

Effect of Sodium Chloride on Ferrous Iron Oxidation and Respiratory Rate by *Sulfobacillus thermosulfidooxidans*

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Biomining is the utilization of acidophilic iron- and/or sulfur-oxidizing microorganisms for metal extraction from low-grade sulfide ores and mineral concentrates. This microbial process of metal recovery has been proven to be a cost-effective and sustainable technology. However, acidophilic microorganisms are extremely sensitive to chloride ions present in biomining operations through the dissolution of silicate minerals or use of saline/seawater. The presence of chloride concentrations inhibits bacterial cell growth and activity, that is attributed to disruption of cell pH homeostasis, lowering of the proton motive force, and effects on the ferrous iron oxidation system. This study aimed to investigate the influence of increasing concentrations of sodium chloride (NaCl) on iron oxidation and respiratory rates of *Sb. thermosulfidooxidans* (DSMZ 9293). The results indicated chloride tolerance of *Sb. thermosulfidooxidans* was pH- dependent. Cells of *Sb. thermosulfidooxidans* showed the ability to tolerate greater concentrations of sodium chloride at higher pH values. At pH 1.4 bio-oxidation of ferrous iron was completely inhibited in the presence of 13 gL⁻¹ NaCl while at pH 2.5 and 3.0 the complete inhibition of iron oxidation occurred in the presence of >35 gL⁻¹ NaCl and > 37 gL⁻¹, respectively. NaCl concentration of \geq 16 g/L inhibited bacterial iron oxidation at pH 1.8.

It is observed that exposure to higher NaCl concentrations resulted in the lower iron oxidation activity of *Sb. thermosulfidooxidans* cells. Comparing to iron oxidation in the assays without the addition of NaCl, iron oxidation rate was 1.5 times lower in the presence of 13 gL⁻¹ NaCl. The bio-respiration in the presence of elevated NaCl concentrations of 0, 5.8, 11, 13, 17, 23, 29, 35 and 40 gL⁻¹ within 30 to 60 min exposure proved capable of adapting or responding only to NaCl concentrations less than 17 gL⁻¹. At higher concentrations, respiration reduced significantly.

In addition, long-term cultivation in the presence of 13 gL⁻¹ NaCl demonstrated no significant improvement of ferrous iron oxidation activity as cells exposed to NaCl. In a similar stress condition, iron oxidation by cells grown in the absence of chloride surpassed iron oxidation by cells grown with chloride; and ferrous iron ions were completely oxidized after 70h, with the iron oxidation rate being 1.9 and 1.2 respectively. The better performance in the iron oxidation of cells historically cultivated in the presence of chloride only occurred within the first 5h of investigating duration. The respiratory rates by cells grown with the addition of NaCl were found to be greater than that of cells grown without NaCl. This confirmed that cells used to expose to NaCl responded better to NaCl than cells suddenly exposed to high concentrations of NaCl within a short stress period. This study has shown that it is possible for *Sb. thermosulfidooxidans* to adapt or habituate to concentrations of 13 gL⁻¹ NaCl, however, this adaptation had a limited improvement in activity.