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Kinetic Modelling of Selenite Reduction by *Enterococcus* spp in batch reactors

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Selenite (SeO32-) is a toxic selenium oxyanion which readily bio-accumulates in the food chain. *Enterococcus* species were found to reduce SeO32- to elemental selenium (Se0) more rapidly (in 1 h) as compared to other known selenite reducing bacteria. The kinetics of thereduction by the *Enterococcus* species was investigated in aerobic batch reactors. The data fitted to the kinetic models was obtained from the reduction of various SeO32- concentrations under established optimum conditions (3.5 h, 35±2 °C, pH ≥ 8). The results of these biological experiments were modelled and the biokinetic parameters were estimated with a first order kinetic model for selenite reduction and elemental selenium accumulation. The AQUASIM software for the simulation of aquatic systems was used for generating the models. The estimated parameters for selenite reduction and elemental selenium formation by the biotic system were the reaction rate constant of k= 0.562 h-1 and yield coefficient, YSe0/Se4+= 0.761. The yield coefficient for the amount of glucose removed per mM of SeO32- , YGlc/Se4+, was not constant and depended of the concentration of selenite being reduced. The model presented in this work was able to fit the experimental data but more work still has to be done in order to develop a more concise model.

* 1. Introduction

The removal of selenite from aquatic systems through bioreduction to elemental selenium is important due to its toxicity to living organisms (Garousi, 2015). Although there are a number of microorganisms capable of achieving SeO32- reduction (Avendaño et al., 2016), *Enterococcus* species used in this study achieved it in a shorter time period with reduction capacity of over 90 % across three different concentrations. *Enterococcus* are lactic acid bacteria (LAB) which can be both commensal and pathogenic but they are usually found in the human alimentary tract (Hanchi et al., 2018). In addition to being Gram-positive, it is their high tolerance to elevated temperatures (5–65 °C), acidic environments (pH 4.5-10), and toxic metals that gives them a competitive edge over other microorganisms (Fisher and Phillips, 2009, Ramos et al., 2020). Besides being toxic at elevated concentrations, selenium is an essential trace element in human nutrition and is important for the functioning of enzymes involved in antioxidant defence, thyroid hormone metabolism and immune response (Kumar et al., 2020). It has previously been proposed that the bioreduction of selenite using *Enterococcus* spp is an efficient and eco-friendly method for the accumulation of elemental selenium (Tendenedzai et al., 2022), however more insight is required before industrial application can be realised. The aim of this study was to also develop a kinetic model to describe the selenite reduction and elemental selenium formation under batch conditions, thereby provided crucial understanding required for scaling.

* 1. Materials, methodology and analytical methods

The microbial characterisation for the *Enterococcus* species used in this study was outsourced to Inqaba Biotechnical Industries (Pty) Ltd. The bacteria were cultivated in Tryptone Soy Broth (TSB) (24 h, 28 °C) and stored in -70 °C storage chamber. To revive the strain, the frozen vials were taken from the storage chamber, streaked onto agar plates. Colonies which had grown on the agar plates were inoculated into fresh TSB for 24 h before harvesting and concentrating by centrifugation. The resultant pellet was added to the batches which had glucose supplemented mineral salt medium described by Tendenedzai et al. (2021), for selenite reduction.

Different selenite concentrations of 1 mM, 3 mM, and 5 mM were added at the start of each experimental run as Na2SeO3 (Sigma-Aldrich, St. Louis, MO). Aerobic batch reduction experiments with a working volume of 200 mL were conducted under already established optimum conditions from a previous study by Tendenedzai et al. (2021) (3.5 h, 120 rpm, 35±2 °C, pH ≥8.5). The SeO32- concentrations were determined using a 940 Professional IC Vario ion chromatograph (Metrohm, Switzerland) with separation column Metrosep C 6 – 250/4.0 (Metrohm, Switzerland) and C 6- eluent- 8 mM oxalic acid (Metrohm, Switzerland).

Se0 was quantified as total selenium using a Varian AA–1275 Series Flame AAS (Perkin Elmar, Varian, Palo Alto, CA, USA) at 196.03 nm wavelength equipped with a 290 mA selenium lamp. The pellet obtained from the centrifugation process comprised of both Se0 and the biomass. It is this combined pellet that was initially acidified with 2 mL of both 70% HNO3 and 32% HCl before digestion in a thermoreactor (60 min, 100 °C) for

re-oxidisation before AA analysis.

The kinetic model was developed using AQUASIM, a computer program for identifying and simulating aquatic systems. AQUASIM enables the user to model multiple substances and offers flexibility in the formulation of transformation processes. Additionally, AQUASIM provides methods for evaluating models, including sensitivity analysis and automatic parameter estimation, which estimate the uncertainty of calculated results (Wanner and Morgenroth, 2004).

* 1. Results and interpretation
		1. Microbial Characterisation

The bacterial colonies that had grown on agar plates containing 100 mM of selenite were analysed for their microbial characteristics. The red colonies (indicating the formation of elemental selenium) were further streaked and sent for analysis to Inqaba Biotechnical Industries. The results were presented in the form of a phylogenetic tree (Figure 1), which showed that the sequence was most similar to *Enterococcus hermanniensis* AY396048 and *Enterococcus gallinarum* MW175593. The BLAST results and 16S report confirmed that the bacteria belonged to the *Enterococcus* species cluster. This species was also identified as being Gram-positive (Tendenedzai et al., 2021).



Figure 1: Phylogenetic tree for the microbial culture results

* + 1. SeO32- reduction and Se0 formation

Reduction of *SeO32-* by the *Enterococcus* spp is shown in Figure 2 (a) and the formation of a reddish colour is an indication of the formation of Se0 which is typically a by-product of this process. The reaction time was rapid in the first hour where over 80 % of the reduction occurred with the total run time being 3.5 h, which is shorter than a previous study for SeO32-reduction (36 h) using *P. stutzeri* NT-I (Tendenedzai J.T. et al., 2021). The rapidness of selenite detoxification by the *Enterococcus* spp compared to other bacteria is a key result of this study and could see these lactic acid bacteria emerging as the preferred microbes for rapid treatment of selenium-laden aquatic bodies. The rapidity of selenite reduction could be due to the increased viability of *Enterococcus* spp as they have an advantage over several bacteria because of their ability to easily adapt to extreme conditions which include high temperatures, highly acidic or alkaline conditions and toxic metals (Tendenedzai et al., 2021, Hanchi et al., 2018).

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| C:\Users\u12317757\OneDrive\Documents\SELENIUM\phD\Enterococcus\AFM 2021\Applied nanoscience\3.5 h.PNG | (c) |
| (b)  | (d) |

*Figure 2*: (a) Colour variations in SeO32- reduction at the start (0 h) and the end (3.5 h) and time courses showing SeO32- reduction, Se0 formation and glucose consumption for the; (b) 1 mM, (c) 3 mM and (d) 5 mM concentrations respectively.

In this study, three SeO32- concentrations were reduced, and none reached zero even after prolonged incubation. The reduction process showed rapid selenite abatement in the first hour and a radical slowing down thereafter. Reduction percentages of 91%, 96%, and 97% were observed for 1 mM, 3 mM, and 5 mM initial concentrations, respectively (Figures 2(b)-(d)). The rapid formation of elemental selenium occurred under optimised laboratory conditions, including high biomass concentration, favourable pH and temperature, and the presence of glucose as a carbon source. The slight lag in Se0 formation was attributed to the formation of metabolic intermediates (Lampis et al., 2017).

* + 1. Kinetic modelling

There are a number of aspects one has to consider when modelling biological systems. One of the aspects involves the determination of the input and output variables as well as the intermediates (if any). Once these have been identified, model formulation becomes another key step.

To model our system, we employed reaction rate equations where the changes in the concentrations were defined as a function of time (Resat et al., 2009). Moreover, a number of assumptions had to be made in order to adopt this model. These included; linearity where the rate of reaction is proportional to the concentration of reactants. This was evident in our study where the reduction rate of the 5 mM SeO32- concentration was faster than the 1 mM concentration. The reversibility of the reactions was an important consideration as well. However, literature suggest that extreme conditions such as low pH and high temperatures would have to occur for Se0 to convert back to its oxyanions (Kim et al., 2017). In our study, we counteracted this by placing the samples in a 15 mM NaOH solution for stabilisation and keeping them at -80°C prior to analysis.

It was also assumed that the rate of reaction was not influenced by the concentration of the product, i.e. elemental selenium. Other assumptions considered were homogeneity and steady state of the batch setup (Resat et al., 2009). Although the process variables in a batch setup can change over time, it is still possible to assume a steady-state for specific stages of the batch process where the process variables are relatively constant. It was further assumed that due to the high concentrations of biomass harvested prior to, and the lack of nitrogen added to the system during the reduction process, the biomass concentration remained constant. Finally, it was assumed that the glucose is oxidised to supply the electrons required for to the reduction of Se4+ (Ji and Wang, 2017).

In our study, it was noted that reduction was at its most rapid in the first hour and thereafter there was a drastic reduction in the rate of the reaction. The universally used Michaelis-Menten kinetic model is one of the best-known approaches to enzyme kinetics as it relates the reaction velocity to the substrate concentration (Klipp et al., 2009). The model can, however, reduce to a first-order kinetic model if the half-saturation constant of the system (KS) is much greater than the substrate concentration ([A]) (Equation 1). The KS parameter indicates the substrate concentration yielding half the maximum consumption rate and is an empirically determined constant related to the body size and physical characteristics of the organism ((Mulder and Hendriks, 2014).

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| $$\frac{d[A]}{dt}=\frac{k\_{max}\left[A\right]}{K\_{s}+\left[A\right]}=-k\left[A\right] \left(for K\_{S}\gg \left[A\right] and k=\frac{k\_{max}}{K\_{s}}\right)$$ | (1) |

where [A] is the concentration of the substance A, kmax is the maximum removal rate of A from solution, KS is the half saturation constant, t is time. The solution to this equation gives the concentration of the substance as a function of time, and the rate constant k can be determined experimentally.

Therefore, as the rate of change of the concentration of the Se4+ over time was observed to be proportional to the concentration of Se4+, the bioreduction of selenite, the accumulation of elemental selenium, and the consumption of glucose were modeled as first order reactions are shown in Equations 2, 3, and 4, respectively:

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| $$\frac{d[Se^{4+}]}{dt}=-k.\left[Se^{4+}\right] $$ | (2) |
| $$\frac{d[Se^{0}]}{dt}=Y\_{\frac{Se^{0}}{Se^{4+}}}.k.[Se^{4+}]$$ | (3) |
| $$\frac{d[Glc]}{dt}=-Y\_{\frac{Glc}{Se^{4+}}}.k.[Se^{4+}]$$ | (4) |

where k is the first order rate constant, [Se4+] is the SeO32-concentration, [Se0] is the elemental selenium concentration, [Glc] is the concentration of glucose in solution, and $Y\_{\frac{Se^{0} }{Se^{4+}}}$and $Y\_{\frac{Glc }{Se^{4+}}}$ are the yield coefficients representing the amount of Se0 produced or glucose removed per mM of SeO32-reduced, respectively. The negative sign in Equations 2 and 4 indicates a concentration decrease with time while the absence of the negative sign in Equation 3 indicates accumulation.

Table 1: Biokinetic parameters for the first order kinetics fitted to SeO32- reduction, Se0 formation and glucose consumption

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| **[Se4+] (mM)** | **k (h-1)** | **YSe0/Se4+** | **YGlc/Se4+** |
| 1 | 0.562 | 0.761 | 93.425 |
| 3 | 33.377 |
| 5 | 15.175 |

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| (a) | (d) |
| (b) | (e) |
| (c) | (f) |

Figure 3: Adapted first order kinetics fitted to measured (a) 1 mM, (b) 3 mM, (c) 5 mM SeO32- reduction and Se0 accumulation data and glucose consumption in the (d) 1 mM, (e) 3 mM, (f) 5 mM batches respectively.

As seen in Figures 3 (a)-(c), the system appears to experience a change in selenium removal mechanism at 1 h, as the model no longer correlates well with data thereafter. It is possible that after 1 h, a distinct mechanism becomes responsible for selenite removal in the solution is activated which rapidly increases the removal rate of SeO32- and the production rate of Se0. This mechanism appears to correspond to a critical glucose concentration around 5 mM – it should be noted that for none of the runs were the glucose completely depleted indicating that this was not a limiting reagent.

* 1. Conclusions

The first-order kinetic model developed in this study provides a useful tool for predicting the elemental selenium accumulation in the bioreduction of selenite using *Enterococcus* spp for the first hour of operation. However, more research still has to be done in order to develop a more concise structured model, likely due to insufficient knowledge of cellular metabolism regarding SeO32- reduction, the diversity of selenite reducing metabolites, and the utilisation of the carbon source. The variations in the consumption of glucose to selenite reduced might due to the fact that most of the glucose is being used for metabolic processes and only partially for the reduction. The cellular process of the species involved in selenium reduction should be investigated with the aim to develop a concise structured model. The results of this study can contribute to the optimisation of bioreduction conditions for the efficient accumulation of elemental selenium.

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