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Polyhydroxyalkanoates (PHAs) Production from the Liquid Fraction of dried/shredded Food Waste

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Abstract An enriched culture of Polyhydroxyalkanoates (PHAs)-forming bacteria was developed using the liquid fraction (condensate) which is generated during drying and shredding of food waste. A mixed enriched culture was used for the production of PHAs, in two Draw-Fill Reactors (DFRs). The culture was developed using cyclic limitation by carbon and nitrogen. Urea was used as nitrogen source and a mixture of volatile fatty acids (VFAs), lactic acid, ethanol and glucose, simulating the composition of the condensate as carbon source. Two cycles of experiments were performed aiming to investigate the effect of the organic loading on the yields and composition of the produced PHAs.
The results showed that the organic loading did not significantly affect the accumulation capacity of PHAs, with the average yield for DFR-1 being 16±5% g PHAs/g DCW (dry cell weight) and for DFR-2 19±3% g PHAs/g DCW. The HV:HB ratio in DFR-1 was estimated to be (19±4):(81±4), whereas in DFR-2 (26±2):(74±2) indicating that the availability of more odd-VFAs may lead to the production of copolymers with higher HV content i.e. a bioplastic with improved properties.

Keywords: PHAs, condensate, food waste, Draw-Fill Reactors (DFRs).

1. Introduction

The worldwide production of petroleum-based plastics has grown exponentially in just a few decades - from 1.5 million tons in 1950 to 359 million tons in 2018– and with it the amount of plastic waste. It is widely known that the exploitation of petroleum for the current demand of plastic materials poses serious environmental concerns, such as global warming, human health risks and ecosystem toxicity [1].

Polyhydroxyalkanoates represent a class of interesting bio-polyesters accumulated by different mixed bacterial cells. Polyhydroxyalkanoates (PHAs) are considered to be a promising alternative to conventional petroleum-based plastics, since these polymers can undergo biodegradation in various environments, including soil, and wastewater, without leaving harmful residues. They are a group of biodegradable polymers that are produced by various microorganisms through a process of bacterial fermentation [2].

PHAs possess a range of properties such as mechanical strength and biocompatibility that make them attractive for various applications and due to their versatility, they can be modified and customized in order to suit specific needs. Thus, the unique properties of PHAs make them a sustainable solution in the quest for greener materials in industries such as packaging, agriculture, and biomedical applications [3].

In recent years, global research attention is focused on the use of different waste materials as carbon sources for microbial synthesis of PHAs in order to reduce production costs. Their production costs are 5–10 times those of traditional petrochemical plastics such as polyethylene, and carbon sources account for 50% of their total costs [4].

In Europe, almost 87.6 million tons of food waste are produced. Despite the high biological value of food waste, traditional management solutions do not consider it a precious resource. Many studies have reported the use of food waste for the production of high added value molecules such as PHAs 5].

Within the framework of a Horizon 2020 project WASTE4think an innovative food waste (FW) valorization approach was developed and implemented. This approach included the drying/shredding process of the food waste, which results in a homogenized solid biomass product named FORBI (Food Residue Biomass) and a liquid fraction (condensate) which is collected by a condenser. The produced condensate is rich in organic carbon, mostly volatile fatty acids (VFAs) but poor in nitrogen, which makes it an ideal substrate for PHAs production [6].

Until now, no attention has been paid to the entire biological process leading to the transformation of the

liquid fraction of food waste, the so called condensate to PHAs

In this study, the use of condensate for PHAs production was investigated as potential carbon source by using a mixed microbial culture, and its efficiency under stress conditions was determined. Under stress conditions such as nitrogen limitation bacterial growth is restricted and carbon surplus is therefore driven to PHAs formation. Alternating carbon and nitrogen limitations were applied, using synthetic condensate and urea as carbon and nitrogen sources, respectively.

2. Materials and methods

2.1 Setup and Operation of bioreactors

Two draw and fill reactors (DFR) of 1L each, made of glass, were used. They operated under non-aseptic conditions, at room temperature (27◦C) with a stirring rate of 250 rpm and a carbon to nitrogen ratio (C/N) of 100. Both DFRs' operation consisted of five distinct phases: (a) aerobic growth phase, with nitrogen addition supply which lasted 47h (external carbon limitation), (b) settling phase during which the aeration and stirring stopped and the suspended biomass settled (1h), (c) withdrawal of 2/3 of the working volume (supernatant withdrawal), (d) aerobic accumulation phase with carbon supply (nitrogen limitation), (e) settling phase during which the aeration and stirring stopped and the suspended biomass settled (1h). Aeration was conducted with moisturized air (Figure 1).

2.2 Microbial Culture

For the first reactor (DFR-1), an enriched aerobic mixed culture, derived from the recirculation of the secondary clarifier of the Wastewater Treatment Plant of Lykovrisi, Attiki, Greece, was used as inoculum. The strategy that was followed for the selection and enrichment of activated sludge in PHA-accumulating bacteria was the alternating cycling between limitation of carbon and nitrogen substrates. Following this procedure, PHAs-accumulating bacteria could adapt under stress conditions and bacteria with high PHAs-accumulation capacity were able to survive longer than their heterotrophic competitors after several cycles. The acclimated culture from DFR-1 was used as inoculum for the second reactor (DFR-2) *(30% v/v).*

2.3 Feedstock

The feedstock used in each cycle consisted of either the carbon or nitrogen source, the basal synthetic medium and a trace element solution. For both reactors, synthetic condensate was used as carbon source, with similar composition as the real one i.e. acetic acid (27% g COD/L), butyric acid (27% g COD/L), propionic acid (6% g COD/L), ethanol (20% g COD/L), glucose (15% g COD/L) and lactic acid (5% g COD/L). DFR-1 operated with 2 g COD/L and DFR-2 with 4 g COD/L. Urea was used as nitrogen source with 20 mg N/L and 40 mg N/L, for DFR-1 and DFR-2 respectively. The composition of the basal synthetic medium used was as follows: 2 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $CaCl_2 \cdot 2H_2O$, 12.5 g/L K_2HPO_4 and 7.5 g/L KH_2PO_4 and 1.0 mL/L of a trace elements solution. The trace elements solution had the following composition: 0.01 g/L $CuCl_2 \cdot 2H2O$, 2 g/L $FeCl_3 \cdot 6H2O$, 0.03 g/L $NaMoO_4 \cdot 2H_2O$, 0.02 g/L $NiCl_2 \cdot 2H_2O$, 1 g/L $ZnSO_4 \cdot 7H_2O$, 0.2 g/L $CoCl_2 \cdot 6H_2O$, 0.3 g/L KI, 0.3 g/L H_3BO_3, 0.03 g/L $MnCl_2 \cdot 4H_2O$ and 2.5 g/L EDTA.

2.3 Analytical Methods

The analysis of soluble COD, TSS and VSS were carried out according to Standard Methods for the Examination of Water and Wastewater [7]. The pH was measured using a digital pH-meter (WTW INOLAB PH720). For the quantification of VFAs, 1 ml of sample was acidified with 30 μL of 20% H_2SO_4 and analyzed via a gas chromatograph (SHIMADZU GC-2010 plus) equipped with a flame ionization detector and a capillary column (Agilent technologies, 30 m x0.53 mm ID x1 μm film, HP-FFAP) using an autosampler (SHIMADZU AOC-20 s). The oven was programmed from 105 °C to 160 °C at a rate of 15 °C·min-1 and subsequently to 225 °C (held for 3 min) at a rate of 20 °C·min-1. Helium was used as the carrier gas at 30 ml·min-1, the injector temperature was set at 230 °C and the detector at 230 °C. Ethanol, glucose and lactic acid were measured via High Performance Liquid Chromatography, HPLC (Agilent1260 Infinity II). Total Nitrogen (TN) was measured with a Shimadzu TN-L analyzer (Shimadzu, Kyoto, Japan; TOC-VCHS and SSM-5000 module). $N\text{-}NH_4^+$ analysis was carried out using the HI-93733-01 Reagents of Hanna Instruments. The PHA detection method was based on the simultaneous extraction and transesterification of PHA. The frozen and lyophilized biomass pellets in glass tubes were used for measuring the PHAs concentration and composition in microbial biomass by gas chromatography with flame ionization detection (GC-FID) after acidic methanolysis as described in Oehmen et al (2005). [8] Pure poly (3-R-hydroxybutyrate-co-3-R-hydroxyvalerate) (PHBV) copolymer with a 3-R-hydroxyvalerate (3HV) content of 8 mol% from Sigma-Aldrich was used for calibration; thus, the contents of 3-R-hydroxybutyrate (3HB) and 3HV were determined; PHA was defined as the sum of 3HB and 3HV.

3. Results and Discussion

The experimental results are shown in Table 1. The mean COD concentration in the feed of DFR-1 was 2025±460 mg O_2/L and the organic nitrogen concentration of 18±6 mg/ L. In the second reactor (DFR-2) the organic loading and concentration of organic nitrogen were doubled (3571±523 mg O_2/L, 36±9 mg/ L) with the aim of

investigating the effect of this increase on the yield and composition of the produced PHAs.

Nitrogen limitation in the carbon phase was achieved in both reactors favoring PHAs accumulation. At the same time, microbial biomass increase was observed, with the concentration in the second one being higher, as in DFR-1. The average value of total and volatile solids was 2.60±1.77 gr TSS /L and 2.13±1.49 g VSS/ L and in DFR-2 4.45±2.14 gr TSS/L and 3.82±1.94 gr VSS/L respectively.

The produced PHAs obtained from both bioreactors were recovered by digestion using chloroform and acidified methanol. The yield was calculated as the ratio of the sum of the monomer's weights over the dry cell weight.

Table 1: Experimental results for both DFR-1 and DFR-2.

Parameter	DFR-1	DFR-2
COD (mg O_2/L)	2025 ± 460	3571 ± 523
TN (mg/L)	18 ± 6	36 ± 9
TSS (mg/L)	2.60 ± 1.77	4.45 ± 2.14
VSS (mg/L)	2.13 ± 1.49	3.82 ± 1.94
PHAs(g)/ DCW (g) (%)	15 ± 5	16 ± 5
HV: HB	(19 ± 4): (81 ± 4)	(26 ± 2): (74 ± 2)

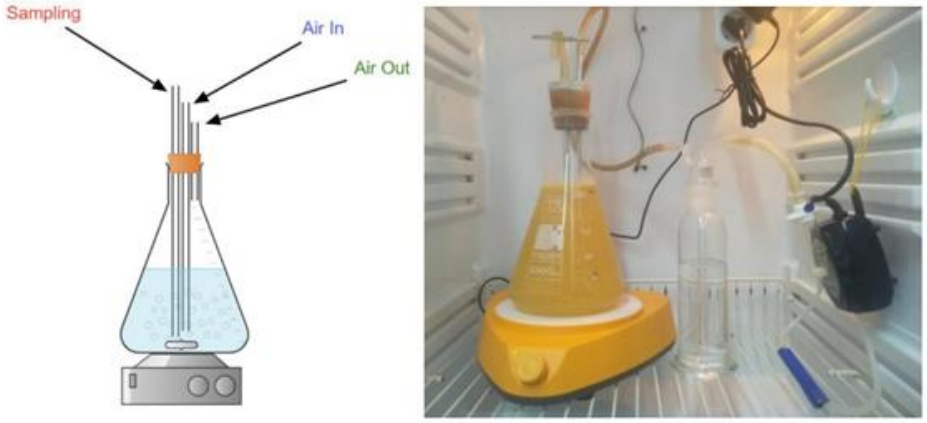

Figure 1. DFRs experimental setup

4. Conclusions

Results obtained in this work can be useful as the starting point for considering the feasibility of PHAs production from the liquid fraction (condensate) from drying food waste as a carbon source.

The two bioreactors showed similar yields, since in DFR-1 and DFR-2 the mean yield was 15±5% and 16±5% PHAs(g)/ DCW (g), respectively. In both experimental cycles, monomers 3HB and 3HV were observed. Therefore, it is deduced that either the copolymer P(3HBco3HV) or a mixture of the aforementioned copolymer and the homopolymers P3HB and P3HV was produced. However, a difference was observed in the PHA composition. More specifically, the ratio HV:HB in DFR-1 and DFR-2 was (19±4):(81±4) and (26±2):(74±2), respectively. This difference is attributed to the doubling of the carbon concentration in DFR-2.

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