

Effect of metabolic state on *Paraclostridium bifermentans* surface properties and the implications for lead removal

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1. Introduction

Pb(II) is a highly soluble, mobile, and toxic ion that continues to cause a variety of human health problems. Exposure to Pb(II) can cause significant harm to most organs [1].

Removal of Pb(II) from wastewater is conventionally achieved with membrane electrodialysis, electrochemical treatment, chemical precipitation, and ion exchange membranes [2].

A promising alternative to conventional technology for the removal of aqueous Pb(II) is bioremediation, where organisms are used to remove or detoxify the heavy metal [3]. Bioremediation is attractive due to the variety of biomaterials applicable (such as algae, fungi, plants, and bacteria) and its potential for low cost and high efficiency operation at low Pb(II) concentrations [4]. Pb(II) removal with organisms has mostly been limited to sorption with biomass [5], the use of plants for phytoextraction [6], and fungi for mycelial biosorption [7]. Some microorganisms have been discovered that reduce the bioavailability and toxicity of Pb(II) by precipitating it out as an insoluble complex.

A consortium of bacteria has been isolated from lead-contaminated soil at a battery recycling plant in Gauteng, South Africa, that has been shown to remove Pb(II) from solution [8]. This lead-resistant consortium has been the subject of many investigations in the pursuit of better understanding the removal mechanisms and possible implementations in the bioremediation and biorecovery of lead in industrial effluent. This includes studies on the influence of lead concentrations [8,9], substrate concentration [8,10], precipitate identification [11,12], influence of other divalent heavy metals [13], and operation in an upward anaerobic sludge bed reactor [14].

Consortium precipitates PbO aerobic and PbS + PbO anaerobic, oxidation-reduction mechanism where Pb(II) acts as terminal electron acceptor [15].

Modelled with fast and then slow stages [16], where the fast has been attributed to biosorption [17]. The role of biosorption also displayed in dead bacteria [18].

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[17] *Paraclostridium bifermentans* and *Klebsiella pneumoniae*

[19]

The chemical composition of bacteria surfaces for both Gram-positive and Gram-negative bacteria are rich in negatively charged functional groups that result in an overall negative surface charge and facilitate the attraction of positively charged metal cations like ionic lead [20]. These functional groups also allow for chemisorption to take place, where hydrogen ions are exchanged for Pb(II) ions (Lu et al., 2012) [21]. Chemisorption not only prevents lead from entering cells, but Functional group complexation can be a prominent adsorption mechanism for some bacteria that use it to concentrate terminal electron accepting ions on the cell wall surface

[22]

Several subterranean anaerobic bacteria have been reported to respire using a range of terminal electron acceptors, including heavy metal pollutants [22]. Respiration involving the reduction of soluble oxidised-metals can lessen the mobility of the metal.

Several authors have used acid-base titration to improve understanding of bacteria surfaces [23] as well as using surface models to predict the effects of pH on metal binding to cell surface [24].

Studies have also been conducted to determine effects of metabolic state on metal adsorption [25,26]

[27]

[26]

[25] Noted that

[28] Highlighted significant hysteresis and time dependence in acid-base titrations of *Shewanella putrefaciens*.

[29]

Lead is a big problem

A consortium has been found: consortium properties like performance: chapter + carla + all the cets. Lateral citation fest.

Is surface complexation a mechanistic step or is? Does rxn happen on surface?

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research.

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92 3. Results

93 This section may be divided by subheadings. It should provide a concise and precise
94 description of the experimental results, their interpretation as well as the experimental
95 conclusions that can be drawn.

96 3.1. Subsection

97 3.1.1. Subsubsection

98 Bulleted lists look like this:

- 99 • First bullet;
- 100 • Second bullet;
- 101 • Third bullet.

102 Numbered lists can be added as follows:

- 103 1. First item;
- 104 2. Second item;
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106 The text continues here.

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108 All figures and tables should be cited in the main text as Figure 1, Table 1, etc.



Figure 1. This is a figure. Schemes follow the same formatting. If there are multiple panels, they should be listed as: (a) Description of what is contained in the first panel. (b) Description of what is contained in the second panel. Figures should be placed in the main text near to the first time they are cited. A caption on a single line should be centered.

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Title 1	Title 2	Title 3
Entry 1	Data	Data
Entry 2	Data	Data

109 Text.

110 Text.

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This is the example 1 of equation:

$$a = 1, \quad (1)$$

112 the text following an equation need not be a new paragraph. Please punctuate equations
113 as regular text.

114 This is the example 2 of equation:

$$a = b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z \quad (2)$$



Figure 2. This is a wide figure.

115 Please punctuate equations as regular text. Theorem-type environments (including
116 propositions, lemmas, corollaries etc.) can be formatted as follows:

117 **Theorem 1.** *Example text of a theorem.*

118 The text continues here. Proofs must be formatted as follows:

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120 if it is clear which theorem is being referred to. □

121 The text continues here.

122 4. Discussion

123 Authors should discuss the results and how they can be interpreted from the
124 perspective of previous studies and of the working hypotheses. The findings and their
125 implications should be discussed in the broadest context possible. Future research
126 directions may also be highlighted.

127 5. Conclusions

128 This section is not mandatory, but can be added to the manuscript if the discussion
129 is unusually long or complex.

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Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

Appendix A

Appendix A.1

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data are shown in the main text can be added here if brief, or as Supplementary Data. Mathematical proofs of results not central to the paper can be added as an appendix.

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Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled, starting with “A”—e.g., Figure A1, Figure A2, etc.

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