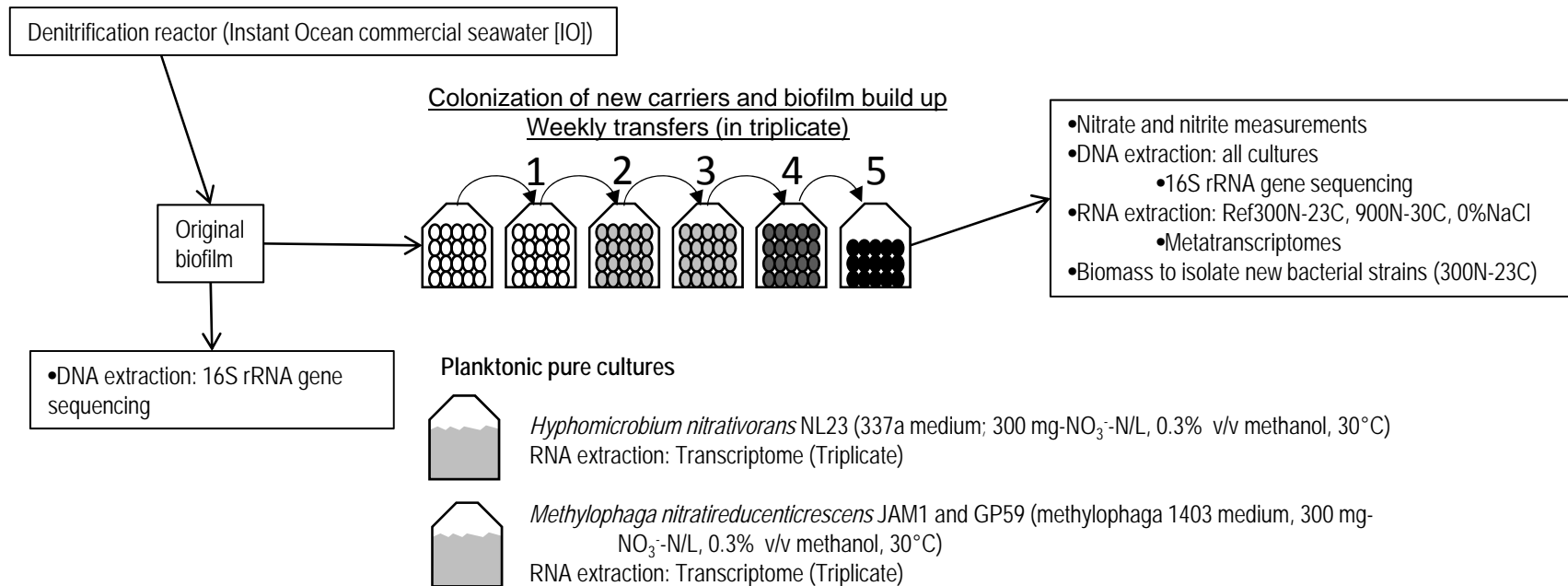


## BIOFILM CULTURE CONDITIONS

### 5 transfers (all under anoxic conditions)

- Ref300N-23C (ASW, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 2.75% NaCl, 23°C): Reference biofilm cultures
- 300N-30C (ASW, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 2.75% NaCl, 30°C)
- 900N-23C (ASW, 900 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.45% v/v methanol, 2.75% NaCl, 23°C)
- 900N-30C (ASW, 900 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.45% v/v methanol, 2.75% NaCl, 30°C)
- 0%NaCl (ASW, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 0% NaCl, 23°C)
- 0.5%NaCl (ASW, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 0.5% NaCl, 23°C)
- 1%NaCl (ASW, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 1.0% NaCl, 23°C)
- IO (Instant Ocean, 300 mg-NO<sub>3</sub><sup>-</sup>-N/L, 0.15% v/v methanol, 23°C)



### Figure S1. Schematic of the experimental assays performed in the study

The original biofilm was thawed, scraped from the carriers, dispersed and distributed in vials containing the ASW medium supplemented with prescribed concentrations of NO<sub>3</sub><sup>-</sup>, methanol and NaCl, or containing the IO medium (Table 1), and 20 free carriers. The vials were incubated at the prescribed temperature under anoxic conditions. The carriers were transferred five times at appr. 1 week interval into fresh prescribed medium and incubated in the same conditions. NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations were measured every 24-48 h. Methanol and NaNO<sub>3</sub> were added when needed if NO<sub>3</sub><sup>-</sup> was completely depleted between transfers. DNA or RNA was extracted from the biofilm at the end of the 5<sup>th</sup> transfer cultures. Planktonic pure cultures were carried out in recommended culture medium for the respective strains.