

P49 Distribution of Biomineralization Ability of Ureolytic Bacteria in Waste Landfills



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Introduction Toxic metals might be immobilized in presence of ureolytic bacteria

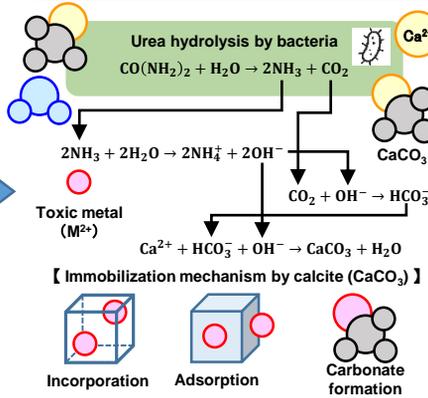
Research background

Landfill Hazardous waste containing toxic metals is mainly disposed in controlled landfill following to metal immobilization treatment.

Minerals Secondary minerals generated by the weathering such as calcite (CaCO₃) can immobilize toxic metals at controlled landfill.

MICP Microbially induced carbonate precipitation (MICP) by ureolytic bacteria inhabiting in nature has been studied owing to effective method for biomineralization of toxic metals.

Biomineralization by ureolytic bacteria



Possibility

Toxic metals in controlled landfill might be immobilized in presence of ureolytic bacteria.

【 Research objectives 】

- To investigate inhabitation of ureolytic bacteria in controlled landfill.
- To evaluate their ability of urea hydrolysis.

Method 1 Selection for candidate of ureolytic bacteria by cultivation

- Fifteen drilled core samples collected from 3 controlled landfills (A, B, and C)

Core sample 1.0 g (wet basis) Sterile water 9.0 mL

Preparing suspension of the sample

1.0 mL of suspension was inoculated into 100 mL of Christensen's medium

Incubated at 30°C for 7 days under shaking condition (100 rpm) : Fig. 1

Color change of the medium from orange to pink is "positive"

Growth of colonies by the agar (Fig. 2) Bacterial cultivation in test tube (Fig. 3)

Table 1 Composition of Christensen's medium

Components	g / L
Peptone	1.0
NaCl	5.0
Glucose	1.0
KH ₂ PO ₄	2.0
Phenol red	0.012
Urea [CO(NH ₂) ₂]	20.0
pH	6.8±0.2

Christensen's medium are supplemented with sterile urea and phenol red as a pH indicator. Hence, when urea hydrolysis occurred by ureolytic bacteria, altering the color of phenol red by pH increasing.

Result 1 Over 70 colonies of candidate of ureolytic bacteria were obtained

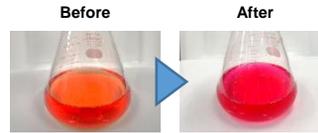


Fig. 1 Color change of the medium

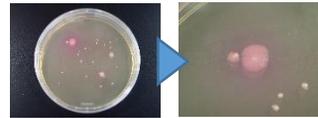


Fig. 2 Colonies on the agar



Fig. 3 Cultivation of obtained colonies

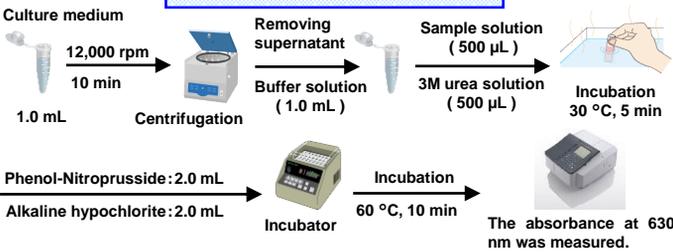
- Ureolytic activity were found in 11 samples out of 15 samples.
- Possibility that prevalence of ureolytic bacteria in controlled landfill.

- Colony formation consisting of ureolytic bacteria whose the surrounding changed pink color.

- Obtaining over 70 colonies which are not losing urease activity after repeating cultivation.
- Measuring of their ureolyzing activity by indophenol method.

Method 2 Measuring of ureolyzing activity

Measuring of NH₄⁺ by indophenol method



Measuring of the number of cells

The number of cells in culture medium was measured by ATP analyzer using ATP detection assay kit.



Definition of "ureolyzing activity"

One unit = The amount of 1 pmol urea hydrolyzed per min per cell (pmol/min/cell)

Result 2 Lower ureolyzing activity

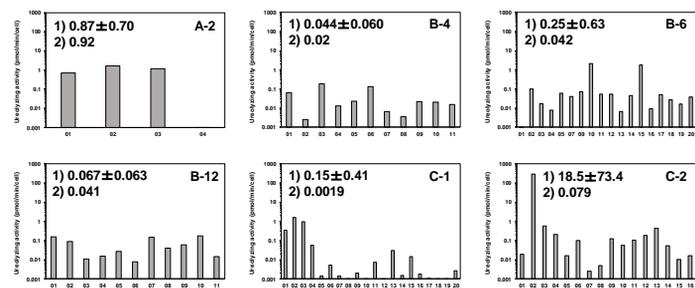


Fig. 4 Ureolyzing activity of each strain obtained from drilled core samples
1) Average ± standard deviation, 2) Median value

- The ureolyzing activity of each strain inhabiting in controlled landfill had significant variations (more than 10,000 times).
- It was also significantly lower than that in previous researches.

Conclusion Biomineralization of toxic metals by ureolytic bacteria might require long periods in controlled landfills owing to lower ureolyzing activity.