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SIMPLE ENTRAPMENT OF ALCALASE IN DIFFERENT SILICA XEROGELS USING THE TWO STEPS SOL-GEL METHOD

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SUMMARY

The present study has focused on the entrapment of Alcalase in different xerogels obtained by using various molar ratios of methyltriethoxysilane, dimethyldietoxisilane and tetraethoxysilane. Silica and their derivatives were characterized with regard to specific surface area (nitrogen adsorbtion), chemical composition (Fourier transform infrared spectroscopy (FT-IR)), weight loss upon heating (thermogravimetric analysis (TGA)) and catalytic activity.

Keywords: Alcalase; sol-gel method; silica; physico/chemical characterization.

INTRODUCTION

The studies on immobilized biomolecules and their applications, particularly in the biochemical, biomedical and food industries proved that they remain very attractive due to their stability and reusability [1,2]. In biotechnologies, stability and reusability of biocatalysts is the key of low production price. The majority of the biotechnologies use enzymes as biocatalysts, but enzymes lose their catalytic activity rapidly in aqueous solutions. That problem can be minimized in two ways: (1) using enzyme friendly reaction media but in that way reusability is impossible, (2) through enzyme immobilization. This

way causes lower enzyme activity than the first way, but higher stability and reusability. The properties of immobilized enzyme are governed by the characteristics of both the enzyme and the carrier material. The interaction between them provides an immobilized enzyme with specific chemical, biochemical, mechanical and kinetic properties. Retention of the biologically active species is achieved by entrapping them in the porous matrix that is created during sol–gel formation [3-5].

Proteases remain the dominant industrial enzyme type, accounting for at least a quarter of the total global enzyme production sales [6]. They are the single class of enzymes which occupy a pivotal position with respect to their applications in the detergent, protein, brewing, meat, photographic, leather and dairy industries [7-9].

In this work, we analyze the effect of the different precursor mixtures on the activity of the immobilized Alcalase. The internal structure of the silica support was determined by nitrogen adsorption, the chemical composition by FT-IR and the thermal stability by thermal analysis.

MATERIALS AND METHODS

Materials: Alcalase (produced by *Bacillus licheniformis*), Folin-Ciocalteu's phenol reagent, L-Tyr and trichloroacetic acid were from Merck, Hammerstein casein, tetraethoxysiliane, methytriethoxysilane, dimethydiethoxysilane were from Fluka. All the other chemicals were commercially available reagents grade products and were used without purification.

Immobilization method: In the first step silica sol was obtained from a mixture of alcoxide, alcohol and water in acid catalysis (silica precursor mixture / solvent / water / HCl = 1 / 0.8 / 1 / 0.004). In the second step, the gels are obtained from sol, enzymatic solution and catalyst (NaF). After gelation the gels were left for aging 24 hours. After that the gels were washed and dried for 24 hours at 4°C. The resulting powders were analyzed with biochemical and physicochemical methods.

Biochemical investigation: Proteolytic activity of immobilized Alcalase was determined at 37 ºC in phosphate buffer, pH 8, using Hammerstein casein as substrate. One unit of protease was equivalent to the amount of enzyme required to release 1 μmol of tyrosine/ml/min. The protein content of the immobilized protease was determined by the Lowry method using the Folin Ciocâlteu`s reagent and a standard calibration curve of bovine serum albumin [10, 11].

Characterization of samples: Specific surface areas were calculated from N_2 adsorption isotherms measured on a Quantachrome NOVA 2000 instrument using the multi-

point BET method. Infrared spectra were recorded on a Mattson Genesis 1 FT-IR spectrometer in 0.1 wt.-% KBr pellets. The themal analysis data were recorded on a MOM Budapest instrument with a heating rate of 10 °C.

RESULTS

Previous works reported that the hydrophobic silica matrices could affect the immobilized enzymes activity [12, 13]. In this work Alcalase was entrapped within sol-gel matrices derived from tetraetoxisilane (TEOS), methyltrietoxysilane (MTES) and dimethyldietoxysilane (DMDES), in different molar ratios. Different precursor mixtures were tested: (I) MTES/TEOS, (II) DMDES/TEOS and (III) DMDES/MTES/TEOS. The results are shown in Figure 1 and Figure 2.

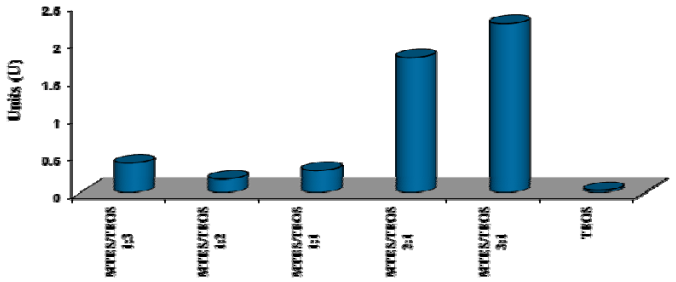

Figure 1. Enzyme entrapment in MTES/TEOS derived silica gel mixtures

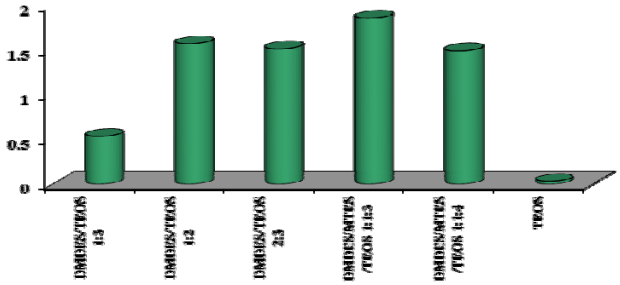

Figure 2. Enzyme entrapment in DMDES/TEOS and DMDES/MTES/TEOS derived silica gel mixtures

The isotherms evolution with the MTES/TEOS molar ratios and the specific surface area are presented in Figure 3 and Figure 4. The thermal analyses data are shown in Figure 5 and Table I while the IR data in Figure 6 and Table II.

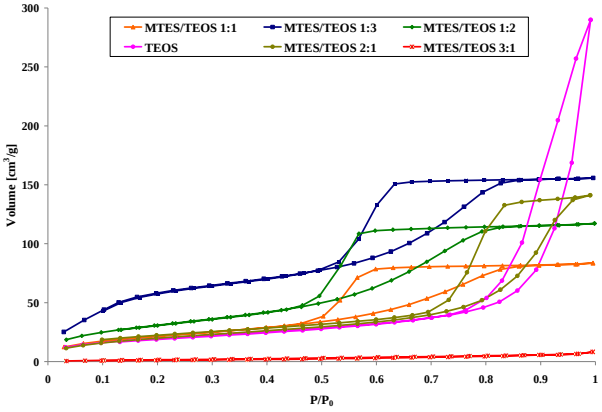

Figure 3. Nitrogen adsorbtion isotherms obtained from MTES/TEOS precursors mixtures

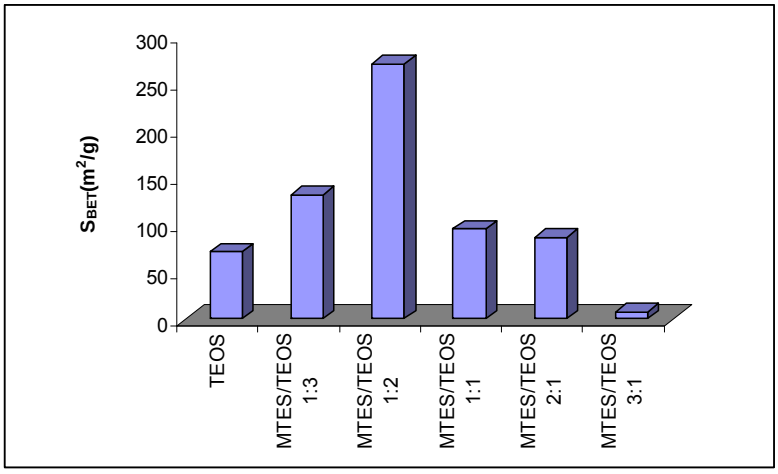

Figure 4. Evolution of the specific surface area determined from the nitrogen adsorbtion isotherms

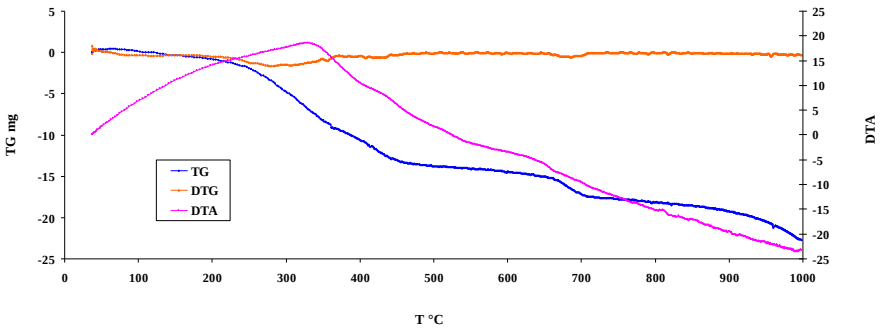

Figure 5. Thermal analysis of MTES/TEOS 1:1 silica gels

Table I. The weight loss of MTES/TEOS 1:1 silica gels

Temperature (°C)	20-220	220-480	480-665	665-1000
Maximum		337		
Weight Loss (%)	1.20	14.63	2.12	8.60
Total weigh loss (%)	23			

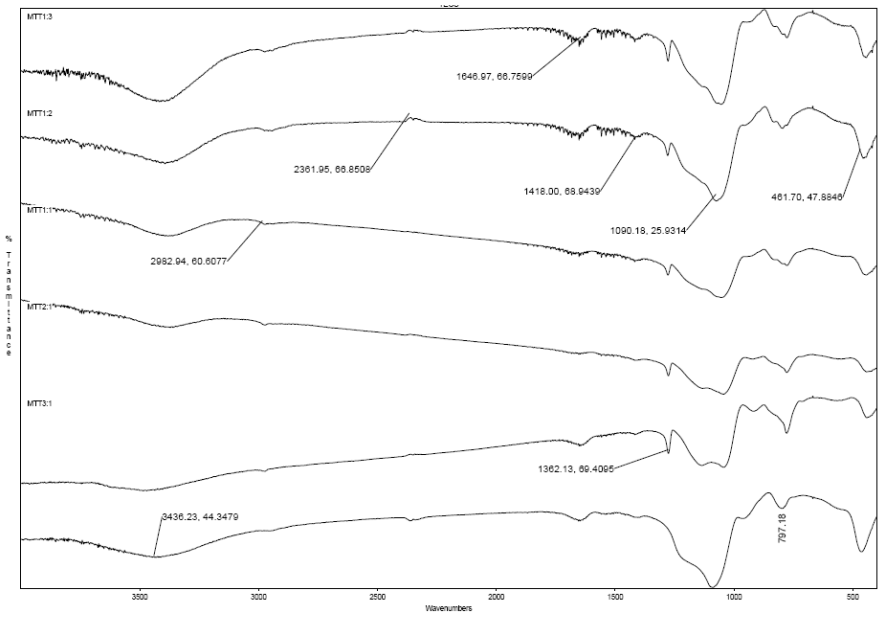

Figure 6. FT-IR spectra of silica gels obtained from MTEOS/TEOS precursor mixtures

Table II. Assignment of IR spectral data of silica xerogels

Frequency (cm^{-1})	Position assessment	Literature value [14]
466	Si-O bond rocking	475 -465
804	OH bending (silanol)	870 - 800
970	Si-OH bond streching	980 -935
1092	Assymetric Si-O-Si streching in SiO_4 tetrahedron	1115 - 1050
1287	υ $SiCH_3$	1250 -1290
1412	CH_2 (υ) scissoring and bending	1470-1350
1646	O-H bending (molecular water)	1625
2359	CO_2	2360
2959	$υ_a$ CH_2, CH_3	2960-2850
3067	υ C-H alchene	3080-3020
3410	O-H streching and adsored water	3800 - 3000

DISCUSSION

Alcalase was entrapped in silica xerogels derived from MTES, DMDES and TEOS using MTES/TEOS, DMDES/TEOS and DMDES/MTES/TEOS precursor mixtures with

different molar ratios. The use of hydrophobic substrates improved the immobilization efficiency. The presence of MTES in the binary or ternary precursor system led to higher activities of the immobilized enzyme. The best results were obtained for the MTES/TEOS mixture with a molar ratio of 3/1 (Figure 1 and Figure 2).

The adsorption–desorption isotherms of all the characterized xerogels described here presented type IV isotherms with hysteresis characteristic for mesoporous materials (Figure 3). The hysteresis type changed with the precursor molar ratio presenting the evolution of the silica carrier texture. The sample derived from TEOS shows a hysteresis of type H3, characteristic for the materials with the cone type porosity, all the other samples presented the H2 type of hysteresis characteristic for the ink bottle type porosity.

All samples gave very similar IR spectra, with only the expected variations in band intensity. The IR spectral assignments of the silica are shown in Table II. The presence of adsorbed water and free surface silanol groups as well as siloxane linkages can easily be conceived from the IR spectra of silica in the range 400–4000 cm^{-1}. In the spectra the broad band located in the range 3000–3800 cm^{-1} corresponds to the fundamental stretching vibration of different hydroxyl groups. The broadness of this band suggests different local environments of the OH groups. The band at 1625 cm^{-1} is assigned to the deformation mode of water molecules, which are probably trapped inside the pores. In the range 400–1500 cm^{-1} the spectrum has several bands. Those located at about 466, 804 and 1092 cm^{-1} are the bond rocking, bond bending and bond stretching vibrations of the Si–O–Si units.

The thermogravimetric analysis of the silica xerogels (Figure 5) indicates a multiple step weight loss: a small weight loss until 220°C – counting for the elimination of the adsorbed water molecules; a substantial weight loss from 200°C-480°C with an exothermic maxima at 337°C associated with an exotermic peak – attributed primarily to the removal of water by dehydroxylation and loss of organic constituents (C,H,O); after 500°C the weight loss can occur due to the final dehydroxylation reactions and definitive carbonization of organic compounds, including the enzyme. The total weigt loss is 23%. The thermal analysis data confirm the IR results.

CONCLUSION

Silica xerogels were obtained from methyltrietoxysilane, dimenthyldiethoxysilane and tetraextoxysilane. The obtained silica matrix was successfully used to immobilize Alcalase. Varying the precursor type and molar ratios the best results were obtained in case of MTES/TEOS 3:1 and DMDES/MTES/TEOS 3:1:1 respectively.

The surface characteristics are adequate for the Alcalase immobilization and the mesoporous silica matrixes make possible the transfer between the macromolecular

substrate and catalyst.

Because glass is chemically inert and thermally stable, it is expected that enzymes immobilized by the present technology can be used as ideal biocatalysts for long-term chemical processing applications involving elevated temperatures and reaction media.

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