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OPTIMIZATION OF ENZYMATIC HYDROLYSIS AND FERMENTATION OF RICE HUSK FOR LACTIC ACID PRODUCTION

Alberto Liguori[1], Mario Daniel Ferrari[1] & Claudia Lareo[1]

[1] *Biochemical Engineering and Bioprocess Department, Chemical Engineering Institute, Universidad de la República, Montevideo, Uruguay.*
** Corresponding author's email address: aliguori@fing.edu.uy*

ABSTRACT

Lactic acid is an industrially important compound due to its versatility in the food, pharmaceutical, and chemical sectors. This study addresses the valorization of rice husk through an integrated process involving sequential autohydrolyzed and alkaline pretreatment, followed by enzymatic hydrolysis and lactic acid fermentation. The effects of solids loading, enzyme dosage, and pH were evaluated using a Box–Behnken experimental design, achieving glucose concentrations of up to 130 g/L. Considering the inhibitory effects observed at high sugar concentrations during fermentation, the hydrolysate was diluted to 40 g/L glucose for subsequent processing. The resulting hydrolysate was fermented by *Heyndrickxia coagulans* DSM 2314 under anaerobic conditions at 50 °C and pH 7.0, reaching a lactic acid concentration of 30.8 g/L after 24 h. The results demonstrate the effectiveness of the process for producing high concentrations of fermentable sugars and their rapid conversion into lactic acid, highlighting its potential for the scalable valorization of lignocellulosic residues.

Keywords: Enzymatic hydrolysis. Rice husk. Lactic acid. Lignocellulosic biomass. Biorefinery.

INTRODUCTION

Lactic acid (LA) is a valuable platform chemical with applications in the food, pharmaceutical, and chemical industry, particularly as a precursor of polylactic acid (PLA), a biodegradable polymer [1]. It can be produced either by chemical synthesis or microbial fermentation. While chemical synthesis relies on petrochemical feedstocks and yields a racemic mixture, microbial fermentation enables the production of optically pure LA from renewable substrates, representing a more sustainable alternative [2,3].

Lignocellulosic residues such as rice husk have gained attention as low-cost feedstocks for LA production. Uruguay is a major rice producer, generating significant amounts of rice husk as a byproduct, which is mainly used for energy generation or disposed of, raising environmental concerns [4]. However, due to its complex structure, pretreatment and enzymatic hydrolysis are required to convert its polysaccharides into fermentable sugars.

In this context, the present work focuses on the optimization of enzymatic hydrolysis of the glucan fraction of rice husk as a key step to obtain a hydrolysate rich in glucose suitable for subsequent lactic acid production by *Heyndrickxia coagulans* DSM 2314.

MATERIAL & METHODS

Raw material

The rice husk used as feedstock was provided by Galofer S.A. (Treinta y Tres, Uruguay). The material was conditioned for storage and kept at 4 °C until use. Prior to the experiments, the rice husk was homogenized, and its moisture content and particle size distribution were determined by mechanical sieving. The chemical composition of the rice husk, expressed as grams of component per 100 grams of dry raw material, was 34% glucan, 17% xylan, 21% lignin, and 21% ash.

Pretreatments

The rice husk was subjected to two successive pretreatments to separate and recover both hemicellulose and lignin for subsequent valorization and to obtain a glucan-rich residue suitable for enzymatic hydrolysis. For the first pretreatment, autohydrolysis was carried out in a 2-L high-pressure Parr reactor (180°C, 30 min). The solid obtained was then subjected to an alkaline treatment with NaOH in a 300-mL cylindrical reactor equipped with an oil bath (24% NaOH, 150°C, 90 min). In both treatments, 5 g water per 1 g of dry solids were used and the solids obtained were centrifuged and thoroughly washed with deionized water. The chemical composition of solid obtained after alkaline treatment, expressed as grams of component per 100 grams of dry raw material, was 76% glucan, 4% xylan, and 18% lignin.

Enzymatic hydrolysis

The pretreated solid was subjected to enzymatic hydrolysis using the commercial enzyme preparation Cellic® CTec2 (Sigma Aldrich®). A Box–Behnken design (17 experimental runs, including five center points) was employed to evaluate the effects of solids concentration (8–16% w/w of pretreated dry solid per 100 g of slurry), enzyme loading (15–25 FPU/g glucan), and pH (4.8–6.0) on hydrolysis efficiency and the resulting glucose concentration. Enzymatic hydrolysis assays were performed in 250-mL Erlenmeyer flasks containing 25 g of slurry. The flasks were incubated in an orbital shaker at 150 rpm and 50 °C for 72 h. The pH of the slurries was maintained using 50 mM citrate buffer. At the end of the hydrolysis, the samples were heated at 100 °C for 15 min to inactivate the enzyme and subsequently centrifuged at 6000 rpm for 15 min. The resulting supernatants were collected and analyzed to determine sugar content.

Fermentation

Fermentation was carried out in a BIOSTAT B bioreactor (Sartorius, Germany; 1 L total volume, 300 mL working volume) using the enzymatic hydrolysate supplemented with mineral salts as substrate. An initial glucose concentration of 40 g/L was selected to minimize potential inhibitory effects associated with higher hydrolysate concentrations. The system was operated in batch mode under anaerobic conditions at 50 °C and 150 rpm. *H. coagulans* DSM 2314 was inoculated at 10% (v/v). The pH was controlled at 7.0 by automatic addition of 5 M NaOH.

Analytical methods

The sugar concentration in the characterization of the raw material, pretreatment, enzymatic hydrolysis assays, and fermentation products was determined using a high-performance liquid chromatography (HPLC) system from Shimadzu, equipped with a refractive index detector (RID-10A) and an Aminex HPX-87H column.

The cellulase activity of the commercial enzyme and the carbohydrate content of the material were analyzed following the analytical method established by NREL [5,6].

Statistical analysis

Statistical significance ($p \leq 0.05$) was assessed by analysis of variance (ANOVA), and response surface analyses were performed using Statgraphics (Centurion 19 ® (Version 19.4.01; Statgraphics Technologies, Inc., The Plains, Virginia, USA).

RESULTS & DISCUSSION

Optimization of enzymatic hydrolysate

Enzymatic hydrolysis of pretreated rice husk was evaluated using a Box–Behnken experimental design, to assess the combined effects of solids loading, enzyme dosage, and pH on three responses: glucose concentration, hydrolysis efficiency, and overall glucose yield. The experimental results are summarized in Table 1.

As shown in Table 1, enzymatic hydrolysis was significantly affected by the evaluated factors, with clear interactions among them. Solids loading was identified as the most influential parameter affecting glucose concentration, increasing glucose titers from ~57 g/L at 8% solids to ~129 g/L at 16% solids. However, this increase was accompanied by a reduction in hydrolysis efficiency, which can be attributed to mass transfer limitations, reduced free water availability, and product inhibition effects [7]. From a process perspective, operating at high solids loadings is advantageous, as it enables the production of more concentrated sugar streams for downstream fermentation while reducing dilution and separation costs.

Enzyme loading exhibited a significant positive effect on all response variables. Increasing the enzyme dosage resulted in higher glucose concentrations, improved hydrolysis efficiency, and greater overall glucose yields. This behavior suggests that enzyme accessibility limitations associated with high-solids operation can be partially mitigated by increasing enzyme dosage [7].

Within the studied range, pH did not show a consistent positive effect on hydrolysis efficiency. Although it was identified as a statistically significant factor in the ANOVA models, increasing pH did not enhance enzymatic conversion and, in several experimental conditions, led to lower hydrolysis efficiencies compared to values near the optimal range. These results indicate that operating close to the pH range recommended by the enzyme supplier resulted in more robust hydrolysis performance for pretreated rice husk.

Although previous studies have reported shifts in the apparent optimal pH at high solids due to local pH gradients and lignin surface charge effects [8,9], the present results indicate that these effects did not translate into improved hydrolysis efficiency at higher pH values for this substrate.

Table 1. Enzymatic hydrolysis performance of pretreated rice husk solids under high-solids conditions. Center points were performed in quintuplicate.

Solid content (%)	Enzyme dosage (FPU/g_{glucan})	Initial pH	Final pH	Glucose[a] (g/L)	Hydrolysis efficiency[b] (%)	Overall glucose production yield[c] (%)
8	15	5.4	5.0	56.7 ± 4.0	79.7 ± 2.6	64.6 ± 2.6
8	20	4.8	4.8	56.2 ± 4.0	80.1 ± 2.5	64.9 ± 2.5
8	20	6.0	5.0	58.5 ± 3.8	84.2 ± 2.4	65.5 ± 2.5
8	25	5.4	5.1	67.2 ± 3.3	99.8 ± 2.0	74.7 ± 2.2
12	15	4.8	4.8	83.3 ± 2.7	78.6 ± 2.6	63.7 ± 2.6
12	15	6.0	5.2	76.8 ± 2.9	75.9 ± 2.7	56.8 ± 2.9
12	20	5.4	4.9	93.3 ± 2.4	87.9 ± 2.3	71.2 ± 2.3
12	20	5.4	4.8	93.4 ± 2.4	87.7 ± 2.3	71.1 ± 2.3
12	20	5.4	5.0	98.1 ± 2.3	92.0 ± 2.2	74.6 ± 2.2
12	20	5.4	5.0	92.8 ± 2.4	87.1 ± 2.3	70.6 ± 2.3
12	20	5.4	5.1	93.0 ± 2.4	87.4 ± 2.3	70.8 ± 2.3
12	25	4.8	4.7	95.1 ± 2.4	90.0 ± 2.3	73.0 ± 2.3
12	25	6.0	5.5	84.6 ± 2.7	79.3 ± 2.6	64.3 ± 2.6
16	15	5.4	4.8	95.5 ± 2.3	70.9 ± 2.9	53.1 ± 3.1
16	20	4.8	4.6	128.3 ± 1.7	94.1 ± 2.2	71.7 ± 2.3
16	20	6.0	4.9	99.8 ± 2.2	74.0 ± 2.8	55.5 ± 3.0
16	25	5.4	4.9	129.0 ± 2.4	95.8 ± 2.3	71.7 ± 2.3

[a] Glucose concentration corresponds to the glucose measured in the liquid phase after 72 h of hydrolysis.
[b] Hydrolysis efficiency was calculated based on the glucan content of the alkaline-pretreated solid.
[c] Overall glucose production yield was calculated relative to the glucan content of the raw rice husk.
Values are reported as mean ± standard deviation

Lactic acid fermentation

The fermentation of the enzymatic hydrolysate, as show in Figure 1, exhibited a lag phase of approximately 6 h, which may be related to the presence of inhibitory compounds in the hydrolysate [10]. Once this phase was overcome, *H. coagulans* entered an exponential phase lasting around 6 h, reaching biomass values above 4 g/L, indicating successful adaptation and active growth under the evaluated conditions.

Simultaneously, the glucose concentration in the hydrolysate medium decreased to near-complete consumption within 24 h, reflecting the efficient utilization of glucose as a carbon source for metabolism and cell growth.

Furthermore, LA production increased after the lag phase, reaching a final concentration of 30.8 g/L at 24 h. This increase, correlated with the active growth phase, suggests a growth-associated production pattern, where LA acts as the main product of substrate fermentation.

The achieved LA concentration (~31 g/L) and complete glucose consumption indicate efficient fermentation under the evaluated conditions; however, further improvements in productivity are expected at higher initial sugar concentrations.

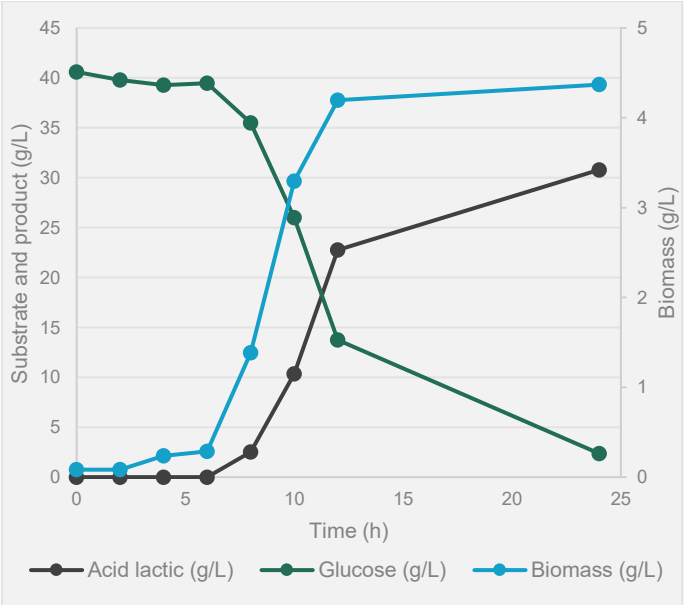

Figure 1. Fermentation of the enzymatic hydrolysate

CONCLUSION

Enzymatic hydrolysis of alkaline-pretreated rice husk enabled glucose concentrations of up to 130 g/L at high solids loadings, with solids loading and enzyme dosage as the main influencing factors. Fermentation by *H. coagulans* achieved 30.8 g/L LA from an initial glucose concentration of 40 g/L, highlighting potential inhibition at higher hydrolysate concentrations.

These results demonstrate the potential of rice husk for LA production, while indicating the need for further optimization to enable fermentation at higher sugar concentrations.

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ACKNOWLEDGEMENTS

The authors acknowledge financial support from ANII (FSE_1_2022_1_175516) and the postgraduate scholarships of ANII awarded to Alberto Liguori. The authors are also grateful to Galofer S.A. for providing the raw material used in this study.