BIOREDUCTIVE SYNTHESIS OF PLATINUM NANOPARTICLES BY *Shewanella oneidensis* MR-1

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Abstract. Currently, different biosynthetic methods that use either microorganisms or plant extracts are being developed as an alternative to the more polluting chemical synthesis procedures for obtaining nanomaterials. Special attention is paid to the biosynthesis of precious metals nanoparticles, in particular to platinum nanoparticles due to their multiple catalytic applications, such as degradation of organic dyes, redox reactions or electrocatalytic and catalytic conversions. In this work, we propose an environmentally friendly method for producing platinum nanoparticles using the metal ion-reducing *Shewanella oneidensis* MR-1. Resting cells of *S. oneidensis* MR-1 were used to reduce aqueous Pt_4^+ ions and deposit them as metallic platinum nanoparticles (Pt^0), at room temperature and at neutral pH. Both lactate and succinate were used as electron donors. Different anaerobic techniques for the cultivation and manipulation of the bacterial cells were used instead of an anaerobic chamber, which seems to be a shortcoming for larger applications. Biosynthesized platinum nanoparticles were characterized through UV-VIS spectroscopy, fluorimetry techniques, and TEM.

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

Rezumat. Sinteza bioreductivă a nanoparticulelor de platină de către *Shewanella oneidensis* MR-1. În prezent, sunt dezvoltate diferite metode biosintetice care utilizează fie microorganisme, fie extracte din plante ca alternativă la procedurile de sinteză chimică mai poluante pentru obținerea nanomaterialelor. O atenție specială este acordată biosintezei nanoparticulelor de metale prețioase, în special a nanoparticulelor de platină datorită multiplelor lor aplicații catalitice, cum ar fi degradarea coloranților organici, reacții redox sau conversii electrocatalitice și catalitice. În această lucrare, propunem o metodă ecologică pentru producerea de nanoparticule de platină utilizând bacteria metal-reducatoare *Shewanella oneidensis* MR-1. Celulele de *S. oneidensis* MR-1 aflate în repaus au fost utilizate pentru a reduce ionii de Pt_4^+ și depunerea lor ca nanoparticule metalice de platină (Pt^0), la temperatura camerei și la pH neutru. Atât lactatul cât și succinatul au fost folosiți ca donori de electroni. Au fost utilizate diferite tehnici anaerobe pentru cultivarea și manipularea celulelor bacteriene în locul unei camere anaerobe, care pare a fi un neajuns pentru aplicații mai mari. Nanoparticulele de platină biosintetizate au fost caracterizate prin tehnici de spectroscopie UV-VIS, fluorimetrie și microscopie TEM.

Cuvinte cheie: biomineralizare, nanoparticule, platină, *Shewanella oneidensis* MR-1.

INTRODUCTION

Nanoparticles (NPs) have properties that differ from the bulk material (JEE VANANDAM et al., 2018) with sizes ranging from 1 nm to 100 nm in at least one dimension (POTOCNIK, 2011; BATISTA et al., 2015), they can be produced synthetically by chemical, physical and biological methods (JEE VANANDAM et al., 2018). The metallic NPs have many potential applications, especially those of noble metals, with uses in medicine, industry, technology, etc. (BUZEA et al., 2004; DANIEL & ASTRUC, 2004; HOLT & BARD, 2005; CHEN et al., 2010; AHMAD et al., 2016; KIM et al., 2002; CHEN et al., 2010; SALDAN et al., 2015; SIDDIQI & HUSEN, 2016). Taking these reasons into consideration, researchers are currently looking for various environmentally friendly methods for producing NPs, one of these methods being the use of microorganisms such as bacteria to reduce different metals (e.g., platinum, palladium, gold, etc.) and to produce metallic NPs. Bacteria are an interesting choice because they are very abundant in nature, they grow fast, are easy to handle, do not require expensive growth media, and the cultivation conditions (i.e., temperature, stirring, oxygen levels, incubation time, etc.) can be easily controlled (PANTIDOS and HORSFALL, 2014). There are numerous reports of bacteria being used to produce precious metallic NPs: NANGIA et al. (2009) used *Stenotrophomona maltophillia* to produce gold NPs; HUSSEINY et al. (2007) used *Pseudomonas aeruginosa* to produce gold NPs; YATES et al. (2013) produced palladium NPs with *Geobacter sulfurreducens*; ATTARD et al. (2012) produced platinum NPs with *Escherichia coli* MC4100; HE et al. (2007) controlled the shape and size of gold NPs produced by *Rhodopseudomonas capsulata*, varying the pH between 7 and 4.

Shewanella oneidensis MR-1 is a Gram-negative, facultative anaerobe, heterotrophic bacterium, recognized for its ability to use a wide variety of metals as final electron acceptors. Its ability to reduce highly toxic heavy metals when found in soluble form to a less toxic insoluble form (i.e., metallic NPs) makes this bacterium of great interest for bioremediation studies (XIONG et al., 2005a; b; SURESH et al., 2011). There are numerous reports that have demonstrated the ability of this bacterium to produce NPs of noble metals. For example, HUANG et al. (2019) showed the ability of *S. oniedensis* MR-1 to produce gold NPs in the presence of light. WIM DE WINDT et al. (2006) used *S. oneidensis* MR-1 to produce NPs from the platinum metal group, such as palladium NPs, using H_2 and sodium formate as electron donors. In another study, XU et al. (2019) used *S. oneidensis* MR-1 to produce NPs from a platinum-palladium alloy using sodium formate as an electron donor and tested their catalytic properties to reduce azo matrices and nitrophenol.

The aim of this study was to use aerobically grown *S. oneidensis* MR-1 cells to reduce Pt_4^+ ions to zero platinum (Pt^0) with the formation of metallic platinum NPs, using sodium formate as electron donor, as an environmentally friendly method of recovering noble metals through the process of biomineralization.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *Shewanella oneidensis* MR-1 (LMG 19005) was cultured aerobically in Luria Bertani (LB) broth for biomass production, at 30°C with orbital stirring for 24 h. The resulted biomass was collected by centrifugation at 7600 rpm for 10 min and washed two times with bicarbonate buffer (30 mM).

Production and purification of Pt-NPs. For Pt-NPs biosynthesis, the washed biomass was resuspended in bicarbonate buffer with 25 mM Na-formate as electron donor and 1 mM H_2PtCl_6 (≥99.9%, Alfa Aesar) as electron acceptor. The wet biomass had a final concentration of 2 g/L. The reaction mixture was placed in butyl-rubber stoppered serum bottles filled to the brim, to remove as much oxygen as possible and ensure "near" anaerobic conditions for Pt-NPs production. The mixture was incubated in dark conditions at 30°C without stirring or shaking, for 10 days for the bio-reduction process to take place. Control experiments were conducted, in the absence of either the metal precursor or Na-formate to confirm the role of bacterial cells to produce the nanoparticles. To extract the prepared Pt-NPs, the bacterial cells were pelleted by centrifugation at 7600 rpm for 10 min, washed with phosphate buffer saline (PBS) and hydrolysed with 1 M HCl at 90ºC for 60 minutes. The Pt-NPs were recovered by centrifugation at 14000 rpm for 10 min, and washed two times with Milli-Q water to remove the residual HCl.

UV-VIS spectroscopy and fluorimetry. Biosynthesis of Pt-NPs was monitored with a Specord 210 Plus spectrophotometer (Analytik Jena) within the 220–800 nm absorbance range. For this purpose, each sample was dispersed in 1 cm path length quartz cuvettes and used to obtain the UV–visible absorption spectrum. The emission spectra of the extracted nanoparticles was measured with a FP8300 spectrofluorometer (Jasco) using the following parameters: PMT 500 V, Ex 335 nm, reading at 350-500.

Electron microscopy examinations. Transmission electron microscopy (TEM) studies were performed on both whole cells and extracted Pt-NPs. The platinised cells of *S. oneidensis* MR-1 were fixed overnight at 4°C in 2.5% glutaraldehyde amended PBS, dehydrated in a graded series of ethanol (10, 30, 50, 70, 90, and 2 × 100% with 15 min each change). To determine the size and shape of the extracted Pt-NPs, each sample was dispersed in Milli-Q water, then all the prepared samples were visualized using a JEOL JEM-1400 operated at 80 kV accelerating voltage. For statistical analysis of the extracted Pt-NPs sizes, at least 100 particles were measured. The crystal outlines were digitized and their dimensions calculated using the ImageJ software (https://imagej.nih.gov/ij/).

Dark-filed microscopy and hyperspectral imaging (DMHI). CytoViva Hyperspectral Enhanced Dark-field Microscope (USA), consists of an enhanced dark-field illumination system attached to a standard light microscope, for probing and characterizing biosynthesized nanoscale materials, as small as 10 nm. The unstained cells were examined with a 100X oil immersion objective. Spectral data within each pixel of the scanned field of view were captured with a CytoViva spectrophotometer and integrated charged-coupled device (CCD) camera. The spectral resolution was 1.5 nm and the pixel size was 6.45 μm. Spectral data were analysed by using the ENVI 4.8 Image Analysis Software (IDL Available).

RESULTS AND DISSCUTIONS

Pt-NPs biosynthesis. The biosynthesis of Pt-NPs has long been investigated using various microorganisms (SRIVASTAVA & CONSTANTI, 2012; ATTARD et al., 2012; RIDDIN et al., 2010; LENGKE et al., 2006; GAIDHANI et al., 2014). *Shewanella* sp. have always been considered an appropriate candidate due to their respiratory diversity illustrated by their ability to use a wide range of final electron acceptors (e.g., oxygen, nitrate, metals and sulphur compounds) as well as their capability to realize extracellular electron transfer (BABAUTA et al., 2012; KOTLOSKI & GRALNICK, 2013). Despite its wide use for various metal NPs biosynthesis, there are only few reports on Pt-NPs produced by *Shewanella* sp. (KONISHI et al., 2007; AHMED et al., 2018; XU et al., 2019).

In this work, we developed an environmentally friendly method for producing Pt-NPs using *S. oneidensis* MR-1. The biosynthesis of Pt-NPs was monitored by visual inspection at first, the reduction of Pt_4^+ ions being indicated by the change in the colour of the cell suspension, from pale yellow to yellowish-grey. As expected, the colour change provided a visual signature for the formation of the black metallic platinum (i.e., Pt-NPs) inside bacterial cells, which can be better observed after centrifugation, when the colour shift from pink to brownish-black of the concentrated biomass becomes more evident (Fig. 1a). Also, this change of colour was monitored by recording the increase in absorbance of the cell suspension incubated for 10 days in the presence of platinum (Fig. 1b). Although the process of bio-reduction took longer than other reported studies (probably due to oxygen traces in the reaction mixture), the *S. oneidensis* MR-1 cells were able to reduce and deposit Pt-NPs in the presence of formate as an electron donor. No colour change and consequently Pt-NPs synthesis were observed in tested controls.

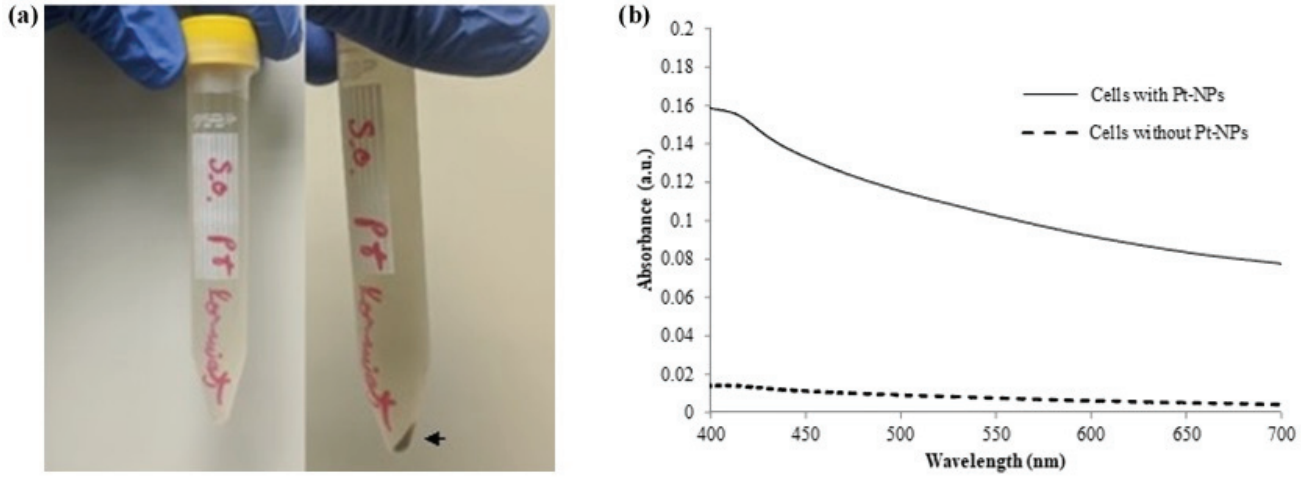

Figure 1. (a) *S. oneidensis* MR-1 in bicarbonate buffer with 1 mM H_2PtCl_6 at the beginning of the experiment (left) and after 10 days (right). (b) Absorbance spectra of cells with and without Pt-NPs in bicarbonate buffer, after 10 days of incubation.

UV-VIS spectroscopy is one of the simplest and easiest techniques for monitoring the reduction of Pt_4^+ to Pt^0, based on the differences between the absorption spectrum of H_2PtCl_6 solution which shows a peak at around 260 nm due to the presence of Pt_4^+ in the water (NISHANTHI et al., 2019) as compared with Pt-NPs solution, where this absorption peak disappears indicating the reduction of metal precursor salt to zero-valent platinum (RIDDIN et al., 2010) (Fig. 2a).

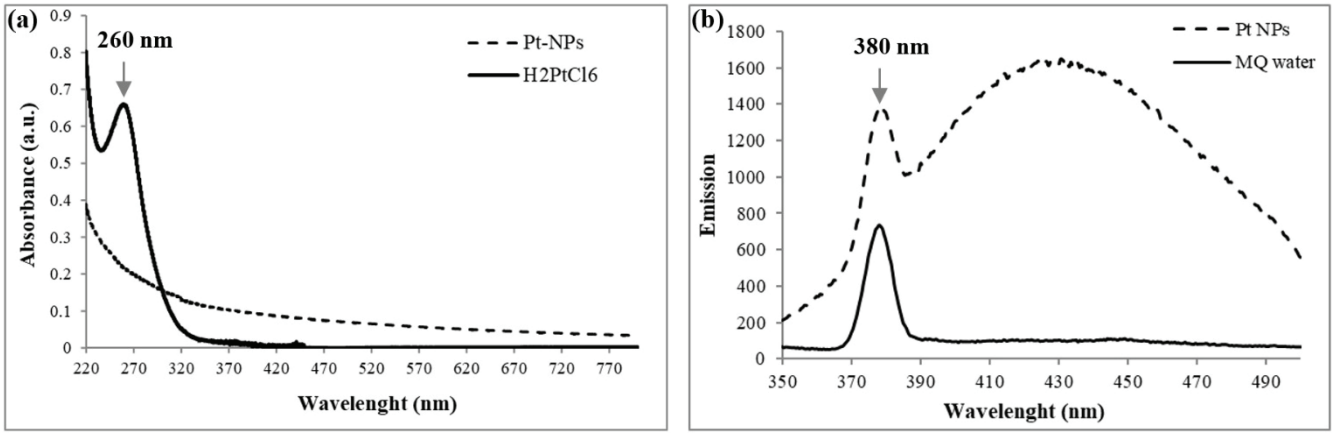

Figure 2. (a) UV–VIS spectrum of H_2PtCl_6 solution showing the characteristic peak at 260 nm and absorbance spectrum of the extracted Pt-NPs. (b) Plasmon emission of the Pt-NPs.

The Pt-NPs plasmon emission was also investigated using the FP8300 spectrofluorometer (Jasco) with the following parameters: PMT 500 V, Ex 335 nm, reading at 350-500. The Pt-NPs were suspended in Milli-Q water which has a visible peak at ~380 nm, and the Pt-NPs suspension show a rise in emission between 390 and 430 (Fig. 2b).

TEM characterization. To confirm the reductive deposition of platinum, *S. oneidensis* MR-1 cells were observed using transmission electron microscopy (TEM) after exposure to an aqueous H_2PtCl_6 solution for 10 days. As shown in Fig. 3a, *S. oneidensis* MR-1 is a rod-shaped bacterium approximately 0.4 µm wide and 1.7 µm long. When exposed to the H_2PtCl_6 solution, a black precipitate was observed on the bacterial cells, supposedly Pt-NPs. The bright-field TEM image of extracted NPs (Fig. 3b) shows a cluster of nearly spherical nanoparticles, with sizes between 15 to 65 nm, and an average diameter of 36 nm (Fig. 3a-insert). It should be noted that size is a crucial factor influencing the properties of NPs, leading to a special geometric and electronic structure, thus significantly influencing their catalytic activity. One possible explanation could be that smaller NPs have a higher surface-to-volume ratio and yield more active sites (KUMARI et al., 2016; LI et al., 2011), which is of great importance for their potential applications. By comparison with other microorganisms, *Shewanella* sp. appears to produce smaller nanoparticles (AHMED et a., 2018; XU et al., 2019) probably due to their increased metal reducing capacity.

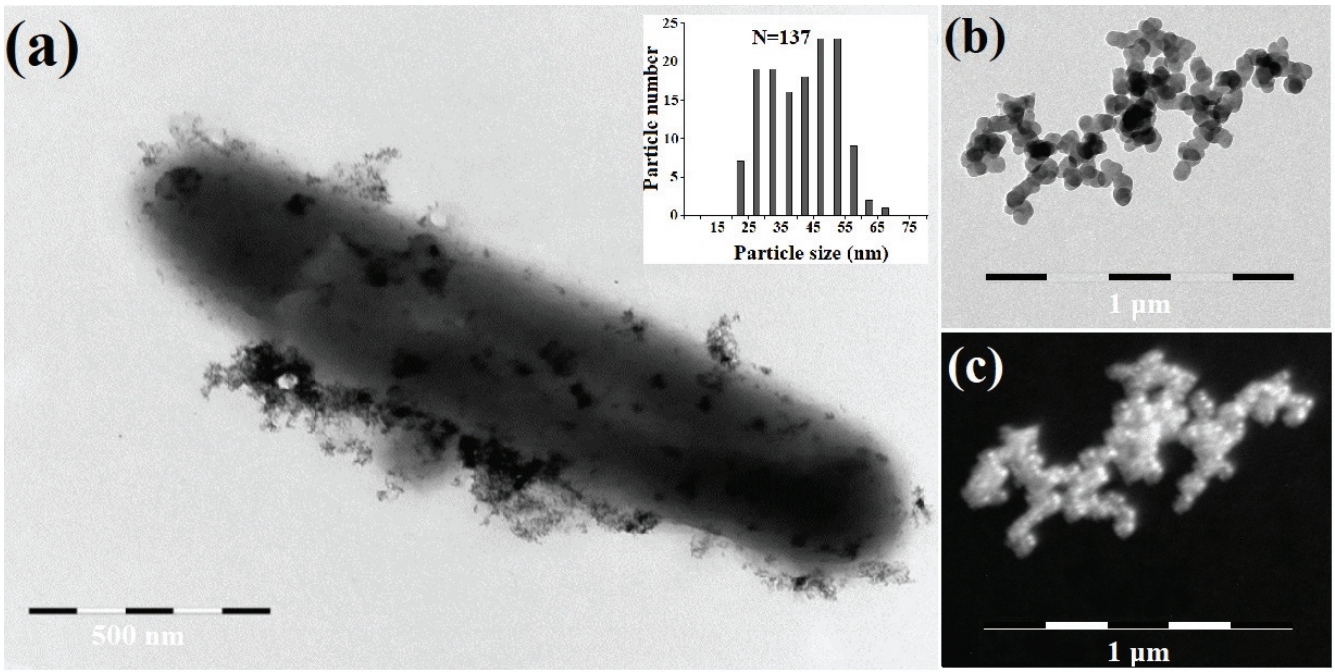

Figure 3. (a) Bright-field TEM image of a *S. oneidensis* MR-1 cell after exposure to a 1mM aqueous H_2PtCl_6 solution. Pt-NPs can be seen as a black precipitate on the cell surface. Size distribution of the extracted Pt-NPs (insert). (b) Bright-field and (c) the corresponding dark-field TEM images of biogenic Pt NPs extracted by acidic hydrolysis.

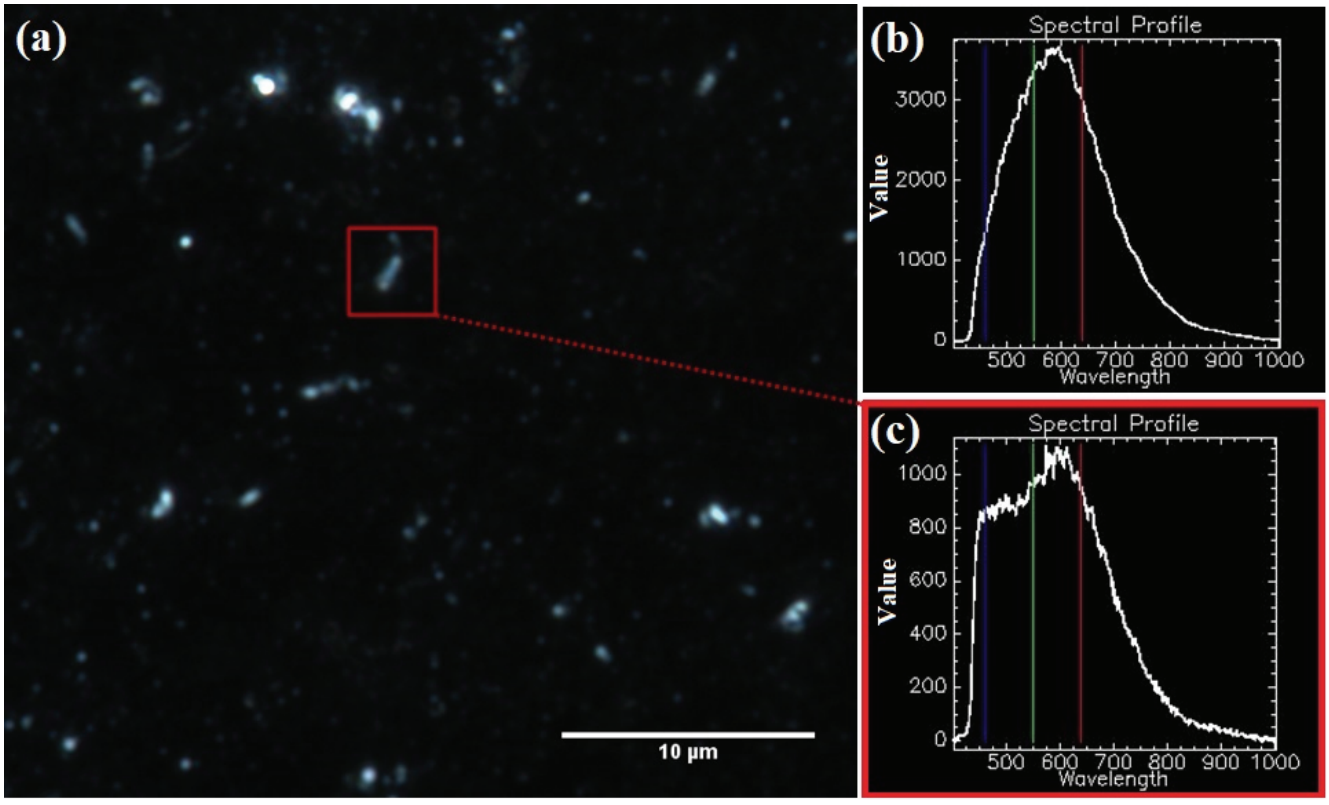

Figure 4. (a) Enhanced dark-field hyperspectral image of bacterial cells with Pt-NPs; (b) Spectral response of control bacteria and (c) cell with Pt-NPs.

Dark-field TEM is a known technique used to identify crystalline NPs in biological matrices (KLEIN et al., 2015). Dark-field TEM reduces the visual noise from bright-field imaging allowing a bright crystalline contrast for an easy identification of NPs. When comparing the bright-field image from Fig. 3b with its dark-field equivalent from Fig. 3c, the NPs appear as much brighter individual spots, as monodisperse and discrete single-crystals, revealing the crystalline nature of the extracted Pt-NPs.

Because the JEOL JEM-1400 used in this experiment was not equipped with an energy dispersive X-ray (EDX) device, we were unable to perform EDX analyses and obtain a map of platinum distribution. However, given that H_2PtCl_6 was the only metal precursor used in the reaction mixture, we can safely say that the metal NPs deposited on the cell surface are Pt-NPs.

Hyperspectral imaging. The CytoViva Hyperspectral Imaging System permits the visualization and hyperspectral characterization of NPs, without the need of any fluorescent labelling or pre-treatment of the samples, the nanomaterials appearing brightly lit against a dark background. Fig. 4a shows a representative hyperspectral dark-field image of a whole-cell of *S. oneidensis* MR-1 brightly lit, indicating the presence of nanomaterials in the cell exterior, associated with the cell membrane, identified as bright spots, suggesting that *S. oneidensis* MR-1 cells are able to reduce Pt^{4+} ions into elemental platinum (Pt^0). The spectral response of a control and a platinized cell was also recorded (Fig. 4b and c). The presence of Pt-NPs on the cell membrane induced changes in the peak morphology of *S. oneidensis* MR-1 cells, while the profile of the control cell (Fig. 4b) significantly differs from that of the cell with Pt-NPs (Fig. 4c). The control cell has a more prominent peak in the 600 nm region as compared to the cell with Pt-NPs which has a wider band with a maximum peak between 600-700 nm and a shoulder in the 500-600 nm region.

Efforts are still required to fully elucidate the mechanism of Pt-NPs biosynthesis. It was proposed that *S. oneidensis* MR-1 could play an important role in both reducing metal ions (NG et al., 2013) and stabilizing the metal NPs (SURESH et al., 2011; MARTINS et al., 2017) by intra- and extra-cellular biomacromolecules such as proteins and polysaccharides which act as capping material to stabilize the NPs and prevent their aggregation.

CONCLUSIONS

In this study we demonstrated that the resting cells of *S. oneidensis* MR-1 incubated in bicarbonate buffer with formate as electron donor and platinum as electron acceptor, are able to transfer electrons to Pt_4^+ ions, reducing it to elemental platinum (Pt^0), as demonstrated by spectroscopy and fluorimetry measurements, TEM, and enhanced dark field microscopy. Quantitative data on crystallinity, structure and composition of the synthesized Pt-NPs should be further demonstrated by investigations such as high-resolution TEM (HR-TEM) micrographs, selected area electron diffraction (SAED) patterns, energy dispersive spectroscopy (EDS) spectra, combined with standard powder X-ray diffraction (XRD).

In conclusion, a green and facile method using *S. oneidensis* MR-1 cells was developed for biosynthesis of Pt-NPs at 30°C and neutral pH without using an anaerobic chamber, which is a drawback for larger applications. The proposed method involves the use of various anaerobic techniques for the cultivation and manipulation of bacterial cells, which are simple, inexpensive and available to anyone.

AUTHORS CONTRIBUTIONS

GHINEA Adrian, MOISESCU Cristina have equally contributed to the conception and drafting of the manuscript and to its important intellectual content.

MOROSANU Ana Maria carried out the TEM analyses.

ARDELEAN Ioan I. and MOISESCU Cristina critically revised and gave their approval of the final version of the manuscript to be submitted.

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