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ACID-BASE PROPERTIES OF MONTMORILLONITE AND INTERACTIONS WITH ENZYMES THROUGH REM INVESTIGATIONS

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Abstract

Interactions of montmorillonite-rich clay materials with enzymes were investigated. A 2:1 layer type raw clay mineral was purified, then ion-exchanged with Na^+, K^+ (Na-Mt and K-Mt), Ca^{2+} and Mg^{2+} (Ca-Mt and Mg-Mt), and further contacted with various enzyme suspensions containing α-amylase, β-amylase, cellulase, invertase, lipase, peroxidase, pectinase, urease, protease or tripsine. The acid and base properties were measured via thermal programmed desorption of NH_3 and CO_2, respectively. It was found that Ca-Mt and Mg-Mt exert stronger interactions with enzymes, generating coarser flake-shaped clay particles, as compared to Na-Mt and K-Mt. This specific behaviour is explained in terms of clay affinity towards enzymes, and correlated to the clay acid-base properties, that could have a great interest for soil chemistry. The shape and the size of clay particles were also discussed through reflection electron microscopy.

***Keywords**: montmorillonite, clay intercalation, ion-exchanged clays, NH_3-TPD, CO_2-TPD, acid-base properties, alkaline, alkaline earths, enzymes, environmental, coagulation-flocculation, immobilization.*

Résumé

Le présent travail traite des interactions de certaines enzymes avec la surface d'une argile riche en montmorillonite. Un matériau minéral à base d'argile de type 2:1 a été préalablement purifié, et ensuite échangé ioniquement par Na^+, K^+ (Na-Mt et K-Mt), Ca^{2+} et Mg^{2+} (Ca-Mt et Mg-Mt), et finalement mis en contact avec diverses suspensions contenant des enzymes de type α-amylase, β-amylase, cellulase, invertase, lipase, peroxydase, pectinase, uréase, protéase ou trypsine. Les propriétés acido-basiques ont été mesurées par voie de thermodésorption programmée de NH_3 et CO_2, respectivement. Il a été établi que les échantillons Ca-Mt et Mg-Mt obtenus manifestent de fortes interactions avec les enzymes, donnant naissance à des particules d'argiles floconneuses et difformes, par comparaison aux échantillons Na-Mt et K-Mt. Ce comportement spécifique est expliqué en terme d'affinité d'une argile contenant des actions alcalino-terreux envers les enzymes, et pourrait être corrélé aux propriétés acido-basiques induites par ces ions. Ce résultat est d'une grande importance pour la chimie des sols. La forme et la taille des particules d'argile sont analysées par microscopie électronique à réflexion.

***Mots clés**: montmorillonite, intercalation des argiles, échange d'ions, thermodésorption programmée de NH_3, TPD de CO_2, propriétés acido-basiques, alcalins, alcalino-terreux, environnement, immobilisation des enzymes.*

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ملخص

يعالج هذا العمل تفاعلات بعض الأنزيمات مع سطح قطعة صلصل غنية بـ montmorillonite. مادة معدنية ذات قاعدة صلصالية من نمط 1:2 قد تم تنقيتها مسبقا، ثم استبدلت أيونات Na^+ و K^+ (Na-Mt و K-Mt)، Ca^+ و Mg^{2+} (Ca-Mt و Mg-Mt)، وأخيرا أجري لها اتصال مع معاليق تحتوي على مختلف أنزيمات. لقد ثبت أن العينات Ca-Mt و Mg-Mt المحصل عليها أظهرت تفاعلات قوية مع الأنزيمات، مولودة جزيئات من الصلصال قطنية (المظهر) ومشوهة، مقارنة بالعينات Na-Mt و K-Mt. هذا السلوك الخصوصي يفسر على أنه تجاذب للصلصال الذي يحتوي على أسهم ألكالينية – ترابية – تجاه الأنزيمات، ويمكن أن تخضع لترابط مع الخواص الحمضية- القاعدية التي حثتها الأيونات. هذه النتيجة كبيرة الأهمية بالنسبة لكيمياء الأتربة. وقد تم تحليل شكل وقامة جزيئات الصلصال بالمجهر الإلكتروني الانعكاسي.

***الكلمات المفتاحية:** Montmorillonite، تداخل الصلصال، تبادل الأيونات، المج الحراري، الخواص الحمضية القاعدية، الكالين، الكالينية الترابية، البيئة.*

Due to their ionic structure, clays have surface charges and exert electrostatic forces on the species present in the surrounding medium. This makes them to be regarded also as pH-modifying agents and interesting supports for enzyme immobilization in soil. There exists a narrow similarity between clays interactions with enzymes in water and those that occur in soil. Many researches [1-4] have already provided a general overview of the interactions occurring between soil clays and enzymes, which still remain to be thoroughly elucidated. In nature, according to the moisture level and the cations present, enzymes can diffuse into the soil pore network more or less freely, but upon desiccation, a sort of enzyme immobilization takes place, conferring to the enzymes specific configuration. The behaviour of these so-called "naturally immobilized enzymes" could be of great interest for environmental purposes. In this regard, clays are well known to be effective coagulating-flocculating agents for both proteic and cationic pollutants in wastewater treatment.

Enzyme interactions with a clay surface will be governed by both electrostatic and hydrophobic considerations, and, here, the clay nature plays a predominant role. For instance, montmorillonite displays marked swelling properties making it suitable for enzyme immobilization, without significant activity depletion, as compared to kaolinite [4]. This is why our interest has been focused towards montmorillonite.

Besides, the enzyme nature, pH and the presence of cations on the clay surface or in the surrounding water may also influence the strength of

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these interactions. Increasing cation contents in water can induce ion-exchange on the clay sheets, and, consequently, change in both the clay properties and pH. According to the counter ion, pH increases can also occur, thereby inducing enzyme inactivation, due to changes in the structure and orientation of the adsorbed enzymes [5-9]. When mixed with proteins or enzymes in aqueous phase, clays behave either as a base or an acid, according to both the charge-compensating ion and free cations. The resulting electrostatic forces can lead to coagulation-flocculation or to dispersion of the clay, according to the interaction strength. Here, one must expect that the presence of any impurity, either between the clay sheets or in the surrounding water, must play a certain role in the flocculation ability of the clay, expressed in terms of the critical coagulation concentrations (ccc). These findings provide evidence that the interactions occurring between clay sheets and a given enzyme can be greatly influenced by the presence of cations at the clay surface, presumably due to the effect of the acid-base properties of the clay surface, as it will be examined further.

The concept of acidity-basicity will become probably one of the most useful criterion for estimating the dispersivity of a given clay and its possible interactions with enzymes in aqueous phase [10]. For this purpose, one has investigated the effects of purification of montmorillonite-containing clays and of the ion-exchange with alkaline and alkaline earth's metals upon the acid-base properties of montmorillonite. Clay interactions with enzymes in aqueous media must be reflected by the way enzymes intercalate clay sheets upon dehydration, and insights through reflection electron microscopy could be very useful in this regard.

EXPERIMENTAL

1. Purification, ion exchange and interlayering with enzymes

A 2:1 layer type raw clay mineral was obtained from the Source Repository (Valea Chioarului, Romania), and purified till the disappearance of the non-desired X-ray diffraction (xrd) lines, according to a procedure fully described elsewhere [10,11]. The as-purified montmorillonite-rich material (Mt) has a Si:Al ratio of 2.30, a surface area of ca. 300 $m^2.g^{-1}$ and a cation exchange capacity (CEC) of 0.37 $mmole.g^{-1}$. This material was further ion-exchanged into the Na-Mt form, and then repeatedly impregnated in nitrate salt aqueous solutions containing K^+, Ca^{2+} and Mg^{2+} cations, under stirring, for 5-6 hours at 80°C, so as to be fully cation-exchanged into the respective forms. Removal of the remaining physically adsorbed salts was achieved through dialysis in hot deionized water (40-50°C).

A clay suspension (clay:water ratio of 5:100 w/w) was further contacted with various enzymes (enzyme:clay ratio = 1:100 w/w) and benzene (benzene:ethanol:water= 0.5:1:100). Enzymes such as α-amylase, β-amylase, cellulase, invertase, lipase, peroxidase, pectinase, urease, protease and tripsine were supplied by ICA-Baneasa, Romania). The experiments were performed in a perfectly neutral buffer solution of pH = 7.0 (0.1 M Na citrate/phosphate, with a buffer: clays suspension ratio = 1:1 v/v, at room temperature for 48 h. The resulting interlayered clays were then centrifuged and repeatedly washed in deionized water, and centrifuged once again and air- dried overnight at 25°C.

2. Clay characterization

The interlayered clays were characterized through xrd analysis using a Philips PW 1710 diffractometer with a CuK_α radiation (λ = 1.54051 Å). Furthermore, in order to provide the topography of the clay surfaces, insights through reflection electron microscopy using a REM-LEO 1530 GEMINI instrument. The samples were not sputtered (covered with carbon or gold) to avoid artefacts. The analysis was focused on the Si reflection, and the images were digitally enhanced. In this technique, heavy elements will display higher reflection than the light ones.

Acid-base measurements were carried out via Thermal Programmed Desorption (TPD) of NH_3 or CO_2, in the temperature range 150-575 ºC, using nitrogen as the carrier gas. The strength distributions of the acid and base sites were estimated by TPD pattern deconvolution, assuming that each peak obeys the Gauss normal distribution [12].

RESULTS

1. Effect of clay purification

Before purification, the crude clay material shows a broad xrd *001* line, due to the wide variety of species sandwiched between the clay sheets. In this case, the clay sheets have various orientations that determine an expanded structure, as shown by figure 1. A thorough purification must involve several consecutive steps such as ultrasound exposure (removal of quartz, cristoballite and some oxides), acid washing (oxides), repeated dispersion (salts), calcination at 500°C (organic compounds, etc.), ion-exchange with the same cation and dialysis (to remove the physically adsorbed salt).

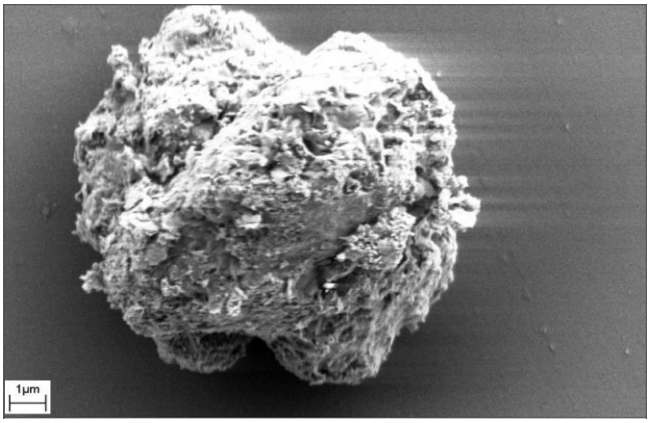

Figure 1: RE micrograph of crude clay.

The resulting Na-Mt sample was identified as being nearly pure montmorillonite devoided of any amorphous phases, and a sharp *001* xrd line was obtained, as previously reported [10]. In this case, the clay has a compact structure containing only parallel sheets, as illustrated by figure 2.

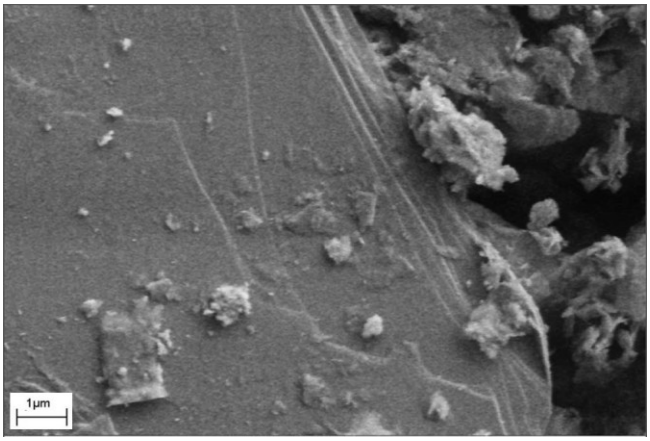

Figure 2: RE micrograph of purified Na-Mt.

Thus, clay purification reduces the clay dispersion in water, and removal of organic matter seems to be the most preponderant factor, to the pronounced hydrophobic behaviour. Evidence in this regard was provided by further impregnation of purified Na-Mt with benzene, generating bulky clusters formed by small benzene drops surrounded by clay scales (Fig. 3). Here, one must mention that Na-Mt acts as any tensioactive agent in the presence of organic compounds. This effect was observed only with alkali-exchanged montmorillonite.

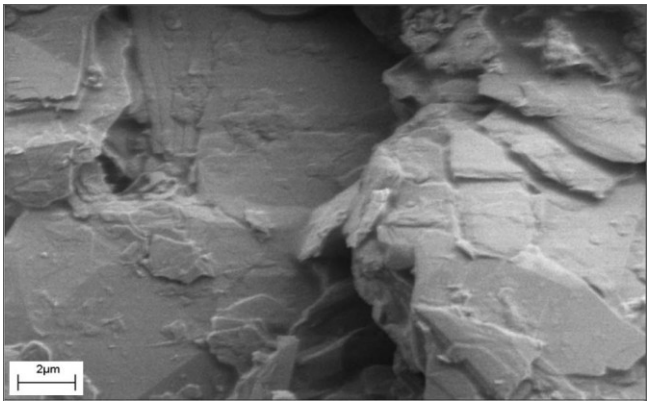

Figure 3: RE micrograph of benzene-Na-Mt clusters.

Furthermore, it was also found that removal of crystalline metal oxides increased the coagulation tendency, while removal of amorphous oxides has no significant influence on clay dispersivity. After a thorough purification and prior to Na-ion exchange, clays display the highest flocculation ability and the lowest coagulation tendency.

2. Effect of ion-exchange

As compared to Na-Mt and K-Mt, alkaline earth-exchanged montmorillonites (Ca-Mt and Mg-Mt) show also perfectly parallel sheets but with higher interlamellar distance, as illustrated by figure 4. As a general tendency, the interlamellar distance must increase with increasing cation solvatation capacity [10], and one must expect that Na-Mt and K-Mt will display the weakest dispersivities in water.

In aqueous medium, bivalent cations induce positive charges in both faces of the clay sheets. It results in a repulsion effect occurs between the clay sheets, and water molecules are attracted through their oxygen atoms by the bivalent cations fixed on the clay sheet. According to both the pH of the surrounding medium and the acid-base properties of the clay sheets, this water layer could more or less thin. This will certainly have an influence upon the sedimentation time of the clay. Indeed, qualitative observations showed that the average time needed for a total clay sedimentation increased according to the following sequence: Na-Mt (8-12 hours) < K-Mt (12-18 days) < Ca-Mt (2-3 days) < Mg-Mt (3-4 days). This sequence is similar to that of increasing acidity, suggesting, thereby, a possible involvement of the acid-base properties of the ion-exchanged clays, as it will be examined later.

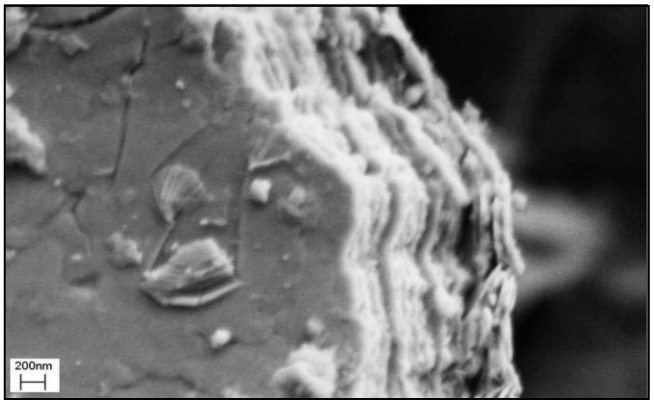

Figure 4: RE micrograph of Ca-Mt particle.

In other words, clay dispersion increased with increased mass and charge of the cation present at the surface. Such a result is of a great importance for flocculation processes, since clay dispersivity could be predicted according to the cation present on the sheet surface or in the surrounding water.

3. Acid-base properties induced by ion-exchange

The acid-base properties are regarded as being the main factor that can influence significantly the clay behaviour [13]. The latter can include many properties, e.g. dispersivity, swelling, possible interactions with enzymes in aqueous phase etc. TPD measurements showed that, as a general feature, both ammonia and carbon dioxide adsorb at relatively low temperature (120 and 70°C, respectively) and desorb from clay surface upon heating, regardless to the exchangeable cation.

Xrd analyses showed that no modifications of the clay structure were caused by TPD, indicating that the adsorption-desorption phenomenon is reversible, and that clays display both acid and base properties. One assumes that the acid-base properties of the clay are mainly due to montmorillonite, because the remaining traces quartz and cristoballite have no influence on the acid strength distribution, as shown by preliminary TPD measurements performed on pure [quartz + cristoballite] mixtures [10].

Ion-exchange with different cations induces various acid-base-properties, as illustrated by figures 5 and 6. The total number of acid or base sites was expressed in terms of

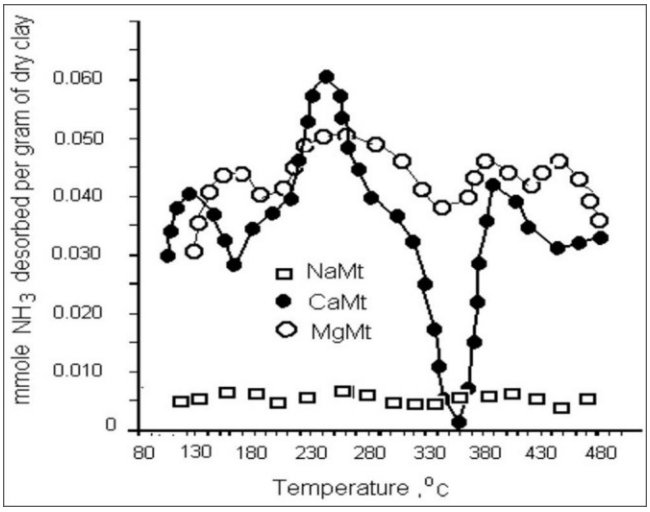

Figure 5: NH_3-TPD patterns of montmorillonite according to the exchangeable cation.

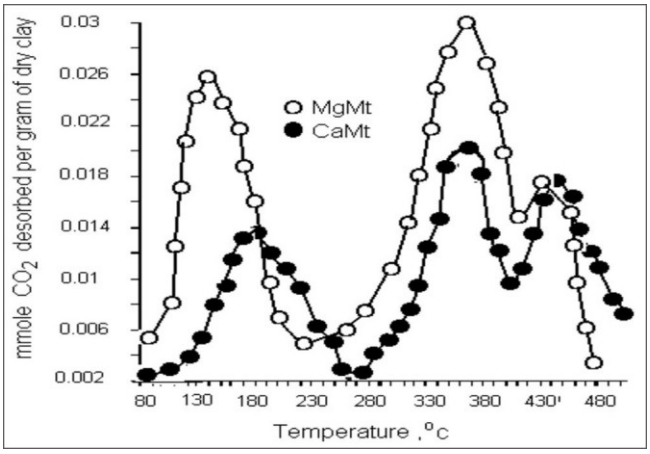

Figure 6: CO_2-TPD patterns of montmorillonite according to the exchangeable cation.

mmoles of ammonia (or carbon dioxide, respectively) desorbed per gram of dry clay upon increasing temperature.

One must emphasize that the shapes of both the NH_3-TPD and CO_2-TPD patterns for all cation-exchanged clays showed various peaks, which correspond to different acid or base strengths. Nevertheless, these acid-base strength distributions vary according to the cation introduced. The ranges of desorption temperature are 80-500°C for CO_2-TPD, and 120-550°C for NH_3-TPD, indicating that on such clay surface, acidity is slightly higher than basicity. It was also found that Ca-Mt and Mg-Mt are more acidic (in terms of mmoles NH_3 desorbed) but less basic (in terms of mmoles CO_2 desorbed) than -Mt and Na-Mt. As a general feature, the amount of desorbed CO_2 decreases with increasing amount of desorbed NH_3 (Fig. 7).

In other words, the clay acidity increases with decreasing base character and inversely. As shown by figure 7, there exists a certain reverse proportionality between the acid and base properties of clays. Thus, one must expect that basicity can be totally shaded by pronounced acidity, and reversely, as already reported [10,14,15].

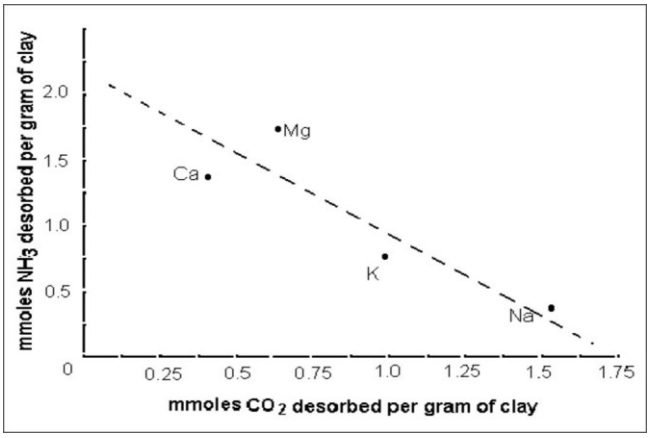

Figure 7: Reverse proportionality between the acid and base properties of clays according to the exchangeable cation.

According to the results obtained, it appears that there are no pure acidic clays without bases properties. Regardless to the acidity level for a given clay, the base properties may arise unavoidably from the oxygen atoms surrounding the respective cations, as already asserted by some authors [16].

The acid strengths of exchanged clays were expressed in terms of temperature of the peak desorption within a certain range, and the strength distribution was estimated by estimating each peak area. As shown in Table 1, ion-exchange with Na^+ and K^+ cations induces mostly weak to medium acidity and basicity. This is manly due to their small polarizing effect, as previously reported [17,18]. In contrast, Ca^{2+} and Mg^{2+} display slightly stronger acidity and basicity.

Table 1: Distribution of the acid strength via NH_3-TPD and basicity via CO_2-TPD.

Sample	Distribution of the acid strength (%)		Distribution of the base strength (%)	
	Weak to medium 120-300°C	Medium to strong 300-500 °C	Weak to medium 80-380 °C	Strong 380-500 °C
NaMt	95	5	75	25
KMt	85	15	72	28
MgMt	75	25	70	30
CaMt	70	30	68	32

However, the acidity level is higher than that of basicity, regardless to the cation introduced on the clay surface. As a general feature, it appears that alkaline cations confer to clays low acidity, and weak acid strength, as compared to alkaline-earths. These findings are of a great importance for explaining the role of the exchangeable cation in the interactions occurring between clays and enzymes in aqueous phase.

4. Clay interlayering with enzymes

In order to elucidate the interactions occurring between clays and enzymes, one must understand, in a first step, what effects clays might have on the colloidal forces, and then, the rheological behaviour of a clay phase. For this

purpose, Reflection Electron Microscopy (rem) has been used to study the effect of a model clay colloid (montmorillonite) on the interparticle forces acting between enzymes and clay surface (Fig. 8 to Fig. 17). In this study, one also assumes that the acid-base properties in water will be proportional to those measured through TPD.

Fig. 8 to Fig. 17, show that clays interact differently with various enzymes, in the presence of different cations. When dispersed in water, Na-Mt is supposed to contain ca. 10 times more water layers between clay sheets than Ca-Mt [9], preventing, thereby, from enzyme diffusion towards clay surface. Thus, one must expect that enzyme interactions with Ca-Mt and Mg-Mt will be stronger than with Na-Mt and K-Mt. In addition, enzyme diffusion towards clay surface must be governed mainly by electrostatic attractions due between clay and enzyme. Consequently, the positive charges on the alkaline earth-exchanged clay surface will enhance these interactions, making that larger amounts of enzymes must adsorb on these materials, as compared with Na-Mt and K-Mt (nearly neutral surface). This is why coarser flake-shaped clay particles with expanded structures were obtained with Ca-Mt upon dehydration, as compared with Na-Mt (Fig. 8 and 9). This seems to be a general tendency that reflects the behaviour difference between alkaline and alkaline earth-exchanged clays, as well supported by the results summarized in Table 2. Unless, the enzyme undergoes shape modifications due to the clay acid-base properties, one assumes that these expanded clay structures arise mainly from various enzyme orientations on the support.

Upon dehydration-rehydration, Ca-Mt and Mg-Mt display higher swelling capacity, as compared to Na-Mt and K-Mt, in agreement with previous results [13]. The swelling capacity was improved by intercalation with enzymes, suggesting an effective contribution of enzymes in retaining water, due to their increased hydrophilic character.

Here, one must quote that the shapes of interlayered clays particles vary according to the hydrophilic character of the species sandwiched between clay sheets. With organic hydrophobic compounds such as benzene, montmorillonite (Mt) generates small clusters of irregular shape (edge-to-edge), while with hydrophilic enzymes, ondulating mosaic sheets or flake-shaped structures are formed. The shape and size of the particle changes according to the nature of the exchangeable cation. For instance, Na-Mt and K-Mt appear as very fine gel-like particles. This is consistent with the statement according to which, in water, Na causes compact clay sludges, with impermeable layers. [19]. Thus, Na reduces porosity as well, making the interlayer space difficult to be entered by water and to enzyme. This is why the amount of fixed enzyme was relatively smaller over alkali-exchanged Mt, as compared to Ca-Mt and Mg-Mt.

Reversely, in the presence of enzymes, Ca-Mt and Mg-Mt give rise to coarser flake-shaped particles, with surface area exceeding 400-600m^2 per gram of dry clay. In this regard, the most probable explanation is that Ca^{2+} cations may provide bridges between the negative charges of both the clay surface and the enzyme functional groups [20]. Consequently, one can state that alkaline-earths improve not only the interaction of enzyme with the clay surface, but also the stability of the [enzyme-Ca-clay surface] complexes formed. If one takes into account that the permanent negative charges of montmorillonite surface are not affected by pH [20], one can conclude that the interactions between enzyme and clay depend only on the number and the strength of such bridges. Anyway, in aqueous phase, clays behave as buffer according to the nature of the cation introduced. Besides, the medium composition seems to influence greatly the strength of the interactions between clay sheets themselves, and between clay sheets and other surrounding species, since increasing the clay concentration increases the magnitude of the attraction, while increasing the electrolyte content of the system induces a reverse phenomenon [21].

DISCUSSION

In cationic 2:1 smectite minerals, such as bentonite or bedeillite, the clay sheets display negative charges on both

Table 2: Effect of enzyme and cation upon the particle shape and clay intercalation.

Species interacted	Swelling capacity				Clay interlayering			
	Na-Mt	K-Mt	Mg-Mt	Ca-Mt	Na-Mt	K-Mt	Mg-Mt	Ca-Mt
Cations	very low	low	high	high	face-to-face	face-to-face	expanded face-to-face	expanded face-to-face
Benzene	very low	very low	very low	low	edge-to-edge	edge-to-edge	edge-to-edge	edge-to-edge
α-amylase	very low	low	high	high	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
β-amylase	very low	low	high	high	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
Cellulase	very low	low	high	high	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
Invertase	very low	low	high	high	flake-shaped	flake-shaped	flake-shaped	coarser flake-shaped
Lipase	very low	low	high	high	edge-to-edge	edge-to-edge	edge-to-edge	slightly flake-shaped
Peroxidase	very low	low	high	high	edge-to-edge	edge-to-edge	slightly flake-shaped	flake-shaped
Urease	very low	low	high	High	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
Pectinase	very low	low	high	high	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
Protease	very low	low	high	high	flake-shaped	flake-shaped	coarser flake-shaped	coarser flake-shaped
Tripsine	very low	low	high	high	face-to-face	face-to-face	flake-shaped	flake-shaped

* Flake-shaped: expanded structure including both face-to-face and edge-to-edge clay intercalation
** Face-to-face structure: parallel clay sheets
***edge-to-edge structure: non parallel clays sheets covering hydrophobic organic clusters.

Figure 8: α-amylase-interlayered Na-Mt.

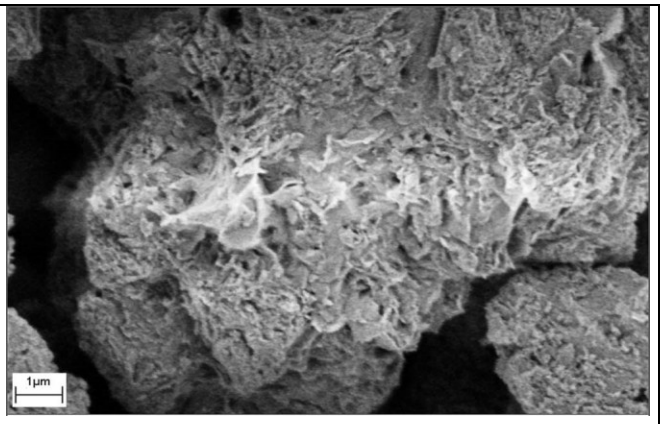

Figure 9: α-amylase-interlayered Ca-Mt.

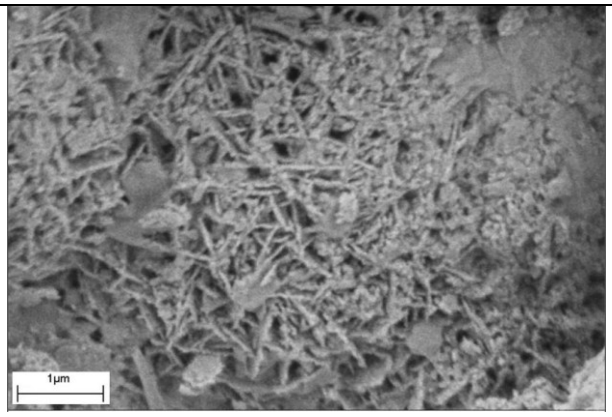

Figure 10: Cellulase-interlayered Ca-Mt.

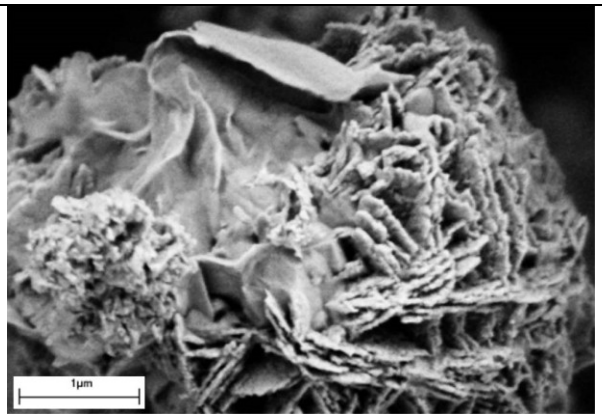

Figure 11: Protease-interlayered Ca-Mt.

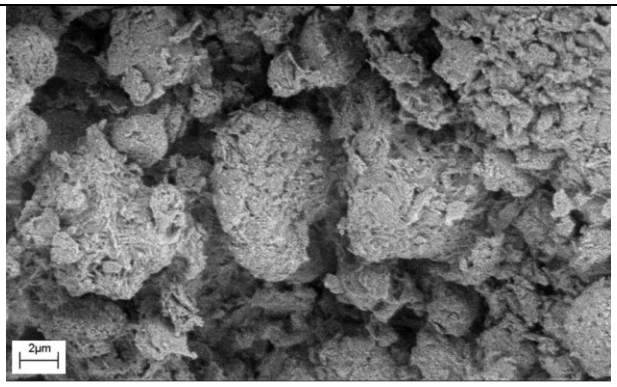

Figure 12: Lipase-interlayered Ca-Mt.

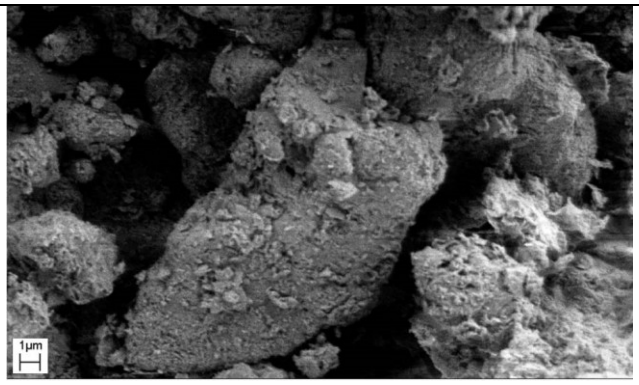

Figure13: Peroxidase-interlayered Ca-Mt.

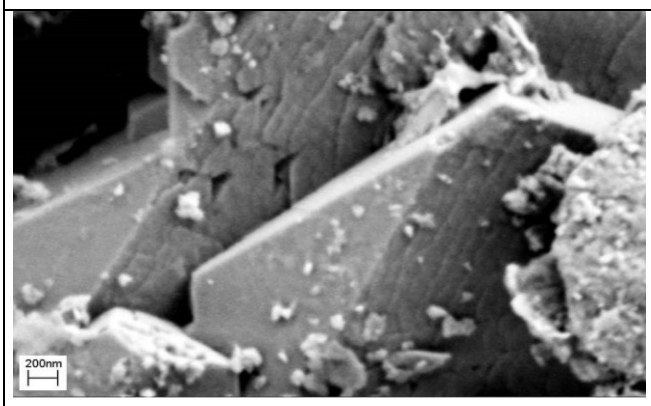

Figure 14: β-Amylase-interlayered Ca-Mt.

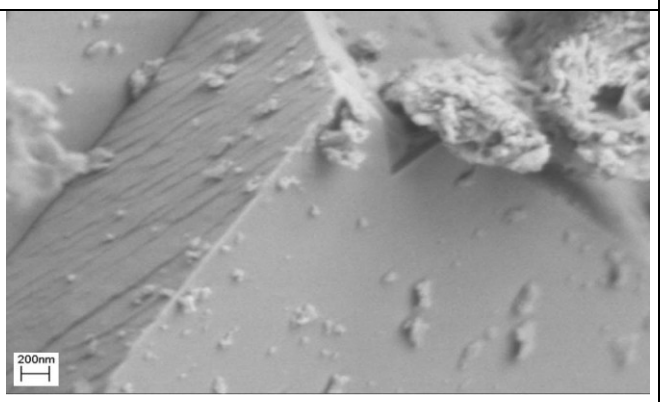

Figure 15: Tripsine--interlayered Ca-Mt.

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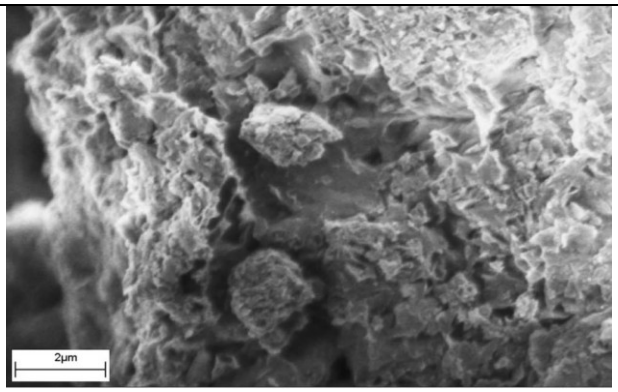

Figure 16: Urease-interlayered Ca-Mt.

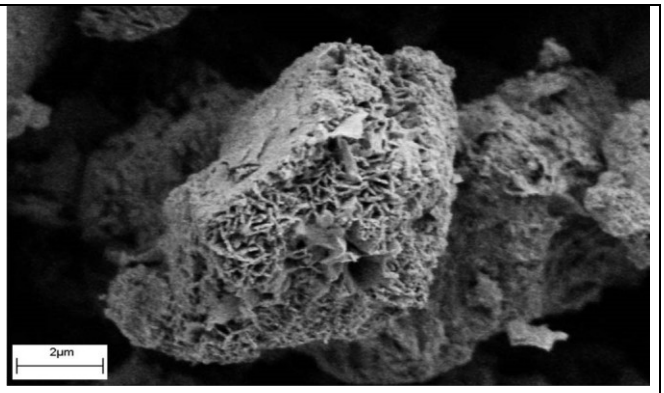

Figure 17: Pectinase-interlayered Ca-Mt.

faces. According to the charge-compensating cation, clays acquire specific acid-base properties, that can greatly influence their interaction with a given enzyme. In the surrounding aqueous medium, the electrostatic forces involved can be more or less modified, inducing even changes in the stability of enzymes present in the medium. Thus, strong interactions can occur between the anionic sheet structure of the dispersed clay and the surrounding species. The resulting [clay-counter ion-water-enzyme] complex will present an expanded porous structure, because of the intercalation of bulky enzyme molecules between the clay sheets, producing either edge-to-edge or face-to-face interlayering, due to the coagulation capacity, and to the various orientations of enzymes on the clay surface, as well. Evidences of such phenomenon were provided by reflectance electronic microscopy insights.

Fundamentals about colloids and interfacial chemistry have already been reported in the literature [22-29]. Colloids have particle size comprised between 0.0001 and 1µm, and are generally classified as hydrophilic and hydrophobic. On one hand, proteins and enzymes are hydrophilic colloids, having polar groups on their surfaces, that determine a high affinity for water, through the easy formation of water-protein bonds. In contact with water, the protonation-deprotonation behaviour of a clay surface can be understood in terms of the acid–base properties, that can be correlated to an electrochemical equilibrium model of the solid–solution interface, where structure and electrostatic interactions play key roles [30].

On the other hand, cationic clays minerals are very important hydrophobic colloidal layered structure with negatively charged surface. The latter can be neutralized by cationic species, forming a double layer, and generating, thereby, a colloidal structure. The charged surface keeps particles from coalescing, but dissolved salts seem to disrupt the double layer and affect the colloid structure. Clays have also high surface area and large CEC values that make them particularly good absorbers for metal ions and enzymes. Under certain conditions, interactions take place between clay surfaces and other chemical species, dispersed in a surrounding medium, through physicochemical processes of a great interest for environmental applications [31-34]. This is why, in our opinion, the way enzymes intercalate clay sheets upon dehydration can reflect the clay interactions with enzymes in aqueous phases. In such processes, clay behaviour can be explained, by analogy to soil physicochemistry in terms of water content [1-5] and, subsequently, in terms of acid-base properties. In regard to the surface area and cation exchange capacity (CEC), clays play a predominant role and influence greatly water retention, aggregation, and interactions with free enzymes [6-9]. In the presence of free or fixed cations on the clay surface, the acid-base properties of a given clay can be dramatically modified. Indeed, it appears that a relatively high amount of Ca and neutral pH on clay surface ought to increase immobilization of enzymes.

In nature, montmorillonite (with the following unit cell formula: $Al_2(OH)_2Si_4O_{10}$), kaolinite ($Al_2(OH)_4Si_2O_5$), nontronite ($Fe_2(OH)_2Si_4O_{10}$) and hydrous Mica ($KAl_2(OH)_2(AlSi_3)O_{10}$) are very important clay minerals, but montmorillonite play the most significant role in the physicochemistry of prairie soils, due to its relatively higher cation exchange capacities and higher swelling when wetted than many other clay minerals. Upon wetting, the montmorillonite layered structure absorbs water and expand, the degree of expansion being dependent on the type of cation fixed. Moreover, their high surface area makes them particularly good absorbers for heavy metals, organic matter, enzymes and other species.

Montmorillonite interacts with a given enzyme in the same way as in coagulation-flocculation processes in wetted soil or during treatment of wastewaters containing biological residues and heavy metal cations. In such processes the clay acid-base properties play a predominant role. This is why, in our opinion, differences in both shape and size of clay flakes were observed according to the acid properties induced by the cation fixed, but also according to the base character (mainly of Lewis type), induced unavoidably by the electronegativity of oxygen atoms in the surrounding vicinity [14,15].

One has also demonstrated that increasing clay acidity induce decreasing base character around the charge-compensating cation fixed on the clay surface. Our results agree with those previously reported [14-16], since it appears that, like for liquid solutions, there exists a certain inverse proportionality between acidity and the base character, in terms of number and strength of sites. In this regard, the specifically low acidity of the alkali-exchanged

clays can be explained only by the compensation of its relatively stronger base tendency, as compared to that of alkaline earth-exchanged clays [16]. Thus, evidences are provided in favour of interpretations based on the effects of the acid-base properties of the clay surface on the orientation of the clay sheets with adsorbed enzymes. A consequence of this new interpretation is a conceptual basis for a possible explanation of some aspects dealing with enzyme immobilization in soils or metal coagulation-flocculation in the presence of enzyme. These aspects still remain to be elucidated, being of a great interest for enzyme immobilization and environmental purposes.

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