

CHARACTERIZATION OF MICROBIOTA AND MICROBIOME DURING PHA BIOPRODUCTION IN LAB-SCALE REACTOR

Leandro Di Gloria, Serena Falcioni, Giulio Munz, Matteo Ramazzotti



INTRODUCTION

Poly-hydroxyl-alkanoates (**PHA**) are organic polymers synthetized from various carbon sources (such as short aliphatic compounds) by certain prokaryotes and algae as reserve stock of energy and carbon.

Considering their biodegradability and proprieties similar to plastic, the PHA can be employed as an **alternative to common plastic**.

Moreover, their production can also be coupled with the denitrification in **waste water treatment** serving as substrate to reduce in the process.

To archive this objective, many researches focused on PHA producing microbes but few reported a detailed overview of the whole community taxonomy (**microbiota**) and functionality (microbiome) during its establishment.

METHODS

An aliquot from a PHA producing reactor which has already reach the steady-state through nitrogen-limitation has been inoculated in a new reactor.

Such reactor alternates 3 hours of nourishment through acetic acid and propionic acid (**feast phase**) and 9 hours of total absence of nutrients (**famine phase**) except nitrogen compound to induce a **restrain on phosphate availability**.

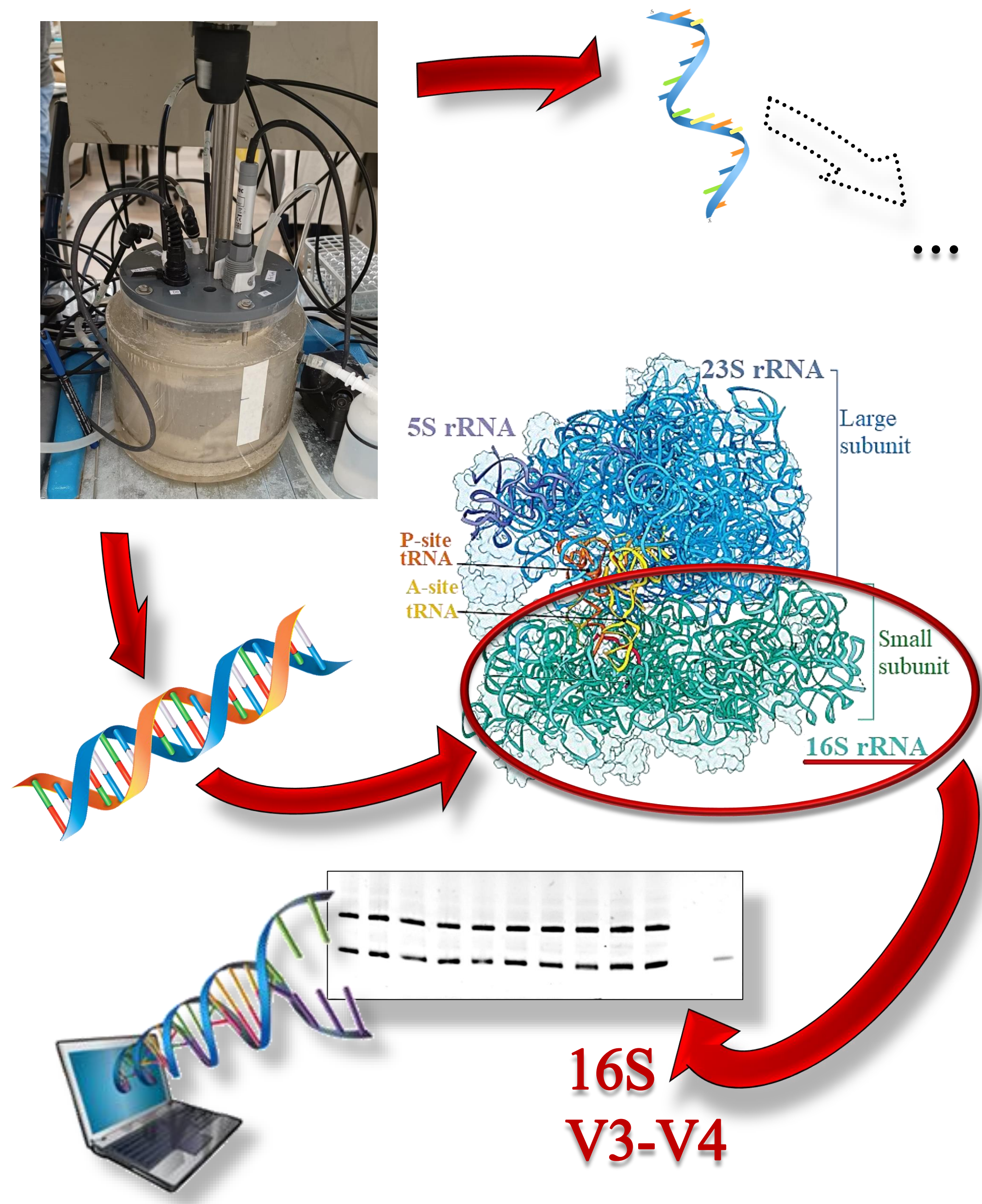
Under this selective pressure, the PHA producing bacteria are selected and enriched.

During three months since the inoculum, 200 mg of suspended biomass from this reactor has been collected, then the total DNA and RNA have been extracted. The **DNA** has been amplified using V3-V4 primers for the **16S gene**, hence the amplicon have been normalized and **sequenced through Illumina MiSeq**.

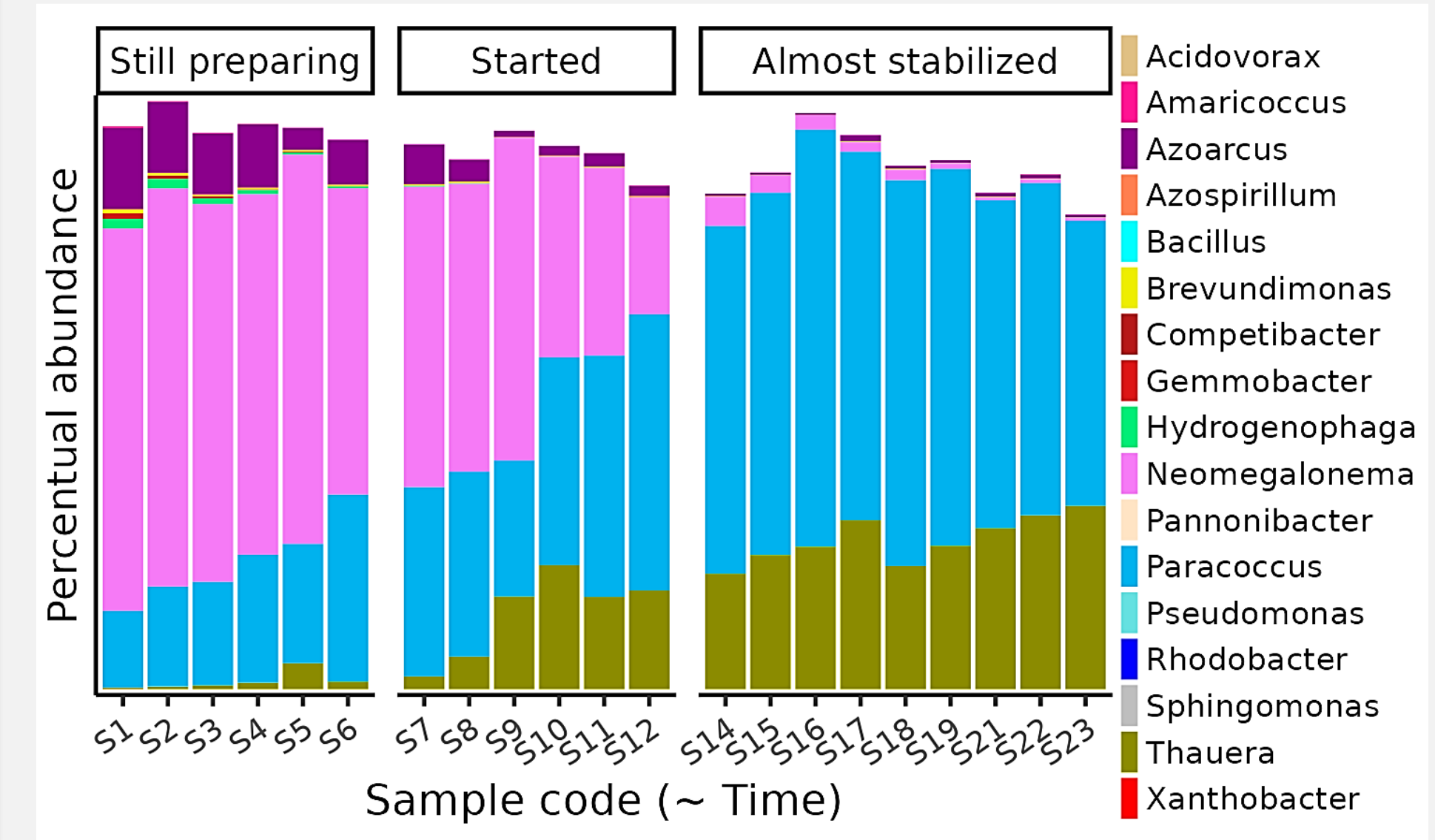
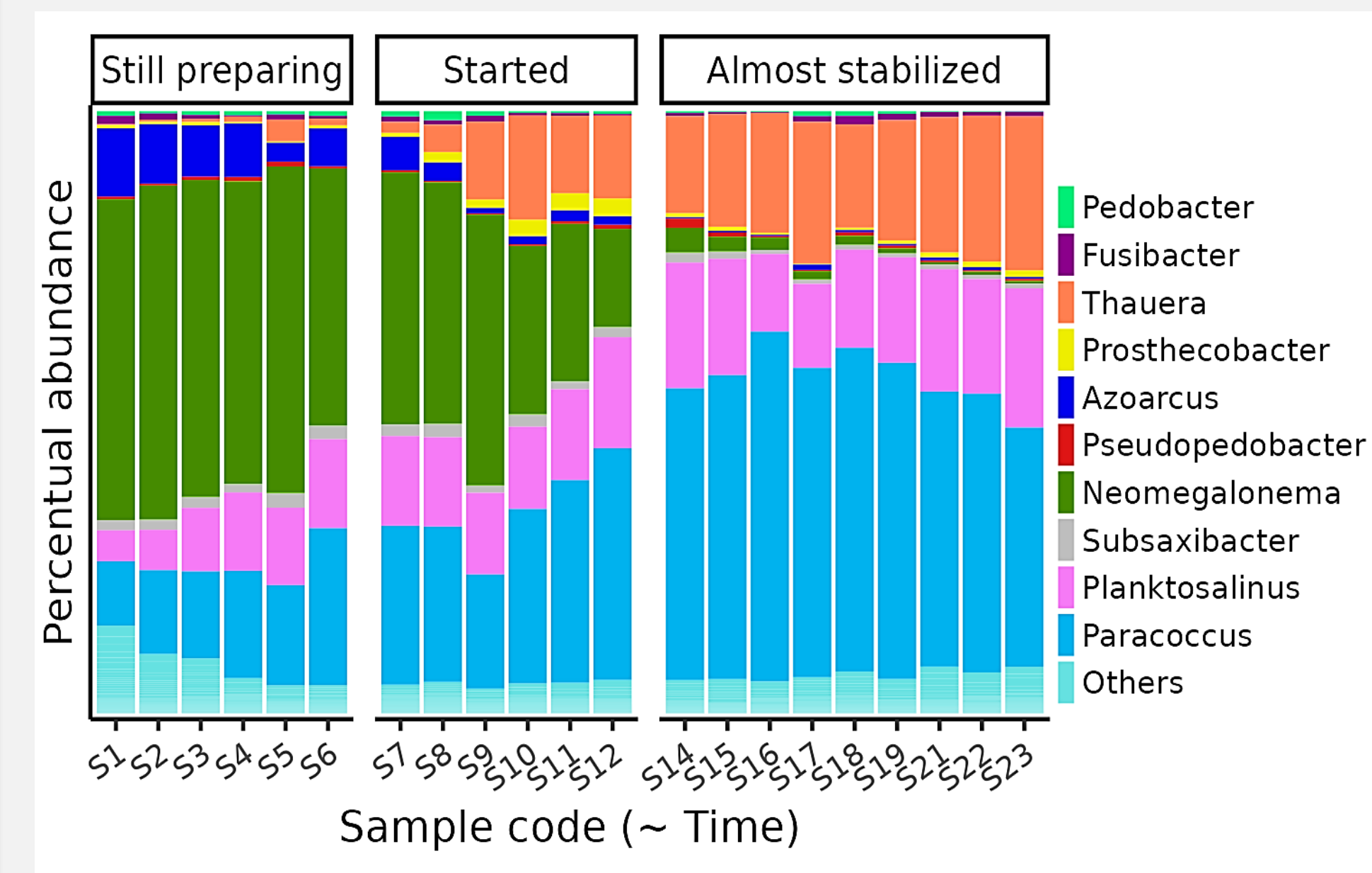
The obtained sequences have been processed and analysed with a specifically optimized bioinformatic pipeline built on Linux bash and R (the scripts are publicly accessible at <https://github.com/LeandroD94>).

AIM OF THE PROJECT

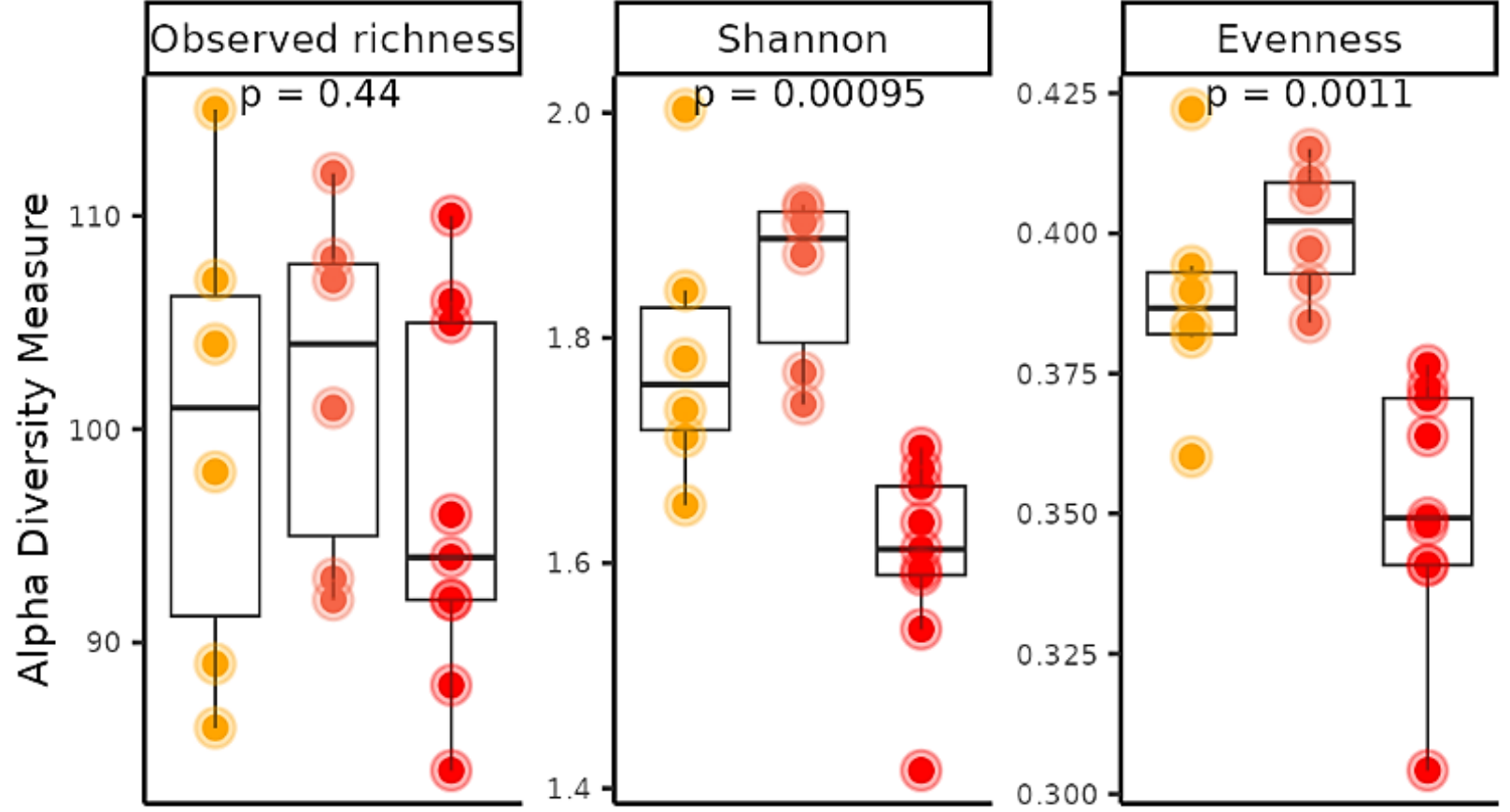
Characterizing the microbiota and microbiome in a laboratory-scale sequencing batch reactor aimed to select and enrich the microbiota required for PHA production through phosphate-limitation.



RESULTS SO FAR

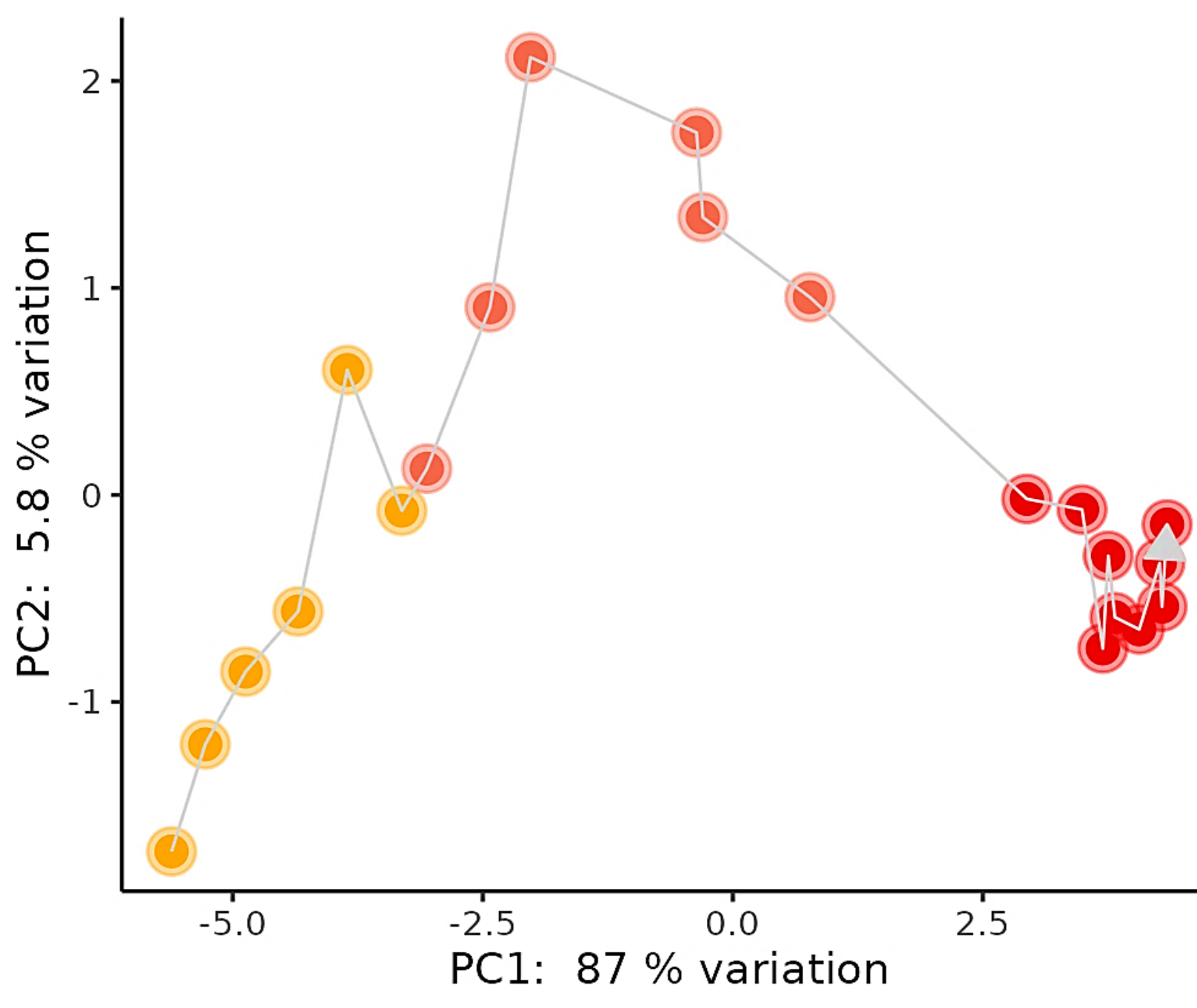


The **alpha diversity** analysis confirms the **lower evenness** of bacterial abundances in a mature reactor characterized by P-limitation (red points) compared to the inoculum microbiota (orange points).



The **beta diversity** analysis (represented using a PCoA obtained with Hellinger distance on genera) shows the **microbiota profile shift** towards the one characteristic of a reactor with P-limitation (evidenced by a grey arrow).

In particular, such profile begins to be almost stabilised **after about 2 months** since the inoculum (estimated according to the lower distance among red points).



The **taxonomic analysis** shows a shift of abundances of the ten most abundant genera in the reactor (top picture) and, in particular, an enrichment of PHA accumulating bacteria (bottom picture) such as **Thauera** and **Paracoccus** (also confirmed by Spearman correlations on log ratio counts).

