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A novel electrically enhanced biosynthesis of copper sulfide Nanoparticles

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ABSTRACT

The slow rate of nanoparticle production is one of the drawbacks of the biological synthesis of nanoparticles. In this study, a novel electro-biosynthesis process was introduced in order to improve the speed of nanoparticle bio-production. Electric current with three different magnitudes was applied to the cultures of *Fusarium oxysporum*, and then these electrified cultures were used for the biosynthesis of copper sulfide nanoparticles from $CuSO_4$ solution. Results proved that although this approach reduced the fungal growth, it significantly tripled the rate of biosynthesis process in contrast to the control culture to which no current was applied. However, the positive influence of electric current on the final amount of the produced nanoparticles was not statistically significant. Finally, the biosynthesized nanoparticles were characterized using Thermo Gravimetry (TG), Differential Scanning Calorimetry (DSC), Mass Spectrometry (MS), Energy Dispersive X-ray spectroscopy (EDX), Scanning (SEM) and Transmission Electron Microscopy (TEM).

1. Introduction

The biosynthesis of metallic nanoparticles that is one of the nanotechnology and biotechnology junctions has recently caught the scientist's interest. Considering the environmental, health and social aspects, there is a need to develop these biological approaches for nanomaterial synthesis [1,2]. Microorganisms produce metallic nanoparticles using an enzymatic process via intra or extracellular mechanisms [3,4]. These mechanisms involve efflux systems, alteration of solubility and toxicity via oxidation/reduction, biosorption, bioaccumulation, complexation or precipitation of metals [5]. Advantages of biological methods include the generation of biocompatible particles, avoiding the toxic surfactants or organic solvents, and lower production cost [6,7]. However, these methods generate the nanoparticles at a much slower rate compared to the chemical techniques. This prolonged reaction is one of the major disadvantages of biological synthesis, and must be fixed to become a successful alternative for chemical procedure of nanoparticle synthesis [8].

Nowadays, the study of chalcogenide nanoparticles like semiconductor metal sulfides is growing rapidly. Among these compounds, copper sulfide as a variable stoichiometric composition attracts considerable attention [9]. This variability causes copper sulfide to exhibit many unusual electronic and optic behaviors that make it a great potential for a wide range of applications [10] such as solar cells, solar controllers, solar radiation absorbers, catalysts, nanometer-scale switches, high-capacity cathode materials in lithium secondary batteries, superconductors at low

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temperature, chemical sensors, and thermoelectric cooling materials [10,11]. Moreover, it is employed in making optical filters, sensors, and architectural glazes [12].

The biosynthesis of copper sulfide nanoparticles using *Fusarium oxysporum* was reported previously by Hosseini et al. [13]. However, as previously mentioned, the slow rate of the process encouraged us to enhance the speed of the biosynthesis process through the application of direct electric current.

The exposure of microorganisms to the electric fields has been studied for decades to identify the loss of viability, mobility, and the resulting changes in microbial metabolism, DNA and protein synthesis, and the membrane permeability [14,15]. The current strength for these processes is often kept below 20 mA in order to prevent the lethal effect of the high current densities [16]. Although, the electrical treatment is relatively simple, the analyses of electrochemical reactions which happen in the electro-microbial systems are very complex. Therefore, there is a high potential for practical application of electro-treatment in microbial processes [14].

The positive and negative influences of electric current depend on the process per se, microorganism type, and the current type and intensity. An example of the negative effect of weak DC is the inactivation of *Thiobacillus ferrooxidans* and *Acidiphilium SJH* in liquid culture [17]. Also, it is observed that the electric current reduced the growth of *Aspergillus niger*, though it improved the HXD degradation in the same time [15]. In addition, stimulating effects in terms of cell growth and the dehydrogenase activity (DHA) were obtained when a DC of 10 mA was applied to the culture of *Enterobacter dissolvens* for 12 h via the platinum electrodes [16].

In the present research work, different intensities of direct current (0, 1, 5, and 10 mA) were applied to the *F. oxysporum* culture. Then, the effects of the different electric current intensities on the cell growth, protein secretion and glucose concentration were investigated and compared to the control experiment. Finally, the electrified cultures were used to produce copper sulfide nanoparticles, and the rate of particle production was compared with the control culture.

2. Materials and methods

2.1. Microorganism

The fungus *F. oxysporum* was kindly provided by The Federal Institute for Geosciences and Natural Resources (BGR), Hannover, Germany, and was maintained on PDA (potato 20% w/v, dextrose 2% w/v, and agar 2% w/v) slants. The fungal spores of 4-day-old slants were inoculated into 300 mL flasks containing 100 mL MGYP medium (malt extract 0.3% w/v, glucose 1.0% w/v, yeast extract 0.3% w/v, and peptone 0.5% w/v) with the concentration of 4×10^6 spore L^{-1}; subsequently, these flasks were placed in a shaker-incubator (Kuhner, LT-X, Switzerland) at 30 °C, and 200 rpm for 120 h. All the experiments were carried out in duplicate, and results were reported with their associated standard errors.

2.2. Electrical treatment

The cultures were subjected to 1, 5, and 10 mA direct electric currents right after the inoculation. The electrochemical cell used in this study was consisted of a 300 mL Erlenmeyer flask equipped with two platinum electrodes pierced through either side of the flask. Each electrode was 5 cm in length and 0.5 mm in diameter. Also, there was a 6 cm space between the electrodes. The electric current was applied by a Solartron Electrochemical Interface (Model: SI 1287) wired to the electrochemical cell which was put in the shaker incubator.

2.3. Biosynthesis of nanoparticles

Having finished the third day of the cultivation period, the mycelia were collected by centrifugation (Sigma, 3–16 PK, Germany) at 5000 × g for 5 min, and washed three times by distilled water under sterile conditions. To initiate the nanoparticle biosynthesis, the mycelia were suspended in 100 mL of 10^{-3} M solution of $CuSO_4$ in 300 mL Erlenmeyer flasks and the pH was set to 5.5. The flasks were put into the shaker-incubator at 30 °C and 200 rpm for 48 h. The biosynthesis experiments performed in triplicate, and the outcomes compared statistically.

2.4. Characterization of nanoparticles

The UV–vis spectra of the solution containing copper sulfide nanoparticles were recorded by a Carry spectrophotometer (Varian, Australia) at a resolution of 1 nm. In addition, the morphology and average particle size of nanoparticles were determined using a Transmission Electron Microscope (TEM, Philips CM20). For SEM investigation, a FEI Quanta 600F equipped with the EDX system Genesis 4000 was employed. Furthermore, thermo-analytical investigations were performed using a Netzsch 449 F3 Jupiter thermo-balance equipped with a DSC/TG sample holder linked to a Netzsch QMS 403 C Aeolus mass spectrometer.

2.5. Analytical methods

To measure the biomass, glucose, and protein concentration of the culture media, and also, the nanoparticle production, samples were taken from the flasks in defined intervals by 2 mL microtubes; then the fungal mycelia were separated from the medium solution by using a Sigma 3–16 PK centrifuge in 3000 rpm for 3 min. The biomass concentration was measured by weighting the cells after drying in 70 °C for 24 h. Also, the filtrate was used for further analyzes. Glucose concentration of the medium was colorimetrically assessed by Nelson and Somogyi method [18,19]. To determine the protein contents of the medium, Bradford method was applied [20]. The concentration of copper ions remained in the flasks was measured by Inductive Coupled Plasma—Optical Emission Spectrometry ICP-OES (Varian 715-ES). The flow rates of Ar for the plasma, and the auxiliary were 15, and 1.5 L min^{-1}, respectively. In addition, the nebulizer