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Analyses of substrates and bacterial genera in biological polyhydroxyalkanoates production performance: A review

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ABSTRACT

Polyhydroxyalkanoates (PHAs) are biocompatible and biodegradable polyesters synthesized by some organisms such as bacteria, fungi, and plants for carbon and energy storage. Among the PHA-producing bacteria, *Bacillus*, *Cupriavidus*, *Pseudomonas*, *Burkholderia*, *Halomonas*, and *Paracoccus* were selected in this review to study the last 10 years of PHA production. PHA yields, carbon source, strains, and their possible relationships were analyzed in 77 articles, finding more than 300 experiments which revealed carbohydrates and fatty acids are the most used substrates, while the highest yields (near 90 % PHAs/biomass), were obtained with engineered *Halomonas* (glucose), *Cupriavidus necator* (fructose:canola oi), and *Bacillus megaterium* (residual glycerol). Considering the extensive but fragmented data across bacterial strains, substrates, and PHA production, which makes systematic comparison difficult, this review aims to serve as a valuable resource by integrating basic information for the optimal selection of carbon sources and/or bacterial strains to produce PHAs efficiently.

1. Introduction

It is estimated that between 4.8 and 12.7 million tonnes of plastic waste generated on land reach the ocean annually, and this figure is expected to rise dramatically by 2025 unless more efficient waste management strategies are implemented (Sabapathy et al., 2020). In response to this environmental challenge, recent years have seen an emphasis on research and development of sustainable alternatives (Katagi et al., 2023).

Since they were discovered in 1926 by the French microbiologist Maurice Lemoigne at the Pasteur Institute in Paris (Lemoigne, 1926), polyhydroxyalkanoates (PHAs) have emerged as a promising solution to replace petrochemical-derived plastics. PHAs represent a family of over 150 aliphatic polyesters with a general structure but diverse polymeric properties (Kosseva and Rusbandi, 2018). These polymers are biodegradable, biocompatible, and capable of being synthesized entirely by microorganisms, making them attractive candidates for sustainable applications using PHAs alone or as a copolymer (Medeiros Garcia Alcântara et al., 2020).

The versatility of PHAs extends to their production from pure carbon sources such as glucose, fructose, or oleic acid to renewable and inexpensive carbon sources, including organic wastes such as vegetable scraps, potato and banana peels, municipal solid waste, wastewater, and algae (Aghaali and Naghavi, 2023).

A PHA molecule commonly comprises 600 to 35,000 (*R*)-hydroxy fatty acid monomers (Fig. 1). PHAs can be categorized into three kinds based on their chain length: short-chain length (scl-PHA), medium-chain length (mcl-PHA), and long-chain length (lcl-PHA), according to the

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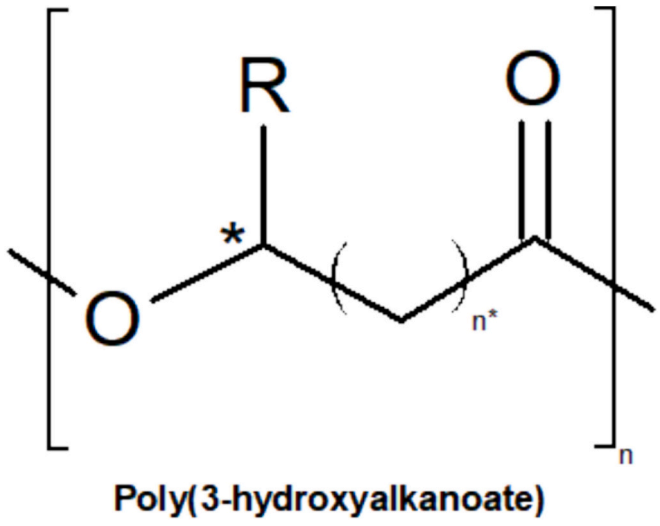

Fig. 1. Chemical structure of PHA. The asterisk represents a chiral center. The nomenclature of PHA is expressed as follows: if R = hydrogen, it is poly (−3-hydroxypropionate); if R = methyl and n* =1, it is poly (3-hydroxybutyrate); if R = ethyl and n* = 1, it is poly (3-hydroxyvalerate). Adapted from Vicente et al. (Vicente et al., 2023).

number of carbon atoms in their monomers (Raza et al., 2018). If the chain comprises 3–5 carbon atoms (C3-C5), it is scl-PHA, where poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV), and its *co*polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3 HV)) are classic examples. PHAs such as poly(3-hydroxyoctanoate) (PHO) and poly(3-hydroxynonanoate) (PHN) are examples of mcl-PHA, which span between 6 and 14 carbon atoms (C6-C14). If they contain more than 14 carbon atoms (> C14), they are lcl-PHA, which are scarcely found in nature (Li et al., 2016; Mezzina et al., 2021); therefore, these are the least common and the least studied of the three classes.

Despite the high diversity of the reported 150 types of R-hydroxyacids, genetic engineering expands the synthesis of novel PHA monomers. Regarding physicochemical properties, scl-PHAs are characterized by a brittle and rigid structure provided by their crystallinity, in contrast to mcl-PHAs, which have greater firmness and elasticity. Several factors influence the productivity, composition, and properties of PHAs, including the carbon source used, the bacterial producer, the composition of the growth medium, the cultivation strategies, and the biopolymer recovery method (Vicente et al., 2023).

In the production of bioplastics, specifically those of the PHA family, bacteria play a crucial role as versatile microorganisms capable of synthesizing these polymers. The most common groups include *Cupriavidus necator* (previously known as *Ralstonia eutropha*), *Bacillus megaterium*, and *Pseudomonas putida*. PHAs are synthesized within the microorganism's cellular structure, and their diameter can vary between 0.2 and 0.5 μm (Raza et al., 2018).

There are two main classifications of bacteria in terms of PHA synthesis. The first group requires stress conditions, such as limitation or depletion of essential compounds including nitrogen or phosphorus, in the presence of carbon excess (Katagi et al., 2023). This group contains bacteria such as *Cupriavidus necator* or *Pseudomonas oleovorans*. These bacteria can use substrates from organic waste, such as food waste or agricultural byproducts, that are rich in carbohydrates or lipids (Nicolescu et al., 2023). In addition, the second group does not require nutrient-deficient conditions to accumulate PHAs as they can synthesize these polymers during their growth phase. Bacteria in this group include *Burkholderia thailandensis* E264 and recombinant *Escherichia coli*.

PHA synthesis involves several integrated and complex regulated metabolic pathways, as presented in Figs. 2 and 3. Pathways I, II, III, and IV generate natural PHAs, in which acetyl-CoA plays a central role (Meng et al., 2014; Tan et al., 2014). In this process, key enzymes such as 3-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC) act together to facilitate the production and accumulation of intracellular PHAs. This polymer acts as a carbon and energy reserve within the bacterial cell in response to changes in environmental conditions (Tan et al., 2014).

At the biochemical level, the process consists of converting central metabolites derived from pathways such as glycolysis (Embden-Meyerhof-Parnas, Pentose phosphate, and Entner-Doudoroff pathways), the

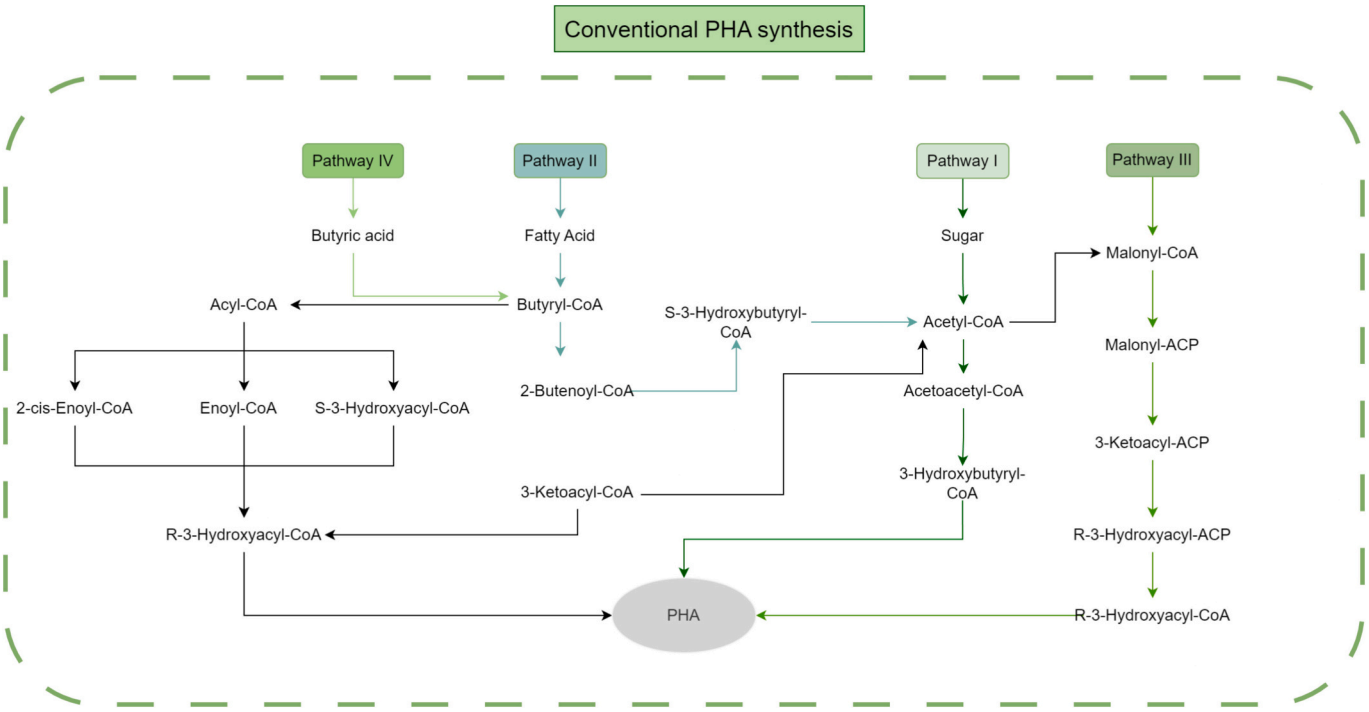

Fig. 2. Conventional metabolic pathways leading to PHA synthesis. Adapted from Meng et al. (Meng et al., 2014) and Tan et al. (Tan et al., 2014).

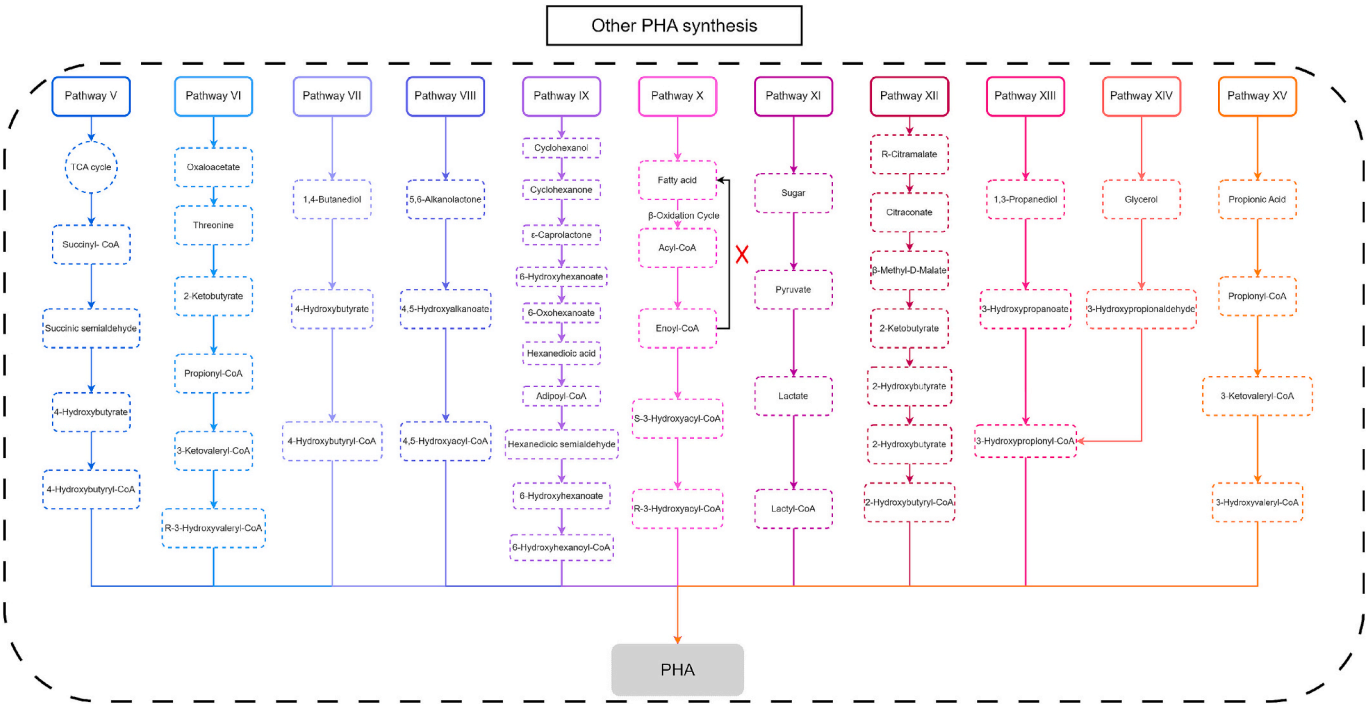

Fig. 3. Additional metabolic pathways leading to PHA synthesis. X represents the metabolic engineering of *P. entomophila* L48 for producing PHA through *β*-oxidation. Adapted from Meng et al. (Meng et al., 2014) and Tan et al. (Tan et al., 2014).

Krebs cycle, fatty acid de novo synthesis, and β-oxidation into precursors for PHA synthesis (Alvarez-Santullano et al., 2021). The starting point is acetyl-CoA, a molecule that, under normal growth conditions, is channeled into the Krebs cycle for energy production. However, under nutritional stress conditions, such as nitrogen, phosphate, or magnesium deficiency, carbon excess favors the diversion of acetyl-CoA into the PHA synthesis pathway (Tan et al., 2014).

For instance, the synthesis of PHB, the most common type of PHA, starts with condensing two acetyl-CoA molecules, catalyzed by the enzyme 3-ketothiolase (PhaA), forming acetoacetyl-CoA. This step is crucial as it marks the beginning of accumulating monomers that form PHA chains. In bacteria such as *Cupriavidus necator* and *Burkholderia cepacia*, PhaA acts as a regulator, redirecting the flow of acetyl-CoA toward polymer synthesis instead of its participation in the Krebs cycle (Kosseva and Rusbandi, 2018; Tan et al., 2014).

Subsequently, acetoacetyl-CoA is reduced to (*R*)-3-hydroxybutyryl-CoA by the action of the enzyme acetoacetyl-CoA reductase (PhaB), using NADPH as a cofactor. This step is essential for the generation of PHA monomers, such as 3-hydroxybutyrate (3HB), which is the main constituent of PHB (Raza et al., 2018).

Finally, the PHA synthase (PhaC) catalyzes the polymerization of (*R*)-3-hydroxybutyryl-CoA monomers, forming long PHA chains accumulated as intracellular granules. PhaC is an essential enzyme for biopolymerization as it determines the rate and efficiency of polymer synthesis from the available monomers. The size and composition of PHAs can vary depending on the type of bacteria and the culture conditions used (Kosseva and Rusbandi, 2018; Pena Serna and Lopes Filho, 2015).

Bacteria accumulate PHAs as intracellular granules. These granules are surrounded by proteins such as phasins, polymerases, and depolymerases. In response to carbon deficiency or changes in the pH medium, depolymerases are activated and degrade PHAs, releasing 3-hydroxyalkanoic acids that bacteria can exploit as a carbon and energy source (Shahid et al., 2021).

One of the significant challenges in PHA production is its high cost compared to petrochemical-based polymers. Renewable and low-cost carbon sources, including agricultural residues, waste oils, and industrial wastewater, have been explored to address this issue. For instance, glycerol, a byproduct of the biodiesel industry, has shown promising results, achieving yields of up to 83 % in bacteria like *Pseudomonas putida* KT2440 (Nguyen et al., 2023). Similarly, lignocellulosic residues, including fruit peels and sugarcane bagasse, represent economical and abundant carbon sources, promoting more sustainable production (Aghaali and Naghavi, 2023). These strategies reduce production costs and encourage the reuse of residual materials, aligning with the principles of the circular economy.

Despite its benefits, PHA production faces several obstacles that hinder its industrial scalability. One critical issue is the efficiency of bacterial producers. While species like *Cupriavidus necator* and *Pseudomonas oleovorans* exhibit high PHA accumulation capability, their yield varies significantly depending on medium conditions and substrate availability (Katagi et al., 2023). Additionally, the costs associated with polymer extraction and purification remain high, using toxic chemical solvents and energy-intensive techniques (Vicente et al., 2023). Another significant challenge is the limited diversity of available monomers, which restricts the final physicochemical properties of the biopolymer. Recent advancements in genetic engineering and synthetic biology have enabled the development of recombinant strains of bacteria that increase PHA yields and expand monomer diversity, unlocking new possibilities to tailor PHA production. These challenges highlight the need for technological innovations to make PHAs a more competitive and accessible solution.

To our knowledge, this is the first study focusing on the direct relationship between PHA production performance linked and the type of substrate and bacterial genera used. In addition, as far as we know, no comparative cost analyses regarding biological PHA production using different organic substrates are available in the literature. Considering all the aforementioned information, this review aims to explore various substrates for their effectiveness in optimizing PHA production across key bacterial genera and determine the impact of substrate type and concentration on biomass production and PHA yield, alongside a comparative analysis of PHA production costs.

2. Methods

The authors used the Scopus database to search for articles related to "Carbon sources associated with the production of PHA by bacteria." The search query was designed to align with the bibliographic review and was as follows: ABS (("PHA" OR "polyhydroxyalkanoate") AND ("substrate" OR "carbon source") AND ("Cupriavidus" OR "*Ralstonia eutropha*" OR "Halomonas" OR "Pseudomonas" OR "Bacillus" OR "Burkholderia" OR "Paracoccus") AND ("yield"). This search yielded 3976 documents, a PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart presented in Fig. 5 includes the screening of articles considering inclusion and exclusion criteria.

Publications from 2014 onwards and studies focused explicitly on carbon sources were included. Opinion pieces, qualitative studies, and research lacking precise substrate data were excluded. In addition, publications not focused on the specified genera, studies on protein optimization in PHA synthesis, research using mixed bacterial cultures for PHA production, studies without specified or traceable carbon sources, and studies lacking sufficient or relevant data such as substrate concentration, biomass obtained, or PHA produced were also excluded.

The bacterial genera *Bacillus*, *Cupriavidus*, *Pseudomonas*, and *Burkholderia* were selected because they have been extensively studied among more than 300 microbial PHA producers (Adebayo Oyewole et al., 2024; Chen et al., 2020; Lim et al., 2023). *Halomonas* and *Paracoccus* were also included because they are the most studied extremophile genera for PHA production (Możejko-Ciesielska et al., 2023). The data obtained were classified, tabulated, and graphed using the software "Origin 2024", and the online tool "SankeyMATIC.com".

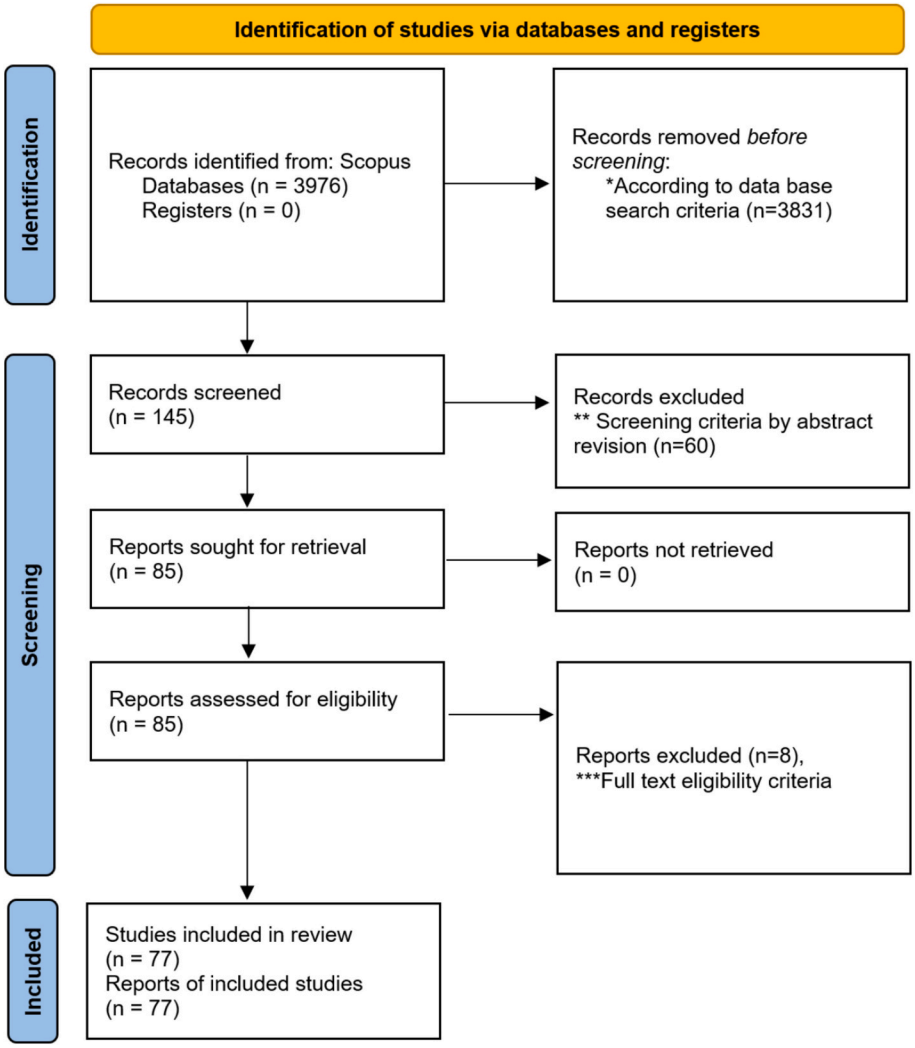

Fig. 5. Inclusion and exclusion criteria for article selection described with PRISMA 2020 flowchart.

*__Data base search criteria:__ Publication period: From 2014 onwards. Document type (Inclusion): Peer-reviewed journal articles and review articles. Document Type (Exclusion): Opinion pieces, editorials, and conference abstracts. Language: English. Knowledge areas (Exclusion): Physics and Astronomy, Computer Science, Mathematics, Economics, Econometrics, Finance or Earth and Planetary Sciences.

**__Screening by abstract revision__: Microbial Scope: Studies were required to explicitly mention at least one of the genera of interest (e.g., Cupriavidus, Halomonas, Pseudomonas). Topic-Based Exclusion: Studies with a primary emphasis on phytohemagglutinin, biodiesel, costs, or amino acids were excluded. Study Design: The abstract needed to indicate an empirical, rather than qualitative, study design.

***__Full text revision and eligibility criteria:__ Protein Optimization: Studies were excluded if their primary focus was on protein engineering or optimization in PHA synthesis. Mixed Cultures: Studies that utilized mixed bacterial inocula for PHA production were excluded. Non-Specified Carbon Sources: Studies using undefined substrates, such as not described plant, algal, or animal extracts, were excluded. Data Sufficiency and Precision: Papers were excluded if they lacked precise quantitative substrate or yield data, or if they presented insufficient experimental or analytical detail for the purposes of this review. Out-of-Scope Objectives: Articles were excluded if their stated objectives did not align with the variables under study in this review. Scope Limited to Carbon Sources: Studies focusing on other factors (e.g., nitrogen levels, C/N ratio, oxygen tension, or salt concentration) without examining carbon sources were excluded.

3. Results

After applying search criteria and exclusion filters, 77 research papers were selected to analyze bacteria, epithet, carbon source, and their relationship with PHA yield. Seventy-six strains were studied in more than 300 experiments (Table 1), including variations in carbon source, concentration, pH, nitrogen source, culture medium, and feeding strategies.

Since PHAs were first discovered in *Bacillus megaterium* (Lemoigne, 1926); *Bacillus*, *Pseudomonas*, and *Cupriavidus* are the most studied genera. In this study, they reached 88 % of all the papers reviewed because of the known capability of these genera to produce and intracellularly accumulate PHAs with a high growth rate (Chandra et al., 2023; Lim et al., 2023; Morlino et al., 2023).

3.1. Carbon sources

Each of the 65 carbon sources found is presented in the Sankey scheme of Fig. 6. They were divided into five categories: Carbohydrate, Fatty acid, Volatile fatty acid (VFA), Polyalcohol, and Other (e.g., salts, alkanes, and aromatics). A sixth group named "Mixture" was used later and included any blend between the five aforementioned categories.

Fig. 7 shows the frequency of substrate types used in experiments as carbon sources, either alone or as a part of a mixture, for each bacterial genus within the analyzed documents. *Cupriavidus* and *Pseudomonas* genera stand out because they were studied with the five groups of substrates, while *Bacillus* and *Burkholderia* prefer the metabolic pathway from carbohydrates (e.g., glucose and fructose).

The most studied metabolic pathways for PHA production (Fig. 2) are those starting from carbohydrates and fatty acids; thus, they are the most used carbon sources in experiments analyzed in this review (76.2 %) followed by VFA (9.4 %), Polyalcohol (9.4 %) and Other (5.0 %). Concerning the articles that used each substrate, the most studied carbon sources are carbohydrates with 45 articles, 24 articles studied fatty acids, 15 studied polyalcohol, 10 studied VFAs, 10 studied other, and 9 studied mixtures.

3.1.1. Carbohydrate

Carbohydrates are biomolecules composed of carbon, hydrogen, and oxygen, mainly synthesized by plants and bacteria through photosynthesis. Carbohydrates are classified as monosaccharides, oligosaccharides, and polysaccharides. Polysaccharides such as starch or hemicellulose are commonly hydrolyzed to be fermented as simpler sugars like glucose, fructose, xylose, sucrose, or lactose, which have been widely studied for PHA production. Thus, every bacterial genus studied was fed by carbohydrates. Metabolic pathways for PHA production are described in Figs. 2, 3, and 4, where carbohydrates commonly follow pathway I, yielding acetyl-CoA that enters the PHA synthetic pathway. In addition, pathway XI also uses sugars to synthesize lactyl-CoA to produce polylactic acid or poly(hydroxybutyrate-lactate) copolymers (Fig. 3). In this review, glucose is the most used carbon source with 54 appearances in experiments and a wide range of PHA yields. It is mainly used in feeding *Bacillus* and *Burkholderia*, obtaining 31–80 % as PHA/biomass percentage for *Bacillus* and 16–60 % as PHA/biomass percentage for the genus *Burkholderia*. Bacteria harbor multiple catabolic pathways with different metabolic features influencing PHA productivity (Fig. 4). Glycolytic pathways with low protein expenses and high generation of reductive power in the form of NADPH (e.g., Pentose-phosphate and Entner-Doudoroff pathways), which is a co-factor of the PhaB enzyme, enhance PHA synthesis and bacterial growth (Alvarez-Santullano et al., 2021). Glycolysis mediated by Pentose-phosphate and Entner-Doudoroff pathways is highly common in bacteria used for PHA synthesis, such as *Cupriavidus*, *Pseudomonas*, and *Burkholderia*, which may be related to high PHA yields starting from glucose (Alvarez-Santullano et al., 2021).

The recovery and reuse of waste as a carbon source is also considered a means to reduce substrate costs. The most used waste as a carbon source is molasses (26 appearances), a byproduct of the sugar industry with a high reducing sugar content. When molasses is used as a carbon source, maximums of 85 % as a PHA/biomass percentage by *Bacillus* fermentation, 47 % using *Burkholderia*, 31.5 % using *Cupriavidus*, and 14 % by *Pseudomonas* were reached.

Table 1
Number of documents, formulations, and strains studied in this review.

Genus	Documents	Experiments	Strains
Bacillus	25	48	24
Burkholderia	5	40	8
Cupriavidus	20	101	13
Halomonas	2	11	5
Paracoccus	2	6	1
Pseudomonas	23	102	25
Total	77	308	76

3.1.2. Fatty acid

The Fatty acids category includes every carboxylic acid from C_8 onwards and oils from canola, palm, olive, and sunflower, many of which were hydrolyzed to obtain fatty acids mixtures. Fatty acids follow pathway II or X (Figs. 2, 3, and 4) for PHA production. PHA synthetic routes from β-oxidation of fatty acids are redundant and yield scl-PHA and mcl-PHA according to the length of the respective fatty acid and the substrate specificity of the PHA synthase (Fig. 4). Although canola oil was the most used fatty acid, its 21 appearances correspond to just 1 article where it was used as part of a mixture with fructose. *Cupriavidus* is the most used genus for fatty acids fermentation (63 appearances), followed by *Pseudomonas* with 51 experiments. The maximum PHA yields obtained from fatty acids are up to 82 % as PHA/biomass percentage by *Bacillus*, 81 % by *Cupriavidus*, and 69 % by *Pseudomonas*.

In this category, the proposal by Guzik et al. (2021) is notable for deriving fatty acids from non-degradable polyethylene and using them as a carbon source for PHA production by *Pseudomonas putida*, achieving a yield of 59 % in terms of PHA/biomass percentage.

3.1.3. Polyalcohol

Polyalcohols are organic compounds containing two or more hydroxyl groups. Their use as a carbon source for bacteria feeding occurs through pathways VII, XIII, and XIV (Fig. 3) for PHA production. Among them, glycerol (1,2,3-propanetriol) is widely used for PHA production. In this review, glycerol was used in 26 experiments as a carbon source, 11 times for *Pseudomonas*, obtaining 13–83 % as PHA/biomass percentage. At the same time, the maximum PHA yields by the rest of the bacterial genus were 89 % as PHA/biomass percentage from *Bacillus* fed with residual glycerol from biodiesel production, 73 % from *Cupriavidus*, and 46 % from *Paracoccus*.

3.1.4. Volatile fatty acid (VFA)

Volatile fatty acids (VFAs) comprise carboxylic acids from C_2 to C_6 (Annamalai et al., 2020; Sun et al., 2022; Zhang et al., 2022) that can be metabolized to produce PHAs through pathways II, IV, and XV (Figs. 2, 3, and 4). Eight different substrates of VFA were used in 30 formulations, 13 of them include acetic acid (the most used VFA in this review) with PHA yields up to 70 % as PHA/biomass percentage from *Pseudomonas* when used as part of a mixture with decanoic acid and glucose, 79 % from *Cupriavidus*, and 54 % from *Halomonas* when used together with propanoic and butanoic acids.

3.1.5. Other

This category includes alkanes, a carboxylate salt, aromatic compounds, lactones, lignin, and proteins. Some belong to residues used as substrate, while others were added to a pure substrate in a mixture of carbon sources. Moreover, others were used as the sole substrate for PHA production with varied yield results.

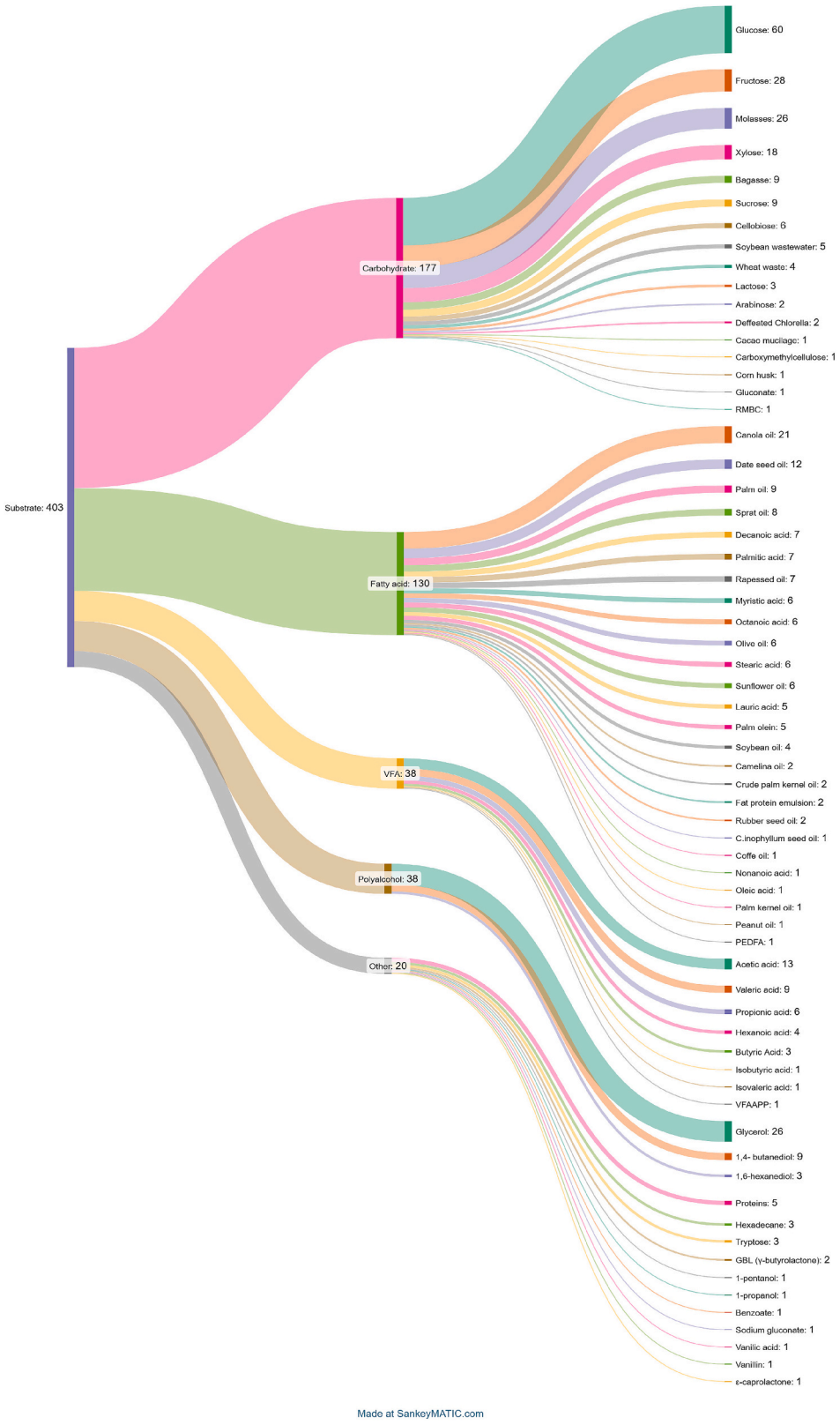

Fig. 6. Categorized substrates used as carbon sources for bacterial fermentation. Each number indicates the number of experiments performed with each substrate/category.

PHA yields produced by different bacteria using the studied substrates are presented in Fig. 8. The category "Other" showed low PHA yields compared with other substrates. In addition, VFA does not exceed PHAs 60 % yield as VFA could be associated with bacterial growth inhibition (Szacherska et al., 2021). The variation on PHA production yield by other carbohydrates does not depend on substrate concentration; however, *Halomonas*, *Bacillus*, and *Cupriavidus* showed the highest yields using these carbon sources. Using fatty acids as a carbon source

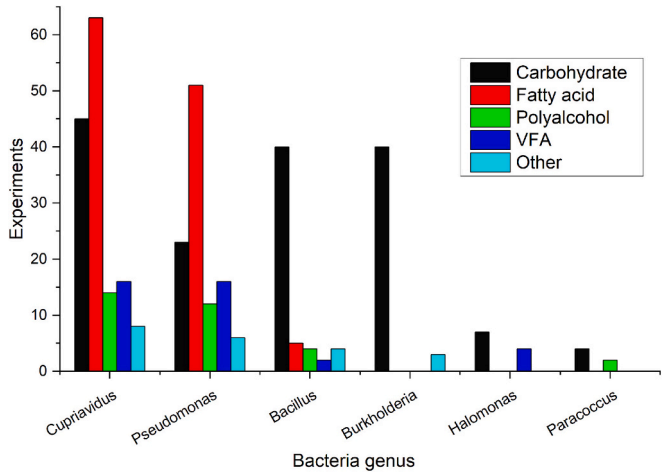

Fig. 7. Frequency of substrate types used in experiments as carbon sources by bacterial genera.

for *Cupriavidus* and some strains of *Bacillus* seems attractive, maintaining relatively low substrate concentrations to obtain PHA yields over 60 %. The bacteria genera with the highest PHA production yield from polyalcohol are *Cupriavidus*, a *Bacillus* strain, and a *Pseudomonas* strain. Since mixtures are different substrate categories combined in various proportions, the requirement of high substrate concentration to improve PHA yields can be assumed.

3.2. Bacterial genera

Biotechnology has studied various microorganisms, especially bacteria, as bioplastic producers. Depending on the available substrate, *Bacillus*, *Burkholderia*, *Cupriavidus*, *Halomonas*, *Paracoccus*, *and Pseudomonas* genera are frequently selected for PHA production. Genetic modifications are also used to enhance their production or control the monomeric composition of copolymers obtained, which makes it possible to direct the process to get a product with the desired physical and mechanical properties.

3.2.1. Genus Bacillus

Due to their genetic stability, *Bacillus* strains have been extensively studied for research and industrial production of PHAs. They can reach high PHA accumulation yields at appropriate conditions of a nutrient imbalance because of the lack of a lipopolysaccharides external layer, which facilitates PHA extraction (Mohapatra et al., 2017). The genus *Bacillus* produces PHAs from different pure and waste substrates and was studied in 25 articles included in this review using 10 specific epithets and 24 strains. Fig. 9 shows strains related to substrate type, concentration, and PHA yield. It is observed that carbohydrates prevail over other carbon sources and that substrate concentration does not ensure a higher PHA production yield. In fact, the yield may depend on various

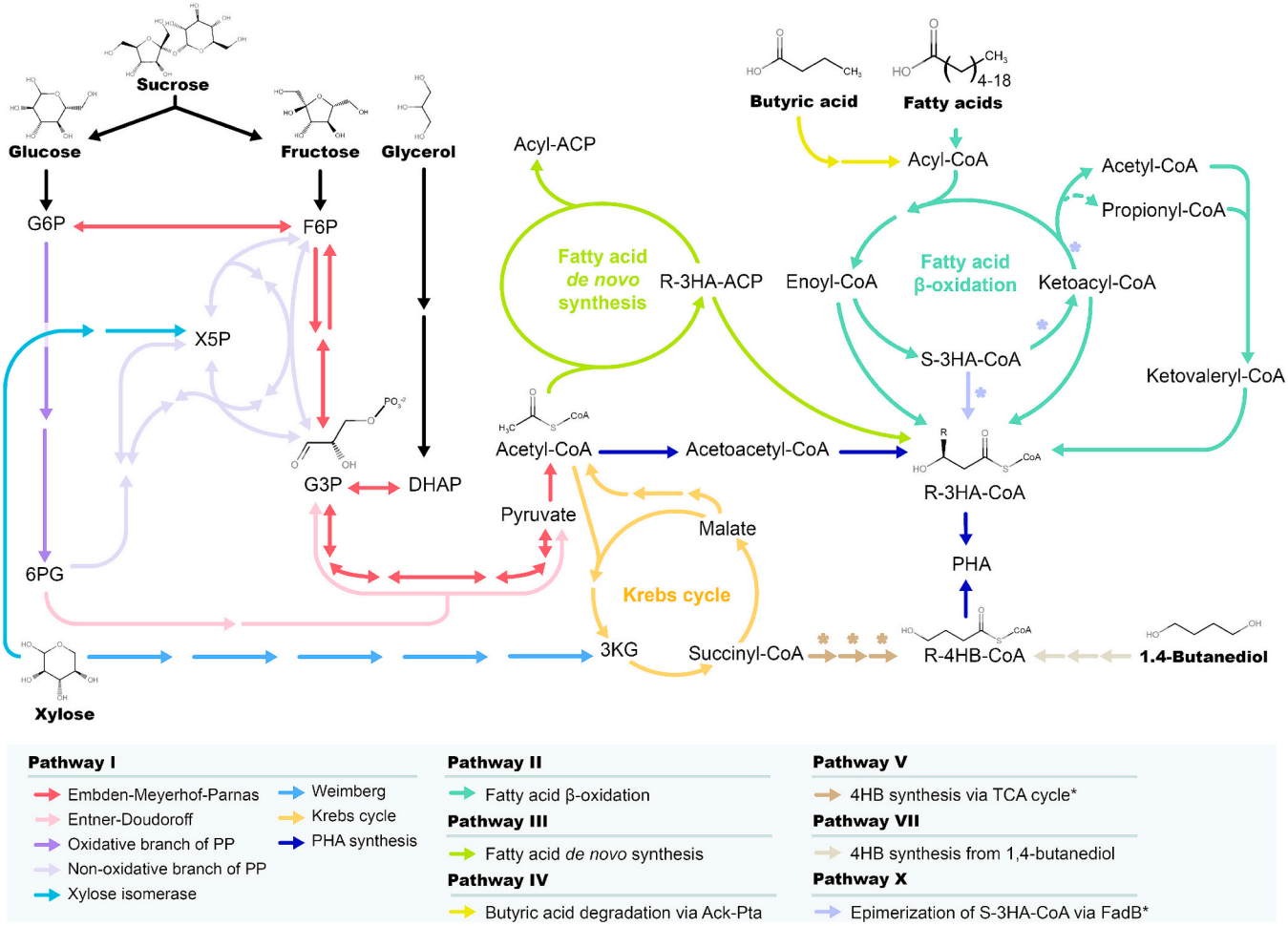

Fig. 4. Detailed metabolic pathways or reactions of the main carbon sources for bacterial PHA synthesis. Each arrow indicates an enzymatic reaction. Asterisks depict essential genetically engineered modifications to accomplish PHA synthesis. X5P, xylulose-5-phosphate. G3P, glyceraldehyde-3-phosphate. DHAP, dihydroxyacetone phosphate. 6PG, 6-phosphogluconate. 3KG, 3-ketogutarate. (*R/S*)-3-HA-CoA, (*R/S*)-3-hydroxyacyl-CoA. R-4HB-CoA, (*R*)-4-hydroxybutyryl-CoA.

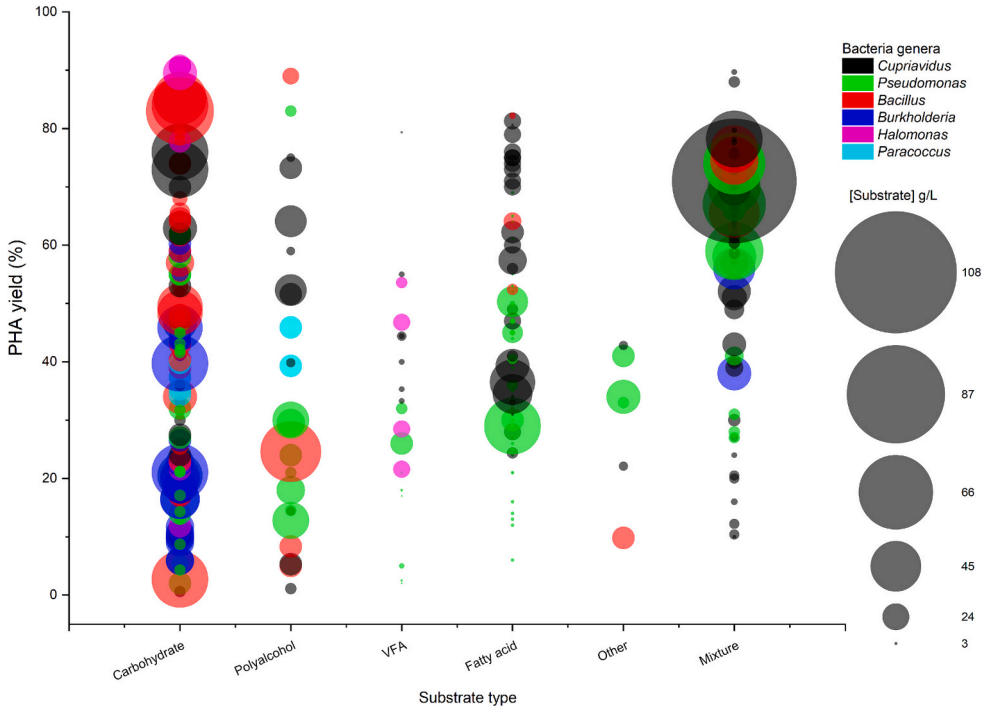

Fig. 8. PHA yield (%) ordered by carbon source. Colors represent the bacterial genus. Circle size refers to substrate concentration ranging from 1 to 110 g/L.

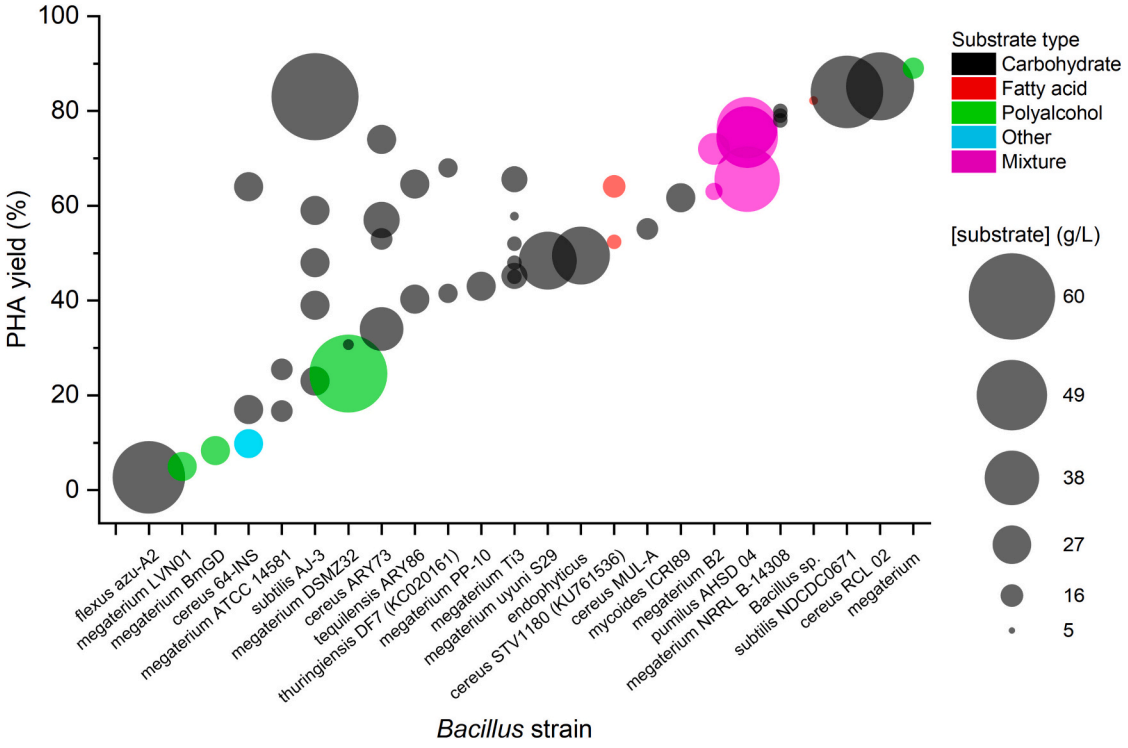

Fig. 9. PHA yield (%) from *Bacillus* bacteria for different substrate types (colors). Circle size increases according to substrate concentration from 5 to 60 g/L.

factors, including the combination of these, such as the selected strain, the specific type of substrate, its concentration, the nitrogen source, incubation time, temperature, and pH. Fig. 9 shows experiments using a specific strain and substrate that could be useful for selecting appropriate carbon sources and working conditions in further research. The analysis of the 25 articles reviewed in this section is presented next.

Katagi et al. (2024) studied *Bacillus* sp. for the production of Polyhydroxybutyrate-*co*-polyhydroxyhexanoate P(HB-*co*-HHx), using 2 g/L of palm oil combined with palm oil effluent (organic wastewater from the palm industry) as a carbon source. The best results were observed at 36 h, after adding 2 g/L of carbon source at 24 and 36 h. The cell dry weight (CDW) reached 4.5 g/L, containing 82.2 % PHAs. *B. endophyticus* was used by Geethu et al. (2021) with sucrose as the sole carbon source in concentrations ranging from 20 to 80 g/L and varying

the amount of Na_2HPO_4 and K_2HPO_4 in an experimental model. For the optimized medium in a bioreactor, they used 40 g/L of sucrose, resulting in a maximum yield of 49.5 % PHAs contained in 1.72 g/L of CDW, considering 1.5 g/L of the mentioned salts. Khattab et al. (2021) used the *B. flexus* azu-A2 strain isolated from wastewater effluents from leather production to obtain PHAs by using cheese whey and lactose fermentation. Cheese whey concentration needs to be more specific to be included in this work; therefore, only lactose fermentation was included. In fact, at 50 g/L lactose, 4.01 g/L of CDW and 2.74 % of poly(3-hydroxybutyrate) (PHB) were produced. Abdelmalek et al. (2022) worked with a cardboard waste hydrolysate as a carbon source with a content of 18.2 g/L glucose. The *B. mycoides* ICRI89 strain was used to produce 3.2 g/L CDW with 65.6 % PHAs, additionally using mineral salt medium (MSM), while 4.26 g/L CDW containing 61.7 % PHAs was obtained with modified MSM with 20 g/L glucose. Das et al. (2022a) produced PHB by the *B. pumilus* AHSD 04 strain, isolated from the peanut plant (*Arachis hypogaea* L.), using 37.7 g/L glucose and 4.3 g/L tryptose as the result of a statistical model. The CDW concentration obtained reached 7.0 g/L with 76.5 % PHAs. To produce the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-co-3 HV), valeric acid was added as a co-substrate in the second step, resulting in a maximum 74.5 % PHAs using 0.5 g/L valeric acid, also obtaining 65.6 % PHAs (with a maximum 4.98 % of 3 HV) with 3 g/L valeric acid. *B. tequilensis* ARY86 was used by Yasin and Al-Mayaly (2021a) with 20 g/L lactose as a carbon source. They measured CDW and %PHAs at 48 and 96 h, obtaining 2.83 g/L CDW with 40.3 % PHAs and 2.57 g/L CDW with 64.6 %PHAs, respectively. Singh et al. (2021) reported the use of *B. thuringiensis* DF7 strain (KC020161) to produce PHAs using 13.3 g/L glucose, obtaining 7.75 g/L CDW with 41.5 % PHAs in batch fermentation and 21.0 g/L CDW with 68 % PHAs through a cyclic fed-batch fermentation after the third cycle.

Bacillus cereus was used in 6 articles. Ali and Jamil (2014) produced PHB from potato starch by the strain 64-INS isolated from domestic sewage sludge. They tested potato starch, glucose, molasses, and sodium gluconate at 20 g/L as carbon sources with different results. Potato starch was not characterized in detail; therefore, it is not included here. Glucose fermentation resulted in the highest yield (64 % PHAs of 2.52 g/L CDW), molasses production resulted in 17 % PHAs of 0,53 g/L CDW, while sodium gluconate resulted in 9.8 % PHAs of 0.75 g/L CDW. Khan et al. (2020) used 5 g/L glucose added to PHA production media with 10 % glucose (PPMG) for 72 h with the MUL-A strain to produce nearly 1.9 g/L CDW containing 55.1 % PHAs. When peptone was added as a nitrogen source to PPMG, the PHA yield increased to 60.5 % from the CDW. Yasin and Al-Mayaly (2021b) used the strain ARY73, isolated from soil, to ferment glucose until 96 h to produce PHAs. The best yields of PHAs were obtained at 96 h. Different concentrations of glucose from 10 to 80 g/L were tested finding that 15 g/L glucose produced 1.83 g/L CDW with 53 % PHAs, 20 g/L allowed to reach 2.67 g/L of CDW containing 74 % PHAs, while using 25 g/L, they reached 1.90 g/L CDW with 57 % PHAs, and 30 g/L produced 1.22 g/L CDW with 34 % PHAs. The copolymer obtained comprised short and medium-length monomer chains (P(3HB-co-3HO)), a rare production and accumulation for a wild-type bacterium. The leaf endophytic RCL 02 strain was isolated from the leaf of *Ricinus communis* and was studied by Das et al. (2022b), who used it to ferment sugarcane molasses. After 62 h of incubation with 47 g/L molasses and 3 g/L nitrogen source, 18.8 g/L of CDW was produced with 85.2 % P(3HB-co-3 HV). Kynadi and Suchithra (2017a, 2017b) used the STV 1180 strain (KU761536) with rubber seed oil, which is a novel carbon source due to its limited commercialization and low cost, containing nearly 80 % unsaturated fatty acids such as oleic, linoleic, and linolenic. Using 10 g/L of the substrate, the STV 1180 strain produced 3.43 g/L of CDW with 52.4 % of PHAs; in contrast, when using 15.7 g/L of the substrate, the CDW and PHA production increased to 3.98 g/L and 64 %, respectively.

Two articles studied *Bacillus subtilis*. Gomaa (2014) used an AJ-3 strain to ferment cane molasses. Crude cane molasses at 20 g/L produced 5.66 g/L CDW with 23 % PHAs, while centrifuged cane molasses reached 7.63 g/L CDW and 39 % PHAs. When 20 g/L cane molasses were treated with calcium phosphate, the CDW resulted in 9.44 g/L and 59 % PHAs, while the treatment with sulfuric acid reached 11.0 g/L CDW with 48 % PHAs. When the cane molasses concentration was increased to 60 g/L with sulfuric acid treatment, it produced 39 g/L CDW with 83 % PHAs. On the other hand, Umesh et al. (2018) synthesized PHA from the NCDC0671 strain using orange peel hydrolysate, obtaining a maximum of 53.3 % PHAs in 4.0 g/L CDW, while the use of 50 g/L sucrose as a carbon source resulted in 6.1 g/L CDW and nearly 84 % PHAs.

Bacillus megaterium is well known for its PHA production capability in sugar substrates, oily substrates, and glycerol. The *megaterium* epithet was used in 10 articles reviewed in this work. Gómez Cardozo et al. (2016) used 14.8 g/L residual glycerol at 25 °C from biodiesel to obtain 3.15 g/L CDW with 89 % PHB. Blunt et al. (2023) examined the ATCC 14581 strain, comparing it with *paraburkholderia sacchari* (previously named *Burkholderia sacchari*) and *Hydrogenophaga pseudoflava*. The chosen substrates were a synthetic mixture of 10 g/L glucose with 5 g/L xylose and 15 g/L of sugars from hardwood hydrolysis called TMP-Bio sugar (C_5 and C_6). The results for the first substrate using *B. megaterium* ATCC 14581, *P. sacchari*, and *H. pseudoflava* were 1.46 g/L CDW with 16.7 % PHAs, 4.33 g/L CDW with 72.8 % PHAs, and 2.82 g/L CDW with 57.9 % PHAs. Moreover, using the TMP-Bio sugar, the *B. megaterium* produced 3.53 g/L CDW with 25.5 % PHAs, *P. sacchari* reached 5.73 g/L CDW with 56.9 % PHAs, and *H. pseudoflava* synthesized 6.32 g/L CDW with a maximum of 84 % PHAs. The authors explained this maximum as a particular affinity of *H. pseudoflava* for other carbon sources present in the TMP-Bio-sugar. The *B. megaterium* strain B2 was tested by Quintero-Silva et al. (2024) using carbohydrates and carboxylic acids from residual medium from bacterial cellulose production (RMBC) and spent cacao mucilage exudate (SCME). The results using 22 g/L RMBC over 12 h were 3.06 g/L CDW and 72 % PHB, whereas using 12 g/L SCME for 20 h produced 3.35 g/L with 63 % PHB. In the study of Gómez Cardozo et al. (2019) they used the LVN01 strain and a recombinant clone B*m*GD with better capabilities to accumulate PHAs. Results using 20 g/L glucose highlight the improvement in CDW production and %PHB for B*m*GD compared to LVN01, being these 3.0 g/L CDW with 8.33 % PHB and 2.0 g/L with 5.0 % PHB, respectively. de Oliveira Schmidt et al. (2022) worked with the DSMZ32 strain for 72 h with 7.5 g/L glucose to obtain 3.88 g/L CDW with 30.7 % PHAs; while using 53.6 g/L of residual glycerol, they obtained 2.68 g/L CDW containing 24.6 % PHAs. Akdoğan and Çelik (2021) studied and compared a wild-type *megaterium* NRRL B-14308 with a recombinant *megaterium* NRRL B-14308 constructed via PHA synthase enzyme (PhaC) overexpression. The obtained PHA resulted in a copolymer P(3HB-co-3 HV) with 58 mol% of 3-hydroxyvalerate (3 HV) monomer. Using 10 g/L glucose, the obtained CDW and PHAs for the batch fermentations were 3.06 g/L and 79 % P(3HB-co-3 HV) (wild-type *B. megaterium)* and 3.52 g/L with 78 % P(3HB-co-3 HV) (rec. *B. megaterium*), while fed-batch fermentation with rec. *B. megaterium* increased the production to 7.68 g/L CDW with 80 % P(3HB-co-3 HV). The PP-10 strain was studied by Bunkaew et al. (2023) to ferment a treated pineapple peel waste (2 %*V*/V) and glucose 20 g/L resulting in 4.24 g/L CDW and 5.4 g/L CDW with 61.1 % PHAs and 43 % PHAs, respectively. Results from pineapple peel were excluded from this analysis because of the difficulty of establishing a real carbon source concentration. de Souza et al. (2020) fermented 7 common sugars and lignocellulosic substrates separately for 24 h using the Ti3 strain. A sugar concentration of 10 g/L resulted in 1.5 g/L CDW with 45 % PHAs (from arabinose), 1.27 g/L CDW with 52 % PHAs (from glucose), and 2.25 g/L CDW with 48 % PHAs (from xylose). The biologically pretreated corn husk hydrolysate with 6 g/L reducing sugars produced 1.73 g/L CDW with 58 % PHAs. In the study of de Souza et al. (2022), the same *megaterium* Ti3 produced PHAs from the hydrolyzed lignocellulosic waste, sugarcane bagasse. They used the lignin-degrading organism *Pycnoporus coccineus* MScMS1 to enhance the

number of available sugars in the hydrolysate used as substrate for PHA production. Following a statistical design to determine the optimum parameters, the amount of biomass and PHAs obtained reached 0.88 g/L CDW with 65.6 % PHAs, while using the reference medium (glucose) at 18 g/L resulted in 1.26 g/L CDW with 45.2 % PHAs. Finally, the *uyuni* S29 strain was used by Schmid et al. (2021) to produce PHAs through sucrose fermentation. The results indicate 48.4 % PHB from 12.4 g/L CDW when using 40 g/L substrate and KCl instead of NaCl under phosphorus limitation.

The genus *Bacillus* clearly tends to produce higher PHA when carbohydrate is used as a carbon source according to specific strains. Moreover, mixtures containing carbohydrates as the main carbon source and agricultural byproducts with a high carbohydrate content produce moderate to high PHA yields. Conversely, the use of palm oil in three stages by a *Bacillus* sp. is highlighted due to the ability to accumulate high levels of PHAs demonstrating *Bacillus* versatility. Finally, regarding extraction methods, solvents like chloroform that dissolve PHAs and their subsequent precipitation with cold methanol remain prevalent due to their high efficiency. However, more sustainable alternatives, such as enzymatic cell disruption or mild detergents, are being explored, which may offer environmental advantages and reduced costs (Muneer et al., 2022; Xu et al., 2021b).

3.2.2. *The genus Cupriavidus*

The members of this Gram-negative rod-shaped genus are versatile bacteria, especially *C. necator*, which can grow autotrophically and heterotrophically using diverse pure and waste substrates (Bellini et al., 2022) to produce mainly scl-PHAs. Particularly in this review, this genus was studied by 20 articles considering 3 specific epithets and 13 different strains. Fig. 10 shows different carbon sources, their concentration used as a substrate for *Cupriavidus* bacteria, and the reached yields. The variety of substrates and low concentration needed to produce high PHA yields when using carbohydrate/fatty acid mixtures are remarkable; moreover, every studied strain produced more than 55 % PHAs in one or more of their experiments, establishing the reputation of *Cupriavidus*.

Obruca et al. (2019) studied the DSM 19379 strain of *Cupriavidus* sp. with different substrates as a carbon source to produce the terpolymer of poly(3HB-*co*-3-HV-*co*-4-HB). Fructose, glucose, glycerol, sunflower oil, γ-butyrolactone (GBL), 1,4-butanediol, ε-caprolactone, and 1,6-hexanediol produced between 0.22 and 10.8 g/L CDW, while their PHA content moved from 5.30 % to 70 % PHAs. Fructose, glucose, and glycerol only produced poly(3-hydroxybutyrate) (PHB); sunflower oil did not demonstrate the presence of PHAs, and the remaining substrates produced poly(3HB-*co*-4HB). Mixtures of GBL or 1,4-butanediol with propionic or valeric acids as 3 HV precursors were used for terpolymer production. The addition of valeric acid increased the production of poly (3HB-*co*-3 HV-*co*-4HB) terpolymer, reaching 7.97 g/L CDW with 10.4 % PHAs, containing 25.9 mol% 4HB and 17.9 mol% 3 HV when used with GBL. Furthermore, adding valeric acid to 1,4-butanediol resulted in 8.68 g/L CDW with 20.5 % PHAs containing 24.7 mol% 4HB and 14.6 mol% 3 HV. A two-stage production process was proposed using glycerol or a mixture of glycerol/1,4-butanediol with 1,4-butanediol or a mixture of 1,4-butanediol/valeric acid as secondary precursors. Once more, valeric acid was an excellent 3 HV precursor mixed with 1,4-butanediol, reaching 2.74 g/L CDW with 52.1 % PHAs containing 29.5 mol% 3 HV when used with glycerol as the primary substrate. Moreover, 5.94 g/L CDW with 69.6 % PHAs containing 17.9 mol% 3 HV were produced when glycerol/1,4-butanediol was used as a substrate. Only 1.06 mol% 3 HV and 0.51 mol% 3 HV were obtained from the same experiments without valeric acid. *Cupriavidus* sp. USMAA1020 was used by Norhafini et al. (2017) to ferment mixtures of two out of four carbon sources, including 1,4-butanediol, 1,6-hexanediol, γ-butyrolactone, and 4-hydroxybutyrate. After studying the effect of mixed substrates and the

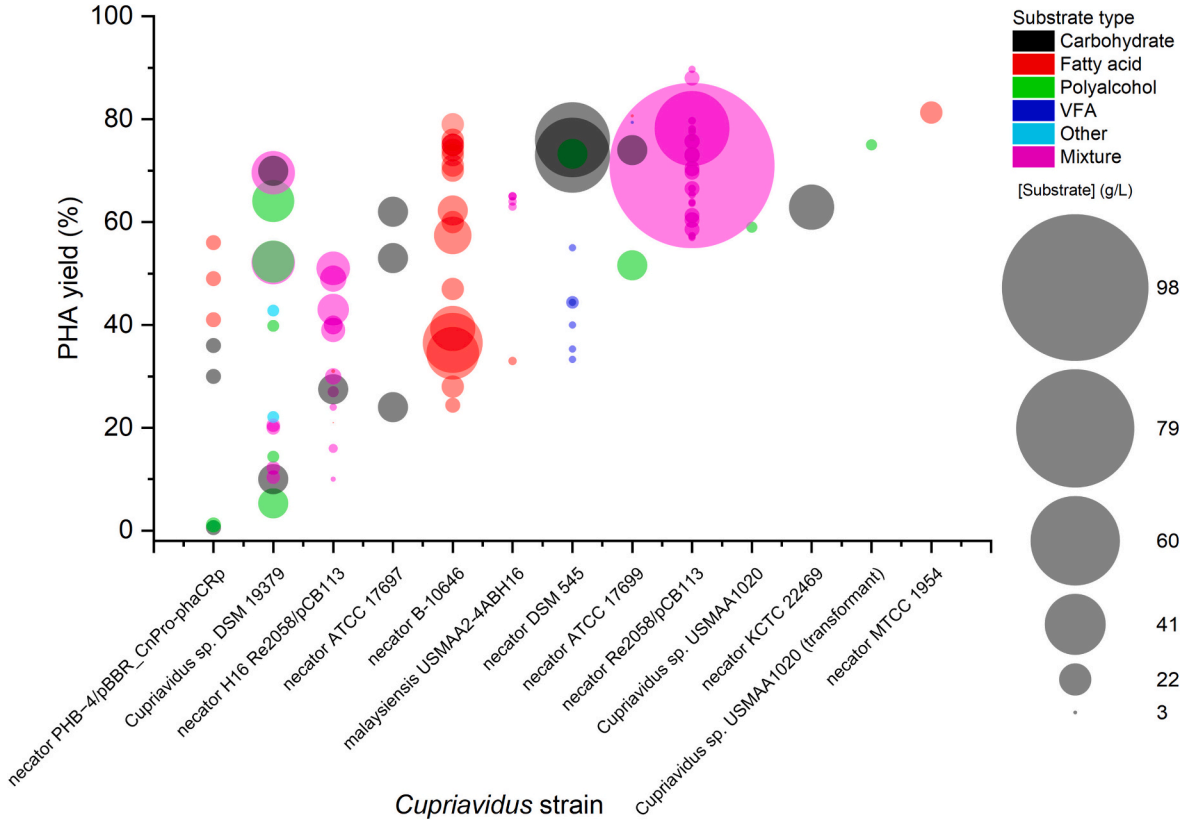

Fig. 10. PHA yield (%) from *Cupriavidus* bacteria for different substrate types (colors). Circle size increases according to substrate concentration, from 1 to 110 g/L. *One data point was excluded from the figure due to its being oversized, but it was analyzed in the text.

total carbon concentration on CDW, percentage of PHA, and mol% of the monomer 4-hydroxybutyrate (4HB), the authors determined the best mixture for 7.5 g/L of 1,6-hexanediol:1,4-butanediol at a 1:5 ratio for a maximum 4HB composition. The optimized experiments in a bioreactor resulted in 4.7 g/L CDW and 59 % PHAs, respectively. A transformant *Cupriavidus* sp. USMAA1020 (with an extra copy of the *pha*C gene) was also used with the same previously described conditions to obtain 24.9 g/L CDW with 75 % PHAs. The obtained 4HB monomer in the P(3HB-*co*-4HB) copolymer for the wild-type strain reached 46 mol% while the transformant strain produced a 4HB composition of 95 mol%. The increase in the 4HB monomer represents an improvement in physicochemical properties, biodegradation, and cell growth. Wong et al. (2022) worked with the *C. malaysiensis* USMA2–4 transformant to evaluate different carbon sources as 3 HV precursors added to 0.6 to 5.0 g/L palm olein. The obtained CDW and PHA content using 1-propanol, 1-pentanol, valeric acid, hexanoic acid and nonanoic acid were 4.7 g/L with 64 % PHAs, 5.2 g/L with 65 % PHAs, 4.6 g/L with 65 % PHAs, 4.2 g/L with 63 % PHAs and 1.8 g/L with 33 % PHAs respectively, showing better results with alcohols and VFAs. The best 3 HV content reached 5.0 mol% when 1-pentanol or nonanoic acid were used as precursors, while the production of this monomer was not reported for hexanoic acid.

Cupriavidus necator is a model bacterium for PHA production (mainly PHB). Formerly *Wautersia eutropha*, *Ralstonia eutropha*, and *Alcaligenes eutrophus*, *C. necator* can accumulate up to 80 % PHAs/biomass when carbon sources are abundant and some nutrients are limited. Seventeen reviewed articles studied *C. necator* with carbohydrates, fatty acids, polyalcohol, volatile fatty acids, and mixtures. *C. necator* ATCC 17697 strain was used by Nygaard et al. (2021) to ferment fructose at 20 g/L, extracting samples at different incubation times. At 24 h, this strain produced 1.37 g/L CDW containing 24 % PHAs; at 48 h incubation time, the production reached 5.77 g/L CDW with 53 % PHAs, while 7.43 g/L CDW with 62 % PHAs was obtained at 72 h. The accumulation stage follows the first growth stage, then, after 72 h, the bacteria use the accumulated PHAs to survive without a carbon source. Three articles describe the use of *C. necator* ATCC 17699 for PHA production. Saratale et al. (2020) used wheat waste hydrolysate pretreated with ultrasound and NaOH to obtain 11.45 g/L CDW with 74 % PHAs; Tanadchangsaeng and Roytrakul (2020) used glycerol as a carbon source to produce 78.9 g/L CDW with 51.6 % PHAs; and Pérez-Camacho et al. (2024) designed a two-stage process for PHA production using 2 g/L propionate or peanut oil obtained 5.55 g/L CDW and 79.4 % PHAs with the first substrate and 6.32 g/L CDW 80.7 % PHAs with the second. Propionate use improves the physical properties of PHA by forming P(3HB-co-3 HV) with a higher 3 HV content than peanut oil. Two articles from the same research group reported using fatty acids as substrates for *C. necator* B-10646. Zhila et al. (2023) used waste sprat oil mostly containing palmitoleic, oleic, and docosahexaenoic acids as a carbon source at different concentrations for 24 h. The CDW reached 1.08, 2.16, 2.35, 2.26, 1.34, 1.39, and 1.51 g/L when the fish oil concentration was evaluated at 10, 15, 20, 25, 30, 35, and 40 g/L, respectively. The accumulated PHA/biomass yield reached 24.4, 60.0, 62.2, 57.4, 39.3, 34.5, and 36.5 %, respectively. The highest biomass and PHA yields were obtained in the 15 to 25 g/L substrate range. Thus, 15 g/L was used in batch fermentation trials, which produced 4.6 g/L CDW with 70 % PHAs. To our knowledge, this was the first report of waste sprat oil used as a carbon source for the biosynthesis of the copolymer poly(3HB-*co*-3 HV-*co*-3HHx). This research group used saturated fatty acids (SFA) and mixtures of SFA as a carbon source, although some mixtures were prepared with oleic acid (Zhila et al., 2024). The CDW produced after 72 h fermentation using lauric, myristic, palmitic, and stearic acids at 15 g/L reached 6.7, 7.5, 3.9, and 2,5 g/L, respectively, while PHB yields resulted in 75, 74, 47, and 28 %, respectively. The five mixtures tested were named from 1 to 5. Mixture 1 used the 4 SFA in equal proportions to reach 15 g/L total and produced 8.9 g/L CDW with 79 % PHB. Mixtures 2 and 3 were prepared with myristic, palmitic, and stearic acids. The composition of mixture 2 was SFA at equal proportions to complete 15 g/L and produced 8.2 g/L CDW with 73 % PHB. In comparison, mixture 3 included 1.5 g/L myristic acid, 11.7 g/L palmitic acid, and 1.8 g/L stearic acid in a total 15 g/L carbon source, producing 8.0 g/L CDW with 71 % PHB. Mixture 4 included the four SFA plus oleic acid, including 4.0 g/L of each fatty acid for the aggregate of 20 g/L, producing 8.6 g/L CDW with 76 % PHB. Finally, Mixture 5 included 1.5 g/L myristic and stearic acids, 9.0 g/L palmitic acid, and 8.0 g/L oleic acid for 20 g/L carbon source. This mixture produced 8.2 g/L CDW containing 75 % PHB. Some characteristics of the obtained PHAs can vary according to the fatty acid chain length. For example, when using fatty acids with 12 carbons, the PHAs presented a molecular mass of 305 kDa. In comparison, using fatty acids with 18 carbons increased the molecular mass to 447 kDa. The DSM 545 strain was studied in 4 articles using glucose, glycerol, and VFAs. Povolo et al. (2015) used this strain to ferment 20 g/L glycerol to obtain 6.35 g/L CDW with 73.3 % PHAs and compared these results to those obtained from the strain with the inactivation of the *phaZ1* gene to enhance the production. The results obtained through the modified strain were similar to the wild-type strain except in the depolymerization phase, where the recombinant strain maintained its PHA content, and the wild-type nearly reduced it to 30 %. Haas et al. (2017) used the *C. necator DSM* 545 strain in a membrane bioreactor with 50 g/L glucose to produce 89.8 g/L CDW containing 73 % PHB after 29 h. When the feed supply was modified, 148 g/L CDW with 76 % PHAs was obtained at 36 h, resulting in a 36 % productivity increase per hour. Five different VFAs were used by Vu et al. (2022) to feed the DSM 545 strain for 24 or 36 h. Using 5 g/L acetic acid as the carbon source, the biomass resulted in 1.8 g/L CDW with 44.4 % P(3HB-*co*-3 HV); the use of propionic acid produced 2.4 g/L CDW containing 33.3 % P(3HB-*co*-3 HV) at 36 h; the use of butyric acid reached the maximum biomass of 2.9 g/L CDW and 55 % P(3HB-*co*-3 HV) content; the use of isobutyric acid produced 2.5 g/L CDW with 40 % P(3HB-*co*-3 HV), and using isovaleric acid for 36 h resulted in 1.7 g/L CDW containing 35.5 % P(3HB-*co*-3 HV). The last article using the DSM 545 strain (Vu et al., 2023) highlights the use of VFAs obtained from the acidogenic fermentation of apple pomace and potato peel liquor (VFAAPP), which mainly contains acetic and butyric acids. The maximum biomass from a semi-continuous fermentation reached 4.5 g/L CDW containing 44.4 % P(3HB-*co*-3 HV). Purama et al. (2018) studied the recombinant *C. necator* H16 Re 2058/pCB113 strain using date seed oil and date molasses at different concentrations to produce P(3HB-*co*-3HHx). Date seed oil (DSO) composition included 39 % saturated FAs, 55 % monounsaturated FAs, and 5 % polyunsaturated FAs. 1 g/L DSO produced 1.7 g/L CDW with 21 % PHAs, while 2.5 g/L produced 2.4 g/L CDW containing 31 % PHAs. The use of date molasses was optimized at 20 g/L total sugars, obtaining 4.0 g/L CDW with 27.5 % PHAs after 48 h. Ten mixtures (Table 2) were prepared to obtain the best combination for a high PHA content and composition of the 3-hidroxyhexanoate (3HHx) monomer, which positively affects the physicochemical properties of the bioplastic. Although using DSO alone

Table 2
PHA yields and 3HHx composition of different substrate combinations. Adapted from Purama et al. (Purama et al., 2018).

DSO (g/L)	total sugars molasses (g/L)	CDW (g/L)	PHA (%)	3HHx composition (mol%)
1.0	2.5	1.7	10	28
1.0	5.0	2.1	16	20
1.0	10.0	3.9	30	4
1.0	15.0	4.2	39	2
1.0	20.0	5.1	43	2
2.5	2.5	2.9	24	22
2.5	5.0	3.7	27	12
2.5	10.0	5.1	40	5
2.5	15.0	6.4	49	4
2.5	20.0	6.9	51	3

DSO: Date Seed Oil, CDW: Cell Dry Weight, PHA: Polyhydroxyalkanoate, 3HHx: 3-hidroxyhexanoate monomer.

produced a higher 3HHx content (more than 35 mol%) in the PHAs, adding sugar molasses increased the amount of obtained PHAs by 40 %.

Lhamo et al. (2024) worked with the KCTC 22469 strain and sucrose to optimize PHA production, reaching 1.47 g/L CDW with 62.9 % PHAs. Arumugam et al. (2018) studied the effect of inoculum size and *Calophyllum inophyllum* oil concentration on PHA production from *C. necator* MTCC 1954. The optimum substrate concentration was 15 g/L, producing 10.15 g/L CDW with 81.3 % PHAs. The inoculum size increase to 50 g/L resulted in CDW and PHAs increases. The engineered strain of *C. necator* PHB^{-}4/pBBR_CnPro-*pha*C_{Rp}, which ports the *pha*C_{Rp} from a *Rhodococcus pyridinivorans strain*, was used by Trakunjae et al. (2022) to ferment different carbon sources in flask experiments. Glucose, sucrose, and glycerol slightly produced biomass with a trifling PHA content, whereas fructose, sugarcane molasses, palm oil (PO), and crude palm kernel oil (CPKO) produced 3.7, 2.1, 5.7, and 6.3 g/L CDW, respectively, with a PHA content of 36, 30, 41, and 49 %. As the best-determined carbon source, 10 g/L CPKO was used in a bioreactor to produce 7.7 g/L CDW containing 56 % PHAs. The fermentation of CPKO specifically enables the production of 2 mol% 3-hydroxyhexanoate monomer composition in the obtained poly(3HB-*co*-3HHx) copolymer. Three articles from the report using the recombinant *C. necator* Re2058/pCB113. Santolin et al. (2021) produced PHAs with a two-stage fed-batch cultivation strategy using fructose and rapeseed oil for accumulation and polymer production, respectively. 124 g/L CDW was produced containing 86 % poly(3-HB-*co*-3-HHx) with 17 mol% content of the 3-HHx monomer. Moreover, Santolin et al. (2023) used fructose and canola oil mixtures to optimize the amount of HHx monomer in the poly(3-HB-*co*-3-HHx). Table 3 shows the experiments and results with different fructose:canola oil proportions and scales. The HHx monomer only appears when oleaginous carbon sources are used, and the increase in oil in the substrate proportion leads to an increase of 3-HHx content. In addition, maximum PHA production yields were reached when using a major proportion of oil compared to experiments where the oil proportion in the substrate composition was lower than 20 %.

Gutschmann et al. (2023) fermented a fat protein emulsion (FPE) from animal byproducts as a carbon source to obtain 7.8 g/L CDW with 78.2 % PHAs when using 50 g/L substrate. The fed-batch cultivations reached 51 g/L CDW containing 71 % PHAs at 60 h using a total of 110 g/L FPE.

Table 3
PHA content and 3HHx composition obtained from different fructose:canola oil proportions. Adapted from Santolin et al. (Santolin et al., 2023).

Fructose: canola oil (g/g)	Volume (mL)	Carbon source (g/L)	CDW (g/L)	PHA (%)	3HHx composition (mol%)
1:0	3	5	3.4	56.9	0.0
10:1	1000	10	9.7	66.5	2.3
5:1	3.0	5	4.1	63.9	3.7
5:1	100	5	3.9	57.3	4.1
5:1	100	10	7.1	61.3	4.4
5:1	1000	10	9.0	58.6	4.3
2:1	3	5	4.8	78.1	5.7
2:1	1000	10	10.5	60.4	6.5
1:1	3	5	5.1	79.7	8.9
1:1	100	5	4.6	63.6	9.2
1:1	100	10	8.1	69.7	8.9
1:1	1000	10	10.9	70.4	7.5
0.5:1	3	5	4.0	76.4	11.2
0.5:1	100	5	4.7	65.3	14.6
0.5:1	100	10	9.0	73.1	12.6
0.5:1	1000	10	12.3	75.7	11.4
0.2:1	3	5	4.6	77.6	14.3
0:1	3	5	4.4	89.7	16.1
0:1	100	5	4.0	66.5	21.9
0:1	100	10	8.6	72.9	16.5
0:1	1000	10	12.9	88	14.3

CDW: Cell Dry Weight, PHA: Polyhydroxyalkanoate, 3HHx: 3-hidroxyhexanoate monomer.

Although the *C. necator* epithet is considered a model bacterium for PHA production, differences were observed for PHA yields using different substrates. Most cases show that using carbohydrates, VFA, or other carbon sources does not favor high PHA yields compared to fatty acids and fatty acid/carbohydrate mixtures. Generally, using carbon sources at concentrations near 20 g/L should be sufficient for a high PHA production.

3.2.3. The genus Pseudomonas

The Gram-negative genus *Pseudomonas* comprises species known for their wide diversity and occurrence in various environments, ranging from arctic to tropical regions, as well as in different habitats such as soil, water, plants, animals, and extreme conditions (Peix et al., 2009). *Pseudomonas* are highly efficient in accumulating PHAs, especially mcl-PHA, from various carbon sources. mcl-PHAs were initially identified in *Pseudomonas oleovorans* (De Smet et al., 1983). This process is a metabolic response to unbalanced growth conditions, such as excess carbon and limiting essential nutrients like nitrogen or phosphorus. *Pseudomonas*, especially *P. putida*, have become an ideal model for PHA biosynthesis due to its tolerance to adverse conditions and ability to metabolize aromatic compounds and fatty acids (Khatami et al., 2021; Mezzina et al., 2021). PHA production by *Pseudomonas* species has been extensively studied across various strains, showcasing their versatility in utilizing diverse carbon sources and optimizing cultivation conditions. This review analyzes the findings from 23 articles covering 12 specific species and 23 distinct strains. Fatty acids, polyalcohols, and a mixture of carbon sources are identified as the primary substrates for PHA production. It is important to highlight that high substrate concentrations do not necessarily lead to increased PHA yields, as this outcome depends on several factors, including bacterial strain, substrate type and concentration, nitrogen source, incubation time, temperature, pH, and other cultivation conditions. Fig. 11 summarizes researchers' experiences on enhancing PHA production yields from *Pseudomonas* strains and different substrates.

Pseudomonas putida KT2440 has emerged as one of the most versatile bacterial strains for bioplastics production, with studies exploring its metabolic flexibility and genetic potential. Nine papers in this review highlight its ability to utilize diverse carbon sources and efficiently produce PHAs. This strain has shown remarkable adaptability to renewable substrates, making it a cornerstone in sustainable bioplastics production research. One of the most significant findings on *P. putida* KT2440 is its ability to metabolize glycerol as a carbon source efficiently. Under optimized conditions using 10 g/L glycerol, the strain achieved a CDW of 7.38 g/L and a PHA yield of 83 % (Nguyen et al., 2023). Additionally, this strain has demonstrated versatility in utilizing mixed carbon sources. For instance, when carbohydrate-lipid-VFA mixtures were used, PHA yields of 70 %, 67 %, and 59 % were achieved, depending on substrate proportions and concentrations (46.6, 55.9, and 1.95 g/L, respectively) (Gao et al., 2018). These results underscore the strain's capacity to optimize substrate utilization for high-yield PHA production. Recent advancements in genetic engineering have further expanded the capabilities of *P. putida* KT2440. The metabolic engineering of *P. putida* KT2440 demonstrated significant improvements in PHA production from lignocellulosic substrates such as xylose and cellobiose. Among the tested strains, 27 A-P13-xylABE-Ptactt achieved a CDW of 3.87 g/L and the highest PHA content of 41.6 % when utilizing xylose as substrate. This may be attributed to adaptive laboratory evolution and optimized transcription of xylose metabolism genes, which minimized byproduct formation. For cellobiose, 27A-P13-bglC-P13-gts produced a CDW of 3.05 g/L with a PHA content of 45.2 %, supported by enhanced β-glucosidase activity and improved transporter performance. These engineered strains were further modified through metabolic engineering approaches, introducing genes from *Escherichia coli* to enable the efficient utilization of lignocellulosic sugars. Key modifications included the incorporation of the *xylABE* operon for xylose metabolism through the xylose isomerase pathway (Fig. 4) and

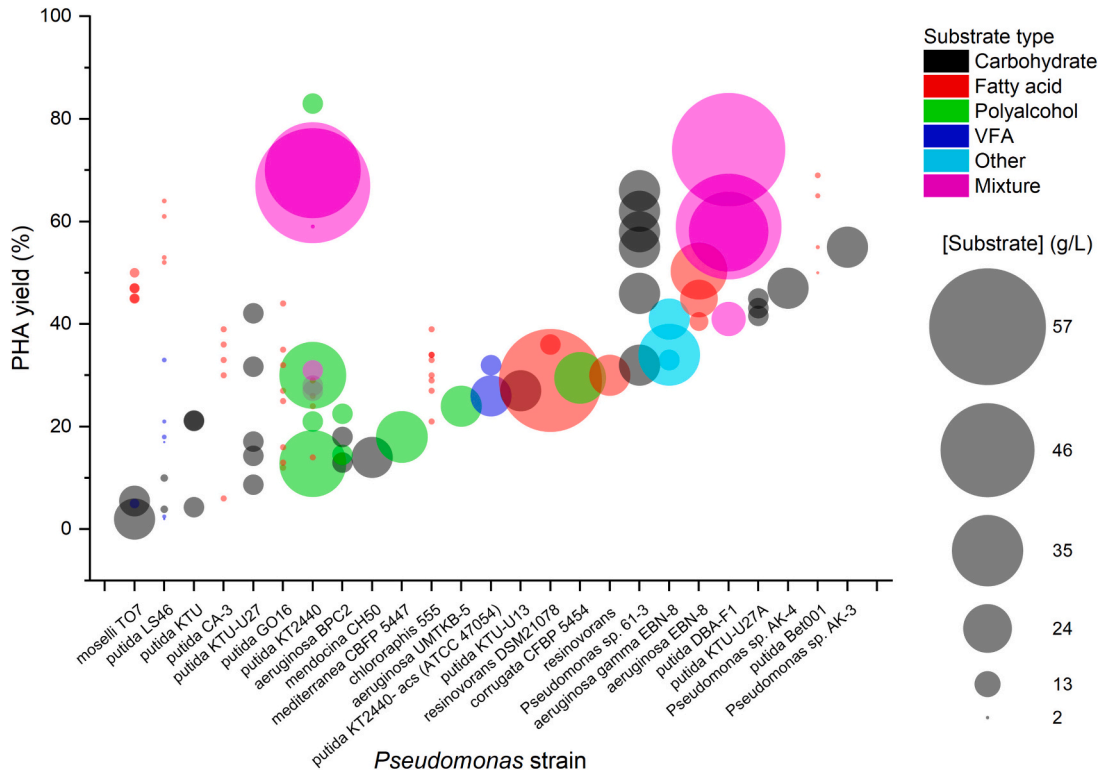

Fig. 11. PHA yield (%) from *Pseudomonas* for different substrate types (colors). Circle size increases according to substrate concentration, from 1.0 to 55.9 g/L. *Two data points were excluded from the figure due to its being oversized, but they were analyzed in the text.

the *bglC* and *gts* genes to improve cellobiose utilization. Combined with genome streamlining and adaptive laboratory evolution, these alterations significantly enhanced substrate assimilation and reduced metabolic bottlenecks (Liang et al., 2020; Liu et al., 2023). Furthermore, co-feeding lignin derivatives (such as vanillin and benzoate) with glycerol significantly increased PHA production by 29 to 63 % while altering monomer composition to favor mcl-PHA production (Xu et al., 2021a). Vegetable oils and fatty acids derived from polyethylene were fermented in 20 L bioreactors in fed-batch mode. With initial carbon concentrations of 0.84 gC/L and controlled feeding, yields of 83 g/L of CDW with a PHA content of 65 % were obtained. These conditions stimulated β-oxidation, generating intermediates such as 3-hydroxyoctanoyl-CoA to synthesize mcl-PHAs (Guzik et al., 2021). Substrate inhibition at high concentrations can limit overall productivity, and economic feasibility remains constrained by substrate and processing costs (Nguyen et al., 2023). Addressing these challenges through metabolic engineering and optimized bioprocesses is critical for improving this strain's productivity. Walsh et al. (Walsh et al., 2015) evaluated *P. chlororaphis* 555, *P. putida* KT2440, *P. putida* CA-3, and *P. putida* GO16 for PHA production using plant oils and hydrolyzed fatty acids as a carbon source. *P. chlororaphis* 555 achieved the highest yields, producing up to 39 % PHA with olive oil hydrolysates and 76 g/L CDW with 17 % PHA in large-scale fermentation using rapeseed oil hydrolysates. *P. putida* GO16, an indigenously isolated strain, exhibited high adaptability to diverse substrates, achieving 44 % PHAs with palm oil hydrolysates. The strain grew efficiently under nitrogen-limited conditions at 30 °C, demonstrating robust metabolic activity when utilizing hydrolyzed fatty acids. *P. putida* CA-3 achieved 39 % PHAs with rapeseed and olive oil hydrolysates, while *P. putida* KT2440 had lower yields, peaking at 28.6 % PHAs with palm oil hydrolysates. These results highlight the potential of these strains, particularly *P. putida* GO16, for optimizing bioplastic production from plant-based substrates. Genetic manipulation of strains derived from *P. putida* KT2440 has allowed further refinement of PHA composition. *P. putida* DBA—F1, a β-oxidation knockout mutant derived from *P. putida KT2440*, exhibits exceptional potential for producing mcl-PHAs, particularly with a high 3-hydroxydecanoate (3HD) content. With a decanoic acid/glucose ratio of 4:6, the strain achieved a CDW concentration of 17 g/L, a PHA content of 58 %, and a productivity of 0.32 g/L/h. By adjusting the substrate ratio to 2:8, CDW increased to 18 g/L, and the PHA content reached 59 %, with an HD content of 100 mol%. These results highlight the potential of *P. putida* DBA-F1 for tailored mcl-PHA production despite challenges such as low biomass yield and metabolic imbalances (Gao et al., 2018).

P. putida LS46 demonstrated its ability to produce mcl-PHAs under oxygen-limited batch-feeding conditions. Using octanoic acid as a carbon source, this strain achieved a CDW of 28.8 g/L, with a PHA content of 61 % and a productivity of 0.66 g/L/h. Pulse-feeding strategies further enhanced PHA synthesis, and mcl-PHAs were mainly composed of C8 (92.9 mol%). These results emphasize the strain's efficiency in utilizing fatty acids for PHA production under optimized conditions (Blunt et al., 2019) and illustrate that manipulating dissolved oxygen levels, adjusting substrate pulses, and maintaining stable fermentation conditions can substantially improve productivity. This opens the door to more efficient processes at the industrial level. Similarly, *P. putida* Bet001, under batch-feeding conditions with oleic acid (C18:1) as a carbon source, accumulated PHAs from 49.7 % to 68.9 % over 48 h at 30 °C. The resulting copolymer consisted of seven different monomers, including even C4 to C14 and odd C7 chain units, demonstrating its ability to produce diverse copolymers (Gumel et al., 2014). This structural variability in the biopolymer makes obtaining materials with customizable mechanical and thermal properties possible.

P. aeruginosa BPC2 revealed that the best PHA yield of 22.5 % was achieved using a glycerol byproduct derived from biodiesel production as a carbon source. The bacterial growth reached a CDW of 7.8 g/L under shake flask culture, incubated for 72 h at 37 °C with 1 % glycerol byproduct. This yield was significantly higher than other tested substrates like glucose, commercial glycerol, and sugarcane molasses (Phukon et al., 2014). However, not all strains have been studied with

refined substrates. *Pseudomonas* sp. AK-3 and AK-4, isolated from landfills, can use cheaper and simpler carbon sources like sucrose. Using 2 % of this sugar yields between 47 % and 50 % PHAs have been achieved (Muneer et al., 2022). In addition, fine-tuning of nitrogen availability can be critical: while a higher nitrogen supply increases biomass, this excess reduces the PHA content. These findings confirm the importance of carefully calibrating C/N ratios and the choice of appropriate nitrogen sources. The recombinant strain of *Pseudomonas* sp. 61–3 showed significant yield variation depending on the concentration of steamed soybean wastewater powder (SWP) and glucose (2 %) as the carbon source. For 72 h of cultivation using 1 g/L SWP, CDW was 0.5 g/L, and PHA content reached 62 %. This condition yielded a high PHA content but low CDW, attributed to limited nitrogen availability. Increasing SWP to 10 g/L resulted in a CDW of 3.4 g/L and a lower PHA content of 38 %, as carbon excess led to a reduced nitrogen-to-carbon ratio. For 50 g/L SWP, CDW peaked at 7.8 g/L, but PHA content dropped to 18 %. This decline may be attributed to nitrogen insufficiency, hindering the copolymer synthesis efficiency. For the NH_4Cl control (0.5 g/L) after 48 h, the CDW reached 0.6 g/L and PHA content 62 %. The high yields obtained under this condition were due to the optimal balance between nitrogen and carbon availability. *Pseudomonas* sp. 61–3 demonstrated that increasing SWP concentration improved CDW but reduced the PHA yield due to nitrogen limitation. Glucose addition was critical in maintaining cellular activity and supporting PHA biosynthesis under nitrogen-limited conditions (Hokamura et al., 2017). *P. putida* KT2440 metabolizes glucose through a cycle generated among the Pentose-phosphate, Entner-Doudoroff, and lower Embden-Meyerhof-Parnas pathways (Fig. 4) that enhance NADPH generation, which is useful for PHA synthesis and for tolerating stressful conditions such as nutrient limitation (Beckers et al., 2016).

PHA production can also benefit from low substrate concentrations, ensuring the appropriate strain is selected and the predominant metabolic pathway is understood. For example, *P. mosselii* TO7, isolated from a vegetable oil factory wastewater treatment plant, performed best with only 0.5 % soybean oil, yielding 49.8 % PHAs and a CDW of 3.76 g/L (Chen et al., 2014). Metabolic studies using inhibitors such as acrylic acid showed that when fatty acids (e.g., octanoate) are used, β-oxidation is essential for PHA synthesis. In contrast, sucrose or gluconate activate different pathways, leaving PHA production relatively insensitive to the inhibition of β-oxidation. However, low yields have also been reported in the literature. For example, *P. corrugata CFBP 5454* yielded 3.12 g/L CDW and a PHA content of 29.5 % in 48 h using glycerol as the sole carbon source. The yield was limited by alginate co-production, which diverted metabolic precursors away from PHA synthesis, highlighting competition for resources under the given conditions. *P. corrugata CFBP 5447*, under similar conditions, produced 2.89 g/L CDW with PAHs content of 18.0 %. Similarly, alginate synthesis significantly impacted carbon flux toward PHA production, reducing the overall yield (Licciardello et al., 2017). *P. mendocina* CH50, cultivated on sugarcane molasses in a 15 L bioreactor, achieved a PHA content of 14.2 % in 48 h. Its inability to metabolize sucrose directly without hydrolysis constrained its efficiency, highlighting the importance of substrate pre-processing for higher yields (Basnett et al., 2020). *P. putida* KT2440 achieved 31.5 % PHAs through enhanced acetate metabolism, improving tolerance and utilization in bioreactor systems (Yang et al., 2019). Under nitrogen-limited conditions, it produced 29.7 % PHAs at low dilution rates, with transcriptomic analysis highlighting the importance of pathway modulation during nitrogen limitation, such as the usage of the Entner-Doudoroff pathway and the glyoxylate shunt, for optimizing PHA biosynthesis (Beckers et al., 2016). *P. resinovorans* demonstrated the utilization of camelina oil under bioreactor-fed batch conditions, achieving a PHA content of 35.6 %, leveraging its innate lipase activity to metabolize unprocessed oils directly (Bustamante et al., 2019). Similarly, spent coffee ground oil served as a substrate for PHA production by *P. resinovorans*, yielding a PHA content of 29.7 % in 24 h under optimized fed-batch culture conditions (Kang et al., 2023). Moreover, comparisons between *P. aeruginosa* mutants reveal that substrate choice and complexity can significantly influence performance. A gamma-irradiated mutant strain, named EBN-8, managed to accumulate more than 50 % PHAs when using 3 % (*v*/v) soybean oil, while another mutant strain EBN-8, using 2 % (v/v) hexadecane, only achieved 40.7 % (Abid et al., 2016; Raza et al., 2016). These differences show that chain length and substrate structure determine the efficiency of β-oxidation and, thus, PHA accumulation. In turn, this has practical implications for selecting suitable substrates according to the capabilities of each strain and the desired final polymer properties. The implementation of feeding pulses has also been fundamental in fed-batch cultures. For example, the continuous use of polyethylene-derived fatty acids facilitated the achievement of a PHA content of 65 % CDW in 20 L cultures in only 25 h. Optimal incubation times vary depending on substrate and culture conditions but are generally between 24 and 144 h among the methodologies reviewed. These periods allow for maximum PHA accumulation without compromising cell growth.

3.2.4. The genus Burkholderia

Burkholderia are Gram-negative bacilli that inhabit soils and water and can accumulate scl-PHAs and mcl-PHAs. Specific *Burkholderia* strains are human pathogens or phytopathogens. The genus *Burkholderia* was studied in 5 articles in this review using 3 specific epithets and 8 strains. Fig. 12 shows those strains related to the substrate type, the substrate concentration, and the PHA yield. It is noted that carbohydrates prevail over other carbon sources, and a higher substrate concentration does not correlate with a higher PHA production yield.

Four strains of *B. cepacia* epithet isolated from various soil sources were studied by Attapong et al. (2023), using glucose at 25 and 35 g/L or a sugarcane bagasse hydrolysate pretreated with sulfuric or phosphoric acid as carbon sources for ASL22, KKR5, SRB1, and SRB3 strains. *B. cepacia* is well known for its ability to ferment different sugar sources and for its tolerance to possible inhibitory compounds. Table 4 shows PHA yields $\leq$23 %, while the usage of sugarcane bagasse enhanced biomass production. Nevertheless, the PHB yield of strains decreased by almost half when bagasse was used instead of glucose.

de Paula et al. (2021) worked with the *B. glumae* MA13 strain to obtain a P(3HB-*co*-3 HV) copolymer. Using 20 g/L sucrose they obtained 4.4 g/L CDW with 26.5 % P(3HB-*co*-3 HV); the use of xylose at 10, 20, 30, 40, and 50 g/L produced between 0.39 and 0.53 g/L CDW (the maximum using 40 g/L xylose), while P(3HB-*co*-3 HV) obtained vary from 18.0 % to 21.1 % (the maximum using 50 g/L xylose). Differences in P(3HB-*co*-3 HV) yields among sucrose and xylose may be explained by the differences in the metabolic pathways (Fig. 4). When using sugarcane molasses at 10, 20, 30, 40, and 50 g/L, the biomass produced by *B. glumae* MA13 moves from 3.3 to 5.8 g/L CDW, while the P(3HB-*co*-3 HV) varied from 26.5 % to 46.6 %. The maximum CDW and P(3HB-*co*-3 HV) were obtained using sugarcane molasses at 20 g/L (5.8 g/L CDW with 46.6 % P(3HB-*co*-3 HV)), and the maximum percentage of de hydroxyvalerate monomer (1.49 mol%) was obtained using 50 g/L xylose as a carbon source. This research group also studied the addition of vinasses to sugarcane molasses and a sugarcane bagasse hydrolysate to produce PHAs, obtaining a maximum of 4.5 g/L CDW with 29.4 % PHA in the first case and 0.61 g/L CDW with 15 % PHAs in the second one. The influence of pH and nitrogen source selection was also analyzed.

P. sacchari DSM17165 accumulates scl-PHAs and mcl-PHAs and has been largely studied for this purpose. Three different strains of the *B. sacchari* epithet were studied in 3 articles. The DSM17165 strain was used by Cesário et al. (2014), obtaining 5.0 g/L CDW with 44 % PHB using 10 g/L glucose and 6.3 g/L CDW with 60.3 % PHB using 20 g/L glucose. 10 and 20 g/L xylose produced 5.2 g/L CDW with 46.7 % PHB and 6.3 g/L CDW with 44.4 % PHB, respectively. When glucose and xylose were combined (10 g/L each), 7.4 g/L CDW and 58.9 % PHB were produced. In addition, 20 g/L arabinose generates 7.4 g/L CDW with 62 % PHB. Different wheat straw lignocellulosic hydrolysates, rich in

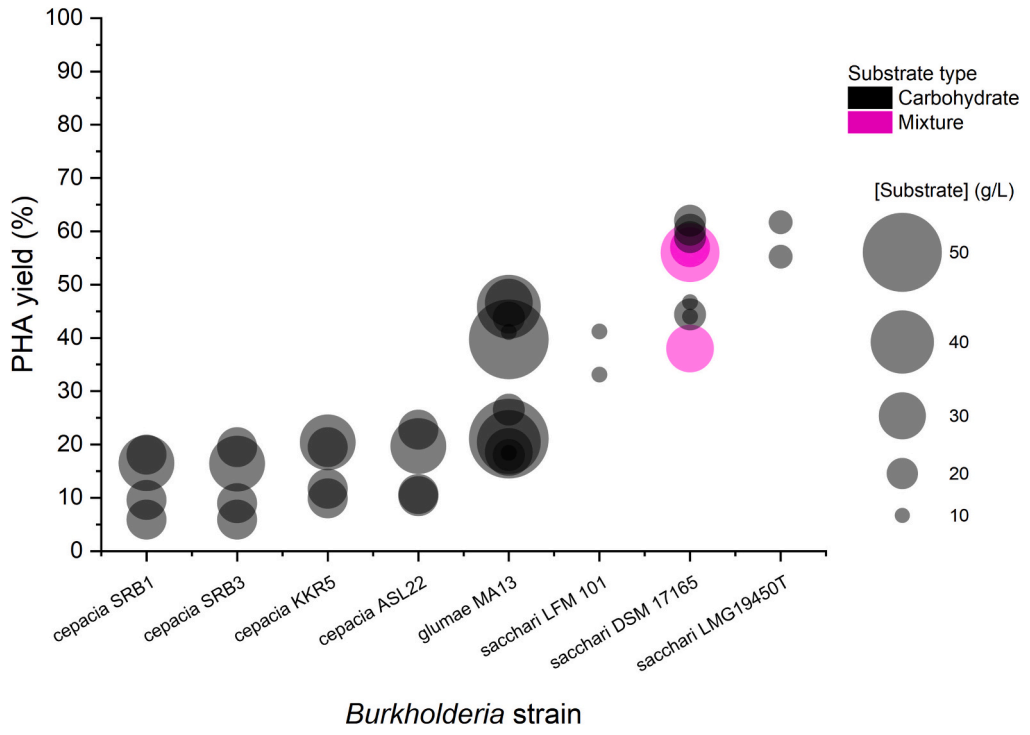

Fig. 12. PHA yield (%) from *Burkholderia* using different substrate types (colors). Circle size increases according to substrate concentration, from 10 to 50 g/L.

Table 4
Comparison of biomass (CDW) and PHB production of ASL22, KKR5, SRB1, and SRB3 strains. Adapted from Attapong et al. (Attapong et al., 2023).

Strain	Substrate	Concentration (g/L)	CDW (g/L)	PHB (%)
ASL22	Glucose	25	4.7	22.8
	Glucose	35	6.5	19.7
	SBH pretreated with 1 % *v*/v sulfuric acid	25	10.0	10.3
	SBH pretreated with 1.5 % *v*/v phosphoric acid	25	11.2	10.7
KKR5	Glucose	25	4.6	19.5
	Glucose	35	7.8	20.4
	SBH pretreated with 1 % v/v sulfuric acid	25	11.7	9.90
	SBH pretreated with 1.5 % v/v phosphoric acid	25	11.6	11.7
SRB1	Glucose	25	3.0	18.1
	Glucose	35	6.2	16.5
	SBH pretreated with 1 % v/v sulfuric acid	25	11.7	5.90
	SBH pretreated with 1.5 % v/v phosphoric acid	25	11.7	9.60
SRB3	Glucose	25	5.0	19.5
	Glucose	35	7.9	16.4
	SBH pretreated with 1 % v/v sulfuric acid	25	12.2	9.00
	SBH pretreated with 1.5 % v/v phosphoric acid	25	11.7	5.90

CDW: Cell Dry Weight, PHB: Polyhydroxybutyrate.

arabinose, glucose, and xylose, delivered by a German biorefinery, were also used as substrates for PHA production. 7.7 g/L CDW with 57 % PHB was obtained from 25 g/L of the named hydrolysate A, 32.4 g/L CDW with 38 % PHB from 30 g/L hydrolysate B and 83 g/L CDW with 56 % PHB using 37 g/L hydrolysate C. Nascimento et al. (2016) worked with the LFM 101 strain using 10 g/L glucose to obtain 2.9 g/L CDW containing 41.2 % PHAs with no significant differences when temperature varied from 30 °C to 35 °C. When 10 g/L sucrose was used at 30 °C, 1.9 g/L CDW with 26 % PHAs was produced in contrast with the 2.65 g/L CDW containing 33.1 % PHAs obtained when the temperature was set at 35 °C. Finally, Oliveira-Filho et al. (2020) used 15 g/L xylose as the sole carbon source to feed the DSM17165 strain in fed-batch fermentation with different nutrient limitation strategies to reach 13.1 g/L CDW with 61.7 % PHAs under nitrogen limitation and 29.3 g/L CDW with 55.3 % PHAs under phosphorus limitation. Every reviewed article that studied *Burkholderia* strains for PHA production showed yields below 60 % except for one experiment. Higher production was obtained with *P. sacchari* (33 %–62 % PHAs), which showed excellent results even from wheat straw, a lignocellulosic waste used as a cheap carbon source.

3.2.5. *Halomonas and Paracoccus genera*

Halomonas and *Paracoccus* were selected as the most studied extremophile genera for PHA production. *Halomonas* is a halotolerant genus that can produce PHAs under non-sterile and high-salt conditions (Diankristanti et al., 2024). As carbon sources for *Halomonas*, some carbohydrates and VFA (Fig. 13) were found. It has been reported that some *Paracoccus* also co-produce some valuable compounds with antioxidant or anticarcinogenic properties such as carotenoids or astaxanthin, which represents a more cost-effective PHA production through a biorefinery scheme (Khomlaem et al., 2021; Muhammad et al., 2020). The types of carbon sources for *Halomonas* reported here were carbohydrates (glucose and carboxymethylcellulose) and VFA, while *Paracoccus* used carbohydrates and polyalcohol (Fig. 13).

The *Halomonas elongata* A1 strain, isolated from the saline alkali soil of Daqing (China), was studied by Liu et al. (2021), who modified a wild-type *H. elongata* A1 to three different recombinant strains called *H. elongata* P0, P1, and P2. Experiments with the A1 strain using 20 g/L carboxymethylcellulose as a carbon source produced 4.17 g/L CDW with 11.8 % PHB, while using 20 g/L glucose reached 6.75 g/L CDW containing 22.8 % PHB. The CDW and %PHB of recombinant *H. elongata* P0, P1, and P2 using glucose as a carbon source resulted in 6.7 g/L with 21.6 % PHB, 6.8 g/L CDW with 45.6 % PHB, and 6.7 g/L CDW containing 90.8 % PHB, respectively. Using the *H. elongata* P2 increased almost 4 times the percentage of PHB compared to the wild-type bacteria. (Diankristanti et al., 2024; Yin et al., 2023) worked with the *Halomonas* sp. YJ01 and various substrates to produce P(3HB-*co*-3 HV). Glucose at

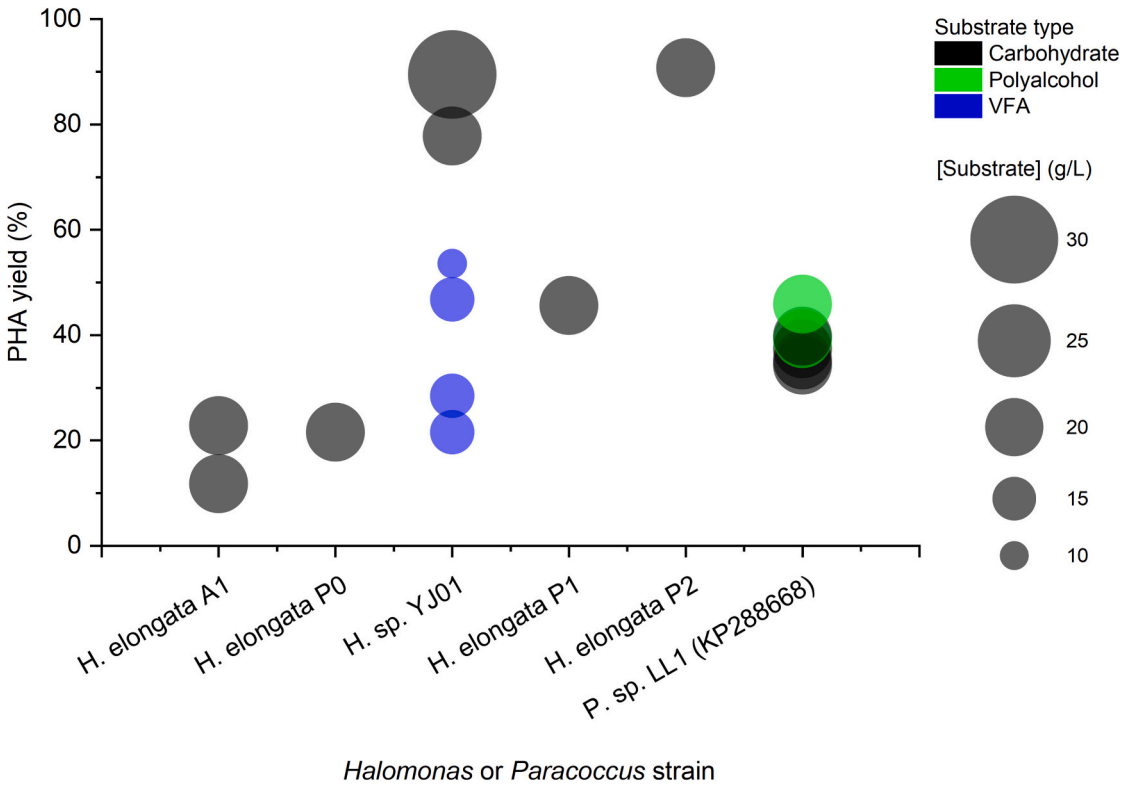

Fig. 13. PHA yield (%) from *Halomonas* and *Paracoccus* bacteria for different substrate types (colors). Circle size increases according to substrate concentration. Substrate concentration for *Halomonas* moves from 10 to 30 g/L, and for Paracoccus, it remains at 20 g/L.

20 and 30 g/L produced 6.6 g/L CDW with 77.8 % P(3HB-*co*-3 HV) and 6.37 g/L CDW with 89.5 % P(3HB-*co*-3 HV). The fermentation of VFA was tested with 15 g/L acetic acid, 15 g/L propionic acid, and 10 g/L butyric acid, which produced 2.6 g/L CDW with 21.6 % PHB, 3.0 g/L CDW with 28.5 % P(3HB-co-3 HV), and 4.0 g/L CDW containing 53.6 % PHB, respectively. The fraction of 3-HV from propionic acid was 29 mol %. Finally, different mixtures of acetic, propionic, and butyric acids were tested, finding that the strain YJ01 with M5 mixture (acetate: propionate:butyrate 1:4:2) produced better results, reaching 3.54 g/L CDW with 46.8 % P(3HB-co-3 HV), including a 3-hydroxyvalerate fraction of 19 mol%.

The *Paracoccus* sp. LL1 (KP288668) strain isolated from Lake Lonar (India) was used in 2 articles to complete 6 experiments. Kumar et al. (2018) used glycerol at 20 g/L to obtain 2.54 g/L CDW with 45.9 % PHAs after 96 h in a batch culture and 24 g/L CDW with 39.3 % PHAs after 120 h in a cell retention culture. The co-production of PHAs and carotenoids is possible with this strain; therefore, total carotenoids obtained reached 1.25 mg/L in the first case and 7.14 mg/L in the second. Khomlaem et al. (2020) used the mentioned LL1 strain to produce PHAs in a flask fermentation to obtain 3.75 g/L CDW with 34.3 % PHAs when using 20 g/L glucose and 3.96 g/L CDW with 37.4 % PHAs when using defatted biomass of *Chlorella* (a variety of microalgae) as substrate. The total carotenoids obtained reached 6.02 mg/L from glucose and 6.08 mg/L from defatted chlorella biomass. A lab-scale bioreactor was used to repeat the experiments, achieving 5.35 g/L CDW with 35.3 % PHAs and 7.60 mg/L of carotenoids using glucose and 9.1 g/L CDW with 39.8 % PHAs and 11.7 mg/L of carotenoids using defatted chlorella biomass as substrate. The bioreactor allows an increase in PHAs and carotenoid yields in less time. Additionally, diluted acid pretreatment to degrade algal carbohydrates into fermentable sugars as an inexpensive carbon source may moderate PHA production costs.

The PHA production yields of the reviewed experiments with *Paracoccus* did not reach 50 %; however, using glucose with the LL1 strain gave the best results. The ability of some *Paracoccus* strains to co-produce PHAs and valuable compounds is highlighted because it represents an opportunity for the production of bioplastics that may reduce production costs in addition to expanding market options. On the other hand, the studied *Halomonas* strains reached a remarkable 90 % PHAs of CDW when using glucose as a carbon source.

3.3. General appreciations

The highest PHA yields per substrate type of every specific epithet studied in this review are shown in Fig. 14 (a and b), which includes a total of 54 experiments. Carbon source concentration (Fig. 14a) and cell dry weight (CDW) production (Fig. 14b) are compared. The relationship of substrate concentration with PHAs or CDW yields remains unknown. However, despite the variability of the data, PHA yields above 60 % (27 experiments) were obtained with an average substrate concentration of 21.2 g/L, while the 27 experiments producing below 60 % PHA used an average of 18.2 g/L substrate. In addition, there seems to be a relationship between CDW and PHA yield as the 27 experiments with high PHA yields (over 60 %) also produced an average of 13.4 g/L CDW. In contrast, the remaining 27 experiments (PHA yields below 60 %) made an average of 4.3 g/L CDW, meaning average PHA productions of 10.1 g/L against 1.57 g/L. This apparent relationship should be considered a general approximation due to variations by strain (synthesis pathways) and fermentation conditions to optimize PHA production. Some reviewed experiments were conducted in two phases to promote growth under nutritionally balanced conditions and accumulation under nutrient-limiting conditions separately. In contrast, others added an extra feed in the second phase using the initial or a different substrate.

Fig. 14 also confirms that PHA production yields are not significant when using different substrates (compared to the 4 main groups including Carbohydrate, Fatty acid, Polyalcohol, and VFA); however, using appropriate mixtures could be an option to explore higher performances at lower costs.

Considering the 308 experiments, only 13 reached PHA yields above

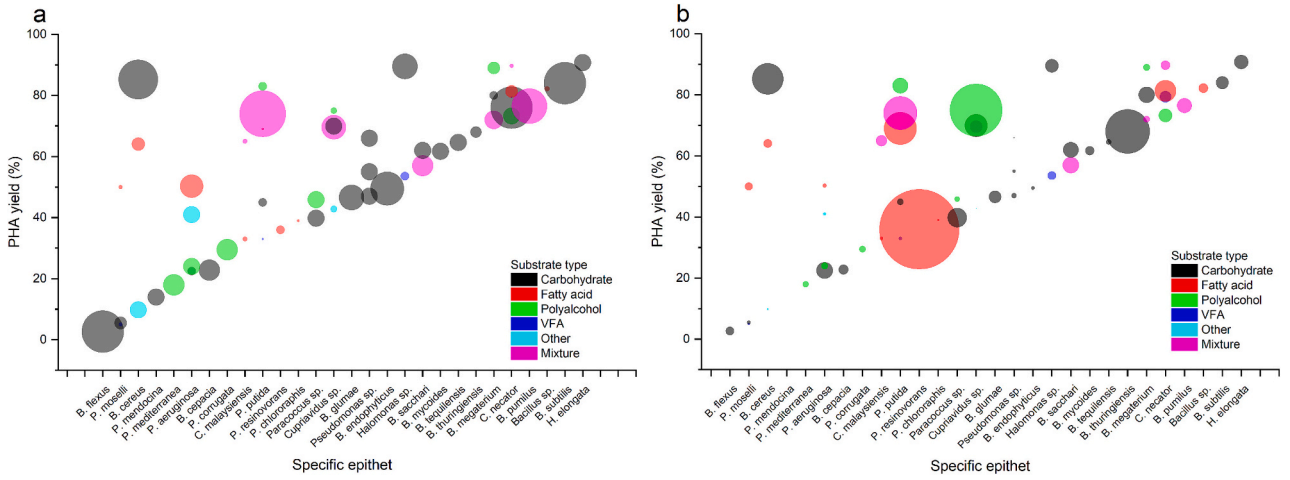

Fig. 14. Highest PHA yield (%) from each specific epithet by substrate type (colors). Circle size increases according to (a) substrate concentration from 1 to 55 g/L and (b) CDW from 0.41 to 38 g/L. *One point of outlier data was excluded from the fig. (148 g/L CDW).

80 %; 6 were produced by *Bacillus*, 4 by *Cupriavidus*, 2 by *Halomonas*, and 1 by *Pseudomonas*. Carbohydrates resulted in the most used substrate (6 experiments), 3 experiments used fatty acids, 2 used polyalcohol, and 2 used a fatty acid/carbohydrate mixture with different fermentation strategies. Among the best results, the most common bacteria/substrate combination was *Bacillus*/carbohydrate (4 experiments).

Reducing PHA production costs is one of the biggest challenges today, since actual costs are almost 10–15 times higher than that of producing conventional polymers, according to Kosseva and Rusbandi (2018). Karan et al. (2019) reported the cost of 2.90 USD/kg PHA, which is relatively high compared to corn starch (0.40 USD/kg) or polylactic acid (2.0 USD/kg). However, fluctuating petroleum prices coupled with advances in biotechnology such as agro-industrial waste as a carbon source for bacteria, which lower production costs and reduce land use, are expected to narrow this gap. For example, Leong et al. (2016) reported a 25 % reduction in production costs when using crude glycerol as a carbon source instead of refined glycerol and a 28 % reduction in production costs when using waste cooking oil (WCO) instead of soybean oil. In addition, the production of PHA also implies energy savings, as PHB requires significantly less energy (44.7 MJ kg^{-1}) compared to petrochemical polymers (e.g., polypropylene needs nearly 86 MJ kg^{-1}), offering both environmental and financial benefits (Costa et al., 2023).

Future research and development to enhance PHA production performance and competitive costs should include the specific PHAs produced in each system and residual organic biomass as substrate for such purposes. In addition, genetically modified bacteria should be further studied to enhance production yields and the physicochemical properties of PHAs by producing short- and medium-chain PHA copolymers.

4. Conclusions

This review examines recent advancements in the production of polyhydroxyalkanoates (PHAs) as potential sustainable biopolymers compared to petrochemical plastics. The work evaluates strategies to enhance PHA production yield and key findings from prominent bacterial genera. An overall summary of the diverse pathways for PHA synthesis in bacteria reveals important metabolic targets related to polymer yields, customized monomeric composition, or obtaining novel monomers using a wide range of carbon sources. Seventy-seven scientific studies were reviewed, including more than 300 experiments involving 76 bacterial strains. The analysis considered metrics such as the percentage of PHA accumulation relative to cell dry weight, efficiency in utilizing various carbon sources, yields under different culture conditions, and metabolic engineering applications for monomer diversification.

Among the significant findings, *Cupriavidus necator* stood out for its ability to accumulate up to 80 % of its cell dry weight in PHAs when using substrates like glucose, glycerol, and vegetable oils. *Pseudomonas putida* was notable for synthesizing medium-chain PHAs with yields reaching up to 83 %, utilizing fatty acids and lignocellulosic residues as substrate. The genus *Bacillus* also demonstrated remarkable adaptability to cost-effective substrates like molasses or glycerol from biodiesel production, achieving PHA yields above 85 %. The extremophilic genera *Halomonas* and *Paracoccus* provided advantages by operating under non-sterile conditions and co-producing added-value compounds, such as carotenoids, enhancing the sustainability and competitiveness of PHA production. However, the experiments studied using *Paracoccus* presented a low output (among 34–46 % PHAs) compared to *Halomonas* (among 12–91 % PHAs).

Future research in this field should focus on addressing the challenges of industrial scalability by optimizing bioprocesses and integrating sustainable systems that utilize waste as the substrate. Moreover, it will be crucial to diversify the properties of PHAs through innovations in genetic and metabolic engineering, which will expand their industrial applications and strengthen their role in the transition toward a more efficient and sustainable circular economy.

CRediT authorship contribution statement

Ariel Vilchez: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Gabriela Guajardo:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Mario Sepúlveda:** Writing – review & editing, Visualization. **Michael Seeger:** Writing – review & editing, Supervision, Funding acquisition. **Francisca Acevedo:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Rodrigo Navia:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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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Data availability

The data analyzed were extracted from 77 published works and can be found following the search criteria described in the methodology.

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