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## **Feedback on a comparative metatranscriptomic analysis**

Cedric Midoux, Tiago P. Delforno, Thais Z. Macedo, Gileno V. Lacerda,  
Olivier Rué, Mahendra Mariadassou, Maria B.A. Varesche, Théodore  
Bouchez, Ariane Bize, Valéria M. Oliveira, et al.

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## Context

The progress of next generation sequencing favors the development of more comprehensive ecosystem studies thanks to metatranscriptomic approaches. These latter can indeed provide access to functional information at a good analysis depth. Through a study of anaerobic digesters treating wastewater contaminated by an anionic surfactant (namely the *linear alkylbenzene sulfonate*, LAS), we developed a bioinformatics pipeline to perform RNAseq analysis of the metatranscriptomics data.



## Data

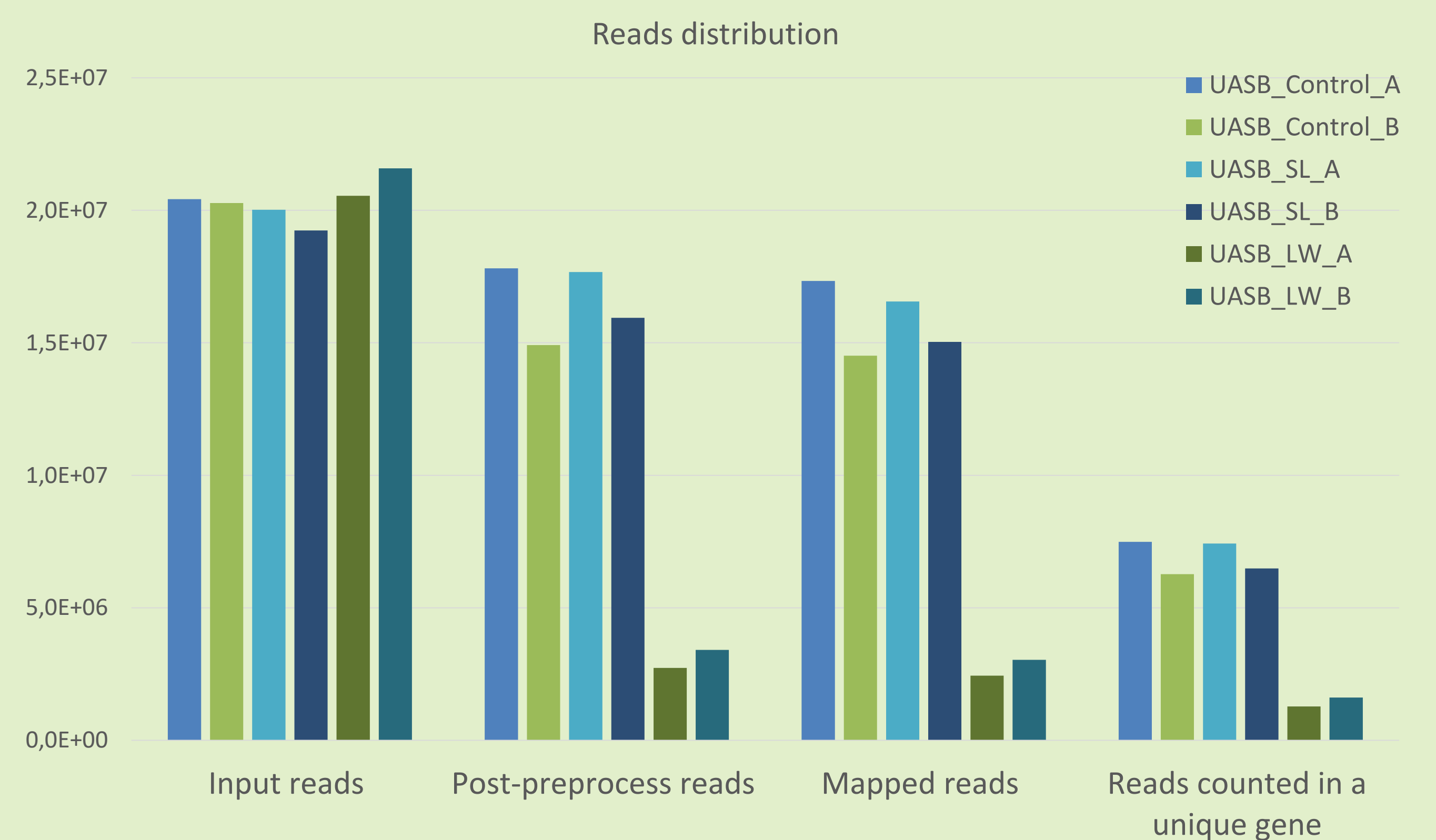
The samples originated from distinct biological reactors :

- fed with synthetic medium (UASB\_Control)
- fed with synthetic medium + LAS (UASB\_SL)
- fed with real laundry wastewater (UASB\_LW)

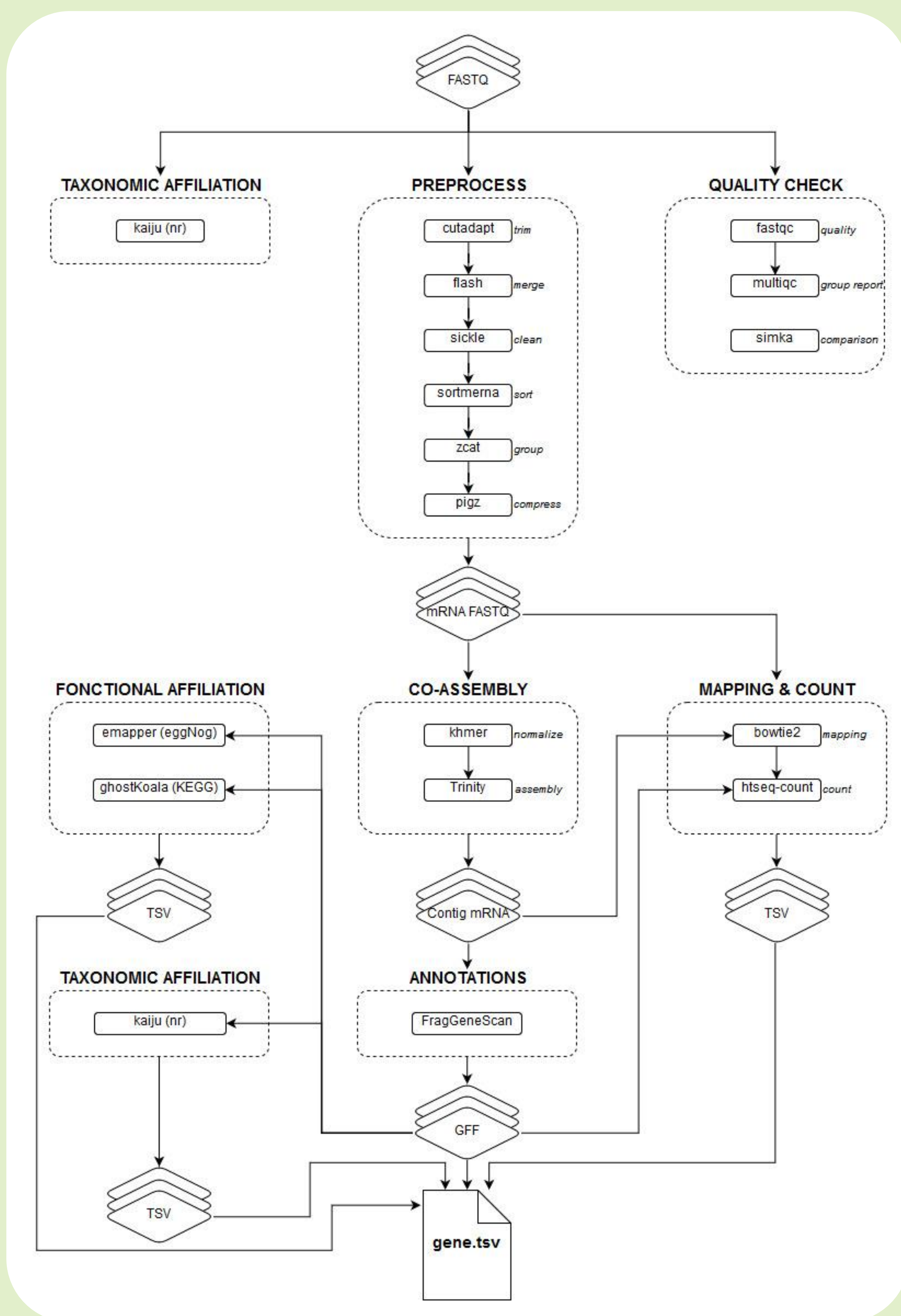
These samples were sequenced in duplicates with *Illumina HiSeq* platform (2 x 100bp ; ~20M reads/sample )



## Metrics



## Tools and workflow



- All results, counts and affiliation, were gathered together in a table.
- The tables and metadata were imported as R Phyloseq objects.
- Thresholds enabling to discard the genes with non-reproducible counts were determined for each sample thanks to the technical replicates, by scatterplot analysis. These genes corresponded to transcripts detected at low levels.
- Study of the biological information corresponding to genes with highly different expression levels were performed on the two-by-two comparison of the samples using the Z-distribution

## Results

- Percentages of LAS removal (~50%) were similar among the three reactors.
- Rarefaction analysis revealed that two million reads were sufficient to access to the main functional capacity.
- In the first step of LAS biodegradation pathway, fumarate reductase subunit C was detected and taxonomically assigned to the genus *Syntrophobacter*.
- In the second step, many enzymes related to beta-oxidation were expressed preferentially in UASB\_SL and UASB\_LW. They were taxonomically related to *Smithella*, *Acinetobacter* and *Syntrophorhabdus*.
- Finally, the adenylylsulfate reductase, taxonomically related to *Desulfovibrio* and *Desulfomonile*, was observed in the desulfonation step with the highest relative abundance in UASB\_LW.

## Conclusions

- ➔ This study enabled detecting active genes likely involved in each step of LAS biodegradation and exploring the microbial active core related to LAS degradation (Delforno *et al.*, 2019).
- ➔ Based on this positive experience, we developed a snakemake pipeline including all this features for use in future metatranscriptomic projects.

Delforno, T.P., *et al.*, Comparative metatranscriptomic analysis of anaerobic digesters treating anionic surfactant contaminated wastewater. *Sci Total Environ*, **649**: p. 482-494 (2019).