MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Optimization of nutritional conditions for the synthesis of TG enzymes by fermentation by *Streptomyces mobaraensis***

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Wang Hanping

1. Thesis topic **Optimization of nutritional conditions for the synthesis of TG enzymes by fermentation by *Streptomyces mobaraensis***
Scientific supervisor Olena Okhmat, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

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EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Hanping WANG

Scientific supervisor _____ Olena OKHMAT

)

SUMMARY

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The purpose of this study was to optimize the nutritional conditions for the fermentation and synthesis of transglutaminase (TGase) by *Streptomyces mobaraensis*, so as to improve the yield and activity of TG enzyme. *Streptomyces mobaraensis* is a microorganism widely used in food processing and bioengineering with important potential for the production of transglutaminase (TG). In order to improve the efficiency of TG enzyme synthesis by *Streptomyces mobaraensis*, a series of single factor optimization experiments and response surface method were used to explore the effects of different nutritional conditions on the yield of TG enzyme.

The experimental results show that *Streptomyces mobaraensis* produces the best results for TG enzyme at a growth temperature of 30°C and a pH value of 7.0. In terms of the carbon source, glycerol has been proven to have a significant effect on enhancing the yield of TG enzyme, and the highest TG enzyme yield is achieved when the concentration of glycerol is 15g/L. Additionally, the nitrogen source also plays an important role in the production of TG enzyme, and the addition of corn flour can significantly increase the yield of TG enzyme, with the optimal concentration being 20g/L. At the same time, the inoculation of 48-hour liquid seed, an initial pH value of 7.0, a cultivation temperature of 30°C, a shaking speed of 210 r/min, and a cultivation time of 48 hours were also determined to be the optimal fermentation conditions. The above optimization measures, by adjusting the culture medium and fermentation conditions, provide an important reference for the fermentation synthesis of TG enzyme by *Streptomyces mobaraensis*, which is expected to further improve the yield and activity of TG enzyme and promote its application in industrial production. The experimental results show that *Streptomyces*

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Key words: Streptomyces mobaraensis; Glutamine transaminase; Nutritional conditions; optimize

TABLE OF CONTENTS

INTRODUCTION

Transglutaminase (TGase), as an enzyme that catalyzes the inter-acyl transfer reaction of proteins, is widely used in the food industry to improve the texture properties of food due to its unique biocatalytic properties, such as enhancing the elasticity of meat products and improving the stability of dairy products. In addition, TGase has also shown potential applications in various fields such as biopharmaceuticals, textiles and leather processing. Given its important role in industrial applications, increasing the yield and activity of TGase has become the focus of researchers.

Streptomyces mobaraensis is an important TGase-producing organism, and its fermentation and synthesis of TGase is extremely sensitive to nutritional conditions. Optimizing the nutritional conditions during the fermentation process can not only increase the yield of TGase, but also increase its biological activity to a certain extent, so as to meet the growing industrial demand. However, the current research on the optimization of nutritional conditions for the synthesis of TG enzymes by Streptomycete momogena fermentation is insufficient, especially in the systematic study of key factors such as carbon source, nitrogen source, pH value, and temperature.

The purpose of this study was to explore the effects of different nutritional conditions on the synthesis of TGase by Streptomyces momouretale fermentation through single factor optimization experiment and response surface method, in order to find the optimal fermentation conditions. By optimizing the carbon source, nitrogen source, phosphate, initial pH, shaker speed and incubation temperature, this study aims to provide theoretical basis and practical guidance for the efficient synthesis of TGase in industrial production. In addition, this study will further explore the application potential of TGase in food processing and other fields, with a view to expanding its application scope and providing new possibilities for the advancement and sustainable development of biotechnology.

The relevance of the topic is carbon source, nitrogen source, temperature, pH, incubation time and inoculation amount, etc

The purpose of the research include increasing the yield of TG enzymes, increasing the activity of TG enzymes, saving production costs, stabilizing product quality and promoting the sustainable development of fermentation processes.

The objectives of the study is Transglutaminase, *Streptomyces mobaraensis* and their nutritional conditions.

The object of the study is Transglutaminase and *Streptomyces mobaraensis*.

The subject of the study is Transglutaminase and *Streptomyces mobaraensis*.

Research methods is Control single variable method and orthogonal test method.

The scientific novelty is Optimization of nutritional conditions.

The practical significance of the results obtained includes increasing the yield of TG enzymes, increasing the activity of TG enzymes, saving production costs, stabilizing product quality and promoting the sustainable development of fermentation processes.

CHAPTER 1
LITERATURE REVIEW

1.1 An overview of transglutaminase

Transglutaminase (TGase) belongs to a family of transferases that introduce covalent cross-links between glutamine residues and primary amines through acyl transfer reactions[1]. Due to its unique catalytic reaction, TGase has been used to improve the textural properties of protein-based foods [2]. Recently, it has also shown potential applications in pharmacological production, the textile industry, and leather processing[3]. Compared to enzymes derived from plants and animals, the TGase of *Streptomyces mobaraensis* is a Ca^{2+}-independent enzyme that is easier to produce on a large scale [4]. Although Streptomyces TGase has been expressed in a variety of xenohosts, only S. mutaraensistgase has been approved as generally recognized as safe (GRAS)[5]. These advantages are conducive to the application of TGase, making the fermentation of *Streptomyces mobaraensis* the main source of commercial TGase products[6]. Therefore, it is necessary to increase the TG enzyme production of *Streptomyces mobaraensis*.

1.1.1 Catalytic properties and sources of transglutaminase

Transglutaminase (TGase) is mainly involved in the cross-linking and modification reaction of proteins in vivo, and can catalyze the transamination reaction between gamma-amide group on glutamine residue in protein or polypeptide chain and ε- amino group on lysine residue or other amino containing substrates. Or deamination with water[7] (Fig. 1-1)

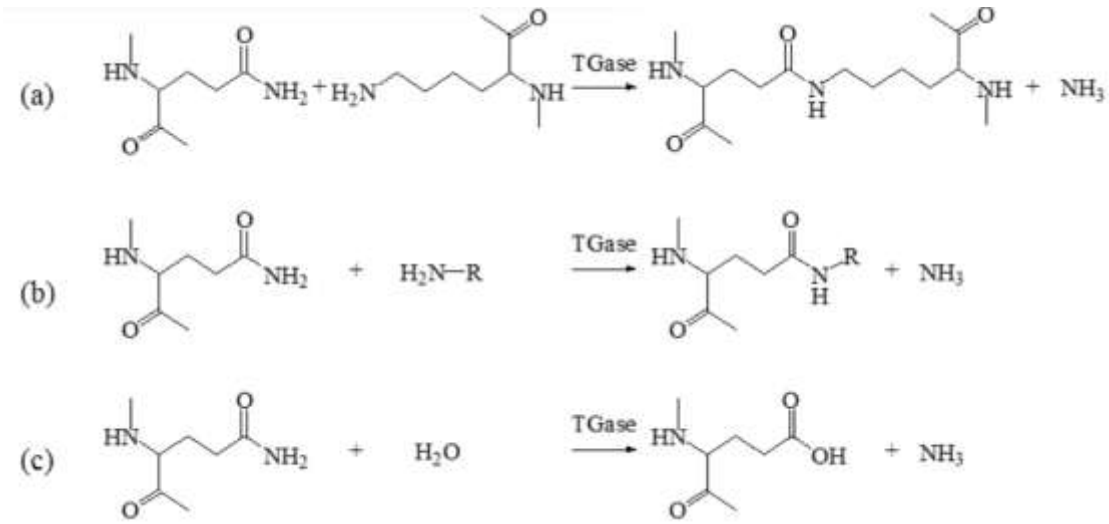


Fig.1.1 – Three reaction types of TGase catalysis

(a)The cross-linking reaction; (b) Incorporation reaction; (c) Deamidation reaction

Transglutaminases belong to a family of transferases that introduce covalent cross-links between glutamine residues and primary amines through acyl transfer reactions[8]. They are widely distributed in various organisms, including humans (glutamine is the most abundant free amino acid in human blood, and the human body can synthesize part of glutamine by itself), animals (animal tissues), plants (such as soybeans), and various microorganisms (bacteria, fungi, etc.)[9]. Glutamine is the most abundant free amino acid in the human blood. In 1959, Clarke et al. first discovered an enzyme that catalyzes transaminase in guinea pig liver and named it transglutaminase [10]. In 1989, Ando et al.[4] screened and obtained a strain called *Streptomyces mobaraensis* that could fermentate to produce glutamine transaminase.

1.1.2 Application of transglutaminase

Streptococcus mobaraensis is involved in a variety of biological metabolic processes by fermentation to synthesize TG enzymes that help decompose and metabolize fatty acids. These biological metabolic processes include energy metabolism and cell membrane structure formation. TGase changes the functional properties of food proteins by catalyzing the cross-linking, deamidation and surface binding of amino acid residues. Transglutaminase plays an important role in the amino acid metabolism pathway, converting glutamine and alpha-ketoacid into

glutamic acid and amino acid, and participating in the metabolic regulation of amino acids. Studies have found that when TGase catalyzes food protein reaction, cross-linking reaction will preferentially occur [11], and it is widely used in the food industry to produce amino acid flavor enhancers, flavor enhancers and other products. TG enzymes have also been found to be able to modify meat, making it more muscular and less likely to lose meat during cooking[12], and can also be used to improve the taste and nutritional value of food. As a protein builder, TG enzymes play an important role in many anabolic processes. During high-intensity strength training, taking glutamine can promote faster muscle growth.[13] Glutamine transaminase can be used in animal feed production to improve protein quality and promote animal growth and development. Transglutaminase can catalyze the transfer of amino acids, and can be used to synthesize a variety of organic molecules, such as carbohydrates, alcohols, ethers, etc. TGase can also be used in the production of dairy products[14]. Casein and whey protein in milk are both amide and amino donor substrates based on TG enzymes, which is conducive to improving the quality of proteins[15]. TGase can be used in cheese production to improve the texture and structure of the product and increase the yield [16]. In the production of yogurt, the viscosity and consistency of yogurt can be improved. The analysis of protein microstructure by electron microscope shows that the cross-linking effect of TGase makes the protein network structure in yogurt more dense[17]. TGase was also found to enhance the antioxidant properties of yogurt products and increase the content of free amino acids[18]. The addition of TGase during the processing of grain products helps to make the dough with good stability, elasticity and water absorption, as well as proper aperture and sufficient volume; TGase can improve the processing performance of wheat flour damaged by protease[19]. TG enzymes also have important applications in the field of enzymatic synthesis, which can be used to produce key substances for organic synthesis, such as the synthesis and processing of fermented food, and improve the performance of food[20]. TG enzyme also has a good substrate for the synthesis of soy protein isolate[21], and TGase can improve the heat resistance and texture of tofu[22]. TGase can also be used for environmental protection and wastewater treatment, using

protein film instead of plastic packaging to reduce garbage pollution, packaging fresh fruits and vegetables[23], with odorless, non-toxic, edible, breathable, degradable organic matter in wastewater, purification of water and other advantages[24].

The synthesis of TG enzymes by the fermentation of Streptococcus mobaraensis has great significance in many fields such as medicine and industry. Therefore, the research and application of TG enzyme synthesis by fermentation of Streptococcus mobaraensis have important scientific and practical value.

1.1.3 Research and development status of transglutaminase at home and abroad

In recent years, TGase has received extensive attention in various fields at home and abroad, including enzyme engineering, cell engineering, pharmaceutical engineering, protein engineering (protein modification to improve properties [25]), wool textile industry and leather processing industry, etc. The research on the synthesis of TG enzyme by fermentation of Streptococcus mobaraensis has received extensive attention at home and abroad. Due to its unique catalytic reaction, TGase has been used to improve the textural properties of protein-based foods [25]. TGase can catalyze site-specific immobilization of proteins or peptides, and the immobilized enzymes made by this method have higher specific activity than those made by chemical method and are more stable than those made by physical adsorption method [26]. Recently, it has also shown potential applications in pharmacological production, textile industry, and leather processing [27]. TGase is highly volatile selective to substrates, especially amido-based donors, and can be modified specifically by TL1HPm, such as pegylation, to alter the stability of the protein and the immunogenicity of the drug protein I2b, 2. In China, some scientific research institutions and universities have carried out relevant research work, including the isolation and identification of Streptococcus centaeus strains, optimization of fermentation conditions, extraction and purification of TG enzymes. At the same time, some domestic enterprises have also carried out certain exploration in the industrial application of TG enzymes. The Food Synthetic Biology and biofufacturing team of

Jiangnan University developed and combined a series of enzymatic modification methods to significantly improve the thermal stability and catalytic activity of S.Mabaren Sistgase. The research work has been funded by the national key research and development project, the National Natural Science Foundation, the National light industry Technology and engineering first-class discipline independent project and other projects. In the late 1980s, the production of transglutaminase by microbial fermentation began to be reported. In 1989, Ando and Motoki et al. reported for the first time the production of transglutaminase by microbial fermentation [3]. They identified Strnepto-verticiliums-112 ,from 5,000 strains, From 5000 strains, they identified Strnepto-verticiliums-112, Streptoverticiliumsp, Streptoverticilium griseocaneun, Steptovertieilium cinnamonen subp, cinnarnoneum and Streptoverticiliun mobaraerse were cinnamonen subp to produce transglutaminase. Zhu Yang, a professor at TTNO Food and Nutrition Research Institute in the Netherlands, and others used the analysis of amino acid metabolism and mass balance to believe that some amino acids are an important basis for bacterial reproduction and secretion of glutamine transaminase. On the basis of cultures designed by Ando et al., appropriate amounts of His, Ile and Met were provided by Professor Zhu Yang. When the fermentation microorganisms were produced in batches using this medium, the yield of glutamine transaminase was increased fourfold. Taiwan Wu Jei - wen and others have been screened strains for Streptoverticilium ladakanurm. According to their study, the enzyme activity of transglutamine was highest when inoculants of 103 to 104 cfu/ml were cultured at 25 ℃ to 28 ℃, or 100 to 150 rpm for four days. The addition of approximately 2 ppm of the antibiotic Colistin can increase the yield by 30%, up to 2.1uml.

Japan Ajinomoto Company has successfully used microbial fermentation to produce enzyme preparations, which have achieved great success after being put into the market, with an output value of more than 30 million US dollars in 2000, accounting for a quarter of the total annual output value of enzymes in Japan, and becoming the most profitable commercial enzyme in a single enzyme type. So far, however, China has not been able to produce the enzyme, and the domestic market is

facing a huge attack from Japanese manufacturers. In addition, MTG also plays an important role in food processing such as meat product processing and aquatic product processing [1]. In addition, the processed cheese with different TG enzymes showed shear thinning. With the increase of TG enzyme content, the viscosity and storage modulus of the material increased. Excessive addition of TG enzymes would lead to excessive cross-linking of proteins in the material, thus reducing the viscosity and appearance smoothness of the printed sample. When the addition of TG enzyme was 0.04%, the sample collapse rate was the lowest (0.26%, 0.33%) before and after 45min of 3D printing, and the 3D printing effect of recycled cheese was the best. Through comparative analysis of 3D printing suitability and molding effect, it is confirmed that TG enzyme can significantly improve the molding stability of 3D printed cheese [28].

In foreign countries, the fermentation of Streptococcus Maoyuanensis to synthesize TG enzyme is also very active. Many foreign research teams have made important progress in the in-depth study of the biochemical properties, molecular mechanisms, genetic engineering improvement and so on. In addition, in terms of the application research of TG enzymes, some foreign food and bioenergy companies are also actively exploring the application potential of TG enzymes in industrial production.

In general, the research on the synthesis of TG enzyme by fermentation of Streptococcus pentagenae is deepening at home and abroad, which is expected to further promote the basic research and industrial application of TG enzyme in the future.

1.1.4 Method for determination of enzyme activity

The determination methods of TG enzyme activity can be mainly divided into two categories: direct determination and indirect determination[29].

1.1.4.1 Direct assay

Enzyme activity is determined by measuring changes in the physical properties of a peptide substrate or a specific protein after cross-linking.

(1) Protein gel flocculation method

At low protein content, the polymer formed by MTG is soluble in water, while at high concentration of organic wastewater, the formation of water-insoluble colloids is promoted by MTG. The lower the surface hydrophobicity of the substrate protein, the greater the sensitivity to MTG, but because the alkali casein is linear in the gel and has a corresponding net charge within the molecule, the surface hydrophobicity is also small, which is conducive to the catalytic effect of MTG. With the increase of enzyme action time, the amount of casein decreased and the amount of biopolymer formed increased [30]. When the substrate is adjusted to a certain concentration, the polymer casein bound by covalent bonds can be precipitated from the solution and can be observed by the naked eye or spectrophotometry[31].

This method can directly show the effect of enzymes in practical applications, and the price is relatively low, but the flocculation polymer can not be accurately measured, so this method can only be used for preliminary screening.

(2) Direct gel electrophoresis method

Faergemand et al.[32] proposed a method using SDS-PAGE to detect the change of physical properties of the substrate, and observed the substrate directly by gel electrophoresis (SDS-PAGE). This method can directly display the effect of the enzyme and can better compare the samples.

(3) Fluorescein amide method

LorandL[33]et al. found that when fluorescein amide is covalently attached to the acyl receptor surface under the action of the enzyme, a fluorescein labeled peptide chain is formed, so the method can measure the activity of the enzyme by measuring its fluorescence intensity. The advantages of this method are high sensitivity and automation.

(4) Radiolabelling assay

The reduction of labeled reactants and the emergence of new products can be determined by chromatographic separation of labeled amines participating in the cold group transfer reaction.

1.1.4.2 Indirect method

After the reaction product is combined with a specific substance, the specific product formed is measured to indirectly determine the size of the enzyme activity.

(1) Colorimetric method

Grosswicz et al. [34] found that MTG catalyzed the reaction of hydroxylamine hydrochloride with acyl donor Z-Gln-Gly to produce γ-glutamyl monohydroxyl, the specific substrate being n-hydroxyphenoxy-l-glutamylglycine (Z-Gln-Gly), and under the action of TCA, the reaction products formed stable brown substances. According to this principle, Hydroxylamine hydrochloride Z-GIn-Gly and reduced glutathione were selected as substrate reagents. After reacting with the solution to be tested under certain conditions, TCA was added quickly to terminate the reaction. Then, the absorbance of the supernatant liquid phase was measured at 525nm. Using L-glutamic acid gamma-hydroxamic acid as the standard, the absorbence-hydroxamic acid ester curve was calibrated.

The method is simple and easy to operate, but the sensitivity is too low and the content of MTG is also low.

(2) Ammonium determination method

TGase catalyzes the substrate and releases an equal amount of ammonium at the same time, and the amount of ammonium released by the enzymatic reaction is determined according to this principle. NicolasD et al. proposed a relatively simple ammonium measurement method. The biggest advantage of this method is that it has a wide selection of acyl donors, and many common primary amines can replace Z-GIn-Gly as acyl donors, which can save a lot of money.

1.2 Synthesis of glutamine transaminase by fermentation of *Streptomyces mobaraensis*

Streptomyces sp., as a kind of microorganism widely used in antibiotic production, has a wide variety of metabolites and has high medicinal value. As one of its important secondary metabolites, TGomycin is often used in clinical treatment such as anti-infection. However, the fermentation process is very sensitive to nutritional conditions, and the optimization of nutritional factors can significantly increase the yield of TGamycin, which has important significance in industrial production.

1.2.1 Activation mechanism of fermentation

Since 1989, Ando et al. [3]screened and obtained a strain of *Streptomyces mobaraensis* capable of secreting TG enzymes, microbial fermentation has become the main way of industrial production of TG enzymes. In the fermentation process of *Streptomyces mobaraensis*, an appropriate medium should be selected first. The inactive pro-TGase is first secreted by *Streptomyces mobaraensis* in the form of zymogen. The zymogen region is located at the N end of the active enzyme with a length of 45aa, which inhibits enzyme activity and plays a very important role in the efficient folding and secretion of the enzyme [35]. Moreover, it can simultaneously secrete two proteases to participate in the activation of the enzyme [3637] : Glutamine transaminase activate protease (Transglutaminaseactivatingmetalloprotease TAMEP) and a kind of calcium ions depend on three peptidyl aminopeptidase (Tripeptidylaminopeptidase, TAP). TAMEP excites the first 41 amino acids of the prozyme region to produce FRAPTGase, and FRAP-TGase is active at this time. TAP excised FRAP and then produced TGase. The results show that the process of TG enzyme synthesis by Streptomyces penetocens fermentation may be regulated by the negative feedback of endogenous metabolites. Zhang Lili [38] added TAMEP inhibitor EDTA and TAP inhibitor PMSF into the fermentation medium of Streptomyces pentapenepentadienes in order to find out which of glutamine transaminase-activated protease and tripeptidyl aminopeptidase had greater influence

on TGase activation. She found that only EDTA could inhibit the increase of TGase activity during the fermentation process. This suggests that TAMEP plays a key role in the activation of TGase. Dong-xu zhang, etc[39]. found Streptomyceshygroscopicus TGase have similar activation process. Shigeru Schmidt, etc[40]. found that the original Streptomyces secretion of a dual protease inhibitors, Streptomyces hay bacillus protease and glutamine transaminase activate protease inhibitors (StreptomycessubtilisinandTAMEPinhibitor, SSTI) is the physiological substrate of its TGase, and it is speculated that the process of TGase activation under physiological conditions is as follows (Fig. 1.2) : Initially, SSTI inhibited TAMEP activity with low activity, and a small amount of pre-TGase was activated to catalyze cross-linked SSTI polymerization. After SSTI polymerization, TAMEP inhibition ability was lost, TAMEP activity was increased, and more pre-TGase was activated, forming a positive feedback process. Long aliphatic chain amino acids similar to N-lauroylsarcosine (LS) can block the glutamine site of SSTI, thereby inhibiting the polymerization of SSTI.

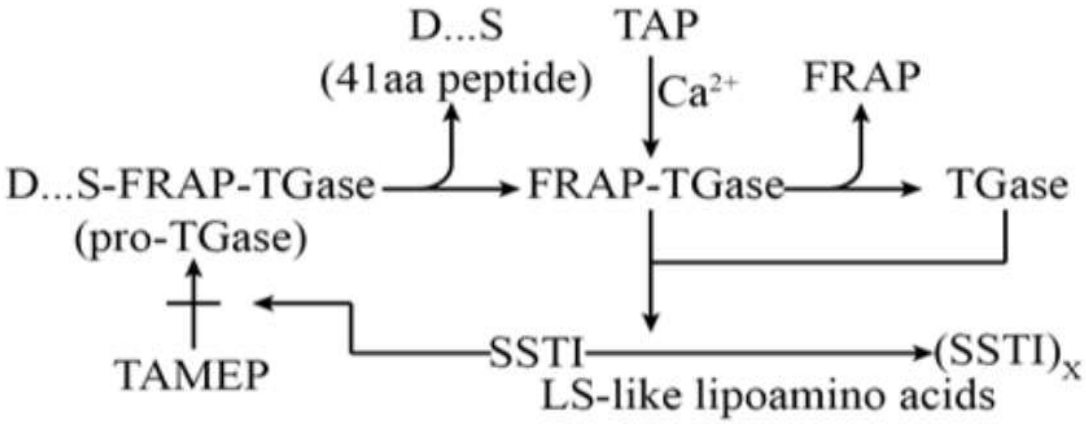


Fig. 1.2 – Activation and regulation mechanisms of TGase from *S.mobaraensis*

1.2.2 Fermentative production of wild *Streptomyces mobaraensis*

Since 1989, Ando et al.[3] screened and obtained a strain *Streptomyces mobaraensis* capable of secreting TGase, microbial fermentation has become the main mode of industrial production of TGase. For years, researchers have promoted the growth of wild Streptomyces by screening strains in the natural environment, mutagenesis breeding, and selecting strains that express TGase more strongly,

Moreover, the quality and yield of TG enzymes of the strain were ensured by optimizing the composition of the medium, inoculation and pre-culture, adding fermentation excipients for fermentation culture, product extraction and purification, product detection and analysis, preparation processing and other steps (Table 1.1). Because the growth rate of wild bacteria is slow, this makes gene editing of Streptomyces very difficult.

Table 1.1 – **Production of TGase using wild type strains**

Expression strain	Optimization scheme	Expression level	Fermentation time
S. mobaraensis	Screening strain	2.5 $U \cdot ml^{-1}$	1985[3]
S. mobaraensis	Medium optimization	1.8 $U \cdot ml^{-1}$	1998[41]
	Optimization of feeding strategy		
S. mobaraensis	Fermentation temperature optimization	2.94 $U \cdot ml^{-1}$	2002[42]
S. mobaraensis	Fermenter mixing control optimization	3.32 $U \cdot ml^{-1}$	2005[43]
S. mobaraensis	Solid state fermentation	0.7 $IU \cdot g^{-1}DW$	2008[44]
S. mobaraensis	Mutagenesis breeding	2.73 $U \cdot ml^{-1}$	2010[45]
S. mobaraensis	Medium optimization	4.3 $U \cdot ml^{-1}$	2012[46]
S. mobaraensis	Medium optimization	5.2 $U \cdot ml^{-1}$	2013[47]
S. mobaraensis	Medium optimization	6.6 $U \cdot ml^{-1}$	2015[48]
S. mobaraensis	Mutagenesis breeding	5.85 $U \cdot ml^{-1}$	2017[49]
S. hygroscopicus	Fermentation additive	5.04 $U \cdot ml^{-1}$	2007[50]
S. hygroscopicus	Fermentation process control optimization	8.19 $U \cdot ml^{-1}$	2009[51]
	Medium optimization		
	Mutagenesis breeding		
S. hygroscopicus	Optimization of feeding strategy	5.79 $U \cdot ml^{-1}$	2009[52]
S. hygroscopicus	Promotion of proenzyme activation by adding exogenous proteases	6.61 $U \cdot ml^{-1}$	2010[53]

1.3 Effects of various nutritional conditions on the fermentation products of *Streptomyces mobaraensis*

Since Ando[3]et al. discovered that Streptomyces pentapentadienes can synthesize TG enzyme by fermentation, many researchers have begun to study the changes in the fermentation of Streptomyces pentadienes under various culture conditions to improve the fermentation yield of Streptomyces pentadienes. According to the research experiments conducted by domestic and foreign scientists in recent years, the yield of TG enzyme largely depends on the growth environmental conditions of microorganisms. Including carbon source, nitrogen source, pH, temperature, dissolved oxygen and so on. To a certain extent, the TG enzyme yield can be increased by adjusting the changes of these factors.

1.3.1 Effect of inoculation amount on fermentation products of bacteria

Inoculation amount refers to the amount of microorganisms added to the medium during the fermentation process. Too low inoculation amount will make the growth of bacteria small and the enzyme activity generated will be small, and the fermentation cycle will be prolonged, which will make the fermentation products more susceptible to the influence of external environment, resulting in changes in yield and quality. However, too much inoculation will lead to too fast growth of bacteria, too thick fermentation solution, insufficient nutrients and dissolved oxygen, and some undesirable secondary metabolites, which is not conducive to fermentation. According to previous reports, the most suitable inoculation amount should be 8%~12%[54].

1.3.2 Effect of species age on fermentation products

According to the previously reported data of the seed medium growth curve of Streptomyces pentapentadienes [54], when the species age was 48h, the enzyme activity and thallus dry weight values were higher, but after more than 60h, although the thallus enzyme activity was still increasing, the thallus dry weight began to decrease. Therefore, the most suitable age for inoculation is 48h.

1.3.3 Effect of pH value on bacterial fermentation products

PH value has a very important impact on the products and composition of bacterial fermentation. According to previous literature references [55], when pH value is below 6.0, the enzyme yield is low; when pH reaches 6.5, the enzyme yield begins to increase; when pH value is 7.0, the enzyme yield reaches the maximum; and then with the continuous increase of pH value, the enzyme yield begins to decline again.

1.3.4 Effect of medium composition on fermentation products

1.3.4.1 Effect of carbon source on bacterial fermentation

Carbon sources are the basis of microbial growth and metabolism, and different carbon sources have different effects on the results of fermentation enzymes. According to previous research papers [54], fermentation medium, soluble starch, fructose, glucose, maltose, glycerol, corn starch and potato starch were used as the only carbon source in this experiment, and the result was that glycerol was the best carbon source, while different studies also obtained that glucose and starch were the best carbon source [56]. This is due to the different strains of Streptococcus pentagenae.

In addition, Zhang Lili et al. [54] also studied the effect of different carbon sources on bacterial fermentation products when starch was the only carbon source. According to the research results, the enzyme activity reached the maximum value when the starch content was 30g/L, while the enzyme activity showed an upward or downward trend when the starch content was lower or higher than 30g/L.

1.3.4.2 Effect of nitrogen source on bacterial fermentation

Zhang Lili et al. studied the fermentation products of several common nitrogen sources: peptone, beef extract, fish meal, soybean meal, yeast powder, ammonium sulfate and potassium nitrate. The results showed that when peptone was used as the only nitrogen source in the experiment, the enzyme activity was the highest, while when beef extract was used as the only nitrogen source, the enzyme activity was 0. When inorganic nitrogen source was added as the only nitrogen source, the enzyme yield of potassium nitrate was higher than that of ammonium sulfate. In addition, the

effects of different amounts of the same nitrogen source on enzyme production were also studied. Polypeptone was used as the only nitrogen source in the study. The results showed that when polypeptone was 30g/L, the enzyme activity was the highest, and when it was lower than or above 30g/L, the enzyme activity showed an upward or downward trend respectively [54].

1.3.4.3 Effect of culture time on the production of enzymes by bacterial fermentation

Culture time is an important factor affecting enzyme activity during fermentation, and different bacteria have different sensitivity to culture time. According to the research results of teacher Zhang Lili et al. [54], the thallus grew slowly during 0-12h of culture, the dry weight of the enzyme began to increase during 12-48h, and reached the maximum at 48h, the enzyme activity began to rise rapidly after 72h of fermentation, and reached the highest at 120h of fermentation.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE RESEARCH

The purpose of the research include increasing the yield of TG enzymes, increasing the activity of TG enzymes, saving production costs, stabilizing product quality and promoting the sustainable development of fermentation processes.

The objectives of the study is Transglutaminase, *Streptomyces mobaraensis* and their nutritional conditions.

The object of the study is Transglutaminase and *Streptomyces mobaraensis*.

The subject of the study is Transglutaminase and *Streptomyces mobaraensis*.

2.1 Experimental material

Strain

Streptomyces mobaraensis (stored in the Laboratory of Biopharmaceutical Engineering, Faculty of Bioengineering, Qilu University of Technology).

Medium

Fermentation medium (g/L) : glycerin 20, peptone 20, yeast powder 5, corn flour 20, K_2HPO_4, 4,KH_2PO_4 2, $MgSO_4 \cdot 7H_2O$ 2, NH_4Cl 3.2, pH 7.0

Seed medium (g/L) : glycerin 20, peptone 20, yeast powder 5, $MgSO_4 \cdot 7H_2O$ 2, KH_2PO_4 2, K_2HPO_4 2, pH 7.0

Primary screening medium (g/L) : starch 15, glucose 15, peptone 20, yeast extract 2, $K_2HPO_4 \cdot 3H_2O$ 2, $KH_2PO_4 \cdot 3H_2O$ 2, $MgSO_4 \cdot 7H_2O$ 1, pH 7.0

GMY medium: Glucose 4.0 g, yeast extract 4.0 g, malt extract 10.0 g, $CaCO_3$ 2.0 g, AGAR 12.0 g, distilled water 1000 ml.

Strain recovery and culture

The cryopreserved Streptomyces pentadienes were resuscitated in GYM solid medium and incubated at 28 ^oC for 5 days.

Seed solution preparation

A colony block of 1cm2 was inoculated into 50mL liquid GYM medium and oscillated for 48 hours at 220rpm and 28 ^oC.

Experimental reagents and instruments (Tab. 2.1-2.2).

Table 2.1 – **Experimental reagents**

Reagent name	Quality	Reagent production company
Glucose, peptone, soluble starch	Analytically pure	Tianjin Kemi Ou Reagent Co., LTD
Malt extract	Analytically pure	OXOID
Glycerin, AGAR powder	Biochemical reagent	Tianjin Kemi Ou Reagent Co., LTD
KH_2PO_4、K_2HPO_4	Analytically pure	Xilong Scientific Co., Ltd.
$MgSO_4 \cdot 7H_2O$、NaCl	Analytically pure	Shanghai Chemical Reagent Co., LTD
$CaCO_3$	Analytically pure	Shanghai Lotus chemical reagent factory
Soybean cake powder	Biochemical reagent	Be available in the market
Yeast extract	Biochemical reagent	Fuyang Hangfu biological products factory
$FeCl_3 \cdot 3H_2O$	Chemically pure	Sinopharm Group
Hydroxylamine hydrochloride	Biochemical reagent	Jiangsu Akovi Technology Co., LTD
L-glutamic acid gamma-monohydroxyexime acid	Chemically pure	Sigma Corporation
CBZ-Gln-Gly	Chemically pure	Sigma Corporation

Table 2.2 – **Experimental instruments**

Instrument name	Instrument type	Producer
Electronic analytical balance	AL204	Mettler-toledo Corporation
pH meter	320	Mettler-toledo Corporation
Vertical electric steam sterilizer	LS-50HD	Shanghai Jingruo Scientific Instrument Co., LTD
Clean table	SW-CG-2F	Shanghai Boxun Industrial Co., LTD. Medical equipment factory
Ultra-low temperature refrigerator	ULT-1386-3V	Kendro laboratory, USA
Constant temperature and humidity incubator	PSX-330H	Ningbo Laifu Technology Co., LTD
Thermostatic oscillator	DHZ-DA	Taicang city experimental equipment factory
Ultraviolet visible spectrophotometer	722S	Shanghai Precision Scientific Instrument Company
centrifuge	H1850	China Xiangyi Co., Ltd
Electric thermostatic air drying oven	DHG-9023A	Hunan Genisez Scientific Instrument Co., LTD

2.2 Culture method

Bevel culture: After the medium solidified, the petri dish was tilted at 45°, and the center of the bevel was coated with Streptomyces penycens with a pipette. The bevel culture dish was placed in a constant temperature incubator at 30 ° C for 6 to 7 days, and the petri dish was observed regularly.

Fermentation culture: According to the needs of Streptomyces pentapentadienes, a fermentation medium containing suitable nutritional conditions is configured, the cultured fermentation strains are inoculated into the prepared fermentation medium and cultured to a suitable active state, the growth situation is regularly detected, the fermentation time and product concentration are centrifugated, and the quality of the extracted products is analyzed.

Seed culture: According to the needs of glutamine transaminase, suitable seed medium was configured, and the seed medium was divided into beakers, test tubes and AGAR petri dishes for high temperature sterilization. The microbial seeds were inoculated into sterile medium using pipette, and then cultured in incubator for a period of time after inoculation. The growth situation was observed regularly, and the cultivated seeds were stored separately.

2.3 Analytical method

2.3.1 Measurement of cell dry weight (DCW)

Take 10ml fermentation liquid and place it in a centrifuge tube after weighing, centrifuge it at a rotational speed of 4000 r/min for 20 min, discard the superserum and wash the sediment with deionized water twice, and dry it in a drying oven at 105 ℃ to constant weight. The dry weight of bacteria is the total weight minus the weight of the centrifuge tube.

2.3.2 Determination of MTG enzyme activity

According to reports[4], using N-CBZ-Gln-Gly as substrate and L-glutamate-γ-oxyamic acid as standard curve, TGase activity was determined by colorimetry. A unit of TGase activity is defined as the amount of enzyme required to produce 1μmol of monooxamic acid per minute at 37 ^{o}C and pH 6.0.

(1) Solution preparation

Reagent A solution: Accurately prepared 0.1M hydroxylamine hydrochloride solution, a small amount of 30mMCBZ-GIn-Gly, 10mM reduced glutathione and 0.2MTris-HCI buffer solution, stored in a refrigerator at 4 ^{o}C.

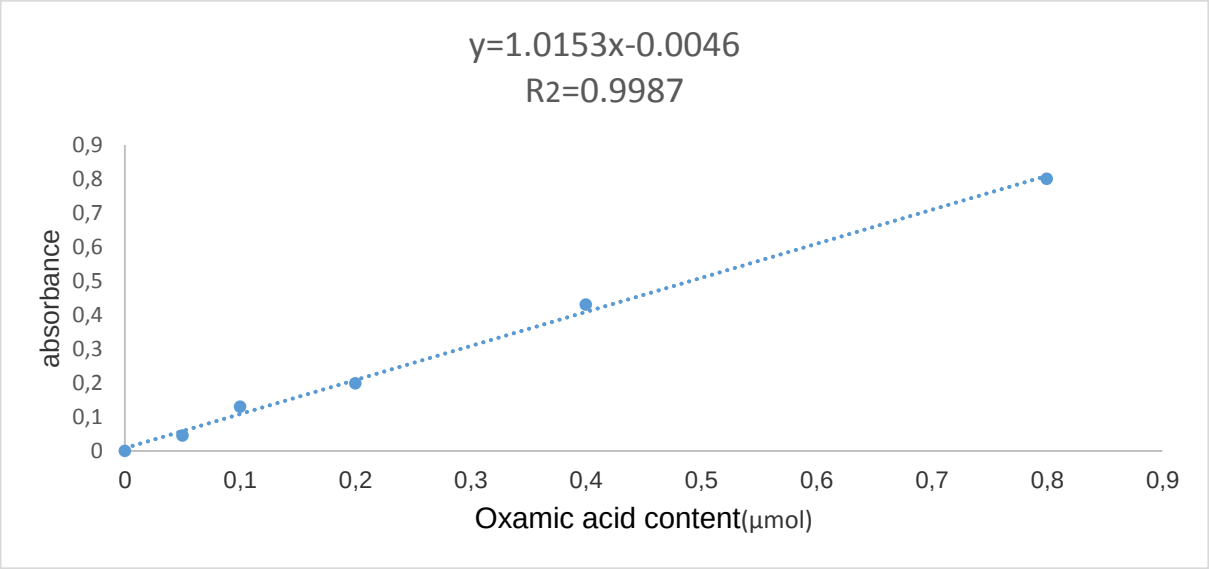
Reagent B solution: 5%FeCl3·6H2O solution was prepared with 0.1MHCl, 12% trichloroacetic acid and 3MHC1 solution were prepared with distilled water, and the three solutions were mixed in equal volume.

(2) Formulate a bar chart

Prepare 0.05, 0.1, 0.2, 0.4, 0.8mM1ml oxyamic acid solution respectively, then extract 0.2ml of each concentration of oxyamic acid solution, add 1mlA solution and 1mlB solution to each solution, mix repeatedly and evenly, centrifuge at 12000rpm, take the supernatant with a wavelength of 525nm, and measure its absorbance. Draw the oxamic acid content - absorbance bar fig. 2.3.

(3) Sample determination

Centrifuge 1ml fermentation product at 12000 rpm for 5 min, then take 0.2 ml supernatant, add 1.0 ml solution A, and bathe in water at 37 ^{o}C for 10min, immediately add reagent B solution until the reaction stops, centrifuge the reactant at 12000rpm for 1min, and extract the supernatant at 525 nm wavelength to determine its absorbance.



y=1.0153x-0.0046

R^2=0.9987

Figure 2.3 – Absorbance bar chart

CHAPTER 3
EXPERIMENTAL PART

3.1 Optimization of fermentation process

3.1.1 Effect of different carbon sources on glutamine transaminase production by bacterial fermentation

In order to analyze the effects of different carbon sources on glutamine transaminase, carbon sources were taken as a single factor variable, glucose, lactose, sucrose, starch, glycerol and mannitol were taken as carbon sources with the concentration of 20g/L, and the rest were added with reference to the fermentation medium in 2.1, cultured in a constant temperature shaking table at 30 ^{o}C and at 200rpm for 48h. The yield of glutamine transaminase was measured after 48h.

3.1.2 Effect of different nitrogen sources on glutamine transaminase production by bacterial fermentation

In order to analyze the effects of different nitrogen sources on glutamine transaminase, nitrogen source was taken as a single factor variable, and soybean powder, peptone, fish meal, corn meal, and mixed solution of peptone + yeast powder were respectively used as nitrogen source at a concentration of 20g/L. The carbon source was the optimal carbon source obtained in the above experiment, and the rest were added according to the fermentation medium in 2.1 in a constant temperature shaking table at 30 ^{o}C. The glutamine transaminase yield was measured after 48 hours of culture at 200 rpm.

3.1.3 Effect of different phosphates on glutamine transaminase production by bacterial fermentation

In order to analyze the effects of different phosphates on glutamine transaminase, phosphate was taken as a single factor variable, and K_2HPO_4, KH_2PO_4 and $(NH_4)_2HPO_4$ were taken as phosphate variables, respectively, with the concentration of 20 g/L. The optimal carbon and nitrogen source was selected

according to the above experimental results, and the rest were added according to the fermentation medium in 2.1 in a constant temperature shaking table at 30 ^{o}C. The glutamine transaminase yield was measured after 48 hours of culture at 200 rpm.

3.1.4 Effect of different initial pH on glutamine transaminase production by bacterial fermentation

In order to analyze the influence of different initial pH on glutamine transaminase, the initial pH was taken as a single factor variable, and the pH values were adjusted by NaOH and HCl to 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. The optimal results of carbon and nitrogen sources and phosphate were added according to the above experiments, and the rest were added according to the fermentation medium in 2.1 in a constant temperature shaking table at 30 ^{o}C. The glutamine transaminase yield was measured after 48 hours of culture at 200 rpm.

3.1.5 Effect of different shaking speed on glutamine transaminase production by bacterial fermentation

In order to analyze the effects of different shaker speeds on glutamine transaminase, the shaker speed was taken as a single factor variable, and the shaker speeds were controlled as 180r/min, 190r/min, 200r/min, 210r/min and 220r/min, and carbon and nitrogen sources, pH value and phosphate were added according to the above optimal experimental values. The rest were added according to the fermentation medium in 2.1 and cultured in a constant temperature shaking table at 30 ^{o}C for 48h. After 48h, the glutamine transaminase yield of the bacteria was measured.

3.1.6 Effect of different culture temperature on glutamine transaminase production by bacterial fermentation

In order to analyze the effects of different culture temperatures on glutamine transaminase, culture temperature was taken as a single factor variable, and culture temperatures were controlled as, ^{o}C: 26, 28, 30, 32 and 34, respectively. Carbon and

nitrogen source, pH value and phosphate were added according to the above optimal experimental values, and the rest were added according to the fermentation medium The glutamine transaminase was cultured at 200rpm at different temperatures for 48h, and the yield of glutamine transaminase was measured after 48h.

3.2 Experimental results and discussion

3.2.1 Determination of species age

In order to obtain a large number of cells with strong metabolic capacity and vigorous growth cycle in a short time and shorten the fermentation delay period, appropriate pre-culture treatment should be carried out on seeds in the early fermentation stage to understand the appropriate growth cycle of cells, so as to improve the production intensity, obtain the growth and survival advantages of production bacteria, and reduce the pollution of hybrid bacteria as much as possible. According to the experimental results of pre-culture dry weight measurement of bacteria, as shown in Figure 3.1, it can be seen that in 0-48h, the dry weight of bacteria continues to increase and the growth rate is fast, but after 48h, the dry weight of bacteria begins to decline slowly and the growth rate slows down. Therefore, inoculation at 48h is most suitable.

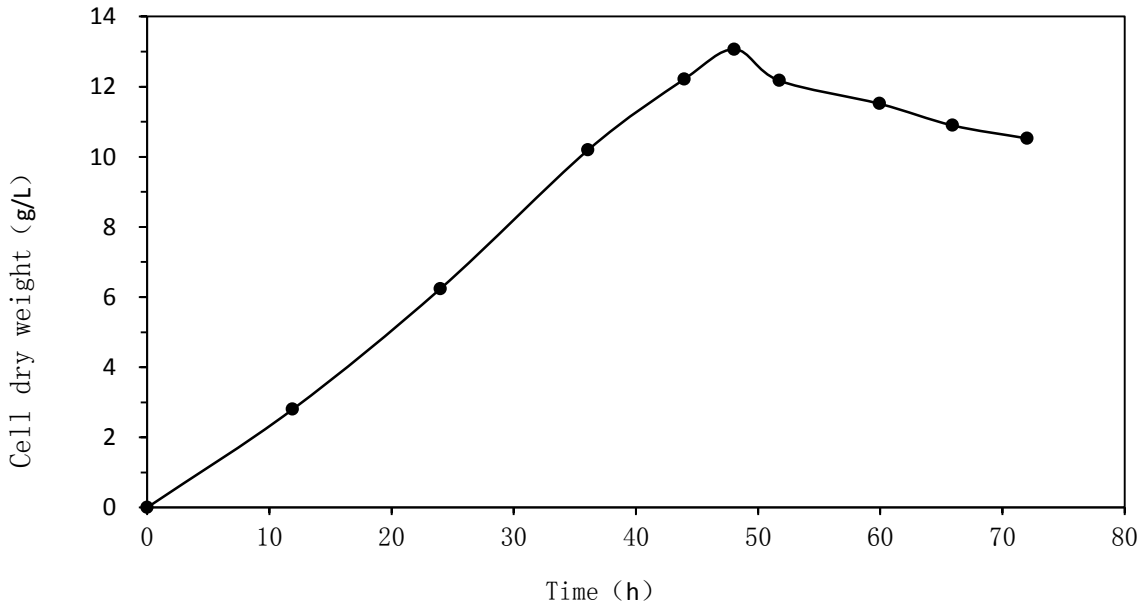


Figure 3.1 – Seed growth curve

3.2.2 Optimization of medium

3.2.2.1 Effects of different nitrogen sources on enzyme production

Nitrogen is commonly found in proteins and nucleic acids in living organisms, and nitrogen sources also provide certain amino acids necessary for cell development and growth components. Common nitrogen sources in the experiment mainly include organic nitrogen sources and inorganic nitrogen sources. Common organic nitrogen sources include beef paste, peptone, yeast powder, soybean powder, corn meal, fish meal, etc., while inorganic nitrogen sources generally refer to some nitrates. Different bacteria have different requirements for nitrogen sources due to different species.

In order to compare the effects of different organic nitrogen sources on TG enzyme production, glycerol 20g/L was selected as the single carbon source, and soybean meal, peptone, fish meal, corn meal, and the mixed solution of peptone + yeast powder were selected as the single nitrogen source, respectively. As shown in the results 2.5, when corn meal was selected as the only nitrogen source, the enzyme activity was the highest and it was the most suitable nitrogen source. Fish meal as the only nitrogen source produced the lowest enzyme activity, the least suitable for nitrogen source, peptone + yeast powder, soybean powder and peptone as the only nitrogen source of enzyme activity yield is similar, but the use of peptone as the only nitrogen source is too volatile yield fluctuate.

As shown in Figure 3.2-3.8, the concentration of corn meal visible to the naked eye after fermentation is the highest, followed by fish meal, and the concentration of the other three groups is lower.

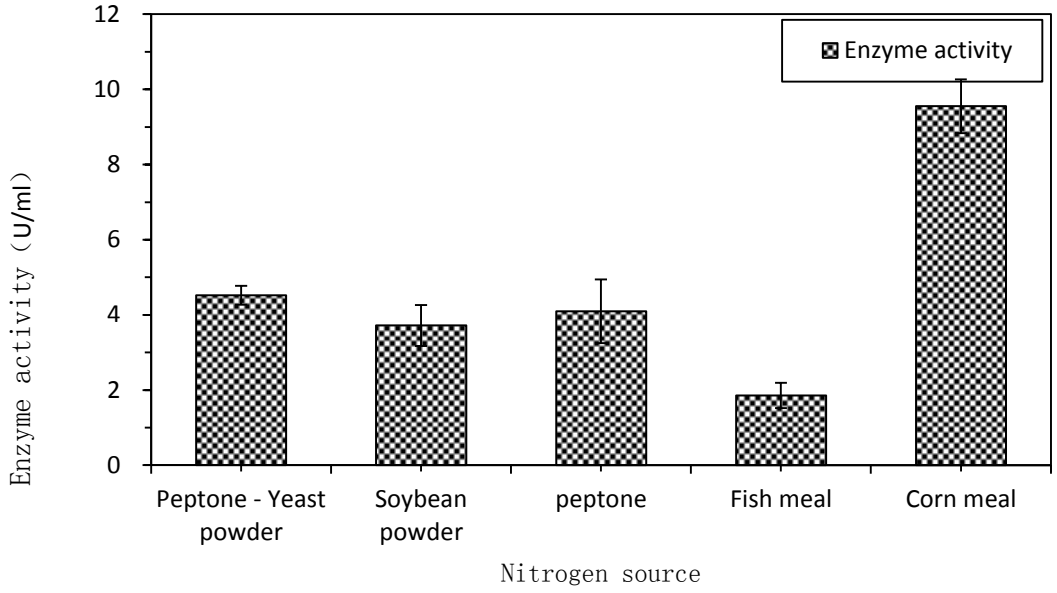


Figure 3.2 – Effects of different nitrogen sources on enzyme production

Figure 3.3 – Comparison of post-fermentation concentrations of different nitrogen sources.

Figure 3.4 – Comparison of concentration of corn meal after fermentation

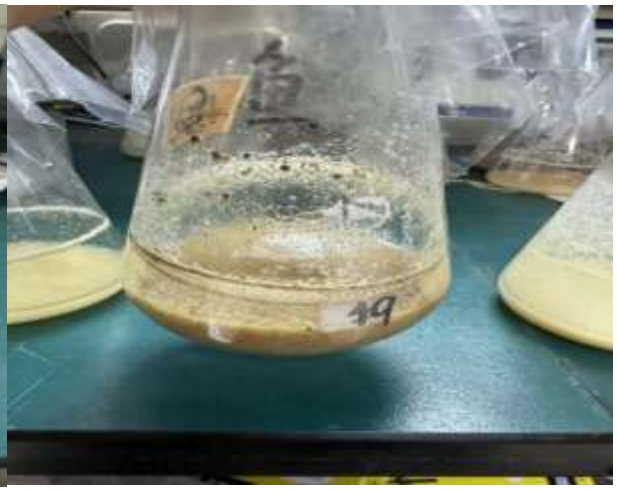


Figure 3.5 – Comparison of concentration of fish meal after fermentation

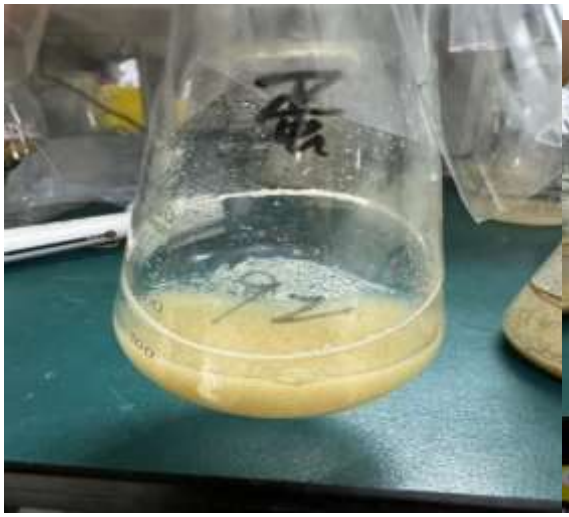


Figure 3.6 – Comparison of the concentration of peptone after fermentation

Figure 3.7 – Comparison of the concentration of soybean powder after fermentation

Figure 3.8 – Comparison of concentration of mixed solution of peptone + yeast powder after fermentation

3.2.2.2 Effects of different amounts of the same nitrogen source on enzyme production

After determining corn meal as the most suitable nitrogen source for bacterial growth, the effects of different corn meal concentrations on enzyme production were further studied. The single carbon source variable was controlled, 10g/L, 15g/L, 20g/L, 25g/L and 30g/L corn flour were added, respectively, and the enzyme activity was observed after 48h. The experimental results are shown in Figure 2-12. The enzyme activity gradually increases at 10~20g/L, reaches the highest level at 20g/L cornmeal concentration, and then gradually decreases from 20~30g/L.

Fig. 3.9-3.11 show the comparison of concentrations of corn flour after fermentation with different concentrations.

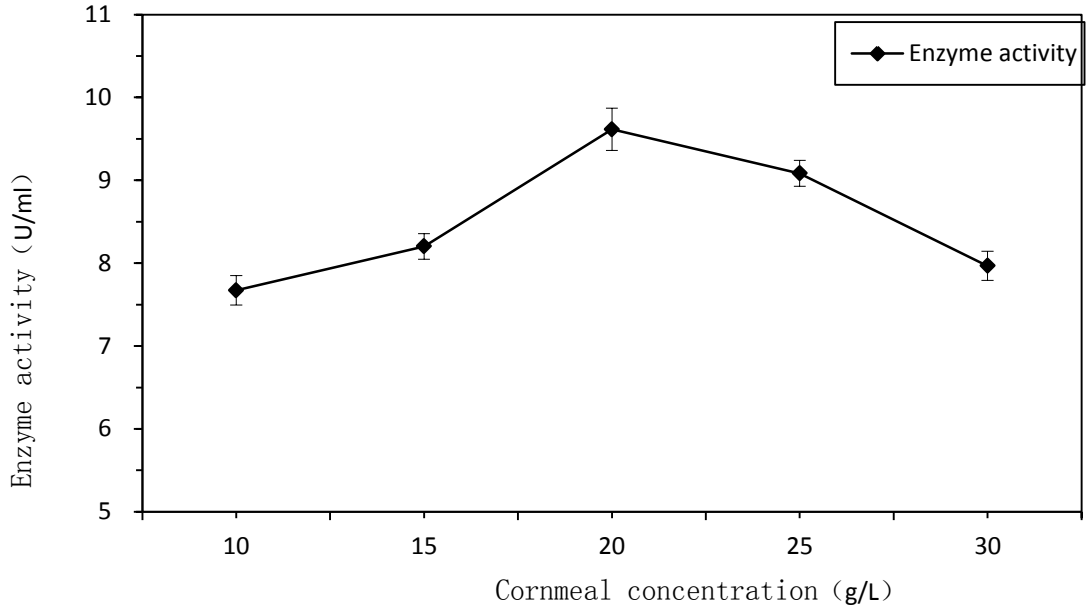


Figure 3.9 – Effect of different concentration of corn meal on enzyme production

Figure 3.10 – Comparison of 10 g/L to 30 g/L corn flour concentrations

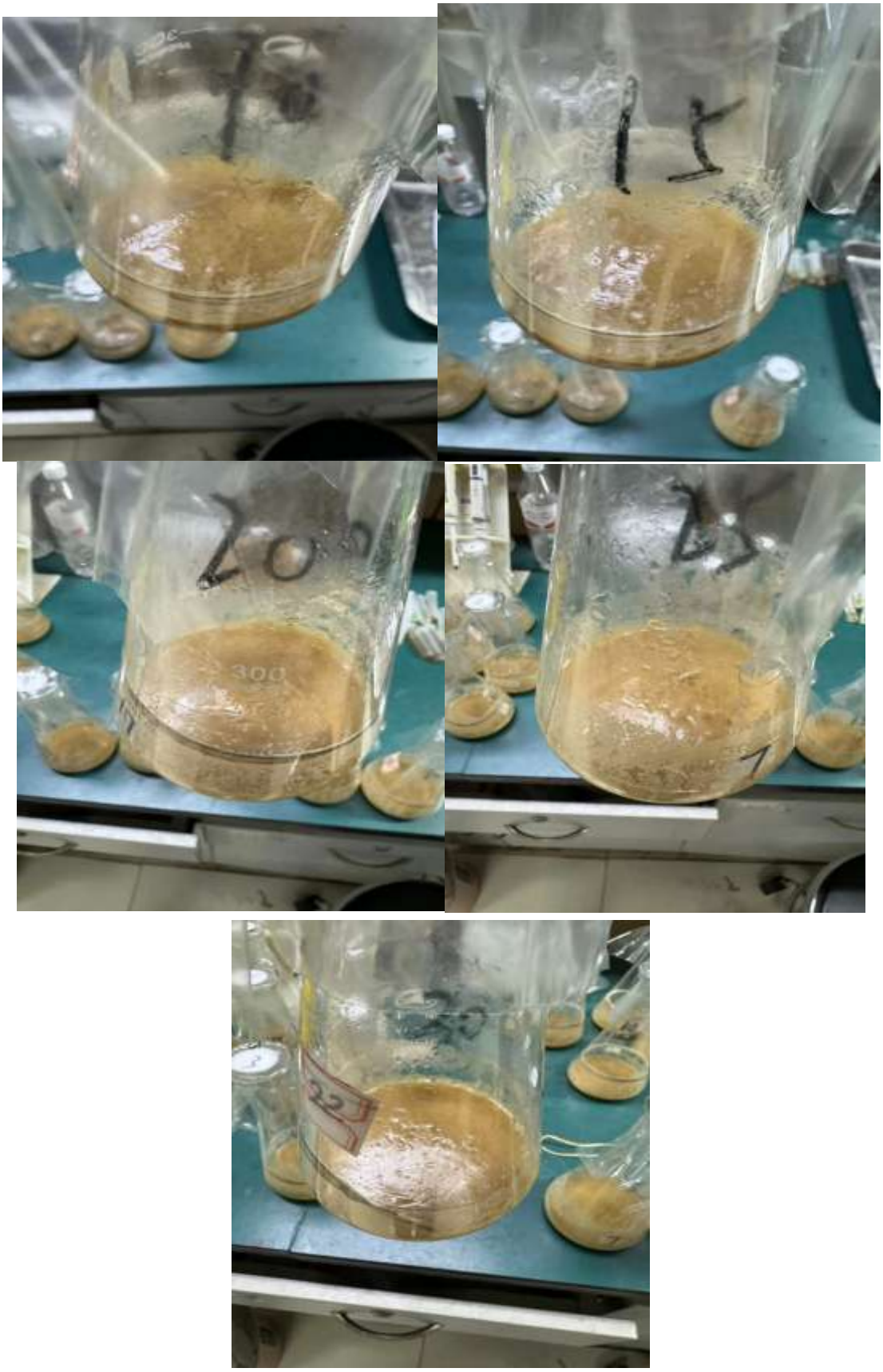


Figure 3.11 – Comparison of corn flour concentration after fermentation

3.2.2.3 Effects of different carbon sources on enzyme production

Carbon source is an important part of fermentation medium, which provides nutrition for the growth and development of microorganisms or the synthesis of various metabolites, and is the main energy source for the survival and growth of many microorganisms. At present, common organic carbon sources mainly include various sugars, alcohols and esters. Carbon sources are used in different forms by different microorganisms, which are determined by their own enzyme systems.

In order to compare the effects of different carbon sources on the TG enzyme activity produced by the fermentation of Streptomyces pentapentadienes, glycerin, mannitol, glucose, lactose, sucrose and starch were respectively used as the only carbon sources, and other variables were controlled for fermentation culture, as shown in Figure 3.12.

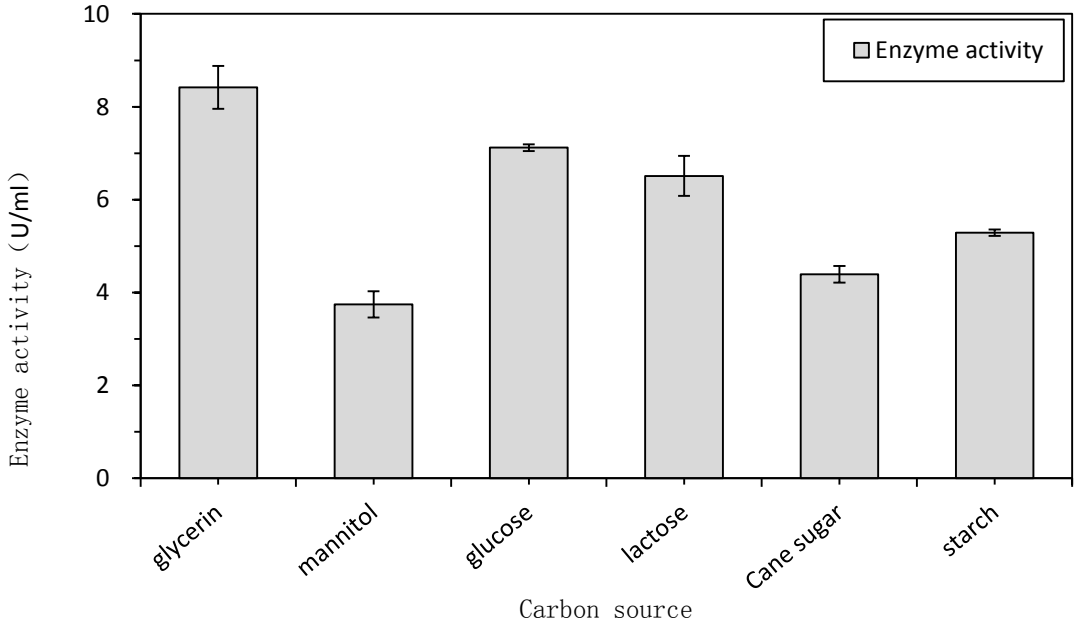


Figure 3.12 – Effects of different carbon sources on enzyme production

As can be seen from Figure 3.12, the final enzyme activity of glycerol is the highest, but it fluctuates greatly, the enzyme activity of glucose and lactose is high, while the activity of mannitase is the lowest, which is obviously the least suitable for use as the only carbon source.

3.2.2.4 Effects of different amounts of the same carbon source on enzyme production

After glycerol was identified as the most suitable carbon source for bacterial growth, the effects of different glycerol concentrations on enzyme production were studied. A single nitrogen source variable was controlled, glycerol of 10g/L, 15g/L, 20g/L, 25g/L and 30g/L were added, respectively, and the enzyme activity was observed after 48h. The experimental results are shown in Figure 3.13. The enzyme activity gradually increased at 10~15g/L, the enzyme activity was the highest at the glycerol concentration of 15g/L, but the fluctuation was large; then the enzyme activity concentration gradually decreased from 20~30g/L, and the enzyme activity was small and high at 20g/L. As shown in the figure, too low glycerol concentration is extremely unfavorable to the fermentation of enzymes.

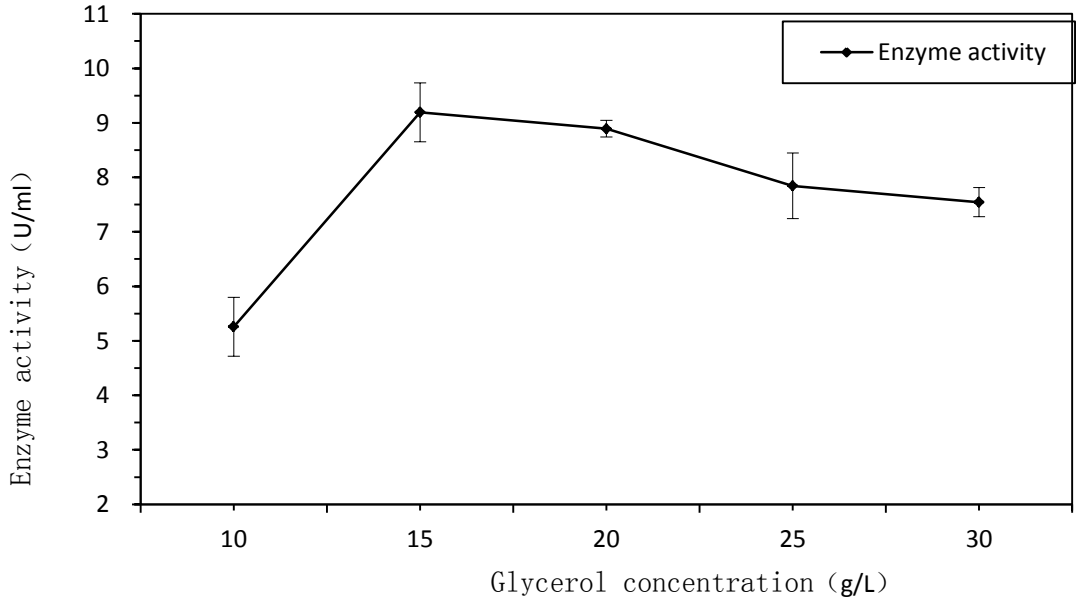


Figure 3.13 – Effects of different glycerol concentrations on enzyme production

3.2.2.5 Determination of the optimal initial pH value

The initial pH value of the medium also has a great impact on the growth of microorganisms, and the pH value is also one of the main reasons for affecting the enzyme activity, which is an important factor affecting the fermentation process of Streptomyces penylocene. Therefore, it is necessary to determine the optimal initial pH value of microbial fermentation. According to the above tests, the carbon source

of the medium was glycerin, and the addition amount was 15g/L. The nitrogen source was corn pulp powder, and the added amount was 20g/L. The single variable was set as pH value, the pH value was adjusted using NaOH and HCl, and the enzyme activity was measured at pH 5.0, 6.0, 7.0, 8.0, 9.0. As shown in Figure 3.14, enzyme activity gradually increases before pH value is 7.0; When pH value is 7.0, the maximum value of enzyme activity is reached. After pH was 7.0, the enzyme activity gradually decreased, and the enzyme activity decreased too much.

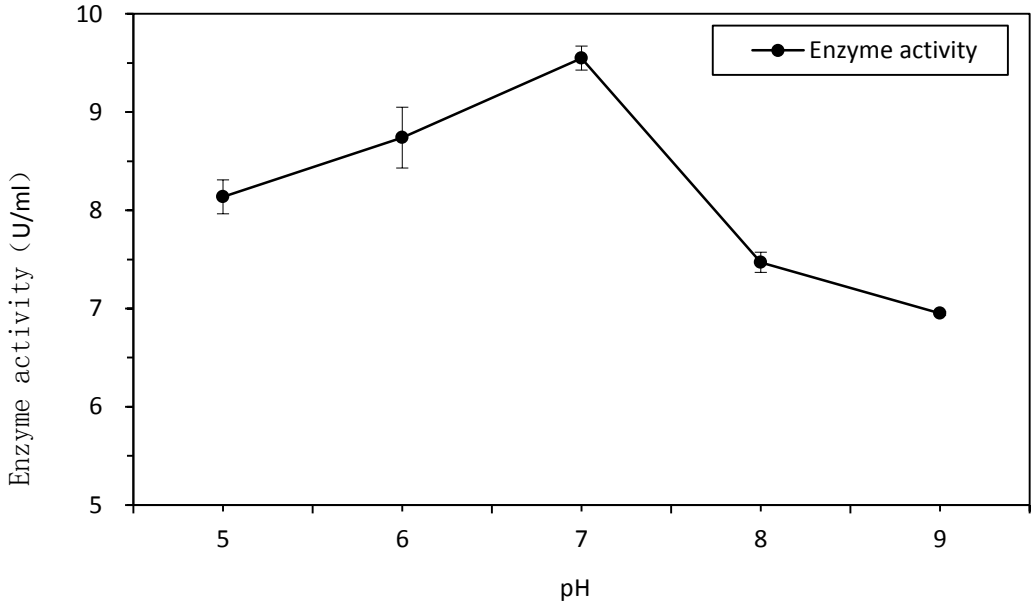


Figure 3.14 – Effects of different pH values on enzyme activity

3.2.2.6 Determination of the optimum temperature

Since all the life activities of microorganisms are biochemical reactions, they need to continuously absorb or release heat, and the activity of enzymes is also greatly affected by temperature. Therefore, temperature is an important factor affecting microbial growth. According to the above tests, the carbon source of the medium was glycerin, and the addition amount was 15g/L. The nitrogen source was corn pulp powder, and the added amount was 20g/L. The single variable was set as temperature to explore the influence of different temperatures on TG enzyme fermentation. The experimental results are shown in Figure 3.15. The results showed that the enzyme activity was the highest when the culture temperature was 30 ^{o}C. When the temperature increases or decreases, the activity of TG enzyme will

decrease to a certain extent. The results indicated that 30 ^{o}C was the most suitable culture temperature for the synthesis of TG enzyme by the fermentation of Streptomyces pentapentadienes.

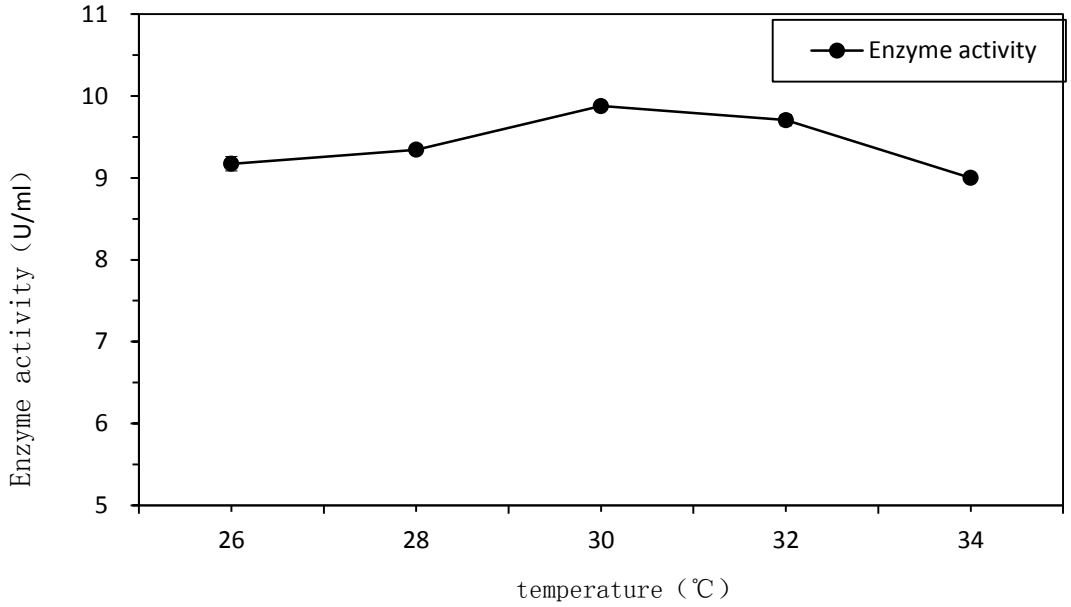


Figure 3.15 – Effects of different temperatures on enzyme activity

3.2.2.7 Effect of rotating speed of shaker on enzyme activity

Streptomyces cyclopentadienes belongs to aerobic bacteria, and the fermentation process is aerobic fermentation, so oxygen is an important factor in the fermentation process. Generally speaking, less dissolved oxygen is beneficial to actinomyces spore germination and mycelium growth, but not conducive to enzyme production. More dissolved oxygen will promote the production of enzymes, but it is not conducive to the growth of bacteria. Oxygen content is therefore crucial in the fermentation process. The rotating speed of the shaking table is an important factor affecting the dissolved oxygen content in the shaking bottle. Too small rotating speed will make the dissolved amount of oxygen in the shaking bottle less, and then easily form mycelium, which is not conducive to the production of enzymes. Too high speed will cause too much dissolved oxygen, will inhibit the growth of bacteria. FIG. 3.16 explored the effects of different rotational speed on enzyme production. Experimental results showed that when the rotational speed was lower than 210r/min, enzyme activity increased with the increase of rotational speed, and the enzyme

activity reached the highest level at 210r/min, while the enzyme activity began to slowly decrease when the rotational speed continued to increase.

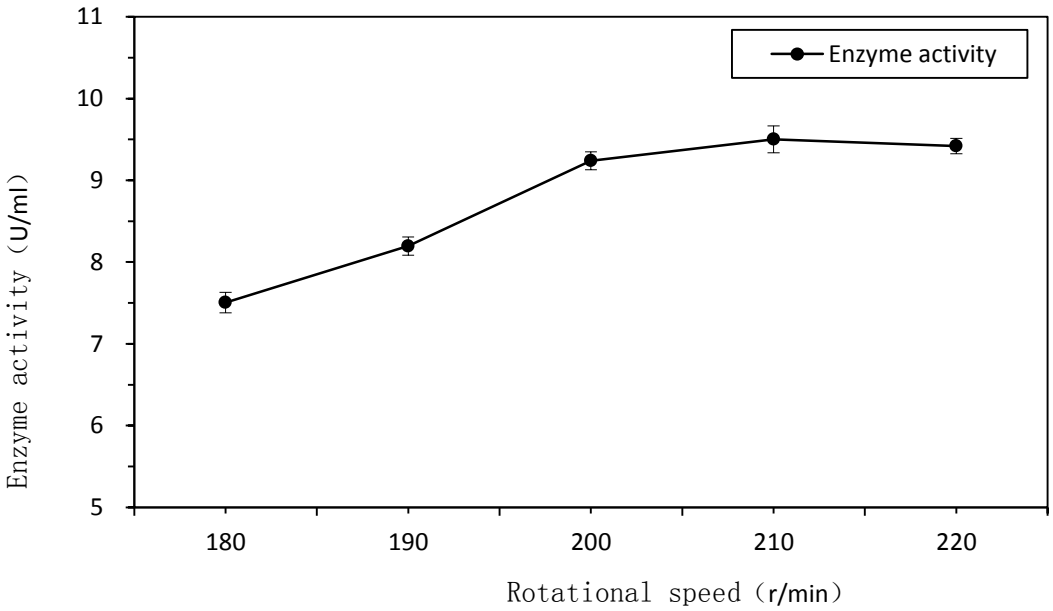


Figure 3.16 – Effect of shaker speed on enzyme activity

3.2.3 Orthogonal test

The optimal medium optimization scheme was studied through the single factor experimental method. However, due to the excessive influence factors in the experiment, it is impossible to explore whether the relationship between each influence factor can be perfectly integrated to form the optimal medium optimization scheme. Therefore, in order to make the experiment more rigorous, it was decided to use the above experimental results to select the best carbon and nitrogen sources for culture medium fermentation, respectively glycerol, glucose, corn flour and peptone + yeast powder mixture, to conduct four-factor and three-level orthogonal test, so as to finally determine the optimal combination of culture medium fermentation. The experimental design and results of the orthogonal experiment are shown in Table 2-20 below.

According to Table 3.1, the concentration of corn meal has the greatest influence on the production of glutamine transaminase, and the mixed solution of peptone + yeast powder has the least influence. The order of influence of the concentration of each factor on the production of glutamine transaminase was corn

meal > glycerin > glucose > peptone + yeast powder mixture. The optimal levels of each factor were glycerol 15 g/L, glucose 15 g/L, corn flour 20 g/L, and peptone + yeast powder mixture 15 g/L, respectively. It is consistent with the experimental results obtained by the previous single-factor control variables. The optimal nutrient conditions of carbon and nitrogen source were obtained: glycerol 15 g/L, glucose 15 g/L, corn flour 20 g/L, peptone + yeast powder mixture 15 g/L. According to this carbon and nitrogen source medium, the fermentation was reconfigured, and the MTG enzyme activity was 9.47 U/ml after fermentation, and the orthogonal test results were verified.

Table 3.1 – **Experimental design and results of orthogonal test**

Test number	glycerin	glucose	Corn meal	Mixed liquor	Enzyme activity
1	1(15)	1(10)	1(15)	1(10)	8.72
2	1	2(15)	2(20)	2(15)	9.45
3	1	3(20)	3(25)	3(20)	8.97
4	2(20)	1	2	3	9.27
5	2	2	3	1	9.11
6	2	3	1	2	8.67
7	3(25)	1	3	2	8.61
8	3	2	1	3	8.17
9	3	3	2	1	8.74
K_1	9.046	8.867	8.520	8.857	
K_2	9.017	8.910	9.153	8.910	
K_3	8.507	8.793	8.897	8.803	
R	0.539	0.117	0.633	0.107	

K1, K2, K3 refers to the indicators and values of each factor at this level.

R is the range, indicating the influence of factors on the test results.

R=Kmax-Kmin

3.3 Summary of this chapter

The best carbon source was glycerol and the best nitrogen source was corn meal by single factor experiment. Through orthogonal test, the correctness of single factor experiment was confirmed, and the optimal carbon and nitrogen source optimization conditions were glycerol 15g/L, glucose 15g/L, corn flour 20g/L, peptone + yeast powder 15g/L. Through the optimization of various variables of fermentation medium, the activity of TG enzyme was greatly improved, and the fermentation culture program was further optimized.

The nutrient conditions of TG enzyme in the fermentation culture of Streptomyces pentapentadienes were further optimized. Liquid seeds that had been cultured for 48 hours were inoculated, and the initial pH value was adjusted to 7.0, the culture temperature was 30 ^{o}C, the shaking speed was 210r/min, and the culture time was 48 hours. By optimizing the fermentation medium and fermentation conditions of Streptomyces pentapentadienes, the TG enzyme activity reached 9.47U/ml.

CONCLUSIONS

1. By comparing the effects of different nutritional conditions on TG enzyme yield, it can be seen that reasonable selection of carbon and nitrogen sources plays a key role in improving product yield. In addition, pH value, temperature, shaker speed and seed age are also important factors affecting the yield of TG enzymes.

2. In this study, the nutritional conditions for the production of TG enzymes by the fermentation of Streptomyces pentoprotocens were systematically optimized. The optimal carbon source was glycerin, the optimal nitrogen source was corn flour, and the optimal culture conditions were inoculation at 48h of seed age, initial pH was 7.0, culture temperature was 30 ^{o}C, and shaking speed was 210r/min. The results were verified by orthogonal experiment, and the optimal carbon and nitrogen source was glycerin 15g/L, glucose 15g/L, corn flour 20g/L, and peptone + yeast powder 15g/L.

3. Glutamine transaminase is a very important enzyme at present. At present, the fermentation process of glutamine transaminase is still immature and the production cost is too high. It is necessary to further construct and improve the engineering strain to improve the stability of the enzyme producing strain and the production efficiency of the enzyme, reduce the production cost, and enhance the stability and activity of the enzyme. Secondly, the application field of glutamine transaminase should be further expanded, involving more fields: biosynthesis, pharmaceutical preparations, food processing, environmental protection, etc., to provide more possibilities for the progress of biotechnology. The use of glutamine transaminase biocatalytic synthesis of polyester, biodegradable plastics and other new materials provides a new way for green sustainable development.

4. Glutamine transaminase fermentation is expected to play a more important role in the field of biotechnology in the future, providing more opportunities and challenges for the progress and application of biotechnology. Through continuous research and innovation, the application prospect of glutamine transaminase will be broader.

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