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Distillable amine-based solvents for effective pretreatment of multiple biomass feedstocks

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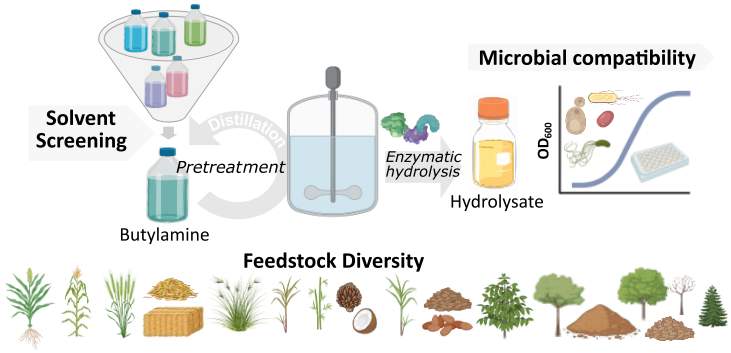
HIGHLIGHTS

- Diverse solvents screened on multiple feedstocks for solvent removal and saccharification.
- Butylamine pretreatment and hydrolysis tested on 22 feedstocks and blends.
- Chemical and lignin composition, including S/G ratio, analyzed for 22 feedstocks.
- Scale-up experiments achieved > 90% glucose release and > 99% butylamine removal.
- Hydrolysates supported microbial growth, showing compatibility with fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

Exploring the potential of advanced distillable solvents as efficient biomass pretreatment agents is critical for biorefineries, enhancing fermentable sugar yields while enabling solvent recovery and recycling without suffering significant losses. Here, we employ distillable amine-based solvents for pretreating a wide range of lignocellulosic feedstocks, aiming to facilitate the industrial release of fermentable sugars from diverse feedstocks through enzymatic hydrolysis. Twenty-two diverse feedstocks, sourced from different geographical regions and representing various biomass categories, were surveyed for chemical (mainly carbohydrates and lignin) and lignin (S, G, and H units) profiles. Several solvents, including ethanolamine, ethanolammonium acetate, butylamine, butylammonium acetate, and triethylamine, were tested for the pretreatment of eight selected biomasses. Among these solvents, butylamine emerged as the most effective due to its favorable sugar release, excellent solvent removal rate, and low boiling point, facilitating solvent recovery and recycling. Extending butylamine pretreatment to all 22 feedstocks demonstrated desirable sugar yields and highly efficient solvent removal in the majority of the biomass sources tested. Agricultural residues and their mixtures showed particularly favorable sugar release. Despite minimal changes in cellulose crystallinity, XRD characterization of sorghum, poplar, and pine before and after butylamine pretreatment showed a decrease in intensity and a slight shift of certain peaks, indicating alterations in cellulose structure. Fourier-transform infrared spectroscopy and thermogravimetric analysis analyses suggested disruption of biomass linkages in hemicellulose and lignin, enhancing enzymatic digestibility. Scale-up experiments of the mixed agricultural feedstocks in a 1 L Parr reactor achieved over 90% glucose liberation and more than 99% butylamine removal, highlighting the scalability of the method. The resulting hydrolysates supported the growth of diverse bacterial and fungal strains, indicating downstream compatibility with commercial fermentation processes. This study presents butylamine as an effective, recoverable pretreatment solvent for a wide range of lignocellulosic feedstocks, offering a promising solution to key biorefinery challenges. The demonstrated scalability and compatibility with various biomass types and blends underscore its potential for industrial application, advancing sustainable biofuel and biochemical production.

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Contents

Abbreviations

FTIR	Fourier-transform infrared spectroscopy
G	Guaiacyl
H	p-hydroxyphenyl
HPLC	High-performance liquid chromatography
LB	Luria-Bertani
pXRD	Powder X-ray diffraction
Py-GC/MS	Pyrolysis-gas chromatography/mass spectrometry
S	Syringyl
TEA	Techno-economic analysis
TGA	Thermogravimetric analysis
YPD	Yeast extract Peptone Dextrose

1. Introduction

Lignocellulosic biomass is one of Earth's most abundant and renewable carbon resources, offering immense potential for the production of biofuels and bioproducts (U.S. Department of Energy, 2024). Converting lignocellulosic sugars into value-added products presents a promising avenue to reduce reliance on petroleum-based resources (Brown et al., 2024; Ding et al., 2024; Long et al., 2024). These structural carbohydrates, primarily composed of cellulose and hemicellulose—the two most abundant biopolymers—combined with lignin, form a biocomposite found in diverse plant sources such as agricultural residues, forestry waste, and dedicated energy crops. Unlike the continued dominance of fermentable sugars derived from food crops, lignocellulosic-based processes avoid competition with food production and can potentially deliver higher carbohydrate outputs per land area (Brown et al., 2024). However, the recalcitrant nature of lignocellulosic biomass poses significant challenges for efficient sugar extraction and utilization.

Over the past several decades, numerous physical, chemical, and biological pretreatment methods, as well as their combination, have been developed to overcome the recalcitrant nature of lignocellulosic biomass (Ding et al., 2024). Notably, most of the leading pretreatment methods fall into the category of chemical methods. A wide range of solvents, including alkalis, dilute acids, organic solvents, ionic liquids, deep and eutectic solvents, have been investigated (Mosier et al., 2005; Zhang et al., 2016; Achinivu et al., 2023; Ullah et al., 2023).

In fact, an ideal pretreatment solvent must be cost-effective, easily recyclable, and work on a wide array of lignocellulosic biomass with high efficiency without undesirable reactions or interferences with enzymatic activity (da Costa Sousa et al., 2009). However, most of the reported pretreatment solvents have shown some shortcomings, including the formation of inhibitor byproducts, the need for special operational conditions like washing the pretreated biomass, and challenges in solvent recovery (Kim and Yoo, 2021). Therefore, it is crucial that pretreatment solvents be readily recovered without requiring additional treatment of the pretreated biomass before enzymatic hydrolysis. In this context, the use of distillable amine-based solvents could be a potential, given the high deconstruction efficiency of amine-based solvents (Ntakirutimana et al., 2022). Although several studies have examined the deconstruction efficiency of amine-based solvents such as alkanolamines (Achinivu et al., 2021), even low-boiling-point butylamine (Tanaka et al., 1985a, b, and c), few have focused on the ideal process by simply recovering the solvents after pretreatment and directly using the pretreated solids for further deconstruction, e.g., enzymatic saccharification, without water washing or other conditioning (**Table 1**).

Another significant challenge in the widespread adoption of most pretreatment methods has been their limited effectiveness across the diverse range of available lignocellulosic feedstocks, contributing to high operational costs and restricted deployment (Li et al., 2015). This feedstock-specific efficacy often necessitates tailored processes for different biomass types, complicating large-scale implementation and reducing the flexibility of biorefinery operations across a wide range of geographical locations. The realization of a biomass pretreatment technology that can generate high yields of fermentable sugars from a diverse range of biomass feedstocks currently available at scale, such as agricultural residues, forestry wastes (hardwoods and softwoods), dedicated bioenergy crops, and mixtures thereof, is a critical step on the journey to realizing a thriving bioeconomy.

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Table 1.
Comparison of major amine-based solvents for lignocellulosic biomass deconstruction.

Solvent	Pretreatment Summary	Major Effects	Solvent Recovery	Ref.
n-Butylamine (n-BA)	Rice straw was pretreated via reflux, autoclave, or vapor circulation with n-BA; solids were either washed or left unwashed depending on method.	Circulation mode gave the highest cumulative sugar yield (70% of theoretical); ~60% delignification; minimal sugar degradation	Evaporation with water	Tanaka et al. (1985c)
Ethylenediamine (EDA)	Corn stover was pretreated with EDA, followed by filtration and water washing.	85.5% lignin removal; 35.2% increase in enzyme adsorption	Washed with water; no recovery	Zhu et al. (2018)
n-Propylamine + ethanol	Corn stover was treated at 140 °C for 40 min; solids were filtered and washed.	81.7% delignification; 83.2% sugar yield; high-quality lignin preserved	Vacuum recovery with ethanol	Tang et al. (2017)
Ethanolamine	Sorghum was treated at 100 °C for 1 h using 75:25 water:ethanolamine; additional water was added for bioconversion without separation	> 99% sugar yield; up to 50% cost reduction in TEA	One-pot; no recovery	Achinivu et al. (2021)
Ethanolammonium acetate	Sorghum was pretreated and solvent was evaporated under vacuum; no washing was applied	High sugar yield; TEA assessed	Vacuum distillation	Achinivu et al. (2024)
Ethanolamine, n-Butylamine, Ethanolammonium acetate, Butylammonium acetate, Triethylamine	22 lignocellulosic feedstocks were pretreated, followed by solvent evaporation under vacuum; no washing was applied	Broad feedstock scope; superior sugar yield; good biocompatibility	Vacuum distillation	**This Study**

This study evaluates the effectiveness of distillable amine-based solvents, including butylamine, for pretreating a broad spectrum of lignocellulosic feedstocks. The feedstocks studied represent diverse categories, including agricultural residues (e.g., corn stover and wheat straw), forestry residues (e.g., poplar and pine), bioenergy crops (e.g., switchgrass and miscanthus), and tropical biomass (e.g., coconut chips and bamboo). The study assessed the performance of various amine-based solvents in terms of fermentable sugar yields and solvent recovery after pretreatment. Screening results revealed the influence of feedstock-specific traits, such as lignin content and composition, on pretreatment outcomes. By integrating compositional data with sugar yield and solvent recovery results as well as structural characterization of representative feedstocks, the work provides insights into how feedstock variability shapes the effectiveness of distillable amine-based solvents, paving the way for identifying optimal solvent–feedstock combinations for next-generation biorefineries.

2. Experimental Section

2.1. Materials

All feedstocks, except for biomass pellets (a pelletized mixture of switchgrass, lodgepole pine, corn stover, and eucalyptus) (Shi et al., 2013), were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) with a 2 mm screen. If the moisture content exceeded 10%, feedstocks were dried at 30 °C in a convection oven (Binder, Bohemia, NY) before grinding. A total of 22 feedstocks were studied here. Details of feedstock sources are summarized in **Table S1** of the supplementary materials, while **Figure S1** presents photographs of the feedstocks in their received state.

The mixed agricultural feedstocks (a.k.a. "Ag mix") were prepared by mixing equal amounts of 2-mm sorghum, corn stover, wheat straw, and hay using a blender with a 40-oz capacity glass container (Model 51BL32, Waring Commercial, Torrington, CT). The Ag mix's chemical composition, measured in triplicate like the individual feedstocks, consisted of 36.1 ± 0.1% glucan, 22.0 ± 0.1% xylan, 19.6 ± 0.0% Klason lignin, 4.9 ± 0.1% acid-soluble lignin, and 6.51 ± 0.67% ash.

Alkanolamines were purchased from Sigma-Aldrich (St. Louis, MO), including ethanolamine (≥99.0% purity), butylamine (99.5% purity), and triethylamine (≥99.0% purity). Ethanolammonium acetate and butylammonium acetate were synthesized as described elsewhere (McCrary et al., 2013; Achinivu et al., 2023).

2.2. Compositional analysis

Raw biomass composition was analyzed using protocols adopted from the National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (LAP) (Sluiter and Sluiter, 2011). Briefly, samples (~0.3 g) underwent two-stage acid hydrolysis: 72% H_2SO_4 at 30 °C for 1 h, followed by dilution with water to 4% and autoclaving at 121°C for 1 h. Acid-insoluble lignin (Klason lignin) was determined gravimetrically using Gooch filtering crucibles (30 mL, medium porosity, DWK Life Sciences Kimble™ KIMAX™, Millville, NJ). Carbohydrates were analyzed using an Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA) with an Aminex HPX-87H column (Bio-Rad, Hercules, CA). Acid-soluble lignin was measured at 240 nm using a Nanodrop 2000 spectrophotometer (Thermo Scientific).

The moisture content of the raw biomass was evaluated by drying 0.5 to 2 g of the sample in porcelain crucibles using a conventional oven (Binder GmbH, Germany) for a minimum of 4 h. The dried biomass was subjected to further heating in a muffle furnace at 575 °C for at least 4 h to determine the ash content. Crucibles were permitted to cool in desiccators, and their weights were recorded between each heating phase.

2.3. Pyrolysis-gas chromatography and mass spectrometry analysis

Ground biomass samples were analyzed using a Pyroprobe 5200 (CDS Analytical, Inc., Oxford, PA) for pyrolysis at 650 °C, as described in the literature (Ralph and Hatfield, 1991). Pyrolysis products were transferred to a Trace GC Ultra and Polaris-Q MS (Thermo Electron Corporation, Waltham, MA) using helium as carrier gas (mean linear velocity: 47.2 cm/sec). The GC was fitted with a Shimadzu SH-Rxi-5Sil column (30 m × 0.25 mm ID × 0.25 µm) and operated in split mode (40 mL/min, split ratio: 10). The GC temperature program ramped from 50 °C (5 min) to 300 °C at 5 °C/min, holding at 300 °C for 5 min. The MS was operated in scan mode, and the ion source was maintained at 200 °C. Pyrolysis products were identified based on their mass spectra using the NIST14 mass spectrum library. Peak areas were calculated using Qual Browser software. Relative quantification was performed by dividing the peak area of each lignin-derived compound by the total peak area, with the S/G ratio determined from the total areas of the S- and G-derived compounds.

2.4. Pretreatment and enzymatic hydrolysis

2.4.1. Glass pressure tube assay

For each assay, 0.3 g of biomass was combined with solvents in a 15 mL Ace glass pressure tube to achieve a 15 wt% biomass loading. The biomass and solvent were mixed, the tubes were sealed, and placed in a pre-heated oil bath at 140 °C for 3 h. After 3 h, the tubes were removed from the oil bath and allowed to cool to ambient temperature before removing caps. The tubes were vacuum-dried at a given temperature (80 °C for ethanolamine, 140 °C for ethanolammonium acetate, 40 °C for butylamine, 120 °C for butylammonium acetate, and 40 °C for triethylamine) in a Binder VDL 115

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oven (Binder, Bohemia, NY) until reaching a constant weight to ensure complete solvent removal, recording weight at each stage.

Following drying, 1.4 mL of deionized water was added, the contents were homogenized, and the pH was adjusted to 5 using sulfuric acid. Then, 0.3 mL of an enzyme mixture containing citrate buffer, sodium azide, and a 9:1 (v/v) cocktail of CTec3/Htec3 (Novozymes, USA) was added to reach a 15 wt% solid loading, a final concentration of 50 mM citrate buffer, 0.1% (w/v) sodium azide, and a protein dosage of 30 mg/g biomass, along with an 8 mm ZrO_2 ball for mixing. Enzymatic saccharification was conducted at 50 °C with constant agitation on an Enviro Genie SI-1200 rotator platform (Scientific Industries, Inc., Bohemia, NY) for 72 h. Sugar release was quantified by HPLC after incubation. Assays were performed in duplicate.

2.4.2. 75 mL Parr Multi-reactor assay

Pretreatment was carried out in the 75 mL Parr Series 5000 multiple reactor system with stir bars. For each assay, 0.9 g of biomass was mixed with solvents at a 15 wt% loading, and the vessels were sealed, stirred at 75 rpm, and heated at 140 °C for 3 h. After pretreatment, the vessels were vacuum-dried at 80 °C, with weights recorded at each stage to assess solvent removal.

To each vessel, 4.2 mL of deionized water was added, the contents were homogenized, and the pH was adjusted to 5 with sulfuric acid. A 0.9 mL mixture containing enzymes, citrate buffer, and sodium azide was added to reach 15 wt% solid loading with a protein dosage of 30 mg/g biomass, 50 mM citrate buffer, and 0.1% sodium azide. Enzymatic saccharification was performed at 50 °C with stirring at 50 rpm for 72 h. Sugar content was determined by high-performance liquid chromatography (HPLC) after incubation. Each experiment was conducted in triplicate.

2.4.3. 1 L Parr reactor assay

The scale-up was performed using a 1 L 4520 Parr bench-top reactor (Parr Instrument Company, Moline, IL) equipped with a three-arm, self-centering anchor stirrer and PTFE wiper blades. Biomass (30 g) was mixed with solvents at 15 wt% loading, and the vessels were sealed, stirred at 75 rpm, and heated at 140 °C for 3 h. After pretreatment, the mixtures were vacuum-dried at 80 °C, with weights recorded at each stage.

Following drying, 140 mL of deionized water was added to rehydrate the solids, and the pH was adjusted to 5 with sulfuric acid. A 30 mL mixture containing enzymes (30 mg of 9CTec3/HTec3 per g biomass) and citrate buffer was added to reach a 15 wt% solid loading. The mixture was incubated at 50 °C with stirring at 50 rpm for 72 h, and sugar release was measured by HPLC. Each experiment was conducted in triplicate.

2.4.4. Determination of solvent removal

Solvent removal was determined using a gravimetric method to calculate the percentage of solvent removed based on the initial amount of solvent added during the pretreatment process. The initial mass of solvent ($m_{solvent,\ initial}$) added during pretreatment was recorded. After vacuum drying, the remaining solvent in the pretreated biomass was determined by subtracting the mass of the dry biomass before pretreatment ($m_{dry\ biomass,\ initial}$) from the mass of dried biomass after pretreatment ($m_{biomass,\ final}$). The amount of solvent removed ($m_{solvent,\ removed}$) was calculated by subtracting the amount of solvent remaining in the biomass after vacuum drying from the total solvent initially added ($m_{solvent,\ initial}$). The solvent removal was estimated following **Equation 1**:

$$Solvent\ removal\ (\%) = \frac{m_{solvent,initial} - (m_{biomass,final} - m_{dry\ biomass,initial})}{m_{solvent,initial}} \times 100 \quad \text{Eq. 1}$$

2.5. Characterization of biomass before and after butylamine pretreatment

2.5.1. Fourier-transform infrared spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectra were collected for untreated samples and samples pretreated with butylamine after solvent removal using a Bruker VERTEX 70/80 system (Bruker Optics, Billerica, MA). A germanium probe tip was used to ensure direct contact with the sample. Each spectrum was acquired by averaging 32 scans at a resolution of 4 cm^{-1}. Spectral data were processed using OPUS software, with baseline correction and normalization applied.

2.5.2. Powder X-ray diffraction analysis

Powder X-ray diffraction (pXRD) analysis was performed on untreated and butylamine-pretreated biomass samples without additional milling or processing. Data were collected at room temperature using a Rigaku MiniFlex 6G bench-top XRD instrument (Rigaku Corp., Japan) equipped with a Cu-Kα source and a HyPix-400MF hybrid pixel array detector. The crystallinity index was calculated from the crystalline and amorphous peak areas of the diffraction patterns using the Segal method (**Eq. 2**) (Segal et al., 1959).

$$CrI\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad \text{Eq. 2}$$

where I_{002} is the intensity of the (002) crystalline plane and I_{am} is the minimum intensity between (002) and (101) peaks, located around 18°.

2.5.3. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was conducted using a Mettler Toledo TGA/DSC 3^+ STAR^e system with a high-throughput autosampler (Mettler-Toledo, Inc., Columbus, OH). Samples (approximately 5 mg) were loaded into 150 µL alumina pans and analyzed under a nitrogen flow rate of 50 mL/min. The temperature was initially increased from 25 to 75 °C at a rate of 10 °C/min, held at 75 °C for 30 min, and then raised from 75 to 725 °C at the same rate of 10 °C/min. Mass loss was continuously recorded, and the weight-loss rate was determined from the first derivative (differential thermogravimetry) of the TGA curves using the STAR^e software suite. The thermal stability and decomposition behavior of untreated and pretreated samples were compared based on decomposition temperature and weight-loss percentage.

2.6. Toxicity assessment of butylamine-pretreated Ag mix hydrolysate

The toxicity of the hydrolysate from the enzymatic hydrolysis of butylamine-pretreated Ag mix was assessed by evaluating its effects on microbial growth and sugar consumption. Filtered hydrolysate supplemented with 10 g/L yeast extract was added to the culture medium in varying concentrations. Optical density and sugar concentration were measured as response variables. Optical density was measured at 600 nm using hydrolysate as the blank, with a well sacrificed for each sample point. For bacterial strains, hydrolysate was diluted in Luria-Bertani (LB) medium, while for yeast strains, yeast extract, peptone, and dextrose (YPD) medium was used as the diluent. LB and YPD without hydrolysate were used as controls. The microbial hosts tested included engineered strains of *Escherichia coli* (JBEI-227476) (Carruthers et al., 2023), *Pseudomonas putida* (IY1449) (Banerjee et al., 2024), and *Saccharomyces cerevisiae* (GAL80L-PPATF1) (Kim et al., 2021), as well as wild-type strain *Rhodosporidium toruloides* (IFO0880) (Coradetti et al., 2018). These strains were selected for their established roles in bio-based production processes and relevance to industrial biomanufacturing.

Experiments were conducted in 24-well plates with a working volume of 5 mL and an inoculum size of 10% (v/v). Cultures were incubated at 30 °C at 200 rpm in a humidity-controlled environment. Bacterial strains were grown in LB-based media, while yeast strains were cultivated in YPD-based media.

2.7. High-performance liquid chromatography analysis

Sugar content in hydrolysates was analyzed using an Agilent Infinity 1260 HPLC equipped with an Aminex HPX-87H column. The mobile phase was 4 mM sulfuric acid at 0.6 mL/min, with a column temperature of 60 °C and a 20-min run time. A refractive index detector was maintained at 35 °C. Peak areas were integrated and compared to calibration curves made with pure glucose and xylose standards. Due to potential co-elution of xylose, galactose, and mannose under the selected column and conditions, which are commonly used for carbohydrate analysis in solutions containing acids,

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alcohols, and metabolic byproducts within a pH range of 1 to 3, mannose from softwoods like pine and minor amounts of galactose were detected as xylose. While specialized carbohydrate analysis columns could achieve better separation and quantification, reporting all hemicellulose-derived monomeric sugars as xylose does not impact the study's conclusions because detailed sugar composition analysis is beyond the study's scope, and only a few softwood samples contain notable amounts of mannose or galactose.

2.8. Statistical analysis

OriginPro 2020b was used for Pearson correlation analysis. The pretreatment and enzymatic hydrolysis in different-scale reactors were analyzed for statistical significance using one-way ANOVA followed by Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1. Composition of various lignocellulosic feedstocks

The chemical composition of lignocellulosic biomass, particularly the proportions of carbohydrates and lignin, is crucial for bioconversion processes. Carbohydrate content sets the limit for the maximum extractable sugars, whereas lignin content influences biomass deconstruction by acting as a physical barrier and interacting with lignocellulose-hydrolyzing enzymes (Chen et al., 2023b and 2024). **Figure 1a** shows the chemical composition of diverse lignocellulosic feedstocks sourced from various geographical and climatic regions. These feedstocks can be categorized into several groups based on their sources and purposes, including agricultural residues (corn stover and wheat straw), forestry residues (poplar and pine), energy crops (switchgrass and miscanthus), and additional waste streams like nut residues and oilseed crops, representing a comprehensive spectrum of materials for bioenergy production.

The composition of these biomass sources varies but is related to the biological origin (type of plant or tree) (Ding et al., 2024). Generally, coniferous species (softwoods) have higher cellulose content (40–45%), higher lignin content (26–34%), and lower pentosan content (7–14%) compared to deciduous species (hardwoods) (cellulose 38–49%, lignin 23–30%, and pentosans 19–26%) (Rowell et al., 2005). However, the hardwood hybrid poplar employed here has higher cellulose content (45.0% glucan) than softwood pine (33.1% glucan) (Rowell et al., 2005). Grass biomass, exemplified by corn stover, typically contains lower lignin content (18.5% Klason lignin) than woody biomass (23.9% for poplar and 33.0% for pine). These compositional data align with the range reported in the Idaho National Laboratory (INL) Bioenergy Feedstock Library (Idaho National Laboratory, 2016). Notably, coconut chips, almond hulls, and pongamia seed cake display markedly lower carbohydrate content compared to other lignocellulosic biomass sources. Coconut chips, derived from the coconut husk, naturally possess a higher lignin content (43.5%) compared to carbohydrates. The high lignin content results in a lower proportion of glucan (24.8%) and xylan (11.5%) in the overall composition. In contrast, the mass closure of almond hulls and pongamia seed cake—summing carbohydrates, lignin, and ash—is lower than other feedstocks, indicating the presence of undetected components like proteins and fatty oils (Muktham et al., 2016; DePeters et al., 2020).

In addition to organic components like carbohydrates and lignin, biomass contains inorganic materials (ash), which can be structural or extractable.

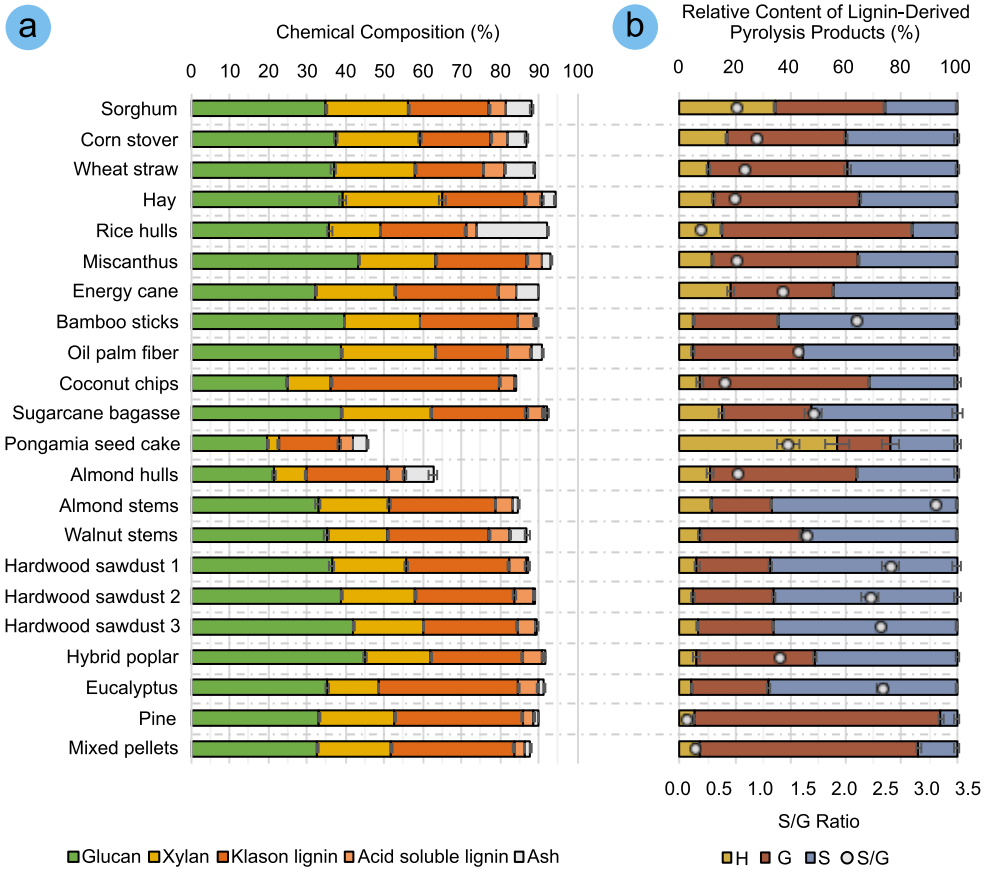

Fig. 1. Chemical and lignin composition of various feedstocks. **(a)** Chemical composition includes carbohydrates, Klason lignin, acid-soluble lignin, and ash content. Bars represent mean values from triplicate measurements, and error bars indicate standard deviations. **(b)** Lignin composition, categorized into syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) units, was determined using Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS). Relative quantification was based on the peak area of each lignin-derived compound relative to the total chromatogram peak area. Bars represent the average of duplicate measurements, with error bars indicating standard deviations.

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Structural ash is bound within the biomass structure, while extractable ash can be removed by washing or extraction, often due to soil contamination (Chen et al., 2023b). As shown in **Figure 1a**, agricultural residues generally have higher ash content than forestry residues, as agricultural crops accumulate more minerals from soil and fertilizers during their shorter growth cycles. Harvesting methods for agricultural residues can also increase ash content due to soil contamination (Puri et al., 2024). For instance, rice hulls have a notably high ash content (18.1%), consistent with literature reports (Puri et al., 2024).

Lignin is one of the major factors influencing the deconstruction of lignocellulosic biomass. Moreover, the monomer composition of lignin, specifically the distribution of hydroxyphenyl (H), guaiacyl (G), and syringyl (S) subunits, can influence biomass recalcitrance depending on the biomass type and the pretreatment used for deconstruction (Ragauskas et al., 2014; Li et al., 2016). For example, a high lignin S/G ratio in eucalyptus biomass is associated with improved cell wall deconstruction and enhanced hydrolysis of cellulose after ionic liquid pretreatment (Papa et al., 2012). However, no correlation could be observed between the lignin S/G ratio and the release of simple sugars from alfalfa and barley biomass after acid and hot water pretreatments, respectively (Chen and Dixon, 2007; Shafiei et al., 2022). In this context, the lignin composition of various feedstocks was analyzed using Py-GC/MS (**Fig. 1b**). As commonly observed, hardwood lignin consists primarily of S and G phenylpropanoid units, with a small proportion of H units. In contrast, softwood species, such as pines, mainly contain G-lignin units, while grasses have lignin composed of S, G, and H units (Happs et al., 2021). The trace amount of S-derived products observed in pine biomass is likely due to minor contamination, possibly originating from the biomass supplier or during sample handling, since gymnosperms are not known to produce S lignin units (Boerjan et al., 2003; Weng and Chapple, 2010).

3.2. Screening of different amine-based solvents on multiple feedstocks

Recent studies have highlighted the effectiveness and versatility of alkanolamines (**Table S2**), such as ethanolamine, in biomass pretreatment, positioning them as a promising solvent group for deconstructing lignocellulosic biomass in commercial biorefineries (Achinivu et al., 2021; Ntakirutimana et al., 2022). When combined with organic acids, these solvents form protic ionic liquids like ethanolammonium acetate, which have demonstrated improved lignin removal, higher glucose yields, and the potential for solvent recovery *via* distillation (Achinivu et al., 2023). Additionally, low-boiling-point amines, such as butylamine, have long been recognized for their superior pretreatment performance, including enhanced reducing sugar yields, delignification, carbohydrate preservation, and solvent recyclability (Tanaka et al., 1983, 1985a, b, and c). Triethylamine, another low-boiling-point amine, has also been blended with ethanol for enzymatic saccharification and high-quality lignin production (Sun et al., 2010). In this study, we examined ethanolamine, butylamine, their corresponding protic ionic liquids (ethanolammonium acetate and butylammonium acetate), as well as triethylamine, for their effectiveness in deconstructing diverse lignocellulosic biomass and solvent recovery. These solvents were tested on a diverse range of lignocellulosic feedstocks, including sorghum, hay, rice hulls, oil palm fiber, coconut chips, hardwood sawdust, and mixed four feedstocks—switchgrass, lodgepole pine, corn stover and eucalyptus—in pellet form (mixed pellets) (Shi et al., 2013), representing tropical, temperate, agricultural, and forestry waste materials.

Solvent removal efficiency post-pretreatment and sugar yields from enzymatic hydrolysis of pretreated biomass varied to different extents with respect to the feedstocks and solvents tested (**Fig. 2**). Ethanolamine yielded high glucose levels for most feedstocks, particularly sorghum (83.3%) and oil palm fiber (81.7%), while rice hulls (57.5%) and mixed pellets (58.4%) exhibited lower yields. A similar trend was observed for xylose yields, although these were considerably lower than the glucose yields for all feedstocks evaluated with ethanolamine. Solvent removal rates for ethanolamine also differed with feedstocks, with rice hulls and oil palm fiber showing higher efficiency (92.8% and 92.7%, respectively). Ethanolammonium acetate generally outperformed ethanolamine in solvent removal (except for sorghum and mixed pellets) and xylose yields (except for coconut chips and mixed pellets). However, glucose yields were notably lower for coconut chips and mixed pellets when treated with ethanolammonium acetate instead of ethanolamine. Differences in the effectiveness of ethanolamine and ethanolammonium acetate for sugar release and solvent removal across various feedstocks reflect the importance of tailoring solvents to specific feedstocks and deconstruction mechanisms (Achinivu et al., 2021 and 2023). Butylamine, butylammonium acetate, and triethylamine demonstrated excellent solvent removal throughout all tested feedstocks. Butylamine pretreatment resulted in glucose and xylose yields exceeding 65% and 50%, respectively, for all feedstocks except mixed pellets (57.7% glucose and 26.8% xylose). In contrast, triethylamine performed poorly, with most feedstocks yielding below 40% glucose and as low as 3.7% for coconut chips. Interestingly, ethanolammonium acetate pretreatment showed marked feedstock dependence, with glucose yields ranging from 16.0% for coconut chips to 86.5% for sorghum. Given its low boiling point, which facilitates recovery through distillation, and its overall performance in total sugar yield and solvent removal, butylamine was selected for further investigations.

3.3. Butylamine pretreatment effectiveness for a wide range of lignocellulosic feedstocks

Comprehensive feedstock compatibility was surveyed to assess butylamine's pretreatment versatility across a broad spectrum of lignocellulosic materials. The capacity to process diverse biomass feedstocks is central to industrial-scale pretreatment technologies (Shi et al., 2013; Li et al., 2015). The surveyed biomass feedstocks cover representative lignocellulosic materials from multiple geographical origins and harvest periods, enabling a rigorous evaluation of the solvent's robustness as a pretreatment agent. **Figure 3** displays the solvent removal following butylamine pretreatment and sugar yields from enzymatic hydrolysis of

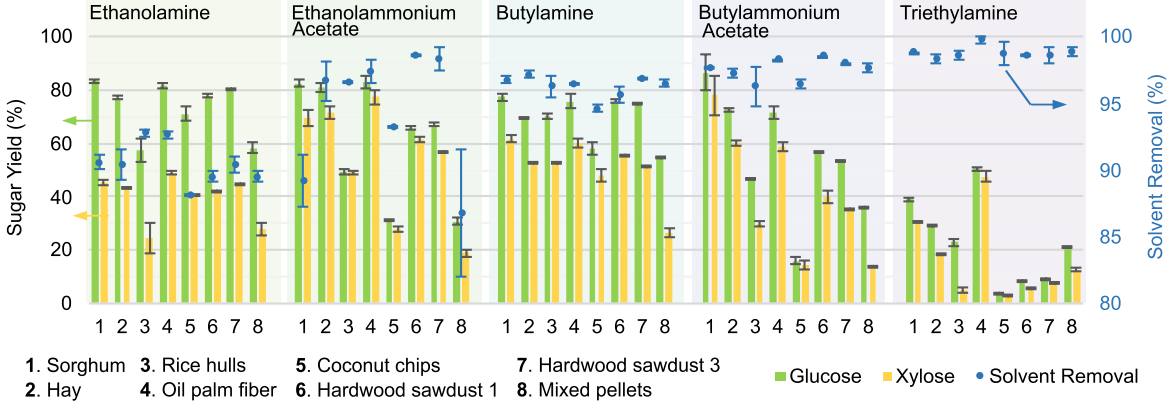

Fig. 2. Different solvents for pretreatment of diverse feedstocks followed by enzymatic hydrolysis.

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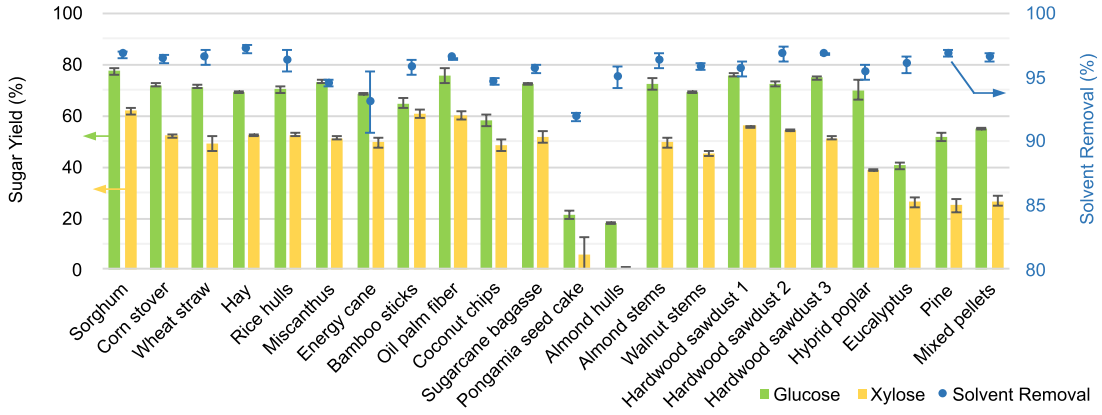

Fig. 3. Butylamine pretreatment of various biomass feedstocks followed by solvent removal and enzymatic hydrolysis to generate xylose and glucose.

pretreated biomass. Solvent removal was consistently high across most feedstocks, typically exceeding 95%, with hay as high as 97%. Glucose yields fall between 65–77% for herbaceous biomass and most hardwoods, with the notable exception of eucalyptus, which yields 40.6% glucose—unexpectedly lower than that of softwood pine (51.7%). Softwoods have traditionally been thought to be more challenging to process because of their higher lignin content and the fact that their lignin, composed primarily of G (guaiacyl) units, is more condensed and complicated to degrade than hardwood lignin, which contains abundant S (syringyl) units (**Fig. 1b**) (Zhu et al., 2010; Dutta et al., 2018). The lower glucose yield observed here from eucalyptus, a hardwood, may be due to its relatively higher lignin content compared to pine (**Fig. 1a**), which differs from prior reports (Dutta et al., 2018). This increased lignin content in eucalyptus could make it more resistant to pretreatment and/or reduce the susceptibility of pretreated biomass to enzymatic hydrolysis, particularly without a washing step, which retains all the lignin-derived inhibitory compounds for enzymes (Chen et al., 2023b).

Also noteworthy is the remarkably low glucose yield for almond hulls and pongamia seed cake, at approximately 20%, with negligible xylose yield. This result can be explained by the unignorable presence of non-lignocellulosic components in almond hulls and pongamia seed cake, such as proteins and fatty oils (Muktham et al., 2016; DePeters et al., 2020), aligning with the compositional data (**Fig. 1**). Nonetheless, the superior sugar yields for most of the feedstocks surveyed, particularly agricultural residues such as sorghum and corn stover, as well as most forestry residues like hardwood sawdust, demonstrate the versatility of butylamine pretreatment across diverse feedstocks.

3.4. Relationship between sugar yield and feedstock composition properties

The impact of lignin content and composition on lignocellulosic biomass deconstruction has been widely recognized (Studer et al., 2011; Papa et al., 2012; Yoo et al., 2017; Dutta et al., 2018). However, there is no significant correlation between sugar yields and lignin characteristics with butylamine pretreatment (**Fig. 4a**). This finding highlights the complex relationship between biomass recalcitrance to pretreatment and lignin content and composition across various feedstock types and species.

To further explore this complexity, we examined the effect of lignin content on sugar yields (**Fig. 4b**). As expected, grasses and hardwoods with lower Klason lignin content had higher glucose yields, while softwoods like pine, with higher Klason lignin content, had lower yields. Similarly, eucalyptus with high Klason lignin also showed low glucose yields, aligning with the negative correlation between total lignin content and enzymatic carbohydrate release observed in *Populus* (16–28% lignin content) (Studer et al., 2011). An exception to this trend is coconut chips, which, despite their high lignin content, yielded favorable glucose amounts, suggesting that biomass recalcitrance depends not only on composition but also on structural factors such as cellulose degree of polymerization and crystallinity (Chen et al., 2022). Low glucose yields from pongamia seed cake and almond hulls, despite low Klason lignin, are likely due to their high non-lignocellulosic compound content (**Fig. 1a**).

Unlike lignin content, the S/G ratio showed neither a significant correlation (**Fig. 4a**) nor a clear trend (**Fig. 4c**) with sugar yields. This observation agrees with previous findings that the impact of the S/G ratio on biomass conversion varies across plant species (Li et al., 2016) and may not affect glucose yield following specific pretreatments (Chen and Dixon, 2007; Papa et al., 2012). Nevertheless, hardwood sawdusts with an S/G ratio > 2.0 yielded more glucose than hybrid poplar with an S/G ratio of 1.2, consistent with reports that an S/G ratio above 2.0 typically favors enzymatic hydrolysis following hot-water pretreatment (Studer et al., 2011) and that *Populus* with an S/G ratio of 2.1 undergoes more efficient conversion than biomass with a ratio of 1.2 during consolidated bioprocessing, as higher S/G ratios contribute to longer, more linear lignin chains and enhanced cellulose accessibility for microbe-bound enzymes (Dumitrache et al., 2016). Additionally, a higher S/G ratio of hardwood sawdusts came with the better deconstruction of hemicellulose (i.e., xylose release) compared to hybrid poplar (**Fig. 1b** and **Fig. 3**), a similar result observed by Davison *et al*. (2006) in their study on dilute acid hydrolysis of *Populus*. They noted that lignin content and composition within a single species affect biomass hydrolyzability. Conversely, grasses like sorghum and corn stover, despite their low S/G ratios (< 1.0), also had high glucose yields (> 70%). Such results could be due to the high proportion of H units, as seen in alfalfa, where the S/G ratio did not strongly correlate with recalcitrance (Chen and Dixon, 2007). Collectively, these findings underscore the need to consider lignin content, composition, and other physicochemical factors when evaluating biomass recalcitrance.

3.5. Characterization of typical biomass before and after butylamine pretreatment

To better understand how butylamine pretreatment modulates biomass recalcitrance by altering physicochemical properties, three representative biomass types, sorghum (grass), poplar (hardwood), and pine (softwood), were selected for further characterization before and after pretreatment (**Fig. S2**). We first measured the pXRD patterns of the untreated and butylamine-pretreated dry biomass to assess changes in cellulose crystallinity. It is important to note that distillable amine pretreatment is unique in that the amorphous lignocellulosic components are not removed but instead remain together with the crystalline cellulose. As shown in **Figure 5a**, the crystallinity index (calculated from pXRD measurements) remained largely unchanged, except in pine, where it decreased from 25.8% to 18.5% after butylamine pretreatment. Nevertheless, the diffraction peak around 2θ 15.3° (assigned to the 101 plane of cellulose I form) exhibited reduced intensity to varying degrees with a slight shift towards higher 2θ values in all three samples after pretreatment, indicating potential alterations in cellulose structure (Park et al., 2010). The changes in crystallinity index and shift in 2θ values corresponding to 101 planes are reflective of the disruption of interunit H-bonding in crystalline cellulose of biomass under study.

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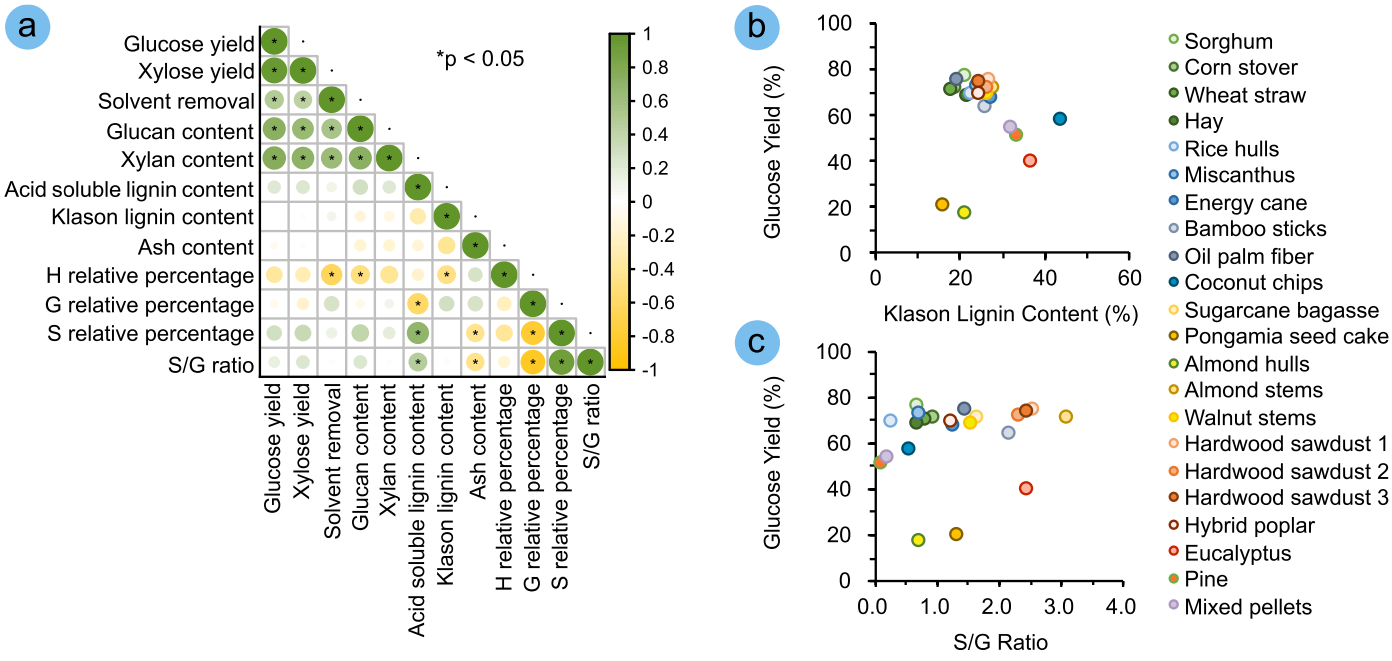

Fig. 4. Relationship between deconstruction efficiency and feedstock composition characteristics. **(a)** Pearson correlation matrix showing coefficients among parameters. Asterisks (*) denote significant correlations ($p < 0.05$). **(b)** Scatter plot illustrating the relationship between glucose yield and Klason lignin content. **(c)** Scatter plot depicting the correlation between glucose yield and S/G ratio.

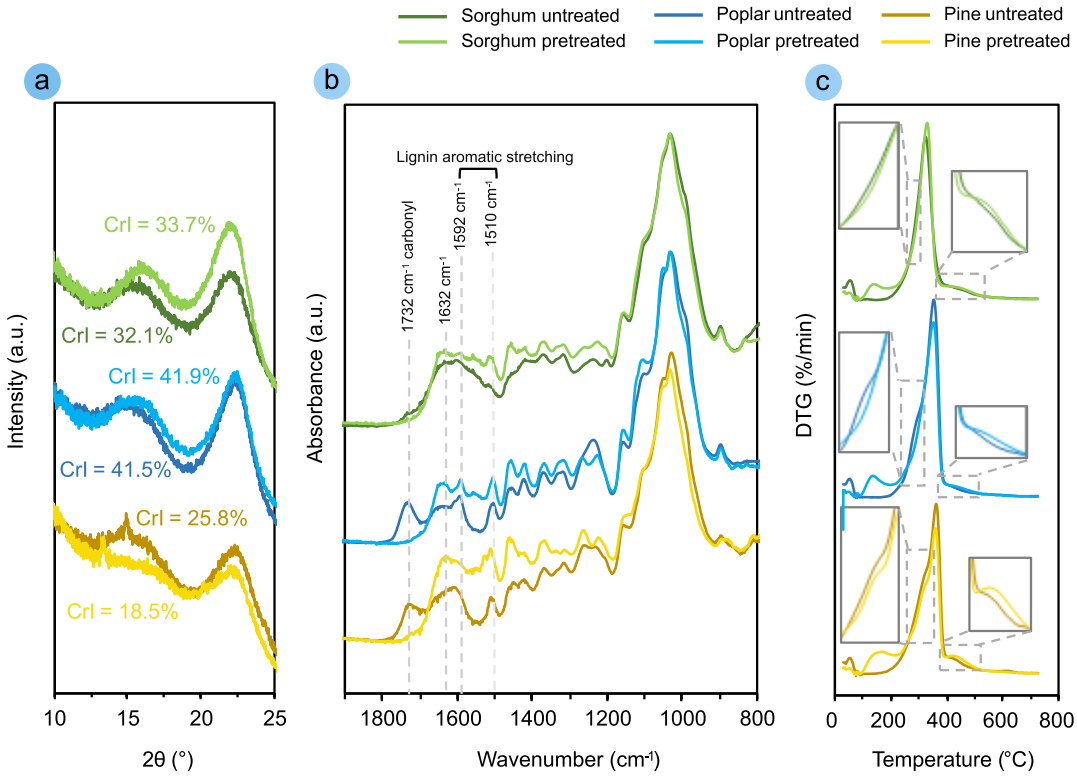

Fig. 5. Characterization of untreated and butylamine-pretreated sorghum, poplar, and pine samples. **(a)** X-ray diffraction (XRD) patterns. **(b)** FTIR spectra. **(c)** Differential thermogravimetry curves.

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FTIR spectroscopy was then conducted on untreated and butylamine-pretreated samples (after solvent removal) to identify chemical modifications in biomass components. Notably, the distinct band at 1732 cm^{-1}, associated with the C=O (carbonyl) stretching vibration of acetyl and uronyl esters in hemicellulose, as well as ester conjugate linkages in lignin (Achinivu et al., 2021), disappeared after pretreatment (Fig. 5b). This disappearance suggests biomass deconstruction through the cleavage of interunit linkages between its components, which reduces structural recalcitrance and enhances enzymatic digestibility. The FTIR spectrum of untreated biomass shows characteristic peaks for lignin's aromatic skeletal stretching at 1510 and 1592 cm^{-1}. Treatment with butylamine, followed by evaporation, had no significant effect on the 1510 cm^{-1} peak in both pine and poplar, indicating that the lignin aromatic structure remained intact after pretreatment. However, in sorghum biomass, this band became more prominent after butylamine treatment, suggesting potential lignin exposure due to changes in biomass structure. These distinct responses among different biomasses can be attributed to variations in lignin content, as evidenced by compositional analysis (Fig. 1). Additionally, all treated biomass samples exhibit a peak at 1632 cm^{-1}, corresponding to the stretching vibration of a carbonyl group within an amide bond (Ji et al., 2020). Such a peak suggests the presence of residual components from the solvent, particularly in the cases of pine and poplar. Although residual butylamine or its derivatives may negatively affect enzymatic or microbial performance (Fig. S5), their impact in lignocellulosic systems is likely complex due to interactions with other components like insoluble lignin and lignin-derived compounds (Chen et al., 2023b). These effects are also influenced by pretreatment conditions, which determine both the quantity and nature of amine residues and the composition of other biomass-derived products. Thus, understanding and managing solvent residue behavior should be considered in tandem with process optimization. Notably, common inhibitors of enzymatic hydrolysis and fermentation, such as 5-hydroxymethylfurfural and furfural, were not detected in the hydrolysates from butylamine-pretreated biomass following enzymatic hydrolysis (Fig. S6).

To further investigate the chemical changes induced by butylamine pretreatment, TGA was performed to assess the thermal stability and decomposition behavior of biomass components. Figure 5c presents the differential thermogravimetry curves, obtained as the first derivative of the TGA data (Fig. S4), which illustrate the rate of weight loss as a function of temperature. Biomass thermogravimetric profiles typically exhibit three major decomposition regions: hemicellulose (245–290 °C), cellulose (290–350 °C), and lignin (350–500 °C) (Singh et al., 2015). For the butylamine-pretreated biomass, a significant weight loss was observed between 150–200 °C compared to untreated biomass, likely due to the introduction of amines or their derivatives, consistent with the amine-related peak in the FTIR spectra (Fig. 5b). Changes in the rate of weight loss in both the hemicellulose and lignin regions for all three biomass types suggest a pronounced molecular interaction of butylamine with hemicellulose and lignin components compared to cellulose, supporting the pXRD and FTIR findings (Figs. 5a and b).

3.6. Initial evaluation of mixed feedstocks and scale-up

Given the excellent sugar yields obtained from butylamine pretreatment of agricultural residues followed by enzymatic hydrolysis, a blend of sorghum, corn stover, wheat straw, and hay—referred to as the Ag mix—was evaluated for butylamine pretreatment (Fig. 6). The Ag mix demonstrated sugar yields competitive with or superior to its individual components (Figs. 3 and 6). This finding aligns with previous reports indicating that mixed feedstocks—such as switchgrass, lodgepole pine, corn stover, and eucalyptus—are efficiently processed using ionic liquid pretreatment, whether in flour or pellet form (Shi et al., 2013). However, when the same mixed pellets were used in the current study for butylamine pretreatment, the sugar yields were lower than expected, yielding 57.7% glucose and 26.8% xylose. The reduced sugar yields may be attributed to differences in pretreatment conditions, including a higher solid loading (15% instead of 10%) in the present study, a lower temperature (140 °C compared to 160 °C), and the absence of mixing in the 15 mL glass pressure tube (in contrast to the automated 500 mL Globe Chemical Reactor system with impeller stirring used by Shi et al. (2013). The lack of agitation likely contributed to less efficient pretreatment of the pellets (Chen et al., 2023a).

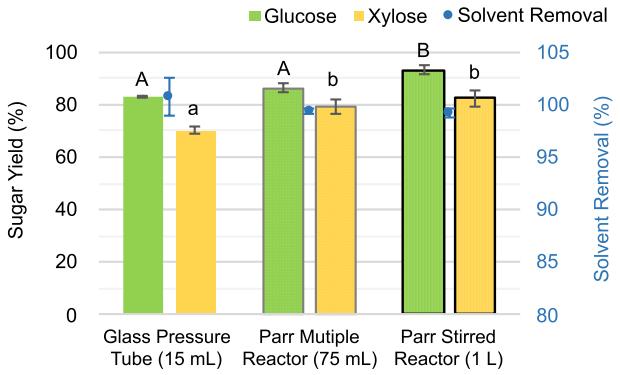

Fig. 6. Initial evaluation of mixed feedstocks and scale-up. Error bars represent the standard deviation of triplicate experiments. Different uppercase letters (for glucose yield) and lowercase letters (for xylose yield) above columns indicate statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post-hoc test.

Accordingly, the performance of the butylamine pretreatment of Ag mix improved as the scale increased with the addition of agitation. Notably, glucose yield from the 1 L stirred reactor was significantly higher ($p < 0.05$) than from the 75 mL multi-Parr reactor and 15 mL glass tube, likely due to improved mixing. The glass pressure tube lacks agitation during pretreatment, while the multi-Parr reactors, though equipped with magnetic stir bars, provide inadequate mixing under the high-solids slurry conditions used. In contrast, the 1 L Parr stirred reactor, equipped with a stirrer suited for high-solids processing, ensures superior mixing, leading to better pretreatment performance. At the 1 L scale, the process liberated > 90% of glucose from the mixed agricultural feedstocks, while achieving > 99% removal of butylamine after pretreatment. These results highlight the potential of blended feedstocks and the scalability of the butylamine pretreatment process, suggesting promising implications for larger-scale biorefinery operations.

A previous TEA conducted by our research group on distillable amine-based ionic liquids (e.g., ethanolammonium acetate) demonstrated that distillability is a key determinant of process costs (Achinivu et al., 2023). Owing to its relatively low boiling point, butylamine represents a promising candidate for improving both affordability and scalability. A more detailed TEA focused specifically on the butylamine-based approach presented here will be reported in forthcoming work.

3.7. Toxicity evaluation and microbial tolerance to butylamine-pretreated Ag mix hydrolysate

The presence of inhibitory compounds in lignocellulosic hydrolysates is well-documented, as these compounds, generated during pretreatment, can negatively impact microbial growth and production. Such compounds include phenolics, organic acids, residual solvents from pretreatment (e.g., butylamine), and other byproducts of biomass processing (Jönsson et al., 2013). The toxicity evaluation of butylamine-pretreated hydrolysate revealed distinct tolerance patterns among the tested microbial hosts. Four strains were tested in total to assess toxicity across a spectrum of prokaryotic and eukaryotic hosts, including two bacterial strains: *Escherichia coli* and *Pseudomonas putida*, and two fungal strains: *Saccharomyces cerevisiae* and *Rhodosporidium toruloides*. Optical density, glucose consumption, and xylose consumption were measured to assess relative toxicity. We did not monitor the titers of specific bioproducts from each microbial strain and focused on growth and sugar consumption as our primary readouts of hydrolysate biocompatibility. Future work will focus on strain engineering and optimization of butylamine hydrolysates to enhance bioproduct titer, rate, and yield. We note that the observed increase in sugar concentrations over time in some conditions can be attributed to water evaporation during the 3-day experiment.

Of the organisms screened, *E. coli* exhibited the lowest overall hydrolysate tolerance, with optimal growth at 5% hydrolysate concentration and substantial reductions at higher concentrations (Fig. 7a). Glucose

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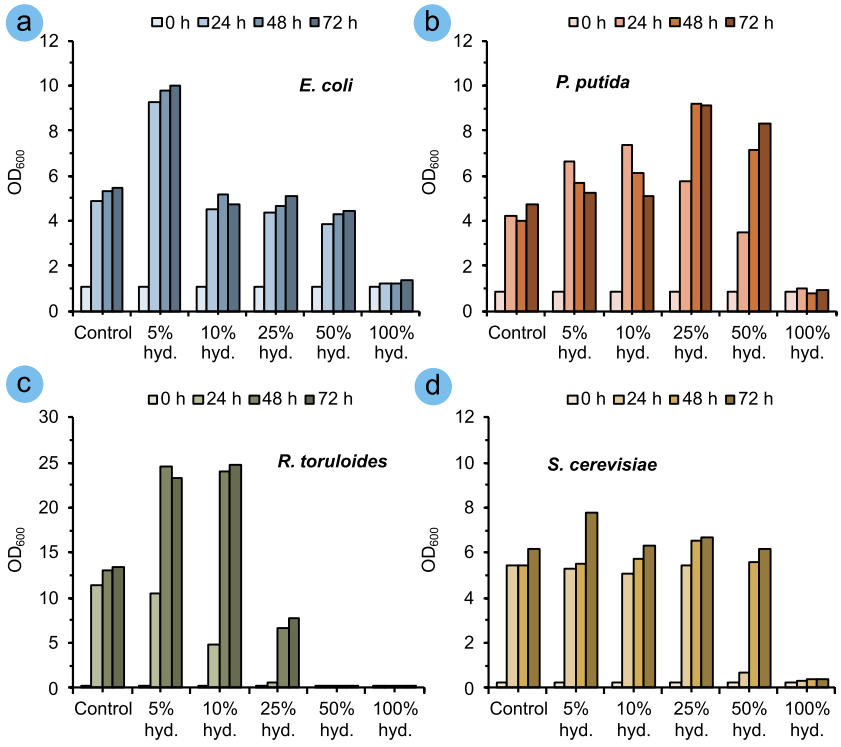

Fig. 7. Evaluation of the toxicity of butylamine-pretreated Ag mix hydrolysate for **(a)** *Escherichia coli*, **(b)** *Pseudomonas putida*, **(c)** *Rhodosporidium toruloides*, and **(d)** *Saccharomyces cerevisiae*.

consumption correlated with the observed optical density findings, showing glucose utilization only at the lowest hydrolysate concentration. Minimal to no xylose consumption was observed across all hydrolysate levels, likely due to the combined effects of inhibitory compounds and osmotic stress at elevated hydrolysate concentrations (Figs. 8a and b). Although glucose is typically a preferred carbon source for *E. coli*, stressful conditions in the hydrolysate can trigger a shift in metabolic priorities. Under such conditions, *E. coli* may rely on pathways that promote rapid survival and growth, such as utilizing simpler, readily available compounds from LB medium and yeast extract, while downregulating sugar metabolism. The amino acids and peptides present in LB and yeast extract can also suppress sugar uptake and metabolism through global regulatory mechanisms, including carbon catabolite repression.

By contrast, *Pseudomonas putida* demonstrated the highest tolerance among the tested strains. It achieved significant cell density at hydrolysate concentrations up to 50%, likely due to the increased sugar availability in the medium. However, growth was minimal at 100% hydrolysate, indicating that undiluted hydrolysate surpassed its metabolic tolerance threshold (Fig. 7b). Glucose was fully consumed up to 50% hydrolysate, reflecting the strain's robustness, but ceased at 100% (Fig. 8c). Xylose utilization was negligible across all conditions, consistent with the strain's carbon source preferences (Fig. 8d). The substantial variation in hydrolysate tolerance between the bacterial strains indicates the presence of species-specific inhibitory factors, rather than broad-spectrum toxicity.

The oleaginous yeast *Rhodosporidium toruloides* displayed moderate hydrolysate tolerance, with growth observed up to 25% hydrolysate. Complete growth cessation was observed at higher concentrations (Fig. 7c). Glucose consumption mirrored these findings, with complete glucose utilization and substantial xylose utilization up to 25% hydrolysate, but none at 50% and above (Figs. 8e and f). While *R. toruloides* typically displays resilience towards hydrolysate toxicity (Sundstrom et al., 2018), the strain's sensitivity to certain inhibitory compounds, particularly phenolics and organic acids, may disrupt cellular homeostasis and impair sugar metabolism. *S. cerevisiae* exhibited the highest tolerance of the two yeast strains (Fig. 7d), sustaining growth and complete glucose consumption up to 50% hydrolysate concentration (Fig. 8g). No significant xylose consumption was observed, as *S. cerevisiae* lacks native xylose-utilization pathways (Fig. 8h). Similar to the bacterial strains, the variation in hydrolysate tolerance between the two yeast strains highlights the species-specific nature of hydrolysate toxicity. Relatively high tolerance in unadapted *S. cerevisiae* under aerobic growth conditions indicates significant promise for butylamine pretreatment bioethanol applications.

While growth was observed at relatively high sugar concentrations, particularly with *P. putida* and *S. cerevisiae*, inhibitory compounds in butylamine-pretreated hydrolysate - including phenolics, organic acids, and residual solvents - still present significant barriers to microbial growth and sugar utilization. The observed variation in species-specific growth responses emphasizes the importance of host selection in bioprocess development. Beyond host selection, strategies such as gradual adaptation in the seed train or fed-batch hydrolysate addition during fermentation could alleviate toxicity and osmotic stress *via* gradual acclimation. Additionally, modification of pretreatment conditions, hydrolysate detoxification, adaptive evolution, and genetic engineering of microbial strains could all improve tolerance to toxic compounds while maintaining efficient glucose metabolism. Given the high tolerance baseline in unadapted strains, these strategies hold significant promise for further process intensification to improve performance and productivity in lignocellulosic biorefineries.

4. Conclusions and Prospects

This study highlights the potential of distillable amine-based solvents, particularly butylamine, for effective lignocellulosic biomass pretreatment. Twenty-two feedstocks were evaluated and screened for deconstruction efficiency. Among the solvents tested, butylamine demonstrated superior performance in achieving high fermentable (e.g., glucose and xylose) sugar yields and efficient solvent recovery across diverse feedstocks. The ability of butylamine to deliver consistent results across multiple biomass types underscores its versatility and suitability for biorefinery applications. Additionally, the feedstock-agnostic nature of butylamine pretreatment minimizes the variability in biomass deconstruction, enabling a more

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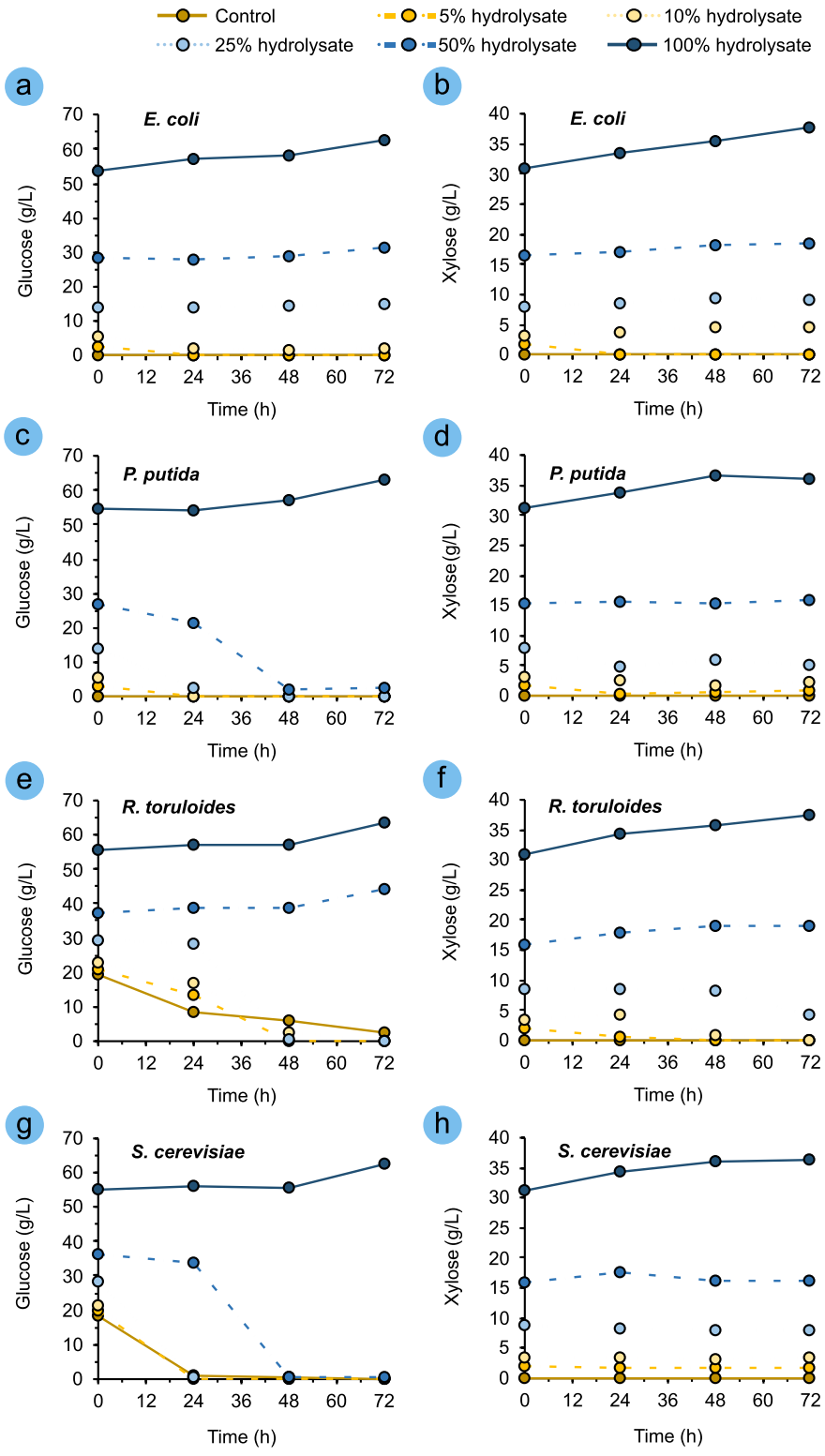

Fig. 8. Glucose and xylose utilization profiles of different microbial strains. Note: The slight increase in sugar concentration under some conditions, particularly over longer incubation times, is likely due to water evaporation.

standardized approach to enzymatic saccharification. The scalability of the butylamine pretreatment process was further validated through successful tests on mixed agricultural feedstocks and larger-scale operations, achieving excellent sugar yields and near-complete solvent recovery. Bioconversion of the resulting hydrolysate was demonstrated in two bacterial and two yeast strains, with high biocompatibility observed for both *S. cerevisiae* and *P.*

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putida. These results emphasize the promise of butylamine as a cost-effective and practical pretreatment agent that aligns with the operational needs of modern biorefineries. It is also important to note that there are safety and environmental considerations in using butylamine at scale, as it is a flammable and toxic solvent. Using it in a closed environment that minimizes release and exposure to both humans and the environment is essential, providing an additional motivation to achieve recovery and recycling efficiencies greater than 99%. Future work should focus on optimizing process parameters for specific feedstocks, reducing energy intensity and consumption, enhancing solvent recovery and recycling systems, and evaluating the long-term economic and environmental feasibility of using butylamine in industrial settings. Such an approach could pave the way for more affordable and efficient biofuel and biochemical production derived from a wide range of lignocellulosic feedstocks. This work demonstrates the transformative potential of butylamine as a versatile and scalable solvent for lignocellulosic biomass pretreatment, addressing critical challenges in biomass deconstruction.

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Author contributions

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Conflict of interest

BAS has a financial interest in Illium Technologies, Caribou Biofuels, and Erg Bio. XC, AK, JMP, VRP, and BAS are named inventors on at least one related patent application. All other authors have no financial interest to disclose.

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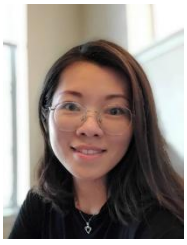

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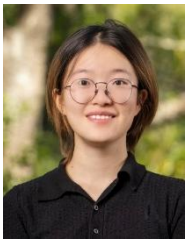

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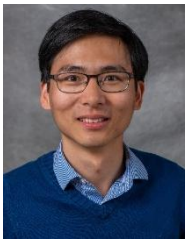

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Dr. Ning Sun is a staff scientist at the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU), part of the Berkeley Lab. She has published over 50 peer-reviewed journal articles with an h-index of 31 and more than 6000 citations. Her research focuses on (1) lignocellulosic biomass conversion to biofuels, (2) polyester waste upgrading, and (3) resource recovery from wastewater. Her Google Scholar profile is available at: https://scholar.google.com/citations?user=ZHT26T0AAAAJ&hl=en

Dr. Eric Sundstrom is a Staff Scientist at Lawrence Berkeley National Laboratory, where he joined the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU) in 2015. As a group leader of the ABPDU Fermentation and Recovery Team, he has directed 25+ industrial partnerships, leveraging national laboratory capabilities to accelerate commercialization of new biofuels, biomaterials, biochemicals, and food products. His research addresses critical barriers to process scale-up, intensification, and integration for novel bioconversions, aiming to speed industrial deployment by reducing process costs, development timelines, and scale-up risks. These projects include a particular focus on intensification of integrated biomass-to-biofuels processing, development of novel process configurations and separations technologies, and characterization of emerging C1 and gaseous bioconversion technologies. He received his Ph.D. in Environmental Engineering from Stanford University in 2013, where he developed microbial community-based approaches for bioconversion of waste methane into renewable bioplastics. His research profile on Google Scholar can be found at the following link: https://scholar.google.com/citations?user=aYV0vuUAAAAJ&hl=en

Dr. Aymerick Eudes is a Biological Engineer Staff Scientist in the Biosciences Area at the Lawrence Berkeley National Laboratory (USA). Dr. Eudes serves as the Director of Bioenergy Crop Engineering at the Joint BioEnergy Institute. He received a PhD in Plant Cellular and Molecular Physiology at the University of Paris-Saclay (France). His research interests include the optimization of crop biomass for bioenergy applications and the engineering of plant traits to enhance crop productivity. His research profile on Google Scholar can be found at the following link: https://scholar.google.com/citations?user=yW8RFOkAAAAJ&hl=en

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Dr. Blake Simmons serves as the Chief Scientific and Technology Officer and Vice President of the Deconstruction Division at the US Department of Energy's Joint BioEnergy Institute (JBEI) in Emeryville. After earning his BS in chemical engineering from the University of Washington, Dr. Simmons continued his studies at Tulane University and received his doctorate in the same field. Dr. Simmons worked as part of the Senior Management team at Sandia National Laboratories for 15 years, most recently serving as the Senior Manager of Advanced Biomanufacturing as well as the Biomass Program Manager. He joined Lawrence Berkeley National Laboratory in February of 2016 as the Division Director of Biological Systems and Engineering. He is an Adjunct Professor at the University of California-Berkeley and the University of Queensland in Australia. His expertise includes advanced biofuels, renewable chemicals, biomanufacturing, ionic liquids, abiotic-biotic interfaces, biomass pretreatment, enzyme engineering, biofuel cells, templated nanomaterials, microfluidics, desalination, and biomineralization. He is the co-founder of three startups, has published more than 450 papers, and holds more than 50 patents. His research profile on Google Scholar can be found at the following link: https://scholar.google.com/citations?user=c-RPud4AAAAJ&hl=en&oi=ao

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Supplementary Information

Table S1.
Feedstock sources used in this study.

Feedstock	Source
Sorghum	Idaho National Laboratory, Idaho Falls, ID
Corn stover	Idaho National Laboratory, Idaho Falls, ID
Wheat straw	Idaho National Laboratory, Idaho Falls, ID
Hay	Rhino Seed & Landscape, LLC, Brighton, MI
Rice hulls	Frontier Ag, Davis, CA
Miscanthus	University of Illinois, Urbana-Champaign, IL
Energy cane	Idaho National Laboratory, Idaho Falls, ID
Bamboo sticks	Nakatoshi Trading Co., Ltd., Tokyo, Japan
Oil palm fiber	Petronas, Malaysia
Coconut chips	ZeeDix Coconut Chips for Reptiles
Sugarcane bagasse	Total Energies, Paris, France
Pongamia seed cake	Terviva Inc., Alameda, CA
Almond hulls	RPAC, Los Banos, CA
Almond stems	RPAC, Los Banos, CA
Walnut stems	Paddock Inc., Oakdale, CA
Hardwood sawdust 1	New River Hardwoods, Mountain City, TN
Hardwood sawdust 2	New River Hardwoods, Mountain City, TN
Hardwood sawdust 3	New River Hardwoods, Mountain City, TN
Hybrid poplar	Idaho National Laboratory, Idaho Falls, ID
Eucalyptus	Idaho National Laboratory, Idaho Falls, ID
Pine	Paddock Inc., Oakdale, CA
Mixed pellets	Idaho National Laboratory, Idaho Falls, ID

Table S2.
Features of selected amine-based solvents for lignocellulose deconstruction.

Solvent	Boiling point	Deconstruction conditions	Major results	Ref.
Ethanolamine	170 °C	25% solvent in water, 100 °C, 1 h, 40% solids (sorghum)	90% glucose, 76% xylose yield; 59% lignin removal; 73% solid recovery	Achinivu et al. (2021)
Ethanolammonium acetate	NA*	Neat PIL**, 140 °C, 3 h, 5% solids (sorghum)	94.5% glucose, 74.3% xylose yield; distillable at 140 °C under vacuum	Achinivu et al. (2023)
Butylamine	77 °C	Vapor-condensate wetting of rice straw, 2.5% w/w butylamine (BA), > 0.8 weight ratio of BA to rice straw	~70% total sugar yield (cellulose + hemicellulose); ~60% lignin removal	Tanaka et al. (1985)
Butylammonium acetate	NA	Used as neat PIL	Effective sugar release; distillable at ~120 °C under vacuum	**This Study**
Triethylamine	89 °C	70% EtOH + 5% triethylamine, 70 °C, 5 h (*Tamarix austromogoliac*)	14.6% lignin solubilization	Sun et al. (2010)

*NA: not available or applicable; **PIL: protic ionic liquid.

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Fig. S1. Photographs of feedstocks in their received state. Images depict the raw physical form of various lignocellulosic materials before any preprocessing steps.

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Fig. S2. Photographs of sorghum, poplar, and pine before and after butylamine pretreatment and solvent removal. Biomass was Wiley-milled and passed through a 2 mm screen before pretreatment.

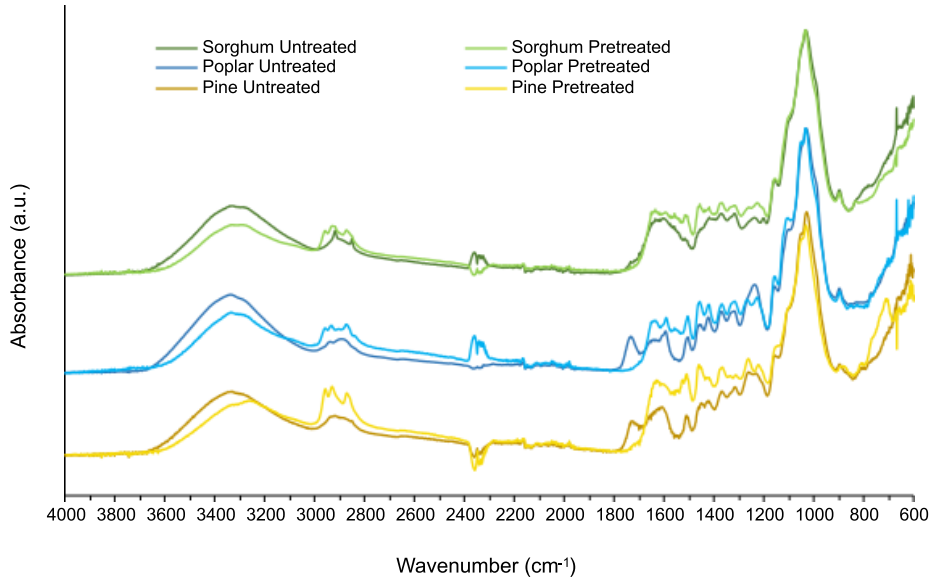

Fig. S3. FTIR spectra of raw and butylamine-pretreated sorghum, poplar, and pine samples.

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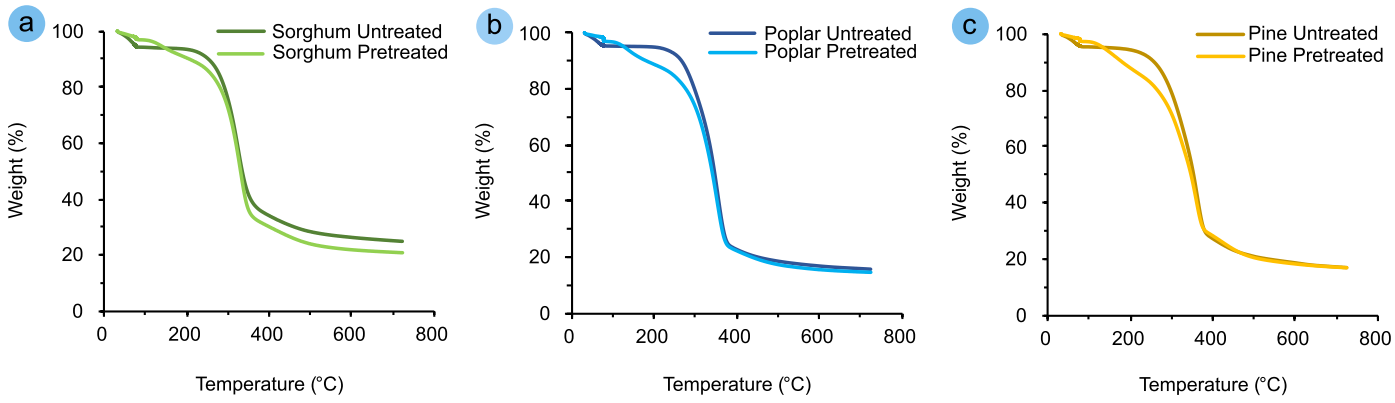

Fig. S4. TGA curves showing the relative weight loss of untreated and butylamine-pretreated samples. **(a)** sorghum, **(b)** poplar, and **(c)** pine.

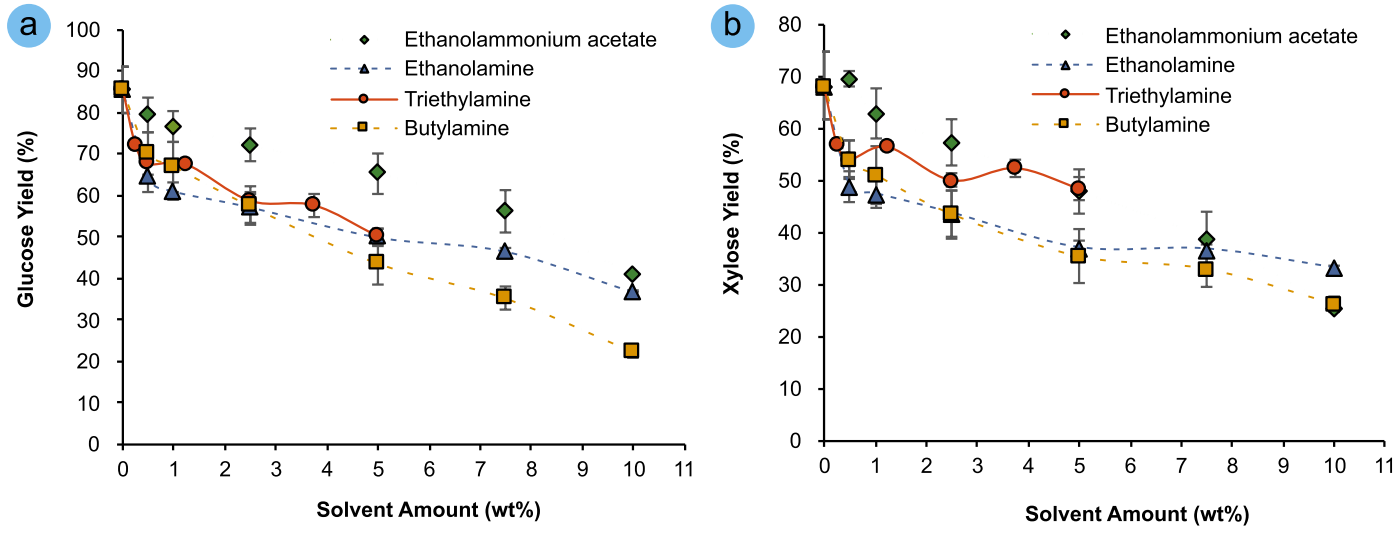

Fig. S5. Effects of amine-based solvents on the enzymatic hydrolysis of cellulose **(a)** and xylan **(b)**. Reactions used 120 mg Avicel and 60 mg xylan in a 1.4 g solution containing amine-based solvent, CTec3/Htec3 (9:1, 50 mg/g substrate), 50 mM citrate buffer (pH 4.8), and 0.1% sodium azide. Hydrolysis was conducted at 50 °C with end-over-end agitation for 72 h.

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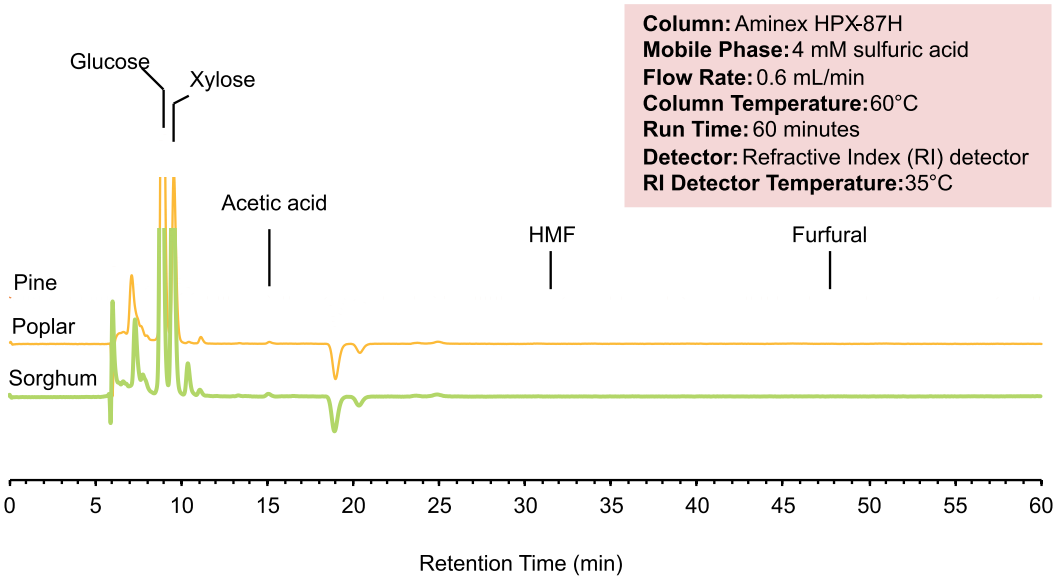

Fig. S6. HPLC chromatograms of hydrolysates generated from enzymatic hydrolysis of butylamine-pretreated sorghum, poplar, and pine.

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