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Morphology-controlled silicon oxide particles produced by red wiggler worms

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ABSTRACT

The preparation of silica particles by vermicomposting has gained increasing attention for use as an inventive alternative to conventional methods. The silica oxides obtained can be used in a number of technological applications. As of yet, these particles cannot be used efficiently because of the lack of research into the relationship between the bioprocess and the shape and size of the particles. The aim of this study is to synthesize silica particles by red wiggler worms using three different grasses: *Equisetum hyemale*, *Zea mays* nixtamalized, and *Otatea ramirezii* and to characterize the obtained particles. However, it is unclear whether the use of diverse systems causes changes in the morphology of the final product. We found that silica particles can be produced by the three studied systems. Furthermore, each system showed a different polymorphism. We demonstrated that the new materials are mesoporous with a low surface area (9–22 $m^2\ g^{-1}$), but each have one specific crystal arrangement. The silicas were characterized by several techniques, namely Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, atomic-force microscopy (AFM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and Brunauer–Emmett–Teller (BET) analysis.

1. Introduction

Grass is a well-known kind of plant that grows naturally over the earth (*Poaceae* or *Gramineae*) [1,2]. It is a versatile, low-growing, green plant life-form that has adapted to diverse climate conditions over millions of years [3]. Additionally, grasses are a valuable source of energy and food for humans and animals [4,5]. The grains of grasses, such as wheat, corn, rice, oats, barley and rye, are the most important food crops for man [6]. Moreover, grasses are also used in the forestry and agricultural industries to produce fuel, thatch, clothing, medicines, paper, and construction materials [7,8]. Consequently, grass by-products cause an increasing disposal problem. Nevertheless, grasses comprise several renewable material resources, which are useful in many technological applications, such as silica (SiO_2) [9,10].

Silica can be found in nature as a ubiquitous material that represents up to 45% of dry weight of soils. In the soil, the silica is chemically combined as silicic acid, which may be taken up by the roots of plants. The mechanism by which plants polymerize silica and produce phytoliths is not fully understood. These microscopic structures are able to transport silicon to the plants' cell walls to perform several roles, such as protective effects, and stronger structural development through the growth, strengthening and reproduction of plant connective tissues. The concentration of silica varies between plant species and also parts of the same plant. Grasses show accumulation of silica up to 4%, which is mostly translocated and stored in the leaves as amorphous silica gel. The decay of the plant recycles silica to the earth, where earthworms help decompose the matter, which is partially transformed into humus [11,12].

In addition, silica can be produced by chemical synthesis [11,12]. However, this approach is not only eco-hazardous and expensive, but also requires severe changes in pressure, pH and temperature [13]. On the other hand, biosilicification is a viable and cheap green approach that can be performed at room temperature. Biogenic silica is, moreover, formed by many terrestrial and aquatic microorganisms, including diatoms, cyanobacteria, sponges, fungus and annelids [14–19].

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The use of earthworms to transform the organic matter in compost is called vermicomposting [20]. This biosilicification process has been used to produce silica nanoparticles via earthworms. Rice husk, sugar husk and sugar cane bagasse were studied as by-products to produce silica particles [17,21,22]. Additionally, silica particles were earlier extracted from the grass *Stenotaphrum secundatum* by biotransformation via annelids. Nevertheless, there is a debate about the relationship among the precursors, the microorganism and the chemical structure of the inorganic material obtained by this innovative method [21,23].

As phytoliths formed by plants can be of different shapes and sizes due to the presence of diverse chemical structures that are characteristic of each system, such as proteins, lignins and polysaccharides, we suggest that silica particles obtained by vermicomposting come in specific crystal arrangements for each system. The lack of existing research into the relationship of the grasses with the morphology of the silica particles that can be obtained by vermicomposting prompted us to propose the use of three systems: *Equisetum hyemale*, *Zea mays* nixtamalized, and *Otatea ramirezii*. We also selected red wiggler worms (*Eisenia foetida*) because they have been demonstrated to be suitable microorganisms for this mesophilic process. The advantages of using red wiggler worms include that they are very prolific, can eat their weight daily, and can excrete more than 50% of their ingestion as humus [24].

On the other hand, *Equisetum hyemale*, also known as rough horsetail, is a native plant that grows within sandy areas of the region of Querétaro (Mexico). From the same region, we collected *Zea mays* and *Otatea ramirezii*. *Zea mays* is commonly harvested in this area, while the *Otatea ramirezii* species is endemic to the Sierra Gorda in Querétaro, Mexico, and belongs to *Bambusoideae* [25–29].

To the best of our knowledge, this study constitutes the first of its kind, in which it is demonstrated that the use of different species of grass provides specific arrangements of the silica particles obtained by the used bioprocess. The aim of this work is the development of a method to produce silica particles with particular morphologies. This approach supports our proposal that the original structure of siliceous plant remains can be modified by vermicasting to produce well-defined structures with potential biotechnological applications. The silica particles obtained can be dispersed in absolute ethanol to recover nanoparticles that preserve a similar morphology to sintered particles. Silica nanoparticles have been used in medicine for the targeting of cancer cells, gene delivery, and immobilization of anticancer drug molecules [30]. The design of silica nanoparticles as biocompatible nanoplatforms depends on a well-designed shape, size, large surface area, and controllable pore size [31–33]. Consequently, it is very important to establish methods that guarantee their use efficiently [34].

2. Experimental

The Grasses *Equisetum hyemale* and *Otatea ramirezii* were collected from the Sierra Gorda in Querétaro savannah, while *Zea mays* nixtamalized was obtained from a commercial source in the same region. The leaves of the plants were mown to feed the earthworms. The corn was wetted, and the grasses' leaves were soaked before use. These by-products were added to the containers separately.

A small-scale system made of hemlock wood of approximately 50 × 35 × 25 cm was built in the laboratory. We used about 1000 red wiggler *Eisenia foetida* earthworms. The containers were prepared at ambient temperature, darkness and aeration, which was prepared with a mesh. The worms were mixed with enough horse dung and water for a month until the environment was favorable for vermicomposting. The studied grasses were gradually added to the container and in exchange for the same amount of dung over approximately twelve months. Consequently, the diet of the earthworms was completely changed into grass. The method of harvesting used was known as "*let the worms do the sorting*". In this method the finished casting is placed every month in one side of the container, and the grass is added to the new space created. As red wiggler earthworms are surface dwellers, they move over to the other side of the bin in search of food. After this, the castings are harvested. The humus collected during the first ten months was discarded because it was contaminated with dung. Next, we harvested the humus of the last two months to ensure that it was composed entirely of grass castings and hence could be used to obtain the silica particles.

The process by which the earthworms transform the food into silica is very complex. The first degradation that the silica-forming bodies of the plants perform is the biodegradation of the $SiO_2 \cdot nH_2O$ in the soil. However, the remains can persist for millions of years. The decay of grasses provides a number of substances, including siliceous phytoliths, which are broken down by the worms to eventually form biolites. Earthworms eat, and the food passes through their pharynx. The pharyngeal glands secrete a substance called mucus (essentially glycoproteins and enzymes), which lubricates and swells the food. In this region, the food is made glutinous before continuing along to the esophagus. Calcium carbonate ($CaCO_3$) neutralizes the food in the esophagus, which moves to the crop, and afterwards to the gizzard. It is in the gizzard that the food is churned and mixed by the earthworm's muscles. The silica and other mineral grains grind against the dirt, and the muscles tear the food into smaller parts until it is fully degraded. In this process, the silica is probably ground to nanometric dimensions by the strong muscular contractions. Then, the churned food moves to the intestine, where is degraded by benign bacteria. The digestion is performed by the enzymes pepsin, cellulase, lipane, amylase, and by drilodefensins which break down the organic matter and protect earthworms against plant polyphenols. The casts are excreted by the earthworm's anus, but the silica is only affected by the mechanical digestion [35,36].

The earthworm's waste, collected as humus, was dried and ground to a mesh size of 200 (74 μm) at ambient temperature. Samples of 11 g of sieved humus were calcined in a crucible. We placed the container inside a muffle furnace (Felisa Model FE363) for heating to 700 °C at a ramp rate of 2 °C. 10 h of heating was allowed to thermally decompose the organic matter, and then the muffle was turned off until it reached 32 °C after 12 h. The weight of the samples had decreased by approximately 50%. The inorganic matter was a sandy brown color in appearance, which indicates the presence of metal oxides and other impurities. Hence, the calcined samples were digested at 40 °C for 4 h using a mixture of hydrochloric acid (HCl)/nitric acid (HNO_3) (Sigma Aldrich (Germany); volume ratio 3:1) with constant stirring, in order to eliminate the impurities. We used 5 mL of the mixture for each gram of sample. The digestion was performed in Nalgene high-density polyethylene (HDPE) bottles. The undesired mixture of acids was removed by decantation from the bottles, and the suspended particles were rinsed with water to neutralize the silica. Subsequent to that, we used a Centurion Scientific K3 series centrifuge for 10 min at 3000 rpm to continue washing the samples until pure silica particles were obtained. The samples were decanted and dried in a stove at 70 °C for 24 h until they reached constant weight.

The silica particles were characterized by dispersive Raman and Fourier-transform infrared (FTIR) spectroscopy. The infrared analysis was performed on a Bruker Vector 33 spectrometer with a resolution of 4 cm^{-1} and 32 scans in transmission mode. The silica was deposited on a support, and the analysis was carried out by attenuated total reflectance with a diamond crystal in the range of 600–3600 cm^{-1}. On the other hand, dispersive Raman was accomplished using a micro-Raman spectrometer (Bruker Senterra, model 910, MA, USA), equipped with a laser light source of 785 nm. The samples were surveyed with 10 s of integration time, and 16 co-additions, with a resolution of 10–15 cm^{-1} and 100 mW. The powder was also placed on the support and the Raman spectra were recorded at room temperature.

The morphology of the silica particles, previously coated with gold, was studied with a scanning electron microscope (SEM) (JEOL-JSM-6060LV) operated at 15 or 20 kV. We used an image magnification of 1000×. A close inspection of the silica samples was conducted by