Whey Valorization for Alcoholic Fermentation

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INTRODUCTION

Whey is the most significant by-product of the dairy industry, generated during cheese production. Approximately 80–90% of the milk entering cheese manufacturing facilities becomes whey. Researchers estimate that globally, 180–190 million tons of whey were produced in 2018, with this quantity projected to increase to 203–241 million tons by 2030, growing annually by 1–2%. Sweet whey typically contains 0.6–0.9% protein, up to 0.3% fat, a relatively large amount of lactose (4.5–5.2%), and 0.02–0.05% minerals. It can be used as an additive in processed foods, bakery products, and animal feed, and is also utilized in the production of nutritional supplements and dietary products. Additionally, whey can be employed in the manufacture of ricotta cheese and alcoholic beverages. However, not all dairy processors utilize whey for such purposes, posing challenges in its disposal as a high-organic-content by-product. Whey's environmental impact is significant (COD values: 0.8–102 g/L, BOD values: 0.6–60 g/L), yet it can serve as a valuable resource for bioethanol production with appropriate biotechnological methods.

In whey, the primary fermentable disaccharide is lactose (β-D-galactopyranosyl-(1→4)-D-glucose), which, when hydrolyzed by lactase enzyme, breaks down into glucose and galactose, suitable substrates for alcohol fermentation by *Saccharomyces cerevisiae*. Whey's lactose content can be economically and effectively increased through ultrafiltration to remove proteins, followed by reverse osmosis (RO), significantly enhancing subsequent fermentation and distillation efficiency.

OBJECTIVES

The main objective of this research was to investigate whether the retentate obtained from the reverse osmosis (RO) filtration of whey is suitable for bioethanol production using both a commercial yeast strain and one of our own isolated strains.

Furthermore, to improve the monitoring of the fermentation process, we intend to track the concentration changes of glucose and galactose (as substrates), ethanol (as the main metabolite), as well as lactic acid and acetic acid (as by-product metabolites) throughout the fermentation.

MATERIALS AND METHODS

Fermentation raw material:

The fermentation substrate was a retentate obtained trough RO of whey sourced from Gordon Prod Ltd. (Bisericani, Romania), with the following composition (% m/m): 0,61% of protein, 0.87% of NPN, 12.55% of lactose, 0.198% of acids, 1.98% of ash, 0.05% of fats, and 16,253% of total solids. Two yeast strains were used: a commercial *Saccharomyces cerevisiae* (CHR-Hansen Viniflora Merit) and a soil-isolated strain collected near the facility's whey outlet.

Strain isolation and identification procedure:

Soil was suspended in physiological saline, homogenized, and plated on modified YPG agar (10 g/L yeast extract, 20 g/L peptone, 20 g/L galactose, 20 g/L agar) to select galactose-metabolizing yeasts. After 72 h incubation at 30 °C, distinct colonies were isolated and purified. MALDI-TOF mass spectrometry (Bruker BioTyper™ MBT™ Smart) and 16S rRNA sequencing (Eurofins BIOMI Ltd.) methods was used for the identification of strains.

Whey preparation:

The whey retentate was supplemented with yeast nutrient (0.1 g/L BACTIV-AID 2.0), pasteurized at 68 °C for 30 min, cooled to 35 °C, then hydrolyzed enzymatically with 0.325% (V/V) NOLA™ Fit 5500 at 35 °C and 200 RPM for 1 h. The enzyme was inactivated at 68 °C for 15 min, then cooled again to 35 °C.

Fermentation:

Triplicate fermentations (in 100 mL Erlenmeyer) were inoculated separately with *S. cerevisiae* and the isolated strain. Fermentation proceeded for 14 days at 30 °C with continuous shaking (200 RPM).

Monitoring:

Samples were taken every 4, 8, and 12 hours. Glucose, galactose, ethanol, lactic acid, and acetic acid concentrations were analyzed via HPLC (Agilent 1260 Infinity).

RESULTS AND DISCUSSIONS

Strain identification:

MALDI-TOF analysis revealed protein profiles consistent with *Pichia fermentans*, a species capable of metabolizing glucose and galactose. Further 16S rRNA sequencing confirmed the isolate as *P. fermentans*, with a 99.09% nucleotide sequence identity to strain *P. fermentans* ATCC 10651.

Fermentation:

The metabolic changes during alcoholic fermentation of whey concentrate using *S. cerevisiae* and *P. fermentans* are shown in Figures 1 and 2, respectively.

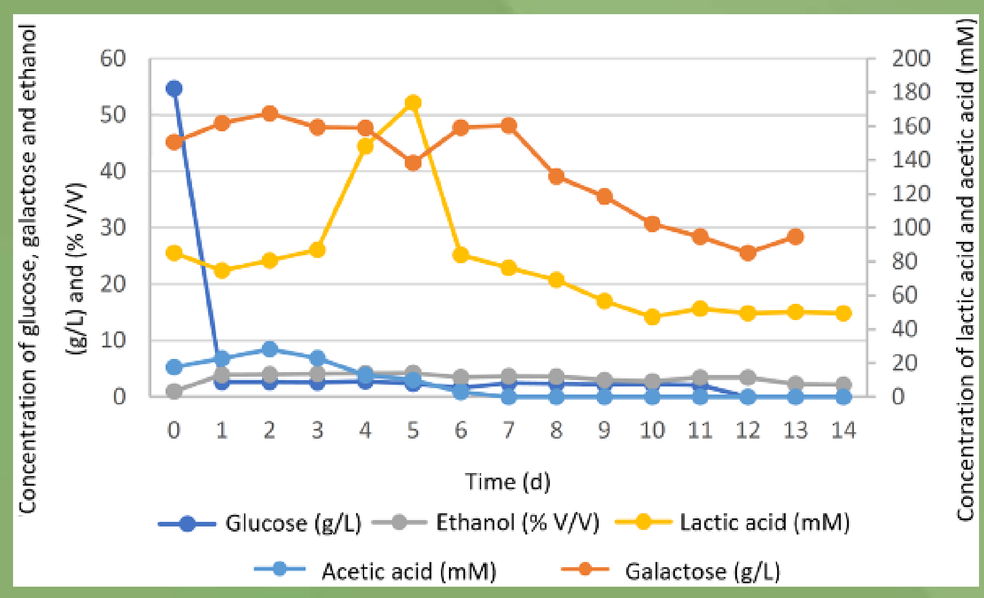

Fig. 1. Metabolic profile of fermentation of whey retentate with *P. fermentans*

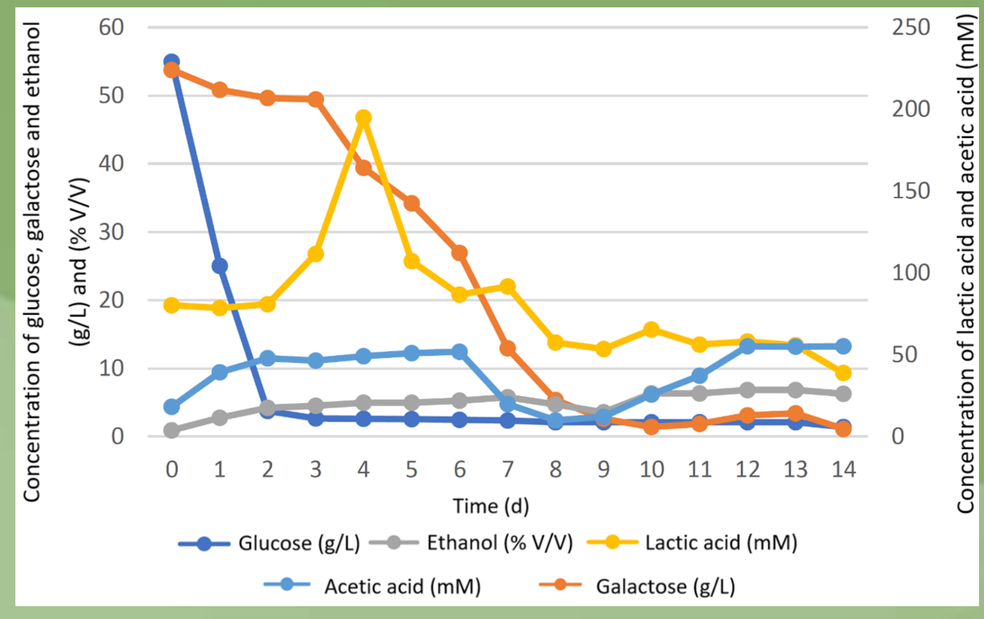

Fig. 2. Metabolic profile of fermentation of whey retentate with *S. cerevisiae*

Data show both yeast strains preferred glucose over galactose, though galactose was metabolized in later fermentation stages. Lactic acid peaked sharply on days 4–5 at 174.03 mM and 194.89 mM, then declined gradually. Acetic acid behavior differed: *P. fermentans* fully consumed it, while *S. cerevisiae* showed an initial drop, then rose to 55.14 mM by day 14. Ethanol production was higher in the case of fermentation with *S. cerevisiae* (6.84% V/V) than with *P. fermentans* (3.45% V/V).

CONCLUSIONS

A yeast strain capable of fermenting whey retentate was successfully isolated, although the commercial *S. cerevisiae* strain exhibited higher ethanol production efficiency. Experimental results clearly indicate that whey retentate is a suitable substrate for bioethanol production and may contribute to the mitigation of potential environmental impacts.

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