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Synthesis of neryl acetate by free lipase-catalyzed transesterification in organic solvents and its kinetics

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Abstract

In this paper, the optimum conditions and kinetics of lipase-catalyzed transesterification reaction of nerol and vinyl acetate for the synthesis of neryl acetate were investigated in a solvent-free system. The optimum conditions were determined as follows: the reaction temperature was 40 °C, the amount of enzyme was 12 mg/mL, and there was no need to add water. Increasing the stirring speed to 200 r/min can eliminate the external diffusion limitations. There was no substrate inhibition when the substrate concentration was lower than 500 mmol/L. However, the experimental results indicated that the product inhibition effect should be considered. The results of the reaction kinetic analysis indicated that the reaction followed the ping-pong double-double reaction mechanism with product inhibition. Finally, the model parameters were calculated by MATLAB software and the results showed that the experimental values could be in good agreement with the simulated values, and the relative error was 6.98%.

Keywords: neryl acetate; lipase; solvent-free system; transesterification; kinetics.

Practical Application: Neryl acetate is one of the raw materials for the production of flavours and fragrances and is the most important species of neryl ester. They are mainly used to blend the essential oils of bergamot, lavender, rose, lily of the valley and neroli, and are widely used in perfumery, cosmetics and soap fragrances.

1 Introduction

Neryl acetate is a colorless liquid with the aroma of fresh fruit. It is the raw material used in the rose flavors, and is the most important variety in neryl esters (Champdoré et al., 1998; Akagić et al., 2021). They are mainly used for blending essential oils such as lemon, lavender, rose, lily of the valley, and are widely used in perfumes, cosmetics, soap flavors, and also for apricot, apple, banana, grape, lemon, and peach (Lee et al., 2020). Neryl acetate is classified by the European Commission as an artificial food flavoring that can be used in food without endangering human health. Therefore, neryl acetate, as an important flavor component, has been widely used in food and beverage industry. Neryl acetate is a natural substance in vegetable oil, which can be prepared by vacuum fractionation. However, the high cost of vacuum fractional distillation and the limited availability of natural raw materials make the process unsuitable for large-scale industrial production (Hou & Sha, 2020). As a result, the application of this natural extraction method is limited. At the same time, the traditional chemical synthesis method for the production of neryl acetate usually involves toxic chemicals. Decomposition reaction or other side reactions are prone to occur under high temperature and pressure conditions (Koeller & Wong, 2001). The existence of reaction by-products will affect the characteristic flavor of neryl acetate, which limits its application in food and beverage industry (Yildiz et al., 2021). The application of new catalyst provides a new idea for the synthesis of neryl acetate. Rubieli et al. (Ze & Rubieli, 2021) synthesized neryl acetate from the esterification reaction of nerol and acetic anhydride by heterogeneous catalysis using ion exchange resins. Through experimental design analysis, the influence of variables on the synthesis of neryl acetate was evaluated. It was concluded that the conversion of nerolidol was more than 98%. However, this reaction system made the separation of neryl acetate more difficult. Therefore, it is an urgent problem to explore a new preparation method of neryl acetate.

Biosynthesis has the advantages of mild reaction conditions, high catalytic efficiency, and strong catalytic specificity. It is a typical "green" environmental protection process (Kumari et al., 2007). Aromatic esters produced by biotransformation have been regarded as natural products by the United States and the European Union (Lan et al., 2001). At present, there are few reports on the biocatalytic synthesis of neryl acetate. Zong et al. (2020) biosynthesized neryl acetate in recombinant E. coli for the first time, and their study provided a new method for biosynthesis of neryl acetate. Using the heterologous expression of alcohol acetyltransferase (ATF1) in E. coli strain, the resulting engineered strain (LZ006) accumulated 0.250 ± 0.014 mg/L of neryl acetate. By improving the metabolic flux of the MVA pathway, the yield of neryl acetate increased 32.2 times. Finally, the yield of neryl acetate was further increased by 46.8 times by dropping pyruvic acid. However, the operation of producing neryl acetate by this method is too complex, and the pyruvate added in the reaction process will also affect the purity of neryl acetate. In addition, transesterification catalyzed by lipase is also an important method to synthesize neryl acetate in non-aqueous phase. Jiang & Cheng (2004) successfully synthesized

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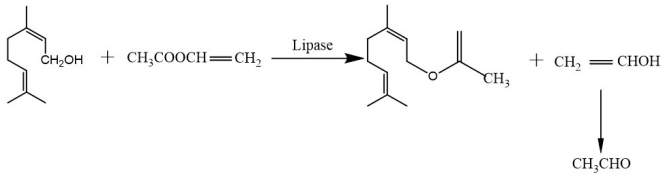
neryl acetate using immobilized *Candida albicans* lipase and modeled the synthesis by central composite design and response surface methodology. $R^2 = 0.9542$ after model fitting. Analysis of variance indicated that the model well reflected and explained the real relationship between reaction parameters. Compared with the single factor method, the conversion rate increased by 2%-3% and finally reached 91.6%. However, no kinetic study was carried out in this report.

Carboxylic acids are persistent organic pollutants, carcinogenic, teratogenic and mutagenic, and can be transported over long distances through various environmental media (atmosphere, water, living organisms, etc.) and remain in the environment for a long time. Some carboxylic acids are difficult to dissolve or extremely unstable in organic solvents, whereas esters can dissolve and remain stable in almost all organic solvents (Yue et al., 2022). Therefore, transesterification of esters is more important than direct esterification of alcohol and acid, which makes enzyme catalyzed transesterification reactions to synthesize perfume ester an important research direction (Shah et al., 2004; Gómez et al., 2020; Febriani et al., 2020). The preparation of neryl acetate by lipase-catalyzed transesterification has obvious environmental advantages. On the one hand, it can reduce the harm of carboxylic acid to the environment. On the other hand, compared with the traditional extraction method, it can reduce the use of vegetation and protect the ecological environment. In the previous research, our laboratory screened and obtained a lipase that can efficiently catalyze the conversion of monoterpene flavor alcohol and vinyl acetate in non-aqueous phase (Xiong et al., 2012). Therefore, in this paper, the lipase is used as a catalyst to catalyze the transesterification reaction of nerol and vinyl acetate to prepare neryl acetate in a non-aqueous phase system. After the transesterification of vinyl acetate with nerol as an acyl donor, the reaction product vinyl alcohol can be isomerized into enol, so that the transesterification reaction can be regarded as an irreversible reaction, accelerate the reaction speed and improve the product yield. The reaction equation is shown in Scheme 1. In addition to investigating the various factors affecting the transesterification reaction, the reaction mechanism is also discussed, the kinetic model is developed and kinetic parameters are solved, so as to provide the necessary basis for its application in industrial production.

2 Materials and methods

2.1 Materials

Nerol (95%) and neryl acetate (95%) were purchased from Sigma-Aldrich, and lipase (99.5%) was purchased from Amano Enzyme Ltd. Analytical grade vinyl acetate, n-hexadecane (98%), toluene, benzene, methylene chloride, acetone and n-hexane were purchased from Sinopharm Chemical Reagent Co., Ltd. Shimadzu GC-2014 gas chromatography was purchased from Shimadzu Corporation, HZ-2111KA constant temperature shaker was purchased from Taicang Hualida Test Equipment Co Ltd, and T-214 electronic balance was purchased from Sartorius AG, Germany.



Scheme 1. Lipase-catalyzed transesterification synthesis of Neryl acetate.

2.2 Experimental procedure

Selection of solvents

The reaction was carried out in a closed screw stopper flask. The concentration of nerol was 100 mmol/L, and the molar ratio of nerol to vinyl acetate was 1 : 5 (except for the reaction with vinyl acetate as solvent). After adding 2 mL organic solvent, it was preheated in a constant temperature shaking table for 10 min, and then 24 mg lipase was added. The reaction started with the addition of lipase. The parameters of the thermostatic shaker were set as follows: reaction temperature 40 °C, shaker speed 200 r/min. After 5 h of reaction, samples were taken with a microsampler, and the yield of neryl acetate was detected by gas chromatography.

Lipase-catalyzed reactions in solvent-free systems

Unless otherwise specified, the reactions were carried out according to the following methods: the reaction vessel was a closed corked flask, the concentration of nerol was 100 mmol/L and 2 mL of vinyl acetate was added as the reactant and solvent. The reactions were preheated in a constant temperature shaker for 10 min, after which 12 mg/mL of lipase was added. The reaction conditions were: reaction temperature 40 °C, shaker speed 200 r/min. Samples were taken at regular intervals using a microsampler and the product yield was measured.

2.3 Analytical method

The product, neryl acetate, was analyzed by gas chromatography with n-hexadecane as the internal standard. The detection conditions were as follows: the chromatographic column was 30 mm x 0.22 mm SGE AC10; N_2 flow rate was 44 mL/min, H_2 flow rate was 40 mL/min, air flow rate was 400 mL/min and the tail blowing was 25 mL/min. The split ratio was 10 : 1. The column temperature was 165 °C, the injector temperature was 280 °C and the detector temperature was 280 °C.

2.4 Determination of the initial velocity of the reaction

The initial rate of reaction is defined as the amount of substance per unit time per unit volume that produces the product neryl acetate with the yield controlled within 5%.

3 Results and discussion

3.1 Screening of different solvents

The characters of the reaction medium are very important for the catalytic activity and selectivity of enzyme. It can affect

the three-dimensional structure of enzyme protein molecule through solvation, thus affecting the catalytic ability of enzyme (van Tol et al., 1995). Because logP is widely used to describe the hydrophobicity of solvents and reflect the characteristics of solvents, six organic solvents with different logP values were selected to investigate the effect of organic solvents on the catalytic reaction. The yield of neryl acetate catalyzed by lipase in different organic solvents is shown in Table 1. It can be seen from the results in Table 1 that the yield of neryl acetate has no obvious relationship with the logP value of organic solvent, which may be due to the complex influence of organic solvent on enzyme catalytic reaction, which is difficult to be measured by a single solvent property. In the first five organic solvents, the yield of neryl acetate was low after 5 h of reaction, whereas when vinyl acetate was used as reactant and solvent, the yield could reach about 98% after 5 h. Another advantage of using vinyl acetate as reactant and solvent is that it simplifies the reaction system and avoids the solvent recovery problem caused by adding other organic solvents. In this experiment, vinyl acetate can be separated and recovered only through simple fractionation. Therefore, in the follow-up experiment, vinyl acetate will be used as reactant and solvent (solvent-free reaction system). This reaction system can reduce the use of solvents, thus reducing the negative impact of organic reactions on the environment.

Table 1. Effect of solvent on the yield of transesterification.

Number	Solvent	logP	Yield (%)
1	Toluene	2.52	53.71 ± 0.20
2	Benzene	2.03	58.51 ± 0.40
3	Dichloromethane	1.25	25.21 ± 0.50
4	Acetone	-0.24	24.13 ± 1.70
5	n-Hexane	3.50	63.21 ± 0.60
6	Vinyl acetate	0.31	98.00 ± 1.20

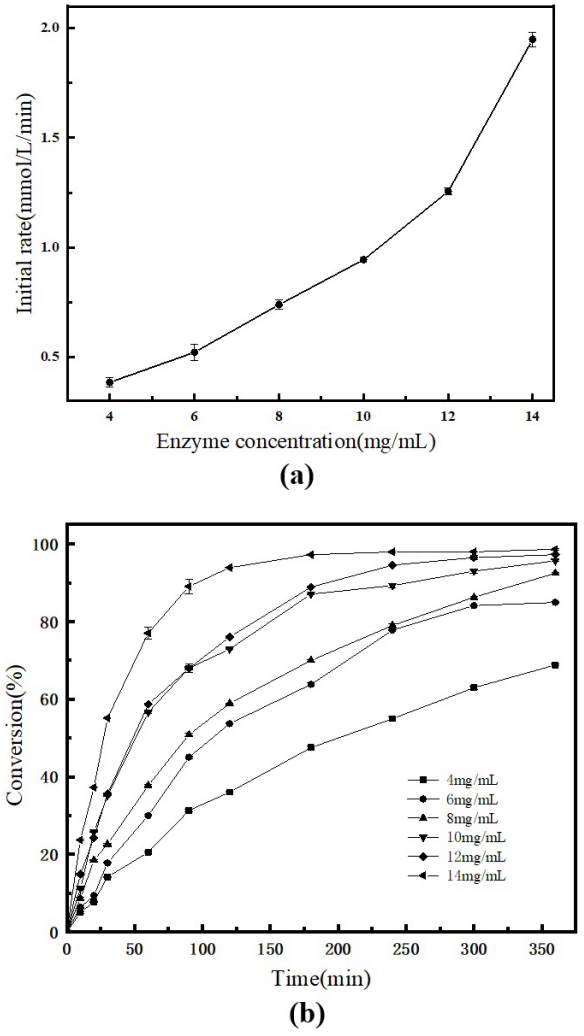


Figure 1. Effect of enzyme concentration on transesterification reactions: (a) Effect of enzyme concentration on initial rate; (b) Process reaction curves at different enzyme concentrations.

3.2 Effect of enzyme concentration

The amount of lipase in the enzyme-catalysed reaction has two main effects on the reaction. On the one hand, the low amount of enzyme cannot play a good catalytic role, the reaction speed is slow and the reaction time is long. On the other hand, the excessive amount of enzyme will cause unnecessary waste and increase the cost. It can be seen from Figure 1a that with the increase of enzyme amount from 4 mg/mL to 12 mg/mL, the initial reaction rate increases linearly, which shows that the reaction system is controlled by kinetics. The influence of internal diffusion can be basically ignored, and the control step of reaction rate is enzyme catalyzed reaction. As can be seen from Figure 1b, after the enzyme amount reaches 12 mg/mL, the increase of yield tends to be flat with the increase of enzyme amount. This may be because within a certain enzyme concentration range, the greater the enzyme concentration, the greater the probability of contact between the enzyme and the substrate and the faster the catalytic speed. However, with the further increase of enzyme amount, the enzyme concentration is close to saturation relative to the substrate concentration, so that the yield cannot be significantly improved. Considering the product yield and economic efficiency, 12 mg/mL was selected as the enzyme dosage in subsequent experiments.

3.3 Effect of temperature

Temperature is an important parameter affecting the enzyme-catalyzed reaction. As can be seen from Figure 2a, when the temperature increases from 20 °C to 40 °C, the initial speed of the reaction keeps increasing, because the increase of temperature will increase the thermal energy of the substrate molecules, improve the probability of contact between substrate and enzyme molecules, and accelerate the speed of the enzyme-catalyzed reaction. When the temperature reached 40 °C, the initial speed of this transesterification reaction was the highest and the yield was over 98% after 6 h. When the temperature exceeded 40 °C, the yield did not increase significantly, but decreased when the temperature was raised to 60 °C (Figure 2b), because the increase of temperature would cause the breaking of non-covalent bonds in enzyme molecule, resulting in denaturation and inactivation of the enzyme. Considering the initial reaction rate and the yield of neryl acetate, 40 °C was finally determined as the subsequent reaction temperature.

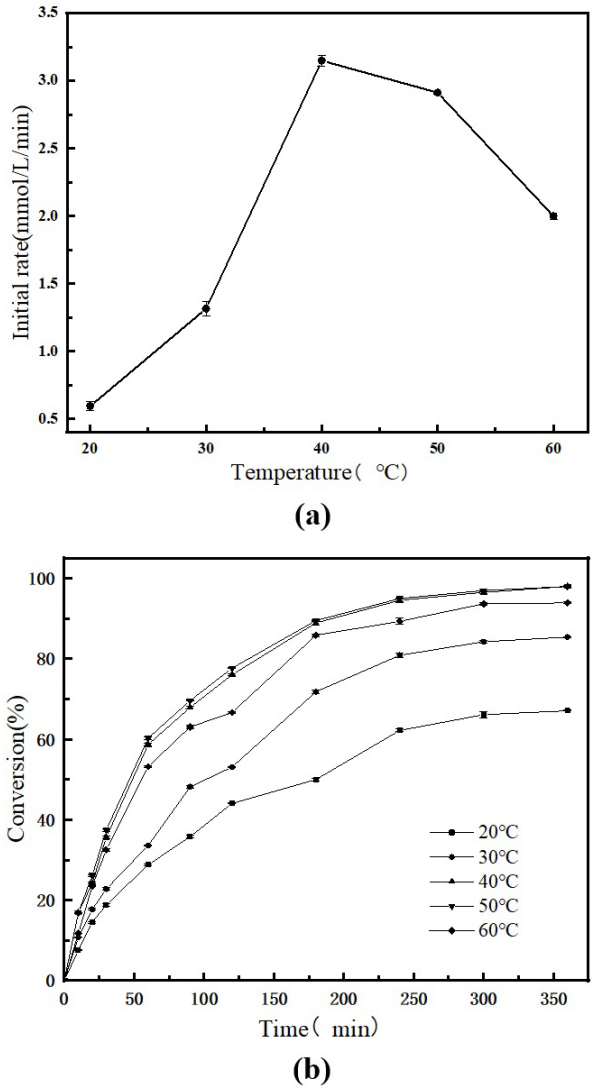


Figure 2. Effect of temperature on transesterification reactions: (a) Effect of temperature concentration on initial rate; (b) Process reaction curves at different temperatures.

According to the Arrhenius formula, $\log V_m$ is plotted against 1/T, as shown in Figure 3. From the graph the slope of the line can be found to be 1554.59 and the calculation gives the activation energy E_a = 30.83 kJ/mol.

3.4 Effect of agitation speed

Since enzymes are insoluble in most organic solvents, the binding of enzymes to substrate molecules is limited by external diffusion (Kalthod & Uckenstein, 1982). It can be seen from Figure 4a and Figure 4b that when the rotating speed of the shaker was less than 200 r/min, the initial reaction rate changed obviously with increasing shaker speed, and the yield also increases, because increasing the rotating speed can effectively reduce the external diffusion resistance. When the rotating speed of the shaker was greater than 200 r/min, both the initial reaction rate and the product yield remain essentially constant, which indicates that the external diffusion limit in the reaction system can be basically

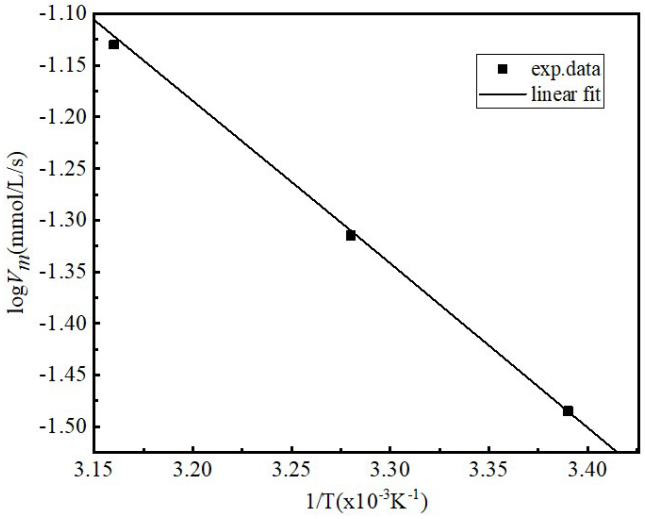


Figure 3. Correlation curve between Log Vm and 1/T.

eliminated when the rotating speed of the shaker was greater than 200 r/min. Considering that the increase of product yield was not significant and the energy consumption was higher when a large rotating speed was adopted, 200 r/min was finally determined as the shaking speed in subsequent experiments.

3.5 Effect of the additional amount of water

In fact, the organic solvent used in the reaction is not absolutely anhydrous, but simply contains less water. At the same time, the micro amount of water molecules that are tightly bound to enzyme molecules are very important for the catalytic activity of enzyme. If the water content of the system is too low, the conformation of enzyme is too "rigid" and the activity of the enzyme is low. Adding the appropriate amount of water makes the centre of the enzyme activity tend to be flexible and the activity of the enzyme is increased, which indicates that the enzyme needs a certain amount of water to maintain the conformation necessary for the catalytic activity of the enzyme molecule (Ke & Klibanov, 1998). Therefore, the catalytic activity of the enzyme can be adjusted by controlling the water content of the system. As can be seen from Figure 5a, when water was not added to the reaction system, the initial reaction rate was very high, which indicated that the necessary water on the original enzyme surface was sufficient to maintain the natural conformation required for the enzyme. However, with the increase of water addition, the initial reaction rate and yield gradually decreased (Figure 5b). This is due to two reasons. One is that excessive water will agglomerate the free enzyme, making it difficult for the hydrophobic substrate to reach the active center of the enzyme, so the mass transfer resistance in the reaction system increases. The other is that the formation of water clusters in the active centre of the enzyme can change the configuration of the enzyme and eventually lead to the decrease of enzyme activity.

3.6 Effect of substrate concentrations

In the organic solvent reaction systems, the enzyme-catalyzed reaction occurs at the interface between the micro aqueous phase and the organic phase adsorbed on the enzyme

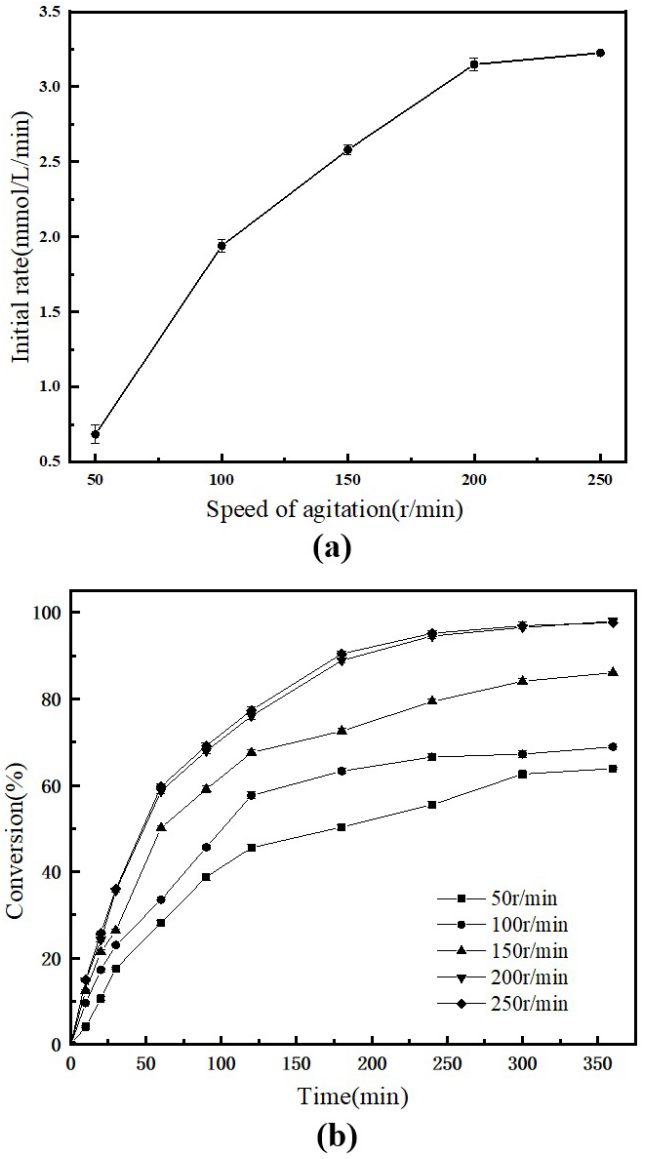


Figure 4. Effect of speed on transesterification reactions: (a) Effect of speed concentration on initial rate; (b) Process reaction curves at different speeds.

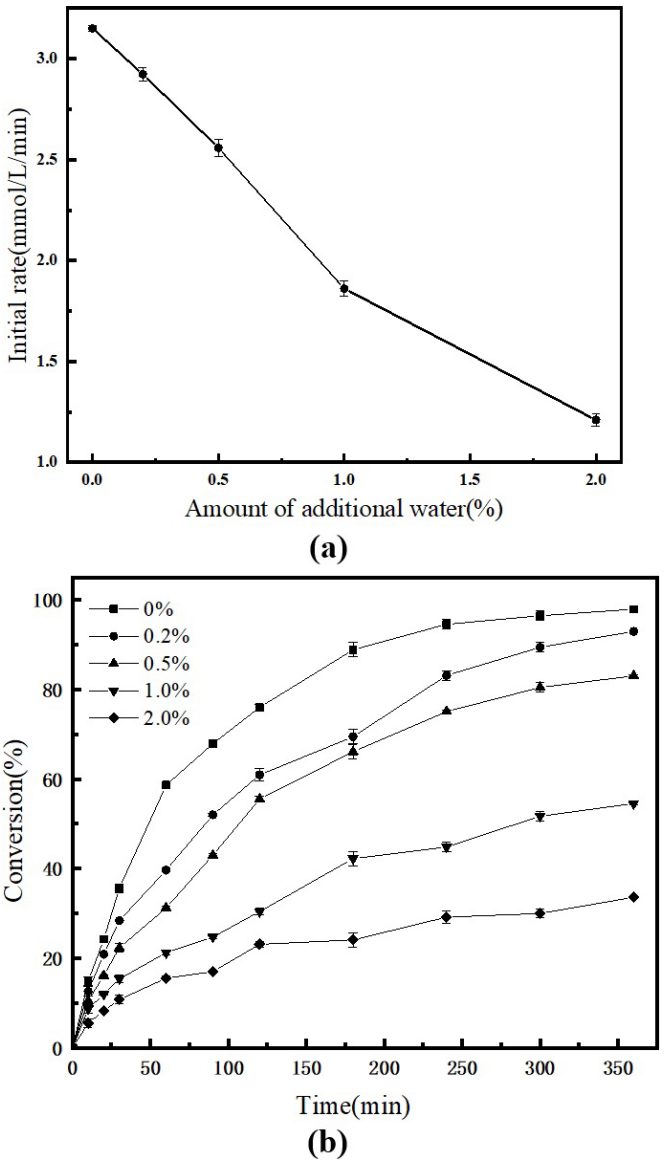


Figure 5. Effect of water addition on transesterification reactions: (a) Effect of water addition concentration on initial rate; (b) Process reaction curves at different water additions.

surface. The substrate molecules in the organic solvent must first diffuse from the organic phase into the micro aqueous phase before they can be catalyzed by the enzyme molecules. When the substrate concentration is too low, the diffusion rate of the substrate molecules into the micro aqueous phase is low, the power of transesterification reaction is small and the yield is low. When the substrate concentration increases, the power of transesterification reaction is high, but the substrate may also inhibit the enzyme. As can be seen from Figure 6a and Figure 6b, the initial rate of the reaction gradually increased with the increase of substrate concentration from 100 mmol/L to 500 mmol/L. According to the double reciprocal curve of substrate concentration and initial velocity, it can be seen that the curve is almost linear, which can confirm that there is no substrate inhibition within the concentration range of the experimental study.

3.7 Effect of product concentration

As can be seen from Figure 7, the initial reaction rate had an obvious downward trend with the increase product concentration, so there is product inhibition in this reaction. This may be due to the product binding to the acyl enzyme molecule generated in the catalytic reaction to form an ineffective dead-end product and occupied special sites on the enzyme molecules, making it difficult for the enzyme to bind to substrates or play the catalytic role.

3.8 Reuse of lipase

The cost of lipase is one of the bottlenecks in the preparation of neryl acetate by biocatalysis method. If lipase has good operational stability during the catalytic reaction and can be reused for many times, the cost of lipase can be reduced. It can

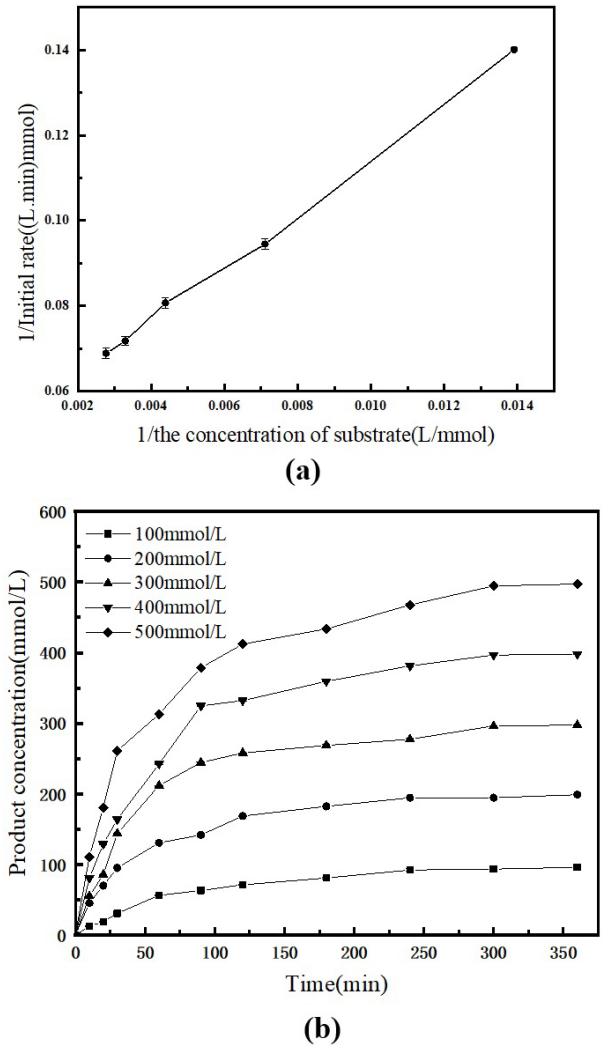


Figure 6. Effect of substrate concentration on transesterification reactions: (a) Double reciprocal plot of substrate concentration and initial rate; (b) Process reaction curves at different substrate concentrations.

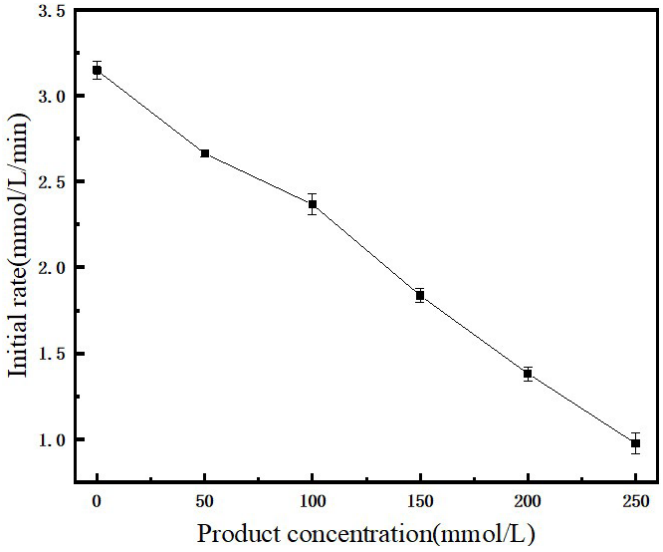


Figure 7. Effect of product concentration on initial rate.

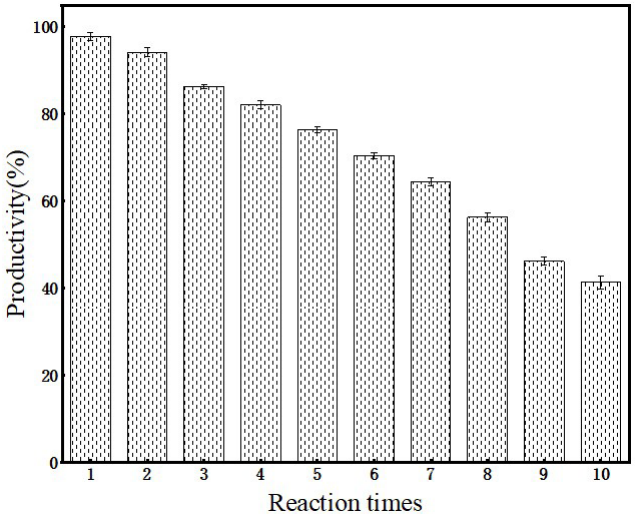


Figure 8. Reusability of lipase.

be seen from Figure 8 that when the enzyme was reused for the 10th time, the yield quickly decreased to 43.36%, indicating that the enzyme inactivated rapidly. The result showed that the stability of the enzyme used in the reaction system was not very good and needed to be improved.

3.9 Kinetic model

Many studies have shown that the transesterification catalyzed by lipase conforms to the ping-pong bi-bi reaction mechanism (Xiong et al., 2012; Wang et al., 2012; Xin et al., 2011). Therefore, the kinetic equation is established by King Altman method in this paper. The reaction sequence is shown in Scheme 2. Vinyl acetate (A) first binds to the free enzyme (E) and forms a non-covalent enzyme–ester complex (EA), which produces the acyl-enzyme intermediate (FP) upon isomerization. The modified enzyme (F) then binds to the released first product and enol (P). The second substrate, nerolidol (B), reacts with the activated enzyme (F) to produce another complex (FB), which produces the ester-enzyme complex (EQ) upon isomerization. EQ finally disassociates into the second product neryl acetate (Q) and the free enzyme (E).

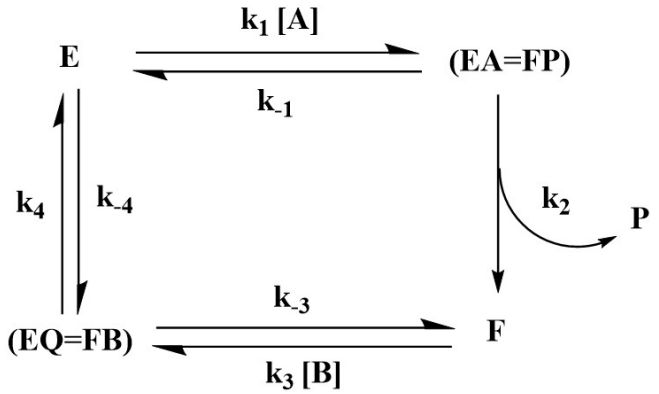
According to King-Altman scheme, the total enzyme in the system is as follows (Equation 1):

$$\begin{aligned}\Sigma = {} & (k_{-3}+k_4)k_1k_2[A]+(k_2+k_{-1})k_3k_4[B]+\\ & (k_2+k_4)k_1k_3[A][B]+(k_2+k_{-1})k_{-3}k_{-4}[Q]+\\ & (k_2+k_{-1})\,k_3k_{-4}[B][Q]= K_A[A]+K_B[B]+\\ & K_{AB}[AB]+K_Q[Q]+K_{BQ}[BQ]\end{aligned} \quad (1)$$

where $K_A = k_1k_2(k_{-3} + k_4)$, $K_B = k_3k_4(k_{-2} + k_{-1})$, $K_{AB} = k_1k_3(k_2 + k_4)$, $K_Q = k_{-3}k_{-4}(k_{-1} + k_2)$, $K_{BQ} = k_3k_{-4}(k_{-1} + k_2)$

The reaction rate can be expressed as follows (Equation 2):

$$V = k_1[E][A]-k_{-1}[EA=FP] \quad (2)$$



Scheme 2. King-Altman scheme.

because (Equations 3-5)

$$\frac{[E]}{[E]'} = \frac{(k_{-1} + k_2)k_3k_4[B]}{\Sigma} \quad (3)$$

$$\frac{[EA = FP]}{[E]'} = \frac{k_1k_3k_4[A][B]}{\Sigma} \quad (4)$$

$$V = \frac{k_1k_{-1}k_3k_4[A][B][E]' + k_1k_2k_3k_4[A][B][E]' - k_{-1}k_1k_3k_{4[A][B][E]'}}{\Sigma} = \frac{V_m[A][B]}{K_A[A] + K_B[B] + K_{AB}[A][B] + K_Q[Q] + K_{BQ}[B][Q]} \quad (5)$$

Since vinyl acetate A acts as a reactant-cum-solvent in this reaction, the concentration of A changes very little during the reaction and [A] can be regarded as a constant, so the above equation can be further simplified as (Equation 6):

$$V = \frac{V_m[B]}{K_A + K_B\frac{[B]}{[A]} + K_{AB}[B] + K_Q\frac{[Q]}{[A]} + K_{BQ}\frac{[B][Q]}{[A]}} = \frac{V_m[B]}{K_A + K_B[B] + K_{QA}[Q] + K_{BQA}[B][Q]} \quad (6)$$

where $V_m = k_1k_2k_3k_4[E]'$, $K_{AB} = \frac{K_B}{[A]}$, $K_{QA} = \frac{K_Q}{[A]}$, $K_{BQA} = \frac{K_{BQ}}{[A]}$, $K_B = K_{BA} + K_{AB}$

From the previous experiments, it is clear that product Q has an inhibitory effect on the enzyme and the equation is modified using the inhibition factor to give (Equation 7).

$$V = \frac{V_m[B]}{K_A + K_B[B] + K_{QA}(1 + \frac{[Q]}{K_{IQ}})[Q] + K_{BQA}[B][Q]} \quad (7)$$

K_{IQ} indicates the inhibition constant of product Q

Equation 7 is the rate equation for the synthesis of nerolides acetate by lipase-catalyzed transesterification in a solvent-free system.

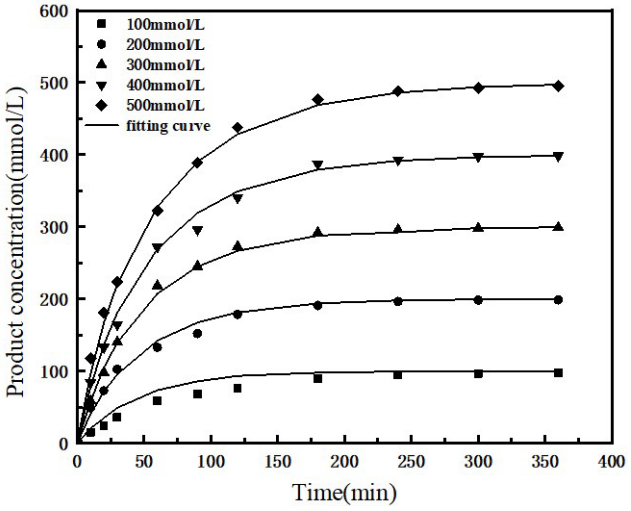


Figure 9. Comparison of simulated values with the experimental data.

Table 2. Parameter values for the simulated rate equation.

Parameters	Numerical values
V_m (mmol/L/min)	1.7×10^{-4}
K_A (mmol/L)	4.496×10^{-1}
K_B	5.1×10^{-3}
K_{QA}	3.001×10^{-1}
K_{BQA} (L/mmol)	16.7736

The model parameters of the reaction kinetic model were fitted by Matlab software and the results are shown in Table 2. The fitted model parameters were substituted into the reaction rete equation. The results showed that the fitted equation values were in good agreement with the experimental values, as shown in Figure 9. The relative error of the model was calculated to be 6.98%. This indicates that the reaction kinetic model is reasonable. Therefore, the kinetic model can better describe the reaction process of the synthesis of neryl acetate by lipase-catalyzed transesterification in the solvent-free system.

4 Conclusions

This paper systematically studied the reaction conditions and reaction mechanism of the synthesis of neryl acetate by lipase catalyzed transesterification in the non-aqueous phase, focused on the effect of lipase on this transesterification reaction under different conditions, established an efficient reaction system, and optimized the reaction conditions. On this basis, a greener and more environmentally friendly reaction system was established. The optimized reaction conditions were as follows: under the conditions of vinyl acetate as acyl donor and solvent, the enzyme amount was 12 mg/mL, the reaction temperature was 40 °C, and the effect of external diffusion could be basically eliminated at 200 r/min in the shaker, and the yield of the reaction could reach more than 98% for 6 h. The reuse of the enzyme under the optimized reaction conditions was investigated, and the

yield decreased to 43.36% after 10 times reuse. The kinetics of the transesterification reaction was studied. The reaction rate equation was deduced by using the ping-pong bi-bi reaction mechanism with product inhibition. The reaction kinetic equation was obtained as follows (Equation 8):

$$V = \frac{\mathbf{V_m}[\mathbf{B}]}{\mathbf{K_A} + \mathbf{K_B}[\mathbf{B}] + \mathbf{K_{QA}}(1 + \frac{[\mathbf{Q}]}{\mathbf{K_{IQ}}})[\mathbf{Q}] + \mathbf{K_{BQA}}[\mathbf{B}][\mathbf{Q}]} \quad (8)$$

The model parameters obtained by MATLAB fitting were: $V_m = 1.7 \times 10^{-4}$ mmol/L/min; $K_A = 4.496 \times 10^{-1}$ mmol/L; $K_B = 5.1 \times 10^{-3}$; $K_{QA} = 3.001 \times 10^{-1}$; $K_{BQA} = 16.7736$ L/mmol; $K_{IQ} = 5.974 \times 10^{-1}$mmol/L .These parameters were substituted into the kinetic equation of the reaction. The results showed that the fitted values were in good agreement with the experimental values, and the relative error of the model was 6.98%.

Abbreviations

A: substrate vinyl acetate. B: substrate nerol. P: product vinyl alcohol. Q: product neryl acetate. E: enzyme. F: acylase. EA: enzyme-substrate complex. EQ: enzyme-product complex. FB, FP: acyl-enzyme intermediate. (EA = FP), (EQ = FB): presence of isomerization of transition state intermediates. V: reaction speed. Vm: max. response speed. ki (i = 1, 2, 3, 4, -1, -2, -3, -4): rate constant. Kx (x = A, B, QA, BQA): model parameters. KIQ: inhibition constant of the product Q.

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