Removal of Selenium Oxyanions in Wastewater by Using a Bacterial Community

**Background**
Selenium is widely used in various industries and is an essential element for human beings. On the contrary, since soluble selenium oxyanions are deadly chemicals toward living things, selenium oxyanions in industrial wastewater are strictly regulated. These industries eliminate wastewater containing selenium in the form of selenate (SeO₄²⁻) and selenite (SeO₃²⁻). At present, chemical precipitation has been used for selenium removal. This method is effective for selenite removal, though the efficiency in selenate removal is rather low. The method needs plenty of chemicals and a large number of sludge is produced. On the other hand, biological technique is capable of reducing both selenate and selenite to insoluble elemental selenium. In the process, bacteria require expensive chemicals as an electron donor for reduction of selenium oxyanions. Therefore, reduction of selenium oxyanions with inexpensive alcohol instead of expensive chemicals is indispensable to practical application. However, there have been no reports on the bacteria that can reduce selenium oxyanions with alcohol.

**Objectives**
To take bacteria that are capable of reducing selenium oxyanions in wastewater by using inexpensive alcohol as an electron donor, investigate the reductive characteristics of the isolated bacteria with regard to selenium oxyanions, and demonstrate a novel method for the removal of selenium oxyanions by using a bacterial community with alcohol as the electron donor.

**Principal Results**
1. **Isolation of bacteria that can reduce selenium oxyanions with ethanol**
A new strain termed 4C-C that was capable of reducing selenium oxyanions was isolated from the sludge in a wastewater treatment facility. This strain was identified as a *Pseudomonas* sp. from its 16S rDNA sequence and from its morphological and physiological characteristics. It was capable of reducing selenate to elemental selenium via selenite formation by using ethanol as an electron donor; however, its potential to reduce selenite was less than its ability to reduce selenate (Table 1).

2. **Reduction of selenium oxyanions using a cell suspension mixture of two bacterial strains**
To complement lesser selenite reduction process, screening was carried out for bacterial strain that was able to reduce selenite with ethanol. The fact that *Paracoccus denitrificans* JCM 6892 could reduce selenite to elemental selenium by using ethanol was found (Table 1). A combination of *Pseudomonas* sp. strain 4C-C and *P. denitrificans* JCM 6892 cells in suspension was investigated for the reduction of selenate to elemental selenium by using ethanol. As a result, this bacterial community enabled the reduction of selenate to insoluble elemental selenium via selenite formation by using ethanol (Fig.1).

3. **Accumulation of elemental selenium by using an immobilized mixture of two bacterial strains**
To simplify the recovery of insoluble elemental selenium, cells from both bacterial strains were immobilized together in a polymeric gel and examined using ethanol. The immobilized bacterial community could also reduce the selenate to insoluble elemental selenium via selenite formation. The immobilized cells in the polymeric gel were pigmented bright red, and there was no red elemental selenium in the wastewater (Fig.2). This meant that the reduced elemental selenium was completely captured in the polymeric gel. These results indicated the potentiality of simplifying the recovery process for insoluble elemental selenium.

**Future Developments**
The availability of this technique at low concentration in selenium oxyanions will be further investigated, and the stable maintenance of the bacterial community will be developed.

**Main Researcher:** Masahiko Morita, Ph. D.,
Research Scientist, Biotechnology Sector, Environmental Science Research Laboratory

**Reference**
2. Environment

### Table 1  Characteristics of bacterial strains concerning the reduction of selenium oxyanions

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Electron donor</th>
<th>SeO$_4^{2-}$ → SeO$_3^{2-}$</th>
<th>SeO$_3^{2-}$ → Se</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> sp. strain 4C-C</td>
<td>Yeast extract</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>(newly isolated strain)</td>
<td>Ethanol</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><em>Paracoccus denitrificans</em> JCM 6892 (known strain)</td>
<td>Yeast extract</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++: possible to reduce selenium oxyanion  
+: possible to reduce selenium oxyanion slightly  
-: impossible to reduce selenium oxyanion

*Pseudomonas* sp. strain 4C-C was capable of reducing selenate to elemental selenium via selenite formation by using ethanol; however, its potential to reduce selenite was less than its ability to reduce selenate. To complement lesser selenite reduction process, *Paracoccus denitrificans* JCM 6892 could reduce selenite to elemental selenium by using ethanol.

![Fig.1](image1.png)  
Reduction of selenium oxyanions using a cell suspension mixture of two bacterial strains

A combination of *Pseudomonas* sp. strain 4C-C and *P. denitrificans* JCM 6892 cells was investigated for the reduction of selenate to elemental selenium by using ethanol. This bacterial community could reduce selenate to insoluble elemental selenium via selenite formation.

![Fig.2](image2.png)  
Accumulation of elemental selenium at nonwoven fabric

The reduction of selenium oxyanions was investigated using a nonwoven fabric on which two bacterial strains were immobilized together with a polymeric gel. The nonwoven fabric was pigmented bright red from ivory yellow, and there was no red elemental selenium in the wastewater. These results indicated the potentiality of simplifying the recovery process for insoluble elemental selenium reduced from selenate and selenite.