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Fermentation development using fruit waste derived mixed sugars for poly(3-hydroxybutyrate) production and property evaluation

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Abstract

Free sugars from fruit wastes were evaluated for the production of poly(3-hydroxybutyrate) (PHB) in *Paraburkholderia sacchari* fed-batch bioreactor fermentations. Different initial sugar concentration, carbon to inorganic phosphorus (C/IP) ratio, IP addition during feeding and volumetric oxygen transfer coefficient (k_La) were evaluated to promote PHB production. The highest intracellular PHB accumulation (66.6%), PHB concentration (108.3 g/L), productivity (3.28 g/L/h) and yield (0.33 g/g) were achieved at 40 g/L initial sugars, C/IP 26.5, 202.6 h^{-1} k_La value and 20% IP supplementation in the feeding solution. The effect of different cell's harvesting time on PHB properties showed no influence in weight average molecular weight and thermal properties. The harvest time influenced the tensile strength that was reduced from 28.7 MPa at 22 h to 13.3 MPa at 36 h. The elongation at break and Young's modulus were in the range 3.6-14.8% and 830-2000 MPa, respectively.

Keywords: *Paraburkholderia sacchari*, poly(3-hydroxybutyrate), fruit waste, fermentation optimisation, thermomechanical properties

1 Introduction

Biopolymers such as polyhydroxyalkanoates (PHAs) are sustainable alternatives for bioplastic production. Poly(3-hydroxybutyrate) (PHB) is a type of PHA and it is intracellularly produced by various prokaryotes when an essential nutrient is depleted from the fermentation broth. Industrial PHAs manufacturing is impeded by the higher manufacturing costs ($5.5/kg) than petroleum-based plastics (less than $1.2/kg) (Crutchik et al., 2020). Currently, commercial PHA are manufactured by pure cultures and carbon sources such as pure carbohydrates (Obruca et al.,

2015). To alleviate this problem, crude biomass has been assessed as feedstock including agricultural residues (Cesário et al., 2014) and food wastes (Sousa et al., 2021).

The bacterial strain *Paraburkholderia sacchari* (Dietrich et al., 2020) has been widely used for PHA production. *P. sacchari* DSM 17165 consumes C5/C6 sugars, organic acids and vegetable oils leading to high cell densities and PHB accumulation (Cesário et al., 2014). Furthermore, *P. sacchari* utilizes crude renewable resources, such as whey and molasses, for PHB production (Cesário et al., 2014; Pradella et al., 2010)

Fruits and vegetables contribute around 40-50% of the annual food waste production. Besides the fruit processing industry, significant quantities of fruits are disposed from open markets and catering services (Ganesh et al., 2022). Such fruit waste could be used for biorefinery development combining the biomass component separation (e.g. phenolics) with the production of fermentation products (e.g. PHB) using the free sugars and the hydrolysate produced from cellulose and hemicellulose. For instance, the free sugar content of apples, peaches and pears is in the range of 40-60% on a dry basis (Karakasova et al., 2009). The development of biorefineries from fruit waste for PHB production and value-added fractions could create a sustainable business model. PHB production could be feasible as a large fraction of fruits is discarded (Gowman et al., 2019). Various fruit waste streams (e.g. pineapple, watermelon, orange, papaya) have been evaluated for PHB production using various bacterial strains (e.g. *Bacillus sp.* SV13, *Klebsiella pneumoniae*) (Suwannasing et al., 2015; Valdez-Calderón et al., 2022). It should be stressed though that high PHB production efficiency that is needed for industrial implementation has been rarely reported.

PHB production at low manufacturing cost can only be achieved by increasing the fermentation efficiency, including PHB concentration, yield, productivity and intracellular PHB content, using at the same time a synthetic medium to avoid the use of expensive complex media. High PHB production from crude renewable resources is dependent on the initial carbon source

concentration, selection of nutrient limitation, initial and feeding media composition, and oxygen supply (Karasavvas and Chatzidoukas, 2020; Mohanakrishnan et al., 2020). Cesário et al. (2014) utilised wheat straw hydrolysate in bioreactor cultures for 105 g/L PHB production at 0.22 g/g yield, 1.6 g/(L·h) productivity and 72% (w/w) intracellular PHB content.

Biorefinery development using fruit wastes should initially begin with recovering marketable components before PHB manufacturing using the sugar-rich fraction. This study focusses on the optimisation of PHB production using free sugars from fruit waste with the bacterial strain *Paraburkholderia sacchari* in bioreactor cultures. The effect of different initial sugar concentrations was investigated in fed-batch bioreactor cultures followed by the evaluation of carbon to inorganic phosphorus (C/IP) ratio, phosphorous addition during feeding and volumetric oxygen transfer coefficient (k_La). PHB properties (thermal, mechanical and molecular weight) were evaluated at different fermentation times. The optimised process for PHB production for fruit waste that is presented in this study will be integrated in a fruit waste biorefinery producing free sugars, hydrolysates from peel residues, antioxidants and pectins.

2 Materials and Methods

2.1 Fruit waste composition and processing

Apples, peaches and pears from local market were macerated and homogenized using a laboratory mill. Free sugars were extracted with distilled water using 1:20 (w/v) solid to liquid ratio at 45°C and 2 h extraction duration. Filtration (Whatman N°1 filter paper) was used to separate the solids from the aqueous solution that contained the sugars. The filtrate was utilized as fermentation medium containing 36.6 g/L fructose, 23.1 g/L glucose, 6.6 g/L sucrose, 0.05 g/L free amino nitrogen (FAN), 0.1 g/L inorganic phosphorus (IP), 0.5 g/L citric acid and 0.3 g/L malic acid. The extracts were frozen at -18ºC until further use.

The fruit composition used in this study is presented in Table 1. Moisture content was determined gravimetrically after drying at 80ºC until constant weight. The determination of ash was performed by combustion in a muffle furnace at 575ºC for at least 4 h. Total carbohydrates and lignin content were determined according to Sluiter et al. (2004). Lipid content was determined by extraction with hexane for 6 h with a Soxhlet apparatus. Protein content was calculated by Total Kjeldahl Nitrogen the extraction of antioxidants and the total phenolic content was measured by Folin–Ciocalteu method according to Filippi et al. (2021). Pectin extraction was performed via aqueous HCl under agitation at 90°C for 120 min (Huang et al., 2018).

2.2 Microorganism and inoculum preparation

The wild-type strain *Paraburkholderia sacchari* DSM 17165 was employed in this work. The inoculum contained 4.5 g/L $Na_2HPO_4 \cdot 2H_2O$, 1 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 1.5 g/L KH_2PO_4, 1 g/L yeast extract, 20 g/L glucose, 1 mL trace elements solution. The latter contained 2.25 g/L $ZnSO_4 \cdot 7H_2O$, 1 g/L $CuSO_4 \cdot 5H_2O$, 10 g/L $FeSO_4 \cdot 7H_2O$, 0.23 g/L $Na_2B_4O_7 \cdot 10H_2O$, 2 g/L $CaCl_2 \cdot 2H_2O$, 0.5 g/L $MnSO_4 \cdot$4-5H_2O, 0.1 g/L $(NH_4)_6Mo_7O_{24}$, 10 mL 35% HCl (Cesário et al., 2014).

Pure glycerol (50%, v/v) and the abovementioned culture medium was used for bacterial cell cryopreservation (−80°C). The precultures were incubated (100 mL) in shake flasks (500 mL) at 30°C for 12–14 h under agitation (250 rpm). At the exponential phase, 10% inoculum at an optical density (600 mn) around 8 was aseptically transferred to the bioreactor.

2.3 Culture medium

The fermentation medium contained 3 g/L KH_2PO_4, 4 g/L $(NH_4)_2SO_4$, 40 mg/L EDTA, 1.7 g/L citric acid, 1.2 g/L $MgSO_4 \cdot 7H_2O$ and 10 mL trace elements solution (Cesário et al., 2014). Sterilisation (121°C, 20 min) of sugars and the rest of the medium was conducted separately.

2.4 Fed-batch bioreactor fermentations for poly(3-hydroxybutyrate) production

Bioreactor fermentations (2 L, Eppendorf, Bioflo120, Germany) were performed at 30°C using 1 L working volume. The fermentation pH was 6.8, while pH was controlled with 2 M HCl and 24% NH_4OH, which also served as nitrogen source. Antifoaming agent (Sigma) was used as needed during fermentation. The aeration used was 2.5 vvm (k_La 224.9 h^{-1}). The C/IP was 22.8 unless otherwise mentioned in specific experiments. Filter sterilisation was used for the air (0.22 μm, Polycap^{TM} AS, Whatman Ltd). The agitation was maintained at 1200 rpm, while the dissolved oxygen (DO) was determined with a DO probe (Mettler Toledo electrode InPro 6000). A concentrated solution from fruit extracted free sugars (~800 g/L) was used as feeding medium that was produced via vacuum evaporation. A continuous feeding strategy was employed using varying feeding rates (3-38 mL/h) to maintain the total sugar concentration at 10-20 g/L. The bioreactor was sterilised at 121°C for 20 min.

Four fed-batch bioreactor cultures were initially performed to assess the influence of different initial sugar concentrations on biopolymer accumulation and bacterial growth. The specific growth rate was calculated in the exponential growth phase by plotting the ln(RCW) against time. Free sugars from fruits were used at different initial concentrations (25-90 g/L). Initial sugar concentration was diluted with deionized water or concentrated under vacuum evaporation to achieve the desirable concentration.

Four fed-batch bioreactor fermentations were subsequently performed to evaluate the influence of different ratios of carbon to inorganic phosphorous (C/IP) using the optimal concentration (ca. 40 g/L) of free sugars. The initial phosphorous concentration in the form of KH_2PO_4 was adjusted in order to achieve C/IP ratios of 24.6, 26.5, and 32.0. In a separate set of fed-batch bioreactor cultures, the supplementation of inorganic phosphorous during feeding was also evaluated by gradually adding 10% and 20% of the initial IP concentration. The main purpose

of IP supplementation during feeding was to prolong the viability of bacterial cell and as a consequence enhance PHB production.

Four fed-batch bioreactor cultures were finally conducted to assess the influence of different values of k_La (157.9–224.9 h^{-1}). The rotation speed of the impeller was 1200 rpm, while varying aeration rates were applied (1-2.5 L_{air}/min). The optimal values of sugar concentration, C/IP ratio and IP supplementation during feeding were used in these fermentations. At the optimal k_La value, a separate fermentation was performed to evaluate the influence of cell harvest time on PHB properties (thermal, mechanical, molecular weight). The k_La was estimated according to the methodology presented by Stylianou et al. (2021).

All experiments were performed in duplicates.

2.5 Poly(3-hydroxybutyrate) extraction

Bacteria cells containing the intracellular polymer were separated from the broth (3000×g, 15 min) and subsequently lyophilized. PHB extraction from the lyophilized biomass was conducted based on the method proposed by Hahn et al. (1994). Bacterial cell disruption was performed with aqueous sodium hypochlorite (20%, v/v) and chloroform (1:1) for 120 min under agitation (150 rpm) at ambient temperature. The suspension was centrifuged (3000×g, 10 min) and three distinct phases were observed. The bottom phase, where is the dissolved PHB in chloroform, was filter separated (Whatman N°1 filter paper) to remove cell debris. Polymer precipitation was performed with ice-cold methanol.

2.6 Polymer properties determination

2.6.1 Differential scanning calorimetry

A Differential Scanning Calorimeter (DSC, Perkin Elmer model Pyris 6, USA) was used. Pure indium was employed for calibration. The measurements were conducted at the Laboratory of Farm Structures, Department of Natural Resources Management and Agricultural Engineering,

Agricultural University of Athens. Specifically, 11 mg of each sample were kept at -20°C for 4 min, then heated from -20°C to 190°C at a rate of 10°C/min and equilibrated at 190°C for 1 min, to avoid the degradation of PHB and eliminate previous thermal history. The samples were, then, cooled to -20°C at 10°C /min and kept at -20°C for 4 min. Then, heating was applied to 190°C at 10°C /min. A nitrogen atmosphere was employed for prevention of thermo-oxidative degradation. The first heating cycle is omitted from the presented data as it represents the behaviour of the material under uncontrollable conditions (e.g. cleaning, drying, pulverisation). The degree of crystallinity X_c (%) was calculated by Equation 1:

$$X_c(\%) = \frac{\Delta H_m}{\Delta H_{m0}} \times 100\%, \textit{ Equation 1}$$

where $\Delta H_{m0,\ 100\%\ \text{crystalline}}$ PHB=146 J/g (Oliveira et al., 2007).

Crystallisation temperature (T_c, °C), crystallisation enthalpy (ΔH_c, J/g), cold crystallisation temperature (T_{cc}, °C), cold crystallisation enthalpy (ΔH_{cc}, J/g), melting point (T_m, °C), and fusion enthalpy (ΔH_m, J/g) were estimated.

2.6.2 Gel permeation chromatography

Molecular weight analysis of the biopolymer was performed by Gel Permeation Chromatography (Shimadzu, Nexera LC-40, Japan) equipped with Agilent pLgel 5um Mixed-C columns (300 × 7.5 mm). Chloroform was employed for sample dissolution (0.25%, w/v) with subsequent filtration through PTFE membrane, 0.22 μm, before further analysis. The mobile phase (chloroform) was employed at 1 mL/min and 30°C column temperature. Universal calibration was performed with polymethyl methacrylate standards ranging from 550 to 2,210,000 g/mol (Agilent Technologies).

2.6.3 Mechanical properties

The ISO 527-3:2018 was employed for PHB films preparation for mechanical properties determination. A universal testing machine (Instron, Model 5900, USA) was employed for the determination of the tensile of the films (10 mm width). Tensile tests were carried out at 10 mm/min separation speed (23°C, 50% relative humidity). Elongation at break, tensile strength and modulus of elasticity were calculated automatically by the Instro 'Bluehill 3' Software. The reported values are the average of five samples that were performed at the same conditions.

2.7 Analytical Methods

A High-Performance Liquid Chromatography equipment (HPLC) (Shimadzu UFLC XR system, Japan) was used for the determination of fructose, glucose, and sucrose as well as malic acid and citric acid. The system was equipped with Phenomenex Rezex ROA-Organic acid H^+ column (300 mm x 7.8 mm) and a Shimadzu refraction index detector. Separation occurred at 65°C and elution was achieved using 10 mM H_2SO_4 aqueous solution as mobile phase (0.6 mL/min). Disaccharides and monosaccharides were also determined using Shodex SP0810 column (8.0 × 300 mm) in the HPLC equipment (Shimadzu UFLC XR system, Japan) with a RI detector. The column was maintained at 80°C and the mobile phase was HPLC grade water with a flow rate of 0.6 mL/min. Diluted samples were filtered (PTFE membrane, 0.22 μm) before analysis and sugar and organic acids quantification was achieved with standard curves developed using commercial sugars and organic acids.

The concentration of ammonium ion during the fermentation was determined based on the phenate method as described by Scheiner (1976). Inorganic phosphorous was measured spectrophotometrically according to Harland and Harland (1980).

Cell dry weight (CDW) measurements was employed for bacterial growth determination. Cells were recovered via centrifugation (10,000×g, 10 min) and the sediment was washed two times with acetone and distilled water. Drying of bacterial pellets was conducted at 50°C.

PHB quantification was carried out by acid propanolysis as previously described by Riis and Mai (1988). Samples were analysed by gas chromatographic analyser (GC) (Shimadzu, Nexis GC 2030, Japan) using automatic sampler (AOC-20i plus), a flame ionization detector (FID) and a Mega-Wax column (30 m × 0.25 mm, film thickness 0.25 μm). The temperature of the oven was initially 100°C for 1 min using subsequently 25°C/min temperature ramp at 160°C, which was kept stable for 1 min before further elevation to 188°C using a 10°C/min ramp. The temperature was subsequently increased to 250°C at 25°C/min with 5 min isothermal period. The carrier gas was helium (1 mL/min). The injector and detector temperatures were 230°C and 250°C respectively. The internal standard was benzoic acid, while the external standard employed was PHB (Sigma-Aldrich).

3 Results and Discussion

This study presents the optimization of PHB production from free sugars obtained from fruit waste. This process will be integrated in a biorefinery that will be presented in detail in a forthcoming publication. The biorefinery begins with the collection of whole fruit wastes (apples, pears and peaches) from open markets (Table 1), which are subsequently macerated and suspended in water to separate the free sugars. After separation of the solids from the liquid fraction, the latter could be treated with absorbent resins to remove polyphenols if it contains high amounts of antioxidants. Macroporous resins have been used for polyphenols separation and purification from various juices (Green et al., 2022). The solids are used for polyphenol extraction with aqueous ethanol and pectin extraction by HCl treatment followed by enzymatic hydrolysis of cellulose and hemicellulose to

produce a sugar-rich hydrolysate. Alternative sequence of unit operations could be employed based on comparative sustainability assessment of different process flow sheets.

3.1 Evaluation of initial sugars concentration

P. sacchari growth and PHB production were evaluated at different initial sugar concentrations in fed-batch bioreactor fermentations. Polymer accumulation was triggered by phosphorous limitation. Bacterial growth, PHB production as well as sugars and inorganic phosphorous consumption were monitored during fermentation (Figure 1). *P. sacchari* consumed all sugars with the rate of fructose consumption being higher than glucose and sucrose.

Feeding was initiated at different times (7, 10, 13, 20 h) when the total sugar concentration was ca. 10-20 g/L. After phosphorous depletion, PHB accumulation was rapidly increased. Consequently, cell growth gradually stopped and the CDW remained constant. The highest PHB concentration (76.5 g/L) with CDW of 133.3 g/L were accomplished at 65 g/L initial sugars concentration, while the highest CDW (149.1 g/L) was attained at 40 g/L initial sugars concentration. Elevating the initial carbon source concentration to 90 g/L caused the reduction of PHB (42.1 g/L), CDW (97.4 g/L), yield (0.23 g/g) and productivity (1.17 g/(L·h)). At the highest sugar concentration (90 g/L), the lag phase was increased showing that bacterial growth is negatively affected at this sugar concentration. The productivity range achieved was 1.17–2.68 g/(L·h), while the highest productivity was attained at 65 g/L initial carbon source concentrations (Table 2).

The lowest lag phase duration (4 h) was observed low initial carbon source concentration (Table 2), while at 65 g/L and 90 g/L carbon source concentration led to higher lag phase duration (6 h and 10.5 h, respectively). The highest specific growth rate (μ=0.5 h^{-1}) was obtained at 40 g/L initial sugars concentration. A high specific growth rate is required to achieve rapid cell growth at high concentrations that will lead to high PHB concentration and accumulation. Thus, the high

specific growth rate attained at 40 g/L initial carbon source concentration is important in industrial bioprocess development. Table 2 shows that the highest residual cell weight (RCW) of 76 g/L was observed at 40 g/L initial carbon source concentration, while the productivity, PHB concentration and yield were similar to those achieved at 65 g/L initial sugar concentration. Therefore, subsequent cultures were carried out at 40 g/L initial sugar concentration where other fermentations parameters (i.e. C/IP ratio, $k_L\alpha$, feeding strategies) were evaluated.

Shake flasks fermentations have been carried out with *P. sacchari* in glucose (10-60 g/L) and xylose (10-30 g/L) with no inhibitory effect (Cesário et al., 2014). The specific growth rate was approximately 0.28 h^{-1} for glucose, while the specific growth rate was reduced (0.21-0.18 h^{-1}) with xylose concentration increase (10-30 g/L) (Cesário et al., 2014). The effect of sugar ratio and sugar concentration (glucose, xylose, arabinose) was investigated by using experimental design. The highest PHB concentration (~2.5 g/L) was attained with glucose and arabinose, while in the case of xylose up to 10 g/L PHB were attained. Mixtures of the above sugars (40 g/L) resulted to ca. 3 g/L PHB (Li et al., 2019).

3.2 Evaluation of C/IP ratio

Four fed-batch bioreactor cultures were performed at varying initial C/IP ratios (32, 26.5, 24.6, 22.8) to evaluate its effect on PHB accumulation (Table 3). Phosphorous limitation was observed at around 15 h in all cases, where PHB accumulation was initiated. Feeding was initiated at 10-13 h when the sugar concentration was around 10 g/L. It was observed that varying C/IP ratios (22.8 - 32) resulted in decreasing PHB concentration (73.1 - 39.2 g/L) and CDW (149.1 - 63.5 g/L). Also, the highest RCW (76 g/L) with 49% (w/w) intracellular PHB content were achieved at the lowest C/IP ratio (22.8) indicating that high phosphorous concentration promotes bacterial growth rather than PHB accumulation. Panda et al. (2006) also showed that high

phosphorus concentration lead to protein synthesis resulting in higher cell production, while low amounts of phosphorus promote PHA production. In the case of 26.5 C/IP ratio, the highest PHB accumulation (67.7%) was observed at 32.5 h with corresponding 0.34 g/g yield and 1.88 g/(L·h) productivity (Table 3). The C/IP ratio of 26.5 was used in subsequent fermentations due to the highest yield and intracellular PHB content (67.7%) achieved in this set of experiments. Although the highest productivity of 2.56 g/(L·h) was achieved at the C/IP ratio of 22.8, this ratio was not selected due to the high RCW produced (76 g/L) that it is not advantageous for industrial bioprocess development targeting PHB production where high RCW leads to lower PHB extraction efficiency.

PHB synthesis by *P. sacchari* has been studied under nitrogen or phosphorus limitation (see supplementary material). Studies on phosphorus limitation mainly evaluate varying initial phosphorous concentration to enhance PHB accumulation. Phosphorous plays an important role in many bioprocesses including nucleic acid and protein synthesis and energy production which are essential for bacterial growth and preservation. Under phosphorous limitation, the reduction of ATP synthase leads to Krebs cycle inhibition, therefore the accumulation of acetyl-CoA favors PHA synthesis (Cavaillé et al., 2013). Higher PHB production could be achieved under phosphorous limitation than nitrogen, probably since phosphorous does not contribute directly to protein synthesis, but it is involved mostly on cell membranes, RNA and nucleotides synthesis (Grousseau et al., 2014). Korkakaki et al., (2017) reported that PHA production by active sludge could be enhanced under low C/P ratio where the carbon consumption rate is higher than phosphorous.

In the case of *P. sacchari*, the effect of nitrogen and phosphorous limitation were evaluated with xylose as carbon source in fed-batch fermentations. Among the two nutrients, higher CDW (29.2 g/L) and PHB production (16.2 g/L) were observed when phosphorous limitation was applied. However, excess of phosphorous concentration achieved higher specific growth rates

($0.19\ h^{-1}$) on xylose suggesting that phosphorous may enhance the growth of *P. sacchari* (Oliveira-Filho et al., 2020). Silva et al. (2004) also showed that phosphorous limitation leads to improved fermentation efficiency than nitrogen limitation. Phosphorus supplementation in the feeding solution has been rarely employed in published studies.

Cesário et al. (2014) achieved 146 g/L biomass concentration, 72% PHB content, 0.22 g/g yield and 1.60 g/(L·h) productivity using wheat straw hydrolysate and 3 g/L KH_2PO_4 as initial phosphorous source. Izaguirre et al. (2020) used hydrolysate from organic biowaste and plum waste juice as carbon source and feeding media, respectively, in fed-batch cultures leading to 43% intracellular PHB content, 71 g/L CDW, 0.59 g/(L·h) productivity and 0.15 g/g yield. The fermentation efficiency achieved with the organic biowaste hydrolysate is lower than the one achieved in this study most probably due to the higher cell growth attributed to the rich nitrogen and phosphorus content of the organic biowaste.

The impact of different feeding strategies on the fermentation efficiency of PHB production by the supplementation of inorganic phosphorous in the feeding solution was investigated in fed-batch *P. sacchari* fermentations (Figure 2). At the optimal initial C/IP ratio (26.5) for enhanced PHB accumulation, two different feeding strategies were implemented, namely supplementation of feeding solution with either 20% or 10% initial IP content. The feeding strategy was continuous and was initiated when ca. 10 g/L sugars were reached. The fermentations duration was 37.5 h in both cases and feeding was initiated at ca. 11 h. The IP added in the fermentation broth during feeding could not be detected during fermentation due to its rapid consumption. The PHB and CDW production as well as the sugar and IP consumption are shown in Figure 2. When the IP content in the feeding solution increased from 10% to 20%, higher PHB production was attained (from 88.4 g/L to 101.6 g/L at 35 h). The highest CDW (142.7 g/L) with 101.6 g/L PHB concentration was also observed when 20% IP feeding solution was used, indicating that IP

addition had a positive effect on both cell growth and PHB synthesis. The productivity was also increased to 2.9 g/(L·h) with a yield on consumed sugars of 0.32 g/g when 20% IP feeding solution was used. These results indicate that IP supplementation during PHB accumulation is necessary in order to prolong bacterial cell viability.

3.3 Evaluation of volumetric oxygen transfer coefficient

The effect of k_La on bacterial growth and PHB production by *P. sacchari* was evaluated under different aeration rates (1, 1.5, 2 and 2.5 L_{air}/min) while the agitation speed was set to 1200 rpm (Figure 3). In all cases, dissolved oxygen was reduced below 3% indicating high metabolic activity. Feeding started at 10-13 h.

It was observed that k_La influence both bacterial growth and PHB production as they were both increased with increasing k_La values (Table 4). The highest total CDW (162.6 g/L) with 108.3 g_{PHB}/L and a productivity of 3.28 g/(L·h) was observed at 33 h in the k_La value 202.6 h^{-1}. At the k_La value of 224.9 h^{-1}, the PHB accumulation (71.1%) was higher than the one (66.6%) achieved in the k_La value of 202.6 h^{-1}, with a yield of 0.32 $g/g_{consumed\ sugars}$ and a productivity of 2.9 g/(L·h). Decreasing the k_La value to 157.9 h^{-1} resulted in the lowest PHB concentration (59.1 g/L) and CDW (111.8 g/L), yield (0.29 g/g) and productivity (1.64 g/(L·h)) (Table 4). The energy required for aeration and agitation in high-cell-density fermentations is one of the main criteria contributing to the high cost of large-scale bioprocesses. Consequently, achieving high productivity and yields are essential in order to reduce utilities consumption (Dietrich et al., 2017). Thus, the k_La value of 202.6 h^{-1} has been selected as the optimal value due to the high productivity achieved.

Most of the microorganisms that produce PHA are obligate aerobes, therefore their need for oxygen for growth and preservation is high (Blunt et al., 2018). The evaluation of k_La has been previously studied in the literature. For example, the supplementation of sufficient oxygen to the cells and maintaining the DO above 10% was achieved by using an airlift bioreactor. Despite the

fact that the CDW was high (150 g/L), the PHB cell content was low (42%), probably because of the oxygen limitation and its association with PHB biosynthesis (Pradella et al., 2010). On the other hand, it was found that the production of PHA in a bioreactor was higher under conditions of uncontrolled pH and high aeration with a PHB accumulation of 40% (Suwannasing et al., 2015).

3.4 Poly(3-hydroxybutyrate) characterization

PHB properties (thermal, mechanical, and molecular weight) were evaluated at different fermentation times (22, 26, 30, 36 and 46 h) conducted at the optimum conditions (C/IP 26.5, k_La 202.6 h^{-1}, 20% phosphorous supplementation in feeding solution). Table 5 summarises the thermal properties of PHB samples taken in this study. Notably, the harvest time did not seem to affect the thermal properties of PHB samples taken at different fermentation durations. It could also be observed that the melting point (T_m), fusion enthalpy (ΔH_m), cold crystallisation temperature (T_{cc}), crystallisation enthalpy (ΔH_c) and cold crystallisation enthalpy (ΔH_{cc}) were within the range reported in literature-cited publications (see supplementary material).

The thermal properties of a polymer are important parameters to consider during thermal processing because they can affect the final product that is formed from biopolymers. Generally, polymers with high molecular weights tend to have lower melting temperatures (T_m) and higher crystallinity compared to biopolymers with lower molecular weights. This means that biopolymers with low molecular weights may be less flexible and more brittle than those with low molecular weights and may also have better biodegradation rates (Arrieta et al., 2016; Hong et al., 2013; Valappil et al., 2007). Domínguez-Díaz et al., (2015) examined various molecular weights of PHB produced from bacterium *Azotobacter vinelandii* resulting that the thermal and mechanical properties of the PHB were largely dependent on its molecular weight, except for samples with a molecular weight above 1400 kDa, which had lower T_m (melting temperature) and crystallinity values. These lower values were thought to be due to molecular entanglements and poor processing

conditions. The study also showed that the physical properties and chemical structure of PHB were also influenced by the bacterial strain.

The polydispersity index (PI), the weight average molecular weight (M_w), the number average molecular weight (Mn) and the mechanical properties of PHB samples taken at different fermentation durations are presented in Table 5. The weight average molecular mass of PHB were in the range of 455.8-484.4 kDa in all cell harvest durations, with similar PI (2.0-2.2). These values are within the range of M_w and PI reported in the literature (see supplementary material). The polydispersity of a polymer refers to the range of chain lengths present in the polymer, showing that fermentation duration has no effect on the average of the polymer chains. A high polydispersity indicates a greater degree of heterogeneity in the lengths of the polymer chains.

High polydispersity indicates high size distribution in the polymer chains. The molecular weight and polydispersity of PHB are controlled by PHA synthase. The molecular weight of PHB is regulated by the concentration and catalytic activity of PHA synthase, the occurrence of chain transfer reaction and PHA degradation during fermentation (Tsuge, 2016). High PHA synthase activity leads to higher molecular weight, while low PHA synthase activity leads to low molecular weight (Stubbe and Tian, 2003).

The tensile strength is decreasing from 28.7 MPa to 13.3 MPa when the fermentation duration increases from 22 h to 36 h. The elongation at break and Young's modulus are in the range of 3.6-14.8% and 827.9 to 1992.7 MPa (Table 6). Decreasing the tensile strength of the polymer would likely have a negative impact on its performance in applications where it is subjected to tensile loads, while increasing the elongation at break of a material would likely have a positive impact for specific applications (e.g. film processing). Materials with a higher elongation at break are more ductile, which means they can stretch or deform more before breaking (McAdam et al., 2020; Nanni and Messori, 2021). The elongation at break of PHB samples taken in this study present similar

values to the literature with the highest value (14.8%) obtained at 30 h (Table 6). The tensile strength was close to the values reported in the literature apart from 30 h and 36 h fermentation where lower values were observed (Table 6).

PHB has thermomechanical properties that are comparable to polypropylene and polyethylene. PHB has some limitations compared to conventional polymers, including low impact strength and elongation at break, making it brittle and stiff. For this reason, blends with other polymers and additives should be developed to overcome these limitations (Yeo et al., 2018). PHB is good candidate for rigid packaging due to its high crystallinity (55-70%) (Garcia-Garcia et al., 2022). The biodegradability of PHB makes it an attractive option for biomedical applications and disposal materials.

4 Conclusions

This study showed that sugars extracted from fruit wastes could be used for highly efficient PHB production by *P. sacchari* cultures. The optimal fermentation efficiency reached ca. 66.6% intracellular PHB content, 162.6 g/L CDW, 3.28 g/(L·h) productivity and 0.33 g/g yield at 40 g/L initial sugars concentration, C/IP ratio of 26.5, k_La value of 202.6 h^{-1} and 20% phosphorus addition in the feeding solution. Future studies should focus on biorefinery development using fruit waste for biopolymer synthesis, antioxidants and pectins. This will enhance the sustainability potential of PHB production within a fruit waste biorefinery.

E-supplementary data for this work can be found in e-version of this paper online

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Conflicts of interest

The authors declare no conflict of interest

Author contributions

Olga Psaki designed and performed the experiments. Ioanna-Georgia I. Athanasoulia, Anastasios Giannoulis prepared and analysed the thermo and mechanical properties. Dimitrios Ladakis, Apostolis Koutinas and Demetres Briassoulis designed and supervised the experiments. Olga Psaki wrote the manuscript. Dimitrios Ladakis, Apostolis Koutinas and Demetres Briassoulis revised the manuscript. All authors have given approval to the final version of the manuscript.

References

1. Arrieta, M.P., López, J., López, D., Kenny, J.M., Peponi, L., 2016. Biodegradable electrospun bionanocomposite fibers based on plasticized PLA–PHB blends reinforced with cellulose nanocrystals. Ind. Crops Prod. 93, 290–301.
2. Blunt, W., Sparling, R., Gapes, D.J., Levin, D.B., Cicek, N., 2018. The role of dissolved oxygen content as a modulator of microbial polyhydroxyalkanoate synthesis. World J. Microbiol. Biotechnol. 34, 1–13.
3. Cavaillé, L., Grousseau, E., Pocquet, M., Lepeuple, A.S., Uribelarrea, J.L., Hernandez-Raquet, G., Paul, E., 2013. Polyhydroxybutyrate production by direct use of waste activated sludge in phosphorus-limited fed-batch culture. Bioresour. Technol. 149, 301–309.

4. Cesário, M. Teresa, Raposo, R.S., de Almeida, M.C.M.D., van Keulen, F., Ferreira, B.S., da Fonseca, M.M.R., 2014. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. N. Biotechnol. 31, 104–113.
5. Crutchik, D., Franchi, O., Caminos, L., Jeison, D., Belmonte, M., Pedrouso, A., Val del Rio, A., Mosquera-Corral, A., Campos, J.L., 2020. Polyhydroxyalkanoates (PHAs) production: A feasible economic option for the treatment of sewage sludge in municipal wastewater treatment plants? Water 12, 1–12.
6. de Sousa Dias, M.M., Koller, M., Puppi, D., Morelli, A., Chiellini, F., Braunegg, G., 2017. Fed-batch synthesis of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) from sucrose and 4-hydroxybutyrate precursors by *Burkholderia sacchari* strain DSM 17165. Bioengineering 4, 36.
7. Dietrich, K., Dumont, M.J., Del Rio, L.F., Orsat, V., 2017. Producing PHAs in the bioeconomy — Towards a sustainable bioplastic. Sustain. Prod. Consum. 9, 58–70.
8. Dietrich, K., Oliveira-Filho, E.R., Dumont, M.J., Gomez, J.G.C., Taciro, M.K., Silva, L.F. da, Orsat, V., Rio, L.F.D., 2020. Increasing PHB production with an industrially scalable hardwood hydrolysate as a carbon source. Ind. Crops Prod. 154, 112703.
9. Domínguez-Díaz, M., Meneses-Acosta, A., Romo-Uribe, A., Peña, C., Segura, D., Espin, G., 2015. Thermo-mechanical properties, microstructure and biocompatibility in poly-β-hydroxybutyrates (PHB) produced by OP and OPN strains of *Azotobacter vinelandii*. Eur. Polym. J. 63, 101–112.
10. Filippi, K., Georgaka, N., Alexandri, M., Papapostolou, H., Koutinas, A., 2021. Valorisation of grape stalks and pomace for the production of bio-based succinic acid by *Actinobacillus succinogenes*. Ind. Crops Prod. 168, 113578.

11. Ganesh, K.S., Sridhar, A., Vishali, S., 2022. Utilization of fruit and vegetable waste to produce value-added products: Conventional utilization and emerging opportunities-A review. Chemosphere 287, 132221.
12. García, A., Pérez, D., Castro, M., Urtuvia, V., Castillo, T., Díaz-Barrera, A., Espín, G., Peña, C., 2019. Production and recovery of poly-3-hydroxybutyrate [P(3HB)] of ultra-high molecular weight using fed-batch cultures of *Azotobacter vinelandii* OPNA strain. J. Chem. Technol. Biotechnol. 94, 1853–1860.
13. Garcia-Garcia, D., Quiles-Carrillo, L., Balart, R., Torres-Giner, S., Arrieta, M.P., 2022. Innovative solutions and challenges to increase the use of Poly(3-hydroxybutyrate) in food packaging and disposables. Eur. Polym. J. 178, 111505.
14. Gowman, A.C., Picard, M.C., Lim, L.T., Misra, M., Mohanty, A.K., 2019. Fruit waste valorization for biodegradable biocomposite applications: A review. BioResources 14, 10047–10092.
15. Green, B. V., Ford, T.W., Goldsborrough, H., Abdelmotalab, M., Ristvey, A.G., Sauder, D.G., Volkis, V. V., 2022. Extraction of Antioxidants from Aronia mitschurinii Juice Using Macroporous Resins. ACS Omega 7, 29877–29885. https://doi.org/10.1021/acsomega.2c02785
16. Grousseau, E., Blanchet, E., Déléris, S., Albuquerque, M.G.E., Paul, E., Uribelarrea, J.L., 2014. Phosphorus limitation strategy to increase propionic acid flux towards 3-hydroxyvaleric acid monomers in *Cupriavidus necator*. Bioresour. Technol. 153, 206–215.
17. Hahn, S.K., Chang, Y.K., Kim, B.S., Chang, H.N., 1994. Optimization of microbial poly(3-hydroxybutyrate) recover using dispersions of sodium hypochlorite solution and chloroform. Biotechnol. Bioeng. 44, 256–261.
18. Harland, B.F., Harland, J., 1980. Fermentative Reduction of Phytate in Rye, White, and Whole Wheat Breads. Cereal Chem. 57, 226-229.

19. Hong, S.G., Hsu, H.W., Ye, M.T., 2013. Thermal properties and applications of low molecular weight polyhydroxybutyrate. J. Therm. Anal. Calorim. 111, 1243–1250.

20. Huang, X., Li, D., Wang, L. 2018. Effect of particle size of sugar beet pulp on the extraction and property of pectin. J. Food Eng. 218, 44–49.

21. ISO 527-3:2018, 2018. Plastics—Determination of tensile properties—Part 3: Test conditions for films and sheets. (Accessed 10 March 2023), https://www.iso.org/standard/70307.html

22. Izaguirre, J.K., da Fonseca, M.M.R., Castañón, S., Villarán, M.C., Cesário, M.T., 2020. Giving credit to residual bioresources: From municipal solid waste hydrolysate and waste plum juice to poly (3-hydroxybutyrate). Waste Manag. 118, 534–540.

23. Karakasova, L., Stefanoski, A., Rafajlovska, V., Klopceska, J., 2009. Technological charasteristics of some apple cultivars. Acta Hortic. 825, 559–564.

24. Karasavvas, E., Chatzidoukas, C., 2020. Model-based dynamic optimization of the fermentative production of polyhydroxyalkanoates (PHAs) in fed-batch and sequence of continuously operating bioreactors. Biochem. Eng. J. 162, 107702.

25. Korkakaki, E., van Loosdrecht, M.C.M., Kleerebezem, R., 2017. Impact of phosphate limitation on PHA production in a feast-famine process. Water Res. 126, 472–480.

26. Li, M., Eskridge, K.M., Wilkins, M.R., 2019. Optimization of polyhydroxybutyrate production by experimental design of combined ternary mixture (glucose, xylose and arabinose) and process variables (sugar concentration, molar C:N ratio). Bioprocess Biosyst. Eng. 42, 1495–1506.

27. Martínez-Herrera, R.E., Alemán-Huerta, M.E., Almaguer-Cantú, V., Rosas-Flores, W., Martínez-Gómez, V.J., Quintero-Zapata, I., Rivera, G., Rutiaga-Quiñones, O.M., 2020. Efficient recovery of thermostable polyhydroxybutyrate (PHB) by a rapid and solvent-free extraction protocol assisted by ultrasound. Int. J. Biol. Macromol. 164, 771–782.

28. McAdam, B., Fournet, M.B., McDonald, P., Mojicevic, M., 2020. Production of polyhydroxybutyrate (PHB) and factors impacting its chemical and mechanical characteristics. Polymers. 12, 1–20.

29. Mohanakrishnan, A.S., Easwaran, S.N., Ravi, D.P., Mahadevan, S., 2020. Understanding the biocalorimetric and respirometric behaviour of co-culture (*R. eutropha, P. putida* and *A. vinelandii*) in poly (3-hydroxybutyrate) batch production. Biochem. Eng. J. 155, 107334.

30. Nanni, A., Messori, M., 2021. Effect of the wine wastes on the thermal stability, mechanical properties, and biodegradation's rate of poly(3-hydroxybutyrate). J. Appl. Polym. Sci. 138, 1–13.

31. Obruca, S., Benesova, P., Marsalek, L., Marova, I., 2015. Use of lignocellulosic materials for PHA production. Chem. Biochem. Eng. Q. 29, 135–144.

32. Oliveira, F.C., Dias, M.L., Castilho, L.R., Freire, D.M.G., 2007. Characterization of poly(3-hydroxybutyrate) produced by *Cupriavidus necator* in solid-state fermentation. Bioresour. Technol. 98, 633–638.

33. Oliveira-Filho, E.R., Gomez, J.G.C., Taciro, M.K., Silva, L.F., 2021. *Burkholderia sacchari* (synonym *Paraburkholderia sacchari*): An industrial and versatile bacterial chassis for sustainable biosynthesis of polyhydroxyalkanoates and other bioproducts. Bioresour. Technol. 337, 125472.

34. Oliveira-Filho, E.R., Silva, J.G.P., de Macedo, M.A., Taciro, M.K., Gomez, J.G.C., Silva, L.F., 2020. Investigating nutrient limitation role on improvement of growth and poly(3-hydroxybutyrate) accumulation by *Burkholderia sacchari* LMG 19450 from xylose as the sole carbon source. Front. Bioeng. Biotechnol. 7, 1–11.

35. Panda, B., Jain, P., Sharma, L., Mallick, N., 2006. Optimization of cultural and nutritional conditions for accumulation of poly-β-hydroxybutyrate in *Synechocystis sp.* PCC 6803. Bioresour. Technol. 97, 1296–1301.

36. Pradella, J.G., Taciro, M.K., Mateus, A.Y.P., 2010. High-cell-density poly (3-hydroxybutyrate) production from sucrose using *Burkholderia sacchari* culture in airlift bioreactor. Bioresour. Technol. 101, 8355–8360.

37. Raposo, R.S., de Almeida, M.C.M.D., de Oliveira, M.D., da Fonseca, M.M., Cesário, M.T., 2017. A *Burkholderia sacchari* cell factory: production of poly-3-hydroxybutyrate, xylitol and xylonic acid from xylose-rich sugar mixtures. N Biotechnol. 34, 12–22.

38. Riis, V., Mai, W., 1988. Gas chromatographic determination of poly-β-hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis. J. Chromatogr. A. 445, 285–289.

39. Rosengart, A., Cesário, M.T., de Almeida, M.C.M.D., Raposo, R.S., Espert, A., de Apodaca, E.D., da Fonseca, M.M., 2015. Efficient P(3HB) extraction from *Burkholderia sacchari* cells using non-chlorinated solvents. Biochem. Eng. J. 103, 39–46.

40. Scheiner, D., 1976. Determination of ammonia and Kjeldahl nitrogen by indophenol method. Water Res. 10, 31–36.

41. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2004. Determination of structural Carbohydrates and Lignin in Biomass. Biomass Anal. Technol. Team Lab. Anal. Proced. 1–14.

42. Sousa, B.V., Silva, F., Reis, M.A.M., Lourenço, N.D., 2021. Monitoring pilot-scale polyhydroxyalkanoate production from fruit pulp waste using near-infrared spectroscopy. Biochem. Eng. J. 176, 108210.

43. Stubbe, J.A., Tian, J., 2003. Polyhydroxyalkanoate (PHA) homeostasis: The role of the PHA synthase. Nat. Prod. Rep. 20, 445–457.

44. Stylianou, E., Pateraki, C., Ladakis, D., Damala, C., Vlysidis, A., Latorre-Sánchez, M., Coll, C., Lin, C.S.K., Koutinas, A., 2021. Bioprocess development using organic biowaste and

sustainability assessment of succinic acid production with engineered *Yarrowia lipolytica* strain. Biochem. Eng. J. 174, 108099.

45. Suwannasing, W., Imai, T., Kaewkannetra, P., 2015. Cost-effective defined medium for the production of polyhydroxyalkanoates using agricultural raw materials. Bioresour. Technol. 194, 67–74.
46. Tsuge, T., 2016. Fundamental factors determining the molecular weight of polyhydroxyalkanoate during biosynthesis. Polym. J. 48, 1051–1057.
47. Valappil, S.P., Misra, S.K., Boccaccini, A.R., Keshavarz, T., Bucke, C., Roy, I., 2007. Large-scale production and efficient recovery of PHB with desirable material properties, from the newly characterised *Bacillus cereus* SPV. J. Biotechnol. 132, 251–258.
48. Valdez-Calderón, A., Barraza-Salas, M., Quezada-Cruz, M., Islas-Ponce, M.A., Angeles-Padilla, A.F., Carrillo-Ibarra, S., Rodríguez, M., Rojas-Avelizapa, N.G., Garrido-Hernández, A., Rivas-Castillo, A.M., 2022. Production of polyhydroxybutyrate (PHB) by a novel *Klebsiella pneumoniae* strain using low-cost media from fruit peel residues. Biomass Convers. Biorefinery 12, 4925–4938.
49. Weinmann, S., Bonten, C., 2019. Thermal and rheological properties of modified polyhydroxybutyrate (PHB). Polym. Eng. Sci. 59, 1057–1064.
50. Yeo, J.C.C., Muiruri, J.K., Thitsartarn, W., Li, Z., He, C., 2018. Recent advances in the development of biodegradable PHB-based toughening materials: Approaches, advantages and applications. Mater. Sci. Eng. C 92, 1092–1116.

Figure Captions

Figure 1 Fed-batch cultures with *P. sacchari* for PHB production in various initial sugar concentrations. (a), IP (b), CDW (c), RCW (d), PHB (e), (f) PHB content. Initial sugar concentrations: (■) 20 g/L, (●) 40 g/L, (△) 65 g/L, (♦) 90 g/L.

Figure 2 Effect of phosphorus supplementation during feeding on PHB production in fed-batch bioreactor cultures. (△) no phosphorus supplementation during feeding, (●) 10% IP supplementation as related to the initial IP concentration, (□) 20% IP supplementation as related to the initial IP concentration. Sugars consumption (a), IP (b), CDW (c), RCW(d), PHB (e), (f) PHB content.

Figure 3 Sugars consumption (a), CDW (b), PHB (c) and (d) RCW, (e) PHB content and (f) Dissolved oxygen during fed-batch fermentations for PHB production under different k_La values. (♦) 157.9 h^{-1}, (●) 180.2 h^{-1}, (■) 202.6 h^{-1}, (△) 224.9 h^{-1}.

Table 1 Composition in apples, pears and peaches used in this study

Composition (%, dry based)	**Apples**	**Pears**	**Peaches**
Moisture content	84.4	83.5	87.2
Free sugars	67.4±3.6	69.5±1.7	72.1±2.1
Protein	2.6±0.3	3.2±0.5	3.5±0.2
Pectin	8.9±0.4	8.4±0.7	6.9±0.5
Glucan	5.3±0.2	4.0±0.6	3.9±0.4
Hemicellulose	4.8±0.2	3.3±0.4	4.1±0.4
Lignin	1.2±0.01	2.8±0.6	2.5±0.3
Oil	0.8±0.1	0.3±0.3	0.3±0.1
Ash	3.3±0.6	2.4±0.3	3.1±0.4
Total phenolic content (g GAE/100 g dry weight)	0.9±0.01	0.6±0.01	0.5±0.01

GAE: Gallic acid equivalents

Table 2 Effect of initial mixed sugar concentration on *P. sacchari* growth and PHB production in fed-batch bioreactor cultures carried out at C/IP 22.8 and k_La 224.9 h^{-1}

Sugar (g/L)	μ (h^{-1})	Lag phase (h)	CDW (g/L)	PHB (g/L)	PHB content (%)	Productivity g/(L·h)	Yield* (g/g)
25	0.46	4	128.9	59.5	46.2	2.02	0.32
40	0.50	4	149.1	73.1	49.0	2.56	0.32
65	0.43	6	133.3	76.5	57.4	2.68	0.31
90	0.36	10.5	97.4	42.1	43.2	1.17	0.23

* g PHB per g consumed sugars

Table 3 Effect of C/IP ratio on *P. sacchari* growth and PHB production in fed-batch bioreactor cultures carried out at 40 g/L initial sugar concentration and k_La 224.9 h^{-1}

C/IP (g/g)	CDW (g/L)	PHB (g/L)	PHB content (%)	Productivity g/(L·h)	Yield* (g/g)
22.8	149.1	73.1	49.0	2.56	0.32
24.6	98.4	60.9	61.9	1.87	0.33
26.5	90.5	61.3	67.7	1.88	0.34
32.0	63.5	39.2	61.7	1.21	0.32

* g PHB per g consumed sugars

Table 4 Effect of k_La ratio on PHB production in fed-batch bioreactor cultures carried out at 40 g/L initial sugar concentration and C/IP 26.5

k_La (h^{-1})	CDW (g/L)	PHB (g/L)	PHB content (%)	Productivity g/(L·h)	Yield* (g/g)
157.9	118.8	59.1	49.7	1.64	0.29
180.2	132.0	79.2	60.0	2.19	0.30
202.6	162.6	108.3	66.6	3.28	0.33
224.9	142.7	101.6	71.1	2.90	0.32

* g PHB per g consumed sugars

Table 5 Thermal properties of PHB produced at different fermentation duration by *P. sacchari*

Fermentation duration (h)	T_m (°C)	T_c (°C)	ΔH_m (J/g)	ΔH_c (J/g)	T_{cc} (°C)	ΔH_{cc} (J/g)	X_c (%)
22	172.1	77.1	83.8	60.4	95.1	5.7	57.3
30	175.6	78.6	86.7	57.9	96.2	6.3	59.3
36	173.7	79.0	85.0	66.7	92.2	9.9	59.6
46	175.1	80.5	88.5	47.8	94.3	10.1	60.6

Table 6 Molecular weight distribution and mechanical properties (tensile strength, elongation at break and Young's Modulus) of the PHB produced at different fermentation durations with *P. sacchari*

Fermentation duration (h)	M_w (kDa)	M_n (kDa)	PI	Tensile strength (MPa)	Elongation at break (%)	Young Modulus (MPa)
22	474.9	225.2	2.1	28.7	3.6	1992.7
26	484.4	221.2	2.2	24.5	7.2	1341.5
30	461.7	221.1	2.1	15.1	14.8	827.9
36	455.8	223.4	2.0	13.3	10.1	1004.8
46	468.9	236.3	2.0	23.9	6.5	1259.8

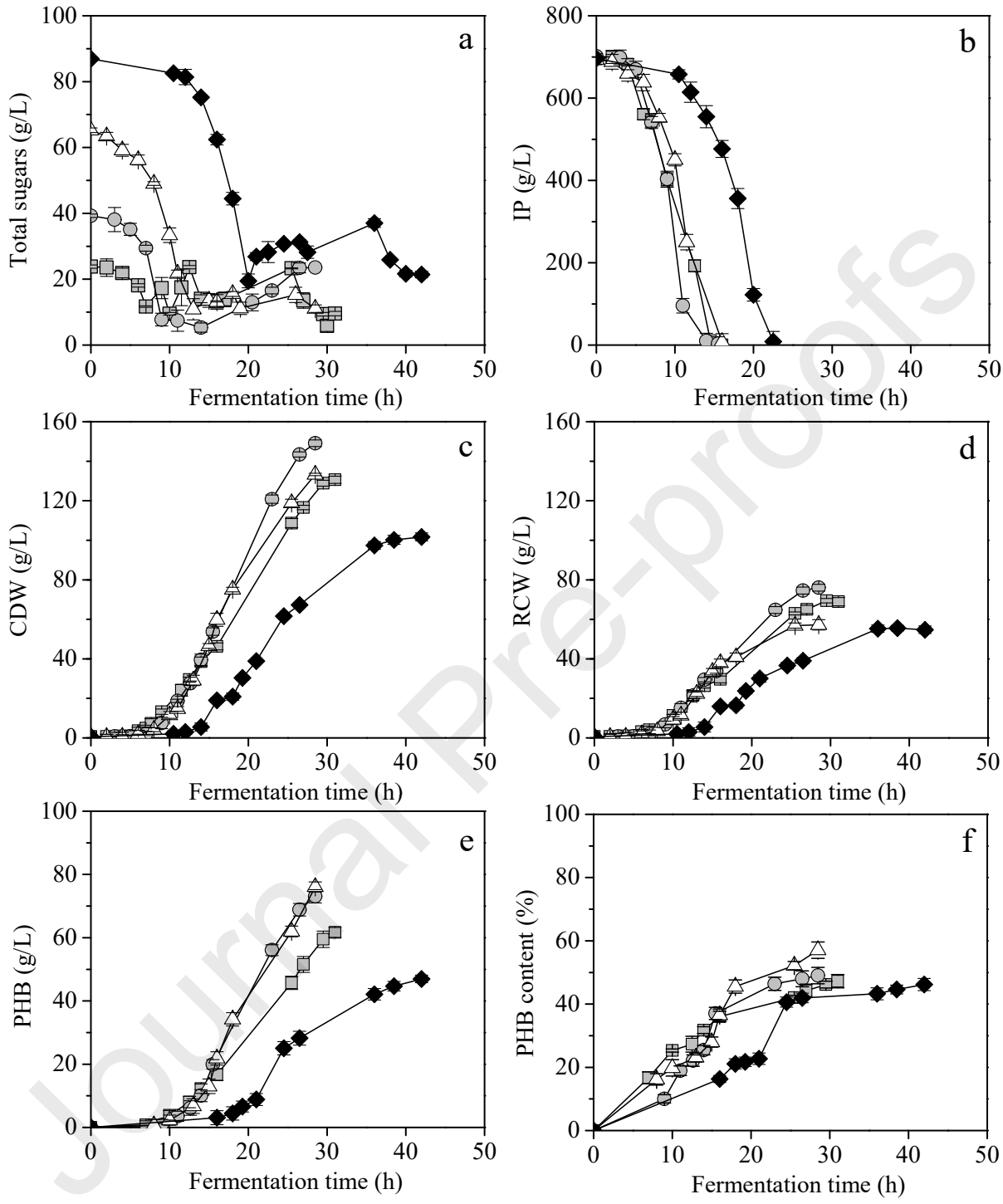
a
Total sugars (g/L)
Fermentation time (h)
b
IP (g/L)
Fermentation time (h)
c
CDW (g/L)
Fermentation time (h)
d
RCW (g/L)
Fermentation time (h)
e
PHB (g/L)
Fermentation time (h)
f
PHB content (%)
Fermentation time (h)

Figure 1

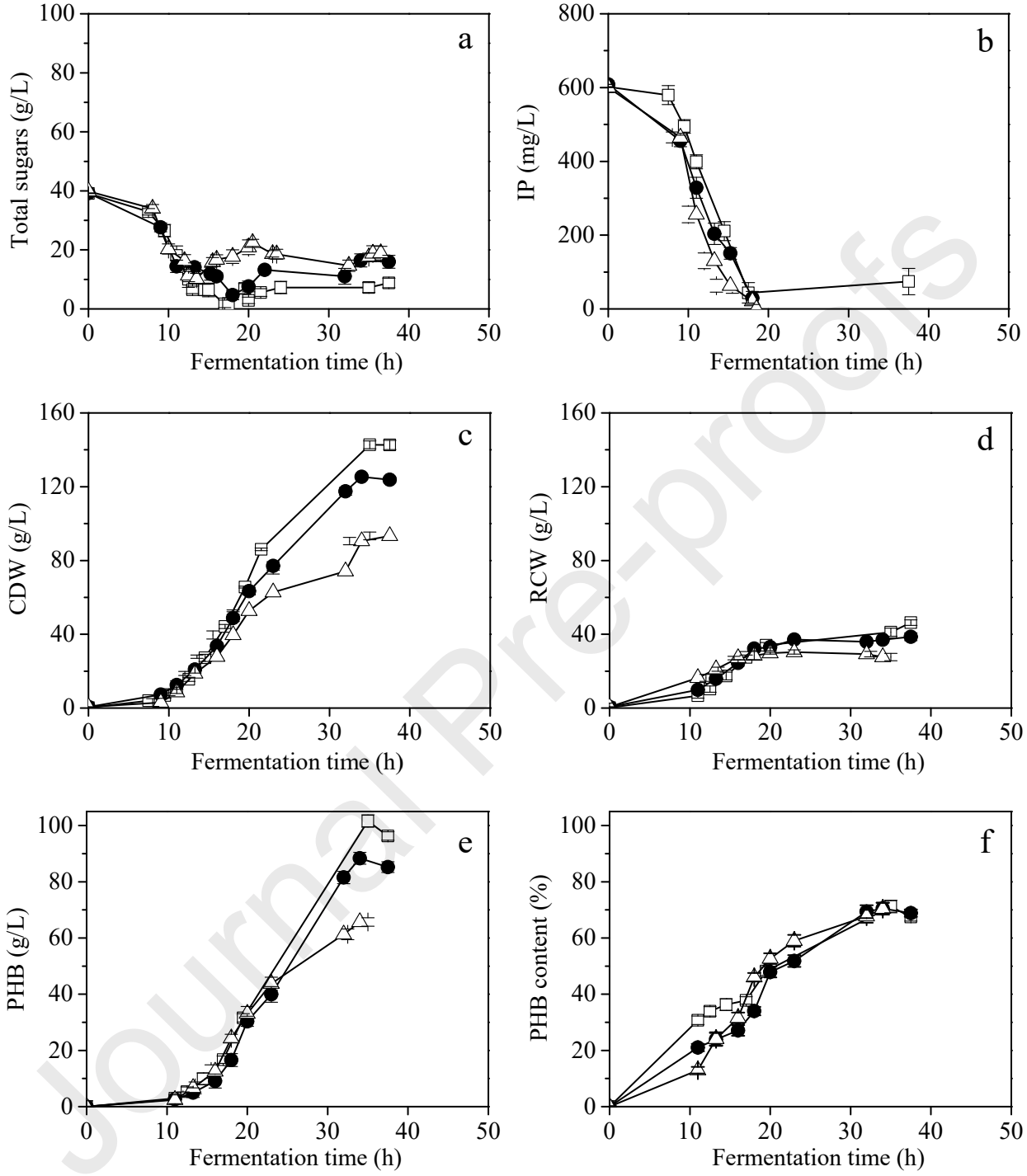

Figure 2

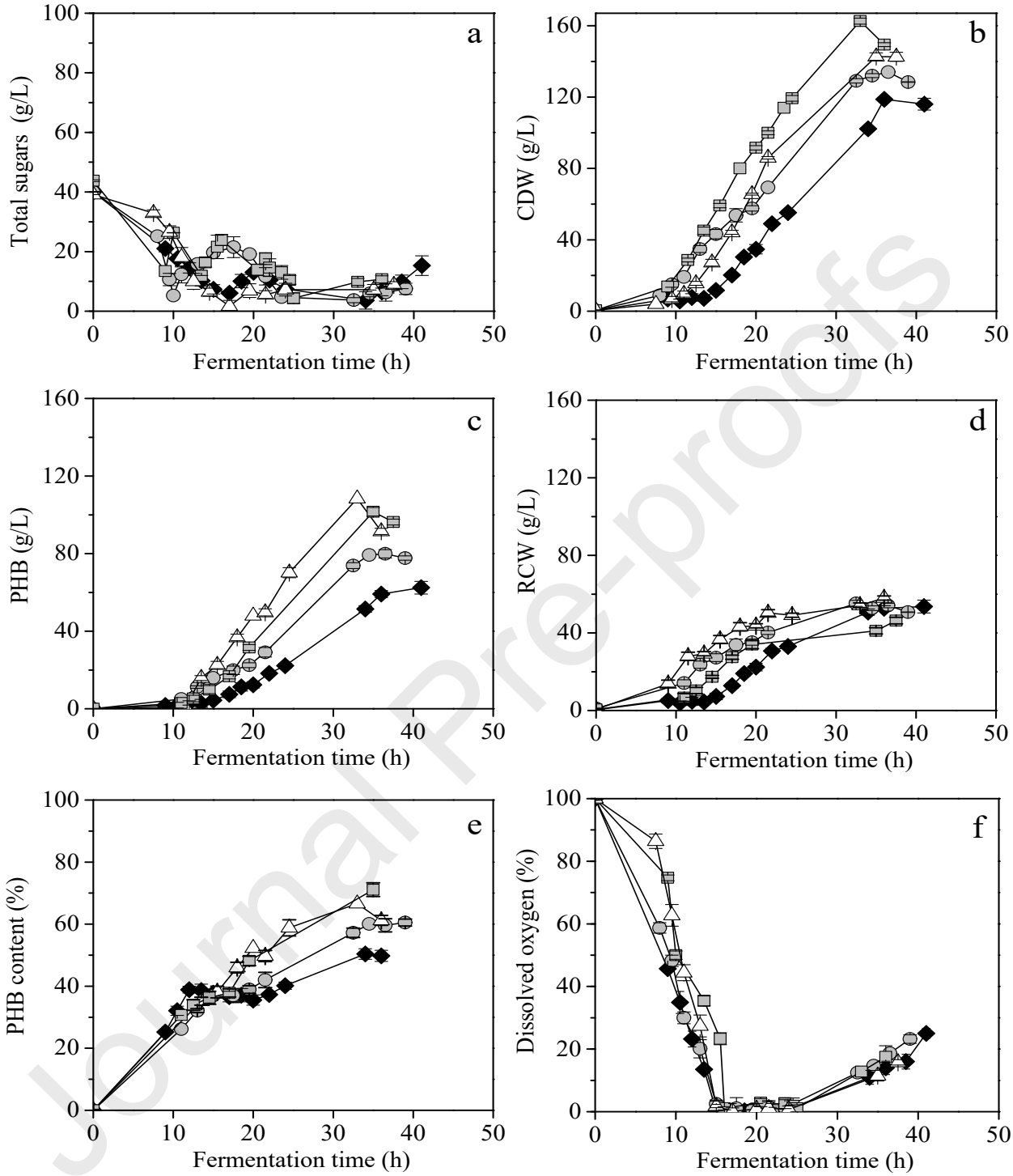

Figure 3

Credit authors statement

Olga Psaki designed and performed the experiments. Ioanna-Georgia I. Athanasoulia, Anastasios Giannoulis prepared and analysed the thermo and mechanical properties. Dimitrios Ladakis, Apostolis Koutinas and Demetres Briassoulis designed and supervised the experiments. Olga Psaki wrote the manuscript. Dimitrios Ladakis, Apostolis Koutinas and Demetres Briassoulis revised the manuscript. All authors have given approval to the final version of the manuscript.

Highlights

- Utilization of free sugars from fruit wastes for PHB production
- Evaluation of fermentation parameters to maximise PHB production efficiency
- Enhanced PHB concentration (108.3 g/L) and productivity (3.28 g/(L·h))
- Molecular weight and thermal properties are not affected by cell harvest time
- Elongation at break and Youngs modulus were 3.6-14.8% and 830-2000 MPa

Free sugars as carbon source

Aqueous extraction of free sugars

Sucrose 10%, Glucose 35% and Fructose 55%

Optimisation of fermentation parameters in fed-batch fermentations

Effect of initial sugar concentration, carbon to phosphorous ratio, feeding strategy, k_La