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Optimizing media for increased productivity of Mutanase through the Single-Factor Optimization approach

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Abstract

Mutanase is an enzyme that has potential for several industrial uses because of its capability to catalyse the breakdown of mutan, which is a vital element in bacterial biofilms and dental plaque. As a result of growing demand within biotechnological processes such as pharmaceuticals or foods, there have been many investigations into producing more of this enzyme. By the submerged fermentation process Mutanase is produced, to make the process cheaper and more effective, numerous factors need to be optimized. The current study focused on both the physical and chemical properties of the mutanase producing medium. Here, optimization examines physical parameters such as time of incubation, temperature, inoculum size, rpm (rotation per minute) and pH. It also covers chemical factors like the supply of carbon, different mineral elements and the source of nitrogen.

The study on optimization showed that the optimal Mutanase was formed after 72 hours of incubation at 35°C temperature, pH 7 and 160 rpm. Chemical parameters optimization study revealed that addition of 0.6% mutan to the media made an immense impact in enzyme production. From the current study the highest Mutanase productivity obtained was 1.16U/ml. After this study the Mutanase production was higher than the previous process which was without adjusted parameters.

Key Words: Mutanase, Temperature, pH, Mutan.

1. Introduction

Mutanase, a specialized enzyme with significant industrial and biomedical applications, has garnered increasing attention due to its ability to hydrolyse mutan, a key component of dental plaque biofilms. This enzymatic activity positions mutanase as a valuable tool in oral healthcare, as well as in the food and pharmaceutical industries. However, the commercial viability of mutanase production is often constrained by low yields, necessitating the development of optimized production strategies to enhance its productivity.

The composition of the growth medium plays a pivotal role in determining the yield and activity of microbial enzymes such as mutanase. Media optimization is a critical step in bioprocess development, as it directly influences microbial growth, enzyme synthesis, and overall process economics. Among the various optimization methodologies, the **Single-Factor Optimization (SFO)** approach remains a widely used and straightforward technique. This method involves systematically varying one factor at a time while keeping all other factors constant, enabling researchers to identify the most influential parameters and their optimal levels. Despite its simplicity, Single Factor Optimization provides valuable insights into the role of individual components in enzyme production, serving as a foundation for more advanced optimization techniques such as Response Surface Methodology (RSM) or Artificial Neural Networks (ANNs).

In the context of mutanase production, the Single Factor Optimization approach has been employed to optimize critical media components, including carbon sources, nitrogen sources, and trace elements, which are essential for microbial metabolism and enzyme biosynthesis. Studies have demonstrated that the choice of carbon source, such as sucrose or glucose, significantly impacts mutanase activity, as these substrates regulate microbial growth and enzyme induction Similarly, nitrogen sources like peptone and yeast extract, along with micronutrients such as magnesium and manganese, have been shown to influence enzyme yield and stability.

This report focuses on the application of the Single-Factor Optimization approach to enhance the

productivity of mutanase by systematically evaluating and optimizing media components. By identifying the most suitable nutrient combinations and concentrations, this study aims to establish a cost-effective and efficient production process for mutanase, thereby contributing to its broader industrial applicability. Introduction to Mutanase and Its Industrial Applications

Overview of Mutanase Enzyme and Its Characteristics

Mutanase (α-1,3-glucanase) is a specialized enzyme that hydrolyses α-1,3-glucan, a polysaccharide predominantly found in the extracellular matrix of microbial biofilms, particularly those formed by cariogenic bacteria like *Streptococcus mutans*. This enzyme specifically cleaves the α-1,3-glycosidic bonds in mutan, resulting in the production of oligosaccharides or glucose. Mutanase is classified under glycoside hydrolase families, with its activity being highly substrate-specific. The enzyme is often produced by microorganisms such as *Trichoderma harzianum*, *Paenibacillus sp.*, and certain strains of *Bacillus*.

Key biochemical properties of mutanase include optimal activity in acidic to neutral pH ranges (pH 5.0–7.0) and temperatures between 30°C and 50°C, depending on the microbial source. Its molecular weight varies between 30 and 60 kDa, and its catalytic activity is influenced by metal ions like Ca^{2+} and Mg^{2+}, which act as cofactors. Mutanase is considered a promising biocatalyst for industrial applications due to its specificity, stability, and ability to degrade biofilms effectively.

Industrial Applications of Mutanase

- **Dental Hygiene and Oral Care Products**

One of the most significant applications of mutanase is in the dental care industry, where it is utilized to combat dental plaque and prevent tooth decay. Dental plaque is primarily composed of mutan, which provides structural integrity to the biofilm formed by *Streptococcus mutans*. Mutanase disrupts this biofilm by hydrolysing the α-1,3-glucan, thereby reducing bacterial adhesion to tooth surfaces.

Mutanase has been incorporated into toothpaste, mouthwashes, and other oral care formulations to enhance their efficacy in preventing dental caries. Studies have shown that mutanase-containing formulations can reduce plaque formation by up to 50% compared to conventional products. This enzymatic approach is particularly advantageous as it targets the biofilm matrix without affecting the viability of beneficial oral microbiota.

- **Food and Beverage Industry**

In the food and beverage sector, mutanase is employed to modify the texture and viscosity of products containing glucans. For instance, in brewing, mutanase can be used to degrade glucans that cause filtration problems during beer production. The enzyme enhances the clarity and stability of the final product by breaking down insoluble glucans that contribute to haze formation.

Additionally, mutanase has potential applications in the production of functional foods and dietary supplements. By hydrolysing α-1,3-glucans into oligosaccharides, mutanase generates prebiotic compounds that promote the growth of beneficial gut bacteria such as *Bifidobacterium* and *Lactobacillus*. These oligosaccharides can be incorporated into health-focused food products to improve gut health and overall well-being.

- **Bioremediation and Environmental Applications**

Mutanase plays a crucial role in bioremediation by degrading biofilms that contribute to industrial fouling and contamination. Biofilms are notorious for their resistance to chemical and physical cleaning methods, posing significant challenges in industries such as water treatment, oil and gas, and food processing. Mutanase-based treatments offer an eco-friendly alternative by enzymatically breaking down the biofilm matrix, thereby enhancing the efficiency of cleaning processes and reducing the need for harsh chemicals.

In addition to biofilm removal, mutanase has been explored for its potential in degrading glucan-based waste materials. For example, agricultural residues rich in α-1,3-glucans can be enzymatically converted into value-added products such as bioethanol or bioplastics, contributing to sustainable waste management practices.

- **Pharmaceutical and Biomedical Applications**

The pharmaceutical industry has shown growing interest in mutanase for its potential applications in drug delivery and wound care. In drug delivery systems, mutanase can be used to degrade glucan-based hydrogels, enabling controlled release of therapeutic agents. This approach is particularly useful for localized drug delivery in biofilm-associated infections, where the enzyme can simultaneously disrupt the biofilm and release antimicrobial agents.

In wound care, mutanase has been investigated for its ability to degrade biofilms formed by pathogenic bacteria in chronic wounds. Biofilm-associated infections are a major impediment to wound healing, and mutanase-based treatments offer a targeted approach to disrupt the biofilm, thereby enhancing the efficacy of antibiotics and promoting tissue regeneration.

- **Research and Development in Biotechnology**

Mutanase is a valuable tool in biotechnology research, particularly in the study of biofilm dynamics and microbial ecology. By selectively

degrading α-1,3-glucans, mutanase enables researchers to investigate the structural and functional roles of these polysaccharides in biofilm formation and maintenance. This knowledge is critical for developing novel strategies to control biofilm-associated problems in various industries.

Furthermore, mutanase is being explored for its potential in synthetic biology and metabolic engineering. For instance, genetically engineered microorganisms capable of producing mutanase with enhanced activity or stability can be developed for specific industrial applications. Advances in protein engineering and directed evolution have facilitated the design of mutanase variants with improved catalytic efficiency, thermal stability, and substrate specificity, paving the way for their broader application in diverse fields.

This property makes it suitable for a variety of industrial applications (Rey *et al*., 2024). The need for this enzyme is increasing in biotechnological processes, such as those that produce foods or medicines, hence efforts to increase its production have been widespread. The most crucial step in improving upon the previous process is figuring out how to optimise the growth media in a way that maximises the amount of mutanase produced while lowering production costs.

Numerous-pronged approaches have historically been employed to maximise the microbial enzyme synthesis process. These approaches involved adjusting various growth-influencing factors, the most frequent ones were pH, temperature, carbon source, and nitrogen source (Singh *et al*., 2017). However, recent advancements have demonstrated that comparable outcomes can be achieved with far less complex techniques focused on single factor optimisation while reducing manufacturing stage time consumption (Maqtari *et al*., 2019).

Using a one-factor-at-a-time strategy, this work focusses on optimising the generation of mutanase by improving the components of growth media. Keeping other variables unchanged while adjusting one key variable for every experiment run allows researchers to pinpoint the exact variable that has the biggest impact on mutanase productivity. Therefore, our goal is to determine the optimal conditions for obtaining high yields of mutanase, which will help us design commercial bioprocesses at a reasonable cost.

Challenges in Scaling Up Single Factor Optimization Results

Limitations of Single-Factor Optimization

While Single Factor Optimization provides valuable insights, it has inherent limitations when transitioning to large-scale production. For instance:

Interaction Effects: Single Factor Optimization does not account for interactions between variables, such as the combined effect of pH and temperature on mutanase activity. This can lead to suboptimal conditions during scale-up

Time and Resource Intensive: Testing each variable individually requires significant time and resources, which may not be feasible for large-scale operations.

Addressing Variability in Large-Scale Systems

Scaling up from laboratory-scale (e.g., 1 L bioreactors) to industrial-scale (e.g., 1000 L fermenters) introduces variability in parameters such as oxygen transfer, mixing efficiency, and heat transfer. For example, oxygen transfer rates (OTR) may decrease by 30% in large fermenters, potentially limiting microbial growth and enzyme production. Strategies such as increasing agitation speed or using oxygen-enriched air can mitigate these challenges.

Future Recommendations for Enhanced Productivity

Integration of Response Surface Methodology (RSM)

To overcome the limitations of Single Factor Optimization, it is recommended to integrate RSM for optimizing multiple variables simultaneously. For example, a central composite design (CCD) can be employed to evaluate the combined effects of pH, temperature, and glucose concentration on mutanase activity. Studies have shown that RSM can improve enzyme yields by up to 40% compared to Single Factor Optimization alone.

Genetic Engineering Approaches

Advances in genetic engineering offer opportunities to enhance mutanase productivity. For instance, overexpression of mutanase genes in recombinant strains can increase enzyme yield by 2-3 fold. Additionally, site-directed mutagenesis can be used to improve the enzyme's catalytic efficiency and stability under industrial conditions.

Continuous Monitoring and Automation

Implementing real-time monitoring systems for parameters such as pH, temperature, and dissolved oxygen (DO) can ensure consistent production. Automation of bioreactors with feedback control loops can further enhance productivity by maintaining optimal conditions throughout the fermentation process.

This work presents a complete study of how to optimize the production of mutanase. In current study, medium optimization was performed utilizing a bacterial Isolate (AK 27), which yielded a peak mutanase output that is 0.68U/ml obtained after incubation time of 72 hours.

Our goal is to offer useful knowledge for improving mutanase output through biotechnology utilization in industry.

2. Materials and Method

2.1 Mutanase producing bacterial culture

A bacterial isolate (AK 27) after screening with Congo red dye, had been used to carry out media optimization for higher mutanase production. For 24 Hrs the culture was kept at 4°C on nutrient agar.

2.2 Preparation of substrate Mutan

The substrate mutan has been produced using *Streptococcus mutans* (MTCC 497). In order to produce mutan, the culture was first cultivated using Brain Heart Infusion (BHI) medium containing 50g/L of sucrose for 72 hours at 37°C temperature and 150 rpm of agitation speed. Following incubation, centrifugation of culture was done for fifteen minutes at 15,000 rpm. Deionized waster was then used to clean the pellet and subjected to a 2 Hrs treatment with 2M KOH at 95°C. Allowing the sample to get cool at room temperature followed by fifteen minutes of centrifugation at 15,000rpm. After completion of centrifugation, the supernatant was stored at 4°C for precipitation overnight after being neutralised to pH 7.0 using glacial acetic acid. Centrifugation was used to separate the precipitates. The participates were cleaned by washing twice with deionised water, then it was lyophilised, and kept for later use (Lemos *et al.*, 2019, Boddapati *et al.*, 2020).

2.3 Mutanase production in submerged fermentation

Seed culture: A 250 ml culture flask was used to grow the mutanase-producing strain AK 27 in 50ml of nutrient broth. The flask was kept at 30°C on an incubator shaker at 120rmp for 24Hrs.

2.4 Production condition

1 ml seed culture was added to 150 ml Mutanase producing medium that contained Mutan, peptone, KNO_3, yeast extract, KH_2PO_4 Inositol, $MgS.O_4.7H_2O$ and NH_4Cl, (pH 7.0 ± 0.2). Culture flasks were then incubated at 30ºC with 150 rpm. Mutanase activity was measured by withdrawing sample at 24 Hrs intervals up to 120 Hrs. (Buddana SK., 2016).

2.5 Estimation of Mutanase

2ml sampling was done at every 24 Hrs intervals and mutanase activity was measured. 1 ml of cell-free broth was incubated with 1ml of 0.2% mutan suspended in 200mM sodium acetate (NaOAc) buffer with pH 5.50 at 50ºC for 30 minutes. Nelson-Somogyi's method used for estimation of reducing sugar with D-Glucose used for standard.

One unit of enzyme activity = Under the specified conditions, the amount of enzyme that catalysed for the release of reducing sugar is equivalent to 1µmol/min. (Nelson, N., 1944), (Buddana *et al.*, 2019).

2.6 Physical parameters optimization

2.6.1 The impact of temperature on Mutanase synthesis

The temperature significantly influences enzyme production, as cultures exhibit optimal efficacy for enzyme synthesis at specific temperatures. In this study, production flasks were incubated at temperatures from 20°C to 50°C in 5°C increments. Samples from each temperature were analysed for enzyme production.

2.6.2 The impact of pH on Mutanase synthesis

The production medium was prepared with pH values that ranged from 4 to 10, with intervals of 1.0. The flasks were incubated for the optimal duration and at the ideal temperature. Enzyme activity was assessed every 24 hours.

2.6.3 Effect of agitation rate on Mutanase production

In order to maintain the medium's rheology which is critical for guaranteeing sufficient dissolved oxygen levels and even medium distribution and maximize enzyme production, rotation per minute (rpm) is required. Enzyme activity was measured every 24 hours while production flasks were incubated at different rpm settings, ranging from 80 rpm to 260 rpm in 20 rpm increments.

2.6.4 Effect of Inoculum age on Mutanase production

The optimum inoculum age plays a critical role for production of enzyme, the production of mutanase enzyme was assessed at various inoculum ages, extending from 6 Hrs to 30 Hrs at different time points, and the enzyme activity was measured at every 6 Hrs interval.

2.6.5 Effect of inoculum size on Mutanase production

To find the ideal culture concentration for optimizing enzyme production, different concentrations of seed inoculum were added to the production medium. Enzyme activity was measured at 48, 72, and 96 hours after different inoculation sizes ranging from 1% to 10%, added to the corresponding production flasks.

2.6.6 Effective incubation period on Mutanase production

It has been noted that certain incubation times are very effective when the culture is most active. Mutanase activity was measured every 24 hours while the production flask was incubated for varied lengths of time, from 12 to 120 hours.

2.7 Chemical parameters optimization

2.7.1 The impact of Carbon source on Mutanase synthesis

Amylose, Amylopectin, Starch, Mutan, Maltose and Inositol were different sources of carbon used in this study to select the best carbon source giving maximum enzyme activity.

2.7.2 The impact of Nitrogen source on Mutanase synthesis

Different nitrogen sources used for the production of mutanase producing strain were ammonium acetate, ammonium sulphate, casein, peptone, sodium nitrate, tryptone and yeast extract. And each sample was analysed for enzyme activity.

2.7.3 The impact of substrate concentration on Mutanase synthesis

Mutan functioned as a carbon source for mutanase synthesis in the production medium; the production of mutanase enzyme was measured at 48, 72, and 96-hour intervals using different concentrations of mutan, ranging from 0.1% to 1.2%.

3. Result and Discussion

3.1 Physical parameters optimization

3.2.1 The impact of temperature on Mutanase synthesis

The production of Mutanase by AK 27 strain was checked at temperatures that varied between 20°C up to 50°C range with 5-degree intervals. Maximum production obtained at 35°C temperature which was 0.63 U/ml mutanase (Figure 1). The enzyme activity started decreasing at 40°C and continuous decrease in enzyme production observed as per increase in the temperature.

The study conducted by Buddana *et al.* (2016) discovered that *Paracoccus mutanolyticus* had the maximum mutanase activity at 30°C temperaturewhen the media composition was the same. *Trichoderma harzianum* have shown maximum mutanase production at 30°C (Wiater and Szczodrak, 2002).

Like the previous investigation, the current study found that mutanase activity increased by twofold at 30°C which then slightly at 35°C, indicating that mutanase synthesis can occur at temperatures 30°C±5°C.

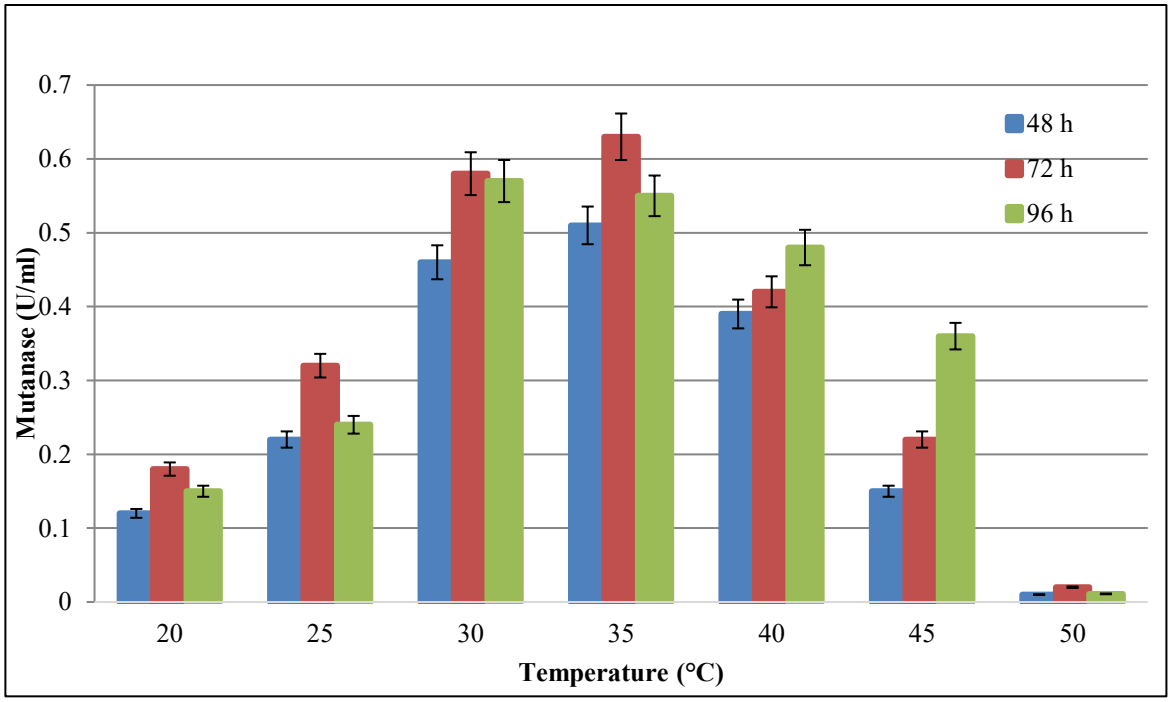

Figure 1: Impact of temperature on mutanase production by AK 27

3.2.2 The impact of pH on Mutanase synthesis

AK 27 culture showed 7.0 pH as an optimum pH for mutanase enzyme production (Figure 2). Under acidic pH range from 4-6, linear increase in activity of mutanase was observed, while from pH 7.0 mutanase activity increased 4.5 fold from pH 4. Beyond 7.0 pH growth of culture decreased linear which resulted into decrease in productivity. Similarly to the obtained result, it is noted that natural pH is the primary environment for mutanase mutanase production (Wiater *et al.*, 2014, Buddana *et al.*, 2016).

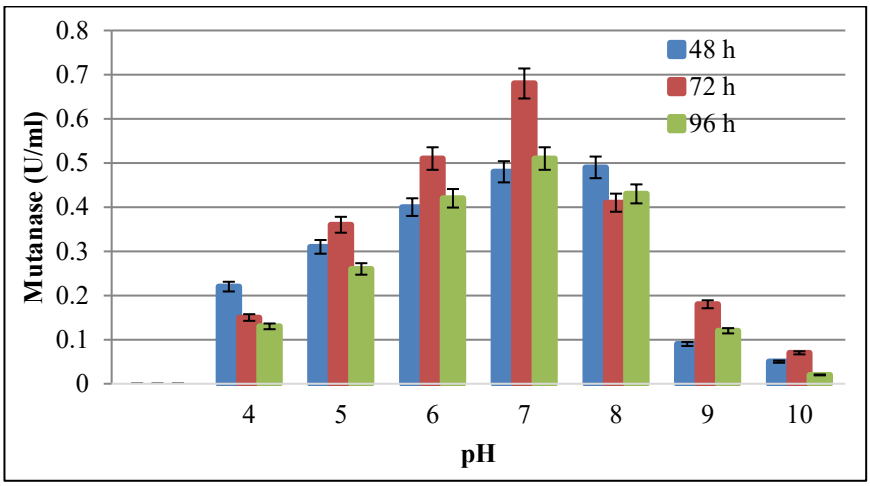

Figure 2: Effect of pH on mutanase Production by AK 27

3.2.3 Effect of agitation rate on Mutanase production

Different agitation rate, ranging from 80 to 260 rpm at intervals of 20 rpm, were used in the current investigation to agitate the bacterial AK 27 culture to obtain higher mutanase production. The highest enzyme activity was reached at 160 rpm (0.79U/ml), indicating maximum mutanase synthesis. Following that, a linear decline in enzyme activity was noted in accordance with an ongoing rpm rise for culture growth (Figure 3).

Wiater *et al.*, 2014 state that in order to boost mutanase production in *Trichoderma harzianum* fungal cultures, agitation speed must be increased to 270 rpm. At high rpm rates, a decrease in activity was seen in the bacterial culture AK 27 in the current experiment. In a similar way, Buddana *et al.*, 2016 employed 150 rpm to produce mutanase from the bacterial strain *Paracoccus mutanolyticus*.

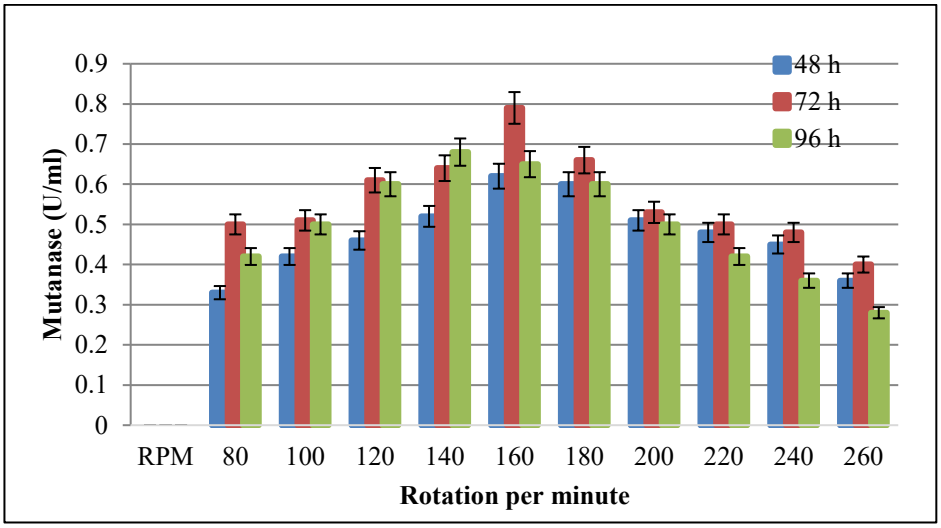

Figure 3: Effect of agitation rate on mutanase production by AK 27

3.2.4 Effect of Inoculum age on mutanase production

Every microbial strain has a different metabolic activity depending on which stage of the microbe's life cycle it is in. The culture with the highest metabolic activity is believed to be in the exponential (log) phase among the several microbial development phases. Studies using several ages of seed culture (6, 12, 18, 24, and 30 hours) were carried out to ascertain how culture age affects the production of mutanase. At 18 hours of inoculum age, the greatest mutanase production (1.1 U/ml) and growth were observed. Subsequent data analysis showed that, up to 18 hours after the inoculum was added, enzyme synthesis increased; beyond that, an increase in inoculum age was associated with a decrease in enzyme production (Figure 4).

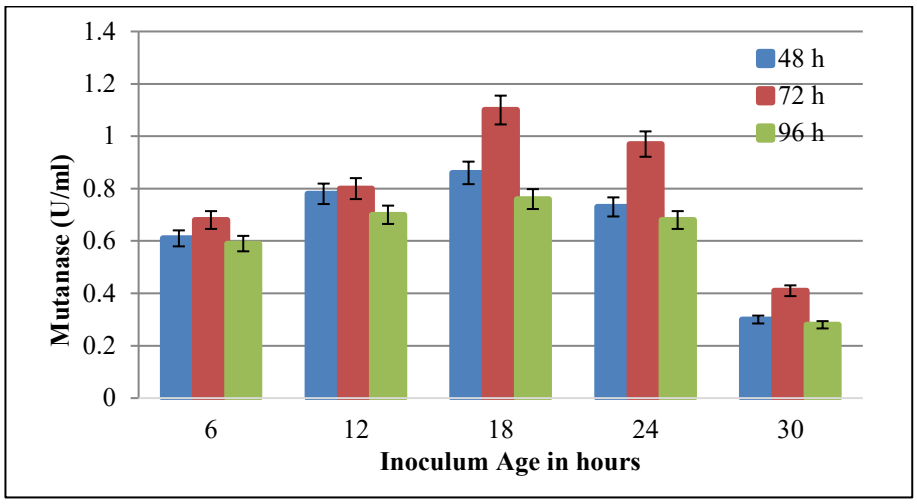

Figure 4: Impact of inoculum age on mutanase synthesis by AK 27

In the research work Wiater and Szczodrak (2002) reported a maximal output of mutanase as 0.38 U/ml from *T. harzianum* F-470 using 48 hours of mycelium as the inoculum. Conversely, Wetter *et al.*, 2005 observed higher activity of mutanase (0.71 U/ml) by using 72 Hrs old culture of *T. harzianum* F-340. Similar investigations were published by Szczodrak *et al.* in 1982, when maximum enzyme production was noted using a 20 hour grown suspension of *A. terreus* mycelium. It follows that optimising the age of the inoculum aids in achieving maximum activity.

3.2.5 Effect of Inoculum size on mutanase production

The size of the inoculum will have an impact on the lag phase, which will then have an impact on the organism's growth and metabolite production. To achieve optimum enzyme yield, the equilibrium between biomass growth and available nutrients must be maintained at an ideal inoculum density. Therefore, knowing the amount of inoculum needed for a particular set of fermentation processes is crucial to reaching optimised productivity values. The mutanase activity profile showed a maximum enzyme yield of 1.16 U/ml at the 5.0% level, which gradually dropped as the inoculum size increased up to 10.0% (Figure 5). Similarly, Pleszczynska *et al.*, 2010 showed that bacterial culture *Paenibacillus sp.* MP-1 could produce the maximum amount of enzyme (0.36 U/ml) when the inoculum size was 5%.

This profile was obtained as a function of inoculum size (1.0 to 10.0%, v/v). Higher inoculum levels may result in lower enzyme yields because the medium's constituents are used more quickly.

Variations in the use of inoculums were shown by literature reports on the influence of inoculum size investigations. In order to produce mutanase from *T. harzianum* F-470, Wiater and Szczodrak (2002) employed 10% mycelium as the inoculum size, which produced the maximum production (0.38 U/ml). While, Wiater *et al.*, (2005) used a 20% inoculum size to produce the highest amount of mutanase (0.71 U/ml) possible using *T. harzianum* F-340.

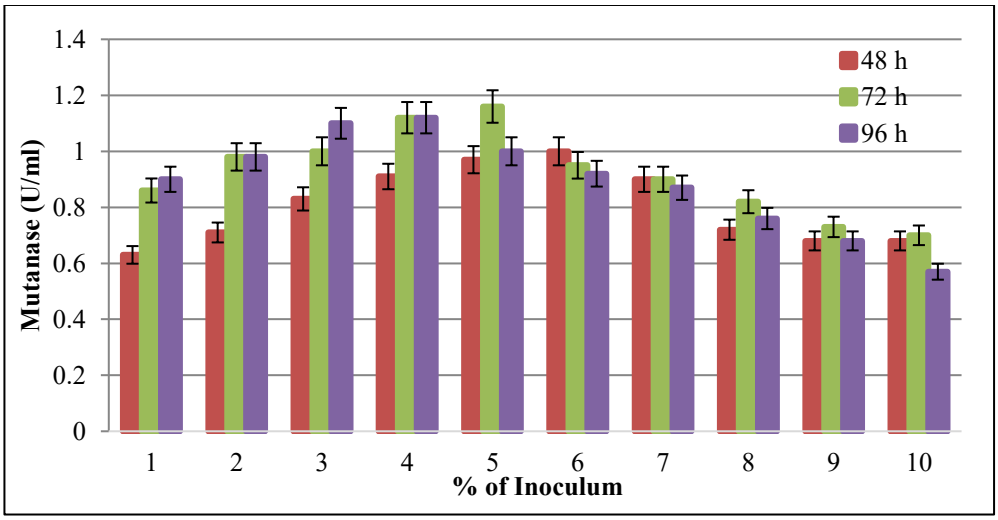

Figure 5: Effect of Inoculum density on mutanase Production by AK 27

3.2.6 Effective incubation period for the production of mutanase by AK 27

To improve growth and mutanase output, additional optimisation of the incubation period was carried out. Every 24 hours, the experiment was observed under optimal conditions and production levels. The growth and mutanase production effects of the incubation time were demonstrated by the results. According to the enzyme production statistics, 1.16 U/ml of mutanase was produced at maximum after 72 hours of incubation (Figure 6). Nonetheless, the pattern of extracellular mutanase synthesis persisted for 120 hours despite a decrease in enzyme titre. The strain's growth study and the pattern of its enzyme production further revealed that bacterial strain AK 27 synthesis of enzymes was related to its growth. Growth analysis of the strain and its enzyme production pattern further suggested that the enzyme production by *P. mutanolyticus* RSP 02 was growth associated (Buddana *et al.*, 2019).

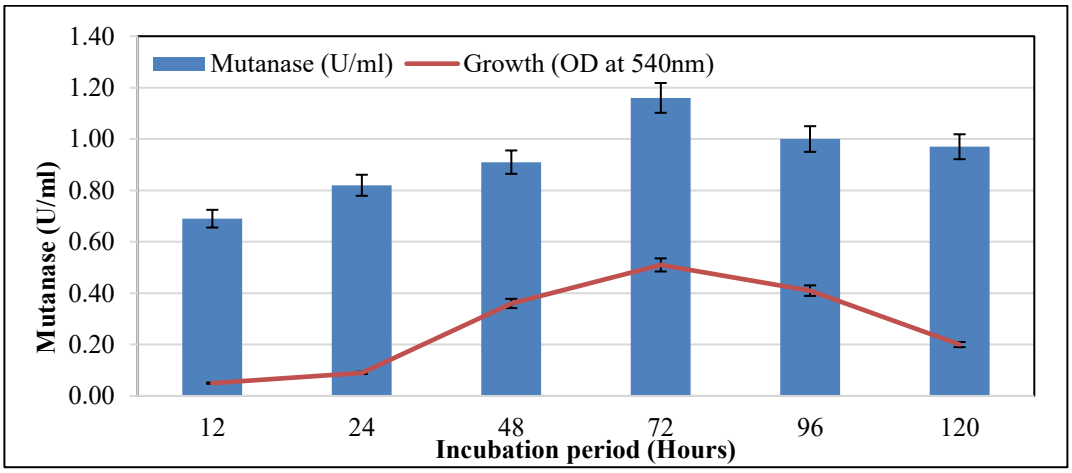

Figure 6: Effective Incubation Period for the production of mutanase by AK 27.

In a comparable investigation, Ebisu *et al.* (1975) documented a synthesis of mutanase at a concentration of 0.003 U/mL after a 24 hour period utilizing *Flavobacterium species*. Matsuda *et al.*, 1997, in their investigation of *B. circulans*, achieved peak enzyme production of 0.17 U/mL following 36 hours of incubation. Quivey and Kriger (1993) documented a peak mutanase production of 0.37 U/mg following 72 hours of incubation with the *T. harzianum* OMZ 779 strain.

3.2 Chemical parameters optimization

3.2.1 The impact of carbon source on Mutanase synthesis

Quivey and Kriger, 1993 studied the determination of additional carbon sources promoting the mutanase induction and found that addition of raffinose induced mutanase production as production of protein is regulated by presence of carbon source.

Similar to above study, For the high production of mutanase different carbon sources were used here including Amylose, Amylopectin, Starch, Mutan, Maltose and Inositol. Among these media containing Mutan as carbon source gave highest enzyme activity which was 1.01 U/ml (Figure 7).

Similar result was observed with *T. harzianum* culture where Mutan proved to be the most precise and potent inducer of enzyme production out of the 16 sugars examined. The highest levels of mutanase activity (0.7 U/mL) were assessed on 72 Hrs of incubation (Wiater *et al.*, 2005).

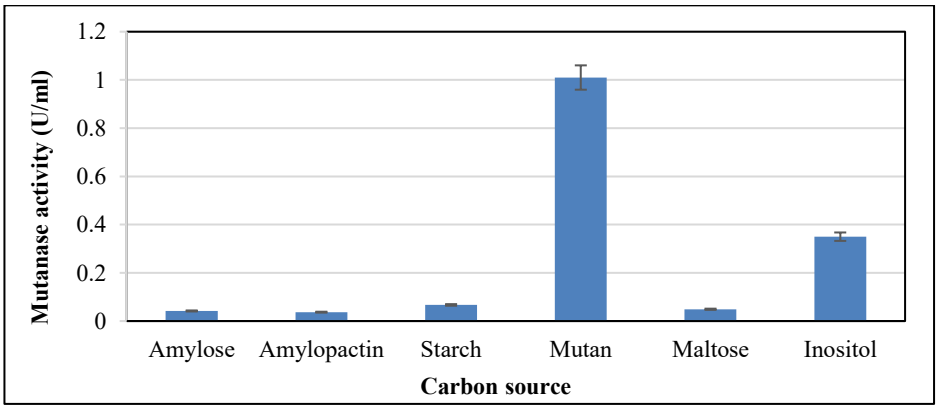

Figure 7: Impact of various carbon sources on mutanase production by AK 27.

3.2.2 The impact of Nitrogen source on Mutanase synthesis

In present study, peptone was found to have a significant impact on productivity of enzyme followed by yeast extract which was 1.09 U/ml and 1.06 U/ml respectively (Figure 8). Other nitrogen sources tried in this study showed less enzyme production than peptone.

Wiater *et al.*, 2005 found that for *Trichoderma harzianum* 0.5% peptone containing media have given sufficient mutanase production. Shimotsuura *et al.*, 2008, have used 0.5% peptone and 0.3% yeast extract for significant production of mutanase enzyme. Wiater *et al.*, 2014 showed that use of soya peptone have highest effect on mutanase production.

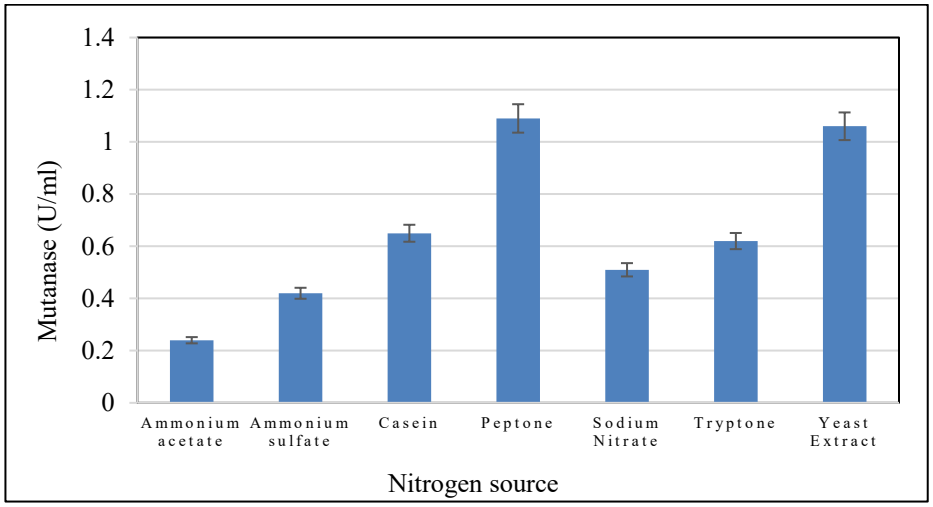

Figure 8: Various nitrogen sources' effect on mutanase production by strain AK 27.

3.2.3 Effect of substrate concentration in production medium

A common and effective inducer of mutanases is mutan which is a mixed-linkage [α-(1→3), α-(1→6)] insoluble D-glucan in water that plays a structural and functional role in oral cavity biofilms (Wiater *et al.*, 2018).

According to the aforementioned study of various carbon and nitrogen sources, mutan is the best carbon source that increases the production of mutanase. Therefore, mutan was studied at different concentrations, ranging from 0.1% to 1.2%, and it was discovered that 0.6% mutan in medium yields the best results (Figure 9). As concentrations of mutan increased after certain level, the activity of mutanase decreased.

A comparable investigation was conducted using *Trichoderma harzianum*, demonstrating that 0.5% mutan yields the highest mutanase activity and that the mutanase activity decreases when the mutan concentration rises (Wiater and Szczodrak, 2002).

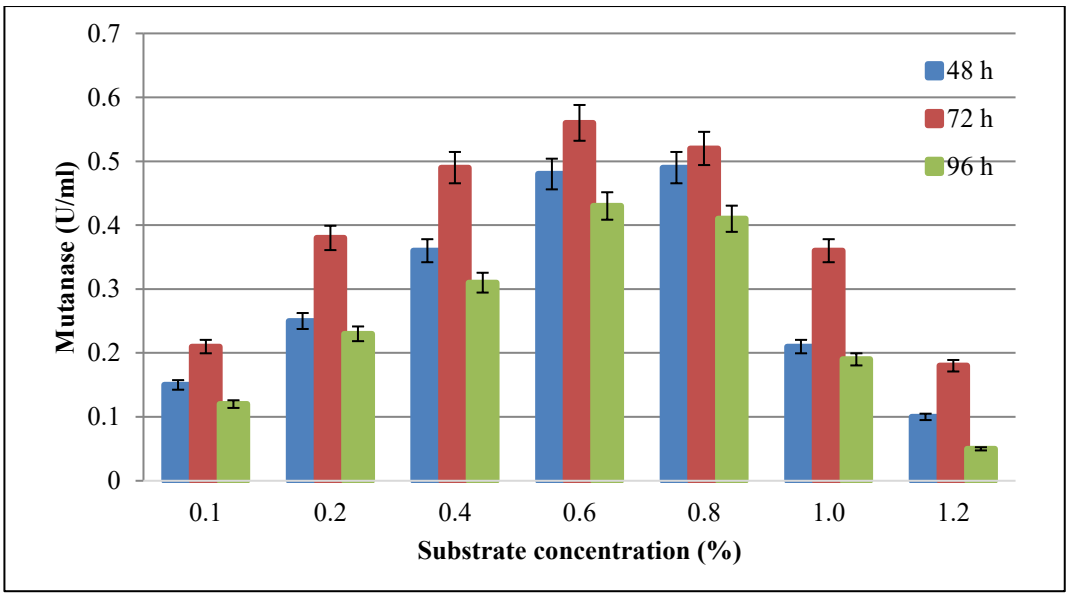

Figure 9: Effect of different substrate concentration on mutanase Production by AK 27.

4. Conclusion

This detailed analysis and set of recommendations provide a comprehensive framework for optimizing mutanase production using the single-factor optimization approach. By addressing the challenges and leveraging advanced techniques, it is possible to achieve significant improvements in enzyme yield and process efficiency.

AK 27 bacterial culture isolated from soil samples was evaluated for mutanase production. Initially, the culture condition was optimized to achieve proper growth of culture for production of mutanase and it was found that AK 27 show 0.68 U/ml mutanase activity under unoptimized condition and after standardization of physical and chemical parameter the activity of mutanase by Ak 27 strain increased to 1.7 fold.

In conclusion, this study demonstrates the utility of the Single Factor Optimization approach in identifying critical factors for mutanase production and provides actionable insights for industrial-scale enzyme production. By addressing the challenges of scale-up and leveraging advanced optimization and genetic engineering techniques, mutanase can be effectively utilized in diverse industries, contributing to eco-friendly and sustainable solutions for biofilm management, waste valorization, and health care. For further details on mutanase applications and optimization strategies.

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