Catal Lett (2008) 122:43–52
DOI 10.1007/s10562-007-9324-1

Hydrolysis of Alkyl Ester on Lipase/Silicalite-1 Catalyst

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Received: 29 August 2007 / Accepted: 22 October 2007 / Published online: 8 November 2007

Abstract Pure silica zeolites have been prepared in powder and pellets form with different surface chemical properties in order to investigate how the binding forces in the lipase enzyme adsorption influence the final conformation of the immobilized enzyme and its catalytic activity. The catalytic activity of the adsorbed lipase have been tested in the alkyl esters hydrolysis reaction. The higher alkyl-esters conversion is observed for the lipase immobilized on the supports prepared in OH^- media compared to the fluoride media supports. The obtained results can be explained by the two different non-covalent interactions between the external surface of zeolites and the enzyme.

Keywords *Rhizomucor miehei* Lipase · Immobilization · Silicalite-1 · Alkyl ester hydrolysis · Self-bonded pellets

1 Introduction

Lipases constitute an important class of high selectivity and stereo-specificity industrial enzymes and are currently used in a wide variety of applications: bio-transformation of fats and oils, polymer synthesis and preparation of esters for food, for cosmetic industry and for diesel engine fuels (*biodiesel ester*) as well as for the production of optically pure compounds for the pharmaceutical industry [1–9].

The biological function of lipases is to catalyze the hydrolysis of esters, but they are also able of catalyzing the reverse reaction, achieving esterification, transesterification in various solvents. In order to use lipases more economically and efficiently immobilization techniques can be applied. In fact the immobilization of enzyme ensures the reusability of immobilized lipase, minimizes the cost of product isolation, provides operational flexibility, and the immobilization also increases enzyme thermal and chemical stability.

Recent studies have demonstrated that the immobilized lipases, adsorbed on hydrophobic supports, exhibit in the alkyl esters hydrolysis reaction a clear hyper-activation compared to the soluble enzyme due to the interfacial activation [10, 11]. This interfacial activation is caused by the conformation change resulting from the adsorption. From X-ray crystallographic studies [12, 13] the crystal and molecular structure of a triacylglycerol lipase has been elucidated. Insofar, the particular and well known conformation of lipase enzyme suggests that the most suitable immobilization method of this enzyme has to involve the "*lid*" region of the same enzyme in order to obtain the activation of its catalytic center.

Several works have studied the lipases immobilization [11, 14–28] on different kinds of support (synthetic and natural zeolites, cellulose, polymers, mesoporous materials, etc.) and by different immobilization routes (e.g., covalent immobilization on epoxy resins and graft copolymers, adsorption on hydrophobic supports and entrapment in photo-crosslinkable resins) but no literature data are available concerning the *Rhizomucor miehei* Lipase (RML) immobilization on zeolite.

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Besides the advantage of an easier recovery of the very expensive bio-catalyst, the use of zeolites as enzymatic supports is of great interest due to the possibility of generating and regulating the acid-base and hydrophobic-hydrophilic properties. Moreover, the zeolite pore structure, their framework composition and their crystals' morphology and size can be easily modified [29–32]. The main drawback of zeolites consists in their small pore sizes, in fact the enzyme cannot enter in the inner channels of the zeolite matrix.

In this work we present a study of the catalytic behavior of Lipase from *Rhizomucor miehei* adsorbed on Silicalite-1 type materials (in pellets and/or powder form) synthesized with different mineralizing agent in ordered to influence the physico–chemical properties of the zeolite surface. Pure silica zeolites are chosen as lipase support due to the high hydrophilic character and strong acid-sites that alumina zeolites show and that may induce some damage to the enzyme, as observed by different authors [33–35] and by our previous studies [36]. The self-bonded zeolite pellets are prepared because supports in this form can be easier to handle than that ones in powdered form that, normally, produce in the industrial application, a very high pressure drop. Moreover, the advantages of self-bonded pellets is avoiding the presence of other non-zeolitic phases that can negatively influence the adsorption and/or the catalytic performance of the enzyme, observed on the support in powdered form.

The main goal of this work is to understand which external surface properties of the zeolite support surface can be modified in order to obtain the lipase enzyme adsorbed in its "activated open conformation." In particular, the effects of the defect groups (Si–OH) and the nature of the cations on the support surface (strongly depending from the mineralizing agent used) have been measured on the enzyme immobilization and its catalytic activity. The catalytic conversion, the stability and the productivity of the supported bio-catalyst have been evaluated in the hydrolysis of methyl myristate to myristic acid.

2 Experimental

2.1 Materials

The enzyme used is the Lipase from *Rhizomucor miehei* (RML), PALATASE 20000L supplied by NOVO (optimum catalytic conditions: pH = 7 and $T = 37$ °C). The reagent used in the preparation of the supports are: silica gel precipitated (s.s.a. 550 m^2/g) or Silica Fumed (s.s.a. 200 m^2/g) as silica sources, tetrapropylammonium bromide (TPABr) as a structure directing agent, sodium hydroxide or sodium fluoride as mineralizing agents and distilled water. The reagents used for the esters hydrolysis reaction are methyl myristate, myristic acid, copper acetate, acetone, and toluene. All these chemicals are commercially available from Sigma Chemical Co., Mississauga ON, Canada.

2.2 Preparation of the Support

The supports in powder form were synthesized by hydrothermal treatment. The molar gel composition was: 1 SiO_2–y NaX–z TPABr–20 H_2O; where $y = 0.04$ and $z = 0.08$ if $X = OH^-$, while $y = 0.12$ and $z = 0.16$ if $X = F^-$. The crystallization temperature was 170 ± 2 °C. The gels obtained in alkaline-system were heated for 1 day (samples S1 and S2), while the gels obtained in F-media were heated for 5 days (samples S3 and S4), each one in autoclave system, in static conditions and autogenous pressure.

The supports in pellet form were prepared from a solid-state mixture. The batch composition of the synthesis system for obtaining self-bonded Silicalite-1 pellets was: 1 SiO_2–0.12 NaX–0.32 TPABr–0.8 H_2O where X is OH^- (sample P1) or F^- (samples P3 and P4, prepared using silica gel precipitated and silica fumed, respectively). The mixtures were prepared by homogenizing in a mortar of the mineralizing agent, structure directing agents, distilled water and silica. The amorphous pastes were prepared in the required pellet form by applying pressures of $70 \div 80$ MPa. The so-obtained pellets were put into a special autoclave [37] in presence of distilled water and heated up to 170 ± 2 °C under autogenous pressure for 24 h. Pellet sample P2 was prepared by mixing the pre-synthesized S2 sample with the sodium silicate solution as a binding agent in weight ratio zeolite : sodium silicate equal to 3:1. The obtained mixture was treated by the usual procedure: pressure treatment at $70 \div 80$ MPa and thermal treatment in autoclave at 170 ± 2 °C. All solid phases were calcined in air flow at 550 °C for 12 h in order to remove the organic compound and characterized by the usual techniques.

2.3 Enzyme Immobilization

A 50 ml of a 0.2 M phosphate buffer solution (pH 7) containing 1 g of RML was added to 0.4 g of the support, in pellet or powder form. If the support was in pellet form, the mixture was shaken for 24 h at predefined temperature in a Dubnoff bath (150 shacking/min), while for the support in powder form, the type of agitation was changed into stirring (250 rpm), but the time and temperature of the procedure were the same. The support with immobilized lipase was washed two times with de-ionized water and

dried at 25 °C overnight. The total protein concentration of the initial solution and the supernatant was calculated using the UV Absorption Methods (at 280 nm) [38]. The calibration curve, determined by a Perkin-Elmer UV Spectrophotometer, was obtained using the BSA (Bovine Serum Albumine) as standard protein. A linear regression equation is applicable to the obtained data: $Concentration = 2.2423 \times Absorbance$ with a correlation coefficient of 0.999. The amount of the total protein adsorbed on the support (W_{IP}, [mg]) was determined from the following mass balance: $W_{IP} = C_0V_0 - C_fV_f$, where C_0 is the initial protein concentration (mg ml^{-1}); V_0 is the volume of the initial solution (ml); C_f and V_f are the protein concentration (mg/ml) and the volume (ml) of the supernatant, respectively.

2.4 Hydrolysis of Methyl Myristate: General Procedure, Repeated Reaction Cycles and Regeneration of the Support

The lipase-support catalyst obtained by the immobilization procedure was added to 5 ml of a 0.2 M phosphate buffer solution (pH 7) containing 0.25 ml of Methyl Myristate 0.7 M in acetone. The mixture was stirred at 37 °C in a thermostatic bath (250 rpm). After the desired reaction time, the produced myristic acid was extracted from the reaction solution with toluene. The amount of the extracted myristic acid has been determined by a colorimetric method proposed by Lowry et al. [39]. The reusability of the lipase-zeolite catalyst was determined using the same immobilized enzyme for a number of cycles after washing them with the phosphate buffer solution (0.2 M, pH 7) thoroughly after every cycle. All the catalytic tests were carried out at 37 °C for a time of 70 h. The support containing the exhaust bio-catalyst can be easily regenerated by a thermal treatment at 380 °C for 5 h. After this regeneration process, in order to verify the complete absence of organic compounds, a thermal analysis on the regenerated support, in air flow (5 ml/min), between 20 and 850 °C, applying a heating rate of 10 °C/min, was carried out.

3 Results and Discussion

3.1 Properties of the Support

Table 1 lists the different reagents used in the supports preparation and their main physical properties. From Figs. 1 and 2, it is possible to observe that the Silicalite-1 type phase is obtained for all prepared supports [40], in powder or pellet form**.** Particularly, by the XRD spectrum of the P2 sample, the presence of an amorphous phase can also be observed. The morphology of the support surface is influenced by the nature of the mineralizing agent used during the synthesis procedure (see Figs. 3, 4b, 5, 6b and Table 1), both for pellets and powder materials. In fact, it is well known that the length-to-width (aspect) ratio of the Silicalite-1 crystals increases with the decrease of the reaction mixture alkalinity [41–43]. The NaF agent leads to the formation of crystals with the typical coffin-shaped morphology (pH of the synthesis is equal to 6), while using the NaOH agent, the resulting crystals have a rounded morphology (pH of the synthesis is equal to 12). The obtained pellets have an irregular morphology shape and a size around 0.7 ÷ 1.4 mm (see Figs. 4a, 6a). If the pellets are prepared using a binding agent (sample P2), the cavities on the pellet surface are not present and the same surface appears more compact with respect to the surface of the self-bonded pellets (see Figs. 4a, 6a). This aspect is also confirmed from the results of the N_2 adsorption/desorption analysis. In fact, the BET area value of the sample P2 (see Table 1) is the only one very different from the typical value of total surface BET of the MFI structure, due to the post-synthesis hydrothermal treatment that promotes the

Table 1 Reagent used and main physical properties of the prepared supports (N_2 Adsorption/Desorption and SEM results)

Sample	Silica source	Mineralizing agent	Total surface BET (m^2/g)	External surface *t*-plot (m^2/g)	Microporous volume (cm^3/g)	Crystal morphology and size (μm) of the pellet surface (length and width)
S1	Fumed	NaOH	345	78	0.15	Rounded 7 × 2
S2	Silica gel	NaOH	325	76	0.14	Rounded 21 × 20
S3	Fumed	NaF	403	81	0.16	Coffin-shape 27 × 5
S4	Silica gel	NaF	415	82	0.17	Coffin-shape 28 × 5
P1	Fumed	NaOH	335	75	0.14	Rounded 19 × 15
P2	Silica gel	NaOH	80	79	Not available	Rounded 16 × 10
P3	Fumed	NaF	357	75	0.13	Coffin-shape 23 × 6
P4	Silica gel	NaF	381	77	0.14	Coffin-shape 20 × 5

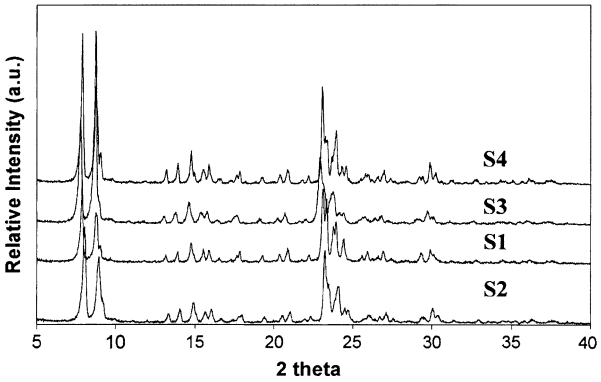


Fig. 1 XRD patterns of the support in powder form

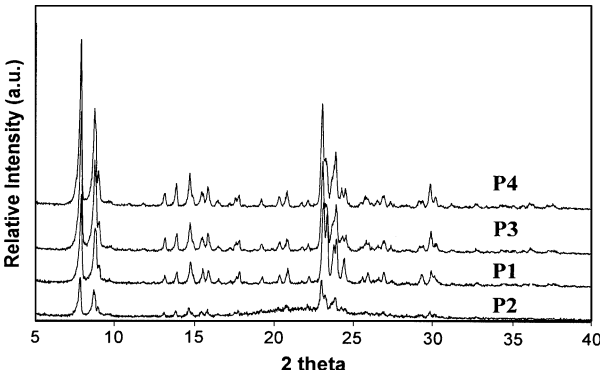


Fig. 2 XRD patterns of the support in pellet form

occlusion of the zeolite mouth pore, whilst the *t*-plot value of external surface is similar for all samples.

The size of the Silicalite-1 crystals obtained by hydrothermal and alkaline synthesis depends on the silica source used. Particularly the crystals' size of the Silicalite-1 type materials obtained with fumed silica is lower than the size of the Silicalite-1 crystals prepared with precipitate silica [44, 45]. In according with these literature data, the crystals' size of the supports S1 is lower than the crystals' size of the S2 sample.

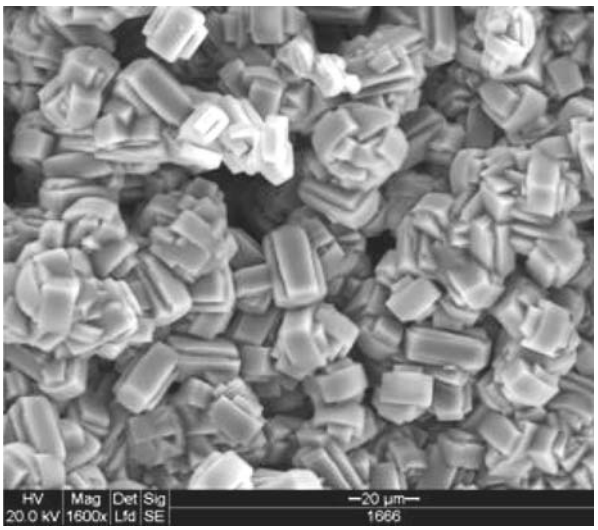


Fig. 3 SEM images of sample P1

Finally, the EDAX analysis show the presence of fluorine ions that most probably remain inside the small cages [$4^1 5^2 6^2$] of the MFI structure after the activation process (Table 2) [46–48]. The fluorine atomic composition of all prepared materials is included in the range between the 1.36 ÷ 2.01 wt.% values, according to Louis and Kiwi-Minsker [46].

3.2 Protein Adsorption

The protein adsorption results independent from the support form, while the proteins are preferably adsorbed on the materials surface synthesized in fluoride media (65 ÷ 68% of an adsorption yield for samples S3 and S4, 65% of an adsorption yield for samples P3 and P4) with respect to the support synthesized in alkaline system (39 ÷ 59 of adsorption yield for samples S1 and S2, 32 ÷ 34% of adsorption yield for samples P1 and P2) (Table 2).

The protein adsorption on Silicalite-1 materials synthesized in fluoride media is not affected by the temperature in the investigated temperature range (Fig. 7), while the protein adsorption on the support synthesized in alkaline system decreases when the temperature increases as it is expected on a thermodynamic based principle (the physisorption is an exothermic process). The pure silica phases, prepared in fluoride media, are strictly hydrophobic due to the very low concentration of the Si–OH groups [49, 50]. So, the high hydrophobic character of these samples (P3, P4, S3, and S4) could result in adsorbing high amount of protein presenting lypophylic properties. Moreover, the presence of a so strongly electrostatic element on the support surface, such as the fluorine ion, has to be considered. Particularly, for these defect-free materials, the presence of five-coordinate silicon $[SiO_{4/2}F]^-$ units [51] might allow creating the strong electrostatic bonds between the support surface and the aminogroups (NH_3^+) of the enzyme (by permanent dipole—induced dipole interactions). These interactions are not affected by the adsorption temperature until 40 °C, but, surely, at higher temperature value these physical bond can be broken.

Oppositely, the surface of the materials synthesized in alkaline media (S1, S2, P1, and P2), is characterized only by the presence of Si–OH *defect groups* (with a weak Brönsted acidity) due to the high pH of the synthesis [49]. These acid sites are the only ones with which the enzyme can interact during the adsorption process, as already

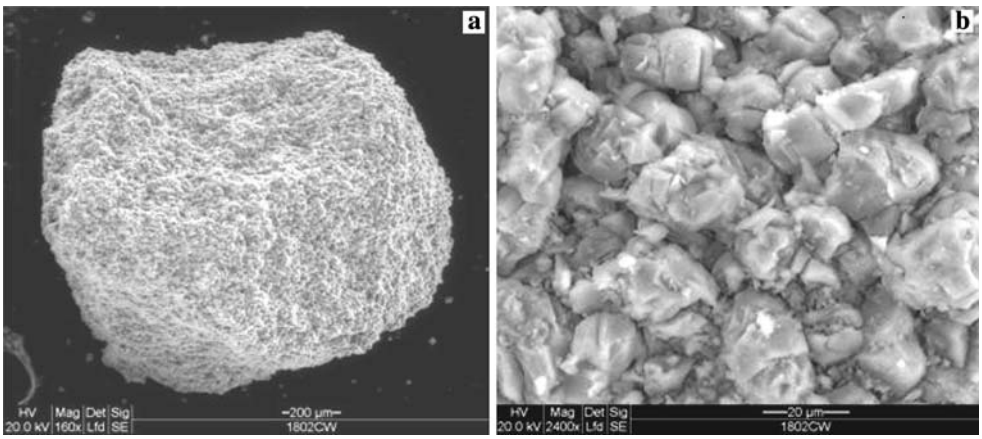


Fig. 4 SEM images of sample P2: (**a**) overview of the pellet; (**b**) particular of the pellet support surface

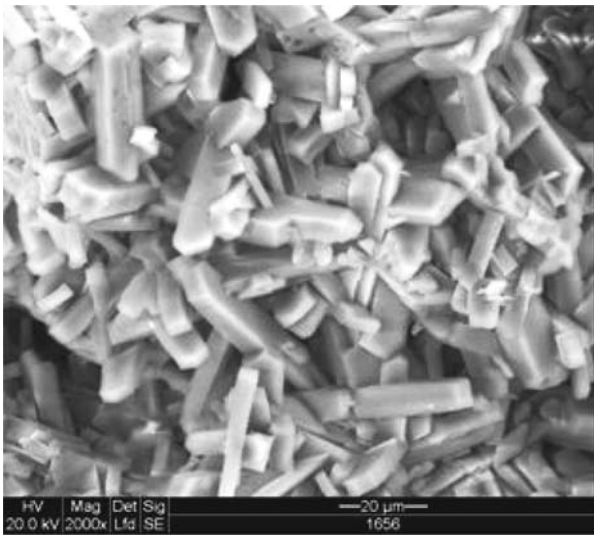


Fig. 5 SEM images of sample P3

observed in our previous studies [36]. Then, we can expect that the interactions between enzyme and support synthesized in alkaline media involve the silanol groups of the support surface and the amino-acidic residues of the enzyme by van der Waals (dipole- induced dipole) interactions. These most probable interactions are weaker than the electrostatic ones, involving the permanent dipole of the fluorine, and are strongly affected by the adsorption temperature just at 25 ÷ 40 °C (Fig. 7).

Finally, the different amounts of the immobilized protein between the S1 and S2 supports surface could be imputable to the different specific surface area of the silica source used. Particularly, the BET s.s.a. of the Silica Fumed, used for the S1 sample synthesis, is 200 m^2/g while the BET s.s.a. of the precipitated silica, used for the S2 sample synthesis, is 550 m^2/g. The density of the silanol defect-groups on the support surface is affected by different synthesis parameters, including the specific surface area of the silica source. Silica with higher specific surface area shows a greater amount of Si–OH groups on its surface than the silica with a lower specific surface area [52, 53]. Probably, for this reason the protein amount adsorbed on the crystals of the S2 sample is higher than the amount adsorbed on the S1 crystals surface. However, this aspect

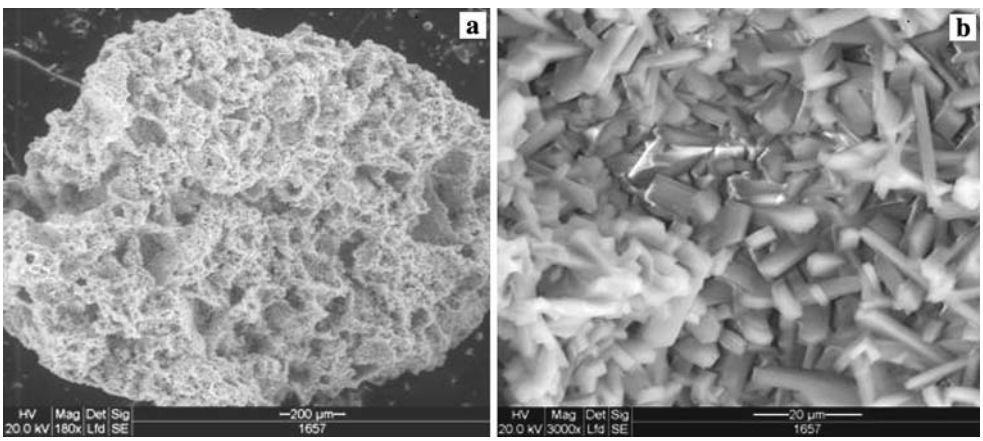


Fig. 6 SEM images of sample P4: (**a**) overview of the pellet support surface; (**b**) particular of the pellet support surface

Table 2 Protein adsorption results on the different kinds of the support (immobilization temperature = 25 °C) and chemical composition of the Silicalite-1 samples obtained by AA analysis

Sample	Mineralizing agent	Fluorine content (wt.%)	Sodium content (wt.%)	Adsorbed protein (mg/g)	Protein adsorption yield (%)
S1	NaOH	–	0.49	151	39
S2	NaOH	–	0.54	200	59
S3	NaF	1.36	0.18	278	65
S4	NaF	1.63	0.25	288	68
P1	NaOH	–	0.52	124	32
P2*	NaOH	–	0.73	145	34
P3	NaF	1.47	0.59	274	65
P4	NaF	2.01	1.30	275	65

All these results are the means of three repeated measurements

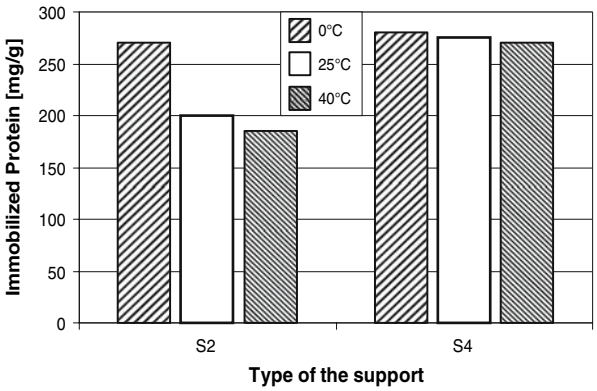


Fig. 7 Enzyme immobilization yield on the support at different temperatures

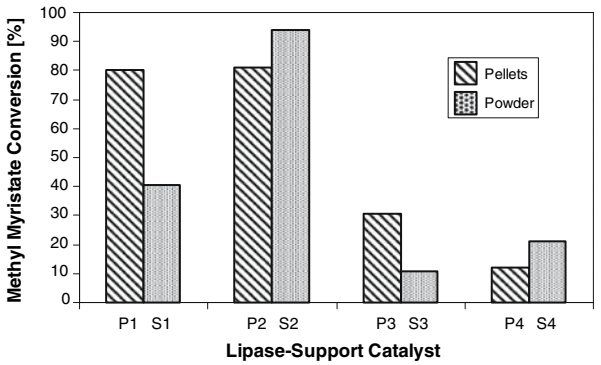


Fig. 8 Methyl myristate conversion. Reaction conditions: 70 h at 37 °C, catalyst amount equal to 0.4 g

should be investigated further by appropriate analysis but it is not the main goal of this work.

3.3 Hydrolysis of Alkyl Ester Reaction by Adsorbed Lipase

The alkyl esters hydrolysis results show that, after 70 h of reaction, the higher methyl myristate conversion is obtained when the lipase was adsorbed on the support prepared in alkaline system (S1, S2, P1, and P2) (Fig. 8) with respect to the conversion value obtained using the enzyme adsorbed on the support prepared in *F*-media (S3, S4, P3 or P4). Particularly, the maximum alkyl ester conversion obtained with the supported biocatalyst is over 90% for the Lipase-S2 catalyst and 81% for the Lipase-P2 catalyst. The different conversion value between the P2 and S2 support could be attributed to the different amount of loaded protein (see Table 2), thus considering the same lipase distribution into the total adsorbed protein.

These results suggest that the different interaction between lipase enzyme and the support strongly influence the final enzyme conformation. In fact, even if our findings show the highest protein adsorption on the supports prepared in *F*-media, the conversion yield values are the lowest. The activity recovery results (observed activity versus expected activity) also show this aspect (Fig. 9). The expected activity represents the activity of the free enzyme in amount equal to that was immobilized on each support. For sample S2 a higher activity than that observed for the correspondent free enzyme is observed. While for sample S4, the higher amount of the immobilized enzyme results very low active with respect to the correspondent free enzyme. The very low catalytic performance of the large amount of the protein adsorbed on the S4 and P4 samples can be attributed to the high electrostatic interactions between the proteins, with which the lipase is co-adsorbed, and the support surface.

Usually, immobilization promotes a dramatic change of the enzyme structure, leading to an apparently more ordered enzymatic structure of the lipase by the adsorption process. The catalytic center of the RML enzyme is made up of three elements: the *Asp-His-Ser* triad, which is responsible for the nucleophilic attack on the substrate [13]. The helical "*lid*" cover the catalytic triad in the close, soluble and inactive lipase form and it is responsible for the

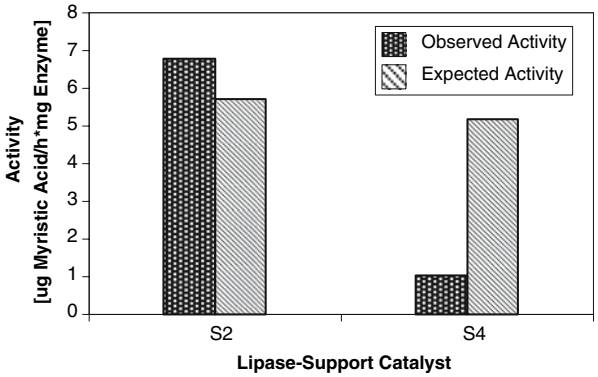


Fig. 9 Activity recovery of the samples Lipase/S2 and Lipase/S4. The expected activity is the activity of the free enzyme: 80 mg for S2 and 115 mg for S4. At the tested reaction time (70 h), both the amount of the free enzyme allow to obtain the total methyl myristate conversion

interfacial activation upon adsorption of the enzyme to oil-water interface. By the "interfacial activation mechanism," the enzyme exhibits its catalytic activity due to the opening of the *lid*-enzyme and the activation of the catalytic center [13]. During the immobilization process, the activation mechanism of the catalytic center is suitable in order to obtain the immobilized open enzyme form. The aminoacids present in the hinge region of the *lid* have an important role both in the enzyme activation mechanism and in the stabilization of the open and immobilized enzyme form. A previous study shows that the hinge region of the *lid*-enzyme is the aminoacid *Arginine* (a basic aminoacid) as R86 residue [54]. By comparing the electrostatic free energies, the same study shows the pH-dependent stability of the closed and open conformation of RML. Particularly, at pH = 7 the R86 residue destabilizes the closed conformation but stabilizes the open one. Moreover, it is also known that the optimum pH range for the hydrolytic activity of RML enzyme is between 7 and 8 [54]. Then, the chemical involvement of the *Arginine* aminoacid of the RML enzyme plays a very important role in the immobilization of this enzyme.

The adsorption mechanism on the support prepared in *F*-media allows to adsorb the greatest enzyme amount in its closed form, while, by contrary, the presence of weak acid

Table 3 Effect of the concentration salt of the buffer on the activity of the immobilized enzyme on the support S2

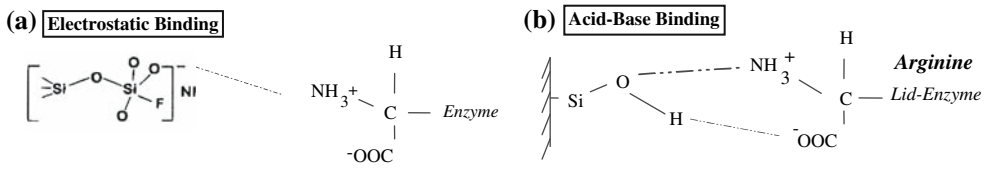
Concentration salt (M)	Protein adsorption yield (%)	Methyl myristate conversion (%)
0.1	37	35
0.2	59	93
0.5	61	70
1	60	63
1.5	58	51

All these results are the means of three repeated measurements

silanol groups on the surface of the support obtained in alkaline system, can selectively interact with the basic aminoacid *Arginine* which constitutes the hinge region of the *lid* (Scheme 1). These interactions seem to allow the opening of the *lid* and, then, the activation of the catalytic center. This is one of the most plausible reason because a lower amount of the adsorbed enzyme allows to achieve a higher methyl myristate conversion. In fact, despite that the structures of both the closed and open conformations of lipases are now known, the mechanism of the lid opening is not yet well clear.

Moreover, it is well known that under particular conditions of pH, temperature and concentration of salt the dimeric state of lipases can be unstable [55–57]. Particularly, the dimeric form of lipases can spontaneously dissociate into a less active monomer. No much information is available about the differences between the dimer and monomer of the lipases enzyme, but it is believed that the dissociation process is irreversible [55]. In Table 3 we report the effect of the concentration salt of the buffer on the immobilized enzyme amount and on the catalytic activity of the system Lipase/S2.

It is possible to observe that, increasing the concentration salt, the adsorption yield of the hydrophobic enzyme increases and remains constant between the values 0.5 and 1.5 M of the concentration salt. At the same time, an enzyme deactivation occurs: the conversion of the methyl myristate decreases from the 93 to 50%. These results suggest that, at 0.2 M of buffer solution, the dimeric and active lipase species have been adsorbed on the S2 support,



Scheme 1 Proposal interaction mechanism between enzyme and prepared supports. (**a**) Electrostatic binding forces between any aminoacid constituent the enzyme and the support prepared in fluoride media. (**b**) Acid-Base binding forces between the weak acid silanol groups of the support and the basic amino-acid *Arginine* constituent the hide region of the enzyme lid

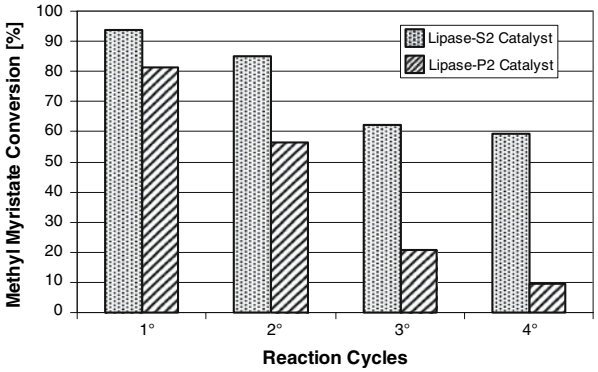


Fig. 10 Reusability of catalysts in the ester hydrolysis reaction. Reaction condition for each cycle: 70 h at 37 °C, initial catalysts amount 0.4 g

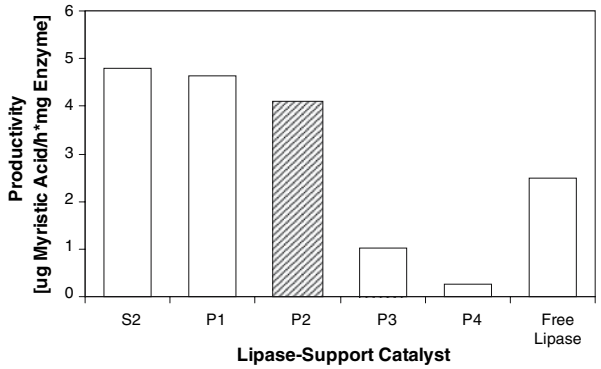


Fig. 11 Productivity after three reaction cycles, each one of 70 h at 37 °C for heterogeneous biocatalyst. For free lipase the quantity used is of 100 mg and each reaction cycle is of 40 h

and are in agreement with previous studies showing that as a dissociation of the enzyme in lower active monomeric species occurs when the concentration of the salt increases [55–57].

On the support containing the strong electrostatic fluorine ions the adsorption of the lipases enzyme in monomeric form could be occurred. This can explain why the highest immobilized enzyme amount is not active.

Finally, the enzyme adsorbed in its active conformation on the supports prepared in alkaline system remains active until four reaction cycles, with an evident decreasing of the methyl myristate conversion due to the inevitable enzyme leaching (Fig. 10). Even if the enzyme activity progressively decreases, the total productivity of the same enzyme, reused for four cycles of reaction, is higher than the total productivity achieved using the free enzyme (100 mg for each reaction cycle). The specific productivity of the immobilized lipase with respect to the free enzyme shows that the zeolitic supports obtained in alkaline media present a higher activity, expressed in μg myristic acid per hour per mg of adsorbed protein (Fig. 11). This aspect confirms that

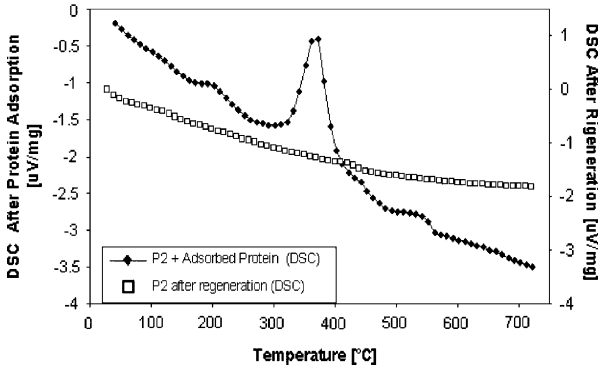


Fig. 12 DSC measurements of the sample P2 after protein adsorption and after regeneration process

the immobilization of lipase enzyme on supports having specific properties could allow a hyper-activation of the same enzyme with respect to its soluble and free state.

3.4 Regeneration Process of the Biocatalytic Support

Finally, since the immobilization of the enzyme has been carried out by physical adsorption, the support containing the exhaust bio-catalyst can be easy regenerated by a simple thermal treatment. As a matter of fact, in Fig. 12 the DSC analysis of the sample after the protein adsorption (sample P2) shows a peak at 367.8 °C, which can be attributed to the protein presence. After the regeneration process (5 h at 380 °C), no peaks are observed in the DSC curve, confirming the complete absence of organic compounds. The regenerated support is then ready for the next enzyme adsorption process. Same support (named RP2) was reused in the protein immobilization and, afterwards, in the hydrolysis reaction. The amount of the adsorbed protein is about the same obtained in the first immobilization (140 mg/g, with respect to 145 mg/g, initially obtained). After 70 h of the reaction, the conversion of the methyl myristate using the Lipase-RP2 catalyst, is 80%, practically the same as the conversion values obtained using the biocatalyst immobilized on the support for the first time. These findings confirm that the support can be used more than once to immobilize lipase, in fact, during the thermal regeneration process, the condensation of the hydroxyl groups (Si–OH) does not occur, probably due to the low-regeneration temperature.

4 Conclusions

Lipase from *Rhizomucor miehei* has been immobilized in its active conformation on the Silicalite-1 type material, both in powder and in pellet form.

The gel or solid-state mixture composition of the prepared materials strongly influence the protein adsorption capacity of the final supports. Concerning the materials prepared in *F*-media, the interfacial activation of the lipase, immobilized on these supports, does not occur and probable the inactive monomeric species of lipase have been adsorbed**.** While, if these materials are obtained in alkaline media, the weak acid Si–OH groups present on the support surface interact with the basic residue of the *lid*-enzyme (Arginine) allowing the immobilization of the enzyme in its open and active form. These kinds of the interaction does not cause the dissociation of the lipase dimeric form into the inactive monomeric species.

The total productivity of the adsorbed lipase enzyme in the hydrolysis of ester reaction has been improved and the hyper-activation of the immobilized enzyme with respect to its free and soluble form occurs. In addition, due to the physical adsorption procedure used, the facility of the supported bio-catalyst preparation and the easy and efficient support regeneration procedure are important aspects that make these heterogeneous bio-catalysts interesting for industrial applications.

The so-prepared "solid and reusable" bio-catalyst can be also used for carrying out the transesterification reaction in continuum, in order to obtain the *biodiesel* product, an interesting renewable energy source.

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