Agrociencia Uruguay 2026 | Volume 30 | Article e1877
DOI: 10.31285/AGRO.30.1877
ISSN 2730-5066

Dipotassium Phosphate Enhances Ethanol Tolerance, Production, and Cell Viability of *Saccharomyces cerevisiae* in Batch Fermentation

Sánchez-Rosales, F. E. [1]; Castellanos-Reyes, K. M. [1]; Vásquez, A. [1]; Herrera, N. [2]; Sanabria-Ortega, H. L. [3]; Martinez, W. A. [1]; Betancourth, J. [1]

[1]*Universidad Nacional de Agricultura, Facultad de Ciencias Tecnológicas, Departamento Académico de Ingeniería de Procesos, Catacamas, Honduras*

[2]*Universidad Nacional de Agricultura, Facultad de Ciencias, Departamento Académico de Biología y Microbiología, Catacamas, Honduras*

[3]*Universidad Nacional de Agricultura, Facultad de Ciencias Agrarias, Laboratorio de Biotecnología, Catacamas, Honduras*

Editor

Gustavo González-Neves
Universidad de la República, Facultad de Agronomía, Montevideo, Uruguay

Received *15 Dec 2025*
Accepted *26 Mar 2026*
Published *20 Apr 2026*

Correspondence

Francisco E. Sánchez-Rosales
fsanchez@unag.edu.hn

Abstract

This study evaluated ethanol production and cell viability in *Saccharomyces cerevisiae* cultures under different concentrations of dipotassium phosphate (K_2HPO_4). Batch fermentations were carried out in shake flasks using an orbital thermostatic incubator at 30 °C, with an initial pH of 5.80. The K_2HPO_4 concentration in the culture medium was adjusted to 1.50, 2.70, and 3.20 g L⁻¹. Cultures supplemented with 3.20 g L⁻¹ K_2HPO_4 achieved the highest biomass concentration (12.10 ± 0.30 g L⁻¹). Moreover, ethanol production reached 16% (v/v) under this condition, whereas only 9% (v/v) was obtained at 1.50 g L⁻¹. Regarding volumetric ethanol productivity, a 1.6-fold increase was estimated when the K_2HPO_4 concentration was increased from 1.50 to 3.20 g L⁻¹. Additionally, under the 3.20 g L⁻¹ condition, cell viability remained above 90%, reaching a maximum of 97%, even when ethanol concentration in the broth reached 16% (v/v). In contrast, cell viability decreased by 10% and 7% at 1.50 and 2.70 g L⁻¹, respectively, when ethanol concentrations exceeded 7% (v/v). Overall, the results demonstrated that kinetic parameters such as cell viability, volumetric productivity, and ethanol concentration were positively affected by increasing K_2HPO_4 concentration, supporting its use as a nutritional strategy in the culture medium to enhance ethanol biosynthesis. These findings are particularly relevant as they highlight the potential of K_2HPO_4 as a strategic and economical nutrient to optimize ethanolic fermentations on an industrial scale, contributing to the design of bioprocesses with improvements in metabolic efficiency and prolonged productive capacity throughout the fermentation cycles.

Keywords: *Saccharomyces cerevisiae*, ethanol, cell viability, potassium phosphate, fermentation

Sánchez-Rosales, F. E., Castellanos-Reyes, K. M., Vásquez, A., Herrera, N., Sanabria-Ortega, H. L., Martinez, W. A., & Betancourth, J. (2026). Dipotassium phosphate enhances ethanol tolerance, production, and cell viability of *Saccharomyces cerevisiae* in batch fermentation. *Agrociencia Uruguay, 30*, Article e1877. https://doi.org/10.31285/AGRO.30.1877

El fosfato dipotásico mejora la tolerancia al etanol, la producción y la viabilidad celular de *Saccharomyces cerevisiae* en fermentación por lotes

Resumen

Este estudio evaluó la producción de etanol y la viabilidad celular en cultivos de *Saccharomyces cerevisiae* bajo diferentes concentraciones de fosfato dipotásico (K_2HPO_4). Las fermentaciones por lote se realizaron en matraces agitados utilizando una incubadora orbital termostático a 30 °C, con un pH inicial de 5,80. La concentración de K_2HPO_4 en el medio de cultivo se ajustó a 1,50, 2,70 y 3,20 g L^{-1}. Los cultivos suplementados con 3,20 g L^{-1} de K_2HPO_4 alcanzaron la mayor concentración de biomasa (12,10 ± 0,30 g L^{-1}). Además, la producción de etanol alcanzó 16 % (v/v) bajo esta condición, mientras que solo se obtuvo 9 % (v/v) a 1,50 g L^{-1}. En cuanto a la productividad volumétrica de etanol, se estimó un incremento de 1.6 veces al aumentar la concentración de K_2HPO_4 de 1,50 a 3,20 g L^{-1}. Adicionalmente, bajo la condición de 3,20 g L^{-1}, la viabilidad celular se mantuvo por encima de 90 %, alcanzando un máximo de 97 %, incluso cuando la concentración de etanol en el caldo llegó a 16 % (v/v). En contraste, la viabilidad celular disminuyó en 10 % y 7 % a 1,50 y 2,70 g L^{-1}, respectivamente, cuando las concentraciones de etanol superaron 7 % (v/v). En conjunto, los resultados demostraron que parámetros cinéticos como la viabilidad celular, la productividad volumétrica y la concentración de etanol se vieron positivamente afectados por el incremento en la concentración de K_2HPO_4, apoyando su uso como estrategia nutricional en el medio de cultivo para mejorar la biosíntesis de etanol. Estos hallazgos son particularmente relevantes ya que resaltan el potencial del K_2HPO_4 como nutriente estratégico y económico para optimizar fermentaciones etanólicas a escala industrial, contribuyendo al diseño de bioprocesos con mejoras de eficiencia metabólica y capacidad productiva prolongada a lo largo de los ciclos de fermentación.

Palabras clave: *Saccharomyces cerevisiae,* etanol, viabilidad celular, fosfato de potasio, fermentación

O fosfato dipotássico aumenta a tolerância ao etanol, a produção e a viabilidade celular de *Saccharomyces cerevisiae* em fermentação em batelada

Resumo

Este estudo avaliou a produção de etanol e a viabilidade celular em culturas de *Saccharomyces cerevisiae* sob diferentes concentrações de fosfato dipotássico (K_2HPO_4). As fermentações em batelada foram realizadas em frascos agitados utilizando um incubador orbital termostatizado a 30 °C, com pH inicial de 5,80. A concentração de K_2HPO_4 no meio de cultura foi ajustada para 1,50, 2,70 e 3,20 g L^{-1}. As culturas suplementadas com 3,20 g L^{-1} de K_2HPO_4 alcançaram a maior concentração de biomassa (12,10 ± 0,30 g L^{-1}). Além disso, a produção de etanol atingiu 16 % (v/v) nessa condição, enquanto apenas 9 % (v/v) foram obtidos a 1,50 g L^{-1}. Quanto à produtividade volumétrica de etanol, estimou-se um aumento de 1,6 vez ao elevar a concentração de K_2HPO_4 de 1,50 para 3,20 g L^{-1}. Adicionalmente, na condição de 3,20 g L^{-1}, a viabilidade celular permaneceu acima de 90 %, alcançando um máximo de 97 %, mesmo quando a concentração de etanol no meio atingiu 16 % (v/v). Em contraste, a viabilidade celular diminuiu 10 % e 7 % nas concentrações de 1,50 e 2,70 g L^{-1}, respectivamente, quando as concentrações de etanol excederam 7 % (v/v). No geral, os resultados demonstraram que parâmetros cinéticos, como viabilidade celular, produtividade volumétrica e concentração de etanol, foram positivamente afetados pelo aumento da concentração de K_2HPO_4, apoiando seu uso como estratégia nutricional no meio de cultura para melhorar a biossíntese de etanol. Essas descobertas são particularmente relevantes, pois destacam o potencial do K_2HPO_4 como um nutriente estratégico e econômico para otimizar as fermentações etanólicas em escala industrial, contribuindo para o desenvolvimento de bioprocessos com melhorias na eficiência metabólica e capacidade produtiva prolongada ao longo dos ciclos de fermentação.

Palavras-chave: *Saccharomyces cerevisiae*, etanol, viabilidade celular, fosfato de potássio, fermentação

1. Introduction

Saccharomyces cerevisiae is the most widely used yeast in fermentative bioprocesses due to its efficient metabolism, ease of genetic manipulation, broad commercial availability, and the fact that its genome has been fully sequenced (Sahana et al., 2024). Its use has been extensively documented in alcoholic fermentation, both in the food industry for the production of alcoholic beverages such as wine and beer (Álvarez et al., 2023; Molinet & Cubillos, 2020; Postigo et al., 2021; Walker & Stewart, 2016), and in the generation of environmentally friendly fuels such as bioethanol (Mohd Azhar et al., 2017). These characteristics make it an ideal model organism for fermentative processes at both laboratory and industrial levels (Sahana et al., 2024).

Although *S. cerevisiae* offers unmatched technological advantages, there are also certain drawbacks related to unfavorable conditions for this yeast that are inherent to alcoholic fermentation, such as osmotic stress, thermal stress, and high ethanol concentrations (Coleman et al., 2007; Henderson & Block, 2014). Therefore, it is essential to investigate alternative fermentation strategies aimed at designing robust bioprocesses that improve the physiological adaptation and tolerance of *S. cerevisiae* to the stressful environments commonly found in fermented broths. Various biotechnological strategies have been proposed, such as genetic modification of strains and the study of nutrients in culture media, as strategies to improve the tolerance and viability of *S. cerevisiae* cell populations (Alfenore et al., 2002; Lam et al., 2014; Varize et al., 2022).

In alcoholic fermentation processes, the availability of nutrients in the culture medium is key, as they are crucial not only for biomass production and ethanol synthesis, but also for improving cell viability (Alminderej et al., 2022; Biswas & Biswas, 2022). There is ample evidence in the literature regarding the study of various components in culture media such as concentrations of carbon sources, nitrogen, and vitamin sources to improve ethanol production (Alfenore et al., 2002; Laluce et al., 2009; Rojo et al., 2023). However, another important nutrient is potassium phosphate (K_2HPO_4), as it plays a dual essential role as a source of both phosphorus and potassium key inorganic ions involved in cell multiplication, osmotic balance maintenance, active nutrient transport across the cell membrane, stimulation of enzymatic reactions involved in ATP anabolism, and the enhancement of cell viability (Barreto et al., 2012; Canadell et al., 2015; Ribeiro-Filho et al., 2022).

Dipotassium phosphate contains the elements phosphorus and potassium, both of which are essential nutrients for metabolic processes in living organisms and for the stability of various chemical systems. In food biotechnology, it is widely used as a phosphorus source for yeasts and bacteria, promoting microbial growth and improving fermentation efficiency in processes such as the production of fermented beverages and starter cultures, thereby demonstrating its relevance in sustainable production and in the development of products with high nutritional and technological value (Barreto et al., 2012; Canadell et al., 2015). Phosphate is known to be incorporated into fermentation media to enhance yeast growth, promote the production of ethanol and aromatic compounds, and help maintain metabolic balance (Ribeiro-Filho et al., 2022). K_2HPO_4 also plays an important role in ethanol tolerance in yeasts, particularly in *Saccharomyces cerevisiae*, the microorganism most widely used in alcoholic fermentations of foods and beverages. During fermentation, increasing ethanol concentrations generate cellular stress, affecting membrane integrity, enzymatic activity, and energy metabolism. The availability of phosphate supports cellular metabolism and contributes to cell survival under these conditions (Ribeiro-Filho et al., 2022). Likewise, K_2HPO_4 is commonly included in fermentation media because it provides inorganic phosphate, which is required for energy metabolism, as well as K^+ ions that are essential for osmotic stability and enzymatic activity, factors that collectively contribute to maintaining cell viability under ethanol stress conditions (Zhao et al., 2023).

Several studies have evaluated K_2HPO_4 as an independent variable in fermentations involving *S. cerevisiae.* For example, Ribeiro-Filho et al. (2022), reported that yeasts require approximately 250 mg/L of phosphate and 500 mg/L of potassium to sustain growth and fermentative metabolism. According to Sun et al. (2024), the presence of KH_2PO_4 and K_2HPO_4 contributed to maintaining phosphate buffer capacity and yeast metabolic activity during fermentation in the selection of a *Saccharomyces cerevisiae* strain with high production of 3-methylthio-1-propanol. Additionally, phosphate supplementation has been shown to increase ergosterol yield by up to 29.5% when optimized with this compound (He et al., 2007). Dipotassium phosphate can therefore be used in relatively low concentrations as both a phosphorus source and a metabolic regulator. In yeast fermentation media, K_2HPO_4 is typically applied in concentrations ranging from 0.1 to 10 g/L, with 0.5 to 2.5 g/L being the most frequently used range to promote cellular growth, while concentrations of 1.8 to 2 g/L have also been reported to maintain the pH close to neutrality during yeast growth (Eliodório et al., 2023; Napitupulu et al., 2021; Ribeiro-Filho et al., 2022; Tan et al., 2025). Although this compound performs key functions in fermentation processes, excessively high concentrations may negatively affect cell growth and fermentative productivity; therefore, it represents an important variable to consider, particularly in industrial-scale processes where cell viability and ethanol tolerance are critical fermentation parameters.

Theoretically, ethanol is a metabolite associated with cell growth, and its synthesis depends on the viability of the cell population. This makes cell viability a critical parameter in evaluating the performance of *S. cerevisiae* under fermentative conditions (Kucharczyk et al., 2025; Sun et al., 2025). Various factors including nutrient concentration and the accumulation of toxic byproducts such as ethanol itself can negatively affect cell viability throughout the process (Mohd Azhar et al., 2017; Sahana et al., 2024; Vamvakas & Kapolos, 2020). For this reason, the aim of the present study was to evaluate the effect of different concentrations of potassium phosphate on the cell viability of *S. cerevisiae* and its impact on ethanol production, in order to improve fermentative conditions and increase process efficiency.

2. Materials and Methods

2.1 Strain and Culture Medium

Saccharomyces cerevisiae was commercially obtained in lyophilized (Fermipan, Mexico). The culture medium proposed by Breisha (2010) was used with some modifications: 15 °Brix of commercial sucrose, 2.0 g L^{-1} of $(NH_4)_2SO_4$ (Merck, Germany), 0.1 g L^{-1} of $MgSO_4 \cdot 7H_2O$ (Merck, Germany) and K_2HPO_4 (Merck, Germany) was adjusted in the medium according to each evaluated condition (A: 1.50 g L^{-1}, B: 2.70 g L^{-1}, and C: 3.20 g L^{-1}). The initial pH of the medium was adjusted to 5.80 using a 0.1 M citrate buffer composed of citric acid and sodium citrate (Merck, Germany). Subsequently, the medium was sterilized at 121 °C for 20 minutes.

2.2 Cultivation Conditions

The inoculum was prepared by taking 5.10 grams of lyophilized yeast and adding it to 170 mL of culture medium at 35 °C, allowing it to rest for 15 minutes. A volume of 10 mL of active cells was then transferred to 250 mL Erlenmeyer flasks containing 140 mL of fresh medium. Batch cultures were carried out at 150 rpm and 30 °C in an orbital shaking incubator with a shaking radius of 25 mm (Biobase BJXP-200B, China). The initial pH of the medium was adjusted to 5.80 using a 0.1 M citrate buffer composed of citric acid and sodium citrate. Finally, culture samples (10 mL) were collected every 6 hours over a 66-hour period.

2.3 Biomass and Soluble Solids Quantification

Biomass was estimated using the turbidimetric method. Culture samples were transferred to 1-cm spectrophotometric cuvettes. The optical density of the culture suspensions was measured at 600 nm using a UV-Visible spectrophotometer (Thermo Scientific™ Evolution™ 201/220, USA), and calibrated with dry cell weight measurements (Rojo et al., 2023). To measure soluble solids content, a digital refractometer (Milwaukee, Germany) was used. Measurements were performed in triplicate, and results were expressed in degrees Brix (°Brix).

2.4 Ethanol Quantification

Ethanol concentration was estimated spectrophotometrically by oxidizing ethanol in a potassium dichromate solution under acidic conditions. The method described by Seo et al. (2009) was used with slight modifications. An oxidative solution was prepared by mixing 0.1 M potassium dichromate (Merck, Germany) with 5 M sulfuric acid (Merck, Germany), stirring vigorously until a homogeneous mixture was obtained. Then, 300 µL of biomass-free supernatant from each culture (diluted when necessary) was mixed with 3000 µL of the oxidative potassium dichromate-sulfuric acid solution. The mixture was stirred vigorously and left to stand for 30 minutes at 30 °C. The optical density of the samples was measured at 595 nm using a UV-Visible spectrophotometer (Thermo Scientific™ Evolution™ 201/220, USA) and calibrated using a standard curve prepared with ethanol solutions ranging from 0.5 to 3.5 % (v/v) (Bennett, 1971; Seo et al., 2009).

2.5 Cell Viability Quantification

Cell viability was determined using the methylene blue staining technique. Dilutions of the culture broth were prepared with sterile water as needed, according to the time points of the growth kinetics. A volume of 500 µL of the diluted culture broth was mixed with 500 µL of sterile methylene blue solution (Merck, Germany). The mixture was agitated, incubated for 5 minutes, and placed in a Neubauer chamber. Microscopic counts (using a 40× objective) were performed to determine the number of stained (non-viable) and unstained (viable) cells in four different fields, with a minimum total of 200 cells. The percentage of cell viability was estimated as the ratio of the total number of unstained (viable) cells to the total number of cells (stained and unstained) (Alfenore et al., 2004; Kwolek-Mirek & Zadrag-Tecza, 2014).

2.6 Determination of pH and Titratable Acidity

The pH of the samples was measured at 25 ± 1 °C using a previously calibrated (OHAUS STARTER 2100, USA) pH meter. The pH was measured in triplicate by inserting the electrode into a beaker containing the sample. Titratable acidity was determined by using phenolphthalein as an indicator, titrating 10 mL of culture broth with a standard 0.1 N NaOH solution. Results were expressed as a percentage of acetic acid (Azbekyan et al., 2025; Woo et al., 2014).

2.7 Specific Growth Rate

The logistic model was used to calculate the specific growth rate (μ h^{-1}) in the different cultures:

$$\frac{dX}{dt} = \mu * X\left(1 - \frac{X}{X_{max}}\right) \qquad \text{Eq. 1}$$

Where X is the biomass concentration ($g{\cdot}L^{-1}$) and X_{max} ($g{\cdot}L^{-1}$) is the maximum biomass concentration. The value of µ was estimated using the Generalized Reduced Gradient (GRG) nonlinear procedure provided by Solver (Microsoft Excel for Office 365 MSO), which applies nonlinear least squares regression using the logistic model (Díaz-Barrera et al., 2021).

2.8 Statistical Analysis

The results are expressed as the mean of three independent cultures, and the standard deviations of the triplicates are provided. All data were analyzed using one-way analysis of variance (ANOVA). Statistical analyses were performed using InfoStat software, version 2013 (National University of Córdoba, Argentina). When significant differences were detected among the evaluated conditions, Tukey's multiple comparison test was applied with a 95% confidence level.

3. Results

Figure 1 shows the biomass and Brix degrees in batch cultures of *S. cerevisiae* carried out at different potassium phosphate concentrations (A: 1.50 g·L^{-1}, B: 2.70 g·L^{-1}, C: 3.20 g·L^{-1}). It was observed that the cultures conducted with 3.20 g·L^{-1} potassium phosphate reached the highest biomass values (12.10 ± 0.30 g·L^{-1}) at 66 hours. In contrast, when 1.50 g·L^{-1} of phosphate was used, biomass was 5.0 g·L^{-1} lower at 42 hours of culture. Under conditions A and B, cultures reached the stationary phase at around 42 hours, whereas in condition C, this phase was reached at 60 hours. All tested conditions started with a concentration of soluble solids close to 15 °Brix, which decreased over time. Finally, the cultures under condition C showed residual values of 1.60 ± 0.14 °Brix at 66 hours, while under condition A, the final soluble solids concentration was higher (3.60 ± 0.14 °Brix at 66 hours of culture). Potassium phosphate is one of the sources that enhances ethanol production by providing essential ions for growth and production in fermentation processes. It is incorporated into the culture medium because it supplies essential nutrients for various cellular processes such as energy metabolism, and the synthesis of nucleic acids and proteins (Ribeiro-Filho et al., 2022).

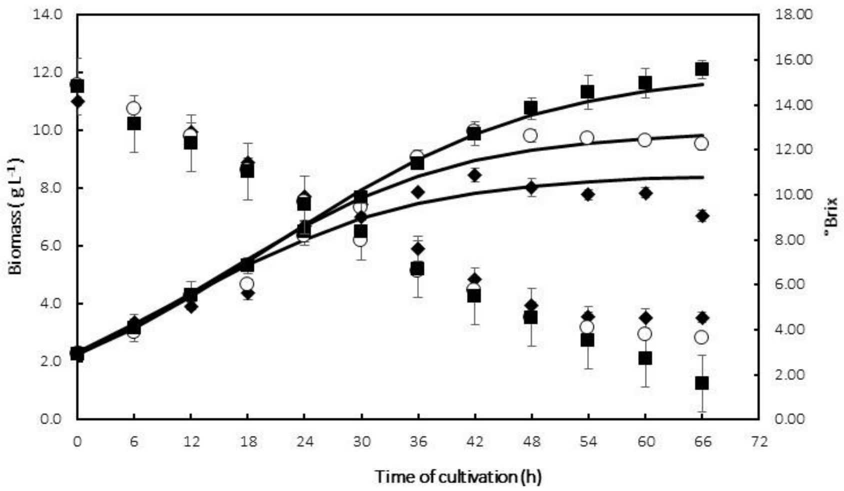

Figure 1. Biomass and °Brix kinetics in batch cultures of *S. cerevisiae* carried out in flasks under different potassium phosphate concentrations. A: 1.5 g L^{-1} (Triangles), B: 2.7 g L^{-1} (Circles), C: 3.2 g L^{-1} (Squares)

Figure 2 shows ethanol concentration in batch cultures of *S. cerevisiae* under the tested potassium phosphate concentrations. Notably, the highest ethanol production, close to 16% (v/v), was obtained at 66 hours in cultures containing 3.20 g·L-1 potassium phosphate. In contrast, when 1.50 g·L^{-1} and 2.70 g·L^{-1} of potassium phosphate were used in the medium, the maximum ethanol production reached was approximately 8% and 10% (v/v), respectively. Under the tested conditions, increasing potassium phosphate concentration (from 1.50 g·L^{-1} to 3.20 g·L^{-1}) enhanced ethanol production, increasing its concentration by 8%. Moreover, when comparing biomass growth kinetics (Fig. 1) with ethanol concentration (Fig. 2), it can be observed that higher ethanol production is associated with higher biomass concentration.

Figure 3 shows the evolution of pH and titratable acidity (TA) in batch cultures of *S. cerevisiae* performed with different concentrations of potassium phosphate. All cultures started with an initial pH of 5.80 (Fig. 3A). Under conditions A and B (1.50 g·L^{-1} and 2.70 g·L^{-1}, respectively), a decrease in pH was observed after 24 hours of cultivation. It is possible that the effect of the final pH value observed in each crop is related to some extent to the concentration of potassium phosphate used. However, more evidence is needed to understand this behavior. Cultures with the lowest concentration (1.50 g·L^{-1}) reached the lowest pH value (4.88) at 66 hours. In contrast, cultures with 3.20 g·L^{-1} of potassium phosphate showed a final pH of 5.62. Titratable acidity was also evaluated in cultures under the three tested conditions (Fig. 3B). The initial titratable acidity was approximately 3% under all conditions and increased to between 4% and 5% in all cultures.

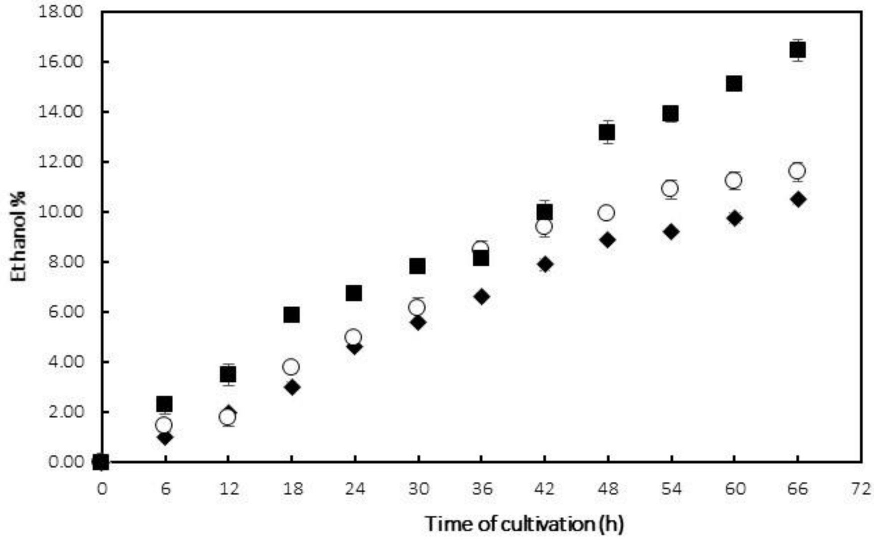

Figure 2. Ethanol kinetics (% v/v) in batch cultures of *S. cerevisiae* carried out in flasks under different potassium phosphate concentrations. A: 1.5 g L^{-1} (Triangles), B: 2.7 g L^{-1} (Circles), C: 3.2 g L^{-1} (Squares)

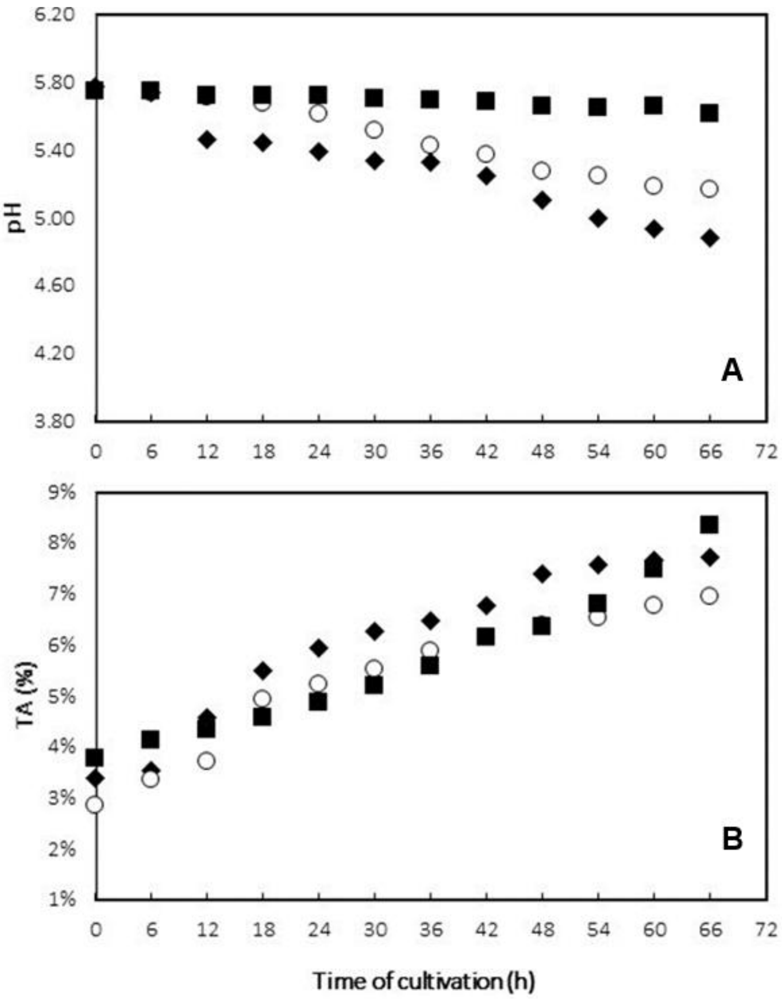

Figure 3. Evolution of pH and titratable acidity in batch cultures of *S. cerevisiae* carried out in flasks under different potassium phosphate concentrations. Medium A: 1.5 g L^{-1} (Triangles), Medium B: 2.7 g L^{-1} (Circles), Medium C: 3.2 g L^{-1} (Squares)

Table 1 presents the fermentation parameters obtained from cultures performed with the different potassium phosphate concentrations. The potassium phosphate concentration had a significant effect on μ, q_p, Q_p, and Q_x. In cultures with 1.50 g·L^{-1} potassium phosphate, μ increased by approximately 1.2 times compared to cultures with 3.20 g·L^{-1}. On the other hand, increasing the concentration from 1.50 g·L^{-1} to 3.20 g·L^{-1} resulted in an approximate 1.6-fold increase in Q_p.

Table 1. Parameters of fermentation obtained in batch cultures of *S. cerevisiae* conducted under different concentrations of potassium phosphate

Treatment	**μ (h^{-1})**	**q_p (g g^{-1} h^{-1})**	**Q_p (g L^{-1} h^{-1})**	**Q_x (g L^{-1} h^{-1})**
A (1.5 g L-1)	0.085 ± 0.00 a	0.18 ± 0.00 a	1.24 ± 0.01 c	0.11 ± 0.01 b
B (2.7 g L-1)	0.080 ± 0.00 a	0.15 ± 0.01 c	1.36 ± 0.02 b	0.13 ± 0.00 a
C (3.2 g L-1)	0.071 ± 0.00 b	0.16 ± 0.00 b	1.97 ± 0.03 a	0.11 ± 0.00 b

Results are presented as means ± SD (n = 3) for each parameter. The same lower-case letters do not differ by Tukey test (5%). Abbreviations: μ (h^{-1}): specific growth rate; q_p (g g^{-1} h^{-1}): product-specific productivity; Q_p (g L^{-1} h^{-1}): volumetric product productivity; Q_x (g L^{-1} h^{-1}): volumetric biomass productivity.

Cell viability was also estimated during *S. cerevisiae* cultivation under the different potassium phosphate concentrations and graphically related to ethanol concentration (Fig. 4). Relatively stable values above 90% viable cells were observed in cultures with 3.20 g·L^{-1} of potassium phosphate, even when ethanol concentration was close to 16% (v/v). In contrast, a decrease of 10% and 7% in cell viability was observed for conditions A (1.50 g·L^{-1}) and B (2.70 g·L^{-1}), respectively, when ethanol concentrations exceeded 7% and 10% (v/v). This decrease in viability observed under condition A coincided with a drop-in biomass concentration (Fig. 1). Finally, the lowest percentage of viable cells (86%) was observed at the end of the cultures with the lowest potassium phosphate concentration.

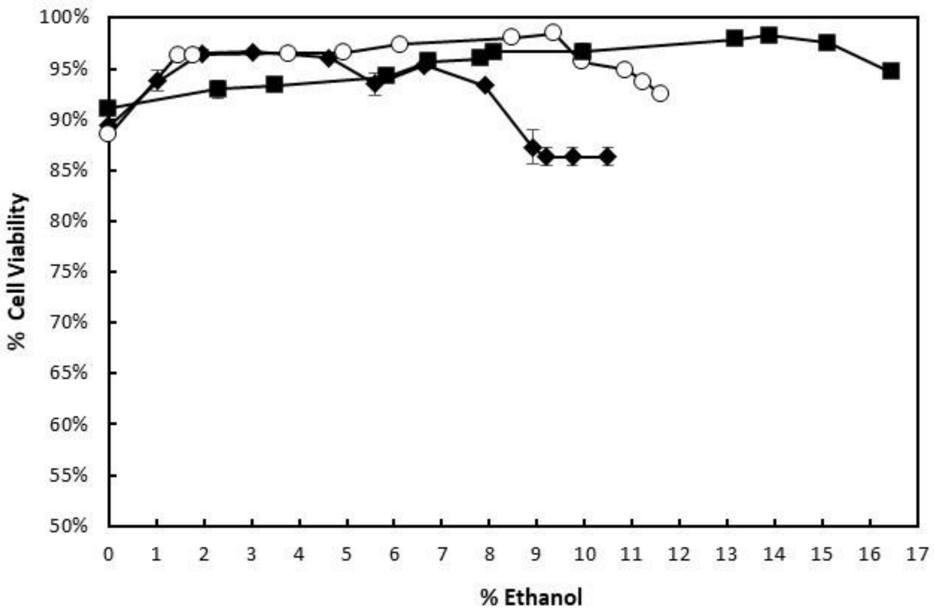

Figure 4. Percentage of *S. cerevisiae* cell viability as a function of ethanol concentration under different potassium phosphate concentrations. A: 1.5 g L^{-1} (Triangles), B: 2.7 g L^{-1} (Circles), C: 3.2 g L^{-1} (Squares)

4. Discussion

Saccharomyces cerevisiae is one of the most widely used industrial strains for alcoholic production. However, the nutritional composition of the medium is key for ethanol production because cells must adjust their metabolism to survive adverse conditions such as high sugar concentrations and ethanol-induced stress environments (Vamvakas & Kapolos, 2020). The results of this study show that, under the evaluated conditions, increasing potassium phosphate concentration promotes biomass production, along with a higher consumption of the carbon source and consequently a lower amount of residual soluble solids (Fig. 1). This evidence suggests a direct relationship between biomass increase and potassium phosphate concentration. This can be explained because this compound supplies inorganic phosphorus and potassium to the medium. Both potassium cations and phosphate anions play fundamental roles in yeast metabolic pathways as they are essential for ATP synthesis (Canadell et al., 2015). Moreover, phosphorus is necessary for cellular anabolism processes such as the synthesis of nucleic acids and phospholipids that form part of the cell membrane (Canadell et al., 2015). Similarly, potassium is linked to primary cellular metabolism functions such as volume regulation and enhancement of the cell growth cycle (Barreto et al., 2012).

Our results indicate that increasing potassium phosphate concentration in the medium favors ethanol production, with an increase of up to 8% (Fig. 2). Previous studies have reported maximum ethanol concentrations between 16 and 19% (v/v) (Alfenore et al., 2002; Breisha, 2010; Laluce et al., 2009; Sahana et al., 2024), which are close to the maximum ethanol concentrations under the conditions evaluated in our study (Fig. 2). Alfenore et al. (2002) reported that when the culture medium was enriched and exponentially fed with a vitamin mixture such as biotin, maximum ethanol concentrations of 19% (v/v) could be reached. Since ethanol is a primary metabolite associated with cell growth, the increased addition of phosphate in the medium may explain the improved alcoholic fermentation, as this compound contributes to increased cell mass, promotes metabolic activity important for ethanol synthesis, and reduces the accumulation of compounds such as acetic acid and short-chain fatty acids (Barreto et al., 2012; Ribeiro-Filho et al., 2022). Additionally, ions of this compound have been used in *S. cerevisiae* as a strategy to reduce ethanol-induced stress environments (Lam et al., 2014).

Regarding the pH values reported in our work, they are similar to those in previous studies and remained within the optimal pH range for *S. cerevisiae* growth (Lin et al., 2012; Liu et al., 2015). For example, Lin et al. (2012) reported that when *S. cerevisiae* BY4742 was grown anaerobically at pH 5, 62% conversion of the carbon source into ethanol was obtained. It is known that inappropriate pH values in *S. cerevisiae* cultures can negatively affect biomass production, specific growth rate, and carbon source consumption (Liu et al., 2015). On the other hand, the increases in titratable acidity percentage observed in our results may be associated with the production and accumulation of non-volatile organic acids in the medium (Thoukis et al., 1965).

The volumetric productivity values obtained in our study are similar and within the range reported in the literature (Laopaiboon et al., 2007; Techaparin et al., 2017). However, it is important to highlight that Laopaiboon et al. (2007) used a culture medium based on sorghum juice for *S. cerevisiae* growth. While it is true that complex media, such as saccharified sorghum liquors, contain various fermentable sugars that favor ethanol production and, consequently, increase volumetric productivity and are less expensive (Wu et al., 2010), they also have the disadvantage of possessing a complex and variable chemical composition, which becomes a challenge for reproducing results. Therefore, it is better to seek strategies that lead to the design of defined media with low-cost nutrient sources.

Furthermore, it is known that *S. cerevisiae* cell viability is affected by ethanol concentration, meaning that growth inhibition occurs due to the product synthesized by the cell itself (Casey & Ingledew, 1985). In our study, it was demonstrated that cell viability was also affected by ethanol concentration, especially when 1.50 $g \cdot L^{-1}$ potassium phosphate was used in the medium. Nevertheless, the results showed that increasing potassium phosphate concentration improved *S. cerevisiae* tolerance up to an ethanol concentration of 16% (v/v) (Fig. 4). Our cell viability results at high ethanol concentrations are close to those reported by Alfenore et al. (2002), who indicated viability values above 90% at approximately 15% (v/v) ethanol (115 g·L^{-1}) when the culture medium was exponentially fed with a vitamin solution. Similarly, other studies have also reported yeast survival in ethanol ranges from 10 to 20% (Jacobus et al., 2021; Lairón-Peris et al., 2021). Conversely, it is known that ethanol accumulation in the medium creates a stressful condition for the cells affecting viability, as it decreases membrane thickness, affecting its permeability and nutrient uptake (Sahana et al., 2024). Additionally, ethanol entry into the cytoplasm alters cytochrome and produces reactive oxygen species (ROS) (Burphan et al., 2018). Lam et al. (2014) proposed the hypothesis that an increase in extracellular potassium, combined with elevated pH, physically reinforces the opposing electrochemical gradients of potassium ions (K^+) and protons (H^+) across the membrane by decreasing ion leakage rates, which constitutes a resistance mechanism against various alcohols. Given that pronounced gradients of K^+ and H^+ ions are generated across the yeast plasma membrane by the K^+ importer TRK1 and the H^+ exporter, these ATP-dependent pumps are designed to modify or strengthen such gradients (Lam et al., 2014).

Rising ethanol concentrations disrupt these gradients by permeabilizing the membrane and increasing ion leakage. However, high concentrations of potassium help maintain the gradients by reducing ion leakage rates, thus allowing the transporters to pump against a differential (Vamvakas & Kapolos, 2020). Therefore, the ethanol threshold concentration is overcome by the increase in these gradients, allowing the cells to maintain viability at higher toxicity levels. In other words, K^+ and H^+ ions contribute to maintaining osmotic balance and membrane potential (Barreto et al., 2012; Ribeiro-Filho et al., 2022). Additionally, it has been reported that increased potassium availability in the medium reduces oxidative stress, as potassium is critical for the expression of genes (CTT1, TSA2, and SOD2) that encode proteins with oxidoreductase activity (Barreto et al., 2012). This imbalance between free radicals and the cell's ability to neutralize them severely impairs biogenesis, affecting growth, viability, and cellular productivity (Barreto et al., 2012). This may help explain the relationship between the increased potassium phosphate and the enhanced cell viability in response to ethanol tolerance observed in our study (Fig. 4).

Finally, the findings of this study demonstrate clear industrial potential for the use of K_2HPO_4 as a strategic nutrient in bioethanol fermentations. Increasing its concentration from 1.50 to 3.20 g·L^{-1} significantly enhanced ethanol production, doubling the final ethanol concentration (from ~8% to ~16% v/v), increasing volumetric productivity (Q_p), and maintaining cell viability above 90% even at elevated ethanol levels. This indicates improved tolerance to ethanol-induced stress, a critical factor in industrial processes where yeast inhibition frequently limits overall performance. These results are particularly relevant for the design of large-scale fermentative bioprocesses, as they suggest enhanced yeast metabolic efficiency and prolonged productive capacity throughout fermentation cycles. From an economic perspective, potassium phosphate is a relatively inexpensive and widely available nutrient source that is compatible with industrial media formulations. Therefore, the observed improvements in ethanol yield, productivity, and fermentation robustness may offset the additional cost associated with supplementation. Collectively, these findings suggest that K_2HPO_4 supplementation at optimized concentrations could improve process efficiency and scalability. Nevertheless, pilot-scale validation and comprehensive techno-economic assessment are recommended to confirm industrial feasibility under continuous or fed-batch fermentation systems.

Despite the limitations of the study, such as the low number of biological replicates, which may reduce the statistical power of the analyses, the use of a colorimetric method for ethanol quantification, and the lack of genetic characterization of the *S. cerevisiae* strain, the results provide valuable evidence on the role of K_2HPO_4 in enhancing ethanol tolerance and fermentative productivity and offer practical insights for optimizing industrial fermentation processes, as well as suggesting future research directions that include larger sample sizes to more rigorously evaluate the assumptions underlying ANOVA, in addition to molecular strain characterization and the use of more specific analytical methods.

5. Conclusions

The adjustment of inorganic nutrients such as potassium phosphate in the culture medium represents a promising biotechnological strategy to improve both ethanol volumetric productivity and cell viability during alcoholic fermentation. Since volumetric productivity depends on the rate of ethanol synthesis and the number of metabolically active cells, maintaining high yeast viability under ethanol stress is essential for efficient fermentation processes. The results of this study demonstrate that doubling the concentration of potassium phosphate increased ethanol production while maintaining high percentages of viable *Saccharomyces cerevisiae* cells, suggesting an improvement in ethanol tolerance mediated by nutritional optimization of the culture medium. From an applied perspective, this strategy could be implemented in industrial fermentation systems, such as large-scale bioethanol production or beverage fermentations, where adjusting potassium levels in the fermentation medium may help sustain yeast activity under stressful conditions and enhance overall process productivity. These findings highlight the potential of nutrient management as a practical tool for process optimization and provide a basis for future studies aimed at validating this strategy at pilot and industrial scales.

Acknowledgements

The authors thank the staff of the Biotechnology and Microbiology Laboratory at the Universidad Nacional de Agricultura (UNAG) for their collaboration in conducting this research. Special thanks are also due to Professor Maria José Talavera for her assistance in translating the manuscript.

Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author Contribution Statement

	FE Sánchez-Rosales	KM Castellanos-Reyes	A Vásquez	N Herrera	HL Sanabria-Ortega	WA Martinez	J Betancourth
Conceptualization				■	■		
Formal analysis		■		■	■		
Funding acquisition	■						
Investigation			■				
Methodology			■				
Project administration	■						
Resources				■			
Software		■					
Supervision	■						
Validation				■			
Visualization		■			■		
Writing – original draft		■				■	■
Writing – review and editing	■						

References

Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C., Uribelarrea, J. L., Goma, G., Molina-Jouve, C., & Guillouet, S. E. (2004). Aeration strategy: A need for very high ethanol performance in Saccharomyces cerevisiae fed-batch process. *Applied Microbiology and Biotechnology*, *63*(5), 537-542. https://doi.org/10.1007/s00253-003-1393-5

Alfenore, S., Molina-Jouve, C., Guillouet, S. E., Uribelarrea, J. L., Goma, G., & Benbadis, L. (2002). Improving ethanol production and viability of Saccharomyces cerevisiae by a vitamin feeding strategy during fed-batch process. *Applied microbiology and biotechnology*, *60*(1-2), 67-72. https://doi.org/10.1007/s00253-002-1092-7

Alminderej, F. M., Hamden, Z., El-Ghoul, Y., Hammami, B., Saleh, S. M., & Majdoub, H. (2022). Impact of calcium and nitrogen addition on bioethanol production by *S. cerevisiae* fermentation from date by-products: Physicochemical characterization and technical design. *Fermentation*, *8*(11), Article 583. https://doi.org/10.3390/fermentation8110583

Álvarez, R., Garces, F., Louis, E. J., Dequin, S., & Camarasa, C. (2023). Beyond *S. cerevisiae* for winemaking: Fermentation-related trait diversity in the genus Saccharomyces. *Food Microbiology*, *113*, Article 104270. https://doi.org/10.1016/j.fm.2023.104270

Azbekyan, G. S., Shirvanyan, A. H., & Trchounian, K. A. (2025). Inhibitory effect of acetic acid on the fermentative metabolism in *Saccharomyces cerevisiae* ATCC 9804 at pH 3.0. *Journal of Innovative Solutions for Eco-Environmental Sustainability*, (SI 1), Article 188. https://doi.org/10.46991/JISEES.2025.SI1.188

Barreto, L., Canadell, D., Valverde-Saubí, D., Casamayor, A., & Ariño, J. (2012). The short-term response of yeast to potassium starvation. *Environmental Microbiology*, *14*(11), 3026-3042. https://doi.org/10.1111/j.1462-2920.2012.02887.x

Bennett, C. (1971). Spectrophotometric acid dichromate method for the determination of ethyl alcohol. *The American Journal of Medical Technology*, *37*(6), 217-220.

Biswas, B., & Biswas, A. B. (2022). Effects of some chemical nutrients on bioethanol production from water hyacinth (*Eichhornia crassipes*) hydrolyzed by heat- and ethanol-resistant strain of *Saccharomyces cerevisiae* AB_{810}. *Journal of the Indian Chemical Society*, *99*(10), Article 100725. https://doi.org/10.1016/j.jics.2022.100725

Breisha, G. Z. (2010). Production of 16% ethanol from 35% sucrose. *Biomass and Bioenergy*, *34*(8), 1243-1249. https://doi.org/10.1016/j.biombioe.2010.03.017

Burphan, T., Tatip, S., Limcharoensuk, T., Kangboonruang, K., Boonchird, C., & Auesukaree, C. (2018). Enhancement of ethanol production in very high gravity fermentation by reducing fermentation-induced oxidative stress in *Saccharomyces cerevisiae*. *Scientific Reports*, *8*(1), Article 13069. https://doi.org/10.1038/s41598-018-31558-4

Canadell, D., González, A., Casado, C., & Ariño, J. (2015). Functional interactions between potassium and phosphate homeostasis in *Saccharomyces cerevisiae*. *Molecular Microbiology*, *95*(3), 555-572. https://doi.org/10.1111/mmi.12886

Casey, G. P., & Ingledew, W. M. (1985). Reevaluation of alcohol synthesis and tolerance in brewer's yeast. *Journal of the American Society of Brewing Chemists*, *43*(2), 75-83. https://doi.org/10.1094/ASBCJ-43-0075

Coleman, M. C., Fish, R., & Block, D. E. (2007). Temperature-dependent kinetic model for nitrogen-limited wine fermentations. *Applied and Environmental Microbiology*, *73*(18), 5875-5884. https://doi.org/10.1128/AEM.00670-07

Díaz-Barrera, A., Sanchez-Rosales, F., Padilla-Córdova, C., Andler, R., & Peña, C. (2021). Molecular weight and guluronic/mannuronic ratio of alginate produced by Azotobacter vinelandii at two bioreactor scales under diazotrophic conditions. *Bioprocess and Biosystems Engineering*, *44*(6), 1275-1287. https://doi.org/10.1007/s00449-021-02532-8

Eliodório, K. P., Cunha, G. C. G. E., Lino, F. S. O., Sommer, M. O. A., Gombert, A. K., Giudici, R., & Basso, T. O. (2023). Physiology of Saccharomyces cerevisiae during growth on industrial sugar cane molasses can be reproduced in a tailor-made defined synthetic medium. *Scientific Reports*, *13*(1), Article 10567. https://doi.org/10.1038/s41598-023-37618-8

He, X., Guo, X., Liu, N., & Zhang, B. (2007). Ergosterol production from molasses by genetically modified *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, *75*(1), 55-60. https://doi.org/10.1007/s00253-006-0807-6

Henderson, C. M., & Block, D. E. (2014). Examining the role of membrane lipid composition in determining the ethanol tolerance of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, *80*(10), 2966-2972. https://doi.org/10.1128/AEM.04151-13

Jacobus, A. P., Gross, J., Evans, J. H., Ceccato-Antonini, S. R., & Gombert, A. K. (2021). *Saccharomyces cerevisiae* strains used industrially for bioethanol production. *Essays in Biochemistry*, *65*(2), 147-161. https://doi.org/10.1042/EBC20200160

Kucharczyk, K., Żyła, K., & Tuszyński, T. (2025). Optimization of fermentation parameters in a brewery: Modulation of yeast growth and yeast cell viability. *Processes*, *13*(3), Article 906. https://doi.org/10.3390/pr13030906

Kwolek-Mirek, M., & Zadrag-Tecza, R. (2014). Comparison of methods used for assessing the viability and vitality of yeast cells. *FEMS Yeast Research*, *14*(7), 1068-1079. https://doi.org/10.1111/1567-1364.12202

Lairón-Peris, M., Routledge, S. J., Linney, J. A., Alonso-Del-Real, J., Spickett, C. M., Pitt, A. R., Guillamón, J. M., Barrio, E., Goddard, A. D., & Querol, A. (2021). Lipid composition analysis reveals mechanisms of ethanol tolerance in the model yeast *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, *87*(12), Article e0044021. https://doi.org/10.1128/AEM.00440-21

Laluce, C., Tognolli, J. O., de Oliveira, K. F., Souza, C. S., & Morais, M. R. (2009). Optimization of temperature, sugar concentration, and inoculum size to maximize ethanol production without significant decrease in yeast cell viability. *Applied Microbiology and Biotechnology*, *83*(4), 627-637. https://doi.org/10.1007/s00253-009-1885-z

Lam, F. H., Ghaderi, A., Fink, G. R., & Stephanopoulos, G. (2014). Engineering alcohol tolerance in yeast. *Science*, *346*(6205), 71-75. https://doi.org/10.1126/science.1257859

Laopaiboon, L., Thanonkeo, P., Jaisil, P., & Laopaiboon, P. (2007). Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnology*, *23*(10), 1497-1501. https://doi.org/10.1007/s11274-007-9383-x

Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S., & Kong, H. (2012). Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. *Biomass and Bioenergy*, *47*, 395-401. https://doi.org/10.1016/j.biombioe.2012.09.019

Liu, X., Jia, B., Sun, X., Ai, J., Wang, L., Wang, C., Zhao, F., Zhan, J., & Huang, W. (2015). Effect of initial pH on growth characteristics and fermentation properties of *Saccharomyces cerevisiae*. *Journal of Food Science*, *80*(4), M800-M808. https://doi.org/10.1111/1750-3841.12813

Mohd Azhar, S. H., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Mohd Faik, A. A., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports*, *10*, 52-61. https://doi.org/10.1016/j.bbrep.2017.03.003

Molinet, J., & Cubillos, F. A. (2020). Wild yeast for the future: Exploring the use of wild strains for wine and beer fermentation. *Frontiers in Genetics*, *11*, Article 589350. https://doi.org/10.3389/fgene.2020.589350

Napitupulu, A. M. M., Suhendra, L., & Gunam, I. B. W. (2021). Effect of Saccharomyces cerevisiae ATCC 9763 concentration and fermentation time on bioethanol content from corn stover crude cellulose substrate. *IOP Conference Series: Earth and Environmental Science*, *913*, Article 012026. https://doi.org/10.1088/1755-1315/913/1/012026

Postigo, V., García, M., Cabellos, J. M., & Arroyo, T. (2021). Wine *Saccharomyces* yeasts for beer fermentation. *Fermentation*, *7*(4), Article 290. https://doi.org/10.3390/fermentation7040290

Ribeiro-Filho, N., Linforth, R., Bora, N., Powell, C. D., & Fisk, I. D. (2022). The role of inorganic phosphate, potassium, and magnesium in yeast flavour formation. *Food Research International*, *162*(Pt A), Article 112044. https://doi.org/10.1016/j.foodres.2022.112044

Rojo, M. C., Talia, P. M., Lerena, M. C., Ponsone, M. L., Gonzalez, M. L., Becerra, L. M., Mercado, L. A., Martín-Arranz, V., Rodríguez-Gómez, F., Arroyo-López, F. N., & Combina, M. (2023). Evaluation of different nitrogen sources on growth and fermentation performance for enhancing ethanol production by wine yeasts. *Heliyon*, *9*(12), Article e22608. https://doi.org/10.1016/j.heliyon.2023.e22608

Sahana, G. R., Balasubramanian, B., Joseph, K. S., Pappuswamy, M., Liu, W.-C., Meyyazhagan, A., Kamyab, H., Chelliapan, S., & Joseph, B. V. (2024). A review on ethanol tolerance mechanisms in yeast: Current knowledge in biotechnological applications and future directions. *Process Biochemistry*, *138*, 1-13. https://doi.org/10.1016/j.procbio.2023.12.024

Seo, H.-B., Kim, H.-J., Lee, O.-K., Ha, J.-H., Lee, H.-Y., & Jung, K.-H. (2009). Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process. *Journal of Industrial Microbiology & Biotechnology*, *36*(2), 285-292. https://doi.org/10.1007/s10295-008-0497-4

Sun, Q., Ma, J., Basit, R. A., Fu, Z., Liu, X., & Fan, G. (2024). Screening of a *Saccharomyces cerevisiae* strain with high 3-methylthio-1-propanol yield and optimization of its fermentation conditions. *Foods (Basel)*, *13*(9), Article 1296. https://doi.org/10.3390/foods13091296

Sun, X., Zhou, X., Yu, R., Zhou, X., Zhang, J., Xu, T., Wang, J., Li, M., Li, X., Zhang, M., Xu, J., & Zhang, J. (2025). Assessing the physiological properties of baker's yeast based on single-cell Raman spectrum technology. *Synthetic and Systems Biotechnology*, *10*(1), 110-118. https://doi.org/10.1016/j.synbio.2024.09.004

Tan, L., Zhang, Y., Liu, P., Wu, Y., Huang, Z., Hu, Z., Liu, Z., Wang, Y., & Zheng, Y. (2025). System metabolic engineering modification of *Saccharomyces cerevisiae* to increase SAM production. *Bioresources and Bioprocessing*, *12*(1), Article 19. https://doi.org/10.1186/s40643-025-00858-9

Techaparin, A., Thanonkeo, P., & Klanrit, P. (2017). High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. *Brazilian Journal of Microbiology*, *48*(3), 461-475. https://doi.org/10.1016/j.bjm.2017.01.006

Thoukis, G., Ueda, M., & Wright, D. (1965). The formation of succinic acid during alcoholic fermentation. *American Journal of Enology and Viticulture*, *16*(1), 1-8. https://doi.org/10.5344/ajev.1965.16.1.1

Vamvakas, S. S., & Kapolos, J. (2020). Factors affecting yeast ethanol tolerance and fermentation efficiency. *World Journal of Microbiology & Biotechnology*, *36*(8), Article 114. https://doi.org/10.1007/s11274-020-02881-8

Varize, C. S., Bücker, A., Lopes, L. D., Christofoleti-Furlan, R. M., Raposo, M. S., Basso, L. C., & Stambuk, B. U. (2022). Increasing ethanol tolerance and ethanol production in an industrial fuel ethanol *Saccharomyces cerevisiae* strain. *Fermentation*, *8*(10), Article 470. https://doi.org/10.3390/fermentation8100470

Walker, G., & Stewart, G. (2016). *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages*, *2*(4), Article 30. https://doi.org/10.3390/beverages2040030

Woo, J.-M., Yang, K.-M., Kim, S.-U., Blank, L.-M., & Park, J.-B. (2014). High temperature stimulates acetic acid accumulation and enhances growth inhibition and ethanol production by *Saccharomyces cerevisiae* under fermenting conditions. *Applied Microbiology and Biotechnology*, *98*(13), 6085-6094. https://doi.org/10.1007/s00253-014-5691-x

Wu, X., Staggenborg, S., Propheter, J. L., Rooney, W. L., Yu, J., & Wang, D. (2010). Features of sweet sorghum juice and their performance in ethanol fermentation. *Industrial Crops and Products*, *31*(1), 164-170. https://doi.org/10.1016/j.indcrop.2009.10.006

Zhao, F., Zhang, Y., Hu, J., Shi, C., Ao, X., Wang, S., Lin, Y., Sun, Z., & Han, S. (2023). Disruption of phosphate metabolism and sterol transport-related genes conferring yeast resistance to vanillin and rapid ethanol production. *Bioresource Technology*, *369*, Article 128489. https://doi.org/10.1016/j.biortech.2022.128489