Microbiological influence of metal ion electrodeposition: Studies using graphite electrodes, [AuCl₄]⁻ and Shewanella putrefaciens

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The microbiological influence of gram negative dissimilative bacteria on the electrodeposition of gold [AuCl₄]⁻ ion is analysed. Previous investigations have shown positive shifts in reversible potentials of gold in the presence of initially live gram negative bacterial cells, Shewanella putrefaciens in the electrolyte bath. This manuscript gives further analysis of the experimental data, with hypothetical influences of bacterial cells on gold nucleation and electrodeposition reaction mechanisms, kinetics and mass transfer as a basis for further investigation and strategies for the engineering of robust bioelectrochemical systems (BES), for novel metal ions recovery from aqueous process streams.

1. Introduction

The interactions of microbiological bacterial cells and electrified interfaces, termed bioelectrochemical systems (BES) have been well documented over the last 20 years [1], with a great deal of interest in their application for recalcitrant wastewater remediation using microbial fuel cells and microbial electrolysis cells [2]. The processes here, involve the reciprocal alliance of microbial cells and electrochemical systems for inorganic wastewater remediation [3,4]. The electrode in these systems acting as an electron donor source (as for H₂ production) or electron donors for respiration, by direct (DET) or mediated (MET) [5,6] electron transfer to microbial cells, coupled with the reduction of the pollutant leading to their reduced mobility and sequestration. The coupling of biological remediation with bacterial substance in BES has shown some promise for perchlorate [4], uranium [7] and nitrate [3] treatment. The inorganic contaminants in systems such as these may act as electron acceptors for dissipatory motives [8,9], reduced in the final step of the biosorption and/or bioaccumulation mechanism [10,11].

Furthermore, the possibility of the cathodic bioelectrocatalysis [12] or process enhancement of metal reduction and deposition, by biological protein enzymes located in the bacterial outer cell wall of gram negative bacteria, or released by bacterial cells [13,14] could be another alternative strategy for bioelectrochemical metal ion remediation. Where bacterial cells influence metal ion electron transfer thermodynamics, kinetics and metal ion mass transfer, leading to their remediation and recovery, with perspective reduction in energy and/or time. This alternative perspective is further investigated here. The possibility of simultaneous biosorption and recovery of metals by live and/or deactivated bacterial cells [15] and bioelectrocatalysis of metal deposition reactions would in principle lead to a versatile remediation and recovery methodology.

Shewanella putrefaciens is chosen here as an atypical electroactive chemolithotrophic gram negative bacterium [16], which adsorbs and transforms a range of metal ions [8,17–22] via implicate interactions of metal ions with the outer membrane lipopolysaccharide layer [23,24] and outer membrane multilaemum type cytochromes MR-1 and OmCA [25]. Reports of AuCl₄⁻ reduction by gram negative bacteria describe the fabrication of a range gold nanoparticles sizes and geometries intracellular and within the periplasmic space for range of pHs [19,20]. Reports by Konishi et al., of the complete reduction of 1 mM AuCl₄⁻ confirmed the process was H₂ dependent, suggesting the involvement of hydrogenases. These protein enzymes may be candidates for bioelectrocatalysis of metal ion electrodeposition.

Furthermore Shewanella produces in-situ redox active flavin mediators [26], able to transfer electrons to a substrate in a cyclic fashion, which may again be of influence. Sorkolov et al., investigated cell surface electrochemical heterogeneity of S. putrificans using acid–base titrations and electrostatic force microscopy report a high degree of cell surface heterogeneity, of a highly differentiated interfacial system capable of supporting multiple intermolecular interactions with a range of solutes and solids [27].
Following on from previous report of bacterial influence on electron transfer thermodynamics of Au^{3+}, Co^{2+} and Fe^{3+} ions [28] this manuscript gives further analysis and modelling of the data of electrochemical experimentation with metal concentrations of 200 ppm AuCl_{4}^{−} in the electrolyte of pH 2, graphite grade G-10 electrodes and initially viable bacterial cells. With analysis of theoretical bacterial influence upon gold electrodeposition kinetics, mass transfer and electronucleation phenomena. Fig. 1 summarises hypothetical biological and electrochemical strategies alluded above within a BES paradigm, of interdisciplinary on-going research by a number of research groups, involving bacterial interactions with metal ions and/or electrified interfaces.

(i) Biosorption and bioaccumulation of metal ions by live cells [10,29–31].
(ii) Bacterial influence on metal ion electron transfer thermodynamics at an electrified interface as reported previously by Varia et al., [28].
(iii) In-situ production of redox mediators by bacterial cells, with hypothesised bioelectrocatalysis [32] of metal ion electron transfer reactions and metal ion mass transfer to the electrified interface. A preliminary evaluation provided in this manuscript.
(iv) Bacterial substance via production of H_{2} as an electron donor and/or MET and DET [1,6] shown of some application in BES for perchlorate, uranium and nitrate remediation [3,4,7].
(v) The recovery and reuse of biogenic metallic products, such as metallic nanoparticles, which could yield high economic return and novel eco-friendly routes for the fabrication of nanomaterials [33–35].

Although the work reported here is an evaluation of theoretical influence of initially viable bacterial cells on metal ion electron transfer phenomena, we believe it contributes in furthering the application of BES for metal ion remediation and recovery, from an electrochemical perspective. Metal deposition phenomena are analysed in the presence of electroactive bacterial cells. Further investigation from a microbial perspective, where bacteria biosorb, bio-accumulate and bio-transform metal ions, with electrode substrates acting as electrode donors for respiration and bacterial substance would be of key significance. All in all, the alliance of the microbial and electrochemical phenomena listed above and described by Fig. 1, would lead, we believe to a creative synergy, of financial gain and environmental service. Thus, this work presents further adjoins preliminary investigations and furthers the unabridged aims of the overall research to develop strategies for the cleaning of metal contaminated aqueous systems such as mine waters or industrial effluents, characterised with low metal ions concentration (<200 ppm) and low pH (<3), to acceptable regulatory levels, by the symbiotic coupling of microbiological and electrochemical phenomena. With perspective for the recovery of the metal contaminant [36] in a form applicable for reuse. For example, the application of bacterial cell walls as “bio-nano-factories” for the manufacture of biogenic nanoparticles [18,19,34,37,38] within specified boundaries of green chemistry principles, carried out at ambient conditions with no further addition of chemicals.

2. Experimental

Aqueous electrolyte solutions of 200 ppm Au^{3+} were prepared with 1000 ppm standard solutions of HAuCl_{4} in 2 M HCl base electrolyte matrix. The pH of electrolyte was adjusted to pH 2
with NaOH or HNO₃, with final conductivities of electrolytes for gold, 32.5 mS cm⁻¹. Solutions were prepared from analytical grade reagents supplied by Fisher Scientific, UK or standard solutions from Spectrofol (VWR, UK). Triple distilled water was used to prepare all solutions. Conductivity and pH were measured using an electrochemical analytical metre (SevenMulti, manufactured by Metter-Toledo GmbH, UK). Electrochemical polarisations were carried out using an EG&G Instruments bi-potentiostat using an automated PCl 100 data acquisition system (Scytype Scientific, UK). Details of apparatus, experimental set up, microbial strain, medium and cultivation can be found elsewhere [28]. Briefly, electrochemical polarisations were carried out using 50 ml of electrolyte solution in each compartment of a glass H-Cell with the anolyte and catholyte sections separated by a glass frit. The working and counter electrodes were three-dimensional G-10 Graphite (1 cm x 1 cm x 1 cm; Ralph Coidan Ltd., UK). An Ag/AgCl reference electrode was used and all potentials are reported with respect to this electrode. 10 ml of bacterial cells were added to the solution with sterile needles using bacterial concentrations of 1 x 10¹⁰ CFU ml⁻¹. Lineal voltammetry experiments, in the absence and presence of bacterial cells were conducted in un-stirred solutions, at scan rate of 0.010 V s⁻¹. After each polarisation, the electrolyte was stirred with a magnetic stirrer and the working electrode polarised at a potential of +1.00 vs. (Ag/AgCl)/V until initial open circuit potential (OCP) was re-established for the metal ion solution under investigation.

3. Results and discussion

The overall reaction for gold electrodeposition from chloride solutions can be described by Eq. (1).

\[ \text{AuCl}_4^- + 3e^- \rightarrow \text{Au}^0 + 4\text{Cl}^- \]  

With previous reports of a reaction mechanism involving two charge transfer steps with a preceding chemical step (c.e.e. mechanism) [39-41], described by Eq. (2a-c) [40], Tafel analysis of \( j \) vs. \( E \), for relatively small overpotentials \( (\eta) < 0.1 \text{V} \), can be applied for the inference of the rate determining step and reaction kinetics [42]. Where \( j \) is the current density and \( E \) is the overpotential. Tafel slopes \( (\lambda) \) of 0.120, 0.060 and 0.025 V per decade would correlate to reaction steps (2a), (2b) or (2c) being the rate determining step of the overall reaction mechanism [42,43].

\[ [\text{AuCl}_4^-] \rightarrow [\text{AuCl}_3^{\text{ads}}] + 2\text{Cl}^- \]  

\[ [\text{AuCl}_3^{\text{ads}}] + 2e^- \rightarrow [\text{AuCl}_2]^{-\text{ads}} \]  

\[ [\text{AuCl}_2]^{-\text{ads}} + e^- \rightarrow \text{Au}^0 + 2\text{Cl}^- \]  

Furthermore, the exchange current density \( (j_0) \) can be used as an indicator of microbial influence on reaction kinetics of gold electrodeposition [44]. An increase in \( j_0 \) would be indicative of faster reaction kinetics. Table 1 summarises the parameters obtained from linear fits of Tafel plots for overpotentials of +0.025 to -0.075V illustrated by Fig. 2, without (a) and with bacterial cells (b). Tafel analysis using a Pt electrode is also included in Table 1, as a basis for further comparison with graphite electrodes.

Reversible potentials \( (E_j) \) for Eq. (2) in the presence of bacterial cells show a positive shift as reported previously [28]. A Tafel slope of 0.059 V per decade for Pt electrode, would suggest an electron transfer step Eq. (2b) to be rate controlling [40]. Higher Tafel slopes are found when graphite was used since Tafel slopes of 0.133 and 0.112 V per decade would suggest AuCl₃⁻ adsorption described by Eq. (2a), to be the rate determining step. For scans with bacterial cells a deviation is seen which would imply bacterial influence on reaction mechanisms. Although dissimilar variation is revealed, this work does show some bacterial influence.

A reduction in \( j_0 \) is also noted and indicative of bacteria lag of electron transfer kinetics. If based on Tafel analysis, reaction Eq. (2a) is the rate determining step, bacteria appear to hinder adsorption of gold ions to the electrode surface. Eq. (3) gives a more comprehensive definition of \( j_0 \) [42] where \( \Delta G^{0\#} \) is the Gibbs free energy of activation, \( h \) is the Planck constant, \( k_B \) is the Boltzmann constant and \( \alpha \) transfer coefficient [43]. A reduction of \( j_0 \) would indicate that bacterial cells or bacterial components increase \( \Delta G^{0\#} \) of electron transfer to gold metal ions located in the IHP. \( \Delta G^{0\#} \) can be further defined by Eq. (4), which would imply that bacteria increase or decrease activation enthalpy or entropy respectively.

\[ j_0 = \frac{k_B T}{h} \int_0^\infty \frac{\exp \left( \frac{-\alpha e \Delta \Phi}{RT} \right)}{\exp \left( \frac{-\Delta G^{0\#}}{RT} \right)} \]  

\[ \Delta G^{0\#} = \Delta H^{0\#} - T \Delta S^{0\#} \]  

Further linear voltammograms under potentiodynamic conditions were carried out for further inference of the system at hand. Fig. 3 illustrates a set of linear voltammograms with varying scan rates (0.010–0.120 V s⁻¹).

Both systems show that the foot of the cathodic peak is steep, implying that nucleation of the metal phase is followed by rapid nuclei growth [45]. The peak potential \( (E_p) \) shifts negatively with increasing the scan rate \( (S) \) which would be most likely be due to an increase in the nucleation overpotential with \( S \). Furthermore, \( E_p \) is proportional to \( S^{1/2} \) (Fig. 4), which would suggest that the metal deposition reaction is diffusion controlled [45]. Similar trends are observed for both systems with and without bacterial cells. It is worth noting that the change in \( E_p \) with respect to \( S^{1/2} \), i.e. the gradient of the line is lower with bacteria present in the electrolyte bath.

A linear relationship of peak current density, \( j_p / \text{A cm}^-² \) vs. \( S^{1/2} \), from Fig. 3, would again point to diffusion limited metal nucleation mechanism. Fig. 5 illustrates such plot, which shows linear correlation and a diffusion-limited process. Linear regression fitting of the data can be used for the determination of metal ion diffusion coefficients \( D_{\text{AuCl}_4} \), using the Randles–Sevcik equation [44], described by Eq. (5) where \( C_{eq} \) is the metal ion concentration and \( n \) is the number of moles of electrons transferred in the reaction. Linear regression fitting yielded diffusion coefficients \( D \) of 0.1379 x 10⁻⁵ and 0.0927 x 10⁻⁵ cm² s⁻¹ for experiments without (a) and with bacteria cells (b), respectively. A reduction
Fig. 2. Tafel plots for Au\(^{3+}\) electrodeposition (scan rate = 0.010V s\(^{-1}\)) on an G-10 graphite electrode at 25 °C from 200 ppm Au\(^{3+}\) in 2 M HCl matrix pH = 2, \(\alpha = 35.2 \text{ mS cm}^{-1}\), (a) without bacterial cells (b) with 10 ml 1 × 10\(^{10}\) CFU ml\(^{-1}\) bacterial cells. The polarisations were carried out 10 min after the addition of bacterial cells.

\[ j_p = (2.69 \times 10^5)n^{3/2}C_oD^{1/2}s^{1/2} \]  

Fig. 3. Set of linear voltammograms for Au\(^{3+}\) (0.010–0.120 V s\(^{-1}\)) on grade G-10 graphite electrode at 25 °C from 200 ppm Au\(^{3+}\) in 2 M HCl matrix, pH=2, \(\alpha = 35.2 \text{ mS cm}^{-1}\). The voltammograms were carried out 10 min after the addition of bacterial cells, (a) without bacterial cells, (b) with 10 ml 1 × 10\(^{10}\) CFU ml\(^{-1}\) bacterial cells.

Fig. 4. \(E_p\) vs. \(S^{1/2}\) for gold electrodeposition on grade G-10, (a) without (Δ symbol) and (b) with 10 ml 1 × 10\(^{10}\), CFU ml\(^{-1}\) bacterial cells (□ symbol).

of the diffusion coefficient would suggest that bacteria reduce the mobility of a metal ion to the electrified interface.

\[ j_p = (2.69 \times 10^5)n^{3/2}C_oD^{1/2}s^{1/2} \]  

Fig. 6 illustrates average steady state current densities \((j_s)\) taken after a 60 s potential step, ranging from 0.6 V to –0.2 vs. (Ag/AgCl)/V. On average lower limiting currents are found when bacteria are added to the electrolyte. This would indicate, as shown in previous investigations [28], that bacteria hinder diffusion phenomena of metal ions to the electrified interface by the attachment and colonisation of bacterial cells to the electrode for defensive and/or dissimilatory [6] motives.

The nucleation and growth phenomena in the initial stages of the gold deposition were investigated by chronoamperometric experimentation as extensively applied for analysis of electronucleation [46,47]. Fig. 7 illustrates the current transients obtained with potential steps of the working electrode of +0.7 to -0.2 V. After each experiment, as with cyclic voltammograms, a potential

Fig. 5. Randles–Sevick plot of \(j_s\) vs. \(S^{1/2}\) for gold electrodeposition on grade G-10, (a) without (Δ symbol) and (b) with (□ symbol) 10 ml 1 × 10\(^{10}\) CFU ml\(^{-1}\) bacterial cells.
of +1.00 V was applied until OCP was re-established (≈0.7 V). The transients with potential steps of +0.7 V exhibit an initial current decay (t < 0.001 s) which would be related to double layer charging. A current decay is not observed for other potential steps. A characteristic current increase is shown for all transients (t > 1 s) which would be due to the nucleation and growth of gold nanoparticles on carbon electrode surface. The observed maximum \( J_M \) resulting from the overlap of growing particles and diffusion zones [48].

The shapes of transients are similar on the whole for both systems without and with bacterial cells (Fig. 7), although much higher currents are observed for low potential steps near the \( E_r \) of AuCl₄⁻ with bacteria (Table 1), which reinforces the analysis of slow scan polarisations. Furthermore, the shape of the transient for potential steps of 0.6 V are significantly different, with a steeper current decay indicating residual currents involving additional electron transfer reactions, or higher rates of gold nucleation. A higher current maximum \( J_M \) and corresponding time maximum \( t_M \) with bacterial cells in the electrolyte is also observed.

The decreasing part of the transients traced in the coordinates of \( j \) vs. \( t^{-1/2} \), define a straight line (Fig. 8) and the diffusion coefficients can be estimated by application of the Cottrell equation (Eq. 6).

\[
j = \frac{nFD^{1/2}C_0}{\pi^{1/2}t^{1/2}}
\]

Table 2 summarizes calculated diffusion coefficients for potential range from 0.6 V to -0.2 V. The average value is shown to be significantly higher to that calculated using the Randles–Sevcik equation (7.91 \( \times \) 10⁻⁵ and 9.67 \( \times \) 10⁻⁵ cm² s⁻¹ without (a) and with bacteria cells (b), respectively) and inconsistent, with higher values of the diffusion coefficient with bacteria in the electrolyte. As discussed previously, for potential steps of 0.6 V, with bacteria in the electrolyte (Fig. 8b), a change in the shape of the current time transient is shown for initial current increase and later current decay. This would suggest that bacterial redox active enzymes are of active influence close to these potentials, and are involved in electron transfer close to onset potentials of the electrodeposition process. Potentiodynamic cyclic voltammetry analysis described a diffusion controlled mechanism, and in light of the graphite substrates, usually having a large number of 3D defects (e.g. pores and steps) [47]. There are several detailed descriptions of the potentiostatic current transients during three dimensional nucleations for diffusion control based on investigations by Hills, Schiffrin and Thompson [49]. They derived expressions for diffusion controlled growth of a single hemispherical nucleus. Developed later by Scharifker and Hills [50] and Scharifker and Mostany [51] with application of the Avrani theorem [52]. This takes into account the overlap of diffusion fields, of a random array of growing centres which follow a uniform model of nucleation sites. Other models have also been put forward by Boso and Rangarajan [53]. A 3D diffusion control model based on investigations by Scharifker and Hills (SH) [50] has been applied extensively, for example studies by Konishi and Staikov regarding the electocrystallization of Au.

![Fig. 6](image-url) Steady state current density \( J_{(a)} \) vs. \( E \), taken after application 60 s potential steps, (a) without bacterial cells, (b) with 10 ml \( 1 \times 10^{10} \) CFU ml⁻¹ bacterial cells (experiment carried out in duplicate), potential step applied after 10 min addition of bacterial cells.

![Table 2](image-url) Diffusion coefficient (D) calculated from the Cottrell (Eq. 6) and Fig. 8, (a) without bacterial cells, and (b) with 10 ml \( 1 \times 10^{10} \) CFU ml⁻¹ bacterial cells.

<table>
<thead>
<tr>
<th>( E ) vs. (Ag/AgCl)/V</th>
<th>0.60</th>
<th>0.50</th>
<th>0.00</th>
<th>-0.20</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( D(\text{cm}^2 \text{s}^{-1}) \times 10^{-5} )</td>
<td>(a)</td>
<td>0.15</td>
<td>0.58</td>
<td>8.18</td>
<td>22.73</td>
<td>7.91</td>
</tr>
<tr>
<td>(b)</td>
<td>n/a</td>
<td>0.91</td>
<td>10.51</td>
<td>17.60</td>
<td>9.67</td>
<td>6.79</td>
</tr>
</tbody>
</table>

![Fig. 7](image-url) Current-time transients for nucleation of gold on graphite carbon for various potential steps, (a) without bacterial cells, (b) with 10 ml \( 1 \times 10^{10} \) CFU ml⁻¹ bacterial cells. Polarisations were carried out after 10 min addition of bacterial cells.
nanoparticles on glass carbon [46]. SH modelling of electronucleation mechanisms and has been utilized here for a simple diagnostic test for the determination of nucleation, growth type and possible influence of bacterial cells. Although the analysis here is theoretical in nature as we were unable to carry out analysis of deposits such as energy-dispersive X-ray spectroscopy or scanning electron microscopy, it gives some insight regarding bacterial influence.

Three dimensional nucleation under diffusion control can be determined from experimentally measured current transients and reduced to non-dimensional form by plotting $j^2 / j_m^2$ versus $(t / t_m)^2$, where $j_m$ and $t_m$ relate to minimum current density and time measurements from the chronoamperometric polarisations, depicted in Fig. 9. According to the SH model, the rising current would correspond to an increase in the electroactive area. This increase in electroactive area might be limited by spherical diffusion around the nuclei due to an increase in the (a) nucleus size and (b) increase in the number of nuclei sites. As the nuclei grow, spherical diffusion zones overlap and mass transfer becomes linear to the planar intersection surface. Instantaneous or progressive nucleation can be seen depending on nucleation rates.

Instantaneous nucleation relates to a high nucleation rate, where all nuclei are immediately created upon application of overpotential. Their number remains constant during the growth process, as described by Eq. (7).

$$j = \frac{zFD^{1/2}}{\pi^{1/2}t^{1/2}} \left[ 1 - \exp(-NrtD) \right]$$

where $z=3$, $F$ is the Faraday constant (96,500 C mol$^{-1}$), $C$ is the [AuCl$_4^-$] concentration (1.015 x 10$^{-6}$ mol cm$^{-3}$), $N$ is the density of nuclei (cm$^{-2}$), and $k$ is the material constant, calculated using Eq. (8).

$$k = \left( \frac{8\pi CM}{\rho} \right)^{1/2}$$

where $M$ is the atomic weight of Au (196.97 g mol$^{-1}$) and $\rho$ is the density of Au (19.30 g cm$^{-3}$). The opposing case of progressive nucleation relates to a low nucleation rate, where nuclei are continuously formed and can be described by Eq. (9).

$$j = \frac{zFD^{1/2}}{\pi^{1/2}t^{1/2}} \left[ 1 - \exp \left( \frac{-AN_0\pi k^2 D t^2}{2} \right) \right]$$

where $k'$ is the respective material constant described by Eq. (10), $N_0$ is the density of active nucleation sites (cm$^{-2}$) and $A$ is the nucleation rate constant (s$^{-1}$).

$$k' = \frac{4}{3} \left( \frac{8\pi CM}{\rho} \right)^{1/2}$$

![Fig. 8. j vs. $t^{-1/2}$ plot from data of transients for nucleation of gold on graphite carbon for various potential steps, (a) without bacterial cells, (b) with 10 ml $1 \times 10^{10}$ CFU ml$^{-1}$ bacterial cells. Polarisations were carried out after 10 min. addition of bacterial cells.](image)

Table 3

<table>
<thead>
<tr>
<th>Units</th>
<th>(a)</th>
<th>(b)</th>
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<tbody>
<tr>
<td>$t_m$</td>
<td>s</td>
<td>3.00 x 10$^{-1}$</td>
</tr>
<tr>
<td>$i_m$</td>
<td>A</td>
<td>4.70 x 10$^{-2}$</td>
</tr>
<tr>
<td>$D$</td>
<td>cm s$^{-1}$</td>
<td>29.49 x 10$^{-5}$</td>
</tr>
<tr>
<td>$A_{N_0}$</td>
<td>2.61 x 10$^4$</td>
<td>1.64 x 10$^5$</td>
</tr>
<tr>
<td>$N_0$</td>
<td>4.53 x 10$^5$</td>
<td>3.23 x 10$^5$</td>
</tr>
</tbody>
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Qualitative characterisation of the nucleation process can be determined by plotting current transients described by Eq. (11) and (12) for instantaneous and progressive nucleation, respectively. If the transients are found to fall in either of these limiting cases, further quantification of nucleation parameters is possible.

$$\frac{j^2}{j_m^2} = \frac{1.9542}{t/t_m} \left[ 1 - \exp \left( -1.2564(t/t_m)^2 \right) \right]^2$$

$$\frac{j^2}{j_m^2} = \frac{1.2254}{t/t_m} \left[ 1 - \exp \left( -2.3367(t/t_m)^2 \right) \right]^2$$

SM fitting for 0.6 V potentials as described by Fig. 7, without bacteria shows an initial instantaneous mechanism which becomes progressive after the current maximum. For electrolytes with bacteria, initially a progressive mechanism is observed becoming instantaneous after the current maximum. The change from instantaneous to progressive is rapid when bacteria are added to the electrolyte. The opposite effect is observed for potentials of 0.5 V, the transient after current maximum becomes progressive for systems without bacterial cells and moving from instantaneous to progressive for systems with bacterial cells. The initial rising transient shows no correlation to either limiting case. For low potentials of 0.0 V and −0.2 V SH curve fittings show good correlation to progressive nucleation models for t < $t_m$ for systems with and without bacterial cells. For a progressive reaction mechanism, the product $AN_0$ can be calculated using Eq. (13).

$$J_m = 0.4615zFcD^{3/4}(kAN_0)^{1/4}$$

while the diffusion coefficient can be estimated from Eq. (14),

$$J_m^2t_m = 0.2598D(zFe)^2$$

and the nuclei saturation $N_0$ (cm$^{-2}$) can be determined from Eq. (15),

$$N_0 = \left( \frac{AN_0}{2kD} \right)^{1/2}$$
Table 3 summarises key values extracted from Fig. 9 for potential steps to 0 vs. (Ag/AgCl)/V, and calculated parameters of $A_N$, $D$ and $N_e$ using Eq. (13) and (15). Based on applied theoretical modelling, bacteria show some, although not significant lagging of electrodeposition kinetics, with a decrease in $A_N$, also observed from Tafel analysis. An increase in mobility of metal ions in their journey to the electrolysed interface is found, with a 19% increase of the diffusion coefficient. In agreement with Cottrell plots (Table 2) obtained at the same potential step to 0.00 vs. (Ag/AgCl)/V (22% increase of the diffusion coefficient). It seems that bacteria increase the diffusion coefficient of metal ions at the electrolysed interfaces from analysis of chronocapacitometric transients using the Cottrell equation and SM modelling, contrary to analysis of potentiodynamic scans using the Randles–Sevcik equation. Further investigation of the effects of bacteria on metal ion mobility at the electrolysed interface would be warrant to resolve these discrepancies. A 40% decrease in the nuclei saturation $N_c$ was also found for electrolytes with bacteria, indicating a lower number of nucleation sites in their presence.

4. Conclusions

The electrodeposition of gold on grade G–10 graphite electrodes was investigated in the presence of electroactive bacterial cells of the Shewanella genus. This upon previous reports of bacterial influence on electron transfer thermodynamics and hypothesised bacterial enhancement of electron transfer phenomena, with the perspective and strategic alliance of electrochemical and microbiological phenomena, for the remediation and recovery of metal ions. Our results have shown some influence of bacterial cells on reaction mechanism from deviation of Tafel slopes ($\lambda$), a lagging of electron transfer kinetics ($j_b$) and influence on mass transfer kinetics ($j_{0b}$). Modelling of chronocapacitometric transients using 3D, diffusion control model based on investigations by Scharifker and Hills was consistent with these observations, although further electrochemical and microscopy experimentation and analysis of bacterial influence on electron metal ion transfer reactions is required before conclusive assertions can be made on their influence. Further experimentation regarding the temporal viability of bacterial cells for systems of low pH and relatively high metal concentrations would be of importance, if the vision for the application of living bacterial cells as eco-friendly nano-factories is to be furthered. We believe the work here provided some insight into how these may be elucidated.

Bacteria applied in this system where initially viable, but in light of the harsh conditions of low pH their viability would be short-lived. Further investigation with other bacterial species or transgenic bacterial cells would be justified in light of monumental advances in molecular biology, genetics and biochemistry, for the bio-engineering of robust electroactive bacterial cell membranes [54] which allow the subsistence of bacterial cells in non-ideallow pH and toxic environments, engineered to over-express implicated bioremediation redox protein enzymes/proteins found to be sympathetic to electrodeposition and biosorption reaction mechanisms, leading to savings in energy and time of the remediation process. Further scientific investigation at the molecular level of the lipopolysaccaride outer leaf bacterial cell membrane and localized electron transfer redox membrane proteins interaction with metal ions and carbon electrodes would also undoubtedly further their applicable technological advancement.

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References
