Advances in Research on Production of 1, 3-PD by immobilized technology

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Abstract. 1,3-propanediol (abbreviated as 1,3-PD) is an important chemical raw material, which is widely used in printing and dyeing, coatings, inks, pharmaceuticals, antifreeze, lubricants, etc. Used as a monomer to synthesize new polyester material PTT (polytrimethylene terephthalate) has attracted wide attention. The production methods of 1,3-PD mainly include chemical synthesis and microbial fermentation. Due to the shortage of petrochemical resources, the production of 1,3-PD by microbial fermentation has become a hotspot at home and abroad. Among them, the application of immobilization technology and the selection of immobilized carrier materials have a good improvement in the efficiency of 1,3-PD produced by fermentation.

Keywords: 1,3-PD; Microbial fermentation; Immobilization.

1. Introduction

In industry, 1,3-PD is one of the bases for the synthesis of new polyester polytrimethylene terephthalate (PTT). At the same time, it is also the main additive of some cosmetics, lubricants, drugs, adhesives and other substances. The structural formula of 1,3-PD is HOCH₂CH₂CH₂OH. It is colorless and odorless, and is a hygroscopic and transparent viscous liquid. It is soluble in certain organic solvents (ethanol, water, ether, etc.). And it is also a low-toxic, non-corrosive substance that can burn when exposed to fire[1, 2].
Table 1. The physical properties of 1,3-PD [3, 4]

| Molecular weight | Melting point (℃) | Boiling point (℃) | Relative density | Saturated vapor pressure (kpa) | Flash point (℃) | Ignition point (℃) |
|------------------|-------------------|-------------------|-----------------|-----------------------------|----------------|-------------------|
| 76.10            | -27               | 210-211           | 1.05 (25℃)     | 0.13 (60℃)                 | 79             | 400               |

The common production measures of 1,3-PD can be divided into chemical synthesis and microbial fermentation. In industrial production, chemical synthesis is widely used, but in recent years, due to the promotion of "energy saving and emission reduction" measures, the production of 1,3-PD through microbial fermentation has gradually become one of the key points of research worldwide[5].

2. Microbial fermentation production 1,3-PD

In the late nineteenth century, it was discovered that 1,3-propylene glycol can be produced by biological fermentation, but it was not until a few years ago that microbial fermentation has been greatly developed. Although the bio-fermentation method is currently in the laboratory and small-scale test stage, it has not yet achieved large-scale industrial production, but compared with the chemical method of producing 1,3-PD, it is more resource-saving and sustainable, which is in line with the 21st century. Development requirements, and equipment investment is small, simple operation, and fewer by-products. Therefore, the microbial fermentation method has great application and development prospects.

2.1. Microorganisms producing 1,3-PD by fermentation

Currently, many microbial strains can use glycerol as a substrate to produce 1,3-PD through fermentation. These strains are shown in Table 2.

Table 2. Strains producing 1,3-PD by fermentation[6]

| Mycobacterium      | Strain                  | Pathogenicity |
|--------------------|-------------------------|---------------|
| Klebsiella         | Klebsiella pneumoniae   | ✓             |
| —                  | Klebsiella TOCA         | ✓             |
| —                  | Klebsiella planticola   | ✓             |
| —                  | Klebsiella aerogenes    | ✓             |
| Citrobacter        | Citrobacter freundii    | ✓             |
| Enterobacter       | agglomerans             | ✓             |
| Lactobacillus      | Lactobacillus polytrophic | —         |
| —                  | Clostridium             | ✓             |
| —                  | Butyric acid            | —             |
| —                  | Bacillus pasteurianum   | —             |
| —                  | Lactobacillus reuteri   | —             |

What deserves our attention is that, with the exception of certain Lactobacillus, most of the above bacteria have certain pathogenicity, and care must be taken in the production process. For now, Klebsiella pneumoniae and Clostridium are the best strains that can produce 1,3-PD. Because of the higher amount of 1,3-PD produced by their metabolism, they attract more attention.
2.2. Metabolic pathway to produce 1,3-propanediol

Studies have shown that glycerin has two main metabolic pathways in microorganisms: oxidation pathway and reduction pathway[7].

*Fig. 1 Metabolic pathway of glycerol*

The oxidation pathway mainly includes the following steps: Glycerol is catalyzed by glycerol dehydrogenase (GDH) to produce 2-hydroxyacetone (DHA). This reaction process requires anaerobic and NADH as a coenzyme; DHA is under the action of ATP and 2-hydroxyacetone kinase, and Generates dihydroxyacetone phosphate (DHAP); DHAP is further metabolized to produce pyruvate, and then enters the TCA cycle through acetyl CoA, or generates other small molecule alcohols, acids and other metabolites. The oxidative pathway generates bioenergy ATP and reducing power NADH, and accompanies the growth of microbial cells. There are two main steps in the reduction pathway: Glycerol dehydratase converts glycerol to the intermediate product 3-hydroxypropanal (3-HPA) in the presence of vitamin B1; In the presence of reducing power NADH, 3-HPA is catalyzed by 1,3-propanediol oxidoreductase to produce 1,3-PD. The reduction pathway consumes excess NADH produced by the oxidation pathway to balance the reducing substances in the microbial cells.

3. Production of 1,3-PD by immobilized technology

Compared with the use of free cell fermentation, immobilized technology is helpful for the recycling of cells, the time of cell culture is greatly reduced, and the feedback inhibition of the fermentation product on the cell will also be improved. It is limited to the limited space of the carrier, and the bacteria will not be lost at will. Both the concentration of the bacteria and the fermentation intensity will be improved. Therefore, using the immobilization method, fermentation cell production of 1,3-PD is one of the most worthwhile methods to try. At present, this technology is rare in the production process of 1,3-PD.

Zhang Xiaomei et al.[8] used calcium alginate immobilized recombinant *E. coli* to produce 1,3-PDO by fermentation. The final concentration of 1,3-PDO was 61.5g/L, the substrate conversion rate reached 76.8%, and the fermentation intensity was 2.57g/(L·H). Compared with the fermentation of free cells, the tolerance of immobilized cells to the substrate is improved to a certain extent.
In the research of Reimann et al.[9], the bacterial cells were intercepted by the hollow fiber membrane filter, the intercepted bacterial cells were returned to the fermentor, and the bacterial cells were recovered. In the later stage of fermentation, the desired product can be obtained without separating the bacteria, saving experiment time. However, under this production condition, the concentration of glycerol remaining in the fermentation broth is relatively high, and the hollow fiber membrane is also more prone to contamination.

3.1. Research on Immobilization Technology
The application of fixed technology and the future of development are relatively good, and the development of fixed technology is also making rapid progress. Researchers have carried out many researches on fixing methods and fixing carrier materials. The four most commonly used methods are adsorption, cross-linking, embedding and covalent bonding (Table 3).

| Method                  | Advantage                                                                 | Disadvantage                                                                 |
|------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Physical adsorption    | The production conditions are mild, simple, low cost, carrier regeneration, and can be used repeatedly | Weak binding force, sensitive to factors such as pH, ionic strength, temperature, etc. |
| Cross-linking method   | There are many cross-linking reagents available, simple technology and high stability | Intense cross-linking conditions and poor mechanical properties |
| Embedding method       | The embedding material and embedding method have a large choice, wide application range and mild preparation conditions | Not suitable for column systems, often with diffusion limitation |
| Covalent bonding       | Carrier and coupling method can be selective; strong binding force, very stable | Fierce coupling conditions can easily cause bacterial inactivation; high cost, some coupling reagents have certain toxicity |

3.2. Selection of fixed materials
Because of the advantages of immobilization technology, and the embedding method is the least harmful to the bacteria, the choice of embedding carrier material is the key item. Wang Wenqiang et al.[11] used carrageenan, guar gum, bentonite (organic and inorganic) to fix yeast and brewed wine, and discussed the performance of immobilized cells in the continuous fermentation process. For Klebsiella pneumoniae, Ren Liangdong, et al. [12] used commonly used immobilized materials: calcium alginate, agar, gelatin, and polyvinyl alcohol. Table 4 shows the performance of the prepared immobilized Klebsiella pneumoniae: at an initial glycerin concentration of 50g / L, fermented at 160r / min in a shaker at 37 °C for 24h, the fermentation performance of different immobilization methods was compared.

| Immobilized materials     | Gelatin | Agar     | Calcium alginate | Polyvinyl alcohol |
|--------------------------|---------|----------|------------------|-------------------|
| Free biomass             | 1.78    | 1.38     | 0.76             | 0.98              |
| Residual glycerin(g/L)   | 2.6     | 5.6      | 3.6              | 7.5               |
| 1,3-PD (g/L)             | 24.6    | 23.06    | 23.6             | 20.9              |
| Fermentation intensity[g/(L-h)] | 1.01  | 0.96     | 0.99             | 0.88              |
| Immobilization of difficulty | easy | easy     | easy             | difficult         |
| Mechanical strength      | low     | low      | strong           | strong            |
| Cost                     | high    | low      | low              | low               |
3.3. New immobilized materials

Cellulose as a natural organic polymer widely exists in nature, is the most abundant natural polymer on earth, and is also the most precious natural renewable resource for human beings. Yao Yijun et al. [13] used a cellulose carrier to immobilize the β-glucosidase system for the deglycosylation of soybean isoflavone in black soybean milk, and evaluated the feasibility of the catalytic system. In addition, M. Gungormusler et al. [14] fixed a strain of Klebsiella pneumoniae with ceramic balls and ceramic rings. The results showed that the use of ceramic cell immobilization increased the yield by nearly 2 times compared with 1,3-PD produced by free cell system.

It is worth noting that with the decreasing coal and oil reserves, as well as the importance of environmental pollution in various countries around the world, cellulose has gradually appeared in people's eyes. Cellulose is non-toxic and exists in many plants, and has great potential for use. The materials prepared using cellulose have a variety of shapes including powder, flake, film, and long and short filaments. However, it is well known that the dissolution of cellulose in water and common organic solvents is almost impossible, mainly due to the existence of a strong hydrogen bonding network between cellulose molecules. In the history of cellulose research and development, whether or not cellulose can be fully dissolved is an extremely important key factor. Through extensive research, it has been found that the main biochemical solvents include: [Cu(NH$_3$)$_4$(H$_2$O)$_2$] solution, LiCl/DMAC solution, NaOH/CH$_3$N$_2$O/H$_2$O solution and ionic liquid, etc [15].

![Fig. 2 General routes for dissolution of cellulose and shaping into microspheres](image)

Stamberg, Kuga and others [16] used different dispersants to study the molding of cellulose particles with binding liquid as the material. As early as the 1980s, He [17] discovered that the researchers mixed the binding liquid into the toluene solution to prepare cellulose particles.
There are many reports on making cellulose materials after dissolving cellulose with ionic liquid, but reports on immobilized carriers are not common.

4. Conclusion and prospect
1,3-Propanediol (1,3-PD) is a very important three-carbon compound that can be polymerized with terephthalic acid to form a polyester-based material, trimethyl terephthalate. Polytrimethylene terephthalate is considered to be a new type of polyester material with development prospects. Microbial fermentation production of 1,3-PD can significantly reduce production costs and reduce the use and waste of non-renewable resources. The application of immobilization technology can greatly improve the efficiency of microbial fermentation production of 1,3-PD. Since immobilized microorganisms can achieve a high volumetric yield in a short time, the resistance to fermentation substrates during fermentation Acceptability and transformation play an important role and are a promising research method. At the same time, the choice of immobilized carrier materials also needs further discussion.

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