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Influence of synthetic carbon grade on the metabolic flux of polyhydroxyalkanoate monomeric constitution synthesized by *Bacillus cereus* AAR-1

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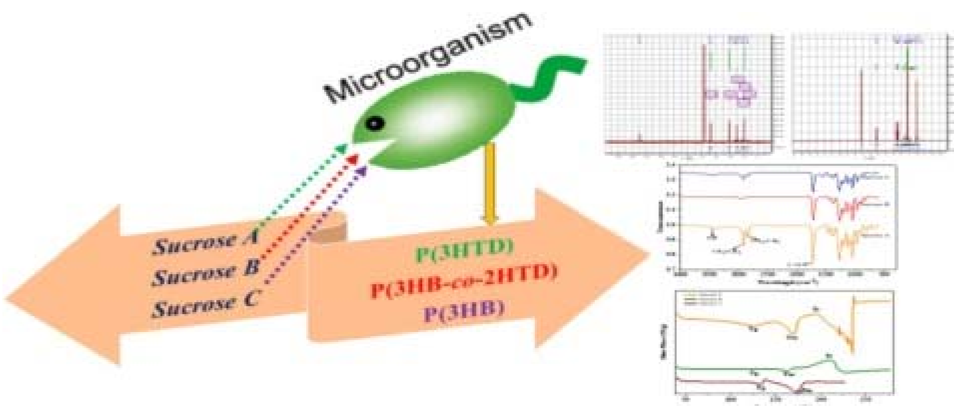
Abstract

Carbon substrate is a pivotal factor influencing polyhydroxyalkanoate (PHA) properties of varied industrial importance. Three synthetic sucrose samples with varying manufacturing purity levels were selected as carbon substrates to synthesize diverse PHAs using a wild-type *Bacillus cereus* AAR-1. Comparative monomeric analyses of the extracted biopolymers revealed Poly (3-hydroxytetradecanoate) (P3HTD), Poly(3-hydroxybutyrate-*co*-2-hydroxytetradecanoate) [P(3HB-*co*-2HTD)], and Poly(3-hydroxybutyrate) (P3HB) with carbon elemental contents that ranged from 39 to 53 % and no nitrogen detected. The decomposition temperature of [P(3HB-*co*-2HTD)] was 279 °C, indicating higher thermal stability than the individual monomeric units. Notably, the homopolymer P3HTD exhibited an increased melting temperature of 172.4 °C and a reduced crystallinity percentage (X_c % = 20.7 %), crucial properties for bioplastics and medical sector applications. All the biopolymers displayed a low specific heat capacity ranging between 0.03 and 0.05 J/g°C, suitable for applications such as thermal storage materials and temperature-regulating textiles. The results suggest that different carbon purity grades influenced homopolymer accumulated in *Bacillus cereus* AAR-1.

Graphical abstract



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Introduction

Polyhydroxyalkanoate is a degradable polymer synthesized and degraded through biological means with more comprehensive applications depending on its monomeric constituents (Koller, 2018; Bhatia et al., 2021). These monomeric components classify PHA into groups based on the number of carbon atoms in the chemical structure (Muneer et al., 2020). The short-chain-length PHAs (scl-PHAs) have monomers with a chain length of 3–5 carbon atoms and are relatively rigid but brittle and crystalline (Choi et al., 2021). They are waterproof with excellent mechanical and thermal properties, making them more applicable as packaging material, agricultural film, and tissue engineering (Reddy et al., 2022). In contrast, medium-chain-length PHAs (mcl-PHAs) with carbon lengths ranging from 6 to 14 are elastic and create a sticky surface, while the long-chain-length PHA (lcl-PHA) has 15 or more carbon atoms in its monomeric unit (Reddy et al., 2022; Koller and Kukherjee, 2022). The unique properties of mcl-PHA provide a thermoelastomeric nature suitable for biomedical applications such as drug delivery systems and skin adhesives. lcl-PHAs are uncommonly produced and less studied PHA groups (Raza et al., 2018). They have decreased modulus tensile strength and a satisfactory elongation breaking point, making them great potential for industrial application (Ribeiro et al., 2016).

Although over 150 distinct hydroxyalkanoates building blocks have been identified as constituents of the PHA biopolyester family, only a restricted range of PHA copolymer types have attained industrial maturity (Koller and Simon, 2022). In large-scale production, most commercial efforts revolve around scl-PHA, predominantly as bulk polymers, with only a limited number of companies currently commercializing mcl-PHA (Koller and Kukherjee, 2022). Poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), commonly manufactured biopolymers, exhibit insufficient impact strength, increased brittleness, and limited flexibility, rendering them unsuitable for numerous industrial and commercial uses (Meereboer et al., 2020). The production of mcl-PHAs has been relatively difficult (Choi et al., 2021), influencing its industrial scalability and impeding high-end applications. Furthermore, in numerous instances, reprogramming microorganisms via genetic reconstruction to generate desired metabolic PHA products leads to disordered cellular activities, potentially causing a substantial reduction in the product yield (Li et al., 2021).

Synthetic media components and complex waste substrates are frequently employed in microbial fermentations. The composition of the media components can fluctuate based on the manufacturing processes, which can significantly influence the outcomes of biological cultivations. Bilinskaya et al. (2020) studied the influence of variable zinc concentrations in commercially available culture broths on the antibiotic susceptibility activities of Enterobacter species. They noted that even slight variations in zinc concentrations of the synthetic culture caused a significant impact on the antimicrobial resistance behavior of Metallo-β-Lactamase-harboring Enterobacteriaceae. Also, Sparviero et al. (2023) reported the yeast extract by different manufacturers enhanced microbial activities such as alginate production and increased the dry cell weight in *Azotobacter vinelandii* ATCC9046. Thus the purity of the carbon substrate is a crucial factor that affect the quality of the biopolymer produced by a wide PHA producer (McAdam et al., 2020). However, the impact of the aforementioned factors in producing the homopolymer mcl-PHA are seldomly reported.

Microorganisms use specific enzymes and metabolic pathways to convert carbon sources into various PHA types (Favaro et al., 2019). High concentrations of certain inorganic impurities, such as salts and heavy metals, adversely affect the metabolic pathways; where it could act as inhibitors or promoters of unwanted side reactions (Samul et al., 2014; Rangel et al., 2020). The strain, *B. cereus* ARY73, produced heteropolymer PHA containing both scl- and mcl-monomers, using glucose as a carbon source (Yasin and Al-Mayaly, 2021). When utilizing molasses containing different sugars, as the sole carbon source, *B. cereus* SPV exclusively produced the 3HB monomeric unit, constituting >50 % of the dry cell weight of the biomass (Akaraonye et al., 2012).

B. cereus ARY73 possess the class IV synthase noted for its broad substrate specificity and diverse metabolic versatility (Valappil et al., 2007). Consequently, additional carbon substrates can stimulate co-monomeric concentration in the organism (Mohammed et al., 2020). Also, *B. cereus* FB11 have been reported to synthesize poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-co-3 HV)] using glucose as the sole carbon source (Masood et al., 2013). And, *B. cereus* FC11, another strain of the *Bacillus cereus* species isolated by Masood et al. (2015), exhibited a substantial increase in 3 HV concentration over the [P(3HB-co-3 HV)] when cultured with glucose supplemented with additional substrates. In this study, various biopolymers synthesized by *B. cereus* AAR-1 obtained from an age-long dumpsite were assessed using sucrose of various purity grades obtained from different manufacturers. The findings thus, contribute valuable insights to PHA biopolymer production where control and optimization of PHA monomeric synthesis for diverse industrial applications could be achieved.

Section snippets

Screening for PHA-producing hydrocarbonoclastic bacteria

The hydrocarbonoclastic (hydrocarbon-degrading) bacteria were enriched using a conventional enrichment technique where crude oil served as the carbon source (Farag et al., 2018). The mineral medium consisted of the following per liter: 1.2 g KH_2PO_4, 1.8 g K_2PO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, and 0.01 g $FeSO_4.7H_2O$ (pH 6.8) (Farag et al., 2018), supplemented with (2 % v/v) crude oil.
For the enrichment process, soil samples were collected from the Abule-Egba municipal dumpsite in

Potential PHA producer

The PHA-producing bacterium used for this study was selected from hydrocarbon-degrading bacteria. In the preliminary investigation following the enrichment studies, all hydrocarbon-degrading bacteria were screened on three carbon sources—glucose, sugarcane molasses, and crude oil—using three PHA screening dyes (results not shown). By leveraging the unique properties and advantages of each carbon source, ranging from readily bioavailable sugar (glucose), industrial waste (sugarcane molasses), to

Conclusion

This study examined how the purity grade of sucrose affects the types of monomeric units accumulated in *B. cereus AAR-1*. This observation therefore underscores the reasons for diverse types of PHAs synthesis when using sucrose-based wastes. *B. cereus* AAR-1 produced a C14 homopolymer mcl-PHA, thus demonstrated the organisms ability to use sucrose as an effective carbon source. The heteropolymer

P(3HB-*co*-2HTD) showed improved thermal stability compared to its homopolymer counterparts. In

CRediT authorship contribution statement

A.R. Akinwumi: Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **O.C. Nwinyi:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **A.O. Ayeni:** Writing – review & editing, Supervision, Project administration, Conceptualization. **S. Venkata Mohan:** Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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