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Original Research Article

Bioremediation of hazardous waste for silver nanoparticles production

Essam A. Makky[1*], Siti H. Mohd Rasdi[1], J.B. Al-Dabbagh[1], G.F.Najmuldeen[2] and Ab Rahim Mohd Hasbi[1]

[1]Faculty of Industrial Sciences & Technology, University Malaysia Pahang, Gambang, 26300 Kuantan, Pahang, Malaysia

[2]Faculty of Chemical & Natural Resources Engineering, University Malaysia Pahang, Gambang, 26300 Kuantan, Pahang, Malaysia

**Corresponding author*

Keywords

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A B S T R A C T

The synthesis of silver nanoparticles (SNPs) was extensively studied by using chemical and physical methods. In this study, the biological methods were used as it gave benefits in research field in the aspect of very low production cost (from waste to wealth) and time saving as well. The study aims to isolate and exploit the microbial power in the production of industrial important by-products in nano-size form with high economic value. It is also to extract the highly valuable materials from hazardous waste, to quantify nanoparticle size and characterization of SNPs by X-Ray Diffraction (XRD) analysis. Disposed X-ray films were used as substrate because it consumes about 1000 tons of total silver, which is chemically produced worldwide annually. The silver was wasted when these films were used and then disposed. Different bacterial isolates were obtained from various sources. Silver was extracted as nanoparticles by microbial power degradation from disposed X-ray film as the sole carbon source for ten days incubation period in darkness. The protein content was added to all the samples and analyse using XRD to characterize the silver (Ag) nanoparticles size in the form of silver nitrite. Bacterial isolates labelled CL4C (isolated from rotten chicken liver) showed the average size of SNPs of about 19.53 nm, GL7 (isolated from Gambang Lake, Kuantan) showed about 52.35 nm and JF Outer 2A (PDA) (isolated from rotten jackfruit) showed 13.52 nm. All bacterial isolates partially identified using Gram's reaction and the results obtained exhibited that belonging to *Bacillus sp*.

Introduction

Increasing number of industrialization and urbanization worldwide had caused serious pollution around the world, especially in the aquatic environment. Wastewaters produced by humans are frequently laden with toxic heavy metals such as copper, silver,

mercury, etc. The soluble form of these heavy metals is very dangerous because it is easily transported and more readily available for plants and animals. For humans, poisoning by these metals can result in severe dysfunction of kidney, reproduction system, liver, brain and central nervous systems (Chen and Lim, 2002). Silver is a precious metal widely used in the photographic, electrical, electronics, chemical and jewellery industries. Even though it is not as expensive as gold or platinum, silver is still only present in limited amounts in nature. This contributes to the need for efficient methods of recycling silver from waste generated by the above industries (Dimeska*et al.,* 2008). Nanoparticles exhibit a completely novel or improved properties compared with larger particles of the size and these new properties are derivative due to the variation in specific characteristics such as size, distribution and morphology of the particles (Mukunthan*et al.,* 2011). A vital aspect of nanotechnology concerns with the growth of experimental processes for the synthesis of nanoparticles of different sizes, shape and proscribed dispersity. With the expansion of new chemical and physical methods, the anxiety for environmental contaminations are also severe as the chemical methods concerned in the synthesis of nanoparticles make a large amount of hazardous products. Thus, there is a need for microbe mediated synthesis that includes a clean, nontoxic and eco-friendly method of nanoparticle synthesis (Mukherjee *et al.,* 2001). Chemical synthesis methods lead to the presence of some toxic chemical, which is absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals (Mukunthan *et al.,* 2011; Thirumurgan *et al.,* 2010). Silver nanoparticles (SNPs) are used in a wide range of applications, including pharmaceuticals, cosmetics, medical devices, food ware, clothing and water purification, among other usage, due to their antimicrobial properties (Craver and Smith, 2008). However, there are few reports on SNP synthesis by bacteria and in this paper, we are reporting the synthesis of SNPs by *Bacillus* isolate. This is also the first report on production of SNPs from disposed X-ray film as the sole carbon source using bacterial power in darkness.

Materials and Methods

Isolation of Bacterial Isolates

The bacteria isolated from water (Gambang Lake, Kuantan) and spoiled food (rotten chicken liver and jackfruit) were grown on nutrient agar under aerobic condition at 37°C. A single colony was isolated from a dozen of Petri-dishes exposed. The isolated bacteria were identified as *Bacillus sp.* through Gram staining. Then, it was sub-cultured in nutrient broth for inoculation.

Pre-treatment of X-ray film sheets

The substrate containing $AgNO_3$ (x-ray film) (Fig. 1) was cut into small pieces (0.2 x 1 cm) and 4N NaOH was added until it covered the substrate surface. The mixture was mixed well using the orbital shaker for overnight at 150 rpm. It was then washed several times using tap water to remove the colour of substrate and the pH was adjusted to pH 7.0. After that, the mixture was left to dried for overnight

Preparation of modified minimal media (MM9)

Three stock solutions need to be prepared, which are solution A, B and C. Solution A

consists of 25.6 g/l of $Na_2HPO_4.7(H_2O)$ (Merck), 6 g/l of KH_2PO_4 (Merck), 1 g/l of NaCl (Merck) and 2 g/l of NH_4Cl (R & M Marketing). All chemicals that have been weighed was put off in 500 ml distilled water, mixed well and top up until it reached the amount of 1 litre. Then, the 500 ml of solution A stock was transferred into another 1 litre beaker and added with 2 ml of solution B (1.0 M $MgSO_4$ solution) and 1 ml of solution C (1.0 M $CaCl_2$ solution). Top up the stock solution with distilled water until reached 1 litre amount.

Synthesis of silver nanoparticles

Fifty ml of complete modified production media (MM9) free carbon source (D-glucose) was added into 100 ml conical flasks. After that, 0.5 g of substrate (pre-treated x-ray film) was added and autoclaved at 121°C for 15 minutes. Then, 5 ml of bacterial inoculum was in pipette into each flask. The controlled mixture was also prepared as free bacterial inoculum, which only contains MM9 with substrate. Finally, all the flasks were incubated at 37°C for ten days in darkness condition. Next, the mixture was centrifuged for 5 min at 5000 rpm and at 4°C to separate the microbial cell with the supernatant.

Assessment and analysis

The protein assay analyse by UV-vis spectrophotometer at 700 nm was used according to Lowry's method (Lowry *et al.*, 1951). Then, the supernatants were transferred into freeze-dry flask and freeze at -20°C for 5 days before undergoing freeze drying process to get the powder for XRD (Rigaku, Miniflex II) analysis.

Result and Discussion

Protein Determination

According to Table 1, the protein content of all the most potent bacterial isolates was higher than the controlled item. The protein content exhibited the highest readings in case of JF Outer 2A (PDA) isolate than other samples.

XRD analysis

The results obtained revealed the seven silver nitrite peaks acquired for silver nanoparticles synthesized of CL4C isolate with an average grain size of about 19.53 nm. The diffraction peaks are at 22.32°, 29.45°, 33.85°, 45.32°, 52.17°, 57.94° and 71.2° (Fig. 2). In the meantime, Figure 3 shows the XRD pattern obtained for silver nanoparticles synthesized of GL7 isolate grown on x-ray sheets at 37°C for the same incubation period. GL7 isolate exhibited that only two silver nitrite peaks obtained in average size of about 52.35 nm, which the diffraction peaks are at 31.18° and32.93°.On the other hand, Figure 4 shows the XRD pattern obtained for silver nanoparticles synthesized of JF Outer 2A (PDA) isolate grown under the same production condition. The results revealed that JF Outer 2A (PDA) obtained 5 silver nitrite peaks with an average size of about 13.52 nm. The diffraction peaks are at 22.39°, 28.4°, 46.38°, 47.31° and 58.22°. In general, JF Outer 2A (PDA) isolate obtained has the smallest size of SNPs, followed by CL4C and GL7 respectively. From the observation, the production of media colour changes after incubation period, as the colour of JF Outer 2A (PDA) samples were brown compared to the controlled item, which is not inoculated (colourless). GL7 isolate is just having colour changes from colourless to pale yellow compared to CL4C. The colour changes affect the size of nanoparticles obtained. The formation of SNPs is able to produce the particular colour in the reaction mixtures due to their specific properties (Udayasoorian *et al.*, 2011). The appearance

of a yellowish brown colour in silver nitrate treated culture supernatant suggested the formation of SNPs. A similar observation was made in the biosynthesis of SNPs by *Fusarium oxysporum* strain in extracellular process (Duran *et al.,* 2005). The brown colour of the medium could be due to excitation of surface Plasmon vibration of SNPs.

There were no previous researchers who used the hazardous wastes, which are x-ray film sheets as the substrate in order to biosynthesise the SNPs. However, in the aspect of bacterial used, some researchers had studied the biosynthesis of SNPs by using *Bacillus sp.* By comparing the results, the substrate they used is 3.5 mM $AgNO_3$ (silver nitrate) and the biogenic nanoparticles 10 – 15 nm in size were observed in the periplasmic space of the bacterial cells between the outer and inner cell membranes using the higher magnification TEM (Pugazhenthiran *et al.,* 2009). Comparing the findings by Kannan and Subbalaxmi, (2011), they used the *Bacillus subtilis* for green synthesis of SNPs. The substrate that they used was silver nitrate at a concentration of 10^{-3} (1% v/v). Based on their investigation, they revealed that the nanoparticles are in polydispersed mixture with the various size ranges from 50 to 80 nm, which was characterized with laser diffraction particle size analyser. The results were also compared to the previous research that used other bacteria. It also showed the various sizes of SNPs obtained but still in the range of 1–100 nm. The bacterial strain *E. coli* was used and suspended in silver nitrate at concentration of 10^{-3} (1% v/v) as the substrate (Natarajan *et al.,* 2010). From the result, the brown colour appeared as the observable formation of SNPs in the reaction mixture and the capable reduction of silver ions occur at extracellular than the intracellular (pellet) culture. They revealed that, the size of SNPs is in the range of 40 - 60 nm. By using suspended *Cassia auriculata* leaf extract with the silver nitrate (99.99 %) aqueous solution as the substrate, the dark yellowish-brown colour appeared as a clear indication of the SNPs formation in the reaction mixture (Udayasoorian *et al.,* 2011). According to the XRD analysis, the average of SNPs size obtained is 20.84 nm. By using the *Pseudomonas aeruginosa* as the source of microorganism for biosynthesis of SNPs, the average size of particle obtained is 50 nm by characterizing it using SEM. The substrate used in this study is silver nitrate at a concentration of 0.1 g/l (Jeevan *et al.,* 2011)

The microbial power in the production of industrial important by-products in nano-scale size with high economic value was successfully isolated and exploited from different sources. Silver nanoparticle was extracted from disposed X-ray film. Bacterial isolated that had successfully reduced silver nitrates to nitrite were identified to be *Bacillus sp*. In general, the size of SNPs was produced and characterized using XRD analysis, which is 19.53 nm by *Bacillus* CL4C, 52.35 nm by *Bacillus* GL7 and 13.52 nm by *Bacillus* JF Outer 2A (PDA).

Table.1 Screening of the most potent isolates for protein content obtained from disposal x-ray film at 37°C

NO.	Isolate Symbol	Protein Content (mg/ml)
		Mean ± SD
1	Control	0.0 ± 0.000
2	CL4C	0.651 ± 0.002
3	GL7	0.673 ± 0.009
4	JF OUTER 2A (PDA)	0.871 ± 0.003

Fig.1 Composition of X-Ray Film

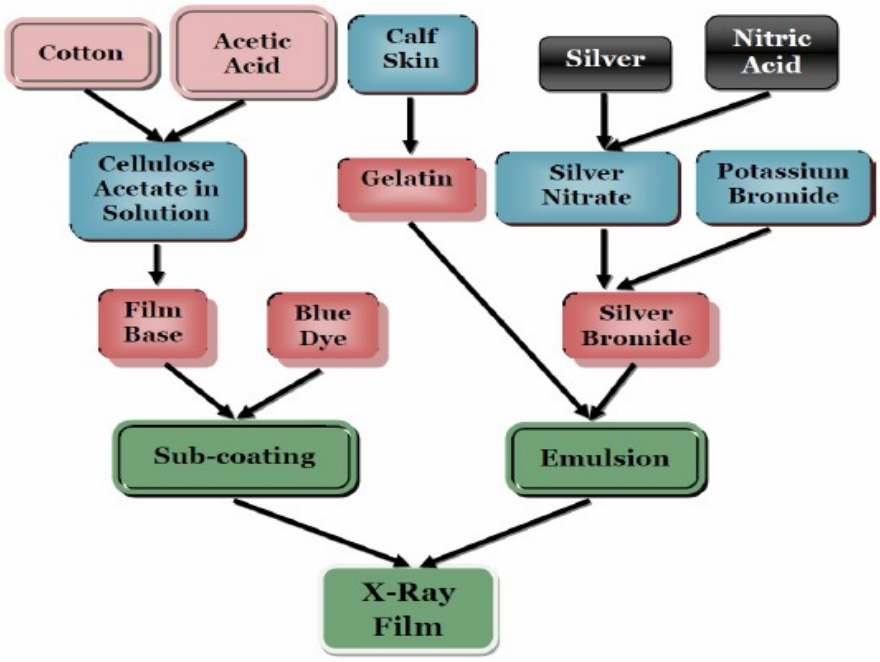


Fig.2 Silver nitrite XRD peaks for CL4C isolate

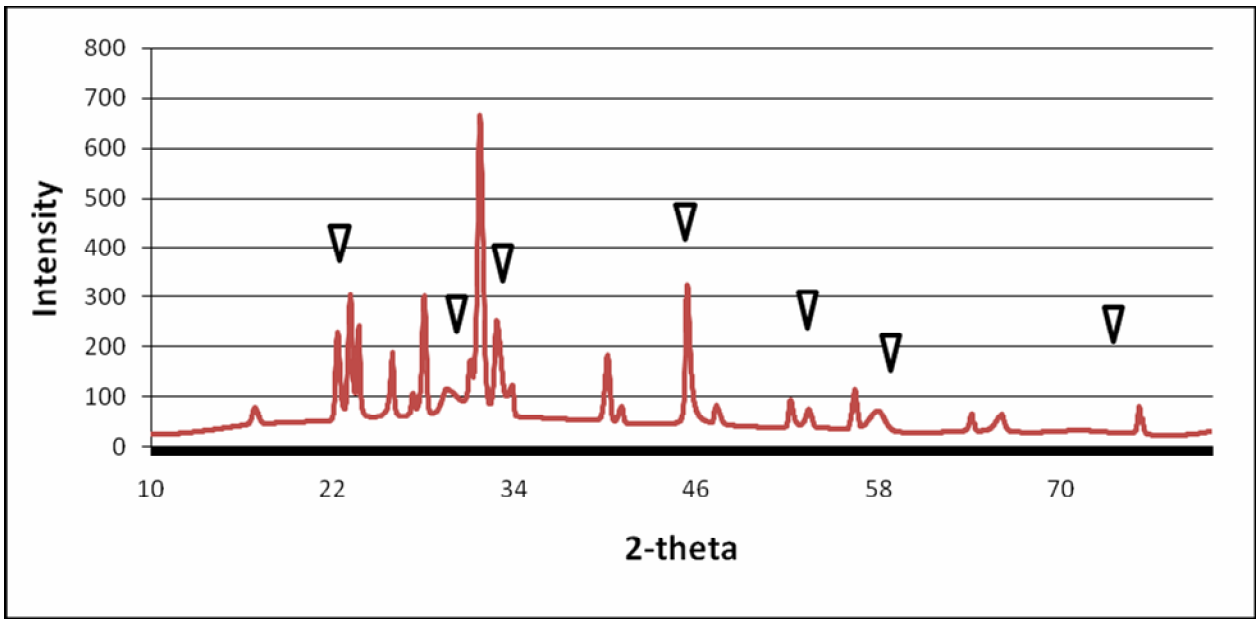


Fig.3 Silver nitrite XRD peaks for GL7 isolate

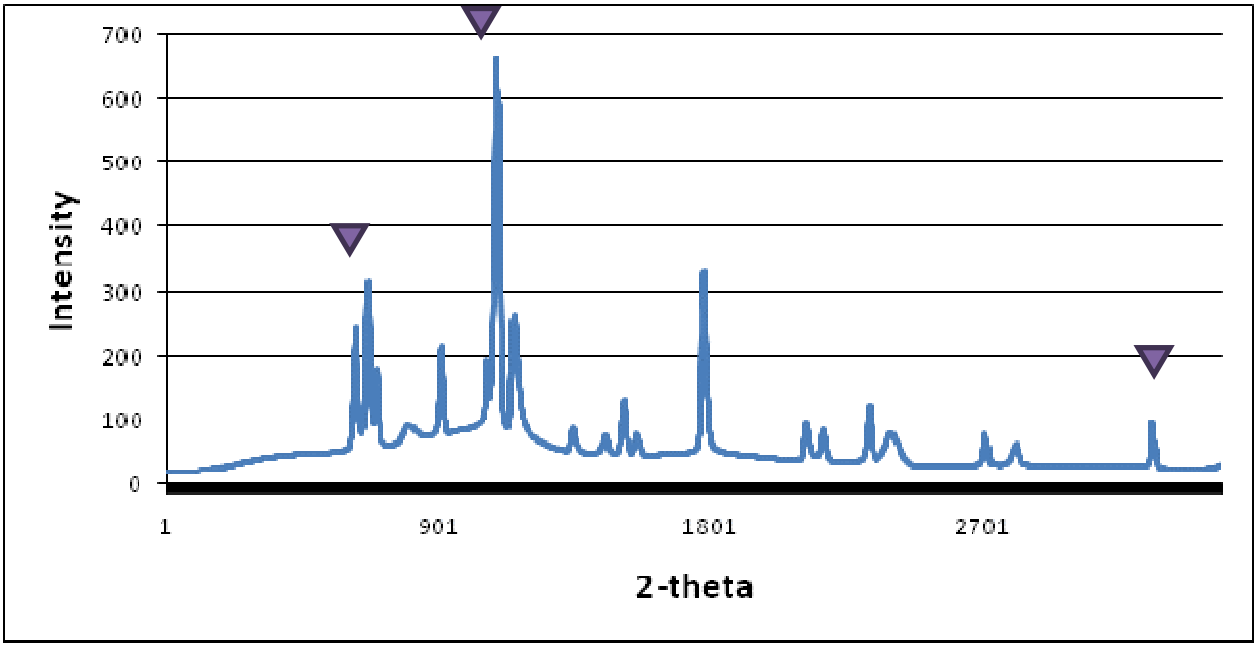
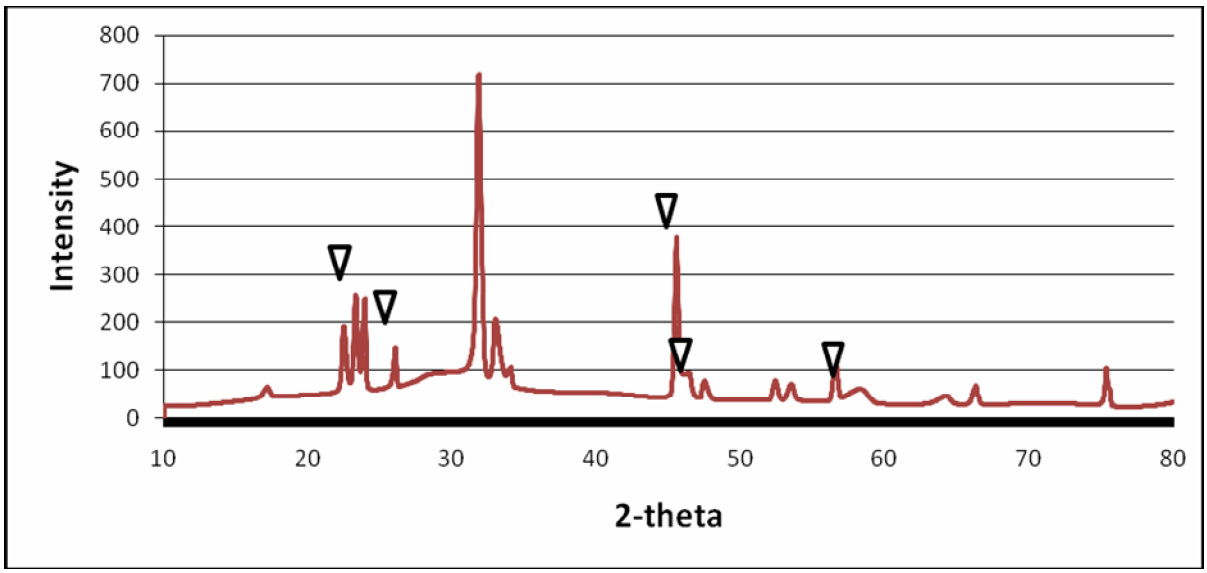


Fig.4 Silver nitrite XRD peaks for JF Outer 2A (PDA) isolate



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