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Efficient Production of Second-Generation Ethanol Through Direct Fermentation Utilising *Saccharomyces cerevisiae* with Sweet Potato Peels, Beet Peels, and Sugarcane Bagasse Juice as Feedstocks

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This study aimed to investigate the potential for producing second-generation (2G) ethanol from sweet potato peels, beet peels, and sugarcane bagasse without hydrolysis. The process involved direct fermentation using *Saccharomyces cerevisiae* for different time intervals (36, 48, 72, and 168 hours). The raw materials were mixed with water and heated up to 105°C for 30 minutes. The fermentation process was carried out in sealed PET bottles kept at 25 °C, using 10 g of *Saccharomyces cerevisiae* and 200 g of biomass (at a mass ratio of 1:20). Measurements of °BRIX and pH were taken at each fermentation interval. After fermentation, the mixture was filtered and distilled to obtain bioethanol. The study found that sweet potato and beet peel biomasses showed significant potential. Regular monitoring of pH, sugar content, alcohol content, and bioethanol percentage was conducted. The mixture was heated to the boiling point of ethanol (78.37 °C) after distillation. The maximum bioethanol weight percentage (wt%) achieved was 18.58% (36 hours), 17.56% (48 hours), and 16.73% (72 hours) for beet peel, sugarcane bagasse juice, and sweet potato peel, respectively. This study highlights the promising prospects of using lignocellulosic biomasses for 2G ethanol production, which contributes to sustainable biofuel processes and paves the way for clean and renewable energy generation.

1. Introduction

The world urgently needs sustainable and renewable energy sources, which is why scientists are exploring second-generation ethanol production as a crucial endeavor to create cleaner, environmentally friendly alternatives (Santos et al., 2023). Recent studies indicate that bioethanol can achieve a remarkable 90% reduction in global CO_2 emissions when compared to fossil fuels. The utilization of lignocellulosic biomass in bioethanol production has undergone laboratory-scale testing in several countries, including Europe, the United States, and Brazil. However, the transition to industrial-scale production for commercial viability introduces significant technological and economic challenges that necessitate careful consideration and resolution. Despite these challenges, the adoption of bioethanol in Europe, the United States, and Brazil has yielded noteworthy economic benefits and fostered new employment opportunities in the agricultural and forestry sectors. Moreover, this transition has played a crucial role in diminishing reliance on imported fossil fuels and enhancing energy security and independence. In the Brazilian context, the potential economic impact is substantial, with projections suggesting that the use of bioethanol could create over 300,000 new jobs by 2030 (IRENA, 2022).
Second-generation ethanol is a significant improvement over its first-generation counterpart because it uses non-food biomass, which avoids competition with food supplies and opens up opportunities for sustainable biofuel production (Antar et al., 2021).

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The first era of ethanol production relied heavily on food crops like corn and sugarcane, which were unsustainable due to their competition with global food supplies (Demirbas, 2007). Second-generation ethanol has gained prominence by utilizing a diverse range of feedstocks that do not compromise food security. These feedstocks include lignocellulosic materials, agricultural residues, and energy crops.

Bioethanol, a biofuel derived from biomass, is pivotal in the global transition from fossil fuels and the fight against climate change. By fermenting sugars from agricultural crops and residues, bioethanol offers a renewable and potentially carbon-neutral alternative to gasoline. Its production not only manages agricultural waste but also boosts rural economies by providing farmers with additional income. Moreover, bioethanol combustion produces cleaner emissions than fossil fuels, cutting down greenhouse gases and particulate matter, which improves air quality and reduces health risks. Advancements in bioethanol production processes, as highlighted in this study, aim to enhance efficiency and cost-effectiveness, establishing bioethanol as a feasible large-scale alternative energy source. This research underscores the potential for innovative use of agricultural waste in biofuel production, contributing to the overarching goal of sustainable energy solutions and a reduced carbon footprint for the biofuel industry.

This paper focuses on direct fermentation, which can improve the economic feasibility of second-generation ethanol production. This work aimed to produce second-generation ethanol using three different biomasses: sweet potato peels, beet peels, and sugarcane bagasse juice, through direct fermentation using *Saccharomyces cerevisiae* as the fermenting agent. This approach optimizes ethanol production while addressing waste valorization and resource efficiency concerns. This study introduces some new approaches to the production of second-generation ethanol (2G) by using unconventional feedstocks like sweet potato and beet peels. These by-products are often overlooked, but they offer the potential for more sustainable bioenergy production.

The study compares the efficiency and viability of sweet potato and beet peels with sugarcane bagasse juice, which is currently the most common feedstock for 2G ethanol production. This comparative analysis provides valuable insights into the use of different biomasses for 2G ethanol synthesis. It also contributes to a better understanding of the environmental and economic considerations involved in sustainable bioenergy production.

Second-generation ethanol is a promising solution to rising global energy demands and environmental imperatives. It avoids food-based sources and aligns with the principles of a circular economy. Biomasses such as sweet potato peels, beet peels, and sugarcane juice present a unique opportunity to use lignocellulosic and sugar-rich materials for efficient bioethanol synthesis. Brazil's advantage in biomass agriculture further enhances the viability of these feedstocks for sustainable bioenergy (Raj et al., 2022; Soltaninejad et al., 2022).

2. Materials and Methods

2.1 Materials

This study investigated the production of ethanol 2G using biomass sources such as beet peel, sweet potato peel, and sugarcane bagasse juice (Figure 01). The beet peels were obtained from Santo Amaro da Purificação in the state of Bahia, Brazil, while sugarcane and sweet potato peels were purchased from a local supplier in Feira de Santana, also in the state of Bahia. The fermentation process involved the use of *Saccharomyces cerevisiae* yeast, and the experiments were carried out at the Laboratory of Energy (LEN) at the Federal University of Reconcavo of Bahia.

Figure 01: Materials used for ethanol 2G production using direct fermentation method.

To monitor the progress of the experiment, it was measured the pH and sugar content (°BRIX) using a pH meter and a refractometer, respectively. Once was reached the ideal temperature, it was filtered the sugarcane bagasse juice and beet juice samples using a funnel and filter paper, and the sweet potato sample was used with a vacuum pump. Throughout the fermentation process, was maintained a mass ratio of 1:20 of *Saccharomyces cerevisiae* to biomass for each experiment. For the distillation step, was used an alcohol micro distiller (TE-012-250) with a 250 ml capacity per cycle. To determine the alcohol content, the density was analyzed, and Table 01 presents the data collected before the distillation process. This methodology ensures precision and reliability in evaluating ethanol 2G production from various biomass sources.

Throughout the study, we utilized periodic sampling and analysis to track the progress of fermentation and ethanol yield. Regular samples were taken to monitor changes in pH, sugar content, and alcohol concentration, allowing us to gain a comprehensive understanding of the fermentation process.

This thorough monitoring approach enabled us to identify the optimal fermentation times and conditions for each type of biomass. By carefully analyzing these parameters, we were able to refine the fermentation process, thus ensuring maximum ethanol production efficiency. This not only demonstrates the potential of using various agricultural residues for bioethanol production but also establishes a standard for future research in optimizing second-generation biofuel production processes.

3. Methods

The production process of ethanol 2G involved three steps: raw material (biomass) treatment, alcoholic fermentation (Figure 02), and distillation, as shown in Figure 03. The first step was to weigh 1 kg of each biomass. The beetroot and sweet potato were manually peeled at the Laboratory of Energy (LEN) at the UFRB. At the same time, the sugarcane bagasse extraction occurred in a sugarcane mill, and the bagasse juice was obtained through pressing. Distilled water and neutral soap were used to sanitize the biomasses, which were then transferred to a beaker with 500 ml of distilled water and heated up to 105 °C for 30 minutes using a heating plate (model SXCM). Sugarcane bagasse juice did not require additional water as it was already in a liquid state. The samples were then cooled to room temperature, and pH (MQuant) and °BRIX (Brix RZ120 refractometer) values were measured. The initial pH of beet peels was difficult to determine due to their reddish color, while sweet potato peels exhibited a pH of 6, and sugarcane bagasse juice showed a pH of 5. The initial °BRIX values for beet peel, sweet potato peel, and sugarcane bagasse juice were 2.4, 3, and 8, respectively (Table 01).

Biomass samples were crushed in a blender and filtered in a vacuum pump. After filtration, 200 g of each sample were transferred to sanitized PET bottles and distributed in triplicates to evaluate 36 h, 48 h, 72 h, and 168 h of fermentation reaction, respectively.

Fermentation was initiated by adding 10 g of the yeast *Saccharomyces cerevisiae* (DONA BENTA FERMIX brand) to each prepared sample (mass ratio 1:20 of yeast:biomass). The PET bottles were then stored at 25 °C to complete the designated fermentation periods (Table 01). Upon reaching the desired reaction period, 80 ml of each fermented must was distilled into a TE-012 micro distiller to carry out the distillation process and measure ethanol 2G production.

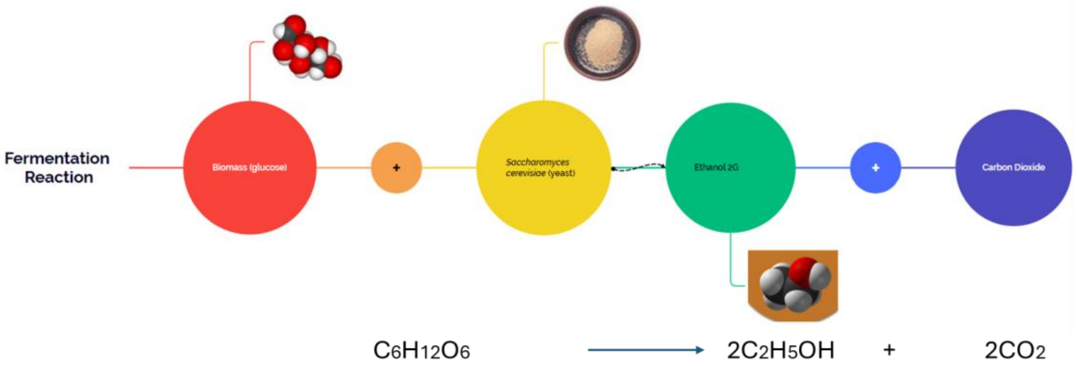

Figure 02: Schematic of fermentation reaction

Table 01: Experimental conditions for fermentation reaction

Experiments	Biomass			
Fermentation time (h)	36	48	72	168
Feedstock (g)	200	200	200	200
Yeast (g)	10	10	10	10
Temperature (°C)	25	25	25	25

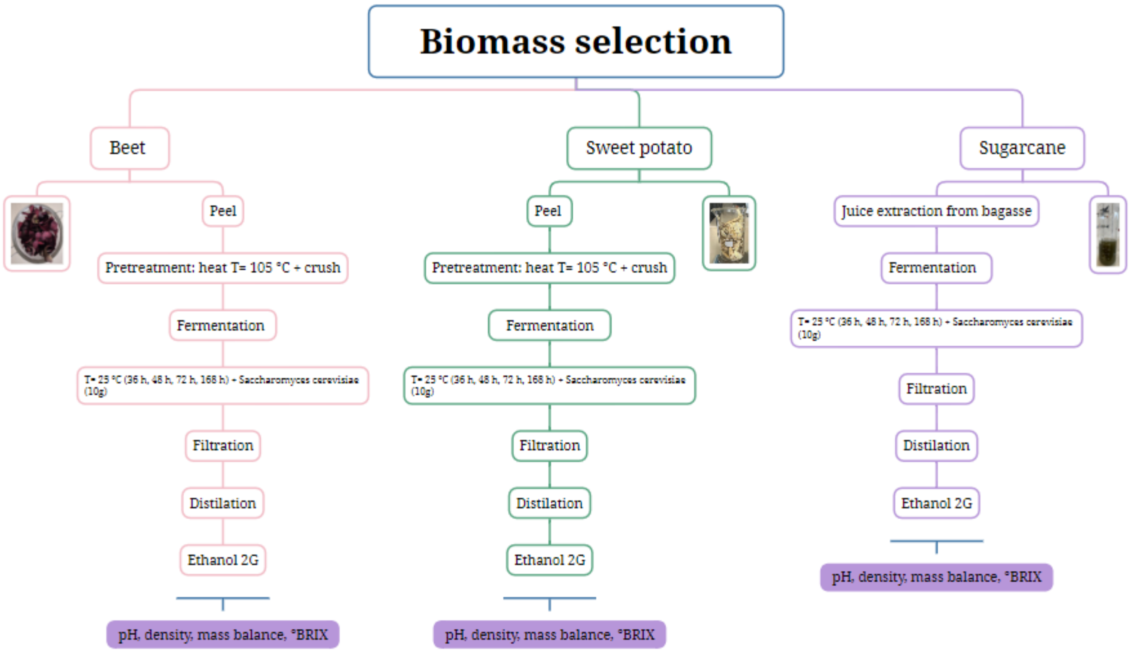

Figure 03: Methodological process for ethanol 2G production

4. Results and discussions

The results presented in Table 02 and Figure 05 show that there are notable variations in the mass percentage of ethanol 2G produced (Figure 04). The fermentation period of 36 hours produced the highest mass percentage of ethanol 2G for both beet peel and sweet potato peel, while sugarcane bagasse juice reached its peak at 48 hours. However, there was a significant decrease at 72 hours, followed by a subsequent increase at 168 hours in the mass percentage of ethanol 2G across all experiments.

No works were found in the literature with the same process characteristics to produce 2G ethanol using direct fermentation with sweet potato and beet peels. Swain et al. (2013) evaluated bioethanol production from sweet potato flour using a co-culture of Trichoderma sp. and *Saccharomyces cerevisiae* in solid-state fermentation (30 °C and 72 h) and the results indicated a bioethanol yield of 47g/100g of sugar consumed. Gadge et al. (2021) evaluated beetroot waste for bioethanol production with *Saccharomyces cerevisiae* under 20-day incubations and the results showed that the highest amount of ethanol was 1.98% after 15 days.

These findings highlight the importance of feedstock selection and fermentation conditions in influencing ethanol production. The study found that temperature, fermentation time, and initial sugar content played a critical role in bioethanol production. The observed fluctuations in ethanol production highlight the dynamic nature of the process, suggesting that a nuanced understanding and careful optimization of these parameters are imperative for achieving efficient ethanol production from the selected raw material sources.

This work emphasizes the importance of fine-tuning these variables to enhance the overall efficiency and yield of ethanol 2G production. Moreover, incorporating these optimized processes into existing agricultural and industrial systems offers a promising opportunity to increase bioethanol production.

By utilizing agricultural by-products such as sweet potato and beet peels, as well as sugarcane bagasse juice, this approach not only adds value to waste materials but also contributes to a more sustainable and circular economy. The knowledge obtained from this study can lay the groundwork for future research and development, leading to more resilient and economically feasible biofuel production technologies.

This progress is crucial in meeting global energy demands and environmental targets, ultimately promoting a cleaner, more sustainable future.

Figure 04: Ethanol 2G produced.

Table 02: Mass balance

Samples	36 h			48 h			72 h			168 h		
	Beet	Sweet Potato	Sugarcane	Beet	Sweet Potato	Sugarcane	Beet	Sweet Potato	Sugarcane	Beet	Sweet Potato	Sugarcane
Total mass in (g)	78.33	78.30	76.80	75.66	76.90	78.00	123.68	128.87	138.43	72.30	73.40	82.70
°BRIX initial (%)	2.40	3.00	8.00	2.20	2.00	8.00	2.10	1.00	7.00	2.00	9.10	10.00
°BRIX final (%)	1.00	1.00	3.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	2.00	3.00
wt% ethanol 2G	18.58	16.73	7.29	14.41	13.78	17.56	6.07	2.39	6.57	11.11	12.53	8.39
Mass of ethanol (g)	14.55	13.10	5.60	10.90	10.60	13.70	7.75	3.08	9.10	8.03	9.20	6.94
pH	5.00	6.00	5.00	5.00	4.00	6.00	5.00	6.00	3.00	5.00	6.00	5.00
Density (g/cm³)	789	789	789	789	789	789	789	789	789	789	789	789

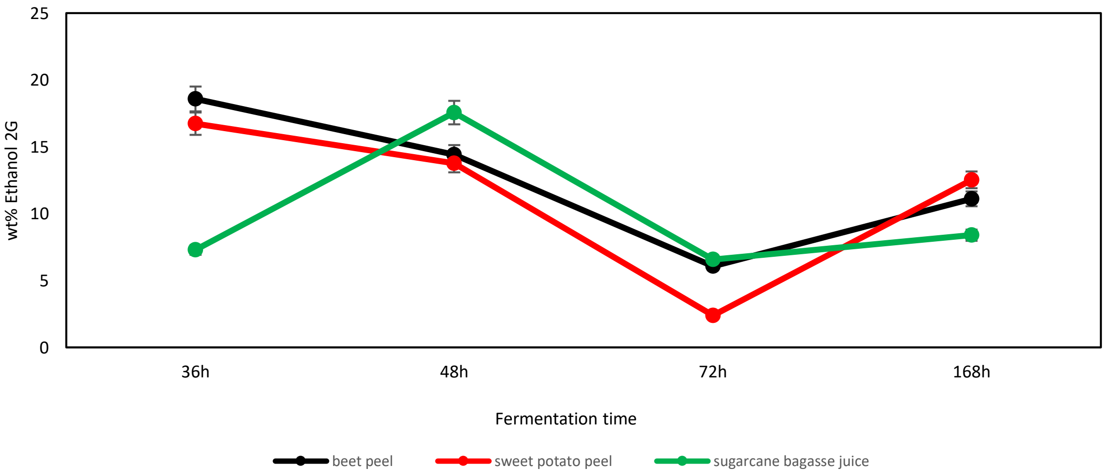

Figure 05: wt% ethanol 2G production

5. Conclusions

Bioethanol, a biofuel produced from biomass, plays a critical role in the global effort to transition away from fossil fuels and combat climate change. Its production harnesses the fermentation of sugars found in crops and residues, offering a renewable and potentially carbon-neutral alternative to traditional gasoline. Utilizing bioethanol not only addresses the management of agricultural waste but also bolsters rural economies by providing farmers with additional income opportunities.

Furthermore, bioethanol combustion results in cleaner emissions compared to fossil fuels, reducing greenhouse gases and particulate matter, thus improving air quality and lowering health risks. Continued advancements in

bioethanol production processes, as demonstrated in this study, promise to enhance efficiency and cost-effectiveness, positioning bioethanol as a viable large-scale alternative energy source.
The experimental conditions for beet peel, sweet potato peel, and sugarcane bagasse juice were all the same in terms of temperature, fermentation time, and raw material quantity. The analysis of results revealed that a 36-hour fermentation time produced the highest percentages of ethanol 2G for beet peel (18.58%), sweet potato peel (16.73%), and 48 hours for sugarcane bagasse juice (17.56%).
During the fermentation process, key parameters such as pH, sugar content, alcohol content, and the mass fraction of bioethanol in each sample were rigorously monitored. pH and °BRIX analysis indicated highly favorable conditions for fermentation throughout all time intervals studied, highlighting the effectiveness of the process. This study significantly contributes to the advancement of more effective and sustainable biofuel production processes by innovatively utilizing agricultural waste and opening new perspectives for generating clean and renewable energy. New experiments will compare untested inoculum types with the results.
This research is fundamental in the ongoing search for environmentally friendly energy alternatives and efforts to reduce the carbon footprint associated with the biofuels industry. It is a remarkable achievement that promises to significantly impact the search for more sustainable energy solutions and our fight against climate change.
Moreover, the integration of bioethanol production into existing agricultural practices can foster a symbiotic relationship between food and energy production, ensuring that both sectors thrive without compromising one another. By utilizing agricultural residues and waste products, bioethanol production can coexist with food production, promoting a more sustainable and balanced use of resources. This approach not only maximizes the utility of biomass but also contributes to the overall sustainability of agricultural systems. As the global community seeks to meet energy needs while preserving the environment, studies like this highlight the critical role that innovative biofuel technologies will play in shaping a greener, more sustainable future.

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