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Gas-Phase Enzymatic Esterification on Immobilized Lipases in MCM-41 Molecular Sieves

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Abstract

Several lipolytic enzymes were immobilized in the pores of MCM-41 and Al-MCM-41 molecular sieves and used as catalysts in the gas-phase esterification of acetic acid with ethanol. The entrapment of enzymes depended on the molecular sieve and the type of enzyme used. The order of enzymatic activity for enzymes entrapped in the pores of MCM-41 and Al-MCM-41 in the esterification reaction was OF (*Rhizopus niveus* lipases) < FAP-15 (*Rhizopus oryzae* lipases) < LEX (*Mucor javanicus* lipases) < PS (*Pseudomonas cepacia* lipases) < AK (*Pseudomonas fluorescens* lipases). Experiments carried out between 298 and 318 K showed no effect of temperature on catalyst yield, suggesting that the enzymes were appropriately immobilized in the pores of the molecular sieves, thus avoiding possible processes such as denaturing or autolysis.

Index Entries: Enzyme; lipase; molecular sieves; MCM-41; ethyl acetate; gas-phase.

Introduction

The application of biologically active materials (mainly enzymes) as catalysts for different chemical reactions has recently attracted interest as a research topic in the area of technologic and scientific development.

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Compared to other catalytic chemical systems, enzymes have several remarkable properties. First, enzymes have a high catalytic capacity (reactions are 10^8 to 10^{12} faster than reactions carried out with no catalyst). Second, enzymes are active in aqueous systems under mild reaction conditions (pH between 5.0 and 8.0, low temperature and pressure), minimizing the probability of side reactions such as decomposition or isomerization. Third, enzymes can be used for almost all types of reaction *(1)*.Finally, reactions catalyzed by enzymes are highly selective, usually with high regio- and/or enantioselectivity. Environmental and economic concerns largely contribute to the recent industrial interest in development of catalysts that minimize the formation of byproducts and residues. However, the use of enzymes has been restricted to high-value-added chemicals owing to important limitations, such as low operational stability and low solubility of reactants in aqueous systems. In the 1980s, Zaks and Klibanov *(2)* and Kazandjian et al. *(3)* found that enzymes could remain active for long periods in systems made of organic solvents as long as a small amount of water is present in the system. Thus, enzymatic reactions in organic solvents have a further advantage since the solubility of reactants and products is high. Important reactions such as synthesis of peptides *(4)*, regioselective esterification of oils and fats *(5)*, industrial synthesis of aspartame *(6)*, and development of organic-phase optical biosensors *(7)* have been studied.

In the search for increased efficiency and further applications for enzymatic systems, several strategies have been developed for efficient heterogeneous immobilization of enzymes. For example, the entrapment of enzymes in sol-gel *(8–10)*, polymers *(11)*, hydroxi matrices *(12)*, and molecular sieves *(13,14)* was studied. The structure of the support (composition and pore dimensions) and the highly heterogeneous surface of solid inorganic supports with multiple adsorption sites (framework oxygen, silanol groups) have a large influence on catalytic activity. In addition, the complex chemical structure of enzymes and the different substrates and solvents result in a complex relationship among solvent, enzyme, and support that ultimately results in a complex dependence of reaction rate on concentrations, water activity, and temperature. For example, the initial rate for the esterification reaction on lipase (produced by *Pseudomonas fluorescens*) *(15)* can vary from 25 mmol/(h·mg of protein) when supported on Celite 545 to 0.07 when supported on glutaraldehyde-activated silica (initial rate of 3.3 mmol/[h·mg] for nonimmobilized enzymes). However, regarding the maintenance of the operational stability of lipases, Ivanov and Schneider *(15)* found that after seven cycles enzymes immobilized on Celite 545 deactivated quickly (<99% of activity after 10 cycles). Similarly, Cao et al. *(16)* observed that enzymatic activity (lipase produced by *Candida antarctica B*) was maintained for six 24-h cycles for the acylation of β-D(+)-glucose with palmitic acid at 333 K.

Studies of gas-phase enzymatic reactions catalyzed by supported enzymes, such as continuous acetaldehyde production *(17)*, ethanol oxida-

tion by alcoholoxidases *(18)*, acetaldehyde reduction by alcohol-dehydrogenase *(19)*, and esterification reactions catalyzed by lipolytic enzymes *(20–22)*, are found in the literature. These studies demonstrate the viability of the application of enzymes as catalysts for gas-phase reactions. Advantages of the use of immobilized enzymes include increased thermal stability and high yield. For example, the use of dry yeast for the conversion of methanol to formaldehyde takes place at an optimum temperature above 350 K *(23)*. Similarly, the yield of an enzymatic reactor in the transesterification of methyl acetate with *n*-propanol is higher than in conventional processes (9 ton/[yr·$L^{-1}_{reactor}$]) *(22)*. Recently, preliminary studies on gas-phase production of ethyl acetate, using lipase from porcine pancreas *(20)* and lipase from *Candida rugosa (24)*, have resulted in small conversions at room temperature with a measured half-life of 386.2 h at 293 K but only 3.8 h at 373 K *(22)*.

Molecular sieves are potentially efficient solid supports for enzymes if their controlled-size pores are large enough to fit an enzyme but small enough for the solid framework to work as "armor" to avoid or diminish denaturation of the enzyme, preserving its ideal conformation for high enzymatic activity. Possible candidates as solid supports for enzymes are MCM-41-type molecular sieves. These solids can be easily synthesized with well-defined pores with diameters in the range of 4.0–10.0 nm. In addition, the control of pore size in MCM-41-type molecular sieves has been extensively studied *(25–28)*. In the present work, we studied the influence of different MCM-41 supports on the catalytic activity of different lipolytic enzymes in the gas-phase esterification of acetic acid with ethanol.

Materials and Methods

Synthesis and Characterization of Molecular Sieves

The molecular sieves were synthesized according to standard procedures described elsewhere *(27,29,30)*. In brief, 2.0 g of cetyltrimethylammonium bromide (CTMABr) (Sigma, St. Louis, MO) and the required amount of aluminum ($Al_2[SO_4]_3$) were added to 210 mL of NH_4OH (25% aqueous solution) and 270 mL of H_2O. After complete dissolution, 10 mL of tetraethyl orthosilicate was added (Aldrich). The solution was stirred continuously at 298 K for 2 h and had the following molar composition ratio: 1 Si: 0.12 CTMABr: 33 NH_4OH: 330 H_2O: x Al (x = 0 for MCM-41 and x = 0.7 for Al-MCM-41). The template was subsequently extracted by digestion in a 1:1 solution of CH_3CH_2OH and H_2O for 2 h. The suspension was then filtered and the solid was dried overnight at 373 K. Subsequently the solid was calcined at 813 K for 1 h in flowing nitrogen and then for 4 h in flowing synthetic air (600 mL/h).

The molecular sieves were characterized by X-ray diffraction, infrared (IR) spectroscopy, ^{29}Si and ^{27}Al nuclear magnetic resonance (NMR) and nitrogen adsorption at 77 K. The X-ray diffraction, was recorded in a Shimadzu XD-3A diffractometer with Cu-Kα radiation. The spectra were

registered between $2\theta = 1.5$ and 10° with a scanning rate of 2°/min, using a current of 30 mA and a voltage of 35 kV. IR spectra were recorded in a Perkin-Elmer 1600 Fourier transform IR (FTIR) spectrophotometer between 400 and 4000 cm^{-1}, equipped with a quartz glass cell coupled to a vacuum/gas-dosing line. The samples were evacuated (about 30 mPa) and heated to 773 K at 20/K min, kept at this temperature for 1 h, and then cooled to 373 K. The samples were then heated to 773 K at 10 K/min to record the reference spectrum. After cooling to room temperature the samples were exposed to about 2 kPa of ammonia for 10 min. Physisorbed ammonia was removed in vacuo at 373 K for 10 min, followed by heating to 773 K at 10 K/min. The IR spectra of the samples containing ammonia were then recorded. Chemical analysis (C/H/N) was performed using a Perki-Elmer 2401 analyzer. NMR spectra of ^{29}Si were recorded on a Bruker AC300/P instrument, using $(CH_3)_4Si$ as reference (MAS-CP, 79.4 MHz for $4\ \mu s \rightarrow \theta = \pi/2$). $^{27}A1$ spectra were obtained on the same instrument, using $[Al(H_2O)_6]^{3+}$ as reference (MAS, 104.3 MHz for $1\ \mu s \rightarrow \theta = \pi/2$). Surface areas and pore diameters (using the BET method with samples previously treated at 578 K in flowing N_2 for 3 h) were measured by nitrogen sorption at 77 K with Micromeritics ASAP-2010 adsorption equipment.

Enzyme Entrapment

Lipases produced by five different microorganisms were used in this work. Lipases produced by *Rhizopus oryzae* (FAP-15), *Rhizopus niveus* (OF) and *Mucor javanicus* (LEX) were from Novo Nordisk A/S (Brazil), and lipases produced by *Ps. Fluorescens* (AK) and *Pseudomonas cepacia* (PS) were from Amano (Japan). Enzyme-containing solutions were added to the molecular sieves by two different procedures: entrapment by sorption *(21)* (ESC) and entrapment by incipient wetness impregnation (WIC). In the sorption procedure about 0.20 g of MCM-41 or Al-MCM-41 was added to 10.0 mL of an enzyme solution (0.05 *M* phosphate buffer, pH 7.5) with a concentration of 0.20 g/L. The mixture was stirred constantly (100 rpm) at 277 K for 2 h and the solids were then filtered out. Another set of catalysts was prepared under similar conditions with potassium hydrogen phthalate buffer (0.05 *M*, pH 4.0). The amount of protein introduced into the solids was quantified by UV-VIS absorption (HP 8452A, diode array), measuring the concentration of protein in solution before and after the impregnation procedure at wavelengths between 180 and 410 nm, depending on the enzyme used. During the incipient wetness procedure, about 1.0 mL of enzyme solution with a concentration of 0.50 g/L at 277 K was slowly added drop by drop to 1.00 g of the support (MCM-41 or Al-MCM-41) with a constant homogenization of the sample. All impregnated supports were maintained in a desiccator for 10 d and then hermetically stored at 283 K until used.

Catalytic Assays

Homogeneous catalytic tests with the five enzymes were carried out according to the following procedure: thirty-four millimoles (2.0-mL) of

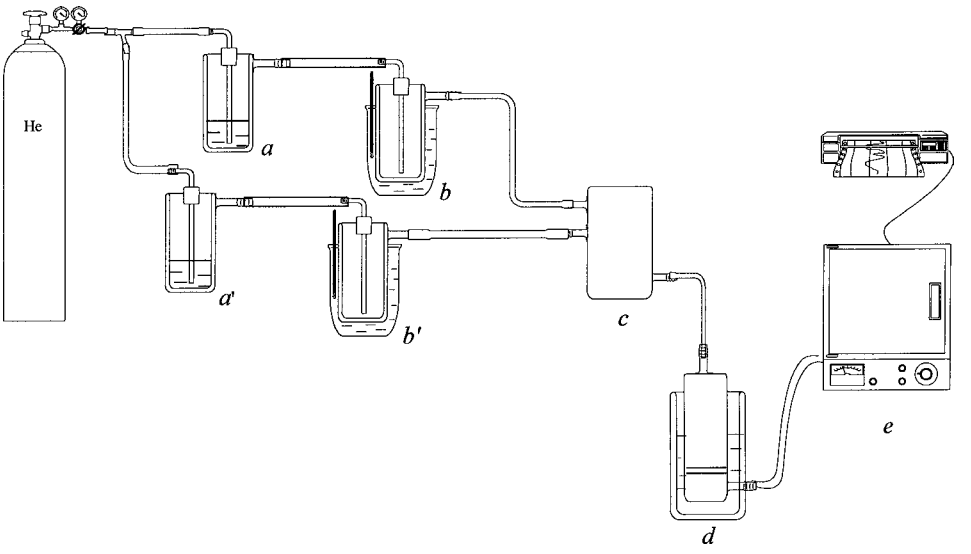

Fig. 1. Gas-phase reaction line, in which *a* and *a'* are the reactant reservoirs, *b* and *b'* are the saturators, *c* the mixer, *d* the fixed-bed reactor and *e* the analysis system.

CH_3CH_2OH and 35 mmol (2.0 mL) of CH_3COOH were added to a 50.0-mL aqueous solution of phosphate buffer (0.05 *M*). Five-milliliters of enzyme solution (0.20 g/L) was then added. The solution was stirred at 298 K for 1 h. Aliquots were removed and analyzed by gas chromatography (HP 5890 II). Liquid-phase reactions with MCM-41- and Al-MCM-41-supported enzymes were also carried out and analyzed under similar experimental conditions (0.10 g of supported enzyme was used as catalyst). For gas-phase reactions, two independent gas lines were used: one for CH_3CH_2OH and one for CH_3COOH (Fig. 1). The reaction system has a reactant reservoir, a temperature-controlled saturator, a mixing chamber, a reactor, and an analyzer. Approximately 20 mL of a liquid reactant (CH_3CH_2OH or CH_3COOH) was added to each reservoir. The carrier gas, He, flowed continuously (20 mL/min), first through the liquid reservoir and then through the saturator. The gas mixture leaving each saturator was He saturated with CH_3CH_2OH or CH_3COOH with the reactant vapor pressure corresponding to the temperature used in each temperature controller. The saturators were connected to the mixing chamber from where the gas mixture was sent to the fixed-bed reactor containing about 0.10 g of catalyst. The system was connected to a gas chromatograph for reactant and product analysis. The gas mixture containing ethanol, acetic acid, ethyl acetate, water, and carrier gas was sampled with an 8.0-mL loop six-way valve maintained at 475 K. Samples were analyzed by a gas chromatograph (HP 5890 II) equipped with a Poropak Q column (5 m, ∅ = 0.3 cm) and a TCD detector. The injector was maintained at 478 K, the column at 348 K, and the detector at 538 K. For the liquid-phase assays, 0.5 µL samples were taken

from the reaction vessel and injected into the gas chromatograph. In the case of the gas-phase assays, calibration curves for reactants and products were prepared by changing the saturator temperature in order to obtain different vapor pressures for each substance. The vapor pressure ratio, P/P_1, was calculated for each substance by using Clapeyron's equation:

$$\ln \frac{P}{P_1} = -\frac{\Delta H_V}{R}\left(\frac{1}{T} - \frac{1}{T_e}\right) \quad (1)$$

in which T is the saturator temperature, T_e is the boiling temperature, ΔH_V is the enthalpy of vaporization and R is the gas constant.

Results and Discussion

Characteristics of Immobilized Enzyme System

Both X-ray diffraction spectra show diffraction patterns typical of those expected from regular hexagonal channel systems (data not shown), indicating that the MCM-41 molecular sieve had a hexagonal structure and a regular distribution of pores *(31–33)*. Average pore diameter was calculated from adsorption measurements and was 4.5 nm for MCM-41 and 4.2 nm for Al-MCM-41 with a pore diameter distribution of 10% for both solids. The surface areas of both solids were also calculated from N_2 adsorption isotherms and were 1238 m^2/g for MCM-41 and and 986 m^2/g for Al-MCM-41. CTMABr template was removed by extraction in a CH_3CH_2OH/H_2O solution, followed by calcination at 813 K in a flow of synthetic air. The template was fully removed after the aforementioned procedure, since <2% residual carbon was observed by chemical analysis (C/H/N). IR spectroscopic analysis confirmed the removal of CTMABr template, since the intensity of bands at 1489, 2844, and 2916 cm^{-1} that refer to the surfactant disappeared after the procedure of digestion followed by calcination (data not shown). 29Si-NMR spectra of the MCM-41 and Al-MCM-41 samples (Fig. 2) show a peak at about –106 ppm, which can be assigned to ($[SiO]_4Si$) groups in the molecular sieve framework. An additional peak in the spectrum of Al-MCM-41 at about –99 ppm was assigned to one Si nucleus bound to one Al atom and three Si atoms ($[SiO]_3[AlO]Si$), suggesting that Al is present in the molecular sieve framework. The ^{27}Al-NMR spectrum of the Al-MCM-41 sample (Fig. 3) showed two peaks at about 53 and 0 ppm, corresponding to tetrahedral and octahedral coordination of Al atoms, respectively. The intensity of the peak in octahedral coordination is very low, suggesting that most Al atoms have a tetrahedral coordination. However, when the Al-MCM-41 sample was calcined at 813 K to remove the template, the ^{27}Al-NMR spectrum showed a small increase in the peak intensity of Al atoms in octahedral coordination. This increase indicates that some of the Al atoms changed their coordination, probably owing to the breaking of Si-O-Al bonds in the molecular sieve framework, resulting in a less crystalline solid. The FTIR spectra showed an

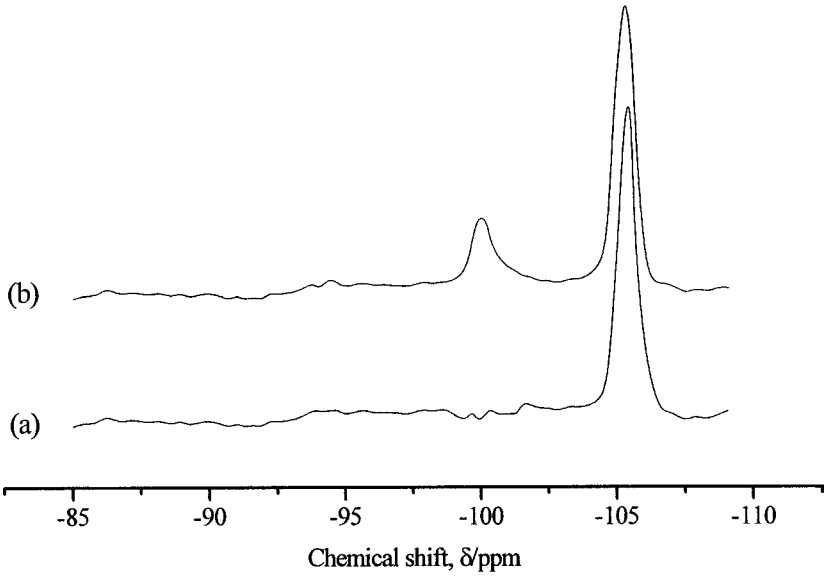

Fig. 2. ^{29}Si-NMR spectra of (a) MCM-41 and (b) Al-MCM-41.

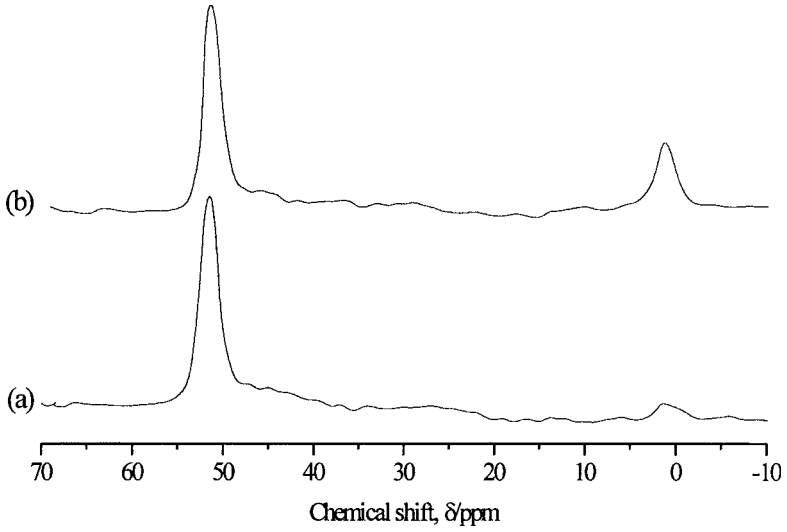

Fig. 3. ^{27}Al-NMR spectra of Al-MCM-41 (a) as synthesized and (b) calcined at 813 K.

intense band at 3747 cm^{-1}, assigned to the vibrations of isolated terminal silanol groups. FTIR difference spectra of ammonia-adsorbed samples show additional weak bands at 3605 and 3590 cm^{-1} that were assigned to bridging Brønsted hydroxy groups [Si-O(H)-Al] *(34)*. Desorption of ammonia from Brønsted sites occurs in the temperature range of 373–700 K, indicating the presence of strong Brønsted sites.

The introduction of enzymes into the support by sorption (ESC) was monitored by UV-VIS analysis (Table 1). The amount of protein intro-

Table 1
Influence of pH on Adsorption of Enzymes into Pores of MCM-41 and Al-MCM-41 Introduced by ESC

	Immobilized enzyme/support (mg/g)			
	pH 4.0		pH 7.5	
Enzyme	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	3.2	2.4	3.5	3.0
FAP-15	2.2	2.1	2.7	2.5
OF	3.6	3.2	4.2	3.9
AK	3.4	3.0	3.8	3.7
PS	4.1	3.7	4.6	4.3

duced into the pores of each support varied within 25% for enzyme solution FAP-15 and 50% for enzyme solution PS. When Al-MCM-41 was used as the support, 15% less protein on average was introduced into the pores of the molecular sieve. This variation could be owing to differences in the size of the pores of MCM-41 (4.5 nm) and Al-MCM-41 (4.2 nm) as well as to differences in the surface area of MCM-41 (1238 m^2/g) and Al-MCM-41 (986 m^2/g). The presence of Al in the molecular sieve framework is also responsible for strong Brønsted sites (as evidenced by the FTIR), which may result in a different interaction between solvent and support or in a change in the protein structure during adsorption. Serralha et al. *(13)* observed a strong influence on the catalytic activity of supported enzymes as the structure and composition of zeolites changed. The pH of the enzyme solutions also has a determining effect on protein load *(15,35)*. Thus, a decrease in pH can result in less protein introduced into the pores of the support. A small increase (<5%) in the amount of protein introduced into the pores of MCM-41 for samples stirred for 8 h compared with those stirred for 2 h was observed. The amount of protein immobilized by the WIC procedure was about six times lower (0.50 mg/g) than that immobilized by the sorption procedure owing to solubility limitations.

Esterification Reaction

To compare the enzyme activity in the esterification reaction of acetic acid with ethanol, it is necessary to use an intensive variable. In this work, the turnover number (TON), for the reaction after 1 h was chosen. The TON is defined as the ratio of milligrams of product formed after 1 h to milligrams of protein used. For a detailed study of enzyme activity, it would be necessary to measure the number of ethyl acetate molecules formed per enzyme molecule per unit of time. However, as preliminary work the use of TON as defined will suffice, since all other experimental conditions were fixed. Thus, changes in TON will reflect changes in enzyme activity as well as in the way the enzymes are positioned inside the pores of the support. For example, because the size of the enzymes used is close to the pore size

Table 2
Ethyl Acetate Formation on Free and Immobilized Enzyme Catalyst (ESC) in Liquid-Phase at 298 K for 1 h

	$[CH_3COOCH_2CH_3]$/mmol (TON)				
		Heterogeneous[a]			
	Homogeneous	pH 4.0[c]		pH 7.5[c]	
Enzyme	pH 7.5[b]	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	0.80 (70)	0.27 (74)	0.33 (121)	0.33 (83)	0.45 (132)
FAP-15	0.94 (83)	0.12 (48)	0.10 (42)	0.21 (69)	0.28 (99)
OF	0.43 (38)	0.03 (7)	0.05 (14)	0.08 (17)	0.10 (23)
AK	1.47 (129)	0.80 (207)	0.83 (243)	0.91 (211)	1.11 (264)
PS	1.22 (107)	0.71 (152)	0.79 (188)	0.76 (145)	0.93 (190)

[a]The numbers in parenthesis are the turnover number, TON.
[b]Reaction pH was maintained at 7.5 for homogeneous and heterogeneous systems.
[c]pH of enzyme entrapment.

of the supports, more than one mode of adsorption of the enzyme inside the pores of the molecular sieves is possible. The enzyme can therefore adsorb with the entrance to the active site facing the interior part of the pores, making access more difficult to substrate molecules. On the other hand, if the entrance to the active site faces the pore mouth, access to a substrate molecule is not affected by entrapment of the enzyme. Because the rate of enzymatic reactions is high and the pore sizes of the molecular sieves used are of the same order of magnitude as those of the enzymes, a higher loading of enzymes into the pores of the solids may result in mass transfer limitations during reaction with inefficient use of the supported enzymes.

Results for liquid-phase homogeneous or heterogeneous esterification reaction of acetic acid with ethanol at 298 K are also expressed as millimoles of ester formed after 1 h per milligram of protein (Table 2). Since the esterification reaction of acetic acid with ethanol is an autocatalytic reaction, blank reactions were carried out in the absence of enzymes. Enzyme activity varied according to the order OF < LEX < FAP-15 < PS < AK for the homogeneous reaction and the order OF < FAP-15 < LEX < PS < AK for the heterogeneous reaction. The change in activity in the homogeneous reaction could be related to two factors: 1) the inherent activity of the enzyme and 2) the optimum pH for the enzyme. The liquid-phase reaction was carried out at pH 7.5, because this is the most favorable pH for most enzymes used in this work.

For gas-phase reactions, only those enzymes that were more active in liquid-phase reactions—AK, PS and LEX—were used. The amounts of ester obtained for gas-phase reaction (Tables 3 and 4) were lower than those for the liquid-phase reaction. However, a comparison between the two systems should be avoided because both the concentration of reactants and the amount of catalyst used were lower in the gas-phase experiments. In

addition, the gas-phase reaction system had a continuous flow of inert gas (He, 20 mL/min), which reduced contact time between substrate molecules and catalyst and constantly removed water from the system, decreasing the water activity of the enzyme. The order of enzyme activity is similar to that in the liquid-phase reactions (AK > PS > LEX). Although the enzymes used in this work were not pure, it was observed that the values for enzyme activities were in agreement with those estimated from the specific activity obtained for the hydrolysis of *p*-nitrophenolpalmitate *(36)*: 34.13, 30.07, and 0.50 nmol/(min·μg) for AK, PS, and LEX, respectively. For catalysts denoted ESC, there was almost no difference between the activity of ethyl acetate formation for enzymes AK and PS when buffer solution at pH 4.0 was used for entrapment. However, when phosphate buffer solution (pH 7.5), was used, the value for activity of enzyme AK was approx 40% higher than that for enzyme PS and 250% higher than that for enzyme LEX (Table 3). Similar results were observed for the Al-MCM-41 support. However, for enzyme AK (pH 7.5) a slightly larger amount of ethyl acetate was observed: 0.032 mmol for MCM-41 and 0.035 mmol for Al-MCM-41. The values of TON for enzyme AK entrapped at pH 7.5 in the pores of Al-MCM-41 by ESC and WIC techniques (called AK[ESC-7.5]/Al-MCM-41 and AK[WIC-7.5]/Al-MCM-41) were 8 and 70, respectively. With other experimental conditions the same, such a large difference in TON is not expected. Similar results were observed for all biocatalysts. Thus, when comparing catalysts prepared by ESC and by WIC, the increase in values of TON is 6- to 13-fold for WIC catalysts since the amount of protein is larger for ESC catalysts than for WIC catalysts. We can only speculate at this point that either the enzymes are too active, making the reaction mass transfer limited, or the enzymes inside the pores of the MCM-41 molecular sieves are not accessible to all substrate molecules.

Except for the catalysts prepared by incipient wefner impregnation (WIC) at pH 4.0, the TON for the enzymes supported on Al-containing supports was always slightly larger than those for the supports without Al atoms (Tables 3 and 4). A possible explanation for this difference is twofold: framework Al atoms are known to increase the acidity of oxides, and the presence of Al atoms on the surface of oxides is known to increase the amount of water adsorbed on the oxides. Thus, an increase in the acidity of the support would increase the rate of reaction since the esterification of acetic acid with ethanol is an acid-catalyzed reaction. In addition, an increase in the amount of water adsorbed on the pores of the molecular sieves would result in an increase in the water activity of the enzyme, rendering it more active. Both phenomena would result in an overall increase in biocatalyst activity.

To study the effect of change over time of the water activity of the enzyme in the continuous reaction system, biocatalysts AK(WIC-7.5)/MCM-41 and AK(WIC-7.5)/Al-MCM-41 were studied by a continuous analysis of the gases (Fig. 4). The amount of ethyl acetate vs. time for both catalysts is shown in Fig. 4. There was an initial increase in the rate of

Table 3
Effect of pH of Impregnation and Framework Al in Support on Formation of Ethyl Acetate in Gas Phase Using ESC-Type Catalysts[a]

	$[CH_3COOCH_2CH_3]$/mmol (TON)[b]			
	pH 4.0		pH 7.5	
Enzyme	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	0.008 (2)	0.009 (3)	0.009 (2)	0.012 (4)
AK	0.019 (5)	0.022 (6)	0.032 (7)	0.035 (8)
PS	0.019 (4)	0.021 (5)	0.023 (4)	0.028 (6)

[a]Conditions were 18.6 kPa of CH_3CH_2OH, 10.7 kPa of CH_3COOH and 0.10 g of catalyst; time = 1 h; gas flow = 20.0 mL/min; and temperature = 298 K.

[b]The numbers in parenthesis are the turnover number, TON.

Table 4
Effect of pH of Impregnation and Framework Al in Support on Formation of Ethyl Acetate in Gas Phase Using WIC-Type Catalysts[a]

	$[CH_3COOCH_2CH_3]$/mmol (TON)[b]			
	pH 4.0		pH 7.5	
Enzyme	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	0.002 (4)	0.001 (2)	0.013 (23)	0.015 (26)
AK	0.014 (25)	0.013 (23)	0.037 (65)	0.040 (70)
PS	0.012 (21)	0.009 (16)	0.030 (53)	0.038 (67)

[a] Conditions were 18.6 kPa of CH_3CH_2OH, 10.7 kPa of CH_3COOH and 0.10 g of catalyst; reaction time = 1 h; gas flow = 20.0 mL/min; and temperature = 298 K.

[b]The numbers in parenthesis are the turnover number, TON.

reaction until a maximum value was reached. After the maximum was reached, after about 1.5 h for AK(WIC-7.5)/Al-MCM-41 and about 4 h for AK(WIC-7.5)/MCM-41, the rate of reaction decreased; a steady-state value was obtained for the lipase supported on the Al-MCM-41. Thus, the higher steady-state rate for the Al-MCM-41-supported biocatalyst compared with the possible steady-state rate obtainable for MCM-41-supported biocatalyst suggests that either the enzyme is more active owing to higher water activity, or enzyme activity is being boosted by the increase in acidity of the support. The same conclusion can be reached for the higher value for maximum rate of reaction.

To study how the enzyme inside the pores of molecular sieves would behave as the reaction temperature was varied, we measured the TON after 1 h at three different temperatures. The experiments were carried out at 298, 308, and 318 K with MCM-41 and Al-MCM-41 WIC-7.5- and ESC-7.5-supported biocatalysts (Tables 5 and 6). Only minor variations in the values

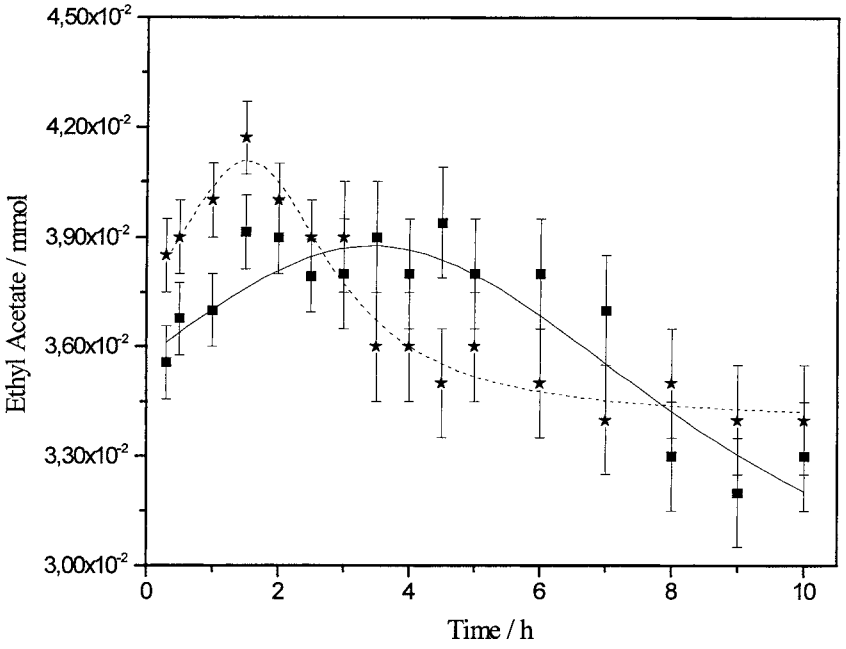

Fig. 4. Kinetic behavior of enzyme AK-WIC-7.5 supported on MCM-41 (■) and Al-MCM-41 (★). Reaction conditions: 18.6 kPa of CH_3CH_2OH, 10.7 kPa of CH_3COOH, and 0.10 g of catalyst; temperature = 298 K and He constant flow = 20.0 mL/min.

Table 5
Effect of Temperature on Formation of Ethyl Acetate Using ESC-7.5-Type Catalysts[a]

	$[CH_3COOCH_2CH_3]$/mmol (TON)[b]					
	298 K		308 K		318 K	
Enzyme	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	0.009 (4)	0.012 (4)	0.010 (4)	0.017 (6)	0.012 (5)	0.018 (7)
AK	0.032 (7)	0.035 (8)	0.030 (7)	0.040 (9)	0.034 (7)	0.042 (10)
PS	0.023 (4)	0.028 (6)	0.025 (4)	0.024 (5)	0.028 (5)	0.027 (6)

[a] Conditions were 18.6 kPa of CH_3CH_2OH, 10.7 kPa of CH_3COOH, and 0.10 g of catalyst; time = 1 h; and gas flow = 20.0 mL/min.

[b] The numbers in parenthesis are the turnover number, TON.

for TON can be observed in all cases. There were even cases in which TON decreased as reaction temperature increased. However, as the amount of water adsorbed in the pores of the molecular sieves decreases with increasing temperature, it is possible that even in those cases what is being observed may be owing to a decrease in water activity at the enzyme microenvironment. Thus, the results for thermostability of enzymes

Table 6
Effect of Temperature on Formation of Ethyl Acetate
Using WIC-7.5-Type Catalysts[a]

	$[CH_3COOCH_2CH_3]$/mmol (TON)[b]					
	298 K		308 K		318 K	
Enzyme	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41	MCM-41	Al-MCM-41
LEX	0.013 (23)	0.015 (26)	0.012 (21)	0.020 (35)	0.016 (28)	0.022 (37)
AK	0.037 (65)	0.040 (70)	0.039 (68)	0.044 (77)	0.040 (70)	0.048 (83)
PS	0.030 (53)	0.038 (67)	0.025 (44)	0.040 (70)	0.026 (46)	0.043 (73)

[a] Conditions were 18.6 kPa of CH_3CH_2OH, 10.7 kPa of CH_3COOH, and 0.10 g of catalyst; time = 1 h; and gas flow = 20.0 mL/min.

[b] The numbers in parenthesis are the turnover number, TON.

inside the pores of MCM-41 molecular sieves are not conclusive at this stage. Nonetheless, current experiments in our laboratories seem to indicate that indeed a change in stability may be expected.

Conclusion

Although preliminary, the results of this work suggest that gas-phase reactions using enzymes supported on controlled pore size solids are an interesting system for study. Introducing enzymes into porous solids with pore diameters close to the size of the enzyme may result in more active, stable biocatalysts with an extended life in the reactor. In addition, enzymes present in the pores of Al-containing solids were more active than those present in the pores of Si-only-containing solids, demonstrating the importance of the structure of the support used for entrapment in gas-phase enzyme systems. For the most active enzymes used in this work, entrapment resulted in an increase in activity for the liquid-phase reaction. Although the results showed that the rate of liquid-phase reactions was higher than that of gas-phase reactions, the influence of water activity was not fully examined, and that will require further study.

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