Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Encapsulation in ZIF-8 as a Biopreservation Strategy and Enhancement of Thermal Tolerance of Saccharomyces cerevisiae

Luana Cristina Camargos Gomes
Federal University of Goiás
Gabriel Luis Castiglioni
Federal University of Goiás
Carlos Alberto Galeano Suarez
Federal University of Goiás
Caridad Noda Perez
carynoda60@ufg.br

Federal University of Goiás

Research Article

Keywords: Saccharomyces cerevisiae, immobilization, ZIF-8, thermal stability, viability, fermentation

Posted Date: March 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8926210/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

This study evaluated ZIF-8 as an immobilization matrix for *Saccharomyces cerevisiae* CAT-1 to determine its effectiveness as a biopreservation strategy for enhancing robustness and performance in ethanol fermentation under thermal and chemical stress. Encapsulated and free cells were compared in sequential batch fermentations to assess operational stability and reusability. Thermal tolerance assays were conducted in YPD medium at 25–50°C, and protective performance was further examined under inhibitory conditions representative of lignocellulosic hydrolysates derived from passion fruit peel. Biomass formation, cell viability, glucose consumption, substrate-to-product conversion yield, volumetric productivity, and fermentation efficiency were evaluated. ZIF-8 encapsulation significantly enhanced thermotolerance and resistance to inhibitory compounds. Encapsulated cells maintained consistently higher viability across the tested temperature range, reaching 96.7% at 50°C compared to 82.9% for free cells. Within the mesophilic range (25–35°C), immobilized cultures demonstrated superior fermentative performance, achieving fermentation efficiency above 93% at 30°C, whereas free cells reached 78.86%. Under hydrolysate stress, encapsulated cells preserved 99.42% viability after 48 h and retained metabolic activity in a subsequent fermentation cycle. Overall, ZIF-8 immobilization improved cellular stability and process resilience, supporting its application in intensified, inhibitor-rich bioprocesses for industrial ethanol production.

Introduction

The use of microorganisms to produce industrially valuable compounds represents a highly relevant biotechnological strategy (Taylor, 2001). For such processes to be effective, factors such as high yield, productivity, operational stability, thermal resistance, and other parameters that ensure process viability and efficiency must be considered. Among the various applications, alcoholic fermentation stands out, with yeasts being the most widely employed microorganisms. *Saccharomyces cerevisiae* is the predominant species used due to its high fermentation efficiency, adaptability to different environments, and robustness under varying process conditions (Lima et al., 2001).

The utilization of agro-industrial residues represents a strategic source of raw materials for multiple industrial sectors. There is an increasing demand for high-quality products and improved production efficiency, combined with the adoption of practices that promote environmental protection and process sustainability. In this context, waste reuse offers significant economic and ecological potential (Velvizhi et al., 2022). Residues generated from passion fruit processing, particularly the peels, are promising feedstocks for biorefineries, especially for ethanol production. These materials are rich in lignocellulosic sugars and represent one of the most advantageous technological routes for the sustainable production of biofuels (Oliveira, 2018). Several factors can influence fermentative performance, with fermentation efficiency being a key parameter. Fermentation efficiency is defined as the percentage of sugar converted into ethanol relative to the theoretical maximum predicted by the Gay-Lussac equation (Fermentec, 1978).

Efficient conversion of lignocellulosic biomass into bioethanol involves three main steps: pretreatment, enzymatic hydrolysis, and fermentation. However, during the hydrolysis stage, a major challenge is the formation of byproducts resulting from the degradation of sugars and lignin. Many of these compounds are toxic to fermentative microorganisms and negatively affect their metabolism (Chandel et al., 2007). In general, the fermentation of lignocellulosic hydrolysates without prior detoxification is associated with slow fermentation kinetics, as well as reduced yields and productivities (Mussatto & Roberto, 2004).

Among the key factors influencing alcoholic fermentation, temperature plays a fundamental role, as it directly affects microbial metabolic activity and the formation of volatile compounds that impact the quality of the final product (Lima, 2009). Moreover, ethanol production is closely associated with fermentation temperature, since carbon dioxide release during fermentation is accompanied by heat generation, which can favor alcohol formation (Mallouchos, 2003). However, elevated temperatures may act as stress factors, impairing yeast growth and viability, reducing ethanol production

efficiency, promoting the proliferation of contaminating bacteria, and increasing yeast susceptibility to the toxic effects of ethanol accumulated in the culture medium (Reis & Ribeiro, 2009; Shen et al., 2020).

Ethanol toxicity in yeast becomes more pronounced at higher temperatures due to increased membrane fluidity, which facilitates the influx of toxic compounds into the cell (Phisalaphong et al., 2006; Fernandes, 2008). In response to elevated temperatures, yeast cells modify the fatty acid composition of their cell membranes as an adaptive mechanism (Wanderley, 1997). According to Amorim et al. (1996), the toxic effects of ethanol on *Saccharomyces cerevisiae* remain relatively constant within the temperature range of 12 to 28°C but intensify at higher temperatures. Therefore, establishing an optimal temperature range prior to fermentation is essential to ensure suitable conditions for microbial growth and to maximize ethanol yield (Ballesteros et al., 2004; Phisalaphong et al., 2006).

For ethanol production, the optimal temperature range generally lies between 26 and 35°C. Cell viability declines at temperatures above 35°C, as these conditions trigger a thermal stress response in yeast cells and disrupt normal physiological functions, including carbohydrate flux and membrane composition (Caspeta et al., 2014). At lower temperatures, higher ethanol yields may be achieved; however, overall productivity tends to decrease due to prolonged fermentation times (Lima et al., 2001; Torija et al., 2003). During fermentation, several stress factors—such as elevated temperature, toxic hydrolysates, high ethanol concentrations, and osmotic pressure—can negatively impact process efficiency (Patrascu et al., 2009). Lin et al. (2012) reported that the highest cell growth rates and ethanol productivity for the *Saccharomyces cerevisiae* BY4742 strain were observed between 30 and 45°C. However, at 50°C, a marked decline in both cell growth and ethanol yield was detected.

Industrial strains of *Saccharomyces cerevisiae* exhibit a certain degree of tolerance to elevated temperatures. However, this tolerance can be compromised under conditions of high ethanol concentration or low pH, negatively affecting cell viability (Reis & Ribeiro, 2009). Yeasts are classified as mesophilic microorganisms, and in industrial practice, the optimal temperature range typically lies between 30 and 35°C (Caldas et al., 2012). In industrial fermentation processes, yeast immobilization is widely applied in the production of beer, wine, and cider. This technological strategy involves encapsulating intact and metabolically active yeast cells within a defined matrix, thereby prolonging metabolite production, including aromatic compounds. In this context, metal–organic frameworks (MOFs) and their derivatives, such as zeolitic imidazolate frameworks (ZIFs), have emerged as promising materials for yeast immobilization. These systems may enhance cellular stability and strain control, while also enabling cell recovery and reuse, facilitating continuous fermentation processes, and supporting other technological advancements (Nedović et al., 2014).

The wide variety of substrates available for MOF synthesis, along with the broad range of possible topologies, enable these materials to be tailored for specific applications (Rogacka and Labus, 2025). MOFs have been applied in the chemical, food, pharmaceutical, and environmental sectors, serving as materials for the capture of toxic industrial chemicals (Islamoglu et al., 2020), gas storage (Ghanbari et al., 2020), chemical catalysis (Gascon et al., 2014), drug delivery systems (Horcajada et al., 2006, 2008), and the immobilization of enzymes and other biocompounds (Rogacka and Labus, 2025). MOFs can be modified through the introduction of different functional groups into their ligands (Deria et al., 2014), by structural modification via ligand exchange or incorporation of new ligands (Tanabe & Cohen, 2011), or by increasing the inertness of the central metal ion. The thermal stability of a MOF depends largely on the strength of the bond between the metal center and the ligand oxygen; as this bond strength increases, so does the material's thermal stability (Tanabe & Cohen, 2011).

Among the various MOF families, zeolitic imidazolate frameworks (ZIFs) are particularly notable for their high porosity, well-defined cavity size, strong interactions with organic polymers, exceptional thermal stability, and outstanding chemical resistance. These features make them especially suitable for addressing key challenges in fermentation processes, such as temperature fluctuations and the presence of toxic compounds (Yang and Chung, 2013).

Therefore, ZIF-8 can be regarded as a promising protective matrix capable of limiting the diffusion of denaturing agents and dissipating heat gradients within the cellular microenvironment. This stress-mitigating effect may contribute to preserving the structural integrity of the cell membrane and organelles, maintaining intracellular homeostasis, and ultimately improving kinetic parameters and overall fermentation productivity. Accordingly, this study proposed the use of ZIF-8 for the immobilization of *S. cerevisiae* cells to evaluate its potential as a biopreservative agent under conditions of thermal stress and in the presence of inhibitory compounds derived from lignocellulosic hydrolysates. The effects of this strategy on cell viability and overall fermentative performance were also investigated.

Despite the well-recognized importance of *S. cerevisiae* and immobilization strategies in enhancing fermentative processes, there remains a need to investigate support materials capable of providing more robust and efficient protection against thermal stress and inhibitory compounds. Such protection is essential to maintain cell viability and alcoholic fermentation performance under elevated temperature conditions. In this context, zeolitic imidazolate frameworks, such as ZIF-8, have emerged as promising candidates. The novelty of this work lies in the in-depth evaluation of ZIF-8 both as an immobilization support and as a biopreservative agent for *S. cerevisiae* cells. This study assessed the capacity of ZIF-8 to enhance cellular protection and optimize fermentative performance over an extended temperature range. Additionally, the efficiency of encapsulation was examined in the presence of inhibitory compounds found in lignocellulosic biomass fermentations derived from passion fruit peel, which may exert toxic effects on yeast cells.

Materials and Methods

MEV/EDS analysis

Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) analyses were performed using a HITACHI TM3030Plus microscope. Yeast samples were vacuum-dried, mounted on aluminum stubs with conductive carbon tape, and placed in the microscope's vacuum chamber. After adjusting the operating conditions, high-resolution images were captured at various magnifications. The analyses were performed at CAITEC, Universidade Estadual de Goiás.

Microorganisms

The microorganism used in this study was the *Saccharomyces cerevisiae* CAT-1 strain. The yeast was supplied by the Biochemical Engineering Laboratory of the Universidade Federal de Goiás. The strain was maintained on solid YPD medium composed of 10 g L^{-1} yeast extract, 20 g L^{-1} glucose, 20 g L^{-1} bacteriological peptone, and 20 g L^{-1} agar.

Inoculum Preparation

Cells were aseptically removed from YPD agar plates using a sterile inoculation loop and transferred into Erlenmeyer flasks containing 17.5 mL of liquid YPD medium composed of 10 g L^{-1} yeast extract, 20 g L^{-1} glucose, and 20 g L^{-1} bacteriological peptone. The pH was adjusted to 5.5. Subsequently, 2.5 mL of a concentrated glucose solution (100 g L^{-1}) was added to the medium. The culture medium was sterilized by autoclaving at 121°C and then incubated at 30°C under agitation at 250 rpm for 12 hours, until the exponential growth phase was reached.

ZIF 8 Preparation and Cell Encapsulation

Yeast immobilization by encapsulation in ZIF-8 capsules was carried out according to the method described by Sun et al. (2019). After inoculum preparation, yeast cells corresponding to 4.5 mg of dry biomass were harvested from the YPD medium by centrifugation and washed three times with deionized water at 3000 rpm for 5 minutes each. The washed cells were then resuspended in 1 mL of an aqueous zinc nitrate solution (0.05 mol L^{-1}), followed by the addition of 1 mL of an aqueous 2-methylimidazole solution (0.2 mol L^{-1}). The reaction mixture was maintained under orbital agitation at 300 rpm and 30°C for 1 hour. Finally, the resulting encapsulated cells were collected and washed three times with deionized water.

Ethanolic Fermentation with Free and Immobilized Cells at Different Temperatures

Encapsulated yeast cells were cultivated under anaerobic conditions in a nutrient medium consisting of 17.5 mL of YPD medium supplemented with 2.5 mL of a concentrated glucose solution (100 g L^{-1}). The initial pH was adjusted to 5.5. Cultivation was performed in Erlenmeyer flasks under agitation at 250 rpm for 24 hours at different temperatures: 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C. Samples were collected at predetermined time intervals, appropriately diluted, and analyzed to monitor metabolite production and substrate consumption.

Ethanolic Fermentation with Free and Immobilized Yeast Cells Using Lignocellulosic Biomass Derived from Passion Fruit Peel

The lignocellulosic biomass underwent acid pretreatment, enzymatic hydrolysis, and fermentation. Pretreatment was performed with 2% (v/v) sulfuric acid at a 1:10 solid-to-liquid ratio and autoclaved at 121°C. After cooling, the pH was adjusted to 4.8 with citrate buffer. The suspension was filtered through cotton cloth, and the recovered solid fraction was dried in a vacuum oven at 50°C for 16 hours, reaching 7.71% final moisture content.

Enzymatic hydrolysis was performed in three 250 mL Erlenmeyer flasks containing dried passion fruit peel (5.4 g), citrate buffer (45 mL, pH 4.8), and diluted enzyme solution (5 mL). The flasks were incubated at 50°C and 250 rpm for 24 hours, with samples collected between 0 and 24 hours. After centrifugation at 3000 rpm for 5 minutes, the supernatant was recovered for glucose determination.

After hydrolysis, the slurry was filtered through cotton cloth to remove solids. The liquid hydrolysate (~ 120 mL) was concentrated to 20 mL in a rotary evaporator, adjusted to pH 5.5 with citrate buffer, and sterilized at 121°C. The resulting acid–enzyme hydrolysate was used as the substrate for ethanol production with free and immobilized *Saccharomyces cerevisiae* CAT-1 cells.

Fermentations were performed in 11 mL glass flasks containing 9 mL of hydrolysate at 30°C without agitation. Free yeast fermentation lasted 24 hours, with samples collected at 12 and 24 hours. Immobilized yeast fermentations were conducted for 48 hours, with samples taken at 24 and 48 hours for analysis.

After 48 hours, immobilized cells were recovered by centrifugation and washed four times with deionized water (3000 rpm, 5 min). Fresh YPD medium (10 g L^{-1} yeast extract, 80 g L^{-1} glucose, and 20 g L^{-1} peptone) was added, the pH adjusted to 5.5, and the volume set to 9 mL to start a new fermentation cycle. This step evaluated cell viability and metabolic activity over an additional 30 hours, as well as the effectiveness of encapsulation after exposure to inhibitory compounds from passion fruit peel hydrolysate.

Validation of Cell Viability

To validate the viability assessment method, free yeast cells were treated with 25% NaCl for 24 hours to induce cell death, which was confirmed using a Neubauer counting chamber. These nonviable cells were then subjected to the immobilization process, and viability was reassessed using methylene blue staining to ensure they remained nonviable. This approach confirmed that ZIF-8 encapsulation did not interfere with the accuracy of viability determination.

Cell concentration

Cell biomass concentration was determined using a calibration curve established by correlating dry cell mass with absorbance at 600 nm. Eq. (1), described by Santos (2017), was applied for the CAT-1 strain:

$$(X_{CAT-1} = 0.3379.ABS - 0.0266) \quad (1)$$

Cell viability

Cell viability was determined using a Neubauer counting chamber and the methylene blue staining method (0.025%). Viable cells do not absorb the dye, whereas nonviable cells appear blue. Samples were diluted in the methylene blue solution, and a 10 µL aliquot was transferred to the Neubauer chamber for accurate cell counting. Live and dead cells were counted under an optical microscope at 40× magnification. Cell viability was calculated according to Eq. (2), as described by Ceccato-Antonini (2012).

$$Viabilidade\ celular\ (\%) = \frac{número\ de\ células\ viáveis}{número\ de\ células\ contadas} \quad (2)$$

Glucose and ethanol concentration

Glucose and ethanol levels were quantified by HPLC. Samples were centrifuged (1075 × g, 2 min), and the supernatant was diluted and filtered through a 0.22 µm nylon membrane before analysis. Separation was performed on a Shimadzu Prominence system equipped with an SCR-102HG Shim-pack column and pre-column. Compounds were detected using refractive index (RID-20A) and UV–Vis (SPD-20A) detectors. The column temperature was set at 50°C, and 5 mM perchloric acid was used as the mobile phase at a flow rate of 0.600 mL min⁻¹.

Fermentation kinetic parameters

The substrate-to-product conversion factor (Y□/□), volumetric productivity (Q□), and fermentation efficiency (η) were calculated according to Equations (3), (4), and (5), respectively.

$$Y_{p/s} = \frac{Pf - Po}{So - Sf}$$

3

$$Q_p = \frac{Pf - Po}{tf} \quad (4)$$

$$\eta = \frac{Y_{p/s}}{0{,}511}\, x\, 100 \quad (5)$$

where Y□/□ (g ethanol·g substrate⁻¹) represents the substrate-to-product conversion factor; S_0 (g substrate·L⁻¹) is the initial substrate concentration; S_f (g substrate·L⁻¹) is the final substrate concentration; P_f (g ethanol·L⁻¹) is the final

ethanol concentration; P_0 (g ethanol·L^{-1}) is the initial ethanol concentration; Q (g ethanol·L^{-1}·h^{-1}) is the volumetric productivity; t_f (h) is the total fermentation time; and η (%) corresponds to the fermentation efficiency.

Results and discussion

The results allowed to evaluate the performance of free and ZIF-8-immobilized yeasts under different fermentation conditions. The samples were collected at predetermined intervals, and the analyses were performed in triplicate. Cell growth, viability, glucose consumption, substrate-to-product conversion, ethanol yield, and fermentation efficiency were assessed for both free and immobilized yeast cultures.

Morphology and composition of ZIF-8 encapsulated cells (SEM/EDS)

Figure 1a displays the typical ellipsoidal morphology of free *Saccharomyces cerevisiae* cells, with visible budding scars characteristic of asexual reproduction. In contrast, Fig. 1b shows successful encapsulation with a ZIF-8 layer, forming a core–shell structure composed of yeast cells coated with ZIF-8. The micrographs reveal a uniform coating, with no partial or uncoated cells, indicating high encapsulation homogeneity. Morphological analysis identified polyhedral particles with a predominantly cubic tendency, including near-cubic and irregular shapes (Fig. 1c).

Furthermore, chemical mapping demonstrated a homogeneous distribution of zinc, oxygen, and carbon on the cell surface, confirming the presence of a continuous ZIF-8 layer surrounding individual yeast cells (Figs. 2–4). These results confirm the effective immobilization of *S. cerevisiae* in ZIF-8, yielding core–shell biohybrid structures with high structural integrity and surface uniformity.

Effect of Temperature on Growth and Glucose Consumption

The growth kinetics of yeast cells during 24 hours of fermentation are presented in Figs. 3a and 3b, allowing evaluation of cellular behavior under different temperatures and immobilization conditions. Prior inoculation was used as a preadaptation strategy, in which the microorganism was cultivated in a medium like that of the main process. This approach stimulates the activation of specific metabolic pathways and the synthesis of adaptation-related proteins, thereby reducing the lag phase. As a result, the onset of exponential growth is accelerated, contributing to improved growth kinetics and a more consistent fermentative process (Cunha, 2023; Hiss, 2001).

This strategy facilitated a faster transition to the active growth phase, as indicated by the marked increase in cell density at the beginning of the stationary phase. The findings suggest that prior inoculation not only shortened the adaptation period but also enhanced the specific growth rate (μ). Such an effect is particularly important in industrial applications, where reduced processing time and increased productivity are highly desirable (Schuler, 2012).

Growth kinetics (Figs. 5a and 5b) were further evaluated to compare the performance of free and immobilized yeast cells at different temperatures. As shown in Fig. 5a, free yeast cells cultivated between 25 and 40°C did not exhibit a distinct lag phase, corroborating the observations reported by Pires (2017). The lag phase is typically characterized by enzyme synthesis and cellular adaptation to the cultivation medium, occurring without measurable cell division (Schmidell et al., 2001). Its absence in this study can be attributed to the use of a medium identical to that employed during inoculum preparation, which facilitated a rapid transition to the exponential phase and promoted an increase in the specific growth rate (μ).

For immobilized yeast cells (Fig. 5b), a delay in process onset was observed. This effect may be attributed to stress imposed by immobilization, which alters the cellular microenvironment and restricts space for growth (Pajić Lijaković et

al., 2017). Despite this initial delay, essential metabolic activities were not compromised, as cell growth and the production of ethanol and carbon dioxide still occurred during fermentation. The observed lag likely corresponds to the period required for cellular adaptation to the new microenvironment and to the specific immobilization method applied.

A delayed onset of cell multiplication was consistently observed in immobilized yeasts, likely resulting from physiological stress imposed by the immobilization matrix. This environment reduces the available space for growth and alters mass transfer conditions, limiting the diffusion of substrates and metabolites (Pajić Lijaković et al., 2017; Verbelen et al., 2006). Furthermore, modifications in the microenvironment may influence gene expression and membrane organization, triggering adaptive responses such as stress-hardening mechanisms (Gasch, 2002). The impact of the support material on cellular physiology should also be considered, as physicochemical interactions between the support surface and the cell wall can alter membrane permeability and metabolic dynamics (Loo et al., 2000).

Bolaños Barbosa et al. (2023) investigated yeast encapsulation in alginate using 0.1 mol L^{-1} calcium chloride as a crosslinking agent. Batch experiments demonstrated a significant reduction in cell viability and mechanical stability, which limited the reuse of the encapsulated systems. In addition, cell leakage from the polymeric matrix through pores, cell proliferation within the capsules, and gas production may further weaken capsule integrity (Simó et al., 2017). Immobilization may also modify the physiological and metabolic properties of cells, as well as the characteristics of the surrounding microenvironment, thereby influencing cellular kinetic behavior. In this regard, the local microenvironment is affected by solute partitioning between the liquid phase and the solid matrix, external mass transfer resistance in the liquid film, and internal diffusion limitations within the immobilization matrix (Merchant, 1987).

These phenomena are described by the solute partition coefficient (Kp), the liquid film mass transfer coefficient (kL), and the effective solute diffusivity within the immobilization matrix (De). The substrate must first diffuse through the bulk liquid and cross the external liquid film surrounding the immobilized particle until it reaches the liquid–solid interface. It then diffuses through the liquid phase inside the matrix, where additional resistance arises from microcolony formation, and finally reaches the interior of the yeast cells, where metabolic reactions occur (Pilkington et al., 1998).

Using a model of cell growth and substrate consumption of *N. agilis* immobilized in carrageenan gel spheres, Gooijer et al. (1991) demonstrated that external mass transfer resistance was the most sensitive parameter affecting the growth of entrapped cells. Similarly, Wang et al. (2020) showed that *Candida antarctica* lipase B immobilized in ZIF-8 exhibited enhanced activity and stability, while the ZIF-8 layer also regulated substrate accessibility to the enzyme interior.

Several studies have reported that immobilized cells behave differently from free cells in solutions, including enhanced metabolic efficiency (Navarro & Durand, 1977) and alterations in metabolic pathways (Brodelius et al., 1979). Mattiasson et al. (1982) proposed that environmental factors such as water activity and oxygen availability play a central role in regulating the metabolism of immobilized cells.

One possible explanation for the metabolic differences between immobilized and free cells is the reduced water activity within gel matrices, likely caused by increased osmotic pressure (Mattiasson et al., 1984). Immobilized systems generally exhibit lower water activity and limited oxygen availability, conditions that can suppress cell growth, metabolic activity, and reproduction.

Galazzo and Bailey (1990) reported enhanced polyphosphate synthesis in immobilized yeasts, possibly induced by reduced water activity. Polyphosphate, an inorganic phosphate polymer, plays multiple biological roles, including energy storage, regulation of metabolic pathways, and participation in stress response and survival mechanisms in microorganisms such as bacteria and yeasts (Júnior et al., 2024).

Accordingly, Shinmyo et al. (1982) reported that Bacillus *amyloliquefaciens* entrapped in κ-carrageenan operated under oxygen-deficient conditions, resulting in reduced cell growth and enhanced α-amylase production. When immobilized

cells sustain high maintenance metabolism at the expense of growth due to reduced water activity, higher yields can be expected for reactions linked to maintenance metabolism and for oxygen-independent processes.

Doran and Bailey (1986) reported that, in alcoholic fermentation with immobilized yeast, substrate consumption was higher than in fermentations with free cells, whereas cell growth and product formation (e.g., ethanol and glycerol) were lower. These findings suggest that immobilization affects microbial growth through interactions between solid support and the cells, as well as cell-to-cell interactions. According to the authors, although the microenvironment of immobilized yeasts—characterized by concentration gradients and osmotic pressure—affects cellular metabolism, reduced growth is primarily attributed to direct physical contact with the immobilization material, whether solid or gel. This contact induces morphological changes and alters cell wall protein expression, thereby impacting overall metabolism.

From a kinetic perspective, the transition from free to immobilized cells can alter the relationship between the maximum specific growth rate (μ_{max}) and the saturation constant (Ks), often reducing μ_{max} due to additional diffusional resistances imposed by the support. In the present study, free cells-initiated growth immediately after inoculation, whereas immobilized cells exhibited a lag phase of up to 8 hours before significant growth, particularly at 25–35°C. These findings suggest that immobilization affects not only kinetic parameters but also physiological aspects of yeast cells, directly influencing their metabolic performance and growth (Kovaleski, 2019).

During the exponential phase, rapid cell multiplication and biomass increase were observed. Free yeast cells cultivated at 25, 30, and 35°C reached concentrations of 2.83, 3.49, and 3.32 g L^{-1} after 24 h, respectively. In contrast, temperatures ≥ 40°C significantly reduced the specific growth rate, leading to cell death at 45 and 50°C. For immobilized cells, although growth ceased at these temperatures, viability was maintained due to the protective effect of ZIF-8. Similar findings were reported by Lin et al. (2012) for *Saccharomyces cerevisiae* BY4742, which showed markedly reduced growth and ethanol production at 50°C. This effect is attributed to thermal stress, as high temperatures induce structural alterations in proteins and plasma membranes, impairing growth, viability, and fermentative capacity (Naves et al., 2010).

For immobilized yeast cells (Fig. 5b), the exponential phase began after approximately 8 h of fermentation, with significant growth observed at 25–35°C. After 24 h, cell concentrations reached 3.30, 3.56, and 3.44 g L^{-1}, respectively. Substrate consumption was maximal during this phase, resulting in complete glucose depletion by 24 h, as it was utilized to meet metabolic demands. Each glucose molecule yields two ATP molecules, supplying the energy required for cellular functions (Schmidell et al., 2001). Accordingly, glucose consumption reached 100% after 24 h of fermentation (Figs. 6a and 6b).

Analysis of Figs. 6a and 6b shows glucose depletion by the end of the exponential phase for both free and immobilized cells, increasing linearly up to 24 h at 25–35°C (Figs. 5a and 5b). In contrast, glucose consumption was markedly reduced at 45 and 50°C in both systems. This reduction reflects the direct effect of temperature on microbial metabolic activity, a key factor in fermentative performance (Lima, 2009). Caspeta et al. (2014) reported that at temperatures above 36°C, yeasts activate the heat stress response, a transcriptional defense mechanism that disrupts carbohydrate metabolism and plasma membrane composition. Although *Saccharomyces cerevisiae*, a mesophilic yeast, can grow at temperatures up to approximately 42°C, prolonged exposure to higher temperatures compromises cell viability (Yamamoto et al., 2008).

Neves (2018) reported that glucose consumption is reduced during the lag phase, as yeast cells adapt to the medium and reorganize their enzymatic machinery in preparation for logarithmic growth. Similarly, Brito (2019) observed negligible initial sugar consumption in *S. cerevisiae* immobilized in calcium alginate, indicating a lag period required for adaptation to the new system. This behavior aligns with the classical microbial growth curve, in which the lag phase represents the time needed for metabolic adjustment to new environmental conditions.

Although the lag period is inherently related to cellular adaptation, mass transfer of substrates and products through polymeric support is another key factor influencing immobilization efficiency. Coutinho (2020) highlighted limited diffusion as a critical parameter in biocatalyst encapsulation, since the porous barrier between the microorganism and the fermentative medium may restrict compound transport into and out of the matrix, impairing fermentation performance. Nevertheless, the present results indicate that despite the initial adaptation phase, immobilization did not compromise overall fermentation efficiency as shown in Table 1.

In addition to diffusion limitations imposed by the polymeric matrix, cell density within the support is a crucial factor affecting immobilization efficiency (Kovaleski, 2019). In that study, *Saccharomyces cerevisiae* immobilized in calcium alginate at densities of 1×10^6, 8×10^6, and 2×10^7 cells mL^{-1} showed distinct sucrose consumption profiles over 54 h of alcoholic fermentation. Beads with the lowest density reached 71% sucrose consumption, with a lag phase during the first 6 h. In contrast, higher densities resulted in lower consumption rates (11% and 29%), with a lag phase observed in one case. These findings suggest that increased cell density promotes internal nutrient competition, limiting growth and impairing substrate consumption and ethanol production (Cha et al., 2014).

Although high cell density may impose metabolic constraints, immobilization also induces physiological changes in yeast cells. Some of these changes enhance fermentative performance, whereas others may be detrimental. Given that *S. cerevisiae* naturally adheres to surfaces in its native habitats, immobilization can be considered a growth mode closer to natural conditions, potentially providing protection against environmental stresses (Verstrepen and Klis, 2006).

However, even under conditions closer to the natural environment, the negative effects observed are mainly related to stress caused by restricted mass transfer. In gel entrapment and inert supports, diffusional limitations depend on the immobilization method. In adsorption-based systems, resistance occurs primarily at the carrier–medium interface, with minimal internal limitations. In encapsulation methods, internal mass transfer is governed by the chemical and mechanical properties of the matrix, such as size, porosity, and texture. Verbelen et al. (2006) cautioned that physiological changes should not be attributed solely to immobilization, as these effects are difficult to separate from mass transfer limitations.

Accordingly, immobilization strongly influences yeast plasma membrane properties and may alter specific solute transport systems (Shen et al., 2003). Comparisons between immobilized and free cells show activation of energy metabolism in immobilized systems, along with increased accumulation of storage polysaccharides (trehalose and glycogen) and structural polysaccharides (glucan and mannan) (Galazzo & Bailey, 1990; Jirku et al., 2000). Their accumulation enhances resistance to environmental stress, supports the production of industrially relevant metabolites, and improves viability under adverse conditions. In *S. cerevisiae*, trehalose and glycogen accumulate in response to unfavorable growth conditions, serving as energy reserves and stress protectants. These compounds are essential for cell cycle progression under reduced growth rates and carbon limitation. Singer and Lindquist (1998) demonstrated that trehalose protects cells during thermal shock by preventing protein denaturation and aggregation, underscoring its key role in cellular protection.

Fermentation Performance and Kinetic Parameters

The ethanol concentrations and principal kinetic parameters obtained during fermentation are summarized in Table 1. Comparative analysis of these parameters highlights significant differences in the fermentative performance of *Saccharomyces cerevisiae* under the tested conditions, for both free and ZIF-8-immobilized cells. Overall, immobilized yeasts exhibited superior performance within the temperature range of 25–35°C, particularly regarding final ethanol concentration, substrate-to-product yield ($Y_{P/S}$), volumetric productivity (Q_P), and fermentation efficiency (η).

Table 1
Fermentation kinetic parameters, substrate-to-product yield (Y□/□), volumetric productivity (Q□), and fermentation efficiency (η), of free *Saccharomyces cerevisiae* cells and cells immobilized in ZIF-8 after 24 hours of fermentation.

Temperature	**25ºC**	**30ºC**	**35ºC**	**40ºC**	**45ºC**	**50ºC**
Final ethanol ($g.L^{-1}$) Lev. Free	10,23	12,89	12,05	7,54	0	0
Final ethanol *($g.L^{-1}$)* Lev. Immobilized	12,29	15,71	13,32	0,32	0	0
$Y_{P/S}$ *($g.L^{-1}$)* Lev. Free	0,306	0,403	0,377	0,236	0	0
$Y_{P/S}$ *($g.L^{-1}$)* Lev. Immobilized	0,371	0,479	0,401	0,075	0	0
Qp ($g.L^{-1}.h^{-1}$) Lev. Free	0,426	0,537	0,482	0,314	0	0
Qp ($g.L^{-1}.h^{-1}$) Lev. Immobilized	0,512	0,655	0,555	0,133	0	0
η (%) Lev. Free	59,88	78,86	73,77	46,18	0	0
η (%) Lev. Immobilized	72,6	93,74	78,47	14,68	0	0

At temperatures considered optimal for yeast metabolism (25 and 30°C), immobilization significantly increased the final ethanol concentration, with gains ranging from 10.54% to 21.89%. This improvement was accompanied by higher substrate-to-product yields (Y□/□), which increased by 6.4% to 21.2% under these conditions. These findings suggest that the ZIF-8 matrix enhances cell viability and metabolic performance, possibly by reducing environmental stress and allowing greater operational stability and cell reuse.

According to Nedović et al. (2014), immobilized cells often exhibit higher glycolytic flux than free cells, resulting in faster glucose consumption and more efficient channeling of substrate toward biomass and ethanol production. The present results support this observation, as the increased ethanol production by immobilized yeasts indicates a shift in fermentative metabolism. This shift may stem from growth limitations imposed by the ZIF-8 matrix, which restricts cell expansion and redirect carbon flux preferentially toward fermentative pathways rather than biosynthesis. Such microenvironmental constraints associated with immobilization may be related to polymer network relaxation around cell aggregates, mechanical forces generated during cell growth within the capsule, and physicochemical interactions among the solvent, matrix, and cells (Pajić Lijaković et al., 2012).

This metabolic modulation is reflected in the average fermentative productivity (Q□), which showed a similar improvement and reached a maximum of 0.655 g L^{-1} h^{-1} for immobilized yeasts at 30°C, approximately 22% higher than for free cells. From an industrial perspective, this increase is significant, as productivity determines the rate of product formation and directly impacts cycle time and process economics.

Consistent with these findings, fermentation efficiency further confirms the superiority of immobilized yeasts under the evaluated conditions. While free cells showed efficiencies of 59.88–78.86% at 25–35°C, immobilized cells reached 72.60-93.74%. At 30°C, efficiency (~ 91%) approached the average value reported for industrial operations (Abreu Cavalheiro & Monteiro, 2013), underscoring the robustness and operational stability of the ZIF-8 system. Overall, these results indicate that immobilization enhances conversion efficiency and productivity while increasing biocatalyst resilience.

Conversely, increasing the temperature to 40°C sharply reduced fermentative performance, with significant declines in final ethanol concentration, substrate-to-product yield (Y□/□), volumetric productivity (Q□), and efficiency (η) in both systems. This result indicates that temperatures above the physiological optimum impair plasma membrane integrity,

enzymatic stability, and intracellular homeostasis, leading to metabolic inhibition and loss of viability. At 45 and 50°C, fermentative activity was completely suppressed, indicating that these temperatures exceed the thermal tolerance of the strain, regardless of immobilization.

Mattiasson et al. (1982) reported improved ethanol productivity and yield using immobilized cells. For free cells in solution, the theoretical maximum ethanol yield from glucose is 51%; however, adsorbed cells have achieved substantially higher yields (Navarro & Durand, 1977), likely due to alterations in metabolic pathways. In the immobilized state, cells are confined within a polymer-rich environment whose macromolecules can organize water and reduce its availability. Even moderate polymer concentrations can induce distinct metabolic effects. For example, adding only 0.5% sodium alginate to batch fermentation with *Saccharomyces cerevisiae* markedly altered cellular productivity (Holcberg & Margalith, 1981).

To evaluate the effect of reduced water activity on *S. cerevisiae* metabolism, batch fermentations were performed at water activities as low as 0.986 by adding increasing concentrations of dextran and polyethylene glycol (Hahn-Hägerdal et al., 1982). Under these conditions, the initial ethanol production rate increased by 50%. Similarly, yeast cells adsorbed onto porous glass showed a 25% increase in ethanol production rate.

Cell Viability and Thermal Stress

The encapsulation process aims to preserve the viability and metabolic functionality of microorganisms by creating a favorable microenvironment for fermentation development. In this context, the use of encapsulation proved to be promising in the present study, as yeasts immobilized in ZIF 8 exhibited superior performance compared to free cells. This improvement was particularly evident in terms of enhanced thermal stability, which allowed satisfactory productivity and fermentation efficiency. This operational advantage may be associated with the intrinsic properties of ZIF 8, whose thermal stability and behavior have been well documented.

Fang et al. (2015) investigated the thermal stability and degradation behavior of ZIF 8 nanocrystals using thermogravimetric analysis under a nitrogen atmosphere and demonstrated that pure ZIF 8 crystals exhibit degradation temperatures above 600°C. This characteristic is especially relevant for applications involving temperature fluctuations, such as gas flow in reactors or industrial ducts, as it ensures durability and structural integrity under severe conditions. Other studies corroborate this thermal robustness. Sung et al. (2019) reported stability up to 550°C, while Yang et al. (2013) observed excellent thermal and separation stability of ZIF 8 PBI nanocomposites under elevated temperatures and hot vapor exposure of up to 230°C during ten days of continuous operation.

This stability was a determining factor in the results obtained in the present study, as illustrated in Figs. 7a and 7b, which show the evolution of cell viability of free and ZIF 8 immobilized *Saccharomyces cerevisiae* over time at different temperatures. Immobilization was effective in maintaining stable cell viability throughout the fermentative process, even under more severe thermal conditions. It is important to note that while the capsule protects cellular integrity, it does not necessarily ensure maximum metabolic activity under highly stressful conditions. After 24 hours of cultivation, immobilized yeasts exhibited cell viability values of 98.4 percent, 97.7 percent, and 96.7 percent, respectively. In contrast, free cells showed more pronounced reductions, reaching 93.7 percent, 88.9 percent, and 82.9 percent.

This behavior was expected, considering that the thermal stability of ZIF 8 greatly exceeds the tolerance limits of the yeast itself, allowing it to function as a physical and protective barrier against thermal stress. These findings are consistent with the observations of Torija et al. (2003), who reported that the initial fermentation rate is maximized between 25°C and 31°C, while cell viability declines significantly above 35°C, particularly in unprotected cultures. Thus, the thermal resistance provided by the ZIF 8 matrix proved sufficient to mitigate the negative effects of heat on encapsulated cells under thermal stress.

The protective effect of immobilization against thermal shock is also supported by physiological studies on yeast. Scientific evidence indicates that cells in the logarithmic phase of rapid growth are more sensitive to thermal stress than slowly dividing cells or those in the stationary phase (Washburne et al., 1993; Lu et al., 2009). Nagarajan et al. (2014) compared the thermotolerance of *S. cerevisiae* confined within or released from calcium alginate matrices with planktonic cells at different physiological stages following exposure to 48°C for two hours. The authors observed that planktonic and immobilized cells in the stationary phase were significantly more tolerant to thermal shock than planktonic cells in the logarithmic phase. The greater resistance observed in immobilized cells resulted not only from their physiological state but also from the physical protection provided by the matrix, as evidenced by the intermediate survival of cells released from the beads. Furthermore, when immobilized, cells ceased division but remained metabolically active and highly fermented, maintaining viability for up to seventeen weeks with viability levels exceeding 95 percent.

The relationship between growth rate and heat resistance was further explored by Lu et al. (2009), who demonstrated that genes involved in the heat stress response and previously associated with thermotolerance exhibit higher expression in slowly growing cells. To test this hypothesis, the authors cultivated cells at different growth rates under steady state conditions in chemostats at 50°C, a lethal temperature for yeast cells growing in rich media. Cells with a doubling time of 2.8 hours exhibited high sensitivity, whereas cells with a doubling time of 13.8 hours were resistant. Cultures with intermediate growth rates, corresponding to a doubling time of 4.6 hours, showed intermediate mortality. As a control, cells cultivated at the intermediate growth rate but at 36°C exhibited resistance like that of slow growing cells. These results indicate that growth deceleration, a characteristic observed in immobilized cells, promotes gene expression patterns that contribute to the enhanced thermal resistance observed in the present work.

Performance in Lignocellulosic Hydrolysate and Tolerance to Inhibitors

After acid pretreatment, passion fruit peels underwent enzymatic hydrolysis at time intervals of 0, 0.5, 1, 2, 4, 8, and 24 hours. As shown in Fig. 8, glucose concentration progressively increased during the process, reaching 32.53 g L^{-1} after 24 hours. Following hydrolysis, the filtrate (approximately 120 mL) was concentrated using a rotary evaporator to enhance sugar content, reducing the volume to 20 mL. Citrate buffer (pH 5.5) was then added to adjust the pH, resulting in a final glucose concentration of 62.05 g L^{-1}. The hydrolysate was subsequently sterilized by autoclaving at 121°C.

The passion fruit peel hydrolysate was used as the fermentation medium for alcoholic fermentation by *Saccharomyces cerevisiae* CAT-1, employing both free and immobilized cells. The results for cell growth, substrate consumption, ethanol production, substrate-to-product yield (Y□/□), volumetric productivity (Q□), and fermentation efficiency (η) are summarized in Tables 2 and 3.

This procedure was designed to evaluate, over an additional 30-hour period, the metabolic activity and viability of the immobilized yeasts, as well as the effectiveness of the ZIF-8 capsule after exposure to inhibitory compounds commonly present in lignocellulosic hydrolysates derived from passion fruit peel, which may exert toxic effects on yeast cells. The results of these tests are shown in Table 4.

Edwards and Doran-Peterson (2012) reviewed the main pectin-rich residues—such as those generated from citrus, sugar beet, and apple processing—that can be used for ethanol production. According to the authors, this type of biomass is characterized by low lignin content and a high pectin fraction, typically ranging from 12% to 35%.

However, a major limitation in using these residues for ethanol production is that galacturonic acid and arabinose are not metabolized by microorganisms traditionally employed in alcoholic fermentation. This constraint helps explain the low fermentative efficiency observed with *Saccharomyces cerevisiae*, since passion fruit peel contains, on average, approximately 25% pectin (Júnior et al., 2006). In addition to its inability to ferment pentoses, this yeast is also incapable

of metabolizing the galacturonic acid released during biomass hydrolysis, further limiting the conversion efficiency of pectin-rich substrates into ethanol.

Table 2
Cell growth, substrate consumption, ethanol production, and fermentation kinetic parameters of *Saccharomyces cerevisiae* immobilized in ZIF-8 during fermentation using lignocellulosic biomass derived from passion fruit peel.

Time (h)	**Cell growth (g/L)**	**Glucose (g/L)**	**Final ethanol (g/L)**	**$Y_{P/S}$ ($g.L^{-1}$)**	**Qp ($g.L^{-1}.h^{-1}$)**	**η (%)**	**Viability (%)**
0 hours	2,5	62,05	0	0	0	0	100
12 hours	2,66	51,92	2,70	0,266	0,225	52,05	68,59
24 hours	2,67	44,24	6,80	0,381	0,283	74,56	19,89

Table 3
Cell growth, substrate consumption, ethanol production, and fermentation kinetic parameters (substrate-to-product yield (Y□/□), volumetric productivity (Q□), and fermentation efficiency (η)) of *Saccharomyces cerevisiae* immobilized in ZIF-8 during fermentation using lignocellulosic biomass derived from passion fruit peel.

Time (h)	**Cell growth (g/L)**	**Glucose (g/L)**	**Final ethanol (g/L)**	**$Y_{P/S}$ ($g.L^{-1}$)**	**Qp ($g.L^{-1}.h^{-1}$)**	**η (%)**	**Viability (%)**
0 hours	2,5	62,05	0	0	0	0	100
24 hours	2,5	57,63	0	0	0	0	100
48 hours	2,5	56,09	0	0	0	0	99,42

Table 4
Cell growth, substrate consumption, ethanol production, and fermentation kinetic parameters (substrate-to-product yield (Y□/□), volumetric productivity (Q□), and fermentation efficiency (η)) of *Saccharomyces cerevisiae* immobilized in ZIF-8 during fermentation in YPD medium.

Time (h)	**Cell growth (g/L)**	**Glucose (g/L)**	**Final ethanol (g/L)**	**$Y_{P/S}$ ($g.L^{-1}$)**	**Qp ($g.L^{-1}.h^{-1}$)**	**η (%)**	**Viability (%)**
0 hours	2,5	85,00	0	0	0	0	100
30 hours	3,5	61,03	10,60	0,442	0,353	86,54	100

When lignocellulosic hydrolysates are used as feedstock, significant amounts of pentose sugars, such as D-xylose and L-arabinose, are released from hemicellulose (Azhar et al., 2017). Ndubuisi et al. (2020), working with the yeast *Pichia kudriavzevii* LC375240, reported that almost no ethanol was produced in pentose-based media due to limited cell growth. Their findings suggest that although the strain was able to assimilate pentose sugars to support growth, it was unable to efficiently ferment them into ethanol.

Acid hydrolysis generates not only sugars but also toxic byproducts that inhibit fermentative microorganisms. These include organic acids (acetic, formic, and levulinic acids), furan derivatives (furfural and 5-hydroxymethylfurfural), and phenolic compounds (Chandel et al., 2007). Acetic acid originates from hemicellulose, furans from pentose and hexose

degradation, and formic and levulinic acids from further furan breakdown (Martín et al., 2007). Lignin is the main source of phenolic compounds, which are considered stronger inhibitors than sugar degradation products (Jönsson et al., 2013, Palmqvist and Hahn Hägerdal, 2000; Rasmussen et al., 2014, Jönsson et al., 1998).

Inhibitory compounds formed during biomass hydrolysis reduce ethanol yield and productivity by impairing microbial metabolism and carbohydrate uptake (Almeida et al., 2007). Furans such as hydroxymethylfurfural and furfural damage DNA, inhibit RNA and protein synthesis, and interfere with key enzymes involved in ethanol production (Modig et al., 2002). Their toxicity is mainly linked to reactive aldehyde groups that disrupt cellular components and membranes (Miller et al., 2010). Organic acids (acetic, formic, and levulinic) inhibit cells in their undissociated forms by diffusing across membranes, while lignin-derived phenolic compounds compromise membrane integrity and enzyme function (Mills et al., 2009, Parawira et al., 2011).

The type and concentration of inhibitory compounds formed during pretreatment depend on the feedstock and the severity of processing conditions (Jönsson et al., 2013; Ko et al., 2015). As a result, purification strategies to reduce inhibitor levels before fermentation are highly important. In general, fermentation of untreated hydrolysates leads to slow kinetics, low productivity, and reduced ethanol yields (Mussatto et al., 2004).

Fermentation converts sugars released during pretreatment and hydrolysis into ethanol. However, the absence of microorganisms capable of efficiently fermenting both hexoses and pentoses into ethanol limits the economic viability of cellulosic ethanol production (Nichols et al., 2014). Although research has advanced in developing wild-type and genetically engineered strains to address this issue, challenges such as incomplete pentose utilization, low ethanol tolerance, and sensitivity to pretreatment-derived inhibitors persist (Kumar et al., 2016).

According to Milessi et al. (2020), cell immobilization on solid supports is a promising strategy to overcome fermentation challenges, as it creates a protective microenvironment that shields yeast from inhibitors present in hydrolysates. This concept supports the use of ZIF-8 immobilization applied in the present study. In addition to enhanced protection, immobilization enables easy recovery and reuse of cells, operation at high cell densities for extended periods, and reduced biomass handling requirements. It also facilitates the industrial application of genetically modified microorganisms, particularly in regions with strict biosafety regulations (Kourkoutas et al., 2004, Milessi et al., 2020).

Fermentations using passion fruit, banana, and coconut hydrolysates commonly show lower ethanol production compared to media containing pure glucose. For example, *Zymomonas mobilis* produced less ethanol when hydrolysates were used as the carbon source. This reduction is attributed to the presence of inorganic compounds and other inhibitory substances in hydrolysates, which can interfere with microbial metabolism (Doelle and Doelle, 1990). Non-purified sugar media may contain impurities that inhibit ethanol production and suppress enzymatic activity, thereby reducing overall fermentation efficiency (Khoja et al., 2015).

Rosa (2021) evaluated fermentation of orange bagasse hydrolysate using *Saccharomyces cerevisiae* G2 104 immobilized in calcium alginate and observed low ethanol yields. After 12 hours, ethanol concentrations were 2.57 g L^{-1} (enzymatic hydrolysate), 1.69 g L^{-1} (acid hydrolysate), and 4.9 g L^{-1} (enzymatic hydrolysate without hydrothermal pretreatment). These findings indicate that enzymatic hydrolysates provide better fermentative performance, likely due to the absence of inhibitory compounds commonly formed during acid hydrolysis. Acid hydrolysates showed reduced sugar consumption and lower ethanol production, probably due to inhibitor formation and generation of non-fermentable sugars. However, acid hydrolysis can still be effective for sugar release if combined with detoxification and supplementation strategies.

To assess the protective effect of the ZIF-8 capsule against inhibitory compounds present in passion fruit peel hydrolysate, encapsulation was evaluated as a protective strategy. The results (Tables 2 and 3; Figs. 9a and 9b and 10) show clear differences between free and immobilized *S. cerevisiae*.

Immobilization effectively preserved cell viability throughout fermentation. After 48 hours at 30°C without agitation, immobilized cells maintained 99.42% viability, while free cells showed a sharp decline to 69.59% at 12 hours and 19.89% at 24 hours. As a result, free-cell fermentations produced low ethanol concentrations (maximum of 6.8 g L^{-1}). In contrast, immobilized cells consumed less glucose, and ethanol productivity was not determined, suggesting that glucose was mainly used for cell maintenance rather than ethanol production.

The enhanced tolerance of immobilized yeasts to toxic compounds may be linked to osmotic stress responses, which stimulate intracellular polyol production for osmotic regulation. This mechanism reduces water activity and increases resistance to harmful chemical substances (Norton and D'Amore, 1994).

Furthermore, data from Tables 3 and 4 confirm the effectiveness of encapsulation as a protective strategy. Immobilized cells remained metabolically active and viable after medium replacement for an additional 30 hours. Fermentation efficiency values reinforced this observation, with immobilized yeasts reaching an efficiency of 86.54%, demonstrating superior stability under the evaluated conditions.

Conclusion

Fermentations conducted at 25–50°C showed clear differences between free and ZIF-8-immobilized yeasts. ZIF-8 acted as a protective barrier, improving cell integrity and fermentative performance at optimal temperatures (25–35°C), with higher $Y_{X/S}$, Q_S, and η values compared to free cells. The capsule also maintained high viability at elevated temperatures, reaching 96.7% at 50°C after 24 hours, although ethanol production was suppressed above 40°C in both conditions.

In fermentations using passion fruit peel hydrolysate, ZIF-8 preserved cell viability against inhibitory compounds; however, ethanol yield remained limited due to the hydrolysate composition, including pentoses, phenolics, organic acids, furfural, and hydroxymethylfurfural. Overall, immobilization in ZIF-8 proved to be an effective biopreservation strategy, enhancing cell protection, robustness, and potential reuse.

Declarations

Author Contribution

C.N.P. conceived and designed the research project and supervised the M.Sc. student L.C.C.G., particularly regarding the immobilization of Saccharomyces cerevisiae in ZIF-8 for application in alcoholic fermentation of lignocellulosic biomass derived from passion fruit peel. C.A.G.S. co-supervised the student and directly guided most of the experimental work involving the yeast. L.C.C.G. performed all laboratory experiments and contributed to data organization and manuscript preparation. G.L.C. conducted and interpreted all high-performance liquid chromatography (HPLC) analyses. All authors reviewed and approved the final version of the manuscript.

Acknowledgement

The authors gratefully acknowledge the financial support provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG). The authors also thank the CAM (IQ-UFG) and CAiTEC (UEG) laboratories and their technical staff for experimental support, as well as colleagues and collaborators who contributed directly or indirectly to the development of this study.

References

1. Abreu-Cavalheiro GM (2013) Solving ethanol production problems with genetically modified yeast strains. Brazilian J Microbiol 44:665–671
2. Almeida JRM, Modig T, Petersson A, Hahn-Hägerdal B, Lidén G, Gorwa-Grauslund MF (2007) Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 82:340–349
3. Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Mohd Faik AA, Rodrigues KF (2017) Yeast in sustainable bioethanol production: A review. Biochem Biophys Rep 10:52–61. https://doi.org/10.1016/j.bbrep.2017.03.003
4. Ballesteros M, Oliva JM, Negro MJ, Manzanares P, Ballesteros I (2004) Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875. Process Biochem 39:1843–1848. https://doi.org/10.1016/j.procbio.2003.09.011
5. Bolaños-Barbosa AD, Rodríguez CF, Acuña OL, Cruz JC, Reyes LH (2023) The impact of yeast encapsulation on wort fermentation and beer flavor profile. Polymers 15:1742. https://doi.org/10.3390/polym15071742
6. Brito JA (2019) Analysis of the use of yeasts immobilized in calcium alginate gel in successive fermentations of apple juice. Undergraduate Thesis, Federal Technological University of Paraná, Ponta Grossa, Brazil. Available at: http://repositorio.utfpr.edu.br/jspui/handle/1/16645 (Accessed 15 July 2025)
7. Brodelius P, Deus B, Mosbach K, Zenk MH (1979) Immobilized plant cells for the production and transformation of natural products. FEBS Lett 103:93–97
8. Caspeta L, Chen Y, Ghiaci P, Feizi A, Buskov S, Hallström BM, Petranovic D, Nielsen J (2014) Altered sterol composition renders yeast thermotolerant. Science 346:1258137. https://doi.org/10.1126/science.1258137
9. Ceccato-Antonini SR (2011) Microbiology of alcoholic fermentation: The importance of microbiological monitoring in distilleries. EdUFSCar, São Carlos, Brazil
10. Chandel AK et al (2007) Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501. Bioresour Technol 98:1947–1950
11. Cha HG et al (2014) Evaluating carriers for immobilizing *Saccharomyces cerevisiae* for ethanol production in a continuous column reactor. Mycobiology 42:249–255
12. Coutinho UTC (2020) Fermentative capacity of *Saccharomyces cerevisiae* immobilized in calcium alginate for beer production. Master's Dissertation, Federal Rural University of Pernambuco, Brazil
13. da Cunha ET, Pedrolo AM, Arisi ACM (2023) Effects of sublethal stress application on the survival of bacterial inoculants: A systematic review. Arch Microbiol 205:190. https://doi.org/10.1007/s00203-023-03542-8
14. Deria P, Mondloch JE, Karagiaridi O, Bury W, Hupp JT, Farha OK (2014) Beyond post-synthesis modification: Evolution of metal–organic frameworks via replacement of building blocks. Chem Soc Rev 43:5896–5912. https://doi.org/10.1039/c4cs00067f
15. Doelle MB, Doelle HW (1990) Sugar-cane molasses fermentation by *Zymomonas mobilis*. Appl Microbiol Biotechnol 33:31–35
16. Doran PM, Bailey JE (1986) Effects of immobilization on growth, fermentation properties, and macromolecular composition of *Saccharomyces cerevisiae* attached to gelatin. Biotechnol Bioeng 28:73–87
17. Edwards MC, Doran-Peterson J (2012) Pectin-rich biomass as feedstock for fuel ethanol production. Appl Microbiol Biotechnol 95:565–575
18. Fang M, Wu C, Yang Z, Wang T, Xia Y, Li J (2015) ZIF-8/PDMS mixed matrix membranes for separation of propane/nitrogen mixtures: Experimental results and permeation model validation. J Membr Sci 474:103–113
19. Fermentec SC, Ltda (1978) Alcohol manufacturing process. Piracicaba, SP, Brazil
20. Galazzo JL, Bailey JE (1990) Fermentation pathway kinetics and metabolic flux control in suspended and immobilized *Saccharomyces cerevisiae*. Enzym Microb Technol 12:162–172

21. Gasch AP (2003) The environmental stress response: A common yeast response to diverse environmental stresses. In: Hohmann, S., Mager, W.H. (Eds.), *Yeast Stress Responses*. Topics in Current Genetics, vol. 1. Springer, Berlin, Heidelberg, pp. 11–70. https://doi.org/10.1007/3-540-45611-2_2
22. Gascon J, Corma A, Kapteijn F, Xamena FXLI (2014) Catalysis of metal–organic frameworks: Quo vadis? ACS Catal 4:361–378. https://doi.org/10.1021/cs400959k
23. Ghanbari T, Abnisa F, Wan Daud WMA (2020) A review on the production of metal–organic frameworks (MOFs) for CO_2 adsorption. Sci Total Environ 707:135090. https://doi.org/10.1016/j.scitotenv.2019.135090
24. de Gooijer CD, Wijffels RH, Tramper J (1991) Growth and substrate consumption of *Nitrobacter agilis* cells immobilized in carrageenan: Part 1. Dynamic modelling. Biotechnol Bioeng 38:224–231
25. Hahn-Hägerdal B, Larsson M, Mattiasson B (1982) Shift in metabolism towards ethanol production in *Saccharomyces cerevisiae* using alterations of the physicochemical environment. *Biotechnology and Bioengineering Symposium* (in press)
26. Hiss H, Lima UA, Aquarone E, Borzani W, Schmidell W (2001) Kinetics of fermentative processes. In: Schmidell W, Lima UA, Aquarone E, Borzani W (eds) Industrial Biotechnology – Volume 2: Biochemical Engineering. Edgard Blücher Ltda., São Paulo, Brazil
27. Holcberg IB, Margalith P (1981) Alcoholic fermentation by immobilized yeast at high sugar concentrations. Eur J Appl Microbiol Biotechnol 13:133–140
28. Horcajada P, Serre C, Maurin G, Ramsahye NA, Balas F, Vallet-Regí M, Sebban M, Taulelle F, Férey G (2008) Flexible porous metal–organic frameworks for controlled drug delivery. J Am Chem Soc 130:6774–6780. https://doi.org/10.1021/ja710973k
29. Islamoglu T, Chen Z, Wasson MC, Buru CT, Kirlikovali KO, Afrin U, Mian MR, Farha OK (2020) Metal–organic frameworks against toxic chemicals. Chem Rev 120:8130–8160. https://doi.org/10.1021/acs.chemrev.9b00828
30. Jirku V, Masak J, Cejkova A (2000) Yeast cell attachment: A tool modulating wall composition and resistance to 5-bromo-6-azauracil. Enzym Microb Technol 26:808–811
31. Jönsson LJ, Palmqvist E, Nilvebrant N, Hahn-Hägerdal B (1998) Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*. Appl Microbiol Biotechnol 49:691–697
32. Jönsson LJ, Alriksson B, Nilvebrant N (2013) Bioconversion of lignocellulose: Inhibitors and detoxification. Biotechnol Biofuels 6:1–10
33. Junior WJFL, Santinello D, Mohammadzadeh S, Treu L, Sant'Ana AS, Campanaro S (2024) Polyphosphate in food systems: Its roles and applications in food and contribution to sustainable processing practices. Trends Food Sci Technol 152:104696. https://doi.org/10.1016/j.tifs.2024.104696
34. Júnior L, Edilton J et al (2006) Physicochemical characterization of by-products obtained from the processing of tropical fruits for their use in animal feed. Revista Ciência Agronômica 37:70–76
35. Kovaleski G (2019) Study of the cellular immobilization of *Saccharomyces cerevisiae* in calcium alginate. Master's Dissertation, Federal Technological University of Paraná, Ponta Grossa, Brazil. Available at: http://repositorio.utfpr.edu.br/jspui/handle/1/4087 (Accessed 10 July 2025)
36. Ko JK, Kim YA, Park Y, Seo J, Kim KH (2015) Compounds inhibiting the bioconversion of hydrothermally pretreated lignocellulose. Appl Microbiol Biotechnol 99:4201–4212
37. Kourkoutas Y, Bekatorou A, Banat IM, Marchant R, Koutinas AA (2004) Immobilization technologies and suitable support materials in the production of alcoholic beverages: A review. Food Microbiol 21:377–397
38. Khoja AH et al (2015) Comparative study of bioethanol production from sugarcane molasses by using *Zymomonas mobilis* and *Saccharomyces cerevisiae*. Afr J Biotechnol 14:2455–2462

39. Kumar R, Tabatabaei M, Karimi K, Horváth IS (2016) Recent updates on ethanol derived from lignocellulosic biomass: A review. Biofuel Res J 9:347–356
40. Lima UA, Aquarone E, Borzani W, Schmidell W (2001) Industrial biotechnology – Fermentative and enzymatic processes, vol 3. Edgard Blücher Ltda., São Paulo, Brazil
41. Lima A (2009) Heat treatment of sugarcane juice for the alcoholic fermentation process: The state of the art. *ABEAS – Brazilian Association of Higher Agricultural Education*, 24: 7–12. Available at: http://www.abeas.com.br/wt/files/3_2009_1.pdf
42. Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H (2012) Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. Biomass Bioenergy 47:395–401. https://doi.org/10.1016/j.biombioe.2012.09.019
43. Loo CY, Corliss DA, Ganeshkumar N (2000) *Streptococcus gordonii* biofilm formation: Identification of genes that code for biofilm phenotypes. J Bacteriol 182:1374–1382. https://doi.org/10.1128/jb.182.5.1374-1382.2000
44. Lu C, Brauer MJ, Botstein D (2009) Slow growth induces heat shock resistance in normal and respiratory-impaired yeasts. Mol Biol Cell 20:891–903
45. Mallouchos A, Komaitis M, Koutinas A, Kanellaki M (2003) Wine fermentations by immobilized and free cells at different temperatures: Effect of immobilization and temperature on volatile by-products. Food Chem 80:109–113. https://doi.org/10.1016/S0308-8146(02)00247-9
46. Mattiasson B, Adlercreutz P, Hahn-Hägerdal B (1982) Interface phenomena in immobilized cellular systems. In: *19th Tutzing Symposium of DECHEMA: Biological properties at interfaces and their importance for biotechnology*, 8–11 March
47. Mattiasson B, Larsson M, Hahn-Hägerdal B (1984) Metabolic behavior of immobilized cells: Effect of some microenvironmental factors. Ann N Y Acad Sci 7:475–478
48. Martín C, Almazán O, Marcet M, Jönsson LJ (2007) A study of three strategies for improving the fermentability of sugarcane bagasse hydrolysates for fuel ethanol production. Int Sugar J 109:33–39
49. Miller EN, Turner PC, Jarboe LR, Ingram LO (2010) Genetic changes that increase 5-hydroxymethylfurfural resistance in ethanol-producing *Escherichia coli* LY180. Biotechnol Lett 32:661–667
50. Milessi TS, Perez CL, Zangirolami TC, Corradini FAS, Sandri JP, Foulquié-Moreno MR, Thevelein JM, Giordano RC, Giordano RLC (2020) Repeated batches as a strategy for high-concentration 2G ethanol production from non-detoxified hemicellulose hydrolysate using immobilized recombinant *Saccharomyces cerevisiae* cells in a fixed-bed reactor. Biotechnol Biofuels 13:85
51. Modig T, Lidén G, Taherzadeh MJ (2002) Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. Biochem J 363:769–776
52. Mussatto SI, Roberto IC (2004) Evaluation of different types of activated carbon in the detoxification of rice straw hydrolysate for xylitol production. Food Sci Technol 24:94–100
53. Nagarajan S, Kruckeberg AL, Schmidt KH et al (2014) Decoupling reproduction from metabolism extends the chronological lifespan of yeast. Proc Natl Acad Sci USA 111:538–547
54. Naves RF, Fernandes FS, Pinto OG, Naves PLF (2010) Microbial contamination in the processing stages and its influence on fermentation yield in an alcohol mill. *Encyclopedia of Biosphere*
55. Navarro JM, Durand G (1977) Modification of yeast metabolism by immobilization onto porous glass. Eur J Appl Microbiol Biotechnol 4:243–254
56. Nedović V, Gibson B, Mantzouridou TF, Bugarski B, Djordjević V, Kalušević A et al (2014) Aroma formation by immobilized yeast cells in fermentation processes. Yeast 32:173–216. https://doi.org/10.1002/yea.3042

57. Neves GT (2018) Metabolic study of the industrial yeast *Saccharomyces cerevisiae* PE-2 and two wild isolates AVE-I and AVE-II. Master's Dissertation, Federal University of Grande Dourados, Faculty of Exact Sciences and Technology, Dourados, Brazil
58. Nichols NN, Hector RE, Saha BC, Frazer SE, Kennedy GJ (2014) Biological reduction of inhibitors in rice husk hydrolysate and fermentation to ethanol using conventional and engineered microbes. Biomass Bioenergy 67:79–88
59. Norton S, D'Amore T (1994) Physiological effects of yeast cell immobilization: Applications in brewing. Enzym Microb Technol 16:365–375. https://doi.org/10.1016/0141-0229(94)90150-3
60. Oliveira AP (2018) Food industry waste for flour preparation: A strategy for utilization. Undergraduate Thesis, Federal University of Paraíba, João Pessoa, Brazil
61. Pajić-Lijaković I, Bugarski B, Plavšić M, Lević S, Kalušević A, Nedović V (2012) Microenvironmentally restricted yeast cell growth on calcium alginate microspheres. Engineering 4:180–183. https://doi.org/10.4236/eng.2012.410B047
62. Pajić-Lijaković I, Milivojević M, Lević S, Trifković K, Stevanović-Dajić Z, Radošević R, Bugarski B (2017) Matrix resistance stress: A key parameter for immobilized cell growth regulation. Process Biochem 52:30–43
63. Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33
64. Patrascu E, Rapeanu G, Hopulele T (2009) Current approaches to efficient biotechnological production of ethanol. Innovative Romanian Food Biotechnol 4:1–11
65. Phisalaphong M, Srirattana N, Tanthapanichakoon W (2006) Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation. Biochem Eng J 28:36–43. https://doi.org/10.1016/j.bej.2005.08.039
66. Pilkington PH, Margaritis A, Mensour NA (1998) Mass transfer characteristics of immobilized cells used in fermentation processes. Crit Rev Biotechnol 18:237–255. https://doi.org/10.1080/07388559891224239
67. Pires JMC (2017) Crabtree effect in *Saccharomyces cerevisiae* and its modulation by titanium dioxide nanoparticles. Doctoral Thesis, University of Évora, Évora, Portugal
68. Rasmussen H, Sørensen HR, Meyer AS (2014) Formation of degradation compounds from lignocellulosic biomass in the biorefinery: Sugar reaction mechanisms. Carbohydr Res 385:45–57
69. Ribeiro E, Reis H (2009) Joint influence of pH, temperature and sulfite concentration in alcoholic fermentation of sucrose musts. In: *Proceedings of the IX Internal Meeting and XIII Seminar of Scientific Initiation*, Federal University of Uberlândia, Uberlândia, Brazil. Available at: https://repositorio.ufu.br/handle/123456789/15135
70. Rogacka J, Labus K (2025) Metal-organic frameworks as highly effective platforms for enzyme immobilization – current developments and future perspectives. Braz J Chem Eng 42:1273–1301. https://doi.org/10.1007/s43153-024-00513-4
71. Santos MV (2017) Study of the competence of *Saccharomyces cerevisiae* in co-culture for ethanol production. Master's Dissertation, Federal University of Goiás, Graduate Program in Chemical Engineering, Goiânia, Brazil
72. Schmidell W, Facciotti MCR, Reginatto V (2001) Bioreactors and fermentative processes. In: Schmidell W, Lima UA, Aquarone E, Borzani W (eds) Industrial Biotechnology: Biochemical Engineering. Edgard Blücher, São Paulo, Brazil, pp 179–192
73. Shen H-Y, Moonjai IN, Verstrepen KJ, Delvaux FR (2003) Impact of attachment immobilization on yeast physiology and fermentation performance. J Am Soc Brew Chem 61:79–87
74. Shen L, Wang Y-T, Tang X-X, Wang K, Wang PM, Sui Y, Zheng DQ (2020) Heat shock causes genomic instability and phenotypic variations in yeast. AMB Express 10:101. https://doi.org/10.1186/s13568-020-01091-7
75. Shinmyo A, Kimura H, Okada H (1982) Physiology of α-amylase production by immobilized *Bacillus amyloliquefaciens*. Eur J Appl Microbiol Biotechnol 14:7–12

76. Schuler MM, Marison IW (2012) Real-time monitoring and control of microbial bioprocesses focusing on specific growth rate: Current status and perspectives. Appl Microbiol Biotechnol 94:1469–1482. https://doi.org/10.1007/s00253-012-4095-z
77. Simó G, Fernández-Fernández E, Vila-Crespo J, Ruipérez V, Rodríguez-Nogales JM (2017) Progress in research on alginate gel polymer coating techniques for cell encapsulation. Carbohydr Polym 170:1–14
78. Singer MA, Lindquist S (1998) Multiple effects of trehalose on protein folding, in vitro and in vivo. Mol Cell 1:639–648
79. Sun C, Chang L, Hou K, Liu S, Tang Z (2019) Encapsulation of live cells by metal-organic frameworks for viability protection. Sci China Mater 62:885–891. https://doi.org/10.1007/s40843-018-9384-8
80. Tanabe KK, Cohen SM (2011) Post-synthetic modification of metal-organic frameworks – a progress report. Chem Soc Rev 40:498–519. https://doi.org/10.1039/C0CS00031K
81. Taylor J (2001) Microorganisms and Biotechnology. Nelson Thornes, Cheltenham, UK
82. Torija MJ, Poblet M, Novo M, Beltran G, Guillamón JM, Mas A, Rozès N (2003) Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. Int J Food Microbiol 85:127–136
83. Velvizhi G et al (2022) Integrated biorefinery processes for conversion of lignocellulosic biomass to value-added materials: Paving a path towards circular economy. Bioresour Technol 343:126151. https://doi.org/10.1016/j.biortech.2021.126151
84. Verbelen PJ, De Schutter DP, Delvaux F, Verstrepen KJ, Delvaux FR (2006) Immobilized yeast cell systems for continuous fermentation applications. Biotechnol Lett 28:1515–1525
85. Verstrepen KJ, Klis FM (2006) Flocculation, adhesion and biofilm formation in yeasts. Mol Microbiol 60:5–15
86. Washburne MW, Braun E, Johnston GC, Singer RA (1993) Stationary phase in the yeast *Saccharomyces cerevisiae*. Microbiol Rev 57:383–401
87. Wang Y, Zhang N, Tan D, Qi Z, Wu C (2020) Facile synthesis of enzyme-embedded metal-organic frameworks for size-selective biocatalysis in organic solvent. Front Bioeng Biotechnol 8:714. https://doi.org/10.3389/fbioe.2020.00714
88. Yamamoto N, Maeda Y, Ikeda A, Sakurai H (2008) Regulation of thermotolerance by stress-induced transcription factors in *Saccharomyces cerevisiae*. Eukaryot Cell 7:783–790. https://doi.org/10.1128/EC.00029-08
89. Yang T, Chung T-S (2013) High-performance ZIF-8/PBI nanocomposite membranes for high-temperature hydrogen separation consisting of carbon monoxide and water vapor. Int J Hydrog Energy 38:229–239. https://doi.org/10.1016/j.ijhydene.2012.10.045

Figures

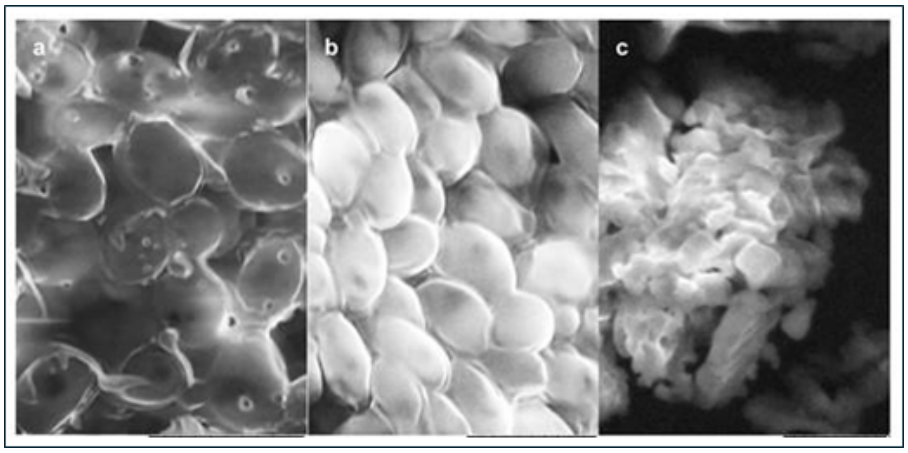


Figure 1

Scanning electron microscopy (SEM) images: (a) free yeast cells, UD10.1x3.0 k, 30 mm; (b) yeast cells immobilized in ZIF-8, UD10.3x2.5k, 30 mm; and (c) ZIF-8 capsules, UD10.3x600k, 100 mm.

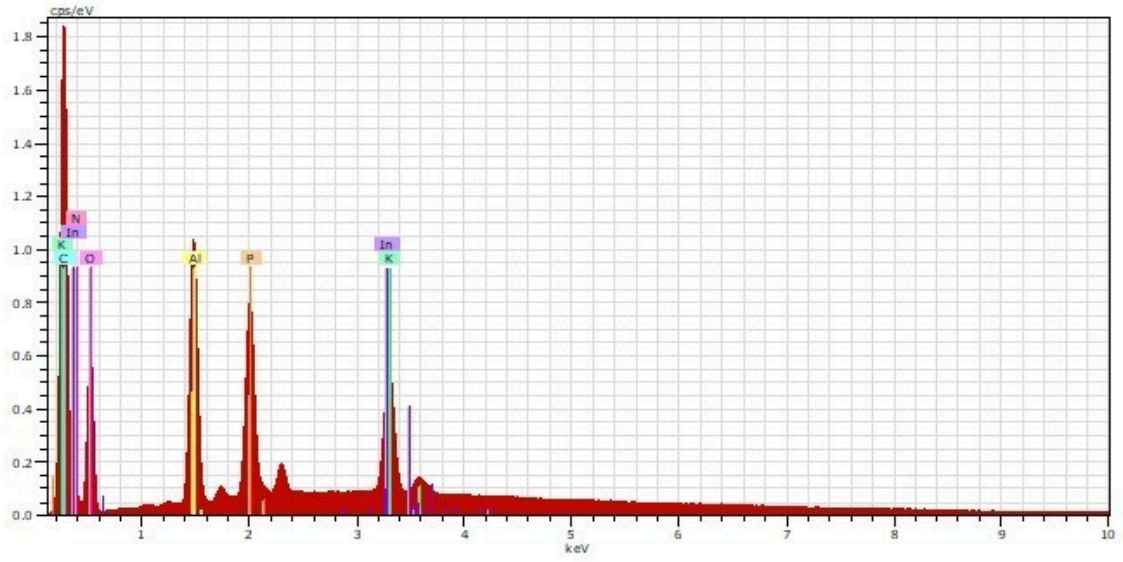


Figure 2

Energy-dispersive spectroscopy (EDS) analysis of free yeast cells.

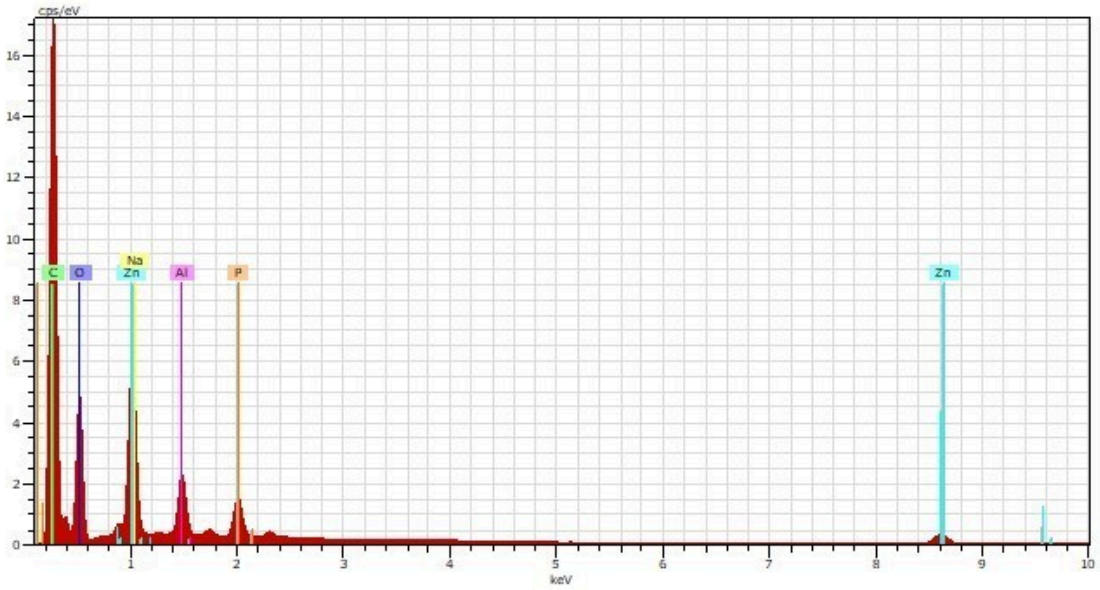


Figure 3

Energy-dispersive spectroscopy (EDS) analysis of yeast cells immobilized in ZIF-8.

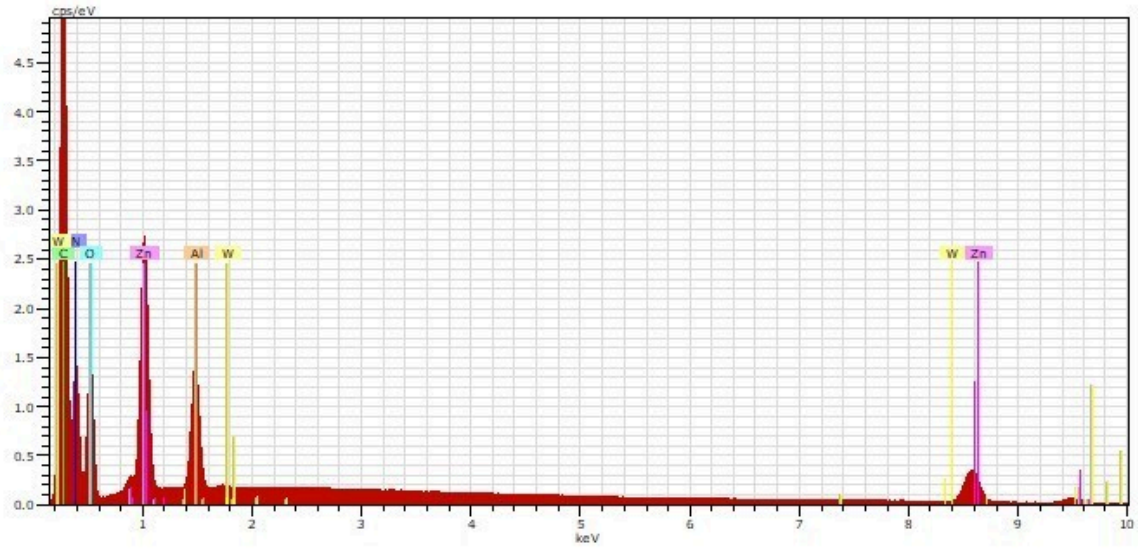


Figure 4

Energy-dispersive spectroscopy (EDS) analysis of ZIF-8 capsules.

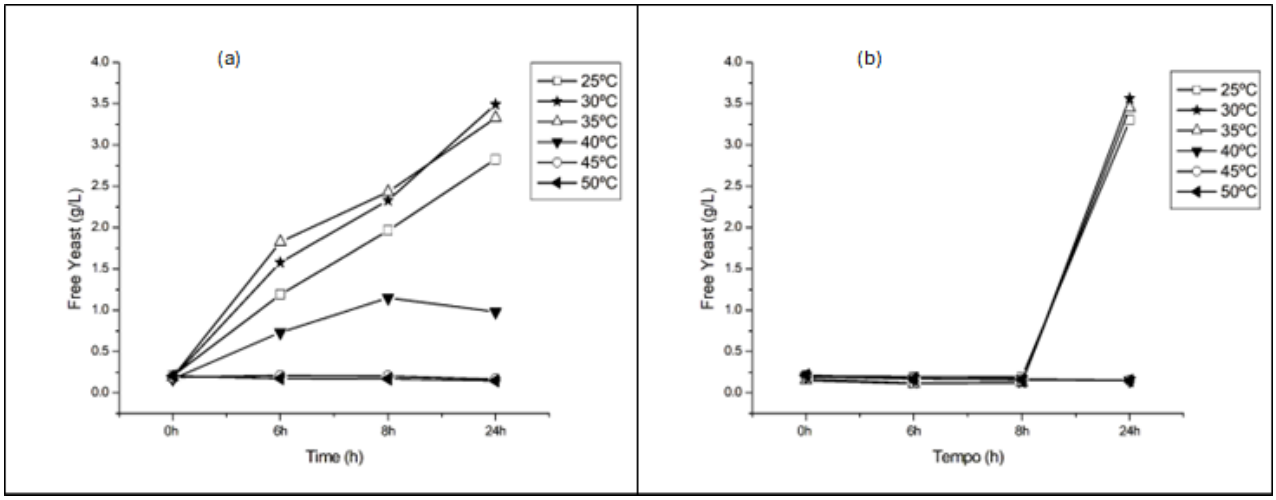


Figure 5

Cell growth profile during aerobic cultivation as a function of time at different temperatures for *Saccharomyces cerevisiae*: (a) free cells; (b) cells immobilized in ZIF 8.

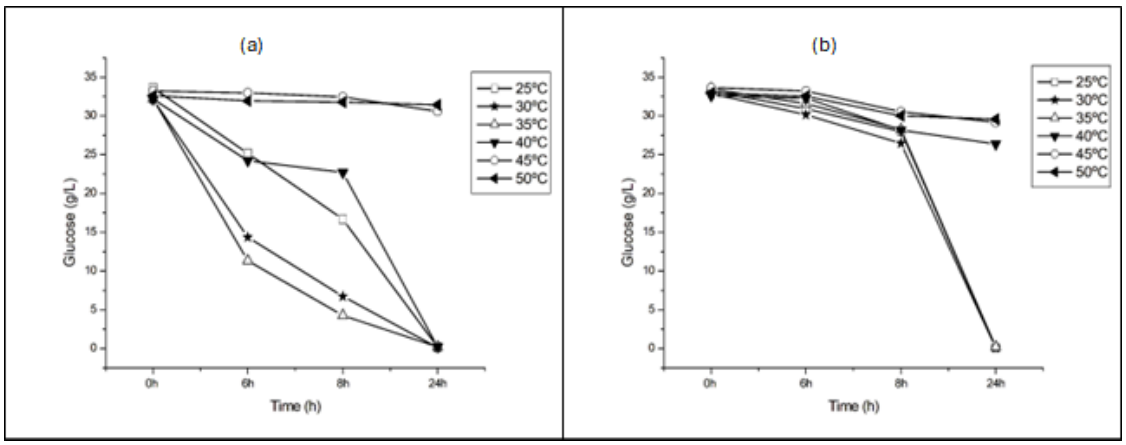


Figure 6

Glucose consumption profile during aerobic cultivation as a function of time at different temperatures for *Saccharomyces cerevisiae*: (a) free cells; (b) cells immobilized in ZIF 8.

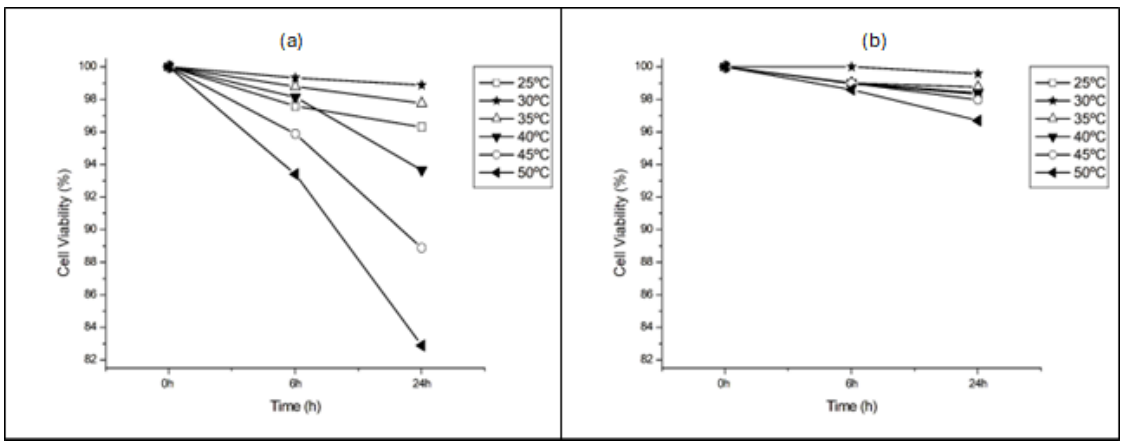


Figure 7

Cell viability as a function of time and temperature for *Saccharomyces cerevisiae*: (a) free cells; (b) cells immobilized in ZIF 8.

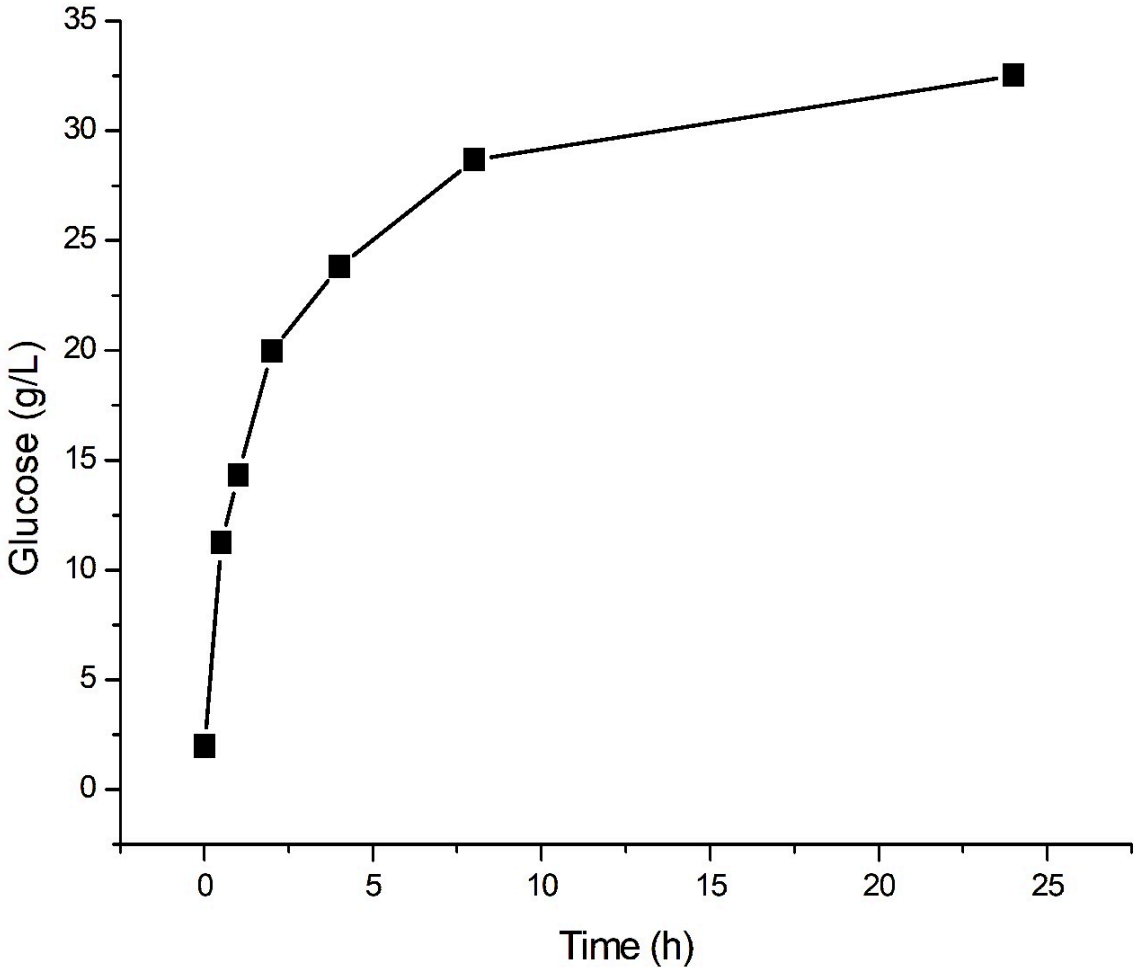


Figure 8

Glucose concentration during enzymatic hydrolysis of pretreated passion fruit peel over time.

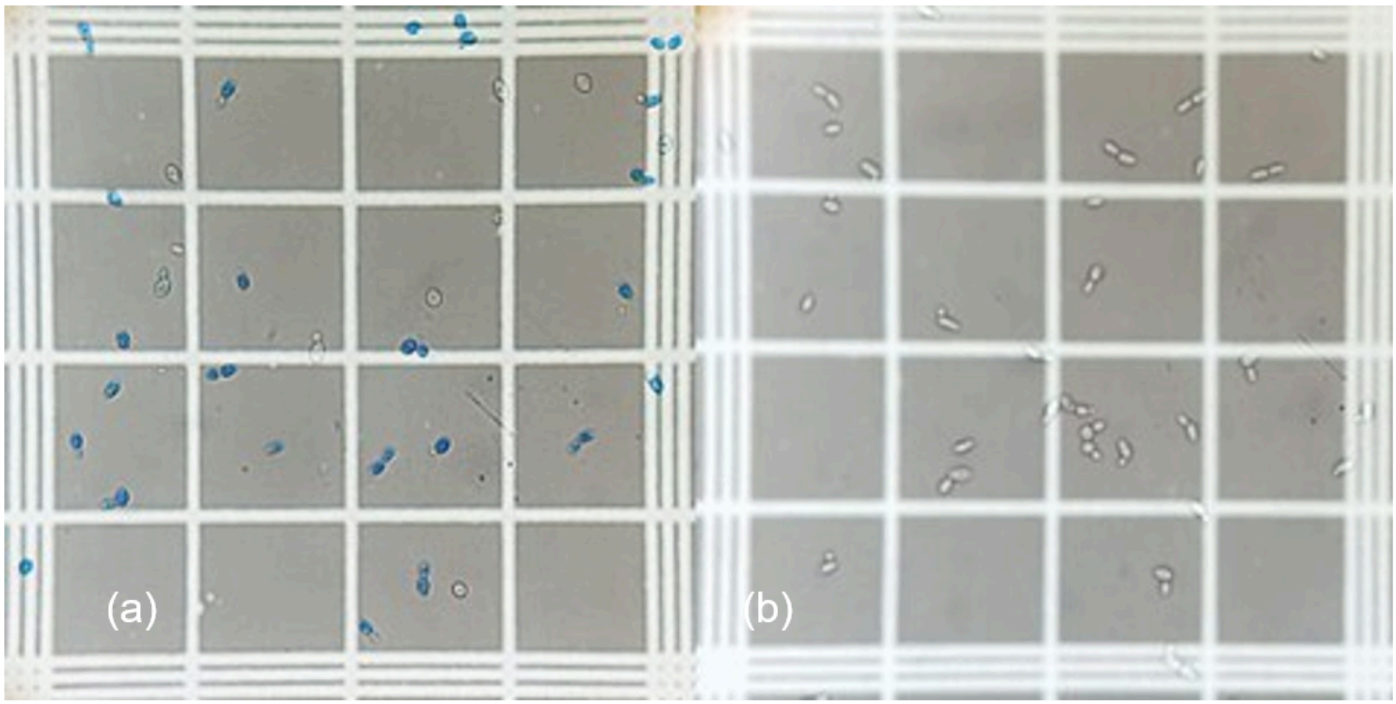


Figure 9

Microscopic images of free and ZIF-8-immobilized *Saccharomyces cerevisiae*: a) Free cells after 24 hours of fermentation b) Immobilized cells after 48 hours of fermentation

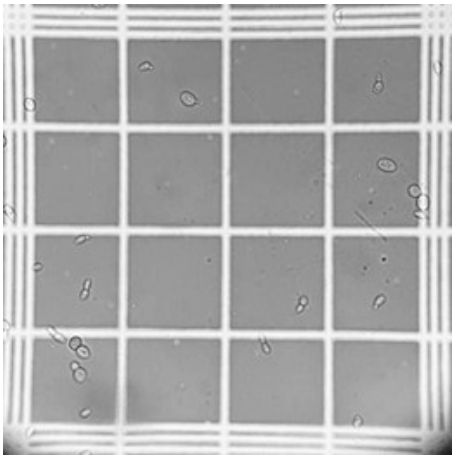


Figure 10

Microscopic image of *Saccharomyces cerevisiae* immobilized in ZIF-8 after 30 hours of fermentation.