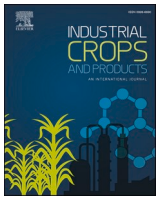
Industrial Crops & Products 223 (2025) 120156

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Performance of mild acetone organosolv fractionation on lignocellulosic feedstocks from new cropping systems for production of advanced bioethanol

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ARTICLE INFO

Keywords:
Agricultural residues
dedicated energy crops
organosolv
biorefinery
bio-ethanol
fermentation

ABSTRACT

Various lignocellulose feedstocks were sourced through an innovative double crop rotation system for production of advanced bioethanol. Dedicated biomass crops that grow with high yields with low cost and greenhouse gas emissions (hemp, biomass sorghum, and sunn hemp) were integrated with the cultivation of conventional food crops to recover agricultural residues (wheat straw, corn stover). These feedstocks with low indirect land use change risk were subjected to a biorefinery approach for the production of advanced ethanol from cellulosic and hemicellulosic sugars and co-production of lignin. The processing steps included tandem pre-extraction and acetone organosolv fractionation, followed by enzymatic hydrolysis of the obtained cellulose-enriched pulp (producing mainly C6 sugars), detoxification of hemicellulose hydrolysate (mainly C5 sugars) and fermentation of C5 and C6 sugars to ethanol. High recovery of cellulose in the pulp (87–100 %), C5 oligomeric and monomeric sugars in the hydrolysate (80–90 %) and isolated lignin (72–84 %) showed that process efficiency can be maintained across the various types of feedstocks. The ethanol production titres and productivity obtained by fermentation by *Saccharomyces cerevisiae* yeast on the C6 sugar enzymatic hydrolysates were robust and similar to those values obtained on model glucose substrate. C5 sugar hydrolysates after detoxification and evaporation were readily converted to ethanol with *Spathaspora passalidarum* CBS 10155. Sunn hemp and hemp feedstocks showed both less optimal enzymatic digestibility of cellulosic pulps and lower ethanol production rates from their hemicellulose hydrolysates. The present work shows the applicability of the aforementioned biorefinery approach for the production of advanced ethanol with a diverse set of lignocellulosic feedstocks obtained from an integrated food and bioenergy production system based on rotating crops. It also highlights that the most critical processing steps for process monitoring and optimisation are the pulp enzymatic hydrolysis of pulps and the detoxification of the C5 sugar hydrolysates.

1. Introduction

Renewable liquid fuels are crucial for the decarbonisation of the transport sector, in particular for aviation and shipping. Biobased alcohols (mainly ethanol) are enabling platform molecules that facilitate the production of sustainable renewable liquid fuels but current mature technologies rely mostly on edible crops. On the other hand, second generation (2 G) biofuels are produced from lignocellulosic feedstocks that include biogenic residues, agricultural and forestry residues, and dedicated and woody crops. Current policy measures, such as the

Abbreviations: 2G, Second generation; C5, Pentoses xylose, arabinose; C6, Hexoses glucose, mannose, galactose, rhamnose; Dw, Dry weight; ILUC, Indirect land use change.

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https://doi.org/10.1016/j.indcrop.2024.120156
Received 4 November 2024; Received in revised form 22 November 2024; Accepted 23 November 2024
Available online 6 December 2024

European Renewable Energy Directive EU/2023/2413, promote the uptake of renewable liquid fuels in particular from 2 G crops to enable decarbonisation of the transport sector.

The use of single lignocellulosic feedstock for the production of advanced ethanol can be challenging from the perspective of the risk of dwindling biomass supply, high costs of the feedstock logistics and impacts of indirect land-use change (iLUC) (Aristizábal-Marulanda and Cardona Alzate, 2019). To avoid such issues, modern agriculture is moving towards the integration of food and bioenergy feedstock production in innovative low iLUC cropping systems. In these systems, dedicated lignocellulosic crops are produced within crop rotation schemes without competing for land or its resources with food crops. The increased diversity of feedstocks will the combination of low-cost feedstocks in suitable proportions (in quantitative and qualitative terms) to meet the conversion process specifications, thus reducing the reliance on a single, usually costly, feedstock source.

Robust and cost-effective technology platforms for conversion of diverse feedstocks into biofuels, bioenergy and biobased products are needed (Sharma et al., 2020). As an example, Zhang et al. (2018) tested the processability of five different herbaceous lignocellulosic feedstocks (corn stover, sorghum biomass, switchgrass, Miscanthus and prairie) through ammonia fibre expansion, enzymatic hydrolysis and fermentation with *Saccharomyces cerevisiae* Y128 yeast and *Zymomonas mobilis* 8b bacteria. In their work, most feedstocks led to a similar range of ethanol yield; however, corn stover hydrolysates contained high amounts of *p*-coumaric and ferulic acids leading to partial fermentation inhibition of xylose. It is thus vital to determine the suitability of feedstocks for a pretreatment technology in order to support feedstock selection and implement processing mitigation strategies.

Pre-treatment processes enable the use of lignocellulosic sugars for advanced ethanol production through enzymatic hydrolysis and fermentation. Conventional pre-treatment approaches include aqueous processes such as steam explosion, dilute acid and ammonia, which are known to lead to highly condensed, low quality lignin (Narron et al., 2016). Organosolv processes use organic solvents in combination with water and/or catalysts (Tofani et al., 2024). In particular, mild aqueous acetone organosolv fractionation is performed at mild temperature (140 °C) using sulphuric acid allowing for the production of cellulose and hemicellulose sugars in high yields, and less condensed, high quality lignin for valorisation in chemicals and materials (Smit and Huijgen, 2017). The mild conditions in this process avoid significant thermal degradation of the solvent (<0.7 %), while the high volatility of acetone reduces energy demands during solvent recovery compared ethanol, acetic acid and glycerol (Smit and Huijgen, 2017). Smit et al. (2022) showed that the integration of a pre-extraction process enables the effective fractionation of different lignocellulosic feedstocks (roadside grass, wheat straw, birch branches and almond shells). Effectively delignified cellulosic pulp in addition to high yield recovery of hemicellulose sugars with low toxicity is important to enable advanced ethanol production from 2 G biomass feedstocks.

There is extensive research towards fermentation of C6 and C5 sugars streams from lignocellulosic biomass to bioethanol. For industrial production of bioethanol, optimized strains of the yeast *Saccharomyces* are being used in edible crops with high efficiency and conversion. However, there are still significant bottlenecks concerning utilization of xylose and C6 sugars other than glucose in lignocellulosic hydrolysates (Liu et al., 2019; Robak and Balcerek, 2020; Su et al., 2020). The ethanol yields obtained by current xylose-fermenting *Saccharomyces* recombinant strains or other developed microbial strains, such as *Zymomonas* strains, are lower than those yields obtained on glucose, and issues regarding strain stability and co-utilization of different sugars remain to be solved (Park et al., 2020; Raj et al., 2022). Alternative ethanol-producing strains are being explored for fermentation of both C6 and C5 sugars since they can show higher tolerance to inhibitors than current commercial yeast strains. Wild-type strains of *Spathasphora passalidarum* have been reported to produce ethanol from xylose at ethanol yields of 0.31 g g^{-1} xylose, and have been cultivated successfully on corn stover hydrolysate (Du et al., 2019) and on beech wood C5 sugar-rich hydrolysates (de Vrije et al., 2024). This opens possibilities for development of efficient bioethanol production from 2 G lignocellulosic sugars.

The present work presents three innovations with respect to current advanced bioethanol processes: 1) Expansion of feedstocks to the use of biomasses sourced through an innovative rotation scheme; 2) Benchmarking of these biomasses at the experimental level an acetone organosolv technology for biomass lignocellulosic biomass fractionation to fermentable sugars and lignin, and 3) Novel fermentation approach for separate fermentation of C6- and detoxified-C5 sugars streams. The biorefinery approach (Fig. 1) implemented in this work includes water-acetone pre-extraction in tandem with mild organosolv fractionation to obtain two sugar streams, namely C5 sugar-rich hydrolysate and C6 sugar-rich pulp. The C5 sugar hydrolysate and C6 sugar pulp are further subjected to detoxification with activated carbon and enzymatic hydrolysis, respectively, to prepare and condition sugars prior to fermentation. The C5 and C6 sugar streams are fermented to ethanol with two yeast strains (*Saccharomyces cerevisiae* Ethanol Red® and *Spathaspora passalidarum* CBS 10155, respectively) to determine their fermentability relative to model sugars.

2. Materials and methods

2.1. Materials

Five feedstocks were used in this study: hemp, sunn hemp, sorghum biomass, corn stover and wheat straw. Each dedicated lignocellulosic crop was sown using a pneumatic planter, with settings adjusted to achieve different sowing densities and depths based on the specific requirements of each crop. The 'Futura 75' hemp variety (*Cannabis sativa* L.) was sown at a density of 157 seeds m^{-2} in April, with 55 kg ha^{-1} of nitrogen applied. The 'Bulldozer' biomass sorghum hybrid (*Sorghum bicolor x Sorghum sudangrass*) was sown in April at 19 seeds m^{-2}, receiving 156 kg ha^{-1} of nitrogen. The 'Ecofix' variety of sunn hemp (*Crotalaria juncea* L.) was sown in May at 52 seeds m^{-2}, fertilized with 36 kg ha^{-1} of nitrogen. Hemp was harvested both manually and mechanically in August, while sunn hemp and biomass sorghum were harvested in September. Wheat and corn (*Zea mays* L.) were planted in late October and March, respectively, and harvested in mid-June and mid-August using a combine harvester. All biomass feedstocks were dried at ambient conditions and milled twice to a particle size of <4 mm using a Retsch SM300 cutter mill equipped with a 4 mm sieve before use in biorefinery tests.

Acetone (Technical grade) was obtained from VWR chemicals. Sulphuric acid (98 %) was obtained from Merck. Acetic acid, furfural, HMF, formic acid, syringic acid, phenol, vanillin, benzoic acid, 4-hydroxybenzoic acid, syringaldehyde, levulinic acid, vanillic acid, D-(+)-xylose were obtained from Sigma-Aldrich. Granular activated carbon HYDRAFFIN CC 1240 SPEZIAL was kindly provided by Donau Carbon GmbH. Enzyme cocktail Cellic CTEC2 was kindly provided by Novozymes and used without further treatment.

The yeast strains used for ethanol production were *Saccharomyces cerevisiae* Ethanol Red® (Leaf - Lesaffre, France) and *Spathaspora passalidarum* CBS 10155 (Westerdijk Fungal Biodiversity Institute, the Netherlands). The yeast strains were stored as 20 % glycerol stocks at −80 °C.

2.2. Pre-extraction and organosolv fractionation

Milled feedstocks were firstly treated in a pre-extraction unit consisting of a double walled glass tube (effective volume 2 L) whose jacket is connected to a heating circulator (Julabo, 300 F) with water at 50 °C. Extraction solvents are fed to the top of the unit using a peristaltic pump (Masterflex, Chem-Durance Bio tubing L/S 25). The solvents were kept

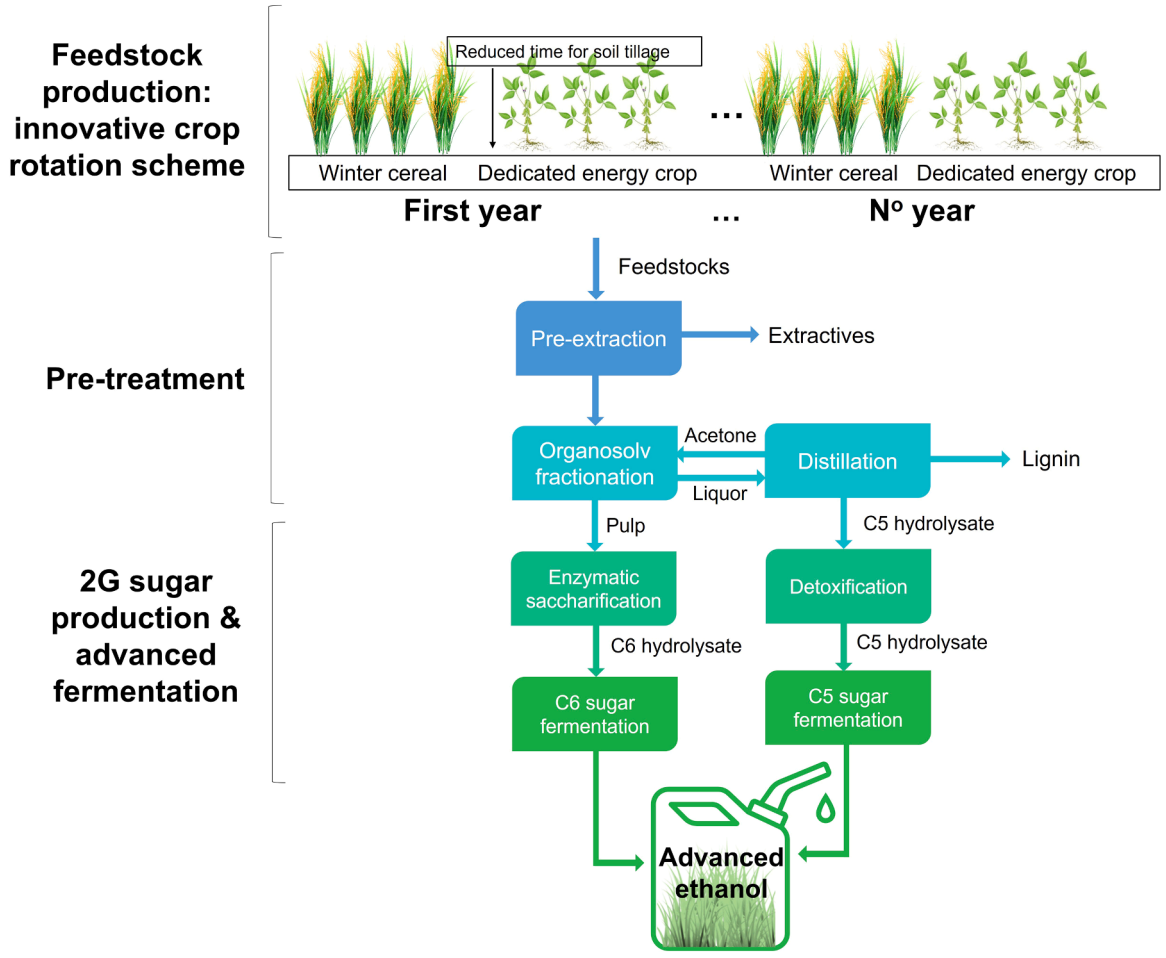

Fig. 1. 2 G biorefinery approach via mild acetone organosolv for advanced ethanol production using multiple feedstocks from innovative cropping systems.

in glass containers and preheated at 50 °C. The pumped solvents are then percolated through the biomass bed by gravity and collected at the bottom of the column in a separate glass container. Before pre-extraction, 350 g dw of the feedstock was mixed with 3 parts of demineralised water before loading into the unit. Initially, 500 g dw of giant reed were used for this test; however, due to slow percolation flow, the amount of biomass for the other tests was reduced to 350 g dw. The wet biomass was allowed to reach the extraction temperature for 1.5 h, after which three solvent fractions are added: (1) extraction with 2.5 parts of demineralised water (Fraction F1); (2) extraction with 4 parts of 50 wt% acetone (Fraction F2); (3) extraction with 4 parts of 100 wt% acetone and 2.5 parts of demineralised water (Fraction F3). After pre-extraction, the biomass is unloaded from the unit and dried overnight at 60 °C before further use. Feedstock pre-extraction performance is quantified through the indicators of feedstock recovery (dw basis), and ash and organic extractives removal percentages (Supplementary materials).

Small scale experiments were performed prior to fractionation to determine the amount of sulphuric acid needed to achieve a pH of 1.8 (Supplementary materials). For the mild acetone organosolv fractionation, between 130 and 185 g dw of pre-extracted feedstock was mixed with 50 wt% acetone to obtain a liquid-to-solid ratio of 6 L kg^{-1} dw. Sulfuric acid was added according to the estimated dose to achieve a pH of 1.8. The reaction mixture was loaded in a 2 L autoclave reactor (Kiloclave, Büchi Glas Uster AG, Switzerland), heated to 140 °C and stirred with an anchor stirrer at 100 rpm for 1 h. After cooling to room temperature, the pH of the slurry was measured, and the mixture filtered over a Whatman GF/D filter. The product pulp was washed with 50 % w/w aqueous acetone (3 L kg^{-1} initial dry biomass), followed by demineralised water (3 L kg^{-1} initial dry biomass). A fraction of the pulp was dried in an oven at 60 °C to determine the dry pulp yield and characterise its biochemical composition. The remainder of the pulp was stored wet at −20 °C for further processing through enzymatic hydrolysis.

The filtered liquor and the first acetone washing of the pulp were combined representing the liquor product of the fractionation. The liquor was evaporated at 60 °C under vacuum to remove acetone and precipitate lignin, which was separated by centrifugation (3488 g, 5 min). The product supernatant is further referred to as C5 sugar-rich hydrolysate. The lignin precipitate was re-dissolved in 60 wt% acetone and evaporated again under vacuum to cause precipitation. The precipitated lignin was then decanted, centrifuged and dried at 60 °C.

Fractionation performance is quantified through the indicators of pulp yield, C6 sugars recovery in pulp, C5 sugar recovery in the product liquor, delignification and lignin yield (Supplementary materials). Herein C6 sugars refer to glucose, mannose, galactose and rhamnose, and C5 sugars refer to xylose and arabinose, or their anhydrous equivalents in solid biomass or pulps.

2.3. Enzymatic hydrolysis of pulps

Pulps were subjected to enzymatic hydrolysis using the commercial enzyme cocktail Cellic CTEC2 (Batch #VCSI0019, Novozymes) to assess their digestibility and the ideal dose to achieve satisfactory glucose yields (i.e. minimum 75 % glucose yield based on available glucose after 48–72 h). For these tests, 5 g dw equivalent of wet pulp were added to 50 mL liquid containing 0.05 M sodium citrate buffer pH 5.0 and 0.02 % w/v sodium azide (corrected for moisture in sample and enzyme dose). The hydrolysing mixtures were incubated at 50 °C under constant mixing (150 rpm). Once at temperature, various amounts of enzyme

cocktail were added to provide doses of 5, 10 and 15 g of enzyme cocktail per 100 g glucan available.

Glucose concentration in the hydrolysate samples was measured using a colorimetric method. For this, 2 mL of reagent (9 % v/v o-toluidine, 1.5 % w/v thiourea in glacial acetic acid) was added to 20 μL of (diluted) hydrolysate sample and heated in a water bath at 90 °C for 8 min. After cooling in tap water for 4 min, the absorbance was measured at 635 nm. Glucose yield was calculated as the weight percentage of the glucan available in the pulp that was measured as monomeric glucose (See Supplementary materials for observed glucose yields over time).

2.4. Hemicellulose sugars detoxification

For the detoxification of hydrolysates from fractionation, a granular activated carbon (HYDRAFFIN CC 1240 SPEZIAL) was used as adsorbent and added to hydrolysates to give a ratio of 4 g activated carbon per 100 g hydrolysate. The mixtures were maintained at 30 °C for 24 h. After this, the carbon was allowed to decant, and the detoxified hydrolysate was centrifuged at 4000 rpm for 10 min. For fermentation, the sugar concentration of the detoxified hydrolysates was increased by evaporating the hydrolysates under vacuum in a rotary evaporator at 60 °C, 150 mbar and 120 rpm.

2.5. Fermentability tests

Precultures were prepared by first growing cells on solid YPG or YPX agar medium. From this, one single colony was transferred to liquid YPG or YPX medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and either 20 g L^{-1} glucose (YPG, *S. cerevisiae*) or 20 g L^{-1} xylose (YPX, *S. passalidarum*). Small scale batch fermentations (30 mL medium in 100 mL Erlenmeyer flasks) were performed in duplicate. C5 substrates were diluted in medium containing 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 1 g L^{-1} KH_2PO_4, 2 g L^{-1} NH_4Cl and 0.3 g L^{-1} $MgSO_4.7H_2O$. The pH was adjusted to 5.5 with 5 M NaOH. The complete medium was centrifuged to remove solids and the supernatant was filter-sterilised (0.2 μm). Medium for the reference culture contained 45 g L^{-1} xylose. C6 substrates were diluted in demineralized water and the pH was adjusted to 5.0 (5 M NaOH or 5 M H_3PO_4). Next, the substrates were sterilised by autoclaving. Sterile stock solutions (0.2 μm filter) were added to concentrations of 10 g L^{-1} yeast extract, 1 g L^{-1} KH_2PO_4, 2 g L^{-1} NH_4Cl and 0.3 g L^{-1} $MgSO_4.7H_2O$. Medium for the reference culture contained 117 g L^{-1} glucose.

Cultivations were started by inoculation of precultures to an optical density at 600 nm (OD600) of 0.5. In general, 5 % v/v of concentrated precultures in saline solution with OD600 of 10 were inoculated. Cultivations were performed at 30 °C with an agitation speed of 150 rpm.

2.6. Analytical methods

Methods for the preparation and characterisation of solid and liquid biomass samples as well as chromatographic techniques are described in detailed in the Supplementary materials.

3. Results and discussion

3.1. Double cropping system based on food and non-food crops for production of bioenergy and food

Dedicated lignocellulosic crops (sorghum biomass, hemp and sunn hemp) and agricultural residues from conventional food crops (corn stover and wheat straw) were included in this study due to their high biomass yields and/or lignocellulosic contents. These dedicated crops can be grown on existing cropland in innovative rotations with conventional food crops in multi cropping systems to provide major savings in terms of cropland use and agronomic inputs while achieving diversified biomass composition and sustainability of the system (Struik et al., 2000; Zegada-Lizarazu et al., 2021). Double cropping systems based on wheat/corn with dedicated lignocellulosic crops in temperate environment were demonstrated to be scalable in field scale tests reported elsewhere (Fig. 2) (Parenti et al., 2024). They were successfully carried out with conventional farm machineries and increased the lignocellulosic biomass production from 60 % to 130 % compared to a conventional mono cropping system over 7-years, without reducing the food production. These systems can potentially start up a biofuel value chain, thus contributing to the sustainability of the transport sector. By managing the species combination ratios, it would be possible to supply low-cost feedstock with close to the required qualitative characteristics of a given biorefining process.

The sustainability aspects and potential for bioethanol production from feedstocks obtained by the specific rotating crops system used for the production the biomasses used in this study have been described in detail elsewhere (Parenti et al., 2024). In this prior work, it was highlighted how high spatial feedstock concentration and mobilisation levels can reduce significantly the environmental (e.g. greenhouse gas emissions) of the sourcing of the biomass. Agricultural residues and dedicated crops like sunn hemp can have relatively low emissions; however, a biomass such as sorghum can be supplied at relatively lower costs. In the present study, the biomasses obtained from such double cropping system field trials have been used as feedstock for the assessment of integrated pretreatment and bioethanol production.

3.2. Composition and fractionation of 2 G lignocellulosic feedstocks

The biochemical composition of the feedstocks used in this work is shown in Table 1. The feedstocks had ash contents between 2.3 % and 8.3 % wt and organic extractives that varied between 5.5 % and 15.8 % wt. Soluble sugars in water extractives of sorghum biomass have been found to correspond mainly to sucrose and to a lesser extent to starch accounting for 15–50 % wt of the biomass (Arai-Sanoh et al., 2011; Xu et al., 2020). The lower contents of soluble (non-structural) sugars observed in the sorghum used in the present study could be related to the specific plant variety as well as the plant parts included in the feedstock (combined leaves and stems). In the case of corn stover, the found non-structural sugars corresponded to 26 % of the total organic extractives, consistent with the ranges reported (1–45 % of water soluble extractives) previously for the various plant fractions that constitute this agricultural residue (Berchem et al., 2017). The amount of C5 and C6 saccharides varied greatly among the various feedstocks. Hemp reported the highest C6 sugar content (55.7 % wt) and sorghum biomass the lowest (27.8 % wt). Total lignin in these biomasses were relatively low between 13.4 % and 20.4 % wt.

Water-acetone pre-extraction of the lignocellulosic feedstocks was implemented in this study with the goal of improving the feedstocks properties for fractionation/biorefining and homogenising materials from different origins. This approach was used previously on similar lignocellulosic biomass and showed a positive impact in terms of less impurities in product streams, lesser acid consumption for the fractionation step and thus less degradation of carbohydrates (Smit et al., 2022). The composition of the feedstocks after pre-extraction is also discussed (Table 1). Application of pre-extraction resulted in an overall increase of the total lignocellulose content of the feedstocks. Relatively similar amounts of organic extractives were ultimately observed in the pre-extracted materials (1.6–3.4 % dw). This was consistent with the fact that similar organic extractives removal was observed for the various feedstocks, in the range of 73–90 % (Supplementary materials). No sugars were detected in organic extractives from the pre-extracted feedstocks. Non-structural polysaccharides were removed during the pre-extraction of sunn hemp (galactan and glucan), sorghum biomass (glucan and fructan) and corn stover (glucan and fructan).

The ash content of the feedstocks also decreased through the pre-extraction process. Pre-extracted crop biomasses resulted in the lowest

Fig. 2. Double cropping systems with lignocellulosic crops planted after the main food crop carried out in Northern Italy. A) planting; B) chopping; C) windrowing; D) baling; and as background, a drone picture of the whole experimental area during the crop growth cycle.

Table 1
Composition of untreated and pre-extracted feedstocks in a dry weight basis. Values between brackets correspond to standard deviations when analyses were performed in duplicates.

Components, % dw	Ash	Organic extractives (ash free, sugar free)	Sugars in extractives	C5 sugars[a]	C6 sugars[a]	Total lignin[b]
Hemp Untreated	2.3 (0.05)	5.5	n.d.[c]	11.3 (0.04)	55.7 (0.56)	17.1 (0.08)
Pre-extracted	1.3 (0.06)	1.6	n.d.	12.1 (0.02)	56.9 (0.29)	18.3 (0.10)
Sunn hemp Untreated	7.0 (0.10)	11.4	1.3	11.7 (0.29)	36.4 (0.64)	17.2 (0.16)
Pre-extracted	3.4 (0.05)	2.7	n.d.	13.5 (0.28)	43.6 (0.03)	20.4 (0.30)
Sorghum Untreated	5.9 (0.01)	15.8	8.0	20.6 (0.17)	27.8 (0.27)	13.4 (0.05)
biomass Pre-extracted	4.5 (0.05)	3.4	n.d.	27.4 (0.22)	36.6 (0.23)	17.7 (0.25)
Corn stover Untreated	7.7 (0.04)	14.4	5.0	19.7 (0.13)	31.4 (0.08)	14.0 (0.24)
Pre-extracted	6.0 (0.02)	2.6	0.5	24.8 (0.02)	39.9 (0.43)	17.7 (0.21)
Wheat straw Untreated	8.3 (0.14)	10.1	n.d.	22.7 (0.51)	36.0 (0.75)	16.7 (0.12)
Pre-extracted	6.4 (0.03)	2.8	n.d.	26.2 (2.41)	40.1 (0.19)	18.6 (0.27)

[a] C5 sugars: Arabinan and xylan; C6 sugars: Glucan, mannan, galactan, rhamnan.
[b] Klason lignin and acid-soluble lignin.
[c] Not detected.

ash contents (1.3–4.5 % dw), whereas the ash content of agricultural residues decreased to a lesser extent. This thus meant that larger variations were observed in terms of ash removal, 33–61 % (Supplementary materials, Figure S1). Prior work has shown that the pre-extraction of biomasses such as wheat straw and roadside grass removes primarily monovalent cation metals (sodium and potassium) and to a lesser extent multivalent cations, such us calcium, magnesium or iron (Jiang et al., 2013; Smit et al., 2022). Furthermore, silicon is also commonly found in straws and herbaceous biomasses and is highly recalcitrant, requiring high pH treatments for effectively leaching this mineral fraction (Khaleghian et al., 2017), thus remaining after the type of pre-extraction performed herein. Furthermore, pre-extraction experiments were carried out with similar amounts of biomass (350 g) and a fixed (solvent) liquid-to-solid ratio, not corrected for the specific organic extractives or ash content of the samples. Due to mass transfer aspects, such as permeability and bulk density of the biomasses, differences in actual contact time of the material with the extracting solvents are expected and can lead to variability in the degree of extractives/ash/dry matter removal. In addition to aspects of mass transfer, the nature of the extractive components and their leachability influence the process.

After pre-extraction, the biomasses were fractionated through mild acetone organosolv fractionation (see Supplementary materials). To guarantee the required process pH of 1.8, sulphuric acid concentrations between 40 and 61 mM were required with the feedstocks, except in the case of sunn hemp which required 93 mM H_2SO_4. As a reference, 20 mM H_2SO_4 can provide the process pH in the absence of biomass. The acid doses required in this study were in the range as previously observed for other feedstocks pre-extracted through this approach (Smit et al., 2022). While there was no direct correlation between the ash content of the pre-extracted feedstocks and the acid requirement, this could be rather

associated to the specific composition of minerals remaining in the pre-extracted samples. Minerals containing cations such as Na, K and Ca can contribute to reducing the concentration of acid from the dissociation of sulphuric acid (Lloyd and Wyman, 2004).

In the present work, we refer to 'pulp' as the solid fraction remaining after fractionation and washing, 'liquor' as the liquid fraction separated from the solid fraction after fractionation combined with the solvent washing of the pulp, and 'hydrolysate' as the aqueous solution remaining after removal of acetone, and the precipitation and separation of lignin. Pulp yields were between 44 % and 60 % dw based on the dry weight of the pre-extracted biomass. The recovery of C6 sugars (mainly glucose) in the pulp was above 83 % for most feedstocks, being lowest for the herbaceous biomasses and highest for the agricultural residues. Delignification percentage, which refers to the removal of total lignin from the pre-extracted biomass, was found to vary between 75 % and 89 %. Between 72 % and 84 % of the removed lignin was recovered via precipitation from the liquor.

To understand the behaviour of the various lignocellulosic fractions in the fractionation, mass distribution of the measured C6 sugars, C5 sugars and lignin across different product fractions are presented in Fig. 3. The mass distributions are shown as percentage of a fraction based on the corresponding total C6 sugars, C5 sugars and lignin contents of the pre-extracted feedstock. In some instances, the mass balance for the components is above 100 % due to uncertainties in the characterisation of the samples, due to e.g. variability in the different biomass matrices of unreacted samples, pulp and liquid fractions. Nonetheless, they are an indication of the overall distribution of products and the degree of degradation of the components. Less than 10 % of the C6 sugars in the feedstocks was hydrolysed during the fractionation with negligible degradation to HMF (<0.4 %). No levulinic acid was detected in the hydrolysates. With regards to C5 sugars, between 6 % and 12 % of these sugars were degraded to furfural. Yields of monomeric C5 sugars was similar across the feedstocks (69–78 %). Since similar pH was attained for all treatments, the variability in furfural formation can be rather associated to the presence of other components such as chlorides, sulphates and other metals such as potassium and aluminium cations that can promote the dehydration of pentoses to furfural (Danon et al., 2014).

Through this organosolv fractionation, between 10 % and 25 % of the lignin remained in the pulp product. High variability in the recovery of precipitated lignin was observed across the feedstocks, between 54 % and 71 % of the lignin available in the pre-extracted feedstocks. The lowest lignin recoveries were observed with hemp and sunn hemp. These yields of precipitated lignin were in general lower than previously reported for other lignocellulosic feedstocks following the same tandem pre-extraction and fractionation (Smit et al., 2022). This can be related to inherent differences in the biomass source/type as well as differences related to processing scale. Additionally, monomeric phenolic products, vanillin and syringaldehyde, were measured and corresponded to 0.2–0.5 % of the lignin available in the pre-extracted feedstock.

The residual lignin in the pulp, precipitated lignin and phenolic products only accounted for 78–87 % of the lignin balance. Water soluble lignin (WSL), oligomeric lignin fragments formed due to the depolymerisation of the lignin during the process can explain the remaining balance. An UV spectroscopy method for the determination of WSL in the hydrolysates was developed by calibration using a reference WSL fraction isolated from wheat straw fractionation (see Supplementary materials). The estimated WSL found in the hydrolysates corresponded to a significant fraction of the lignin in the pre-extracted feedstocks (21–36 %). The distribution of apparent lignin fractions shown herein suggests that oligomeric lignin is formed during this type of mild organosolv fractionation process and it can correspond to a significant fraction of the product hydrolysate even after precipitation of the larger molecular weight lignin as previously discussed (Smit et al., 2022). However, it is important to highlight that the lignin mass balances including WSL were between 100 % and 123 %, and this is likely related to inherent uncertainties in the characterisation techniques. The characterisation of the biochemical composition of residual lignin via acid hydrolysis of the pulp, for instance, cannot fully differentiate actual lignin from other co-precipitated impurities, such as remaining lipophilic extractives. Furthermore, while the WSL determination method is corrected by the presence of feedstock-dependant interfering compounds such as furanics and phenolics, other type of organic extractives can also lead to interference in the measurements from different feedstocks. Advanced characterisation techniques are still needed to fully understand the mechanism of depolymerisation and faith of lignin during biomass fractionation.

3.3. Cellulose enzymatic digestibility and fermentability of C6 sugars

The enzymatic digestibility of the (wet) pulps produced from the feedstocks was evaluated at mL scale with a consistency of 10 g dw pulp per 100 g total liquid using various enzyme doses of commercial enzyme cocktail Cellic® CTEC2. Given that glucan contents varied between 70 % and 84 % dw (Supplementary information), the use of an enzyme dose based on glucan availability in the mixture allows to compare the digestibility of the pulps obtained from the different biomasses. Fig. 4 shows the glucose yields obtained from the different pulps after enzymatic hydrolysis for 48 h with different enzyme doses.

Pulps from sunn hemp and hemp gave significantly lower yields (32–59 %) than the other feedstocks. Sorghum biomass gave the highest yields, reaching over 90 % glucose yield with enzyme doses of 10 and

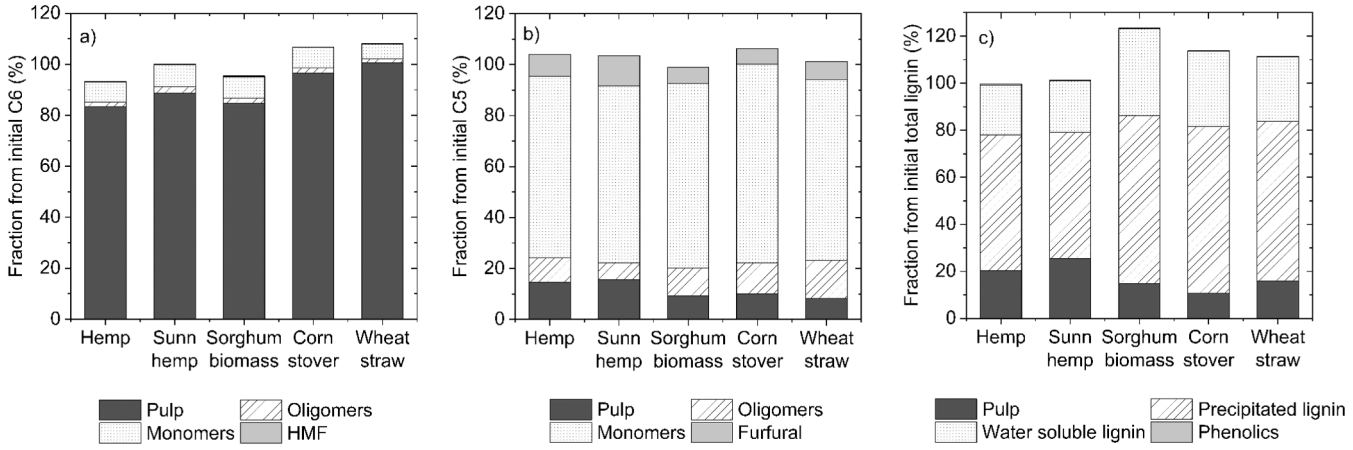

Fig. 3. Distribution of biochemical fractions from pre-extracted feedstocks in the various product fractions after mild acetone organosolv fractionation: a) C6 sugars; b) C5 sugars; c) Total lignin.

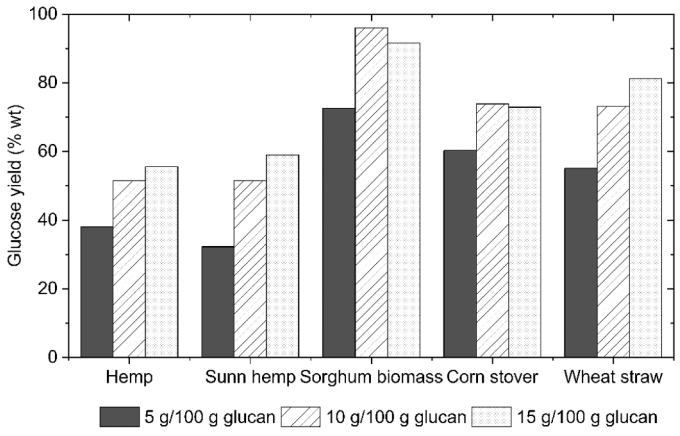

Fig. 4. Glucose yields after 48 h enzymatic hydrolysis of mild acetone organosolv pulps from. Conditions: 10 % consistency, 50 °C, pH 5 (citrate buffer).

15 g per 100 g glucan. Research in literature indicates that certain physico-chemical properties of biomass and pulps dictate their amenability to enzymatic hydrolysis. Among these properties, particle size, (cellulose) crystallinity, delignification degree, surface area availability, among others, have been attributed to contribute to their digestibility. Other aspect of importance is the actual accessibility of cellulose and non-cellulose components to cellulase. This is influenced by the fractionation or pre-treatment severity but can differ from biomass to biomass for the same process (Zhu et al., 2009). For the present set of pulps, there was no clear correlation between the actual contents of lignin and ash with the glucose yields attained at either 5 (limiting enzyme addition) or 10 (medium enzyme dose) g enzyme per 100 g glucan. This suggests that differences in the digestibility in this set of materials was not linked to unselective binding of enzymes. Other causes of this phenomenon can be found in other physical features of the materials. Djajadi et al. (2017) studied the enzymatic digestibility of different pulps obtained from hydrothermal pretreatment and various biomasses. In their study, the glucose yields obtained from the various samples were not correlated to the absolute contents of cellulose, hemicellulose or lignin, but rather to the relative abundance of hemicellulose and lignin on the surface of the pretreated materials (as determined by ATR-FTIR). Hemicellulose removal in particular was found to reduce the hydrophilicity of the biomass and decrease the wettability and in theory the accessibility of the enzymes to the biomass. Huang et al. (2019) found that for post-extracted bamboo residues from diluted acid treatment, the hydrophobicity of the material correlated to its enzymatic digestibility instead of the actual degree of delignification or xylan removal. Further removal of hemicellulose in delignified biomass has also been found to improve enzymatic digestibility by increasing exposure of microfibrils and thus of cellulose to enzymes (Ding et al., 2016). In general, this confirms that despite the fractionation enabling the separation of a C6 sugar-rich pulp from diverse sets of feedstocks, their processability through enzymatic hydrolysis may vary across them due to inherent properties beyond the overall pulp composition.

Samples of enzymatic hydrolysate slurries were centrifuged to remove unhydrolyzed matter and evaporated under vacuum to produce sugar syrups with a total sugar concentration varying between 164 and 225 g kg^{-1}. These C6-rich sugar syrups were used subsequently as C6 substrates for ethanol fermentation tests. Between 90 % and 94 % wt of the available sugars in the substrates corresponded to glucose, while the rest corresponded to xylose and mannose from the remaining available hemicellulose in the fractionated pulps.

The growth performance of *S. cerevisiae* on media derived from these C6 sugar syrups is presented in Fig. 5. The syrups were diluted with water at a 1:1 ratio, resulting in media with total sugar contents between

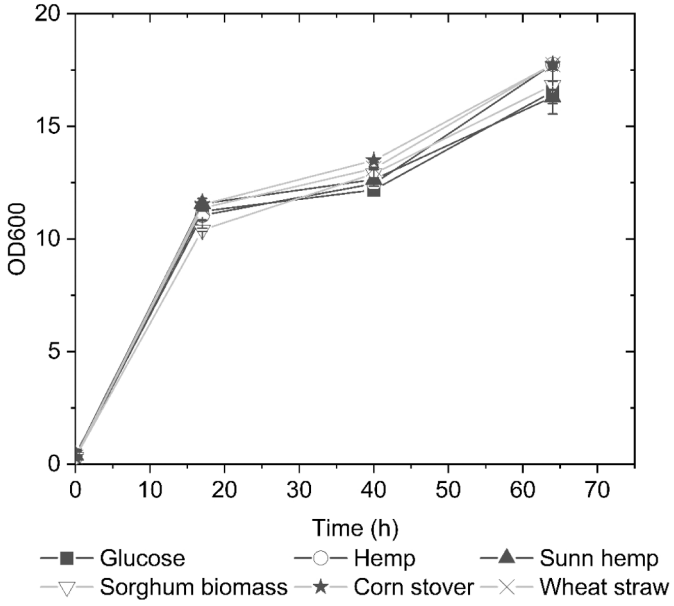

Fig. 5. Growth of *S. cerevisiae* in medium with glucose (117 g L^{-1}) or in medium with C6 substrates from lignocellulosic feedstocks (50 % w/v).

84 and 109 g L^{-1}). The growth performance observed on the lignocellulosic C6 sugar syrups were similar to that on a medium with pure glucose (117 g L^{-1}) (reference culture).

Table 2 presents the sugar consumption and ethanol yields obtained in fermentation tests using C6 sugar substrates. Ethanol was produced by *S. cerevisiae* in all cultures with total sugar concentrations of 33–41 g L^{-1}. The ethanol yield of the reference culture was 0.41 g g^{-1} of total sugar available, i.e. 81 % of the theoretical value of 0.51 g g^{-1} glucose. The ethanol yield on the substrates of the five feedstocks was 92–97 % of the ethanol yield of the reference culture. All glucose from the C6 substrates was fully consumed by *S. cerevisiae*. Minor amounts of xylose remained. Other products of the fermentations were glycerol (in concentrations between 1.7 and 3.8 g L^{-1}) and minor amounts of acetic acid (varying between 0.0 and 0. 6 g L^{-1}).

The high ethanol yields and sugar consumption observed during fermentation of these C6 sugar syrups were consistent with a previous study from our laboratory describing fermentability of the C6 sugar rich stream from beech wood using mild acetone organosolv fractionation (de Vrije et al., 2024). Under similar fermentation conditions, the beech wood C6 sugar syrup was fermented by the same *S. cerevisiae* strain, obtaining ethanol yields of 0.44 g g^{-1} glucose (corresponding to 104 % compared to the yields on control medium on glucose) and ethanol titres of 95 g L^{-1}. These data demonstrate the high suitability and flexibility of the mild acetone organosolv process to produce C6-sugar syrups for fermentation from different types of lignocellulosic biomasses. There is extensive research performed in the last decades on fermentation of lignocellulosic streams to bioethanol (Malik et al., 2022; Patel and Shah, 2021). The most studied pre-treatment technologies for solubilisation of sugars from lignocelluloses for uses in fermentation are based on (diluted) acid hydrolysis (Jain and Kumar, 2024). Sugar streams resulting from these treatments often require extensive detoxification, due to the production of inhibitors during the pre-treatment (mostly furfurals derived from sugars) or to the co-solubilisation of fermentation inhibitors present in the biomass, which is not needed in the case of the C6 sugar syrup through the approach presented herein. The fermentability of the C6 sugar fraction of the different biomasses was comparable to that reported for similar biomasses in previous studies but using other pre-treatments. Fermentability tests of a corn stover sugar stream obtained by diluted acid hydrolysis after biological detoxification resulted in a maximum ethanol yield of approx. 0.37 g g^{-1} sugar

Table 2
Fermentation data of *S. cerevisiae* cultivations in shake flasks on medium with glucose (reference culture) and C6 sugar syrups from lignocellulosic feedstocks (diluted at 50 % w/v). Data are from samples collected at a fermentation time of 17 h when ethanol titres in the broth were the highest.

Sugar / Feedstock	Total sugars in fermentation broth, g L^{-1}	Sugar consumption, % wt	Ethanol production, g L^{-1}	Ethanol yield / total sugars		
				g g^{-1}	% of reference	% of theoretical
Glucose	117.3 (0.1)	99.9 (0.0)	48.3 (0.1)	0.41 (0.00)	100	80
Hemp	84.0 (0.1)	95.7 (0.0)	33.0 (0.0)	0.39 (0.00)	95	76
Sunn hemp	108.8 (0.0)	93.8 (0.0)	41.4 (0.1)	0.38 (0.00)	92	75
Sorghum biomass	88.2 (1.7)	94.9 (0.0)	34.6 (0.2)	0.39 (0.01)	95	76
Corn stover	93.7 (0.3)	94.7 (0.1)	36.6 (0.0)	0.39 (0.00)	95	76
Wheat straw	93.4 (2.0)	95.7 (0.1)	37.5 (0.5)	0.40 (0.00)	97	78

consumed and a final ethanol titre of 25 g L^{-1} (Zhang et al., 2022). Wheat straw treated under acid hydrolysis resulted in streams that showed ethanol yields from sugars between 85 % and 98 % of the theoretical maximum (Talebnia et al., 2010). Different hemp species have been investigated for bioethanol production using different pre-treatments (hot water extraction, acid hydrolysis and alkaline hydrolysis), with best results obtained on alkaline treated biomasses, resulting in ethanol yields of approx. 96 % of the theoretical maximum (Zhao et al., 2020). Sorghum biomass represent a popular feedstock for 2 G bioethanol production, and yields between 76 % and 89 % of the theoretical maximum have been reported for bioethanol production from hydrolysates of sorghum bagasse produced using different pre-treatments (Xiao et al., 2021). These yields are lower than the yields shown in Table 2 for the C6 sugar syrup of sorghum biomass, as in this syrup mostly glucose is present as substrate, while in standard hydrolysates from the literature, both C5 and C6 sugars are present, resulting in lower overall ethanol yields.

3.4. C5 sugar hydrolysate detoxification and fermentability

Prior work showed that inhibitors found in C5 sugar-rich hydrolysate after mild acetone organosolv fractionation of beechwood can impede fermentation with *S. passalidarum* (de Vrije et al., 2024). To allow for fermentability, the C5 sugar-rich hydrolysates obtained from the pre-extracted feedstocks used in this study were treated with a granular activated carbon followed by further evaporation under vacuum until reaching 40–84 g kg^{-1} total sugar content. Approx. 65–80 % of the available sugars in these concentrated C5 sugar-rich syrups corresponded to xylose, while the rest corresponded to other sugars (arabinose, galactose, mannose, rhamnose and glucose) hydrolysed during fractionation.

Fig. 6 presents the concentration of fermentation inhibitors (organic acids, furanics and phenolics) in the hydrolysate obtained after mild acetone organosolv fractionation of the pre-extracted feedstocks, and after detoxification and vacuum evaporation. Organic acids (acetic and formic acid) were found in high concentrations (5600–8700 mg kg^{-1}) in the hydrolysates and no removal of these compounds was observed after detoxification with granular activated carbon. Furanics (HMF and furfural) and phenolics (vanillin and syringaldehyde) were found in lower concentrations ranging 1500–2280 mg kg^{-1} and 69–178 mg kg^{-1}, respectively, in hydrolysates. Between 69 % and 85 % of furanics were removed through the treatment with granular activated carbon and further removal (>93 %) was observed after vacuum evaporation. Between 61 % and 100 % of the detected phenolics were removed through the detoxification treatment; however, the phenolic compounds that remained after detoxification were not further evaporated due to their low volatility under the applied conditions. This explains the apparent higher concentration of phenolics after evaporation (Fig. 6).

The C5 sugar-rich syrups obtained after evaporation were used as substrates for fermentability tests by *Spathasphora passalidarum* as fermenting microorganism. This strain is able to ferment xylose efficiently and have been used successfully for growth in xylose-rich lignocellulosic streams (de Vrije et al., 2024). The growth performance of *S. passalidarum* on media derived from these detoxified C5 sugar syrups (98 % v/v) is presented in Fig. 7a. Growth of *S. passalidarum* was observed in media based on wheat straw, corn stover and sorghum biomass, which contained approximately 54–60 g L^{-1} total sugars. Growth started after a lag phase of 1–2 days while growth in medium with pure xylose (reference culture) started within 1 day (Fig. 7a). In contrast, no or low growth occurred in media based on detoxified C5 sugar-rich syrups from hemp and sunn hemp. When these streams were diluted at a 1:1 ratio (49 % v/v, Fig. 7b), growth started within 1 day in medium with C5 sugar substrate from hemp, and after a lag phase of 3 days also for C5 sugar substrate from sunn hemp.

Table 3 presents the sugar consumption and ethanol yields obtained in fermentation tests using detoxified C5 sugar substrates. Ethanol was produced in all cultures, with the highest production titres of 16–17 g L^{-1} from substrates of corn stover, sorghum and wheat straw, and a lower production from the diluted substrates of sunn hemp and hemp. The observations of poorer fermentability in substrates from sunn hemp and hemp are presumably linked to the larger content of

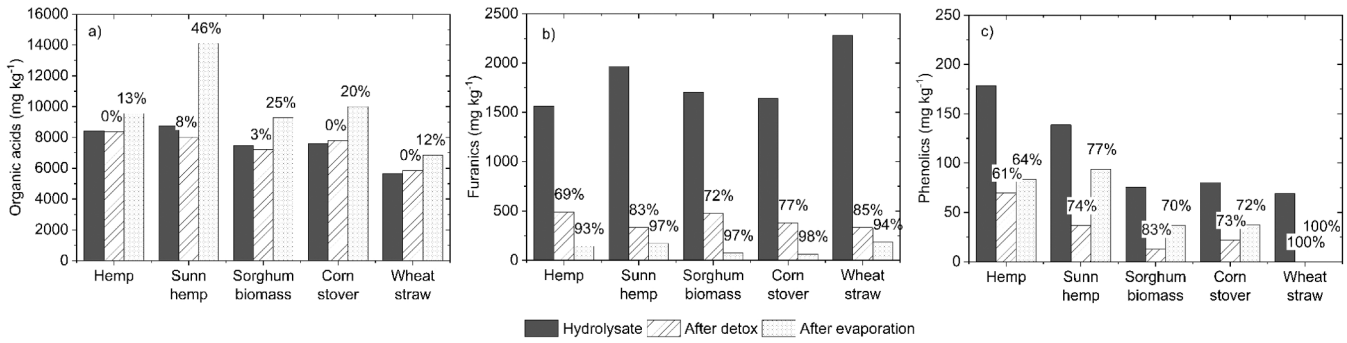

Fig. 6. Concentrations of fermentation inhibitors in hydrolysate before and after detoxification with granular activated carbon and after further evaporation: a) Organic acids (formic and acetic acid); b) furanics (5-hydroxymethylfurfural and 5-furfural); c) phenolics (vanillin and syringaldehyde). Percentage removal is presented on top of bars for concentrations after detoxification and after consecutive evaporation.

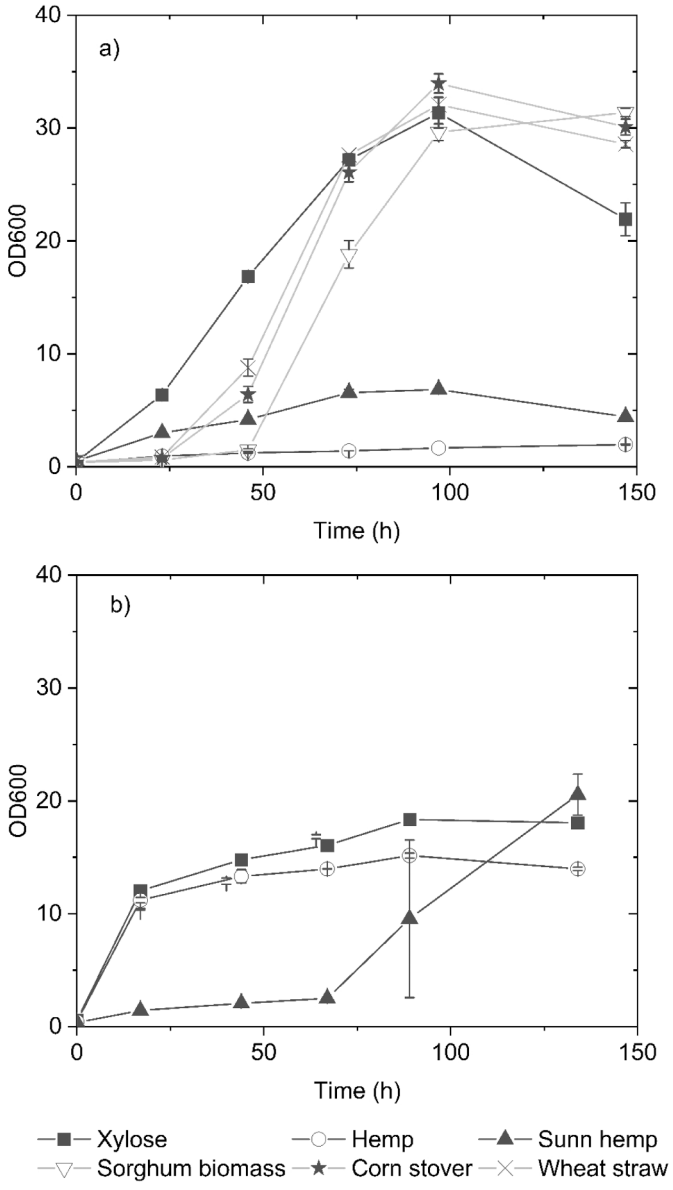

Fig. 7. Growth of *S. passalidarum* in medium with xylose (a) 45 g L^{-1}; b) 22 g L^{-1}) or in medium with detoxified C5 sugar substrates from lignocellulosic feedstocks (**a)** 98 % v/v; b) 49 % v/v).

inhibitory compounds that were still present in the C5 sugar syrups after detoxification and evaporation. Despite the consistent implementation of process conditions, the concentration of furanics and phenolics was at least two times higher in detoxified C5 sugar syrups from sunn hemp and hemp compared to those in syrups from corn stover, sorghum or wheat straw. This suggests that a threshold of maximum toxicity was not achieved for these two biomasses.

The ethanol yield of the reference culture was 0.38 g g^{-1} total sugars which is 74 % of the theoretical value of 0.51 g g^{-1} xylose. The ethanol yields on the substrates of the five feedstocks were 69–88 % of the ethanol yield observed in the reference culture. Xylose was the main sugar (65–80 %) in the C5 sugar syrups and was fully consumed by *S. passalidarum*. Galactose and glucose were consumed for more than 90 %, while consumption of arabinose was on average 50 % of the initial content. In cultures with substrates from hemp and sunn hemp, 50 % of the rhamnose and all of the mannose were consumed. Xylitol production was not detected.

The yields of bioethanol produced on the detoxified C5 sugar-rich syrups in this study (0.26–0.38 g g^{-1} total sugars) are comparable to those reported recently for the fermentation of a detoxified C5 sugar-rich syrup from beech wood produced via mild acetone organosolv fractionation (de Vrije et al., 2024). On laboratory-scale fermentations on beech wood C5 sugar-rich syrup (containing 59 g L^{-1} total sugars), the highest titre of ethanol was 18.7 g L^{-1}, with a yield of 0.32 g g^{-1} consumed sugars (approx. 63 % of the theoretical value of 0.51 g g^{-1} consumed sugars), with the consumption of 97 % of the sugars. The maximum theoretical ethanol yield (0.51 g g^{-1} sugar) is not reached in xylose-to-ethanol fermentations by native yeast strains, such as *S. passalidarum*. This could be related to the minimal requirements of oxygen for xylose fermentation, which need to be optimal for achieving high yields of ethanol by the yeast *Candida shehatae* (Bideaux et al., 2016). The yeast used in this study, *S. passalidarum*, utilizes xylose in control media under aerobic, oxygen-limited and anaerobic conditions, producing ethanol as major product (Veras et al., 2017). The highest yield corresponded to cultures under oxygen-limited conditions producing ethanol yields of 0.44 g g^{-1} xylose consumed. This value is higher than the highest ethanol yield obtained on the reference xylose culture herein (0.38 g g^{-1} xylose, Table 3), indicating that the fermentation conditions used in the present study are not optimal. For optimizing the fermentation conditions on the (detoxified) C5 sugar syrups, further research is needed to determine the most suitable medium composition, aeration conditions and pH range for each syrup. This was out the scope of this study.

3.5. Biorefinery outlook

To reach the goal of increased advanced liquid biofuels production, with 2 G bioethanol as major player, the demand for low-emission fuels would need to double from current levels by 2030. There is a need for a stable and sufficient supply of 2 G feedstocks since none of the main sustainable fuel options are on track for a net zero pathway (IEA, 2024). If this supply can be realised within the EU member states, the resulting advanced biofuels will contribute to enhance energy security, by reducing dependence on other regions for biomass production. Double cropping systems designed to produce food alongside lignocellulosic biomass have been evaluated for their potential to enable advanced bioethanol production (Parenti et al., 2024), thus highlighting an important theoretical contribution to the transition towards a net zero scenario. The cited study highlights how carbon emissions of the supply chain can be optimised when focusing on high spatial concentration and mobilisation levels of a feedstock such as sunn hemp as well as food crop

Table 3
Fermentation data of *S. passalidarum* cultivations in shake flasks on medium with xylose (reference culture) and C5 substrates from lignocellulosic feedstocks.

Sugar / Feedstock	Total sugars in fermentation, g L^{-1}	Sugar consumption, % wt	Ethanol production, g L^{-1}	Ethanol yield / total sugars		
				g g^{-1}	% of reference	% of theoretical
Xylose	45.5 (0.4)	100.0 (0.0)	17.2 (0.4)	0.38 (0.01)	100	75
Hemp	17.2 (0.2)	93.7 (0.1)	5.6 (0.0)	0.33 (0.00)	88	65
Sunn hemp	33.1 (0.0)	96.0 (0.0)	8.1 (0.0)	0.32 (0.00)	87	63
Sorghum biomass	53.9 (0.2)	89.6 (0.2)	16.0 (0.0)	0.30 (0.00)	79	59
Corn stover	60.4 (0.1)	90.8 (0.7)	15.9 (0.5)	0.26 (0.01)	69	51
Wheat straw	54.8 (0.2)	90.9 (1.3)	17.0 (0.3)	0.31 (0.01)	82	51

residues. Regarding bioethanol production, carbon emissions are contributed by bioethanol manufacturing to a lower extent compared to the whole chain emissions (Parenti et al., 2024). However, it is important to clarify that the carbon footprint estimated for the bioethanol manufacturing in the cited study may not directly apply to this biorefinery approach presented herein due to differences in the assumed technologies. Future work should look into effects of technology selection, biorefinery performance both from the technology and feedstock impact, and associated impact of the valorisation of co-products like lignin in applications other than energy recovery.

The biomasses used in this study were produced in a field using standard cultivation and harvesting procedures in double cropping systems. To maximise the valorisation of the components in the biomass, a biorefinery approach was followed, starting with pre-extraction to homogenize the materials and condition them for the further process. The pre-extraction conditions used were the same for all biomasses and were not optimised. The pre-extraction process implemented allowed for the substantial removal of extractives and ash from the feedstocks. Pre-extracted feedstocks required relatively low sulphuric acid doses, within the range required for more conventional woody biomasses through the same mild acetone organosolv treatment approach (Smit and Huijgen, 2017). The removal of organic extractives through pre-extraction also provides potential future opportunities for the valorisation of non-structural components, such as non-structural sugars (sucrose, starch) and other hydrophilic compounds such as uronic acids, proteins, pigments and salts.

In the following step, mild acetone organosolv fractionation was applied to obtain a cellulosic pulp containing mostly glucose and a liquor containing xylose and lignin. In terms of the conversion of cellulosic pulp to ethanol, the major impact of feedstock diversity seems to be related to the enzymatic digestibility of the pulps after fractionation. No direct relationship between pulp composition and amenability to saccharification was observed. While prior art has suggested that differences in hemicellulose and lignin surface distribution affects the accessibility of enzymes to carbohydrates in pulp, there is a need to further investigate how in particular (acetone) organosolv processes affect this accessibility during enzymatic hydrolysis of resulting pulps from different feedstocks. This would allow for creating tools to monitor pulp quality so that conditions during enzymatic hydrolysis can be optimised in real time in multiple-feedstock biorefinery. The present fermentation results show that C6 sugars resulting from this biorefinery approach are quite similar across the various feedstocks and can be consistently fermented by a commercial yeast strain.

Prior work has suggested that detoxification with activated charcoal is more efficient in hydrolysates obtained from pre-extracted feedstocks (Smit et al., 2022). However, there are additional variability in the efficiency of detoxification across the feedstocks studied herein. Particularly high levels of phenolics were found in hydrolysates from hemp and sunn hemp. While this did not completely impede the conversion of the C5 sugars to ethanol in these hydrolysates, it did impact the ethanol productivity rate, which can translate to issues in fermentation scale-up. Optimisation of the detoxification process for the different hydrolysates was outside the scope of this study. However, these results indicate that there is also a need to monitor inhibitor levels in hydrolysates and adjust detoxification conditions / carbon usage when processing certain less conventional feedstocks in a biorefinery process. Alternative detoxification approaches or strategies to deal with the presence of inhibitory compounds may also be desirable to decrease the impact of this downstream step on operation costs (Pan et al., 2022). This may include approaches that apply an additional treatments to hydrolysate streams such as cold plasma treatment, microbial detoxification, microbial immobilization, as well as non-destructive approaches that involve e.g. strain adaptation or microbial co-culture in the fermentation process (Pan et al., 2022; Preethi et al., 2021).

Additionally, the co-production of lignin from such feedstocks through the biorefinery approach presented herein unlock opportunities for biobased products. While residual (low quality) lignin from other aqueous-based pre-treatment methods is typically combusted as energy supply, the organosolv fractionation process allows for concurrent lignin valorisation, specifically in the replacement of fossil based chemicals in polyurethane foams and coatings (de Haro et al., 2019; Smit et al., 2023). Co-production of liquid advanced fuels as well as chemicals is key in the development of robust biorefineries that will integrate into the future circular economy.

4. Conclusions

The present work showed a comparison of the performance of various lignocellulose feedstocks in a biorefinery approach for the production of advanced ethanol via pre-extraction, mild acetone organosolv fractionation, enzymatic hydrolysis, detoxification and fermentation. The feedstocks included in this study were sourced through an innovative low iLUC risk crop rotation system. This system is based on the cultivation of herbaceous crops (sunn hemp, hemp, sorghum biomass) and food crops (wheat, corn) on the same land, in a rotating scheme. Pre-extraction of the biomasses was found to decrease substantially the ash and organic extractives content of the feedstocks, increasing the relative content of lignocellulose components. The sulphuric acid dose required to reach standard pH levels for the acetone organosolv process under mild conditions was within the range seen for more conventional woody biomasses. High recovery of cellulose in the pulp product and of C5 oligomeric and monomeric sugars in the hydrolysate product showed that process efficiency can be maintained across the various types of feedstocks. However, the efficiency of the enzymatic digestibility of the pulps varied significantly for the various feedstocks with no correlation to differences in the compositions of the pulps. Ethanol production from C6 sugar enzymatic hydrolysate was quite robust and similar to that obtained from model glucose substrate with *Saccharomyces cerevisiae* yeast. High levels of phenolics were found in particular in C5 sugar-rich hydrolysates from hemp and sunn hemp after detoxification. This influenced the ethanol formation rate with *Spathaspora passalidarum* CBS 10155 although did not impact significantly the overall ethanol yield per gram of sugar. The present work shows the applicability of the aforementioned biorefinery approach for the production of advanced ethanol with a diverse set of sustainable lignocellulosic feedstocks. However, it highlights that processing steps for special attention for biorefinery scale-up and future deployment are found in the enzymatic hydrolysis of pulps and detoxification of C5 sugar-rich hydrolysates.

Funding

This work was supported by the European Union's Horizon 2020 Research and Innovation Programme (grant agreement No. 744821), the Dutch Ministry of Economic Affairs and the Wageningen University and Research Knowledge Base Program 34, Circular and Climate Neutral Society.

CRediT authorship contribution statement

Andrea Monti: Writing – review & editing, Supervision, Funding acquisition. **Walter Zegada-Lizarazu:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Andrea Parenti:** Writing – review & editing, Visualization, Conceptualization. **Rumeysa Caliskan:** Investigation. **Karla Dussan:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Ana M. López-Contreras:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Arjan T. Smit:** Writing – review & editing, Funding acquisition, Conceptualization. **Petra Bonouvrie:** Methodology, Investigation, Formal analysis. **Rick van de Vondervoort:** Methodology, Investigation, Formal analysis. **Truus de Vrije:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Michiel Hoek: Methodology, Investigation, Formal analysis.

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.

Acknowledgements

K. Dussan, M. Hoek, P. Bonouvrie, R. Caliskan and A.T. Smit thank Ben van Egmond and Karina Vogelpoel-de Wit for their contributions.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.120156.

Data Availability

Data will be made available on request.

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