Entrainment of enzymes in silica aerogels

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Aerogels, the world’s lightest solids, possess extraordinary traits such as very low density, very high surface area, very high porosity and ultra-low heat conductivity. These traits made aerogels favorable in various applications, including high-performance thermal insulators, catalyst supports, electrode materials, random laser matrices, cosmic dust collectors and more. Of the many potential applications of aerogels, one of the most challenging has been the development of a general procedure for bioactive aerogels by the entrainment of enzymes within these air-light materials. The difficulty in reaching this “holy-grail” was dual: The special procedures for obtaining the unique structure of aerogel are destructive to enzymes; and the aerogels are extremely sensitive to any procedural modification. Thus, the use of pure silica aerogel for the entrainment of enzymes was not known. Here we present a generalized, bio-friendly procedure for the entrainment of enzymes in silica aerogel, retaining both the enzymatic activity and the air-light structure of the aerogel. All of the aerogel synthesis steps were modified and optimized for reducing the risk of enzyme denaturation, while preserving the aerogel characteristic structure of the composite. The entrainment of three enzymes of different types was demonstrated: glucose oxidase, acid phosphatase and xylanase. All aerogel-entrapped enzymes showed superior activity over the common method of sol–gel entrainment in xerogels, due to the much wider and open pore network of the former. Michaelis-Menten kinetics was observed for the entrapped enzymes, indicating that the enzymes are highly accessible and diffusional limitations are negligible. The Michaelis-Menten constant, \(K_m\), has remained at the same level, indicating that enzyme-substrate affinity was not affected. Thermal stabilization was observed for entrapped acid phosphatase reaching peak activity at 70 °C. Large molecular weight substrates such as xylan for xylanase, are no obstacle for the aerogel matrix, while completely inapplicable for the xerogel. All of these properties are highly relevant for biotechnological applications.

Introduction

Aerogels hold the world record for the least dense solids on earth, with densities as low as 3 mg/cc [1]. They are also known for their extreme properties, such as ultra-high porosity and large surface areas that can reach over 1000 m²/g. These properties make the aerogels highly useful in many applications, from high-
performance thermal insulators [2] to catalyst supports [3], electrode materials [4], random lasers matrices [5], space micrometeorites collectors [6] and more [7–9]. Of the many potential applications of aerogels, maybe the most overlooked has been the development of a general procedure for enzymatically active aerogels, that is, the entrapment and immobilization of enzymes within these air-light materials. Achieving it has practical importance to the general field of immobilized enzymes, since these are used as key components in many biotechnological processes, such as in the fructose-glucose isomerization [10], in stereoselective drug modifications [11] and many more [12–14]. The immobilization allows easy separation of the biocatalysts from the reaction pot and their reuse, and generally provides a protective environment to the enzyme [15–17]. Enzyme immobilization methods [18] include covalent binding to a support [19], crosslinking [20], entrapment within nanoparticles [21], entrapment within porous supports, both crystalline (MOFs [22]) and amorphous [16,23]. Of the latter approach, entrapped enzymes in sol–gel derived materials [24] has evolved into one of the major routes for enzyme immobilization, practiced on virtually all major enzymes of medical, biotechnological, environmental, synthetic and sensing use [15,25–30]. The generality of the procedures, the ease of recycling, the major increase in enzyme stability to heat [15,31] and to destructive chemicals [16,17], the inertness of the entrapping matrices, have all contributed to the wide scope use of that methodology. The elevated stability is mainly due to the physical confinement that prevents the dynamic conformational motions of denaturing, allowing the enzyme to remain active under extreme conditions [15,16]. A drawback which has accompanied this immobilization is the diffusional limitation on the substrate molecules to reach the buried enzyme, and on the product molecules to diffuse out. This is particularly so in the final dry xerogel form, because the drying itself results in a shrunk enzyme–carrier composite, with smaller pores [23].

It is now clear, why the “holy-grail” of the field of enzymes immobilization has been the development of a general approach of entrapment of enzymes within aerogels: Aerogels should ease the major issue of diffusional limitation by virtue of their wide-open mesoporous network. However, entrapment of enzymes within aerogels has been difficult to achieve, because of the experimental procedures of preparation of sol–gel derived aerogels. The use of alcohols during the sol–gel transition and solvent exchange and the use of supercritical CO2 during drying – are destructive for enzymes, leading to their total deactivation. Of the thousands of enzymes, only the hydrophobic and robust lipase could withstand these standard procedures, and even here, pure oxide aerogels could not be used and hydrophobic derivatization was needed [32–36].

In this study, we set to develop a generalized procedure for the entrapment of enzymes in silica aerogel. The generalized procedure should deal with all potentially denaturing steps of the aerogel synthesis. First, the sol–gel transition itself, that usually requires an alcohol as a co-solvent; second, the solvent exchange needed for the alcogel step; and finally, the supercritical drying. If successful, the entrapment should not only lead to enzymatic activity of the enzyme@SiO2 aerogels, but to improved activity compared to the classical entrapments in silica xerogel, by virtue of alleviating diffusional limitations and elevating enzyme stability.

Three enzymes with distinct different activities have been selected for that purpose: An oxido–reductase – glucose oxidase, a hydrolase – acid phosphatase, and an enzyme operating on

![Scheme 1](image1.png)

**SCHEME 1**

A summary of the generalized procedure for the entrapment of enzymes in silica aerogel.
very large macromolecules – xylanase. Finding the right conditions for the entrapment of the enzymes during the formation of the aerogel required a very delicate fine-tuning of the experimental conditions, and therefore we regard the next Experimental Methods section (2) as part of the main text – it will then be analyzed in the Discussion (3).

Experimental methods

Materials

Tetramethyl orthosilicate ≥98% (TMOS) was obtained from Merck. Glucose oxidase from Aspergillus niger 100–250 units/mg (cat. no. G7141), acid phosphatase from potato 0.5 units/mg (cat. no. P3752), xylanase from Aspergillus oryzae ≥2.5 units/mg (cat. no. X2753), horseradish peroxidase 150–250 units/mg (cat. no. P8250), poly(vinyl alcohol) MW 146,000 (cat. no. G7141), acid phosphatase from potato 1.0 mg/mL, and acid phosphatase 1.0 mg/mL and for xylanase 6.0 mg/mL. The xylanase solution was filtered before use, to eliminate insoluble components. The mixed solutions were casted into molds, allowed to gel (10–60 min) and left for aging at room temperature, overnight. The hydrogels were then transferred to an ethanol bath for 24 h, at 4 °C, with the ethanol replaced once during that time period. The alcogels were then subjected to a rapid supercritical drying process, in a manual "Pelco CPD2" instrument. The drying process, was carried out for the minimal duration possible – 5 h – and consisted of three washing steps in liquid CO2, heating to 35 °C to reach supercritical phase and depressurization at a rate of 25 psi/min. The obtained aerogels (denoted enzyme@SiO2) were sealed and kept at 4 °C for further use. Additional entrapments: For comparative purposes, standard sol–gel entrapments of these enzymes in silica aerogels were carried out by drying the hydrogels overnight in a vacuum desiccator, at room temperature. The effect of partial hydrophobization was tested for GOx by replacing 5% (molar) of the TMOS with MTMS in the sol synthesis procedure. The effect of PVA on the performance of GOx, was tested by using 8.3 mg/mL PVA in the enzyme buffer solution.

Enzymatic activity

**Entrapped glucose oxidase (GOx@SiO2)**

Here and below, 80 mg of the ground aerogel or xerogel were rinsed twice in 10 mL buffer and the supernatant of the second rinse was tested for enzyme leak activity. The samples were incubated at 35 °C, in a 5 mL reaction buffer of 41.4 mM sodium acetate at pH 5.1, with 1.7% wt. of glucose. Glucose oxidation was determined by transferring 0.3 mL of the reaction solution to a 0.6 mL solution containing 0.21 mM o-dianisidine and 2 μM peroxidase, followed by 10 min incubation in 35 °C. Absorbance was measured at 500 nm, and the initial velocity for the reaction, v0, was determined from the slope of the first 30 min, using the unit definition of 1 μmol of oxidized β-D-glucose per minute.

**Entrapped acid phosphatase (AcP@SiO2)**

The samples were incubated at 37 °C in a 5 mL reaction buffer of 45 mM sodium citrate at pH 4.8, with 6.6 mM of pNPP. Phosphate hydrolysis was determined by transferring 0.22 mL of the reaction solution to a 0.78 mL solution of 0.1 M NaOH. Absorbance was measured at 410 nm, and v0 was determined from the slope of the first 60 min, using the unit definition of 1 μmol of hydrolyzed pNPP per minute.

**Entrapped xylanase (Xyl@SiO2)**

The samples were incubated at 40 °C, in a 10 mL reaction buffer of 50 mM sodium citrate at pH 5.3, with 13.5 mg/mL of xylan. Xylan hydrolysis was determined by transferring 0.4 mL of the reaction solution to a 0.6 mL DNSA reagent, containing 1%...
DNSA, 1% NaOH and 0.05% sodium sulfite, boiling the mixture for 5 min and cooling to room temperature. Absorbance was measured at 540 nm, and $v_0$ was determined by the slope of the first 40 min, using the unit definition of 1 nmol of liberated xylose per minute. A calibration curve was made using xylose solutions of different concentrations.

Control activity
In all enzymatic assays, the reported activity of enzyme@SiO$_2$ is after the subtraction of the residual small activity with the buffer only (control). Also, for all enzymatic reactions, catalytic activity in the presence of a blank silica aerogel and xerogel (without the enzymes) was tested – no such background activity was found. Free enzyme reactions were carried out for comparative purposes, using the same amount of enzyme as in the entrapment process. Michaelis-Menten and Arrhenius plots were performed at different substrate concentrations and temperatures, respectively.

Physical characterizations
SEM images were taken with a Magellan 400L XHR instrument, operating at 1–2 kV. The specific surface area, pore volume and pore size distribution were obtained by a N$_2$ adsorption–desorption apparatus (Micromeritics ASAP 2020), at 77 K. All samples were degassed under vacuum at 50 °C for 10 h before analysis. The surface area was calculated using the BET equation, over acquired adsorption data in the $P/P_0$ range of 0.05–0.2 for aerogels and 0.05–0.12 for xerogels. The pore distribution was analyzed using the BJH method, over the acquired desorption data points. Total pore volume was calculated using a single point adsorption at $P/P_0 = 0.97$. The bulk density was determined by measuring the volume and weight of the cylindrical shaped gels.

Results and discussion

Development of the entrapment procedure (Scheme 1)

The development of a general procedure for enzyme entrapment in silica aerogel required the consideration of all potentially enzyme-denaturing steps along the way, and replacing them with more enzyme-friendly steps, while still keeping the aerogel structure, which in itself is very sensitive to preparation conditions. Careful and detailed search for the right conditions of keeping the enzymes alive on one hand and maintaining the unique structure of an aerogel on the other hand, led to the following general procedure: First, the standard sol–gel procedure involves the use of a large amount of an alcohol as co-solvent, in order to homogenize the mixture of water and the hydrophobic silicon alkoxide. Here we utilized an early observation we made, namely that alcohol is not needed for that homogenization, and that the much smaller amount of alcohol released during the hydrolysis stage, is enough for that purpose [37].
A two-step modification of that procedure consisted of a rapid, acid catalyzed hydrolysis of TMOS, to get a homogenous SiO₂ sol and later on the hydrogel, which contains a minimal amount of alcohol from the alkoxide hydrolysis, not enough for shutting down the enzymatic activity. The enzymes were added to the sol after adjusting the pH with a suitable buffer (not used in the standard aerogel procedure). Here we also utilized the fact that buffers accelerate the gelation, so that adding a strong base for the gelling stage was avoided. Gelation was indeed quick: The samples with glucose oxidase (GOx) gelled after 29 ± 4 min, with acid phosphatase (AcP) after 10 ± 1 min and with xylanase (Xyl) after 12 ± 2 min. This fast gelation step is already a key protective measure against denaturing. The second standard step in the aerogel synthesis that needed adjustment was the formation of the alcoigel. This is a standard step of replacement of water from within the gel with ethanol, which is a solvent suitable for the supercritical drying. Although at this stage the enzymes are already protected by the gel cage, prolonged exposure of the enzymes to ethanol may still be harmful. We therefore optimized a minimal duration of the solvent exchange time which still maintains the aerogel final structure, and optimized a significant reduction of the temperature of the process (Supplementary S1). After testing the different parameters (including the use of additives such as glycerol or ethylene glycol, which were found to be unnecessary, see Supplementary S1), just one solvent replacement (instead of the 3 replacements used standardly) was found sufficient, taking 24 h of solvent exchange (instead of 2–5 days) at 4 °C (instead of at least room temperature). The final step of the aerogel synthesis is the supercritical drying of the alcoigel, that is, the replacement with liquid CO₂, transition to supercritical CO₂ and slow depressurization. The whole drying process was completed in less than 5 h and included rinses with liquid CO₂ at room temperature during the first 3 h, warming to 35 °C (compared with 40–45 °C min.) during the next 30 min.

### FIGURE 2
Comparative (a) BET surface areas, (b) BET C-constants and (c) BJH desorption average pore sizes for different enzyme@SiO₂ aerogels and xerogels. AcP1 and AcP2 show typical variability between samples of the same entrapped enzyme.

### TABLE 1
A summary of the physical characterizations of the aerogel and xerogel composites*.

<table>
<thead>
<tr>
<th>Enzyme@SiO₂ Composites</th>
<th>Bulk Density (mg/cm³)</th>
<th>Volume Reduction (%)</th>
<th>BET Surface Area (m²/g)</th>
<th>BET Constant (au)</th>
<th>Average Pore Diameter (nm)</th>
<th>Total Pore Volume (cm³/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerogels</td>
<td>130 ± 10</td>
<td>39 ± 6</td>
<td>870 ± 70</td>
<td>86 ± 6</td>
<td>12.5 ± 0.6</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Xerogels</td>
<td>1400 ± 300</td>
<td>94 ± 2</td>
<td>480 ± 50</td>
<td>340 ± 80</td>
<td>2.16 ± 0.05</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>

* Results are based on the average of all three enzyme composites. See Fig. S4 for specific enzymes data.
and finally evacuation of the supercritical CO$_2$ at a rate of 25 psi/min, which is at the high end for the preparation of aerogels.

**Aerogel characterization**

Yet another challenge has been tailoring enzyme-friendly entrapment procedure, which will not alter the delicate special structure of the aerogel. This has been achieved: Figs. 1, 2, and Table 1 provide material characterizations results of the enzyme@SiO$_2$ aerogels, and the full set of the additional results for all entrapped enzymes is collected in Fig. S2–S4, Supplementary material. These figures and the table contain also comparative data with the conventionally prepared enzyme@SiO$_2$ xerogels. As can be seen, typical aerogels are obtained, quite distinctly different from the parallel xerogels. The physical appearance of the bulk (Figs. 1a, b and S3) and the known “cloud-like” feeling (when held), are those of aerogels, compared with the glassy compact appearance of the xerogels. This visual appearance shows up also in the physical parameters (Table 1; Fig. S3) namely in the low bulk density, the significantly lower volume reduction of the aerogels compared to the xerogels, the much higher surface areas, the much wider average pore sizes, and the much larger pore volume – all clearly pointing to the open porosity of the aerogels. Scanning electron microscopy (SEM) imaging shows the typical rough and porous surface of the aerogels, compared with a smoother microporous one observed in the xerogels (Fig. 1c, d). These different visual

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**SCHEME 2**

The bio-catalyzed reactions and the detection reactions: Glucose oxidase (top), acid phosphatase (middle) and xylanase (bottom). The spectrophotometric color changes are indicated by the circles.
properties show up in the nitrogen adsorption–desorption isotherms of the aerogels, which are totally different from the xerogels: For the aerogels, type IV isotherms, according to IUPAC classification [38], are obtained (Figs. 1e and S3). Such isotherms, with a type H1 hysteresis loop, indicate a pore size distribution which is in the mesopores range. Indeed, BJH porosity analysis of the isotherms, results in an abundance of mesopores in the size range of 10–100 nm (Figs. 1h and S3). For the xerogels, type I isotherms are obtained (Figs. 1f and S3), typical of materials which are rich in narrow micropores. This xerogels’ small radii porosity hampers the diffusion of substrates and products inside the xerogel matrix, as we shall see in the next section. The excellent compliance of the enzymatically doped aerogels with the BET equation (Figs. 1g and S3) is indicative of the homogeneity of the adsorption sites on the molecular level, which, in turn, points to the homogeneity of the doped material as a whole. We also notice a major difference in the C constants of the BET equation of the aerogels compared to those of the xerogels – around 80 compared with around 300, respectively (Table 1, Fig. 2). We recall that the BET constant represents the magnitude of interaction of the adsorbed molecule (N₂) with the material surface. We suggest that the higher BET constant values for the xerogels originate from the abundant of nano and micropores that are typically of higher adsorption energies compared to

![Graphical representation of initial reaction rates of the doped aerogels and xerogels of (a) GOx@SiO₂, (b) AcP@SiO₂ and (c) Xyl@SiO₂. Xyl@SiO₂ xerogel showed no activity. (d) Graphical summary of (a)–(c): The relative activity of the enzyme@SiO₂ aerogels compared with enzyme@SiO₂ xerogels.]

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th><strong>v₀</strong> (units/mg enzyme)</th>
<th><strong>Kₘ</strong></th>
<th><strong>Vₘₐₓ</strong> (units/mg enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerogel</td>
<td>Xerogel</td>
<td>Aerogel</td>
</tr>
<tr>
<td>Glucose Oxidase</td>
<td>0.35 ± 0.17</td>
<td>0.0039 ± 0.0027</td>
<td>7.4 ± 1.1 mg/mL</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>0.014 ± 0.005</td>
<td>0.0010 ± 0.0013</td>
<td>0.58 ± 0.14 mM</td>
</tr>
<tr>
<td>Xylanase</td>
<td>0.63 ± 0.05</td>
<td>–0.017 ± 0.033</td>
<td>3.7 ± 1.7 mg/mL</td>
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mesopores, because of their smaller radii of curvature, resulting in stronger binding energy of the N₂ molecules.

Enzymatic activity (Scheme 2)
The main goal of this study – to develop a general procedure for the entrapment of enzymes in silica aerogel while retaining both their activity and the aerogel structure, has been achieved: All three enzyme@SiO₂ aerogels were found to be active; and all three showed remarkably higher activity compared to the enzyme@SiO₂ xerogels (Fig. 3 and Table 2). GOx@SiO₂ aerogel showed \( \times 89 \) higher activity, AcP@SiO₂ aerogel showed \( \times 13 \) higher activity, and, particularly noticeable, Xyl@SiO₂ was active only in the aerogel, while the xerogel showed no activity at all: the macromolecular substrate – xylan (Scheme 2), with an estimated average molecular weight of 30 kDa [39] – could not diffuse into the micropores of the xerogel, whereas the wide-pores of the aerogel allow the accessibility of the active site of xylanase to that substrate. As already noted in the Experimental details (Section “Control Activity”), the blank pure silica aerogels and xerogels show no activity, and there was no enzyme leakage from the matrices. Thus, the enzymes, on one hand are tightly held within the cages of the aerogel, and on the other hand are accessible to reaction with substrate molecules in-diffusing from the surrounding solution, allowing also the out-diffusion of product molecules.

An important observation is the compliance of all three aerogel-entrapped enzymes with the classical Michaelis-Menten kinetics (Fig. 4). For the xerogels, only AcP@SiO₂ showed apparent compliance to this type of kinetics analysis (Fig. 4c). Note in Fig. 4d the decline of the highest substrate concentration in Xyl@SiO₂ aerogel (average of three repetitions): It indicates the concentration limit of the matrix to process the very large substrate molecules, possibly as a result of increasing blockage of the pores. It is significant that the Michaelis-Menten constants, \( K_m \), of the entrapped enzymes are similar to those of the free enzymes (Table 2). This result stands out, as usually entrapment of enzymes increases the observed \( K_m \) [15,40], as indeed seen in our case for AcP entrapped in the xerogel matrix (Table 2). Such increase in the apparent \( K_m \) values, has been attributed mainly to arise from diffusional limitations, and also to possible conformational changes due to the entrapment [16,19,41,33]. Thus the significance of the observation that the \( K_m \) values in the case of the aerogel entrapments are retained indicates that the entrapped enzymes remain intact from the point of view of their
biochemical activity, and accessible to reaction. The cost of entrapment within a matrix does show up in the $v_{\text{max}}$ values, still much higher than in the xerogel, but lower than the free enzymes (Table 2). In fact, this trend is evident in most cases of enzyme entrapments, and is a general feature in catalysis and bio-catalysis, when changing a homogeneous solution to heterogeneous conditions [23].

Temperature dependence of the enzymatic activity was determined for AcP@SiO$_2$ and Xyl@SiO$_2$ and aerogels; the results are shown in Fig. 5 (GOx was not included because of the temperature instability of H$_2$O$_2$). Xyl is a relatively stable enzyme, which starts to denature at about 60°C both in solution (Fig. 5a) and when entrapped (Fig. 5b). AcP, however, is a delicate enzyme which thermally denatures in solution already around 40°C (Fig. 5c). It was previously shown in several studies that the thermal stability of thermally unstable enzymes is enhanced significantly upon entrapment in oxide sol-gel derived xerogels, particularly of silica [31,42] and alumina [15,43]; indeed we found that the stability of AcP entrapped in sol-gel xerogel jumps to 80°C (Fig. S5). Such stabilizations were attributed mainly to the rigidity of the encasing cages which does not allow conformational motions which accompany the denaturing process [44,45]. Does the open pore aerogel offer such protection? The answer is positive – AcP@SiO$_2$ aerogel is active up to 70°C (Fig. 5d). These observations point to a probable similarity in the early stages of the molecular-level processes of the entrapment of the enzyme molecules in both the aerogels and xerogels: A general unexpected feature of entrapment in sol-gel matrices is that the polycondensation into a rigid inorganic network, does not squeeze the protein by the pressure of the matrix formation; rather, the sol-gel polycondensation process is such that it surrounds this “obstacle”, leaving it intact. Thus, while the entrapping cages in the xerogel and aerogel are apparently similar in their intimate protecting formation around the biomolecule (the early stage of the sol-gel process), these materials differ in their porosity, as described in detail above. This porosity develops at the late stage of the matrix formation, quite different for the xerogels and aerogels.

Next, we checked if the temperature dependence obeys Arrhenius-type behavior of the reaction rate, up to the temperatures of the denaturing point (negative slope; positive slope relates to denaturing [15]) – if obeyed, then apparent activation energies ($E_a$) of the activity of the biomaterial can be elucidated. Fig. 6 shows that the temperature dependence indeed obeys an Arrhenius-type behavior, from which apparent activation energies for the enzymatic processes could be estimated. It is seen (Table 3) that, compared with the free enzymes (Fig. S6a, c), somewhat higher activation energies are needed. These higher activation energies reflect the contribution of the diffusional limitations even in the open pore system of the aerogels: Indeed, the activation energy for AcP@SiO$_2$ xerogel is twice as high as that for the aerogel (Fig. 5b). Comparison of the Arrhenius pre-exponential factors ($A$) provides additional interesting insight (Table 3). We recall that the proposed physical interpretation of $A$ for entrapped enzymes is that it represents the frequency

![FIGURE 5](image-url)

Temperature effect on the initial reaction rates of (a) Xyl in solution, (b) Xyl@SiO$_2$ aerogel, (c) AcP in solution and (d) AcP@SiO$_2$ aerogel.

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of collisions of the substrate with its target in the correct orientation, and that it was found that xerogel porosity which directs the substrate to the active site increases $A$ by orders of magnitude [15]. This is indeed seen when comparing AcP in solution with AcP@SiO$_2$ xerogel (Table 3). However, again a major change is seen when compared with AcP@SiO$_2$ aerogel: $A$ is practically the same as for the free enzyme. Together with the similarity of the $K_m$ values, this point to the fact that aerogels provide a much less restricted environment compared with the xerogels. However, for Xyl@SiO$_2$ aerogel, the picture is different: $A$ of the entrapped enzyme is 3 orders of magnitude larger than that of the free enzyme. The reason of why this effect is seen for Xyl but not for AcP is probably due to the very large difference in the size of the substrate molecules: While for AcP it is the small pNPP, for Xyl it is the very large xylan of 30 kDa. Thus, while the former “enjoys” the wide porosity of the aerogel, the latter, because of its size, is subjected to the frequency-enhancing cage effect.

**Conclusion and outlook**

In this work, we have introduced an enzyme-friendly synthesis procedure for the entrapment of these biocatalysts within silica aerogel. The challenge has been dual: even though modifications from the typical aerogel synthesis procedure were needed, these had to retain the typical aerogel structure. Highly porous light-weight bioactive aerogel-composites were obtained. The resulting enzyme@SiO$_2$ aerogel showed enzymatic activity that far exceeded that of the xerogel composites, supporting our hypothesis that the high porosity of the aerogel will enable faster catalytic rates by reducing the diffusional limitation of the substrates and products. Also, we demonstrated that, in the case of xylanase, the aerogel-entrapped enzyme can catalyze a reaction of a macromolecule, which was not possible when the enzyme was entrapped in a microporous xerogel. The Michaelis-Menten constant of the enzyme@SiO$_2$ aerogels remained similar to the free enzymes, suggesting that the entrapped enzymes remained active and in the same conformation. Finally, thermal stabilization was observed, an attractive feature for industrial use of these composites.

We see the outlook of this study is in two directions: First, extension to oxides which are approved by the regulatory agencies for human use, such as alumina [46] and iron oxide [47]; and second, applying chemical modifications of the pure silica employed in this study. A preliminary exploration of the latter option on GOx@SiO$_2$ aerogel showed its positive potential: The first chemical modification was a partial, small degree of hydrophobization of the matrix with 5% methyltrimethoxysilane (MTMS) replacement of the TMOS; and the second was the use of a hydrophilic polymer, PVA, which was added to the gelation step with the enzyme (see Experimental details “Entrapped glucose oxidase (GOx@SiO$_2$)”)). Both additives showed an enhancement of the initial reaction rate, $\times 3.5$ and $\times 5.8$ times the activity of the unmodified composite aerogel, respectively (Fig. 7). Our hypothesis is that the MTMS adds a hydrophobic character to the aerogel, which helps reduce the interactions with the polar substrates and products, easing their diffusion, while the PVA helps to maintain an aqueous polar environment for the entrapped enzymes and thus further contributes to the preventing of denaturing during the solvent exchange and supercritical drying steps. We believe that by

![Figure 6](image-url)  

**FIGURE 6**  
Arrhenius plots of the activities of (a) AcP@SiO$_2$ aerogel and (b) Xyl@SiO$_2$ aerogel.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$E_a$ (kJ/mol)</th>
<th>$A$ (units/mg enzyme)</th>
</tr>
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<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>50 ± 10</td>
<td>2.4*10$^5$</td>
</tr>
<tr>
<td></td>
<td>100 ± 20</td>
<td>1.1*10$^{15}$</td>
</tr>
<tr>
<td></td>
<td>38 ± 4</td>
<td>1.2*10$^5$</td>
</tr>
<tr>
<td>Xylanase</td>
<td>62 ± 4</td>
<td>5.9*10$^7$</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>3.0*10$^6$</td>
</tr>
<tr>
<td></td>
<td>35 ± 3</td>
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</table>

**TABLE 3**  
Activation energy ($E_a$) and Arrhenius pre-factor ($A$) of two enzymes in the entrapped and free forms.
The effects of additives on the initial reaction rate of GOx@SiO2: 5% molar hydrophobic MTMS; 1% wt. PVA.

careful adjustment of added components, suitable for each enzyme, the activity of other enzyme@SiO2 aerogel composites can be enhanced.

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Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A: Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.mattod.2019.09.021.
