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Visualized study on mass transfer characterization in the process of biological catalytic hydration of acrylonitrile using pendant drop method

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In this work, a pendant drop method was utilized to observe visually the mass transfer process of an acrylonitrile droplet during bio-hydration. At a temperature of 293 K, the droplet radius was reduced from 100 μm to 49.8 μm within 55 seconds and the value of the diffusion coefficient of acrylonitrile in water was 9.51×10^{-10} m^2/s. The reduction of the droplet radius is conducive to the acceleration of the mass transfer rate. Cells without enzyme activity to some degree decrease the mass transfer rate of acrylonitrile. At the same temperature, the droplet radius is reduced from 100 μm to 50.4 μm within 135 seconds at a cell concentration of 0.204 g/L. The bio-hydration reaction can greatly strengthen the mass transfer process, with the intensifying factor ranging from 1.70 to 4.78. The increase of acrylamide concentration shortens the critical droplet radius of acrylonitrile, and hence also strengthens the mass transfer process.

INTRODUCTION

Acrylamide is widely used in various industrial fields and is mainly produced by the hydration of acrylonitrile[1, 2]. Compared with other methods[3-5], bio-hydration catalysed by nitrile hydratase (NHase) is widely used in the process of acrylamide production as it presents various advantages such as low energy consumption, a safe bioprocess, and high purity. As NHase as a crude cell-free extract is remarkably unstable to environmental factors, such as temperature, pH and high concentrations of organic compounds[6], wild-type free cells with NHase activity including *Rhodococcus ruber* TH3 (*R. ruber* TH3)[7] and *Pseudomonas chlororaphis* B23[8] are utilized to convert acrylonitrile to acrylamide in industry.

In the typical industrial process, this bio-catalytic reaction is performed in a stirred tank (**Fig.** 1a), and the limitations remain in industry are the low product concentration and the long reaction time. It generally takes more than 250 mins to reach the concentration of 39 wt% of acrylamide in production, and 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. One important characteristic of the process is that the solubility of acrylonitrile in water is relatively low (7.35 mL/100 mL at 293 K)[9] and undissolved acrylonitrile may lead to an irreversible inactivation of the cells if the contact time between undissolved acrylonitrile and cells is too long[10, 11]. Due to the limitation of the device on one aspect, and to ensure that the cell is not destroyed by the impeller on the other, the agitation

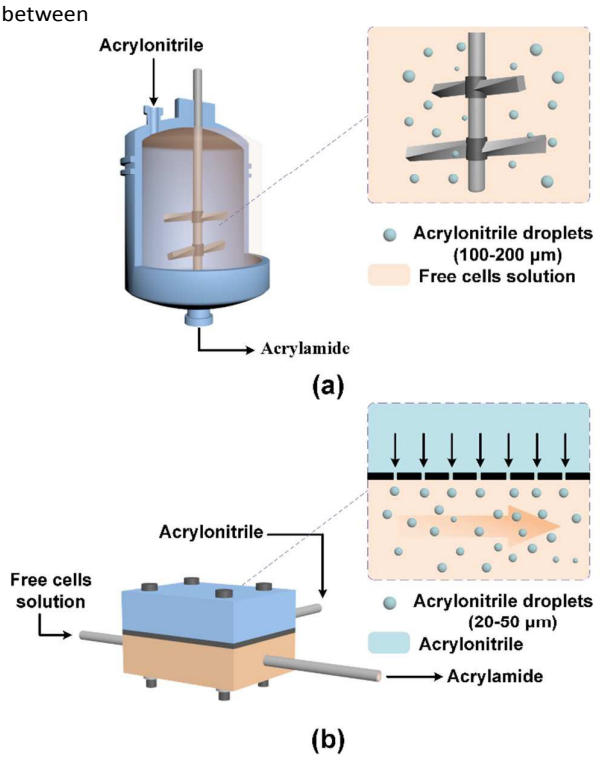

Fig. 1 Reaction process and droplet distribution characteristics in different styles of reactor. (a) Reaction process and droplet distribution characteristics in a stirred tank. (b) Reaction process and droplet distribution characteristics in a membrane dispersion microreactor.

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rates of stirred tank are generally controlled within 100 rpm. As the diameters and diameter distributions of acrylonitrile droplets deeply depends on the agitation rate of impeller (at slower agitation rates, droplet diameter is larger and the distribution is more disperse), the diameters of acrylonitrile droplets mainly range 100-200 μm[12], and the dissolved time is relatively long. Hence acrylonitrile can only be added at a low rate to allow for its dissolution, and the reaction time is extended. In addition, Acrylamide also inhibits the catalytic activity of free cells seriously with the accumulation of concentration, and the acrylonitrile will not be hydrated after a short contact time between the free cells and the high concentration of acrylamide (more than 25 wt%), which led a low production concentration.

To solve the above-mentioned problems, the major step which influences the effect of the hydration reaction process needs further discussion. This process includes four steps[13]: The first step is the dissolution and mass transfer of acrylonitrile in water; the second is the traversing of the substrate across the cell wall and membrane; the third step is the hydration reaction in cells catalysed by NHase; and the final step is the traversing of production from cell to solution. As mentioned above, the dissolution and mass transfer of acrylonitrile in water is important. In previous research[9], a membrane dispersion microreactor was used to strengthen mass transfer rate of acrylonitrile and enable the concentration of acrylamide to reach 45.8 wt% within 35 mins. The advantage of this reactor is to ensure that acrylonitrile can be dispersed into small droplets ranges from 20-50 μm (**Fig.** 1b), hence increased the speed rate of adding acrylonitrile under the premise of rapid dissolution of acrylonitrile. However, due to the invisibility of the bio-reaction device, it was hard to evaluate how different factors, such as the existence of the cells, the strengthening of the reaction and the change of the concentration of acrylamide, influenced the mass transfer process. More persuasive evidence is needed to explain the phenomenon that the improvement of the bio-reaction by the reinforcement of mass transfer became less noticeable at the end of the reaction process. On the other hand, the diffusion coefficient and mass transfer coefficient are important parameters to establish a mass transfer model and design the scale of the reactor in industry with the reaction kinetics data[14]. Hence it is important to measure the diffusion coefficient and mass transfer coefficient of acrylonitrile in the bio-reaction system.

Compared with other measurement methods of the mass transfer coefficient and diffusion coefficient[15-17], the pendant drop method can reflect the influence on the mass transfer by different factors through considering the differences among the relation between the bubble volume and the dissolution time in a more visual way. Nowadays, the pendant drop method has been widely in used in a variety of research fields. Teipel et al. [18] created a drop volume technique to discuss the adsorption behavior of nonionic surfactants on liquid drop and shown that the dripping process of a surfactant solution droplet in air and paraffin oil surroundings causes different types of satellite droplets, the satellite droplet was approximately 10 to 15 times smaller than the primary droplet in air while 50-200 smaller than the primary droplet in paraffin oil; Wang et al.[19] investigated the Marangoni effect induced by the interphase mass transfer in liquid–liquid extraction and reaction processes by the pendant drop method. The experiments on mass transfer of solute acetic acid or acetone from single 1-hexanol drops to continuous water phase and acetic acid from methyl isobutyl ketone drops to water were carried out; Erbil et al.[20] also utilized the same method to examine the evaporation rate of fully spherical liquid drops in relation to their diffusion coefficient, vapour pressure, and drop surface temperature and estimated the diffusion coefficients of heptane, nitromethane and toluene vapours in air were 0.074, 0.093 and 0.089 cm^2/s respectively, which were in good agreement with other literatures. Hence this method may a convenient and visual way to study the mass transfer process in acrylonitrile-water-cells systems.

In this research, the pendant drop method was used to observe the mass transfer phenomenon of acrylonitrile droplet in various solvent environments. First, the dissolution of acrylonitrile was observed in deionized water at different temperatures; the diffusion coefficient of acrylonitrile in water was obtained depending on the steady state of molecular diffusion model in semi-infinite media. Secondly, the observation of mass transfer of acrylonitrile in a solution of R. ruber TH3 free cells solution without NHase enzyme activity was carried out. The mass transfer coefficient (k) was taken into the discussion of the effect of cells without NHase enzyme activity on the mass transfer rate. Thirdly, the mass transfer of acrylonitrile in a solution of R. ruber TH3 free cells solution with different enzyme activity was surveyed, and an intensifying factor (I) was introduced to discuss the reinforced effect on the mass transfer rate by bio-reaction. Finally, the critical diameters of acrylonitrile in various concentrations of acrylamide aqueous solution were measured, illustrating how improving the concentration of acrylamide influences the solution of acrylonitrile as the bio-reaction continues.

The purpose of the research is to gain the diffusion coefficient of acrylonitrile in water and visually show the effect of different factors on the mass transfer of acrylonitrile in the reactant solution where it exists.

EXPERIMENTAL SECTION

Cell culture and preparation of *R. ruber* TH3 free-resting cells

The bacterial strain used in this study was a *R. ruber* TH3 and the culture medium was prepared as reported method[21]. Approximately 10% inoculums of the seed medium were inoculated in 500 mL flasks with 50 mL of culture medium and then cultured at 301 K for 48 h. The cells were harvested by centrifugation (25,000 r/min, 10 mins) and washed with deionized water several times. The treated cells were then re-suspended in the required concentration, resulting in free-resting cells of *R. ruber* TH3 containing NHase. To obtain the R. *ruber* TH3 free-resting cells without NHase enzyme activity,

cobalt chloride was removed from the culture medium, and other steps were the same as mentioned.

The cell concentration was achieved by UV spectro-photometry (HP8453, Aglient) at 460nm, with 500 U/mL of enzyme activity corresponding to 0.51 g/L of cell concentration. Other enzyme activity of cell suspension was achieved by diluting the above-mentioned concentration cell suspension.

GC analysis for acrylamide and acrylonitrile

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

NHase activity assays

The activity of *R. ruber* TH3 free-resting cells containing NHase was assayed by determining the amount of acrylamide produced per volume unit of the fermentation broth. Hydration reaction was carried out as follows: 200 μL of acrylonitrile was added in a conical flask with a reaction mixture containing 100 μL of free cells solution and 4.5 mL of phosphate-buffered saline (pH = 7.0). The reaction was performed at 301 K for 5 mins and then terminated by adding 200 μm of 2.5 M hydrochloric acid (HCl). The mixture was centrifuged and the supernatant was then evaluated by the GC method. One unit (U) of NHase activity was defined as the amount of free cells which could catalyze the formation of acrylamide at a rate of 1 μmol/min.

Pendant drop device for the determination of the mass transfer rate of acrylonitrile

The principle of the pendant drop method (**Fig.** 2) is based on the ability to determine the volume of a droplet forming with a capillary of defined diameter and record the change in volume with time. A capillary tube with an outer diameter of 1.0 mm and an inner diameter of 0.7 mm was tapered with approximately a 10 μm tip by using a micro-pipet puller (P-97, SUTTER Co. Ltd., USA). The capillary tip was connected with a U-shaped stainless steel needle with an outer diameter of 0.45 mm and an inner diameter of 0.24mm. A syringe (SGE - GT 1mL, J&J industries Co. Ltd., Beijing) equipped with the above-mentioned needle was utilized to inject acrylonitrile by using the pendant drop technique (OCAH200, Data Physics Instruments GmbH, Germany). A defined volume of droplet generated in a certain period of time by adjusting the injection time and injection speed. Acrylonitrile droplet was formed in a quartz cuvette (5×10×20mm) containing deionized water or *R. ruber* TH3 free cells solution. The quartz cuvette was wrapped in a transparent heat jacket and the temperature was controlled ranging from 278-298K. The droplet formation time was controlled within 2 seconds at an initial diameter of approximately 250 μm. The dissolving process of an acrylonitrile droplet in this work was all recorded from the diameter of 200 μm by a microscope with a CCD video camera

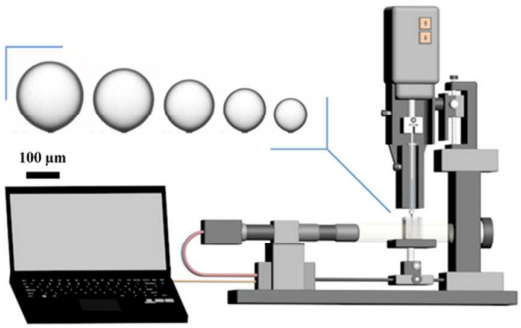

Fig. 2 Device and mechanism for pendant drop method

(PL-A742, PixeLINK, Canada), which was connected to a computer. The collection frequency of images and resolution were 200 images per second and 640 × 480 dpi respectively.

RESULTS AND DISCUSSIONS

Effect of temperature on the mass transfer of acrylonitrile

The dissolution of acrylonitrile was observed and recorded in deionized water at different temperatures (**Fig.** 3a), and **Fig.** 3b shows the change in the radius of the acrylonitrile droplet during the dissolving process. When the temperature was set as 278 K, the droplet was reduced in radius from approximately 100 μm to 81.5 μm within 55 seconds. With the increase in temperature, the dissolution rate of acrylonitrile in water was accelerated and the radius of the droplet was reduced to 34.2 μm during the same period of the dissolving process at a temperature of 298 K. This phenomenon indicated that the mass transfer rate of acrylonitrile in water depends to a great extent on the temperature.

The diffusion coefficients of acrylonitrile in water were obtained by measuring the variation of the radius of the droplet versus the time taken to dissolve, and a model on the steady state of molecular diffusion in semi-infinite media was created. Derived from the expression of the molar flux through the interface of the droplet surface, the following equation was obtained:

$$-dn_A = k(C_{A0} - C_{A\infty})Adt \quad (1)$$

n_A stands for the amount of molar of the acrylonitrile droplet, and is defined as $n_A=V_A\rho_A/M_A$, V_A stands for the volume of the acrylonitrile droplet and equals $4/3\pi R^3$ (R stands for the radius of the acrylonitrile droplet), and ρ_A and M_A stand for the density and the molar mass of acrylonitrile respectively. k stands for the mass transfer coefficient of acrylonitrile; A stands for the surface area of the droplet and *equals* $4\pi R^2$; C_{A0} stands for the concentration of acrylonitrile around the droplet. As it took more than 15 seconds for the generation of acrylonitrile droplet and the reduction of droplet radius from approximately 125 μm to 100μm. It was considered that the concentration of acrylonitrile on the oil-water interface was

steady and C_{A0} could be approximately considered as the saturation concentration of acrylonitrile (C_{AS}) at the corresponding temperature; $C_{A\infty}$ stands for the concentration

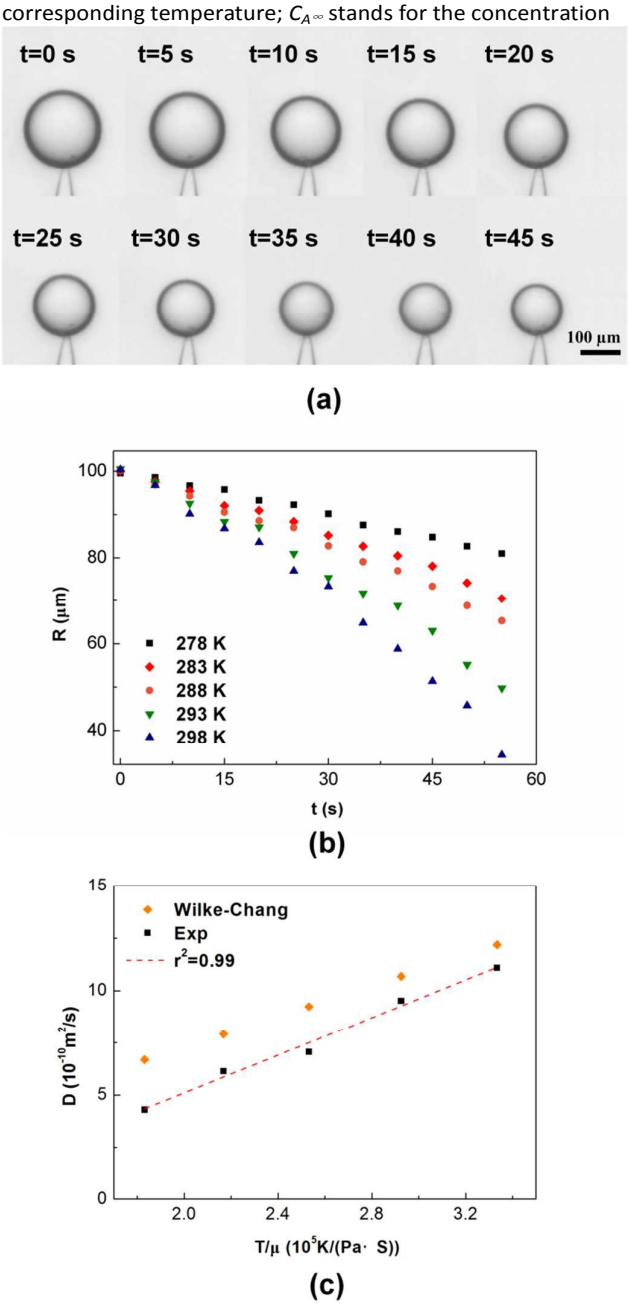

Fig. 3 The effect of temperature on the mass transfer of acrylonitrile: (a) the dissolution of acrylonitrile in deionized water at a temperature of 293 K (b) the radius of acrylonitrile droplet with respect to the dissolution time in water; (c) the diffusion coefficient of acrylonitrile in water with respect to the ratio between temperature and viscosity.

of acrylonitrile around the quartz cuvette, as the volume of water in the quartz cuvette was much larger than the volume of the acrylonitrile droplet, $C_{A\infty}$ was almost equal to zero. Hence Eq. (1) can be simplified to

$$-\frac{\rho_A}{M_A}dR = kC_{AS}dt \quad (2)$$

As the droplet dissolved in the water phase at a slow rate，it was considered that at any moment of the record dissolution process, the values of C_{A0} and $C_{A\infty}$ were constant. As the displacement distance of the interface between the acrylonitrile and bulk phase (approximately 50 μm) was smaller than the scale of the bulk phase (more than 2.5mm), the concentration distribution obeyed a quasi-steady state assumption in this work, which is consistent with a shrinking-core model[22]. Moreover, there was no obvious convection during the dissolution process and on turbulence was observed at the interface, hence the differential equation at the interface of the droplet and boundary conditions are written as:

$$D_{AW}\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC}{dr}\right)=0 \quad (3)$$

$$r = R,\ C = C_{AS};$$

$$r = \infty,\ C = C_{A\infty};$$

D_{AW} stands for the diffusion coefficient of acrylonitrile in water.

The integral of Eq. (3) combined with the boundary conditions is $C=-R(C_{A\infty}-C_{AS})/r+C_{A\infty}$. Furthermore, the molar flux through the interface from the droplet surface to the stationary bulk phase is written as:

$$k(C_{AS}-C_{A\infty}) = -D_{AW}\frac{dC}{dr}\Big|_{r=R} \quad (4)$$

By combining Eq. (3) and Eq. (4), the value of k equals to D_{AW}/R. So Eq. (2) is re-written as:

$$dt = -\frac{\rho_A}{M_A C_{AS} D_{AW}}RdR \quad (5)$$

The theoretical relationship between the droplet radius and the dissolution time can be obtained by the integration of Eq. (5) as:

$$t = -\frac{\rho_A}{2M_A C_{AS} D_{AW}}R^2 + \frac{\rho_A}{2M_A C_{AS} D_{AW}}R_0^2 \quad (6)$$

R_0 stands for the initial radius of the acrylonitrile droplet. By fitting Eq. (6) to the experimental data, the diffusion coefficient of acrylonitrile in water at different temperatures can be identified for the whole experiment.

Common semi-empirical equations about the diffusion coefficient for liquid-liquid system such as the Stocks-Einstein[23] equation and Wilke-Chang equation[24, 25] considered that there is a linear relationship between the diffusion coefficient and ratio of the temperature and the viscosity of the solvent. **Fig.** 3c shows the pattern of variation in the diffusion coefficient with the ratio of the temperature and the viscosity of the solvent (black square). The diffusion coefficients of acrylonitrile in water are 4.30, 6.13, 7.07, 9.51 and 11.09×10^{-10} m^2/s respectively at temperatures ranging from 278 to 298 K. By linear fitting of the experimental data, the relationship between D_{AW} and T/μ follow thus:

$$D_{AW} = 4.51\times10^{-5}T/\mu - 3.91\times10^{-10} \quad (7)$$

and the correlation coefficient is 0.99. The point of the orange rhombus stands for the theoretical prediction values of the diffusion coefficient at different temperature according to the Wilke-Chang equation. The latter equation is widely used in the field of the prediction of the diffusion coefficient for small

molecules in water (the diameter of solute molecule is relatively similar to which of water molecule) and is written as:

$$D_{AW0} = 7.4\times10^{-8}\frac{(\Phi_W M_W)}{\mu_W V_{A*}^{0.6}}\frac{T}{\mu_W} \quad (8)$$

Where D_{AW0} stands for the diffusion coefficient of acrylonitrile in an infinite diluted acrylonitrile aqueous solution, Φ_W, M_w and μ_w stands for the association parameters, molar mass and viscosity of water respectively, and V_{A*} stands for the molar volume of acrylonitrile. It has been shown that the experimental value is relatively smaller than the theoretical value. The reason for the difference between the experimental value and theoretical value may be due to the fact that the theoretical value represents the diffusion coefficient of acrylonitrile in an infinite diluted acrylonitrile aqueous solution, while in this work, the value represents the equivalent diffusion coefficient during the whole mass transfer process. In addition, the value of the diffusion coefficient of n-butyl alcohol in water was measured by the same method. At a temperature of 288 K, the value of the diffusion coefficient is 6.22×10^{-10} m^2/s. Lyons's work[26] showed the value of the diffusion coefficient of n-butyl alcohol in water ranged from 9.55 to 7.55×10^{-10} m^2/s under a molar concentration of n-butyl alcohol ranging from 0.05 to 0.700 mol/L. In our work, the diffusion coefficient of n-butyl alcohol in water was the equivalent diffusion coefficient under a molar concentration of n-butyl alcohol ranging from an infinite dilution to a saturation concentration (approximately 1.04 mol/L). And as the diffusion coefficient decreased with the increase of the n-butyl concentration, 6.22×10^{-10} m^2/s ought to be in the range of the diffusion coefficient of n-butyl alcohol in water under a different n-butyl alcohol concentration. Hence, this method is a convenient and relatively accurate way to predict the diffusion coefficient of acrylonitrile in water, and more studies will be made to modify this model in the future.

Effect of *R. ruber* cells without enzyme activity on the mass transfer process of acrylonitrile

The mass transfer process of acrylonitrile in a solution of *R. ruber* TH3 free cells solution without NHase enzyme activity was observed at a temperature of 293 K. The variation in the radius of acrylonitrile droplet with respect to the dissolution process is shown in **Fig.** 4a. Reduced to the same radius (approximately 50μm) from 100 μm, it took 55 seconds in water, instead of more than 102 seconds in a free cells solution without NHase enzyme activity. With the increase in the concentration of cells, a decreasing variation of the dissolution rate was observed, and this difference can be attributed to the mass transfer situation. Saien et al.[27] applied nanoparticles to enhance the mass transfer in a liquid-liquid system and pointed out that the disturbance field created by the motion of the nanoparticles caused a convective motion and reinforced the mass transfer process. The motion of the nanoparticles introduced by Brownian motion is less strong compared to the motion of cells. However, mass transfer was actually weaker in the liquid-liquid-cells system. A valid explanation of this phenomenon is that *R. ruber* TH3 free cells tended to attached to interface of the droplet and part of the acrylonitrile traversed the cells during the diffusion process, leading to the reduction of the mass transfer rate. A number of studies[28, 29] in fact have shown that bacterial cells adsorb to the oil-water interface and form a robust membrane, which was consistent with the phenomenon described in our work.

The parameter *k* is introduced to account for the mass transfer rate of acrylonitrile in a free cells solution, and

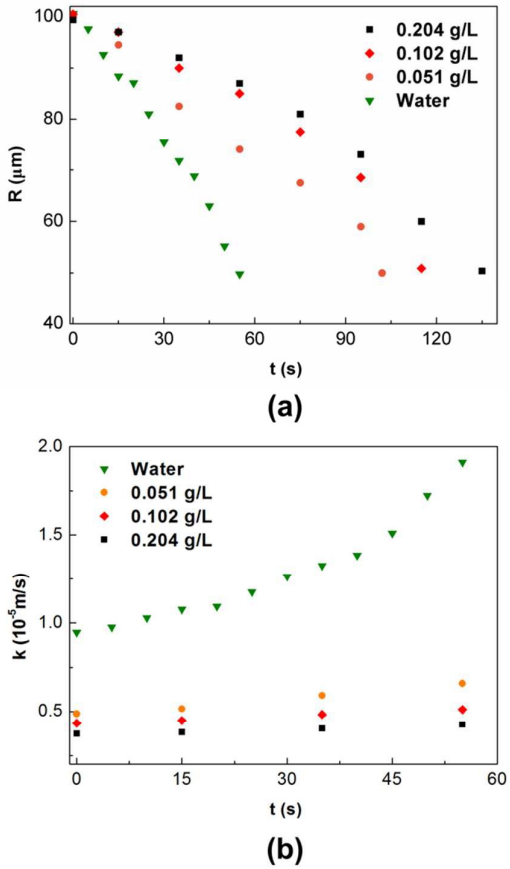

Fig. 4 The effect of R. ruber cells without enzyme activity on the mass transfer process of acrylonitrile: (a) the radius of acrylonitrile droplet with respect to the dissolution time in a free cells solution without enzyme activity; (b) the mass transfer coefficient of acrylonitrile in a free cells solution without enzyme activity with respect to the dissolution time. The cell concentration is 0.051, 0.102 and 0.204 g/L and the temperature is 293 K.

according to Eq. (2), the value of k was obtained by recording the variation in the radius of acrylonitrile droplet with respect to the dissolution time and calculating the slope of the *R-t* curve. **Fig.** 4b shows that the mass transfer rate of acrylonitrile in water is significantly greater than that of acrylonitrile in a cells solution without NHase enzyme activity. With the dissolution of the droplet, the value of *k* of acrylonitrile in water increased from 0.95×10^{-5} to 1.91×10^{-5} m/s within 55 seconds. The rules for the changes of *k* for acrylonitrile in the free cells solution, though not obvious as that in water, showed the same trend at a different concentration of cells, indicating that the reduction of the radius of the droplet is conducive to the acceleration of the mass transfer rate. This conclusion is consistent with the previous work with respect to the hydration of acrylonitrile in a membrane dispersion microreactor.

Effect of bio-hydration reaction on the mass transfer process of acrylonitrile

The influence of the bio-hydration reaction on the mass transfer process of acrylonitrile with different enzyme activity was discussed. The experiment was carried out under a temperature of 293 K, and enzyme activity ranged from 100 U/mL to 400 U/mL. **Fig.** 5a shows the pattern of variation in the radius of acrylonitrile droplet as the dissolution process continued. The radius of the droplet was reduced from 100 µm to 50.9 µm in approximately 30 seconds at enzyme activity of 100 U/mL (the red curve). Meanwhile, the radius of the acrylonitrile droplet dissolved in water was 75.4 µm from the same initial radius. With the improvement of enzyme activity, the reduction of the radius of the acrylonitrile droplet became more remarkable within the same dissolution time, indicating that the bio-hydration reaction can greatly strengthen the mass transfer rate of acrylonitrile. This intensification mainly benefitted from the consumption of acrylonitrile by cells containing NHase, leading to an increase in the concentration gradient of acrylonitrile in the reaction solution around the cells.

The patterns of variation in k as the dissolution time continued at different rates of enzyme activity were recorded in **Fig.** 5b. It is shown that the mass transfer rate for acrylonitrile increased with dissolution time in all enzyme activity during the process of dissolution. This trend is the same as with the dissolution of acrylonitrile droplet in a free cells solution without NHase enzyme activity and mainly caused by the reduction of the diameter of the droplet. The value of k increased with the increase of enzyme activity. With enzyme activity of 400 U/mL, the maximum value of k could reach 4.78×10^{-5} m/s at 30 seconds, which was significantly greater than the value of k in water (1.26×10^{-5} m/s) with the same dissolution time.

To illustrate the reinforced effects on the mass transfer rate by bio-reaction more quantitatively, I was introduced and defined as $I=k_r/k_0$, where k_0 and k_r are the mass transfer coefficients of acrylonitrile in a water and *R. ruber* free cells solution. **Fig.** 5c shows that I increased considerably as the enzyme activity increased and ranged from 1.70 to 4.78 in all the concentration ranges, indicating that the mass transfer rate can be greatly improved by the bio-reaction under experimental conditions. In addition, with the same enzyme activity, I increased as the dissolution process continued. The causes of this trend are due to the reinforcement of the bio-reaction on the mass transfer of acrylonitrile (consumption of acrylonitrile by cells) and the fact that the diameter of acrylonitrile droplet was smaller in a free cells solution than in pure water at the same time, and the mass transfer rate would be accelerated with the decrease in the diameter of the droplet. In conclusion, the hydration reaction contributed significantly to the dissolution of acrylonitrile, and with the increasing of the enzyme activity, the coefficient increases multiply.

Effect of concentration of acrylamide on the critical radius of acrylonitrile droplet

During the biological hydration process, the concentration of acrylamide increased with the reaction continued. Hence it is significant to have a deep understanding on the effect of the increase of acrylamide concentration on the mass transfer process of acrylonitrile. As the reduction of the radius of the droplet is conducive to the acceleration of the mass transfer rate, the critical diameters of acrylonitrile droplet formed in various concentrations of an acrylamide aqueous solution were first studied.

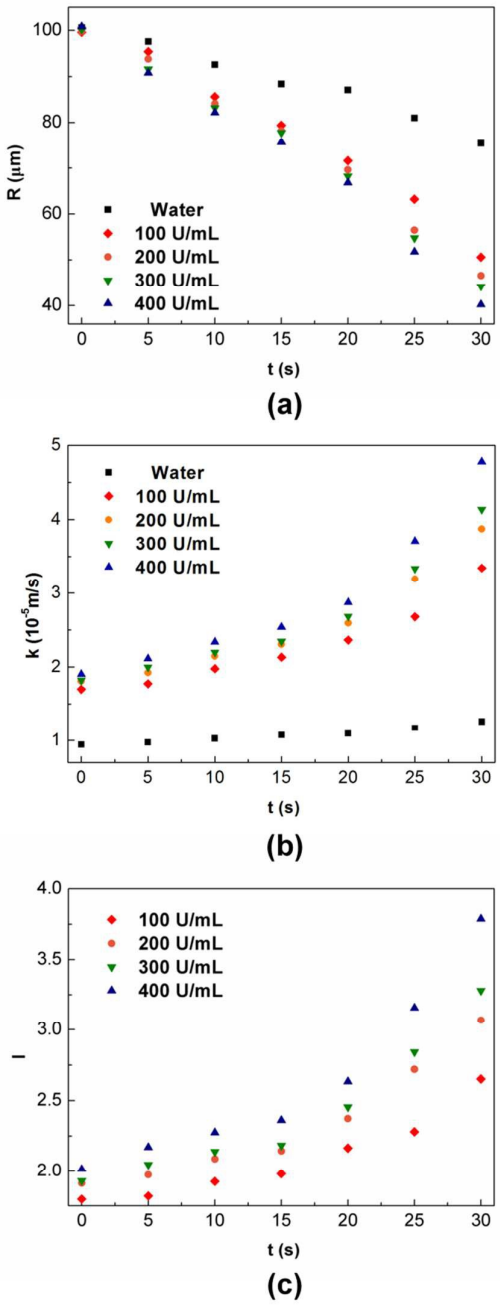

Fig. 5 The effect of bio-hydration reaction on the mass transfer process of acrylonitrile: (a) the radius of acrylonitrile droplet with respect to the dissolution time in a free cells solution with enzyme activity; (b) the mass transfer coefficient of acrylonitrile in in a free cells solution with enzyme activity with respect to the dissolution time. (c) the intensifying factor with respect to the dissolution time in a free cells solution with enzyme activity. The enzyme activity of NHase is 100, 200,300 and 400 U/mL and the temperature is 293 K.

The critical diameters of acrylonitrile droplet formed in various concentrations of an acrylamide aqueous solution were defined as the limit after which the drop separated from the capillary, and were measured to discuss how far the

improvement of concentration of acrylamide influenced the dissolution of acrylonitrile as the bio-reaction continued. As the influence of acrylamide on the bio-reaction became much more serious after the concentration exceed 25 wt%, the

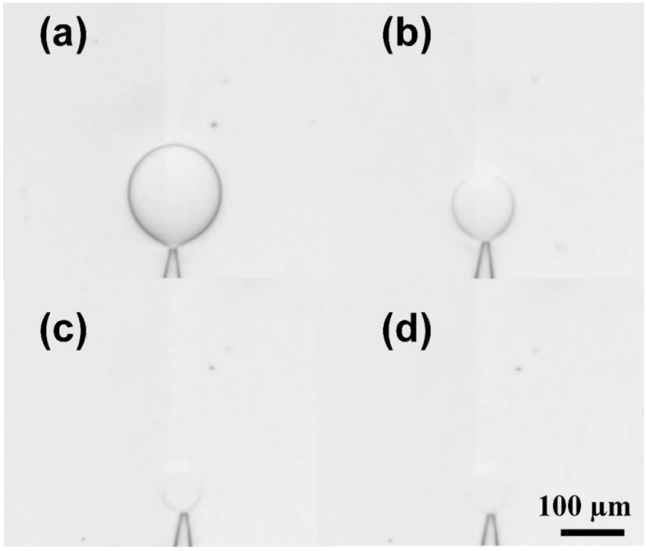

Fig. 6 The critical acrylonitrile droplet formed in different concentrations of acrylamide aqueous solution: (a) the concentration of acrylamide equals to 25 wt%; (b) the concentration of acrylamide equals to 30 wt%; (c) the concentration of acrylamide equals to 35 wt%; (d) the concentration of acrylamide equals to 40 wt%.

experimental concentrations of acrylamide were set as 25 wt%, 30 wt%, 35 wt% and 40 wt% respectively, and the temperature was fixed at 293 K. The critical acrylonitrile droplet was recorded in **Fig.** 6. It is shown that the critical diameters were approximately 147, 94 and 62 μm with respect to acrylamide concentrations of 25 wt%, 30 wt% and 35 wt% respectively. When the concentration reached 40 wt%, the droplet of acrylonitrile was in practice hard to form in the acrylamide aqueous solution. In order to account for this phenomenon, the element that influenced the critical diameter of the droplet needs to be discussed.

The droplet formed at the tip of capillary is subjected to the competing effects of gravity, buoyancy and interfacial tension. After exceeding its critical volume, the droplet separates from the capillary, hence the following force balance that exists at the instant the droplet separates is as follows:

$$\pi r_t^2 \frac{2\gamma}{R_c} = \frac{4}{3}\pi R_c^3 \Delta\rho g \quad (9)$$

According to Young-Laplace equation, the left hand side of Eq. (9) is the effect of interfacial tension, and r_t is the radius of the capillary tip, γ is the interfacial tension between the adjoining phases, and R_c is the critical radius of acrylonitrile droplet. The right hand side of Eq. (9) is the effect of gravity and buoyancy. Where $\Delta\rho$ is the density difference between acrylonitrile and the acrylamide aqueous solution, g is the gravitational acceleration. Hence R_c can be obtained by Eq. (9) and equals $(3r_t^2\gamma/2\Delta\rho)^{0.25}$. As the intermolecular forces between acrylonitrile and acrylamide are significantly smaller than those between acrylonitrile and water, the interfacial tension between the acrylonitrile and acrylamide aqueous solutions will be reduced with the increase in the concentration of acrylamide, as does the critical radius of the acrylonitrile droplet.

It has been proved that the reduction of the radius of droplet is conducive to the acceleration of the mass transfer rate; hence the improvement to the bio-reaction by the reinforcement of mass transfer became less noticeable at a high concentration of acrylamide. In addition, the solubility of acrylonitrile in the acrylamide aqueous solution was 12.2 wt%, 17.7 wt%, 19.1 wt% and 23.5 wt% with an acrylamide concentration of 25 wt%, 30 wt%, 35 wt% and 40 wt% respectively. The increase of solubility led to an increase of the concentration gradient of acrylonitrile in the solution, which also intensified the mass transfer rate of acrylonitrile. More studies will be is made with regard to this field, including a mass transfer model of the ternary system and the optimization of the bio-process based on this conclusion.

Conclusions

A pendant drop method was used to observe the mass transfer phenomenon of acrylonitrile droplet under various solvent environments. The values of D_{AW} were 4.30, 6.13, 7.07, 9.51 and 11.09×10^{-10} m^2/s respectively at temperatures ranging from 278 to 298 K, where D_{AW} and T/μ obeyed a linear relationship. The reduction of the radius of the droplet was conducive to the acceleration of the mass transfer rate and cells without enzyme activity weakened the mass transfer rate of acrylonitrile to some extent. Both the bio-hydration reaction and the increase in the acrylamide concentration greatly strengthened the mass transfer process of acrylonitrile.

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