Refuse derived bio-organics and immobilized soybean peroxidase for green chemical technology

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Giuliana Magnacca, Enzo Laurenti, Erika Vigna, Flavia Franzoso, Lorenzo Tomasso, Enzo Montoneri, Vittorio Boffa

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*Dipartimento di Chimica, NIS Centre of Excellence, Università di Torino, Via P.Giuria 7, I-10125 Torino, Italy, Fax: +39 011 670 7855; Tel: +39 011 670 7543; e-mail: giuliana.magnacca@unito.it

[a] Dipartimento di Chimica, Università di Torino, Via P.Giuria 7, I-10125 Torino, Italy; enzo.laurenti@unito.it; enzo.montoneri@unito.it

[b] Section of Chemistry, Aalborg University, Shongårdsholmsvej 57, 9000 DK Aalborg, Denmark, vb@bio.aau.dk
Abstract

A silica monolith was prepared from commercial silica powder dispersed in water containing polymeric water soluble bio-organics (SBO) isolated from composted urban vegetable wastes. The monolith and the pristine powder were characterized for their morphology and reactivity for immobilizing Soybean Peroxidase (SBP). Compared to the pristine powder, the monolith exhibited lower specific surface area (about 30% less), total pore volume and pore size (of about 200 Å of width), and bond less SBP under the same experimental conditions. The immobilized SBP products were tested for their catalytic activity in the reaction of hydrogen peroxide, 3-(dimethylamino)benzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrazone (MBTH), by comparison with the same reaction performed with native SBP in solution. The reaction performed in the presence of immobilized SBP was slower than that catalyzed by native SBP in solution. However, in spite of its lower SBP content, monolith immobilized SBP (M-SBP) was found kinetically more active than the powder immobilized SBP (P-SBP). Also, M-SBP allowed to achieve the same reagents conversion as native SBP (95% of reagent conversion), although in longer time, whereas the maximum reagent conversion achieved with P-SBP was much lower (75% of reagent conversion). The M-SBP was more easily recovered from the reaction medium and found more stable than P-SBP upon repeated catalyst recycling (after 20 cycles 75-80% of the initial activity was retained by both immobilized samples, slightly higher in the case of M-SBP).

Keywords:
Biocatalysis, Biomass, Monolith preparation, Oxidoreductasis, Porous material
**Introduction**

Developing new immobilized biocatalysts is a current trend in green chemistry to perform reactions in heterogeneous media rather than in homogeneous media where the catalyst recovery is economically and environmentally critical. Also, valorization of biowastes as source of chemicals is a mean to cope with increasing amount of wastes. Within these scopes, the present work exemplifies urban refuse as sustainable renewable resource for the development of cost effective innovative technology to establish new, greener and safer chemical processes. Previous work has indeed demonstrated the potential of urban bio-wastes (UBW) to be a cost-effective exploitable source of soluble bioorganics (SBO) to recycle to the chemical and consumer’s market.[1] Indeed, as result of increased production due to population urbanization, UBW are concentrated in confined areas by municipal collection. Also, they are a rather rich source of bio-organic matter. This, depending on the source nature and waste management process conditions, may run up to 40–60% concentration and, in principle, provides a wide variety of products fitting a number of diversified specific technological requirements and consumer’s needs. The above SBO are described as mixtures of macromolecules with average molecular weight (Mw) values ranging from 67 to 463 kg mol$^{-1}$ and number average molecular weight (Mn) yielding polydispersity index values (Mw/Mn) in the 6 to 53 range. Chemical composition data also show that these macromolecules contain several functional groups and C types of different polarity. They appear to be formed by long aliphatic C chains substituted by
aromatic rings and several functional groups as COOH, CON, C=O, PhOH, O-alkyl, OAr, OCO, OMe, and NRR', with R and R' being alkyl C or H as represented in the molecular fragment shown in Figure 1. Although entirely virtual, this molecular fragment helps to memorize analytical chemical data and to expect chemical-physical properties. Indeed, consistently with Figure 1 representations, SBO have been found to exhibit typical properties of anionic surfactants and polyelectrolytes. In essence, they are capable to lower the surface tension of water and to change their molecular conformation in solution or yield macromolecular aggregates with 100-300 nm or larger hydrodynamic diameter as a function of concentration. The solution behavior of SBO has allowed their use as templates for fabricating mesoporous silica powders by sol-gel reaction starting from tetraethylorthosilicate (TEOS) and 3-aminopropyltriethoxysilane (APTES). On the other hand, by the presence of the above polar functional groups, SBO have been reported capable to interact with inorganic oxides and perform as dispersants or flocculants of these materials depending on the experimental conditions. These results have offered the scope for testing further the potential of SBO in the field of catalysts preparations.

The present work was undertaken with two specific aims: (i) to find out if the same previously reported silica material could be obtained in presence of SBO by an alternative procedure not involving the use of silane reagents and organic solvent (see, for instance, ref. 4), and (ii) to test the silica product in heterogeneous biocatalysis. To this purpose a commercial silica (obtained by precipitation in aqueous medium) was dispersed in water in the presence of SBO derived from
composted urban vegetable residues, dried and heated to 500°C to yield a mesoporous monolith. This material was used as support to immobilize Soybean Peroxidase (SBP).

The reaction of hydrogen peroxide, 3-(dimethylamino)benzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrazone (MBTH), catalyzed by Soybean Peroxidase (SBP) was chosen to evaluate the performance of the immobilized catalyst versus the free catalyst dissolved in a homogeneous reaction medium. The choice of SBP as active probe in this work is due to the ability of this enzyme to catalyze the oxidation of a large number of organic and inorganic substrates by means of hydrogen peroxide,[5,6] and its high resistance to thermal and chemical denaturation.[7,8] This makes SBP very interesting for biotechnological applications, in particular when the immobilization on solid support is requested in order to favor the removal of the enzyme from the reaction bath and its reuse for many reaction cycles. The hereinafter reported results will show the importance of the morphological changes induced in silica prepared in the presence of SBO for the immobilized SBP stability and performance.

**Experimental**

**Materials**

The SBO in 10% w/w yield was obtained from park home gardening residues and public park trimmings composted for 230 days at the Acea Pinerolese plant in Pinerolo, Torino, Italy. According to a previously reported procedure,[1] the composted material was further processed in a pilot plant made available by
Studio Chiono e Associati in Rivarolo Canavese, Italy. This comprises an electrically heated mechanically stirred 500 L reactor, a 102 cm long x 10.1 cm diameter (7.9 m² of surface) polysulfone ultrafiltration (UF) membrane with 5 kD molecular weight cut-off supplied by Idea Engineering s.r.l. from Lessona (Bi), Italy, and a forced ventilation drying oven. According to the operating experimental conditions, the compost was reacted 4 h with pH 13 water at 60 °C and 4 L/kg water/solid ratio. The liquid/solid mix was allowed to settle to separate the supernatant liquid phase containing the soluble compost hydrolyzate from the insoluble residue. The recovered liquid phase was circulated at 40 L h⁻¹ flow rate through the UF membrane operating with tangential flow at 7 bar inlet and 4.5 bar outlet pressure to yield a retentate with 5-10 % dry matter content. The concentrated retentate was finally dried at 60 °C. The final product obtained in 15 % w/w yield, relatively to the starting compost dry matter, was isolated as a black solid with dry matter containing 38.25 % w/w carbon, 4.01 % w/w nitrogen and 27.1 % w/w ashes. It was further characterized by ¹³C NMR and potentiometric titration, and by surface tension measurements, according to a previously reported analytical protocol.

The silica powder (FK320) was purchased from Degussa. This product is claimed by the vendor to be obtained by precipitation from sodium silicate in water acidified with sulphuric acid. Soybean peroxidase (SBP, EC 1.11.1.7) RZ =2.0 was purchased from Bioresearch Products Inc. (Iowa, USA) and used without further purification. Hydrogen peroxide 30%, 3-(dimethylamino)benzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrazone (MBTH) were purchased from Sigma-Aldrich Italia.
Monolith preparation

SBO (1 g) was kept under stirring in 15 ml water for 2 hours. Silica particles (4 g) were added to the black SBO solutions. More water (10 ml) was then added to favor mixing of the reagents under stirring for two hours. The system showed thixotropic behavior, and vigorous stirring favored the formation of a fluid phase with low viscosity. After relaxing for two hours, the mixture became thicker to yield finally a black wet pellet with the shape of the container in which the preparation was carried out. This material was let drying at room temperature for three days, removed afterwards from the container and calcined in oven at 500°C for four hours in order to remove all organics and yield a porous monolith.

Immobilization of SBP on silica

One gram of pristine silica powder or monolith was suspended in 0.075 L of a 10% v/v solution of 3-aminopropyltriethoxysilane in water. The pH was adjusted to 4.0 with HCl and the mixture was heated at 80°C for three hours in a water bath. The resulting suspension was filtered on a buchner funnel and the product was washed with bidistilled water and dried at 120°C. Successively, 0.5 g of silanized product were suspended in 0.050 L of glutaraldehyde 2.5% v/v in 0.1 M phosphate buffer solution at pH 7.0, and allowed to react for 1 h in the dark at room temperature. The resulting suspension was then filtered. The recovered solid was washed four times, added to 0.005 L of 2.5 mg mL⁻¹ soybean peroxidase solution in 0.1 M phosphate buffer at pH 7.5 and left to react at 4°C for 20 h. The final product, obtained by filtration, washing four times with the same buffer and gentle drying, was stored at 4°C. The amount of SBP
immobilized was calculated as the difference between the initial amount of enzyme and that recovered in the washing liquid. The concentration of SBP in solution was determined by means of UV-Visible spectroscopy, measuring the absorbance at 403 nm as previously reported\(^9\).

**Materials characterization methods**

N\(_2\) gas-volumetric adsorption at 77 K (ASAP2020 by Micromeritics) for the determination of specific surface area (BET model)\(^{10}\) and porosity (BJH model)\(^{11}\) were performed according to the cited literature on powder and monolith samples activated in vacuum (residual pressure 10\(^{-2}\) mbar) at 60°C prior to analysis.

High-Resolution Transmission Electron Microscopy was performed on a JEOL JEM 3010UHR (300 kV) TEM fitted with a single crystal LaB\(_6\) filament. All samples were dry deposited on Cu “holey” carbon grids (200 mesh).

**Kinetic measurements**

The catalytic properties of immobilized SBP materials were tested by using the DMAB-MBTH reaction\(^{12}\). Before to start the kinetic measurements, silica powder and monolith were incubated for 1 h in acetate buffer 0.1 M pH 5.4.

Then, 6 mg of immobilized SBP samples (on powder and monolith, indicated as P-SBP and M-SBP, respectively), or 10 \(\mu\)L of SBP in solution, were placed in a reactor (pyrex beaker) together with 6 mL of a solution of the same buffer containing 3-(dimethylamino)benzoic acid (DMAB) 5.0 \(\times\) 10\(^{-4}\) M and 3-methyl-2-benzothiazolinone hydrazone (MBTH) 2.1 \(\times\) 10\(^{-5}\) M. The SBP amounts were:
2.5 \times 10^{-9} \text{ mol on P-SBP, } 1.8 \times 10^{-9} \text{ mol on M-SBP, and } 8.3 \times 10^{-11} \text{ mol for the free protein, respectively.}

The reaction was started by adding hydrogen peroxide 6.4 \times 10^{-5} \text{ M. During the tests, the reaction mixture was continuously stirred and maintained at 25 °C.}

The reaction was followed by recording the absorbance at 590 nm (\epsilon_{590 \text{ nm}} = 47600 \text{ M}^{-1}\text{cm}^{-1}) at different reaction times for 4 hours. For each test-time, the stirring was stopped for about one minute, ~3 ml of supernatant was transferred in a quartz cuvette for spectroscopic analysis and afterwards put back in the reactor.

In the case of free-enzyme experiments, ~3 ml of the solution were analyzed without separating SBP from substrate. In no cases, SBP signals interfered with 590 nm band.

The conversion rates were calculated with respect to the stoichiometric amount of the DMAB-MBTH product that can be obtained in these experimental conditions.

Results

Chemical and physico-chemical characterization of SBO

The SBO material used in this work is a product of biological origin. It is sourced from urban private yard residues and public park trimmings which have undergone aerobic biodegradation for 230 days. It has quite complex chemical composition. Assessment of its chemical nature is rather difficult, owing to the broad distribution of molecular weight and to the content of many organic moieties from the main constituents of vegetable matter which are not
completely mineralized by biodegradation. At best, it may be characterized for its content of organic matter obtained from the weight loss after calcination at 650°C, its C and N elemental analytical data, and its organic moieties and functional groups content obtained by $^{13}$C NMR spectroscopy as reported in Table 1. Organic matter in SBO can be virtually represented by molecular fragments such as that shown in Figure 1, where aliphatic and aromatic C moieties and functional groups fit analytical data. Besides the organic content, SBO exhibits some minor inorganic components which are reported and quantified in Table 2.

Water surface tension ($\gamma$) vs. SBO concentration (Cs) data, such as those reported in Fig. 2, are a useful tool to understand the behavior of the above SBO in solution and suggest potential uses for it. The experimental data show a clear trend of $\gamma$ to decrease upon increasing Cs. This feature has been reported for other similar substances isolated from different urban biowastes.[2] The observed slope changes in the $\gamma$-Cs plots have been explained to arise from conformational changes occurring for the polymeric substances in aqueous solution. These are capable to assume coil conformation and/or form large macromolecular aggregates in pseudo-micellar fashion. In both cases, polar functional groups are likely pointed to the external water phase, while the lipophilic C moieties are held in the inner structural core. For their chemical nature assessed by Table 1 data and their solution behavior indicated by Fig. 2, the SBO used in this work qualified as dispersants in water phase capable to interact with silica particles with their polar functional groups, to organize the bonded silica particles around their pseudo-micellar structural assembly and to allow, upon removal of the organic phase by calcinations, coalescence of the
pristine silica particles into a silica network following the shape of the organic dispersant.

Figure 2 reports the water surface tension ($\gamma$) vs. SBO concentration ($C_s$).

**Physico-chemical characterization of silica prepared in the presence of SBO**

Under the experimental conditions of this work (see Experimental section) the commercial silica powder was added to water containing SBO under stirring. The resulting suspension was then allowed to thicken to a pellet which was dried and calcined at 500°C to remove all organic material. This procedure has been found to yield monolithic materials which are compact, assume the shape of the container in which they are produced, can be handled without breaking or disaggregating in powder form and maintain dimensional stability in water over the 1-11 pH range. Figure 3 shows the images relative to the monolith preparation steps into cylindrical shape by performing the above procedures in a beaker: i.e. the black pellet formed by SiO$_2$ and SBO dried at room temperature (left section) and the white material after calcination (right section).

Analyses carried out by transmission electron microscopy (TEM) showed (Figure 4, left image) that the pristine silica powder is made up of small particles of ~ 10 nm width or less, producing large interparticle void spaces, whereas the monolith (Figure 4, right image) exhibits less and smaller interparticle void spaces.

Gas-volumetric analysis of N$_2$ adsorbed at 77K confirmed that the monoliths had pore sizes distribution peaking at smaller diameter levels than for the powdery pristine silica material. For both powder and monolith silica, Figure 5
reports the adsorption isotherms (Figure 5 A) and the pore size distribution (Figure 5 B) as obtained applying BJH model to the desorption experimental data. Table 3 reports the relative specific surface area calculated via BET model and the BJH-desorption total pore volume.

The adsorption-desorption isotherms (Figure 5 A) show hysteresis loops at high values of relative pressure indicating the presence of large pores. The extent of porosity changes passing from the powder to the monolith sample.

The BJH model applied to the isotherms desorption branch indicates that the powder is macroporous and that the monolith formation occurs with reduction of the pores width. Indeed, Figure 5 B shows that the pore size distribution peaks at 400 Å for the powder and at 330 Å for the monolith. At the same time, Table 3 shows that the BET specific surface area and the total pore volume decrease consistently (24% and 39% respectively) from the powder to the monolith.

Composition and activity of silica-immobilized Soybean Peroxidase (SBP) biocatalyst

As described in more details in the Experimental section, SBP was immobilized on both powder and monolith following a known procedure.[13] In this procedure, the product of the reaction of silica and 3-aminopropyltriethoxysilane is first obtained and then reacted with glutaraldehyde and SBP according to the reaction scheme reported in Figure 6.

Table 4 reports the reaction yield of the immobilization process and the SBP content in the final product for silica in powder and in monolith forms.

It may be observed that the SBP concentration in the powder is significantly higher than in the monolith, and this seems consistent with the higher specific
surface area of the powder compared to the monolith.

The immobilized SBP on powder (P-SBP) and monolith silica (M-SBP) were tested for their catalytic activity by the DMAB-MBTH reaction (exemplified in Figure 7) in comparison with the same reaction performed with free SBP in solution and in the absence of SBP.

The results are reported in Figure 8. As expected, the experimental data demonstrate that the immobilized SBP allows a slower conversion of the substrate to the reaction product. The initial reaction rate decreases in the order free SBP > M-SBP > P-SBP > control. However, after two hours the M-SBP catalyst allows to achieve the same 95% reagent conversion as free SBP in solution, whereas the P-SBP catalyst is slower and less efficient. Furthermore, P-SBP does not exceed 75% conversion of the reagent even after four hours of reaction (data not shown). To test performance stability upon repeated recovery and recycling, the immobilized catalysts were recovered from the reaction medium after two hours, simply taking the M-SBP sample out from the reaction mixture, or by filtration the suspension of P-SBP. Both samples were washed with acetate buffer to remove product and reagents residues from the reaction medium physically adsorbed on the catalyst, and used again to start a new reaction with fresh reagents. This procedure was repeated twenty times. At each cycle, the monolith immobilized catalyst (M-SBP) was easily removed from the reaction medium by simply picking it out, whereas the powder had to be filtered. Figure 9 reports the biocatalyst activity measured as % of retained activity as function of the number of reaction cycles.

It may be observed that, after an initial activity decline, both the monolith and the powder tend to remain at a constant value, at about 75-80% of the initial
activity. This appears slightly higher in the case of M-SBP.

Discussion

It has been found in this work that the polymeric bio-organic substances (SBO) isolated from urban vegetable refuse allow to fabricate a mesoporous silica monolith (Figure 3) starting from commercial silica powder. Compared to the powder, the silica monolith exhibits (Table 3 and Figures 4-5) lower specific surface area, total pore volume and pore size, mostly concentrated at 330 Å width. The pore size distribution peaking at 250-350 Å was observed also for silica prepared by sol-gel reaction in the presence of a similar SBO material as used in the present work.[3] This validates the expectation (see characterization of SBO section) that, either in the sol-gel formation reaction and in the dispersion of commercial silica, both carried out in the presence of SBO, particles might form and/or coalesce into a silica network following the shape of the organic polymeric substance. The quasi coincidence of the pore size distribution peak of sol-gel and dispersed silica suggests that either SBO used in the present work and the polymeric bio-organic substances used in previous work,[3] although sourced from different biowastes, have the same behavior in water solution and yield pseudo-micellar assemblies of similar size. The results of this work obtained with commercial silica, therefore, and those of previous work[3] obtained with sol-gel synthesized silica, confirm the property of polymeric bio-organic substances isolated from urban bio-wastes to perform as templates for the fabrication of mesoporous inorganic oxides. This property stems from their solution behavior to form large pseudo-micelles either by their molecular conformation and/or by intermolecular aggregation. With silica, the template
function seems effective either during sol-gel formation of silica and in the process of dispersing preformed silica.

In the present work, the additional property of SBO to perform as binder for the formation of silica monoliths has been evidenced. This is certainly due to the presence of the inorganic residues (specifically SiO₂ and salts) which can act as melting mixture. In fact, the fabrication of the monolith fails in the presence of ash-free SBO (i.e., SBO treated with HF in order to eliminate silica). In this case only aggregated powder is obtained and no modifications of morphological features is observed with respect to the original FK320 powder.

While monoliths formation, as exemplified in Figure 3, might propose the use of SBO for the fabrication of porous ceramics and other materials in aqueous media and using commercial preformed powdery particles, for heterogeneous biocatalysis, specifically addressed in the present work, the use of a monolith-like material, coupled to its mesoporosity features, is quite relevant for the following reason. Recovery of the immobilized biocatalyst in monolith form from the reaction medium is much easier and more efficient than in the case of the powdery biocatalyst. The results in Figure 9 show that repeated recovery of the monolith biocatalyst is possible with a limited activity loss occurring only in the first three cycles and being inferior to the activity loss for the powdery biocatalyst.

In addition to the improved performance upon being repeatedly cycled, the monolith immobilized biocatalyst appears to exhibit higher kinetic activity (Figure 8) than the powder immobilized biocatalyst, in spite of the fact that the latter contains more SBP (Table 3). This suggests that, for the specific DMAB-MBTH probe reaction used in this work, the morphological features of the
immobilized catalyst are more important than the amount of immobilized
catalyst. The higher performance of the monolith could be due to the presence
of mesopores where the active peroxidase is concentrated in confined spaces
yielding well-accessible micro-reactors and/or allowing better contact among
the reagents than in the case of the powder. These morphological features,
coupled to higher dimensional stability, may also be the reason of the higher
activity stability exhibited by the monolith compared to the powder catalyst when
recycled repeatedly. The results certainly encourage further studies on
polymeric organic substances isolated from different sources to establish
property-chemical nature-source relationships for the development of green
chemical technology.

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SBO production test facility.

References

Biochemenergy: a project to turn a urban wastes treatment plant into
biorefinery for the production of energy, chemicals and consumer’s products


Figure captions
Figure 1. Virtual molecular fragments for SBO isolated from UBW.

Figure 2. Surface tensione (γ) versus SBO concentration (Cs) in water.

Figure 3. Silica monolith after drying at room temperature (left image) and after calcination at 500°C for 4 hours (right image).

Figure 4 - TEM image of the monolith (right section) compared to the powder (left section).

Figure 5 - N₂ adsorption-desorption isotherms at 77K (left section A) and pore size distribution calculated via BJH model on desorption data (right section B) for silica powder (solid line, no symbols) and for silica monolith (line with triangle symbols).

Figure 6. Reaction scheme for the immobilization of SBP on silica.

Figure 7. Test reaction catalyzed by SBP in homogeneous solution or immobilized on silica[12].

Figure 8. Conversion rate of the DMAB-MBTH reaction at 25 °C in the absence (control) and presence of SBP, either free or immobilized on silica powder (P-SBP) and monolith (M-SBP).

Figure 9. Kinetic tests of SBP immobilized on silica powder (P-SBP) and monolith (M-SBP) for repeated reaction cycled. The activities were measured as % decrease of absorbance at 590 nm after 120 min of reaction time.
Table 1. Content of C moieties and functional groups as C meq g⁻¹ in SBO

<table>
<thead>
<tr>
<th></th>
<th>Cala</th>
<th>NRb</th>
<th>ORb</th>
<th>OCO</th>
<th>Phc</th>
<th>PhOYd</th>
<th>PhOH</th>
<th>COOH</th>
<th>CON</th>
<th>C=O</th>
</tr>
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<tbody>
<tr>
<td>11.7</td>
<td>2.32</td>
<td>4.20</td>
<td>0.13</td>
<td>4.26</td>
<td>0.52</td>
<td>1.64</td>
<td>3.84</td>
<td>0.45</td>
<td>1.49</td>
<td></td>
</tr>
</tbody>
</table>

a aliphatic C; b R = alkyl C; c Ph = aromatic C, except PhO C; d PhO = aromatic C bonded to O as in dyaryl ethers and alkyl aryl ethers according to Y = R, Ph.

Table 2. Percentages (w/w) of inorganic components and ash[^1]

<table>
<thead>
<tr>
<th></th>
<th>SiO₂</th>
<th>Fe₂O₃</th>
<th>Al₂O₃</th>
<th>MgO</th>
<th>CaCO₃</th>
<th>K₂CO₃</th>
<th>Na₂CO₃</th>
<th>Total</th>
<th>Asha</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.45</td>
<td>1.10</td>
<td>0.93</td>
<td>1.87</td>
<td>15.16</td>
<td>6.34</td>
<td>0.37</td>
<td>31.2</td>
<td>27.9</td>
<td></td>
</tr>
</tbody>
</table>

a from residue after calcination at 650°C

Table 3 - Morphological data for silica powder and monolith

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET (m²g⁻¹)</th>
<th>Total Pore Volume (cm³g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>powder</td>
<td>164</td>
<td>1.61</td>
</tr>
<tr>
<td>monolith</td>
<td>124</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Table 4. Yield and product data for SBP immobilization reaction according to Figure 6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% immobilization[^a]</th>
<th>mg SBP/g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-SBP[^b]</td>
<td>81.2</td>
<td>15.6</td>
</tr>
<tr>
<td>M-SBP[^c]</td>
<td>59.4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

[^a] The % immobilization was calculated as the difference between the initial amount of enzyme and that recovered in the washing liquid in respect to the initial amount of SBP. The concentration of SBP in solution was determined by measuring the absorbance at 403 nm;[^b] powder immobilized SBP;[^c] monolith immobilized SBP.
Figure 2

![Graph showing the relationship between \( \gamma \) (mN/m) and \( C_s \) (g/L). The graph displays a decrease in surface tension with increasing concentration.]
Figure 3
Figure 5

(A) Adsorbed volume (cm³ g⁻¹ STP) vs. relative pressure p/p°

(B) Pore volume (cm³ g⁻¹ Å⁻¹) vs. pore width (Å)
Figure 6

1. Monolith + C₂H₅O⁻Si(CH₂)₃⁻NH₄⁺ + C₂H₅O⁻Si(CH₂)₃⁻NH₂ → Monolith + H₂O

2. Monolith + Glutaraldehyde → Monolith

3. Monolith + SBP → M-SBP
Figure 7

\[
\begin{align*}
\text{MBTH} + \text{H}_{2}\text{O}_2 & \rightarrow \text{SBP} + \text{2 H}_2\text{O} \\
\text{DMAB} & \rightarrow \text{[product]}^+
\end{align*}
\]