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A Study on the Lipase-Catalayzed Esterification in Organic Solvent

Mustafa Ümit ÜNAL

Çukurova Üniversitesi, Ziraat Fakültesi, Gıda Mühendisliği Bölümü, Adana-TURKEY

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Abstract: This study was undertaken to investigate effect of molecular sieves on the esterification reaction between lauric acid and geraniol in isooctane catalyzed by immobilized lipase in a batch and continuous-loop packed-bed reactor. Molecular sieves were used to reduce the inhibitive effect of water formed during the reaction by removing it as it formed. Results in a batch reactor showed that, for the molecular sieves to have a pronounced effect on the performance of the enzyme, a ratio of at least 2:1 with respect to the weight of the immobilized enzyme must be used. It was also found that the ability of molecular sieves to reduce inhibition by water is more noticeable when working in a packed-bed reactor. The physical contact between the molecular sieves and enzymes is of utmost importance for the adsorption of water since water is markedly insoluble in the organic solvent.

Organik Çözgende Lipaz Enzimince Katalizlenen Esterleşme Reaksiyonu Üzerinde Bir Araştırma

Özet: Bu çalışmada, moleküler süzgeçlerin laurik asit ve geraniol arasındaki esterleşme reaksiyonu üzerine etkisi araştırılmıştır. Reaktör olarak kesikli ve sürekli paketlenmiş-yatak reaktörleri kullanılmıştır. Reaksiyon izooktan çözgeni içerisinde gerçekleştirilmiş ve tutuklanmış lipaz enzimince katalizlenmiştir. Moleküler süzgeçler, reaksiyon sırasında oluşan suyu uzaklaştırarak suyun reaksiyon üzerine olan inhibe edici etkisini azaltmak için kullanılmıştır. Kesikli reaktörlerde yapılan deneylerden elde edilen sonuçlar, moleküler süzgeçlerin enzimlerin performansı üzerine belirgin bir etkiye sahip olabilmesi için, moleküler süzgeç enzim oranının en az 2:1 (ağırlık üzerinden) olması gerektiğini göstermiştir. Ayrıca, moleküler süzgeçlerin suyun inhibe edici etkisini azaltması paketli yatak reaktöründe çok daha belirgin olmuştur. Su, organik çözgende çözünmediği için, moleküler süzgeçlerle enzimler arasındaki fiziksel temas suyun adsorbe edilmesi açısından çok önemlidir.

Introduction

The majority of enzymatic catalysis is performed in water, because it is widely believed that enzymes can work only in aqueous solutions due to the fact that enzymes function in aqueous environments in nature. However, biocatalysis in organic solvents has become an actively investigated field of enzyme technology because of developments in this area (1, 2). Lipases are of current industrial interest for production of fatty acids by hydrolysis of triglycerides and for interesterification of triglycerides. Because of commercial and scientific interest in lipases, many studies have been performed on lipase catalyzed esterifications in organic media in the past years (3, 4, 5, 6, 7, 8, 9, 10, 11). The lipase-catalyzed esterification between lauric acid and geraniol is as follows:

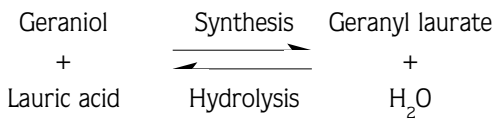

Figure 1. Esterification between lauric acid and geraniol

Water has several effects on the reaction and enzyme activity and performance. Esterification is generally a water limited reaction because the equilibria catalyzed by hydrolytic enzymes is in favour of hydrolysis (12). Secondly, water inhibits the reaction. Lastly, when an immobilized enzyme with a support which has hydrolphilic nature is used water causes aggregation of support pellets resulting in a drop in the rate of enzyme activity (13). The inhibitive effect of the water on the esterification reaction between geraniol and lauric acid in isooctane was demonstrated by Vazquez-lima *et al* (14).

In this study, molecular sieves were used so as to prevent inhibitive effects of water on the esterification reaction between lauric acid and geraniol in iso-octane catalyzed by immobilized lipase. Molecular sieves are an important class of synthetic adsorbents which posses high porosity, with pores of uniform size and essentially molecular dimensions (15). They adsorb small molecules only and have a particular affinity for unsaturated and polar molecules.

Materials and Methods

Materials

The enzyme (lipase) was obtained from Novo Enzymes Ltd in Copenhagen in Denmark. It was a commercial preparation ('Lipozyme IM 20') from *Mucor miehei* and immobilized on a microporous anion exchange resin with a nominal diameter 250-600 μm. Its activity was 29 BIU/G. Geraniol and lauric acid were from Sigma Chemicals. The solvent, iso-octane was purchased from BDH Ltd. Molecular sieves were obtained from Sigma Chemicals. Two types of molecular sieves were used. One was 0.079-0.127 cm beads (8x12 mesh beads) with a nominal pore diameter of 10 °A and water capacity of approximately 28% by weight. The other was 0.127-0.2 cm beads (4x8 mesh beads) with a nominal pore diameter of 3 °A and water capacity of approximately 20% by weight. All chemicals were of reagent grade.

Methods

In order to estimate the degree of conversion, successive samples were removed at time intervals and titrated with 0.1 N NaOH solutions, with phenolphtalein as the indicator. The extent of conversion was calculated as follows:

$$\text{Conversion}(\%) = \frac{(AV)_{inlet} - (AV)_{outlet}}{(AV)_{inlet}} \times 100$$

where AV is the calculated acid value at inlet and outlet.

Batch experiments were performed in a magnetically stirred 125 ml erlenmeyer flask (Figure 2). The speed of the magnetic stirrer was kept constant in all experiments.

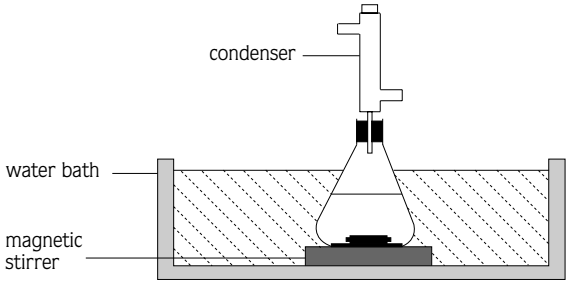

Figure 2. Diagram of equipment for batch reactor experiments

The packed-bed reactor was a glass column with a diameter of 3.4 cm and a total height of 10.5 cm, with a sintered glass distributor (Figure 3).

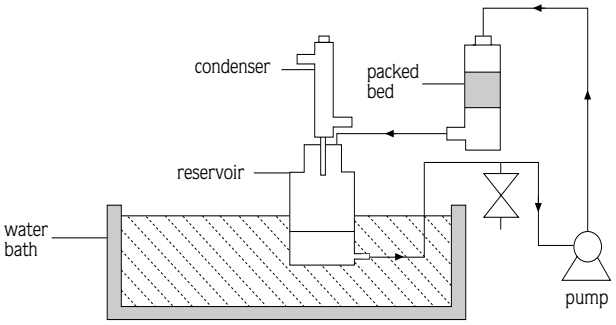

Figure 3. Diagram of equipment for packed-bed experiments

The substrate/solvent mixture was recirculated using a peristaltic pump with speed control (Watson-Marlow 603 U). The flow rate was maintained constant (10.4 L/hr) in all reactions. The flow direction of the solvent was downwards from the top of the column. In order to provide maximum possible contact between enzyme pellets and molecular sieves, the packed-bed column was arranged in such a way that one layer of molecular sieves and one layer of the enzyme pellets were placed (Figure 4). On the bottom and top of the column were the layers of molecular sieves. Glass beads were packed on top of the column.

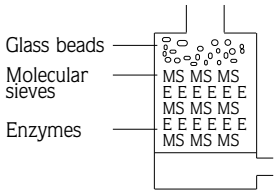

Figure 4. Configuration of the packed-bed

Reaction temperature was maintained at 55°C throughout the reaction using a water bath in both batch and packed-bed reactors. So as to prevent evaporation of the solvent and volatiles the erlenmeyer flask in the batch reactor and reservoir in packed-bed reactor were equipped with a total condenser. In all experiments stoichiometric mixtures of substrates of 0.25 M were used. The working volume was 50 ml and 150 ml in the batch and packed-bed experiments, respectively. Unless otherwise reported 0.03 g of immobilized enzymes per ml solvent were used.

Results and Discussion

Batch Results

Figure 5 shows the effect of molecular sieves on conversion of lauric acid in a batch reactor. The molecular sieves employed were 0.079-0.127 cm beads (8x12 mesh beads) with a pore diameter of 10 °A and with a water capacity of approximately 28% by weight. In order to examine effect of molecular sieves, different amount of molecular sieves were used. Approximately 0.8 g of molecular sieves are required to remove the stoichiometric amount of water formed during the reaction, assuming 100% lauric acid conversion to water.

From Figure 5, it is clear that the use of molecular sieves resulted in higher degrees of lauric acid conversion as compared to the experiment in which no molecular sieve was used. Virtually no difference in conversion was observed when 5.3 and 2.6 g of molecular sieves were used. The same conversion, 86%, was achieved in both experiments. As the amount of molecular sieves employed dropped from 2.6 g to 0.6 g, which was less than the amount of molecular sieves required to remove the stoichiometric amount of water, the achieved conversion was very close to the conversion in the experiment in which no molecular sieve was used.

The use of the molecular sieves with 3 °A pore diameter {water capacity of 20% and the size of 0.127-0.2 cm beads (4x8 mesh beads) } in the batch reactor increased lauric acid conversion, too (Figure 6). Ca. 84% conversion was with the experiment of 2.6 g molecular

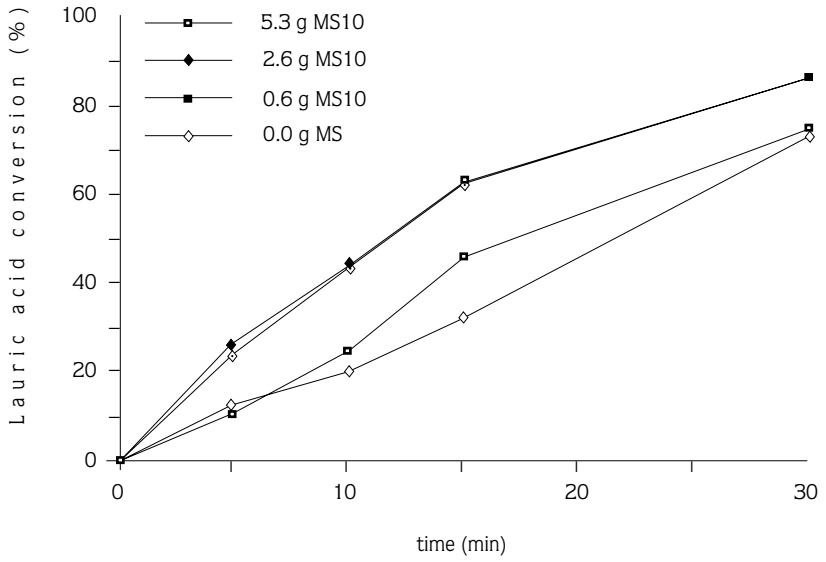

Figure 5. Effect of molecular sieves on lauric acid conversion MS10: Molecular sieve with a pore diameter of 10 °A

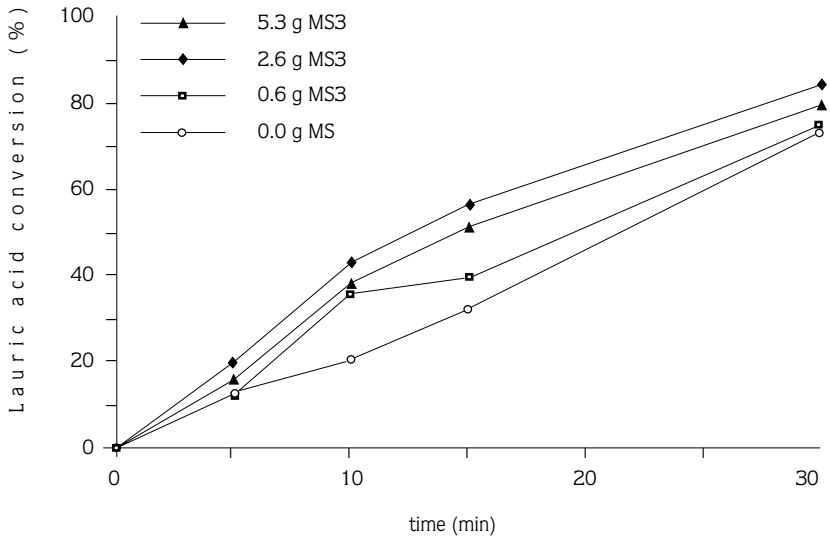

Figure 6. Effect of molecular sieves on lauric acid conversion MS3: Molecular sieve with a pore diameter of 3 °A

sieves. The conversion with 5.3 g molecular sieves was, however, less than the conversion with 2.6 g, ca. 80%. The conversion with 0.6 g molecular sieves was 75%. This was close to that which was obtained with the experiment in which no molecular sieve was used because the amount of molecular sieves used was less than the required amount to remove the stoichiometric amount of water.

Figure 7 depicts a comparison of the effect of molecular sieves with 3 and 10 °A pore diameters. Higher degrees of conversion were brought about by the molecular sieves with 10 °A in comparison to the ones with 3 °A pore diameter. One possible explanation of this is that the molecular sieves of 10 °A pore diameter have a higher water capacity. Secodly, the molecular sieves of 10 °A pore diameter are smaller in size {0.079-0.127 cm beads (8x12 mesh beads)}, that is, they have more surface area, which can contact to more enzymes and adsorb more water since water can only diffuse from enzyme pellets to melecular sieves when two species are in physically contact because isooctane is water immiscible.

Packed-bed Results

The results from the experiments with the molecular sieves of 10 °A pore diameter are shown in Figure 8.

The effect of the molecular sieves on the conversion can clearly be seen from the Figure 8. The use of molecular sieves increased the conversion and the conversion increased with an increase in the amount of molecular sieves used.

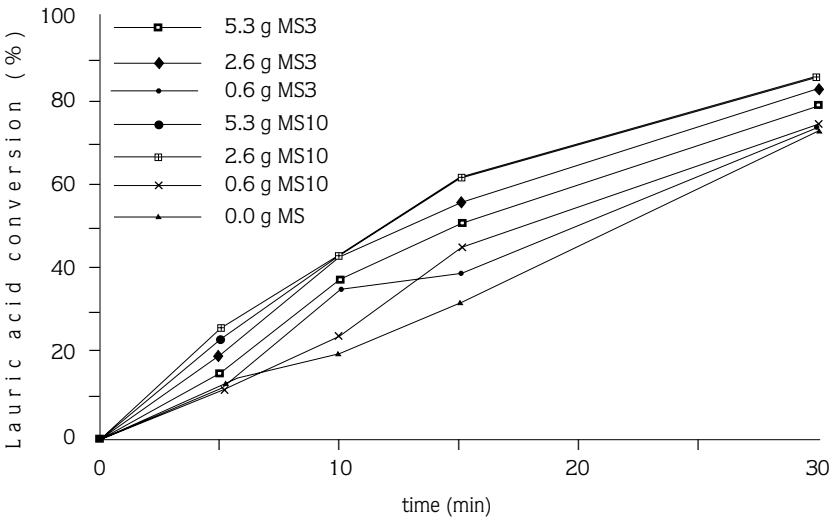

Figure 7. Effect of molecular sieves on lauric acid conversion MS3: Molecular sieve with a pore diameter of 3 °A MS10: Molecular sieve with a pore diameter of 10 °A

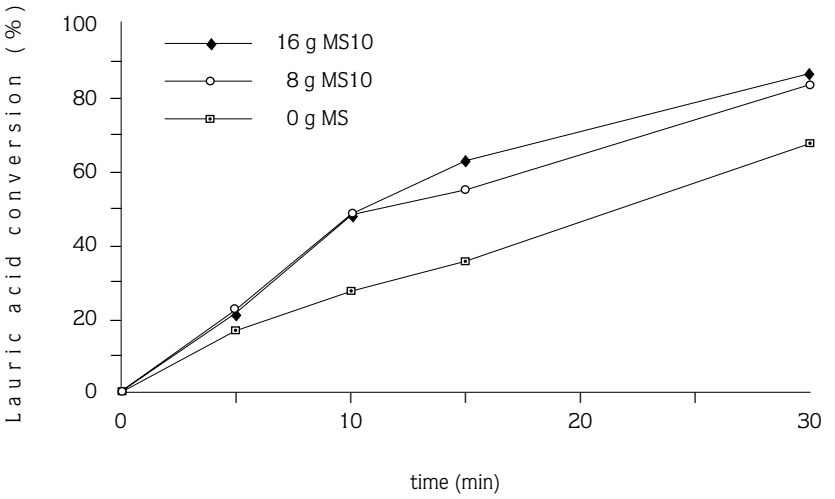

Figure 8. Effect of molecular sieves on lauric acid conversion MS10: Molecular sieve with a pore diameter of 10 °A

Figure 9 shows the results from the experiments with the molecular sieves of 3 °A pore diameter. These results are similar to those with the molecular sieves of 10 °A pore diameter. The use of molecular sieves increased the lauric acid conversion.

A comparison of the molecular sieves of 3 and 10 °A diameter is given in Figure 10. The initial conversion rates were higher with the molecular sieves of 10 °A pore diameter. However, the final conversion was similar in both types of melcular sieves. The achieved conversions in packed-bed experiments were higher as compared with the batch results. The reason for this could be that packed-bed configuration provided a higher contact between the molecular sieves and enzymes. whereas, in batch experiments the contact between the two species was the result of random movement.

Conclusions

It was found that the use of molecular sieves increased the lauric acid conversion in batch and packed-bed reactors by adsorbing the water formed during the reaction. However, in batch experiments when the amount of molecular sieves used was below the amount required to remove stoichiometric amount of water formed during the reaction the results were similar to the experiments in which no molecular sieve was used. In the batch experiments the molecular sieves of 10 °A pore diameter resulted in higher degrees of conversion than the molecular sieves of 3 °A pore diameter. The reasons can be that firstly, the molecular sieves of 10 °A pore diameter have a higher water capacity, and secondly, they have more surface area since they are smaller in size. In the packed-bed experiments there were no significant

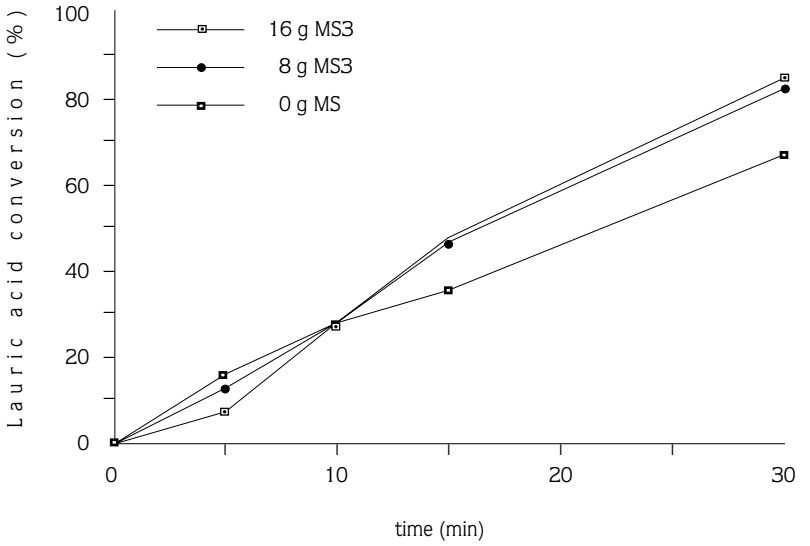

Figure 9. Effect of molecular sieves on lauric acid conversion MS3: Molecular sieve with a pore diameter of 3 °A

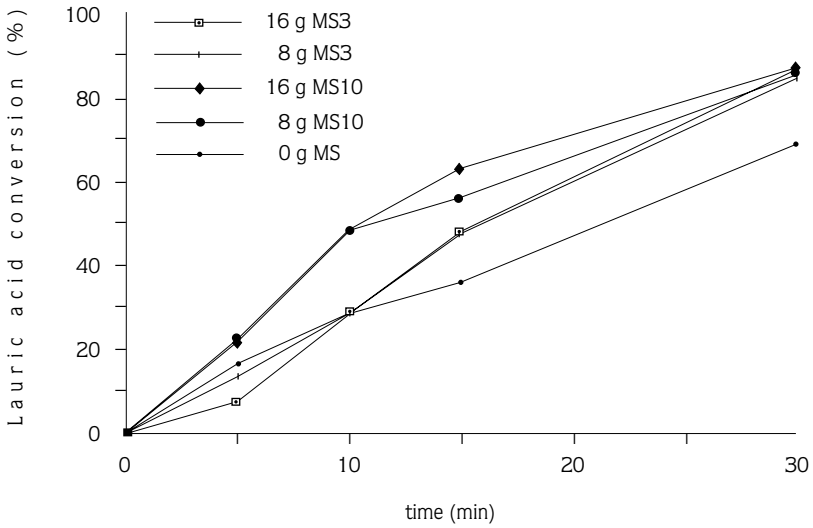

Figure 10. Effect of molecular sieves on lauric acid conversion MS3: Molecular sieve with a pore diameter of 3 °A MS10: Molecular sieve with a pore diameter of 10 °A

differences between the two types of molecular sieves in terms of final conversion. Furthermore, in the packed-bed experiments the achieved conversions were higher as compared to the batch results. The reason for this is that the packed-bed configuration provided a higher contact between the molecular sieves and enzymes. Whereas, in the batch experiments the contact between molecular sieves and enzymes was the result of random movements. As far as water adsorption is concerned the contact between molecular sieves and enzymes is of utmost importance since the organic solvent used (isooctane) is water immiscible. From industrial point of view, the use of molecular sieves in large scale processes is feasible since separation of molecular sieves from enzymes is easily possible because molecular sieves are greater in size than the catalyst.

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