Original Article

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# Integrated bioconversion of starchy and hemicellulosic fractions of macauba oil presscake into ethanol by nonconventional yeasts

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Abstract: Co-fermentation of hexoses and pentoses from plant biomass to ethanol is a strategy to increase ethanol production from lignocellulosic materials and add value to residues from agroindustrial chains. The macauba (*Acrocomia aculeata*) is an oleaginous palm-tree species considered to contribute to development in some regions of Brazil. Oil extraction from its fruits generates large amounts of presscake, which is rich in starch and hemicellulose. In this work, the production of second-generation ethanol from sugars released by acid hydrolysis of these polysaccharides was examined. The strains *Sungouiella akabanensis* UFVJM R131, *Spathaspora passalidarum* NRRL Y-27907, and *Kluyveromyces marxianus* CCT 7735 were evaluated as unconventional pentose-fermenting yeasts. Hydrolysis of macauba presscake with dilute $H_2SO_4$ produced a solution containing 17.2 g $L^{-1}$ glucose, 15.0 g $L^{-1}$ xylose, and 2.1 g $L^{-1}$ arabinose. All yeast strains were able to utilize glucose and xylose, with clear differences in sugar conversion kinetics. The 5-hydroxymethylfurfural (HMF) in the hydrolysate delayed sugar consumption by *S. akabanensis* and *S. passalidarum*. However, all HMF and furfural were biotransformed by all strains after 12 h. Ethanol yields ($Y_{P/S}$) were not significantly different among strains, averaging 0.38 g $g^{-1}$, which was 25% higher than the theoretical ethanol yield associated exclusively with glucose conversion. The *K. marxianus* CCT 7735 lineage showed about twice the volumetric productivity of the others, probably

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due to its tolerance of HMF. © 2025 The Author(s). *Biofuels, Bioproducts and Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: *Acrocomia aculeata*; xylose; fermentation; acid hydrolysis; *Candida akabanensis*; bioethanol; *Kluyveromyces*

## Introduction

The use of biologically based sources for the development of new products and processes has paved the way for an alternative economy that moves away from reliance on fossil or mineral resources. This approach has become increasingly relevant across various sectors, including food, pharmaceuticals, fine chemicals, polymers, energy, and biofuels.[1,2] In the biofuels sector, for instance, biomass sourced from living organisms across all kingdoms offers the potential to produce alternative fuels, replacing those derived from fossil resources like oil, natural gas, and coal.[3] Plant-based biomass has played a key role in guaranteeing the supply of the main commercial biofuels, such as biodiesel and ethanol.[4]

Selecting a biological source to establish a viable bioethanol production chain is complex and ultimately depends on supply and cost considerations.[5] For each region of the world, there will be distinct and sustainable opportunities for using plant biomass reflecting different environmental, social, and economic realities.[6] The presence of a variety of potential biomass feedstocks, and years of successful biofuel development, have provided Brazil with the infrastructure to be an important player in biomass-to-ethanol conversion.

Among the alternative feedstocks, the macauba palm (*Acrocomia aculeata*) offers strong potential in the biofuel industry due to its high agronomic productivity and the substantial oil and carbohydrate content of its fruits.[7–11] Oil extraction from fruit harvested on 1 ha yields approximately 10 tons of macauba pulp oil presscake each year.[12] Macauba oil presscake contains notable amounts of starch, cellulose, hemicellulose, and soluble sugars, accounting for over 50% of its dry weight.[13] This composition makes macauba oil presscake an attractive option for ethanol production. Challenges persist, however, in achieving efficient saccharification of its polysaccharides and in converting both hexoses and pentoses into ethanol.[7]

Recent advances in bioethanol production have introduced technologies designed to improve the co-fermentation of pentose and hexose sugars. *Saccharomyces cerevisiae* is one of the more efficient yeasts for hexose fermentation, particularly for glucose, the most common hexose in lignocellulosic hydrolysates. However, *Scheffersomyces* sp. and *Candida* sp. are more commonly applied for pentose fermentation as they present the enzymatic machinery to ferment pentoses into ethanol.[14–16] Conventional approaches to second-generation (2G) ethanol production have been based on industrially established *S. cerevisiae* strains, genetically modified to ferment pentoses and hexoses.[17]

Although the genetic modification of established organisms has become a common strategy for addressing pentose fermentation limitations, research on native and unusual microorganisms remains essential. Such microorganisms may offer efficient fermentation capabilities and serve as valuable sources of new genes or evolutionary mechanisms. By addressing the diversity of microorganisms, new studies may improve the efficiency of hexose and pentose fermentation and, ultimately, enhance 2G ethanol production using unconventional microorganisms.[18,19]

Despite previous reports on ethanol production from the hemicellulosic fraction of macauba oil presscake,[7] no study has yet addressed the simultaneous conversion of its starchy and hemicellulosic fractions using nondetoxified hydrolysates and unconventional, nonengineered yeasts. This integrated approach exploits the full carbohydrate potential of macauba biomass and evaluates native yeasts for their tolerance to inhibitors and capacity for co-fermenting C5 and C6 sugars.

In this scenario, the current study assessed three unconventional yeast species capable of co-fermenting hexoses and pentoses: *Sungouiella (Candida) akabanensis* UFVJM-R131,[20] *Spathaspora passalidarum* NRRL Y-27907,[21] and *Kluyveromyces marxianus* CCT 7735. This study therefore provides new insights into the valorization of macauba residues and the simplification of 2G ethanol production processes.

## Material and methods

### Chemical characterization of macauba pulp oil presscake

Macauba oil presscake, obtained by expeller processing of fruit pulp from S.Oleum Brasil Agro Industrial S.A. (Joao Pinheiro-MG, Brazil), was dried overnight at 60 °C and crushed in a knife mill model SL-31 (Solab, Piracicaba-SP, Brazil) equipped with a 2 mm diameter sieve. Cellulose, hemicellulose, and lignin content was quantified by gravimetric analysis according to the method proposed by Van Soest,[22] as adapted


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by Mongeau and Brassard.[23] Moisture, total protein, total lipids, and ash were determined following Adolfo Lutz Institute (IAL) protocols.[24] Total soluble sugar concentration was evaluated using the phenol-sulfuric acid method proposed by DuBois *et al.*,[25] and the starch content was quantified using the anthrone–sulfuric acid method proposed by McCready *et al.*[26]

## Acid hydrolysis of macauba pulp oil presscake

The hydrolysate of macauba pulp oil presscake was obtained as described by Gonçalves *et al.*[7] The acid hydrolysis was carried out in a conical flask containing the macauba oil presscake and 0.8% (v/v) sulfuric acid p.a, ACS (Dinamica, Indaiatuba-SP, Brazil) solution with a solid:liquid ratio of 1:10, which was subjected to 121 °C for 60 min in a vertical autoclave (Phoenix Luferco, Araraquara-SP, Brazil). After chemical hydrolysis, the solid residue was separated by vacuum filtration using Whatman no. 1 filter paper and reserved for further analysis. The soluble fraction recovered (hydrolysate) was adjusted to pH 5.5 with CaO p.a., ACS (Dinamica, Indaiatuba-SP, Brazil) and then subjected again to filtration on Whatman no. 1 filter paper to remove insoluble $CaSO_4$ formed during the pH adjustment. It was then stored frozen at −4 °C for further analysis and fermentation.

### Chemical characterization of macauba pulp oil presscake hydrolysate

The hydrolysate recovered after filtration was characterized chemically by high-performance liquid chromatography (HPLC) on an LC 20A Prominence system (Shimadzu, Kyoto, Japan). Sample injections of 5 µL were delivered by a programmed autoinjector through a Rezex ROA–Organic Acid $H^+$ column (300 × 7.8 mm) (Phenomenex, Torrance-Ca, USA), maintained at 60 °C. The analytes were eluted with 0.0025 mol $L^{-1}$ $H_2SO_4$ at a flow rate of 0.6 mL $min^{-1}$. Sugars and alcohols were detected using a refractive index detector (RID-20A) (Shimadzu), and acetic acid, furfural, and 5-hydroxymethylfurfural were detected with a ultraviolet-visible (UV–visible) detector (SPD-10AV) (Shimadzu) at 210 nm. Identification and quantification were based on analytical curves constructed with external standards of chromatographic purity (Sigma-Aldrich, Cotia-SP, Brazil) using LabSolutions LC software (Shimadzu).

## Detoxification of macauba pulp oil presscake hydrolysate

The hydrolysate was subjected to a process for removing furans, called detoxification. This process was carried out by adding p.a., ACS activated charcoal (Isofar, Duque de Caxias-RJ, Brazil) to the hydrolysate in a proportion of 5% (w/v).[27] The mixture was homogenized vigorously on a magnetic stirrer for 30 min at 50 °C. This was followed by vacuum filtration to remove activated carbon residues. The detoxified hydrolysate was reserved for further analysis and fermentation tests.

## Pentose fermenting yeasts

*Spathaspora passalidarum* NRRL Y-27907 and *Kluyveromyces marxianus* CCT 7735, both from the Collection of Microorganisms, DNA and Cells of the Federal University of Minas Gerais (CM-UFMG), were provided by Dr Carlos Augusto Rosa, from the Department of Microbiology, Institute of Biological Sciences (ICB/UFMG), Belo Horizonte-MG, Brazil. The strain *S. akabanensis* UFVJM R131 (Basionym: *Candida akabanensis*), registration SISGEN A18446E, from the Collection of Microorganisms of the Bioprocesses and Biotransformation Laboratory of the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM), Diamantina-MG, Brazil, was provided by the microbial prospection and bioprocesses research group from the same institution.

Yeasts were stored at −18 °C ± 2 °C in yeast extract–peptone–malt extract–dextrose (YEPMD) medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, D-glucose) (Kasvi, Pinhais-PR, Brazil) with 10% glycerol in 2.0 mL cryotubes. The strains were inoculated by surface spreading on YEPMD agar and incubated at 28 °C for 48 h. Macro and microscopic observations confirmed colony purity. The inocula were prepared by cultivating the strains in 20 mL of YEPMD liquid medium at 28 °C ± 2 °C, under orbital shaking at 150 rpm, until an optical density of 1.0 at 610 nm was reached.

## Fermentability tests

Fermentability tests were carried out in two stages. The first (Test I) evaluated the behavior of *S. passalidarum* NRRL Y-27907, *K. marxianus* CCT 7735, and *S. akabanensis* UFVJM R131, against detoxified and nondetoxified hydrolysates. The second (Test II) evaluated the fermentation kinetics of these yeasts, focusing on their response to hydrolysate detoxification for ethanol production.

### Test I

The fermentative tests were conducted in a batch system, in 50 mL conical flasks containing 30 mL of hydrolysate inoculated with free medium yeast cells from 10 mL inoculum medium, recovered by centrifugation at 9300 *g*

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for 10 min. Fermentation was carried out for 36 h under orbital agitation at 150 rpm, at 28 °C ± 2 °C. The process was monitored at 12 h intervals for cell growth and consumption of reducing sugars. At the end of the process, the ethanol content was determined. The fermentative assays were carried out in three replications.

### Test II

Fermentations for evaluating performance parameters were conducted as in Test I, with monitoring of cell growth, sugar consumption, and ethanol production at 2 h intervals during the first 14 h and subsequently every 12 h.

## Cell growth and fermentative profile analysis

Cell growth was monitored by measuring turbidity (optical density, OD). Aliquots of 1 mL from the fermentation medium were centrifuged at 9300 *g* for 10 min, and the resulting pellet was resuspended in the same volume of distilled water, and measured spectrophotometrically at 610 nm (spectrophotometer Biospetro SP-22, Shangai, China) in a 10 mm glass cuvette. Cell concentration was expressed on a dry basis ($g\,L^{-1}$) using a correlation curve between optical density and cell dry mass. The supernatant was reserved for chemical analysis by HPLC under the operating conditions described above.

## Calculation of fermentative parameters

Xylose consumption ($\Delta_{xyl}$), glucose consumption ($\Delta_{glu}$), hydroxymethylfurfural consumption ($\Delta_{hmf}$), and acetic acid consumption ($\Delta_{ace}$) were calculated as the difference between their initial and final concentrations. Ethanol ($\Delta_{eth}$), glycerol ($\Delta_{Gly}$), and cellular biomass ($\Delta_{cell}$) produced were calculated as the difference between their final and initial concentrations. Ethanol yield based on substrate consumption, $Y_{P/S}$ ($g\,g^{-1}$), was calculated by dividing the ethanol produced ($\Delta_{eth}$) by the sugars consumed ($\Delta_{glu} + \Delta_{xyl}$). Ethanol yield based on cell biomass produced, $Y_{P/X}$ ($g\,g^{-1}$) was calculated by dividing the ethanol produced ($\Delta_{eth}$) by the biomass produced ($\Delta_{cell}$). Cell biomass yield based on substrate consumed, $Y_{X/S}$ ($g\,g^{-1}$) was calculated by dividing the cellular biomass produced ($\Delta_{cell}$) by the sugars consumed ($\Delta_{glu} + \Delta_{xyl}$). Volumetric productivity, $Q_P$ ($g\,L^{-1}\,h^{-1}$) was determined by division between the maximum ethanol concentration achieved and its corresponding fermentation time.

Xylose consumption rate ($r_{xyl}$), glucose consumption rate ($r_{glu}$), 5-hydroxymethylfurfural consumption rate ($r_{hmf}$), acetic acid consumption rate ($r_{ace}$), ethanol production rate ($r_{eth}$), glycerol production rate ($r_{gly}$), and maximum specific cell growth rate ($\mu_{MAX}$, $h^{-1}$) were calculated by the ratio of the variation in concentration of each component to the time interval at the maximum inclination of the progress curves.

## Statistics

All fermentative and analytical assays were carried out in three replications. Analysis of variance (ANOVA) was applied to all results, and the means were compared using the Tukey test at $P = 0.05$.

# Results and discussion

## Chemical characterization of macauba pulp oil presscake

Table 1 presents the results of the chemical characterization of the macauba pulp oil presscake. The starch, hemicellulose, cellulose, and soluble sugar content exceeded 50% of biomass dry weight.

The contents of cellulose, hemicellulose, and total soluble sugars (TSS) determined here (Table 1) were close to the values reported by Santos *et al.*,[28] who worked with macauba pulp oil presscake and observed values of 11.5%, 9.6%, and 11.5%, respectively. Regarding the starch concentration (33.75%), the value was higher than that reported by Santos *et al.*[28] (who reported 23.1%) and Munhoz *et al.*[29] (who reported 23.6%). The differences noted may be related to the fruit ripening stage or edaphoclimatic conditions; both factors influence the chemical characteristics of macauba fruits.[30,31] Unlike other agroindustrial residues commonly used to produce 2G ethanol, such as sugarcane bagasse or corn crop residues,[32] macauba oil presscake is distinguishable by its high starch content.

The lignin content was notably lower than those reported for other biomasses in the literature, such as sunflower at

**Table 1. Chemical composition of macauba pulp oil presscake.**

| Component | g 100 $g^{-1}$ |
|---|---|
| Moisture | 4.43 ± 0.05 |
| Ash content | 2.80 ± 0.12 |
| Fat content | 24.33 ± 1.09 |
| Protein | 6.19 ± 0.09 |
| TSS | 10.05 ± 0.67 |
| Starch | 33.75 ± 2.52 |
| Cellulose | 7.72 ± 0.54 |
| Hemicellulose | 10.66 ± 0.68 |
| Lignin | 1.99 ± 0.52 |

TSS, total soluble sugars.


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4%,[33] buriti endocarp at 11.79%,[34] and jatropha oil presscake at 15.98%.[35]

Based on the proximal composition of macauba oil presscake (Table 1), ethanol production can be projected using stoichiometric estimates for carbohydrate conversion. Considering an expected oil presscake yield of 10 t per hectare in macauba plantations,[12] starch conversion alone could theoretically produce 2400 L of ethanol per hectare. Inclusion of total soluble sugars, hemicellulose, and cellulose increases this projection to 4350 L per hectare. Even assuming carbohydrate-to-ethanol conversion efficiencies of 70% to 90%, the projected volumes of ethanol would still be significant compared with the productivity of ethanol from corn grains (2000–3500 L $ha^{-1}$),[36] for example.

## Chemical characterization of detoxified and nondetoxified hydrolysates

Table 2 presents the concentration values of carbohydrates (glucose, xylose, and arabinose), glycerol, and inhibitors (5-hydroxymethylfurfural, furfural, and acetic acid) in the hydrolysates obtained after biomass pretreatment with diluted sulfuric acid, both detoxified and nondetoxified.

The hydrolysis of the macauba oil presscake, before detoxification with activated charcoal, resulted in a solution (hydrolysate) containing 17.16 g $L^{-1}$ of glucose, 15.05 g $L^{-1}$ of xylose, and 2.1 g $L^{-1}$ of arabinose. The presence of acetic acid, furfural, and 5-hydroxymethylfurfural (HMF) was also observed. These compounds have been termed 'inhibitory' due to their adverse effects on microorganism growth and ethanol production.[37] The detoxification of the hydrolysate using activated charcoal led to a 15% reduction in acetic acid content and completely removed the inhibitory furan compounds (furfural and HMF), indicating the efficiency of this process. On the other hand, there was also a reduction of up to 13% in glucose, xylose, and arabinose concentrations, the main sugars found in the hydrolysate. Sarawan *et al.*[38] associated this phenomenon with the adsorption of compounds onto activated charcoal.

**Table 2. Chemical composition of the soluble fraction of the macauba pulp oil presscake hydrolysate obtained by hydrolysis with diluted sulfuric acid and detoxified with activated charcoal.**

| Component | Nondetoxified hydrolysate | Detoxified hydrolysate |
|---|---|---|
| | g $L^{-1}$ | |
| Glucose | 17.16 ± 0.65 | 15.35 ± 0.32 |
| Xylose | 15.05 ± 0.63 | 14.26 ± 1.98 |
| Arabinose | 2.14 ± 0.24 | 1.67 ± 0.03 |
| Glycerol | 0.69 ± 0.15 | 0.51 ± 0.00 |
| HMF | 0.53 ± 0.02 | ND |
| Furfural | 0.02 ± 0.01 | ND |
| Acetic acid | 2.42 ± 0.09 | 2.06 ± 0.04 |

ND, Not detected; HMF, 5-hydroxymethylfurfural.

## Fermentation processes carried out with nondetoxified and detoxified macauba oil presscake hydrolysates

Figures 1–3 show the metabolic profiles of the three yeasts studied in fermentation trials with detoxified and nondetoxified macauba oil presscake hydrolysates. It is possible to follow the consumption of sugars, the production of ethanol and cell growth, and the biotransformation of furans and acetic acid.

Glucose was consumed entirely after 12 h of fermentation with the nondetoxified hydrolysate for all the yeasts evaluated. In contrast, the total glucose consumption in the detoxified hydrolysate was observed only after 24 h of fermentation.

Xylose consumption was slower and less complete than glucose consumption under the conditions tested. In the fermentations with the nondetoxified hydrolysate, xylose consumption was faster than in the detoxified hydrolysate. It was possible to observe in the fermentations performed by the three yeasts evaluated that xylose consumption suddenly slowed down and stopped when the glucose has been exhausted.

Arabinose was not consumed in either case, maintaining its initial values.

The fermentative profiles described in the paragraphs above were similar to those observed by Matos *et al.*,[32] when studying the fermentation of sunflower seed (*Helianthus annuus*) oil presscake hydrolysate using *S. akabanensis* UFVJM R131.

The highest ethanol productions were observed after a 12 h fermentation period in nondetoxified hydrolysates (11.31 g $L^{-1}$), and after 24 h in detoxified hydrolysates (9.38 g $L^{-1}$). The yield values ($Y_{P/S}$) in the fermentative processes carried out with nondetoxified and detoxified hydrolysates did not differ significantly ($P < 0.05$), presenting an average of 0.38 (g $g^{-1}$) (Table 3). Matos *et al.*,[33] in their studies with *S. akabanensis* UFVJM-R131, obtained a lower yield of 0.27 g $g^{-1}$. Batista *et al.*,[39] using the yeast strain *Scheffersomyces stipitis* HM 44.1 in the fermentation of detoxified macauba hydrolysate, achieved an ethanol yield ($Y_{P/S}$) of 0.37 g $g^{-1}$, close to that obtained in this study using the *S. akabanensis* UFVJM R131 strain, 0.36 g $g^{-1}$.

All the yeasts evaluated produced glycerol when fermenting the nondetoxified and detoxified hydrolysates (Table 3). Developing glycerol using yeast is usually associated with the

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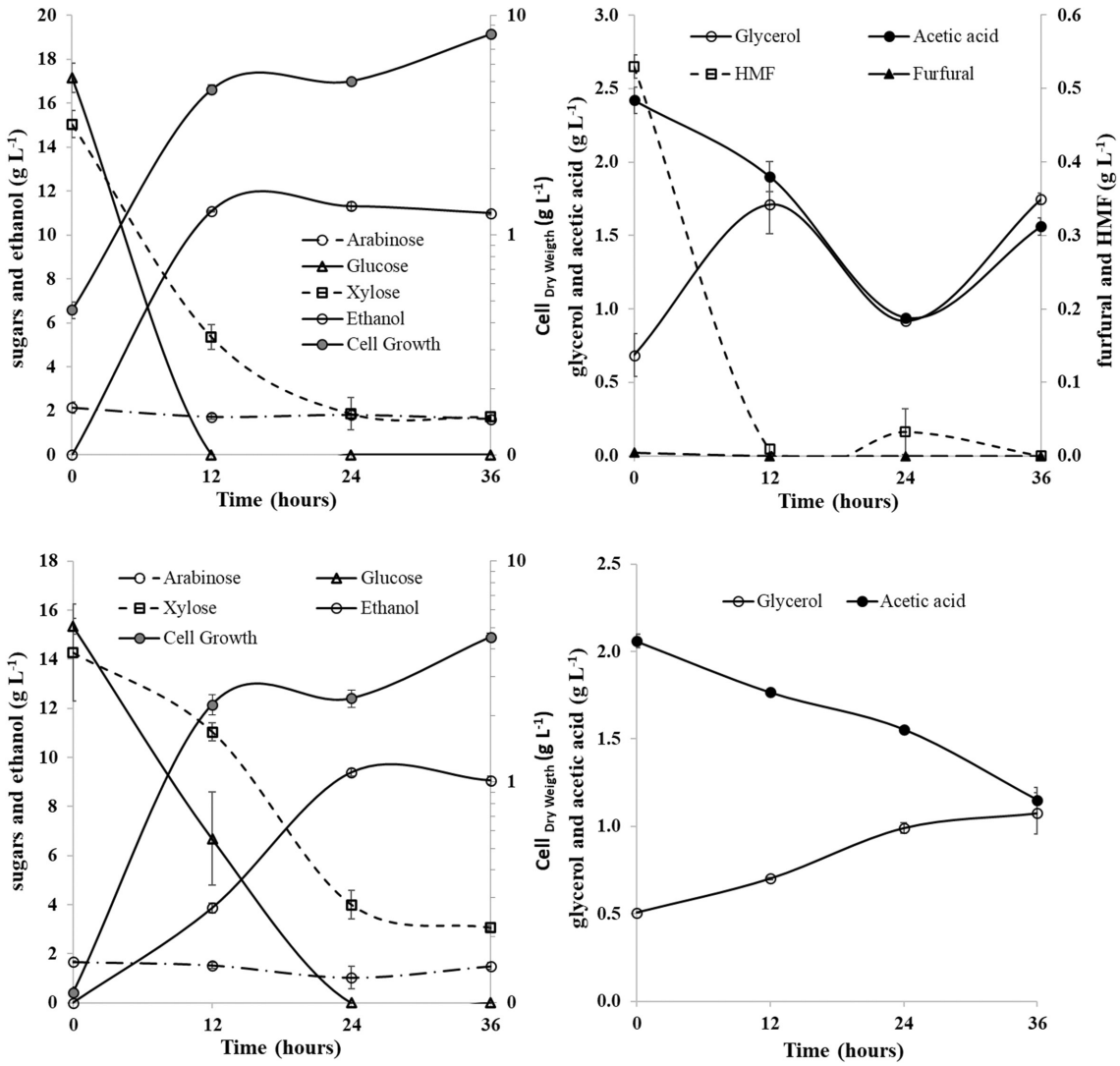



Figure 1. Fermentation progress curves of the macauba pulp oil presscake hydrolysate, nondetoxified (top) and detoxified with activated charcoal (bottom), using *Sungouiella akabanensis* UFVJM-R131.

regeneration of the $NAD^+$ required for glycolytic pathway progress, or as an osmoregulatory solute under osmotic stress conditions.[40] However, diverting the metabolism towards glycerol also reduces the ethanol production yield.

The furfural and HMF content was reduced significantly within 12 h in the nondetoxified hydrolysate processes carried out with all yeasts (Table 3). This observation can be associated with the ability of some yeasts to biotransform furans through catalytic action of NAD(P)H-dependent oxidoreductases in their respective alcohols, with lower or no toxicity.[41]

Acetic acid content in the nondetoxified and detoxified hydrolysates remained practically unaffected by the fermentative processes performed with *S. passalidarum* NRRL Y-27907 and *K. marxianus* CCT 7735. However, *S. akabanensis* UFVJM R131 proved capable of reducing the concentration of this aliphatic acid in the hydrolysates significantly within the first 24 h.

The cell growth of the three yeast species studied in the first 12 h was about twice as high in the nondetoxified medium in comparison with the value obtained in the process conducted with the detoxified fermentation medium (Table 3).

The concentrations of furans and acetic acid in the nondetoxified hydrolysate of macauba oil presscake were less harmful to the alcoholic fermentation promoted by the yeasts evaluated than the hydrolysate composition alteration caused by detoxification with activated carbon.

Table 3 shows the fermentation parameters – ethanol yield ($Y_{P/S}$), specific ethanol yield relative to cell mass ($Y_{P/X}$), biomass yield ($Y_{X/S}$), sugar consumption, and metabolite production after 12 h – for both hydrolysates and all three strains.


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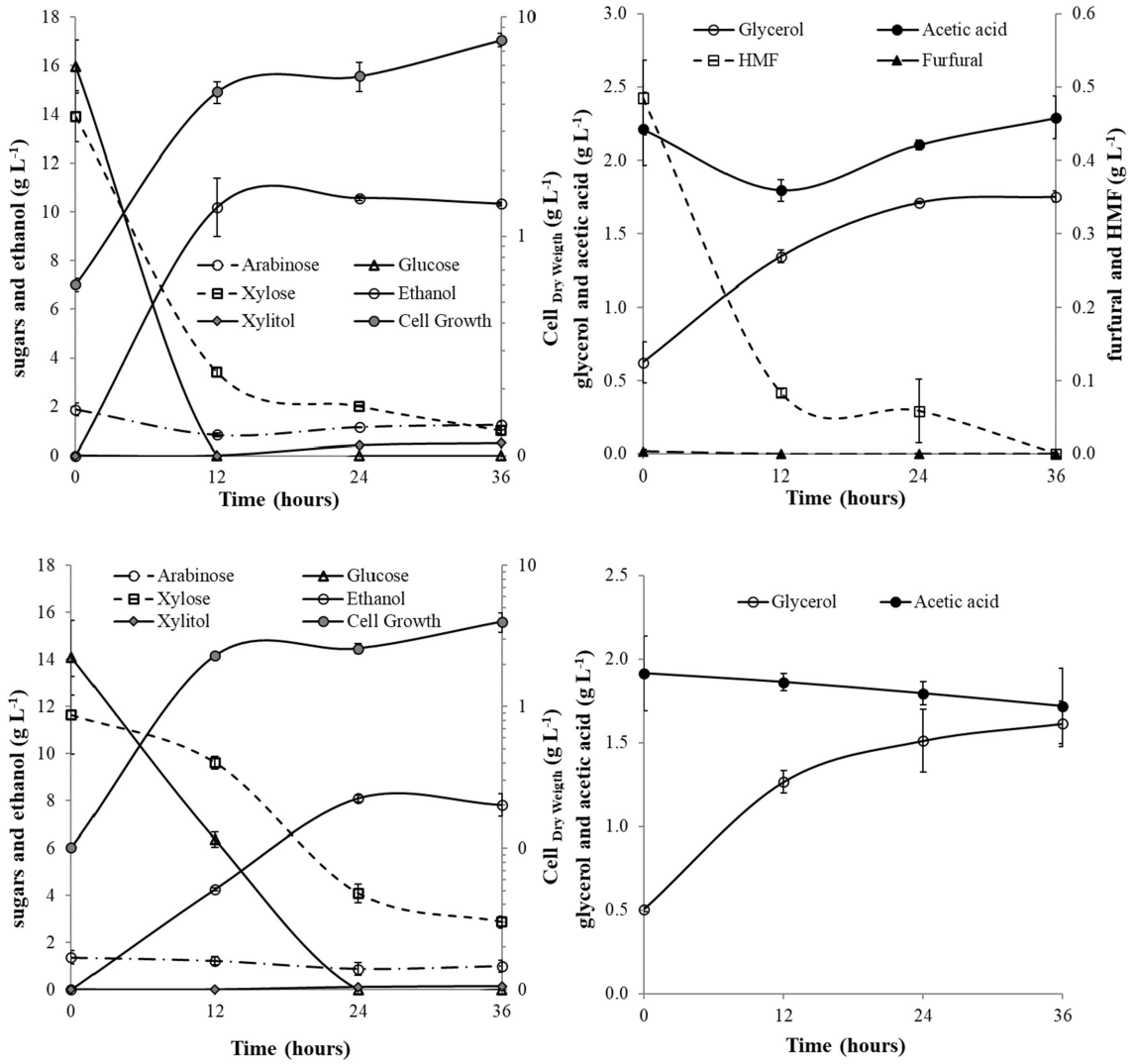



Figure 2. Fermentation progress curves of the macauba pulp oil presscake hydrolysate, nondetoxified (top) and detoxified with activated charcoal (bottom), using *Spathaspora passalidarum* NRRL Y-27907.

The data presented in Table 3 show that there were no significant differences ($P < 0.05$) between ethanol yield values ($Y_{P/S}$), regardless of the medium condition, detoxified or nondetoxified, or the yeast strain tested (*S. akabanensis* UFVJM R131, *K. marxianus* CCT 7735, and *S. passalidarum* NRRL Y-27907). Conversely, the volumetric productivity values ($Q_P$) were affected by the detoxification of the hydrolysate but not by the yeast strains evaluated. The $Q_P$ values were at least 2.4 times higher when the fermentation processes were carried out with the nondetoxified hydrolysate.

The cell biomass yields ($Y_{X/S}$) obtained in the processes conducted with the nondetoxified medium differed significantly between *K. marxianus* CCT 7735 and *S. passalidarum* NRRLY 27907, whereas no significant difference was found when using the detoxified medium. In the processes conducted with the *S. akabanensis* UFVJM R131, the $Y_{X/S}$ values did not differ significantly, regardless of the detoxification process.

The ethanol yield as a function of cell biomass ($Y_{P/X}$) did not differ significantly between the fermentation processes carried out with the *S. akabanensis* UFVJM R131 and *S. passalidarum* NRRL Y-27907 strains in both detoxified and nondetoxified media. In the processes conducted with the *K. marxianus* yeast, regardless of the hydrolyzed medium, $Y_{P/X}$ values were significantly lower than those observed in the bioprocesses conducted with the *S. akabanensis* UFVJM R131 and *S. passalidarum* NRRL Y-27907 strains. Although the *K. marxianus* strain performed better in population growth, therefore, this did not increase ethanol productivity.

All three evaluated yeast strains performed better on nondetoxified macauba oil presscake hydrolysate, mainly

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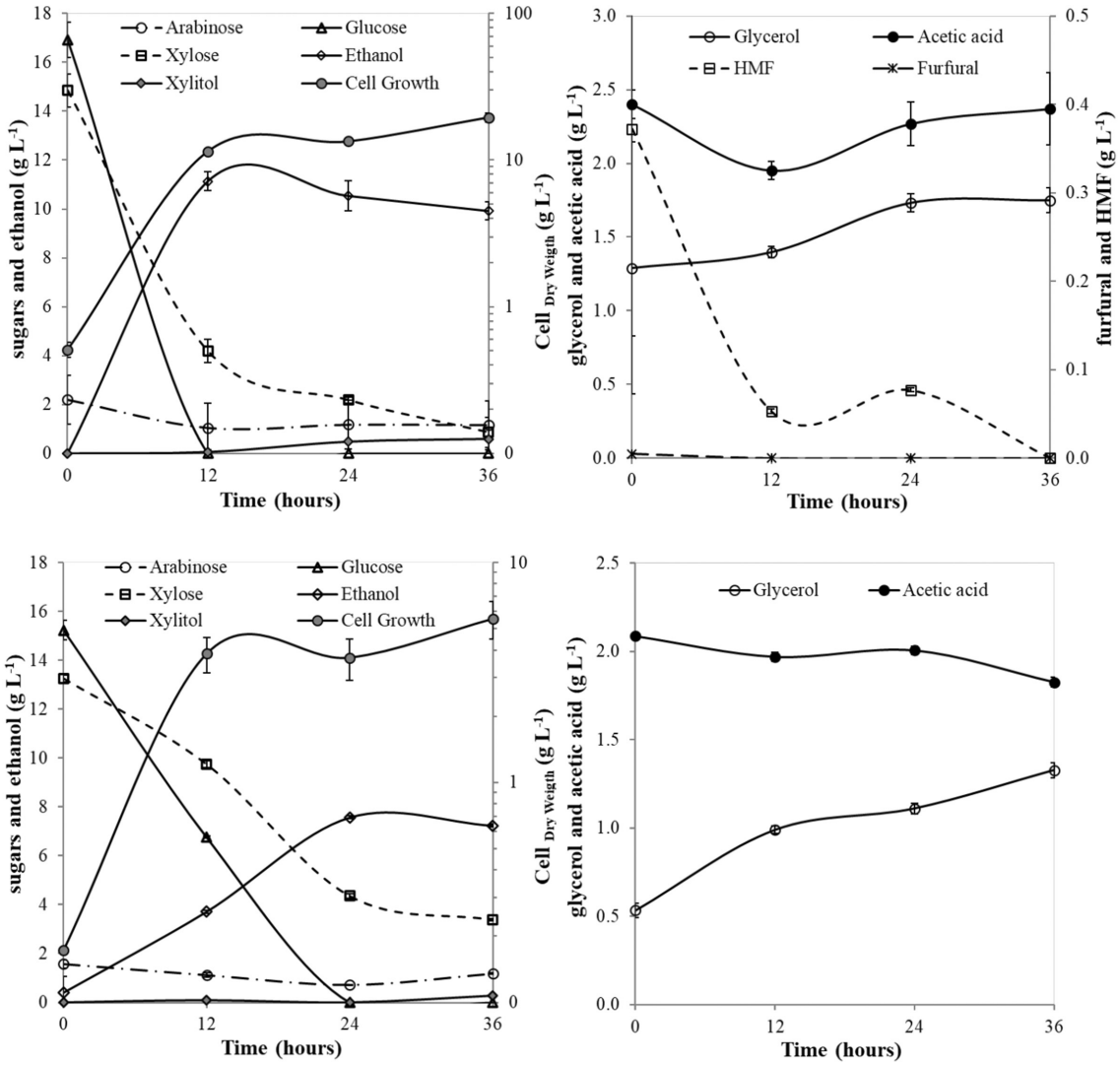



Figure 3. Fermentation progress curves of the macauba pulp oil presscake hydrolysate, nondetoxified (top) and detoxified with activated charcoal (bottom), using *Kluyveromyces marxianus* CCT 7735.

regarding ethanol production and productivity (Table 3). The removal of furfural and HMF promoted by activated carbon treatment did not benefit the fermentations tested in any way. Given the nature of the adsorbent used and its known ability to capture metals,[42] it is possible that the detoxification process of the hydrolysate also removed nutrients, like minerals, important for the physiology of the evaluated yeasts, compromising their performance.

## Fermentation performance on nondetoxified macauba cake hydrolysate

To achieve the precise determination of performance parameters on the fermentation process carried out with the nondetoxified hydrolysate medium, the processes were monitored every 2 h for the first 14 h. Figure 4 presents the fermentative profiles of the three pentose-fermenting yeast strains in nondetoxified macauba oil presscake hydrolysate.

A delay in the consumption of glucose and xylose was observed in all fermentative trials. The delay continued until the HMF was exhausted. Sukwong *et al.*[43] reported a lag in glucose consumption during the alcoholic fermentation of macroalgae hydrolysate by *K. marxianus* and *Scheffersomyces stipitis* associated with HMF. It is known that furans promote the downregulation of many enzymes that act in the Embden–Meyerhof–Parnas pathway and the redox imbalance that affects sugar assimilation.[44]

Under the fermentation conditions in this study, none of the evaluated yeasts exhibited the classic catabolic repression in which the presence of glucose suppresses the use of other sugars. Instead, they co-fermented glucose and xylose, albeit at different consumption rates. Glucose depletion, however,


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**Table 3. Yield coefficients and variation of substrates, products, and cellular concentrations after 12 h of fermentation processes performed with nondetoxified (NDT) and detoxified (DT) macauba pulp oil presscake hydrolysates.**

| Parameters | *Sungouiella akabanensis* UFVJM R131 | | *Spathaspora passalidarum* NRRL Y-27907 | | *Kluyveromyces marxianus* CCT 7735 | |
|---|---|---|---|---|---|---|
| | NDT | DT | NDT | DT | NDT | DT |
| $Y_{P/S}$ ($g\,g^{-1}$) | 0.41 ± 0.02a | 0.33 ± 0.08a | 0.37 ± 0.07a | 0.47 ± 0.16a | 0.39 ± 0.02a | 0.30 ± 0.02a |
| $Y_{P/X}$ ($g\,g^{-1}$) | 2.59 ± 0.24a | 1.80 ± 0.27b | 2.59 ± 0.20a | 1.95 ± 0.05b | 1.01 ± 0.04c | 0.76 ± 0.09c |
| $Y_{X/S}$ ($g\,g^{-1}$) | 0.16 ± 0.02ab | 0.23 ± 0.11ab | 0.15 ± 0.03b | 0.24 ± 0.09ab | 0.38 ± 0.01a | 0.36 ± 0.14ab |
| $Q_P$ ($g\,L^{-1}\,h^{-1}$) | 0.92 ± 0.00a | 0.32 ± 0.02b | 0.85 ± 0.10a | 0.36 ± 0.00b | 0.93 ± 0.03a | 0.31 ± 0.01b |
| $\Delta_{cells}$ ($g\,L^{-1}$) | 4.15 ± 0.18b | 2.13 ± 0.23c | 3.95 ± 0.49b | 2.19 ± 0.06c | 11.0 ± 0.12a | 4.91 ± 0.69b |
| $\Delta_{xyl}$ ($g\,L^{-1}$) | −9.69 ± 0.23a | −1.98 ± 0.31b | −10.5 ± 1.07a | −2.02 ± 1.58b | −10.7 ± 0.25a | −3.50 ± 0.23b |
| $\Delta_{glu}$ ($g\,L^{-1}$) | −17.2 ± 0.65a | −7.74 ± 0.21b | −15.9 ± 1.10a | −7.71 ± 1.62b | −16.9 ± 0.73a | −8.47 ± 0.44b |
| $\Delta_{hmf}$ ($g\,L^{-1}$) | −0.52 ± 0.02b | 0.00 ± 0.00a | −0.46 ± 0.13b | 0.00 ± 0.00a | −0.39 ± 0.30b | 0.00 ± 0.00a |
| $\Delta_{ace}$ ($g\,L^{-1}$) | −0.52 ± 0.15a | −0.29 ± 0.03a | −0.42 ± 0.29a | −1.32 ± 2.32a | −0.45 ± 0.14a | −0.12 ± 0.03a |
| $\Delta_{eth}$ ($g\,L^{-1}$) | 11.1 ± 0.03a | 3.86 ± 0.18b | 10.2 ± 1.21a | 4.26 ± 0.06b | 11.1 ± 0.38a | 3.71 ± 0.07b |
| $\Delta_{gly}$ ($g\,L^{-1}$) | 0.94 ± 0.20a | 0.20 ± 0.01d | 0.72 ± 0.18abc | 0.76 ± 0.07ab | 0.58 ± 0.02bc | 0.46 ± 0.02 cd |

The values followed by the same letter in the same row do not present a statistically significant difference according to the Tukey test ($P = 0.05$). The parameters assessed in the fermentation processes are as follows: $Y_{P/S}$ refers to the ethanol yield coefficient; $Y_{P/X}$ represents the specific ethanol yield coefficient relative to the biomass; $Y_{X/S}$ is the biomass yield coefficient; $Q_P$ refers to the volumetric ethanol productivity. The consumption of sugars and formation of metabolites after 12 h of fermentation are represented by several parameters: $\Delta_{xil}$ shows the consumption of xylose; $\Delta_{glu}$ refers to glucose consumption; $\Delta_{hmf}$ is the consumption of 5-hydroxymethylfurfural, and $\Delta_{ace}$ is the consumption of acetic acid. The formation of products was also analyzed with $\Delta_{eth}$ representing the formation of ethanol and $\Delta_{gly}$ indicating the formation of glycerol after 12 h of fermentation.

impaired xylose utilization, which either slowed markedly or ceased altogether, as observed for *K. marxianus* (Fig. 4 – lower graphs).

Fermentation profiles revealed that glucose was the preferred carbohydrate for *S. akabanensis* (upper graphs), as its assimilation was completed within 24 h of the bioprocess, with a maximum consumption rate of $-2.35\,g\,L^{-1}\,h^{-1}$. Xylose was consumed at a rate of $-1.32\,g\,L^{-1}\,h^{-1}$ and was completely consumed within 48 h, with most of the consumption occurring in the first 24 h, at which point only 28% of the initial xylose remained. For arabinose, there was no consumption throughout the 48 h fermentation. 5-Hydroxymethylfurfural and acetic acid were degraded during the process; HMF could not be detected after 10 h of fermentation and acetic acid was reduced from $2.38\,g\,L^{-1}$ to $1.13\,g\,L^{-1}$ after 48 h. Glycerol accumulated during the process, with its concentration tripling from $0.56\,g\,L^{-1}$ to $1.53\,g\,L^{-1}$. The $Y_{P/S}$ value achieved was $0.37\,g\,g^{-1}$, reflecting 72.40% of fermentation efficiency. In a study conducted by Perez *et al.*,[45] using a synthetic medium and the *S. akabanensis* 15-02C yeast, a $Y_{P/S}$ of $0.12\,g\,g^{-1}$ was achieved.

The *S. passalidarum* strain (Fig. 4 – middle graphs) preferred glucose as the main carbon source, with a consumption rate of $-1.54\,g\,L^{-1}\,h^{-1}$. This strain completely consumed glucose after 24 h of fermentation. Xylose was consumed entirely after 48 h, with the highest consumption rate, $-0.86\,g\,L^{-1}\,h^{-1}$, recorded between 12 and 24 h. The HMF profile was similar to that observed for *S. akabanensis*, as described above. At the end of the process, acetic acid decreased to a concentration of $1.90\,g\,L^{-1}$, from the initial $2.38\,g\,L^{-1}$. The accumulation of glycerol was also observed, with a maximum concentration of $1.57\,g\,L^{-1}$, a value three times higher than the initial concentration recorded. The highest ethanol concentration was observed after 36 h of fermentation ($10.66\,g\,L^{-1}$), with a maximum production rate of $0.45\,g\,L^{-1}\,h^{-1}$. The yeast strain *S. passalidarum* showed a higher ethanol yield ($Y_{P/S}$) than that reported by Lima *et al.*,[46] who obtained a $Y_{P/S}$ of $0.28\,g\,g^{-1}$ when fermenting sugarcane bagasse hydrolysate supplemented with $KH_2PO_4$, $MgSO_4{\cdot}7H_2O$, urea, and yeast extract.

The yeast species *K. marxianus* preferentially consumed glucose, with a metabolization rate of $-2.69\,g\,L^{-1}\,h^{-1}$, consuming all glucose after 12 h of fermentation. Regarding xylose, the maximum consumption rate obtained was the highest among the yeast strains studied, reaching $-2.20\,g\,L^{-1}\,h^{-1}$. Although it was not the main object of the study, it was noted that the *K. marxianus* strain produced xylitol in addition to ethanol. This was observed at a maximum concentration of $0.57\,g\,L^{-1}$ after 36 h of fermentation. The ability of *K. marxianus* to produce

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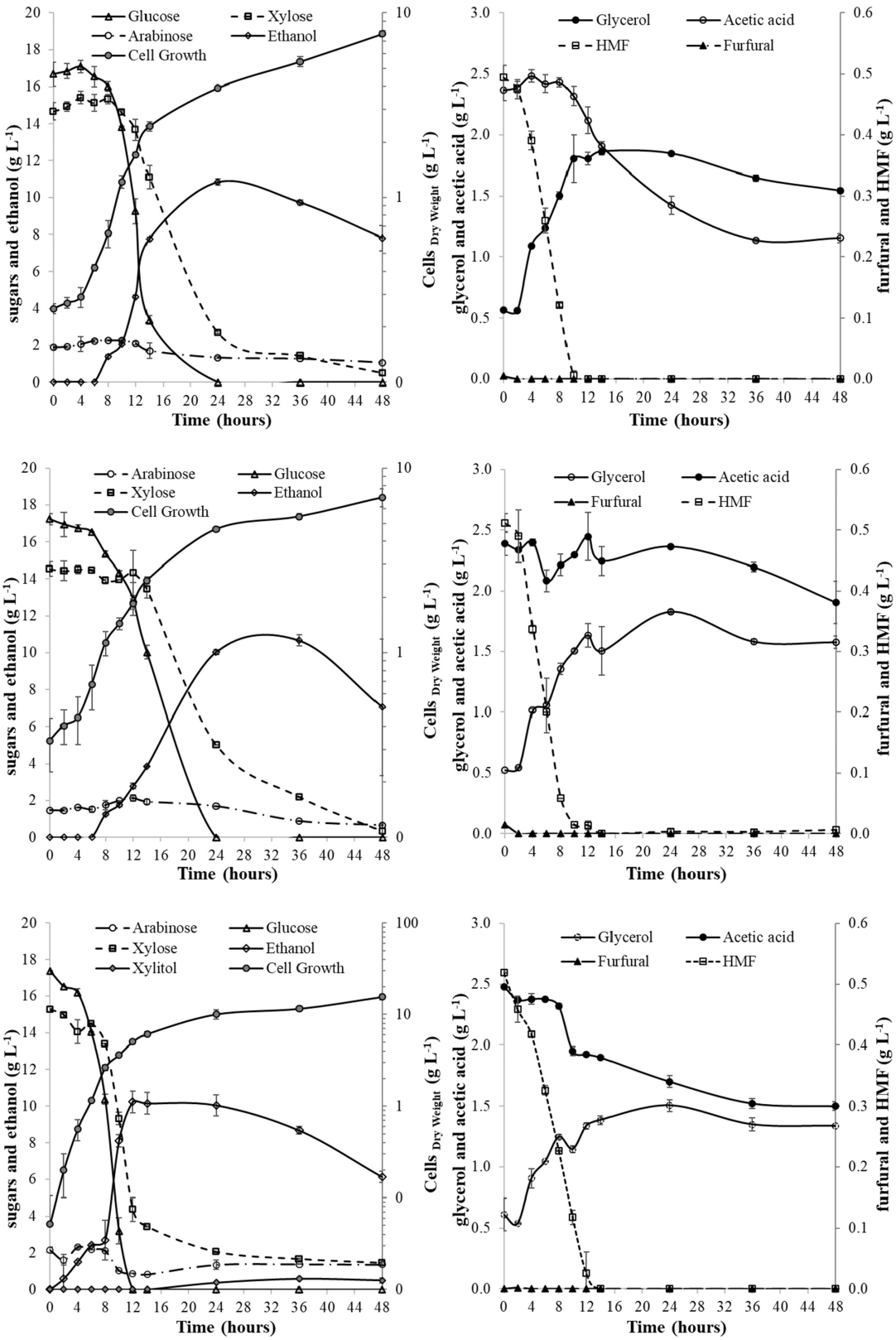



Figure 4. Fermentation progress curves of the nondetoxified macauba pulp oil presscake hydrolysate carried out with different pentose-fermenting yeast strains: *Sungouiella akabanensis* UFVJM R131 (upper graphs), *Spathaspora passalidarum* NRRL Y-27907 (middle graphs), and *Kluyveromyces marxianus* CCT 7735 (lower graphs).


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xylitol was described by Baptista and Domingues[47] in a study involving the valorization of lignocellulosic biomass. Glycerol was produced at a rate of 0.12 g $L^{-1}$ $h^{-1}$, with its maximum concentration (1.50 g $L^{-1}$) detected after 24 h of fermentation. The production of glycerol during the fermentation process constitutes a metabolic response due to the increase in ethanol concentration in the medium, which protects the cells.[48]

The acetic acid present in the hydrolysate was degraded by the yeast *K. marxianus* throughout the entire fermentation process, reaching degradation rates of −0.17 g $L^{-1}$ $h^{-1}$ – the highest among all the yeasts studied. 5-Hydroxymethylfurfural was degraded entirely within 14 h of fermentation, reaching a degradation rate of −0.05 g $L^{-1}$ $h^{-1}$, the lowest among the three yeasts. Furfural was detected at a concentration of 0.002 g $L^{-1}$ only during the second hour of fermentation, after which it was no longer detected. Oliva *et al.*,[49] studying the impacts of furan compounds on the *K. marxianus* CECT 10875 strain, observed that the concentrations of furfural and HMF required to inhibit ethanol production were 2.53 g $L^{-1}$ and 4.01 g $L^{-1}$, respectively. Similarly, Liu *et al.*[50] reported on the adaptive response of microorganisms to the inhibitory compounds furfural and HMF and elucidated the biotransformation capacity of these compounds into less toxic metabolites, allowing the microorganism to achieve high fermentation rates and cellular growth again.

The ethanol yield (0.35 g $g^{-1}$) was close to that obtained in a study conducted by Du *et al.*,[51] with the *K. marxianus* CICC 1727–5 strain, which, in a fermentation process with saccharification and simultaneous co-fermentation (SSCF) using corn cobs, achieved a $Y_{P/S}$ of 0.41 g $g^{-1}$. Tinôco *et al.*,[52] studying a process with simultaneous saccharification and fermentation (SSF) of sweet sorghum bagasse by *K. marxianus* CCT 7735, achieved a $Y_{P/S}$ of 0.25.

Table 4 shows that there was no significant difference ($P < 0.05$) in the ethanol yield values ($Y_{P/S}$) in any of the processes conducted, regardless of the yeast strain used. As for the volumetric productivity ($Q_P$), the values were only significantly different for the process that used *K. marxianus*, which reached rates of 0.87 g $L^{-1}$ $h^{-1}$ – a value about twice as large as the other two yeasts. The biomass yield values ($Y_{X/S}$) differed significantly among the three strains, with the highest value observed for the *K. marxianus* yeast at 0.35 g $g^{-1}$ and the lowest for *S. akabanensis* at 0.12 g $g^{-1}$. Ethanol yield values as a function of biomass ($Y_{P/X}$) were statistically similar for two strains, *K. marxianus* CCT 7735 and *S. passalidarum* NRRLY 27907, presenting $Y_{P/X}$ values of 2.17 g $g^{-1}$ and 2.32 g $g^{-1}$, respectively. *S. akabanensis* UFVJM R131 strain exhibited the highest ethanol yield per biomass, with a $Y_{P/X}$ of 2.99 g $g^{-1}$.

**Table 4. Kinetic and production parameters of macauba pulp oil presscake hydrolysate fermentation carried out with different pentose-fermenting yeasts.**

| Kinetics and production responses | *Sungouiella akabanensis* UFVJM R131 | *Spathaspora passalidarum* NRRL Y-27907 | *Kluyveromyces marxianus* CCT 7735 |
|---|---|---|---|
| $\mu_{max}$ ($h^{-1}$) | 0.24 ± 0.01a | 0.24 ± 0.08a | 0.51 ± 0.03b |
| $t_g$ (h) | 2.90 ± 0.17a | 3.14 ± 1.01a | 1.35 ± 0.10b |
| $Y_{P/S}$ (g $g^{-1}$) | 0.37 ± 0.02a | 0.38 ± 0.01a | 0.35 ± 0.02a |
| $Y_{P/X}$ (g $g^{-1}$) | 2.99 ± 0.13a | 2.32 ± 0.05b | 2.17 ± 0.19b |
| $Y_{X/S}$ (g $g^{-1}$) | 0.12 ± 0.00a | 0.17 ± 0.00b | 0.35 ± 0.02c |
| $Q_P$ (g $L^{-1}$ $h^{-1}$) | 0.46 ± 0.01a | 0.42 ± 0.00a | 0.87 ± 0.05b |
| $r_{xyl}$ (g $L^{-1}$ $h^{-1}$) | −1.32 ± 0.05a | −0.86 ± 0.04b | −2.20 ± 0.16c |
| $r_{glu}$ (g $L^{-1}$ $h^{-1}$) | −2.35 ± 0.45ab | −1.54 ± 0.43a | −2.69 ± 0.12b |
| $r_{hmf}$ (g $L^{-1}$ $h^{-1}$) | −0.07 ± 0.00a | −0.07 ± 0.01a | −0.05 ± 0.00b |
| $r_{ace}$ (g $L^{-1}$ $h^{-1}$) | −0.10 ± 0.01a | −0.13 ± 0.04a | −0.17 ± 0.02a |
| $r_{eth}$ (g $L^{-1}$ $h^{-1}$) | 1.52 ± 0.16a | 0.45 ± 0.12b | 1.81 ± 0.16a |
| $r_{gly}$ (g $L^{-1}$ $h^{-1}$) | 0.26 ± 0.01a | 0.08 ± 0.00b | 0.12 ± 0.01c |

Values sharing the same letter within a row are not significantly different according to the Tukey test ($P = 0.05$).
$Q_P$, ethanol volumetric productivity; $r_{ace}$, acetic acid uptake rate; $r_{eth}$, ethanol formation rate; $r_{glu}$, glucose uptake rate; $r_{gly}$, glycerol formation rate; $r_{hmf}$, 5-hydroxymethylfurfural uptake rate; $r_{xyl}$, xylose uptake rate; $t_g$, generation time; $Y_{P/S}$, ethanol yield coefficient; $Y_{P/X}$, specific ethanol yield coefficient; $Y_{X/S}$, cell biomass yield coefficient; $\mu_{max}$, maximum specific growth rate.

The ethanol production profiles followed glucose consumption, showing that the yeasts relied primarily on this carbohydrate. Even so, the peak ethanol production achieved in all fermentation systems with the nonconventional yeasts can be explained only by the combined consumption of glucose and xylose. After the glucose was completely consumed, the yeasts continued to grow slowly. At the same time, it was observed that once the glucose was depleted, ethanol, residual xylose, acetic acid, and glycerol were partially consumed, suggesting diauxic growth.

## Conclusion

This study demonstrated an integrated bioconversion of the starchy and hemicellulosic fractions of macauba (*A. aculeata*) oil presscake into ethanol using three nonconventional yeasts: *S. akabanensis*, *S. passalidarum*, and *K. marxianus*. Unlike previous reports, which evaluated only the hemicellulosic fraction under detoxified and nutrient-supplemented conditions, these results show that

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ethanol production is feasible directly from nondetoxified hydrolysates, achieving comparable yields while significantly reducing process complexity. Activated carbon detoxification, although effective in removing furans, negatively affected sugar availability and yeast performance, indicating that detoxification may not be necessary for macauba hydrolysates. Among the yeasts studied, *S. akabanensis* UFVJM R131, recently discovered as a Brazilian xylose-fermenting yeast, displayed competitive ethanol yields ($0.37\,g\,g^{-1}$) and effective *in situ* HMF conversion, highlighting its potential for biorefinery applications. Overall, this work presents a simplified, previously unreported bioprocess that valorizes the complete carbohydrate potential of macauba oil presscake, using unconventional yeasts, offering a pathway to the development of cost-effective and sustainable second-generation ethanol production.

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