

# Training an AGS reactor to treat high salinity wastewater

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## Introduction

**Aerobic granular sludge (AGS)** is a promising technology for treating industrial wastewater, with higher biomass retention and tolerance to toxic substrates than conventional activated sludge systems. The presence of **extracellular polymeric substances (EPS)** in the AGS structure increases the bacterial protection and stability of the granules. **Several industrial wastewaters contain high salt content** in their composition that often inhibit the bacteria responsible for nutrients removal. A strategy to prepare the system to high salinity is the gradual adaptation of the aerobic granules to increased salt concentrations. The **objective** of this work is to evaluate the **AGS process performance** for treating saline wastewater whilst following the temporal **bacterial community changes to determine on the relationship between functional microbiome and reactor performance**.

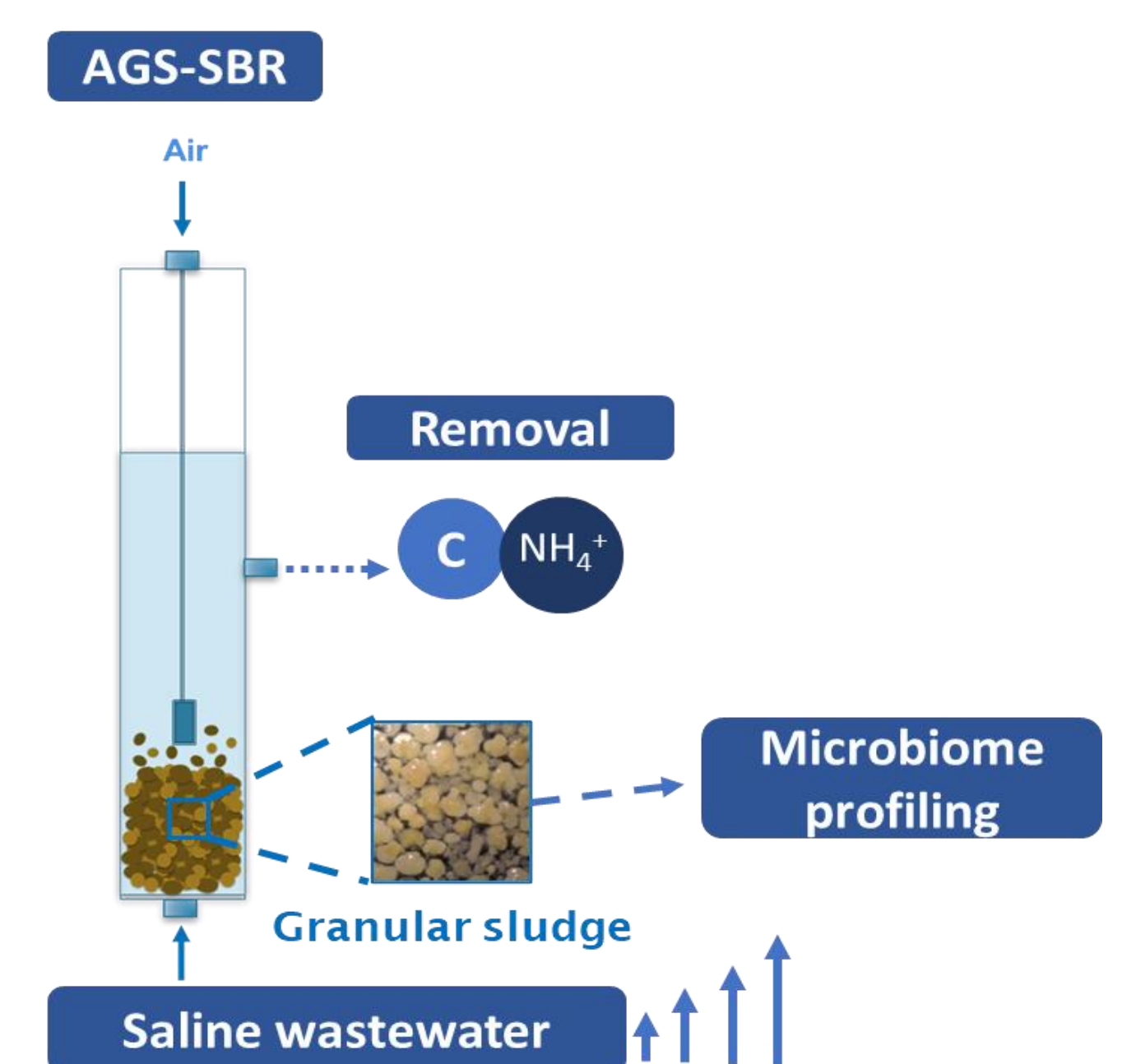
## Methods

A lab-scale AGS-SBR (sequential batch reactor) was operated for 248 days and fed with synthetic wastewater containing ammonium, phosphate and acetate (40, 20 and 680 mg L<sup>-1</sup>, respectively) in its composition. Over operation, a stepwise addition of NaCl to the wastewater from 0 to 14 g L<sup>-1</sup> was performed, splitting the operation into IX phases (Table 1). The performance of the AGS-SBR was assessed for COD, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> removal. The microbial composition of the AGS during saline wastewater treatment was monitored using next-generation sequencing (NGS).

Table 1 – AGS reactor operational phases

Phases	I	II	III	IV	V	VI	VII	VIII	IX
Days	0-7	8-21	22-35	36-64	65-90	91-124	125-168	169-203	204-248
NaCl (g L <sup>-1</sup> )*	0	2,7	4,4	6,0	6,8	8,5	10,0	11,6	14,0

\*Average values



## Results

### AGS performance

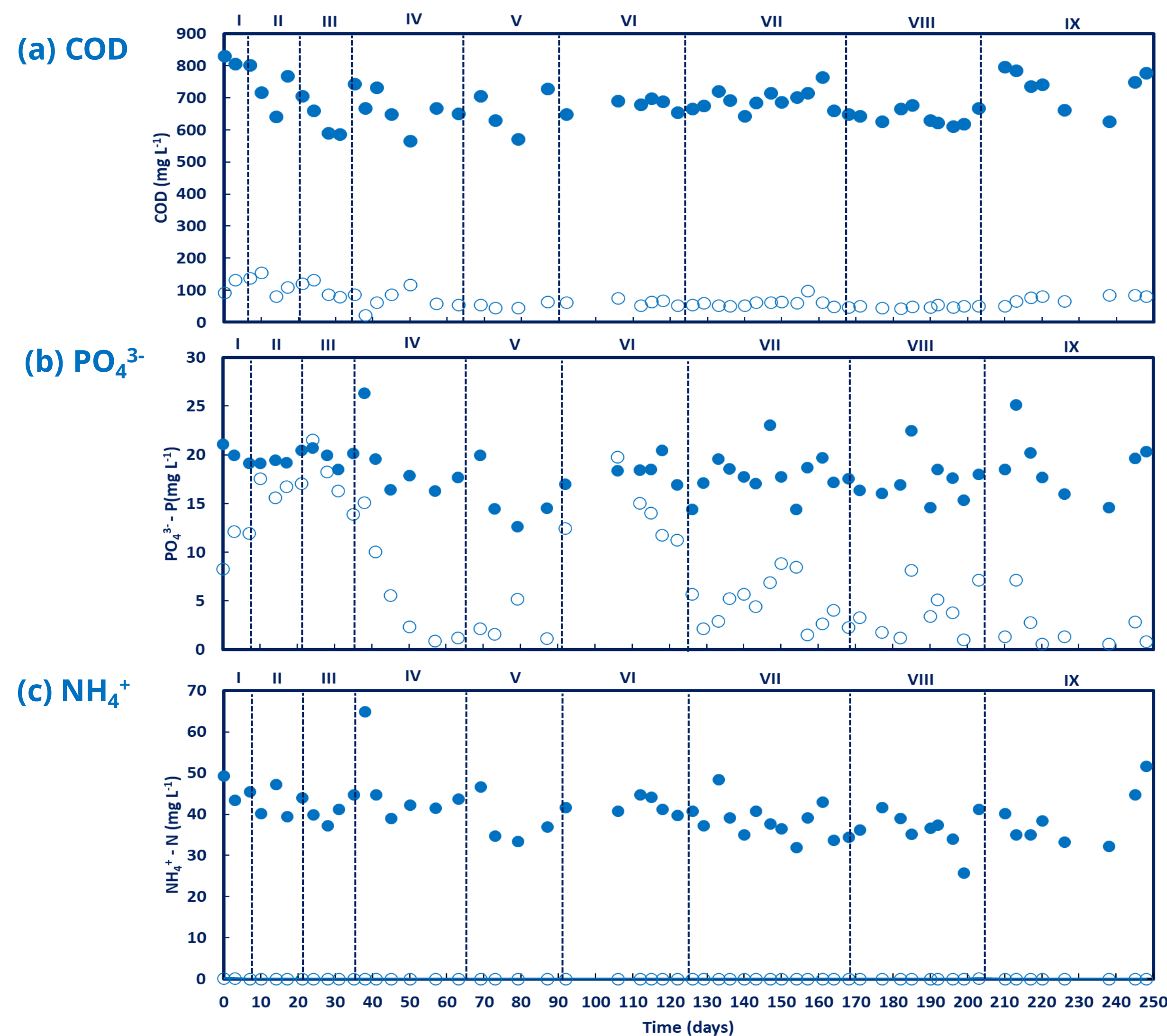


Figure 1. COD (a), PO<sub>4</sub><sup>3-</sup> (b) and NH<sub>4</sub><sup>+</sup> (c) concentrations profiles at the reactor inlet (●) and outlet (○).

- COD concentration at the outlet reached less than 100 mg O<sub>2</sub> L<sup>-1</sup> throughout the operation;
- Initially phosphate removal was reduced (phases I, II and III) but improved during phases IV and V; during phase VI, phosphate removal decreased, but resumed until the end of reactor operation;
- Ammonium removal was stable and very efficient throughout reactor operation, with ammonium levels near zero at the effluent.

### AGS microbiome

#### (a) Phylum

Phyla	Phase I d0	Phase II d14	Phase III d36	Phase IV d50	Phase V d63	Phase V d79	Phase V d90	Phase VI d122	Phase VII d154	Phase VII d168	Phase VIII d199	Phase IX d248
Proteobacteria	49,2	55,9	86,2	100,0	98,4	64,6	77,6	80,9	85,5	86,4	86,1	80,2
Acidobacteria	2,7	3,8	0,0	0,0	0,0	0,0	0,0	0,5	0,7	0,0	0,0	1,8
Actinobacteria	7,9	6,3	13,8	0,0	1,6	6,9	0,0	4,3	2,0	4,1	5,4	5,5
Bacteroidetes	31,1	21,5	0,0	0,0	0,0	21,5	25,4	12,2	9,2	8,5	7,7	11,2
Cyanobacteria	1,6	1,6	0,0	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0
Gemmatimonadetes	3,4	3,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,3
Ignavibacteriae	0,5	1,5	0,0	0,0	0,0	1,7	0,0	1,1	1,3	0,9	0,7	0,3
Nitrospirae	1,0	3,1	0,0	0,0	0,0	4,6	0,0	1,0	1,1	0,0	0,0	0,6
Other	2,7	3,2	0,0	0,0	0,0	0,0	0,0	0,2	0,2	0,1	0,1	0,2

#### (b) Genus

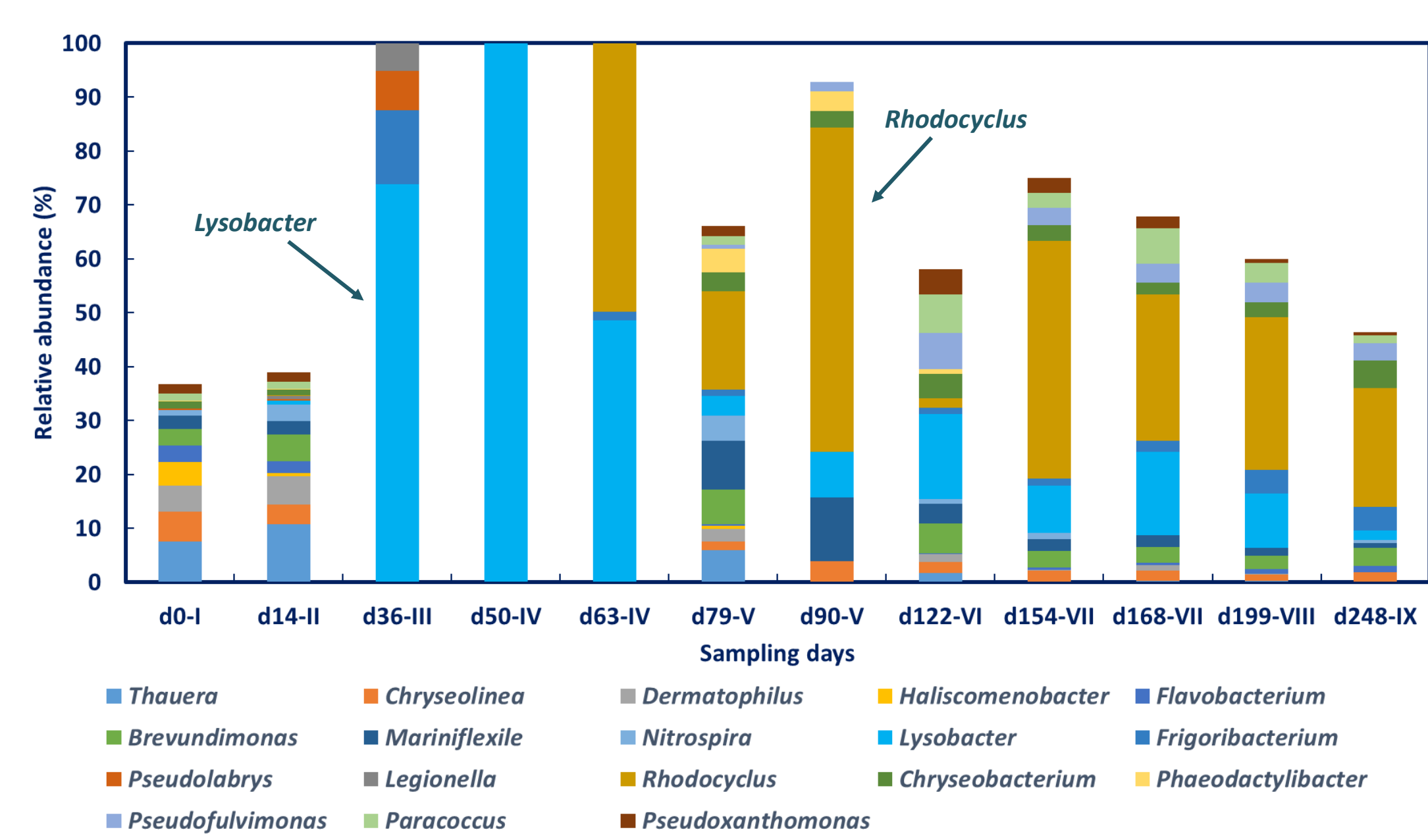


Figure 2. Heatmap of the relative abundance of the bacterial phyla (a) and bacterial genera presenting the highest relative abundances (b) in the AGS biomass along the reactor operational phases.

- Higher bacterial phyla and genera diversity observed while treating wastewater up to 3 g NaCl L<sup>-1</sup>; a greater diversity decrease was observed while treating 6 g NaCl L<sup>-1</sup> wastewater;
- For wastewater with salinity levels up to 6 g NaCl L<sup>-1</sup>:
  - Proteobacteria dominated the bacterial phyla and *Lysobacter* dominated the bacterial genera.
- Wastewater with salinity levels higher than 6 g NaCl L<sup>-1</sup>:
  - Proteobacteria kept dominating the bacterial phyla and *Rhodocyclus* dominated the bacterial genera.

## Conclusions

The AGS-SBR presented good COD and NH<sub>4</sub><sup>+</sup> removal during the adaptation to increased salinity in the wastewater. PO<sub>4</sub><sup>3-</sup> removal was initially affected by salt increase but resumed during the adaptation to higher salt concentration. A pronounced bacterial diversity decrease was observed in the AGS microbiome while treating wastewater containing 6 g NaCl L<sup>-1</sup>. The stepwise addition of salt to the wastewater allowed the gradual shaping of the microbial community, without causing detrimental effects on the biological removal performance. This strategy can be valuable for adapting the treatment of high salinity wastewater as those produced by industry.

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