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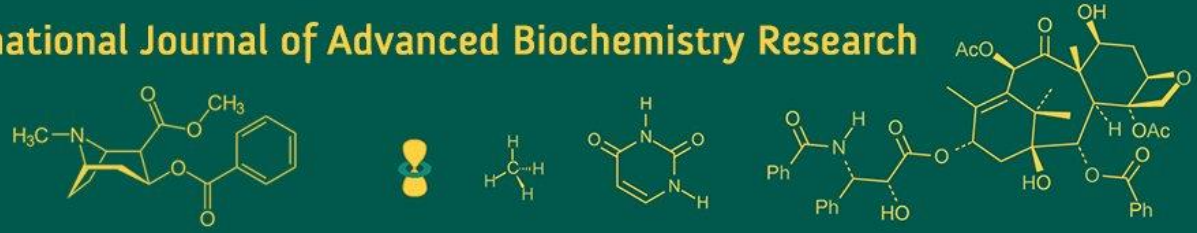

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Production and optimization of pullulan biopolymer using *Auerobasidium pullulans* under controlled fermentation conditions

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Abstract
This study explores the optimization of pullulan production by *Aureobasidium pullulans* under various fermentation conditions. The impact of various fermentation parameters, such as temperature, pH, inoculum size, carbon, and nitrogen sources, was systematically evaluated. The optimal temperature for pullulan production was found to be 28 °C, with a marked decrease in yield at higher temperatures due to thermal stress. The optimal pH was determined to be 5, with significant reductions in pullulan yield observed at both acidic and alkaline extremes. Inoculum size experiments revealed that a 1.5% inoculum resulted in the highest pullulan production, while larger inoculum sizes caused overcrowding effects, reducing yields. Among the carbon sources tested, glucose was the most effective, leading to the highest pullulan production. Yeast extract was identified as the most favourable nitrogen source, with a concentration of 0.5% optimizing pullulan synthesis. The time course of pullulan production revealed a peak yield of 7.850 g/L on Day 7 (168 hours), after which a decline in production was noted, likely due to nutrient depletion and accumulation of metabolic by-products. Sugar utilization patterns also demonstrated near-complete consumption by 168 hours, marking the end of optimal fermentation conditions. These findings provide valuable insights into the key parameters for optimizing pullulan production, contributing to enhanced yields and more efficient industrial-scale biopolymer production.

Keywords: *Aureobasidium pullulans*, pullulan production, fermentation optimization, biopolymer, industrial-scale production

Introduction
Pullulan is a versatile, extracellular polysaccharide produced by the fungus *Aureobasidium pullulans*. It is composed of repeating maltotriose units linked by α-(1→4) and α-(1→6) glycosidic bonds, giving it unique physicochemical properties. These include excellent film-forming ability, high solubility in water, and non-toxicity, which have established pullulan as a valuable biopolymer in various industries. Its applications span food, pharmaceuticals, cosmetics, and packaging, where it serves as an edible coating, stabilizer, and environmentally friendly alternative to synthetic plastics. Additionally, its biocompatibility and biodegradability make it a promising material for emerging fields such as tissue engineering and drug delivery (Souza *et al*., 2023) [7]. In the food industry, pullulan is widely used as an edible film and coating to preserve freshness and extend shelf life. Its non-toxic and tasteless nature makes it ideal for encapsulating flavors, enhancing the appeal of various food products. In pharmaceuticals, pullulan finds applications in creating capsules and drug delivery systems due to its biocompatibility and ability to form hydrophilic films. It is also employed in wound dressings and tissue scaffolds, highlighting its significance in medical and healthcare applications. The cosmetics industry benefits from pullulan's film-forming properties, using it in skin-care products and facial masks to provide an instant tightening effect. Furthermore, pullulan serves as a biodegradable and compostable alternative to synthetic plastics in the packaging industry, addressing the global challenge of plastic waste. Its role in agriculture includes applications as seed coatings and biodegradable films, promoting sustainable farming practices (Prajapati *et al*., 2013) [14].

Despite its extensive applications, the production of pullulan faces challenges, primarily due to the high costs associated with its fermentation process. Optimizing pullulan production is crucial to overcoming these challenges and ensuring its economic feasibility. Fine-tuning critical parameters such as pH, temperature, carbon and nitrogen sources, and agitation speed can significantly enhance the yield and reduce production costs. Such optimization not only makes pullulan more accessible for industrial applications but also supports the growing demand for sustainable and biodegradable materials (Badhwar *et al*., 2019) [2].

The importance of optimizing pullulan production extends beyond economic factors. With industries increasingly shifting toward greener alternatives, pullulan emerges as a key material supporting sustainable development. Its efficient production can drive innovation in various sectors, contributing to a circular economy and addressing pressing environmental challenges. Research focused on enhancing pullulan yield is therefore essential, laying the foundation for its broader adoption and advancing its role in creating a sustainable future (Eldadamony *et al*., 2024) [8].

Material and Methods

Microorganism and inoculum maintenance

The yeast-like fungal strain *Auerobasidium pullulans* MTCC 2013 was sourced from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The strain was stored on Yeast Malt Agar (YMA) plates at 4 °C and sub cultured every two weeks to maintain viability.

Fermentation conditions

A loopful of *A. pullulans* culture, grown on YMA plates, was transferred into 250 ml Erlenmeyer flasks containing 100 ml of sterile seed medium prepared with Yeast Malt Broth (YMB). The seed medium was composed of glucose (25 g/L), yeast extract (0.4 g/L), dipotassium hydrogen phosphate (K_2HPO_4, 5 g/L), sodium chloride (NaCl, 1 g/L), and magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, 0.4 g/L). The flasks were incubated in an orbital shaker at 180 rpm and maintained at 25 ± 2 °C for 72 hours. The resultant culture, referred to as the seed culture, was subsequently utilized to inoculate the fermentation media prepared according to the experimental design.

To determine fungal biomass, 10 ml of the fermented broth was centrifuged at 12,000 rpm for 10 minutes at 4 °C. The resulting cell pellet was washed twice with sterilized distilled water and centrifuged again to ensure the removal of impurities. The purified pellet was dried overnight at 80 °C before being weighed (Singh *et al*., 2009) [16].

Optimization of fermentation conditions

Pullulan production was optimized by independently analyzing the influence of various process parameters in a sequential manner. The optimized conditions obtained for one parameter were applied in subsequent experiments to determine the next optimized parameter. Initially, the basal fermentation medium comprised glucose (2.5%), ammonium sulfate (0.06%), dipotassium hydrogen orthophosphate (0.5%), sodium chloride (0.1%), magnesium sulfate (0.04%), and yeast extract (0.04%), with the pH adjusted to 5.0. This medium was inoculated with *Auerobasidium pullulans* at a concentration of 5 x 10^8 CFU/ml and incubated for varying periods, such as 24, 48, 72, 96, 120, 144, 168 and 192 hours, under different temperatures (30, 37, 43, 50, and 60 °C). The pullulan production was carried out via shake flask fermentation. To evaluate the effect of pH, the basal medium's pH was adjusted to 2.0, 3.0, 5.0, 7.0, and 9.0 using either 1N HCl or 1N NaOH. For optimizing pullulan production, the strain's growth medium was supplemented with varying carbon sources, including glucose, sucrose, and maltose (each at a concentration of 2%), and nitrogen sources such as ammonium sulfate, yeast extract, and sodium nitrate (each at 0.5%). Fermentation was conducted in 250 ml Erlenmeyer flasks with a working volume of 100 ml. The fermentation medium was sterilized at 121 °C for 15 minutes prior to inoculation. Incubation was carried out at 30 °C under the previously optimized conditions. This approach allowed for systematic optimization of fermentation conditions to achieve enhanced pullulan production.

Pullulan extraction

The supernatant obtained after separating fungal biomass was used for pullulan isolation. As illustrated in Figure 1, the process involved adding 95% ice-cold ethanol in a 2:1 ratio (ethanol: supernatant) to precipitate pullulan. The mixture was incubated at 4 °C for 24 hours to facilitate crude pullulan precipitation. The precipitated material was collected by centrifugation at 12,000 rpm for 20 minutes at 4 °C, followed by drying at 80 °C overnight. The dried pullulan was then weighed (Chen *et al*., 2012b) [4].

Assay of reducing sugar

Residual sugar levels in the fermentation broth were quantified using the DNS (3,5-dinitrosalicylic acid) method as described by Miller (Miller, 1959).

Statistical analysis

The experimental design followed a Completely Randomized Design (CRD). All experiments were conducted in triplicate, and the results were reported as mean values along with their respective standard deviations to ensure reliability and accuracy of the data.

Results and Discussion

The colony morphology of *Aureobasidium pullulans* grown on Yeast Malt Agar (YMA) medium is shown in Figure 1.

Fig 1: Colony morphology of *Aureobasidium pullulans* on Yeast Malt Agar (YMA)

The colonies exhibit a characteristic brownish-black pigmentation, typical of this fungal strain. This coloration is indicative of the natural pigmentation of the fungal cells, which is often associated with the production of secondary metabolites during growth.

The precipitation of pullulan biopolymer, as shown in Figure 2, occurs following the addition of chilled ethanol to the fermentation supernatant. This leads to the formation of a distinct white, cotton-like precipitate, confirming the successful biosynthesis and recovery of the pullulan biopolymer.

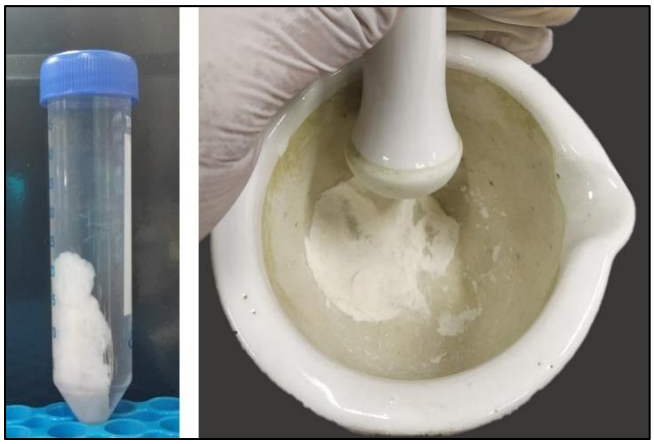

Fig 2: Pullulan precipitation and dried pullulan powder

The appearance of this white mass is a result of the polymer's high molecular weight and solubility properties under the ethanol precipitation conditions, demonstrating the efficient synthesis of pullulan by *A. pullulans* during the fermentation process. Following pullulan formation, various optimization strategies were implemented to further enhance production, including adjustments in fermentation parameters such as carbon source concentration, pH, temperature, and nutrient levels.

Effect of temperature and pullulan production

The effect of temperature on pullulan production was studied by varying the temperature from 25 °C to 60 °C, and the corresponding pullulan yields were recorded. The results are presented in Figure 3, which shows the pullulan production at different temperatures during fermentation.

As indicated in Figure 3, pullulan production was observed to be optimal at 28 °C, where the highest yield of 4.267 g/L was recorded. At this temperature, the microbial activity was likely at its peak, as it supports optimal enzymatic activity and metabolic processes for pullulan synthesis. A further increase in temperature to 32 °C resulted in a significant decrease in pullulan production, with a yield of 2.431 g/L. This decline could be attributed to the fact that higher temperatures may stress the microorganisms, affecting their growth and metabolic pathways. At 37 °C, the pullulan yield continued to decrease to 1.212 g/L, and with temperatures increasing further, the yield continued to drop significantly. At temperatures above 42 °C, the production of pullulan decreased drastically, with yields at 50 °C (0.197 g/L) and 60 °C (0.087 g/L) being the lowest, indicating that elevated temperatures are detrimental to the growth and activity of *Auerobasidium pullulans* in the production of pullulan.

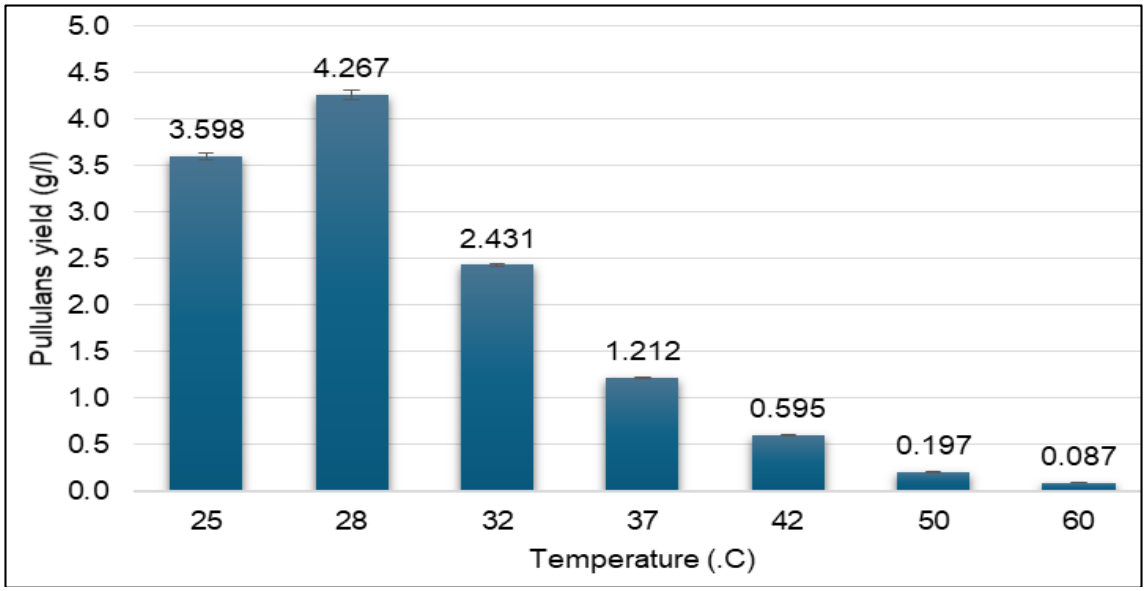

Fig 3: Effect of different temperature on pullulan production

This data suggests that temperature plays a critical role in pullulan production, with moderate temperatures (around 28 °C) being most favorable for maximizing yield. Temperatures higher than 32 °C, especially those above 40 °C, inhibit microbial growth and pullulan synthesis, likely due to thermal stress on the microbial cells or a reduction in enzyme activity essential for biopolymer production. However, the optimal temperature may vary depending on the *A. pullulans* strain employed; for instance, Chen *et al.* (2019a) [3] and Cheng *et al.* (2009) [5] reported 30 °C as the ideal temperature for maximizing pullulan yield, while Gaur *et al.* (2010) [9] identified 28 °C as the optimal temperature for production.

Effect of initial pH on pullulan production

The effect of pH on pullulan production was examined by varying the pH levels from 2 to 9, and the corresponding pullulan yields were recorded. The results are presented in Figure 4, shows the pullulan production at different pH values during fermentation.

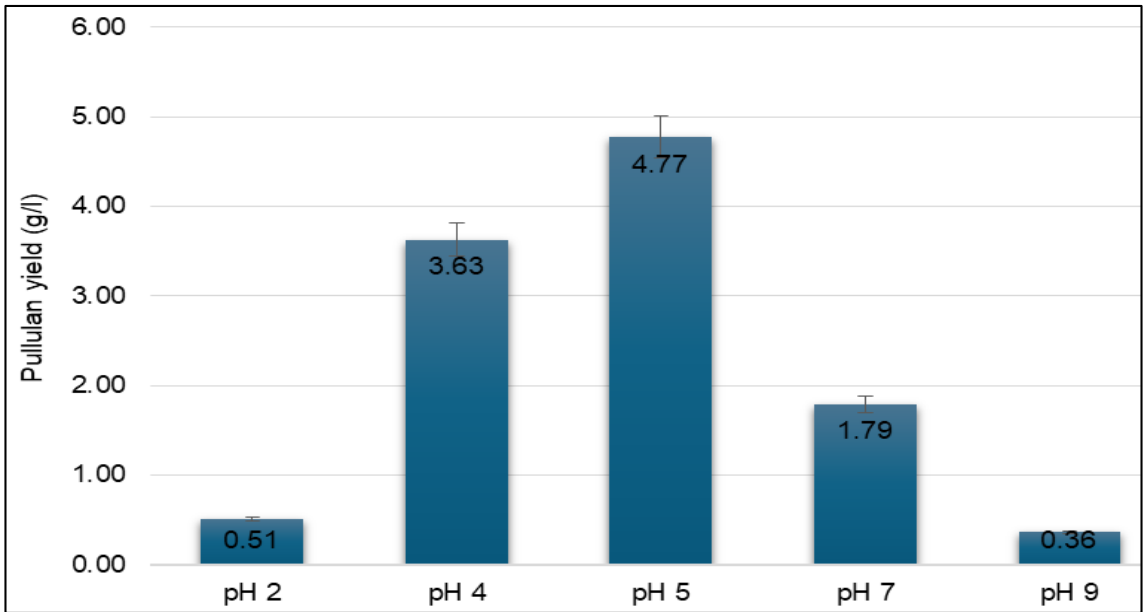

Fig 4: Effect of pH on pullulan production

As shown in Figure 4, the highest pullulan yield of 4.77 g/L was achieved at pH 5, indicating that this slightly acidic pH condition is optimal for pullulan production. At pH 4, the pullulan yield was still relatively high at 3.63 g/L, but the yield decreased as the pH moved away from this optimal value. At pH 7 (neutral), the pullulan yield was 1.79 g/L, which represents a significant drop compared to pH 4 and 5. At the extreme pH values, both low (pH 2) and high (pH 9), the pullulan yield was considerably reduced, with values of 0.51 g/L and 0.36 g/L, respectively. This suggests that the production of pullulan is highly sensitive to deviations from the optimal pH range. Acidic conditions (pH 2) may inhibit microbial growth and enzyme activity, while alkaline conditions (pH 9) likely cause stress on the cells, reducing their metabolic activity and, consequently, pullulan synthesis.

The data clearly indicates that the optimal pH for pullulan production lies between 4 and 5. Various studies support this observation, with Sugumaran & Ponnusami (2015) [18] and Gaur *et al*. (2010) [9] reporting pH 5 as the ideal condition for maximizing pullulan yield. Outside this range, the production of pullulan is significantly reduced, which can be attributed to the effect of pH on the microorganism's growth and enzymatic functions. In highly acidic or alkaline environments, the integrity of microbial cells may be compromised, and enzymes responsible for pullulan biosynthesis may become less efficient or inactive.

Effect of different nitrogen sources on pullulan production

The effect of different nitrogen sources, including sodium nitrate, yeast extract, and ammonium sulfate, on pullulan production by *Auerobasidium pullulans* was investigated. The results showed distinct variations in pullulan yields depending on the nitrogen source used. The pullulan yield for each nitrogen source was measured and the results are summarized in Figure 5.

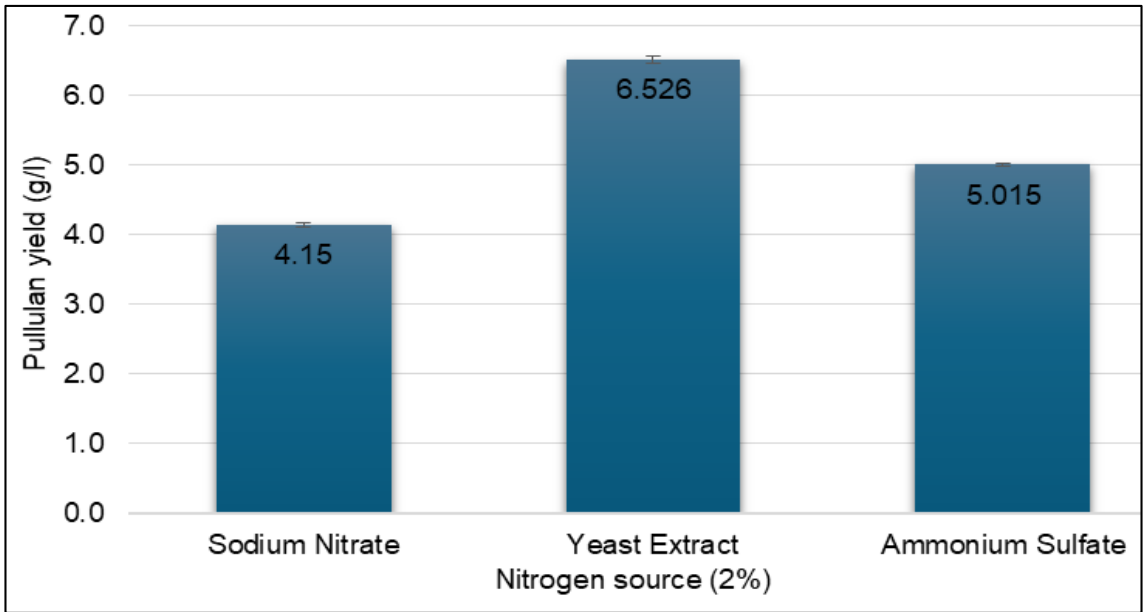

Fig 5: Effect of different nitrogen sources on pullulan production

From the data presented in Figure 5, it is evident that yeast extract yielded the highest pullulan production of 6.526 g/L, followed by ammonium sulfate at 5.015 g/L. Sodium nitrate, an inorganic nitrogen source, produced the lowest yield of 4.150 g/L. These results suggest that organic nitrogen sources, particularly yeast extract, provide more favorable conditions for pullulan production than inorganic sources like sodium nitrate. Yeast extract, being rich in amino acids, peptides, and vitamins, likely supports optimal microbial growth and enhances enzymatic activity, which is crucial for the biosynthesis of pullulan. In contrast, sodium nitrate, despite being a common nitrogen source in many microbial fermentations, resulted in the lowest yield in this study, likely due to its less efficient assimilation by *Auerobasidium pullulans* for pullulan production. Ammonium sulfate, another inorganic nitrogen source, gave a moderate yield, indicating that it could also support growth and pullulan synthesis but not as effectively as yeast extract.

Effect of yeast extract concentration on pullulan production

To further optimize pullulan production, various concentrations of yeast extract were tested. The results are presented in Figure 6.

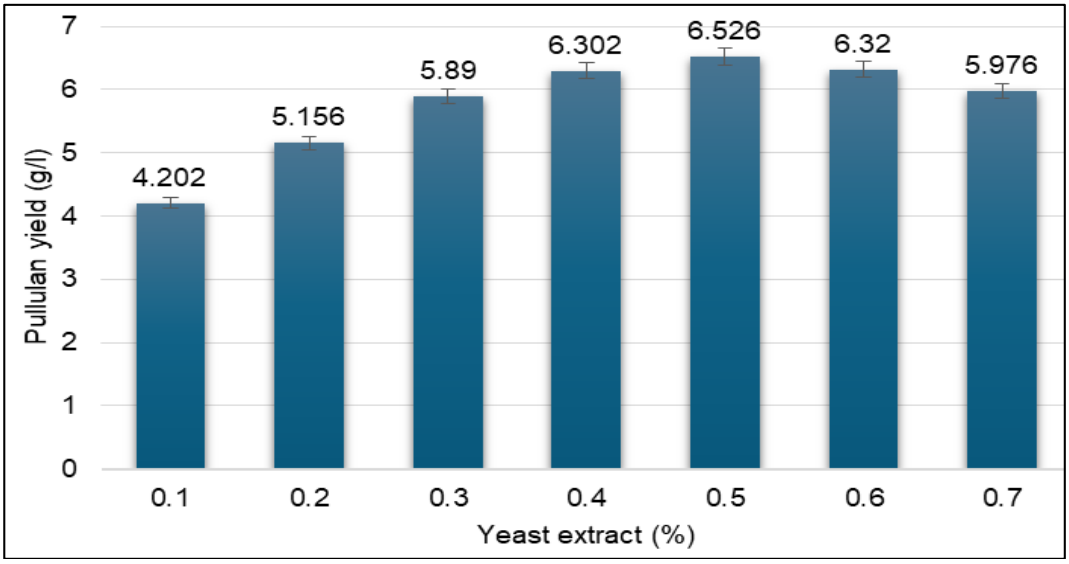

Fig 6: Effect of yeast extract concentration on pullulan production

As shown in Figure 6, the highest pullulan yield of 6.526 g/L was achieved with a 0.5% yeast extract concentration. This result aligns with the findings of Cheng *et al*. (2009) [5], who reported that yeast extract at this concentration provides optimal conditions for pullulan production, likely due to its balanced supply of nitrogen and growth factors essential for microbial activity. At concentrations below 0.5% (0.1%, 0.2%), the pullulan yield was lower, indicating that insufficient nitrogen may limit microbial growth. At concentrations above 0.5% (0.6%, 0.7%), a slight decline in pullulan production was observed, suggesting that excess nitrogen could negatively impact the biosynthesis of pullulan due to metabolic imbalances or inhibitory effects.

Based on the comparison of different nitrogen sources, it can be concluded that yeast extract is the most effective nitrogen source for pullulan production, yielding the highest concentration of pullulan. While ammonium sulfate and sodium nitrate can support growth to some extent, they are less efficient in promoting pullulan synthesis compared to yeast extract. Further optimization of yeast extract concentration revealed that 0.5% yeast extract provides the highest yield, and concentrations above this level result in a slight decrease in pullulan production. Therefore, yeast extract at 0.5% is the optimal nitrogen source and concentration for maximizing pullulan production in fermentation with *Aureobasidium pullulans*.

Effect of inoculum size on pullulan production

The effect of inoculum size on pullulan production by *Auerobasidium pullulans* was investigated by varying the inoculum volume (v/v%) in non-stirred flask fermentation. The results are presented in Figure 7, which shows the pullulan yield corresponding to different inoculum sizes.

From the data presented in Figure 6, it is evident that inoculum sizes of 1.25% and 1.5% resulted in the highest pullulan production, with yields of 7.061 g/L and 7.254 g/L, respectively. These inoculum sizes appear to provide an optimal balance for maximizing pullulan synthesis. At lower inoculum sizes (0.2%, 0.4%, and 0.6%), the pullulan yield was considerably lower, indicating that insufficient microbial inoculum may limit the fermentation process, reducing the rate of growth and pullulan production. The yield increased progressively with inoculum size up to 1.5%, suggesting that larger inoculum sizes support more rapid growth and an enhanced ability to produce pullulan. However, beyond 1.5% inoculum size (1.75% and 2%), a decrease in pullulan yield was observed. The yield at 1.75% was 6.761 g/L, and at 2.0%, it was 6.088 g/L. This decline in pullulan production at higher inoculum sizes may be attributed to the overcrowding of the culture medium, leading to nutrient depletion and oxygen limitation, which could affect microbial growth and pullulan biosynthesis.

In conclusion, inoculum sizes of 1.25% and 1.5% resulted in the highest pullulan production, indicating that these inoculum sizes are optimal for maximizing pullulan synthesis in *Aureobasidium pullulans*. Lower inoculum sizes (0.2% to 0.6%) resulted in suboptimal yields, while higher inoculum sizes (1.75% and 2%) led to a decline in production, likely due to the adverse effects of overcrowding. Therefore, an inoculum size of 1.5% is recommended for optimizing pullulan production in fermentation with *Aureobasidium pullulans*.

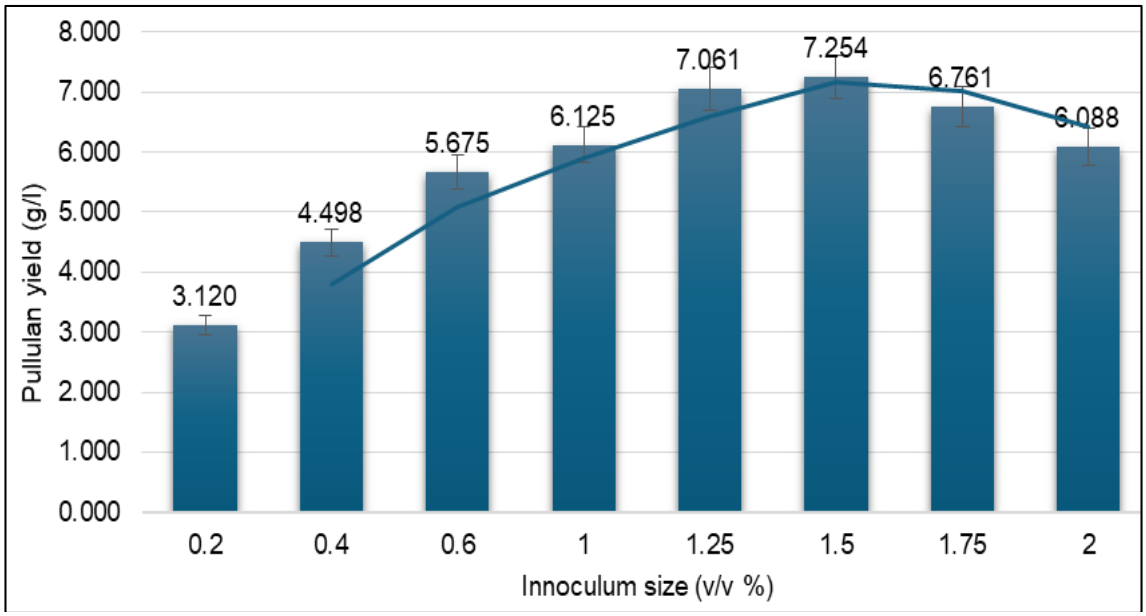

Figure 7: Impact of inoculum size on pullulan yield during fermentation

Effect of different carbon sources and concentration on pullulan production

The impact of different carbon sources on pullulan production was investigated by using glucose, maltose, and sucrose as the carbon sources, and the corresponding pullulan yields were measured. The results are presented in Figure 8, which shows the pullulan yield for each carbon source.

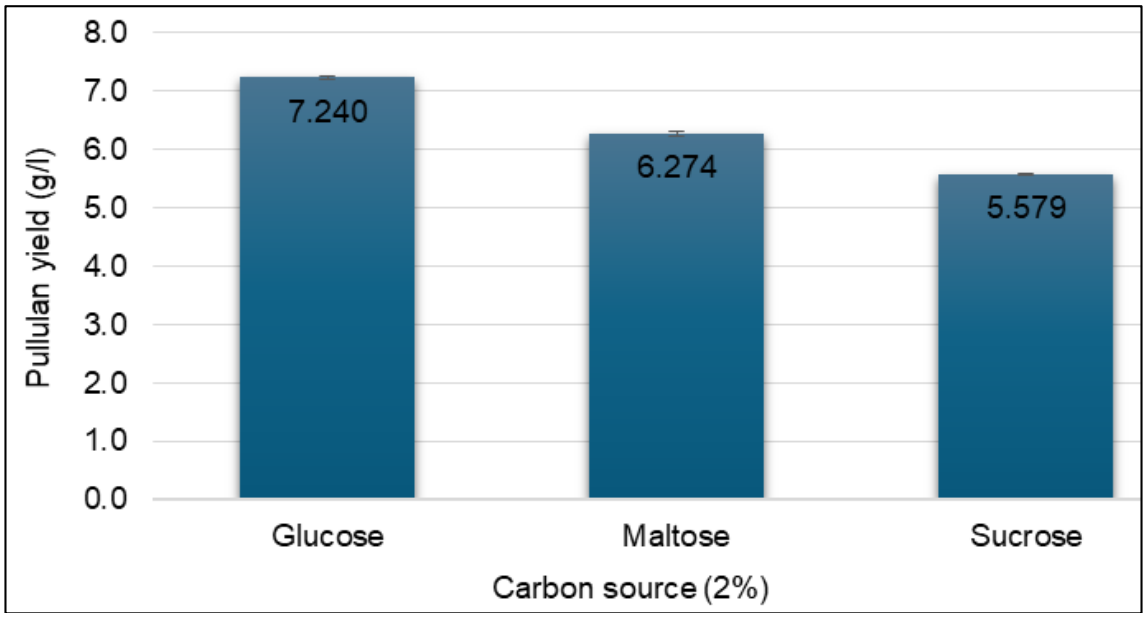

Fig 8: Effect of different carbon sources on pullulan production

As observed, glucose resulted in the highest pullulan yield of 7.240 g/L, followed by maltose at 6.274 g/L and sucrose at 5.579 g/L. The superior performance of glucose as a carbon source for pullulan production can be attributed to its availability as a readily metabolizable sugar for *Aureobasidium pullulans*, supporting optimal growth and pullulan synthesis. Maltose, while also a fermentable sugar, produced slightly lower yields, indicating that the microbial strain may metabolize glucose more efficiently than maltose. Sucrose, though an effective carbon source in many microbial fermentation processes, yielded the lowest amount of pullulan in this study, suggesting that it may be less efficiently utilized by *Auerobasidium pullulans* for pullulan production.

The results indicate that glucose is the most favourable carbon source for pullulan production among the sugars tested. The efficiency of pullulan production appears to be linked to the ease with which the carbon source is metabolized by the microbial strain, with glucose being the preferred choice. Similar findings were reported by He *et al*. (2021), [10] and Chi & Zhao (2003) [6], further validating glucose as an optimal substrate for pullulan synthesis. These studies emphasize glucose's effectiveness as a carbon source, promoting efficient microbial metabolism and enhancing pullulan production. However, G. Chen *et al*. (2019) [3] identified sucrose as the optimal substrate, highlighting that the choice of carbon source can vary depending on the *A. pullulans* strain employed.

Effect of Glucose Concentration on Pullulan Production

The effect of varying glucose concentrations on pullulan production was also studied, with glucose concentrations ranging from 1% to 9%. The corresponding pullulan yields are presented in Figure 9.

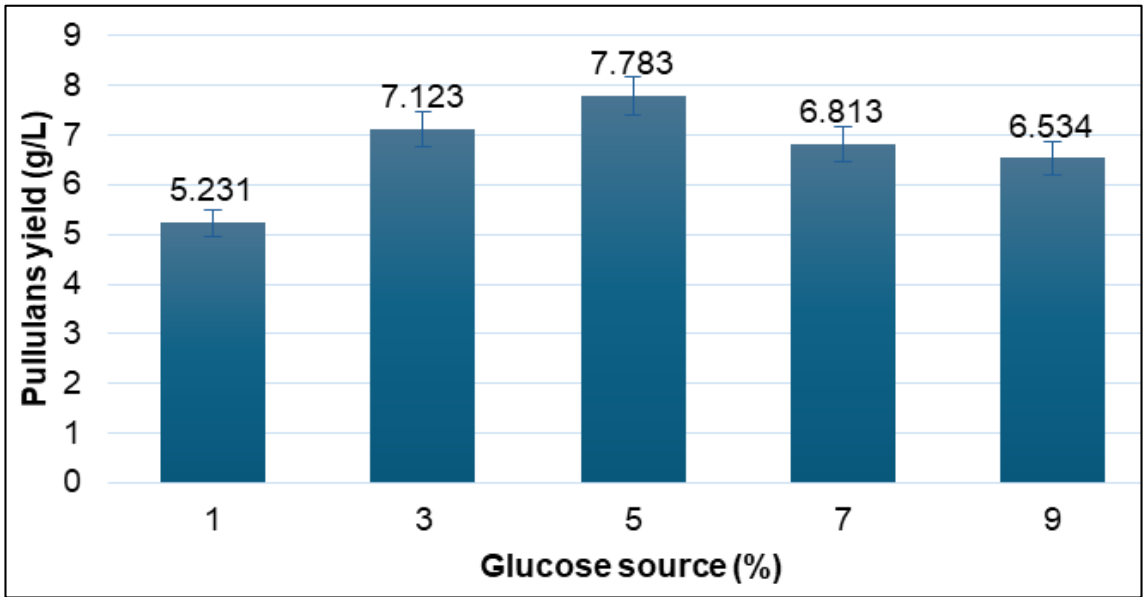

Fig 9: Effect of different glucose concentration on pullulan production

The highest pullulan yield of 7.783 g/L was observed at a glucose concentration of 5%. This concentration seems to provide an optimal balance between sufficient glucose availability for microbial growth and pullulan synthesis while avoiding inhibitory effects at higher concentrations. At 3% glucose, the yield was also relatively high (7.123 g/L), indicating that lower glucose concentrations can still support significant pullulan production. However, when the glucose concentration was increased to 7% and 9%, the yield decreased to 6.813 g/L and 6.534 g/L, respectively. This suggests that higher glucose concentrations may lead to osmotic stress or an imbalance in nutrient availability, which can negatively affect the growth and metabolic activity of *Aureobasidium pullulans*. The reduced yield at higher concentrations of glucose may be attributed to the accumulation of by-products, such as organic acids, which can inhibit microbial growth and pullulan biosynthesis.

These findings highlight the importance of selecting the appropriate carbon source and concentration to optimize pullulan production during fermentation.

Time course of pullulan production during fermentation

The optimization of pullulan production was carried out over a period of eight days, with the production being monitored at 24-hour intervals as shown in Figure 10. As observed, pullulan production began and after 24 hours (Day 1), the pullulan yield was 1.441 g/L, showing an initial increase as the microbial fermentation progressed. Over the subsequent days, the production continued to rise, with a notable increase observed by Day 3, where the yield reached 5.940 g/L. The maximum pullulan production of 7.850 g/L was achieved by Day 7 (168 hours). However, a slight decrease in the pullulan yield was observed on Day 8 (192 hours), where the mean yield dropped to 7.053 g/L. This decrease could be attributed to the onset of substrate depletion or the accumulation of fermentation by-products, which may have inhibited further growth and production of pullulan, this decrease in pullulan production beyond Day 7 may be attributed to nutrient depletion in the medium, the accumulation of metabolic by-products, or the onset of microbial growth limitations. Similar results for maximum pullulan production have been reported in previous studies, with by Singh *et al*. (2021) [17] achieving peak production on Day 6, while Ranjan *et al*. (2012) [15] observed the highest yield on Day 4, reflecting variations in production timelines depending on the strain used and experimental conditions.

These findings align with previously reported studies, where similar trends in polysaccharide production were noted due to the exhaustion of available carbon and nitrogen sources during extended fermentation periods (Aasen *et al*., 2000; Leroy & De Vuyst, 2001; Ozacar & San Keskin, 2024) [1, 11, 13]. The data suggests that pullulan production is time-dependent, with an exponential increase during the early fermentation stages, followed by a plateau phase as the culture reached a near-optimal state for pullulan production. The drop in yield at the end of the experiment indicates the need for careful management of the fermentation time to maximize yield and avoid any negative effects of prolonged cultivation. This optimization study highlights the importance of time as a critical parameter for pullulan production and provides useful insights into the temporal trends of pullulan yield in fermentation processes. The results further suggest that an optimal fermentation time of approximately 7 days may be most efficient for maximizing pullulan production, with any further increase in duration potentially leading to diminished returns.

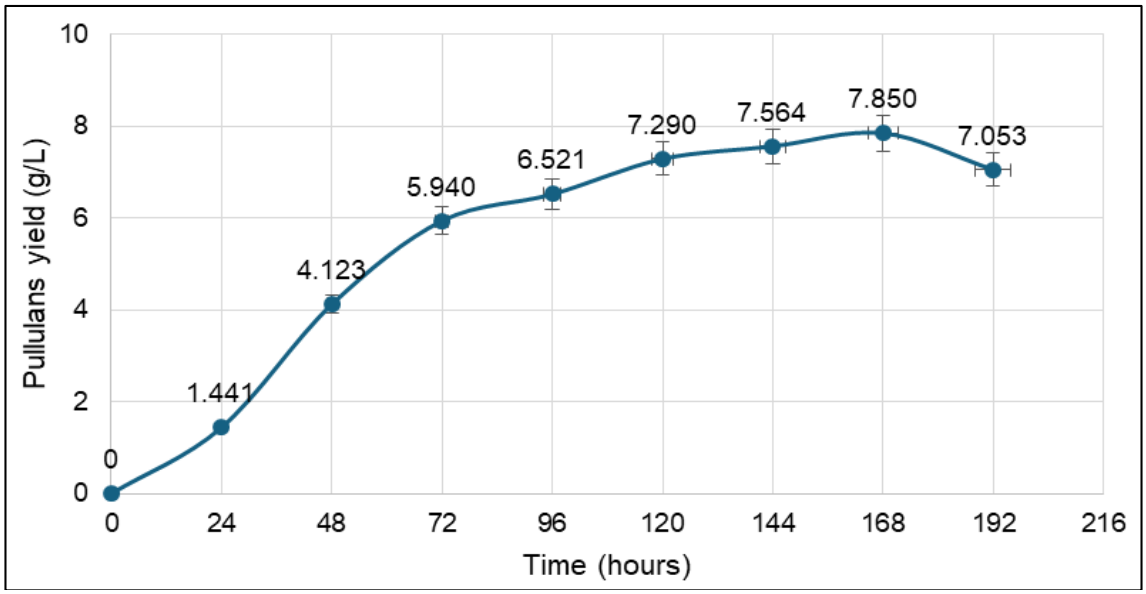

Fig 10: Time course of pullulan production and biomass yield during fermentation

Sugar consumption and its impact on pullulan synthesis

As the fermentation progresses, the pattern of sugar consumption indicates how *A. pullulans* adapt to the available carbon source. In the initial stages of fermentation, there is a rapid uptake of sugar, which aligns with the high metabolic activity of the microorganism during the exponential growth phase. In this phase, the sugar is primarily used for cell growth and the synthesis of pullulan. As the fermentation continues, the rate of sugar consumption begins to slow. This slowdown is likely due to the depletion of readily available sugars and the accumulation of metabolic by-products. The slowing consumption reflects the transition to the stationary phase, where the growth rate stabilizes and the organisms shift towards sustaining production. This is also the point at which pullulan production reached its maximum, indicating that the synthesis of the biopolymer continued at a stable rate despite reduced sugar availability.

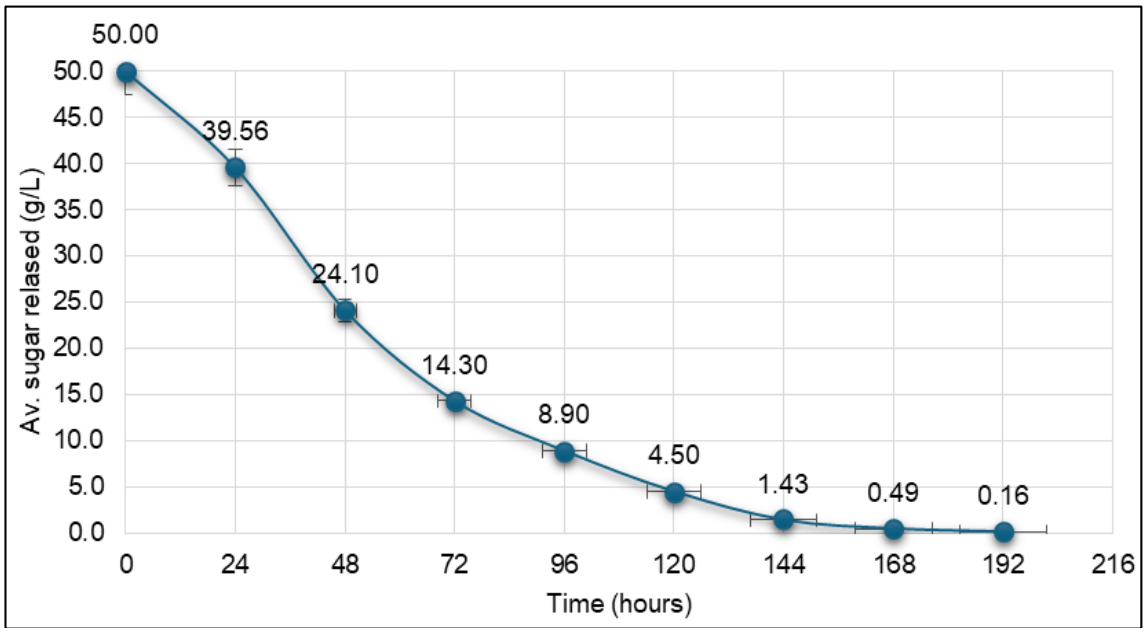

Fig 11: Dynamics of sugar utilization in pullulan production

As illustrated in Figure 11, by Day 7 (168 hours), the sugar concentration had drastically decreased to 0.16 g/L, indicating near-complete consumption of the available substrate. This depletion likely constrained further microbial activity and pullulan synthesis, accounting for the decline in production observed beyond this point. These findings suggest that terminating fermentation at this stage could enhance process efficiency by optimizing resource utilization and preventing unnecessary extension of fermentation time.

These findings highlight the importance of optimizing fermentation conditions, such as sugar concentration and fermentation time, to maximize pullulan production while preventing inefficient use of the carbon source.

Conclusion and Future Perspectives

In summary, this optimization study highlights key factors influencing the production of pullulan by *Auerobasidium pullulans* in fermentation, namely inoculum size, temperature, pH, carbon sources, and nitrogen sources. The

results confirm that moderate conditions such as an inoculum size of 1.5%, a temperature of 28 °C, pH 5, glucose as the preferred carbon source, and yeast extract as the optimal nitrogen source to maximize pullulan production. These findings provide invaluable insights into the conditions that foster optimal biopolymer synthesis and present avenues for scaling up production processes. The identification of these optimal parameters serves as a foundational step toward more sustainable and efficient pullulan production, offering significant potential for various industrial applications.

Future research can build upon these findings by exploring additional optimization strategies, such as the integration of advanced fermentation technologies like fed-batch or continuous culture systems, to further enhance pullulan yields. Moreover, the potential for genetic and metabolic engineering of *Auerobasidium pullulans* to increase production efficiency and reduce fermentation costs warrants investigation. Additionally, examining the impact of alternative, more cost-effective raw materials and the application of sustainable biorefinery concepts could open up new avenues for economically viable pullulan production. Studies focused on the optimization of fermentation medium composition, as well as the exploration of novel, non-conventional carbon and nitrogen sources, will be crucial for future advancements.

In conclusion, the data presented in this study provides a comprehensive framework for optimizing pullulan production, while also setting the stage for further research and innovation in this field. As biotechnological advances continue to evolve, *Auerobasidium pullulans* holds great promise as a sustainable source of pullulan, paving the way for the development of green biopolymers that can meet the needs of a growing global population while minimizing environmental impact.

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