofuels* 5, 26.
- Capolupo, L., Faraco, V., 2016. Green methods of lignocellulose pretreatment for biorefinery development. *Appl. Microbiol. Biotechnol.* 100, 9451–9467.
- Centi, G., Perathoner, S., 2021. Redesign chemical processes to substitute the use of fossil fuels: a viewpoint of the implications on catalysis. *Catal. Today* 387 (1), 216–223.
- Chen, G.-Q., 2009. A microbial polyhydroxyalkanoates (PHA) based bio-and materials industry. *Chem. Soc. Rev.* 38, 2434–2446.
- Chen, Y., Stevens, M.A., Zhu, Y., Holmes, J., Xu, H., 2013. Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. *Biotechnol. Biofuels* 6, 8.
- Chundawat, S.P.S., Sousa, L.C., Roy, S., Yang, Z., Gupta, S., Pal, R., Zhao, C., Liu, S.-H., Petridis, L., O'Neill, H., Pingali, S.V., 2020. Ammonia-salt solvent promotes cellulosic biomass deconstruction under ambient pretreatment conditions to enable rapid soluble sugar production at ultra-low enzyme loadings. *Green Chem.* 22, 204–218.
- Das, L., Li, W., Dodge, L.A., Stevens, J.C., Williams, D.W., Hu, H., Li, C., Ray, A.E., Shi, J., 2020. Comparative evaluation of industrial hemp cultivars: agronomical practices, feedstock characterization, and potential for biofuels and bioproducts. *ACS Sustain. Chem. Eng.* 8, 6200–6210.

- Davis, R., Kataria, R., Cerrone, F., Woods, T., Kenny, S., O'Donovan, A., Guzik, M., Shaikh, H., Duane, G., Gupta, V.K., 2013. Conversion of grass biomass into fermentable sugars and its utilization for medium chain length polyhydroxyalkanoate (mcl-PHA) production by *Pseudomonas* strains. *Bioresour. Technol.* 150, 202–209.
- Den, W., Sharma, V.K., Lee, M., Nadadur, G., Varma, R.S., 2018. Lignocellulosic biomass transformations via greener oxidative pretreatment processes: access to energy and value-added chemicals. *Front. Chem.* 6, 141.
- Dong, J., Chen, Y., Benites, V.T., Baidoo, E.E.K., Petzold, C.J., Beller, H.R., Eudes, A., Scheller, H.V., Adams, P.D., Mukhopadhyay, A., 2019. Methyl ketone production by *Pseudomonas putida* is enhanced by plant-derived amino acids. *Biotechnol. Bioeng.* 116, 1909–1922.
- Fulkerson, W., Judkins, R.R., Sanghvi, M.K., 1990. Energy from fossil fuels. *Sci. Am.* 263, 128–135.
- Galbe, M., Zacchi, G., 2012. Pretreatment: the key to efficient utilization of lignocellulosic materials. *Biomass Bioenergy* 46, 70–78.
- Gharpuray, M.M., Lee, Y.H., Fan, L.T., 1983. Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. *Biotechnol. Bioeng.* 25, 157–172.
- Gopi, S., Kontopoulou, M., Ramsay, B.A., Ramsay, J.A., 2018. Manipulating the structure of medium-chain-length polyhydroxyalkanoate (MCL-PHA) to enhance thermal properties and crystallization kinetics. *Int. J. Biol. Macromol.* 119, 1248–1255.
- Gu, Y., Zhang, Y., Zhou, X., 2015. Effect of Ca(OH)₂ pretreatment on extruded rice straw anaerobic digestion. *Bioresour. Technol.* 196, 116–122.
- Guarnieri, M.T., Franden, M.A., Johnson, C.W., Beckham, G.T., 2017. Conversion and assimilation of furfural and 5-(hydroxymethyl) furfural by *Pseudomonas putida* KT2440. *Metab. Eng. Commun.* 4, 22–28.
- Hazer, D.B., Kılıçay, E., Hazer, B., 2012. Poly(3-hydroxyalkanoate)s: Diversification and biomedical applications: A state of the art review. *Mater. Sci. Eng., C* 32, 637–647.
- Heggsset, E.B., Syverud, K., Øyaas, K., 2016. Novel pretreatment pathways for dissolution of lignocellulosic biomass based on ionic liquid and low temperature alkaline treatment. *Biomass Bioenergy* 93, 194–200.
- Horlamus, F., Wang, Y., Steinbach, D., Vahidinasab, M., Wittgens, A., Rosenau, F., Henkel, M., Hausmann, R., 2019. Potential of biotechnological conversion of lignocellulose hydrolyzates by *Pseudomonas putida* KT2440 as a model organism for a bio-based economy. *GCB Bioenergy* 11, 1421–1434.
- Hossain, M., Rahaman, M., Lee, D., Phung, T., Canlas, C., Simmons, B., Rennecker, S., Reynolds, W., George, A., Tulaphol, S., Sathitsuksanoh, N., 2019. Enhanced softwood cellulose accessibility by H₃PO₄ pretreatment: high sugar yield without compromising lignin integrity. *Ind. Eng. Chem. Res.* 59 (2), 1010–1024.
- Hossain, M.A., Rahaman, M.S., Yelle, D., Shang, H., Sun, Z., Rennecker, S., Dong, J., Tulaphol, S., Sathitsuksanoh, N., 2021. Effects of polyol-based deep eutectic solvents on the efficiency of rice straw enzymatic hydrolysis. *Ind. Crops Prod.* 167, 113480.
- Isikgor, F.H., Becer, C.R., 2015. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polym. Chem.* 6, 4497–4559.
- Islam, M.K., Wang, H., Rehman, S., Dong, C., Hsu, H.-Y., Lin, C.S.K., Leu, S.-Y., 2020. Sustainability metrics of pretreatment processes in a waste derived lignocellulosic biomass biorefinery. *Bioresour. Technol.* 298, 122558.
- Jimenez-Gutierrez, J.M., Verlinden, R.A.J., van der Meer, P.C., van der Wielen, L.A.M., Straathof, A.J.J., 2021. Liquid hot water pretreatment of lignocellulosic biomass at lab and pilot scale. *Processes* 9, 1518.
- Jung, W., Savithri, D., Sharma-Shivappa, R., Kolar, P., 2020. Effect of sodium hydroxide pretreatment on lignin monomeric components of miscanthus × giganteus and enzymatic hydrolysis. *Waste Biomass Valoriz.* 11, 5891–5900.
- Kainthola, J., Shariq, M., Kalamdhad, A.S., Goud, V.V., 2021. Comparative study of different thermal pretreatment techniques for accelerated methane production from rice straw. *Biomass Convers. Biorefin.* 11, 1145–1154.
- Kim, H.W., Chung, C.W., Hwang, S.J., Rhee, Y.H., 2005. Drug release from and hydrolytic degradation of a poly(ethylene glycol) grafted poly(3-hydroxyoctanoate). *Int. J. Biol. Macromol.* 36, 84–89.
- Kim, I., Han, J.-I., 2012. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. *Biomass Bioenergy* 46, 210–217.
- Kim, J.S., Lee, Y.Y., Kim, T.H., 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* 199, 42–48.
- Kurth, N., Renard, E., Brachet, F., Robic, D., Guerin, P., Bourbouze, R., 2002. Poly(3-hydroxyoctanoate) containing pendant carboxylic groups for the preparation of nanoparticles aimed at drug transport and release. *Polymer* 43, 1095–1101.
- Lehto, J., Louhelainen, J., Kłosińska, T., Drożdżek, M., Alén, R., 2018. Characterization of alkali-extracted wood by FTIR-ATR spectroscopy. *Biomass Convers. Biorefin.* 8, 847–855.
- Lenz, R.W., Marchessault, R.H., 2005. Bacterial polyesters: biosynthesis, biodegradable plastics and biotechnology. *Biomacromolecules* 6, 1–8.
- Li, F.-H., Hu, H.-J., Yao, R.-S., Wang, H., Li, M.-M., 2012. Structure and saccharification of rice straw pretreated with microwave-assisted dilute lye. *Ind. Eng. Chem. Res.* 51, 6270–6274.
- Li, Z., Yang, J., Loh, X.J., 2016. Polyhydroxyalkanoates: opening doors for a sustainable future. *NPG Asia Mater.* 8, e265.
- Liu, E., Li, M., Das, L., Pu, Y., Frazier, T., Zhao, B., Crocker, M., Ragauskas, A.J., Shi, J., 2018. Understanding lignin fractionation and characterization from engineered switchgrass treated by an aqueous ionic liquid. *ACS Sustain. Chem. Eng.* 6, 6612–6623.
- Liu, Z.-H., Shinde, S., Xie, S., Hao, N., Lin, F., Li, M., Yoo, C., Ragauskas, A., Yuan, J., 2019. Cooperative valorization of lignin and residual sugar to polyhydroxyalkanoate (PHA) for enhanced yield and carbon utilization in biorefineries. *Sustain. Energy Fuels* 3, 2024–2037.
- López González, L.M., Heiermann, M., 2021. Effect of liquid hot water pretreatment on hydrolysates composition and methane yield of rice processing residue. *Energies* 14, 3254.
- Lynd, L.R., Wyman, C.E., Gerngross, T.U., 1999. Biocommodity engineering. *Biotechnol. Prog.* 15, 777–793.
- Lyu, Q., Chen, X., Zhang, Y., Yu, H., Han, L., Xiao, W., 2021. One-pot fractionation of corn stover with peracetic acid and maleic acid. *Bioresour. Technol.* 320, 124306.
- Marchessault, R.H., Liang, C.Y., 1962. The infrared spectra of crystalline polysaccharides. VIII. Xylans. *J. Polym. Sci.* 59, 357.
- Marcia, Md.O., 2009. Feruloylation in grasses: current and future perspectives. *Mol. Plant* 2, 861–872.
- Mirmohamadsadeghi, S., Chen, Z., Wan, C., 2016. Reducing biomass recalcitrance via mild sodium carbonate pretreatment. *Bioresour. Technol.* 209, 386–390.
- Molaverdi, M., Karimi, K., Mirmohamadsadeghi, S., Galbe, M., 2021. High efficient ethanol production from corn stover by modified mild alkaline pretreatment. *Renew. Energy* 170, 714–723.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Biotechnol. Bioeng.* 96, 673–686.
- Mozejko-Ciesielska, J., Szacherska, K., Marciniak, P., 2019. *Pseudomonas* species as producers of eco-friendly polyhydroxyalkanoates. *J. Polym. Environ.* 27, 1151–1166.
- Nguyen Van, H., Sander, B.O., Quilty, J., Balingbing, C., Castalone, A.G., Romasanta, R., Alberto, M.C.R., Sandro, J.M., Jamieson, C., Gummert, M., 2019. An assessment of irrigated rice production energy efficiency and environmental footprint with in-field and off-field rice straw management practices. *Sci. Rep.* 9, 16887.
- Ouyang, S.P., Liu, Q., Fang, L., Chen, G.Q., 2007. Construction of pha-operon-defined knockout mutants of *Pseudomonas putida* KT2442 and their applications in poly(hydroxyalkanoate) production. *Macromol. Biosci.* 7, 227–233.
- Pal, S., Joy, S., Kumbhar, P., Trimukhe, K.D., Varma, A.J., Padmanabhan, S., 2016. Effect of mixed acid catalysis on pretreatment and enzymatic digestibility of sugar cane bagasse. *Energy Fuels* 30, 7310–7318.
- Pérez, J.A., Ballesteros, I., Ballesteros, M., Sáez, F., Negro, M.J., Manzanares, P., 2008. Optimizing Liquid Hot Water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. *Fuel* 87, 3640–3647.
- Pileidis, F.D., Titirici, M.M., 2016. Levulinic acid biorefineries: new challenges for efficient utilization of biomass. *ChemSusChem* 9, 562–582.
- Rai, R., Boccaccini, A.R., Knowles, J.C., Mordon, N., Salihi, V., Locke, I.C., Moshrefi-Torbati, M., Keshavarz, T., Roy, I., 2011. The homopolymer poly(3-hydroxyoctanoate) as a matrix material for soft tissue engineering. *J. Appl. Polym. Sci.* 122, 3606–3617.
- Reinoso, F.A.M., Rencoret, J., Gutiérrez, A., Milagres, A.M.F., del Río, J.C., Ferraz, A., 2018. Fate of p-hydroxycinnamates and structural characteristics of residual hemicelluloses and lignin during alkaline-sulfite chemithermomechanical pretreatment of sugarcane bagasse. *Biotechnol. Biofuels* 11, 153.
- Ríos-González, L.J., Medina-Morales, M.A., Rodríguez-De la Garza, J.A., Romero-Galarza, A., Medina, D.D., Morales-Martínez, T.K., 2021. Comparison of dilute acid pretreatment of agave assisted by microwave versus ultrasound to enhance enzymatic hydrolysis. *Bioresour. Technol.* 319, 124099.
- Robinson, R., 1996. *The Great Book of Hemp: The Complete Guide to the Environmental, Commercial, and Medicinal Uses of the World's Most Extraordinary Plant*. Inner Traditions/Bear & Co.
- Rocha, R.C.S., da Silva, L.F., Taciro, M.K., Pradella, J.G.C., 2008. Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) with a broad range of 3HV content at high yields by *Burkholderia sacchari* IPT 189. *World J. Microbiol. Biotechnol.* 24, 427–431.
- Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochem.* 40, 3693–3700.
- Sánchez-Pascuala, A., Fernández-Cabezón, L., de Lorenzo, V., Nikel, P.I., 2019. Functional implementation of a linear glycolysis for sugar catabolism in *Pseudomonas putida*. *Metab. Eng.* 54, 200–211.
- Sathitsuksanoh, N., Zhu, Z., Wi, S., Zhang, Y.-H.P., 2011a. Cellulose solvent based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. *Biotechnol. Bioeng.* 108, 521–529.
- Sathitsuksanoh, N., Zhu, Z., Wi, S., Zhang, Y.-H.P., 2011b. Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. *Biotechnol. Bioeng.* 108, 521–529.
- Sathitsuksanoh, N., Zhu, Z., Zhang, Y.-H.P., 2011c. Cellulose solvent-based biomass pretreatment: concentrated phosphoric acid vs. ionic liquid. *Abstr. Pap. Am. Chem. Soc.* 241.
- Sathitsuksanoh, N., Zhu, Z., Zhang, Y.-H.P., 2012. Cellulose solvent-based pretreatment for corn stover and avicel: concentrated phosphoric acid versus ionic liquid [BMIM] Cl. *Cellulose* 19, 1161–1172.
- Satlewal, A., Agrawal, R., Bhagia, S., Das, P., Ragauskas, A.J., 2018. Rice straw as a feedstock for biofuels: availability, recalcitrance, and chemical properties. *Biofuels Bioprod. Biorefin.* 12, 83–107.
- Segal, L., Creely, J.J., Martin Jr., A.E., Conrad, C.M., 1959. An empirical method for estimating the degree of crystallinity of native. *Cellul. Using X-ray Diffractom. Text. Res. J.* 29, 786.
- Seidl, P.R., Goulart, A.K., 2016. Pretreatment processes for lignocellulosic biomass conversion to biofuels and bioproducts. *Curr. Opin. Green Sustain. Chem.* 2, 48–53.
- Sheldon, R.A., 2011. Utilisation of biomass for sustainable fuels and chemicals: molecules, methods and metrics. *Catal. Today* 167, 3–13.

- Sheng, Y., Tan, X., Gu, Y., Zhou, X., Tu, M., Xu, Y., 2021. Effect of ascorbic acid assisted dilute acid pretreatment on lignin removal and enzyme digestibility of agricultural residues. *Renew. Energy* 163, 732–739.
- Shi, S., Guan, W., Bliersch, D., Li, J., 2021. Improving the enzymatic digestibility of alkaline-pretreated lignocellulosic biomass using polyDADMAC. *Ind. Crops Prod.* 162, 113244.
- Sills, D.L., Gossett, J.M., 2012. Using FTIR to predict saccharification from enzymatic hydrolysis of alkali-pretreated biomasses. *Biotechnol. Bioeng.* 109, 353–362.
- Sindhu, R., Silviya, N., Binod, P., Pandey, A., 2013. Pentose-rich hydrolysate from acid pretreated rice straw as a carbon source for the production of poly-3-hydroxybutyrate. *Biochem. Eng. J.* 78, 67–72.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP). Available: (<http://www.nrel.gov/biomass/pdfs/42618.pdf>). Accessed 2019 November 2., Technical Report. NREL/TP-510-42618.
- Socha, A.M., Parthasarathi, R., Shi, J., Pattathil, S., Whyte, D., Bergeron, M., George, A., Tran, K., Stavila, V., Venkatachalam, S., Hahn, M.G., Simmons, B.A., Singh, S., 2014. Efficient biomass pretreatment using ionic liquids derived from lignin and hemicellulose. *PNAS* 111, E3587–E3595.
- Soltanian, S., Aghbashlo, M., Almasi, F., Hosseinzadeh-Bandbafha, H., Nizami, A.-S., Ok, Y.S., Lam, S.S., Tabatabaei, M., 2020. A critical review of the effects of pretreatment methods on the exergetic aspects of lignocellulosic biofuels. *Energy Convers. Manag.* 212, 112792.
- Stevulova, N., Cigasova, J., Estokova, A., Terpakova, E., Geffert, A., Kacik, F., Singovszka, E., Holub, M., 2014. Properties characterization of chemically modified hemp hurds. *Materials* 7, 8131–8150.
- Stevulova, N., Cigasova, J., Purcz, P., Schwarzova, I., Kacik, F., Geffert, A., 2015. Water absorption behavior of hemp hurds composites. *Materials* 8, 2243–2257.
- Thomsen, A.B., Rasmussen, S., Bohn, V., Nielsen, K.V., Thygesen, A., 2005. Hemp Raw Material: The Effect of Cultivar, Growth Conditions and Pretreatment on the Chemical Composition of the Fibres. Risø DTU-National Laboratory for Sustainable Energy Roskilde, Denmark.
- Tian, D., Guo, Y., Hu, J., Yang, G., Zhang, J., Luo, L., Xiao, Y., Deng, S., Deng, O., Zhou, W., Shen, F., 2020. Acidic deep eutectic solvents pretreatment for selective lignocellulosic biomass fractionation with enhanced cellulose reactivity. *Int. J. Biol. Macromol.* 142, 288–297.
- Tulaphol, S., Hossain, M.A., Rahaman, M.S., Liu, L.-Y., Phung, T.K., Rennecker, S., Grisdanurak, N., Sathitsuksanoh, N., 2019. Direct production of levulinic acid in one pot from hemp hurd by dilute acid in ionic liquids. *Energy Fuels* 32, 1764–1772.
- Tulaphol, S., Sun, Z., Sathitsuksanoh, N., 2021. Chapter six - biofuels and bioproducts from industrial hemp. In: Li, Y., Zhou, W. (Eds.), *Advances in Bioenergy*. Elsevier, pp. 301–338.
- Tyagi, P., Gutierrez, J.N., Nathani, V., Lucia, L.A., Rojas, O.J., Hubbe, M.A., Pal, L., 2021. Hydrothermal and mechanically generated hemp hurd nanofibers for sustainable barrier coatings/films. *Ind. Crops Prod.* 168, 113582.
- Wang, F.-L., Li, S., Sun, Y.-X., Han, H.-Y., Zhang, B.-X., Hu, B.-Z., Gao, Y.-F., Hu, X.-M., 2017. Ionic liquids as efficient pretreatment solvents for lignocellulosic biomass. *RSC Adv.* 7, 47990–47998.
- Wang, R., Wang, K., Zhou, M., Xu, J., Jiang, J., 2021. Efficient fractionation of moso bamboo by synergistic hydrothermal-deep eutectic solvents pretreatment. *Bioresour. Technol.* 328, 124873.
- Wongsirichot, P., Gonzalez-Miquel, M., Winterburn, J., 2020. Integrated biorefining approach for the production of polyhydroxyalkanoates from enzymatically hydrolyzed rapeseed meal under nitrogen-limited conditions. *ACS Sustain. Chem. Eng.* 8, 8362–8372.
- Wongsirichot, P., Muanruksa, P., Kaewkannetra, P., Winterburn, J., 2021. Comprehensive optimization of tropical biomass hydrolysis for nitrogen-limited medium-chain polyhydroxyalkanoate synthesis. *Waste Manag.* 128, 221–231.
- Wyman, C.E., 1999. Biomass ethanol: technical progress, opportunities, and commercial challenges. *Annu. Rev. Energy Environ.* 24, 189–226.
- Yaegashi, J., Kirby, J., Ito, M., Sun, J., Dutta, T., Mirsiaghi, M., Sundstrom, E.R., Rodriguez, A., Baidoo, E., Tanjore, D., Pray, T., Sale, K., Singh, S., Keasling, J.D., Simmons, B.A., Singer, S.W., Magnuson, J.K., Arkin, A.P., Skerker, J.M., Gladden, J.M., 2017. *Rhodospiridium toruloides*: a new platform organism for conversion of lignocellulose into terpene biofuels and bioproducts. *Biotechnol. Biofuels* 10, 241.
- Yang, B., Tao, L., Wyman, C.E., 2018. Strengths, challenges, and opportunities for hydrothermal pretreatment in lignocellulosic biorefineries. *Biofuels Bioprod. Bioref.* 12, 125–138.
- Yu, J., Zhang, J., He, J., Liu, Z., Yu, Z., 2009. Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull. *Bioresour. Technol.* 100, 903–908.
- Yuan, Y., Jiang, B., Chen, H., Wu, W., Wu, S., Jin, Y., Xiao, H., 2021. Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis. *Biotechnol. Biofuels* 14, 205.
- Zan, F., Huang, H., Guo, G., Chen, G., 2021. Sulfite pretreatment enhances the biodegradability of primary sludge and waste activated sludge towards cost-effective and carbon-neutral sludge treatment. *Sci. Total Environ.* 780, 146634.
- Zeng, Z.K., Jang, J.C., Shurson, G.C., Thakral, S., Urriola, P.E., 2021. Ammonia fiber expansion increases in vitro digestibility and fermentability of corn distillers dried grains with solubles with or without carbohydrases. *Anim. Feed Sci. Technol.* 273, 114824.
- Zhang, J., Zhang, X., Yang, M., Singh, S., Cheng, G., 2021. Transforming lignocellulosic biomass into biofuels enabled by ionic liquid pretreatment. *Bioresour. Technol.* 322, 124522.
- Zhao, X., Moates, G.K., Elliston, A., Wilson, D.R., Coleman, M.J., Waldron, K.W., 2015. Simultaneous saccharification and fermentation of steam exploded duckweed: improvement of the ethanol yield by increasing yeast titre. *Bioresour. Technol.* 194, 263–269.
- Zhao, Y., Wang, Y., Zhu, J.Y., Ragauskas, A.J., Deng, Y., 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. *Biotechnol. Bioeng.* 99, 1320–1328.
- Zheng, Y., Pan, Z., Zhang, R., 2009. Overview of biomass pretreatment for cellulosic ethanol production. *Int. J. Agric. Biol. Eng.* 2, 51–68.
- Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* 100, 2411–2418.