

IMPACT OF THE INOCULUM COMPOSITION ON READY BIODEGRADABILITY TESTS

Cyril Sweetlove¹, Jean-Charles Chenèble¹, Yves Barthel², Marina Moletta-Denat³, Doris Brockmann³, Jacques L'Haridon¹, Gérald Thouand⁴

¹ Environmental Research Department, L'Oréal Research & Innovation, Aulnay-sous-Bois, France, ² Ecotoxicology Department, Eurofins Expertises Environnementales, Mazierville, France

³ INRA Transfert, Narbonne, France, ⁴ UMR CNRS 6144 GEPEA CBAC lab, University of Nantes, La Roche sur Yon, France

Email: csweetlove@rd.loreal.com - Phone number: +33 1 58 31 75 18

INTRODUCTION

Next generation sequencing provides better understanding of the structural and functional diversity of microorganisms. In order to determine the ready biodegradability of chemicals, laboratory tests were performed with sludge from a domestic waste water treatment plant (WWTP; Maxéville, France) that had undergone washing and dilution treatments before being used as inoculum. Our interest is focused on the impact of these pretreatments on the inoculum diversity prior to testing. In this context, metagenomic analyses were applied to follow the evolution of inoculum bacterial populations from sampling to the end of the test.

MATERIAL AND METHODS

Six tests were performed with a RespiCond VI® respirometer between May and November 2015 according to OECD ready biodegradation standards [1]. Different stages of activated sludge were assessed: raw activated sludge (Raw AS), washed (washed AS), diluted (Inoculum D0) and inoculum at the end of the test (Inoculum D28) were sequenced by MiSeq sequencer (Illumina). The primers used target the V4-V5 variable regions of the 16S ribosomal RNA sequence of prokaryotes. The analysis allowing the phylogenetic affiliation was performed with Mothur® (version 1.33.1) and identification on the SILVA taxonomy basis (version 119) for bacteria.

RESULTS

1 Diversity Index

The Shannon diversity index of all the sludge samples and for the different conditions are between 4.5 and 5.4 (Figure 1), synonymous, as expected, of great diversity. The ANOVA analysis indicates a difference in diversity for each condition of the same test, but also for each condition between the different tests. The Post-hoc Tukey tests revealed significant differences mainly between Raw AS, washed AS and Inoculum D0 between 2 groups T1 to T3 and T4 to T6. It can also be noted that the Inoculum D28 condition generates a higher variability which does not make it possible to distinguish significant differences as for the other conditions. The two test groups (T1 to T3 and T4 to T6) are distinct up to Inoculum D0. It is now necessary to verify the impact of these conditions on the bacterial compositions.

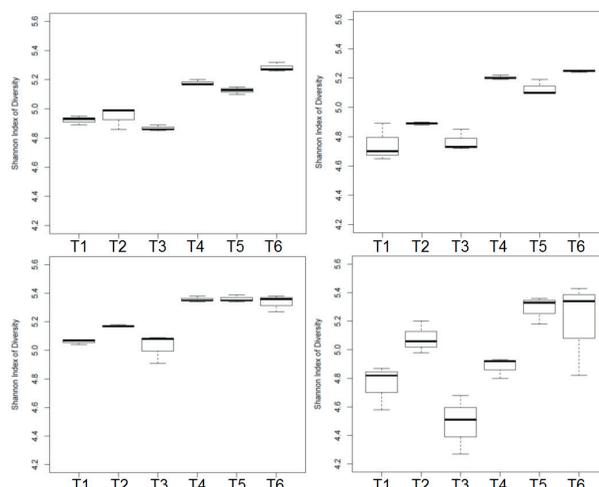


Fig. 1: Boxplot of Shannon index of diversity for 6 tests of biodegradability (T1 to T6) for conditions (A) Raw AS, (B) Washed AS, (C) Inoculum D0 and (D) Inoculum D28

2 Inoculum composition

The major families in Raw AS, washed AS and inoculum D0, exceeding 5% in at least one sample and presented in descending order, are (Figure 2): Saprospiraceae (Bacteroidetes), Chitinophagaceae (Bacteroidetes), Rhodocyclaceae (Betaproteobacteria), unclassified bacteria, Methylophilaceae (Betaproteobacteria) except for D0 and SJA-28 (Chlorobi). Despite some differences, there is a marked homogeneity in the abundance of the major families for the different Raw AS, washed AS and inoculum D0 except for SJA-28 (Chlorobi), which is in fact only overexpressed in the T2 test only. The T28 inocula controls in the mineral medium are very different from the other conditions and also between them. The family Saprospiraceae (Bacteroidetes), predominant for the other conditions, does not seem to adapt to the test medium or agitation condition because at D28, this family is poorly represented whatever the test.

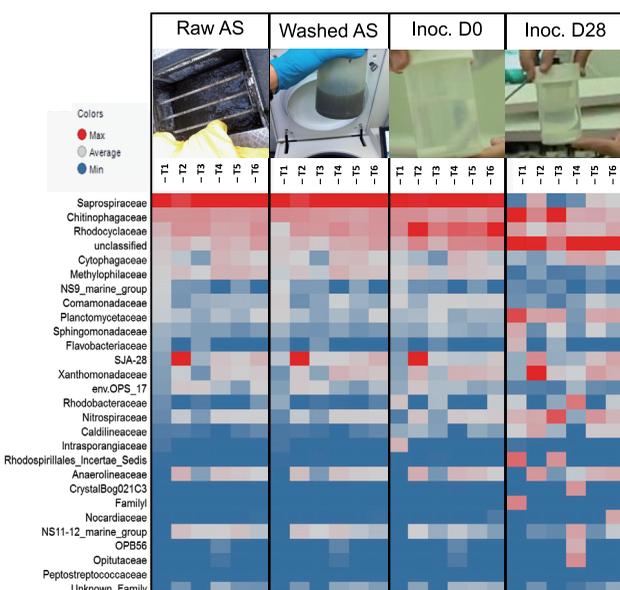


Fig. 2: Heat map of the 28 major families in the inocula of the 6 different tests per condition

CONCLUSION

On the basis of these analyses and contrary to what might be expected:

- There is no visible decrease in the diversity of bacteria as the inoculum treatment progresses
- There is an homogeneity in the major families from sampling (Raw AS) to the start of the test (T0) and also between the 6 sludge samples from the Maxéville WWTP sampled between May 2015 and November 2015. For the 6 tests, in spite of a bacterial population close at the start of the test (T0 controls) and a non-nutritive and reproducible mineral medium, the T28 controls have a very different bacterial composition.

REFERENCES

[1] OECD, 1992. OECD guideline for testing of chemicals - Ready Biodegradability 301, adopted by the council on 17th July 1992, OECD

Acknowledgement - The authors would like to express their thanks to Marie Thomas for her help.

PERSPECTIVES

- Determine the impact of positive reference and poorly water soluble substances on the inoculum composition in ready biodegradability test
- Evaluate further the effect of bioavailability improvement methods on the inoculum composition in tests with poorly water soluble substances