

Enhancing Second-Generation Ethanol Production Through Acid Hydrolysis and *Saccharomyces cerevisiae* Fermentation: A Sustainable Approach Using Cassava Peel and Manipueira Biomass

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The biofuel derived from controlled fermentation and subsequent distillation of lignocellulosic biomass is commonly referred to as second-generation ethanol (2G). This energy production pathway has experienced exponential growth due to its dual role as an alternative additive to fossil fuels and a potential replacement for this environmentally detrimental energy source in the future. This study aimed to produce second-generation ethanol by utilizing cassava peel and manipueira through acid hydrolysis, followed by a 10-day fermentation process using the yeast *Saccharomyces cerevisiae* the fermenting microorganism. The process began with the mechanical pulverization of the biomass, followed by a triple round of acid hydrolysis using sulfuric acid at 100 °C for 10 minutes. Subsequently, fermentation occurred within sealed PET bottles, with each sample containing 10 g of yeast and 25 g of biomass, maintaining a 1:2.5 mass ratio of yeast to biomass, at an average room temperature of 35 °C for 10 days. After this incubation period, the fermented solutions were evaluated for °Brix and pH levels, then filtered using a vacuum pump and distilled in a micro distillation apparatus to obtain ethanol 2G. The obtained results demonstrated the significant potential of cassava peel (*Manihot esculenta*) and manipueira biomass for second-generation ethanol production, with °Brix values of 4 % for cassava peel and 3% for manipueira, respectively. During the fermentation process, regular assessments of pH, sugar content, alcohol concentration, and corresponding ethanol mass percentage were conducted for each sample. These analyses revealed a gradual increase in alcohol levels over time, reaching a peak of 26.2 % for cassava peel and 24 % for manipueira after 10 days. These findings suggest that cassava and manipueira peels, as sources of lignocellulosic biomass, hold promise for second-generation ethanol production. This research directly contributes to the development of more efficient and sustainable processes for generating biofuels from agricultural waste, paving the way to produce clean and renewable energy.

1. Introduction

The growing concern about climate change, driven by the escalating greenhouse gas (GHG) emissions and global interest in reducing reliance on oil, has led many nations, including Brazil, to explore alternatives to fulfill their energy needs. This seeks for sustainable energy sources has intensified research in the field of biofuels. Biofuels, as defined by the National Oil and Gas Agency (ANP), encompass fuels derived from biomass sources, such as plants, agricultural residues, and even microorganisms. In Brazil, ethanol has emerged as an extensively studied alternative, notably propelled by the National Alcohol Program (Proálcool) initiated in 1975

in response to the first major oil crisis. Proálcool positioned itself as a renewable alternative, experiencing substantial growth and exhibiting promising projections for the future, according to Cuchi and Galão (2022). To explore alternative raw materials for ethanol production, reduce costs, and enhance sustainability, this study employed two types of biomasses, manipueira, and cassava peel, for second-generation ethanol production. These natural resources, beyond serving technological advancements, harbor significant potential for minimizing environmental and economic impacts. They offer a promising avenue for reducing dependence on fossil fuels and mitigating associated greenhouse gas emissions. Cassava, a starch-rich root, has gained prominence in ethanol production, as highlighted by Cuchi and Galão (2022). Leão (2023) underscores the global significance of cassava as the fifth most relevant basic crop, with an annual production of approximately 285 million tons of unprocessed roots. The industrialization process generates by-products, with solid waste presenting opportunities for use as fodder, solid fermentation substrates, and even bioreactors for energy production. However, liquid waste (manipueira), predominant in quantity, poses a considerable pollution risk. Among the cassava processing by-products, cassava peel, generated in the initial processing stage, is composed of peel, bark, and tips (Teixeira et al., 2011). Martinez (2016) provides physicochemical characteristics, indicating that approximately 80 % comprises starch, 15 % fiber, 1.6 % ash, 2 % protein, 1 % sugar, and 0.8 % fatty matter. pH values vary due to environmental and geographic factors, making it a more comprehensive resource than other residues like sugarcane bagasse. Manipueira, the liquid residue from cassava starch and flour processing, is recognized for its high polluting potential. Suman et al (2011), note that for each ton of processed roots, the industry generates 300 L of manipueira with a chemical oxygen demand (COD) of 60 g/L. Its composition includes 58.18 g/L of carbon, with 37.96 g/L as total sugars, 14.90 g/L as reducing sugars, and 20.22 g/L as non-reducing sugars. Some studies have been conducted to evaluate the potential of cassava as a viable alternative for alcohol production, driven by its impressive starch storage capacity in the roots. These characteristics position cassava as a favorable option for traditional communities, as well as small and medium-sized producers, thereby fostering industrialization. The widespread cultivation of cassava across the entire Brazilian territory further accentuates its suitability for such endeavors. The local establishments known as "houses of flour," prevalent in small communities, serve as the epicenters for processing the raw material essential for flour production. Given that these small communities typically consist of low-income populations, engaging in cassava cultivation emerges as a pragmatic means of livelihood (SUMAN et al., 2011). This strategic shift not only renders these communities self-sufficient in biofuel production but also propels them toward the development of innovative technologies for a resilient and sustainable production matrix. Considering possible processes for second-generation ethanol production, fermentation emerges as the optimal route for lignocellulosic biomasses. Barros (2022) emphasizes the necessity of delignification processes to separate cellulose and hemicellulose fractions, followed by acid or enzymatic hydrolysis to break down lignin structures and yield sugars for fermentation. Starch hydrolysis involves the stability of glycosidic bonds in alkaline conditions, leading to the complete unfolding of amylose molecules in acidic conditions. This process results in the gradual transformation of amylose into simpler dextrin and the eventual formation of glucose (Oliveira, 2009). Fermentation, using *Saccharomyces cerevisiae* as the primary enzyme, converts the sugars derived from cassava starch hydrolysis into alcohol, releasing carbon dioxide. This work aimed to produce second-generation ethanol using cassava peel and manipueira as biomass, with *Saccharomyces cerevisiae* catalyzing the fermentation reaction in 10 days at 35 °C.

2. Materials and methods

2.1 Materials

The experimental tests employed cassava-derived materials procured from a local flour mill in the Santo Antônio community, situated in the city of Irará, Bahia. These materials encompass cassava peel obtained during the vegetable scraping process and manipueira extracted by filtering cassava mass as part of the liquid removal process during flour production. During the experiments, distilled water, 2M sodium hydroxide, 2% phosphoric acid, and *Saccharomyces cerevisiae* were used for acid hydrolysis, fermentation, and distillation. Conducting the experimental phase of this study required a diverse array of instruments, all of which were available at the Energy Laboratory (LEN) at UFRB. These instruments included an analytical balance, paper filter, glass funnel, heating blanket, thermometer, autoclave, pH meter, pet bottles, °BRIX refractometer, micro distiller, blender, and sieve.

2.2 Methods

Cassava peels:

The alcohol production from cassava peel involved four distinct phases: raw material preparation, acid hydrolysis, alcoholic fermentation, and distillation (Figure 03). In the initial stage of raw material preparation, the thoroughly dried cassava peel underwent a crushing process in a conventional blender, followed by sieving and weighing (Figure 01). For this experiment, 150 g of this material was utilized. Moving to the second stage, to enhance cellulose breakdown, the crushed peel underwent an acid hydrolysis treatment. Three samples were prepared with a ratio of 330 mL of phosphoric acid 2 % for every 25 g of crushed peel, heated at 121 °C for 10 minutes. Following this, the samples were cooled, and the pH was neutralized with the addition of NaOH 2M, reaching a pH between 6.5 and 7.5. The °BRIX was also measured using a refractometer.

In the third stage, alcoholic fermentation took place. The hydrolysate was placed in PET bottles, and 10 g of biological yeast was added. The fermentation reaction was monitored daily by observing the gas released when opening the bottles, continuing until its release ceased, after 10 days. After this step, the °BRIX degree was measured. To assess the impact of pre-treatment with acid hydrolysis in the fourth and final step, the material underwent filtration and distillation, and the °BRIX was measured again. In a control version, the procedures were replicated, excluding step 2 and substituting the hydrolyzing reagents with distilled water in the same proportion.



Figure 1: Cassava peels crushed.

Manipueira:

Concerning the procedures applied to manipueira (Figure 04), the methodology closely followed the previously described standard. In this phase, an amount of 150 g of biomass was utilized in the reaction medium, mirroring the conditions outlined earlier. To enhance the cellulose degradation process, an acid hydrolysis treatment was implemented. This involved creating a mixture comprising 330 mL of phosphoric acid 2 % for every 25 g of manipueira, resulting in the generation of three samples, each packaged in PET bottles, as illustrated in Figure 2. Subsequently, the material underwent heating at 121 °C for 10 minutes, followed by a cooling period in preparation for the subsequent step. To neutralize the pH of the samples, NaOH 2M was added, and the °BRIX was measured using a refractometer. The third and fourth phases of the methodology applied to the bark were replicated for the manipueira. To examine the influence of pre-treatment, the same procedures were duplicated, apart from step 2. Instead of using hydrolyzing reagents, water was added in the same proportion, serving as a control group in the experiment. Figure 3 shows the process used to obtain manipueira residue from cassava root. This method is largely used in the northeast of Brazil and includes 5 steps (Ferreira et al., 2001).



Figure 2: Manipueira sample

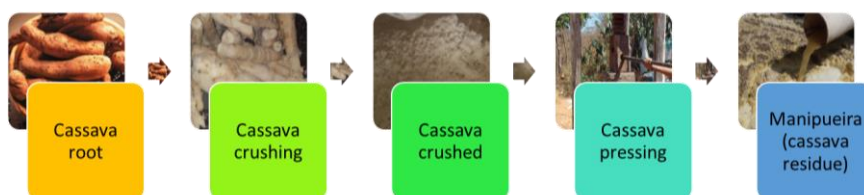


Figure 3: Method to produce manipueira from cassava root (adapted from Ferreira et al., 2001)

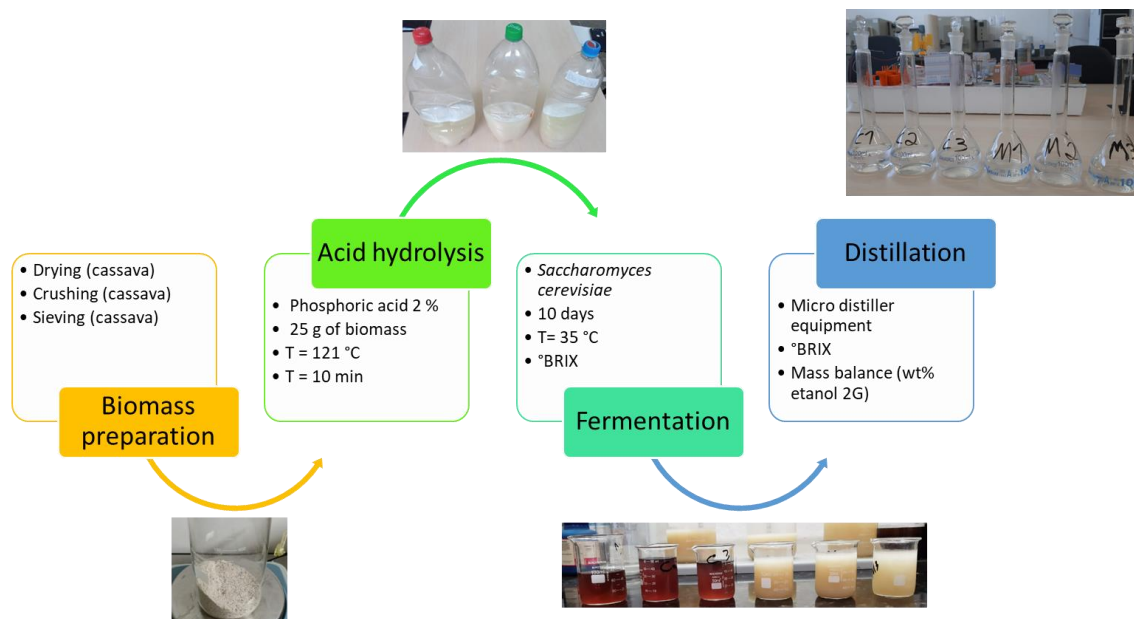


Figure 4: Schematic of ethanol 2G production from cassava peel and manipueira

2. Results and discussions

The comprehensive distillation, carried out using the TE-012-250 micro distiller at the Laboratory of Energy (LEN), played a crucial role in assessing the efficiency of ethanol 2G production from cassava peel and manipueira hydrolysates. Upon completion of the process, the quantity of alcohol generated was measured based on the initial mass introduced into the equipment. The obtained results showed promising conversion rates for ethanol 2G, particularly noteworthy in the case of cassava peel. Mass data indicated an impressive yield of 26.20 % of ethanol 2G from cassava peel, underscoring the viability of this biomass as a robust source for renewable ethanol production (Table 01, Figures 5 and 6). All triplicates were conducted under controlled temperature conditions, maintaining at 35 °C, with daily measurements taken during the 10-day fermentation period. This stringent temperature control is imperative to ensure optimal enzyme activity throughout the fermentation process, thereby ensuring consistent and reliable outcomes.

The consistent monitoring of temperatures during fermentation was critical for comprehending and interpreting the results. Variations within the specified temperature range can significantly impact microbial activity, directly influencing ethanol production. Therefore, maintaining temperatures within these precise parameters enhances the reliability of the obtained results. The selection of a 10-day fermentation period was based on practical considerations and process efficiency. This duration allows ample time for yeast to convert sugars present in the hydrolysates into ethanol, striking a balance between the necessity for sufficient time and the pragmatic demands of an efficient process.

In their 2021 study, Adegbehingbe et al. investigated the production of bioethanol from fermented cassava peel using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. They discovered that inoculating cassava peel with *Saccharomyces cerevisiae* resulted in a high yield of 30% and an ethanol volume of 45 mL. The study also

recorded the flash point of both fermented samples inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Jos and Kumoro (2017) researched the simultaneous saccharification and fermentation of sweet and bitter cassava using *Saccharomyces cerevisiae* for bioethanol production. They found that sweet cassava produced more ethanol compared to bitter cassava. This is because bitter cassava usually contains high levels of cyanide, while sweet cassava has low levels of cyanide (Sarkiyayi and Agar, 2010), which affects ethanol production. Cassava peels, on the other hand, contain starch, which is a cheap and abundant renewable energy source that can be easily accessed to produce fermentable glucose syrups and dextrans (Adeleke et al., 2017). The results suggest that cassava peel and manipueira stand out as promising sources for ethanol 2G production, with a notable emphasis on cassava peel, indicating high yield. These findings underscore the potential of these biomasses as sustainable alternatives in biofuel production, contributing to the quest for renewable energy sources and the mitigation of greenhouse gas emissions. The weight percentage (wt%) of second-generation ethanol was calculated from the mass balance, considering all input (fermented wort) and output (distilled alcoholic composition) variables.

Table 1: Mass Balance

Sample	°BRIX (initial)	°BRIX (final)	wt% ethanol 2G
Cassava 01	5.0	0.4	25.80
Cassava 02	4.2	0.4	26.20
Cassava 03	4.0	0.4	23.20
Manipueira 01	3.4	0.4	24.10
Manipueira 02	3.2	0.4	20.20
Manipueira 03	2.0	0.2	23.70

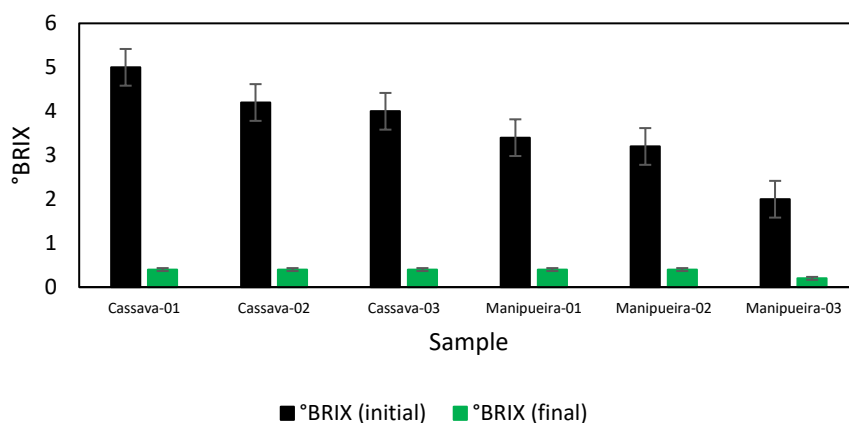


Figure 5: Evaluation of °BRIX before and after the distillation process

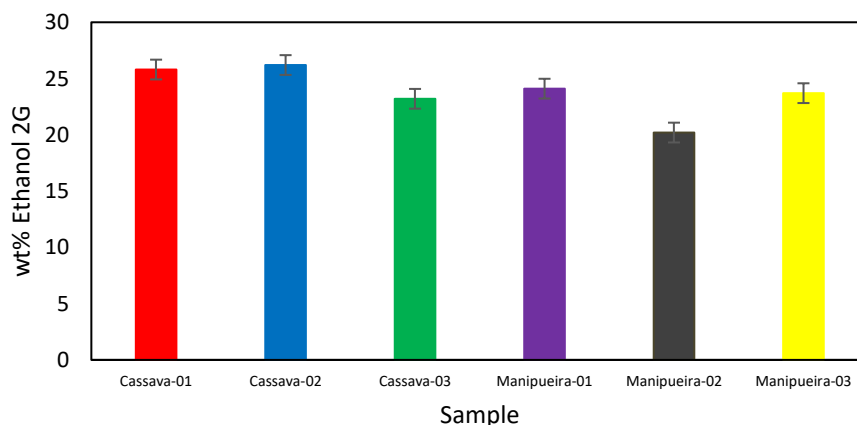


Figure 6: wt% of ethanol 2G produced from Cassava peels and manipueira biomasses using acid hydrolysis and a 10-day fermentation process.

3. Conclusions

Manipueira and cassava peel biomasses reveal a substantial presence of fermentable sugars, indicating the potential of these biomasses as promising sources for second-generation ethanol production. The distillation process played a pivotal role in separating ethanol from the mixture, contributing to the acquisition of results that mirror the quality of the final product. When assessing the initial concentration of sugars in non-distilled manipueira samples, a °Brix of 2 to 3.4 was observed, subsequently reduced to 0.2 to 0.4 after distillation. Conversely, non-distilled cassava peel samples exhibited a higher sugar content, with Brix indices ranging from 4 to 5, similarly reduced to 0.4 °Brix after distillation. This reduction can be attributed to the breakdown of lignin through acid hydrolysis. The specific alcohol percentage results obtained indicated noteworthy variations, with the manipueira sample presenting percentages in the range of 20.2 % to 24 %, and the cassava peel sample varying from 23.2 % to 26.2 %. It is crucial to note that distillation may not be entirely efficient, permitting the presence of other impurities in distilled samples of cassava and manipueira peels.

These findings highlight the promising utilization of cassava and manipueira peels as sources of lignocellulosic biomass for second-generation ethanol production. The presence of fermentable sugars in these biomasses suggests the potential for efficient production of high-quality ethanol through distillation. This study, by contributing to the development of more efficient and sustainable processes in biofuel production from agricultural waste, distinguishes itself by avoiding competition with food production and reducing greenhouse gas emissions. Moreover, it opens new perspectives for the generation of clean and renewable energy, solidifying its significance as a substantial advancement in the quest for environmentally friendly and economically viable solutions. The next steps in this research involve evaluating yeast growth kinetics and optimizing the production process. This phase aims to understand yeast behavior and enhance overall efficiency in bioethanol production from cassava.

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