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Hydration of acrylonitrile to produce acrylamide using biocatalyst in a membrane dispersion microreactor

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HIGHLIGHTS

- Membrane dispersion microreactor was used for the hydration of acrylonitrile.
- The concentration of acrylamide reached 45.8 wt% within 35 min.
- Few amounts of acrylonitrile were accumulated.
- Heat transfer efficiency was intensified.

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ABSTRACT

In this work, a membrane dispersion microreactor was utilized for the hydration of acrylonitrile to produce acrylamide. Through observation using a microscopy, it was found that the acrylonitrile was dispersed into the continuous phase (the aqueous phase contains nitrile hydratase (NHase)) as droplets with a diameter ranged from 25 to 35 μm, hence the mass transfer specific surface area was significantly increased, and the concentration of acrylamide reached 52.5 wt% within 50 min. By contrast, in stirred tanks, the concentration of acrylamide only got 39.5 wt% within 245 min. Moreover, only a few amounts of acrylonitrile were accumulated in this microreactor system. Through optimizing the flow rate, the concentration of acrylamide reached 45.8 wt% within 35 min, the short reaction time greatly weakened the inhibition of acrylonitrile and acrylamide on the enzyme activity, which is suitable for prolonging the life of free cell.

1. Introduction

Acrylamide is an important raw material used to manufacture various polymers (Li, 2010; Martinez et al., 2014; Wang et al., 2014). In recent years, compared with the chemical catalysis technique (Onda et al., 2007; Tilvez et al., 2013), the bioconversion of acrylonitrile to acrylamide catalyzed by NHase is attracting more attention because it is performed under mild reaction conditions and presents various advantages such as the low energy consumption, safe bioprocess, and high purity (Feng et al., 2008; Ma and Yu, 2012; Zhang and Pierce, 2009).

The bioconversion of acrylamide is mainly catalyzed by NHase obtained from rhodococcus ruber TH3 (*R. ruber* TH3) free cells (Jie and Pierce, 2009; Raj et al., 2006), and bioreaction is generally performed in a stirring tank. There are two shortcomings in the producing process (Tudorascu et al., 2009). One is the low product concentration, the production of acrylamide only could reach about 30 wt%. As the solubility of acrylamide is about 55.7 wt% at 288 K, which will cost much energy to obtain the acrylamide powder through the distillation and crystallization. The other limitation is the long reaction time. Due to the low mass and heat transfer rate of the stirred tank, the acrylonitrile can only be dropped slowly into stirred tanks, and it takes 250 min to reach the concentration of 39 wt% of acrylamide. More seriously, because of the inhibition of acrylamide on free cells (Xudong et al., 2004), the life of biocatalyst will shorten greatly in the reaction process, therefore free cells must be added to maintain the reaction rate until the proper production concentration is achieved. Therefore, it is very important to develop a new reactor to intensify the mass and heat transfer rates to improve the productivity of this bioreaction.

This hydration process is controlled by three major factors, which are the main problems of free cell biocatalysts. First, acrylonitrile substrate significantly inhibits the enzyme activity of NHase because acrylonitrile is an organic solvent, which likely causes NHase denaturation (Mersinger et al., 2005). At an optimum reaction temperature, the concentration of acrylonitrile should not exceed 60.69 g/L (solubility of acrylonitrile under 293 K). Hence,

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acrylonitrile should be mixed with aqueous free cell solution at high ratios of the two phases to reduce the inhibitory effect of acrylonitrile on enzyme activity; the consumption of acrylonitrile should also be accelerated to obtain the same result. Second, acrylamide also inhibits enzyme activities when concentration exceeds 25 wt% (Sun et al., 2009). As a result, low acrylamide concentration is produced, thereby requiring additional energy via an enrichment process to obtain pure acrylamide and produce polyacrylamide. Hence, a shortened contact time between high acrylamide concentration and free cells can significantly improve product concentration. Third, the kinetics of acrylonitrile hydration to produce acrylamide catalyzed by NHase is relatively fast and reaction heat is high (Cantarella et al., 2013); as a result, temperature during hydration is substantially increased. An increased temperature then inactivates NHase and byproducts from side reactions. Ice-brine bath should be used to maintain the temperature range from 283 K to 293 K (van Pelt et al., 2008), which likely increases energy consumption. Reaction rate should also be limited to ensure that heat is removed.

In our previous works, a membrane dispersion microreactor was developed to improve mass and heat transfer rates (Sahoo et al., 2007; Wang et al., 2006). In this reactor, one fluid is dispersed into another phase as very small droplets with the diameters of micrometers. The mass transfer specific surface area was increased significantly, and a homogeneous mixing could be reached in one second (Zhao et al., 2007). This microreactor has been applied in the organic synthesis and the inorganic precipitation with fast kinetics (Miyazaki and Maeda, 2006). For example, Zhang et al. (2012) developed the Beckmann rearrangement in a microstructured chemical system to prepare caprolactam because this process results in good performance in terms of mass and heat transfer. Huang et al. (2013) also showed that a membrane dispersion microreactor can be applied to prepare ZnO nanoparticles with the diameter of 7 nm with high dispersibility and uniform particle size. However, few studies have applied microsystems in acrylonitrile hydration.

In this study, a membrane dispersion microreactor was utilized to perform acrylonitrile bioconversion. The velocities of the two phases and the flow rate ratio of acrylonitrile and free cells in aqueous solution were discussed to optimize hydration process. Heat transfer efficiency was also preliminarily evaluated to characterize the performance of this membrane dispersion microreactor.

2. Methods

2.1. Bacterial strains and preparation of free-resting cells containing NHase

R. ruber TH3 was cultured in this study according to previously described methods (Ma et al., 2010). Approximately 5 mL of the seed medium was inoculated in 500 mL flasks containing 50 mL of culture medium and then cultured at 301 K for 48 h. The cells were washed with deionized water and harvested by centrifugation. The treated cells were resuspended in the required concentration and free-resting cells of *R. ruber* TH3 containing NHase were obtained.

2.2. NHase activity assays

Free-resting cells containing NHase were used in enzyme activity assay. Hydration was performed as follows: 200 μL of acrylonitrile was added to 4.6 mL of the reaction mixture containing 100 μL of free cells and 4.5 mL of phosphate-buffered saline (pH = 7.0). The reaction was performed at 301 K for 5 min and then terminated by adding 200 μm of 2.5 M hydrochloric acid (HCl). The mixture was centrifuged for 10 min and the supernatant was evaluated by gas chromatography (GC) method. One unit (U) of NHase activity was defined as the amount of free cells which can catalyze the formation of acrylamide at a rate of 1 μmol/min.

2.3. GC analysis for acrylamide and acrylonitrile

The concentrations of acrylamide and acrylonitrile in the product mixture were determined using GC (7890A, Agilent, USA) equipped with a polyethylene glycol polymer capillary column (30 m × 0.25 mm × 2 μm) and a flame ionization detector under the following conditions: injection and detector temperature were set at 533 K; column temperature was set at 433 K; and flow rate of carrier gas (nitrogen) was 2.5 mL/min. Acetamide was utilized as an internal standard.

2.4. Preparation of acrylamide in a traditional stirring tank

Acrylonitrile hydration was performed with a traditional stirring tank. Approximately 200 mL of free-resting cells was diluted with deionized water in a round-bottom flask to obtain a final volume of 1 L and then placed in an iced water bath. Acrylonitrile was slowly added dropwise to the reaction mixture with vigorous stirring (250 r/min) to ensure that temperature was controlled at 293 K. The reaction was terminated by adding 2.5 M HCl when the concentration of acrylonitrile reached 1% (V/V). The mixture was centrifuged and the supernatant was determined by GC method.

2.5. Preparation of acrylamide in a membrane dispersion microreactor

A membrane dispersion microreactor was used as the devices of the hydration reaction in this study and the structure of the device was the same with previous work (Wang et al., 2006). Analytic-reagent (AR) acrylonitrile was used as a dispersed phase. A pure free-resting cell solution was set as an initial continuous phase and placed in a three-necked, round-bottomed flask with continuous stirring. The two phases were delivered by metering pumps (Beijing Satellite Co., Ltd.) in a series of flow rates and then mixed in the membrane dispersion microreactor. The reaction mixture was then delivered via a long tube (2.5 m) with a diameter of 3 mm, which was connected to the microreactor and used to transport the mixture to the three-necked, round-bottomed flask. The mixture delivered in the above flask was then set as new continuous phase. All of the devices were settled in an iced water bath, and the temperature of the system was controlled at 273 K. Enzymatic bioreaction was performed in the membrane dispersion microreactor and the long tube.

The microreactor was composed of stainless steel (type 316) and the inner structure comprised a distribution room, a stainless steel sintering membrane, and a cross flow channel. The size of the cross flow channel was 10 × 1 × 0.6 mm. The membrane (3 × 1 mm) with a porosity of 70% and a size of 5 μm was placed between the distribution room and the cross-flow channel; the dispersed phase was then distributed into small droplets under the shear effect of the continuous phase. As a result, a greater specific surface area was obtained to perform bioreaction and mass transfer rate was enhanced.

3. Results and discussion

3.1. Effect of the velocities of the two phases on the concentration of acrylamide

Fig. 1 shows the influence of the velocities of two phases on the concentration of acrylamide. The flow rate ratio of pure