Poly-hydroxyalkanoates production from mixed microbial cultures: effect of dissolved oxygen and pH.

KORA E.[1,2], NTAIKOU I.[1*] and LYBERATOS G.[1,3]

[1]Institute of Chemical Engineering Sciences, Foundation for Research and Technology, GR 26504, Patras, Greece

[2]Department of Environmental Engineering, University of Patras, 2 Seferi st., GR 30100, Agrinio, Greece

[3]School of Chemical Engineering, National Technical University of Athens, Zografou Campus, GR 15780, Athens, Greece

*corresponding author: Ioanna Ntaikou

e-mail: ntaikou@iceht.forth.gr

Abstract

The accumulation potential of poly-hydroxyalkanoates (PHAs) produced from an acclimated mixed microbial culture (MMC) under different dissolve oxygen (DO) levels and pH was investigated, using volatile fatty acids (VFAs) as carbon source. Specifically, the PHAs production potential of the MMC was evaluated in batch mode in a 5L bioreactor under controlled high aeration (DO level set at 25% saturation, DO25) and low aeration (DO level set at 5% saturation, DO5). The effect of the controlled DO level on the PHAs yield expressed in % PHAs/DCW (w/w) and also the monomeric composition was evaluated in comparison to the results obtained during constant aeration at 2L/min/L rector i.e. with highly varying DO (DOv). The optimal yield of 79.5±0.14% PHAs/DCW was achieved for DO25, while in the cases of DO5 results were similar to those from DOv, i.e. 60 % PHAs/DCW and 65 % PHAs/DCW, respectively . In all cases, P(3HB-co-3HV) was produced whereas the assimilation of propionate to HV in the polymers did not seem to be correlated to the DO changes, with HV content being above 5% in all cases. The subsequent simultaneous advantageous for the process, indicating that control of DO and not of pH proved to be even more advantageous for the process enhancing the assimilation rates as well, indicating that the production of PHAs from MMCs may be quite efficient if the cultivation conditions are properly controlled.

Keywords: polyhydroxyalkanoates, dissolved oxygen, mixed cultures, pH, nutrients limitation

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 indeed minimal culturing requirements. It is indeed reported that cultivating MMCs instead of pure cultures may lead to halving the upstream production cost. (Catherine et al., 2022) However, PHAs yields from MMCs are generally quite lower than those of pure cultures (Kora et al., 2021). Towards the effort to identify optimal cultural strategies for efficient PHAs production from MMCs different operational parameters have been studied, among which dissolved oxygen (DO) and pH are quite crucial.

2. Materials and methods

2.1. MMC enrichment

An already enriched mixed microbial culture from a previous study (Kora et al., 2023) was cultivated in sequential nutrient supply of C and N in a borosilicate bioreactor using volatile fatty acids (VFAs) i.e. propionate and butyrate as the sole carbon sources and NH_4Cl as nitrogen source. The reactor was operated at draw-fill mode (DFR), with 5L working volume, at 27 ± 1 ºC. For the startup of the reactor, only carbon sources was provided in concentrations corresponding to 1700 mg/L chemical oxygen demand (COD), whereas throughout the following period of sequential limitation of carbon and nitrogen feeds with 1500 mg/L COD, and 75 mg/L $N\text{-}NH_4^+$, respectively for two weeks, following the increase of the concentration of both feeds at 3000 mg/L COD, and 150 mg/L $N\text{-}NH_4^+$ up to the end of the experiment. Both feeds were also supplemented with minerals, trace elements and phosphate buffer as described by (Ntaikou et al., 2018).

2.2. Optimization of operational efficiency of MMC at batch mode

To investigate the effect of DO on the PHAs accumulation capacity of the MMC in batch mode, the Dolevel was set either to 25% of the saturation value (DO25), or 5% (DO5) and also was not preset but the aeration rate was constant at 2L/min/L (Dofree). The pH values that were studed were 7.5 (pH7.5) and 8.5 (pH8.5) All tests were performed in an automated bioreactor (BioBench, 30L, Biostream international, BV) equiped withon line controlled air spurger, stirrer, pH and DO sensors. The working volume was 5 L and the initial COD concentration was adjusted to approximately 10g/L using volatile fatty acids (propionate and butyrate) as the sole carbon and the media were supplemented with NH_4Cl and phosphate buffer for the experiments that studed the DO level, the optimal value of C/N was selected from a previous study (Kora et al., 2023). Inoculation was performed as with MMC collected at the end of a growth phase of the DFR and cultures were incubated at 27±1 ºC for approximatelly 48h. For the experiments studying the effect of the DO the initial pH was set at 7.5 whereas for the experiments studying the effect of pH, the DO levels was set considering the best performance of the DO batches. Samplings were performed throughtout the batch experiment, during which the chemical oxygen demand (COD), N-NH_4^+, total and volatile suspended solids, pHa and the PHAs content of the biomass and the composition of extracted PHAs were measured.

2.3. Extraction of PHAs from the bacterial biomass and estimation of yields

For the PHAs extraction from the microbial a soxlet apparatus was used according to (Ntaikou et al., 2018).

The PHAs accumulation yield, $Y_{PHAs/DCW}$ was estimated according to the equation:

$$Y_{PHAs/DCW} = \frac{PHAs}{DCW} * 100\%$$

where PHAs is the sum of HB and HV content in gr, and DCW the lyophilized biomass in grams.

The PHAs yield in terms of organic carbon uptake, expressed as consumed COD, $Y_{PHAs/CODcons.}$ was estimated according to the equation:

$$Y_{PHAs/CODcons.} = \frac{PHAs}{CODcons.} * 100\%$$

the estimated PHA concentration in expressed (g), and CODcons. is the concentration of consumed COD (g).

2.4. Analytical Methods

COD, and N-NH_4^+ were quantified as described by (Ntaikou et al., 2009). VFAs was 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 Dissolve oxygen (DO)

To investigate the contribution of DO concentration to PHAs accumulation three batch experiments were conducted, specifically DO25, DO5 and DOv were studied. It was shown that nitrogen was fully consumed after 24h in the case of DO25 and DOv, while in the case of low DO i.e. DO5, nitrogen was consumed with a lower rate being exhausted after 33h. In terms of the COD removal, in DO25 91±2.0 % consumption was noted after 30 h, whereas for DO5 and DOv the removal ratio reached 83.5±0.7% and 78.3±0.3% COD, respectively. Biomass concentration didn't differ among the experiments for the three DO levels studied being ~3.8 g/L TSS and ~3.3 g/L VSS. Regarding to PHAs accumulation the best yield referring to PHAs/DCW and PHAs/CODcons. was achieved at 79.5±0.14% and 27.2±0.22% respectively for DO25. DOv and DO5 yields of PHAs/CODcons. did not show much difference while, the PHAs/DCW yield of DOv was slightly higher than DO5 as presented at **figure 1**. Madhusoodanan et al. (2022) reported that PHA production by using a pure culture B. endophyticus was affected by DO stress, resolving in a drastic increased through the oxygen absence or low DO. Further analysis indicated that PHAs production decrease at DO concentrations higher than 2.5 mg/L (31% saturation) would led the MMC to use different metabolic pathways, thus the accumulation of other compounds may be favored (Pinto-Ibieta et al. 2021).

3.2. Effect of pH

To investigate the contribution of pH regulation to accumulation of pH=7.5 and pH=8.5 were studied. It was shownthat nitrogen was fully consumed quite quickly, i.e. after 10h for pH8.5 and after 24h for pH7.5. COD was fully consumed at pH7.5 and partially at pH8.5, whereas the contrary biomass concentration was approximately the same in both cases. Regarding to PHAs accumulation referring to PHA/DCW and PHAs/CODcons. pH7.5 was slightly higher at 69.8±1.0% and 26.3±0.6% as presented at figure 1, and the 3HV content was 6.0±0.1% (Table 1) Concerning pH effect on PHAs accumulation, research has shown that pH values close to neutral are more suitable. Spesifically, Mahato et al. (2021) investigated the potential of a pure culture, *Pseudomonas aeruginosa* strain:EO1, to produce PHAs at different pH and reported that the best environment was at pH 7. On the other hand, Catherine et al. (2022) investigated the PHAs potential of glycogen accumulating organisms and reported that the different pH (between 6.8 to 8.8) didn't affect the microrganisms ability to PHAs accumulation.

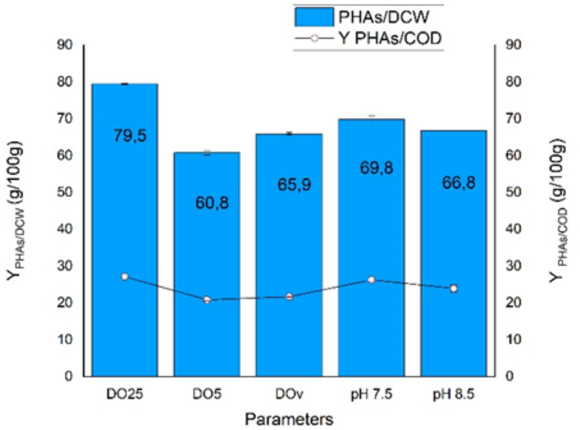

Figure 1. PHAs accumulation referring to PHA/DCW and PHAs/CODcons for the cases of DO25, DO5, Dov, pH7.5 and pH8.5.

Table 1. COD consumption, mean molecular ratio (%) of HB and HV monomeric units in the PHAs produced from the MMC and TSS, VSS concentration.

Case	COD cons.(%)	3HB (%)	3HV (%)	TSS (g/L)	VSS (g/L)
DO25	91.0±2.0	93.7±0.2	6.3±0.2	3.84±0.1	3.25±0.2
DO5	83.5±0.7	93.6±0.2	6.4±0.2	3.79±0.0	3.31±0.0
DO_v	78.3±0.3	92.3±0.1	7.7±0.1	3.94±0.1	3.32±0.1
pH 7.5	96.8±0.1	94.0±0.1	6.0±0.1	5.02±0.5	4.66±0.5
pH 8.5	65.9±0.3	96.8±0.6	3.2±0.6	5.59±0.1	4.99±0.1

4. Conclusions

The results of the present study demonstrate that the dissolve oxygen concentratation and the pH values are crucial for PHAs acumulation among MMC. PHAs accumulation referring to PHA/DCW yield and PHAs/CODcons. yield were suficient in all cases. Nevertheless the best case senario was proven to be DO25 with 79.5±0.14% PHA/DCW and 27.2±0.22% PHAs/CODcons. Regarding to pH optimazation, stable pH7.5 shown better results comparing to pH8.5. However comparing to DO25, (pH was set initially at 7.5 without constant adjustment), PHAs accumulation yield was higher indicating that constant adjustment of pH do not enhance PHAs accumulation.

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