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A kinetic study of the biological catalytic hydration of acrylonitrile to acrylamide

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HIGHLIGHTS

- The kinetics of biocatalyzed hydration of acrylonitrile to acrylamide was studied.
- The conversion rate in micro-reactor reached approximately 90% of the max rate.
- The free cells tended to adsorb onto the oil phase.

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ABSTRACT

In this study, the kinetic characteristics of the bio-hydration of acrylonitrile by free cell catalysts were studied. Due to the aggregation phenomenon, free cell catalysts showed different characteristic when compared with free enzyme catalysts. The Michaelis constants were obtained through the homogeneous reaction. Both the values of V_m and K_m increased with the increase in enzymatic activity, ranging from 100 to 500 U/mL, and the variation trend of K_m was not consistent with the rule of enzyme-catalyzed reaction. To reduce the external diffusion resistance of acrylonitrile to free cells, a membrane dispersion micro-reactor was used, and the conversion rates of acrylonitrile in the membrane dispersion micro-reactor under all of the experimental conditions reached approximately 90% of the max conversion rates. The reinforcement of the apparent reaction rate by the micro-reactor may have benefitted from the significant increase in the specific surface area of the acrylonitrile droplets and the adsorption of the free cell catalysts on the undissolved droplets.

1. Introduction

Acrylamide is an important basic chemical product and is widely used in various industrial fields [1–3]. Currently, the biological transformation of acrylonitrile to acrylamide catalyzed by nitrile hydratase (NHase) has received increasing attention as it presents various advantages, such as low energy consumption, a safe bioprocess, and high purity [4–6]. As NHase is remarkably unstable with regard to environmental factors, such as temperature, pH and high concentrations of organic compounds [7], wild-type free cells with NHase activity, including *Rhodococcus ruber* [8] and *Pseudomonas chlororaphis* [9] cells, are widely utilized in industry to convert acrylonitrile to acrylamide.

In the bio-hydration process mentioned above, free cells can be regarded as shell-core catalysts and the cell wall and membrane can be treated as the shell of the biocatalyst, which has no catalytic activity and increases the resistance of reactant to the internal NHase to some extent. Meanwhile, NHase inside the free cells is the core of the bio-catalyst, which is the active site of the free cell. For the homogeneous aqueous acrylonitrile solution with free cell catalysts, acrylonitrile molecules have to first migrate to the surface of the free cells and then traverse across the cell wall and membrane. Finally, the bio-hydration of acrylonitrile in cells takes place when the reactant binds to NHase [10]. The whole mass transfer process includes the external diffusion process of acrylonitrile to the free cell catalysts and the internal diffusion process of acrylonitrile to NHase. As shown in Fig. 1(a), due to the low solid content of free cell catalysts, most of the reactant is unable to participate in the reaction and the external diffusion process of acrylonitrile to the free cells reduces the apparent reaction rate. In addition, as the solubility of acrylonitrile in water is relatively low (7.35 mL/100 mL at 293 K) [11], the concentration of acrylonitrile in free cells is lower, which also reduces the apparent reaction rate.

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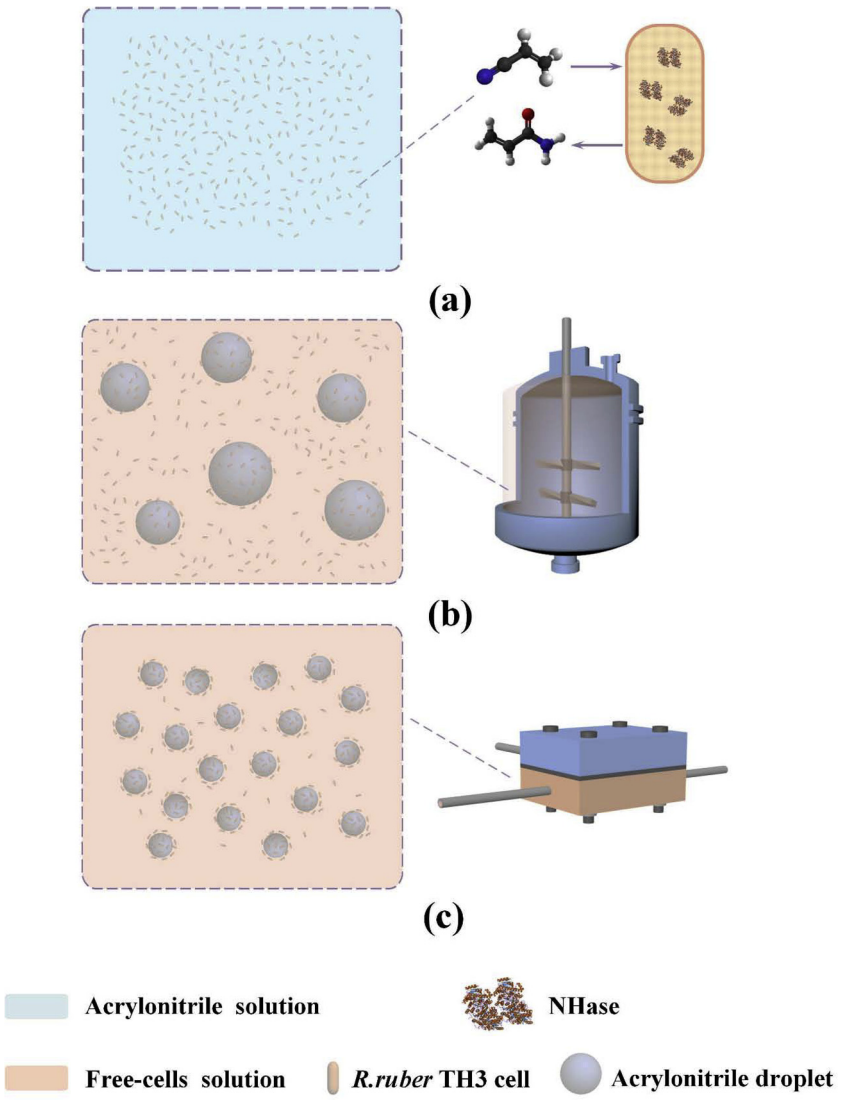

Fig. 1. Characteristics of the bio-hydration of acrylonitrile under various mixing states. (a) Homogeneous mixing system; (b) The mixing system in a stirred tank (the droplet size ranges from 100 to 200 μm); (c) The mixing system in a micro-reactor (the droplet size ranges from 20 to 50 μm.

In a typical industrial process, this bio-hydration reaction is normally performed in a stirred tank [12]. Free cell catalyst solution diluted to a specific enzyme activity (approximately 200–300 U/mL) is first fed into the stirred tank, and the bio-reaction is then performed with acrylonitrile added to the reaction mixture. As the dissolution rate of acrylonitrile in water is slow, the reactant is actually dispersed in the stirred tank in the form of droplets. Meanwhile, the presence of mycolic acids in the cell wall makes the cells hydrophobic and likely to attach to the interface of the undissolved acrylonitrile droplets [13,14]. Hence, acrylonitrile actually attaches to free cells in the form of oil droplets at the initial stage, and then, the radius of the acrylonitrile droplet decreases as the reaction continues. The contact between the free cell catalysts and the undissolved acrylonitrile can decrease the external diffusion resistance of acrylonitrile to free cells and also increase the concentration of acrylonitrile in free cells, thereby contributing to the acceleration of the apparent reaction rate. However, to ensure that the cell is not destroyed by the impeller of the stirred tank, the agitation rates of the stirred tank are generally controlled within 100 rpm, and the diameters of the acrylonitrile droplets mainly range from 100 to 200 μm [15]. Previous research [16] has shown that the dissolution rate increases with an increase in the diameter of the undissolved droplet, and a lengthy contact time between the free cells and a high concentration of acrylonitrile will lead to the irreversible inactivation of the catalytic activity [17–19]. Another problem resulting from the larger droplet diameter is that acrylonitrile is unable to provide enough specific surface area for all of the free cells to attach, resulting in a relatively low utilization of free cells (Fig. 1(b)). Therefore, the production of acrylonitrile requires a long reaction time (generally more than 250 min) and results in a low concentration of product (approximately 39 wt%) [12].

To reduce both the external diffusion resistance of acrylonitrile to free cells and the time of exposure of undissolved acrylonitrile to free cells, a membrane dispersion micro-reactor was used for the bio-hydration reaction process to ensure that the acrylonitrile was dispersed into small droplets ranging from 20 to 50 μm [11,20]. The advantage of this reactor is to increase the dissolution speed of acrylonitrile and provide enough specific surface area for all of the free cells to attach, resulting in the acceleration of the apparent reaction rate due to the high catalytic activity of the free cells. In our previous research [11], acrylonitrile was designated as the dispersed phase and pure free-cell solution was designated as the continuous phase, and a membrane dispersion micro-reactor