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ORIGINAL PAPER

Nonsterile Lactic Acid Production from Pulse Husks

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Abstract

The production of lactic acid from agricultural by-products has gained significant attention due to its potential for value-added products. This study investigates the feasibility of producing lactic acid from soybean, pea, and faba bean husks through various pretreatment methods and fermentation strategies. Our results show that soybean and pea husks can be effectively converted into lactic acid, with yields of 0.25 g/g and 0.34 g/g, respectively. In contrast, no lactic acid production was observed from faba bean husks, suggesting that this material may be more recalcitrant to degradation. Dilute acid pretreatment and enzymatic hydrolysis were found to be effective in releasing significant amounts of sugars from soybean and pea husks, but not from faba bean husks. Our findings provide valuable insights into the potential of agricultural by-products as feedstocks for lactic acid production and highlight the need for further research into the optimization of pretreatment and fermentation strategies.

Highlights

- Different hydrolysis conditions for soybean, pea and faba bean husk were tested.
- Fermentative lactic acid production from hydrolysates was investigated.
- Lactic acid was produced from soybean (0.25 g/g) and pea husk (0.34 g/g).
- No lactic acid was produced from faba bean husk.

Keywords Lactic Acid · Legumes · Lignocellulosic Residues · Utilization · Fermentation

Introduction

Pulses, such as beans, lentils, chickpeas and peas, are lately experiencing a sharp increase in interest due to their nutritional value, environmental benefits and role in sustainable food systems. For human consumption, pulses are usually dehulled, leaving behind the husks as a by-product. In faba beans e.g., the husks constitute about 5 to 10% (w/w, dry matter) of the total plant biomass [1]. For soybeans, a global production of 18–29 million metric tons was estimated [2].

Pulse husks are currently largely unused. They are a lignocellulosic material, mainly consisting of cellulose, hemicellulose and low proportions of lignin. This makes them a suitable starting material for a variety of biotechnological conversion processes based on carbohydrates such as lactic acid (LA) production.

LA is a versatile platform chemical that has a wide range of applications, be it in the food (e.g., food preservative, acidulant, flavour enhancer) or non-food sector (e.g., cosmetics and pharmaceutical industry, production of polylactic acid, environmental management, waste treatment) [3–6]. The demand for LA is projected to rise steadily in the upcoming years [7], creating a need for new and sustainable substrate sources. Industrially, about 90% of the LA is produced via microbial fermentation [3].

Residual lignocellulosic biomass offers a promising alternative to non-renewable fossil resources and synthetic media as it is an abundant, cost-effective and renewable source of carbohydrates. Numerous lignocellulosic substrates have already been successfully tested for lactic acid production. These include agricultural residues such as straw [8] and corn stover [9], agro-industrial by-products like rice bran [10] and sugarcane bagasse [11] as well as industrial residues such as fibre sludge [12].

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LA production from lignocellulosic biomass is typically carried out in four steps: (1) chemical or mechanical pretreatment to break down the lignocellulosic structure, (2) enzymatic hydrolysis to yield fermentable sugars, (3) fermentation to metabolize sugars to LA and (4) separation and purification of LA [13].

To make use of the increasing amounts of residues, in this study, the husks of green pea, faba bean and soybean were investigated as feedstock for the fermentative production of LA. A dilute sulfuric acid pretreatment was employed aiming at the solubilization of the hemicellulose fraction followed by enzymatic hydrolysis to target the cellulose fraction. A pre-trial was carried out to determine the appropriate sulfuric acid concentration to release the highest amounts of metabolizable sugars.

There is a wide range of microorganisms able to produce lactic acid including different species of bacteria like lactic acid bacteria or *Bacillus* strains but also filamentous fungi, yeasts or microalgae and cyanobacteria [14]. The organism chosen for the fermentation step was the *Bacillus coagulans*. *Bacillus spp.* have multiple advantages for LA production. *B. coagulans* produces L-LA with high levels of isomeric purity [11]. *Bacillus spp.* can use both pentose and hexose sugars and therefore metabolize all sugars derived from lignocellulosic biomass [15]. The fermentation can be carried out at 50 °C so that the process does not necessarily have to be kept sterile [15]. By omitting the sterile conditions, the fermentation processes become more economical feasible on an industrial scale [16]. In addition, *B. coagulans* is relatively tolerant to inhibitors usually present in lignocellulosic hydrolysates, such as hydroxymethyl furfural (HMF), furfural and organic acids [17, 18]. In conclusion, its metabolic versatility with the possibility to produce isomeric pure LA makes *B. coagulans* particularly interesting for the conversion of lignocellulosic biomass.

The aim of this research is to explore the feasibility of producing lactic acid from soybean, faba bean and pea husks through a combined acidic and enzymatic hydrolysis process. The results of this study are expected to contribute to a utilization of currently underutilized residues and to an establishment of efficient bioprocesses.

Materials and Methods

Biomass

Faba bean husks were generously provided by Roland Beans GmbH (Germany) and pea and soya husks by HiWeiss GmbH (Italy). Any remaining seeds were removed from the received biomass by hand. The husks were ground to a particle size < 3 mm using a coffee grinder (Rommelsbacher EGK 200, Germany).

Microorganism

B. coagulans strain (DSM 2314) used in the fermentations was kindly provided by the Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam, Germany. The bacteria were provided as agar slants, and a cryogenic culture was prepared for further use. For the fermentation inoculum, two cryobeads were cultivated with 25 mL MRS broth in a 100 mL flask. Cultivation was carried out at 37 °C for 16 h at an initial pH of 6.2.

Hydrolysis

For the pre-screening trials, hydrolysis of 9–10% (w/w) of each of the biomaterials was first carried out at 121 °C for 20 min in presence of 0.1, 0.5 and 1% (w/w) H_2SO_4. Afterwards, the pH was manually adjusted to 5.0 by adding NaOH. In a second step, the pre-treated materials were enzymatically hydrolysed. Cellulase (Cellic CTec2, Sigma Aldrich, Germany), Protease (Protease S-02, ASA Spezialenzyme GmbH, Germany, activity > 50 U/mL) and Glucoamylase (Glucoamylase AN, ASA Spezialenzyme GmbH, Germany, activity 1200 U/mL) were added at a dose of 2 mL/L. The reaction was carried out at 50 °C for 20 h in a horizontal shaker at 120 rpm. After hydrolysis, the hydrolysate was separated from the solid residue by centrifugation at 14,025 g for 10 min (Centrifuge 5804, Eppendorf, Germany). A sample was taken after the acidic pre-treatment and at the end of the enzymatic hydrolysis. Samples were sterile-filtered with an 0.2 µm filter (Chromafil ®) and stored at -21 °C until analysis.

Based on the results of the pre-screening, the acid concentration for the hydrolysis of each substrate was chosen. The hydrolysis followed the same procedure as described above. The hydrolysate was stored at -21 °C until further use for fermentation.

Fermentation

The fermentations were carried out in an Eloferm bioreactor (Biotronix GmbH, Germany) with a working volume of 1 L. For each fermentation, 300 mL of husk hydrolysate were used and inoculated with 6% (v/v) of the *B. coagulans* preculture (see Sect. 2.2). The hydrolysates produced were used without any other additives. Fermentations were carried out in triplicates at 50 °C and pH 6. The pH was kept constant by automatic addition of 5 M NaOH. Samples were taken regularly during the fermentation, sterile filtered with

an 0.2 µm filter (Chromafil ®) and stored at -21 °C until analysis.

Analytics

Phosphate

The phosphate content was measured using the photometric ascorbic acid method. At first, four separate solutions were prepared: (I) sulfuric acid (2.5 M), (II) potassium antimonyl tartrate solution (0.2743 g $C_8H_4K_2O_{12}Sb$ x 3 H_2O in 50 mL demineralised water), (III) ammonium molybdate solution (0.4 g $(NH_4)_6$ $Mo_7O_{24} \times 4$ H_2O in 10 mL demineralised water) and (IV) ascorbic acid solution (1.76 g ascorbic acid in 100 mL demineralised water). Molybdenum reagent (V) was prepared by combining 2.5 mL (I), 0.25 mL (II), 0.75 mL (III) and 1.5 mL (IV). Sample (100 µL), 900 µL demineralised water, 10 µL (III) and 160 µL (V) were mixed. After incubating at 60 °C for 15 min, absorption was measured at 880 nm using a plate reader (Infinite 200 PRO, TECAN, Switzerland). A calibration curve with KH_2PO_4 as a standard was used as a reference.

Free Amino Nitrogen (FAN)

Free amino nitrogen (FAN) was determined following the modified EBC-ninhydrin method. Two reagents were prepared. For reagent I, 1 g Na_2HPO_4 x $12H_2O$, 0.6 g KH_2PO_4, 0.05 g ninhydrin, and 0.03 g fructose were dissolved in 10 mL demineralised water. Reagent II contained 0.2 g KIO_3, 60 mL demineralised water, and 40 mL absolute ethanol. For analysis, 20 µL sample, 50 µL (I), and 30 µL demineralised water were combined and heated at 90 °C for 5 min. Then 900 µL of reagent II were added, and absorption at 570 nm was measured using a plate reader (Infinite 200 PRO, TECAN, Switzerland). A calibration curve with glycine as a standard was used as a reference.

Qualitative Sugar Determination

A qualitative determination of the sugars in the hydrolysates was carried out by high performance thin layer chromatography (HPTLC). 1–5 µL of the samples and different sugar standards were placed on a HTPLC plate (Silica gel 60, 20 × 10 cm, Supelco) covered with silica gel using an autosampler (CAMAG® Automatic TLC Sampler 4, Switzerland). After drying, the plate was placed in a horizontal developing chamber filled with the migration solvent (ethyl acetate (68%, v/v), methanol (23%, v/v), water (9%, v/v)). For better separation, the plate was run twice. The plate was stained with naphthoresorcinol reagent (0.1 g napthoresorcinol, 95 mL ethanol, 5 mL conc. H_2SO_4) by dipping, drying and heating to 120 °C for 10 min. Glucose, xylose and arabinose were identified in the samples and subsequently measured quantitatively by HPLC.

Sugars and Organic Acids

Organic acid (acetic acid, formic acid and LA) and sugar (arabinose, glucose, xylose) content in hydrolysates and fermentation samples were determined using HPLC (U3000, ThermoFisher Scientific, US: LPG-3400SD pump, WPS-3000 L auto-sampler, TCC-3000RS oven, RefractoMax521 refractive index detector). 20 µL of sample was injected into an Aminex HPX-87 H column (300 mm × 7.8 mm) and eluted isocratically with 0.4 mL/minute 5 mM H_2SO_4 at 50 °C. Calibration curves were generated with pure solutions of known concentration.

HMF and Furfural

HMF and furfural were determined using HPLC (Shimadzu Deutschland GmbH, Germany) with the following setup: degasser Knauer DG-1300, pump Shimadzu LC-20AT, auto-sampler Shimadzu SIL 10AF, column oven Shimadzu CTO-10AS VP, detector Shimadzu SPD-20 A. 10 µL of the sample was injected into the column (Prodigy™ ODS-3, Phenomenex Ltd. Deutschland, Germany) and the column temperature was 35 °C. As eluents, acetic acid (0.75%, v/v, in water) (A) and acetonitrile (B) were used with a flow rate of 0.5 ml/minute. The solvent composition (v/v) was changed in several stages during the measurement: 0 min: 2.5% B; 10 min: 30% B; 20 min: 65% B; 22 min: 90% B; 26 min: 2.5% B. The wavelength of the detector was 270 nm. Calibration curves were generated with pure solutions of known concentrations.

Results and Discussion

Hydrolysis Pre-Screening

The yields of free amino nitrogen (FAN), phosphate and sugars (sum of xylose, arabinose and glucose) of the pre-screening trials are shown in Fig. 1. Hydrolysis of soybean husks yielded the highest concentrations of phosphate (4.6-6.0 mg/g substrate) and FAN (1.6–3.4 mg/g substrate). Pea husks had the highest sugar yields with up to 0.39 g/g substrate. Faba bean husks had overall the lowest yields. A consistent trend of yields across all parameters depending on the acid concentration however could not be observed.

The enzymatic hydrolysis without acidic pre-treatment showed the lowest sugar yields across all substrates. Arabinose was hardly released by enzymatic hydrolysis only

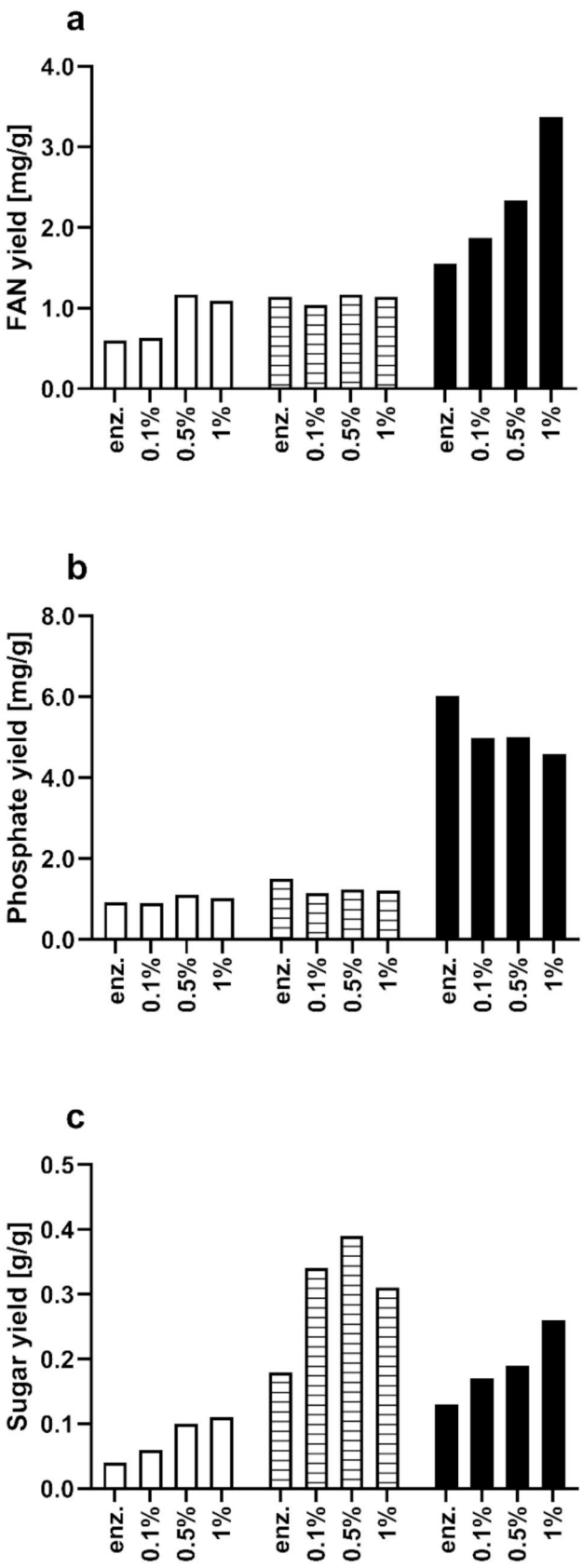

Fig. 1 FAN (**a**), phosphate (**b**) and sugar yield (**c**) of the pre-screening trials using faba bean (white), pea (striped) and soybean husks (black). Hydrolysis was carried out either only enzymatically (enz.) or started with a dilute sulfuric acid pre-treatment (0.1, 0.5 and 1%, w/w, H_2SO_4) followed by enzymatic hydrolysis

(Table 1). The addition of the sulfuric acid pre-treatment significantly increased the xylose and arabinose yield, especially when using concentrations of 0.5 and 1% (w/w). The overall glucose yield was also significantly higher when combining acid and enzymatic hydrolysis. Dilute acid hydrolysis primarily targets hemicellulose while the majority of lignin and cellulose remains in the acid insoluble residues [19], thus the pre-treatment mainly releases arabinose and xylose. After the removal of hemicellulose, cellulose is more accessible to the enzymatic attack, leading to increased glucose yields.

Under acidic conditions, the generation of by-products that may inhibit microorganisms poses a significant risk. Pentoses can be broken down to furfural and hexoses can degrade into 5-HMF. Both HMF and furfural can be further degraded to formic and levulinic acids. Additionally, acetic acid may form from the hydrolysis of acetyl groups of hemicellulose [20]. The concentrations of 5-HMF and furfural were below 0.1 g/L in all hydrolysates. The concentration of formic and acetic acid was below 2 g/L in all the studied hydrolysates (data not shown). *B. coagulans* DSM 2314 was reported to be able to withstand significantly higher concentrations of inhibitors without being negatively affected. Cubas-Cano et al. [17] found that a concentration of 5 g/L of furans led to a 75% growth rate inhibition. Van der Pol et al. [18] reported that the presence of 0.75 g/L furfural and 2.5 g/L 5-HMF caused inhibition in *B. coagulans.* For carboxylic acids, concentrations up to 20 g/L did not cause a negative effect in LA yield in the study of Cubas-Cano et al. [17], whereas van der Pol et al. [18] found that growth inhibition started already at 2.5 g/L formic acid and 7.5 g/L acetic acid. Thus, in this study, the concentration of potential inhibitors did not affect the choice of acid concentration.

The selection of acid concentration was primarily based on maximizing the sugar yield. The highest sugar yield in pea husk hydrolysis (0.39 g/g) was achieved with 0.5% (w/w) sulfuric acid. For soybean husk, the highest sugar yield (0.26 g/g) occurred at 1% (w/w) sulfuric acid. In the case of faba bean husk, the sugar yield was nearly identical at both 0.5% and 1% (w/w) sulfuric acid. Therefore, the decision was made based on the highest yield of phosphate and FAN resulting in a chosen sulfuric acid concentration of 0.5% (w/w).

The hydrolysis of the husks revealed considerable differences in the yields, despite all feedstocks being part of the same product group. Table 2 presents a summary of the composition of the studied husks, highlighting a

Table 1 Sugar yield (g per 100 g substrate) obtained after the hydrolysis of pulse husks. A: enzymatic hydrolysis, B-D: acid hydrolysis using 0.1% (w/w, B), 0.5% (w/w, C) and 1% (w/w, D) sulfuric acid, followed by enzymatic hydrolysis. T_A: yield after acid hydrolysis, T_E: yield after enzymatic hydrolysis

		Arabinose		Glucose		Xylose	
		T_A	T_E	T_A	T_E	T_A	T_E
Pea husks	A	-	0.81	-	10.05	-	5.65
	B	0.19	1.41	0.47	26.24	0.72	7.07
	C	3.79	3.86	0.56	26.45	3.37	8.89
	D	4.09	3.82	1.36	17.44	8.70	9.86
Faba bean husks	A	-	0.41	-	2.61	-	1.11
	B	0.77	0.92	0.69	3.63	0.79	2.07
	C	1.76	1.54	1.50	3.83	3.66	5.23
	D	1.57	1.18	2.22	3.14	5.90	6.37
Soybean husks	A	-	0.23	-	5.04	-	7.72
	B	0.18	0.86	1.43	9.33	1.01	7.23
	C	5.45	5.07	2.00	7.61	3.12	6.55
	D	5.86	5.72	2.23	9.32	9.88	10.81

Table 2 Cellulose, hemicellulose, lignin and protein content (%, w/w) of the studied pulse husks

	Cellulose	Hemicellulose	Lignin	Proteins	Reference
Pea husk	22.7–68.8	7-19.3	1.4–22.8	0.3–13.6	[22–26]
Faba bean husk	43.5–74.8	1.2–10.9	0.6–17.5	4.3–20.8	[1]
Soybean husk	28.6–52.3	18.5–33.8	2.3–13.1	9.4–15.4	[2]

notable diversity in the proportions. This variability may be attributed to several factors such as genetic differences, cultivation practices and processing conditions [2, 21]. Nevertheless, it is evident that the biomass primarily comprises hemicellulose and cellulose enabling high theoretical sugar yields. Compared to other lignocellulosic materials, legume husks can contain higher level of proteins, promising high FAN yields.

The sugar yields obtained in this study, however, are significantly below the theoretical potential based on the composition of the husks, indicating that cellulose and hemicellulose are not fully degraded during hydrolysis. This is especially evident with faba bean husks, which released only approximately 0.1 g of sugar per gram of biomass. The selected hydrolysis method seems inadequate for completely releasing all the sugars contained in the material. Future studies could explore alternative pre-treatment methods, such as citric acid pre-treatment. Bittencourt et al. [27] compared sulfuric and citric acid pre-treatments of soybean husks, concluding that citric acid is significantly more effective than sulfuric acid, achieving higher yields in the subsequent enzymatic hydrolysis.

There is limited research on the hydrolysis of pulse husks, besides from soybean husk. Cortivo et al. [28] and Schirmer-Michel et al. [29] reported similar sugars yields from the dilute acid hydrolysis of soybean husks using 1% (w/w) sulfuric acid and 10% (w/v) solid content at 121 °C, with 0.19 g of sugars per gram of biomass. In this study, 0.18 g/g were released. Additionally, Ourique et al. [30] reported a proportion of 55.1% (w/v) xylose, 33.4% (w/v) and 11.4% (w/v) glucose in the hydrolysate, closely aligning with our findings of 54.8% (w/v), 32.5% (w/v) and 12.4% (w/v), respectively.

As previously mentioned, pre-treatment is crucial for enhancing cellulose conversion efficiency. Rojas et al. [31] found that enzymatic hydrolysis alone could only convert 4% of cellulose, but this was increased to 40% with the addition of acid pretreatment. Similarly, Yoo et al. [32] reported a 70% increase in glucose yield of enzymatic hydrolysis following dilute acid pre-treatment. They observed that without pre-treatment, enzymatic hydrolysis yielded only 0.16 g glucose per g of biomass, whereas after acid pre-treatment, the yield increased to 0.27 g/g. In this study, the glucose yield was significantly lower, yet it could be enhanced by 85% by acid pre-treatment. Overall, the total sugar yield from all three hydrolyzed husks in this study was increased by more than 100% through acid hydrolysis, clearly highlighting the effectiveness of pre-treatment. The produced hydrolysates were then used as nutrient source for fermentative LA-production.

Lactic Acid Fermentation

The results of the LA fermentations are given in Fig. 2. Hydrolysis of pea husks yielded about 0.16 g/L FAN, 0.22 g/L phosphate and 39.2 g/L sugars (28.5 g/L glucose, 8.5 g/L xylose and 2.2 g/L arabinose). Three hours after the inoculation with *B. coagulans*, the production of LA started. Glucose was metabolised first, followed later by xylose and eventually arabinose. The sugars were completely

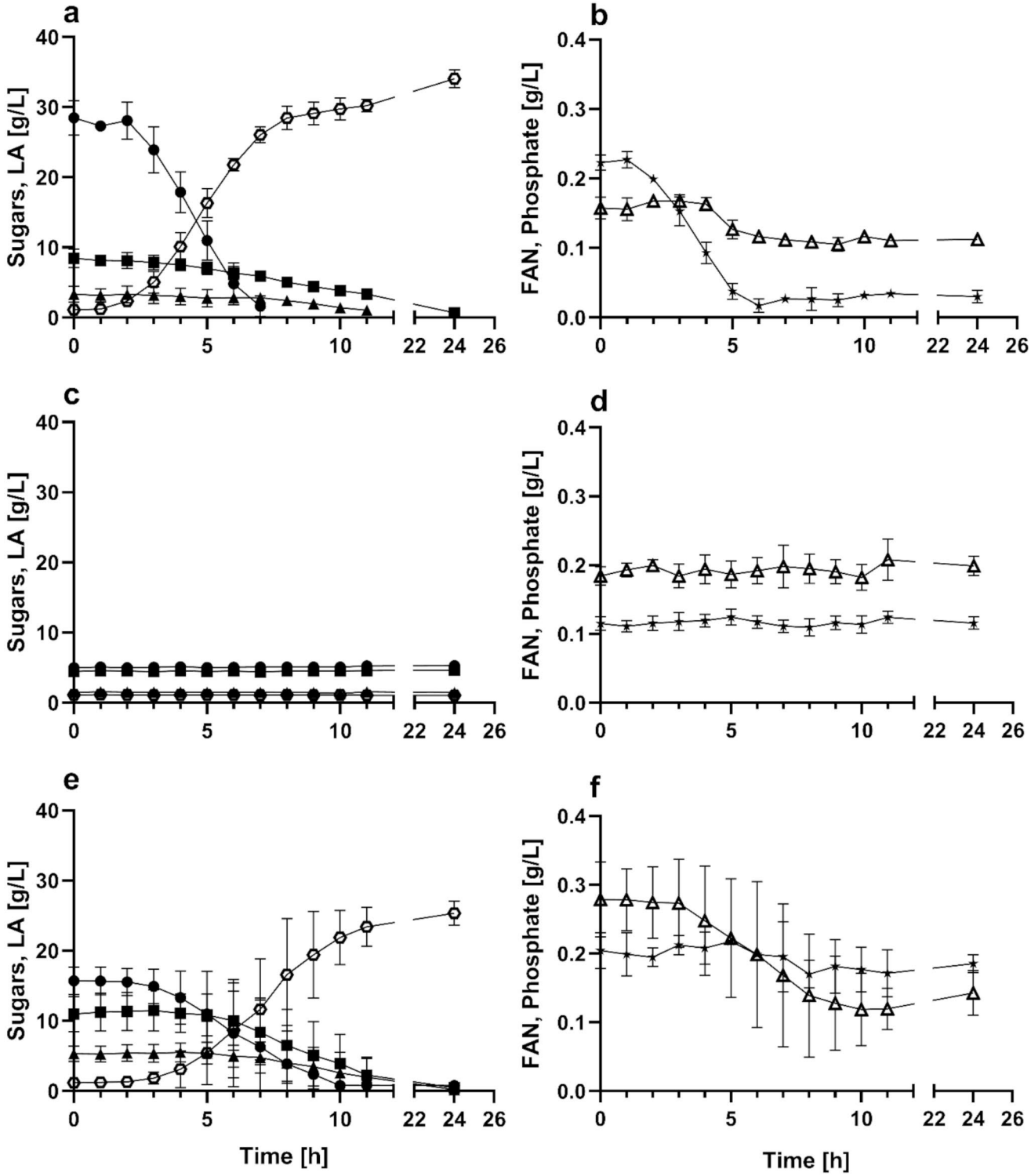

Fig. 2 LA fermentation profiles including changes in concentration of glucose (●), xylose (■), arabinose (▲), LA (○), phosphate (★), and FAN (Δ) from pea husk hydrolysate (**a-b**), faba bean husk hydrolysate (**c-d**) and soybean husk hydrolysate (**e-f**). The standard deviation is represented by the vertical lines

metabolized by the end of the fermentation. The concentration of FAN decreased only slightly during fermentation whereas phosphate was almost completely consumed. After 24 h of fermentation, a final LA concentration of 34 g/L was obtained.

Hydrolysis of faba bean husks yielded about 0.12 g/L FAN, 0.18 g/L phosphate and 10.9 g/L sugars (4.4 g/L glucose, 5.0 g/L xylose and 1.5 g/L arabinose). However, the bacteria did not produce LA. FAN, phosphate and sugar concentrations remained at the same level during the experiment.

Hydrolysis of soybean husks yielded about 0.2 g/L FAN, 0.27 g/L phosphate and 32.0 g/L sugars (15.7 g/L glucose, 11.0 g/L xylose, 5.3 g/L arabinose). The lag-phase lasted between three and seven hours in the three fermentations, which accounted for the high standard deviations of the measured concentrations. However, at the end of the fermentations, the concentrations evened out again. After 24 h, the sugar was completely used up in all experiments and the final LA concentration was 25.3 g/L on average. As for the pea husk fermentation, glucose was metabolized first, followed by xylose and eventually arabinose. The concentration of FAN decreased slightly whereas the phosphate concentration was reduced by almost 50%.

Table 3 displays the yields and productivities of LA production. The yield of LA from pea husks was higher than that from soybean husks, both in relation to the initial substrate and to the sugar content. Consequently, the productivity was also elevated. In contrast, faba bean husk hydrolysate did not yield any LA. The maximum productivity was observed at five to six hours for pea husks and at 8 to 9 h for soybean husks, after which productivity declined steadily. Between 11 and 24 h of fermentation, the LA concentration increased only marginally, as the sugar had already been nearly fully metabolized.

In all fermentations, glucose was the preferred carbon source, followed by xylose and eventually arabinose. This phenomenon, known as glucose repression, has also been noted in *B. coagulans* by other researchers [33, 34]. Specifically, Glaser and Venus [35] found that the consumption of pentose sugars started when the glucose concentration dropped to levels comparable to the initial concentrations of xylose and arabinose concentration. Subsequently, simultaneous consumption of all sugars was observed. In our study, glucose and xylose were also metabolized simultaneously in soybean husk hydrolysate once the glucose concentration was reduced to the level of xylose.

Table 3 Yield and productivity of the LA fermentations (average of three fermentations) after 24 h

Substrate	Yield [g/g substrate]	Yield [g/g sugar]	Productivity [g/L×h]	Maximum productivity [g/L×h]
Pea husk	0.34	0.87	1.42	6.43
Faba bean husk	-	-	-	-
Soybean husk	0.25	0.80	1.06	5.26

Lignocellulosic hydrolysates often consist mainly of sugars but do not contain enough nitrogen, which is essential for microbial growth and product formation. Typically, other nitrogen sources such as yeast extract are added, representing a high additional cost factor in the process [36]. Schroedter et al. [37] explored various alternative and low-cost nitrogen sources for *B. coagulans* 14–300. They found that without additional nitrogen, no LA was produced from reed hydrolysate. In contrast, the inclusion of baker's yeast, lucerne green juice and corn steep liquor resulted in yields ranging from 75 to 92% after 48 h. Our trials demonstrated that LA fermentation of pea and soybean husk could proceed without the need for extra nitrogen.

Studies on LA fermentation using pulse husk hydrolysate are rare. Bittencourt et al. [38] investigated the production of LA using a culture of *Lactobacillus pentosus*. A hydrothermal pretreatment with citric acid as a catalyst was followed by enzymatic hydrolysis of the solid residue. From 100 g of dry soybean husk, 27.8 g of glucose and 7.4 g of xylose were recovered in the hydrolysate, resulting in a final yield of 25.1 g of LA. Although the sugar concentration in this study was slightly lower, the final LA yield was comparable, at 0.25 g/g soybean husk.

Several studies have already been carried out with other lignocellulosic materials using different strains of *B. coagulans*. An overview of previous studies is given in Table 4. The reported productivities and yields span a wide range, depending on the substrate and the process design. Productivities have been observed to vary from as low as 0.2 to over 6 g/L and hour, with the productivities in this study falling on the lower end of the spectrum. However, the comparison of productivity has limited significance, as some studies focus only on the exponential growth phase while others consider the entire trial period. If the productivities in this study were based solely on the first 11 h of the trials, during which most of LA was produced, the productivities would be 2.7 g/L and hour for pea husks and 2.1 g/L and hour for soybean husks.

The yield related to the sugar content varies from approximately 55% to over 100%, with the yield of this study falling within the upper half of the range. In one case, a yield exceeding 100% is reported, which may be attributed to the metabolization of sugars that are not being monitored or to additional saccharification occurring during the fermentation process.

The yield based on biomass ranges from as low as 9% to nearly 50%. The results obtained in this study are comparable to those from other research utilizing separate hydrolysis and fermentation (SHF) or simultaneous saccharification

Table 4 Overview of the previous research on LA fermentation from lignocellulosic substrates using strains of *B. Coagulans*. SHF = separate hydrolysis and fermentation, SSF = simultaneous saccharification and fermentation, SSCF = simultaneous saccharification and co-fermentation, CSL = corn steep liquor, YE = yeast extract

Substrate	Pre-treatment	Enzymatic hydrolysis	Fermentation process	Nitrogen source	Strain	Productivity [g/L×h]	Yield [g/g sugar]	Yield [g/g biomass]	Reference
Sugarcane bagasse	Dilute acid	-	Batch, SHF	-	14–300	1.7	0.87		[11]
Sugarcane bagasse	Steam explosion	+	Batch, SHF	-	DSM2314	2.03–2.68	0.55–0.88		[42]
Sugarcane bagasse	Dilute acid	+	Batch, SHF	YE	NCIM 5648	1.04	0.90	0.09	[43]
	Dilute alkali					2.86	0.92	0.26	
Common reed	Dilute alkali	+	Batch, SHF	various	14–300	0.53–0.59	0.75–0.92		[37]
Common reed	Dilute acid	+	Batch, SSCF	-	IPE22			0.41	[41]
Corn stover	Dilute acid	-	Batch, SHF	-	NL01		0.73	0.27	[44]
Corn stover	Steam explosion	+	Fed-batch, SHF	-	NL01	1.04	0.75		[45]
Wheat straw	Steam explosion	+	Batch, SSF	-	MA-13	1.74		0.27	[46]
Wheat straw	Dilute acid	+	Batch, SSCF	CSL	IPE22			0.46	[40]
Wheat straw	Lime	+	Fed batch, SSF	YE	DSM 2314		0.81	0.25	[47]
Coffee pulp	Dilute acid	+	Batch, SHF	YE	Not specified	3.6-4.0	0.54–0.78	0.21–0.27	[33]
Empty fruit bunch	Dilute acid	-	Batch, SHF	YE	JI12	6.23	0.97		[48]
Empty fruit bunch	Dilute acid	+	Batch, SSCF	YE	JI12	3.4		0.49	[39]
			Batch, SHF			1.5	1.09		
Walnut shell	Microwave-assisted autohydrolysis	-	Batch, SHF	YE, peptone	DSM 2314	0.2	0.81		[49]
Pea husk	Dilute acid	+	Batch, SHF	-	DSM 2314	1.42	0.87	0.34	This study
Soybean husk	Dilute acid	+	Batch, SHF	-	DSM 2314	1.06	0.80	0.25	This study

and fermentation (SSF) processes. Notably, yields exceeding 40% were only achieved in simultaneous saccharification and co-fermentation (SSCF) trials [39–41]. Unlike most of the other studies, which either fermented only the liquid fraction after pre-treatment or used only the solid residue for enzymatic hydrolysis and fermentation, in the SSCF process, the whole slurry after pre-treatment was subjected to fermentation. Zhang et al. [41] demonstrated that fermentation of the liquid hydrolysate from dilute acid pre-treatment of common red yielded 17.9 g of LA per 100 g biomass, while SSF of the solid residue produced 20.7 g LA, and SSCF achieved 41.1 g LA per 100 g biomass.

In this study, the entire biomass subjected to pre-treatment was used for enzymatic hydrolysis, followed by an SHF process. The findings of Ye et al. and Zhang et al. [39–41] indicate that employing an integrated SSCF process could potentially enhance LA yield. Due to its thermophilic characteristics, its ability to metabolize pentose sugars, and its strong resistance to inhibitors, *B. coagulans* is a particularly suitable organism for SSCF.

Conclusion

In this study, acidic and enzymatic hydrolysis were carried out in the same matrix without separation of the liquid phase after the acidic treatment. This approach carries the potential for the presence of inhibitors resulting from the pre-treatment; however, these inhibitors were found to be present only at low concentrations. Simultaneously, this method eliminates an additional processing step, resulting in a reduced total volume and potentially higher concentrations of sugars. The results of this study have shown that pea and soybean husks can be used as nutrients for LA formation and thus may path the way to new utilization approaches.

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Data Availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

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