Stabilization of chromium by reductase enzyme treatment

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Sources of Chromium Pollution

- Metal Finishing Industry
- Petroleum Refinery
- Leather Tannery
- Iron & Steel Industry
- Inorganic chemical production
- Pulp producing processes
- Production of chromium metal
- Explosives

All the above wastes are also important source for bacterial screening
# Chromium Limit values for compliance test using BS EN 12457-3 at L/S 10 Kg-1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inert Waste Landfill (mg Kg-1)</th>
<th>Stable Non-reactive Hazardous Waste in Non-hazardous Landfill (mg Kg-1)</th>
<th>Hazardous Waste Landfill (mg Kg-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium (Total)</td>
<td>0.5</td>
<td>10</td>
<td>70</td>
</tr>
</tbody>
</table>

Reduction of chromate by *Bacillus sp.*

- **Cr (VI) (soluble, toxic)** \( \rightarrow \) **Cr (III) (insoluble, less toxic)**

\[ \text{Bacillus sp} \quad \text{K}_2\text{CrO}_4 \quad \text{reductase} \quad \text{Cr(OH)}_3 \]

- Reduction depends on the type and concentration of the organic substrate used for bacterial growth

- Diphenylcarbazide method (APHA, 1989) using UV Visible Spectrophotometer - Cr(VI) estimation

- Later confirmed with X-ray absorption spectroscopy

- Laboratory experiments were conducted to find out the reduction mechanism in bacteria
Relative rate of Cr(VI) reduction

<table>
<thead>
<tr>
<th>Cr(VI) Concentration (mM)</th>
<th>Relative rate of reduction (μM/h) ± Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Lactate</td>
</tr>
<tr>
<td>0.1</td>
<td>0.85 ± 0.14</td>
</tr>
<tr>
<td>0.2</td>
<td>1.48 ± 0.46</td>
</tr>
<tr>
<td>0.5</td>
<td>2.50 ± 0.13</td>
</tr>
<tr>
<td>0.75</td>
<td>2.70 ± 0.24</td>
</tr>
<tr>
<td>1.0</td>
<td>2.60 ± 0.31</td>
</tr>
<tr>
<td>2.0</td>
<td>2.20 ± 0.48</td>
</tr>
</tbody>
</table>

Relative rates for time (h) vs percent reduction of Cr(VI) showed maximum Cr(VI) reduction with glucose, followed by lactate & acetate.
Substrate addition

Addition of same substrate at stationary phase increased the rate of bacterial growth as well as Cr(VI) reduction
Extraction of reductase enzyme

Bacillus culture

MSM + glucose + 0.1mM Cr(VI)

37°C incubation

Mid exponential phase (72h)

Centrifuged at 12000 rpm

Pellet (bacterial cells) washed with buffer (pH 7)

Pellet resuspended in buffer

Ultrasonication in sonicator (2 h)
Cell suspension

Centrifuged at 4000 rpm → Cell Pellet
discarded

Supernatant

Centrifuged at 150,000 rpm for 2h at 4°C

Cell pellet       Supernatant

(Cytoplasmic fraction)

Resuspended in buffer

(Cell membrane fraction)
Chromium (VI) reduction by cytoplasmic and cell membrane fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>0.1 mM Cr(VI)</th>
<th>0.2 mM Cr(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction rate (µM/h) at 0.5 h</td>
<td>Reduction rate (µM/h) after 12 h</td>
</tr>
<tr>
<td>Membrane</td>
<td>6.4 ± 0.4^A</td>
<td>10.3 ± 0.9</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>8.8 ± 0.9</td>
<td>16.9 ± 1.7</td>
</tr>
</tbody>
</table>

^ Standard error
Cr(VI) Reduction in the presence of sulfate & thiosulfate

- Experiments conducted at different chromate concentrations (0.5-1.0mM) in the presence of different concentrations of sulfate & thiosulfate (0.05-1.0mM)

- In contrast to sulfate, raising the thiosulfate concentration in the medium doubled the reduction rate under similar conditions
Bacterial reduction of toxic Cr(VI) to non-toxic Cr(III)

Chromate Reductase

Active uptake

Enhances biosynthesis of Chromate Reductase

Cr(VI) (chromate ion)

Active uptake

Cr(III) (soluble organic complex?)

Cr(III) (Insoluble hydroxide?)

Cell wall & membrane

Active Expulsion

Thiosulfate

Thiosulfate
Applications

1. Direct

- Washing chromate contaminated soil with reductase enzyme precipitates Cr, it could be recycled and reused in industrial process

- Landfill cost would be reduced for the waste producers.
2. Indirect

Chromate contaminated soil/land

Pretreatment (spray) with reductase

Precipitate and concentrate metals in land (Stabilization)

Excavate the soil

Soil washing

Cr separated from soil aggregates

Recycled Cr - back to industry
Sustainable solution

• Bacteria are widely distributed in nature, screening of reductase enzyme producing strains from the contaminated source is not a difficult task

• Since bacterial growth is rapid, we could produce the reductase enzyme in 72h – by providing nutrient and trace amount of chromate

• Industrial symbiosis approach would be a feasible option - waste water from food industries could be used as energy source for bacterial growth and reductase production
Chromium and Health

- Chromium is an essential trace mineral (~200 mcg/day) required by humans for health.

- It is involved in the metabolism of carbohydrates, fats, and proteins.
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