Study of the basic laws and parameters of immobilization of yeast cells on various carriers for the production of bioethanol

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Abstract. Over the past few years, due to a sharp increase in oil and gas prices, as well as a number of environmental problems, the world has seen a rapid increase in the consumption of bioethanol, the production of which o based on the use of biorenewable raw materials. Today, over 90% of bioethanol is produced, as a rule, using yeast cells (Saccharomyces cerevisiae) as catalysts. With the help of microorganisms immobilized by cells, it is possible to organize economically efficient production of ethanol based on whey. A bioreactor with immobilized yeast cells allows for a continuous process of whey fermentation under optimal conditions with a high yield of bioethanol. Immobilized yeast cells have been found to be superior to free yeast cells because immobilized cells are more ethanol resistant and have lower substrate inhibition. Various researchers have concluded that immobilized S. cerevisiae produces more ethanol compared to free cells, although the immobilizing agents used varied.

1 Introduction

Use The industrial-scale production of cellulosic ethanol faces significant hurdles, primarily due to the high costs associated with processing [1]. A key cost driver is the substantial energy consumption, particularly steam, required for distilling the low ethanol content broth obtained from lignocellulosic materials. Despite these challenges, cost-effective ethanol production from lignocellulosic sources is feasible with the utilization of S. cerevisiae [2]. Several strategies have been developed to increase cellulose content in the fermentation system, leading to higher ethanol yields and reduced expenses. The fermentation process, which serves as the cornerstone of ethanol production, involves a variety of microorganisms such as fungi, yeast, and bacteria [3]. Among these, *S. cerevisiae* stands out as one of the most widely utilized yeast species in industrial production. It is renowned for its ability to produce ethyl alcohol, the primary product of fermentation.

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Compared to other yeast fungi and bacteria, *S. cerevisiae* demonstrates superior physiological properties during ethyl alcohol production. Notably, it exhibits resilience across a broad range of acidity levels, facilitating enzymatic activity, and displays higher resistance to ethanol compared to many other bacteria [5]. *S. cerevisiae* is classified as a safe yeast, rendering it more advantageous for human applications compared to many other bacteria and fungi. This article delves into recent developments in ethanol production employing S. cerevisiae from various angles, including substrates, inhibitors, restoration of biomass hydrolysates, growth conditions, co-cultivation with other bacteria, and a range of immobilization techniques. It specifically aims to assess the present status of ethanol production utilizing S. cerevisiae cells, with a particular emphasis on factors like substrates, inhibitors, growth conditions, interactions in co-cultivation with other bacteria, and different methods of immobilization. Sodium or calcium alginate and agar-agar cubes are among the most commonly employed immobilizing agents in these processes [5]. In pursuit of alternatives, numerous studies have explored novel immobilizing agents characterized by their affordability and simplicity of use. These include sugar cake, alginate-chitosan granules, fragments of corn cobs, sweet sorghum core, a composite matrix comprised of crushed tissue from alginate-corn stalks, cashew and apple pulp, lyophilized cellulose gel, dried spongy onions (Luacylindrica L.), carboxymethyl cellulose, N-vinyl-pyrrolidone, and sodium alginate [6]. Yeast cell immobilization is regarded as a promising technique for improving ethanol production. Immobilizing yeast cells helps mitigate contamination risks and facilitates the separation of biomass from the liquid phase, thereby enhancing process efficiency. This method contributes to maintaining the stability of cellular activity, resulting in heightened production levels. Moreover, it offers cost-effectiveness, enables the reuse of the biocatalyst, shortens fermentation duration, and shields cells from inhibitors [7]. Several studies have demonstrated that immobilized *S. cerevisiae* cells yield higher ethanol quantities compared to their free counterparts. The concentration of yeast cells plays a vital role in ethanol production during immobilization, with elevated concentrations of primary yeast cells correlating with increased rates of sugar consumption and ethanol production. Furthermore, *S. cerevisiae* immobilized using sodium alginate cross-linked with N-vinyl-2-pyrrolidone exhibits greater ethanol production than when solely using sodium alginate as the immobilization agent [8].

Over the past two decades, significant research efforts have been dedicated to developing novel types of biocatalysts, particularly immobilized microorganism cells [9]. Studies indicate that utilizing immobilized microbial cells offers a viable means to economically produce ethanol from whey. Whey, being a low-cost substrate, presents a favorable economic advantage. Bioreactors employing immobilized microbial cells demonstrate significantly higher productivity compared to traditional ones utilizing free cells, with reported increases ranging from 3 to 50 times by various authors. The majority of publications detail bioreactors incorporating yeast cells within hydrophilic gels, although there are also mentions of sorption and chemical immobilization techniques [10]. As demonstrated by the examples provided, the utilization of immobilized yeast cells enables the establishment of a continuous whey fermentation process under optimal conditions, resulting in a high yield of bioethanol.

2 Materials and Methods

2.1 Isolation of pure culture

When investigating yeast growth activity in whey cultures, research methods such as the Koch method, optical density determination, and microscopy were employed to study growth dynamics and optical density changes [11].

2.2 Immobilization method

The immobilization method involves yeast being immobilized within sodium alginate gels. This process begins with suspending yeast biomass in a 3.3% solution of sodium alginate, followed by introduction into a 2% solution of calcium chloride. This results in the formation of spherical biocatalysts, approximately 3 mm in diameter, which are left in the calcium chloride solution for 60 minutes. Afterward, the biocatalyst is washed with running water and utilized as intended. Notably, no free yeast cells remain due to fermentation. However, a drawback of this method is that when sodium alginate gel particles containing yeast cells are coated with an additional layer of sodium alginate, the flow of nutrients to and from inactive cells is significantly hindered, negatively impacting the quality of the final product, particularly its organoleptic properties.

An alternative method involves immobilization in cryogels using polyvinyl alcohol (PVA) and carrageenan. This process begins with purifying microorganisms from the environment using a 0.9% sodium chloride solution. Gels with concentrations of 8% PVA and 1% carrageenan are prepared, and yeast is immobilized within these gels. The mixture is rapidly frozen in a freezer, reaching temperatures ranging from -8°C to -25°C, which facilitates the cross-linking of yeast cells while maintaining a semi-frozen state, suspended in an unfrozen liquid microphase. This process ensures the creation of robust cryogels with yeast cells embedded within. These cryogels are kept at temperatures of -8°C to -25°C for 24 hours before being thawed at room temperature. This cycle can be repeated to further reinforce the structure. The viability of yeast cells post-freezing and cryogel formation is assessed using the methylene blue staining method [12].

2.5 Statistical analysis

Statistical data processing was conducted using RStudio software (version 1.3.959, RStudio PBC, 2020). Two-way repeated measures analysis of variance (RM ANOVA) was carried out to detect a statistically significant difference between the values of cell number during cultivation (strain × time). In cases where a significant difference was demonstrated by ANOVA, Tukey's HSD test was performed for pairwise comparison. Treatments were categorized (by letters in descending gradation) according to the results of this test, and box plots/graphs were created.

3 Results and Discussion

Study of the biochemical activity of yeast cells. Milk whey is a valuable source of raw materials for protein synthesis due to its richness in carbohydrates (lactose), minerals, and vitamins. Yeast is recognized as one of the most beneficial sources of protein within whey, capable of utilizing lactose as a nutrient medium. Dry yeast typically comprises 45–50% nitrogenous substances, 2–5% fat, 25–35% carbohydrates, and 6–8% ash. The nitrogen compounds in yeast are deemed to have complete biological value as they contain a plentiful supply of essential amino acids crucial for maintaining health [13]. In this research work, the dynamics of the growth of yeast and lactic acid bacteria on whey samples were studied. The results are presented in Figure 1.

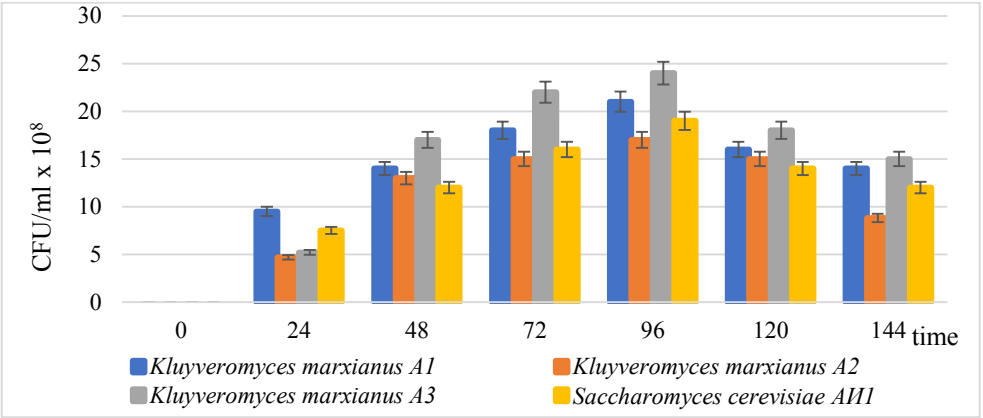

Fig. 1. Dynamics of growth of yeast and lactic acid bacteria cultures on milk whey

As depicted in Figure 4, the investigation into the growth dynamics of yeast cultures on whey revealed significant biomass accumulation activity across all strains. Notably, among the yeast strains examined, *Kluyveromyces marxianus* A3 exhibited the highest biochemical activity towards whey, with a cell count of $2.4x10^9$ CFU/ml. *Saccharomyces cerevisiae AI1* and *Kluyveromyces marxianus A2* demonstrated cell counts ranging from $1.7x10^9$ to $1.9x10^9$ CFU/ml. Additionally, the *Kluyveromyces marxianus A1* strain displayed comparatively high activity, with a cell count of $2.1x10^9$ CFU/ml.

According to literature sources, the yeast *Kluyveromyces marxianus* showcases the capability to utilize lactose and inulin as carbon sources, alongside producing highly active β-fructofuranosidase. Renowned for their rapid growth rate, these yeast strains find widespread application in biotechnological production, particularly in protein synthesis [14]. These data are confirmed by our research results.

Throughout the growth process, yeast utilizes whey lactose and lactic acid as energy sources, converting mineral nitrogen-containing salts into complete cellular protein. Literature sources indicate that yeast-derived whey exhibits a significantly higher protein content compared to the original whey [15]. The availability of easily digestible carbon sources and growth factors within whey is viewed as a promising resource in biotechnological processes [16]. Furthermore, the issue of fully and efficiently utilizing whey is pertinent from both economic and environmental perspectives.

Various methods for processing whey are known, but among them, whey fermentation with different lactose-fermenting yeast cultures for ethanol production remains relevant and is of great interest to researchers. The study of immobilized yeast cells has revealed important regularities and parameters. Immobilization of microorganism cells is seen as an effective approach to enhancing and making modern biotechnological processes more economically attractive. This method offers several advantages, including long-term stability of cells, enhanced molecular selectivity, greater resistance to inhibition, improved cell protection from the environment, increased surface area of the biocatalyst per unit volume of the bioreactor, minimal loss of activity during immobilization and fermentation, reduced lag phase, and shorter reaction times. Immobilized microorganism cells can be utilized for extended durations with high yields of target products, simplifying technical solutions in comparison to processes employing free cells. Moreover, they maintain high cell densities throughout the process, thereby lowering biotechnological process costs.

During the research, the process of yeast cell immobilization on various carriers was investigated, examining patterns and parameters. Methods for immobilization, carrier properties and characteristics, as well as the properties of the immobilized yeast, were also

determined. Carriers such as silica gel, glass beads, polyvinyl alcohol (PVA), carrageenan, and sodium alginate were utilized. Various yeast strains, including *Kluyveromyces marxianus A1, Kluyveromyces marxianus A2, Kluyveromyces marxianus A3*, and *Saccharomyces cerevisiae AI1*, were studied.

In this study, carriers were selected and modified based on yeast cells isolated from different substrates. Among the immobilization methods, capture or encapsulation of cells in a gel or adsorption on a solid carrier were found to be common and effective. Polymer cryogels, formed from frozen solutions of polymer or monomer precursors, were chosen for further research. The selected carriers for yeast cell immobilization included polyvinyl alcohol (PVA), carrageenan, and sodium alginate. Yeast cultures were immobilized on various polymer cryogels—polyvinyl alcohol (PVA), carrageenan, and sodium alginate. The yeast strains studied included *Kluyveromyces marxianus A1, Kluyveromyces marxianus A2, Kluyveromyces marxianus A3*, and *Saccharomyces cerevisiae AI1*.

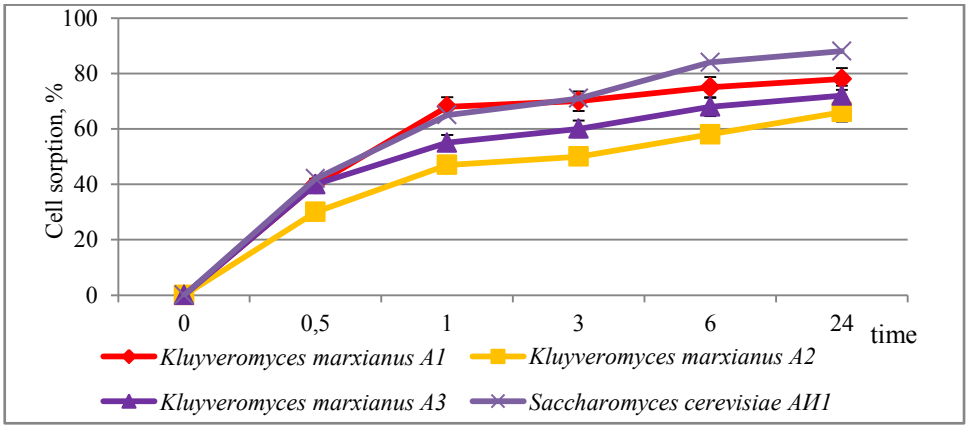

Fig. 2. Adsorption of yeast cells onto polyvinyl alcohol

The yeast strains *Kluyveromyces marxianus A1, Kluyveromyces marxianus A2* and *Kluyveromyces marxianus A3* demonstrated notable sorption activity on PVA, with sorption percentages of 88%, 90%, 87%, and 78%, respectively. In contrast, the yeast strain *Saccharomyces cerevisiae AI1* exhibited lower sorption activity towards PVA, with sorption percentages of 66% and 72%.

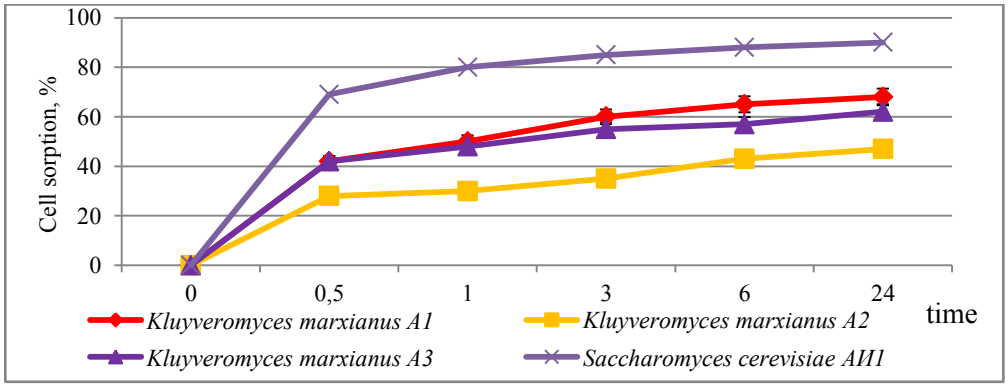

Fig. 3. Adsorption of yeast cells on carrageenan

The findings from the immobilization of yeast cells on carrageenan revealed that the *Saccharomyces cerevisiae AI1* strain exhibited a higher number of sorbed cells compared to other yeast cultures. Sorption percentages of yeast cells on carrageenan ranged from 69% to 90%.

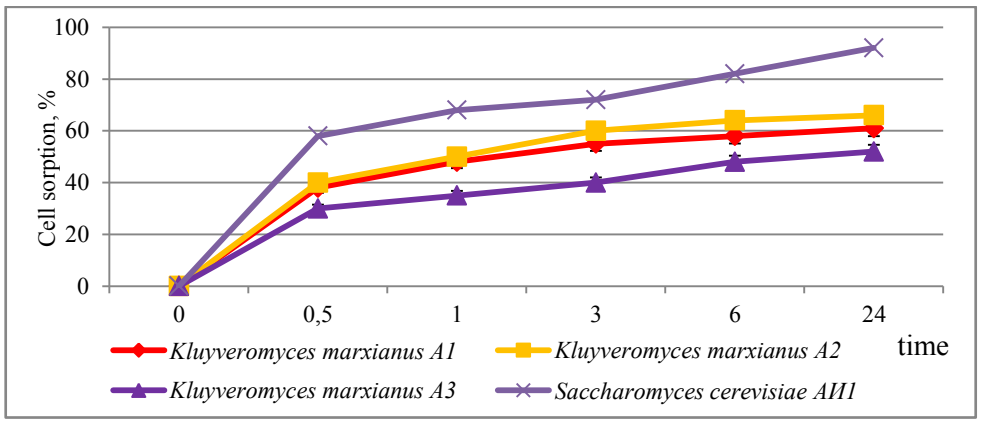

Fig. 4. Adsorption of yeast cells on sodium alginate

The *Saccharomyces cerevisiae* yeast strains *AI1* exhibited notable sorption activity for sodium alginate, with cell sorption ranging from 58% to 92%. In contrast, the strains *Kluyveromyces marxianus A1, Kluyveromyces marxianus A2*, and *Kluyveromyces marxianus A3* displayed lower sorption activity, with sorption percentages in the range of 30% to 66%.

Overall, the immobilization of yeast cells on various carriers demonstrated high sorption capacity for yeast strains *Kluyveromyces marxianus A1, Kluyveromyces marxianus A2, Kluyveromyces marxianus A3*, and *Saccharomyces cerevisiae AI1* across all sorbents used.

4 Conclusion

The investigation into the growth dynamics of immobilized yeast cell cultures in whey revealed vigorous growth and robust vitality indicators, suggesting that carriers composed of polymer cryogels exhibit non-toxic properties for microbial cells. These findings underscore the potential of utilizing immobilized yeast cells for efficient bioethanol production.

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Authors' contribution

"Conceptualization, G.A. and P.S.; Methodology, G.A.; Software, A.M.; Formal Analysis, A.M.; Investigation, A.A.; Resources, A.A; Writing – Original Draft Preparation, G.A.;

Writing – Review & Editing, A.M.; Visualization, A.A.; Supervision, A.S; Project Administration, G.A.;

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