

Acclimation of microbial consortia on acidogenic effluents for enhanced production of polyhydroxyalkanoates.

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Abstract

The aim of the present study was to develop efficient mixed microbial cultures (MMCs) for polyhydroxyalkanoates (PHAs) production, via their acclimation to a mixture of volatile fatty acids (VFAs), sugars, and lactate i.e. the acidogenic effluents of a hydrogen-generating bioreactor processing food wastes. Acclimation was conducted under sequential carbon (C) and nitrogen (N) limitation in draw fill reactors (DFRs) with organic loading 3 g/L (in terms of the chemical oxygen demand (COD)) in the C phase and ammonium chloride as N source in the N phase. Two DFRs were operated simultaneously with different carbon-to-nitrogen ratios (C/N) i.e. 10 g COD/g N-NH₄⁺ and 50 g COD/g N-NH₄⁺ and differences in the operational efficiency and microbial structure were assessed. Subsequently, batch experiments were conducted with the acclimated MMCs and with different initial substrate concentrations (6 -24 g COD/L) and different C/N ratios (50-200 g COD/g N-NH₄⁺). It was shown that the feeding strategy during acclimation resulted in the domination of different microbial genera of Betaproteobacteria and Bacteroidota and affected greatly the yields and production rates of PHAs at batch mode, with the highest obtained yield exceeding 0.7 g PHAs/ g microbial biomass.

1. 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 cultures (MMCs), which have minimal culturing requirements and also can utilize

various acidified wastes and wastewaters as carbon sources (Kora et al., 2023).

In the current study the development and evaluation of MMCs of PHAs producers, using acidogenic effluents (rich in volatile fatty acids (VFAs) fermentation broths generated during dark fermentation of food wastes) as carbon (C) 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 (N), for the development of the MMCs draw and fill reactors (DFRs) were used, in which the supply of a C-rich feed (acidogenic effluents) and a C-free feed that contained only a N source in the form of ammonium sulphate was alternated at a mode of 48h or 72h batch cycles. For the 72h operational cycle the concentration of the feeds was not changed 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 C feed but different N 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.

2. Materials and methods

2.1. Mixture of acidogenic effluents

The acidogenic effluents that were used as carbon source were the effluents of a hydrogen producing continuous bioreactor fed with FORBI extract i.e. a sugar rich solution produced from the extraction of dried and shredded household biowastes (Antonopoulou et al., 2020). After the removal of solids via centrifugation, a

mixture of different effluents was prepared and it was diluted to substrate concentration of 3 g/L s-COD (soluble Chemical Oxygen Demand) after supplementation with minerals and trace elements as described by Kora et al (2023). The composition of the final mixture that was used as carbon source for all experiments was the following (in mg/L): sugars, 600; acetate, 200; propionate, 20; butyrate, 750; valerate, 65; caproate, 50; ethanol, 15; lactate, 100 and proteins 100.

2.2. Enrichment of PHAs producers

Two identical DFRs with different carbon-to-nitrogen ratios (C/N) i.e. 10 g COD/g N-NH₄⁺ and 50 g COD/g N-NH₄⁺, were operated for 37 days, during which they were monitored in terms of C and N consumption, pH and suspended solids (volatile, VSS and total, TSS) daily. The PHAs intracellular content of selected biomass samples at the end of C phases was also estimated, whereas the structure of the microbial communities was also analysed in the inoculum and at the end of the operational period of the reactors R1 and R2.

2.3. Batch experiments

At the end of the operational period of R1 and R2 reactors, the acclimated MMCs that were developed, were further accessed as biocatalysts for the targeted PHAs production from the acidified effluents at batch mode, during which C and N are supplied to the culture simultaneously, allowing for the maximum PHAs accumulation. In those experiments the effect of the C/N ratio (50, 100 and 200 g COD/g N-NH₄⁺) and the initial substrate concentration, S₀ (6, 12 and 24 g/L COD) on the yields and productivities of PHAs were assessed.

2.4. Analytical Methods

COD, and N-NH₄⁺, VFAs and PHAs were quantified as described by Dounavis et al. (2016). The monomeric composition of the produced PHAs was identified by measuring its methyl-ester derivatives using a Shimadzu Nexis GC 2030 gas chromatograph equipped with a flame ionization detector (FID) and a MEGA-5H (INC. 30 m × 0.25 mm I.D. × 0.25 μm film) capillary column as described at Kora et al. (2023).

3. Results

3.1. Effect of C/N on the operation of the reactors, the PHAs yields and the microbial structure

During the operation of R1 and R2 complete consumption of C and N was achieved into the 48 h cycles. The behaviour of the MMCs was differentiated during the N phase solely since for R1 thought, since it was shown that N uptake was complete after only ~8 h, whereas for R2 after ~30 h due to the higher availability of N. The carbon uptake profiling is the same i.e. 90 % uptake after ~8 h and 100 % uptake after ~30 h for both reactors. The difference in the N availability and uptake profiles has also led to the different pH evolution patterns

between reactors, during the N cycles. As such for R1 the pH remained at 7.5, but in R2 (having a lower C/N ratio, i.e. higher N amount) the pH dropped during the N consumption to acidic values, ranging thus from 5.9 - 7. This different availability of N with subsequent different pH evolution patterns LED to the dominance of different microbial taxa, with different PHAs producing capacity and consequently to different PHAs yields as well. Those differences were studied by analysing the relevant distribution of the microbial population of the MMCs at different taxa level via Next Generation sequencing (NGS) i.e. sequencing the 16S regions V4 and V5 of rDNA, after extracting the whole bacterial DNA of the MMCs. It was shown that the MMCs were significantly differentiated, with the phylum of Proteobacteria which was of extreme dominance (~ 90%) in the inoculum decreasing in R1 with considerable increase of Bacteroidota, whereas in R2 there was a smaller decrease of Proteobacteria and also a small decrease of the population of Bacteroidota. Further analysis of the microbial distribution at genus level (Table 1) revealed that the dominant Proteobacterium *Acinetobacter* in the initial culture was severely suppressed. Instead the top genera dominated in R1 were the Bacteroidota genera of the Saprospiraceae family, followed by the Proteobacterium *Thauera*, the sum of which was ~26% of the total population. In R2 the top genera dominated were ones from the Comamonadaceae family (Proteobacteria), with a relative abundance of 41.5%, followed by *Thauera*.

Table 1. Relative abundance (%) of genera at the inoculum and the end of operation of R1, R2 reactors.

Genus	Inoc.	R1	R2
<i>Acinetobacter (PB)</i>	55.59	0.90	0.92
<i>Aeromonas (PB)</i>	6.99	0.26	0.25
<i>Comamonadaceae genera (PB)</i>	6.05	6.37	41.50
<i>Thauera (PB)</i>	3.21	12.33	18.74
<i>Enterobacteriaceae genera (PB)</i>	4.30	0.47	0.96
<i>Brevundimonas (PB)</i>	0.23	3.12	0.16
<i>Saprospiraceae genera (BD)</i>	0.18	14.31	0.13
<i>Leadbetterella (BD)</i>	0.19	4.25	0.45
<i>Flavobacterium (BD)</i>	0.53	7.49	0.05
<i>Pajaroellobacter</i>	0.16	4.81	0.24
<i>Rhodococcus</i>	0.24	3.06	1.76
<i>Nakamurella</i>	0.14	3.09	0.56
<i>Caldilineaceae genera</i>	0.16	3.86	0.39

Due to the high dominance of the Proteobacteria and specifically one genus in the R2 reactor, compared with the higher heterogeneity of the population of the R1 reactor it can be assumed that 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.

In order to assess the PHAs production capacity of the two systems, quantification of the intracellular concentration of the PHAs content in the microbial biomass was carried out, through extraction with organic solvents and analysis by gas chromatography. As shown in Fig. 1, R1 has exhibited a higher yield reaching 48% PHAs in the microbial biomass (expressed as cell dry weight, CDW) in both cases, while R2 exhibited yields of 9.5 - 11%.

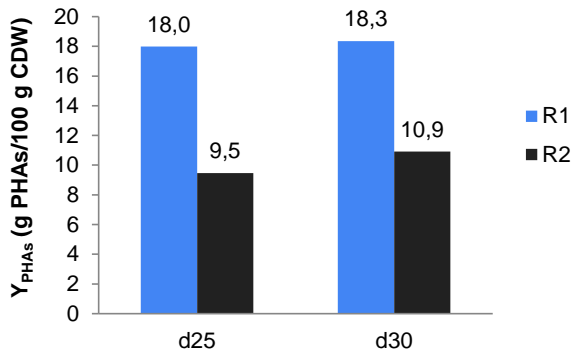


Figure 1. PHAs intracellular accumulation at the end of C phase at days 25 and 35 of the operation of R1 and R2 reactors.

3.2. Effect of C/N and S_0 on batch production of PHAs from acidogenic effluents

Batch cultures were incubated for 48 h, during which the COD and N consumption were monitored and the PHAs yields were also estimated. It was shown that for both R1 and R2 cultures the maximum COD consumption was noted for the C/N 50 i.e. the highest availability of nitrogen, reaching >90 % and 85 % respectively. Regarding the PHAs yields (Fig.2) in terms of the intracellular accumulation capacity, the highest value for R1 culture was ~36% noted for the C/N 50, whereas in the case of R2 culture the C/N ratio of 50 resulted to the lowest PHAs yield, 13 % whereas the ratios of 100 and 200 resulted to quite higher and almost the same PHAs yields i.e. ~32 %. Those results indicate that indeed that at batch mode the PHAs productivity maximizes compared to DFR (since the carbon is used for growth and accumulation mainly and not for maintenance mainly), the extra nitrogen availability (lower C/N ratio) leads to higher and PHAs yields for R1 culture. Regarding the effect of S_0 on the yields of PHAs it was shown that the consumption of COD for the lower concentrations of substrate 24-48 h of incubation were sufficient, whereas for the sufficient uptake of COD in

References

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the case of the highest S_0 concentration, 24 g/L 72 h of incubation is required. As also shown from the second graph, the accumulation capacity of the R1 culture is highly affected by the initial carbon concentration, with S_0 6g/L leading to a similar yield with that of the previous experiments at the same conditions (C/N 50, S_0 6 g/L), i.e. ~35 % and increasing tendency for higher S_0 . The highest yield achieved was 55 % PHAs/CDW, which due to the high TSS achieved as well led to a PHAs concentration led to the fourfolding of PHAs production capacity.

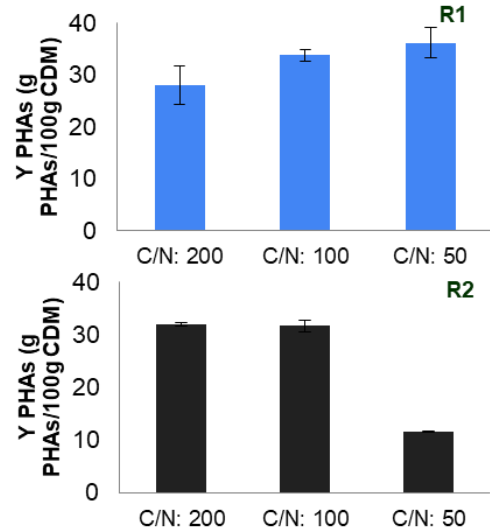


Figure 2. PHAs intracellular accumulation after 48h of batch cultures with R1 and R2 MMCs, using different C/N ratios.

4. 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 development of biocatalysts with distinct characteristics in terms of their adaptation capacity and PHAs production potential.

Acknowledgments

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via a robust bacterial consortium dominated by *Zoogloea* sp. using acidified discarded fruit juices as carbon source. *International Journal of Biological Macromolecules*, **226**, 1500–1514.