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Six Harvests of Bioethanol: Performance of the Native Yeast Isolated from the Process

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Abstract **- Brazil holds a prominent position in bioethanol production, ranking as the world's second-largest producer. In 2024, the country achieved a record production of 36.83 billion liters, with significant growth in corn-based ethanol. Ethanol's competitiveness improved, with its price parity with gasoline reaching 65.3%, boosting domestic consumption.**
The sector has benefited from public policies such as the RenovaBio program and the Future Fuel initiative, promoting a low-carbon economy. However, one of the challenges in ethanol production is the selection of yeast strains used in fermentation. Yeasts play a crucial role in the process, and introducing selected strains can enhance efficiency. Studies, however, indicate that these strains may gradually be replaced by native yeasts from the industrial environment.
A promising alternative is the selection of native strains adapted to process conditions, ensuring greater persistence throughout harvest seasons. Research conducted by Steckelberg revealed that using native yeasts isolated from the industrial process offers advantages over commercial strains, as they are better suited to the fermentation environment. The study tracked six consecutive harvests at a bioethanol production facility, analyzing the permanence and dominance of these yeasts.
Given this landscape, ethanol production in Brazil continues to advance, driven by new technologies and strategies to optimize fermentation, ensuring greater efficiency and sustainability for this biofuel.

Index Terms **- Bioethanol Native Yeast, alcoholic fermentation, performance**

I. Introduction

Bioethanol plays an important role in mitigating climate change, as it is a sustainable alternative to fossil fuels. Brazil remains the second-largest ethanol producer in the world, behind only the United States. Ethanol's competitiveness has also improved, with the price parity with gasoline reaching 65.3%, the best since 2010. This has stimulated internal biofuel consumption [1].

The current outlook for bioethanol production in Brazil is quite promising. In 2024, Brazil recorded its highest ethanol production in history, totaling 36.83 billion liters, a 4.4% increase compared to 2023. Of this total, 7.7 billion liters were produced from corn, representing a growth of 32.8% [1], [2].

Additionally, the bioenergy sector has benefited from new laws and public policies, such as the Combustível do Futuro (Fuel of the Future) initiative and the RenovaBio program, which promote sustainable mobility and a low-carbon economy [3].

In summary, Brazil is in a strong position in the bioethanol sector, with significant advances in production, consumption, and public policies. The future looks promising for the continued growth and sustainability of biofuels in the country. Ethanol production as a sustainable fuel also deserves attention for its significant environmental benefits, such as reducing greenhouse gas emissions and the high efficiency of carbon dioxide capture and fixation by sugarcane itself [4]. Given all these prospects, several technologies have been tested to increase ethanol production in Brazil. However, some barriers remain to be overcome, with yeast selection being the main challenge.

Yeasts are undoubtedly an essential component in ethanol production, and understanding their needs and behavior is crucial to optimizing the fermentation process.

The use of selected yeasts as inoculants in industrial fermentations is often recommended to ensure greater efficiency in the process [5]. However, recent studies indicate that even with the introduction of these specific strains, they can gradually be replaced by native strains present in the fermentative environment [6], [7].

According to Steckelberg et al. [6], the permanence of a selected strain throughout the entire harvest in fermentation vats is unlikely. Despite this, initiating the fermentation process with selected yeasts can offer benefits, as these strains are more adapted to the adverse conditions of the vats, such as high acidity, rising temperatures, and high alcohol content. However, it is essential to consider the cost of this approach compared to the use of commercial yeasts, such as baker's yeast, before making a decision.

One strategy adopted by industries to optimize the fermentation process is the identification and isolation of native strains already present in the industrial environment, ensuring that only the most adapted ones are used at the beginning of the next harvest [8], [9]. The selection of these strains considers specific aspects of fermentation, going beyond ethanol yields to include essential factors for survival under adverse conditions.

A strain's ability to prevail in the fermentative environment, known as dominance, and its continuity throughout the process, persistence, are directly related to various characteristics, such as kinetic efficiency in production, growth rate, and resistance to environmental stresses. These factors determine which strains will be most suitable for industrial fermentation over time [10].

Steckelberg [11] found that the practice of using selected native yeast strains as inoculants for the beginning of the harvest proved to be more promising compared to the use of selected strains available on the market, such as PE, CAT, and FT.

According to Steckelberg [12], [11], the use of selected native yeasts isolated from the process is a recommended

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practice, as native yeasts persist throughout harvests, even though each harvest has its own behavioral profile. This finding is supported by the results obtained in Steckelberg's work [11].

This study aimed to monitor six consecutive harvests of a bioethanol production unit using the same native strain isolated from the process as an inoculant to assess its persistence and dominance over the harvests.

II. Materials and Methods

Samples

Samples of fermented broth were collected during two consecutive seasons from three industrial units that produce bioethanol from sugarcane and its byproducts. Samples were collected at non-regular intervals, spaced no more than 40 days apart, during the 2018 and 2019 seasons. Samples were previously diluted in 0.9% saline solution and cultured on WLN differential medium (DIFCO no. 0424) supplemented with 100 ppm monensin for inhibition of bacterial growth. Plates were prepared by the spread-plate method and incubated at 32 °C for 7 days for selection of different colony morphologies. Biotype identification was based on the morphological characteristics of colonies (size, color, and texture). Different biotypes were subcultured, in duplicate, purified, and maintained on PDA (potato–dextrose agar) slants.

Yeast identification

Yeasts were identified molecularly by karyotyping. Chromosome isolation was performed by modifying the protocol proposed by Blond and Vezinhét [15]. Chromosomes were separated by pulsed-field agarose gel electrophoresis using a Bio-Rad CHEF III equipment. The gel was stained with ethidium bromide in TAFE solution (0.5 mL/L) and analyzed under ultraviolet light (UVP BioImagem System). The chromosomal profile was analyzed in duplicate for each biotype (colony morphology).

III. Results and Discussion

Figures 1, 2, 3, 4, 5, and 6 show the yeast population dynamics during the 2018, 2019, 2021, 2022, 2023, and 2024 harvests in a bioethanol production unit.

The bioethanol production unit selected a native yeast, *Saccharomyces cerevisiae*, in 2017 to be used as the starter culture in the following harvests alongside commercially known yeasts.

The unit initiated fermentation with the selected yeasts PE, ANGEST, and CAT, along with the native yeast isolated from its own process in 2017, designated as CEV1. The native yeast CEV1 was able to remain in the process until the end of the harvest seasons in 2018, 2019, 2021, 2022, and 2023, as it exhibits efficient technological characteristics and persistence in the process.

The 2024 harvest was atypical, as excessive heat and a large fire that affected sugarcane production negatively impacted fermentation performance.

In 2018 (Fig.1), five different yeasts were present in the process during the harvest, three originating from the initial inoculum and two native strains. As observed, the CEV1 strain started at a low concentration but, after 20 days of harvest, began to dominate the process until the end of the season.

It was noted that at 85, 120, 145, 175, and 210 days, the native yeast CEV1 was the only strain inhabiting the process. By the end of the harvest, after 235 days, CEV1 remained in the process at a high concentration, sharing the vat with just one other yeast. The selected yeasts, PE and CAT, were completely eliminated from the process after 20 days of harvest.

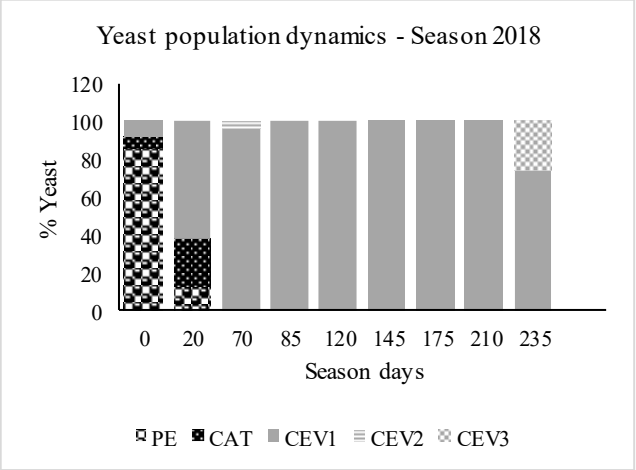

Fig. 1: Yeast population dynamics in the fermentation process during the 2018 season

During the 2019 harvest (Fig. 2), five different yeast strains were present in the process, three originating from the initial inoculum and two native strains. As observed, after 60 days, the CEV1 strain had already reached a concentration equal to that of the selected yeast CAT.After 90 days of fermentation, the native yeast CEV1 dominated the process, with only the selected yeast CAT1 remaining. By 120 days of harvest, the selected yeasts were no longer present in the process, and CEV1 was the predominant strain in the vat.At 150 and 180 days, only the native yeast CEV1 inhabited the vat. At 210 and 240 days of fermentation, CEV1 remained the most concentrated yeast in the vat, sharing the environment with CEV2 and CEV3, which were present in low concentrations and had previously inhabited the vat during the 2018 harvest.

During the 2021 harvest (Fig. 3), the selected yeasts PE2 and Angest were used as starter cultures, along with the selected native yeast CEV1. Only at the beginning of the harvest did CEV1, which was already at a higher concentration, share the vat with the PE yeast. From 40 days into the harvest onward, CEV1 completely dominated the process until the end of the season

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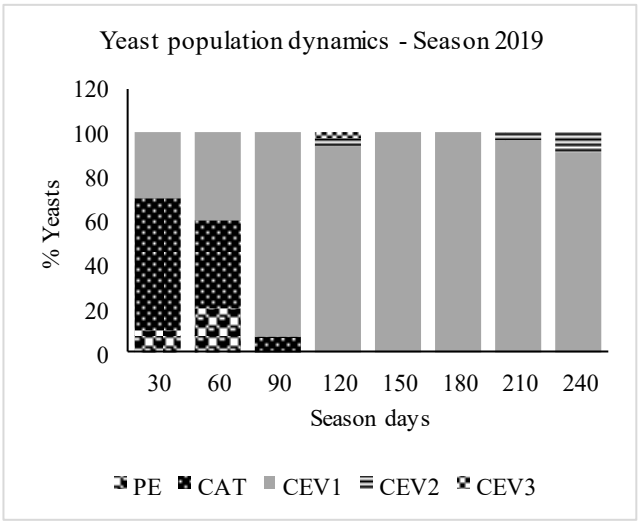


Fig. 2: Yeast population dynamics in the fermentation process during the 2019 season

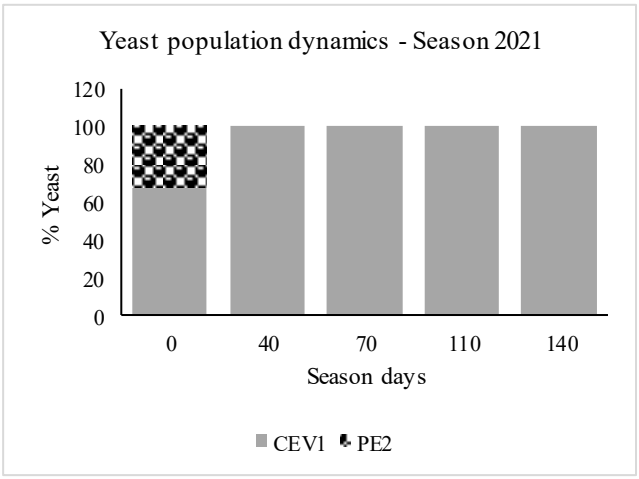


Fig. 3: Yeast population dynamics in the fermentation process during the 2021 season

During the 2022 harvest (Fig. 4), the bioethanol production unit started the season with the selected yeasts ANGEST and PE2, along with the selected native yeast CEV1. It was observed that from the beginning, the native yeast CEV1 was present 100% in the process. Only after 195 days of harvest did it share the process with the yeast CEV4.

The 2023 harvest began with the selected yeasts CAT, PE, ANGEST, and the selected native yeast CEV1. During this harvest (Fig. 5), a total of 10 different yeast strains inhabited the process—two from the initial inoculum and eight native strains. As observed, the CEV1 strain was present in all samplings, initially sharing the vat with the selected yeast PE and dominating the process in the samplings taken at 30, 60, 90, 120, and 150 days. At 180 days into the harvest, the vat was shared between the selected yeast CEV1 and five native yeasts: CEV5, CEV6, CEV7, CEV8, and CEV9. By 210 days, CEV1 was present at a lower concentration, sharing the vat with the native yeasts CEV5 and CEV10. In the final sampling, in addition to the selected native yeast CEV1, the native yeasts CEV5, CEV10, and CEV11 were also present. Although CEV1 remained present throughout the harvest and dominated the process for most months, the emergence of native yeasts that had not appeared in previous harvests was observed. This occurrence can be attributed to the abundant rainfall at the end of the harvest season.

Fig. 4: Yeast population dynamics in the fermentation process during the 2022 season

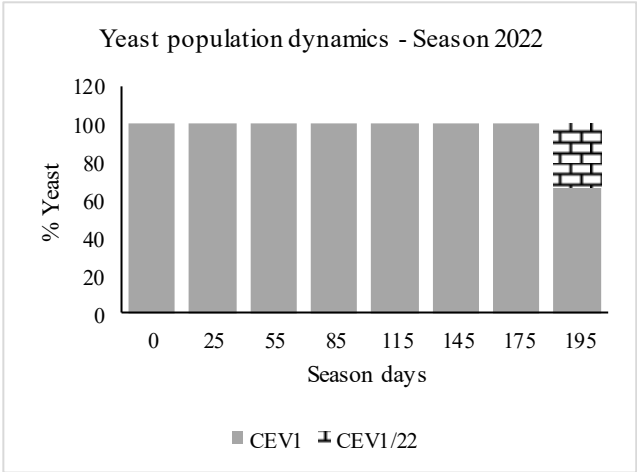
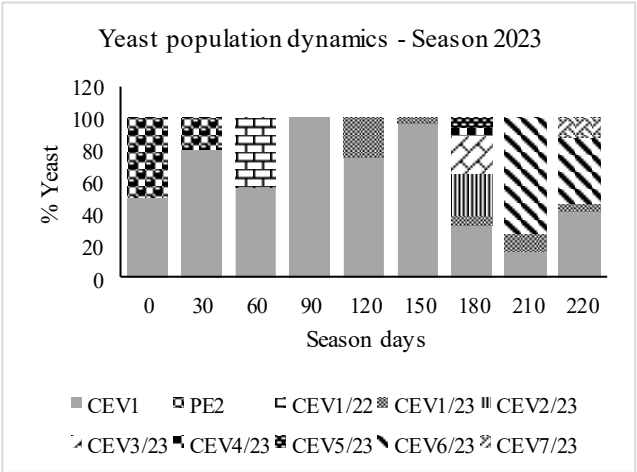


Fig. 5: Yeast population dynamics in the fermentation process during the 2023 season



During the 2024 harvest (Figure 6), eight different yeast strains inhabited the process—three from the initial inoculum and five native strains.As observed, the CEV1 strain was present in five of the seven analyzed samplings. At the beginning of the harvest, the selected native yeast CEV1 had a higher concentration than the selected yeasts PE and CAT. At 40 and 70 days, the CEV1 yeast completely dominated the process, and by 70 days, the CEV12 yeast was detected. After 100 days of harvest, the selected native yeast CEV1 shared the vat with the yeasts CEV12, CEV13, and CEV14. At 130 days, CEV1 was observed at a low concentration (6.3%), while the yeasts CEV12, CEV13, CEV15, and CEV16 were present in the vat. By 170 and 200 days of harvest, the selected native yeast had been eliminated from the process, and the native yeasts CEV12, CEV16, CEV17, CEV18, CEV14, and CEV19 inhabited the fermentation environment. The dynamics of this harvest can be explained by the severe fire that affected the sugarcane fields, which led to an increased entry of native yeasts and contributed to the elimination of the selected native yeast CEV1.

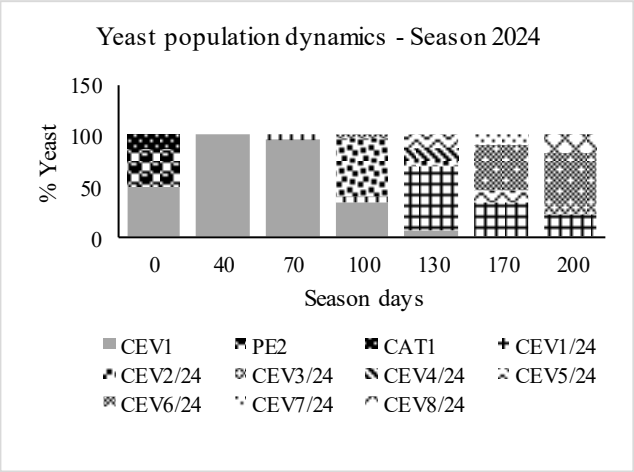


Fig. 6: Yeast population dynamics in the fermentation process during the 2024 season

IV. Conclusion

The data presented corroborate Steckelberg's argument [6], indicating that the probability of a selected strain persisting throughout the harvest during fermentation is low. However, the selected native strain demonstrated a significant capacity for maintenance, remaining for five consecutive harvests until the end of the fermentation cycle, being eliminated only in the 2024 harvest due to climatic adversities.

The temporal analysis of the harvests reveals the predominance of yeast CEV1, which exerted dominance over the fermentation process throughout all the evaluated harvests. In 2021, yeast CEV1 shared the fermentation environment with the selected strain PE2 during the first month, later assuming absolute control of the process. In the 2022 harvest, yeast CEV1 began the period with predominance, sharing the fermentation environment only in the last month with a native yeast.

Based on the monitoring of six harvests, it is observed that the adoption of native yeasts selected from the fermentation process itself results in substantial benefits. Among the observed advantages, the increased process stability, the reduction in input consumption, and the improvement in fermentation yield stand out, highlighting the relevance of this approach for sustainability and production efficiency.

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