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Green synthesis, Optimization and Characterization of SiO_2 nanoparticles using *Aspergillus tubingensis* F20 isolated from drinking water

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Abstract

This study aimed to demonstrates a positive correlation between silica metal tolerance ability of a drinking water fungi and its potential for the synthesis of silica oxide (SiO_2) nanoparticles (NPs). Metal oxide NPs can be synthesized biologically by different methods including; microorganisms, plant extracts and\ or plant biomass. These methods in some time are better alternatives to the chemical and physical methods through an environmentally route. In the present work, twenty fungal strains were isolated from eight potable water samples and tested for producing silica nanoparticles (SiO_2NPs), using precursor salt Dipotassium fluorosilicate (K_2SiF_6). Out of these twenty fungal strains, only one fungal isolate had the potency to reduce metal salt into metal NPs, which was identified by a molecular assay as *Aspergillus tubingensis* F20, and was assigned an Accession number of (MK226258.1) using the NCBI GenBank database. The factors affecting mono-dispersed production of SiO_2 NPs such as; reaction times, incubation temperatures, hydrogen ion concentrations (pH) and salt concentrations were optimized. It is revealed that 10^{-3} M precursor salt concentration, 72 h of reaction time at pH 3, and an incubation temperature 28°C are the optimum conditions for the production of smaller size NPs. The biosynthesized NPs was characterized using several techniques including; Dynamic light scattering (DLS), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy dispersive X-ray spectroscopy (EDX). It is observed that the shape of SiO_2NPs is spherical with an average size of 8 nm, and surface charge of - 8.19 mv, which indicates that SiO_2NPs is more stable.

Keywords: *Aspergillus tubingensis*, Silica nanoparticles, Microbial synthesis, Fungi, Drinking water

1. Introduction

In the last decade, the nanoscience and technology attracted the scientist's attention in various fields. Nanoscience is rapidly increasing and growing, primarily because of the new applied techniques,

methods of synthesis of the nanomaterials, in addition to the new tools of the characterization and assessment. The application of nanoscale and nanostructure materials within range of 1-100 nm is an emerging area of nanoscience and nanotechnology. Previously, Sharma *et al*., (2009) reported that in the last few years nanomaterials became a preferable solution to many technological and environmental challenges in several fields including; medicine, water treatment, solar energy conversion and catalysis.

There are different methods for preparing NPs such as physical, chemical and biological methods. Unfortunately, each method may show some disadvantages. Generally, the physical methods produce low yields. Whereas the chemical methods exhibited many difficulties due to the toxicity of some used solvents, contamination due to precursor chemicals, and the generation of non-preferred by-products (Wang *et al*., 2007). Hence, Zonooz and Salouti, (2011) revealed that there is general trend to use ecofriendly, safe and clean methods for NPs preparation that does not produce toxic by-products due to the process of synthesis. In a previous study, Bruins *et al*., (2000) pointed that the metal microbe interactions have an important role in several biotechnological applications including; bioremediation, bio-mineralization, bioleaching and microbial corrosion. Peto *et al*., (2002); Kumar *et al*., (2003); Sastry *et al*., (2004) added that the inorganic materials either intracellular or extracellular are recorded to be produced by unicellular and multicellular microorganisms.

According to Ghorbani *et al*., (2015), SiO_2 NPs have gained a greater attention because of its highly reactive surface area to volume ratio, chemical and physical stability, low toxicity and straight forward surface chemistry. Moreover, Kasaai, (2015) reported that it is frequently used in industrial manufacturing, packaging, ceramic and synthesis of high molecule composites material, drug delivery, cancer therapy, disease labeling, biosensor, food and agriculture. In the present work, an attempt was made to synthesis SiO_2 NPs using eco-friendly, rapid, and low cost method by challenging *A. tubingensis* with the aqueous solution of K_2SiF_6. Moreover, the characterization and control of NPs synthesis are significant to appreciate.

2. Material and methods

2.1. Sampling

About eight samples from the plants treatment and distribution systems of the potable water in Al-Giza governorate, Egypt, were collected and analyzed. Tap water from pipes were collected after sterilization of the tap using ethyl alcohol (70%), flamed and then left to flash for 5 min. The water samples were kept in 1000 ml clean, sterilized polypropylene plastic bottles with screw caps that were sealed, transferred to the laboratory in ice box, and then kept at 4°C. Examination of these water samples was carried out within 6-8 h after sampling. Sodium-thiosulphate (4.7% w/w) was added to the water samples to inactivate the residual free chlorine (Gonçalves *et al*., 2006; Sammon *et al*., 2010).

2.2. Isolation of fungi from the water samples

The water samples were then filtered and analyzed according to the standard method (WHO. 2017). Briefly, 100 ml of each sample was filtered through a sterile 0.45 µm membrane cellulose nitrate filters under sterile conditions in a Laminar flow cabinet. With the aid of a sterile forceps, the filter was transferred to a petri plate containing sterilized solidified Sabouraud Dextrose agar (SDA) medium supplemented with Gentamycine (25 mg/l) after autoclaving (Mbata *et al*., 2008). Three replicates were used for each water sample. The plates were incubated at 25- 28°C for 5-7 d, and were monitored daily for appearance of fungal colonies. The number of developing colonies was recorded, and the single spore fungal isolates were sub-cultured separately for identification.

2.3. Metal tolerance profiles of the fungal isolates

The maximum tolerable concentration (MTC) assay was performed to determine the silica metal tolerance ability of the fungal isolates (Ahmad *et al*., 2006). SDA plates containing different concentrations of K_2SiF_6 (10-200 ppm) were prepared, and then inocula of the tested fungi were spotted individually on the surface of media, while the control plates were free of the metal. The plates were incubated at 28°C for at least 5 d, and examined daily for the fungal growth. Maximum concentration of the metal ions in the medium which allowed growth of the fungus was considered as MTC.

2.4. Molecular identification of the isolated fungus

The most promising fungal isolate showing tolerance to the metal ions was identified using molecular technique based on 18s-rRNA (Stephen *et al*., 1990). The ribosomal RNA was extracted using GeneJet Plant genomic DNA purification Kit (Thermo) according to the manufacturer protocol. Primers ITS1-F (50TCC GTA GGT GAA CCT TGC GG 30) and ITS4-B (50TCC TCC GCT TAT TGA TAT GC 30) have been used for isolation of the 18s-rRNA. The Polymerase Chain Reaction (PCR) product has been cleaned up using Gene JETTM PCR Purification Kit (Thermo). Sequencing of the PCR product was carried out at GATC Company using ABI 3730xl DNA sequencer. The sequence results were processed and compared with the NCBI/ GenBank database.

2.5. Extracellular biosynthesis of SiO_2NPs by the promising fungal isolate

The fungal isolate showing the highest MTC value was selected for extracellular synthesis of SiO_2NPs in reference to Vipul *et al*., (2005). For this fungal isolate, conidia were scrapped from cultures which were grown on SDA slants for 5 d at 30°C, and then suspended in sterile dist. water. An aliquots of 3 ml of this suspension were used to inoculate 250 ml Erlenmeyer flasks each containing 100 ml 0of Malt extract glucose yeast extract peptone (MGYP) media, composed of malt extract (0.3%), glucose (1.0%), yeast extract (0.3%) and peptone (0.5%). They were then incubated at 25–28°C, under shaking (200 rpm) for 72 h. The mycelial mass was then separated from the culture broth by centrifugation (5000 rpm) at 10°C for 20 min., and then the settled mycelia were washed thrice with sterile dist. water 3 times. The harvested mycelial mass (20 g wet weight) was then re-suspended in 100 ml aqueous solutions of K_2SiF_6 (10-3 M) at pH 6.8, and kept on a shaker (200 rpm) at 28°C for 72 h. After incubation, NPs containing fungal mycelia were filtered inside laminar flow through Whatman No. 1 filter paper. The control treatment was only metal ions without the fungal biomass. The bio-transformed product was collected periodically for characterization.

2.6. Optimization of the physio–chemical parameters for NPs biosynthesis

The size of SiO_2NPs was optimized by varying the biosynthesis parameters one at a time, such as temperature (18, 28, 38, 48°C), pH (3, 5, 7, 9), substrate concentration (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} M K_2SiF_6), and reaction time (24, 48, 72, 96 h). The particle size of the biosynthesized SiO_2NPs was determined using the Dynamic light scattering (DLS) technique.

2.7. Characterization of the biosynthesized SiO_2 NPs

2.7.1. Dynamic light scattering (DLS)

The particle size distribution of the SiO_2NPs was evaluated using DLS measurements (Yang *et al*., 2014). This technique was also used to measure the surface charge of the particles known as Zeta potential (ZP), through a Zeta sizer NANO-ZS (Malvern Instruments Ltd., United Kingdom). Zeta potential determines whether the particle within a liquid will tend to flocculate or not.

2.7.2. Transmission electron microscope (TEM)

The TEM was used to confirm the size and shape of SiO_2NPs using the drop coating method (Su, 2017).

The NPs sample was irradiated by an electron beam of equal prevailing density, and then TEM micrographs was taken using JEOL Transmission Electron Microscope (JEM-1230, Japan).

2.7.3. Scanning Electron Microscopy (SEM)

To characterize the surface topology, the SiO_2 NPs were coated with heavy metal gold and then subjected to SEM imaging (Ponce *et al*., 2012). These images of biosynthesized NPs were obtained using SEM (Inspect FEI Ltd, Holland).

2.7.4. Energy Dispersive X-Ray (EDX)

The elemental analysis of the NPs was carried out using Thermo-Noran EDX attachment (Scimeca *et al*., 2018), equipped with SEM (Inspect FEI Ltd, Holland).

2.8. Statistical analysis

Data of the 18s-rRNA sequence was analyzed using Mega software ver. 7 (Kumar *et al*., 2016). Results were cleaned and prepared for the multivariate analysis and similarity assessment to the NCBI/ GenBank database records. Hierarchical cluster analysis technique has been used for such purpose. Euclidean distance has been used as a similarity assessment tool, while nearest neighbor was the linkage method of the cluster analysis. Phylogenetic tree produced from the analysis showed the similarity between our species and similar records obtained from the NCBI/ GenBank.

3. Results

3.1. Isolation and screening of fungi for synthesis of SiO_2NPs

Analysis of the different silica MTC (10-200 ppm) showed that the fungus F15 (Fig. 1) exhibited the highest resistance to silica among the isolated recovered from the drinking water samples (Table 1). Thus, this isolate has been subjected to identification and then characterization using a molecular technique.

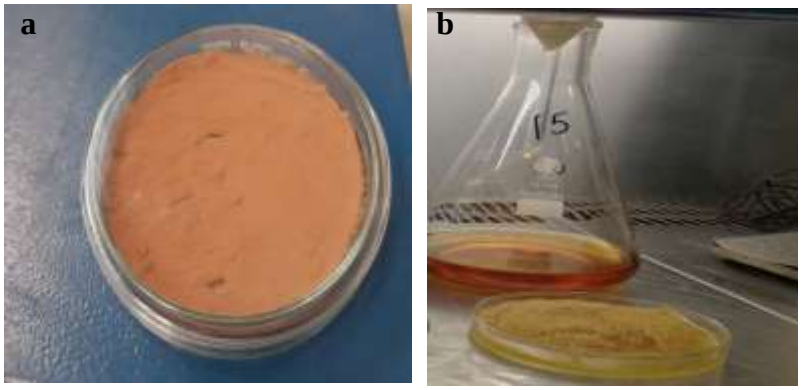


Fig. 1. (a) The fungus F15 growing on SDA, **(b)** The fungal biomass

Table 1: Growth of the fungal isolates on SDA medium supplemented with different concentrations of K_2SiF_6

Isolate Code	Fungal growth on SDA medium supplemented with different K_2SiF_6 concentrations (ppm)				
	10	**50**	**100**	**150**	**200**
F1	+++ve	+++ve	++ve	-ve	-ve
F2	+++ve	+++ve	++ve	-ve	-ve
F3	+++ve	+++ve	++ve	-ve	-ve
F4	+++ve	+++ve	++ve	+ve	-ve
F5	+++ve	+++ve	++ve	-ve	-ve
F6	+++ve	+++ve	++ve	-ve	-ve
F7	+++ve	+++ve	++ve	-ve	-ve
F8	+++ve	+++ve	++ve	-ve	-ve
F9	+++ve	+++ve	++ve	-ve	-ve
F10	+++ve	+++ve	++ve	-ve	-ve
F11	+++ve	+++ve	++ve	-ve	-ve
F12	+++ve	+++ve	++ve	-ve	-ve
F13	+++ve	+++ve	++ve	-ve	-ve
F14	+++ve	+++ve	++ve	-ve	-ve
F15	+++ve	+++ve	++ve	++ve	+ve
F16	+++ve	+++ve	++ve	-ve	-ve
F17	+++ve	+++ve	++ve	-ve	-ve
F18	+++ve	+++ve	++ve	-ve	-ve
F19	+++ve	+++ve	++ve	-ve	-ve
F20	F20	-ve	-ve	-ve	-ve

* Where; +: good growth, ++: very good growth, +++: excellent growth

3.2. Molecular identification of the promising fungal isolate

The most efficient fungal isolate for production of SiO_2NPs (F15) was identified as *A. tubingensis* strain F20, based on 18s-rRNA and was assigned the accession number of (MK226258.1) by the GenBank database. The phylogenetic analysis of this isolate (F15) using 18s-rRNA sequence data and GenBank database showed high similarity of 100% to *A. tubingensis* (Fig. 2).

3.3. Optimization of the physio–chemical parameters for SiO_2NPs biosynthesis

In an attempt to achieve better size control, optimization of the biosynthesis parameters such as; reaction times, incubation temperatures, hydrogen ion concentrations (pH) and different salt concentrations, were studied by changing one parameter at a time, keeping the other test conditions the same.

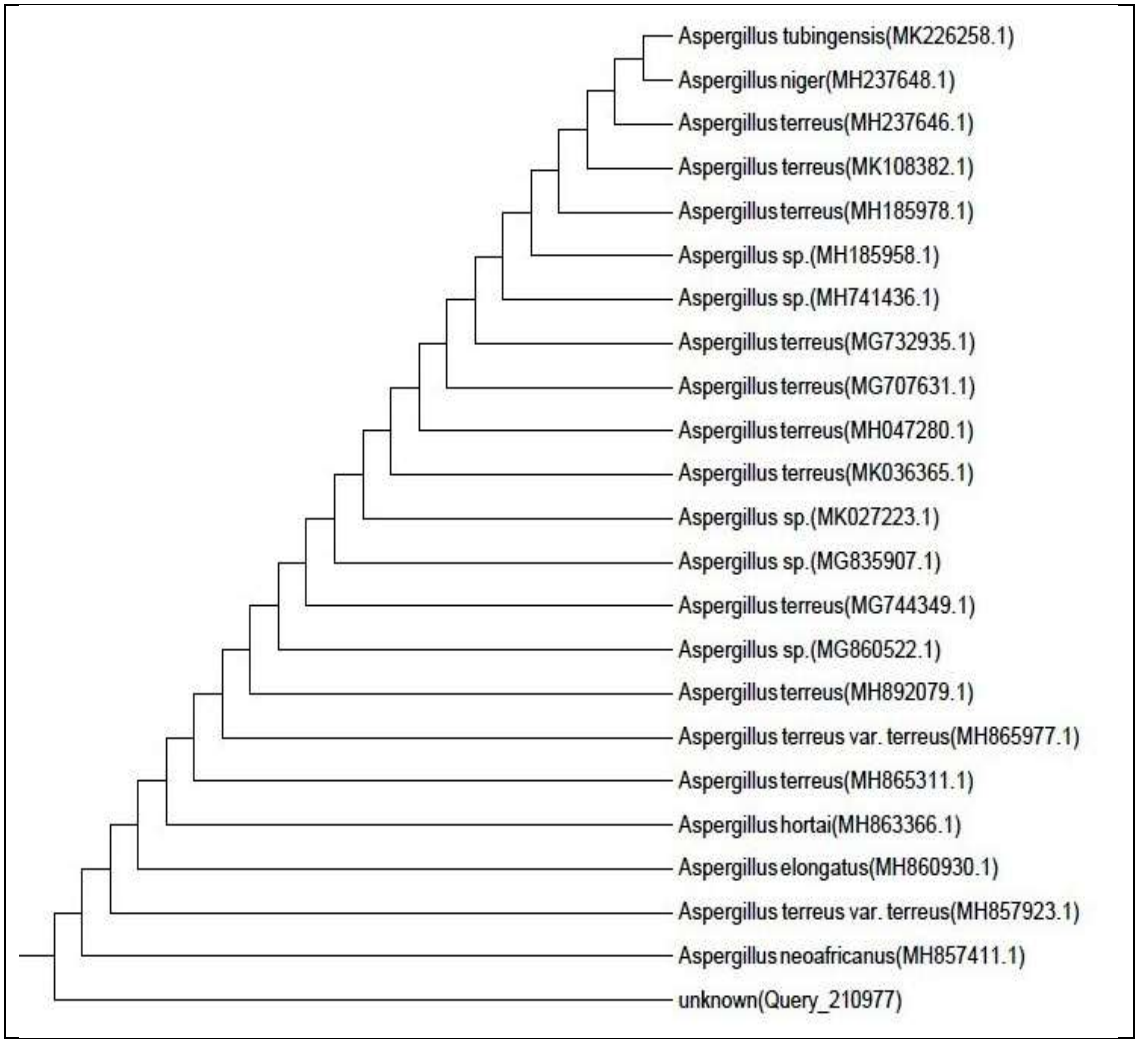


Fig. 2. The phylogenetic tree of the F15 isolate based on 18s-rRNA gene sequences

3.3.1. Effect of reaction time

The DLS analysis shows that the fungal precursor compound gave its best yield of NPs with the smallest size of 32 nm after 72 h of incubation; however, incubation for 96 h gave the same results (Fig. 3a).

3.3.2. Influence of different incubation temperatures

The DLS assay reveals that the average distribution size of the SiO_2NPs increased when the temperature deviated from 28ºC. At the temperature of 28ºC, the average size of NPs has the smallest size of 68 nm (Fig. 3b).

3.3.3. Dependence of SiO_2NPs biosynthesis on the hydrogen ion concentrations (pH)

The DLS technique reported that there is a direct relationship between the pH of the reaction mixture and size of the NPs. By increasing the pH, the size of NPs increases (Fig. 3c). It is observed that at pH =3, the NPs has the smallest size of 27 nm.

3.3.4. Effect of the salt concentrations

The DLS method recorded that the size of SiO_2 NPs reduced with the decrease in the concentrations of the precursor compound from (10^{-1} M to 10^{-4} M). However, it is obvious that the concentration of 10^{-3} M has the smallest size 31 nm, whereas 10^{-1} M has the largest size 240 nm (Fig. 3d).

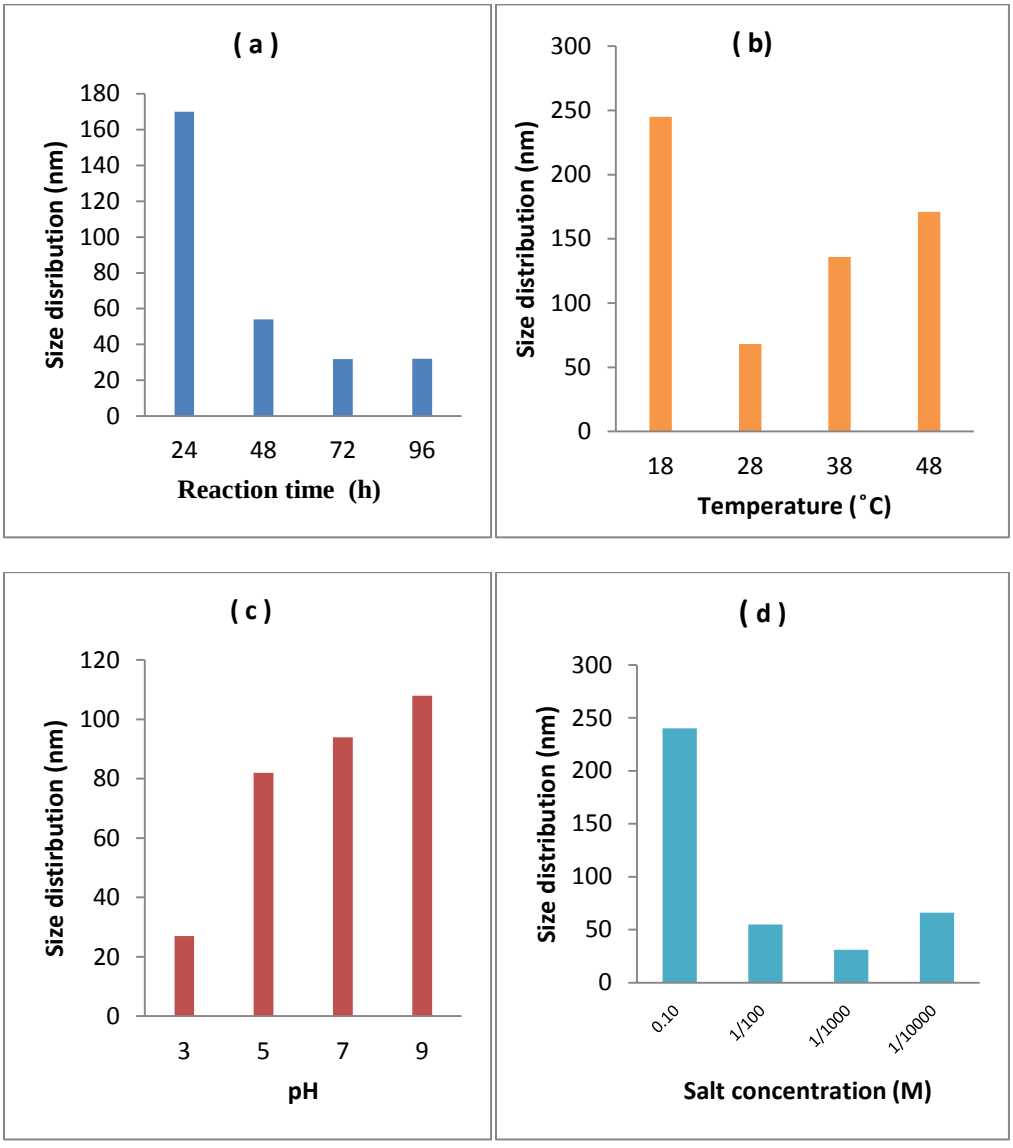


Fig. 3. The parameters used to control the size of the biosynthesized SiO_2NPs; (a) Effect of reaction time, (b) Effect of temperature, (c) Effect of pH, (d) Effect of salt concentration

3.4. Characterization of the SiO_2NPs

3.4.1. Dynamic light scattering (DLS)

The particle size of the biosynthesized SiO_2NPs was analyzed using the DLS technique (Fig.4a). The DLS histogram shows that SiO_2NPs possesses an average size of 8 nm, and the Poly-dispersity index (PDI) was 0.348, which reflects the mono-dispersed nature of the particles. The surface charge of the SiO_2 NPs is measured as Zeta potential of -8.19 mV (Fig. 4b) using the DLS technique.

3.4.2. Transmission Electron Microscope (TEM)

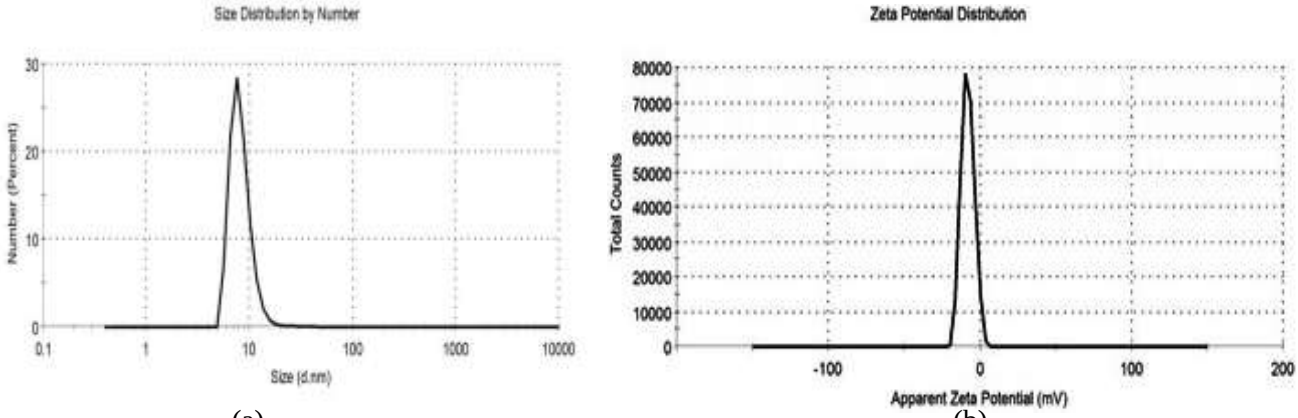
The low magnification TEM image at 50 nm scale bar (Fig. 5), shows well dispersion of SiO_2.

3.4.3. Scanning Electron Microscope (SEM)

The SEM image demonstrates the morphology of the biosynthesized NPs, which possess a spherical shape with a size range of 1-15 nm (Fig. 6a).

3.4.4. Energy Dispersive X-ray (EDX)

The spectroscopic analysis of the NPs sample demonstrates the presence of silicon (Si) and Oxygen (O) composition in the biosynthesized SiO_2NPs. The percentage weights of silica and oxygen are 53.35% and 46.15%, respectively (Fig. 6b).



(a) (b)

Fig. 4. DLS measurements of biosynthesized SiO_2NPs: **(a)** Particle size distribution, **(b)** Zeta potential

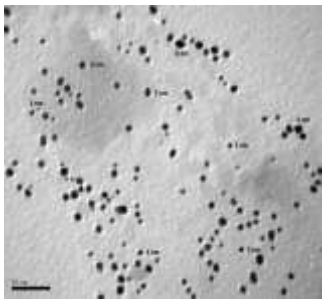
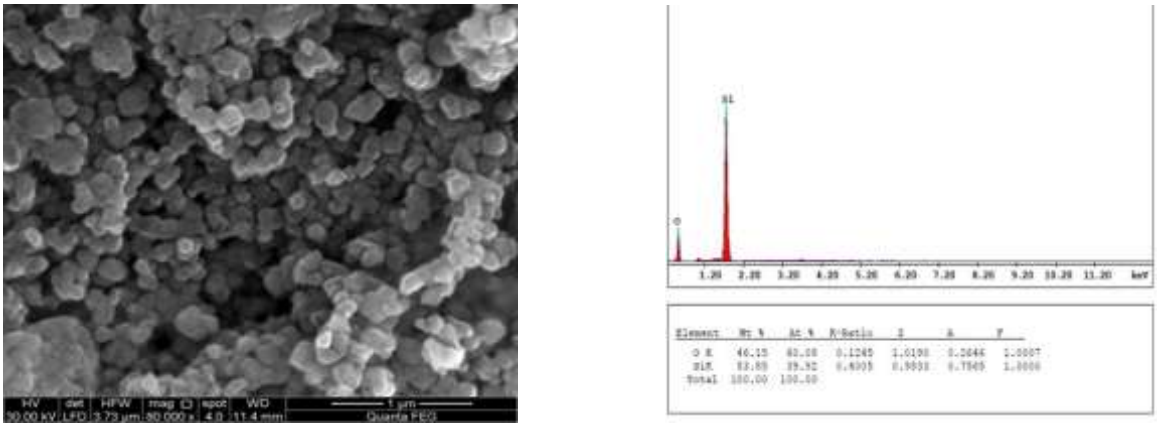


Fig. 5. TEM micrograph shows the spherical shape of the biosynthesized SiO_2NPs, and its size is about 3-9 nm, at scale bar of 50 nm



(a) (b)

Fig. 6. **(a)** SEM image of the biosynthesized SiO_2NPs showing spherical shape, **(b)** Energy Dispersive X-ray spectrum of the biosynthesized SiO_2NPs showing the presence of silicon and oxygen elements with average weights of 53.35 %, and 46.15 %, respectively.

4. Discussion

Silica NPs are more hydrophilic and biocompatible, as they are not subject to microbial assault, and no swelling or porosity alters with the change in pH. Suganya *et al*., (2016) reported that they are favorable since they are cheap, simple to create, easy to separate by means of centrifugation, and have surface hydroxide groups that make them easy to functionalize.

The present study aimed to select new candidates for the production of SiO_2NPs from a precursor source K_2SiF_6. In the current study, it is found that only one fungal isolate out of twenty isolates recovered from potable water samples showed high potential to produce SiO_2NPs. According to Prasad and Anal, (2009); Raliya and Tarafdar, (2013), microorganisms act as trigger for the biosynthesis of metal and metal oxide NPs, due to their negative electro kinetic potential which make them able to attracts the cations. They added that the extracellular secretion of enzymes by fungi exhibit the advantage of getting clear and monodisperse NPs, which are free from cellular components associated with the microorganisms, in addition to the ease of the process of down-streaming.

On the basis of a molecular method, this fungal isolate is identified as *A. tubingensis* strain F20. In general, Bakri *et al*., (2010) reported that the molecular methods are highly sensitive and selective compared to the morphological and physiological characterization, which are sensitive to the various environmental conditions and thus turned out to be more difficult. Few previous studies reported the synthesis of NPs using *A. tubingensis* (Tarafdar *et al*., 2013; Ramesh *et al*., 2014).

Results of optimization of the physio–chemical parameters for the biosynthesis of SiO_2NPs showed that the production of the NPs with diameters below 100 nm increased with; a)-The decrease in the concentration of salt from 10^{-1} M to 10^{-4} M, where the smallest NPs size was obtained at salt

concentration of 10^{-3} M. The formation of particles with large size may be attributed to the presence of large amount of silica in small volume of the salt solution (Gerick and Pinches, 2006). b)-Incubation for 48 h onwards. It is also observed that further incubation beyond 48 h, up to 96 h decreased the size of NPs, where the smallest size was recorded at 72 h of incubation (Tarafdar *et al*., 2013). c)-The concentration of hydrogen ions in the reaction medium plays crucial role in determining the NPs size. Similar to the current results, Kathiresan *et al*., (2009) demonstrated that as the pH of the reaction mixture changes, the catalytic activity of the enzymes secreted by the fungus also has changed. d)-The size of the NPs increased when the temperature deviated from 28°C. Suvith and Philip, (2014) attributed this increase in the particle size at high temperature to the agglomeration of particles. The current results of optimization the different parameters for the biosynthesis of SiO_2NPs are in accordance with previous results of Tarafdar *et al*., (2013).

The size of the biosynthesized SiO_2NPs was primarily measured by the DLS technique. The histograms of SiO_2NPs recorded by the DLS clearly demonstrated that its mean diameter is 8 nm. The DLS also measured the surface charge of particles expressed as zeta potential. According to Tarafdar *et al*., (2015), the surface charge of the NPs plays an important role during interaction with molecules of the other biological systems such as the plant, which should generally be in the range of -30 to + 30 mv. It can be observed through the low magnification of the TEM image that all SiO_2NPs is present in mono-disperse stage. SEM image showed that the SiO_2 NPs is not in direct contact and is well distributed, indicating stabilization of the NPs by the protein capping agent. The elemental composition of SiO_2 NPs by the EDX technique displayed that only peaks of Si and O atoms are detected, which confirm the absence of impurities during the synthesis of SiO_2 NPs.

Conclusion

The current results revealed that the fungus *A. tubingensis* F20 (NCBI GenBank Accession No. (MK226258.1) is able to synthesize SiO_2 NPs with the average diameter of 8 nm. Factors responsible for more production of mono-dispersed SiO_2NPs are optimized. It is concluded that; 10^{-3} M precursor salt concentration, 72 h of incubation at pH 3, and incubation temperature of 28°C, resulted in the yield of the smallest NPs diameters. The SiO_2NPs synthesized by such biological method is considered economically and environmentally safe, because it is naturally encapsulated by the fungal protein which is water soluble.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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