Turkish Journal of Engineering

Turkish Journal of Engineering (TUJE)
Vol.3, Issue 2, pp. 92-96, April 2019
ISSN 2587-1366, Turkey
DOI: 10.31127/tuje.429072
Research Article

THE BIOSYNTHESIS OF SILVER NANOPARTICLES BY CYTOPLASMIC FLUID OF CORIOLUS VERSICOLOR

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Received: 31/05/2018 Accepted: 21/09/2018

ABSTRACT
The aim of this study is to investigate the production conditions of silver nanoparticles (NPs) in the presence of $AgNO_3$ with the fungal cytoplasmic fluid (FCF) of white rot fungus *Coriolus versicolor*. In this study, parameters such as pH, $AgNO_3$ concentration and FCF ratio of *C. versicolor* were optimized. *C. versicolor* was grown in SBM and then kept in ultrapure water to obtain FCF for synthesis of the nanoparticle. Nanoparticle formation was monitored by UV spectrophotometry at 420 nm wavelength and the silver nanoparticles were imaged by SEM. In the optimization study, it was found that at pH 5.0, 1.5 mM $AgNO_3$ and 50% FCF containing medium was found to provide optimal conditions for the synthesis of the silver nanoparticle. The nanoparticles were spherical and varied between 15-35 nm.

Keywords: *Nanoparticle, Silver, Coriolus Versicolor, Biosynthesis*

1. INTRODUCTION

Applications in which materials with dimensions less than 100 nm are used form the basis of nanotechnology (Gurmen & Ebin, 2008). Nanoparticles (NPs) have superior properties like high surface/volume ratio, character of surface atoms, quantum size effects, dimensional dependence of electronic structure when compared to materials in macro-size (Shukla *et al.*, 2012). Their superior properties enable them to be used in optical applications and in the production of many materials such as highly active catalysts, super conducting materials, surface-active materials (Gurmen & Ebin, 2008). Numerous advantages and wide application areas make nanoparticle synthesis one of the most interesting topics of the last few decades.

Nanoparticle synthesis is carried out by different chemical methods (such as chemical/photochemical reduction, electrochemical techniques) or by physical methods (Pantidos & Horsfall, 2014). However, these methods have disadvantages such as the use of toxic chemicals or high cost (Shanmugasundaram *et al.*, 2013). For this reason, alternative biological methods called "green nanotechnology/green synthesis" have been developed. Green synthesis applications are usually performed using microorganisms (such as algae, bacteria, fungi) or plant extracts. Especially microorganisms can be optimized for different conditions because they have high adaptability ability (Pantidos & Horsfall, 2014).

Compared with bacteria, fungi with micelles providing large surface area offer great advantages in the synthesis of metallic nanoparticles (Mukherjee *et al.*, 2001). This large area can be used to accelerate the interaction of metal ions with the fungal reducing material, and thus the conversion of ions to metallic nanoparticles can be increased (Pantidos & Horsfall, 2014).

Fungi can synthesize molecules in protein structure that can increase the rate of nanoparticle synthesis (Pantidos & Horsfall, 2014).

Silver, with good conductivity, chemical stability, catalytic, antibacterial and healing activity, is among the attracting metallic nanoparticles (Pantidos & Horsfall, 2014; Sharma *et al.*, 2009).

Investigations on the synthesis of silver nanoparticles (AgNPs) by biological methods show that microorganisms have great potential. Biomolecules such as amino acids, enzymes, proteins, polysaccharides, vitamins found in extracts reduce the amount of Ag+ ions in wastewater. Although it is a chemically complex reaction, it makes Ag+ ions harmless to the environment (Sharma *et al.*, 2009).

In this study, the synthesis of AgNPs was optimized by biosynthesis method**,** developed as an alternative to physical and chemical NP synthesis methods. Optimization parameters were determined as pH, $AgNO_3$ concentration and fungal cytoplasmic fluid ratio.

2. MATERIALS AND METHOD

2.1. Material

White rot fungus *C. versicolor* was obtained from the Microbiology Laboratory of the Department of Environmental Engineering, Mersin University. $AgNO_3$ and other chemicals have analytical purity.

2.2. Method

2.2.1. Preparation of Fungal Cytoplasmic Fluid (CV-FCF)

C. versicolor grown on SDA plates at 30°C was transferred to modified Stock Basal Medium (mSBM) with different pH values (5.0; 6.0) to obtain cytoplasmic fluid (Table 1) (Mazmanci *et al.*, 2002).

The fungus was then incubated at 30 °C, in the dark, at 160 rpm for 10 days. At the end of the incubation, the pellets were rinsed with sterile ultrapure water to remove the metabolite formed during the incubation. Subsequently, the pellets were re-suspended in sterile ultrapure water-containing tubes (1:1 v/v) and incubated for 5 days at 30 °C in the dark, at 160 rpm to obtain fungal cytoplasmic fluid (CV-FCF). After the incubation, the pellets were removed from the medium by centrifugation and the CV-FCF was stored at +4 °C for study.

Table 1. Modified Stock Basal Medium (mSBM)

Material	g/L
KH_2PO_4	13.60
K_2HPO_4	17.41
$CaCl_2.2H_2O$	0.05
$MgSO_4.7H_2O$	0.05
$NH_4(H_2PO_4)$	1.00
Glucose	10.00
pH	5.0; 6.0

2.2.2. Optimization Studies in AgNPs Biosynthesis

$AgNO_3$ solution was added to CV-FCF obtained from pellets grown at different pH values (5.0; 6.0) as final concentration on 1.00 mM. CV-FCF-free $AgNO_3$ solution and $AgNO_3$-free CV-FCF medium were used as control groups for pH optimization.

Optimization of the $AgNO_3$ concentration was performed in medium containing 0.50, 0.75, 1.00, 1.50 mM $AgNO_3$. CV-FCF medium without $AgNO_3$ and medium without CV-FCF for each $AgNO_3$ concentration were used as control groups.

Optimization of CV-FCF ratio was performed using media containing 25%, 50%, 75% and 100% CV-FCF. $AgNO_3$-free media containing 25, 50, 75 and 100% CV-FCF and $AgNO_3$ solution without CV-FCF were used as control groups.

Optimization studies were carried out at 30 °C, in dark conditions and at 160 rpm. The formation of AgNPs was observed with an increase in the medium color at 420 nm in the UV spectrophotometer at two-day intervals for 20 days.

Characterization of AgNPs in precipitates obtained from centrifugation was performed by scanning electron microscopy (SEM) after washing with ultrapure water and drying.

3. RESULTS

For the growth of the *C. versicolor*, optimum pH range has been reported to be 5.0-5.8 (Jo *et al.*, 2010). FCF obtained from *C. versicolor* cultivated in pH 5.0 and 6.0 conditions were studied to show the effect of medium pH. It was observed that the fungus pellets grown in pH 5.0 were larger than those grown in pH 6.0 (data not shown).

The effects of CV-FCF obtained from the fungus pellets grown at pH 5.0 and 6.0 were shown in Fig. 1a. It was observed that CV-FCF at pH 5.0 produced higher concentrations of AgNP and reached the highest absorbance value at day 18 (Fig. 1b).

Results of $AgNO_3$ concentration optimization were presented in Figs. 2a and b. The highest absorbance value was reached on day 16 in medium containing 1.50 mM $AgNO_3$.

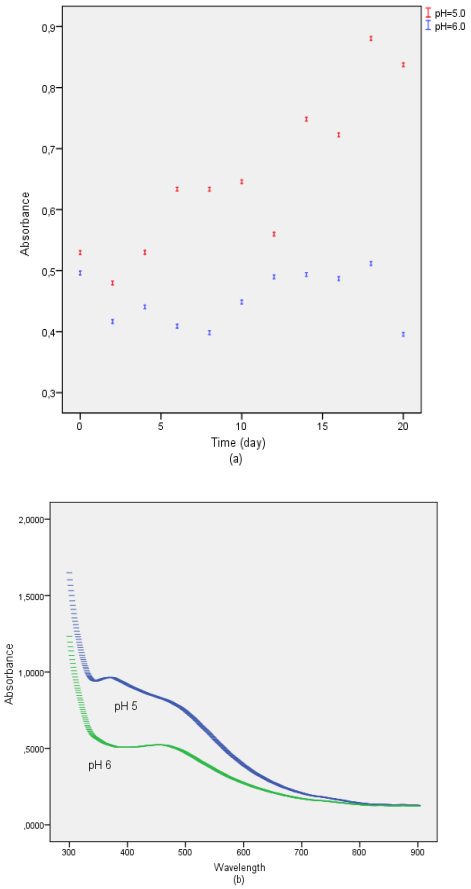

Fig. 1. Effect of pH on AgNPs biosynthesis. (a) Change in time-dependent absorbance value, (b) Spectra of the day reaching the highest absorbance value

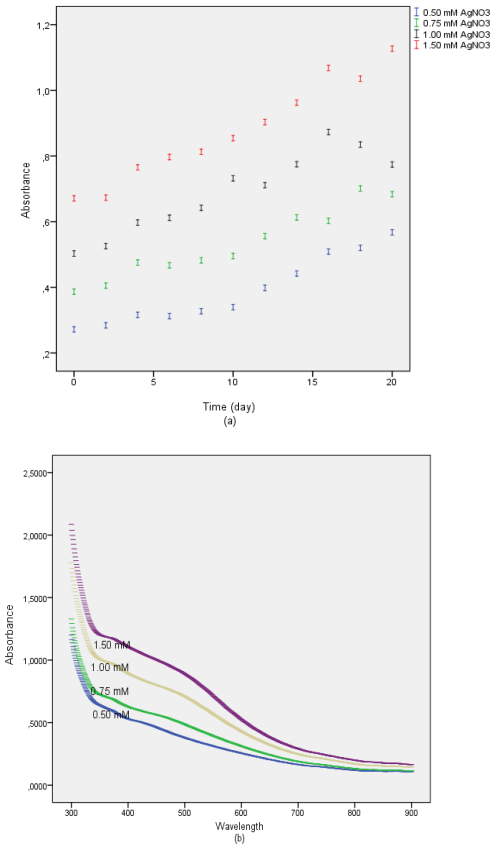

Fig. 2. Effect of $AgNO_3$ concentration on AgNP biosynthesis. (a) Change in time-dependent absorbance value, (b) Spectra of the day reaching the highest absorbance value.

The CV-FCF ratio optimization studies demonstrated that the highest absorbance value was reached on day 14 for 100% CV-FCF containing medium. (Fig. 3a and b).

In the SEM analysis, the AgNPs obtained from the solution with the highest absorbance value (the pH 5.0, 1,50 mM $AgNO_3$, 100% CV-CFC) were used. Results have shown that they were spherical and their size varied between 15-35 nm (Fig. 4).

4. DISCUSSION

Microorganisms, like every living thing, have various defense mechanisms to maintain their lives. Most metal ions are toxic to bacteria, therefore, converting toxic metal ions into water-insoluble form is a defense mechanism developed by bacteria (Sastry *et al.*, 2003; Prathna *et al.*, 2010). They carry out this transformation either inside or outside the cell with the biomolecules they produce. Many studies have been conducted with fungi in this regard and it has been shown that fungi, which are exposed to toxic conditions, may exhibit similar behaviors by producing extracellular or intracellular metabolites (Khan *et al.*, 2018).

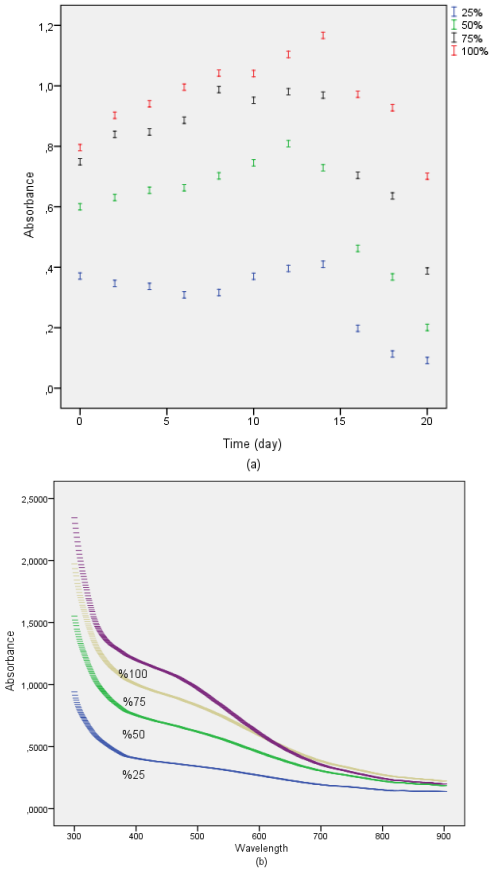

Fig. 3. Effect of CV-FCF dilution ratio on AgNP biosynthesis. (a) Change in time-dependent absorbance value, (b) Spectra of the day reaching the highest absorbance value.

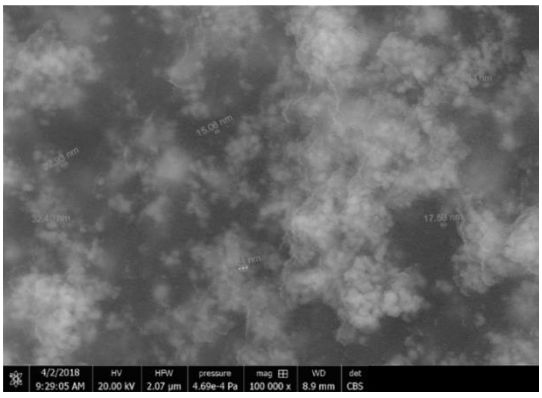

Fig. 4. Scanning electron micrograph of silver nanoparticles synthesized by CV-FCF.

Silver NPs exhibit remarkable colors (yellow to brown) due to the excitation of surface plasma vibrations in the particles, and their formation can be qualitatively determined (Sastry *et al.*, 2003). During the study, same color changes were observed when $AgNO_3$ solution was added to CV-FCF. This result indicated that Ag+ ions are reduced very quickly. According to the results, it can be predicted that the fungus may convert silver toxicity to a non-toxic metallic form despite the use of fungal cytoplasmic fluid (Sastry *et al.*, 2003; Ahmad *et al.*, 2003; Khan *et al.*, 2018).

Gudikandula *et al.* synthesized the AgNPs using the media of white rot fungus grown at pH 6.0 (Gudikandula *et al.*, 2017). In this study, FSF obtained from fungus grown at pH 5.0 and 6.0 was used. CV-FCF obtained from *C. versicolor* grown at pH 5.0 was found to be more efficient in AgNPs synthesis. This suggests that pH is an important parameter. Similarly, Kathiresan *et al.* optimized the pH value (5.0-9.0) of the cytoplasmic fluid obtained from *Penicillium fulvutanum*. They obtained the best optical density at pH 6.0 (Kathiresan *et al.*, 2009). This indicates that the pH value of both fungi growth environment and FCF have a significant effect on AgNPs synthesis.

The final concentration of $AgNO_3$ was used as 1.00 mM for nanoparticle synthesis in the previous studies (Vigneshwaran *et al.*, 2006; Zonooz&Salouti, 2011; Abdelrahim *et al.*, 2017). In this study, $AgNO_3$ concentration varied between 0.50 - 1.50 mM. AgNP formation increased with increasing $AgNO_3$ concentration and the highest absorbance value was reached at 1.50 mM. Kathiresan *et al.* (2009) and Gudikandula *et al.* (2017) reported the similar results. Adıgüzel *et al.* synthesized AgNPs with bacterial cytoplasmic fluid (BCF) obtained from newly isolated Streptomyces genus. They studied $AgNO_3$ concentrations ranged from 0.50 to 1.50 mM and reported the highest absorbance value at 1.00 mM $AgNO_3$ concentration (Adiguzel *et al.*, 2018).

In the optimization of the CV-FCF ratio, the absorbance values reached on day 12 were 0.390, 0.809, 0.980 and 1.103 for the 25, 50, 75 and 100% CV-FCF, respectively. Formation of AgNP was increased with increasing CV-FCF ratio in media. This suggests that the amount of AgNP synthesized depends on the amount of metabolite. When the results were compared as proportionally, it was seen that the amount of nanoparticle produced in the medium that contains 50% CV-FCF was higher than the others. Although the highest absorbance value was reached (day 14; 1,160 abs) in the medium containing 100% CV-FCF, 50% CV-FCF was found to be more efficient in AgNP biosynthesis (day 12; 0,809 ABS). Therefore, the optimum value was accepted as 50%.

As a result of SEM analysis, it was observed that the synthesized NPs were in spherical shape and in dimensions ranging from 15-35 nm. Previous studies supported these findings (Kathiresan *et al.*, 2009; Syed *et al.*, 2013; Das *et al.*, 2012).

Ahmad *et al.* reported that AgNP synthesis takes place outside the cell because biomass remains in its original color after the reaction (Ahmad *et al.*, 2003). However, Sastry *et al.* determined that AgNP synthesis takes place in the cell because of the color change in the biomass (Sastry *et al.*, 2003). Bacteria or fungi can synthesize silver nanoparticles both in- and outside the cell. Results obtained in this study demonstrate that fungal biomass is not needed for AgNP synthesis and this process can be performed with metabolites. On the other hand, the reaction catalyzed by CV-FCF does not provide evidence of which of the intracellular metabolites are involved in nanoparticle synthesis.

5. CONCLUSION

Fungal cytoplasmic fluid of *Coriolus versicolor* obtained from different conditions (pH 5.0 and 6.0, 0.50-1.50 mM $AgNO_3$ concentration, 25-100% CV-FCF) was investigated for silver nanoparticle biosynthesis. Maximum AgNPs production was carried out in media obtained from *C. versicolor* grown at pH 5, containing 1.50 mM $AgNO_3$ and 100% CV-FCF. But, it has been found that 50% CV-FCF is more effective in AgNPs biosynthesis. SEM analysis of AgNPs showed they were spherical and their dimensions varied between 15-35 nm.

ACKNOWLEDGEMENTS

This paper has been presented orally at the 1st International Symposium on Innovative Approaches in Scientific Studies in Antalya.

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