

# Aerobic Granular Sludge system under chiral pharmaceutical exposure: insights on nutrient removal performance and microbiome composition

Catarina Miranda<sup>1\*</sup>, Maria Elizabeth Tiritan<sup>2,3</sup>, Paula M.L. Castro<sup>1</sup>, Catarina L. Amorim<sup>1</sup>

<sup>1</sup> Universidade Católica Portuguesa, Centro de Biotecnologia e Química Fina – Escola Superior de Biotecnologia, Porto, Portugal

<sup>2</sup> Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia do Porto, Portugal

<sup>3</sup> Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Portugal

\* cmiranda@ucp.pt



## Introduction

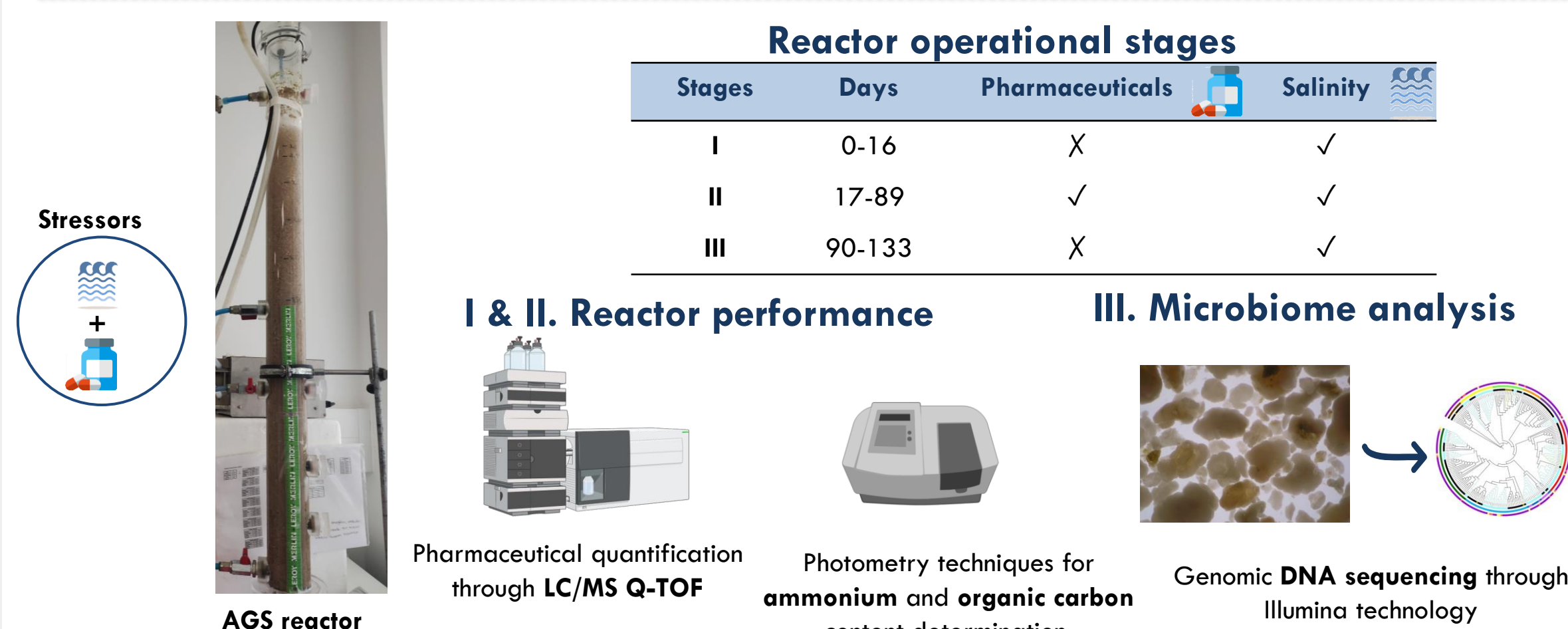
Currently, the extensive use of pharmaceutical compounds coupled with the limited efficacy of conventional wastewater treatment plants (WWTPs) has intensified the persistence of these compounds in environmental matrices;

Aerobic granular sludge (AGS) is a low-footprint and sustainable technology acclaimed for its robust ability to withstand several pollutants, including pharmaceutical compounds;

Tramadol and venlafaxine are two widely consumed pharmaceuticals that can exhibit enantiomeric-specific behavior, therefore unveiling their fate in AGS systems is of utmost relevance;

Our work explored the impacts of two distinct stress scenarios - pharmaceutical exposure and oscillating salinity levels throughout a day - on AGS nutrient removal performance and microbial community composition.

## Experimental



## Results and Discussion

### I. The fate of pharmaceuticals in AGS reactor

- During stage II the reactor intermittently received wastewater containing five pharmaceutical compounds at concentrations near those found in the environment.

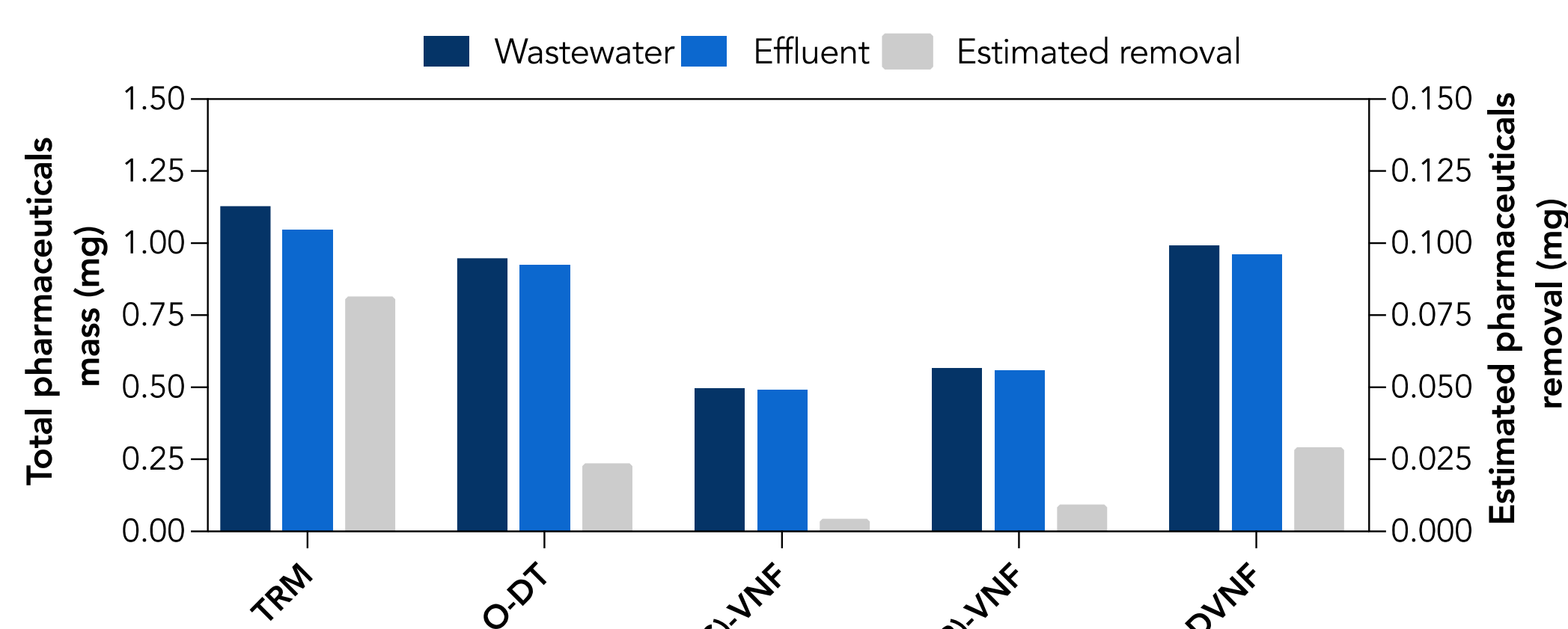


Figure 1. Estimated pharmaceutical mass balance for five analytes during stage II. Total pharmaceutical mass (mg) in wastewater and effluent. The pharmaceutical removal was estimated based on the pharmaceutical mass in the wastewater and effluent. Abbreviations for tramadol (TRM); O-desmethyltramadol (O-DT); (S)- and (R)-Venlafaxine (VNF); O-desmethyltramadol (O-DT).

- Overall, the AGS system revealed a limited capacity to remove the pharmaceuticals in the wastewater;
- An additional treatment step should be considered to remove the persistent pollutants from the treated effluent effectively.

### II. Reactor removal performance

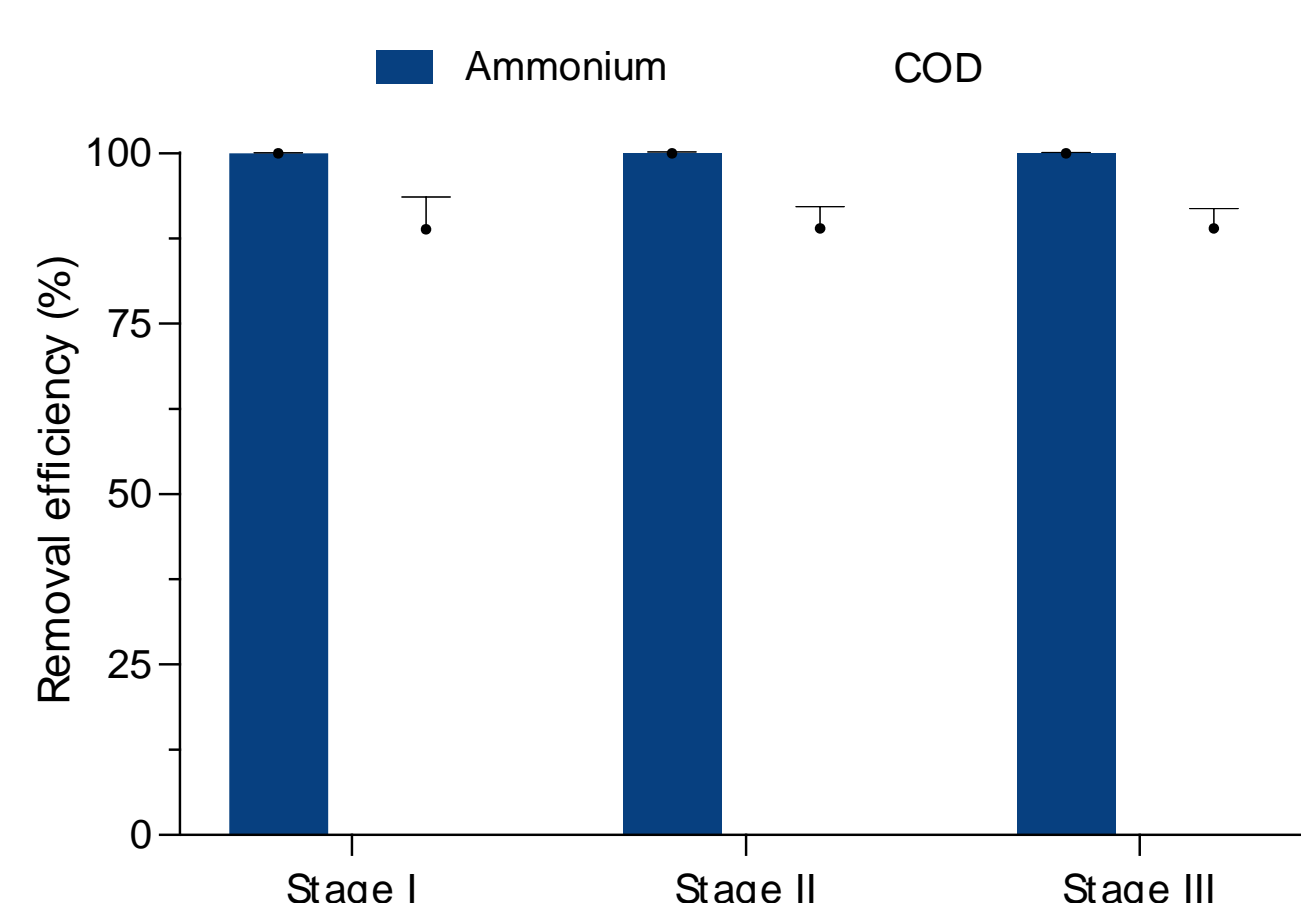


Figure 2. Removal efficiency (%) for ammonium and chemical oxygen demand (COD) over reactor operation.

- Neither ammonium nor COD removal was impaired by the oscillating salt content and the presence of pharmaceuticals in the wastewater;
- Ammonium and COD removal performance remained identical across all operational stages.

### III. AGS microbiome composition

- The taxonomic composition was assessed to follow the dynamics of the AGS microbiome over operation.

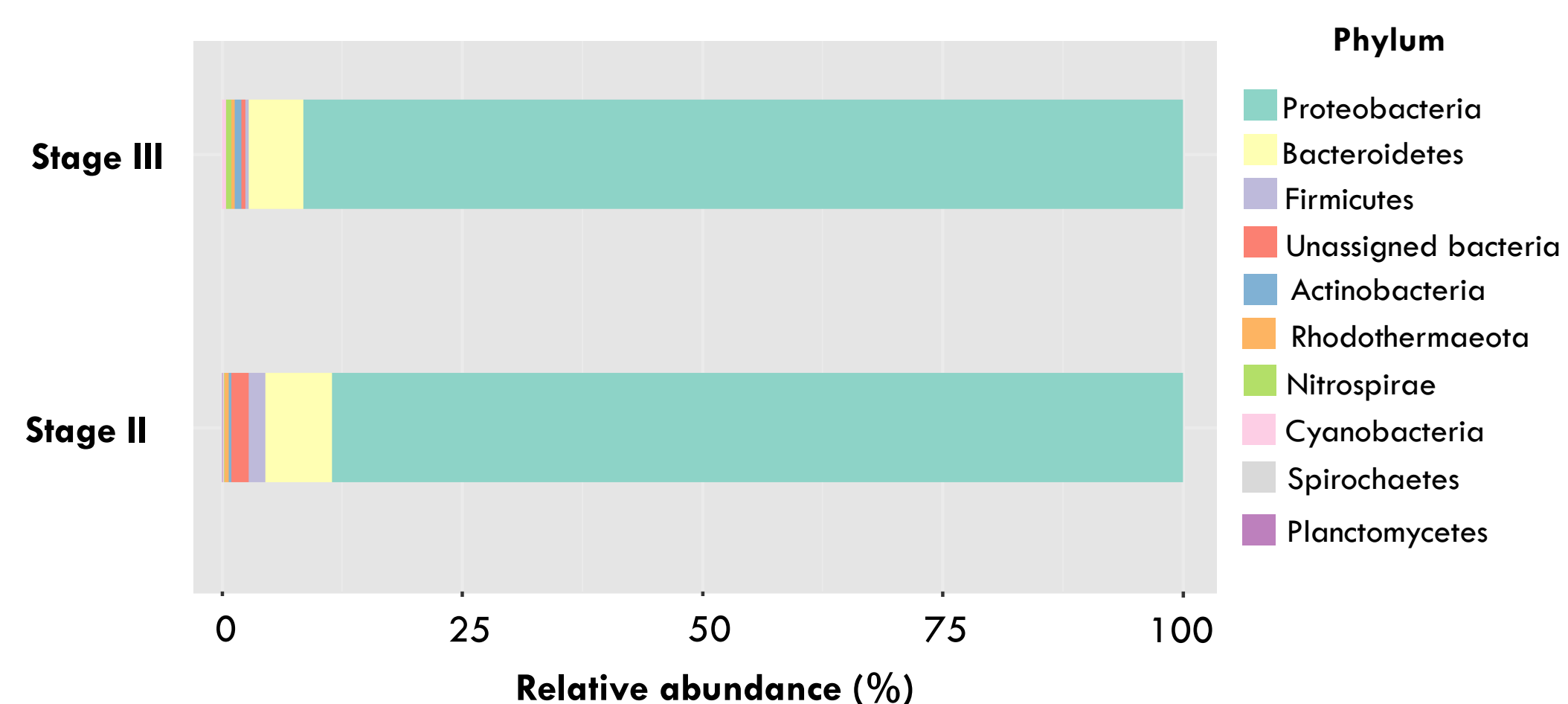


Figure 3. AGS taxonomic composition in terms of phylum over stages II and III of operation.

- Proteobacteria and Bacteroidetes were the predominant phyla during the whole reactor operation;
- Differences found in the relative abundance of certain phyla from stage II to III may reveal that the microbiome was dynamic and adaptive after facing the stressors.

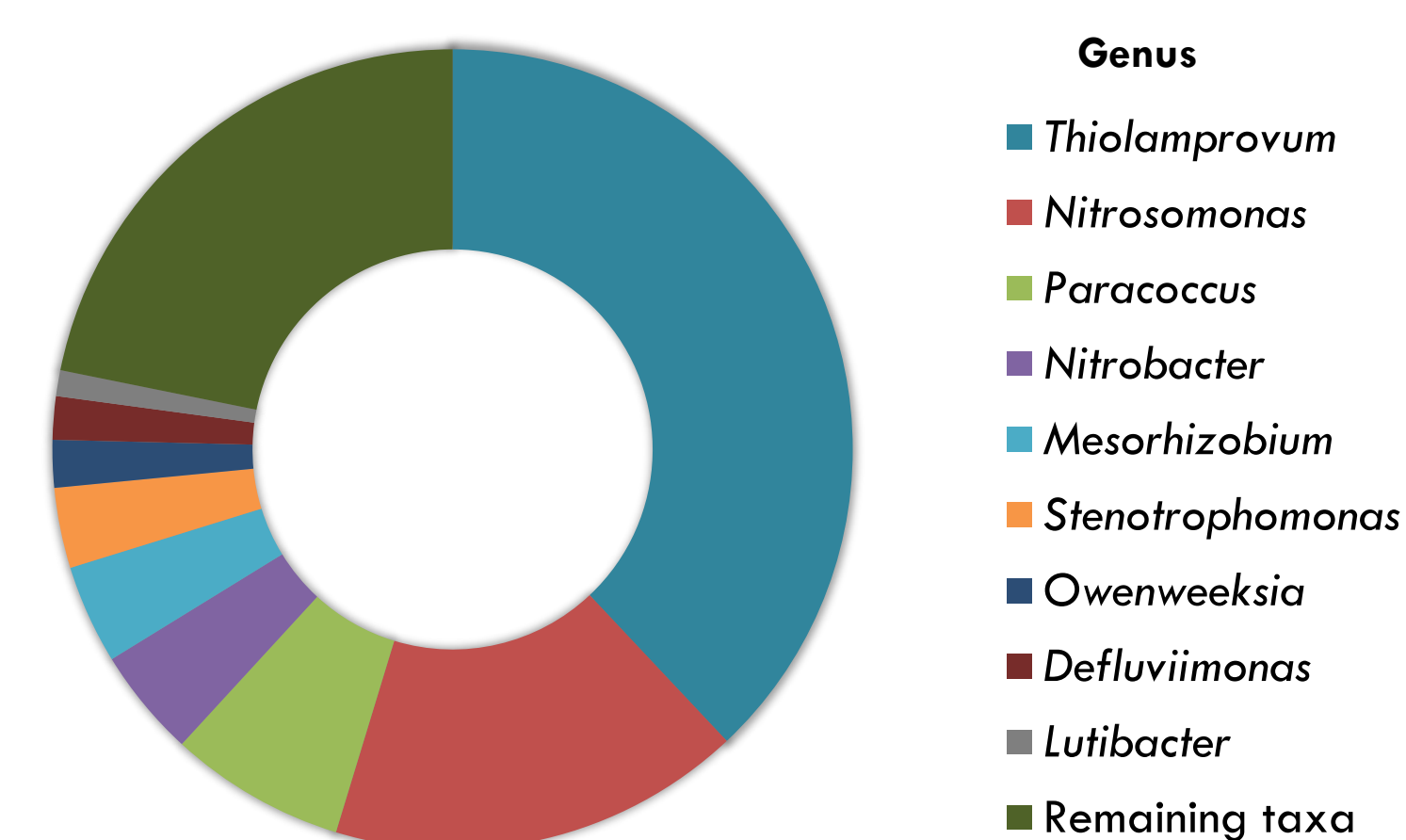


Figure 4. Top 10 most dominant bacterial genera over stage II of operation.

- Genera involved in nitrification, denitrification, and EPS production were found at relevant abundances in the AGS microbiome;
- The presence of genera related to the reactor performance and the stability of bacterial granules likely helped the biomass withstand stress scenarios, ensuring that removal performance remained unaffected during these periods.

## Conclusions

- AGS technology is a robust system effective in coping with stressful scenarios, such as saline intrusion events and the presence of pharmaceuticals in wastewater;
- Although the AGS system showed reduced capacity to remove pharmaceuticals from wastewater, the biomass exhibited effectiveness in treating wastewater, with COD effluent levels consistently within the permissible discharge limit under the EU directive;
- AGS microbiome was diverse and dynamic, harboring several bacterial genera associated with nitrogen removal performance and granular formation/stability, thus highlighting the potential of this technology to withstand adverse scenarios.

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