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Review on the effect of various factors on cellulase production by *Aspergillus niger* using solid-state fermentation

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Abstract: Cellulases are important enzymes used in various industrial applications, like production of biofuels and also used in paper industries and textile industries. Aspergillus niger, a well-studied fungus that is filamentous. It is significantly used for cellulase due to its ability to degrade lignocellulosic biomass effectively. Solid state fermentation (SSF) provides a cost effective and environmentally conscious for enzyme production. In this review we discuss the effect of different factors such as temperature, pH, moisture level, substrate, incubation duration, inoculum volume, aeration (oxygen level), and metal-ions on the production of cellulase by using A. niger under the condition of solid-state fermentation. This review majorly focuses on optimal conditions of various factors, enzymatic reactions, and the industrial significance.

Key words: Aspergillus niger, Solid-state fermentation (SSF), cellulase production, factors, cost-effective

1. INTRODUCTION:

Cellulase is a hydrolytic enzyme responsible for breaking down cellulose into simple sugars¹. This enzyme plays a significant role in production of bioethanol, waste management, and various biotechnological applications. *Aspergillus niger* is a well-studied microorganism for the production of cellulase due to its ability to grow on various agro-industrial wastes². Solid state fermentation (SSF) is a well-studied method for enzyme production as it provides natural fungal growth conditions, reduces water usage, and utilizes agricultural substrates like wheat bran, sugarcane bagasse, and rice husk³. Different factors influence cellulase production, such as pH, temperature, moisture level, substrate level, incubation period, inoculum volume, oxygen level, and metal ions. Studying these factors is important for optimization condition for high enzyme production and its industrial applications.

2. SOLID-STATE FERMENTATION FOR CELLULASE PRODUCTION:

Solid state fermentation is a method which microorganisms like fungi grow on solid substrate with moist environment. With addition of nutrients the fermentation may be enhanced. It is a technique best for fungi like *Aspergillus niger*, which needs low water content. The important step in SSF is selection of suitable substates, optimizing fermentation condition to enhance the cellulase production in large quantity.

3. COMMON SUBSTRATES USED IN SOLID STATE FERMENTATION:

Wheat bran is easily available substrate which provides all kind of essential nutrients for fermentation⁴. Rice husk contains components such as lignocellulosic which is good source for fungal growth and enzyme production⁵. Sugarcane waste is rich in cellulose and it is mostly used for cellulase production³. An agricultural residue such as corn stover enhances fungal growth and enzyme production⁶. Fruit peel is an abundant waste which is rich in polysaccharides, it supports fungal growth⁷.

4. FACTORS AFFECTING CELLULASE PRODUCTION:

Various factors impact cellulase production in solid state fermentation and each influence the fungal growth, metabolism and enzyme production.



Effect of temperature

Temperature is an important parameter which influence fungal growth and enzyme production².

Optimum temperature: Studies shows that *A. niger* produce maximum cellulase at temperature range of $30-35^{\circ}C^{8}$. **Higher temperature (>40°C):** High temperature results in, reduced fungal growth, substrate drying and enzyme

Higher temperature (>40°C): High temperature results in, reduced fungal growth, substrate drying and enzyme denaturation⁹.

Lower temperature (<25°C): Low temperature slows down the fermentation reaction, enzyme production and leading to prolonged fermentation period¹⁰.

Effect of pH

Enzyme stability and microbial activity are influenced by the pH of the fermentation medium¹¹.

Optimal pH: Studies indicate that the optimal pH range for cellulase production is 4.5-6.0¹².

Acidic conditions: At low pH, below 4.0 is not suitable for enzyme production and fungal growth¹³.

Alkaline conditions: The decline in enzyme activity was observed above pH 7.0 due to protein denaturation¹⁴.

Effect of Moisture Content

Moisture content is a vital factor in solid state fermentation. It influences nutrient diffusion and fungal growth¹⁵.

Optimum moisture content: The most favourable moisture content level for *A. niger* is 50-70%⁶.

Low moisture content (below 50%): Low moisture content results in restriction of fungal metabolism and enzyme production⁷.

High moisture content (above 70%): High moisture content causes anaerobic environment, inhibition of enzyme secretion and leads to contamination⁹.

Effect of Inoculum Concentration

Inoculum concentration plays an important role in fungal growth and enzyme production in solid state fermentation⁶. **Optimal inoculum volume:** Research shows the maximum cellulase production is present in 1×10^6 to 1×10^7 inoculum concentration¹¹.

Low inoculum concentration (below $1x10^6$ spores/mL): Low inoculum concentration leads to insufficient fungal biomass and reduced fungal enzyme production⁷.

High inoculum concentration (above 1x10⁷ spores/mL): Results in excessive mycelial growth and nutrient depletion, leading to decreased enzyme yield¹⁰.

Effect of Substrate Composition

The selection of substrate for SSF influence the enzyme biomass as it provides both carbon and nutrient source¹⁶. Lignocellulosic substrates: Lignocellulosic substrates enhance cellulase production since it has high cellulose content³.

Simple sugars: Simple sugars such as glucose, sucrose leads to catabolite repression and reduced enzyme production¹¹. Effect of Incubation Period

The duration of incubation plays a vital role in production of cellulase and its metabolism rates⁴.

Optimum incubation time: The maximum growth of *A. niger* needs 3-7 days of incubation for cellulose production¹². **Short duration:** Below 5 days of incubation leading to less enzyme production due to low fungal growth and substrate utilization³.

Long duration: Above 7 days of incubation leads to enzyme degradation due to cell death and contamination⁷.

5. CONCLUSION:

Cellulase production by microorganism through fermentation is affected by various factors such as pH, temperature, moisture level, substrate, incubation time, inoculum concentration, oxygen level, and metal ions. Optimizing these factors improves enzyme production through solid state fermentation. One such microorganism A. *niger* is used for the cellulase production in industries. Future development in genetic engineering, microbiology and bioprocess technology can improve the enzyme production in mass quantity and its industrial applicability.

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