Reference number – ADS-017

Encapsulated pyridazine Cr(III) complexes prepared from biosorbents supported in zeolites

H. Figueiredo[a], M.M.M. Raposo[a], A.M. Fonseca[a], I.C. Neves*[a], C. Quintelas[b] and T. Tavares[b]

[a]Departamento de Química e Centro de Química, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal. E-mail*:ineves@química.uminho.pt

[b]Departamento de Engenharia Biológica, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal.

Key words: Zeolites; *Arthrobacter viscosus*; Biosorvents; Pyridazine Cr(III) complexes; Encapsulation.

The encapsulation of a pyridazine Cr(III) complex was prepared from a robust biosorption system consisting of a bacterial biofilm supported on NaY or NaX zeolites. The maximum removal efficiency was 20% for Cr in both systems based in NaY or NaX. The bacterial biofilm, *Arthrobacter viscosus*, supported on the zeolite reduce Cr(VI) to Cr(III). The Cr(III) is retained in the zeolite by ion exchange. These occluded complexes were characterized by chemical analysis, spectroscopic methods (FTIR and UV/Vis) and surface analysis (DRX). The various techniques of characterization used show that the Cr(III) complex was effectively encapsulated in the zeolite and this process does not modified the morphology and structure of the NaY/NaX zeolites. These materials have potential applications in heterogeneous catalysis in mild conditions.

1. INTRODUCTION

Heavy metal such as cadmium (Cd), chromium (Cr) and lead (Pb) are toxic priority pollutants in aqueous waste streams of many industries, such as metal plating facilities, mining operations and tanneries. The soils are contaminated and pose a risk of metals groundwater and surface water contamination. Numerous processes exist for removing these metals including chemical precipitation, ion exchange, membrane filtration and carbon adsorption [1].

Cost effective alternative technologies or sorbents for treatment of metals contaminated waste streams are needed. In this regard, the zeolites have a great potential for removing heavy metal from industrial wastewater. The existence of a net negative structural charge in the structure promotes a strong affinity for metal cations which give the adsorption properties of these supports. Sodium, potassium and other positively charged exchangeable ions occupy the channels within the three-dimensional structure and can be replaced by heavy metals [2].

Biosorption is the accumulation of metals by biological materials without active uptake and can be considered as a collective term for a number of passive accumulation processes which may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation [3]. Other authors [4] referred that biosorption is the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediation or by purely physico-chemical pathways of uptake.

Bacteria are quite adequate for heavy metals biosorption, due to their ability to sorb metal ions, suitability for natural environments and low cost. *Arthrobacter viscosus* is a good exopolysaccharide producer, which, by itself, would allow foreseeing good qualities for support adhesion and for metal ions entrapment [5]. The new systems combine the biosorption properties of the microorganism with some characteristics of the heterogeneous catalysts, such ion exchange properties and shape selectivity.

Among different heavy metals that may be removed from liquid solutions by biosorption, chromium demands special attention as it may present several oxidation states. The chromium was removed from $K_2Cr_2O_7$ liquid solutions with different initial concentrations. A possible reduction of $Cr_2O_7^{2-}$ may be performed by the biofilm itself. The metabolic reduction has studied and modelled for different pure bacterial cultures [6]. A*rthrobacter viscosus* bacterium supported on the zeolite reduce Cr(VI) to Cr(III) and the Cr(III) is retained in the zeolite by ion exchange.

One of the approaches for the preparation of redox-active zeolite catalysts is the encapsulation of metal complexes in the zeolite channels where the general idea is to combine the solution like activity with shape-selective control induced by the zeolite. The heterogenisation of transition metal complexes in zeolites [7], clays [8] and hybrid inorganic-organic matrix obtained by sol-gel technique [9] is specially interesting and it was shown that the methodologies used for encapsulation/immobilization of the metal complexes are largely determined by the supports. The present work associate the biosorption studies to the encapsulation of metal complexes in zeolites for the applications in heterogeneous catalysis in mild conditions. In order to prepare Y and X zeolites with entrapped chromium complex, the diffusion of functionalized ligands into the zeolite through the pores was promoted, where form complexes with the intrazeolite metal ion, obtained by biosorption method [10-11].

Cr(III) complex with pyridazine ligand is typically four coordinate with a planar geometry around the metal centre. This low coordination geometry allows the metal centre to axially coordinate extra ligands, which can be the zeolite framework (inducing a more effective complex encapsulation) and leaving the other coordination site for the oxidant in the catalytic reactions [12]. The pyridazine skeletons are commonly found in compounds exhibiting a wide range of biological activity [13]. The derivatives of pyridazines could also find application as ligands in supramolecular chemistry and in metallic complexes which exhibit catalytic properties [14-15].

In this work we report the encapsulation of a pyridazine Cr(III) complex prepared from a robust biosorption system consisting of a bacterial biofilm supported on faujasite (FAU) zeolites. The NaY or NaX was placed as a support in inoculated medium with *Arthrobacter viscosus* bacterium, in batch experiments. The new system, the biosorbent supported in zeolites was tested with low concentration of chromium. Total metal cations concentrations were measured with an atomic absorption spectrophotometer. The results showed that the maximum removal efficiency was 20% for Cr in both systems based in NaY or NaX, and the *Arthrobacter viscosus* bacterium supported in zeolite reduce Cr(VI) to Cr(III). The Cr(III) is retained in the zeolite by ion exchange and after the intrazeolite metal ion was coordinated with pyridazine ligand.

2. EXPERIMENTAL

2.1. Materials and Reagents

Arthrobacter viscosus was obtained from the Spanish Type Culture Collection of the University of Valência. Aqueous chromium solutions were prepared by diluting $K_2Cr_2O_7$ (Aldrich) in distillated water. The faujasite zeolites NaY and NaX were obtained from W.R. Grace. The zeolites were calcinated at 500 ^oC during 8 hours under a dry air stream prior to use. The 3,6-dichloropyridazine and all the other chemicals used for the reaction synthesis of the ligand were purchased from Aldrich and used as received.

2.2. Methods

2.2.1. Preparation of the biofilm supported in zeolites

All experimental work was conducted in triplicate. 1.0 g of the Y or X zeolites was placed in a 250 mL Erlenmeyer flask to which 150 mL of the different dichromate solutions (50, 100 and 150 mg_{Cr}/L) and 15 mL of *Arthrobacter viscosus* culture media were added. For the microorganism growth it was used a media with 5 g/L of peptone, 3 g/L of malt extract, 3g/L of yeast extract and 10 g/L of glucose, sterilized at 120^oC for 20 min. The Erlenmeyer flasks were kept at 28 ^oC, with moderate stirring. Samples (1 mL) were taken, centrifuged and analyzed for metals using atomic absorption spectrophotometry (AAS).

2.2.2. Encapsulation of the complex in zeolites

Step 1. Synthesis of ligand 3-ethoxy-6-choropyridazine

3,6-Dichloropyridazine (6.7 mmol) was heated at reflux with NaOEt (8.1 mmol) in ethanol (100 mL) for 3 hours, then cooled and the solvent was removed under reduced pressure to give an oily solid. This solid was poured into water (100 mL) and neutralized with a solution of HCl (10%). The reaction mixture was then extracted with dichloromethane (2x50 mL). The organic extract was dried with $MgSO_4$ and the solvent was evaporated under reduced pressure to give the crude product which was purified by "flash" chromatography on silica with increasing amounts of ether in petrol ether (b.p. 40-60 ^oC) as eluent.

3-Ethoxy-6-chloropyridazine was obtained in 62 % yield as a colourless solid, mp 49-51 ^oC; ^1H NMR ($CDCl_3$) δ 1.45 (t, 3H, J = 7.2 Hz, $OCH_2C\underline{H}_3$), 4.56 (q, 2H, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 6.94 (d, 1H, J = 9.0 Hz, 4-H), 7.36 (d, 1H, J = 9.0 Hz, 5-H); IR (Nujol) ν 2924, 1587, 1529, 1484, 1424, 1364, 1279, 1182, 1139, 1112, 1094, 1070, 1031, 916, 900, 855, 821, 778, 701, 668 cm^{-1}.

Step 2. Encapsulation of the Cr(III) complex.

Two Cr-zeolites samples obtained from 100 mg_{Cr}/L solution in biosorption method were used for the encapsulation of pyridazine Cr(III) complex. The modified zeolites with metal complex were designated as $[CrL_n]$Cl-Y or $[CrL_n]$Cl-X where L represents the ligand $[C_6H_7N_2ClO]$.

The Cr-Y and Cr-X were calcinated at 500 ^oC during 6 hours under a dry air stream before the encapsulation in order to remove the organic matter of the *Arthrobacter viscosus* bacterium. An amount of 0.63 mmol (100 mg) of ligand was dissolved in 100 mL of Et_2O. After complete dissolution, this mixture was added to 0.5 g of Cr-zeolites and was refluxed for 24 hours. The green solid was collected, washed with ethanol and dried in an oven at 60 °C for 8 hours. The samples were treated with 0.01 M solution of NaCl for 8 hours and

subsequently filtered and washed with hot distilled water. The samples were purified with ethanol (6 hours) using Soxhlet extraction to remove the residual metal complex physically adsorbed on the external surface. Finally, the materials were dried in an oven at 90 °C, under vacuum, for 12 hours.

2.3. Characterization procedures

Total metal cations concentrations in biosorption method were measured using a Varian Spectra AA-400, an Atomic Absorption Spectrophotometer, AAS. ^{1}H spectra were recorded using a Varian Unity Plus spectrometer at 300 MHz, chemical shifts being given in ppm. The UV-visible absorption spectra of samples were obtained using a Shimadzu UV/2501PC spectrophotometer using quartz cells. Room temperature FTIR spectra of the samples were recorded on a Bomem MB104 spectrometer. The transmission spectra of the powdered samples were obtained using KBr pellets over the range 4000-600 cm^{-1} by averaging 20 scans at a maximum resolution of 4 cm^{-1}. X-ray diffraction patterns were recorded using a Philips Analytical X-Ray model PW1710 BASED diffractometer system. The solids samples were exposed to the Cu Kα radiation at room temperature in a 2θ range between 20 and 60^{o}. Thermogravimetric analysis of samples were carried out using TGA 50 Shimadzu instrument under high purity helium supplied at a constant 50 mL min^{-1} flow rate. All samples were subjected to a 6 °C min^{-1} heating rate and were characterized between 25 and 600 ^{o}C. The elemental chemical analysis (Si, Al, Na and Cr) were performed by University of Minho, Departamento de Ciências da Terra, using inductively coupled plasma atomic emission spectroscopy.

3. RESULTS AND DISCUSSION

3.1. Biosorption method

Figure 1 shows the removal of chromium by a biofilm of *Arthrobacter viscosus* supported on NaY and NaX zeolites. In steady-state conditions no difference between the two supports is detected, the same happening at higher initial concentrations. The maximum removal efficiency was 20% for chromium in both systems.

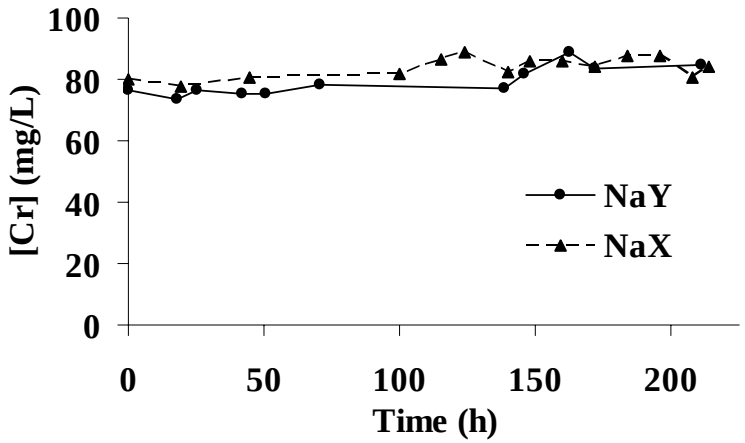
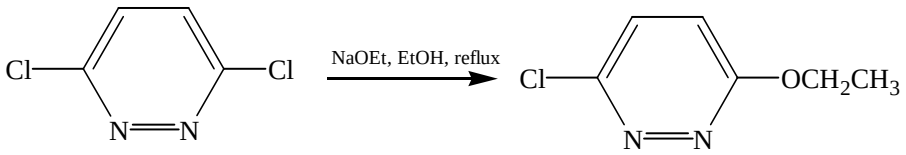


Fig. 1. Removal of chromium by a biofilm of *Arthrobacter viscosus* supported on Y and X zeolites

The removal of chromium in both systems was fast and presented a typical biosorption kinetics, which includes two phases: the first one is associated with the external cell surface, biosorption itself, and the second one is an intra-cellular accumulation/reaction, depending on the cellular metabolism [16]. The relatively low maximum removal efficiency seems to be connected with the no affinity between the anionic charge of the metal ion and the anionic charge of the bacteria and with the high ionic radius of the chromium ion. Although, zeolites have high surfaces areas (500-700 m^2g^{-1}), most of this area is internal. These limitations probably reduce the adhesion of the *Arthrobacter viscosus* bacterium on the support.

3.2. Encapsulation of pyridazine Cr(III) complexes

This work constitutes the preliminaries studies in which it was found that the chromium complex with heterocyclic functionalized ligands present good properties for catalysis. The 3-ethoxy-6-chloropyridazine ligand was synthesized through a nucleophilic aromatic substitution, from 3,6-dichloropyridazine when refluxed with the alkoxyde in the corresponding alcohol (Scheme 1).



Scheme 1. Synthesis of 3-ethoxy-6-chloropyridazine ligand

The synthesis of Cr(III) complexes encapsulated in supercages of Y and X zeolites was carried out by a flexible ligand method which involves the reaction of 3-ethoxy-6-choropyridazine ligand with chromium exchanged zeolite by biosorption method. Complexation of $[C_6H_7N_2ClO]$ with Cr(III) were accompanied by the color change. After the encapsulation, the samples became green. The remaining uncomplexed metal ions in the Y or X zeolite were removed by exchanging with NaCl solution. Soxhlet extraction removed excess ligand and probably metal complex formed on the surface of the zeolite due to leaching [12]. After extensive extraction, no change in colour in the modified zeolites was observed. In order to characterise the resulting catalysts and to assess the efficiency of the encapsulation process, the parents zeolites, the Cr-Y and Cr-X zeolites obtained from biosorption method, and samples of $[CrL_n]Cl$-Y and $[CrL_n]Cl$-X, were studied by several techniques and the obtained results were compared.

The mole Si/Al ratio, the percentage of Cr, determined before and after encapsulation, obtained by bulk chemical analysis and estimated number of metal molecules per unit cell are presented in Table 1.

In both faujasite zeolites, the decrease in the Si/Al ratio between NaY to NaX is due to the Na^+ increase in NaX which means that free space will be available for adsorption or encapsulation of the complexes [10, 17]. This increase in Na^+ must be responsible for the amount of chromium in NaX after biosorption method.

Table 1
Chemical analysis of the zeolite samples

	Si/Al	Cr content (wt %)	Number of metal per unit cell
NaY	2.88	--	--
NaX	1.63	--	--
Cr-NaY[(a)]	2.88	0.14	0.45
Cr-NaX[(b)]	1.63	0.19	0.67
$[CrL_n]Cl$-Y[(c)]	2.88	0.13	0.42
$[CrL_n]Cl$-X[(d)]	1.63	0.16	0.57

[(a) and (b)] samples obtained from biosorption method
[(c) and (d)] samples obtained after encapsulation process

A comparison of the Si/Al ratio in the different stages of the preparation of theses catalysts suggests that these procedures do not modified the structure of the zeolites. After encapsulation procedure in both systems a decrease in amount of chromium content was observed. The leaching of the complex is more pronounced in the case of the NaX.

The powder X-ray diffraction patterns of NaY or NaX, Cr-Y and Cr-X and encapsulated metal complexes were recorded at 2θ values between 5 and 70. No significant variations were observed in the diffraction patterns due to the structure of zeolites before and after encapsulation procedure as well as before and after biosorption method. In fact, a comparison of the X-ray diffractograms of the parent zeolites with those of encapsulated metal indicates that the structure of the zeolites is retained upon metal complex encapsulation. No new peaks due to neat complex were detected in the encapsulated zeolite due to probably very low percent loading of metal complexes.

Complementary studies using a thermal analysis (TGA) contributed to a better understanding of the effect of the metal complex on the thermal properties of framework zeolite. The zeolites with microorganism present the same behavior of the original support. The TGA curve shows a weight loss at 120 ^{o}C which may be attributed to the removal of intrazeolite water. During the encapsulation of Cr(III) complex in Y and X zeolites, the weight loss occurs in two major stages in the broad temperature range. The first stage presented a weight loss in the temperature above 130 ^{o}C. This loss is due to removal of intrazeolite water and Cl^- ions as well as water associated with encapsulated complex. In the second stage of TGA curve, 3.0 % weight loss in the temperature at 480 ^{o}C is observed for $[CrL_n]Cl$-X and 1.5 % at 550 ^{o}C for $[CrL_n]Cl$-Y which corresponds to the decomposition of organic matter in the encapsulated complexes. The difference in values of weight loss and temperature of the decomposition of the encapsulated complexes suggests: (i) different interactions of the complex with the framework zeolites or (ii) different coordination of the ligand with chromium inside the zeolites. In fact, the X-zeolites suffer a reduction in the free space of the FAU supercages due an increase in the Na^+ content. Thus, it is expected that the Cr(III) complexes in X zeolite exhibit some distortions relative to the complex in Y zeolite due to steric hindrance caused by the reduction in free volume [17, 19].

The data obtained by vibrational spectroscopy (FTIR) were used to provide structural information of the framework zeolite and the encapsulated chromium complex. In both

systems with Y and X zeolites, the spectra of the parent zeolites and modified zeolites are dominated by the strong zeolite bands: broad band at 3700-3300 cm^{-1} is attributed to surface hydroxylic groups and bands corresponding to the lattice vibrations are observed in the spectral region between 1300-450 cm^{-1} [18]. No shift or broadening of these FAU zeolites vibrations are observed upon inclusion of the complexes, which provides further evidence that the framework zeolite remains unchanged. The bands due to the encapsulated Cr(III) complexes are weaker, as they are diluted in the FAU zeolites, and can only be observed in the region 1650-1200 cm^{-1}, where the zeolite does not absorb.

In the range of 1650-1200 cm^{-1} the infrared spectra of entrapped complexes in the FAU zeolites are different. In the case of the Cr(III) complex in Y zeolite, no bands attributed to neat complex was detected in the encapsulated complex in zeolite due to probably very low percent loading of metal complex. These results are in accordance to the TGA observations.

The infrared spectra of NaX (A), Cr-NaX (B) and $[CrL_n]Cl$-X (C) in the range 1800-1000 cm^{-1} are presented in Fig. 2. The IR spectra of the Cr-NaX (B) exhibit a band at 1385 cm^{-1} which are assigned to the presence of chromium after biosorption method. The entrapped complexes exhibit IR bands at 1255 and 1208 cm^{-1} which are shifted from those shown by the free ligand in the bands assigned to the X zeolite. The presence of these bands indicates that the metal complex has been encapsulated in the X zeolite, but also suggests that its structure is not identical to those formed with encapsulated complex in Y zeolite.

These observations in FTIR analysis in both systems Y and X zeolites can also be attributed to: (i) distortions of the complexes, and/or to (ii) interactions with zeolite structure, very weak in the case of Y.

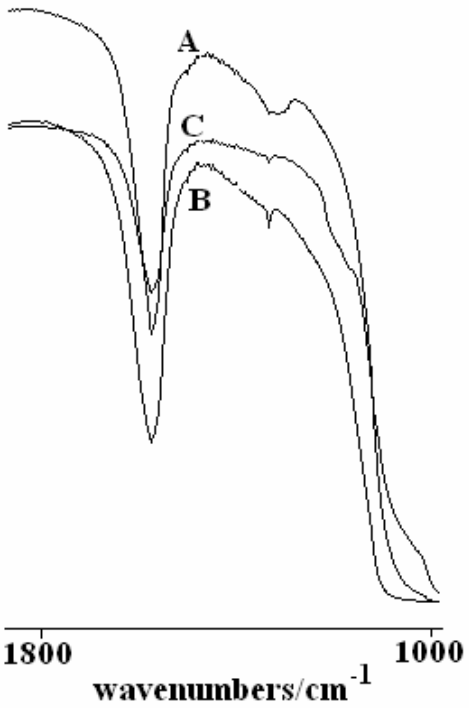


Fig. 2. Infrared spectra in the range 1800-1000 cm^{-1}; (A) NaX, (B) Cr-X and (C) $[CrL_n]Cl$-X.

In order to get the maximum information about encapsulation procedure, the residual solvents obtained in the different stages of the Cr(III) complex encapsulation were analyzed by UV/Vis spectroscopy.

The electronic spectrum of the ligand exhibits two bands at 280 and 217 nm due to n-π^* and π-π^* transitions of the pyridazine group.

UV/Vis spectra measurement performed in the residual solvent before Soxhlet extraction for both systems with Y and X zeolites, exhibit a band at 280 nm attributed to the presence of the ligand. This residual ligand detected show that partial amount of ligand was coordinated with chromium inside the zeolite. However, in the residual solvents from the treatment with NaCl solution and Soxhlet extraction no electronic bands due to the ligand could be detected in the UV/Vis spectra.

4. CONCLUSION

A biofilm of *Arthrobacter viscosus* supported on Y and X zeolites is able to remove chromium from dilute solutions and can be applied in wastewater remediation. The reduction of Cr(VI) to Cr(III) is performed by the biofilm itself. This metal is exchanged in the zeolite and coordinated with pyridazine ligand.

The results obtained for the encapsulation process confirm that the metal complex can be encapsulated in the FAU supercages, without damage to the original matrix or loss of its crystallinity. The data indicate that chromium complexes in NaX exhibit structural and electronic properties different from those of the same complexes in NaY. The present results are very encouraging and warrant further research on heterogeneous catalysis in mild conditions.

ACKNOWLEDGMENTS

We thank Dr. A.S. Azevedo for collecting the powder diffraction data and Dr. C. Ribeiro for chemical analysis from Departamento de Ciências da Terra of Universidade do Minho. This work was supported by the FCT (Portugal) through project POCTI and FEDER (ref: POCTI/37816/QUI/2001 and POCTI/44840/QUI/2002).

REFERENCES

[1] S.E. Bailey, T.J. Olin, R.M. Bricka and D.D. Adrian, Water Res., Vol. 33, No. 11 (1999) 2469.
[2] A. Corma and H. Garcia, Eur. J. Inorg. Chem. (2004) 1143.
[3] J.R. Duncan, D. Brady and A. Stoll, Environ. Technol. No. 15 (1994) 429.
[4] G.M. Woodburn, , Q. Yu and J.T. Matheickal, Water Res., 32 (1999) 400.
[5] C. Quintelas and T. Tavares, Biotecnol. Letters, Vol. 23 (2001) 1349.
[6] Y.T. Wang and H. Shen, Water Res., 7 (1997) 727.
[7] I. Neves, C. Freire, A.N. Zakharov, B. Castro and J.L. Figueiredo, Colloids Surf. A. Physicochem. Eng. Aspects 115 (1996) 249.
[8] F. Costa, C.J.R. Silva, M.M.M. Raposo, A.M. Fonseca, I.C. Neves, A.P. Carvalho and J. Pires, Micropor. Mesopor. Mat., 72 (2004) 111.
[9] I. Montinho, V. Boev, A.M. Fonseca, C.J.R. Silva and I.C. Neves, Sol. Sta. Sci., 5 (2003) 203.
[10] P.P. Knops-Gerrits, D.E. DeVos, F. Thibault-Starzyk and P.A. Jacobs, Nature 369 (1994) 543.
[11] D.E. DeVos, P.P. Knops-Gerrits, R.F. Parton, B.M. Weckhuysen, P.A. Jacobs and R.A. Schoonheydt, J. Inclusion Phenom. Mol. Recog. Chem. 21 (1995) 159.
[12] M.R. Maurya, S.J.J. Titinchi and S. Chand, J. Mol. Catal. A: Chem, Vol. 24, 2 (2004) 257.
[13] A.R. Katritzky, C.W. Rees and E.F.V. Scriven (eds.), Comprehensive Heterocyclic Chemistry II, Vol. 6, Pergamon Press, Oxford, 1996.
[14] B.G. Harvey, A.M. Arif and R.D. Ernst, Polyhedron, 23 (2004) 2725.
[15] S. Brooker, Eur. J. Inorg. Chem., 10 (2002) 2535 and references cited therein.
[16] M.T. Tavares, C. Martins and P. Neto, In: A.K. Sengupta (eds.), Hazardous and Industrial Wastes, Lancaster: Tecnomics Publishing Co., 1995, pp. 223.
[17] P.P. Knops-Gerrits, F.C. De Schryver, M. van der Auweraer, H. van Mingroot, X.Y. Li and P.A. Jacobs, Chem. Eur. J. 2 (1996) 5.
[18] B. Imelik and J.V. Vedrine (eds.), Catalyst Characterization, Plenum Press, New York, 1994.
[19] R. Ferreira, M. Silva, C. Freire, B. Castro and J.L. Figueiredo, Micropor. Mesopor. Mat., 38 (2000) 391.