BIOGENIC AND SYNTHETIC AMORPHOUS SILICA – COMPARE AND INTERACTION WITH BACTERIAL SYSTEMS

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Abstract

The aim of the work is to compare the biogenic silica nanoparticles, which have been isolated from rice husks with synthetically produced silica brand Cab-O-Sil LM-150. The comparison is based on an evaluation of the two systems from the point of view of chemical composition, particle size and structure, ability to form clusters of particles and interactions with selected bacterial systems. Methods used for comparison were: SEM, EDX, TEM, FTIR, ICPOES and bacteriological tests. Husks are standardly contaminated by accompanying ions that are important for plant growth, but undesirable for obtaining a quality product. Rice husk used for the silica isolation were purified by boiling in 10% HCl for 2 hours. Rice husks were then washed with distilled water until pH 7. The dried and purified husks were burned in an oven at 650°C of ramping temperature 10°C/min during 2 hours. The obtained product has a chemical composition analogous to the synthetic product. Biogenic particles reach a size of about 20 nm, about 10 nm synthetic. Both materials have an amorphous structure. Interaction with bacterial systems was performed with Gram-negative bacteria *Escherichia coli* W3110 strain and Gram-positive bacteria *Bacillus cereus*. In both cases analogous behaviour was observed depending on the concentration of nanoparticles and reducing the growth rate of cultures of 8% for *E. coli* and 5% for *B. cereus* resulted nanoparticles at the concentration of 150 mg/ litter of medium.

Keywords: biogenic silica, synthetic silica, chemical composition, particle size, bacterial systems

1. INTRODUCTION

Amorphous silicon dioxide have already reached considerable field of application. Due to its chemical composition and particle size it is used as an additive: for improving powders flow-ability, rheological parameters correlation, influencing friction behaviour. Silicon dioxide filler is an integral part of different pharmaceutical, cosmetic, food or polymer products. Synthetic amorphous SiO_2 nanoparticles e.g. type Cab-O-Sil is produced by continuous process of silicon tetrachloride flame hydrolysis at temperature about 1700°C. Silicon dioxide nanoparticles can be found in certain type of plant too [1, 2]. Silicon is absorbed by plants root system in form of colloid ortho-silicic acid [3]. Due to a number of biochemical processes and consequently photosynthesis the overall process leads to formation of SiO_2 nanoparticles. These nanoparticles are primary shaped in surface layers of plant parts (leaves, stalks, husks) and creates barriers several microns thick. They are firmly connected with organic phase – e.g. cellulose in these barriers. Silica has an important role from the point of view of plant reinforcement and a surrounding environment protection. In these systems we can find accompanying ions of calcium, potassium, sodium, magnesium, aluminium and others. These ions are essential for the growth of plants and their amount is related to the plant species and a soil composition in which plants grow. Rice husks can be considered as the largest potential source of silica from an agriculture waste due to high silica content up to 23 wt. %. The most common isolation method of this compound from rice husks is their burning at 700°C with a chemical pre-treatment with hydrochloric acid to ensure the removal of accompanying ions [4, 5, 6]. Silicon dioxide that is formed in plants due to biochemical processes and photosynthesis is known as biogenic or biomorphous. Biogenic silica has a great potential for use in biomedicine, pharmaceutical, cosmetic and food industry [7,8].

2. EXPERIMENTAL PART

2.1 Materials and methods

Rice husk (obtained from Oryza sativa L.) imported from Vietnam, province Khanh Hoa, were used for experiments. Commercial product brand Cab-O-Sil LM -150 (Cabot GmbH), synthetically produced amorphous fumed silica was used as a comparative material. Hydrochloric acid (Penta Chemicals) and deionized filtered water were used for a purification process of rice hulls. To remove dust and other impurities, obtained hulls were washed with deionized water, next dried (60 °C for 3h) and purified in 10% hydrochloric acid under reflux for 2h. Acidic residues were removed by washing samples with deionised water. After that a dried sample was calcinated in a laboratory furnace for 2 h at 650 °C in air. The heating rate was 10 °C per minute from an ambient temperature (25 °C).

Biogenic silicon dioxide nanoparticles isolated from rice husks and synthetic silicon dioxide nanoparticles type Cab-O-Sil LM-150 were characterized by a scanning electron microscope (SEM), FE SEM ZEISS ULTRA PLUS. The samples were prepared by depositing dried materials onto the microscope holder. Map of the chemical composition was obtained by EDX analysis performed using Oxford Aztec Energy detector.
ATR-FTIR spectroscopy was performed to analyse the functional groups present in the prepared samples using a Nicolet iZ10 spectrometer, Thermo Scientific (USA), with DTGS detector, technique: ATR Ge crystal, number of sample/background scans: 32/128, resolution: 4 cm^{-1}, spectral range: 4000–700 cm^{-1}, apodization: Happ-Genzel.
A chemical composition was evaluated using an inductively coupled plasma optical emission spectrometer (ICP OES, OPTIMA 2100 DV, Perkin Elmer) for the determination of Ca, Mg, K, Na, Al and Fe ions in digests. Rice husk sample was prepared by microwave digestion using Multiwave PRO, Anton Paar, Austria. equipped with high-pressure teflon vessels. Decomposition was carried out for 45 min at 150 °C in nitric acid under the pressure of 60 bar.
Transmission Electron Microscopy (TEM) was used to determine the shape and size of nanoparticles. Samples were suspended in anhydrous ethanol and subsequently ultra-sonified using an ultrasonic homogenizer (20% amplitude) and next those suspensions were centrifuged for 5 min at 6000 rpm. Supernatants from each sample were dropped on copper grid with holey carbon film and dried on the air. Transmission Electron Microscopy analysis was done on JEOL JEM 2010F at 160kV of accelerating voltage.

Microorganisms *Escherichia coli* W3110 [9] and *Bacillus cereus*, a soil isolate obtained in the Laboratory of Enzyme Technology, Prague, were grown in Luria Bertani (LB) medium (in g/L): tryptone 10, yeast extract 5, NaCl 5, pH 7.2. For all experiments, *E. coli* was cultivated at 37°C and *B. cereus* at 28°C. To prepare the inoculum, a stock culture was transferred onto agar plates with LB medium and cultivated for about 16 h. A single colony was used to inoculate 20 mL of LB medium and the flask was shaken on a rotary incubator at 200 rpm. One ml of the inoculum was then used to inoculate batch cultures in 500-mL flasks containing 100 mL of LB medium supplemented with the nanoparticles, leaving one as a control to track the normal growth of the microbial culture in the absence of nanoparticles. The cultures were shaken for 24 h. Nanoparticles powder 5, 10 or 15 mg was dispersed in 1 ml of sterile Milli-Q water, vigorously mixed for 5 min and transferred to bacterial cultures to get final concentration 50, 100, or 150 mg of nanoparticles per litre. Biomass concentration was assayed spectrophotometrically measuring the optical density of culture samples at λ of 600 nm (OD_{600}). Cultures were sampled every thirty minutes for eight hours to record the growth. The specific growth rate μ (h^{-1}) was calculated from five consecutive OD_{600} measurements ($\mu=\Delta \ln OD_{600}/\Delta t$, where t is time) during the exponential phase of growth.

2.2 Results and discussion

Biogenic silicon dioxide was obtained by a chemical purification of rice hulls and a subsequent calcination of the purified intermediate product as described above. The ultra fine powder of biogenic silica was compared with a synthetic CAB-O-Sil LM – 150 in point of material properties and a biological interactions. From scanning electron microscope observation the formations of biogenic silica appears to be more compact in

comparison to the synthetic product. Both of them have a tendency to form the agglomerates. The size of these structures which were shown on **Fig. 1** are in dozen of nanometers. Transmission electron microscopy allows determine the size, shape and morphology both of investigated materials. Size of biogenic silicon dioxide nanoparticles obtained from rice husks are ranged from 20 to 30 nm, see **Fig. 2A**. The size of the pyrogenic amorphous nanoparticles of silicon dioxide produced at 1700°C is about 10 nm. TEM images clearly show the round shape of nanoparticles. Characteristic feature of the synthetic product is the formation of a chain (chain-like) structure, as is evident from **Fig. 2B**. In both cases, the nanoparticles have an amorphous structure which was confirmed by selected area electron diffraction (SAED).

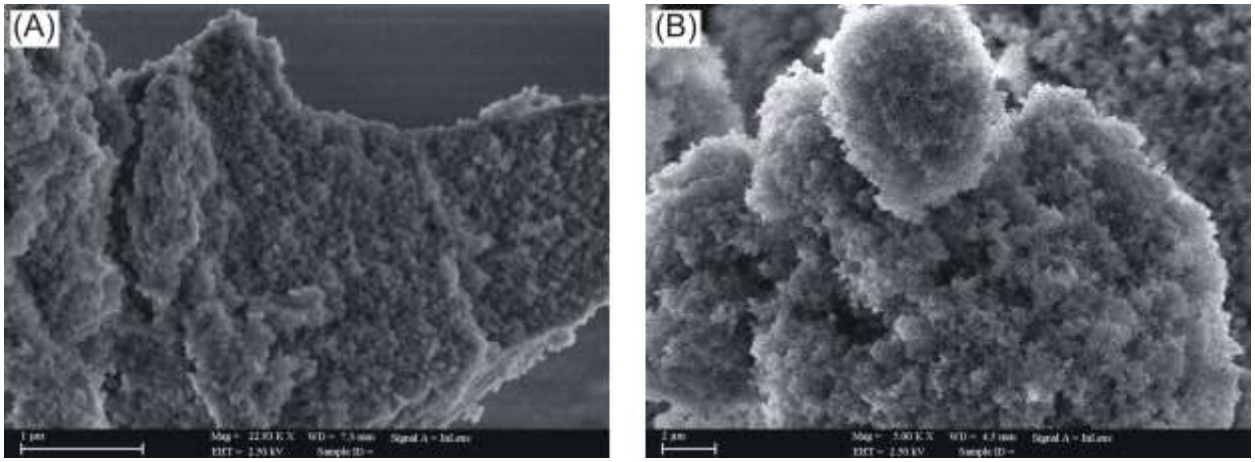

Fig. 1 SEM images of the biogenic silicon dioxide nanoparticles isolated from rice husks **(A)**, synthetic silicon dioxide nanoparticles type Cab-O-Sil LM-150 **(B)**

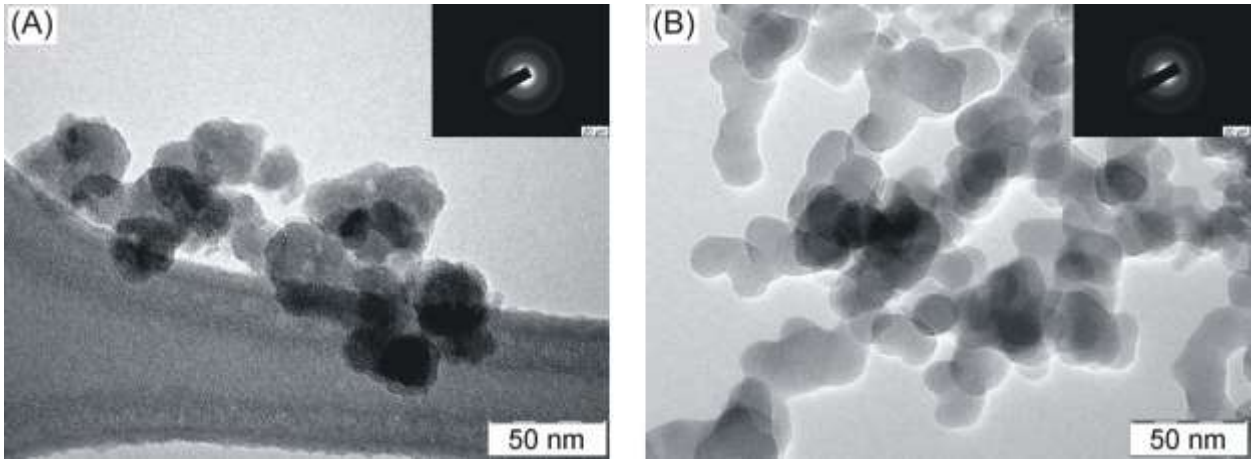

Fig. 2 TEM images of the biogenic silicon dioxide nanoparticles isolated from rice husks **(A)**, synthetic silicon dioxide nanoparticles type Cab-O-Sil LM-150 **(B)**. Selected Area Electron Diffraction (SAED) showing a diffraction ring – inset

The basis of purification process is removal of the major accompanying magnesium, sodium, calcium, potassium, aluminum and iron ions. The presence of these ions significantly affect on the quality of the obtained silicon dioxide after combustion. It is necessary to remove the highest content of these ions in order to get the purest product. **Fig. 3A** shows an overview EDX analysis of the chemical composition of raw rice husk before and after removal of undesired accompanying ions. The analysis confirmed that the raw material is characterised by a high content of silicon, carbon, oxygen and additional ions which plants need for growth. Samples after purification (**Fig. 3A** inset) show only the presence of three main components (Si,C,O). Small amount of carbon which is characterized by occurrence of the carbon peak is negligible, because mainly comes from the method of performing EDX analysis (carbon tape).

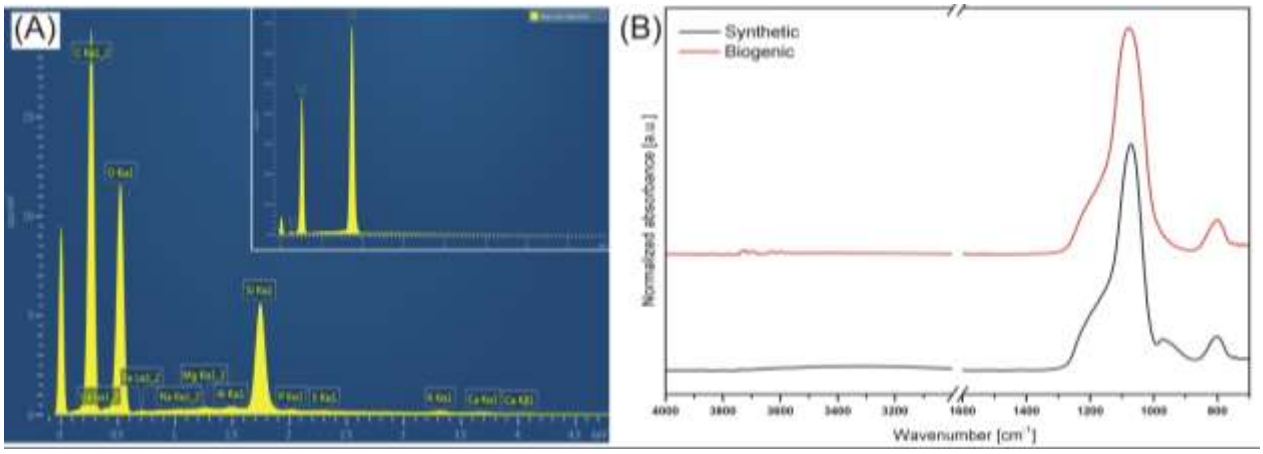

Fig. 3 EDX analysis of rice husks before and after (inset) the process of chemical pre-treatment **(A)**, FT–IR spectroscopy analysis – a comparison between isolated biogenic and synthetic silicon dioxide nanoparticles **(B)**

Inductively Coupled Plasma Optical Emision Spectrometry (ICPOES) was used to determine the exact content of ions in rice husk sample. Selected purification method removes impurities up to almost 100% as you can see from **Table 1**.

Table 1 Ionic compositionin of rice husk samples *(Oryza sativa L.)* before and after purification in 10% hydrochloric acid

Oryza sativa L.	Ionic composition [mg·kg^{-1}]					
	Mg	**Ca**	**Na**	**K**	**Al**	**Fe**
Before	360	847	88	2787	685	297
After	**2.62**	**<2**	**8.32**	**<2**	**51.1**	**6.95**

Infrared spectroscopy was used for comparison of chemical bonds in the biogenic and synthetic silicon dioxide nanoparticles. **Figure 3B** shows the ATR-FTIR spectra of investigated samples. Spectra of synthetic silicon dioxide illustrate the three characteristic peaks at wavenumbers of 1072 cm^{-1}, 950 cm^{-1} and 800 cm^{-1}. Expanded bond appeared near 1072 cm^{-1} is attributed to the asymmetric stretching vibrations of the Si-O-Si bonds. Another characteristic signal at 800 cm^{-1} corresponds to the presence of the symmetric stretching vibrations of Si-O-Si bonds [8,10,11]. According to literature, band apparent at 950 cm^{-1} indicates the presence of the silanol bonds, Si-OH, and it is closely related to the presence of the hydroxyl groups in the range of 3400-3200 cm^{-1} [8]. Biogenic silicon dioxide obtained via process describe above differ from synthetic product. In the tested samples the hydroxyl groups are not visible (absence of peaks near 950 cm^{-1}, 3400-3200 cm^{-1}).

Biogenic silicon dioxide isolated from rice husks was also compared with synthetic product in terms of interaction with bacterial systems. Comparative study of bacterial growth of gram-negative *E. coli* and gram-positive *B. cereus* exposed to silica nanoparticles was made in LB medium containing biogenic nanoparticles or synthetic Cab-O-Sil in concentrations of 50, 100 and 150 mg/liter. No toxic effect of both types of particles on growth of cultures was observed. All characteristic phases of the growth curve were clearly visible in the course of 24 h and no differences in final OD were determined: final OD_{600} for *E. coli* and *B. cereus* cultures was 4.35±0.1 and 11.2±0.3, respectively. Specific growth rates calculated for *E. coli* and *B. cereus* cultures with and without silica particles are in **Fig 4.** The growth of *E. coli* and *B. cereus* cultures exposed to synthetic silica nanoparticles was not inhibited at any concentration. The growth rate in the presence of biogenic nanoparticles was slightly decreasing with increasing concentration of nanoparticles. The growth rate reduction of about 8% for *E. coli* was determined at the highest concentration of (150 mg/liter) of

biogenic nanoparticles. As regards *B. cereus*, the reduction was about 5% at the highest concentration of biogenic nanoparticles.

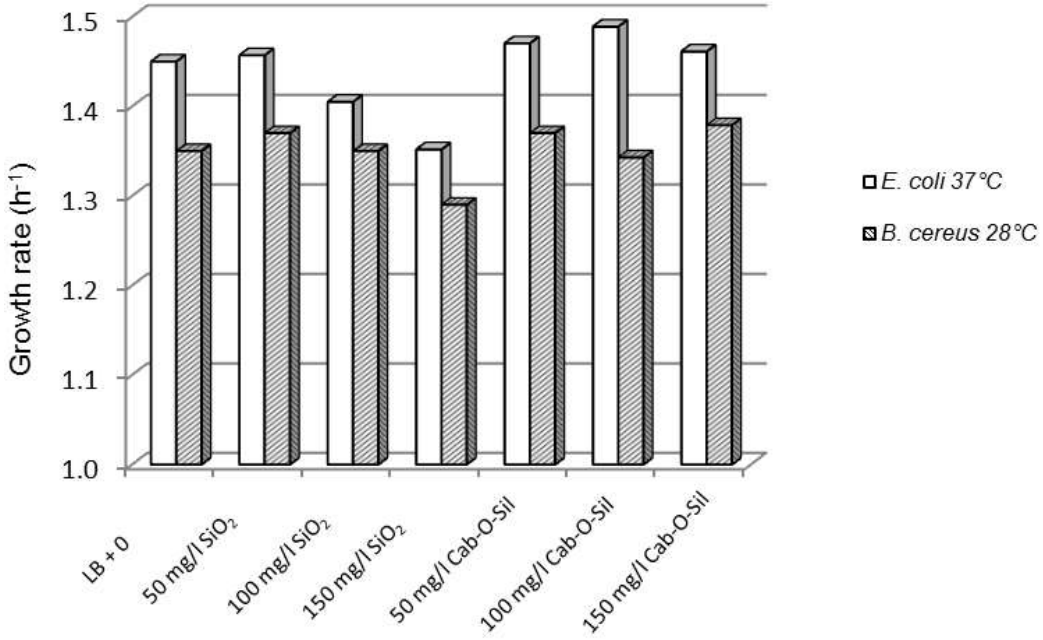

Fig. 4 Concentration effect of biogenic (SiO_2) and synthetic silicon nanoparticles, (Cab-O-Sil) on the specific growth rate of *E. coli* W3110 and *B. cereus* grown in batch cultures in LB medium at 28°C (*B. cereus*) or 37°C (*E. coli*) for 24 h. The specific growth rate μ (h^{-1}) was calculated as the slope of five adjacent points from exponential growth phase

3. CONCLUSION

The present study demonstrate that it is possible to obtain pure silicon dioxide, SiO_2 in form of nanoparticles from rice husks, which is one of the largest agricultural waste material. The size of biogenic silica nanoparticles obtained after purification and calcination processes are in the range from 20 to 30 nm. These nanoparticles have a spherical shape, amorphous structure and have tendency to agglomeration. Using the suitable purification method of raw material (rice hulls) were efficiently removed additional ions (up to 100%) which have significant impact on the morphology and purity of final product. Nanoparticles of commercially available amorphous silicon dioxide brand Cab-O-Sil LM 150 have 10 nm size of individual particle and tendency to easily form agglomerates with chain-like structures. An infrared spectroscopy shows only one difference between biogenic and synthetic silica, that is lack of silanol bonds in the nature product. The main reason of this dissimilarity is probably the process of preparing silica powder from rice husks. Biogenic silica nanoparticles may be regarded as analogous to synthetic product in point of view interaction with bacterial systems. No deleterious effect of the nanoparticles on culture growth of gram-negative (*E. coli*) and gram-positive (*B. cereus*) bacteria was observed. Similar results were described by Williams et al. [12]. Although the slow and steady decrease of the growth rate in the presence of biogenic particles was determined, the final biomass concentrations achieved in cultures after 24 h of growth were the same as in nanoparticles-free control cultures.

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