The Influence of Salinity and Ammonium Levels on *amoA* mRNA Expression of Ammonia Oxidizing Prokaryotes

T. Fukushima\(^1\), Y. J. Wu\(^1\) and L.M. Whang\(^1,2,\)

\(^1\)Department of Environmental Engineering,  
\(^2\)Sustainable Environment Research Center (SERC), National Cheng Kung University
Factor in shaping ammonia oxidizing archaeal (AOA)/bacterial (AOB) community

Ammonium

Temp.

Sulfide

Phosphate

Salinity

Organic carbon

$pH$

DO

Factor in shaping ammonia oxidizing archaeal (AOA)/ bacterial (AOB) community

Ammonium

Salinity

Difficulties of AOA/AOB research

• Only few AOA isolates
• Slow growing

The *amoA* mRNA-based analysis may allow us to understand AOA-AOB competition in a short amount of time.
Investigate the influence of salinity and ammonium levels on AOA-AOB competition by using amoA mRNA based analysis
Materials and Methods
## Experimental overview

### Mini-CSTR operation

![Image of Mini-CSTR operation](image)

### Sample preparation

- **RNA extraction**
- **Reverse transcription (RT)**

<table>
<thead>
<tr>
<th>RNA</th>
<th>cDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>cDNA</td>
</tr>
</tbody>
</table>

### Sample preparation

- **RNA extraction**
- **Reverse transcription (RT)**

### Quantitative analysis

#### Influence on AOA-AOB competition

![Graph showing influence on AOA-AOB competition](image)

#### Real-time PCR

![Real-time PCR graph](image)

### AOA Community analysis

#### Influence on AOA community

![Graph showing influence on AOA community](image)

#### T-RFLP

![T-RFLP analysis](image)

#### PCR

![PCR analysis](image)
Mini-continuous stirred tank reactor (Mini-CSTR) Operation

Working vol.: 1.0L  
HRT: 10 days  
pH: 7.2±0.1

<table>
<thead>
<tr>
<th>Seeding sludge</th>
<th>Ammonium loading (mgN/L/day)</th>
<th>Salinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. A</td>
<td>AOA sludge (lab-scale reactor)</td>
<td>2mgN/L/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7%</td>
</tr>
<tr>
<td>Exp. B</td>
<td>AOA sludge + AOB sludge (full scale WWTP)</td>
<td>3mgN/L/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7%</td>
</tr>
<tr>
<td>Exp. C</td>
<td>AOA sludge + AOB sludge</td>
<td>2 mgN/L/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mgN/L/day</td>
</tr>
</tbody>
</table>
Results and Discussion
Effect of salinity level on ammonia oxidation

Exp. A
(AOA sludge) (2mgN/L/day)

Exp. B
(AOA + AOB sludges) (3mgN/L/day)

Ammonia oxidation was slightly inhibited under high salinity condition
Effect of salinity level on AOA-AOB competition

 amoA mRNA expressions by both of AOB and AOA were repressed under high salinity condition

High salinity

Low salinity

Exp. A

Exp. B
Effect of salinity level on AOA community

Influence of salinity levels may be different among AOA species.
Effect of salinity level

- High salinity level inhibited ammonia oxidization.
- High salinity level repressed both of archaeal and bacterial *amoA* mRNA expression.
- T-RFLP results implied that influence of salinity levels was different among AOA species.
Effect of ammonium levels on ammonia oxidization

Exp. C (AOA + AOB sludges)

Ammonia accumulation was observed under high ammonium loading condition.
amoA mRNA expressions by AOA was repressed under high ammonium condition, while that by AOB was not affected.
Effect of ammonium levels on AOA community

Influence of ammonium levels may be different among AOA species

10mgN/L/day

2mgN/L/day
Conclusions

- Mini-CSTR operation and mRNA-based analyses allowed us to understand the influence of environmental factors on AOA/AOB in a short time.

- High salinity and ammonium levels repressed archaeal \textit{amoA} mRNA expression, while only high salinity level repressed bacterial \textit{amoA} mRNA expression.

- T-RFLP results implied that the influence of salinity and ammonium levels were different among AOA species.
Acknowledgement

This work was supported by Sumitomo Foundation.