

Bio-enzymatic treatment and decarboxylation applications in catalytic synthesis

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Abstract. Bio-enzymatic processes have been recognized as a green production process that meets the requirements of environmental protection. The pretreatment, enzyme preparation and decarboxylation process of bio-enzyme were carried out through bio-enzyme catalysis. It shows that under the conditions suitable for bio-enzymatic conversion, high-efficiency catalysis can be carried out, and the environmental problems such as hazardous waste and sewage caused by chemical synthesis are reduced. This program has solved many problems in food manufacturing and pharmaceutical manufacturing and is worth promoting. At the same time, we have a certain outlook on the existing problems and future development trends of biological enzymes.

1. Introduction

Biotechnology is a high technology in the 21st century. Enzyme engineering and cell (or organelles) are used to complete the products needed for catalytic function - enzyme engineering technology as an important branch of biotechnology, with the characteristics of high efficiency, specificity, safety, and mild catalytic conditions, it has been more and more widely used in various fields of food industry and pharmaceutical industry. Enzymes have high biological activity and are 10⁷-10¹³ times more than inorganic catalysts [1] have brought new development ideas for the food industry and the pharmaceutical industry. With the gradual expansion of enzyme research and application fields, the achievements of enzymes in the industrialization process are gradually changing the entire industrial manufacturing field.

The sources of enzymes are mainly animals, plants and microorganisms. They have a wide range of different properties. According to the types of their catalytic reactions, the International Association of Biochemistry classifies enzymes into six types: oxidoreductases, lyases, isomerases, transferases, and hydrolase and synthetase. These six enzymes are important catalysts for the enzymatic preparation of keto-acids such as keto-leucine, the enzymatic preparation of chiral products such as calcium-D-pantothenate, and the enzymatic synthesis of peptide synthesis products such as alanyl glutamine and enzymes. The preparation of amino acid derivatives such as cracking, transfer, and hydrolysis products has an extremely wide range of applications.

In recent years, with the promotion of genetic engineering, bioengineering, immobilized cell technology and immobilized enzyme reactor technology, the application of enzymes in new products has been promoted once again. With the increasing environmental protection threshold and product quality, bio-enzymes have been given new functions according to people's wishes, and have



constructed new species in various forms, bringing huge economic benefits to the food and pharmaceutical fields.

2. Pretreatment of enzymes

The engineered strains that have been specifically engineered can be crushed by physical, mechanical, chemical or enzymatic treatments after fermentation or fixed transcription, generally including crude enzyme preparation and refining processes. That is, the solid-liquid separation of the cells or organelles carrying the biological enzyme is carried out. After the cells are broken up, dialysis or a centrifuge is used to separate the various small molecular substances in the enzyme and the extract, and then according to the biological enzyme molecular weight, primary structure and three-dimensional structure filtration, sedimentation, adsorption and desorption, chromatographic flow, electrophoresis, molecular sieve and other methods for further purification.

3. Application of enzyme preparation

The enzymes are bound in a certain area to play a catalytic role in a specific range. The commonly used methods are immobilization techniques and crystallization, and the immobilization technology is to immobilize biological enzymes or cells that have undergone pretreatment. The enzyme is further purified by different vectors, and the immobilized enzyme and immobilized cells can be firmly fixed in the carrier without being lost. Crystallization is the crystallization of the purified enzyme to further freeze-dry or concentrate and obtain the characteristic enzyme crystals. In either way, high-purity characteristic enzymes will be obtained.

4. Application of enzymes in decarboxylation

4.1. Decarboxylation Mechanism of Enzymes

Amino acid decarboxylase is an important amino acid decarboxylase that catalyzes the removal of a certain amino acid from the carboxyl group. The generic term for the corresponding amine lyase is $\text{NH}_2\text{CHR}\text{COOH} \rightarrow \text{NH}_2\text{CH}_2\text{R} + \text{CO}_2$. For example, after lysine decarboxylation, cadaverine, tyrosine decarboxylation to obtain tyramine, glutamic acid decarboxylation to obtain γ -aminobutyric acid, etc., these enzymes use pyridoxal phosphate as a coenzyme, which has been widely used in industrial production. Many of the amines produced by the decarboxylation of amino acids play an important role physiologically and pharmacologically in animals. For example, decarboxylation of arginine to obtain agmatine can enhance the analgesic effect of opioids and slow down the occurrence of drug resistance [2]. In this experiment, the decarboxylation of L-aspartic acid to β -alanine under the action of L-aspartate α -decarboxylase was used as an example to illustrate the decarboxylation process of biological enzymes.

4.2. Drugs and Reagents

B-Alanine (β -ara) Standard: China National Institute for the Control of Pharmaceutical and Biological Products

L-aspartate (L-asp) Standard: China National Institute for the Control of Pharmaceutical and Biological Products

L-aspartic acid: industrial products; potassium dihydrogen phosphate, glutaric acid, ammonium sulfate, calcium chloride as analytical grade

4.3. Instruments and Equipment

Agilent 2160series high-performance liquid chromatograph, 101s electric magnetic stirrer, one ten-thousandth scale, dragon 100 μ l autosampler.

Liquid conditions: column temperature of amino-bonded silica gel column (250mm*4.6mm, 5 μ m), 30°C, 0.05mol/L phosphate buffered saline as mobile phase, flow rate 1.0ml/min, wavelength 205nm, injection volume 20 μ l [3].

4.4. Enzyme immobilization and catalytic conversion

Polyvinyl chloride, gelatin as a solidified carrier, potassium dihydrogen phosphate, sodium chloride, hexamethylenediamine, potassium chloride, glutaraldehyde, ammonium sulfate and calcium chloride as auxiliary reagents, using IMC technology for L-day door After the solidification of the tyrosine α -decarboxylase, after 12-24 hours of treatment, the immobilized cells are rinsed to obtain an immobilized biological enzyme catalyst.

Using sodium carbonate and L-aspartic acid with a 0.045 mol/L sodium aspartate aqueous solution, controlling the temperature at 33-35°C, adding the immobilized cells with a total enzyme activity of 1800 U for catalytic conversion, and sampling after 24 hours of reaction. The product is shown in Figure (2):

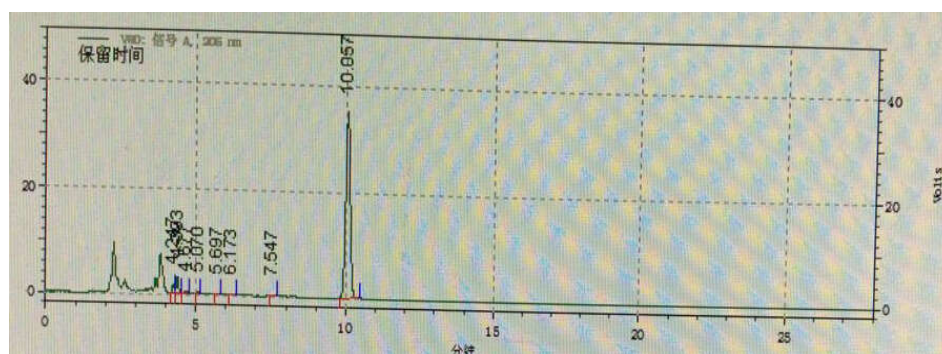


Figure 1. Contrast map

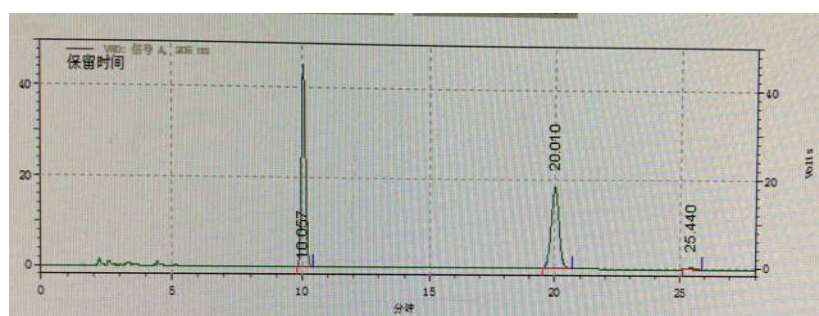


Figure 2. Transformational 24-hour map

It can be seen from the above figure that after bio-enzyme efficient catalysis, the substrate L-aspartic acid (about 20min out of the peak position) and L-aspartate α -decarboxylase under the action of decarboxylation conversion rate of 100%, it is completely converted to completely transformed into Beta-alanine (Figure 1: about 10 minutes at the front).

After the conversion is complete, the resulting conversion solution is de-enzymed by centrifugation. After the activated carbon is decolorized, it is directly distilled under reduced pressure. The resulting fraction condensate can be directly recycled to dispose of sodium carbonate and L-aspartic acid, thus recycling the water resources. The recovery rate was 87.5% or more. After the obtained biological enzyme is detected, the enzyme activity can be directly applied to the next batch, and the recovery rate is above 95%. The obtained activated carbon is recycled by a qualified recycler and can be reused to recycle activated carbon.

The above is only a simple example of the decarboxylation process of a biological enzyme. We know that a lot of chemicals come from biological systems, because bacteria and their ancestors have been in the world for 3.6 billion years. Such a long time is enough to evolve many new enzymes. There are now approximately 5×10^{30} prokaryotic organisms on our planet [4], each carrying a unique catalytic enzyme, so the development of biological enzyme systems is an inexhaustible treasure house.

In the development of new bio-enzyme, through genetic engineering and cell engineering techniques, the selection of suitable bio-enzyme carriers, bio-enzyme toxicity, bio-enzyme three-dimensional structure, bio-enzyme catalytic mechanism, bio-enzyme self-degradation and regeneration, bio-enzyme highly purified or crystallization are still the focus of research.

4.5. Problems

In the L-aspartate α -decarboxylase catalysis example, when the substrate L-aspartic acid (amino acid) is used as a raw material, a very slight amount of impurities is generated through the catalytic conversion of bio-enzyme, as shown in Fig. 2, impurities of about 4.0 minutes may be other substances newly generated under the action of miscellaneous enzymes during the conversion process. They can be removed through the activated carbon adsorption process and the crystallization process. If the purity requirements are high, they can be refined repeatedly to achieve high purity target.

Through the bio-enzyme catalyzed process to achieve the preparation of new substances, it is difficult to avoid the introduction of new proteins or nucleic acid fragments, how to effectively remove and ensure that the safety of the prepared product in the field of food, medical care is still the current need to continue investment in research and development one of the hotspots. At the same time, a large number of biological enzyme residues will inevitably bring about new problems. How to perform effective degradation or reuse is also a major task facing us.

5. The development trend of bio-enzymes

With the introduction of the national concept of manufacturing 2025, the combination of intelligent industry and emerging bio-enzymatic catalysis technology is expected to realize a variety of emerging technologies in the near future: such as a single bio-enzyme nutritional supplement can achieve targeted localization of time-limited enzyme release and supplement of physiological lesions. It is expected to realize new-type medicine in patients with hormone or enzyme deficiency such as diabetes and hypertension. Bio-enzyme coupling technology can link bio-enzymatic molecules with electronic and optical equipment systems, and transfer bio-enzyme conversion processes through signals to realize electronic bio-enzymes or optical bio-enzymes. Bio-enzyme biomimetic technology infinitely enlarges the functions of a specific tissue or organ of an animal or plant, and continuously obtains the target product. Bio-enzyme chip technology is also expected to be the first to be implemented and applied to the medical field. The information technology and bio-enzymatic technology can be effectively linked to create a chip that can achieve memory function to treat genetic defects that are currently difficult to solve. The urgent need for the development of self-degrading products of biological enzymes can also solve problems such as urban solid waste and industrial waste that are plaguing human development.

We have reason to believe that in the future development of new bioenzymatic technologies, agriculture, light industry, medical and health care, new energy, new materials, recyclable resources, and other aspects will all change with each passing day, and have long plagued us. Medical problems, biomass energy and environmental pollution will surely be solved quickly.

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