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Novel membrane-based biotechnological alternative process for succinic acid production and chemical synthesis of bio-based poly (butylene succinate)

Caixia Wang [a,b], Wei Ming [c], Daojiang Yan [a,b], Congcong Zhang [c], Maohua Yang [a], Yilan Liu [a,b], Yu Zhang [d], Baohua Guo [d], Yinhua Wan [a], Jianmin Xing [a,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, PR China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Department of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, PR China
[d] Department of Chemical Engineering, Institute of Polymer Science and Engineering, Tsinghua University, Beijing 100084, PR China

HIGHLIGHTS

- Novel membrane-based whole process for succinic acid production was developed.
- Ultrafiltration was integrated to fermenter to improve succinic acid fermentation.
- Succinic acid crystal was purified with 99.4% purity in this study.
- The separation process reached 90% maximum recovery rate.
- PBS was synthesized using succinic acid directly purified in this study.

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ABSTRACT

Succinic acid was produced in a novel membrane-based fermentation and separation integrated system. With this integrated system, product inhibition was alleviated by removing acids and replenishing fresh broth. High cell density maintain for a longer time from 75 to 130 h and succinic acid concentration increased from 53 to 73 g/L. In the developed separation process, succinic acid was crystallized at a recovery of 85–90%. The purity of the obtained succinic acid crystals reached 99.4% as found by HPLC and ^{1}H NMR analysis. A crystallization experiment indicated that among by-products glucose had a negative effect on succinic acid crystallization. Poly (butylene succinate) (PBS) was synthesized using the purified succinic acid and ^{1}H NMR analysis confirmed that the composition of the synthesized PBS is in agreement with that from petro-based succinic acid.

1. Introduction

Succinic acid (1,4-butanedioic acid) is important for the synthesis of some valuable chemical derivatives, which can be used in food and pharmaceutical products, solvents, biodegradable polymers, surfactants and detergents (Zeikus et al., 1999; Bozell and Petersen, 2010). Due to its independence from petroleum, environmental benefit and CO_2 sequestration, biological production of succinic acid has been investigated intensively during recent years (Song and Lee, 2006; Cheng et al., 2012b).

Great efforts have been devoted to develop fermentation technology to produce succinic acid as replacement of the petrochemical route (Guettler et al., 1999). Efforts mainly focus on three aspects: development of efficient biocatalysts with higher succinic acid productivity (Kim et al., 2008; Liang et al., 2013), fermentation control (Li et al., 2010b) and downstream process improvement (Cheng et al., 2012a) aimed to separate succinic acid effectively from fermentation broth. Among succinic acid producing bacteria, *Actinobacillus succinogenes* and genetically engineered *Escherichia coli* have been mostly studied and showed great advantages in succinic acid production (Guettler et al., 1999; Millard et al., 1996). However, product inhibition was observed both in *A. succinogenes* and *E. coli* because of the acids accumulation and nutrient depletion, which limits the bacterial growth rate and productivity. For *A. succinogenes*, cell growth was inhibited at concentration levels of 8.8–17.6 g/L formate, 10–40 g/L acetate, 9–18 g/L lactate, and

* Corresponding author. Address: Institute of Process Engineering, Chinese Academy of Sciences, P.O. Box 353, Zhongguancun Bei-er-tiao 1, Haidian District, Beijing 100190, PR China. Tel./fax: +86 10 62550913.
E-mail address: jmxing@home.ipe.ac.cn (J. Xing).

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10–80 g/L succinate (Li et al., 2010a). *E. coli* is more resistant to acidic products than A. succinogenes but still suffers from products inhibition. In this situation, novel bioreactors and integrated fermentation and separation processes which can remove produced acids and replenish new fermentation media should be developed.

The downstream process consists of preliminary removal of biomass and larger molecules, decolourization, conversion of succinate salts into free acid, and crystallization. The removal of biomass and lager molecules such as proteins is crucial to the following purification step. In recent years, ultrafiltration has been widely used in various physicochemical and biochemical processes for the separation of solids from liquid at low operational cost, low energy consumption and with elimination of filter aids. It has been demonstrated that clarification of succinic acid fermentation broth by ultrafiltration is possible (Wang et al., 2012b). Compared to the traditional centrifugation, clearer fermentation broth can be achieved by ultrafiltration. Our previous study indicated that 100% cells and 90% protein can be getting out fermentation broth while for centrifugation only 92% cell and 53% protein can be removed (Wang et al., 2012a). More importantly, membrane systems can be connected to the fermentation bioreactor to alleviate product inhibition and achieve high cell density. Permeation was drawn off continuously and then fresh media was added by the coupled system. It is reported that the submerged membrane fermentation could double lactic acid production and cell density (Ramchandran et al., 2012). An integrated membrane-bioreactor-electrodialysis system was also built for succinic acid production at high concentration, productivity and yield. Under the optimized conditions, biomass concentration and succinate concentration reached 42 g/L and 14.8 g/L, which are respectively 28 and 20 times higher compared to batch cultures (Meynial-Salles et al., 2008). In this study, ultrafiltration membrane was applied to connect fermentation and separation process, and to clarify the fermentation broth.

The key challenges in the separation process are the low concentration of succinic acid in the aqueous broth, the presence of the byproducts such as lactic acid, acetic acid and formic acid with physicochemical properties similar to succinic acid. Up to now, several recovery technologies such as adsorption, extraction and electrolysis have been investigated for the recovery of succinic acid (Pratiwi et al., 2013; Orjuela et al., 2013). Crystallization has been proved to be effective in getting succinic acid from the aqueous broth even in the presence of formic acid and acetic acid. However, direct crystallization by acidification was proven to yield low recoveries and purities (Lin et al., 2012). Resin-based crystallisation could improve the yield and purity for the separation process (Lin et al., 2010). In order to get succinic acid of higher purity, fermentation broth should be treated before crystallization. To develop a cost-effective downstream process, not only a single operation unit but also the compatibility of these operation units especially the connection possibility of these units should be considered.

Succinic acid can be transformed into a wide range of chemicals and polymers (Delhomme et al., 2009). Among them, PBS has attracted much attention from both academia and industry because of its biodegradability, excellent thermal ability and good mechanical properties (Sinha Ray et al., 2003) PBS exhibits ecological advantages over non-biodegradable polymeric material. Currently, commercially available PBS is efficiently synthesised through condensation polymerization from the starting materials of 1,4-butanol and succinic acid, which was all derived from petrochemical process. Condensation polymerization of PBS requires succinic acid of higher purity (at least above 98%), which challenges the separation process of bio-succinic acid production. Effective recovery process of succinic acid need to be developed to fulfill the PBS synthesis using bio-succinic acid.

This study aims to provide an integrated process for biotechnological production of succinic acid which can be used for further PBS synthesis or chemical transformation. An effective separation process was developed and integrated to the fermentation by membrane unit to improve the succinic acid production. PBS was synthesized using succinic acid directly purified from its broth by the developed process in this study.

2. Methods

2.1. Bacterial strains, fermentation media and cultivation

E. coli MG-PYC (pTrchisA-pyc, △ldhA) was used for succinic acid fermentation. In this strain, the ldhA gene involved in the lactic acid synthesis pathway was deleted and heterologous pyruvate carboxylase (pTrchisA-pyc) was over-expressed for increased succinic acid production. Fermentation media contained per liter: 20 g glucose, 20 g tryptone, 10 g yeast extract, 0.15 g $MgSO_4$, 0.2 g $CaCl_2$, 0.02 g $MnCl_2$, 0.45 g $Na_2HPO_4{\cdot}12H_2O$, 6 g $NaH_2PO_4{\cdot}2H_2O$ and 3 g $(NH_4)_2SO_4{\cdot}7H_2O$. Dual phase fed-batch fermentation with or without ultrafiltration were conducted including an aerobic and an anaerobic phase at 37 °C. Glucose was added intermittently when it was used up. During the aerobic phase, *E. coli* grew to get high cell density. Air was sparged to the media and pH was maintained at 7.0 with 10 M NaOH during this period. During the anaerobic phase, CO_2 was sparged into the media and the strain started to produce succinic acid. pH was controlled at 6.7 with 10 M NaOH during the anaerobic phase. IPTG (Isopropyl-β-D-thio-galactoside) was added at 23.8 mg/L to the medium to induce expression of pyruvate carboxylase. All the fermentation media was sterilized at 121 °C for 20 min and glucose was sterilized separately at 115 °C for 30 min.

Fermentation experiments were conducted in a 7.5 L bioreactor with 3 L fermentation media initially. In the fed-batch fermentation integrated with ultrafiltration, 1.5 L fermentation media was pumped out of fermenter after 55 h cultivation, and same 1.5 L fresh media was supplemented. The amount of seed inoculum was 5% relative to the fermentation media. During the strain construction and seed cultivation, the cultures were grown aerobically at 37 °C in Luria–Bertani (LB) medium (10 g tryptone, 5 g yeast extract, and 5 g NaCl/L).

The purchased succinic acid was from Guangdong Guanghua Chemical Factory Co., Ltd (China). The chemicals used were of analytical grade and purchased from either OXOID (England) or Sinopharm Chemical Reagent Beijing Co., Ltd (China) unless otherwise described.

2.2. Membrane process

Hollow fiber ultrafiltration equipment with membrane of PVDF 50 kDa (Tianjin MOTIMO Membrane Technology Co., Ltd. China) having an effective membrane area of 0.25 m^2 was used in this study. Water flux under different pressures was measured for 20 min to figure out the critical water flux and helped the selection of optimal operation pressure before integration. The desired trans-membrane pressure was maintained by controlling the attached diaphragm valves.

When integration, this ultrafiltration membrane connected with fermenter by peristaltic pump (Tianjin MOTIMO Membrane Technology Co., Ltd. China) and pipeline. After 42 h anaerobic cultivation, the pump started and the broth was pumped into the ultrafiltration membrane to remove cells, proteins and obtain the clear fermentation broth for 15 min. Membrane flux from the permeate loop side was detected during this period. Cells retained on the membrane were flushed backed into fermenter. Then same amount of fresh media was replenished after coupling. After fermentation finished, fermentation broth was filtrated by a same