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Synthesis of citronella acetate by lipase catalyzed transesterification in ionic liquid and its kinetics

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Abstract

In this paper, the lipase catalyzed transesterification of citronellol with vinyl acetate was investigated in ionic liquid. Firstly, six kinds of ionic liquids and six kinds of lipases from different sources were screened. The results showed that the lipase from *Pseudomonas fluorescens* had the best catalytic effect in the ionic liquid [bmimn][TF2]. The factors affecting the transesterification were optimized, and the optimal reaction conditions were as follows: the molar ratio of vinyl acetate to citronellol was 3:1; the reaction temperature was 40 °C; the amount of enzyme was 10 mg/mL; the rotating speed of the shaker was 200 r/min. When the concentration of substrate was below 500 mmol/L, no inhibition of substrate was found, while the inhibition of product was not negligible. The reuse of the lipase was studied, and the results showed that the catalytic activity of the lipase decreased by 20% after 7 times. The kinetic study showed that the reaction was a ping-pong bi-bi reaction mechanism with the inhibition of citronellyl acetate. The reaction kinetics model was established, and the parameters of the model were fitted by MATLAB software. The fitting values and the experimental values were in good agreement, and the relative error was only 7.75%.

Keywords: citronellyl acetate; lipase; ionic liquid; transesterification; kinetics.

Practical Application: Citronella acetate is one of the raw materials for the production of flavours and fragrances and is the most important species of citronella ester. It is widely used in perfumery, cosmetics and soap fragrances.

1 Introduction

Citronellyl acetate is a colorless liquid with the odor of the lemon and an important part of the flavor and fragrance. Therefore, citronellyl acetate is widely used in food, beverage, cosmetics, perfume and pharmaceutical industries (Dabiri et al., 2012) and has great application value. The traditional production methods of citronellyl acetate include natural extraction and chemical synthesis (Geraschenko et al., 2019). However, due to the lack of natural raw materials and high distillation cost, it is not suitable for large-scale industrial production (Dreistadt et al., 2022). Therefore, the application of natural extraction method is limited. In traditional chemical synthesis method takes a large number of inorganic acids as catalysts for esterification reaction to synthesize citronellyl acetate, so a large number of acidic waste materials will be produced in the production process, which seriously pollutes the environment. At the same time, under high temperature and pressure conditions, other side reactions are easy to occur, which will affect the characteristic fragrance of citronella acetate and limit the application of this method in food and beverage industry (Sun et al., 2011). At present, people are seeking catalysts of low energy consumption, high catalytic efficiency and green solvents to replace the traditional catalyst and organic solvents which used for making citronellyl acetate. In view of the defects of natural extraction and chemical synthesis, we need to find a new method to produce natural flavor compounds. The catalytic synthesis of enzyme has the advantages of mild reaction conditions, high catalytic efficiency and strong catalytic specificity,which is a typical green and environment-friendly process for the synthesis of perfume (Yue et al., 2022).

Because of its low melting point, non-flammability, high thermal stability and good solubility, ionic liquids are green solvents and have the potential to replace traditional organic solvent (Marullo & D'Anna, 2021; Dabiri at al., 2012). In general, enzymatic reaction will use some strong polar solvent to dissolve the substrate of the strong polarity. In general, some strongly polar solvents are used to dissolve strongly polar substrates in enzymatic reactions (Yeler & Nas, 2021), but enzymes are often inactivated in some strongly polar organic solvents, while ionic liquids with the same polarity can maintain their catalytic activity and stability. Therefore, ionic liquids are more suitable as solvents for enzyme-catalyzed reactions than organic solvents. Recently, more and more researchers have studied the lipase-catalyzed reaction in ionic liquids. Yang et al. (2021) studied Novozym 435-catalyzed interesterification of ethyl ferulate with phosphatidylcholine in a two-phase system consisting of an ionic liquid and toluene was optimized to prepare feruloylated lysophospholipids (FLPs). After optimizing the conditions, two FLPs products were obtained, and the conversion rates were all over 50%. However, the yields of this conversion method are not high and further improvements to the reaction system are required to increase yields. Wang et al. (2021) achieved the

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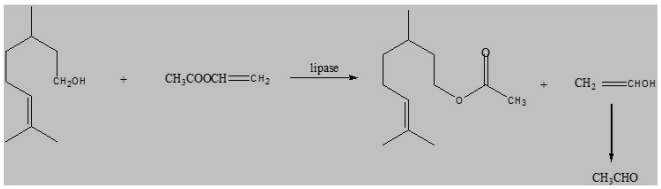
first Knoevenagel condensation of acetyl acetone and aromatic aldehydes in ionic liquids and deep eutectic solvents, catalyzed by Porcine pancreatic lipase and Porcine pancreatic α-amylase. The highest yield can reach 96.1%. However, the mixture of Porcine pancreatic lipase and Porcine pancreatic alpha-amylase makes the separation of the products very difficult. Liu et al. (2013) studied the synthesis of biodiesel catalyzed by lipase in ionic liquid [EMIM] [TFO]. The results showed that the yield of biodiesel could reach over 99%, which indicated that lipase showed the best catalytic activity in [EMIM] [TFO]. At the same time, the time dynamic study found that lipase showed a positive synergistic effect and eliminated the rate limiting step. Therefore, ionic liquids have great potential as reaction media in lipase-catalyzed reaction.

At present, there are few reports about the lipase-catalyzed synthesis of citronellyl acetate in non-aqueous solvents. Yuan et al. (2022) based on the activation mechanism of lipase at the water/oil (organic phase) interface, inexpensive raceme straw was processed into powder and filaments on which Pseudomonas fluorescens lipase was immobilised by physical adsorption to synthesise citronellol via ester exchange of citronellol and vinyl acetate. The results showed that 99.8% conversion could be achieved after 12 hours at 37 °C and 160 r/min. However, the reaction time of this reaction system was excessively long. Yadav & Borkar (2009) studied the ester exchange reaction of citronellyl acetate catalyzed by the lipase Novozym 435 under conventional and microwave-assisted heating conditions in toluene, with 93% and 90% conversions after 3 h of reaction, respectively. Similarly, they analysed the reaction kinetics. However, their study was carried out in toluene, which not only affected the activity of the lipase but also made downstream separation more difficult. Wang et al. (2010) studied the synthesis of citronellyl esters catalyzed by Rhizobium chinense lipase (RCL) in a non-aqueo systems. Citronellyl acetate was synthesized in hexaheptane catalyzed by RCL lipase using ethyl acetate, ethyl acetate, butyl acetate and isoamyl acetate as donors. The results showed that the highest reaction conversion was achieved when vinyl ester was used as the acyl donor, with 98% conversion after 96 h. However, the reaction was slow and the reaction time was long. Kutyła et al. (2022) investigated the direct esterification of β-citronellol and acetic acid in organic solvents by biomass-bound lipase from the cardiophilic bacterium Chrysosporium pannorum A-1 to prepare citronellyl acetate. A maximum molar conversion value of 98% was obtained in 24 h using a 2:1 alcohol to acid molar ratio and a 3% w/v biocatalyst in hexane at 30 °C. However, the study had a long reaction time and was not subjected to kinetic modelling. However, the preparation of citronellyl acetate by transesterification in ionic liquids has not been reported.

In this paper, solvents and lipases from different organisms were screened for the highest catalytic activity for the transesterification synthesis of citronellyl acetate in the ionic liquid (Scheme 1). The effects of various factors were studied to establish an efficient biosynthetic system. On this basis, the mechanism of the transesterification reaction was studied and the kinetic model was established, which provided the necessary basis for industrial production.



Scheme 1. Enzyme-catalyzed transesterification synthesis of citronellyl acetate.

2 Materials and methods

2.1 Chemicals

All lipases used in the experiments were purchased from *Amano Enzyme Ltd,* which were derived from *Pseudomonas fluorescens, Aspergillus niger, Candida rugosa, Rhizopus oryzae, Mucor javanicus, and Burkholderia cepacia.* All ionic liquids were prepared in laboratory. The citronellol and citronellyl acetate were obtained from *Sigma-Aldrich and Tokyo Chemical Industry Co, Ltd*, respectively. Other chemicals and solvents are commercially available analytical grade reagents. All chemicals and lipases were used without further modification.

2.2 Experimental method

The reaction was carried out in a plugged bottle, which was placed in a constant temperature shaker, so as to keep the temperature constant at an ideal temperature and achieve a good mass transfer effect. Unless otherwise specified, the reactions were carried out according to the following methods. 100 mmol/L citronellol and 300 mmol/L vinyl acetate were diluted to 3 mL with ionic liquid as the solvent. The reactants were placed in a constant-temperature shaker and preheated for 10 min. After adding 10 mg/mL lipase to the reactants, the reaction was initiated at 40 °C and 200 r/min. The samples were periodically withdrawn from the reaction mixture. The samples were extracted with n-hexane and the upper organic phase were detected by gas chromatography (GC) to analyze the amount of citronellyl acetate produced in the reaction process.

2.3 Analytical method

The liquid samples were analyzed by gas chromatography equipped with flame ionization detector and 30 m × 0.22 mm SGE AC10 capillary column. Hexadecane is the internal standard substance and citronellyl acetate was quantified by internal standard method. Specific test conditions were as follows: the N_2 flow rate was 30 mL/min; the H_2 flow rate was 40 mL/min; the air flow rate was 400 mL/min; the split ratio was 1:39; the column temperature was kept at 150 °C, the injector temperature was 220 °C, and the detector temperature was 250 °C.

3 Results and discussion

3.1 Screening of different ionic liquids

In the catalytic reaction of lipase, a small amount of water molecules is needed to maintain the spatial conformation of lipase. The hydrophobic effect of ionic liquids makes it difficult to separate water molecules from enzymes, thus making the spatial structure

stable. The stronger the hydrophobicity of ionic liquids is, the stronger the force between water molecule and enzyme is, and the more stable spatial structure is (Zhao et al., 2009). However,when the hydrophobicity exceeds the critical value, the hydrophobicity of ionic liquids can inhibit the contact between the substrate and the enzyme and reduce the catalytic effect of lipase (Zhang et al., 2011). Therefore, the catalytic effects of lipase in ionic liquids with different hydrophobicity are different. The catalytic effects of *Pseudomonas fluorescens* lipase were investigated in different ionic liquids. The results were shown in Figure 1. It could be seen that the catalytic effect of lipase was the best when ionic liquid [BMIM] [NTF_2] is used as solvent, while the catalytic effect of lipase was very poor when ionic liquid with [BF_4] was used as solvent, which may be related to the hydrophilicity of [BF_4]. The strong hydrophilicity will absorb the water on the surface of lipase, destroy the spatial structure of lipase and reduce the catalytic activity of lipase. Therefore, [BMIM] [NTF_2] ionic liquid was selected as the reaction medium for subsequent experiments.

3.2 Screening of lipases from different microorganisms

The lipases from different microorganisms have different catalytic properties. In this paper, the transesterification of vinyl acetate and citronellol catalyzed by six free lipases from different sources to synthesize was studied. The process curves of different lipase-catalyzed reactions were shown in Figure 2. Under the same experimental condition, Pseudomonas fluorescens lipase had the best catalytic effect, and the yield of citronellyl acetate can reach 99.46% after 6 h. The catalytic effect of Burkholderia cepacia lipase was the second and the yield of citronellyl acetate can reach 74.58% after 10 h. Candida rugosa and Mucor javanicus lipases had poor catalytic effect on the reaction, which could only reach 7.39% and 6.64% after 10 h, respectively. The lipases from Aspergillus niger and Rhizopus oryzae had negligible catalytic effect on this reaction. Consequently, Pseudomonas fluorescens lipase was chosen as the optimum enzyme and used for subsequent reactions.

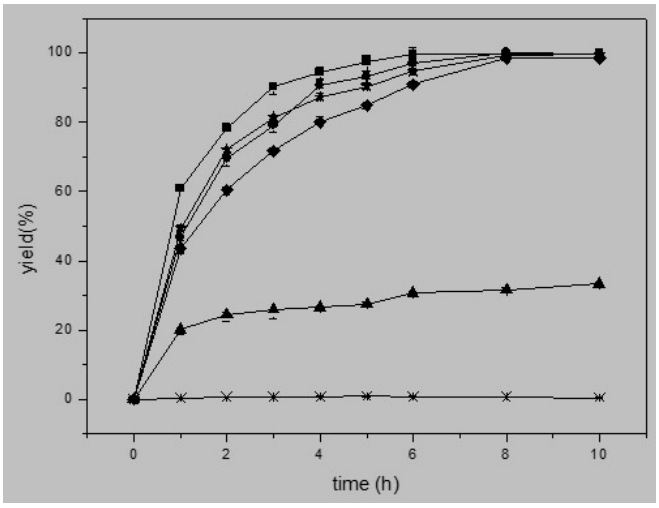


Figure 1. Effect of different ionic liquids. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min; (▪) [BMIM][NTF_2] (1-Butyl-3-MethylImidazolium bis(trifluoromethylsulfonyl)imide), (●) [BMIM][OTF] (1-Butyl-3-methylimidazolium trifluoromethanesulfonate), (▲) [BMIM][BF_4] (1-Butyl-3-methylimidazolium tetrafluoroborate), (♦) [BMIM][PF_6] (1-Butyl-3-methylimidazolium tetrafluoroborate), (★) [EOEIM][PF_6] (1- ethoxyethyl -3- methylimidazole hexafluoroborate), (×) [EOEIM] [BF_4] (1- ethoxyethyl -3- methylimidazole tetrafluoroborate).

3.3 Effect of molar ratio of substrate

Generally, increasing the mole ratio of substrate can not only change the equilibrium of the reaction, but also can increase the reaction rate and shorten the reaction time. In this paper, the reaction curves of different molar ratios of vinyl acetate to citronellol were investigated (Figure 3). When the molar ratio of

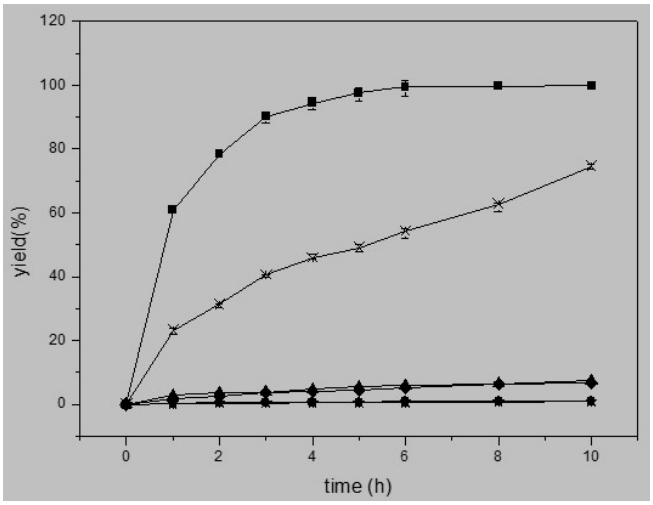


Figure 2. Effect of different lipases. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min; (▪) *Pseudomonas fluorescens*, (●) *Aspergillus niger*, (▲) *Candida rugosa*, (★) *Rhizopus oryzae*, (♦) *Mucor javanicus*, (×) *Burkholderia cepacia*.

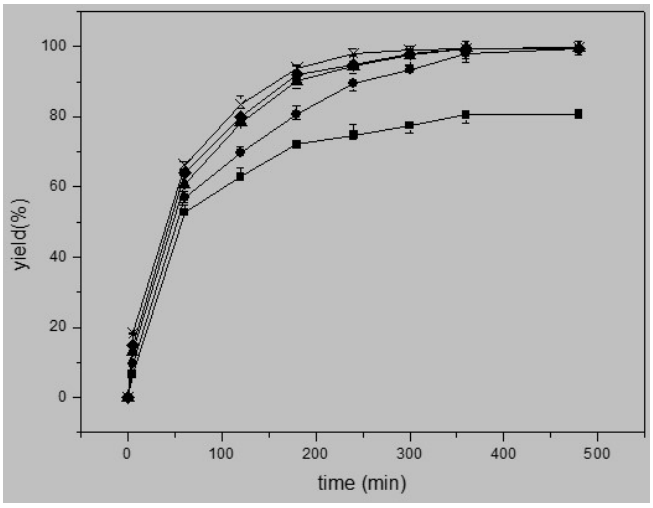


Figure 3. Effect of molar ratio of vinyl acetate to citronellol. Reaction conditions: citronellol, 100 mmol/L; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min; (▪) 1:1, (●) 2:1, (▲) 3:1, (♦) 4:1, (×) 5:1.

vinyl acetate to citronellol was varied from 1:1 to 5:1, the reaction rate increased with the increase of mole ratio. However, when the molar ratio increased from 1:1 to 3:1, the increased yield of the reaction rate was higher than that when the molar ratio increases from 3: 1 to 5: 1. Therefore, the optimal mole ratio of vinyl acetate to citronellol was 3:1.

3.4 Effect of temperature

Temperature is an important factor affecting the reaction kinetics, which not only has an influence on the catalytic activity of lipase, but also affects the mass transfer between reactants and the permeation velocity of substances on the enzyme. The effects of five temperatures on the reaction process were investigated. The reaction process curves at each temperature were shown in Figure 4. In the experimental temperature range, with the increase of temperature, the reaction rate increased and the time required for the reaction to reach the end point shortened. It also showed that no enzyme deactivation was found. From the Figure 4, when temperature increased from 20 °C to 40 °C, the reaction rate increased rapidly, and the time required to complete the reaction was shortened from 10 h to 6 h; when the temperature continued to rise to 60 °C, the reaction rate could keep increasing, but the increase was slow and the time for completing the reaction was shortened from 6 h to 4 h. The increase of catalytic effect is not obvious when temperature increased from 40 °C to 60 °C. In addition, due to the limited heat resistance of the enzyme, high temperature was not conducive to the stability of lipase conformation. Taking into account the catalytic effect, the consumption of energy and the stability of the lipase, 40 °C was the optimum reaction temperature.

3.5 Effect of enzyme loading

Enzyme loading directly affected the rate of reaction and the final conversion of transesterification. As can be seen from Figure 5, when the enzyme loading was less than 10 mg/mL, with the increase of enzyme loading, the rate of catalytic reaction increased significantly; but with the increase of enzyme loading from 10 mg/mL to 12.5 mg/mL, the increase of reaction rate and yield tended to be flat. The reason might be that in a certain range of enzyme loading, with the increase of enzyme concentration, the probability of substrate getting in touch with enzyme increased, the effect of catalytic transesterification got better and the rate of reaction was faster. However, with the further increase of enzyme loading, the increase of enzyme concentration led to the decrease of effective collision probability between reactant molecules. Relative to the substrate concentration, the enzyme concentration was close to the saturated state, so as to make the reaction rate no longer increased. Considering the cost of catalyst and catalytic effect, 10 mg/mL was finally determined as the most suitable enzyme loading.

3.6 Effect of the speed of agitation

Lipase-catalyzed transesterification reaction was studied at five different rotating speeds, and the curves of reaction process was shown in Figure 6. From the figure, when the rotating speeds were 50 r/min and 100 r/min, the rate of reaction was slow and the yield of citronellyl acetate could reach 73.34% and 88.34% after 6 h, respectively. The reason was that the viscosity of ionic liquid was high. This was because the viscosity of ionic liquids was high, and lipase cannot be well dispersed in the reaction system at low shaking speed, and the contact between enzyme and substrate was insufficient, resulting in low yield. When the speed of agitation increased from 100 r/min to 200 r/min, the reaction rate and yield increased significantly, and the yield reached 99.46% after 6 h. This was because that when the speed increased, the mass transfer of the reaction system was improved, and lipase and substrate were well dispersed in ionic liquid, thus obtaining higher yield. Continuing to increase the rotating speed to 250 r/min, the reaction rate and yield were

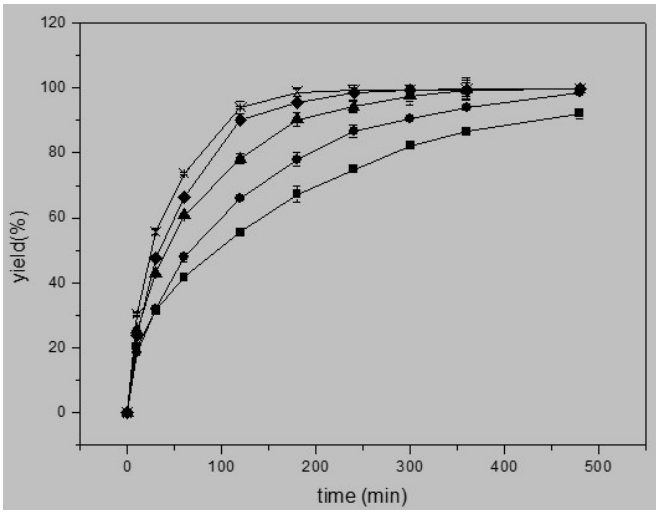


Figure 4. Effect of temperature. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; speed of agitation, 200 r/min; (▪) 20 °C, (●) 30 °C, (▲) 40 °C, (⬥) 50 °C, (×)60 °C.

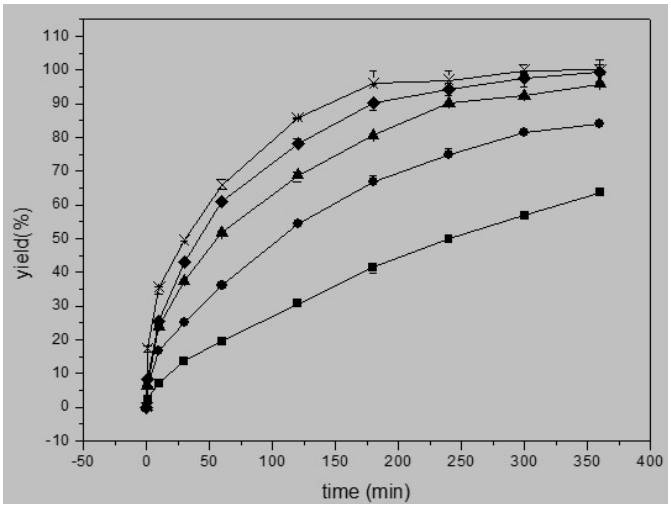


Figure 5. Effect of enzyme loading. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; temperature, 40 °C; speed of agitation, 200 r/min; (▪) 2.5 mg/mL, (●) 5 mg/mL, (▲) 7.5 mg/mL, (⬥) 10 mg/mL, (×) 12.5 mg/mL.

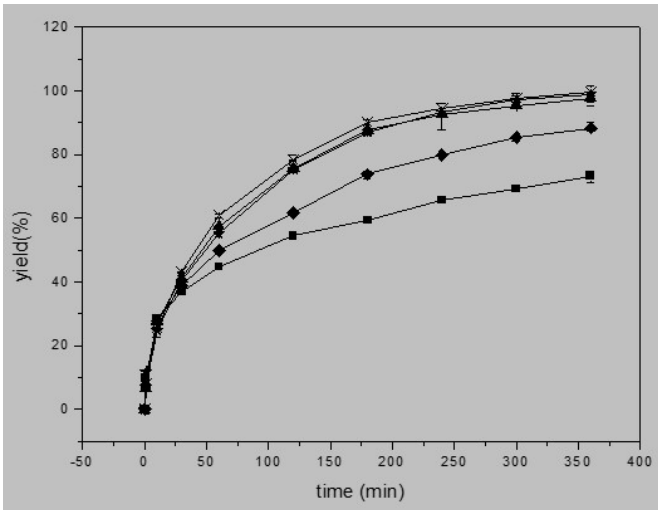


Figure 6. Effect of speed of agitation. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; (▪) 50 r/min, (♦) 100 r/min, (▲) 150 r/min, (×) 200 r/min, (★) 250 r/min.

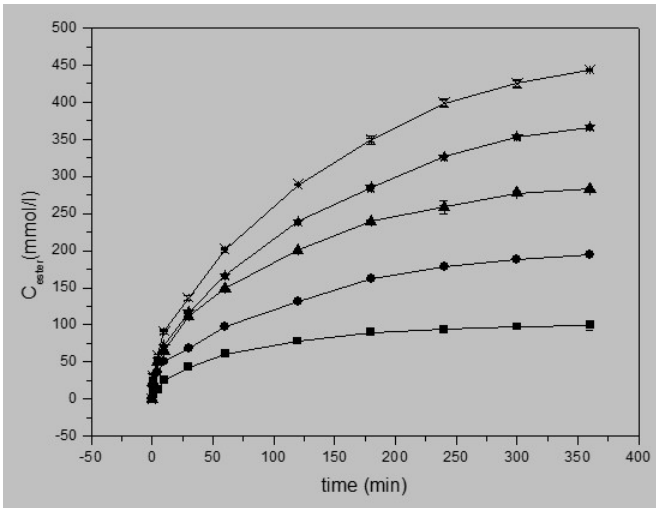


Figure 7. Effect of concentration of substrate. Reaction conditions: molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 rpm; (▪) 100 mmol/L, (●) 200 mmol/L, (▲) 300 mmol/L, (★) 400 mmol/L, (×) 500 mmol/L.

not improved. Therefore, the optimal speed of agitation was determined at 200 r/min.

3.7 Effect of substrate concentration

The effect of substrate concentration was investigated and the results were shown in Figure 7. With the increase of substrate concentration, the reaction rate gradually increased. In enzyme-catalytzed reactions, due to the affinity between enzyme and substrate molecule, substrate molecule has a trend to close to the active center of the enzyme, and finally combined with active center of the enzyme, which increases the effective concentration of substrate in the active center of enzyme. With the increase of substrate concentration, the effective collision increased between the catalytic site of the enzyme and substrate molecule. The proximity effect was gradually strengthened, enzyme-catalyzed activity increased and the rate of reaction increased. According to the double reciprocal curve of the concentration of citronellol and initial rate (Figure 8), the curve is basically linear, which showed that there was no substrate inhibition in the range of the concentration of substrate studied in this experiment.

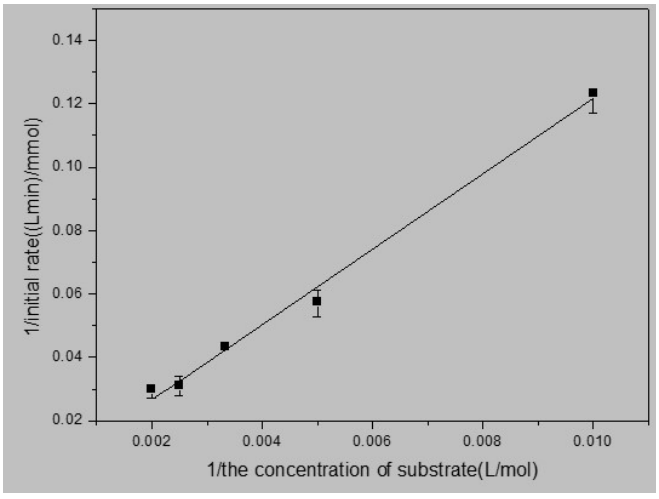


Figure 8. Lineweaver-Burk plot. Reaction conditions: molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min.

3.8 Effect of product concentration

In enzyme-catalyzed reaction, the products sometimes produce allosteric effect on the enzyme, which changes the conformation of lipase to a certain extent, thus reducing the catalytic activity of the enzyme. The effect of product concentration on the transesterification reaction was investigated within the range of 0 mmol/L to 250 mmol/L, and the results were showed in Figure 9. It can be seen from the figure that the reaction rate and yield decreased obviously with the increase of product concentration, which indicated that citronella acetate inhibited lipase. The reason may be that the product molecules had combination with acyl enzyme molecule which was formed in the process of enzyme-catalyzed reaction to cause the change of the space-conformation of enzyme protein, which inhibited the activity of lipase.

3.9 Effect of the additional amount of water

The best catalytic activity is shown when lipase molecule only has certain space-conformation. The space-conformation of enzyme molecules is maintained by non-covalent forces, and water molecule directly or indirectly participate in these forces. Therefore, water is an important part of maintaining the molecular structure of lipase, and the catalytic activity of lipase is closely related to the water molecules adsorbed on lipase. The reaction process under different water addition amounts

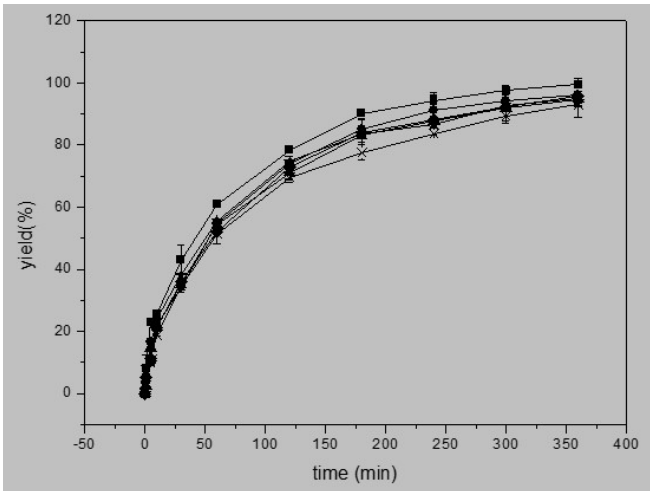


Figure 9. Effect of concentration of product. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 rpm; (▪) 0 mmol/L, (●) 50 mmol/L, (▲) 100 mmol/L, (♦) 150 mmol/L, (★) 200 mmol/L, (×) 250 mmol/L.

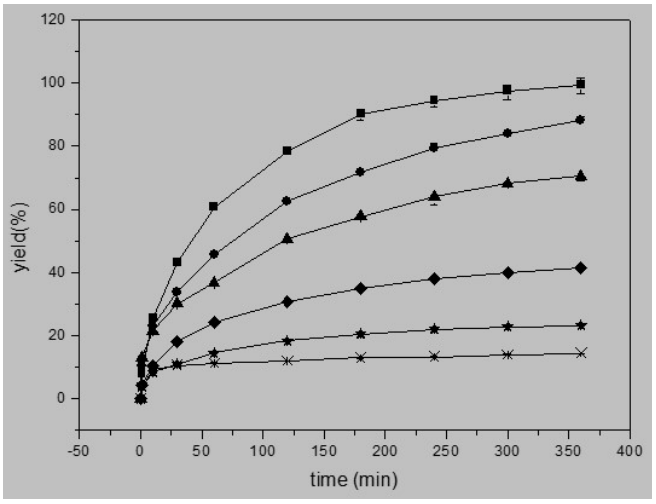


Figure 10. Effect of additional water. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 rpm; (▪) 0% (v/v), (●) 0.2% (v/v), (▲) 0.5% (v/v), (♦) 1% (v/v), (★) 2% (v/v), (×) 5% (v/v).

is shown in Figure 10. It can be seen that with the increase of water content, the transesterification reaction rate gradually decreases. This downward trend may come from three aspects. First of all, the water in whole system was sufficient to maintain the conformation of enzyme. At this time, adding the amount of water led to increase the conformational flexibility of enzyme and the change of the structure of enzyme, which led to the inactivation of lipase. Secondly, excessive water molecules were adsorbed on the enzyme and hindered the contact between enzyme and substrate, which limited the internal diffusion of substrate on the lipase. Lastly, too much water strengthened the interaction between enzymes, and enzyme were absorbed together through water, which was not conducive to dispersion of lipase in the reaction and reduced the probability of the contact between enzymes and substrate.

3.10 Reusability of the enzyme

Microbial lipase is an important source of industrial lipase. The process of producing microbial lipase is very complex, including fermentation and purification, so the cost of lipase is high. If lipase can't have good stability in the catalytic reaction, the development of enzyme-catalyzed preparation of citronella acetate would be restricted. Therefore, whether lipase can be recycled is an important condition for lipase to be applied to industrial production. Because of the high viscosity of ionic liquids, it is impossible to separate lipase from mixture by filtration. In this experiment, lipase was separated by sedimentation, and then new ionic liquids and substrates were added for the next batch of reaction. From the Figure 11, the yield of citronella acetate decreased slightly during the reuse of lipase, and decreased to 79.69% after 7 times of reuse, which indicated that the stability of lipase was poor in this reaction system and needed to be further improved.

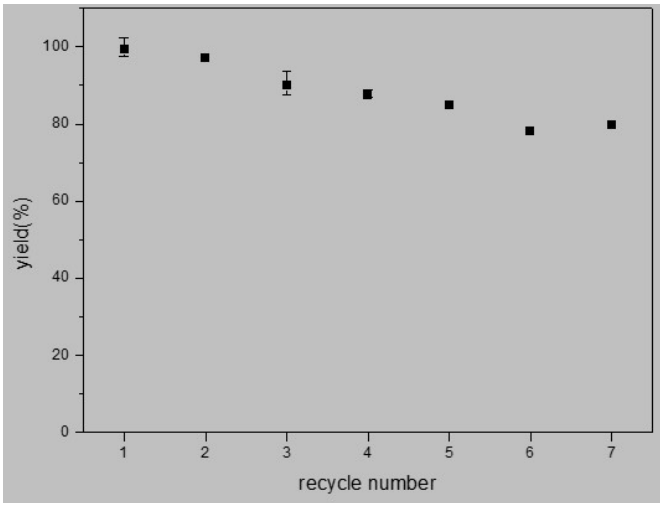


Figure 11. Reuseability of lipase. Reaction conditions: citronellol, 100 mmol/L; molar ratio of vinyl acetate to citronellol, 3:1; lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min; reaction time, 6 h.

3.11 Kinetic study

The kinetic model of lipase-catalyzed transesterification of citronellol can be investigated by using the initial rate data under the same conditions (Jiao et al., 2022). From the double reciprocal curve in Figure 12, it can be seen that by changing the initial concentration of vinyl acetate, the reciprocal of the initial rate is parallel to the fitting line of the reciprocal of the initial concentration of vinyl acetate at different initial concentrations of citronellol, which shows that the reaction follows the ping-pong reaction mechanism (Vieira et al., 2022; Varma & Madras, 2010; Gómez et al., 2020) and can be described by the ping-pong bi-bi reaction kinetic model. It can be seen from the above

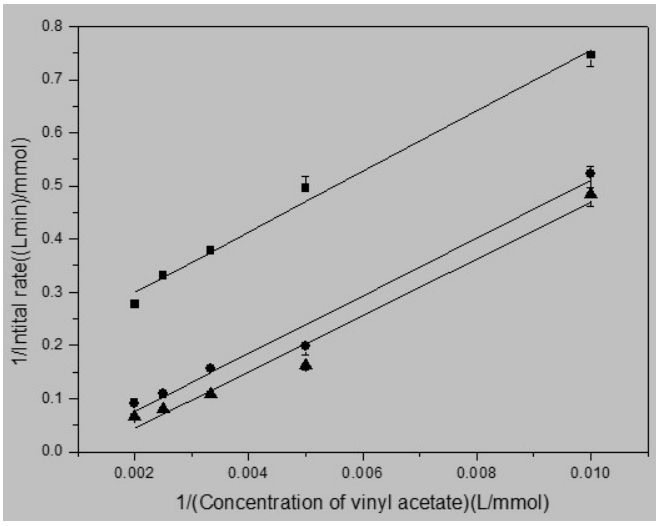


Figure 12. Lineweaver-Burk plot. Reaction conditions: lipase, 10 mg/mL; temperature, 40 °C; speed of agitation, 200 r/min; citronellol, (▪) 100 mmol/L, (●) 300 mmol/L, (▲) 500 mmol/L.

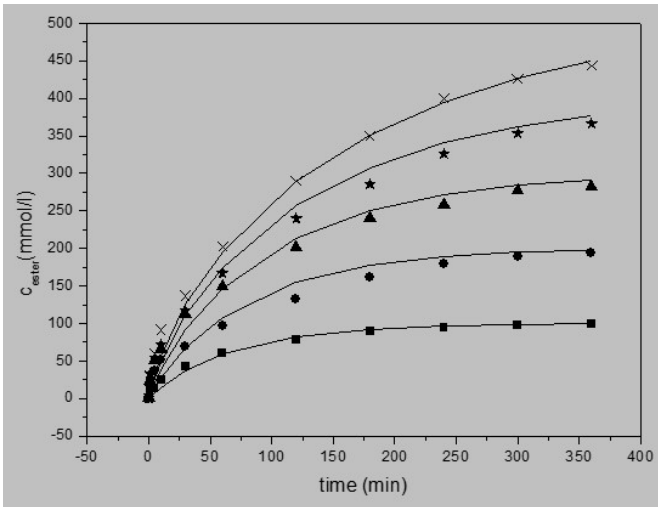
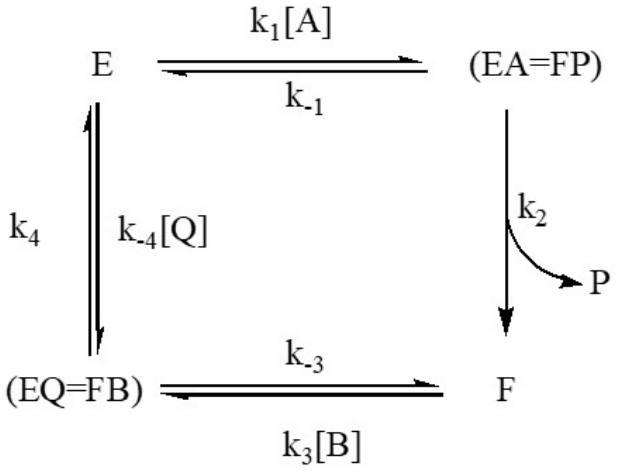


Figure 13. Comparison of simulated values with the experimental data. For the initial concentration of citronellol, the experimental values are represented as points: (▪) 100 mmol/L, (●) 200 mmol/L, (▲) 300 mmol/L, (★) 400 mmol/L, (×) 500 mmol/L. The simulated values are represented by the trend lines.



Scheme 2. King-Altman scheme of the enzyme-catalyzed bi-bi transesterification.

Table 1. Parameter values for the simulated rate equation.

Parameter	Value
V_m (mmol/L/min)	6.15×10^{-1}
K_A (mmol/L)	2.11×10^{3}
K_B	3.68×10^{-1}
K_{QA}	1.93
K_{BQA} (L/mmol)	5.66×10^{-2}
K_{IQ} (mmol/L)	2.20×10^{2}

section that citronellyl acetate inhibits the reaction. Therefore, the kinetics of lipase-catalyzed transesterification to citronellyl acetate in this ionic liquid is a ping-pong double-double reaction mechanism inhibited by citronellyl acetate. In this model, vinyl acetate (A) and the free enzyme (E) have a complexation to form a non-covalent enzyme-ester complex (EA), which is converted into the acyl-enzyme intermediate (FP) upon isomerization. At the same time, the first product and enol (P) was released to produce the modified enzyme (F). Citronellol (B) reacts with the activated enzyme (F) to produce another complex (FB), which produces the ester-enzyme complex (EQ) upon isomerization. EQ finally decomposes into citronellyl acetate (Q) and the free enzyme (E). The reaction sequence was shown in King-Altman scheme (as shown in Scheme 2).

The transesterification reaction adopts ping-pong bi-bi reaction kinetics model with product inhibition, and the reaction rate equation can be obtained as follows (Xiong et al., 2012) (Equation 1):

$$V=\frac{V_m[A][B]}{K_A[A]+K_B[B]+K_{AB}[A][B]+K_Q(1+\frac{[Q]}{K_{IQ}})[Q]+K_{BQ}[B][Q]} \quad (1)$$

Where K_{IQ} is the inhibition constant of Q.

This equation is the rate equation of the lipase-catalyzed transesterification synthesis of citronellyl acetate.

According to the experimental data, the optimal model parameters were fitted by Matlab software, and the results were shown in Table 1. The results showed that the fitting values were in good agreement with the experimental values, and the relative error of the calculated model was 7.75%, as shown in Figure 13. Therefore, the kinetic model can be used to describe the reaction process of Lipase-catalyzed transesterification to citronellyl acetate in ionic liquids.

4 Conclusion

The synthesis of citronellyl acetate by lipase-catalyzed transesterification in ionic liquids was studied systematically and the parameters were optimized. It was found that in the ionic liquid [BMIM] [NTF_2], *Pseudomonas fluorescens* lipase had the best results in lipase catalytic reaction. Under the optimized reaction conditions, the yield of citronella acetate can reach over 99.46% after 6 h. After reusing the lipase for 7 times, the catalytic activity of the lipase decreased by 20%. The reaction mechanism was studied and it was determined that the reaction was a ping-pong bi-bi reaction mechanism inhibited by citronellyl acetate. The kinetic model of the reaction was established and the model parameters were fitted. The relative error between the fitting value and the experimental value was 7.75%, which indicated that the reaction kinetic model was reasonable. In this paper, an efficient synthesis of citronellyl acetate was achieved using an ionic liquid as solvent and a lipase-catalyzed transesterification reaction, providing a new method for the green synthesis of citronellyl acetate. The study of the reaction kinetics provides a valuable reference for the industrial production of the method.

Abbreviations

A: substrate, vinyl acetate. B: substrate, citronellal. E: enzyme. EA: enzyme- vinyl acetate compound. EQ: enzyme- citronellyl acetate compound. F: acyl enzyme. FB、FP: acyl-enzyme intermediate. k_i: rate constant (i = ± 1, ± 2, ± 3, ± 4). K_{IQ}: inhibition constant of citronellyl acetate. K_x: parameters of kinetics modeling (x = A, B, QA, BQA). P: product, vinyl alcohol. Q: product, citronellyl acetate. V: reaction rate. V_m: maximal rate of reaction.

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