Synthesis of Ultra-Small Platinum, Palladium and Gold Nanoparticles by *Shewanella loihica* PV-4 Electrochemically Active Biofilm and Their Enhanced Catalytic Activities

Elaf Ahmed, Shafeer Kalathil, Le Shi, Ohoud Alharbi, Renyuan Li, Noor Zaouri, Peng Wang

PII: S1319-6103(18)30019-X
DOI: https://doi.org/10.1016/j.jscs.2018.02.002
Reference: JSCS 945

To appear in: *Journal of Saudi Chemical Society*

Received Date: 24 November 2017
Revised Date: 6 February 2018
Accepted Date: 8 February 2018


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Synthesis of Ultra-Small Platinum, Palladium and Gold Nanoparticles by *Shewanella loihica* PV-4 Electrochemically Active Biofilm and Their Enhanced Catalytic Activities

Elaf Ahmed, Shafeer Kalathil, Le Shi, Ohoud Alharbi, Renyuan Li, Noor Zaouri, Peng Wang

Water Desalination and Reuse Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

E-mail: Peng.Wang@kaust.edu.sa

Keywords: (Electrochemically Active Biofilm, Ultra-Small Nanoparticles, *Shewanella loihica*)

Abstract

Ultra-small nanoparticles (USNPs) of noble metals have a great potential in a variety of applications due to their high surface areas and high reactivity. This work employed electrochemically active biofilms (EABs) composed of a single bacterium strain of *Shewanella loihica* PV-4 and successfully synthesized USNPs of noble metal Au, Pd, and Pt. The synthesized USNPs had a size range between 2 and 7 nm and exhibited excellent catalytic performance in dye decomposition. The results of this work shine lights on the use of EABs in nanoparticle synthesis.

1. Introduction

Ultra-small nanoparticles (USNPs), defined as a class of particles having size of 1-10 nm [1, 2], due to their size, lie between complete molecular dispersions and large-sized nanoparticles (NPs) and accordingly reveal intermediate structural, optical [3], electrical [4], catalytic [5], and magnetic properties [6], often different from large NPs of the same material [1, 2, 7]. For example, noble metal USNPs, such as gold (Au), silver (Ag), palladium (Pd) and platinum (Pt) show attenuated surface plasmon resonance and exhibit molecular-like optical properties due to loss of their metallic properties [8-10].
USNPs have applications in diverse fields, including catalysis [11], photocatalysis [12, 13], energy conversion and storage, chemical manufacturing, biological applications, biomedicines [14-21], semiconductors [22], and environmental treatments [14]. Especially, the catalytic activity of USNPs particularly of noble metals represents a rich resource for chemical processes [23, 24].

There are a myriad of chemical synthesis methods available in literature to synthesize USNPs of noble metals, including reduction, thermal decomposition, and sol-gel processes [8, 24-27]. However, all of them involve intensive use of chemical agents [8, 10, 28], making them environmentally unfriendly. Furthermore, the addition of surfactant, polymeric stabilizer, and other capping agents to stabilize these USNPs make the synthesis process complicated and severely limit the activity of these USNPs as catalysts [28-31].

Recently, biological approaches to nanoparticles synthesis using microorganisms, especially bacteria, offer certain attractions [32]. Over their evolution, bacteria have developed mechanisms which involve altering the chemical natures of some toxic metals to eliminate their toxicity and thus allow themselves to survive and grow properly [33]. This change in the chemical nature sometimes leads to formation of metal nanoparticles. Hence, the formation of the metal nanoparticles is literally an incidental by-product of a bacterial resistance mechanism against a specific metal, but has been gradually developed into an alternative way of purposefully producing nanoparticles [34].

In this regard, many microorganisms, such as bacteria, fungi, and yeast, have been exploited to synthesize metal nanoparticles [35]. Among these microorganisms, bacteria are typically favorable choices due to their abundant availability, ease of handling, easy genetic manipulation, and the fact that studies on one bacterium can be easily extrapolated to others [36, 37].
Electrochemically active biofilm (EAB) is the biofilm that is grown on any artificial electrodes [38, 39]. The utility and performance of EAB have been intensively studied in the past decades especially within microbial fuel cells (MFCs) systems to harvest energy from various environmental wastewater [40, 41]. In the past decade, the use of EABs for purposeful nanoparticle synthesis and modification has grown considerably as the EAB-assisted nanoparticle synthesis involves less chemical usage and mild synthesis conditions that are amicable to biofilm residing bacteria [8, 30, 36, 42, 43]. Thus, EAB holds certain potential to be an emerging platform for metal nanoparticle synthesis in future. In literature, mixed bacterial cultures were extensively seen and used in metal nanoparticle synthesis while the use of a single strain bacteria was rarely investigated for the same purpose [35]. In this work, EAB composed of a single bacteria strain, *Shewanella loihica* PV-4, was cultivated and used so to investigate its capability of synthesizing UNSPs of noble metal. The results show that the EAB of *S. loihica* PV-4 leads to a successful synthesis of USNPs of Au, Pd, and Pt and the synthesized USNPs possess high catalytic activities toward degradation of a common dye. The results of this work shows promising potential of single-strain EAB assisted USNP synthesis and thus invites more research efforts into this emerging field.

2. Experimental method and characterization

2.1 Biofilm preparation and growth

*S. loihica* PV-4 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) bacterium collection under the number DSM 17748. Literature conditions were used to grow the bacteria [44].

Commercial carbon paper was used as a support for the biofilm to grow on (Fuel cells ETC 6354706) and the carbon paper was cut into uniform pieces of (2 × 3 cm). The bioreactor for
EAB growth, which was sealable 100 ml glass bottle, was first filled with 88 ml of the sterilized nutrient media, then 10 mM sodium lactate as an electron donor and 10 mM sodium fumarate as an electron acceptor. The solution was mixed very well to get a homogeneous mixture and the bioreactor was initially purged with nitrogen gas for 30 minutes to remove all the dissolved oxygen to maintain anaerobic conditions.

An aliquot of 10 mL of *S. loihica* PV-4 single strain inoculum with an optical density (OD) of 2 in nutrient media was then added to the bioreactor, followed by immediately putting the bioreactor inside a constant-temperature chamber of 30 °C. The carbon paper was quickly immersed into the media and the bottle was tightly sealed to ensure that there was no oxygen infused into the solution. The medium was continuously stirred for one week, by the end of which a living EAB would have been developed on the surface of the carbon paper as illustrated in Figure 1. It is important to mention that there was no potential involved in the formation of the biofilm on the surface of the carbon paper.

![Figure 1: Schematic growing procedure of EAB on the surface of a carbon paper.](image)
The electrochemical activity of the EAB formed on the carbon paper was investigated using cyclic voltammetry (CV) measurement. The CV analysis was performed by Autolab Potentiostat, using a standard three-electrode system, with Ag/AgCl as the reference electrode, Pt mesh as the counter electrode and the EAB formed on the carbon paper as the working electrode. The artificial media, containing 10 mM sodium lactate was used as the electrolyte during the CV measurements. The scan rate used was 5 mV/S.

2.2 EAB-assisted synthesis of Au, Pd and Pt USNPs

Gold (III) chloride (99%, AuCl₃, Sigma-Aldrich), chloroplatinic acid (hydrate (>99.9% trace metals basis H₂PtCl₆·xH₂O, Sigma-Aldrich), palladium (II) chloride (PdCl₂ 99%, Sigma-Aldrich) were used as the precursors to Au, Pt, and Pd USNPs in this work. The metal precursor solution of 1 mM was first prepared by using DI water and 10 mM sodium lactate as the electron donor within the glass bottle of the same dimension as the bioreactors in the previous step. The prepared precursor solution was purged with nitrogen gas for 30 minutes to ensure an anaerobic condition. To investigate the effect of pH on the nanoparticle synthesis, a set of the precursor solutions with different pH values of 4, 7 and 9 were prepared for each metal.

The carbon paper with the EAB prepared previously was then carefully transferred into the metal precursor solution reactor. The reactor was then tightly sealed and continuously stirred for 48 hours in the constant temperature chamber of 30 °C.

After 48 hours, the synthesized nanoparticles were collected by centrifuging the solution containing the particles at 13,500 RPM for 20 minutes. The supernatant was carefully emptied out and the precipitate solid was mixed with acetone for 5 minutes followed by centrifuging at 13,500 RPM for another 20 minutes. The supernatant was emptied out again and 10 mL of
ethanol was used then to store the USNPs before use. Control experiments were conducted using the same metal precursor and sodium lactate in the absence of EAB to confirm the role of the EBA to produce the nanoparticles.

2.3 Dye degradation experiment
The as-synthesized Pd USNPs were tested for their catalytic activities to accelerate the degradation of a synthetic dye: methyl orange (C_{14}H_{14}N_{3}NaO_{3}S, Sigma-Aldrich). In conducting dye degradation experiment, a 50 µL of the stock solution of methyl orange dye with 0.15 mM concentration was transferred to a 5 ml glass bottle, followed by adding 1.0 ml DI water. 100 µL sodium tetrahydridoborate (NaBH₄) with a concentration of 0.1 M was then added to the solution as reductant. An aliquot of 100 µL of the as the synthesized Pd USNPs (0.3 mg/mL) in ethanol solution was added to the prepared dye solution containing NaBH₄. An immediate color change was observed as soon as the metal nanoparticles were added to the dye solution. The dye concentration was measured by the UV-Vis absorbance at 468 nm.

2.4 Material characterizations
Scanning electron microscopy (SEM) (FEI Instruments) was used to characterize the surface morphology of the carbon paper and the carbon paper covered by the EAB. In conducting SEM observation, the sample was fixed using 3% of glutaraldehyde in cacodylate buffer (pH ~6.8) as a fixative. The fixation was done overnight to ensure a complete infiltration of the fixative into the sample matrix. Prior to SEM, samples were coated with a 4 nm thin layer of Pd/Pt metals using K575X sputter.

Transmission electron microscopy (TEM) was used to study the morphology, crystal structure, and the elemental distributions of the USNPs samples. TEM analyses of the samples were performed using a TitanG2 80-300 CT from FEI Instruments that was equipped with a field-
emission-gun and a GIF Tridiem 863 energy-filter from Gatan, Inc. Before the TEM analysis, the samples were prepared by diluting 250 µL of the nanoparticle solution in 750 µL ethanol then sonicated for 5 minutes to ensure the homogeneous solution and prevent any aggregation. Then the sample was placed on a copper grid for the TEM analysis.

The UV–Vis adsorption spectra were recorded on a spectrophotometer (Shimadzu, UV 2550), using DI water as a reference. X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advanced A25 diffractometer equipped with a Cu X-ray tube (Cu Kα; λ = 0.154 nm) operated at 40 kV and 40 mA from 30 to 80 degree.

3. Results and discussions

3.1 EAB characterizations

The *Shewanella* strains bacteria have attracted great attention because of their diverse respiratory capacities, illustrated by their ability to utilize a wide range of terminal electron acceptors, including oxygen, nitrate, metals and sulfur compounds, and also due to their confirmed extracellular electron transfer (EET) capability [38, 45].

In this work, carbon paper was chosen as a support for the biofilm because of its mechanical stability, high electric conductivity, and biocompatibility, low cost and wide availability. Figure 2a presents SEM images of the plain carbon paper. As can be seen, the carbon paper has an interwoven fiber structure with a uniform fiber diameter around 10 µm and a smooth surface. The visual change on the surface of the carbon paper before and after the growth of the biofilms was very significant. It can be clearly seen that there was a sticky pinkish layer grown on the carbon paper fibers with a total coverage of the surface after seven days biofilm growth and the layer represented the EAB as shown in Figure S1.
From Figure 2b to 2d, it can be clearly seen that there was a total coverage of the bacteria cells on the surface of the carbon paper and each fiber of the paper was fully wrap-covered. These are good signs showing that the bacteria in the EAB were in healthy conditions. The bacteria in the EAB were condensed and formed a thick layer with individual bacteria in close contact to one another and being very well connected. Some free bacterial cells could be located and from these bacteria the size and the structure of the bacteria were recognized. The length of the individual bacteria is around 1-3 µm and diameter of 0.8 µm, which is very well consistent with the literature as shown in Figure S2 [46, 47].

Figure 2: (a) SEM images of plain carbon paper, (b-d) and EAB grown on the surface of carbon paper at different magnifications.
Cyclic voltammetry (CV), a type of potential sweep method, is probably the most widely used analytical tool for examining the EET process in EABs for detecting the activity of EABs. Although in this work, the Ag/AgCl reference electrode was used in the measurement, for the convenience of comparison, all the potential were converted to be relative to reversible hydrogen electrode (RHE) using Equation 1.

$$E_{RHE} = E_{Ag/AgCl}^0 + 0.059 \, pH + E_{Ag/AgCl}^\circ$$  (Equation 1)

Where $E_{RHE}$ is the converted potential vs. RHE, $E_{Ag/AgCl}^0 = 0.1976$ at 25 °C, and $E_{Ag/AgCl}^\circ$ is the experimentally measured potential against Ag/AgCl reference electrode.

The CV measurements were conducted with EAB formed on the carbon paper as the working electrode immersed with artificial media containing 10 mM sodium lactate as the electrolyte. Figure 3 compares the EAB grown for different time courses, 0, 1, 4, and 7 days.

From the results in Figure 3, it can be noted that by increasing the duration of the EAB growth, the recorded current was increased. The EAB grown for 7 days period, produced the highest negative current among all samples under the same conditions. The results can be explained by the fact that EAB grown in 7 days had the highest population of bacteria in the biofilm. The bacteria grown on the carbon paper responded to the applied potential and increased the produced current. Among all samples, the plain carbon paper shows the lowest current response and this is due to the lack of bacterial cells on its surface. Thus, the positive relationship between the electrochemical responses of the EABs and their growth duration implies reactivity and viability of the biofilms on the carbon paper.
The electrochemically active bacteria present in the EAB would biologically oxidize sodium lactate (electron donor) and produces extracellular electrons. Electrochemically active bacteria have the capability to transfer electrons extracellularly through different mechanisms. Direct electron transfer (DET) and mediated electron transfer (MET) mechanisms [47]. It has been reported that electrochemically active bacteria have self-secreted mediators such as Flavin. These mediators have the ability to carry electrons from the bacterial cell to the external acceptor in the media [48, 49].

Electrons are a strong reducing agent, which can then reduce metal precursors that are present abundantly in the surrounding media to produce nanoparticles as illustrated in Figure 4. In the
following sections, the effects of pH and metal precursor concentrations on the USNPs formation are investigated.

![Figure 4: Schematic illustration of the synthesis of UNSPs by EAB.](image)

3.2 Effect of pH the USNPs formation

In studying the effect of the pH on the USNPs synthesis, three different pHs, namely 4, 7, 9 of the metal precursor solutions with precursor concentration fixed at 1 mM were tested. It was quickly found that at pH 4, there was no production of metal nanoparticles due to the intolerance of *S. loihica* PV-4 to the acidic medium of pH 4. Figure S3 shows SEM images of dead *S. loihica* PV-4 cells after placing the carbon paper with living biofilm in the precursor solution of pH 4 for 48 hours. Some damaged and ruptured cell structures are clearly seen, which confirms that the EAB poorly responded to acidic conditions and that the biofilm was a factor in unsuccessful synthesis of nanoparticles at pH 4. In the cases with pH 7 and 9, successful nanoparticle synthesis were obtained.
Figure 5: TEM image (a) and particle size distribution (b) of as-synthesized Au NPs at pH 7; TEM image (c) and particle size distribution (d) of as-synthesized Au NPs at pH 9; (e) EDX spectra of the Au NPs.

Figure 5a and 5c presents the TEM of Au nanoparticles synthesized at pH 7 and pH 9. As can be seen, the produced particles were fairly uniform and had a size of USNPs ranging between 2-7 nm in the case of pH 7 and 2-15 nm in pH 9. Figure 5b and 5d show the size distribution histogram of the synthesized Au USNPs, which was manually measured from TEM images with more than 100 particles. EDX spectrum in Figure 5-e clearly confirms that these nanoparticles
were Au. The successful production of Au USNPs confirms the role of the EAB in the experiments as well as the effect of pH value.

Figure 6: TEM image (a) and particle size distribution (b) of as-synthesized Pd NPs at pH 7; TEM image (c) and particle size distribution (d) of as-synthesized Pd NPs at pH 9; (e) EDX spectra of the Pd NPs.

Figure 6a and 6b present TEM images and size distribution histogram of the synthesized Pd nanoparticles at pH 7. The produced Pd nanoparticles were fairly uniform and had a size of USNPs ranging between 2-6 nm. At pH 9, the USNPs of Pd were also produced with slightly bigger size ranging 2-12 nm as shown in Figure 6c and 6d. Figure 6e confirms the USNPs being Pd using EDX spectra. For Pt, at pH 7 the produced nanoparticles were uniform with sizes of 2-6 nm (Figure 7a and 7b). At pH 9, the USNPs of Pt were also successfully produced with the size
between 2-10 nm (Figure 7c and 7d). EDX spectra confirm that the particles were Pt NPs in Figure 7e.

![Figure 7: TEM image (a) and particle size distribution (b) of as-synthesized Pt NPs at pH 7; TEM image (c) and particle size distribution (d) of as-synthesized Pt NPs at pH 9; (e) EDX spectra of the Pt NPs.](image)

Thus, when pH is either 7 or 9, relatively uniform USNPs of all three metal, Au, Pd, and Pt were successfully synthesized and the sizes of the particles fell into the range of USNPs with good size distribution, with the size range at pH 9 a bit wider than that at pH 7. The above results confirm successful production of USNPs of noble metals by EAB.
3.3 Effect of metal precursor concentrations on the USNPs formation

To study the effect of concentrations of the metal precursors on the formation of the nanoparticles, four concentrations of metal precursors were investigated for each metal, 0.1, 0.5, 1.0 and 5.0 mM. In all cases, the concentrations of 0.1 mM of three metal precursor solutions failed to produce any particles while, in comparison, with the concentrations being increased to 0.5, 1 and 5.0 mM, the USNPs were successfully formed as shown in the TEM images Figure 8. For all of these three metals, no large metal particles (> 20 nm) were observed, indicating this is a quite robust synthesis method for ultra-small noble metals.

Figure 8a shows an HRTEM image for the Pd nanoparticles synthesis from 0.5 mM precursor. It clearly shows that each particle is highly crystalized with single crystalline nature. The interplanar spacing values measured from the HRTEM agree well with the theoretical values calculated from the crystal cell parameters of Pd [50].

Figure 8b and Figure 8c show an HRTEM image for the Pd nanoparticles synthesized from 1.0 mM and 5.0 mM precursor. No obvious difference can be directly found. However, the statistically average particles sizes are varied for them with the same evolution trend. As the concentration of the precursor increased, the average particle size of the products slightly increased in all three metal cases. For example, the average particle size was 2.62, 6.92 and 8.03 nm when the concentration of the precursor was 0.5, 1.0 and 5.0 mM for Pd NPs (Figure 9-a).
Figure 8: TEM images of (a-c) Pd NPs synthesized with the precursor concentration of (a) 0.5 mM, (b) 1 mM and (c) 5 mM; (d-f) Pt USNPs synthesized with the precursor concentration of (d) 0.5 mM, (e) 1 mM and (f) 5 mM; (g-i) Au USNPs synthesized with the precursor concentration of (g) 0.5 mM, (h) 1 mM and (i) 5 mM.

Figure 8d to Figure 8f, show HRTEM images of the Pt nanoparticles synthesized at 0.5, 1.0 and 5.0 mM precursor solutions. As can be seen, USNPs were formed at all concentrations, with the average particle size being 2.84, 4.27 and 8.57 nm for 0.5, 1.0 and 5 mM respectively (Figure 9b). The Au NPs show the same trend as the other two noble metals. Figure 8g to Figure 8i presents the TEM images of the Au NPs and their average particle size was 3.42, 4.02 and 6.19 nm for the 0.5, 1.0 and 5.0 mM precursor concentrations respectively Figure 9c.
Figure 9: The average sizes of the USNPs with different precursor concentrations (a) Au, (b) Pd, and (c) Pt NPs.

The clear relationship in all three metal cases between their precursor concentrations and their particle sizes provide an easy approach to control the average particle size during EAB-assisted noble metal nanoparticles. The XRD further confirms the crystallinity of the synthesized USNPs as shown in Figure S4.

A control experiment was conducted in this work, where the otherwise same reaction was run separately to produce USNPs of the metals in the absence of the EAB. This test was conducted to ensure that the EAB is responsible for the formation of USNPs. As expected, the growth of USNPs in the reactor was unsuccessful in the absence of EAB.

In summary, the USNPs produced using *S. loihica* PV-4 had ultra-small size of 2-7 nm with the optimized pH and precursor concentration. When comparing the species of *Shewanella* with other bacteria, it is found that *Shewanella* tends to produce nanoparticles smaller than those produced by other microorganisms as reported in the literature. For example, *Pseudomonas stutzeri* reportedly produced metal nanoparticles with particle size around 46 nm while *Lactobacillus sp.*, *Bacillus licheniformis*, and *Verticillium sp.* produced nanoparticles of 20, 50, and 25 nm respectively [51]. Presumably, the smaller particle size may be attributed to the higher reduction ability of *Shewanella* comparing to other bacteria.
3.4 Dye degradation performance by the UNSPs

Dye degradation experiments were performed to test the effect of the produced USNPs. In this experiment the Pd USNPs produced at pH 7 were collected and used as a catalyst for methyl orange (MO) degradation.

![Figure 10: (a) MO dye degradation using Pd USNPs. (b) Plot of ln(C_t/C_0) versus time for the reduction of MO using Pd USNPs.](image)

As a control, with only NaBH₄ being in the MO solution, no noticeable degradation occurred during the experiment as shown in Figure S5. As can be seen from Figure 10, using Pd USNPs in the presence of NaBH₄ shows an instant MO degradation. As a matter of a fact, Pd USNPs exhibited excellent catalytic performances in MO degradation. The degradation of MO using USNPs took around 50 seconds to see a drastic color change of the solution. Figure 10b shows that the MO degradation in this case follows the first-order kinetics.

4. Conclusion

In this work, EAB of *S. loihica* PV-4 was employed to successfully synthesize USNPs of Au, Pd and Pt. This synthesis method involves mild operating conditions, consumes less chemicals, and is more environmentally friendly. The synthesized USNPs all show good catalytic activity on the
degradation of methyl orange dye. This work shines light on the use of EAB for effective and controllable synthesis of USNPs.

Acknowledgement

This work was supported by the King Abdullah University of Science and Technology (KAUST) center competitive fund (CCF) awarded to Water Desalination and Reuse Center (WDRC). The authors are grateful to the other members of the KAUST Environmental Nanotechnology group for the helpful discussions.

References:


Supplementary Information

Synthesis of Ultra-Small Platinum, Palladium and Gold Nanoparticles by *Shewanella loihica* PV-4 Electrochemically Active Biofilms and Their Enhanced Catalytic Activities

Elaf Ahmed, Shafeer Kalathil, Le Shi, Renyuan Li, Ohoud Alharbi, Noor Zaouri, Peng Wang

Water Desalination and Reuse Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia
E-mail: Peng.Wang@kaust.edu.sa

Keywords: (Electrochemically Active Biofilm, Ultra-Small Nanoparticles, *Shewanella loihica*)

Figure S1: Digital photos of (a) plain carbon paper, (b) carbon paper with successful EAB growth on the surface.
Figure S2: SEM images for a single cell Shewanella *Loihica* bacteria.

Figure S3: SEM images of dead cells and shrunk/ruptured bacterial cell on the surface of the carbon paper after placing the biofilm in a solution of pH 4.
Figure S4: X-ray diffraction spectrum of the USNPs of Au.

Figure S5: Digital image of an instant MO dye degradation once Pd USNPs were added to the solution.