ACCLIMATION OF MICROBIAL CONSORTIA ON ACIDOCENIC EFFLUENTS FOR ENHANCED PRODUCTION OF POLYHYDROXYALKANOATES

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Introduction

Polyhydroxyalkanoates (PHAs) are microbial bioplastics that can replace conventional petrochemical plastics contributing to the tackling of plastic pollution. Various microorganisms, mainly bacteria, and cyanobacteria have been recognized as efficient PHAs producers from various carbon sources. The maintenance of pure cultures however has high energy requirements thus contributing to the high cost of the final products. In recent years emphasis has been placed on the production of PHAs from mixed microbial culture (MMCs) which have minimal culturing requirements and also can utilize various acidified wastes and wastewaters as carbon sources.

In the current study the development and evaluation of MMC of PHAs producers, using acidogenic effluents as carbon source and induced at different degree of nutritional stress, was evaluated. In particular, since PHAs are known to be produced during the limitation of a basic nutrient such as nitrogen, for the development of the MMCs, draw and fill reactors (DFRs) were used, in which the supply of a carbon-rich feed (acidogenic effluents i.e. effluents from dark fermentative hydrogen producing reactors) and a carbon-free feed that contained only a nitrogen source in the form of ammonium sulphate was alternated at a mode of 48 h or 72 h batch cycles. For the 72 h operational cycle the concentration of the feeds was not changes and as such during this cycle the MMCs were subjected to expended nutrient pressure to which they should adjust. Two identical reactors were operated simultaneously in which the same carbon feed but different nitrogen feed was used so that the C/N ratio between the switching cycles was varied having the value 50 for R1 and 10 for R2, i.e. R2 in the nitrogen phase was supplied with 5 times the amount of ammonium nitrogen compared to R1.

The effect of the different degree of nutritional stress was then assessed in terms of the operational efficiency, the structure of the MMCs, and the achieved PHAs yields both at continuous and batch mode.

Scope & Methodology

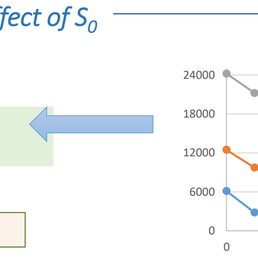
Development of PHAs-producing MMCs via nutrient limitation acclimated to acidogenic effluents

- ✓ *Assess differences in operation*
- ✓ *Assess differences in microbial structure*
- ✓ *Select best performing MMC in terms of PHAs production*

Composition of C feed (mg/L)	
Carbohydrates	600
Acetic Acid	200
Propionic Acid	20
Butyric Acid	750
Valeric acid	65
Caproic acid	50
Ethanol	15
Lactic acid	100
Proteins	100

2 identical lab-scale bioreactors operated at Draw & Fill mode

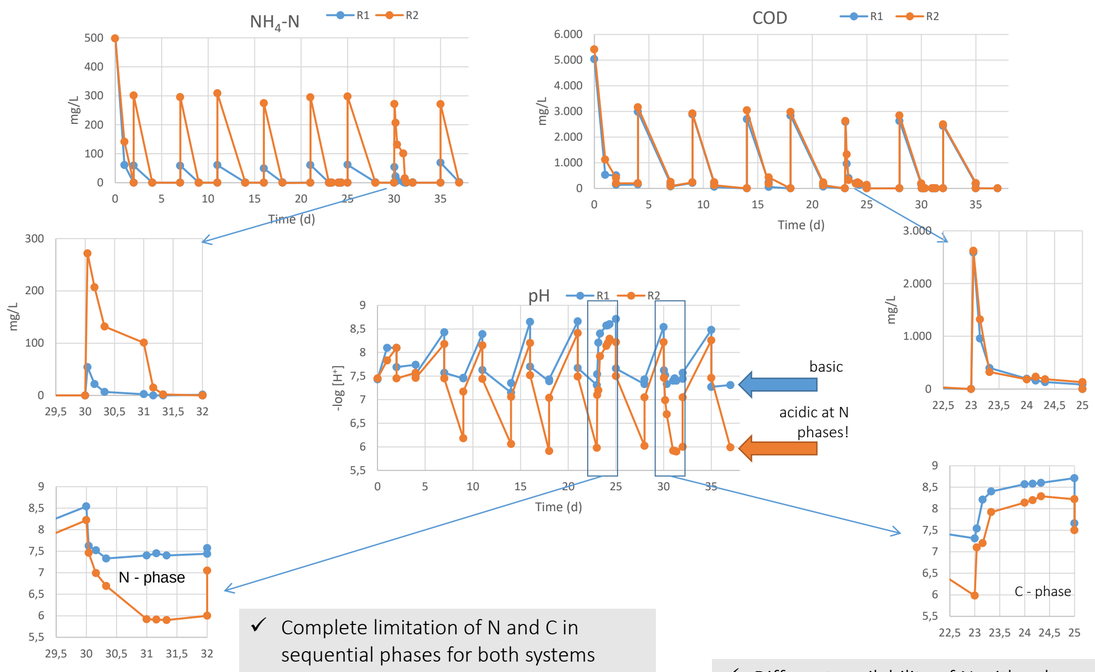
	R1	R2
V_w, mL	1000	1000
COD, mg/L	3000	3000
NH_4^+, mg/L	60	300
C/N, g COD/g TN	50	10
Operational mode	2d- 2d- 3d	2d- 2d- 3d



Composition of N feed (mg/L)	
Ammonium	R1: 197 g/L
Sulphate	R2: 985 g/L

Development and acclimation of MMCs in DFRs

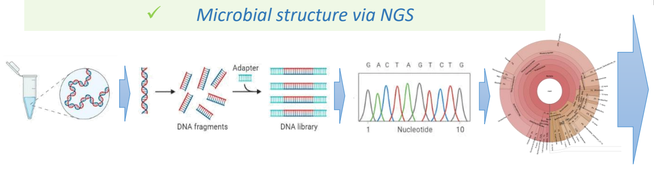
Monitoring of DFRs operation

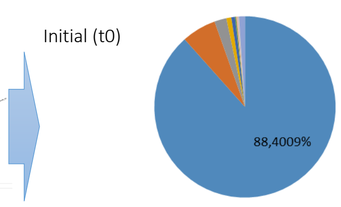


- ✓ Complete limitation of N and C in sequential phases for both systems
  - *N- R1: 100% uptake after ~8 h*
  - *N- R2: 100% uptake after ~30 h*
  - *C- R1/R2: 90% uptake after ~8 h*
  - *C- R1/R2: 100% uptake after ~30 h*
- ✓ Different availability of N with subsequent different pH evolution patterns are expected to lead to
  - *dominance of different microbial species*
  - *different PHAs yields*

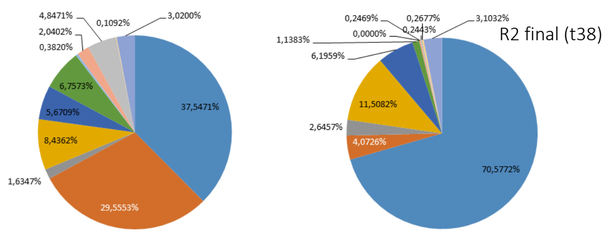
Analysis of Microbial Structure of the Acclimated MMCs

- Evaluation of acclimation conditions
  - ✓ *Microbial structure via NGS*

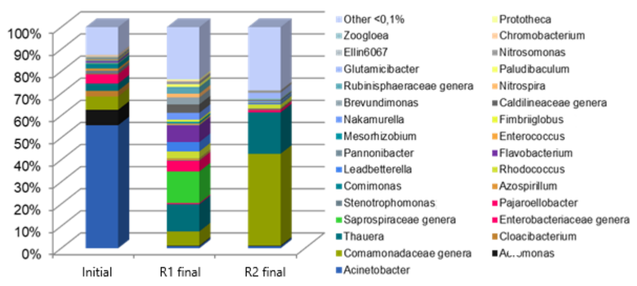




- ✓ Different C/N ratio and pH led to *dominance of different microbial taxa*
- ✓ *At PHYLUM level*
  - *R1: Bacteroidota & Proteobacteria*
  - *R2: Proteobacteria mainly*

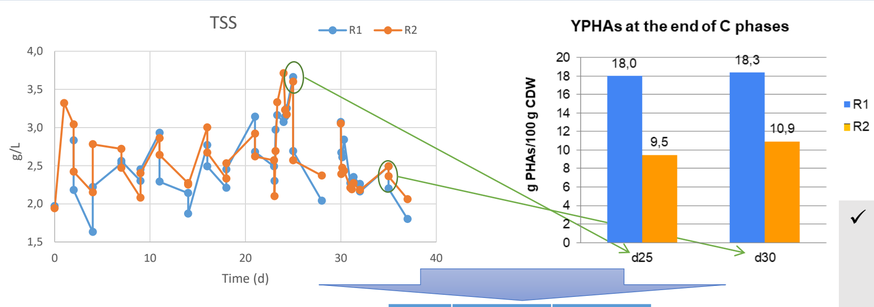


Dominant genera



- ✓ High dominance of Proteobacteria over Bactariodota, i.e. the genus *Thauera* and Comamonadaceae genera in the R2 reactor
- ✓ Higher heterogeneity of the population of the R1 reactor
  - *The conditions of R1 reactor allow the maintenance of a greater range of PHAs producing microorganisms*
  - *This could be considered as an advantage of the MMC because it may allow easier adaptation to new environments with different carbon sources.*

PHAs yields



Day	R1	R2
25	0.65 g/L	0.35 g/L
35	0.46 g/L	0.27 g/L

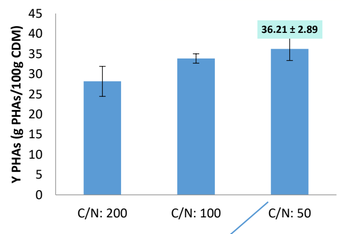
- ✓ Different C/N ratio leads to
  - *Differentiation in operation*
  - *Differentiation in microbial structure*
  - *Differences in PHAs yields and concentrations*

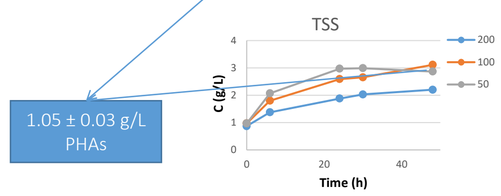
Assessment of PHAs production from the acclimated MMCs at batch mode

Effect of C/N

R1 derived MMC

COD reduction : ~70-80 % for 200 and 100 at 48 h; >90 % for 50 at 48 h

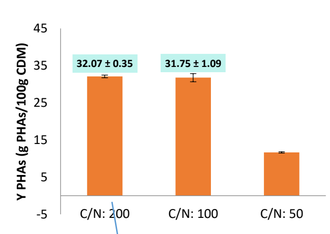


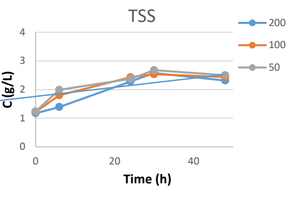


1.05 ± 0.03 g/L PHAs

R2 derived MMC

COD reduction : ~65-80 % for 200 and 100 at 48 h; >85 % for 50 at 48h



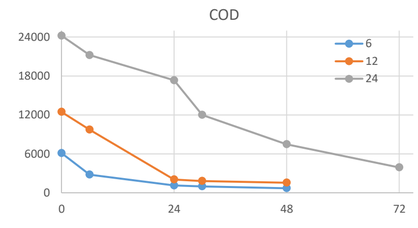


0.87 ± 0.00 g/L PHAs

Effect of S_0

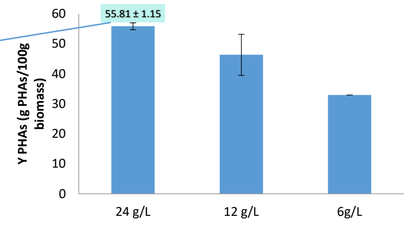
R1 derived MMC

COD reduction : >90% for 6 g/L and 12g/L at 48 h; ~85% for 24 g/L at 72 h



8 g/L TSS was achieved for **24 g/L at 72 h**

4.47 ± 0.09 g/L PHAs



- ✓ at batch mode the PHAs productivity maximizes compared to DFR **C is used for growth and accumulation mainly and not for maintenance)**
  - *the extra nitrogen availability (lower C/N ratio) leads to higher TSS and PHAs yields for R1 culture*
  - *the extra nitrogen availability (lower C/N ratio) leads to the same TSS and lower PHAs yields for R2 culture*
  - *Higher initial substrate concentration lead to higher accumulation capacity and also higher PHAs concentration, requires though longer culturing periods*

Conclusions

The results of the present study demonstrate that subjecting MMCs to different conditions during their acclimation to the same waste highly affects its structure, leading thus to the deveolpment of biocalatysts with distrinct characteristics in terms of their adaptation capacity and PHAs production potential. In overall it was shown that:

- ✓ During adaptation of the initial MMC
  - *the more strict limitation of N results to the higher heterogeneity of PHAs producers, which may be contributed to the maintenance of the pH at favorable levels for more genera*
  - *the heterogeneity of the MMC favors PHAs accumulation*
- ✓ On the contrary upon formation of the consortia
  - *higher amount of provided N at batch mode leads to higher PHAs accumulation capacity yields*
  - *higher substrate concentration leads to higher accumulation capacity and also higher PHAs concentration, indicating that the effectiveness of the production depends also on the availability of C and is not inhibited up to the limits tested*

Acknowledgments

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