A rapid analysis of tungsten in a tungsten-mixed portland cement by use of sulfur-oxidizing bacteria

（硫黄酸化細菌を利用したタングステン含有コンクリート中のタングステンの迅速分析）

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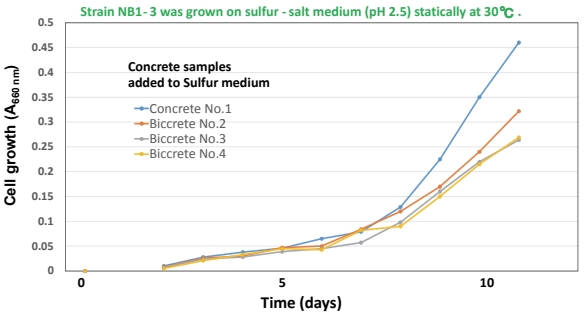
Abstract Corrosion of concrete structures, especially, in sewer system and sewage treatment plants, has become a big social problem. In the process of concrete corrosion, hydrogen sulfide produced in sewage pipes by the action of sulfate-reducing bacteria is oxidized by sulfur-oxidizing bacterium *Acidithiobacillus thiooxidans* to produce sulfuric acid, Portland cement contains calcium oxide, calcium hydroxide, and calcium carbonate, and these compounds react with sulfuric acid to give a sandy calcium sulfate (gypsum). Gypsum production being dependent on *A. thiooxidans* markedly weakens strength of concrete structures. Author has clarified that tungsten (W) strongly inhibits growth of *A. thiooxidans* under acidic conditions and nickel (Ni) inhibits bacterial growth at neutral and slightly alkaline pHs. A portland cement mixed with tungsten and nickel as a bacteriostatic agent was produced to protect concrete corrosion. An effectiveness of the concrete supplemented with W and Ni on concrete corrosion has been reported. In this paper we tried to develop a rapid method to analyze tungsten in a tungsten-mixed portland cement by an activity of sulfur-oxidizing bacteria to produce sulfuric acid.

Method & Results Analysis of tungsten was carried out in a transparent plastic tube with screw cap. A portland cements that contains tungsten (0, 4, 8, and 20 kg/m^3) were prepared and powdered. The reaction mixture (25.5 ml) contains powdered concrete (100 mg), 9K salt medium pH 3.5 (20 ml), 0.1 M β-alanine buffer pH 2.0 (3.0 ml), EDTA-2Na (2 µmol), elemental sulfur (250 mg), tetrathionate-K (6.6 µmol); thiosulfate-Na (0.4 mmol), 0.001% bromocresol green (1.0 ml), and cell suspensions of sulfur-oxidizing bacteria (*A. thiooxidans*, *Thiomonas intermedia, Paracoccus versutus, Starkeya novella*). The reaction was started by addition of concrete. Both pH and color changes were traced every 30 min for 2 h. After 2 h, the reaction mixture containing a cement without tungsten showed greenish yellow (pH 3.92). In contrast, reaction mixtures containing 4, 8, and 20 kg tungsten/m^3 showed greenish blue (pH 4.22), blue (pH 4.57), and blue (pH 4.68), respectively, indicating that this analysis is useful to distinguish rapidly whether a concrete sample contains tungsten or not. Addition of sodium cyanide or sodium tungstate (inhibitor for sulfur oxidase of *A. thiooxidans*) to reaction mixtures increased the speed of blue color development.

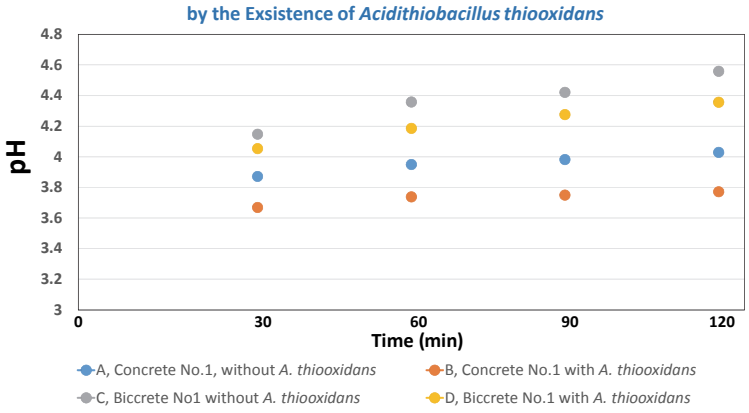
Tungsten Oxide, Nickel, and Cement Contents in Concrete Samples Used in this Study

Concrete	Nickel (mg/kg)	Tungsten oxide (mg/kg)	Cement (kg/m^3)
Concrete No.1	14.8	2.4	387
Concrete No.2	-	-	440
Concrete No.3	16.0	4.2	440
Concrete No.4	-	-	435
Biccrete No.1	102	163	387
Biccrete No.2	206	202	387
Biccrete No.3	458	484	387
Biccrete No.4	936	1010	387

Growth of *Acidithiobacillus thiooxidans* strain NB1-3 in Sulfur Medium Supplemented with 25 mg of Concrete Sample



pH Increase Caused by Concrete Addition wasRepressed by the Exsistence of *Acidithiobacillus thiooxidans*



Composition of a Reaction Mixture used for Analysis of Tungsten in Concrete

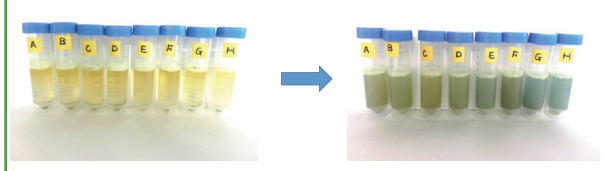
The analysis of tungsten in concrete sample was done in a transparent plastic tube with screw cap.
The composition of a reaction mixture was shown below. The total volume was 25.5 ml.

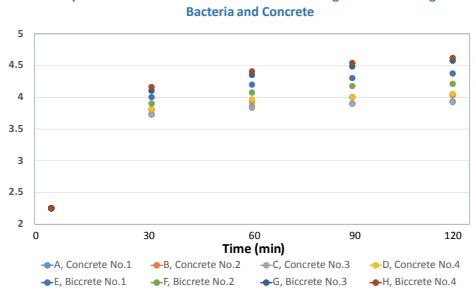
1. Sulfur-Oxidizing Bacteria
   - *Acidithiobacillus thiooxidans* NB1-3 ····· 0.1 ml
   - *Thiomonas intermedia* NBRC 14564 ····· 0.2 ml
   - *Paracoccus versutus* NBRC 14568 ····· 0.2 ml
   - *Starkeya novella* NBRC 14993 ····· 0.2 ml
2. Energy Source for sulfur-oxidizing bacteria
   - Elemental sulfur ····· 250 mg
   - Patassium tetrathionate ····· 6.6 µ mol
   - Sodium thiosulfate ····· 0.4 mmol
3. 0.1 M β-alanine-SO_4^{2-} buffer (pH 2.0) ····· 3.4 ml
4. 9K basal salt medium (pH 2.5) ····· 20 ml
5. EDTA-2Na ····· 0.002 mmol
6. 0.001% Bromocresol green solution ····· 1.0 ml
7. Concrete sample powdered ····· 100 mg

Color Change of a Reaction Mixture after 120 min of Incubation

Concrete sample used
A, Concrete No.1 — E, Biccrete No.1
B, Concrete No.2 — F, Biccrete No.2
C, Concrete No.3 — G, Biccrete No.3
D, Concrete No.4 — H, Biccrete No.4

0 min

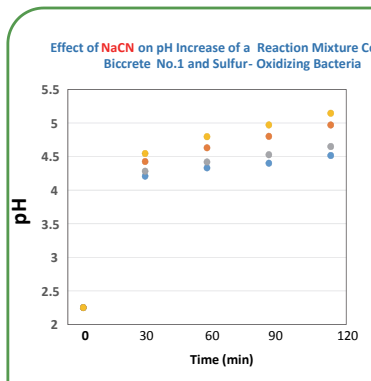




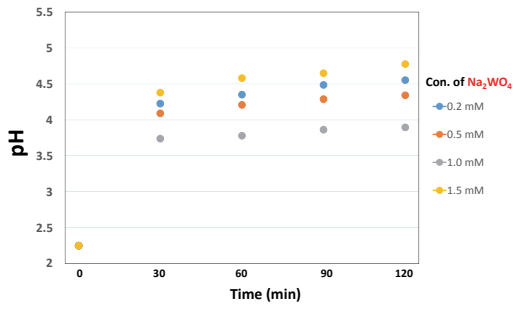
Sulfur-oxidizing bacteria used

Acidithiobacillus thiooxidans
Thiomonas intermedia
Paracoccus versutus
Starkeya novella

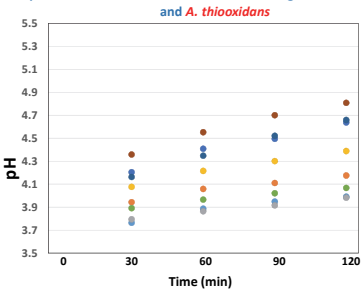
Effect of NaCN on pH Increase of a Reaction Mixture Containing Biccrete No.1 and Sulfur- Oxidizing Bacteria



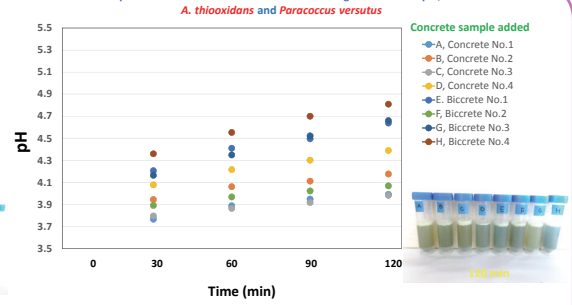
Effect of Na_2WO_4 on pH Increase of a Reaction Mixture Supplemented with Biccrete No.1, *A. thiooxidans, and Starkeya novella*



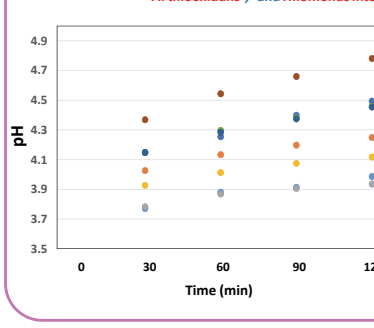
pH Increase of a Reaction Mixture Containing Concrete Sample and *A. thiooxidans*



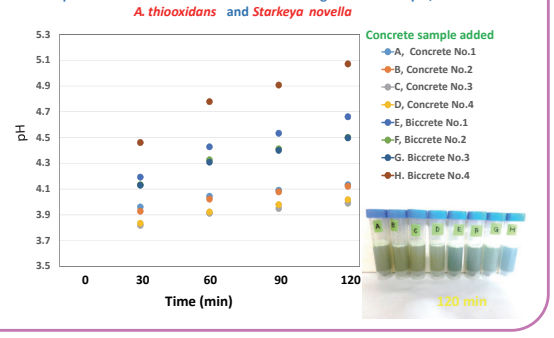
pH Increase of a Reaction Mixture Containing Concrete Sample, *A. thiooxidans* and *Paracoccus versutus*



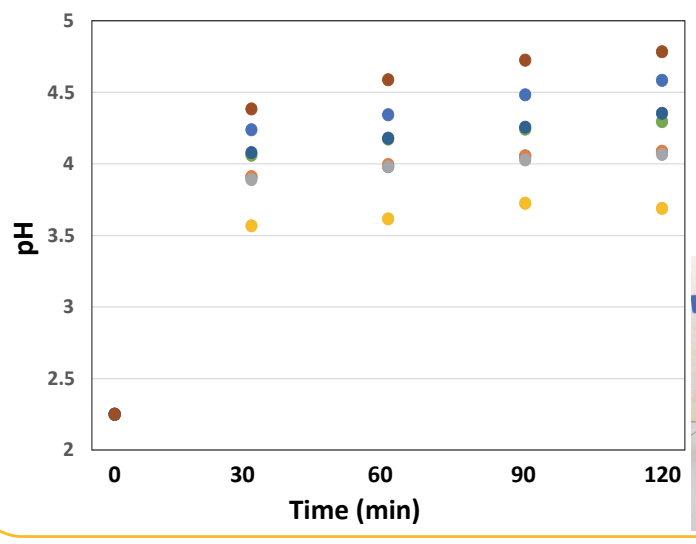
pH Increase of a Reaction Mixture Containing Concrete Sample, *A. thiooxidans* , and *Thiomonas intermedia*



pH Increase of a Reaction Mixture Containing Concrete Sample, *A. thiooxidans* and *Starkeya novella*



pH Increase of a Reaction Mixture Containing Concrete Sample, *A. thiooxidans* , *Thiomonas intermedia* and *Starkeya novella*



Conclusion

1. Four plain concretes (Concrete No.1, 2, 3 and 4) and four concretes supplemented with tungsten and nickel (Biccretes No. 1, 2, 3 and 4) were prepared. These eight concretes were powdered.
   Each of the powdered concretes (100 mg) was added to the acid solution (25.5 ml) containing an acid-base indicator (BCG), sulfur-oxidizing bacteria, energy sources for sulfur-oxidizing bacteria, and EDTA-2Na.
   After a vigorous shaking in a transparent plastic tube with a screw cap, both color and pH changes of these acid solutions were traced every 30 min for 2 h.
2. We found the best condition to distinguish a plain concrete from Biccrete by use of a color change of acid-base indicator (BCG). The results obtained from color change were confirmed by use of a pH meter.
   After incubation of eight concrete samples for 2 h, four plain concretes gave greenish yellow (pH 3.8～ 4.1).
   In contrast, four Biccretes containing tungsten gave blue (pH 4.4～5.1).
3. The following results indicate that a rapid analysis of tungsten in tungsten-mixed portland cements is based on a microbiological activity of sulfur-oxidizing bacteria to produce sulfuric acid from inorganic sulfur compounds and this bacterial activity is inhibited by tungsten dissolved from a tungsten mixed concrete, but not from a plain concrete without tungsten.
   (a) pH increase or blue color development in an acid solution was accelerated by 2 mM of sodium cyanide and 1.0 mM of tungstate.
   These chemicals are known as a potent inhibitor for the enzymes that is involved in sulfuric acid production in *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans.*
   (b) It was found that *A. thiooxidans*, *Thiomonas intermedia*, *Starkeya novella*, but not *Paracoccus versutus*, are most preferable sulfur-oxidizing bacteria for the analysis of tungsten in concrete.