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# Comammox, selfish predator for urea

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## Introduction

The discovery of complete ammonia oxidizer (comammox) *Nitrospira* revolutionized our understanding of nitrification. However, despite the identification of comammox genomes with potential urea hydrolysis capabilities, direct evidence of their ability to hydrolyze urea remains elusive. This raises questions about their competition with heterotrophic microorganisms, which also possess urease genes in mixed cultures. To address these gaps in knowledge, this study aims to achieve the following objectives:

### Objectives

1. Cultivation of urea-hydrolyzing and -oxidizing community.
2. Validation of comammox enrichment.
3. Show comammox dominance in urea utilization.

## Methods and Results

1. Two long-term reactors for comparative enrichment were operated for >400 days, fed with ammonia and urea at pH 6, respectively (Figure 1). Nitrogen-species and 16S rRNA detections were perform regularly.

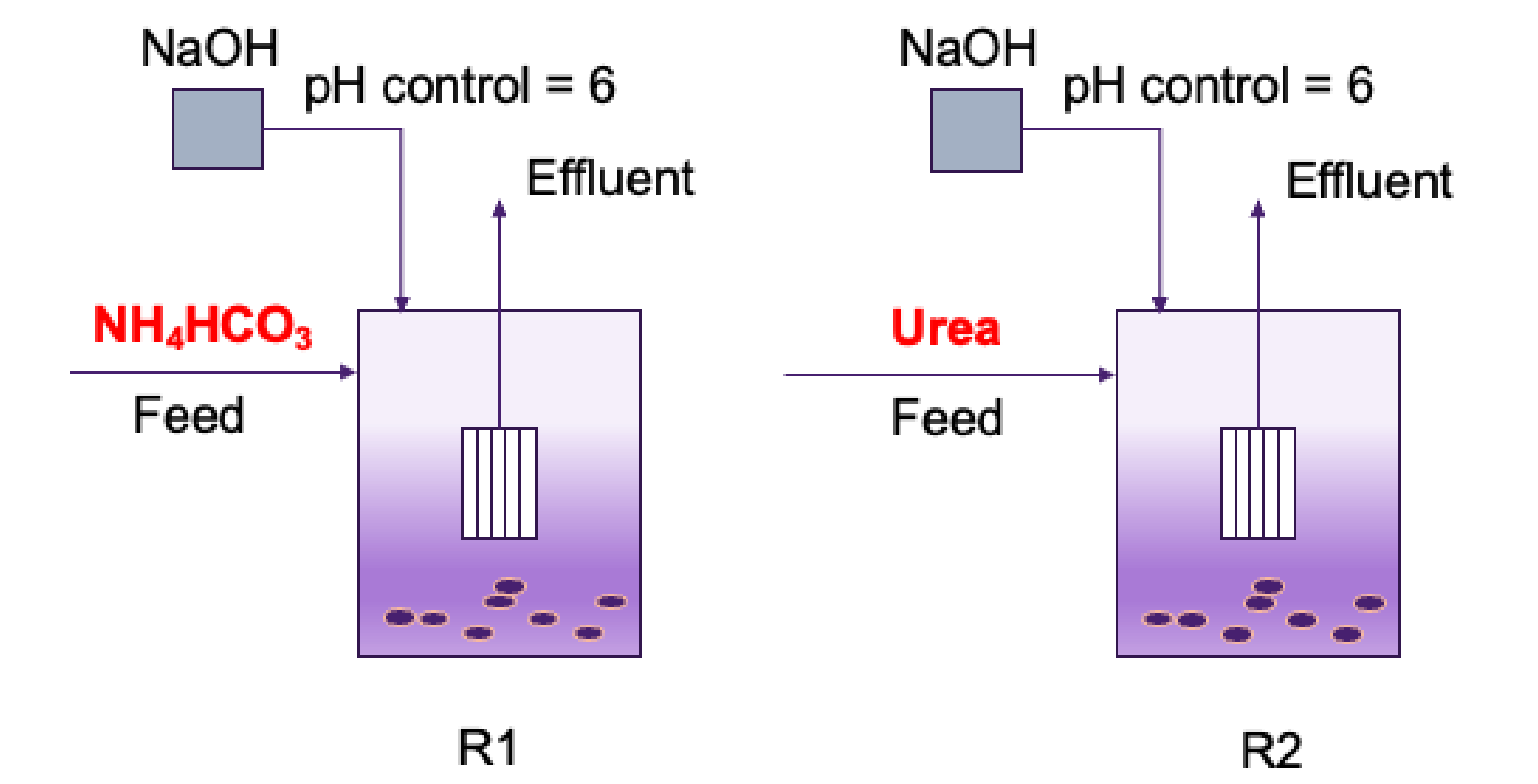
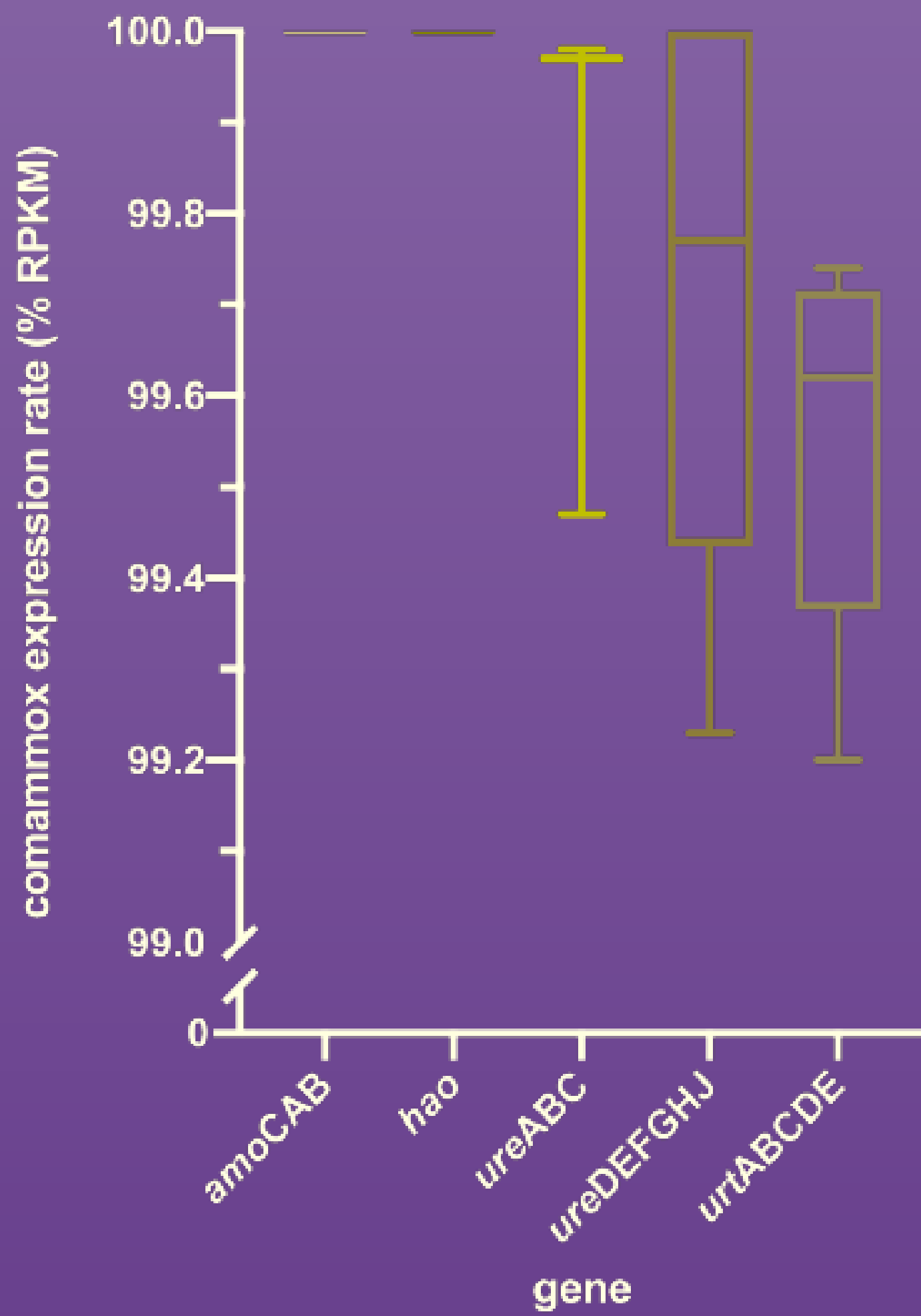


Figure 1: Reactors for comparative enrichment

# Comammox can dominate the use of urea.



2. Confirm comammox enrichment by 16S rRNA (Figure 2a), FISH (Figure 2b) and metagenomic analysis (Figure 2c). In the urea-fed reactor, Nitrospira eventually dominated the community, where 82% of nitrifiers belonged to comammox.

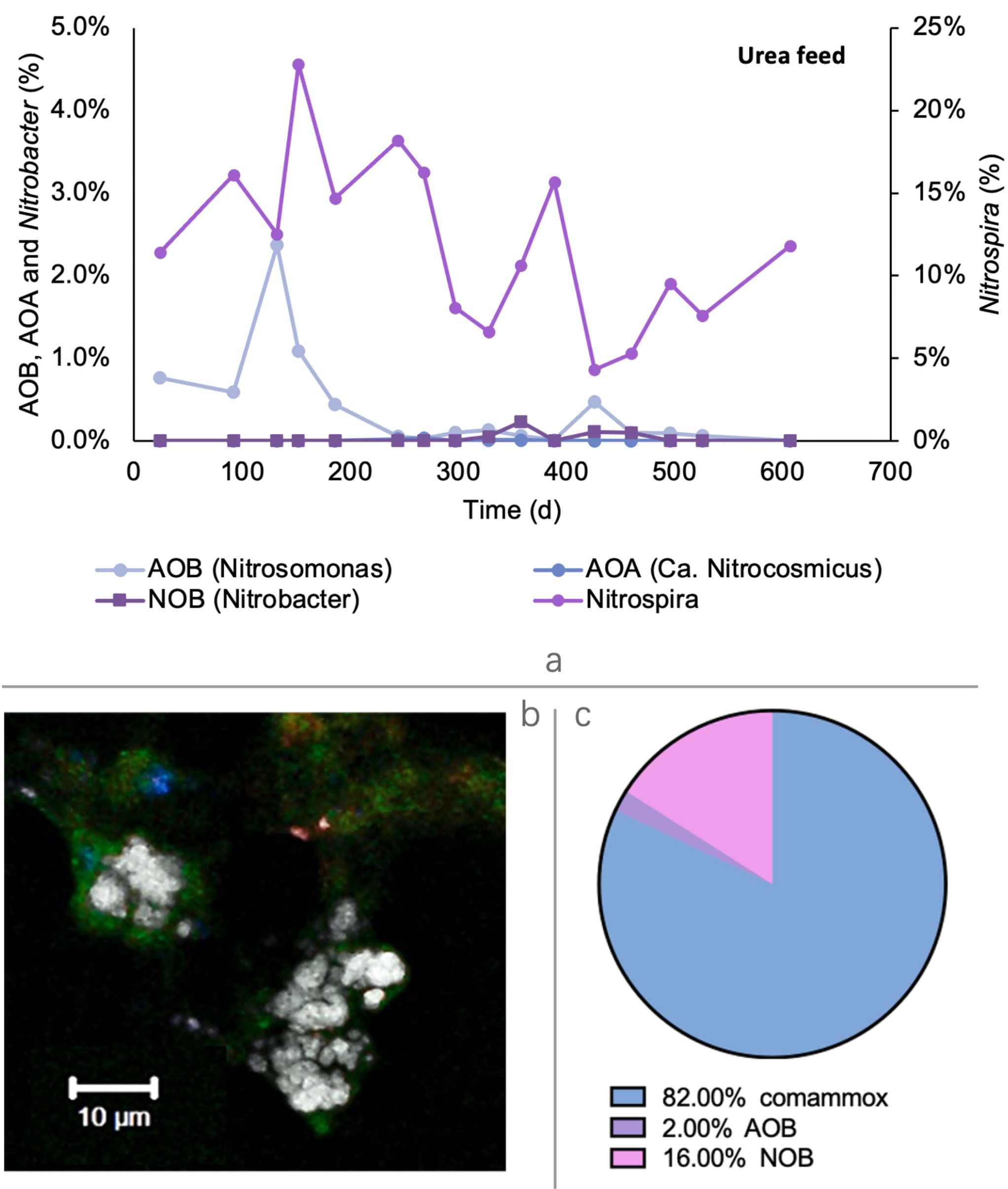


Figure 2: 16S rRNA result during long-term cultivation

3. Examination of the metagenomic-assembled genomes (MAGs) based on functional genes (Figure 3). 5 high-quality comammox MAGs were recovered in total, with complete/incomplete set of nxr, hao and amo genes.

Gene	NxrA	NxrB	hao	amoA	amoB	amoC
comammox1	3	4	2	1	1	7
comammox2	2	2	1	1	0	2
comammox3	3	2	1	1	1	6
comammox4	2	2	1	1	1	5
comammox5	1	1	1	1	1	0
NOB1	2	2	0	0	0	0
NOB2	1	3	0	0	0	0
NOB3	2	1	0	0	0	1
NOB4	2	2	0	0	0	0

Figure 3: Differentiating comammox Nitrospira and NOB Nitrospira by functional gene copy numbers.

## Take away message

1. Feeding urea is a strategy of comammox enrichment.
2. Comammox can dominate the use of urea in such community.
3. In this case, comammox enrichment belonged to clade A comammox.



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