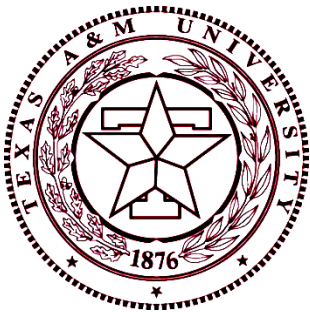


# Isolation and Characterization of Nitroguanidine(NQ)-degrading Bacteria



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# Project Team (ER19-1198)

## Membrane Bioreactor System for Cost Effective Treatment of Munitions Constituents Manufacturing Wastes

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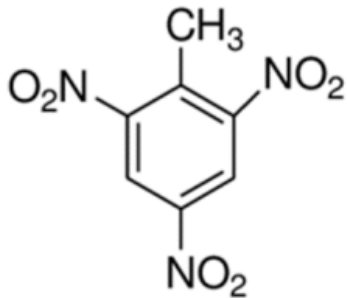
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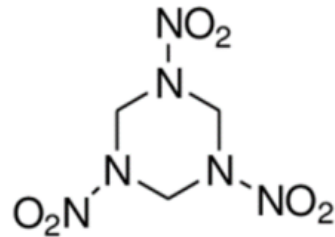
# Munitions



## Legacy and current munitions



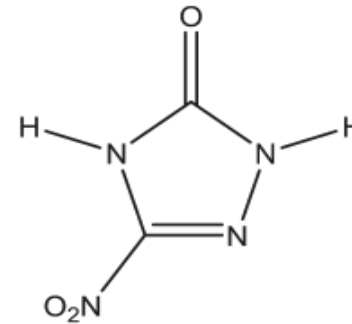
**TNT**



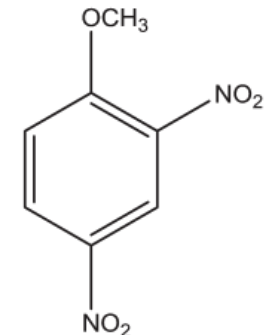
**RDX**

Sensitive	Toxicity	Property
<b>TNT</b> (before WWII)	Carcinogen	<ul style="list-style-type: none"><li>• Sorbs to soils</li></ul>
<b>RDX</b> (after WWII)	Possible carcinogen	<ul style="list-style-type: none"><li>• Sorbs poorly to soils.</li><li>• A common GW contaminant</li></ul>

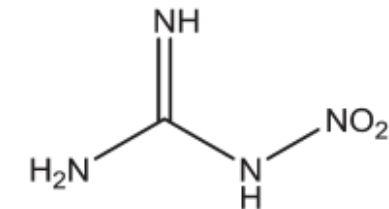
## Insensitive munitions



**NTO**



**DNAN**

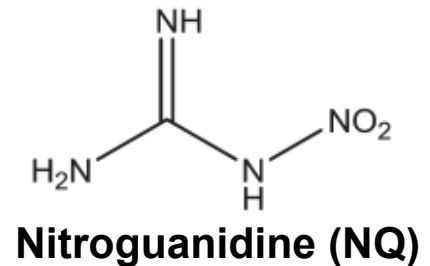


**Nitroguanidine (NQ)**

- being developed to
  - ✓ resist external impact, friction, heat, or sparks from unexpected events
  - ✓ minimize the acute hazards

# Nitroguanidine (NQ)

- colorless, crystalline solid
- melts at 257 °C and decomposes at 254 °C.
- Nitroguanidine is an extremely insensitive but powerful high explosive.
- Other uses:
  - As precursors for herbicides
- Acute, and chronic toxicity against mice, aquatic organisms, and plants
- Ammonium nitrate as residual (~10%) could be left during NQ synthesis waste streams



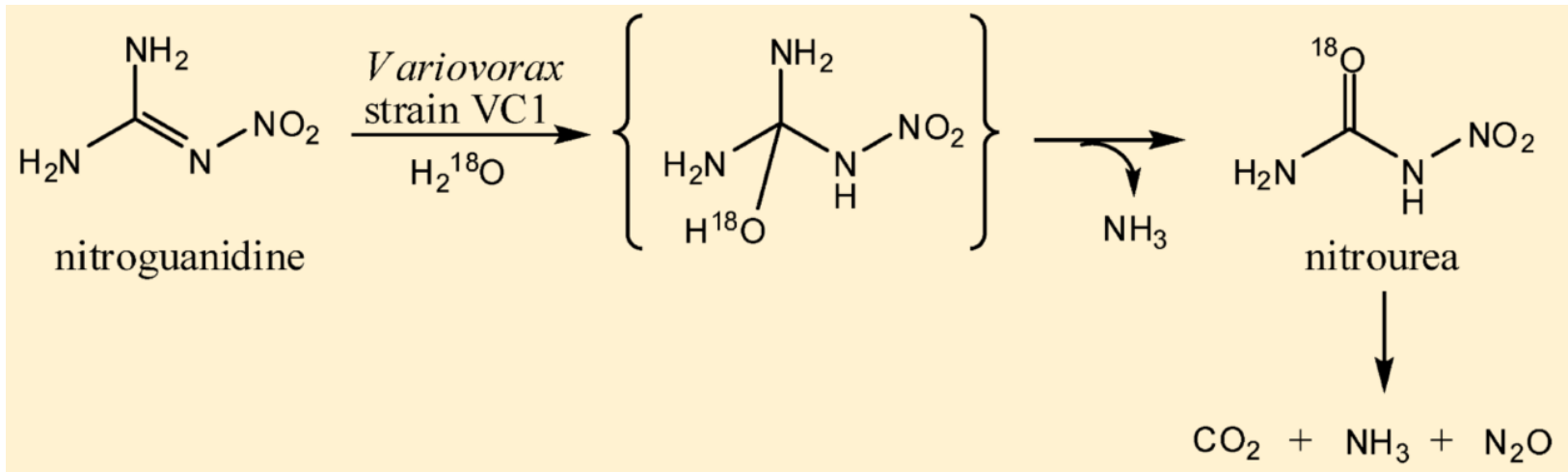
## **Abiotic NQ degradation:**

- **photolysis and catalytic reactions**
- **Reductive degradation by Iron-based minerals**

**=> more toxic metabolites**

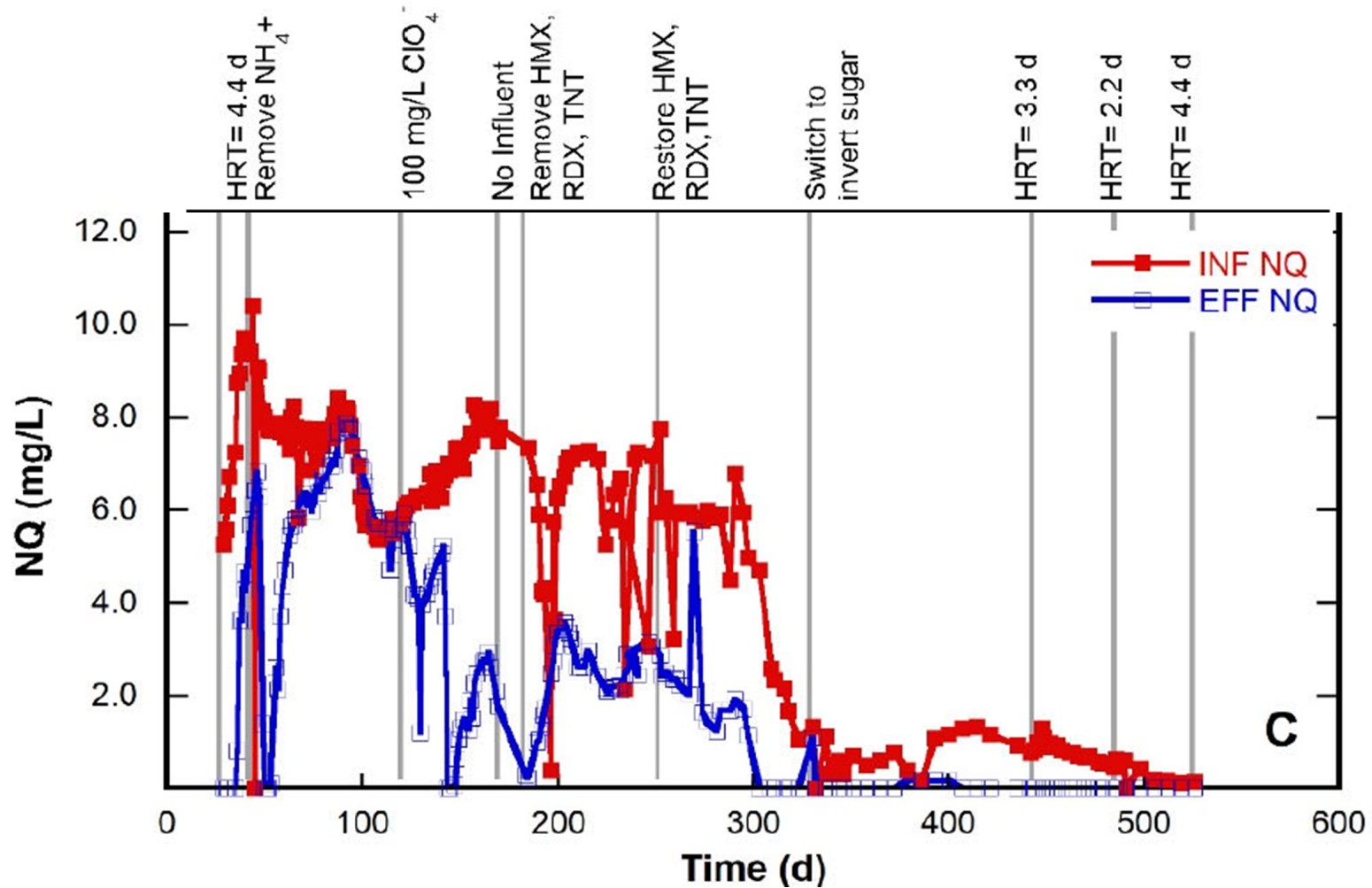
# Biodegradation of Nitroguanidine (NQ)

## Aerobic biodegradation of NQ by *Variovorax* strain VC1

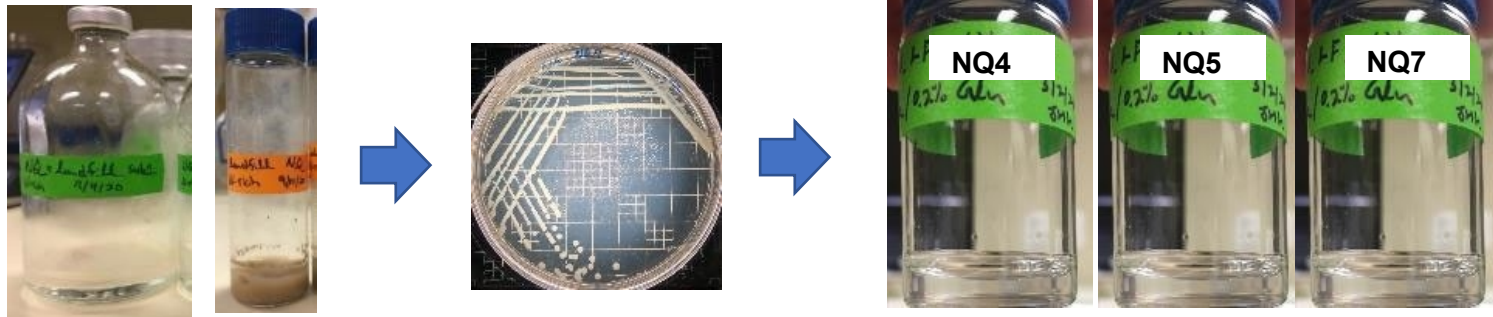


- Only 1 bacterium has been identified and characterized. Strain VC1 is an aerobic bacterium capable of using NQ as a sole nitrogen source

# Complete NQ degradation in Aerobic Membrane Bioreactor (MBR)



# Enrichment and Isolation of NQ-degraders



## Enrichment sources

- Sediments from Galveston Bay, TX
- AFFF-impacted soils from San Antonio, TX
- Biomass from a NQ-degrading membrane bioreactor

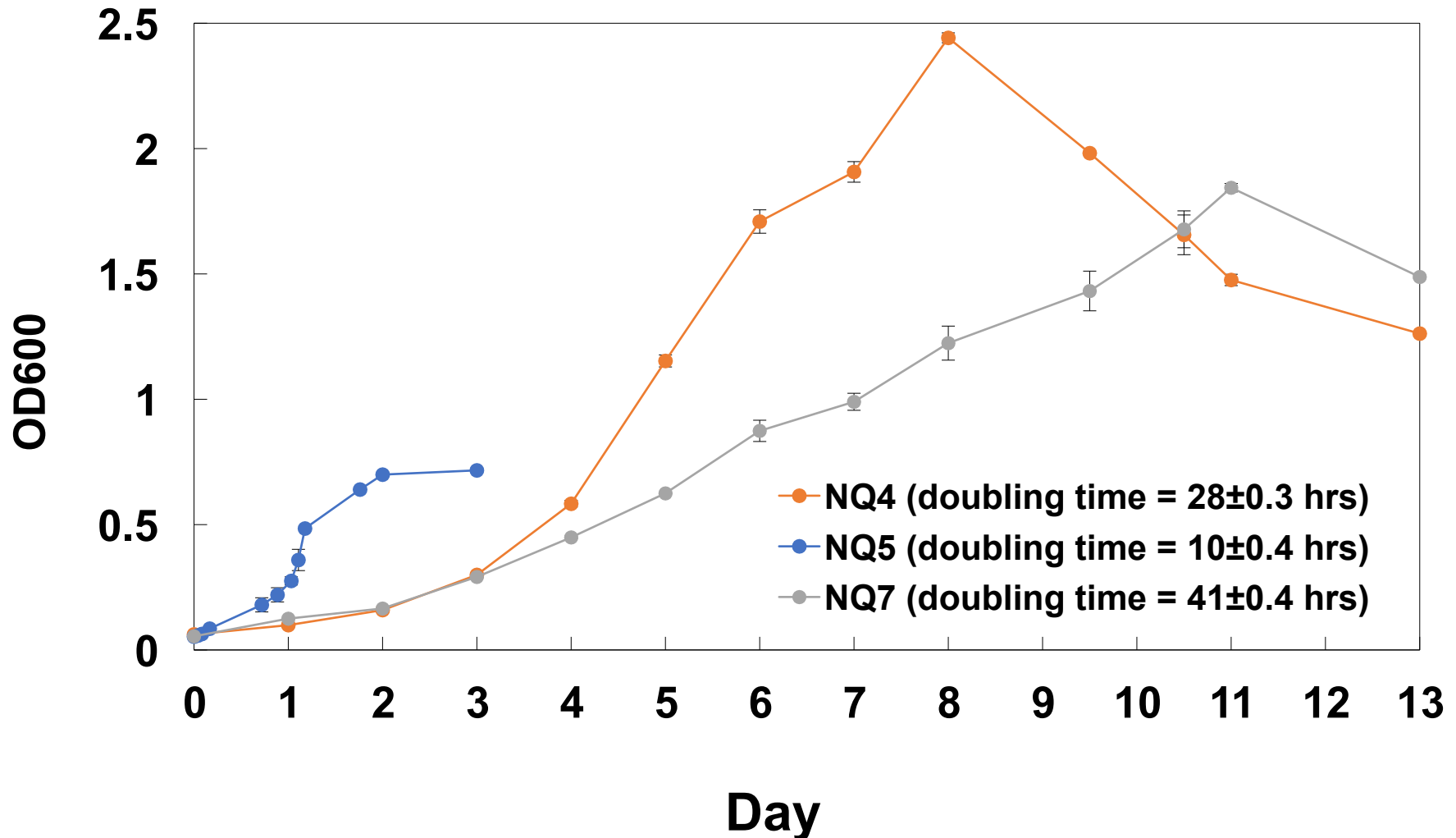
**N-source:** NQ (2 mM)

**C-source:** glucose (11 mM)

**Nitrogen-free mineral salts medium**

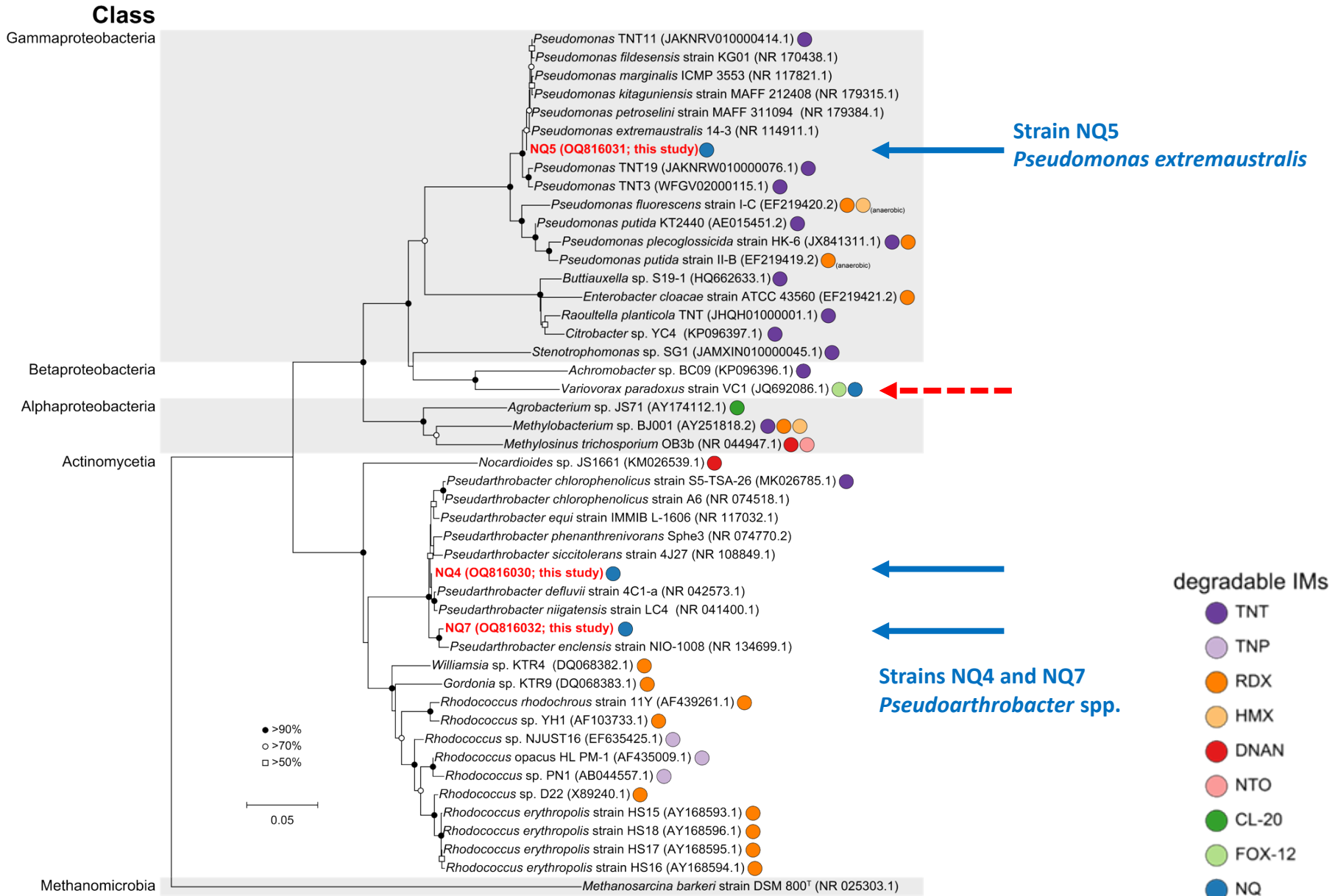
# Growth Curves of 3 NQ-degrading Cultures

## NQ as a Sole Nitrogen Source





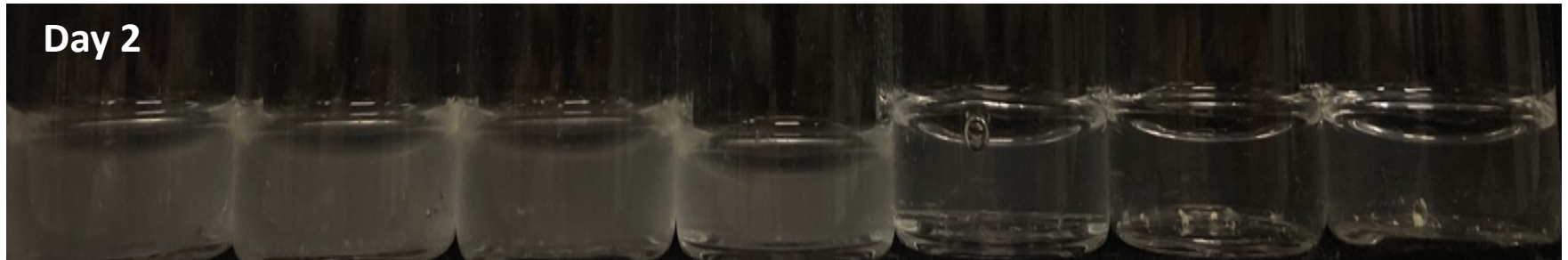
# Identification of 3 Isolates



# Is strain NQ5 salt tolerant?

Positive control

No NaCl	NaCl 0.5%	NaCl 1 %	NaCl 2 %	NaCl 5%	NaCl 10 %	NaCl 15 %
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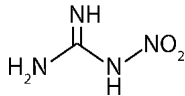
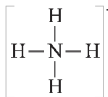

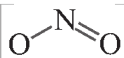
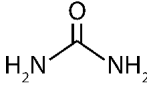
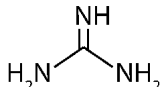
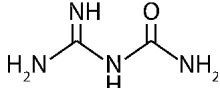
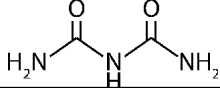
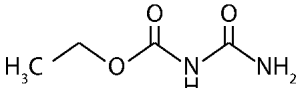
Growth	+	+	+	+	-	-	-
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Positive control: strain NQ5 in N-free MSM + 0.2% glucose+ 2mM NQ

**=> Strain NQ5 has a potential to treat NQ in saline wastewater**

# Can isolates grow on different N sources?

## Growth on inorganic nitrogen and NQ analogs

As N-source	Chemical structure	NQ4 (soil)	NQ7 (sediment)	NQ5 (MBR)
<b>Nitroguanidine (NQ)</b>		+	+	++
<b>Ammonium</b>		++	++	++
<b>Nitrate</b>		++	++	++
<b>Nitrite</b>		++	++	-
<b>Urea</b>		++	++	++
<b>Guanidine</b>		+	+	++
<b>Guanylyurea</b>		-	-	+
<b>Biuret</b>		-	-	-
<b>Ethyl allophanate</b>		-	-	++

- **NQ5 can degrade**

- ✓ Nitroguanidine
- ✓ Guanidine
- ✓ Guanylyurea
- ✓ Ethyl allophanate

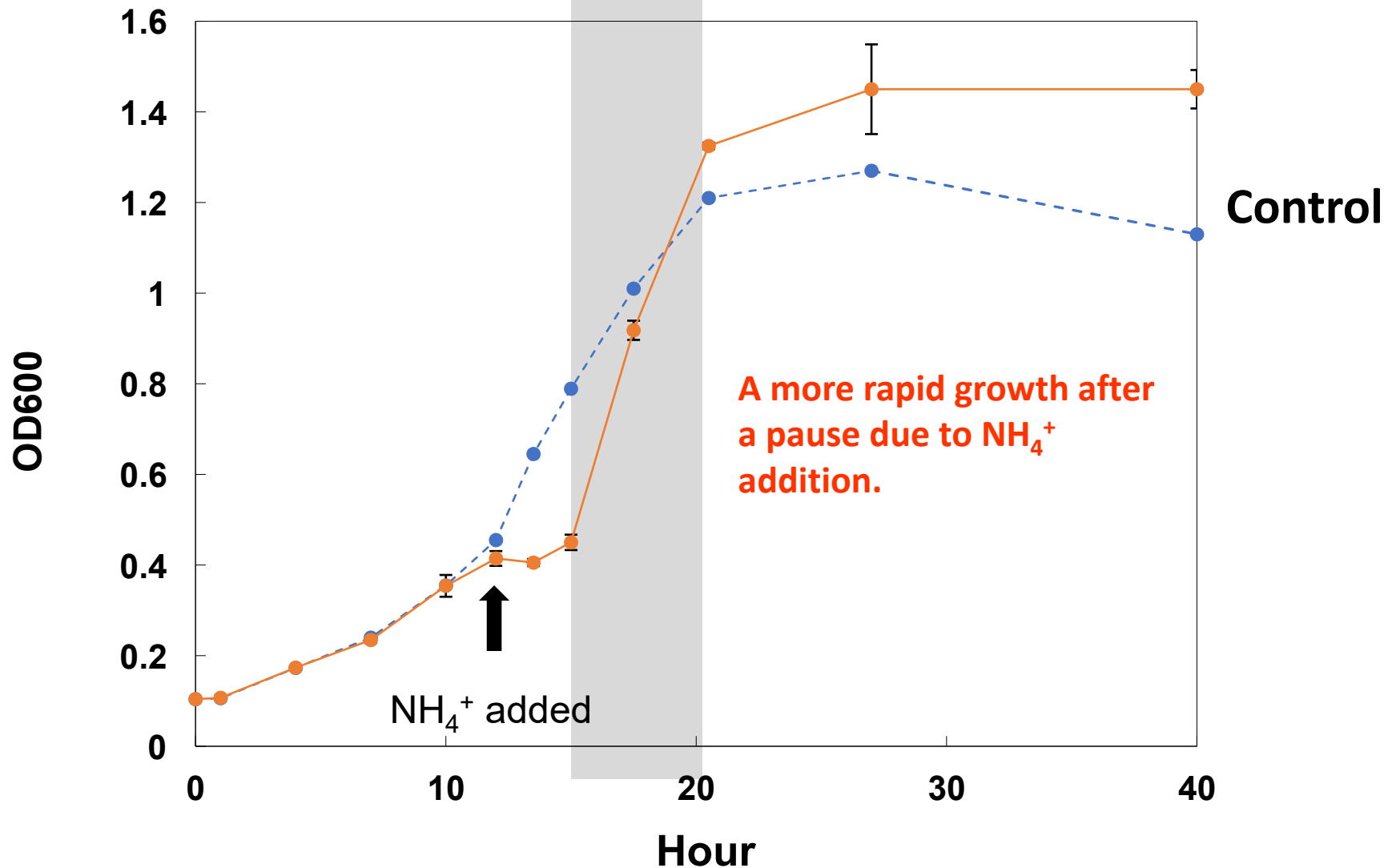
• NQ5 can't use nitrite.

• Biuret can't be used by all three isolates.

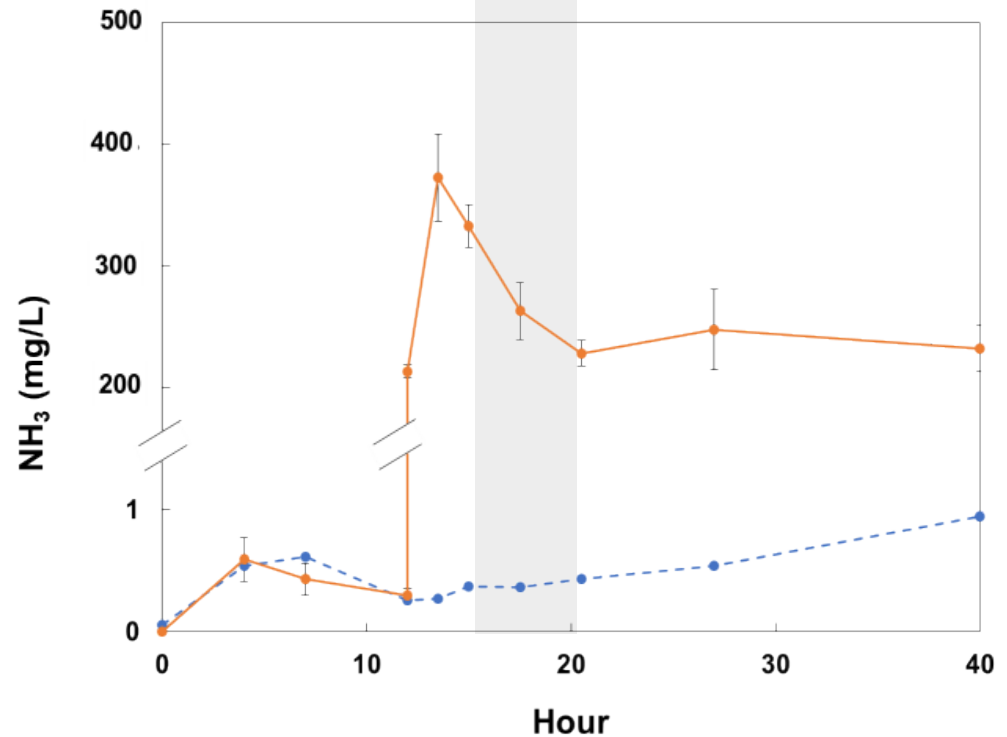
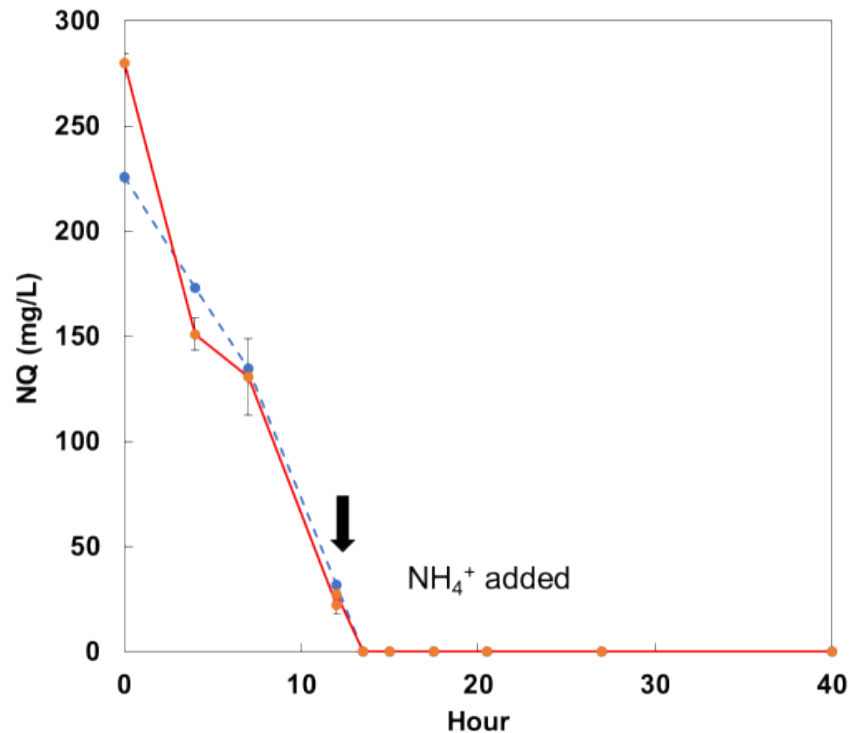
Do different nitrogen sources affect NQ degradation by strain NQ5?

# Effects of $\text{NH}_4^+$ on NQ degradation

## NQ-grown Strain NQ5



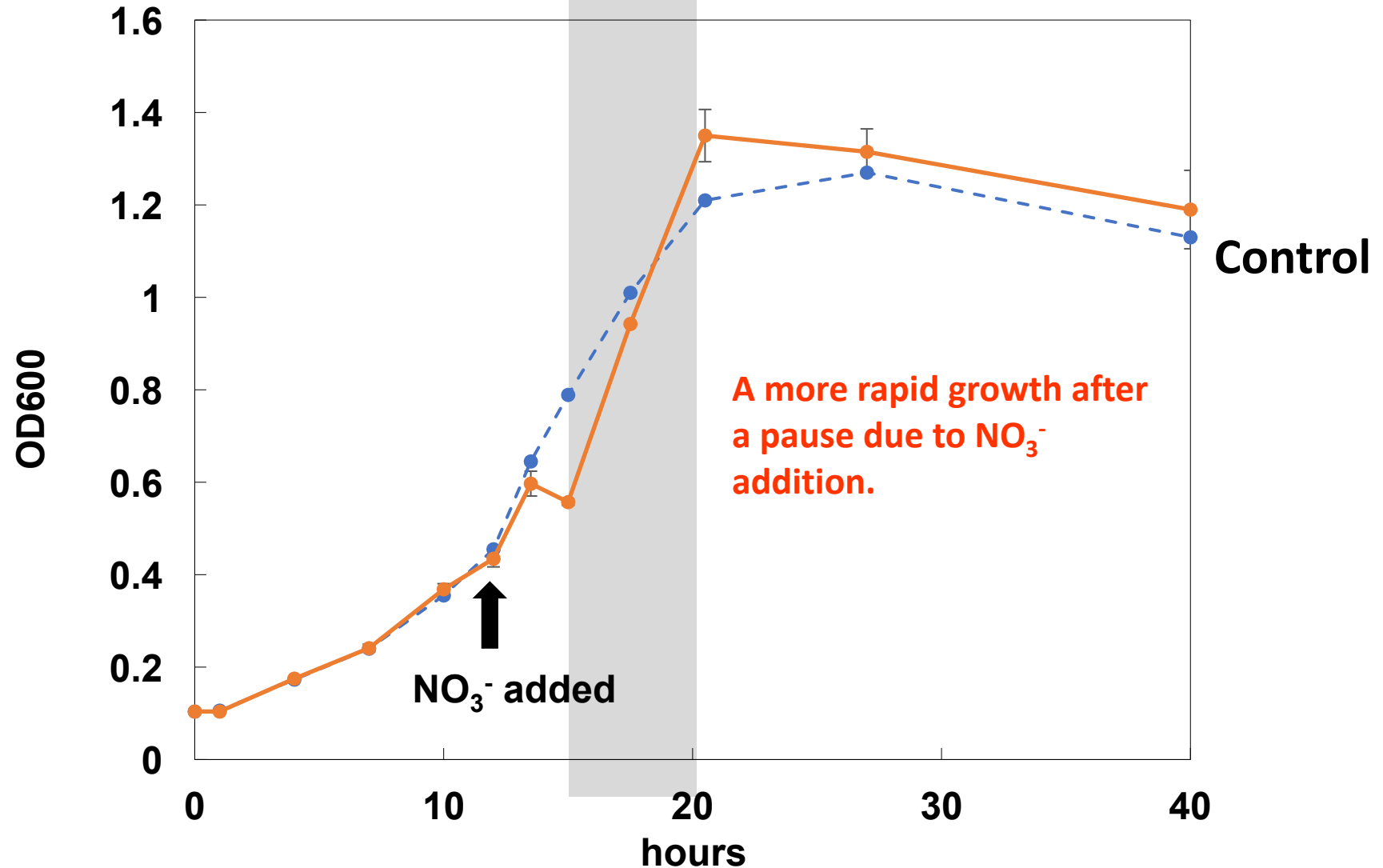
# Effects of $\text{NH}_4^+$ on NQ degradation



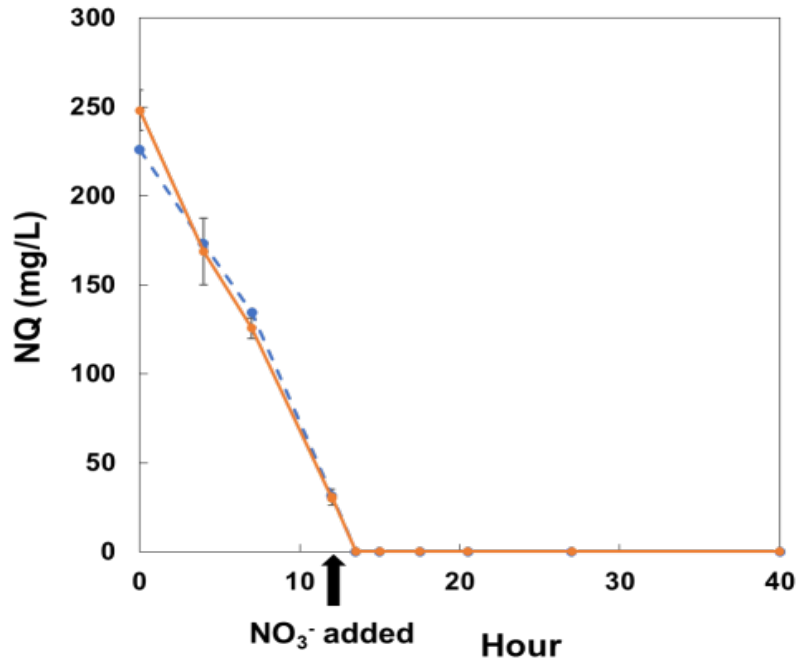
- When  $\text{NH}_4^+$  was spiked at hour 12, strain NQ5 continued degrading NQ until it was depleted. However, no cell growth was observed during shortly after  $\text{NH}_4^+$  addition.
- No  $\text{NO}_3^-$  was not detected.

# Effects of $\text{NO}_3^-$ on NQ degradation

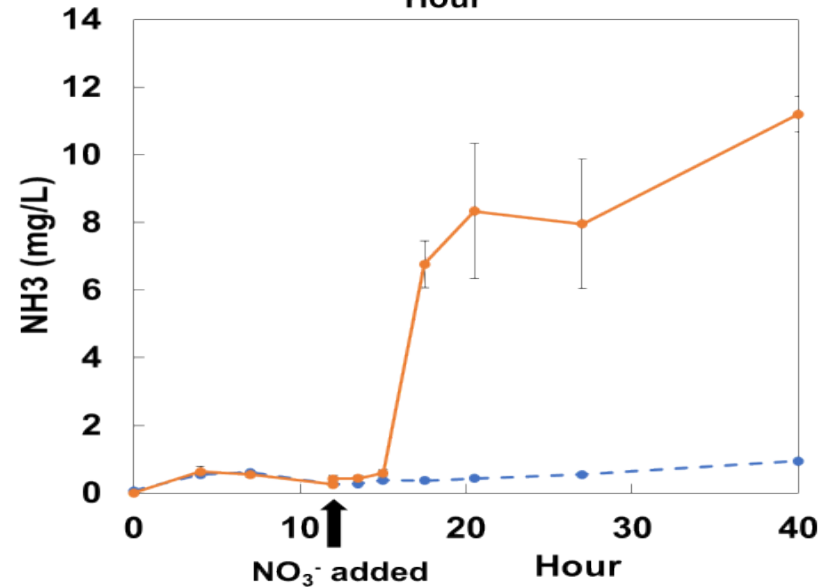
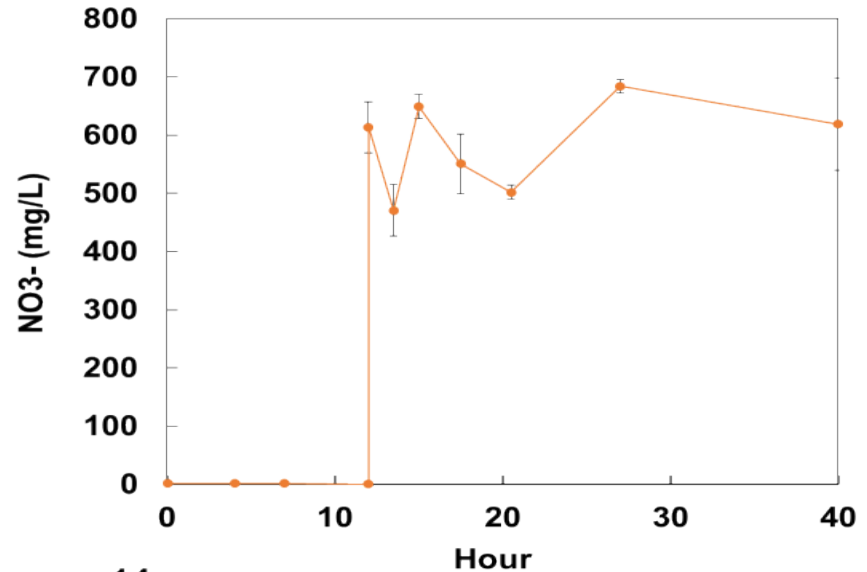
## NQ-grown Strain NQ5



# Effects of $\text{NO}_3^-$ on NQ degradation



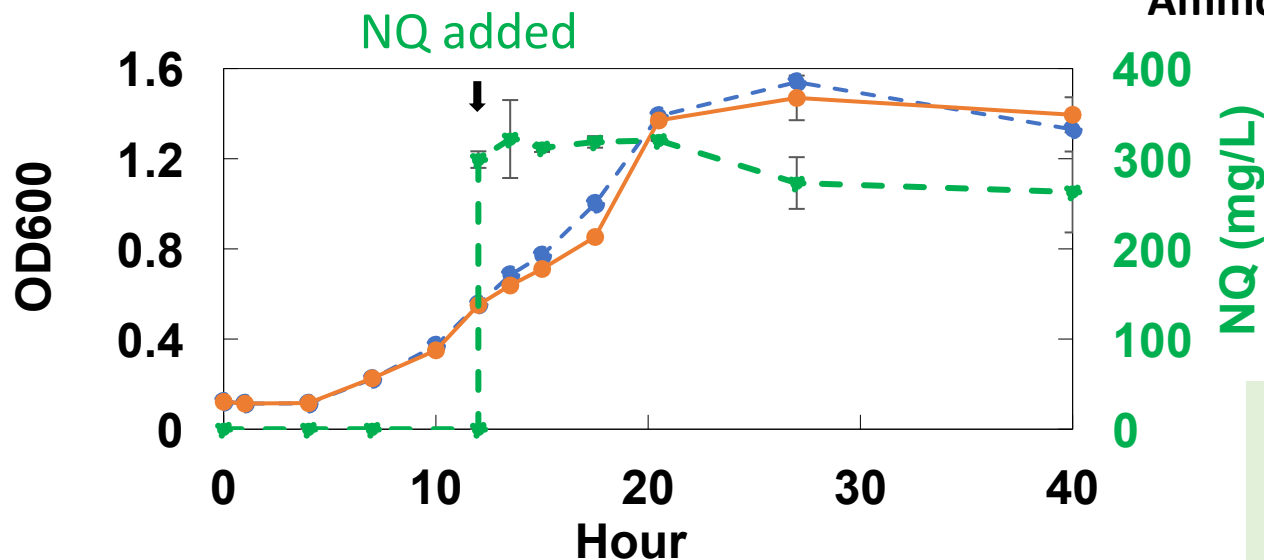
- When  $\text{NO}_3^-$  was spiked at hour 12, strain NQ5 continued degrading NQ until it was depleted. No immediate pause of cell growth until NQ was depleted.
- Accumulation of  $\text{NH}_4^+$  was observed around hour 4-15.



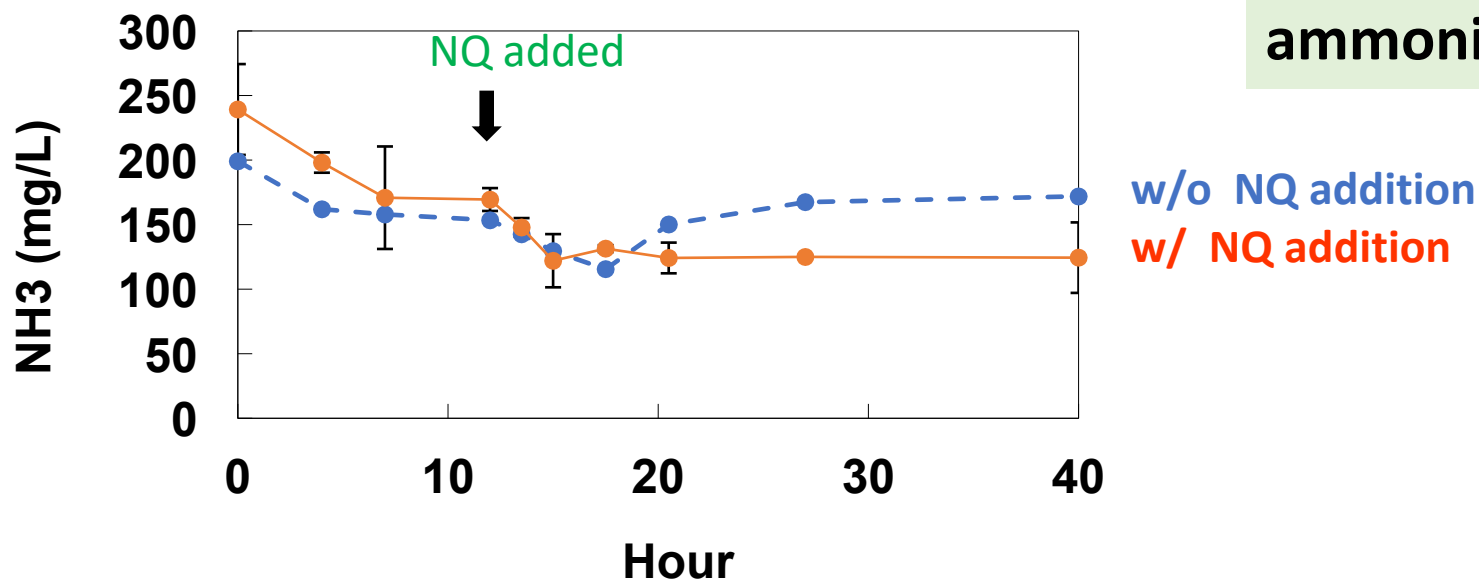


# Can NQ be degraded by $\text{NH}_4^+$ -grown strain NQ5 ?

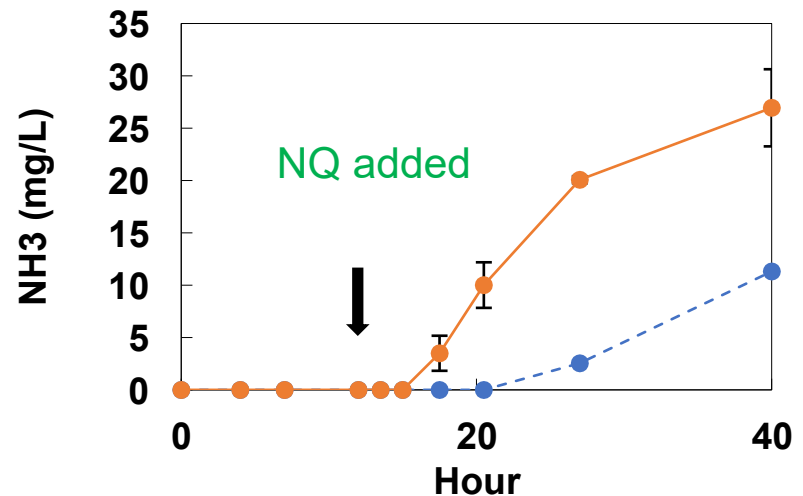
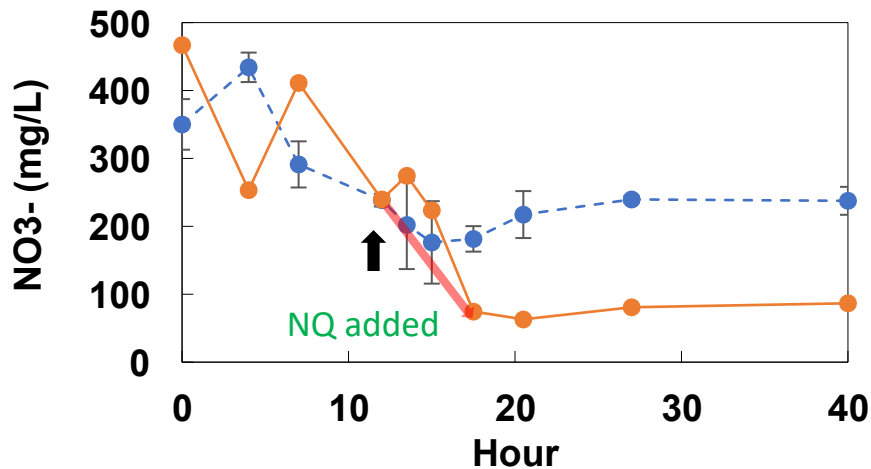
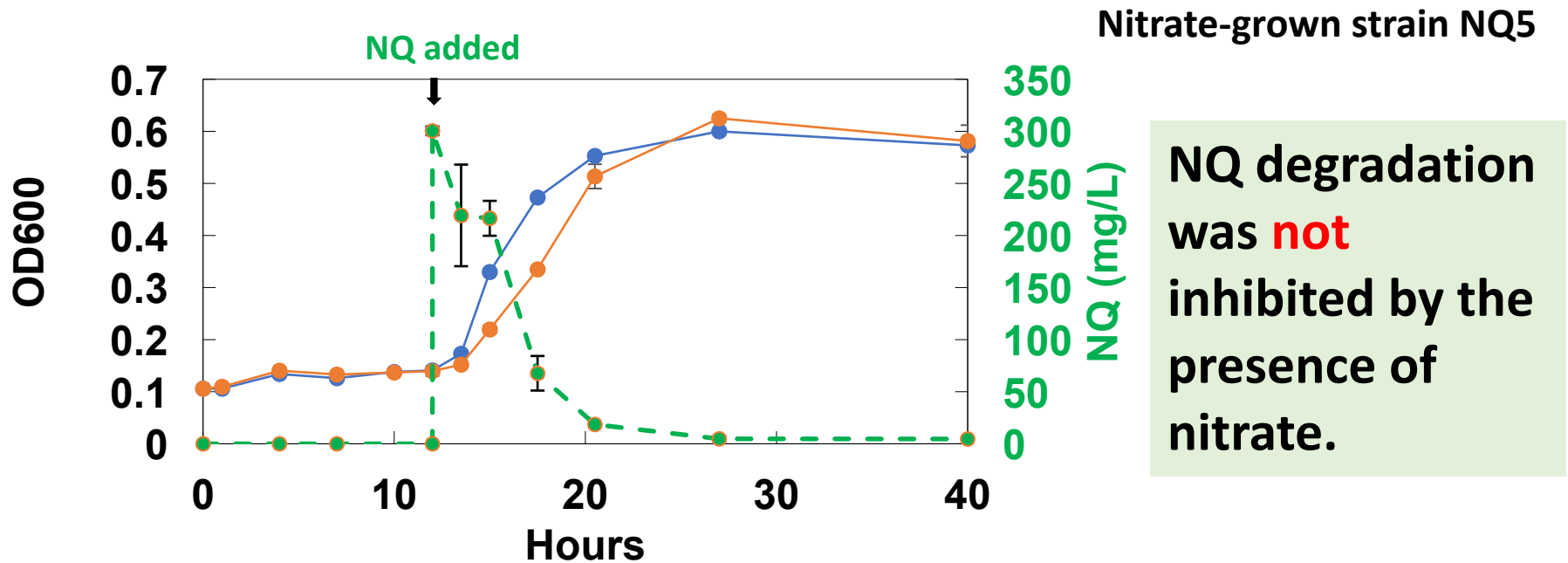
Ammonium-grown strain NQ5



NQ degradation was inhibited by the presence of ammonium.



# Can NQ be degraded by $\text{NO}_3^-$ -grown strain NQ5 ?



# Take Home Messages

- Three NQ-utilizing bacteria, strains NQ4, NQ5, and NQ7, were isolated from soil, an aerobic MBR, and marine sediment, respectively. All three strains can utilize NQ as a sole nitrogen source.
- Strain NQ5
  - exhibited a 4-fold faster NQ degradation compared to that of the only known NQ degrader (*Variovorax* strain VC1),
  - can tolerate up to 2% NaCl.
  - can utilize guanidine, guanylurea, and ethyl allophanate, but not nitrite as N source
- NQ degradation was not inhibited by the presence of nitrate, but ammonium.

# Future Works

- Identification of functional genes in the involving in NQ degradation in strains NQ4, 5, and 7.

## XenA and XenB genes in NQ5

XenA (CDS 87.49% similarity with *Psuedomonas putida* XenA)

XenB (CDS 84.25% similarity with *Psuedomonas putida* XenB)

- Assess cometabolic degradation of other types of IMs by strain NQ5
- Assess feasibility of bioaugmentation with NQ-degraders for enhanced NQ removal.

THANK YOU 😊

