The Relationship of Genus Bacillus, Sulfate Reducing Bacteria and Sulfur Oxidation Bacteria in the Activated Sludge

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Abstract
Corrosion of sewer pipe due to sulfuric acid produced by sulfate reducing bacteria (SRB) is a serious problem today. We have studied that the number of SRB is reduced in the activated sludge of a B. subtilis -dominated. The purpose of the present investigation is to prove the interaction between B. subtilis, SRB and sulfur oxidation bacteria (SOB), and to develop the technique for corrosion protection of concrete.

We began with the number of B. subtilis, SRB and SOB in the activated sludge. There were about $10^7$ B. subtilis, SRB and SOB per gram in the activated sludge. The results showed that there were not a few SRB. This experiment seems to prove that there is some correlation between B. subtilis and SRB. It is evident that more work needs to be done by using different approaches to know what is really happen.

Introduction
SRB and SOB are widely detected in the environment. These bacteria play an important role on carbon, sulfur and nitrogen cycles. About 10 to 1,000 mg/l of sulfate are contained in sewage. Sulfate reduction causes concrete corrosion and unpleasant odors in the sewerage system. On the other hand, aerobic digestion treatment of the night soil at sanitation enter-facility has been operated on effective condition and there was not odor (Murakami et al., 1995). Bacteria in the digestion tank were isolated, a many of them was B. subtilis.

In this study, the number of SRB, SOB and B. subtilis. colonies was examined in the night soil.

Materials and methods
Activated sludge
The activated sludge was collected at Okutone Amenity Park, and it was reported that B. subtilis was dominating in the activated sludge. Okutone Amenity Park is one of the night soil treatment plants, and adopts the membrane separation method. In our experiment, we used both wet and freeze-dried sample.

Measurement of the number of Bacillus colonies
Firstly, liquid sample 20ml was stirred for 10 seconds by using a homogenizer. Then, the samples were diluted at $10^3$ to $10^7$ times by the 0.85% physiological salt solution, and each diluted water sample (0.1ml) was applied on nutrient agar plate culture medium. Then, the applied culture medium was cultivated in 30°C at dark place. After cultivation, the colonies of Bacillus, which appeared on the culture medium, were counted.
Measurement of the number of SRB

SRB was cultivated in a medium containing the following ingredients: 10.0 g C_{17}H_{21}NO_{4} BrH, 5.0g C_{3}H_{2}NO_{3}, 0.5 g Na_{2}SO_{3}, 2.0 g MgSO_{4} 7H_{2}O, 0.5 g FeSO_{4} 7H_{2}O and 0.5g C_{6}H_{10}FeNO_{7}. Then, the medium was autoclaved at 120°C for 15 min. The SRB seeds were incubated at 37°C. The number of SRB (MPN method with m-ISA medium) (Mara and Williams, 1970) were measured.

Measurement of the number of SOB

SOB were cultured in Starkey medium containing the following ingredients: 0.3 g (NH_{4})_{2}SO_{2}, 4.0 g K_{3}HPO_{4}, 1.5g KH_{2}PO_{4}, 0.5g MgSO_{4}7H_{2}O, trace metallic solution, 0.3 g Yeast extract, 1% the thiosulfuric acid. After the shaking culture, the number of SOB, which was below 4.5 pH, was counted.

Results and discussion

Table 1 shows that the number of the B. subtilis, SRB and SOB. This data were compared with the already reported result. [1] is the test result of the night soil treatment plant (Ubukata, 2010) and [2] is data of municipal wastewater disposal plant (Miyazato, 2005). The number of the B. subtilis was about 10^{7} cells per gram, and it was the same range as existing data. On the other hand, the number of SRB was also about 10^{7} cells per gram, which was comparable with preceding data. Accordingly, the number of SRB was not small. However, this activated sludge did not have odor like the nigh soil. Therefore, the activity of SRB was inhibited for some reason.

Table 1: The number of the colonies

<table>
<thead>
<tr>
<th>Okutone Amenity Park</th>
<th>B. subtilis(cells/g)</th>
<th>SRB(cells/g)</th>
<th>SOB(cells/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>freeze-dried sample</td>
<td>6.6×10^{7}</td>
<td>7.9×10^{7}</td>
<td></td>
</tr>
<tr>
<td>liquid sample</td>
<td>5.0×10^{7}</td>
<td>9.2×10^{7}</td>
<td>6.1×10^{4}</td>
</tr>
<tr>
<td>data from preceding study</td>
<td>8.9×10^{7}~5.43×10^{8} [1]</td>
<td>0.5×10^{7}~32.6×10^{7} [2]</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

We counted B. subtilis, SRB and SOB in the activated sludge of a B. subtilis -dominated. These results suggest that SRB was not small especially. The result that the activity of SRB was limited is not explained. Thus, we need to research about other activated sludge of a B. subtilis -dominated including experiment with model of the night soil treatment plant.

References