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Process analysis of AuCl_3^- sorption leading to gold nanoparticle synthesis by *Shewanella putrefaciens*

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Abstract

A process evaluation of AuCl_4^- (< 300 mg/L, < pH 3, H_2 e-donor) sorption kinetics and mass transfer analysis by initially viable *Shewanella putrefaciens* is presented here. Following on from previous reports of a thermodynamically spontaneous reaction, high sorption capacity of 1346 mg/g and significant evidence of zero valent gold nanoparticles (AuNPs) deposited within the bacterial cell wall. Linear and non-linearized kinetic modelling of the data shows a good fit to a pseudo second-order model and theoretical analysis of diffusional limiting steps suggest diffusion on the surface or through the cell wall to be the process rate limiting step. Process analysis of microbial gold synthesis, provides a foundation for the design of smart reactor systems for process intensification.

1. Introduction

A variety of phenomena has been reported regarding the interactions of bacterial cells and metal ions such as adsorption, absorption, ion exchange, entrapment, reduction/oxidation and methylation [1]. Such phenomena could be combined in series or in parallel and usually grouped under the umbrella term, biosorption [2]. Technological application from these investigations has focused principally on (a) environmental remediation of heavy metals and radioactive pollutants in aqueous waters [3]; and (b) state of art biohydrometallurgical methods. Bacteria applied principally to oxidise and leach metals from primary ores, labelled “microbial mining” [4, 5].

In contrast the microbial-mediated sorption and transformation by faradaic reduction of metal ions, to metallic nanoparticle (NP) zero-valent and oxide precipitates, has been reported extensively [6]. The reduction of precious metal ions such as AuCl_4^- studied here to zero-valent Au^0 NPs (AuNPs) is ubiquitous in the microbiological kingdom from fungi [7], yeast [8], archaea [9], algae [10] and especially for bacteria. Including proteobacteria [11-13], cyanobacteria [14], actinobacteria [15], firmicutes [16] and thermotogae [9].

Such phenomena have technological and monetary importance from two overlapping perspectives. First, from the recovery perspective. Microbial reduction and precipitation of metal ions can be capitalised for metal recovery from urban mining leachates and industrial and mining effluents. These aqueous solutions typically of low pH and low metal ion concentration ($< 1 \text{ g L}^{-1}$) [17]. Secondary, from NP synthesis perspectives. Here microbial cells are applied as green eco-friendly “nano-factories”, following a green bottom-up methodology [18]. Processes in this case, carried out at ambient temperatures without the need for chemical additives such as capping or stabilising agents. For example in all cases microbial synthesis lead to biogenic NP surrounded by a proteinaceous capping/stabilizing agent [19].

From nanotechnological standpoints AuNPs are focused on here in light of their exceptional optical, chemical, photochemical and electronic properties [20]. AuNPs have a long history, with scientific reports dating back to the legendary 1857 lecture by Michael Faraday [21]. From an anthropogenic standpoint one can go even further back. With application of gold nanoparticles in gold bhasma [22] used for medical purposed in the Indian continent as far as the Vedic period and for decorative purposes by Roman artisans from the 4th century [23]. In the contemporary era they have shown vast

present and potential future application as ideal building blocks for future nanotechnological self-assembling systems [20].

The *Shewanella* Genus applied in this study was chosen as an archetypal dissimilative metal reducing bacterium [24]. Reported to reduce not only AuCl_3^- [25-27] but also other precious metals such as Ag^+ [28], PGMs such as Pt^{4+} [29] and Pd^{2+} [30] and chalcogens such as Se^{4+} [31] to biogenic zero valent nanoparticles (NPs), predominantly in the bacterial cell wall. Providing for ease for their separation using mild detergents and/or sonication [16].

Mechanistic studies of AuCl_4^- sorption and AuNP synthesis by the *Shewanella* Genus have been reported to be mediated with an electron donor such as H_2 or formate [25, 32]. Solution pH and metal ion concentration was also shown to effect AuNP synthesis location, size and geometry. Further understanding and elucidation of metal nucleation and NP formation mechanisms would be interesting. Firstly, from scientific perspectives in understanding the quintessence of the phenomena and secondly for the formulation of protocols for synthesis of NPs with diverse physicochemical properties.

This study nethertheless aims to provide a process analysis of the sorption process, shown to lead to AuNP synthesis. Under specific conditions of low pH (< 3), with H_2 as the electron donor and low metal ion concentration (< 300 mg/L). As a basis for further process optimisation and intensification of the specific case. For these conditions, high removal capacity up to 1346 mg/g-cells and thermodynamically spontaneous ($\Delta G \approx -24$ kJ/mol) process has been reported [27]. Furthermore as illustrated by Fig. 1, AuCl_4^- where precipitated predominantly in the cell wall periplasm with average diameters of 7.42 ± 0.16 nm (No. NPs = 401) [27, 33]. Off note bacterial cell membrane are not ruptured after 24 hours' incubation with solutions of low pH though the outer leaf is compromised. This would suggest that bacterial cells may retain some viability for solutions of low pH (<3) for extended periods of time.

The methodology described here would be applicable for the recovery of other precious and PGM metals by other microbial cells for various in-vitro conditions. Laying foundations for simulation and design of smart methodologies and reactors for efficient and intensified recovery of metals and/or synthesis of NPs. The vision for such a process would be for a "one pot" green methodology uniting paradigms of metal remediation, recovery and synthesis.

Furthermore, although the synthesis of AuNPs by deactivated cells has been reported [27, 32], our vision would be the application of live cells [34]. The long term viability of bacterial cells is undoubtedly called into question in aqueous systems of low pH, but analysis of initial metal removal (0 - 4 hrs) should provide some clues as to sorption and transformation process kinetics and mass transfer by initially viable bacterial cells. This in-lieu of prospective future application of synthetic bacterial cells. Designed and programmed [35] from the “bottom up” in function and utility and able to sustain life in a range of in-vitro environments.

2. Materials and methods

Details of apparatus, experimental set up, microbial strain, medium and cultivation can be found elsewhere [27]. Briefly *S. putrefaciens* CN32 (American Type Culture Collection) were revived under aerobic conditions. Grown to a stationary growth phase, harvested, concentrated in 0.070 L, 0.9 % w/v NaCl and purged for 0.5 hr with N₂. Metal solutions were prepared from stock solutions, diluted to final volumes of 0.050 L, adjusted to a pH between 1 – 3, poured into 0.100 L vials and autoclaved at 121°C for 15 min. Aqueous metal solutions and washed bacterial cells suspensions were made anoxic by bubbling for 1 hr with N₂ and then purged with H₂ for approximately 20 seconds, prior to the addition of bacterial cells. Typically 0.005 L of 1 x 10¹⁰ cells/L was added to metal solutions, placed in an incubator at 25°C and gently shaken. Samples were taken periodically, filtered through 0.2 µm filters and analysed for AuCl₄⁻ using ICP-OES (Vista-MPX, UK). Assays were also performed to check for changes in solution pH in the presence of bacteria cells.

2.1 Statistical analysis

To sufficiently use the theoretical assumptions behind applied mathematical equations, used to model the data, error functions were used to adequately measure goodness of fit. In this study the correlation of determination (R²) Eq. 1 was applied for linearized curve fitting of model kinetic equations. Linear fitting and non-linearized optimization were also evaluated using a chi-squared statistic test (χ²) (Eq. 2), sum of squared errors (SSE) (Eq. 3) and average relative error (ARE) (Eq. 4). Appropriately applied in similar investigations [27, 36].

$$R^2 = \frac{(q_{cal} - \bar{q})}{\sum_{i=1}^n (q_{cal} - \bar{q}) + \sum_{i=1}^n (q - \bar{q})} \quad (1)$$

$$\chi^2 = \sum_{i=1}^n \frac{(q_{cal} - q)^2}{q} \quad (2)$$

$$SSE = \sum_i^n (q_{cal} - q)^2 \quad (3)$$

$$ARE = \frac{100}{n} \sum_i^n \left| \frac{q - q_{cal}}{q} \right| \quad (4)$$

3. Results and discussion

Understanding of pertaining reaction process and limiting steps for specific conditions would be important for further process intensification, optimization, manipulation and/or imitation. For example, the selection of a unit operation to conduct this process will be done on the basis of the limitations shown on its kinetics. A process that is diffusion limited emphasises the need for efficient mixing, while a process limited a rate of reaction could benefit from external input energy to increase its performance. Fig. 2 illustrates the microbiological interface, with basic architectural aspects of the cell wall [24]. A basic mechanism was first proposed by pioneering work of Beveridge and co-workers investigating bacterial metal mineralization [37]. They designated a two-step mechanism, involving electrostatic interactions with anionic sites in the cell wall, consequently acting as spots for metal nucleation, leading to metal nucleation and precipitation within the cell wall. This shows some multidisciplinary parallel to investigations of electrochemical gold deposition [38] and nucleation [39], extensively modelled using a three dimensional diffusion controlled mechanism [40]. Accordingly, we propose four key steps of an overall reaction process illustrated in Fig. 2.

Step 1: Film diffusion - Transport of the AuCl_4^- ion from the bulk solution, through a stagnant aqueous thin film bordering the bacterial cell wall.

Step 2: Pore diffusion - Transport of the ion through the cell wall (lipopolysaccharide (LPS) layer, OM, PS, IM and/or cytoplasm. To protein enzyme metal recognition peptide motifs sorption and nucleation sites.

Step 3: AuCl_4^- reduction - Electron transfer to metal ions located at redox active site of membrane proteins such as cytochromes or hydrogenase leading to a phase change of AuCl_4^- to Au^0 .

Step 4: AuNP nucleation - Reduced Au^0 behaving as nucleation sites for formation of Au^0 crystals and finally nanoscale particles.

If the last steps are assumed rapid [41, 42], either mass transfer steps 1 and 2 could be rate limiting. Analysis of sorption dynamics can provide valuable insight into reaction pathways [43, 44]. Furthermore, sorption kinetics allows prediction of solute uptake rate, which is important for process

intensification, engineering scale up and process scheduling. Sorption kinetics were fitted with a pseudo first-order model of Lagergren [45], a pseudo second-order model [43] and intra-particle diffusion model [46].

3.1 Solution pH

Solution pH has been implicated as one of the most important factors in the bio-sorption process. Protons in solution would affect properties of the cell wall such as charged ionic groups in outer leaf of the OM, composed largely of lipopolysaccharide (LPS). LPS of the *Shewanella* genus contain acid function groups [47] such as -OH^- , -COO^- , $\text{-H}_2\text{PO}_4^-$, and -NH_4^+ , which would play an important role in metal binding [48]. The pH would also effect the speciation of the Au^{3+} ion [49]. With AuCl_4^- the dominate species bellow pH 6. No buffers were applied in this study to represent of real wastewater effluents. As illustrated by Fig. 3 pH of metal assays where not significantly affected.

3.2 Sorption kinetics

The pseudo first and second-order model have been used extensively in the literature to describe metal ion uptake under non-equilibrium conditions [50]. Especially for classical adsorbents such as activated carbon [51]. Here bacterial cells could be perceived as living activated carbon particles. Although some attempts have been made to give a theoretical bases to the equations [50]. Our current understanding is limited in comparison to that of uptake thermodynamics. The intra-particle diffusion model, also used here to fit the data, has also shown some relevance [51-53] and is based on the assumption that film diffusion is negligible and intraparticle diffusion to be rate limiting.

3.3 Linear modelling of the pseudo first-order, pseudo second-order and intra-particle diffusion model

The amount of metal ions adsorbed for a given volume of solvent and mass of bacterial cells is described by Eq. 5. Where q_t (mg/g) is the amount of metal ions sorbed at time t , C_0 and C_t is the initial and final concentration at time t of metal ions in solution and V (0.050 L) is the volume of the solution. M is the mass of adsorbent added to solution based on a cell weight of 1.281×10^{-13} g/cell, evaluated previously using allometric correlations [27].

$$q_t = (C_0 - C_t) \frac{V}{M} \quad (5)$$

The pseudo first order model can be described by Eq. 6, where q_e (mg/g) is the amount of metal ions sorbed at equilibrium and k_1 (1/hr) is the first order rate constant. The pseudo second order model can be described by Eq. 7, where k_2 (g / (mg hr)) is the second order constant.

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (6)$$

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (7)$$

Integrating Eq. 6 and 7 using the boundary conditions $t = 0, q_t = 0$ and $t = t, q = q_t$, yields Eq. 8 and 9 respectively. Linear modelling can thus be carried out with plots of $\text{Log}(q_e - q_t)$ vs. $1/t$ and $1/q_t$ vs. t for the pseudo first-order or pseudo second-order model respectively. Such plots should be straight if the models fit the data and allow estimation of model parameters k_1, k_2 and q_e .

$$\text{Log}(q_e - q_t) = \text{Log}(q_e) - \frac{k_1}{2.303t} \quad (8)$$

$$\frac{t}{q_t} = \frac{1}{q_e} + \frac{1}{q_e^2 k_2 t} \quad (9)$$

The intra-particle model was first proposed by Weber and Morris [46] (Eq. 10) and further developed for various sorption systems [54]. Linearization of Eq. 10, yields Eq. 11, a plot of $\text{Log}(q_t)$ vs. $0.5 \text{Log}(t)$, should give a straight line if this model can be applied.

$$q_t = k_{ip} t^{0.5} \quad (10)$$

$$\text{Log}(q_t) = \text{Log}(k_{ip}) + 0.5 \text{Log}(t) \quad (11)$$

3.4 Non-Linear modelling of kinetic models

Linear regression assumes a Gaussian distribution of error at each point. However linearization of the data implicitly alters the error structure and may also violate the error variance and normality assumption [55]. Therefore a non-linear optimization method was also applied in this study, optimization was performed using the solver add-in of Microsoft Excel® which uses a generalized reduced gradient algorithm [56].

3.5 Model of best fit

Non-linearized modelling of the data presented, on the whole gave better fits as summarized in Table 1. The pseudo second order equation in general showed the highest overall correlation ($X^2 < 25.5$, ARE < 6.99) and fit among the three kinetic models evaluated, with the first order second best ($X^2 < 25.5$, ARE < 15.1) and intraparticle model last ($X^2 > 249$, ARE > 15.6). Fig. 4-5 illustrates non-linear 2nd order modelling of gold sorption assays. For assay of 247 mg/L AuCl₄⁻, pH 2, the sudden drop in q from 866 mg/L to 515 mg/L (Fig. 2) from 1 hr to 2 hr could be due to significant changes in cell viability for such systems of low pH. Modelling of this assay was only carried out for measurements up to 1 hr based on this assumption. Values of the rate constant k_2 between 0.009 and 0.451 mg/(g hr) compares well with previous reports of metal ion removal of 200 mg/L Cr⁶⁺ by *Rhizopus arrhizus* [57] of 0.04 mg/(g hr) and 200 mg/L Pt²⁺ removal by immobilized *Saccharomyces cerevisiae* [58] of 0.13 mg/(g hr). Furthermore, Ho and McKay report a pseudo second order model to point to processes of chemical sorption or chemisorption involving valence forces through sharing or exchange of electrons between the sorbent and sorbate [43]. This is compelling in light of the evident AuNPs nucleation within and extracellular of the cell wall [27].

3.6 Diffusion process

On the assumption that reaction steps 3 and 4 are rapid two potential rate limiting steps are evident. Namely (a) related to diffusion of metal ions through a stagnant aqueous thin film surrounding the bacterial cell wall (Step 1, Fig. 2) and (b) related to diffusion of metal ion at or through the cell wall to metal recognition sorption and nucleation sites (Step 2, Fig. 2). Attempts were made therefore to calculate coefficients of respective processes. Using methods described by Bhattacharya et al., [51] for removal of cadmium by activated carbon and applied by Mohan, Ramanaiah and Sarma [42] for biosorption of azo dye by *Spirogyra* sp. Such analysis was first elucidated by Helfferich [59] and is based on the work of Cranks [60]. Assuming an approximation of sorbent spherical geometry, the overall rate constant of the process can be correlated with the pore and film diffusion coefficient independently as described by Eq. 12 and 13 respectively.

$$D_p = 0.03 \left[\frac{R_p^2}{t^{1/2}} \right] \quad (12)$$

$$D_f = 0.23 \left[\frac{R_p \zeta C_e}{t^{1/2} C_0} \right] \quad (13)$$

Where the radius (R_p) of the sorbent bacterial cell taken as $0.387 \mu\text{m}$ from allometric analysis of 12 bacterial cells [27], based on an average bacteria volume of $0.243 \mu\text{m}^3$. The film thickness (ζ) was estimated as $10.0 \mu\text{m}$ [59]. The half time of removal ($t_{1/2}$) was calculated using parameters obtained from best fit model, pseudo second-order Eq. 7.

If film diffusion was to be the rate limiting step than D_f values should be of the range 10^{-10} to $10^{-12} \text{m}^2/\text{s}$ while if pore diffusion is the rate limiting step D_p should be in the range 10^{-15} to $10^{-18} \text{m}^2/\text{s}$ [42]. Magnitudes of evaluated coefficients summarized in Table 2, clearly suggest a pore diffusion to be the rate-limiting step since D_p values range from $2.49 \times 10^{-16} \text{m}^2/\text{s}$ to 7.24×10^{-18} . This would indicate that metal ion diffusion through or at the surface of the cell wall “bottlenecks” the overall reaction rate.

Modification via genic engineering of the cell wall interface would be an astute step forward to enhance the case here [61]. Furthermore, electroactive bacteria such as that of the *Shewanella* Genus could be grown as biofilms on carbon electrodes. As applied extensively in microbial electrochemical technologies [62]. In most cases electroactive bacteria including the *Shewanella* Genus have been applied as catalysis of organic substrates in the anode compartment [63]. In contrast, bacterial cells grown on cathodes could be applied for AuNP recovery and/or synthesis. Here electrodes stepped to negative potentials could be applied to support cells. For example, as direct electron donors to bacterial cells or the in-situ production of electron donors such as H_2 . Furthermore, diffusion of metal ions to bacterial cells immobilized on electrodes could be improved by capacitive deionisation [64]. Finally, acid functional groups or cell wall enzymes implicated in metal sorption and reduction could be influenced by applied potential. Providing a basis for selective sorption and the metal reduction process.

4. Conclusions

This study completes an analysis of process thermodynamic (previous study), kinetic and mass transfer (this study). For AuCl_4^- removal and AuNP synthesis by *S. putrefaciens*, for specific conditions of solution low pH and metal ion concentrates with H_2 present as the electron donor. Analysis and modelling of initial metal ion removal kinetics indicate a strong fit to a pseudo second-order model.

Evaluation of diffusional coefficients deemed metal ion diffusion at/through the bacterial cell wall (pore diffusion) to be the rate limiting step of the proposed process. This work provides quantitative foundations of the process for specific conditions. With the intension of future process optimization and intensification. Further studies of bacterial viability, separation methods and yield of AuNPs from the cell wall would be a sensible continuation, building on the elementary process analysis for the *Shewanella* case, provided in this study. Leading to the commercial application of metal sorption, recovery and microbial nanoparticles synthesis.

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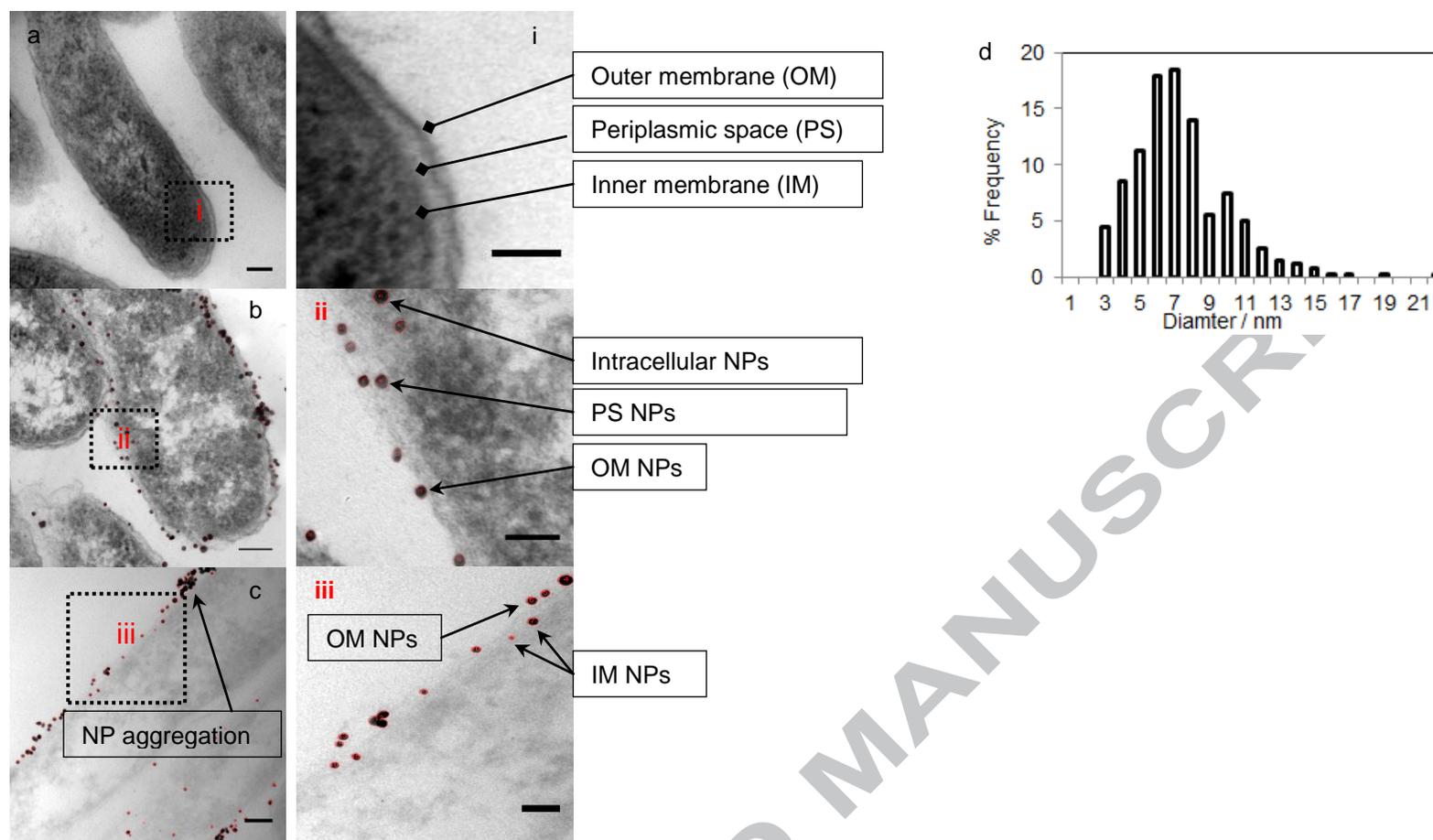


Figure 1 : TEM images (a-c) with respective close-up of the CW (i) – (iii) of *S. putrefaciens* before (a) and after (b) – (c) incubation with 170 mg/L AuCl_3^- , pH 3 after 24 hrs. Scale bar for (a) – (c) = 100 nm, for (i) – (iii) = 50 nm. Fig. 5(d) histogram % frequency NPs sizes determined using ImageJ precipitated intra- and extra cellular by initially *S. putrefaciens* cells after 24hrs incubation with 170 mg/L AuCl_4^- , pH 3

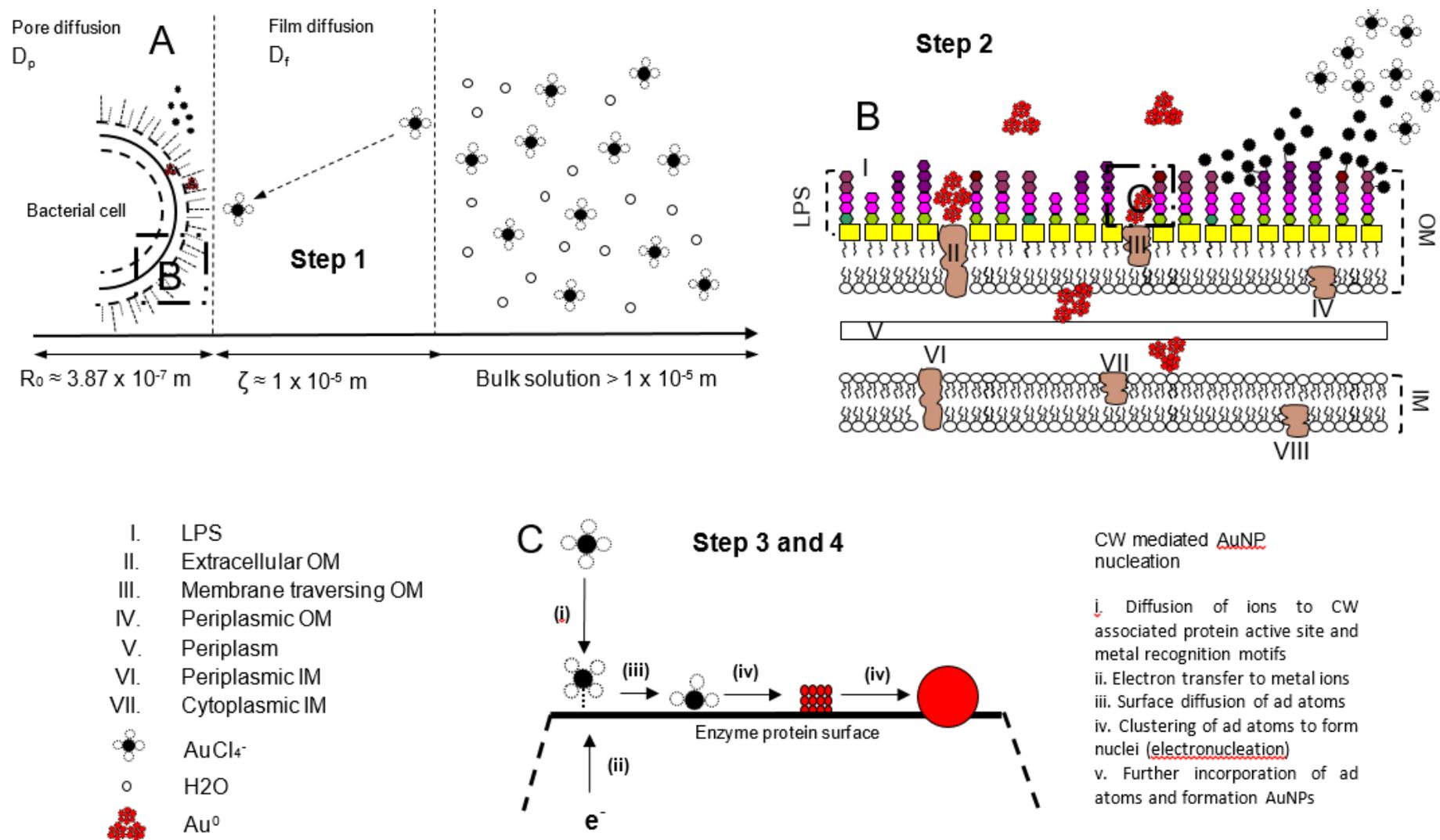


Figure 2 : Illustration of the microbial reduction interface and metal sorption and transformation process steps, taken from different levels of heirirical resolution. (a) Bacterial cell interface, (b) cell wall interface and (c) protein interface.

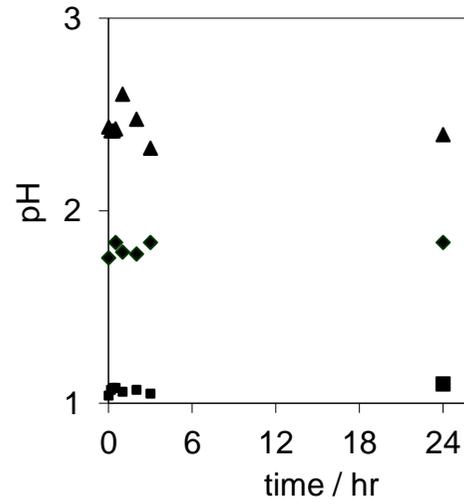


Figure 3: Changes in pH of metal solutions injected with 5ml of 1×10^{10} CFU/ml *S. putrefaciens* cells.

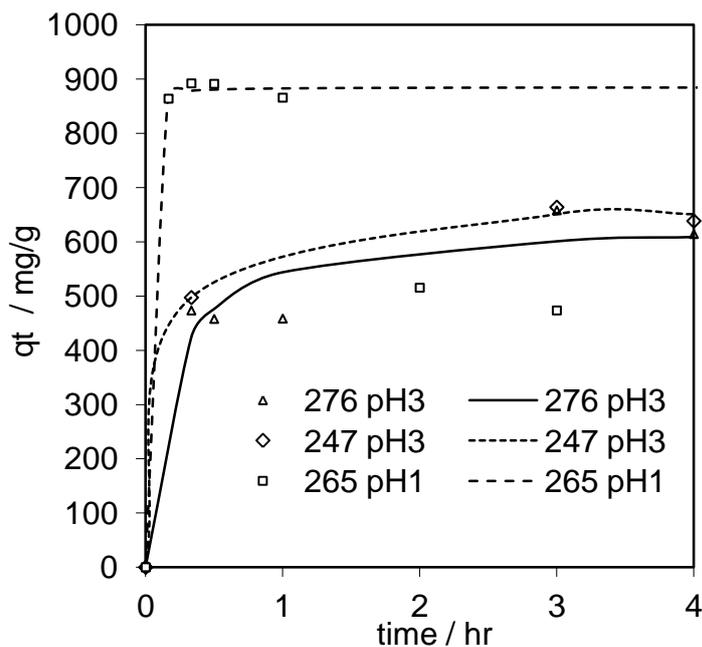


Figure 4 : Non-linearized fit of pseudo second-order to initial metal removal for assays of concentration > 200 mg/L of various pH. Fitting of assay 265 mg/L AuCl_4^- is for only 0 – 1 hr. Data points replicated from a preliminary publication [1]

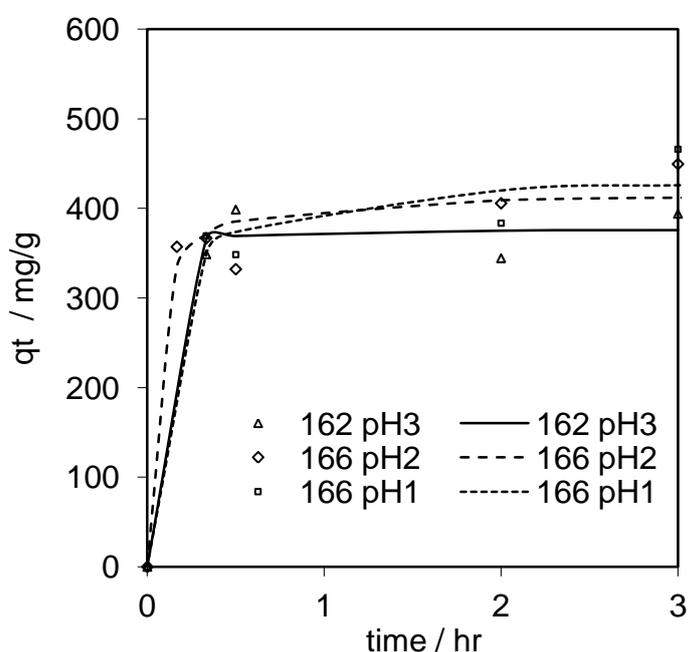


Figure 5 : Non-linearized fit of pseudo second-order to initial metal removal for assay of concentration ≈ 160 mg/L, of pH 1, 2 and 3. Data points replicated from a preliminary publication [1]

[1] Varia J, Zegeye A, Roy S, Yahaya S, Bull S. *Shewanella putrefaciens* for the remediation of Au³⁺, Co²⁺ and Fe³⁺ metal ions from aqueous systems. *Biochemical Engineering Journal*. 2014;85:101-9.

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		C_0 mg/L	pH	SSE	χ^2	ARE	R^2	Rate constant	q_e g/mg	
Pseudo first-order	Non-linearized	276	3	2.35×10^4	43.4	15.1	n/a	K_1 1/hr	3.69	586
		247	2	3.23×10^2	0.497	0.98			4.33	651
		265	1	7.24×10^2	0.824	0.87			80.6	878
		162	3	2.11×10^3	5.61	4.55			8.81	378
		166	1	5.60×10^3	14.0	6.14			5.29	417
		166	2	7.50×10^3	19.2	6.90			13.7	391
	Linearized	276	3	9.49×10^5	1.83×10^3	104	0.816	6.75×10^{-2}	908	
		247	2	1.71×10^5	340	25.3	0.667	2.95×10^{-1}	1.01×10^3	
		265	1		n/a					
		162	3	2.78×10^5	755	53.6	0.282	9.14×10^{-2}	1.02×10^3	
		166	1	2.62×10^5	692	37.4	0.740	6.15×10^{-1}	282	
		166	2	5.51×10^5	1.45×10^3	73.0	0.902	3.47×10^{-1}	172	
Pseudo second-order	Non-linearized	276	3	1.34×10^4	25.5	6.99	n/a	K_2 mg / (g hr)	9.49×10^{-3}	634
		247	2	4.99×10^2	0.768	1.25			1.27×10^{-2}	672
		265	1	6.56×10^2	0.746	0.80			4.51×10^{-1}	884
		162	3	2.42×10^3	6.50	5.14			2.48×10^{-1}	377
		166	1	4.03×10^3	9.95	5.18			2.65×10^{-2}	438
		166	2	4.82×10^3	12.5	5.48			5.72×10^{-2}	418
	Linearized	276	3	1.79×10^4	38.2	8.10	0.990	5.63×10^{-3}	667	
		247	2	3.83×10^3	7.49	4.05	0.998	2.25×10^{-2}	667	
		265	1	9.90×10^5	113×10^3	31.4	1.000	3.38×10^{-3}	769	
		162	3	2.58×10^3	6.93	4.79	0.992	9.66×10^{-2}	385	
		166	1	7.37×10^3	19.7	5.18	0.947	1.10×10^{-2}	476	
		166	2	7.73×10^3	21.5	7.51	0.995	2.42×10^{-2}	455	
Intraparticle model	Non-linearized	276	3	7.98×10^4	249	15.8	n/a	K_{ip} mg/(g hr ^{0.5})	454	
		247	2	6.86×10^4	257	15.6			431	
		265	1	2.93×10^5	454	42.4			1.18×10^3	
		162	3	8.62×10^4	433	28.8			283	
		166	1	6.10×10^4	295	19.7			310	
		166	2	1.09×10^5	629	30.1			324	
	Linearized	276	3	1.04×10^5	224	17.9	0.874	525		
		247	2	1.28×10^5	250	20.5	0.948	565		
		265	1	6.70×10^5	1.13×10^3	69.0	0.716	747		
		162	3	1.38×10^5	419	38.3	0.637	378		
		166	1	9.77×10^4	286	24.7	0.645	389		
		166	2	1.36×10^5	554	32.9	0.854	391		

Table 1 : Summary of data evaluated by different kinetic models

C_0 mg/L	pH	$t_{(1/2)}$ 1/hr	D_p m ² /s	D_f m ² /s
276	3	0.332	3.76×10^{-18}	5.52×10^{-14}
247	2	0.234	5.34×10^{-18}	8.31×10^{-14}
265	1	0.005	2.49×10^{-16}	5.10×10^{-12}
162	3	0.021	5.84×10^{-17}	5.10×10^{-13}
166	1	0.172	7.24×10^{-18}	7.34×10^{-14}
166	2	0.084	1.49×10^{-17}	1.44×10^{-13}

Table 2 : Summary of evaluated diffusion coefficients using parameters fitted from the pseudo second-order model

Highlights

- Kinetic modelling of AuCl_4^- uptake by *Shewanella putrefaciens*
- Theoretical analysis of plausible diffusional rate limiting steps
- Geometrical analysis of observed biogenic AuNPs

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