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SUCCINIC ACID PRODUCTION FROM RICE HUSK HEMICELLULOSIC LIQUOR: EVALUATION OF FERMENTATION INHIBITORS

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ABSTRACT

Lignocellulosic hydrolysates typically contain inhibitors that limit microbial fermentation. Based on the characterization of rice husk autohydrolysis liquor, this study assessed the individual and combined effects of the main inhibitors identified—acetic acid, formic acid and furfural—on succinic acid fermentation. Batch experiments were performed using a defined synthetic medium containing glucose and xylose as the carbon sources. A control fermentation (without inhibitors addition) was compared against media supplemented with each inhibitor separately and with the three inhibitors simultaneously, at concentrations representative of those found in the liquor. In addition, a fermentation using rice husk autohydrolysis liquor as the carbon source was performed. Results showed that acetic acid (~3.0–3.3 g/L) suppressed succinic acid fermentation in synthetic medium. However, when the hemicellulosic hydrolysate was evaluated, *A. succinogenes* DSM 22257 was capable of fermenting after a detoxification step resulting in ~8–9 g/L succinic acid.

Keywords: *Actinobacillus succinogenes*, fermentation, inhibitors, rice husk, succinic acid.

INTRODUCTION

Succinic acid can be produced either from fossil resources from petrochemical routes, such as the hydration of maleic acid, or via microbial fermentation. The biological route has attracted growing interest because it can rely on renewable feedstocks and, depending on the metabolic pathway, may involve net CO_2 fixation during fermentation[1]. In some systems, the overall CO_2 uptake has been reported to approach ~1 mol CO_2 per mol succinic acid, which may help reduce the net greenhouse-gas footprint. Succinic acid (SA) is an important building-block chemical used in food products as a pH modifier, flavouring, and antimicrobial agent, and in the manufacture of pharmaceuticals, antibiotics, amino acids, vitamins, dyes, perfumes, lacquers, photographic chemicals, and polymer films. In addition, SA is a precursor for high-value chemicals such as adipic acid, tetrahydrofuran, and 1,4-butanediol, supporting applications across biodegradable plastics, food, and pharmaceuticals[2]. From an industrial perspective, the choice of carbon source is critical: fermentation on pure glucose is costly, motivating the use of lower-cost substrates such as industrial sugar streams, starchy materials, or lignocellulosic biomass. Replacing fossil resources with biomass is therefore a key strategy for advancing the bioeconomy, especially when the feedstock is an abundant residue whose conventional management options have technical or environmental limitations.

Rice husk is a representative example of such residue. It is produced in large quantities and contains structural carbohydrates (cellulose and hemicelluloses) embedded in a lignin matrix, together with minor fractions of proteins, starch, extractives, and a significant inorganic content. In Uruguay, rice husks are commonly used as a soil amendment, as animal bedding (especially in the poultry industry), and as a solid fuel in rice-processing facilities for steam and electricity generation. However, their high ash- and silica-related content can complicate combustion operation (e.g., fouling and accelerated equipment deterioration), and the same lignin–silica features may slow biodegradation when applied to soil[3]. Because rice husk still contains a substantial fraction of polysaccharides—values around 34.0 ± 1.3 wt% glucan and 16.8 ± 0.9 wt% hemicelluloses—this biomass is a promising low-cost feedstock for bioconversion routes. Importantly, fermentation-based production requires converting polymeric carbohydrates into soluble sugars; therefore, hydrolysis and pretreatment steps are needed to obtain fermentable streams from rice husk.

Most SA-producing microorganisms have been isolated from the rumen, and commonly used wild-type strains include *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, and *Anaerobiospirillum succiniciproducens*[4]. The aim of this work was to evaluate the effect of inhibitors commonly present in rice husk hemicellulosic liquor on SA fermentation by *A. succinogenes* DSM 22257. To validate the results obtained in semi-synthetic media, a fermentation using rice husk-derived liquor as the carbon source was carried out after conditioning and detoxification.

MATERIAL & METHODS

Feedstock
Rice husk supplied by Galofer S.A. (Treinta y Tres, Uruguay) was used as the lignocellulosic feedstock. The material was conditioned for storage and handling and stored at 4 °C until use.

Autohydrolysis pretreatment
Autohydrolysis was performed in a 2-L high-pressure Parr reactor. Rice husk was mixed with water at a liquid-to-solid ratio of 5 g water per g dry husk. The reactor was heated to 180 °C and held for 30 min after reaching the target temperature. After pretreatment, the solid and liquid fractions were separated by centrifugation at 1315 × g.

Analytical methods
The composition of the raw biomass and the pretreated solids (extractives, ash, structural carbohydrates, and lignin) was determined following NREL Laboratory Analytical Procedures (TP-510-42619, NREL/TP-510-42622, NREL/TP-510-42618 and NREL/TP-510-42623). The chemical composition of the pretreatment liquors was analyzed following NREL procedures (NREL/TP-510-42623). All analyses were performed in triplicate.
Glucose, xylose, arabinose, furfural and organic acids (acetic and formic acids) were quantified by high-performance liquid chromatography (HPLC).

Microorganism and inoculum preparation
A. succinogenes DSM 22257 was obtained from the DSMZ culture collection (Leibniz Institute DSMZ, Germany). Seed cultures were prepared in 250-mL bottles containing 125 mL of BHI medium supplemented with 0.7% (w/v) xylose and 0.2% (w/v) glucose. The pH was adjusted to 7.4 ± 0.2, and cultures were incubated at 37 °C and 150 rpm for 20–24 h. Media were sterilized at 121 °C for 15 min and inoculated after cooling.

Semi-synthetic fermentation media and inhibitor evaluation
A semi-synthetic fermentation medium was formulated to reproduce the main features of the rice husk liquor in terms of sugar level and inhibitor presence. Xylose was set to 18.9 g L^{-1} (matching the concentration measured in the liquor). Magnesium carbonate ($MgCO_3$, 15 g L^{-1}) was added for pH control. The base medium was sterilized by autoclaving (121 °C, 15 min), and sterile salts and phosphate solutions were then added as nutrients[5]. Yeast extract (YE) was prepared separately as a concentrated solution (125 g L^{-1}; 25×) to minimize Maillard reactions during sterilization and was added to the medium at 4% (v/v) (final YE concentration: 5 g L^{-1}). Fermentations were inoculated with seed culture at 10% (v/v) to reach an initial OD_{600} of 0.09-0.1. Acetic acid, formic acid and furfural were added at concentrations representative of those measured in the autohydrolysis liquor. The control condition (sugars without inhibitors) was compared to single-inhibitor conditions and to a combined condition containing the three inhibitors. Cultures were incubated at 37 °C and 200 rpm. Semi-synthetic fermentations were conducted in duplicate, and samples were collected periodically for sugar and product analysis by HPLC.

Fermentation using rice husk liquor as carbon source: acid hydrolysis, detoxification, and fermentation
The liquor was first subjected to acid hydrolysis in an autoclave at 121 °C using 1% (w/w) H_2SO_4. The hydrolyzed liquor was adjusted to pH 2–3 and detoxified with granular activated carbon (GAC) in a shaker for 10 min at 25 °C and 150 rpm, using a GAC-to-liquor ratio of 1:20. After detoxification, the liquor pH was first increased to 5–6 using ~5 M NaOH to minimize CO_2 release and foaming during subsequent $MgCO_3$ addition. $MgCO_3$ was then added for buffering, and the pH was finally adjusted to 7.2 ± 0.2. The conditioned liquor was sterilized (121 °C, 15 min), after which YE (added from a 125 g L^{-1} stock solution, 5 mL per flask), phosphate and salt solutions were aseptically added, and the medium was inoculated. Fermentations were performed in a shaker at 37 °C and 200 rpm for 144 h.

RESULTS & DISCUSSION

The liquor composition is reported after dilute-acid post-hydrolysis, since this is the stream used for detoxification and fermentation. Post-hydrolysis increased the proportion of monomeric sugars but also promoted partial pentose dehydration, explaining the lower xylose level compared with the liquor before post-hydrolysis and the presence of furfural. Glucose was the predominant sugar in the post-hydrolysis liquor, followed by xylose, and while acetic acid was the dominant inhibitor. These values were used to define inhibitor supplementation levels for the semi-synthetic media.

Table 1 Composition of rice husk autohydrolysis liquor.

Compound	Concentration (g/L)
Glucose	21.66 ± 0.40
Xylose	4.25 ± 0.02

Arabinose	2.01 ± 0.07
Acetic acid	1.11 ± 0.03
Formic acid	3.54 ± 0.03
Furfural	1.95 ± 0.09

In the inhibitor screening experiments, the inhibitor-free control (glucose + xylose) showed clear fermentation progress, with substantial sugar consumption and SA accumulation by ~144 h. When the three inhibitors were combined (acetic acid + formic acid + furfural), only minor changes in sugar concentrations were observed and SA did not increase relative to the initial measurement, indicating strongly limited fermentation under the tested conditions. In the formic acid condition, fermentation was achieved but with a pronounced lag phase: succinic acid remained near its initial level at the intermediate sampling point and increased markedly only by ~144 h. In contrast, acetic acid was the only inhibitor that fully suppressed fermentation within the evaluated concentration range, as sugars remained essentially unchanged and succinic acid did not accumulate over time. The furfural condition showed divergent behavior between replicates: one bottle displayed no productive fermentation, while the other exhibited a lag phase followed by strong SA production at ~144 h and a decrease in furfural from 0.60 to −0.01 g/L. This observation is consistent with previous reports describing furfural conversion by succinate-producing bacteria[4], supporting the idea that adaptation and/or in situ detoxification may enable fermentation in the presence of furfural.

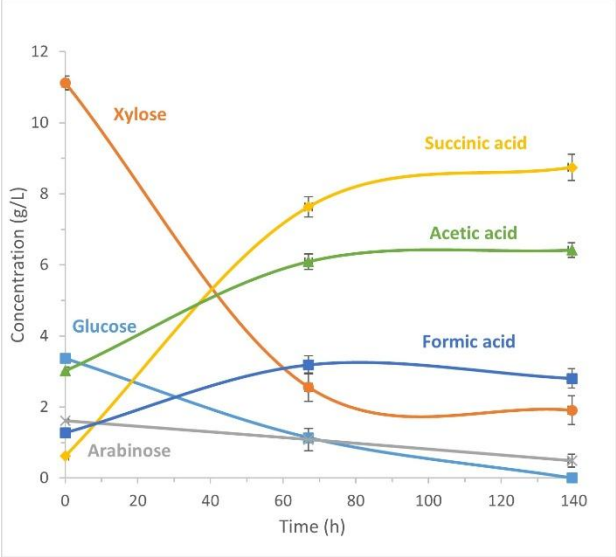

Figure 1. Fermentation profiles of succinic acid fermentation from detoxified rice husk liquor by *A. succinogenes*.

The detoxified rice husk liquor supported *A. succinogenes* growth and SA production, reaching ~8–9 g/L after ~140 h. Glucose was depleted and xylose was substantially consumed during the fermentation, confirming active fermentation on the liquor-derived sugars. However, the time required to achieve these titers was relatively long, suggesting that further optimization is needed to improve productivity, for example by increasing fermentable sugar concentration and/or improving process or strain adaptation to the liquor matrix.

CONCLUSION

Acetic acid was identified as a strong inhibitor, completely suppressing SA fermentation within the tested concentration range (~3.0–3.3 g/L). In contrast, after conditioning and detoxification, rice husk liquor supported *A. succinogenes* fermentation, achieving ~8–9 g/L SA after ~140 h, demonstrating the potential of this stream as a substrate for SA production.

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