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硕士学位论文

丁酸梭菌代谢粗甘油产1,3-丙二醇的工艺优化以及电渗析脱盐的研究

Studies on the Optimization of the Process for 1,3-Propanediol from Raw Glycerol by Clostridium Butyricum and Electrodialysis Desalting

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摘 要

1，3-丙二醇（1，3-PD）是一种重要的化工原料，具有广泛的应用领域，最主要的用途是合成PTT（聚对苯二甲酸丙二醇酯）。目前，生产1，3-PD的主要方法有化学合成法和微生物发酵法。发酵法生产1，3-PD以其利用可再生资源和对环境友好等优点日益受到重视。

本论文用丁酸梭状芽孢杆菌（Clostridium butyricum）发酵工业粗甘油生产1，3-PD，以降低发酵法生产1，3-PD的成本。研究内容主要包括补料分批发酵过程优化、菌株代谢进化、放大实验的考察以及电渗析脱盐的实验。

一、通过控制甘油浓度（20 g/L）的补料方式进行补料分批发酵，分别考察接种量、搅拌转速、初始甘油浓度以及灭菌操作对补料分批发酵的影响。研究结果如下：

（1）提高接种量对1，3-PD的最终浓度、转化率以及生产强度影响不大；

（2）增大搅拌转速可提高菌体浓度，但不影响1，3-PD的终浓度、转化率以及生产强度。而无搅拌会延长发酵时间，使得产物生产强度很低，仅为0.36 g/L/h。因此，搅拌转速为100-150 rpm最为合适。

（3）实验表明，初始甘油过高会抑制菌体的生长以及1，3-PD的生成。初始甘油浓度45 g/L左右的发酵结果较佳，1，3-PD 的浓度为32.8 g/L，转化率为0.44 g/g。在搅拌和通氮气的条件下，培养基是否灭菌对厌氧发酵结果影响不大。

二、首先，通过代谢进化得到能耐受高浓度甘油（100 g/L）的C. butyricum M80。结果表明，发酵时间显著缩短，由原来的60 h缩短至43 h；菌体浓度由2.4 g/L增大到2.9 g/L；1，3-PD的浓度由32.8 g/L提高到41.5 g/L；生产强度由0.55 g/L/h增大到0.97 g/L/h。其次，考察补料液甘油浓度和培养温度对补料分批发酵的影响。结果表明，增大补料液甘油浓度使1，3-PD由原来的41.5 g/L增大到46.64 g/L。降低培养温度虽能提高产物浓度，但由于发酵时间的延长，使得1，3-PD的生产强度反而降低了，由1.09 g/L/h减小至0.88 g/L/h。

三、基于5 L发酵罐的发酵工艺，进行50 L发酵反应器的放大实验。对比发现，50 L发酵罐的发酵结果优于5 L发酵罐的发酵结果。C. butyricum M80对50 g/L甘油进行补料分批发酵得到59.36 g/L的1，3-PD，发酵时间仅为23.5 h，转化率和生产强

度分别为0.53 g/g和2.52 g/L/h。

四、通过陶瓷膜和超滤的处理，发酵液菌体和蛋白质去除率分别为99.0% 和98.1%。再通过电渗析装置考察了不同因素对发酵液脱盐的影响。结果发现，最佳的操作电压为10 V；淡室和浓室电导率的最佳比值为1:1。在该操作条件下，发酵液的脱盐率达99% 以上，1，3-PD的损失率为3.9%。

关键词： 1，3-丙二醇 粗甘油 丁酸梭菌 代谢进化 电渗析

关键词：1,3-丙二醇；粗甘油；丁酸梭菌；代谢进化；电渗析

Abstract

1,3-Propanediol (1,3-PD) is an important chemical compound with wide range of applications. It is mainly used as a monomer for the synthesis of PTT. Nowadays, the main methods of producing 1,3-PD are chemical synthesis and microbial fermentation. Microbial production of 1,3-PD has attracted wide attentions as it uses renewable resources and dose not generate pollutants.

In this paper, industrial raw glycerol was used as the substrate to produce 1,3-PD by Clostridium butyricum, in order to reduce the cost of 1,3-PD production. The contents of paper include fed-batch cultures progress optimization, metabolic evolution, scale-up fermentation and electrodialysis desalting.

1. Fed-batch cultures were carried out by controlling the glycerol concentration under 20 g/L. The effects of inoculum rates, stirring speed, initial glycerol concentration and sterilization operation on fed-batch cultures were investigated. Results are as follows:

(1) Increasing inoculum rates didn't affect the final concentration, yield and productivity of 1,3-PD;

(2) Though increase the stirring speed could increase the biomass, it didn't affect the final concentration, yield and productivity of 1,3-PD. While the time of fed-batch cultures grew longer and reduced the productivity of 1,3-PD when without stirring. The productivity of 1,3-PD was 0.36 g/L/h merely. Therefore, the stirring speed of 100-150 rpm was the most suitable to fed-batch cultures.

(3) Experiments showed that too high initial glycerol concentration was disadvantage to the biomass, or the generation of 1,3-PD. The results of initial glycerol concentration of about 45 g/L were the best.The final concentration and yield of 1,3-PD was 32.8 g/L and 0.44 g/g. What's more,sterilization or not had not obvious influence on Clostridium butyricum anaerobic fermentation system under stirring and nitrogen.

2. Firstly, we obtained the stain Clostridium butyricum M80 through the metabolic evolution, which could tolerate up to 100 g/L of raw glycerol. As a result, the tolerance and growth efficiency of strains improved significantly. The fermentation time of M80 was shortened from 60 h to 43 h. And the biomass was enhanced from 2.4 g/L to 2.9 g/L. The concentration of 1,3-PD was enhanced from 32.8 g/L to 41.5 g/L. The productivity were improved from 0.55 g/L/h to 0.97 g/L/h. Secondly, the effects of increase glycerol concentration in feeding broth and temperature on fed-batch cultures were investigated. As a result, increase glycerol concentration in feeding broth could enhance 1,3-PD concentration from 41.5 g/L to 46.64 g/L. The results of fed-batch cultures indicated that reducing temperature could improve the 1,3-PD concentration a little, but the fermentation time of fed-batch culture grew longer and the productivity of 1,3-PD was reduced from 1.09 g/L/h to 0.88 g/L/h.

3. Through investigate scale-up fermentation in 50 L fermentator basic on the fermentation progress of 5 L fermentator, we drew the conclusion that the results of 50 L fermentator were better than 5 L fermentator. Then, 50 g/L of glycerol was used as the substrate to produce 1,3-PD by C. butyricum M80. The results of fed-batch cultures were that the concentration, yield and productivity of 1,3-PD were 59.36 g/L, 0.53 g/g and 2.52 g/L/h, respectively. And the fermentation time was only 23.5 h.

4. Firstly, the rates of cell and protein removing respectively were 99% 98.1% by ceramic membrane and ultrafiltration. Secondly, the equipment of electrodialysis desalting was operated to test the performance of the salt removing effect in the ferment with different factors. The results indicated that the best operation voltage was 10 V and the best ratio of conductivity in dilute and concentrated compartment was 1:1. The results of electrodialysis indicated that the desalination rate of fermentation broth was above 99% and loss rate of 1,3-PD was 3.90% under these operating conditions.

Key words: 1,3-propanediol; raw glycerol; Clostridium butyricum; metabolic evolution; electrodialysis

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