Ni Stress to Sulphate Reducing Bacteria Enhances Ni Complexation:
Opportunity for Ni-Co Separation from wastewater

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Abstract: This study assesses the Ni complexation in both batch and continuous bioreactors that contain sulfate reducing bacteria (SRB). Results showed that in the batch systems, more than 50% of Ni remained soluble while almost all Co precipitated with sulfide in the range of 50-200 mg/L metal supplementation. Besides, Ni complexation occurred in the continuously stirred tank reactor (CSTR) rather than the up-flow fixed bed reactor (UFBR), although UFBR has higher SRB activity than CSTR. This could be due to the difference in biomass retention mode (suspended or attached), as biofilms protect microorganisms against metal stress and therefore a need for Ni complexation is discouraged.

Keywords: Nickel; Protein; Sulphate reducing bacteria

 Session –Sulfur cycle technology

1. Introduction

Sulfate reducing bacteria (SRB) has been widely applied for the treatment of metal- and sulfate-containing waste streams, in which biologically produced sulfide can precipitate with metals and recover these resources. However, metal-microbial interactions by SRB go beyond sulfide generation to precipitate metals. Desulfotomaculum sp. (DF-1), for instance, were reported to facilitate the production of Ni-protein complex over its precipitation with sulfide (Fortin et al., 1994). This is a protection mechanism against metals and occurs by an alteration of their protein expression profiles (Gillan, 2016). The effect of these extracellular proteins can be unique for each metal, which can be used as a strategy for selective recovery of metals. This is particularly important for metals that require high amounts of reagents and chemicals for separation due to similar chemical behavior, as is the case of nickel (Ni) and cobalt (Co).

Ni and Co demand and prices have increased significantly, thus opening the need to exploit secondary resources (Hennebel et al., 2015). Wastewater from Ni mining often contain high concentrations of unrecovered Ni and Co due to inefficient extraction (Crundwell et al., 2011). Ni complexation induced by SRB could be used as a biotechnological strategy to separate this metal over others such as Co in wastewater. To date, the study of these mechanisms is limited to pure cultures, while little research has been carried out in engineered systems aiming to control these processes. Therefore, the aim of this study was to assess Ni complexation in batch and continuous bioreactors containing SRB.

2. Material and Methods

Sulfate reducing activity (SRA) tests were carried out using serum bottles (120 mL) with a working volume of 100 mL at 120 rpm and 30 °C. SRB biomass from a lab reactor (0.2g VSS/L), lactate (1388 mg/L COD), sulfate (2127 mg/L SO₄) and nutrients (Alexander et al., 1980) were added to the serum bottles. The following metals concentrations were evaluated in triplicate: 1) 0, 10, 50, 100, 200 and 500 mg/L Ni²⁺ or Co²⁺; (2) a mixture of 100 mg/L Ni²⁺ with 10, 50 and 100 mg/L Co²⁺. A continuously stirred tank reactor (CSTR) and an up-flow fixed bed reactor (UFBR)
fixed with polyurethane foam (PUF) were operated for more than 100 days at room temperature and COD/SO4 ratio of 0.67 (g/g). The reactors were fed with synthetic medium containing lactate, sulfate, micro and macro-nutrients. After steady state conditions (HRT: CSTR 7 days and UFBR 2 days), 100 mg/L Ni was also supplemented. Sulfide, sulfate, COD, pH and metals were measured in both batch and continuous experiments. Qualitative analysis of Ni complexation was done by adding 2% HNO3 to the filtered reactor effluent or liquid media of the batch experiments. The acid denaturizes the complexing protein and results in the precipitation of black Ni precipitates (Fortin et al., 1994). Protein identification and microbial community structure were conducted using Q-Exactive HF-X and Illumina platform, respectively.

3. Results and conclusions

3.1 Sulfate reducing activity tests

In SRA tests without metal addition, sulfide production rate was around 26 mg/(L.d) (Figure 3.1a) and COD removal was 97%, indicating the suitability of the inoculum for sulfate reduction. With increased metal addition, soluble Ni was much higher than that of Co (e.g. >50% for Ni and <10% for Co in the range of 10-200 mg/L), even when both metals were present in the experiments (Figure 3.1b). Both Ni and Co at 500 mg/L were inhibitory to SRB so a significant decrease in sulfide was observed.

![Figure 3.1 Sulfate reducing activity with Ni and Co addition: (a) Remaining sulphide (Δ) and total sulphide (o) production (=remaining sulphide + sulphide in metal sulphide precipitates); (b) Soluble Ni and Co remained in the liquid phase at the end of SRA tests.](image)

Six dominant proteins, who had maximum number of peptides to bind metals were exclusively encountered in Ni experiments (Figure 3.2). Most of these characteristic proteins are produced by Desulfomicrobium baculatum (DSM4028) (Figure 3.2), the dominant species in the inoculum. [NiFe] hydrogenases, a metalloenzyme that obviously harbor Ni and can be produced by DSM4028, were also found to be dominant proteins in Ni systems.

![Figure 3.2 Dominant proteins encountered in the sulfate reducing activity tests (Ni 10-100 mg/L, Co 10-100 mg/L, Ni 100 + Co 10-100 mg/L), classified based on “Accession” of the proteins](image)
3.2 Sulfate reducing bioreactors

During the first 100 days of CSTR, COD and sulfate concentration fluctuated severely (Figure 3.3 a, b) due to the continuous biomass lose and sulfide inhibition that reached up to 2500 mg/L sulfide. At steady state, COD and sulfate removal were relatively low in CSTR (40% and 27%, respectively). The addition of 100 mg/L Ni on day 284 did not affect the COD and sulfate removal. Though low, Ni complexation was detected in the CSTR operation (around 3.5 mg/L soluble Ni) despite that sulfide concentration was sufficient for precipitation (~300 mg/L sulfide). By contrast, in UFBR, the removal of COD and sulfate significantly increased after 70 days of operation and the addition of Ni (100 mg/L) (Table 3.1), which can be attributed to the establishment of the SRB biofilm and lower inhibitory sulfide concentrations resulted from the metal sulfide precipitation. In contrast to the CSTR operation, Ni complexation was not observed in UFBR, instead, full Ni precipitation in the reactor bottom occurred (>99%). The difference was mainly attributed to the biofilm protection against Ni stress that may affect the expression of Ni complexing proteins. Nevertheless, other operational conditions that could favor Ni complexation should be explored.

![Figure 3.3](image)

**Figure 3.3** COD and sulfate variation in the operation of the CSTR (a, b) and UFBR (c, d). Falcon tubes on the up right corner are the filtered effluent from the two reactors and that with addition of 2% HNO$_3$.

<table>
<thead>
<tr>
<th>100 mg/L Ni</th>
<th>CSTR</th>
<th>UFBR</th>
</tr>
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<tbody>
<tr>
<td>COD removal</td>
<td>43.5±0.9%</td>
<td>77.4±1.1%</td>
</tr>
<tr>
<td>Sulfate removal</td>
<td>28.4±1.4%</td>
<td>67.0±1.3%</td>
</tr>
<tr>
<td>Ni removal</td>
<td>96.8±1.0%</td>
<td>99.7±0.1%</td>
</tr>
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**Table 3.1** COD, sulfate removal and Ni precipitation percentage of two continuous sulfate reducing bioreactors (CSTR vs. UFBR) with or without Ni supplementation
References


