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THE INFLUENCE OF LIGHT ON THE BIOSYNTHESIS OF PT AND AU NANOPARTICLES BY *SHEWANELLA ONEIDENSIS* MR-1

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Abstract:
Shewanella oneidensis MR-1 is a metal-reducing bacterium widely used in dissimilatory reduction and recovery of precious metals. It has previously been used for the photo-induced reduction of Au(III) to Au(0), but to date there are no reports for the photo-induced reduction of Pt(IV). This study investigated whether light could be used to stimulate the synthesis of Pt nanoparticles (PtNPs) in S. oneidensis MR-1. The synthesis of AuNPs and PtNPs by S. oneidensis MR-1 both at dark and under white light was verified by UV-Vis spectroscopy and transmission electron microscopy (TEM). The results indicated that light significantly influences the synthesis of both Au and Pt nanoparticles. Under light conditions, the synthesis of AuNPs and PtNPs was induced within 24 hours, and the concentration increased exponentially over time. While being exposed to dark conditions, the reduction appeared to be a slower process (i.e., 48 hours for Au and more than 72 hours for Pt). The size and cellular localization of the NPs synthesized in above mentioned conditions also differed.
To the best of our knowledge, this is the first report that the synthesis of PtNPs by S. oneidensis MR-1 can be induced by light, which could be a cost-effective and an environmentally friendly alternative to chemical synthesis.

Keywords: biomineralization, gold, nanoparticles, platinum, Shewanella oneidensis MR-1.

1. INTRODUCTION

Gold (Au) and platinum (Pt) are precious metals of special importance due to the fact that they present a low chemical reactivity, are highly corrosion-resistant, have high malleability, very high melting points and a very good electrical conductivity. Due to their chemical and physical properties, their malleability, and their appearance, these metals are used for decorative purposes, for jewellery manufacturing, as well as in the electronic parts industry, medicine and for the catalysis of certain reactions (Spitzer and Bertazzoli 2004; Ramesh et al., 2008). Nanoparticles (NPs) of these metals have innumerable applications in various fields. Platinum nanoparticles (PtNPs) are used in the medical field due to their antimicrobial, antioxidant and anti-cancer properties, photothermal therapy, drug administration (Bloch et al., 2021), in optical applications and for reducing pollutants from car emissions due to their catalytic activity (Saif et al., 2022). Gold nanoparticles (AuNPs) are used in dark field microscopy, for the detection of microbial cells and their metabolites, in bio-imaging of tumorous cells and the receptors from their surface, for air purification, including the elimination of unpleasant odours and carbon monoxide in rooms, in emission management, water purification, electrochemical cells (York et al., 2007; Bickford et al., 2008; Hu et al., 2009; Wang et al., 2010;

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Dykman and Khlebtsov, 2011; Ma et al., 2016). Because of their small size (1-100 nm), AuNP can penetrate the tissues with ease and attack certain cells, such as lymphoid tissues, making them potentially useful in immunotherapy (Ahmad et al., 2017; Hammami et al., 2021).
Due to the multiple applications of these precious metals and their nanoparticles, there is an increasing interest in obtaining them. There are two categories of technologies for the manufacture of these nanoparticles, chemical and biological.
Traditionally, chemical methods used to recover noble metals (Au and Pt) include cyanide leaching, precipitation, filtration, and electrochemical treatments (Mata et al., 2009). Although these methods are very effective, they present limited selectivity and use large quantities of toxic chemicals in this process, thus causing pollution (He et al., 2005). Compared to traditional methods, biological methods using microorganisms for the extraction and recovery of metals can successfully replace some phases of these classical methods, making them more attractive due to their simplicity and to the elimination of the need to use toxic chemicals (Mishra et al., 2014). In the last decade, more and more studies on the use of different microorganisms have been included (e.g.: bacteria, yeasts, fungi) as "nanofactories" for the synthesis of metallic NPs (Li et al., 2011; Zou et al., 2021). Regarding the use of microorganisms for the biosynthesis of NPs on a large scale, for commercial applications, it is important to ensure optimum conditions by strictly monitoring certain parameters such as pH, incubation time and concentration of metal ions (Narayanan and Sakthivel, 2010; Wu and Ng, 2017). However, these biological methods, together with the advantage of being simple, energy-efficient, cost-effective, mild, and environmentally friendly, being referred to as 'green chemistry', also have the capacity to control the size and shape of nanoparticles (Bai et al., 2009; Sathishkumar et al., 2010). As one of the most abundant biological resources, microorganisms have adapted to environments contaminated with toxic metals by developing various tactics, one of them being the biosynthesis of metallic NPs.
In this respect, a general interest has emerged towards the group of metal-reducing bacteria, with particular attention to the genera *Shewanella* sp. and *Geobacter* sp., bacteria capable of carrying out a special extracellular electron transfer (EET). Due to their unique EET characteristics, metal-reducing bacteria have gained increasing interest in the last two decades, leading to the development of a number of new technologies in interdisciplinary areas such as biogeochemistry, bioelectrochemistry and nanotechnology. In the absence of intracellular electron acceptors, these bacteria can oxidize anaerobically various organic substances and then transfer the electrons from the intracellular environment, passing through membrane barriers, to compounds with redox activity (electron acceptors) in the extracellular environment. Bacteria with the ability to perform EET is called electro-active bacteria, *S. oneidensis* MR-1 and *G. sulfurreducens* being the two most known model strains.
Shewanella oneidensis MR-1 is a facultative anaerobic Gram-negative bacterium that can use a wide range of metal ions in the process of anaerobic respiration as electron acceptors, this ability to reduce metal ions, making this bacterium of particular interest in studies on the biosynthesis of metal NPs (Burgos et al., 2008; Suresh et al., 2011; Huang et al., 2019; Xu et al., 2019; Ghinea et al., 2021).
The studies published so far have shown that there are three important routes by which *S. oneidensis* can reduce metals in the anaerobic respiration process: enzyme route (metal-reducing enzymes), the route of redox mediators, and by the production of nanowires (Beliaev et al., 2001; 2002; Marsili et al., 2008; Kotloski and Gralnick 2013; Wee et al., 2014; Pirbadian et al., 2014; Saffarini et al., 2015). An important factor that may have an effect on the activity of enzymes involved in the metal reduction process is light. A number of studies have already reported that in the production of AuNPs by *S.*

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oneidensis MR-1, light plays an important role, the metal reduction rate being much higher under light conditions than in the dark (Wu and Ng, 2017; Huang et al., 2019).
To date, to the best of our knowledge, there are no reports on the effect of light on the production of PtNPs and considering the promising results obtained in the case of Au, the present study aims to investigate the effect of light and darkness on both the production of AuNPs and of PtNPs as well as the efficiency of the process according to these parameters.

2. MATERIALS AND METHODS

2.1. Bacterial strain and growth conditions

In this study we used the metal-reducing bacteria *Shewanella oneidensis* MR-1 (LMG 19005), purchased from the BCCM/LMG Bacterium Collection (Gent, Belgium). The cultivation of the bacterial strain was performed in LB (Luria-Bertani) medium for all experiments. The cells used as inoculum were pre-grown until they reached the exponential phase (24 h) in 100 mL LB, pH 7.0, containing yeast extract (5 g/L), sodium chloride (10 g/L) and tryptone (10 g/L). After 24h of cultivation, at 30°C and 150 rpm (rotation per minute), the cells were collected by centrifugation, at 7500 rpm for 10 minutes and washed two times with bicarbonate buffer (30 mM) pH 7.0, after each wash the cells were recovered by centrifugation.

2.2. Nanoparticles synthesis

The nanoparticles (NPs) synthesis experiments were performed in anaerobic conditions in two variants: under light and under dark conditions. For the PtNPs synthesis, the washed cells were resuspended in Falcon tubes filled with 15 mL bicarbonate buffer (30 mM) with 25 mM sodium formate (HCOONa) and 1mM platinum hexachloride ($H_2PtCl_6 \cdot xH_2O$). For the AuNPs synthesis the cells were resuspended in bicarbonate buffer (30 mM) with 30 mM sodium lactate ($NaC_3H_5O_3$) and 1mM sodium tetrachloroaurate ($NaAuCl_4 \cdot 2H_2O$). The cells concentration in each tube was 2g/L wet biomass. To ensure the anaerobic conditions, all solutions used were bubbled with nitrogen for 15 minutes using an air filter to maintain the sterility of the solutions. The tubes with the bacterial suspensions were incubated with the cap slightly loose in airtight GENbags for anaerobiosis (BioMerieux, France) for 6 days at 30ºC, under light or dark conditions.

2.3. UV-Vis Spectroscopy

For determining the absorption spectra of the Pt and Au solutions at the initial moment and at the end of the incubation time, each sample was diluted 20 times and the spectral analysis was recorded between 220 and 600 nm using a Specord 210 Plus (Analytik Jena) spectrophotometer.

2.4. Transmission Electron Microscopy (TEM)

The ultrastructural investigation of *S. oneidensis* cells, PtNPs, and AuNPs synthesized under light and dark conditions was performed on ultrathin sections. For this, the cells were pre-fixed overnight in 3% glutaraldehyde in 0.05 M cacodylate buffer pH 7.4 at 4°C, included in 3% agar later on and further processed according to the routine protocol (Mirancea et al., 2007). After 6 successive washes with 0.05 M cacodylate buffer, the samples were postfixed with 4% OsO4 (in 0.1 M cacodylate buffer) for 2h at room temperature, followed by serial washes, and then dehydrated in a graded series of ethanol (30%, 50%, 70%, 90%, and 100%). After treatment with propylene oxide (PO) and pre-embedment of samples (PO and Epon), the final embedment (Epon) at 60° C for at least 24 hours followed. The sectioning was performed with an Ultrotome III LKB ultramicrotome with a glass

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knife, the ultrafine sections (70-90 nm thick) were double counterstained with Uranyless and lead citrate. All the prepared grid samples were analyzed using a JEOL JEM-1400 TEM operated at 80 kV accelerating voltage and visualized with Quemesa CCD camera (Olympus Soft Imaging Solutions).

2.5. Statistical analysis

To quantify the shape and size distribution of PtNPs and AuNPs, a statistical analysis of the size of the crystals present inside the bacterial cells was performed using digital TEM images. The outlines of at least 100 crystals were digitized and their dimensions calculated using ImageJ software (https://imagej.nih.gov/ij/). The crystal dimensions were estimated by calculating the best fit of an ellipse to the contours of the crystals. The particle size (particle diameter) is reported as the average of length (L) and half-length width (W) of the measured ellipse (Devouard et al., 1998). The shape factors, describing the elongation of the crystals, were calculated from their width to length ratio, so that $W/L \leq 1$.

3. RESULTS AND DISCUSSIONS

3.1. Nanoparticles biosynthesis

The process through which bacteria (and other organisms) produce nanomaterials is called biomineralization. The process of biomineralization is not a unique process, but it represents an amalgam of different biochemical processes that can be observed in all life's domains, both at small and big scale. At small scale, bacterial cells can form crystals in the interior, on the surface or in the vicinity of the cells by biomineralization (Man, 2001). *S. oneidensis* MR-1 is a metal-reducing bacterium capable of synthesizing different types of NPs both inside and outside the cell (Kim et al., 2018). In this study we investigated the effect of light and darkness on the biosynthesis of AuNPs and PtNPs by *S. oneidensis* MR-1.

The formation of PtNPs and AuNPs by *S. oneidensis* MR-1 under standard white light illumination (400-700 nm) over 6 days was monitored at first visually and the results are present in Figure 1. After 24 hours of incubation, a colour change was observed, samples with Au turned pink and those with Pt started to turn black. In the case of samples incubated under dark, a slight colour change appeared only after 48 hours, both for Au and for Pt. At the end of the incubation period (i.e., 6 days) the Au samples incubated under light conditions were deep purple and the Pt samples black, while the samples incubated in the dark had a light pink colour in the case of Au and dark grey in the case of Pt (Figure 1). This colour difference between the samples incubated under light and those in the dark was a first indication that the rate of the metal reduction reaction in the light is higher than that of the samples incubated in the dark. The change of suspension colour indicates the initiation of the metal reduction process and that the NPs begin to be synthesized, PtNPs having a black colour while AuNPs colour varying from pink to dark purple depending on the NPs size (Amendola et al., 2017; He et al., 2005). The intensity of the colour is also an indication of the NPs concentration in the sample.

Figure 1. Visual examination of the colour shift of (a) Pt samples in the dark; (b) Pt in light; (c) Au in the dark; (d) Au at light, at the end of the incubation period; (e) Pt at the initial moment; (f) Au at the initial moment.

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3.2. UV-Vis spectroscopy

The formation of PtNPs and AuNPs by *S. oneidensis* MR-1 was also verified by UV-Vis spectroscopy (Figure 2). Platinum hexachloride (H_2PtCl_6•xH_2O) shows an absorption peak at 260 nm and as Pt is reduced from Pt(IV) to Pt(0) with the formation of PtNPs, it flattens until it disappears completely (Georgieva and Andonovsky, 2003). In Figure 2a, it can be observed a maximum absorption peak of the Pt solution at 265 nm at the initial moment and its disappearance at the final moment, in the case of the solution incubated in the light. In the case of the dark Pt sample, the absorption spectrum of Pt did not completely flatten, which indicates that Pt was not completely reduced.

The AuNP's interaction with light is determined by their shape and size. The electric field of a light ray induces in the free electrons of a NP an oscillatory motion with a well-defined frequency, this is known as surface plasmon resonance. The plasmonic resonance determines the wavelength at which a NP absorbs or emits light. Due to the surface plasmonic resonance, AuNPs shows a maximum absorbance peak at 530 nm (visually perceptible as the colour dark purple) (Figure 2c), this may vary depending on the shape and size of the NPs (He et al., 2005). Thus, depending on these parameters, the colour and absorbance of the AuNPs suspension may vary. For example, if the NPs have a diameter between 12 and 41 nm, then the absorption peak will be at 520-530 nm, the wavelength of the maximum absorbance peak increasing with the NP diameter (He et al., 2005; Amendola et al., 2017). Following the UV-Vis spectroscopy analysis, in the case of samples incubated in the light, a maximum absorption peak was observed at 565 nm (Figure 2c) while in the case of those incubated under dark conditions, no peak was observed (Figure 2d), probably due to the low concentration or absence of NPs.

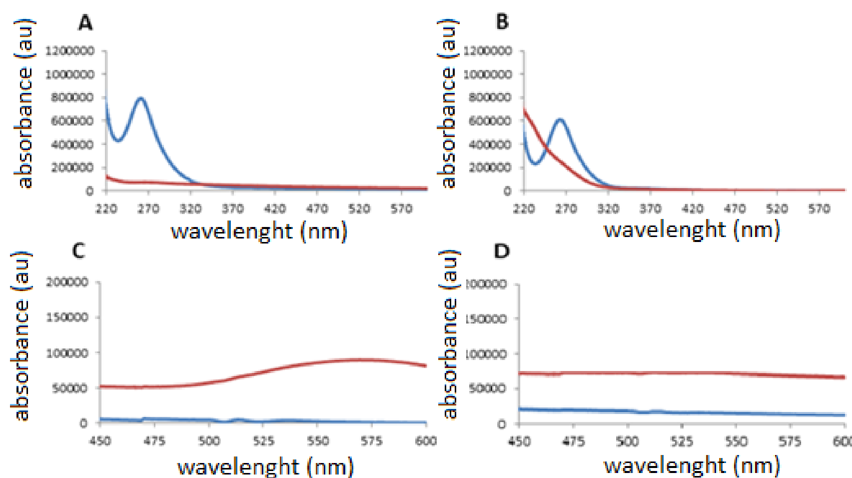

Figure 2. The UV-Vis absorption spectrum of the Pt solution (A) incubated in the light and (B) in the dark, and of the Au solution (C) incubated in the light and (D) in the dark.

3.3. NPs characterization

Related to the location, morphology, and diameter of monometallic NPs synthesized by the metal-reducing bacterium *S. oneidensis* MR-1, the TEM technique was used to characterize PtNPs and AuNPs, respectively. Figure 3a, b shows that in both cases, of cells incubated with Pt under light and under dark conditions, the synthesized NPs are mainly located outside the cell, more precisely on the external cell membrane but also on the internal membrane and in the periplasmic space. The presence

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of PtNPs on the internal membrane and in the periplasmic space indicates that the bacterium is directly implicated in the synthesis of these NPs, membrane proteins with a role in electron transfer being probably involved in this process. In the literature, there are studies that demonstrated that cytochromes with redox activity (e.g., MtrC and OmcA) located in the cell membrane of *S. oneidensis* MR-1 and with a function in anaerobic respiration also play an essential role in NPs production (Beliaev et al., 2002; Saffarini et al., 2015). A previous study also showed that Pt(0) NPs produced by *S. algae* are located not only on the cell surface but also in the periplasmic space (Konishi et al., 2007).

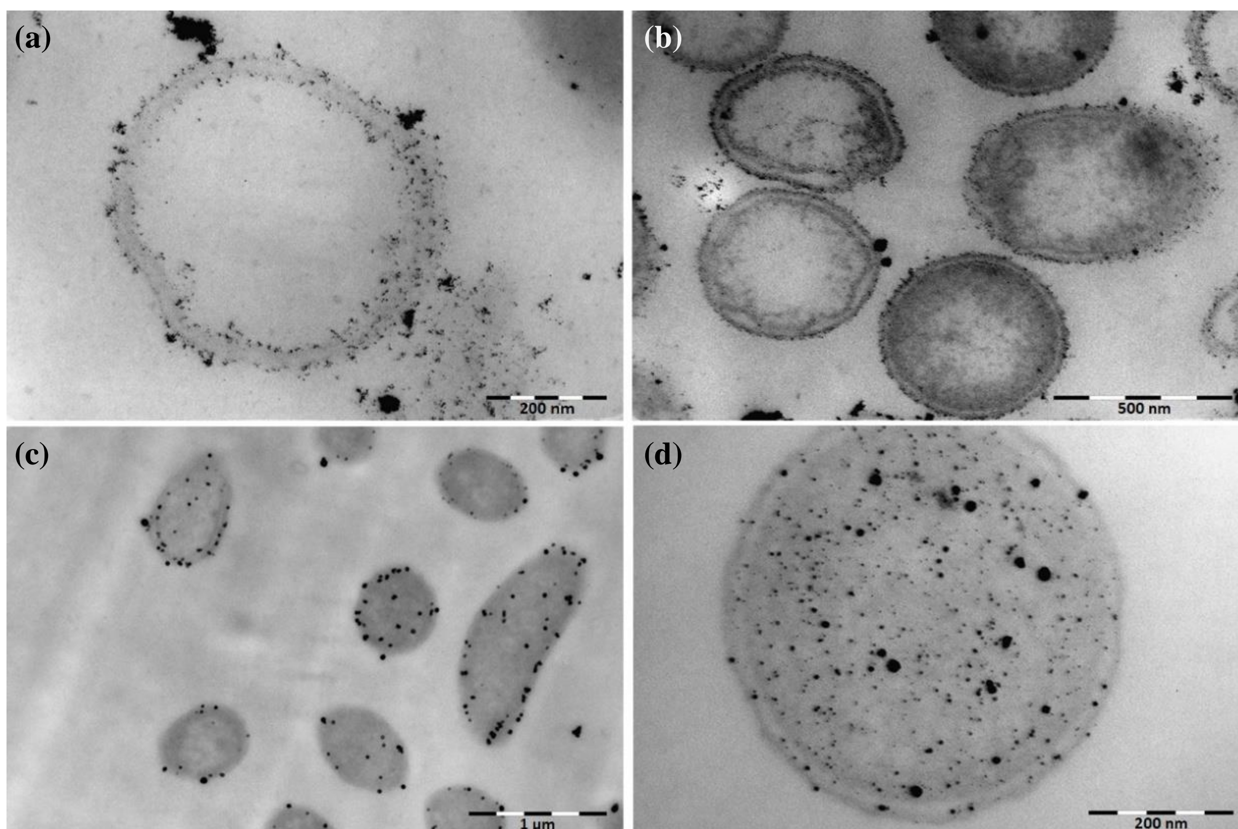

Figure 3. TEM images of S. oneidensis MR-1cells from the samples (a) Pt light; (b) Pt dark; (c) Au light; (d) Au dark.

Dark-field TEM imaging is a conventional technique used to identify crystal structure. In dark field mode, a single diffracted beam is used to form the TEM image. This causes specimens that do not have a crystalline structure to be represented as very dark in the final image, whereas those with a crystalline structure appear as bright spots, thus allowing visual phase differentiation in TEM. Figure 4 shows the TEM images in bright field and the correspondent in dark field, of different types of NPs synthesized by *S. oneidensis* MR-1. These images confirm that the electron-dense particles observed in the bright field TEM images are single crystals (nanoparticles) and not an amorphous precipitate. Thus, the crystalline nature of the synthesized PtNPs and AuNPs is confirmed.

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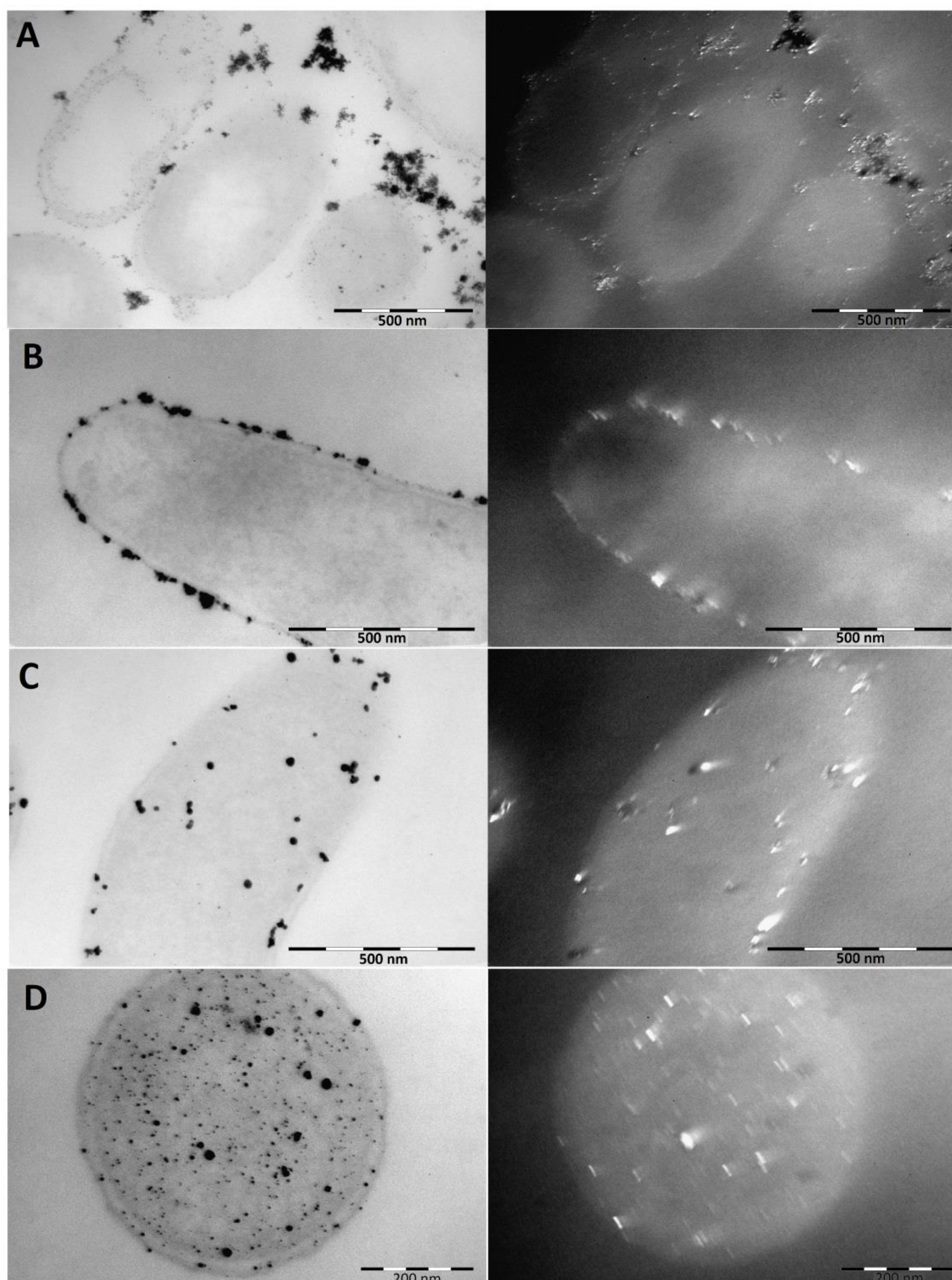

Figure 4. Bright-field and dark-field TEM images of S. oneidensis MR-1 cells with (A) PtNPs under light, (B) PtNPs under dark, (C) AuNPs under light, and (D) AuNPs under dark conditions.

The ImageJ measurements showed that the obtained PtNPs are very small in size, with an average diameter of 2 nm for PtNPs synthesized in light (Fig. 5 a) and approximately 1 nm for those synthesized in dark (Fig. 5 b), these values being similar to those reported in the literature (e.g., 5 nm) (Tuo et al., 2016; Xu et al. 2019). Regarding the PtNPs morphology, this was also similar to that reported by Tuo et al. (2016).

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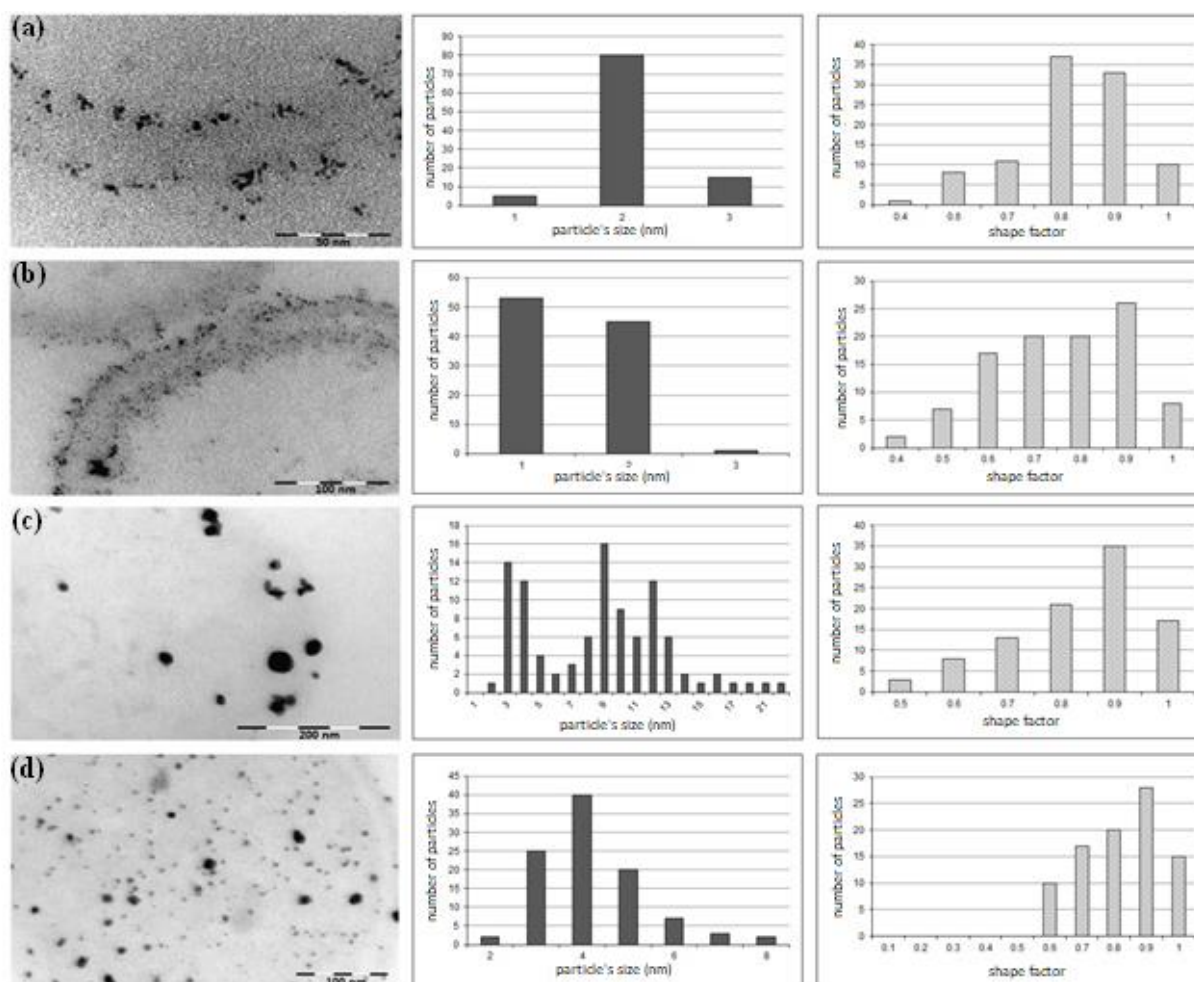

Figure 5. Size and shape distributions of Pt NPs incubated in (a) light and (b) dark, and of Au NPs incubated in (c) light and (d) dark, synthesized by S. oneidensis MR-1 cells.

Some of these small PtNPs agglomerate into small groups and are arranged in the form of "snowflake" shaped spots on the cell surface. In the case of snowflake-shaped spots, the average size was 13 nm. Also, PtNPs agglomerations were observed in the vicinity of the cells, in the extracellular environment. These extracellular NPs are most likely the result of the activity of some organic molecules with the role of some flavin-type redox mediators involved in the extracellular transport of electrons ("electron shuttles") (Pirbadian et al., 2014; Saffarini et al., 2015).
In the case of AuNPs, it was observed that they are usually located both in the area of the cell membrane and inside the cell (Figure 5 c, d), this could be due to different metabolic pathways used in the reduction of Au compared to those used for Pt reduction. The size of AuNPs synthesized by *S. oneidensis* MR-1 in this experiment it was between 2 and 30 nm (Figure 5 c, d), these values being similar to those reported in the literature (i.e., 2-50 nm) (Suresh et al., 2011; Wu and Ng, 2017; Huang et al., 2019).

4. CONCLUSIONS

The light's influence in the process of metals reduction can be observed, initially, by simply comparing the colour of the samples. The light exposed samples changed their colour with a

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significantly greater velocity than the samples kept in the dark. The difference between the two incubation parameters is even more visible following the spectrophotometer analysis by comparing the absorption spectra of the two metals, the samples exposed to light having a greater quantity of reduced metal. Also, in this study, differences were observed both in terms of the localization of PtNPs compared to AuNPs and in terms of the size of NPs synthesized under light versus dark conditions, for both metals. The presence of NPs on the cell membrane shows that the metal was reduced by the active participation of the cell during the process of anaerobic respiration. The NPs found outside of the cell could be the result of the extracellular electron transfer mechanism where soluble electron transport molecules or nanowires could be involved.

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