Science of the Total Environment 756 (2021) 143729

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

A review of biopolymer (Poly-β-hydroxybutyrate) synthesis in microbes cultivated on wastewater

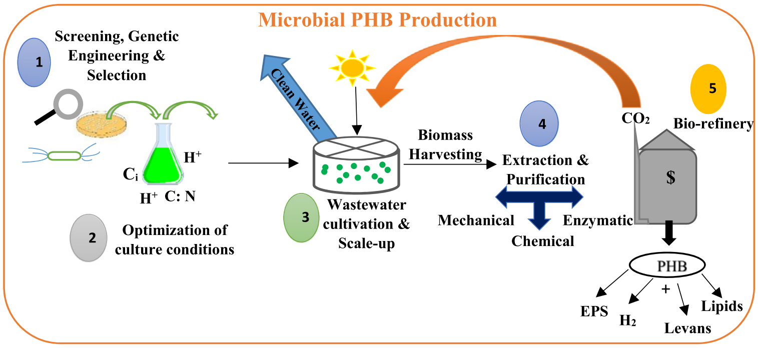
Ayesha Algade Amadu [a], Shuang Qiu [a], Shijian Ge [a,*], Gloria Naa Dzama Addico [b], Gabriel Komla Ameka [c], Ziwei Yu [a], Wenhao Xia [a], Abdul-Wahab Abbew [a], Dadong Shao [a], Pascale Champagne [d], Sufeng Wang [e]

[a] *Jiangsu Key Laboratory of Chemical Pollution Control and Resources Reuse, School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Xiao Ling Wei 200, Nanjing 210094, PR China*
[b] *Council for Scientific and Industrial Research (CSIR) – Water Research Institute (WRI), P.O. Box AH 38, Achimota Greater Accra, Ghana*
[c] *Department of Botany, University of Ghana, P.O. Box LG55, Legon, Accra, Ghana*
[d] *Department of Civil Engineering, Queen's University, Kingston, Ontario K7L 3N6, Canada*
[e] *School of Economics and Management, Anhui Jianzhu University, Hefei, Anhui 230601, PR China*

HIGHLIGHTS

- Criteria for selecting appropriate PHB-producing microbes were proposed.
- Biosynthesis and degradation pathway of PHB was illustrated.
- Optimization of culture conditions for improved PHB yield was highlighted.
- Feasibility of producing other metabolites using wastewater was evaluated.
- Environmentally friendly extraction methods and application of PHB were reviewed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 July 2020
Received in revised form 4 November 2020
Accepted 5 November 2020
Available online 25 November 2020

Editor: Yifeng Zhang

Keywords:
Microbe
Poly-β-hydroxybutyrates
Resource recovery
PHA
PHB
Wastewater

ABSTRACT

The large quantities of non-degradable single use plastics, production and disposal, in addition to increasing amounts of municipal and industrial wastewaters are among the major global issues known today. Biodegradable plastics from biopolymers such as Poly-β-hydroxybutyrates (PHB) produced by microorganisms are potential substitutes for non-degradable petroleum-based plastics. This paper reviews the current status of wastewater-cultivated microbes utilized in PHB production, including the various types of wastewaters suitable for either pure or mixed culture PHB production. PHB-producing strains that have the potential for commercialization are also highlighted with proposed selection criteria for choosing the appropriate PHB microbe for optimization of processes. The biosynthetic pathways involved in producing microbial PHB are also discussed to highlight the advancements in genetic engineering techniques. Additionally, the paper outlines the factors influencing PHB production while exploring other metabolic pathways and metabolites simultaneously produced along with PHB in a bio-refinery context. Furthermore, the paper explores the effects of extraction methods on PHB yield and quality to ultimately facilitate the commercial production of biodegradable plastics. This review uniquely discusses the developments in research on microbial biopolymers, specifically PHB and also gives an overview of current commercial PHB companies making strides in cutting down plastic pollution and greenhouse gases.

---

* Corresponding author.
E-mail address: geshijian1221@njust.edu.cn (S. Ge).

https://doi.org/10.1016/j.scitotenv.2020.143729
0048-9697/

Contents

1. Introduction . . . 2
   1.1. Overview of biopolymer production routes . . . 3
2. Research tendencies in Poly-β-hydroxybutyrate production . . . 3
   2.1. Current developments . . . 4
3. Selection of Poly-β-hydroxybutyrate-producing microbes suitable for wastewater cultivation . . . 5
4. Utilization of wastewater for Poly-β-hydroxybutyrate production. . . . 5
   4.1. Poly-β-hydroxybutyrate production from industrial wastewater . . . 6
   4.2. Poly-β-hydroxybutyrate production from municipal wastewater . . . 8
   4.3. Co-production of Poly-β-hydroxybutyrate and other metabolites in wastewater . . . 8
   4.4. Biosynthesis and metabolic engineering of microbial Poly-β-hydroxybutyrate . . . 8
      4.4.1. Gene manipulation – nutrition . . . 9
      4.4.2. Gene manipulation – morphology . . . 9
      4.4.3. Gene manipulation - non-native host . . . 9
5. Factors influencing the accumulation and composition of Poly-β-hydroxybutyrate . . . 9
   5.1. Strain . . . 9
   5.2. Nutrients . . . 10
   5.3. Feeding mechanism . . . 12
   5.4. pH . . . 12
   5.5. Photoperiod . . . 12
   5.6. Cycle length . . . 13
6. Effects of extraction methods on Poly-β-hydroxybutyrate yield and purity . . . 13
7. Challenges and future prospects . . . 14
   7.1. Optimizing production . . . 14
   7.2. Acclimatization of microbes for wastewater cultivation . . . 14
   7.3. Improving yield . . . 14
8. Conclusions . . . 15

CRediT authorship contribution statement . . . 15
Declaration of competing interest . . . 15
Acknowledgement . . . 15
References . . . 15

1. Introduction

Wastewater (WW) generation is an unavoidable aspect of daily life and a consequence of industrialization and urbanization. Almost all the water used in our homes and industries end up as municipal or industrial WW and, when released into the environment, creates a significant footprint such as eutrophication (Ge et al., 2015; Ranade and Bhandari, 2014; Qiu et al., 2020b). These WW effluents are often loaded with contaminants that make it unsafe for discharge into the aquatic environment. However, some WWs have been found to be excellent nutrient sources for certain microbes (Das et al., 2018). Fast growing eukaryotic algae, cyanobacteria and bacteria (hereafter collectively referred to as microbes) are the promising solution to biological nutrient removal such as activated sludge system in wastewater treatment plants (WWTPs) (Ge et al., 2014; Qiu et al., 2020b; X. Xu et al., 2020; M. Xu et al., 2020). Not only is microbial WWT eco-friendly (Arroyo and Molinos-Senante, 2018), it ensures the use of fewer resources for microbial growth, and also offers opportunities for resource recovery (Gabriel et al., 2018; Ge et al., 2017). Countless algal species used in WWT are excellent bio-fixers (Demirbas, 2011; Kassim and Meng, 2017), thus they are able to remove carbon dioxide from the atmosphere as well as inorganic nitrogen and phosphorus from the aquatic environment. These nutrients support microbial growth (Chen et al., 2020; Ge et al., 2018a; Ge and Champagne, 2017; Ge and Champagne, 2016) while producing valuable energy alternatives such as biomethane and bio-fuel, natural antioxidants, insect feed additives (Ge et al., 2018b; Jochum et al., 2018; Qiu et al., 2019b, 2020a; Shen et al., 2020), bio-fertilizers (Shweta and Samuel, 2015), or biopolymers (Meixner et al., 2017).

Bio-plastics are plastics obtained from renewable biomass, unlike their petroleum counterparts, and have been produced from first generation feed-stocks such as corn, sugar beet, or second-generation feedstock like lignocellulose materials in the past. Recently, however, more attention is paid to the usage of third generation feedstock such as microbes that do not compete with human food or animal feed, arable land or freshwater (Ge et al., 2017; Qiu et al., 2019a). Microbial bioplastics are polymers synthesized within the cytoplasm of some cells as water-insoluble “inclusion bodies” (Jiang et al., 2015). These inclusion bodies have been described as “entirely natural and biodegradable” (Hankermeyer and Tjeerdema, 1999; Sedlacek et al., 2019). They serve as a carbon reserve material especially when cells are grown under stress (Kamravamanesh et al., 2018; Mendhulkar and Shetye, 2017). Notwithstanding their role as storage material, they help microbial cells maintain their integrity, particularly through protection against sudden osmotic imbalances (Sedlacek et al., 2019). Polyhydroxyalkanoate (PHA) is an umbrella term that describes a group of naturally occurring polymers of which Poly-3-hydroxybutyrate (P3HB) is the most studied (Jiang et al., 2015; Mathuriya and Yakhmi, 2019; Urtuvia et al., 2014). Microbial polymers have material properties that are suitable in many industrial applications due to their similarity to conventional plastics. For instance, good barrier properties have allowed for their use in the food industry for packaging purposes (Khosravi-Darani, 2015; Markl et al., 2018). These properties are based on their melting point (175 °C), glass transition temperature (15 °C), molecular weight (5×105 Da), density (1.25 g/cm^3), tensile strength (40 MPa) and young's modulus (3.5 GPa) (Carofiglio et al., 2017; Hempel et al., 2011; Sathya et al., 2018).

It is estimated that the production cost for PHB is four to nine times higher than the price of polyethylene (Hempel et al., 2011; Kamravamanesh et al., 2017). High preference for pure culture fermentation, as well as substrate requirements and various culture conditions also compound the high production cost problem. Culture conditions like temperature, pH, light fluxes, nutrients and cycle length must be optimized to achieve significant yields, while not overlooking the important role the carbon source plays in the production process (Sedlacek et al., 2019). Most of the carbon sources utilized in the production of conventional PHB are raw material-based, comprising solely of carbohydrates, such as sucrose, maltose, glucose, starch and fatty acids along with their derivatives, methanol and alkanes (Kamravamanesh et al., 2017).

In order to overcome the challenge of finding suitable non-competitive and cost-effective carbon sources for PHB production, recent research efforts have concentrated on coupling WWT with PHB production. Wastewater from various sources is among the options that have been investigated (Muhammadi et al., 2015; Raza et al., 2018; Roland-holst and Heft-neal, 2013). The challenge, however, is that, although sufficient amounts of PHB production from industrial WW (both synthetic and natural) have been reported in some studies, others have also noted that enrichment of the WW medium was necessary to achieve similar results (Yuan et al., 2015). Nevertheless, coupling microbial WWT with resource recovery is a viable solution to reducing the WW footprint, as well as reducing the cost of production of beneficial microbial bio-products such as bio-plastics.

1.1. Overview of biopolymer production routes

PHA synthesis occurs via different metabolic routes. Heterotrophic bacteria for instance, are able to control metabolite flux for PHA accumulation by using extracellular substrates. Contrarily, photoautotrophs such as cyanobacteria have to begin with CO_2 to synthesize their metabolites (Asada et al., 1999; Carpine et al., 2015). These organisms draw energy from sunlight to produce ATP without the need for oxygen. Such anaerobic conditions contribute to cost reduction in culture systems due to the elimination of aeration (Fradinho et al., 2019). Under nitrogen-starved conditions, amino acid synthesis is reduced, thus leading to an increase in acetyl-CoA flux. Another enzyme, phosphoacetyltransferase activity increases and finally PHB synthesis takes place due to the activation of PHB synthase.

The photoautotrophic approach of producing PHB occurs in microbes such as *Chlorella* sp., *Calothrix* sp., purple- and green-non sulphur bacteria that use light as an energy source for photosynthesis. Chemoautotrophs on the other hand utilize CO_2 and energy from chemical reactions. Methanotrophs are chemoautotrophic microbes widely used in WWT and PHB production (AlSayed et al., 2018). Contrarily, the heterotrophic route of PHB production involves microbes such as *Synechocystis* sp., *Spirulina* sp. and halophilic archaea that utilize waste organic matter for PHB synthesis (Salgaonkar and Bragança, 2017). Chemoheterotrophs use chemical energy and organic matter to synthesize PHB but it should be noted, that the chemoheterotrophic method of PHB production in bacterial system is expensive due to costly carbon sources such as acetate (a PHB precursor) needed for growth (Narancic et al., 2016). In contrast, the production of PHB through the photoautotrophic cyanobacteria or microalgae systems is a feasible alternative for low-cost PHB production due to the inexpensive feedstock from light and CO_2 (AlSayed et al., 2018; Carpine et al., 2020; Löwe et al., 2017). There are instances where PHB is produced both autotrophically and heterotrophically. For instance, X. Xu et al. (2020) and M. Xu et al. (2020) demonstrated the ability of *Cupriavidus necator* to produce PHB via heterotrophic and autotrophic approaches. Heterotrophic growth in *Cupriavidus necator* takes place through the utilization of organic substrates (Garcia-Gonzalez et al., 2015). Oxygen and NH_4^+ are consumed during growth while producing CO_2 as a side-product. At this stage, PHB production is suppressed by excess NH_4^+ supply but under nutrient limitation, the organic carbon source is used for PHB production (Mozumder et al., 2014). Ranaivoarisoa et al. (2019) also evaluated the PHB performance of *Rhodopseudomonas palustris* TIE-1 under photoautotrophic, chemoheterotrophic and photoheterotrophic conditions. The high PHB yield under chemoheterotrophic conditions (aerobic) was attributed to the supply of amino acids from peptone supplementation. Overall, photoelectroautotrophy and photoferroautotrophy showed the highest PHB electron yield and the highest specific PHB productivity, respectively. These results demonstrate the ability of *R. palustris* to yield the highest specific PHB productivity using Fe(II) as an electron donor for photoautotrophy through new routes. These new routes serve as potential substitutes for PHB bioproduction (Ranaivoarisoa et al., 2019). Similarly, the growth performance of *Chlorella vulgaris* (a photoautotrophic microbe) measured under different CO_2 concentrations and light intensities using a novel microdroplet photobioreactor proved to be better than that of a flask culture because of the reduced shading effects and improved mass transfer (Sung et al., 2016). However, strains such as *Calothrix scytonemicola*, *Nostoc muscorum* and *Spirulina* sp. LEB 18 perform poorly in photobioreactors due to biofilm formation (Carpine et al., 2020). These scenarios prove that, aside the type of microbial strain, reactor type also influences PHB production to a large extent.

Using mixed microbial cultures (MMC) for WW treatment coupled with PHB production offers numerous advantages such as reduction in cost associated with production (Fradinho et al., 2019; Yuan et al., 2015), and utilization of complex substrates (Aslan et al., 2016; Chen and Jiang, 2017) among others. To achieve this, various genetic engineering and molecular biology techniques have been adopted. To enhance PHB production in microorganisms, techniques such as overexpressing genes in natural producers (Ben et al., 2016) or introduction or deletion of genes in non-PHB-producing microbes have been successfully accomplished (Wu et al., 2016). Advances in this area have allowed for the alteration of biosynthetic pathways involved in PHB production, which subsequently led to significantly higher PHB yields. Additionally, these alterations have provided opportunities for the utilization of a broader substrate range, otherwise unsuitable for microbial cultivation. Thus, allowing successful exploitation of resources initially considered as waste. However, due to the high cost associated with sterilization, it is economically unwise to cultivate genetically engineered organisms on wastewater. There have also been successful attempts at transferring the capability for PHB synthesis from bacteria to higher plants (Poirier et al., 1995; Suriyamongkol et al., 2007). Although this approach has been demonstrated to be successful in many laboratory scale projects, commercialization has yet to be cost-competitive.

The paper presents the biosynthesis and biodegradation pathway of the biopolymer, PHB which is produced by microbes cultivated on waste effluents, either in pure or mixed cultures. The conditions associated with wastewater cultivation such as nutrients, pH, light, cycle length and strain type are also discussed along with some genetic engineering approaches that have been successfully employed in the production of PHB. The paper also highlights the commercial aspect of PHB production and the challenges faced by the industry.

2. Research tendencies in Poly-β-hydroxybutyrate production

As far back as 1519, interests in the study of microalgae arose from the discovery of *Spirulina* sp. in Spain due to its nutritional properties (Soni et al., 2017). Since then, numerous research has gone into understanding the potential benefits of microalgae to man. This led to the discovery of the PHB homopolymer by Lemoigne in 1920's (Kosseva and Rusbandi, 2018). Many years down the line, in the 1950's Oswald et al. (1957) pioneered the research to propose the use of microalgae in wastewater bioremediation. This has set the pace for wastewater cultivation of microbes for resource recovery. In this paper, the authors have attempted to show the trends in PHB research over a 20-year period through author keywords such as PHB, microbial bioplastics and biosynthesis as topic searches for indexed articles published from 1999 to 2020. This summarized information provides an indication of developments in research interests from a temporal perspective. ELSEVIER ScienceDirect, SpringerLink, Taylor & Francis and WileyOnline Library databases were used and data obtained from each database using the keywords was analysed. The analysis revealed that out of the searched literature, PHAs were the most frequently mentioned keyword for the 20-year period (50%). These results indicate the level of interest of research activities within the scientific community. Interestingly, degradation declined from 5% in 1999–2003 period to 1% in the 2014–2020 period which could indicate a shift from a focus on single use plastic degradation to a more sustainable point of view of durability and re-usability for an eco-friendly bio-economy. Research in industrial applications of PHB increased from 2% in 1999–2003 period, doubled in 2004–2013 and continued to increase. Currently research interest in 2020 stands at 7% in comparison to other keywords used in this research (Fig. 1).

2.1. Current developments

Spirulina sp. and *Chlorella* sp. are common species that are commercially cultivated worldwide. They are mostly used as protein supplements for human food, aquaculture feed and pigments in the cosmetics industry. *Dunaliella salina* and *Haematococcus pluvialis* are also very popular due to their pigments and antioxidants like carotenoids, astaxanthins and beta carotene (de Jesus et al., 2018). Likewise, microbial biopolymers are of much interest due to their environmental and economic potential. The global PHA market is said to reach US $ 93.5 million in 2021, from an evaluated US $ 73.6 million in 2016 (Singh et al., 2019). This market size has the potential to grow by US $ 18.66 million during 2020–2024 period (Technavio, 2020). Currently, several biopolymer companies exist across Asia, Europe and the Americas, producing PHA and its variants. Most of these companies sell PHA as raw materials in resin or powder form under brand names such as Sogreen™ (by Tianjin GreenBio, China), Mirel® (by Metabolix, USA), Nodax™ (by MHG Bio, USA), Biocycle (by Biocycles, Brazil), MINERV-PHA™ (by Bio-On, Italy) and VersaMer™ (by Polyferm, Canada) among others. Metabolix, which is now Yield 10 Bioscience has demonstrated the feasibility of using PHA polymers to enhance the performance of a widely used polymer such as polylactic acid (PLA). Amorphous PHA copolymers have been used as plasticizers to strengthen PLA which is often brittle, thus, bringing both ductility and toughness. Another advantage of this blend is that, it does not compromise the compostability of the polymer (Bioplastic News, n.d.). Another exemplary company is Mango Materials, based in San Francisco, USA.

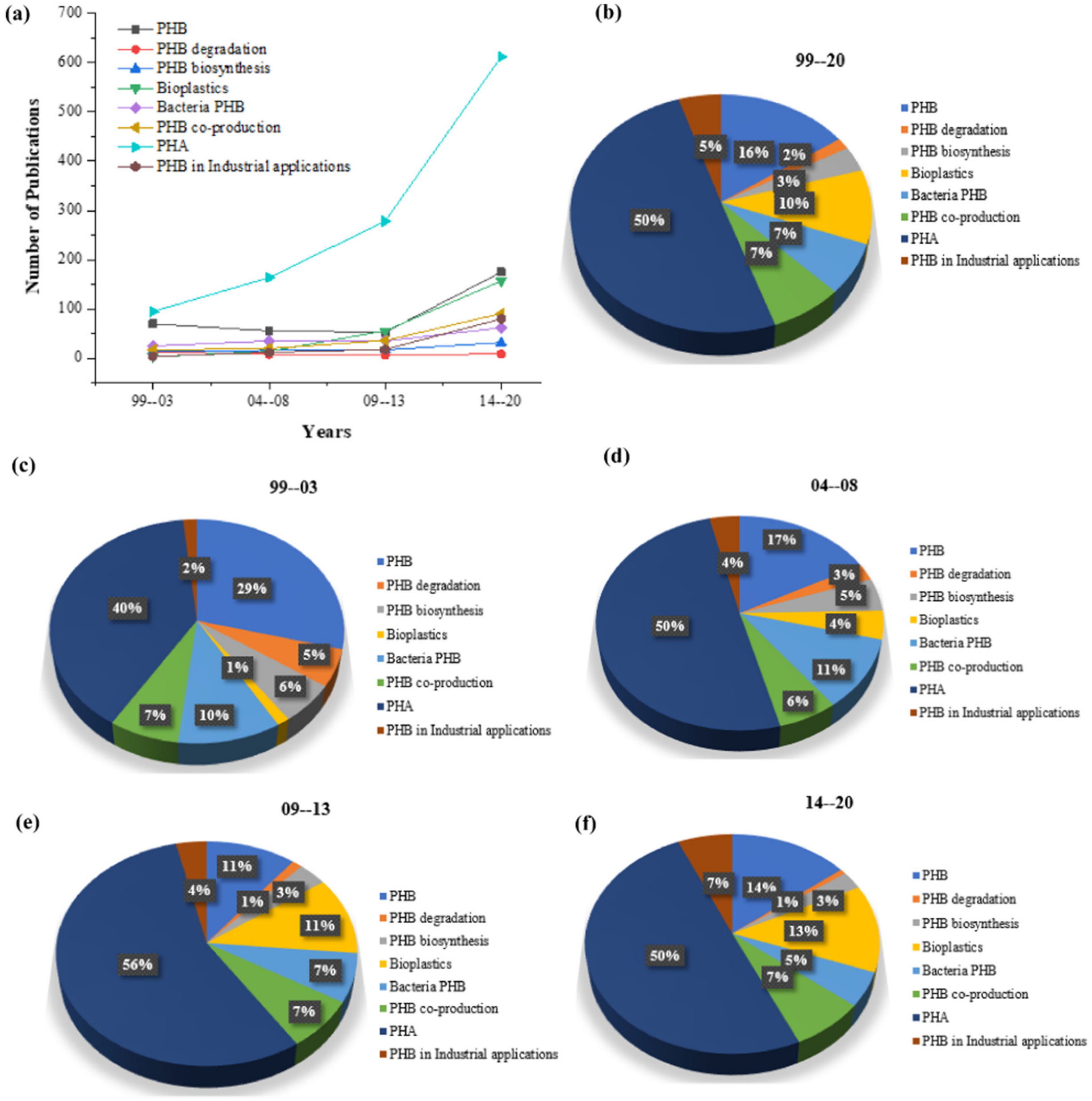


Fig. 1. Trends in research related to microbial bioplastics from polyhydroxyalkanoates (PHA) and Poly-β-hydroxybutyrate (PHB), their biosynthesis, degradation, co-production and industrial applications with reference to the (a) increments of published literature, and percentage research interest from (b) 1999 to 2020, (c) 1999 to 2003, (d) 2004 to 2008, (e) 2009 to 2013, and (f) 2014 to 2020.

The company has been in existence since 2012, producing PHA pellets under the brand name YOPP and YOPP+. The company situates its factories next to existing methane production facilities such as landfills, WWTPs and agricultural facilities in order to convert the methane into PHA, specifically, P3HB. This conversion is done by the natural PHA accumulation ability of methanotrophs that are naturally selected in the cultivation system and not genetically engineered (Mango Materials, n.d.). The consortia of methanotrophs are ancient, robust bacteria that withstand invasion by other microorganisms and hence, do not require expensive sterilization processes. The use of methane as feedstock also adds to cost reduction as well as scalability of the entire system. Currently, the company works with biogas from the Silicon Valley Clean Water WWTP in the San Francisco Bay Area to validate and scale their PHA production process. Across board, it has been observed that the main challenges faced by most companies are improving strain characteristics through genetic techniques, developing efficient methods of cultivation in terms open ponds or closed reactors, contamination control and optimizing harvesting processes. Biomass harvesting alone, contributes between 20 and 30% of the overall biomass production price (Fasaei et al., 2018). Energy-intensive processes such as drying also contribute significantly (20%) towards the overall cost of production. It is important to also bear in mind the type and volume of solvents used in the extraction processes. Not only are the solvents usually toxic, they are also volatile, making it difficult to recover and reuse. Novel extraction techniques such as enzymatic or bioextraction systems are promising approaches for environmentally friendly cell disruption (Costa et al., 2018).

3. Selection of Poly-β-hydroxybutyrate-producing microbes suitable for wastewater cultivation

The high diversity among PHB-producing microbes is attributed to their existence in various ecological niches due to their ability to withstand various environmental conditions. Both prokaryotic and eukaryotic PHB-producing microbes dwell in high organic matter habitats such as dairy waste conditions (Obruca et al., 2011; Rodriguez-Perez et al., 2018), oil processing wastes (Carofiglio et al., 2017), waste from pulp and paper mill processes (Bengtsson et al., 2008; Bhuwal et al., 2013; Jiang et al., 2012), agricultural wastes and wastewater treatment plant (WWTP) activated sludge (Khardenavis et al., 2007; Sangyoka et al., 2012). Among PHB-producing microbes, halophiles have been favoured in PHB studies since they eliminate the need to maintain aseptic conditions (Tohme et al., 2018), as well as their robustness in harsher environments. PHBs have also been found to aid in cell integrity by providing protection against sudden osmotic imbalances (Sedlacek et al., 2019). Thus, microbes that are naturally exposed to harsh environmental conditions could store more PHB. Identifying PHB-producing microbes in nature, involves collection and rapid screening using phenotypic- and genotypic-based screening (Muhammadi et al., 2015). Viable colony staining techniques have been proposed as a method of phenotypic-screening of PHB-accumulating bacteria. Gram-staining methods were used to characterise PHB-producing microbes from soil pelagia and paper mill effluents (Bhuwal et al., 2013; Biradar et al., 2015) which allows rapid detection of PHB-accumulating colonies through PCR techniques (Gasser et al., 2009; Wang et al., 2014). Genotypic-screening methods were subsequently developed as more appropriate tools to circumvent the numerous drawbacks of phenotypic-screening methods (Biradar et al., 2015). Two of the most widely exploited microbes in PHB research are the photosynthetic cyanobacteria, *Synechococcus* and *Synechocystis* spp. (Arias et al., 2018b; Kamravamanesh et al., 2018; Kavitha et al., 2016b) due to their PHB accumulating efficiency while growing on WW (Burnap, 2015; Hollinshead et al., 2014), and the ease of genetic manipulation due to the availability of their full genome sequence (Kanesaki et al., 2012).

Apart from distinguishing microbes based on their natural ecological niches and cell wall structure, they can be grouped further into two major categories based on the required culture conditions for polymer synthesis (Khanna and Srivastava, 2005). The first group requires that an essential nutrient like magnesium, nitrogen, phosphorus or sulphur becomes limiting for PHA synthesis from an excess carbon source. This includes bacteria species such as: *Ralstonia eutropha* (now *Cupriaviduc necator*), *Protomonas extorquens* and *Pseudomonas oleovorans*. The second group accumulates the polymer during growth phase, thus, devoid of nutrient limitation. *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii* and the recombinant *Escherichia coli* (Khanna and Srivastava, 2005) fall within this category. Six nutrient limiting approaches based on nitrogen (N) and phosphorus (P) in *Halomonas smyrnensis* were tested. The highest levan and PHB yields were achieved under unlimiting conditions (Tohme et al., 2018), indicating that, *H. smyrnensis* belongs to the group of PHB-producers that do not require limitation of a nutrient for PHB accumulation.

It is also important to consider the nutritional requirements of the microbe in terms of metabolism. That is, whether the microbe is a photoautotroph, chemoautotroph, photoheterotroph or chemoheterotroph. Thus, in order to select the most appropriate microbe for optimal PHB production, a roadmap is proposed in Fig. 2 based on; (1) cell wall structure (2) nutritional requirements (3) ecological niche and (4) number of carbon atoms. The cell wall structure of microbes confers unique properties on the microbe. Most PHB-producing bacteria in literature have been found to be gram-negative, compared to the limited number of Gram-positive bacteria (Muhammadi et al., 2015). A major advantage of using gram-positive species for PHA production is the absence of the immunogenic lipopolysaccharides (Philip et al., 2009). Lipopolysaccharides make up majority of the impurities in purified PHAs from gram-negative bacteria and have been known to induce strong immunogenic reactions. Therefore, their absence in gram-positive PHA is a major advantage in medical applications. The number of carbon atoms in the monomeric units of PHA can also be used as a criterion to group microbes based on carbon chain length. PHA production is strain specific and sometimes dependent on carbon substrate the microbe is exposed to. The first group produces short-chain-length PHAs (SCL-PHAs – C3-C5), while a second group, can accumulate medium-chain-length PHAs (MCL-PHAs – C6-C14). Although the majority of bacteria accumulate either SCL- or MCL-PHAs, a third group of bacteria have been found to synthesize PHA *co*-polymers containing both SCL- and MCL-PHA (C3-C14) (Goh and Tan, 2012). The fourth group is capable of synthesizing long-chain length PHAs (LCL-PHAs >C14) (Timm et al., 2004) and these typically include the pseudomonads. Knowing the specific carbon-chain length produced by the microbe will also inform the choice of solvent used in the extraction process, as solvent properties such as concentration, action time and working temperature are known to affect the molecular weight of the polymer (Chen, 2009). Taking these factors into consideration will facilitate optimized cultivation, harvesting and extraction conditions for efficient decision making in the event of scale-up.

4. Utilization of wastewater for Poly-β-hydroxybutyrate production

Microbial WW cultivation has been performed since the 1950s (Hoffmann, 1998). This technology allows the integration of WWT with microalgal biomass generation for resource recovery. Wastewater has been used as a source of substrate for the production of biofuels, lipids and biopolymers for decades (Monshupanee and Incharoensakdi, 2014; Patel et al., 2018; Rahman et al., 2015; Takeshita et al., 2014). Biopolymers such as PHBs have been successfully produced with various waste streams on a laboratory scale (Kamravamanesh et al., 2017; Meixner et al., 2016). Although the price of producing biopolymers largely depends on the substrate cost (Roland-holst and Heft-neal, 2013), several reports have also estimated that the total cost of producing these polymers also depends on microbial yield and productivity, culture conditions such as temperature, aeration, pH-value etc., and the recovery and purification processes. The carbon source alone is said to account for 25–45% of the total production costs (Nath et al., 2008).

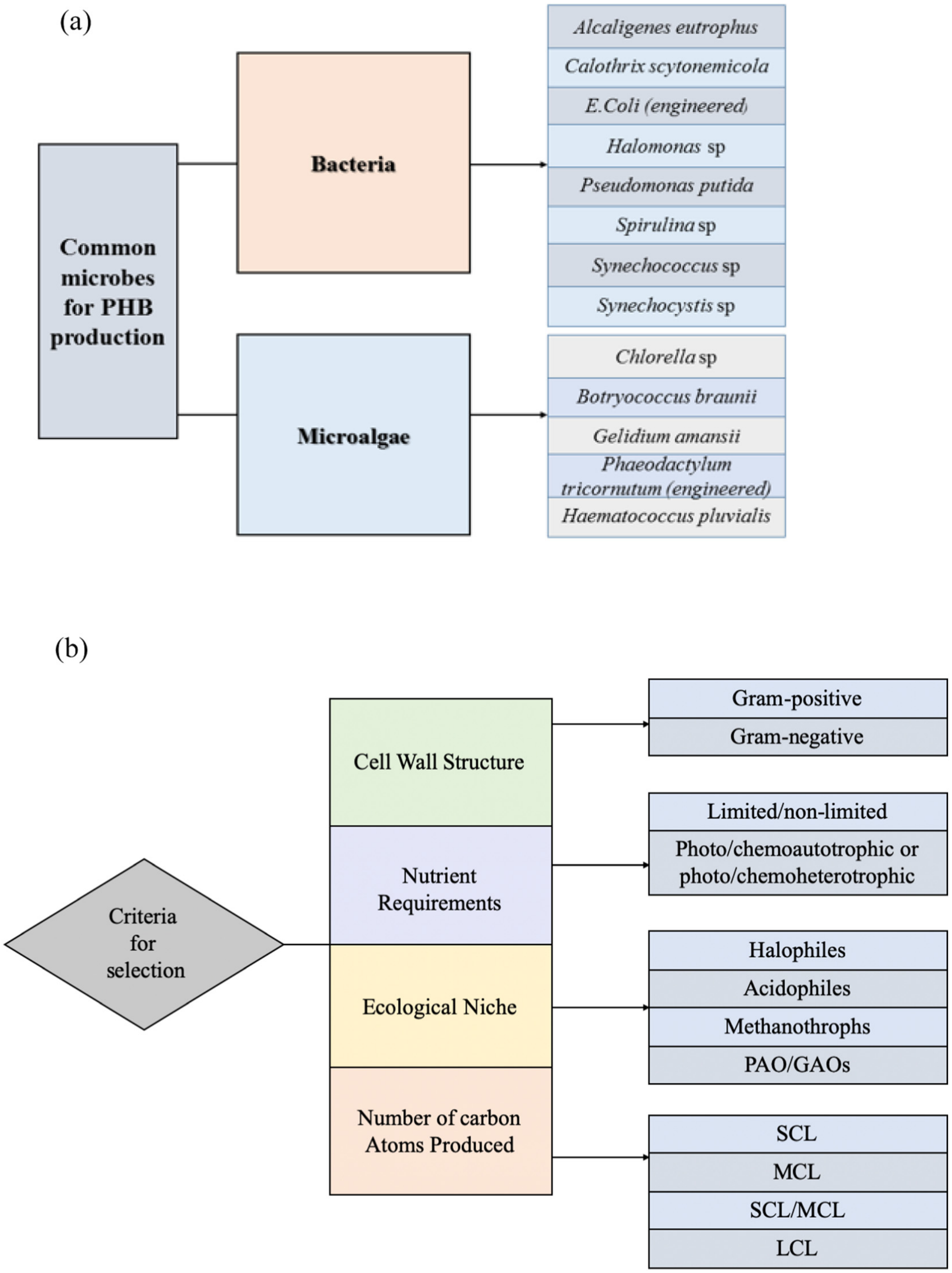


Fig. 2. (a) Common microalgal and bacterial strains (wild and engineered types) utilized in research and industrial applications for enhanced Poly-β-hydroxybutyrate production (b) criteria for selecting Poly-β-hydroxybutyrate-storing microbes based on ecological, morphological, physiological and metabolic factors.

Thus commercially produced polymers such as PHAs have utilized relatively inexpensive substrates such as methanol, cheese whey, molasses, olive oil mill WW, poultry waste etc. (Pisco et al., 2009) which can be broadly classified under industrial and municipal WW sources. Essentially, there are two principal routes to produce PHB from WW. These are: (1) cultivation of pure culture of a microbe using the WW as carbon source; and (2) utilization of open mixed microbial culture (MMC) that is enriched in PHB-producing microbes by the selective pressure imposed on the culture. The latter approach allows the integration of PHB production with WW treatment by adjusting already widely used principles of biological WW treatment such as activated sludge processes. Environmentally friendly methods of waste management such as anaerobic digestion produces effluents like digestate supernatant which provide carbon, nitrogen and phosphorus sources for microalgal cultivation (Hollinshead et al., 2014; Kovalcik et al., 2017; Meixner et al., 2016). The effluent quality improvement parameters highlighted in Table 1 are with emphasis on nitrogen, phosphorus and COD reduction.

4.1. Poly-β-hydroxybutyrate production from industrial wastewater

Food processing WW is often rich in fermentable nutrients like lactose, lipids and soluble proteins, and can therefore serve as an

Table 1
Microbial cultivation with wastewater as substrate for biopolymer production.

Strain	Source	Substrate	Culture conditions	Organic Nutrient Removal	PHB content	General comments & References
Chlorella pyrenoidosa[a]	National Collection of Industrial Microorganisms (NCIM), India	Cheese whey WW[b]	Stationary phase	94.2% (N), 92.54% (P)	6.54g/L (79.8%)	Reduced COD[c] & BOD[d] while fixing CO$_2$ (Sathya et al., 2018)
Pseudomonas aeruginosa	National Chemical Laboratory, Pune, India	Sugar refinery waste (cane molasses)	N/A	N/A	62.44% CDW[e] PHB	Economically improved productivity of 0.11 g/L/h (Tripathi et al., 2012)
Cupriavidus necator	Soil	Cane final molasses	Exponential-Stationary Phase	N/A	2.86 ± 0.82 g 27%	Hydrothermal acid pre-treated molasses as carbon source led to highest growth (Sen et al., 2019)
Synechocystis cf. *salina* Wislouch (No. 192)	Culture Collection of Autotrophic Organisms (CCALA)	Digestate supernatant	Exponential Phase	N/A	78% PHB	Nutrient concentrations (TN[f], P[g]) after harvesting, were below detection limits (Meixner et al., 2016)
Bacillus subtilis nG220	Soil samples from Haryana and Uttar Pradesh (India)	Sugar industry WW	Stationary Phase	N/A	51.80%	PHB yield up to 4.991 g/L with sugar industry WW as sole nutrient source (Singh et al., 2013)
MMC[h] (Activated sludge)	Tuam WWTP[i] Galway, Ireland	Synthetic WW (acetate, yeast extract)	N/A	Effluent PO4–P concentrations below 1 mg/L	28.8–50%	Maximum PHB - 28% (anaerobic) & 50% (aerobic conditions) (Rodgers and Wu, 2010)
Activated sludge	Combined dairy and food processing industry WWTP	Rice & Jowar grain-based distillery spent wash	N/A	N/A	40% - 42.3%	Addition of DAHP increased PHB (67%) (Khardenavis et al., 2007)
Azohydromonas lata DSMZ 1123	N/A	Dairy industrial WW (Cheese Whey)	Mid-exponential phase	N/A	P(3HB) 1.21 g/L; P(3HV) 0.45 g/L	Pre-treated whey, suitable substrate for PHBs & PHVs[j] production (Sharifzadeh et al., 2010)
Sludge	Sludge from the UC Davis WWTP oxidation ditch	Cheese WW	N/A	83% COD removal efficiency	3% by MLVSS[k]	More glycogen & PHB produced by SF[l] type than RF[m] type (Goffredo et al., 2009)
E. coli	City of Logan, WWT facility	Hydrolyzed microalgae Supernatant with standard *E. coli*M9 growth media	Stationary phase	N/A	31% PHB	Maximum PHB accumulation up to 31 ± 8.9% (Rahman et al., 2015)
Activated Sludge	Winnipeg South End Water Pollution Control Centre	1.MWW[n] 2.Beef extract 3. Acetate 4. Glucose	N/A	Phosphorus uptake (mg/L) MWW (33.2) Beef Extract (BE) (23.2) Acetate (A) (83.0) Glucose (G) (54.4)	PHB from MMW (15%) BE (13%) A (42%) G (40%)	MWW used with carbon-rich industrial waste for PHB obtainment (Yuan et al., 2015)
Activated sludge	N/A	Food processing industrial WW (acetic)	N/A	N/A	33%	C/N[o] ratio of 144 led to maximum PHB (Kumar et al., 2004)
Activated sludge	Kayseri domestic wastewater treatment plant (Turkey)	Simulated WW(Acetate)	N/A	N/A	55%	PHB storage increased as the cycle length decreased (Ozdemir et al., 2014)
Bacillus megaterium CCM2037	Czech Collection of Microorganisms (Brno, Czech Republic)	Cheese whey	Stationary phase	N/A	51.57%	PHB yields improved by 40% after 1% introduction of ethanol (Obruca et al., 2011)
Pseudodonghicola xiamenensis	Red sea, Saudi Arabia	Date syrup	Stationary phase	N/A	38.85%	4% NaCl, and peptone was the preferred nitrogen source (Mostafa et al., 2020)
Purple non-sulfur bacteria (mixed consortium)	N/A	Winery WW	N/A	COD & N reduction	203 mg/L	Co-production of H$_2$ (468 mL/L) and PHB (203 mg/L) (Policastro et al., 2020)
Aulosira fertilissima	N/A	Aquaculture WW	Log phase	Ammonia, nitrite, and phosphate reduction	92 g/m^2 (summer), 89 g/m^2 (rainy), 80 g/m^2 (winter)	Recirculatory WWT and PHB production (Samantaray et al., 2011)

[a] Algae,
[b] WW – Wastewater,
[c] COD – Chemical oxygen demand,
[d] BOD – Biological oxygen demand,
[e] CDW – Cell dry weight,
[f] TN – Total nitrogen,
[g] P – Phosphorus,
[h] MMC – Mixed microbial culture,
[i] WWTP– Wastewater treatment plant,
[j] PHV – Polyhydroxyvalerate,
[k] MLVSS – Mixed liquor volatile suspended solids,
[l] SF - static fill,
[m] RF – react fill,
[n] MWW – Municipal wastewater,
[o] C/N – Carbon-to-nitrogen ratio.

inexpensive substrate for microalgal cultivation (Raza et al., 2018). Mixed cultures allow the exploitation of complex substrates. In the WWT industry, microorganisms employed for phosphorus removal, also known as phosphorus-accumulating organisms (PAOs), have the capability to synthesize PHB as their source of energy (Yuan et al., 2015). The reuse of organic matter in producing PHB from industrial WW highly rich in carbon such as agricultural waste, brewery waste and municipal WW, may considerably lower the cost (Anterrieu et al., 2014). Furthermore, biopolymer production has been integrated into a sugar factory WW treatment by mimicking factory processes in two parallel sequencing batch reactors (SBRs). Both SBRs produced biomass along with PHA production while maintaining WWT standards with respect to carbon, nitrogen and phosphorus for the factory effluents. Cassava starch manufacturing WW (CSW) has been successfully used by a chemoautotrophic bacteria species, *Cupriavidus* sp. KKU38 as a substrate suitable for PHB production. Acidogenic fermentation of CSW to obtain volatile fatty acids (VFAs) was first conducted by Sangyoka et al. (2012) and was found to be more efficient in producing PHB than raw CSW. Variations in chemical oxygen demand:nitrogen:phosphorus ratio (COD:N:P ratio) were also investigated, and an optimum ratio of 100:0.5:11 resulted in maximum PHB (85.53%). Clearly, apart from the suitability of WW for PHB production, culture conditions have to be well optimized in order to achieve maximum results.

4.2. Poly-β-hydroxybutyrate production from municipal wastewater

To a large extent, the concentration of WW constituents influences PHB production (Kavitha et al., 2016a). For instance, in an instance where glucose was the only substrate used, majority of it was converted by the succinate-propionate pathway to propionyl-CoA, resulting in poly-3-hydroxyvalerate (PHV) (Ahn et al., 2009) with relatively low PHB production. It was thus suggested that a mixture of municipal WW and certain carbon-rich industrial wastes could be suitable substrates for PHB production. The feasibility of mixing municipal wastewater (MW) and magnesium (Mg^{2+})-enriched nickel laterite ore wastewater (NLOWW) on the growth, cellular composition, photosynthetic activities, nutrient and Mg^{2+} removal ability of *Chlorella sorokiniana* was demonstrated by Chen et al. (2020). This approach was demonstrated as economically feasible with revenue of $75.6 per kilogram biomass which could be applied in PHB research. Low PHB production has been observed with beef extract 13% cell dry weight (CDW) suggesting that PAO in this system could not effectively utilize beef extract and that substrates high in amino acid were therefore not ideal for PHB production (Yuan et al., 2015). Again, a method for the treatment of municipal WW was developed by Basset et al. (2016), incorporating the selection of biopolymer-storing microorganisms under aerobic conditions with nitritation/denitritation. The selection of these microorganisms was successful and internally stored PHA facilitated denitritation in the famine phase.

The challenge with using mixed cultures for PHB production is the variation in carbon source preference, which sometimes leads to overall low PHB productivity due to the presence of certain species. Under carefully selected limiting conditions, dominance of a specific strain(s) subsequently results in higher PHB yields. Thus, majority of research on PHB production in mixed cultures usually enrich the cultures to eliminate the non-PHB storing population, leaving a mixture of a few high yielding species which sometimes leads to an almost-pure culture. For instance, Kourmentza et al. (2009) reported that after several cycles of alternating between carbon and nitrogen limitation, *Pseudomonas* sp. dominated the culture. However, the enriched mixed culture was found to be more promising for PHAs production from short-chain fatty acids compared to the individual productivity of two isolated strains. The enriched culture also led to higher yields of PHAs per VFAs consumed. Another challenge with utilizing municipal wastewater (MWW) for PHA production is the relatively low VFA content. Despite this challenge of VFA-poor MWW streams, a feast–famine approach has proven to be feasible at laboratory and pilot-scale studies (Morgan-Sagastume et al., 2014). The advantage of using mixed cultures in wastewater PHB production is the ability to maintain the cultures under non-sterile conditions. Pure cultures require stringent conditions in order to maintain only the desired population. Nonetheless, techno-environmental assessment of MMC PHA production from Municipal WWTP has a potential of delivering more valuable renewable raw materials than the known biogas and bioenergy of current technologies (Morgan-Sagastume et al., 2016). Table 1 highlights some key research works done on microbial PHBs achieved through WW cultivation. These key works demonstrate the importance of simultaneous water quality improvements and valuable biopolymer production.

4.3. Co-production of Poly-β-hydroxybutyrate and other metabolites in wastewater

Aside the sole production of PHB with WW as nutrient source, other valuable by-products can be obtained through a cell factory concept of co-production. Co-production of PHB and other metabolites is a common phenomenon in a typical microbial system. It is feasible to use the PHB pathway to manipulate other metabolic pathways (Kang et al., 2010; Xu et al., 2016). However, the yield of one product over another is dependent on the microbial strain, the culture conditions as well as to a large extent, the research goals. Integrated approaches aimed at producing high yields of multiple bio-products can be successfully achieved, as long as the biosynthesis pathways of the individual products do not compete for substrates (Kumar and Kim, 2018). Depending on which bio-product is desired, growth conditions can be manipulated to stimulate the accumulation of a specific bio-product over another (Quagliano and Miyazaki, 1999). To make bio-processing easier, one of the desired products must be “membrane-bound, secretory or extracellular” (Kumar and Kim, 2018) in order to allow for the maximum utilization of resources, as well as easy downstream processing especially with regard to the simultaneous extraction of various metabolites.

Carotenoids such as astaxanthins and β-carotene are undoubtedly important metabolites of high market value that can be co-produced along with PHBs. Several industrial applications exist for the use of these pigments in nutritional supplements, alternative medicine, food etc. thus, showing a great potential in lowering manufacturing costs due to this zero waste approach. The bacterium *Rhodobacter sphaeroides* is capable of utilizing waste effluents in PHB and hydrogen production while concomitantly reducing COD levels (Eroğlu et al., 2004; Ghimire et al., 2016). Similarly, high hydrogen production was achieved through co-production with PHB by *R. palustris* CGA676 utilizing agroindustrial waste. The highest hydrogen production was observed in wheat bran effluents (648.6 mL/L), while the highest PHB yield was obtained with olive pomace (11.53% TS) (Corneli et al., 2016). The ability to obtain various products from a batch culture emphasizes the feasibility of cost reduction through coproduction of metabolites.

4.4. Biosynthesis and metabolic engineering of microbial Poly-β-hydroxybutyrate

Biosynthesis of PHB occurs via a variety of well-established routes. The most common PHB production route takes place: (i) within microbial cell systems via a PHB-polymerase catalysed reaction through genetically engineered recombinant microbes, (ii) during the anaerobic digestion of biological wastes or (iii) through the utilization of transgenic plants (Hahn et al., 1999; Zinn et al., 2001). Of these, microbial production is considered the major source of PHB. Due to the large chemical diversity of the biopolymer, biosynthesis in microorganisms varies widely (Suriyamongkol et al., 2007). Among the numerous classes of microorganisms, bacteria are the most studied group of PHB-producing microbes. Although other classes of microorganisms such as green algae (Arias et al., 2019; Sathya et al., 2018) and diatoms

(Hempel et al., 2011) are known, bacteria, either wild type or genetically engineered types, have been reported to produce higher polymer contents. Thus, majority of literature on the biosynthesis pathways and genetic manipulations have focused on bacterial strains.

Under certain nutritional and environmental conditions, microbes accumulate PHB through the hydrolytic activity of PHA depolymerase (PhaZ) (Uchino et al., 2008). Although factors such as oxidative stress (Koskimäki et al., 2016), protein synthesis rate and cellular energy demand (Handrick et al., 2000) have been identified as possible stimulants of PHB accumulation in microbes, till date, the mechanisms controlling the accumulation process are yet to be fully understood (Teixeira et al., 2019). The balance between these stimulants and the accumulation process, is a crucial metabolic cycle (PHB cycle) (Trainer and Charles, 2006) which is very necessary for microbial survival in the environment (Sedlacek et al., 2019). Several studies have correlated PHB accumulation with microbial survival under UV radiation, high temperature and osmotic shock (Koskimäki et al., 2016; Ruiz et al., 2001; Sedlacek et al., 2019). The intracellularly accumulated PHB is coated by an abundance of structural proteins called Phasins that regulate the number and size of the PHB granules within microbial cells (Jendrossek, 2009; Mezzina and Pettinari, 2016).

The aim of many genetic manipulation approaches has been to increase polymer content, to reduce cost or improve polymer quality. These approaches have been improved upon over many years to meet biotechnological needs.

4.4.1. Gene manipulation – nutrition

To overcome certain production hurdles for potential industrialization, several studies have successfully engineered certain microbial strains to produce significant amounts of PHBs by broadening substrate ranges (Wang et al., 2018), or engineering bacteria morphology for easy downstream separation (Chen and Jiang, 2017; Liu et al., 2011). PHB is synthesized through a three-step pathway in bacteria (Steinbüchel and Füchtenbusch, 1998) involving three key enzymes, namely β-ketothiolase, NADP-specific acetoacetyl-CoA reductase, and PHB synthase which are coded by *phb*A, *phb*B, and *phb*C, respectively.

By changing the fatty acid concentrations, co-monomer compositions were easily regulated in *Pseudomonas putida* KTQQ20 which subsequently produced "a novel diblock copolymer P3HHx-b-P(3HD-co-3HDD) made up of 49mol% P3HHx and 51mol% P (3HD-co-3HDD)" (Tripathi et al., 2013). Thus, a platform was established to produce a biopolymer with adjustable monomer compositions through this β-oxidation-weakened *P. putida*.

4.4.2. Gene manipulation – morphology

Engineering attempts have also been made towards modifying the morphology of cells in terms of size and form modification, to increase accumulation of inclusion bodies such as PHBs. The size and shape of *E. coli* JM109SG cells were successfully modified by making them larger and less fragile. Typical *E. coli* cells of about 0.5–2 μm increased their diameters to 10 μm and changed from typical rod shapes to spherical, leading to increased PHB production. However, these new cells grew poorly compared to their parent cells (Jiang and Chen, 2015). Jiang et al. (2015) observed that, cells reverted to rod shape upon *mreB* allele insertion, thus, recommended that shape change induction be done after the cells have already grown to high enough densities. Again, typical binary fission in *E. coli* was modified to multiple fission by removing fission-related genes *minC* and *minD* (Wu et al., 2016) (Fig. 3). Multiple fission rings known as Z-rings were formed at several locations of a lengthened cell, thus achieving more than the usual two daughter cells through cell division. This led to higher CDW and over 80% PHB accumulation compared to control cells with normal binary fission. Enlarged morphology is generally known to increase PHB synthesis and also promote separation of cells through gravity from the fermentation broth (Jiang and Chen, 2015). Enhanced downstream processing and subsequent cost reduction can be achieved through this technology.

4.4.3. Gene manipulation - non-native host

Apart from gene manipulation within PHB-producers, *phb* genes can be incorporated directly into non-native hosts. The successful incorporation of PHB genes from *Ralstonia eutropha* into *Chlamydomonas reinhardtii* demonstrated the assimilation of part of a native biopolymer synthesis pathway into a non-native host. PCR results confirmed the integration of both *phbB* and *phbC* genes into nuclear DNA of *C. reinhardtii*, thus the double transgenic microalgae harbouring *phbB* and *phbC* genes was obtained (Chaogang et al., 2010). Similarly, the full PHB pathway of a bacteria (*R. eutropha* H16) was successfully integrated into a diatom (*Phaeodactylum tricornutum*), achieving about 10.6% PHB (% CDW) (Hempel et al., 2011). These successful examples highlight the possibility of inserting biochemical pathways into non-native hosts, to broaden the avenues available for PHB production. With current engineering technologies such as clustered regularly interspaced short palindromic repeat (CRISPR), cell factories can be engineered using ideal microbial strains to efficiently synthesize PHBs. Microbe-derived bio-plastics can therefore become economically attractive through a bio-refinery model, where multiple bio-products will be produced from a single microbial source, particularly if WW is utilized as the carbon source, thereby valorizing the entire process. Fig. 4 illustrates the biosynthesis, application and degradation of PHB (Hankermeyer and Tjeerdema, 1999; Pakalapati et al., 2018; Steinbüchel and Füchtenbusch, 1998).

5. Factors influencing the accumulation and composition of Poly-β-hydroxybutyrate

Physiological processes within microbial cells are temperature, light and nutrient dependent. Optimal growth yield occurs within specific ranges which are successfully controlled in closed systems, but prove challenging in open pond systems (Ge et al., 2017). Nutrient availability, feeding mechanism, pH, cycle length, temperature and light are factors that influence the ability of PHB-producing microbes to accumulate substantial amounts of the biopolymer, as well as influence its composition. The inherent ability of the microbes to assimilate certain nutrients, synthesize specific metabolites, as well as strain specificity should also be considered in accounting for PHB yield. The molecular mass of PHB produced in the cells of the bacteria, *E. coli* for instance depends strongly on culture conditions (Suriyamongkol et al., 2007).

5.1. Strain

Apart from the fact that substrate cost is a major bottleneck in PHB production (Chen and Jiang, 2017; Lee et al., 1999; Roland-holst and Heft-neal, 2013), the mode of application and type of substrate, has a huge impact on the performance of the microbial community. For instance, Roja et al. (2019) observed faster growth rate in cyaonbacteria species (*Synechococcus*, *Leptolyngbya* and *Oscillatoria*) than in green microalgae (*Chlorella*) cultivated on ASN III medium. The maximum growth of the cyaonbacteria species occurred on day 18 compared to day 21 for green algae. The difference in the thermal stability of the extracted PHA also buttresses the point about strain specificity in situations where cultures conditions are kept the same. The use of MMCs in activated sludge systems for PHB production is an approach that reduces the production cost as well as having the advantage of higher biodegradability (87%). This is due to the consortium of organisms compared to a single organism system in pure cultures (Moita and Lemos, 2012; Shalin et al., 2014). In a mixed system, cultures are usually subjected to enrichment conditions in order to favour the microbes with a higher biopolymer production rate. For instance, the impact of a non-storing biomass on PHA production in a mixed culture was investigated and the results revealed that, although *Plasticicumulans acidivorans*, a known PHA producer has the potential of accumulating a good amount of PHA, the presence of the non-storing population (*Methylobacillus flagellates*) reduced the maximum PHA content of the culture to 66 wt% from more than 80 wt% in an SBR (Marang et al., 2014). Between 84

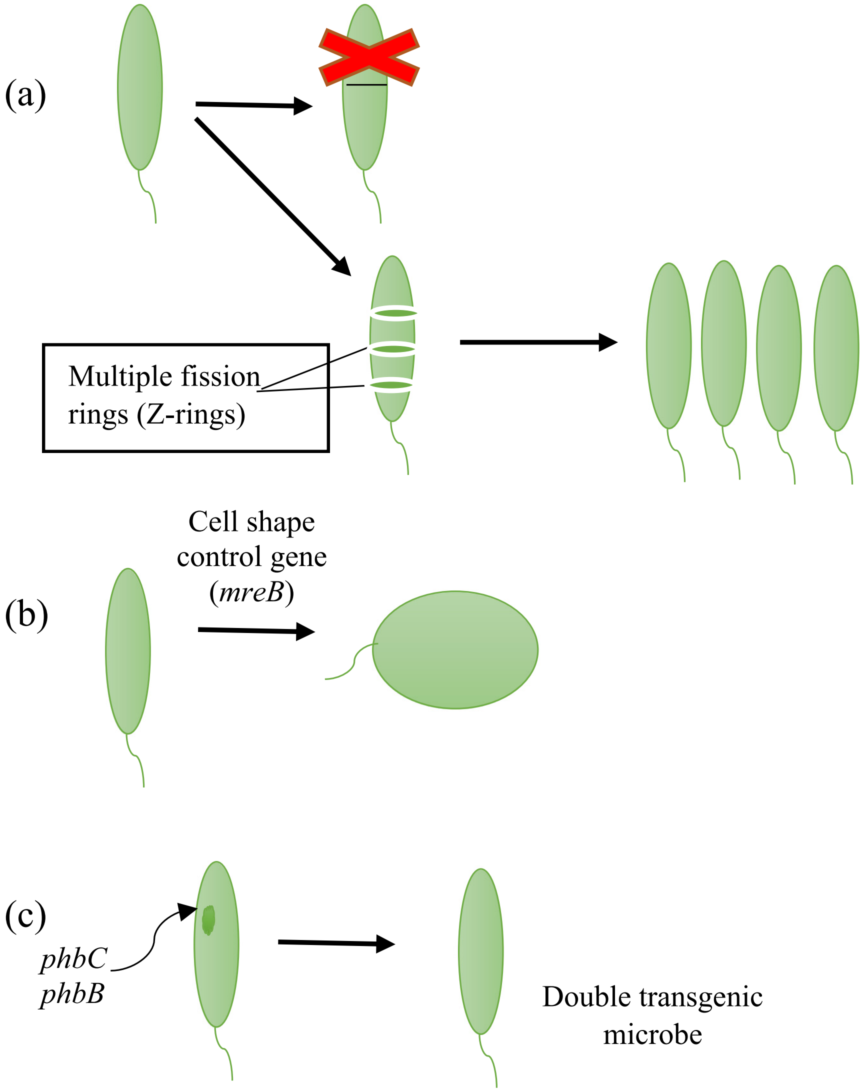


Fig. 3. Enhanced Poly-β-hydroxybutyrate production through genetic modifications for cost-effective bio-plastic production. (a) Modification of typical binary fission to multiple fission-Four daughter cells instead of two. (b) Changing from typical rod shapes to spherical-Enhanced cell size. (c) Introduction or deletion of genes in non-PHB-producing microbes.

and 90 wt% PHB content has also been achieved in an MMC system dominated by proteobacteria, *Plasticicumulans acidivorans* and *Thauera selenatis* (Jiang et al., 2011a). A feast/famine approach was used with acetate and lactate as substrates. This approach gives an indication of the selective pressure of MMCs on microbial stains in PHB production. In another study, the effect of the influent substrate concentration (30–60 Cmmol VFA/L) on the selection of a PHA-storing culture was assessed, using fermented sugar molasses. An influent substrate concentration of 45 Cmmol VFA/L proved to be the best PHA-storing capacity, yielding about 74.6% due to a highly enriched PHA-storing population of 88% (Albuquerque et al., 2010). The fact that neither substrate concentration nor feast to famine ratio was limiting factors in those conditions is noteworthy. Meaning that the PHA yield achieved was as a result of the dominating population of PHA-producers.

5.2. Nutrients

Nutrient limitation is widely known to affect PHB production (Kaewbai-ngam et al., 2016; Kamravamanesh et al., 2017). Particularly, nitrogen (N) and phosphorus (P) limitation, which are common natural stress conditions encountered by microbes have been well documented and known to influence the accumulation of PHBs (Dutt and Srivastava, 2018; Kamravamanesh et al., 2018; Monshupanee and Incharoensakdi, 2014; Takeshita et al., 2014). The combined effects of N and P deficiency have resulted in the highest PHB accumulation in unicellular cyanobacterium *Synechocystis* sp. PCC 6714 under photoautotrophic conditions (Kamravamanesh et al., 2017), and have also led to dominance of cyanobacteria over green algae *Scenedesmus* sp. in an SBR with mixed consortia of microalgae (Arias et al., 2019). Further confirming the influence of nutrients on strain type selectivity. Nutrient deprivation, especially nitrogen deprivation, leads to a significant metabolic reorganization. Evidently, bacterial PHB synthesis can be induced by limiting oxygen or an essential nutrient like nitrogen, phosphate, sulphate, magnesium, or potassium (Ansari and Fatma, 2016; Basset et al., 2016; S. S. Costa et al., 2018a; Nakaya et al., 2015; Nath et al., 2008). In their absence, microorganisms cannot produce amino acids or proteins, but instead synthesize and accumulate PHB as discrete granules in the presence of excess carbon (Muhammadi et al., 2015). They store this excess carbon until the limitation is removed, at which time they may degrade and metabolize the stored PHB (Hankermeyer and Tjeerdema, 1999). Metabolic reorganization that occurs within the cells includes; loss of chlorophyll (chlorosis) (Sauer et al., 2001), a decrease in protein levels (Depraetere et al., 2015) and increase in storage polymers like glycogen and PHB in a sequential manner (Damrow et al., 2016). Generally, nutrient limitation, is more favourable for PHB accumulation

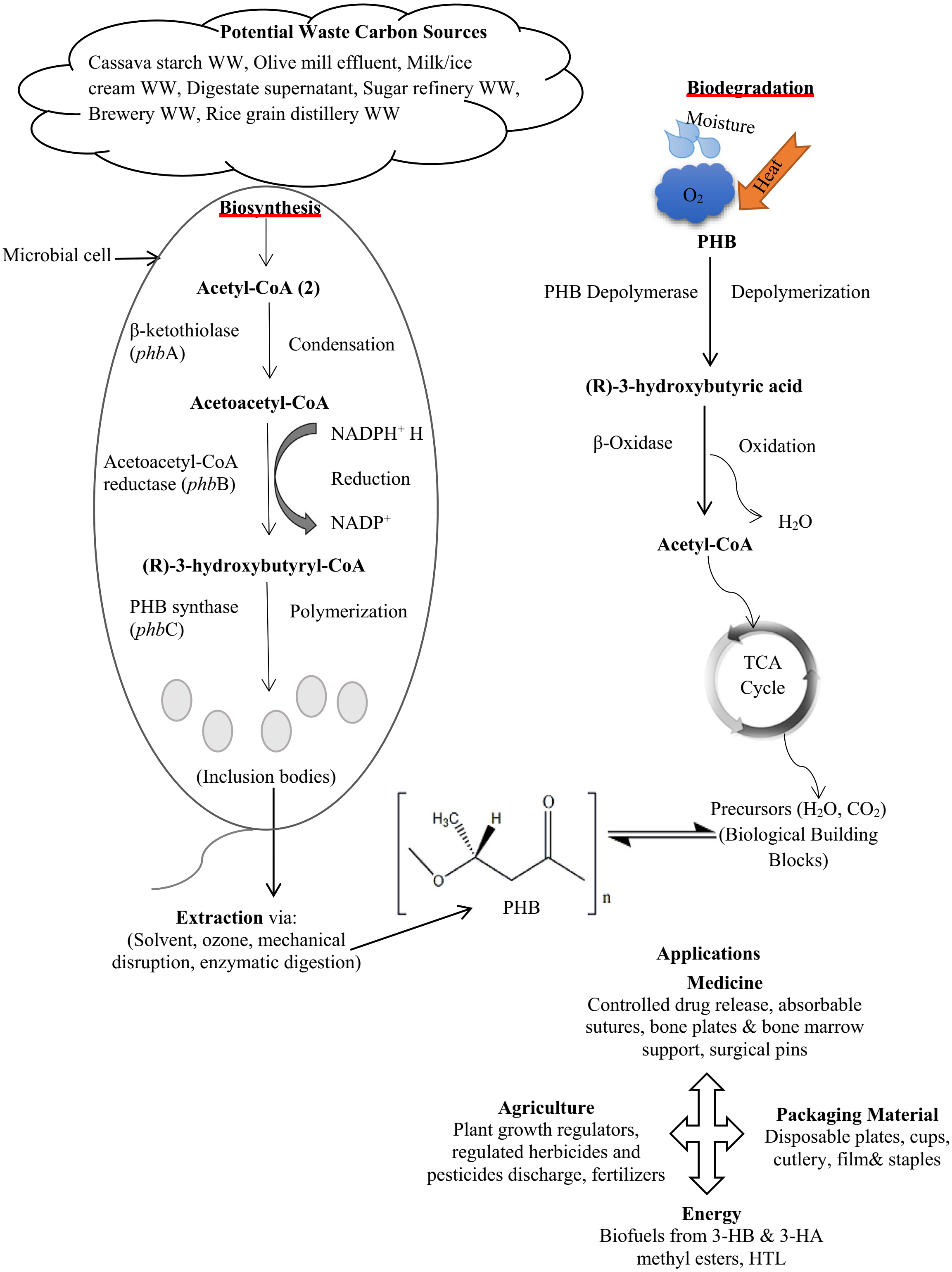


Fig. 4. Microbial Poly-β-hydroxybutyrate biosynthesis and degradation pathway with potential wastewater carbon sources and industrial applications.

than their complete absence (Arias et al., 2018a; Cavaillé et al., 2016), thus majority of studies in this area make reference to nutrient limitation rather than deprivation.

Numerous reports on impacts of various forms of nitrogen on microbial growth (Carpine et al., 2015; Costa et al., 2018a; Dionisi et al., 2005; Manna et al., 1999; Montiel-Jarillo et al., 2017; Nakaya et al., 2015; Tavernier et al., 1997; Tohme et al., 2018) have reported higher PHB yield with nitrate than with ammonia as the limiting nutrient (Kamravamanesh et al., 2017). Higher PHB yield was observed with organic nitrogen source (peptone) than with inorganic nitrogen (NH_4Cl) (Mostafa et al., 2020). The PHB contents of 134 PHB-producing strains of bacteria studied appeared to be subtly noticeable under normal growth conditions. However, this significantly increased in 63 strains which were put under nitrogen deprivation (—N). Higher than with phosphate deprivation, and/or potassium and an all-nutrient deprivation (Kaewbai-ngam et al., 2016). Usually, microalgae cellular growth decreases in nitrogen-limited medium, indicating that nitrogen deficiency affects metabolic activity of microorganisms negatively and this subsequently translates to low biomass production, as well as alteration of the biochemical composition (Costa et al., 2018a). To overcome the bottleneck of reduced biomass growth while trying to achieve high PHB content, additional carbon sources are introduced to the medium during nitrogen limitation. For instance, acetate supplementation under N-deprivation has been shown to increase PHB accumulation. In cyanobacteria, *Synechosystis* PCC 6803, a twofold increase was observed after addition of acetate. Glucose supplementation significantly improved cellular growth rate and, thus, improved PHB productivity (Ansari and Fatma, 2016; Monshupanee et al., 2016). Adding acetate at the beginning of nitrogen deprivation doubled the PHB levels within the cells and contributed 44–48% to PHB synthesis, further demonstrating this (Dutt and Srivastava, 2018).

Gene expression related to assimilation of nitrogen and nitrate or the transport of nitrite is known to be reduced by overexpression of either *Sig*E or *Rre*37 after nitrogen starvation. *Sig*E and *Rre*37 are transcriptional regulators whose transcript and protein levels increase during nitrogen starvation (Nakaya et al., 2015). Nutritional influence on gene expression can further be understood through the biosynthetic pathway of PHB, discussed in Section 4.4.1. In a mixed WW-borne microbial culture, phosphorus limitation resulted in a cyanobacteria-dominated culture and noticeably higher levels of carbohydrate content (43%–48%) than cultures with high loads of nitrogen and phosphorus and carbon limitation (29%). Carbon uptake and the resultant production of polymers from cyanobacteria are shown to be improved through nutrient feeding strategies (Arias et al., 2018a).

5.3. Feeding mechanism

The way in which microbes are fed in a controlled cultivation system has a great influence on their bio-product accumulation capacity. Ge et al. (2018b) compared single-dose initial feeding, multiple-dose step feeding and single-dose exponential feeding under mixotrophic conditions and noticed that feeding glycerol at the late exponential growth stage resulted in the highest biomass and lipid productivities with varied lipid compositions. Likewise, PHB production is usually more efficient in a two-stage culture (Monshupanee et al., 2016; Ozdemir et al., 2014). Cells are first grown in an enriched medium containing suitable nutrients, once they generate enough biomass, they are centrifuged, washed and transferred to a nitrogen-free medium. During step two, absence of nitrogen or phosphorus, or both, leads to PHB accumulation at the expense of further biomass generation. Conversely, in a one-step culture condition, cells grown in a medium with glucose as the only carbon source were used as inoculum in a nitrogen-limited medium containing different carbon sources. The amount of nitrogen supplied leads to limitations in cell multiplication thus, the biopolymer is formed from the excess of carbon source available (Silva-queiroz et al., 2009).

Aerobic dynamic feeding (ADF) conditions also showed promise of significant capacity to store polyhydroxybutyrate (PHB). In a study (Serafim et al., 2004), high substrate concentration supplied at once, proved to be inhibitory for PHB storage mechanism in a mixed culture. To avoid substrate inhibition, acetate was supplied differently including 180 Cmmol/L continuously fed and three pulses of 60 Cmmol/L each. This approach increased the specific PHB storage rate in both cases, yielding 56.2% and 78.5% PHB content respectively, indicating that pulse feeding has a positive impact on PHB storage. Similarly, Fradinho et al. (2014) observed that out of the six tested organic acids (malate, citrate, lactate, acetate, propionate and butyrate), only three of the VFAs enabled PHA production in a mixed photosynthetic culture. Acetate and butyrate led to the formation of PHB while propionate produced a HB:HV copolymer with a 51% fraction of HV. These examples reiterate the fact that feeding strategy could be optimized to achieve specific polymer properties and obtain significant yields especially in a mixed culture, by stimulating the dominance of a high polymer-storing population.

WWTPs operated under anaerobic and aerobic cycles are capable of PHA production due to the presence of glycogen accumulating Organisms (GAOs) and PAOs. These microbes cycle PHA as part of their metabolism by taking up carbon substrates for PHA synthesis while consuming glycogen. Under anaerobic conditions, PAOs release phosphate, thus, acquiring energy for PHA accumulation. Meanwhile, during aerobic conditions, phosphate is taken up in excess for the replenishment of polyphosphate pool, and PHA is degraded for storing energy (Serafim et al., 2008). Thus, in the presence of oxygen, both PAOs and GAOs use stored PHA for growth, maintenance and glycogen pool replenishment.

The behaviour of microbes subjected to feast-famine regime was first proposed by Daigger and Grady (1982). The authors explained that, when there is an absence of external substrate for a significant period, the amount of intracellular components such as RNA and enzymes required for cell growth decreases. After such long starvation period, in the event that the culture is dosed with an excess of carbon, the amount of the available intracellular enzymes is much lower than that required to reach the maximum growth rate. This is usually observed as slow growth response. In such situations, PHA storage becomes the dominant response mechanism.

5.4. pH

Physical growth parameters also play a crucial role in biopolymer accumulation capacity of microbes. The parameters mostly known to influence maximum PHB production include pH usually between 7.0 and 7.5 and an incubation temperature of 30 °C (Lathwal et al., 2015). A pH of about 7.5 has been reported to be the optimal pH for many microorganisms (Ansari and Fatma, 2016; Kavitha et al., 2016a; Montiel-Jarillo et al., 2017; Touloupakis et al., 2016). Outside this range, microalgae capacity to absorb CO_2 is drastically reduced and the cell's ability to maintain the activity of the RuBisCO enzyme is interfered (Sutherland et al., 2015). Such unfavourable conditions translate into poor cell growth which subsequently affects the PHB storage capacity. This is because, in a microbial culture system, cells would firstly channel their energy into increasing biomass before accumulating storage products like PHB. This phenomenon has been demonstrated in several studies. For instance, acidic pH is known to be unfavourable for PHB accumulation in *Bacillus cereus* SPV. The maximum optical density (OD) was 0.05 under acidic conditions (pH 3.0). However, at pH 6.8, the OD was as high as 5.9 which yielded PHB 23% CDW. Getachew and Woldesenbet (2016) also reported that the best PHB production from *Bacillus* sp. was observed at an optimum pH of 7 at 37 °C. Similarly, Mostafa et al. (2020) observed that the highest PHB accumulation by *Pseudodonghicola xiamenensis* was achieved at pH 7.5–8.0 and pH 7–9 for PHB-producing bacteria (*Bacillus* sp.) (Thapa et al., 2019). Outside this range, PHB accumulation reduced drastically. However, to overcome certain cultivation issues, such as contamination, pH is usually increased to saline concentrations. The effect of pH on *Synechocystis* sp. PCC 6803 was observed by raising the pH to as high as 11 in mass cultures. Although this technique led to contamination-free cultures, it also led to reduced lipid and increased carbohydrate contents (Touloupakis et al., 2016). In a co-culture system where other by-products such as lipids and starch are desired, this technique might not be favourable.

5.5. Photoperiod

Due to the ability of photosynthetic microbes to utilize sunlight as an energy source, sunlight has been proposed as a cheap source of illumination for phototrophic mixed culture (PMC) production of PHA for cost reduction (Fradinho et al., 2019). Light-harvesting complexes allow certain microbes to capture enough light energy for biomass production. This subsequently leads to accumulation of storage compounds like PHB. Low light intensity was used to select PHA-producers in the selection step and high light intensity of about 20 W/g was used to achieve maximum PHA productivity rate (2.21 ± 0.07 Cmol PHB) in the accumulation step (Fradinho et al., 2019). Similarly, Monshupanee and Incharoensakdi (2014) also observed that high light intensity (200 μmol photon m^{-2} s^{-1}) was optimal for the co-production of GL, LP and PHB in *Synechocystis* sp. In a cyanobacteria-dominated mixed culture, maximum concentration of PHB (104 mg/L) was achieved under continuous illumination in comparison with 12 h light/dark alternation (Arias et al., 2018b). Similarly, 26.37% w/w PHB was obtained faster (on day 7 instead of day 21) by applying 10:14 h light/dark photoperiod conditions together with other optimized physicochemical conditions such as pH of 7.5 and temperature of 30 °C in a study with cyanobacteria (Ansari and Fatma, 2016). Contrarily, PHB production was tripled in a dark fermentation culture under 30:30 min light/dark conditions compared to continuous illumination (Montiel Corona et al., 2017). Similarly, in another study alternating light/dark rhythm under shaken conditions improved intracellular PHB accumulation in *Synechocystis* sp. compared to continuous light (Koch et al., 2020). These studies prove that, the effect of light (in terms of duration and intensity) on PHB productivity and yield is species-specific. Much like a feast-famine regime, light can also be alternated in order to achieve the desired results in a two-stage system of selection and accumulation.

5.6. Cycle length

The duration of a culture influences the rate of biopolymer accumulation. Several studies have demonstrated the effect of cultivation cycle and length on PHB yield. For instance, aerobic microorganisms from activated sludge mixed culture were reported to store 16, 18, 42, and 55% PHB content after 12, 8, 4, and 2 hour cycles respectively (Ozdemir et al., 2014). Similarly, an experiment of a mixed culture with a cycle length range of 1–18 h at 20 °C and 30 °C, revealed that, over 75% (CDW) PHB was accumulated by two dominating microbes; *Zoogloea* and *P. acidivorans*. This correlated well with cycle length at a constant solid retention time (SRT) (Jiang et al., 2011b). Both studies indicate that, to achieve high PHB content, the cycle length should be decreased. Contrarily, Moralejo-Gárate et al. (2013) revealed that a longer cycle length of 24 h was ideal for PHA production, whereas a shorter cycle length of 6 h favoured polyglucose production over PHA in the same dominating microbe in the mixed culture. This phenomenon suggested a metabolic rather than a microbial competition response since two metabolic products were simultaneously achieved from the SBR. From these studies, it can be inferred that the effect of cultivation length on PHB productivity and final yield is dependent on the microbial community and the reactor setup.

6. Effects of extraction methods on Poly-β-hydroxybutyrate yield and purity

PHB-producing microbes can accumulate up to 90% of their own weight as biopolymer (Bhuwal et al., 2013; Ozdemir et al., 2014) but the polymer, within the cell is difficult to extract. Downstream processing such as harvesting and extraction, are said to be responsible for 60–80% of the total production cost (Jacquel et al., 2008). Thus, in order to achieve a significant recovery yield with the desired polymer properties, extraction methods have to be optimized. Conventional methods of PHB extraction include solvent, chemical, mechanical, enzyme and surfactant-chelate extraction. Over the years, different solvents and cell disruption techniques have been developed and applied in lysing cells to release the desired product.

Solvent extraction usually involves the soaking of microbial biomass in a cocktail of solvents in a stepwise manner and the subsequent recovery of the polymer through precipitation. Cell wall strength plays a significant role in the disruption of microbial cells for biopolymer recovery and manipulation of the growth medium composition can lead to alterations in microbial cell wall structure. Processing conditions such as the type, concentration, action time and working temperature of solvent have significant effects on the molecular weight, as well as extraction rate and purity of a biopolymer (Chen, 2009). These conditions affect the costs, characteristics, and biopolymer monomeric composition, which subsequently impacts their applications in industry (Costa et al., 2018b). Applications of the polymer in the medical industry for instance, require toxin-free polymer with high purity compared to applications in single-use plastic bags. Extraction methods are also known to significantly ($p < 0.05$) affect the molecular mass, degree of crystallinity and monomeric composition of the biopolymer (Costa et al., 2018b), indicating that the extraction method is crucial in polymer recovery as well as obtaining the desired characteristics for the intended industrial applications. For instance, PHB extracted with a mixture of sodium hypochlorite, diethyl ether and hot chloroform was used in cancer detection. The breast cancer cells (T47D) appeared to have a stronger attachment for the PHB sheets compared to normal epithelial cells (PCS-600-010) (Sabarinathan et al., 2018). This biocompatibility with mammalian cells has allowed the application of PHA polymers especially PHB in surgical tools, wound dressing, bone repair and drug delivery (Bonartsev et al., 2019; Bunster, 2016). It is also widely used in the agricultural sector (Tan et al., 2019). Generally, higher acid concentrations have led to desirable mechanical strength properties. Although the end results might be good, it is important to take note of the negative environmental and economic implications of the use of harsh chemicals in extraction.

Due to the disadvantages of the aforementioned methods such as toxicity, time-consumption and costliness, environmentally friendly methods such as biological extraction (known as bioextraction) and hydrothermal conversion are being exploited. Bioextraction methods include bacteriophage-mediated lysis systems, predation systems and mealworm digestion systems. These methods promise of cost reduction and reduced harmfulness to the environment and human health (Haddadi et al., 2019). Coupling WWT with PHB recovery is a step in the right direction. Ceyhan and Ozdemir (2011) utilized hypochlorite method of extracting PHB from *Enterobacter aerogenes* cultivated in domestic wastewater and obtained a yield as high as 96.25%. Again, PHB-containing biomass utilized in WWT was successfully transformed into propylene through hydrothermal conversion (Li and Strathmann, 2019). Extraction solvents such as anisole, cyclohexanone and phenetole have also been tested by Rosengart et al. (2015) as sustainable industrial solvents. Biopolymer recovery yields of 97% and 93% were achieved with anisole and cyclohexanone, respectively, which were very similar (96–98%) to yields obtained by chloroform extraction.

After the successful extraction of the biopolymer components from cell biomass, it is necessary to purify the extract to eliminate impurities such as solvents, bacteria, colour and odour. This also allows application of the biopolymer in sensitive areas such as the medical industry as mentioned earlier. Purification methods usually involve enzymes or chelating agents in combination with hydrogen peroxide treatment (Jacquel et al., 2008) and ozone (Horowitz and Brennan, 2005). Ozone treatment has many advantages such as bleaching, deodorization, and solubilization of impurities from the biopolymer. This could eventually replace hydrogen peroxide treatment. Klasener et al. (2018) also proposed an environmentally friendly approach to handling the biopolymer extraction WW by utilizing it in further cultivation of microbes that is, re-utilizing the aqueous phase; a technique currently gaining grounds in microalgae research. This was demonstrated with *Spirulina* LEB 18 which was successfully cultivated in extraction WW in their study. Optimization of the extraction and purification processes of PHB should consider the type of polymer-producing strain, expected standard of purity, intended industrial application as well as type and composition of the desired biopolymer. It is necessary to also consider the use of green and recyclable harvesting techniques such as the use of crystalline nanocellulose to reduce cost associated with harvesting microalgae (Qiu et al., 2019a). Table 2 summarizes some key works on

Table 2
Effects of extraction methods on yield and purity of PHBs.

Method	Strain	Extractant	Cultivation conditions	Yield	Purity (%)	References
Organic solvent extraction	*Spirulina* sp.	Sodium hypochlorite, methanol, chloroform, acetone	Vertical tubular reactor with continuous agitation at 30 °C	6.1–9.8% w/w	63.51 and 93.62	(Costa et al., 2018b)
	Synechocystis sp. *PCC 6803*	KOH, ethanol, sodium acetate, amyloglucosidase, chloroform, methanol, KCL, acidic dichromate, H_2SO_4	Autophototrophic/heterophototrophic growth in BG-11 50/200 μmol photon m^{-2} s^{-1} at 28 °C	Autophototrophic Ndeprivation (2.4–13.5% w/w DW) heterophototrophic 0.4% (w/v) glucose addition (3.3–9.2% w/w DW)	N/A	(Monshupanee and Incharoensakdi, 2014)
	Cupriavidus necator	Acetone/ethanol/propylene carbonate (A/E/P, 1:1:1 v/v/v)	Autotrophic (CO_2 as substrate and H_2 as electron acceptor)	83–92%	92–93	(Fei et al., 2016)
	Nostoc muscorum NCCU-442	Pre-treatment of biomass with methanol:acetone: water:dimethylformamide [40:40:18:2 (MAD-I)] with 2 h magnetic bar stirring followed by 30 h continuous chloroform soxhlet extraction	10:14 h light:dark periods with 0.4% glucose (as additional carbon source), 30 °C	NaCl and P deficiency yielded 26.37% PHB	N/A	(Ansari and Fatma, 2016)
	Cupriavidus necator H16	Cyclohexanone, 120 °C 3 min	Tryptic soy broth with vegetable oil as sole carbon source, 35 °C, 72 h	82.3%	95	(Jiang et al., 2018)
Mechanical disruption	*Bacillus flexus*	SDS sonication	Modified basal mineral (MBM) medium	96.7	≥96	(Arikawa et al., 2017)
	Pseudomonas putida Bet001	Sonication and heptane (marginal non-solvent)	Octanoic acid as sole carbon & energy source	29.5/37.1%	N/A	(Ishak et al., 2015)
Enzyme extraction	*Cupriavidus necator*	CelumaxVR BC	Citrus molasses as carbon source and a supply of propionic acid during N limitation	93.2%	94	(Neves and Müller, 2012)
Bioextraction						
Bacteria extraction system	*Pseudomonas putida*	*Bdellovibrio bacteriovorus* (mutant strain)	30 °C Sodium octanoate as carbon source	0.65–0.87 g/L	N/A	(Martínez et al., 2016)
Phage lysis system	*Pseudomonas oleovorans*	Bacteriophage Ke14	Modified P1 ammonium mineral salts medium contains with crude glycerol, 30 °C	0.84 g/L	N/A	(Hand et al., 2016)
Animal digestive system	*Cupriavidus necator*	Yellow mealworm (*Tenebrio molitor*)	Palm olein (plant oil) & waste animal fats	55 wt% - from palm olein 60 wt% - from waste animal fats	94	(Ong et al., 2018)

biopolymer extraction and Fig. 5 demonstrates the production stream of PHB from wastewater-cultivated microbes through a bio-refinery concept.

7. Challenges and future prospects

The major challenge faced in the PHB industry is of an economic nature. Current commercial PHB production is done in batch fermenters using bacteria that require large amounts of organic carbon sources and salts, contributing to about 50% of production cost (Costa et al., 2019). Microalgae are promising microbes in this field due to the fact that they are photoautotrophic, utilize CO_2 and light as their main energy source, thus, contributing to greenhouse gas emission reductions (Garcia-Gonzalez et al., 2015). In addition to the fact that landfill degradation of conventional plastics is slow and incineration of plastics generates toxic by-products, life cycle assessments (LCAs) mostly conclude that PHB production using WW is economically feasible but still remains uncompetitive with conventional bioplastics (Troschl et al., 2018; Yates and Barlow, 2013). Thus, in order to successfully reduce bio-plastics cost, and increase its competitiveness, a few issues have to be addressed.

7.1. Optimizing production

Developing a zero-waste process such as a bio-refinery where multiple products are co-produced during cultivation, extraction WW is reused and resultant biomass is utilized as biochar for soil conditioning. These techniques are likely to lower production price. However, in doing so, consideration has to be given to cultivation conditions, reactor type, yield, quality, monomer properties and microbial strain especially with regard to genetically modified microbes which are sensitive and require specifically controlled conditions. Studies show that genes involved in PHB production are post-transcriptionally regulated because there is no differential expression in PHB biosynthesis genes during the production of PHB under varied growth conditions. Certain microbes such as *Calothrix scytonemicola* TISTR 8095, *Nostoc muscorum* CCAP 1453/9, and *Spirulina* sp. LEB 18, are not ideal for cultivation in photobioreactors due to the formation of biofilms during growth. Thus, reactor set-up is an important factor to consider for optimized PHB production. To ensure the sustainability of PHB production, microbes that are capable of growing under different growth conditions should be prioritized in order to enhance the viability of production processes. For instance, *Rhodopseudomonas palustris* TIE-1 has demonstrated metabolic flexibility because it can grow under chemoheterotrophic, photoheterotrophic and photoautotrophic conditions or *Cupriavidus necator* that can produce PHB both heterotrophically and autotrophically. Also the ability of the microbe to synthesize PHB under nutrient-limited conditions (e.g. *Synechocystis* sp.) or under normal growth (e.g. *Cupriavidus necator*) should be considered due to their unique ability of phenotypic heterogeneity.

7.2. Acclimatization of microbes for wastewater cultivation

In trying to reduce production cost, open-pond systems would have been ideal to incorporate with WWTP, but this is challenging as it would limit the type of PHB-producing microbes that could be used as well as influence the downstream processing. Thus, it is important to select WW sources for specific microbes that are naturally inclined to thrive well under those conditions.

7.3. Improving yield

Currently, novel strategies in the field include the use of biological methods in PHB recovery through entomology and botany. The former involves the use of insects by feeding them on PHB-accumulating microbes and recovering the polymer via excretion. These insects could

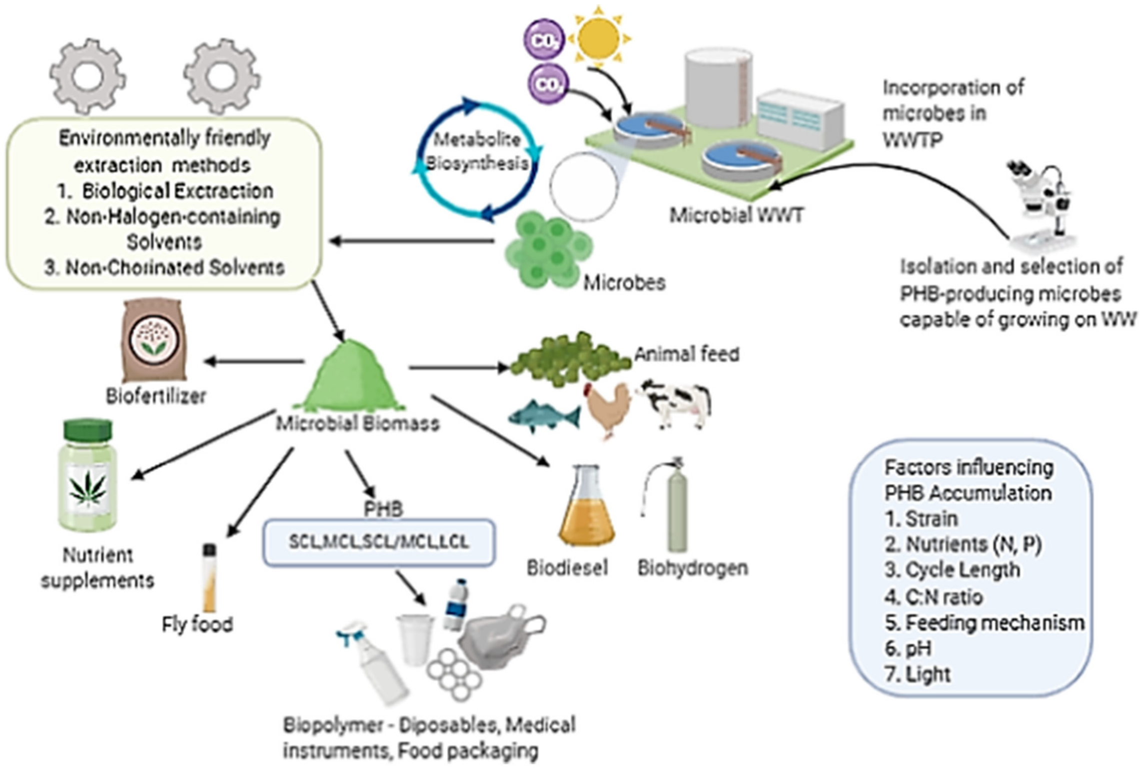


Fig. 5. Utilization of wastewater-cultivated microbial biomass in a bio-refinery concept for value added products, simultaneous wastewater treatment and carbon capture for a better climate and bio-economy.

subsequently be incorporated in fish feed, bio-fertilizers or other useful bio-products. The latter involves the use of transgenic plants to produce PHBs. Plant parts such as roots, stems, leaves and fruits should continue to be exploited. Another successful approach that has been well documented and holds future prospects lies with the use of halophiles which can withstand harsh environmental stresses as well as contamination, potentially reducing the cost of production.

8. Conclusions

With regard to substrate choice, studies have shown that substrates high in VFAs are suitable for bacterial PHB production. Microalgae such as *Chlorella* sp. are also known to have high tolerance for VFAs. Thus waste substrates such as anaerobic digester liquor with high VFA content would be ideal for PHB production in microalgae or in mixed cultures and agro-industrial wastes like ensiled maize, which are high in easily fermentable carbohydrates, would be a better substrate option for hydrogen production in a co-production system. Producing biopolymers such as PHB from wastewater-cultivated microbes for bioplastic production can be regarded as a sustainable approach to wastewater treatment. Majority of the plastic pollution seen worldwide, is as a result of single-use plastics such as shopping bags which do not require stringent sterilization efforts during production unlike those required in medical applications. Thus, adopting mixed microbial cultures capable of growing on wastewater seems like a reasonable approach to tackling the plastic pollution problem due to the biodegradability of the polymer. Additionally, the ability of autotrophic microalgae to utilize inorganic carbon sources adds more value to the production chain as the microbes can thrive on flue gases such as CO_2 from industrial plants. Aside cost-reduction, this also plays a critical role in sustainable environmental bioremediation through the improvement of effluent quality.

It would be beneficial from an environmental and economic perspective for future commercial PHB-producing plants to consider integrating waste effluents rich in organic matter for simultaneous WWT and PHB production. A two-stage system that takes advantage of metabolically versatile microbes would be ideal. Such microbes are commercially viable because they can grow under various conditions by increasing their biomass within a short period when conditions are favourable and subsequently accumulating PHB when nutrients are limited. This approach has the potential to reduce cost through the utilization of abundant resources like CO_2 and light for autotrophic purposes and waste organic matter for heterotrophic methods and a combination of various approaches.

CRediT authorship contribution statement

Amadu, Ayesha Algade: Writing- original draft, Visualization, Data curation, Investigation. **Qiu, Shuang:** Writing - review & editing, Visualization, Funding acquisition. **Ge, Shijian:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **Addico, Gloria Naa Dzama:** Writing - review & editing. **Ameka, Gabriel Komla:** Writing - review & editing. **Yu, Ziwei:** Data curation, Investigation. **Xia, Wenhao:** Data curation, Investigation. **Abbew, Abdul-Wahab:** Data curation, Investigation. **Shao, Dadong:** Writing - review & editing. **Champagne, Pascale:** Writing - review & editing. **Wang, Sufeng:** Writing - review & editing.

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.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (52000103, 51708294, and 21976089), Natural Science Foundation of Jiangsu Province for Distinguished Young Scholars (BK20190022), Natural Science Foundation of Jiangsu Province (BK20181303), China Postdoctoral Science Foundation (2020M671402), and Fundamental

Research Funds for the Central Universities (30920021117). Dr. Shijian Ge acknowledges the support of Distinguished Professorship of Jiangsu Province and China Association for Science and Technology.

References

Ahn, C.H., Park, J.K., Wang, J., 2009. Changes in polyhydroxy-alkanoates (PHAs) during enhanced biological phosphorus removal with dairy industrial wastewater. J. Environ. Eng. 135, 1213–1220. https://doi.org/10.1061/(asce)0733-9372(2009)135:11(1213).

Albuquerque, M.G.E., Torres, C.A.V., Reis, M.A.M., 2010. Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection. Water Res. 44, 3419–3433. https://doi.org/10.1016/j.watres.2010.03.021.

AlSayed, A., Fergala, A., Eldyasti, A., 2018. Sustainable biogas mitigation and value-added resources recovery using methanotrophs intergrated into wastewater treatment plants. Reviews in Environmental Science and Biotechnology (Vol. 17, Issue 2). Springer Netherlands https://doi.org/10.1007/s11157-018-9464-3.

Ansari, S., Fatma, T., 2016. Cyanobacterial polyhydroxybutyrate (PHB): screening, optimization and characterization. PLoS One 11 (6), e0158168. https://doi.org/10.1371/journal.pone.0158168.

Anterrieu, S., Quadri, L., Geurkink, B., Dinkla, I., Bengtsson, S., Arcos-Hernandez, M., Alexandersson, T., Morgan-Sagastume, F., Karlsson, A., Hjort, M., Karabegovic, L., Magnusson, P., Johansson, P., Christensson, M., Werker, A., 2014. Integration of biopolymer production with process water treatment at a sugar factory. New Biotechnol. 31, 308–323. https://doi.org/10.1016/j.nbt.2013.11.008.

Arias, D.M., Fradinho, J.C., Uggetti, E., García, J., Oehmen, A., Reis, M.A.M., 2018a. Polymer accumulation in mixed cyanobacterial cultures selected under the feast and famine strategy. Algal Res. 33, 99–108. https://doi.org/10.1016/j.algal.2018.04.027.

Arias, D.M., Uggetti, E., García-Galán, M.J., García, J., 2018b. Production of polyhydroxybutyrates and carbohydrates in a mixed cyanobacterial culture: effect of nutrients limitation and photoperiods. New Biotechnol. 42, 1–11. https://doi.org/10.1016/j.nbt.2018.01.001.

Arias, D.M., Rueda, E., García-Galán, M.J., Uggetti, E., García, J., 2019. Selection of cyanobacteria over green algae in a photo-sequencing batch bioreactor fed with wastewater. Sci. Total Environ. 653, 485–495. https://doi.org/10.1016/j.scitotenv.2018.10.342.

Arikawa, H., Sato, S., Fujiki, T., Matsumoto, K., 2017. Simple and rapid method for isolation and quantitation of polyhydroxyalkanoate by SDS-sonication treatment. Journal of Bioscience and Bioengineering 124 (2), 250–254. https://doi.org/10.1016/j.jbiosc.2017.03.003.

Arroyo, P., Molinos-Senante, M., 2018. Selecting appropriate wastewater treatment technologies using a choosing-by-advantages approach. Sci. Total Environ. 625, 819–827. https://doi.org/10.1016/j.scitotenv.2017.12.331.

Asada, Y., Miyake, M., Miyake, J., Kurane, R., Tokiwa, Y., 1999. Photosynthetic accumulation of poly-(hydroxybutyrate) by cyanobacteria - the metabolism and potential for CO2 recycling. Int. J. Biol. Macromol. 25, 37–42. https://doi.org/10.1016/S0141-8130(99)00013-6.

Aslan, A.K.H.N., Ali, M.D.M., Morad, N.A., Tamunaidu, P., 2016. Polyhydroxyalkanoates production from waste biomass. IOP Conf. Ser. Earth Environ. Sci. 36, 012040.

Basset, N., Katsou, E., Frison, N., Malamis, S., Dosta, J., Fatone, F., 2016. Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. Bioresour. Technol. 200, 820–829. https://doi.org/10.1016/j.biortech.2015.10.063.

Ben, M., Kennes, C., Veiga, M.C., 2016. Optimization of polyhydroxyalkanoate storage using mixed cultures and brewery wastewater. J. Chem. Technol. Biotechnol. 91, 2817–2826. https://doi.org/10.1002/jctb.4891.

Bengtsson, S., Werker, A., Welander, T., 2008. Production of polyhydroxyalkanoates by glycogen accumulating organisms treating a paper mill wastewater. Water Sci. Technol. 58, 323–330. https://doi.org/10.2166/wst.2008.381.

Bhuwal, A.K., Singh, G., Aggarwal, N.K., Goyal, V., Yadav, A., 2013. Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper, and cardboard industry wastes. International Journal of Biomaterials 2013. https://doi.org/10.1155/2013/752821.

Bioplastic News, n.d. Metabolix develops polyhydroxalkanoate (PHA) copolymer technology – bioplastics news [WWW document]. URL https://bioplasticsnews.com/2014/05/30/metabolix-polyhydroxalkanoate-pha-copolymer/.

Biradar, G.G., Shivasharana, C.T., Kaliwal, B.B., 2015. Isolation and characterization of polyhydroxybutyrate (PHB) producing *Bacillus* species from agricultural soil. Euro. J. Exp. Bio. 5, 58–65.

Bonartsev, A.P., Bonartseva, G.A., Reshetov, I.V., Kirpichnikov, M.P., Shaitan, K.V., 2019. Application of polyhydroxyalkanoates in medicine and the biological activity of natural poly (3-hydroxybutyrate). Acta Nat. 11 (2), 4–16. https://doi.org/10.32607/20758251-2019-11-2-4-16.

Bunster, G.F., 2016. Polyhydroxyalkanoates: Production and Use. Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. April. , pp. 6412–6421 https://doi.org/10.1081/e-ebpp-120049915.

Burnap, R.L., 2015. Systems and photosystems: cellular limits of autotrophic productivity in cyanobacteria. Front. Bioeng. Biotechnol. 3, 1–13. https://doi.org/10.3389/fbioe.2015.00001.

Carofiglio, V.E., Stufano, P., Cancelli, N., De Benedictis, V.M., Centrone, D., De Benedetto, E., Cataldo, A., Sannino, A., Demitri, C., 2017. Novel PHB/Olive mill wastewater residue composite based film: thermal, mechanical and degradation properties. J. Environ. Chem. Eng. 5, 6001–6007. https://doi.org/10.1016/j.jece.2017.11.013.

Carpine, R., Olivieri, G., Hellingwerf, K., Pollio, A., Marzocchella, A., 2015. The cyanobacterial route to produce poly-β-hydroxybutyrate. Chem. Eng. Trans. 43, 289–294. https://doi.org/10.3303/CET1543049.

Carpine, R., Olivieri, G., Hellingwerf, K.J., Pollio, A., Marzocchella, A., 2020. Industrial production of poly-β-hydroxybutyrate from CO2: can cyanobacteria meet this challenge? Processes 8 (3), 1–23. https://doi.org/10.3390/pr8030323.

Cavaillé, L., Albuquerque, M., Grousseau, E., Lepeuple, A.S., Uribelarrea, J.L., Hernandez-Raquet, G., Paul, E., 2016. Understanding of polyhydroxybutyrate production under carbon and phosphorus-limited growth conditions in non-axenic continuous culture. Bioresour. Technol. 201, 65–73. https://doi.org/10.1016/j.biortech.2015.11.003.

Ceyhan, N.G., Ozdemir, G., 2011. Poly-β-hydroxybutyrate (PHB) production from domestic wastewater using *Enterobacter aerogenes*. African J. Microbiol. Res 5, 690–702. https://doi.org/10.5897/AJMR10.864.

Chaogang, W., Zhangli, H., Anping, L., Baohui, J., 2010. Biosynthesis of Poly-3-hydroxybutyrate (PHB) in the transgenic green alga *Chlamydomonas reinhardtii*. J. Phycol. 46, 396–402. https://doi.org/10.1111/j.1529-8817.2009.00789.x.

Chen, G.Q., 2009. A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. Chem. Soc. Rev. 38, 2434–2446. https://doi.org/10.1039/b812677c.

Chen, G.Q., Jiang, X.R., 2017. Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis. Synth. Syst. Biotechnol. 2, 192–197. https://doi.org/10.1016/j.synbio.2017.09.001.

Chen, Z., Qiu, S., Amadu, A.A., Shen, Y., Wang, L., Wu, Z., Ge, S., 2020. Simultaneous improvements on nutrient and Mg recoveries of microalgal bioremediation for municipal wastewater and nickel laterite ore wastewater. Bioresour. Technol. 297, 122517. https://doi.org/10.1016/j.biortech.2019.122517.

Corneli, E., Adessi, A., Dragoni, F., Ragaglini, G., Bonari, E., De Philippis, R., 2016. Agroindustrial residues and energy crops for the production of hydrogen and poly-β-hydroxybutyrate via photofermentation. Bioresour. Technol. 216, 941–947. https://doi.org/10.1016/j.biortech.2016.06.046.

Costa, J.A.V., Moreira, J.B., Lucas, B.F., Da Silva Braga, V., Cassuriaga, A.P.A., De Morais, M.G., 2018. Recent advances and future perspectives of PHB production by cyanobacteria. Ind. Biotechnol. 14, 249–256. https://doi.org/10.1089/ind.2018.0017.

Costa, S.S., Miranda, A.L., Andrade, B.B., Assis, D. de J., Souza, C.O., de Morais, M.G., Costa, J.A.V., Druzian, J.I., 2018a. Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae. Int. J. Biol. Macromol. 116, 552–562. https://doi.org/10.1016/j.ijbiomac.2018.05.064.

Costa, S.S., Miranda, A.L., Assis, D. de J., Souza, C.O., de Morais, M.G., Costa, J.A.V., Druzian, J.I., 2018b. Efficacy of *Spirulina* sp. polyhydroxyalkanoates extraction methods and influence on polymer properties and composition. Algal Res. 33, 231–238. https://doi.org/10.1016/j.algal.2018.05.016.

Costa, S.S., Miranda, A.L., de Morais, M.G., Costa, J.A.V., Druzian, J.I., 2019. Microalgae as source of polyhydroxyalkanoates (PHAs) — a review. Int. J. Biol. Macromol. 131, 536–547. https://doi.org/10.1016/j.ijbiomac.2019.03.099.

Daigger, G.T., Grady, C.P.L., 1982. The dynamics of microbial growth on soluble substrates. A unifying theory. Water Res. 16, 365–382. https://doi.org/10.1016/0043-1354(82)90159-2.

Damrow, R., Maldener, I., Zilliges, Y., 2016. The multiple functions of common microbial carbon polymers, glycogen and PHB, during stress responses in the non-diazotrophic cyanobacterium *Synechocystis* sp. PCC 6803. Front. Microbiol. 7, 1–10. https://doi.org/10.3389/fmicb.2016.00966.

Das, S.K., Sathish, A., Stanley, J., 2018. Production of biofuel and bioplastic from *Chlorella pyrenoidosa*. Mater. Today Proc. 5, 16774–16781. https://doi.org/10.1016/j.matpr.2018.06.020.

de Jesus, C.S., da Silva Uebel, L., Costa, S.S., Miranda, A.L., de Morais, E.G., de Morais, M.G., Costa, J.A.V., Nunes, I.L., de Souza Ferreira, E., Druzian, J.I., 2018. Outdoor pilot-scale cultivation of *Spirulina* sp. LEB-18 in different geographic locations for evaluating its growth and chemical composition. Bioresour. Technol. 256, 86–94. https://doi.org/10.1016/j.biortech.2018.01.149.

Demirbas, A., 2011. Biodiesel from oilgae, biofixation of carbon dioxide by microalgae: a solution to pollution problems. Appl. Energy 88, 3541–3547. https://doi.org/10.1016/j.apenergy.2010.12.050.

Depraetere, O., Deschoenmaeker, F., Badri, H., Monsieurs, P., Foubert, I., Leys, N., Wattiez, R., Muylaert, K., 2015. Trade-off between growth and carbohydrate accumulation in nutrient-limited *Arthrospira* sp. PCC 8005 studied by integrating transcriptomic and proteomic approaches. PLoS One 10, 1–19. https://doi.org/10.1371/journal.pone.0132461.

Dionisi, D., Beccari, M., Gregorio, S.D., Majone, M., Papini, M.P., Vallini, G., 2005. Storage of biodegradable polymers by an enriched microbial community in a sequencing batch reactor operated at high organic load rate. J. Chem. Technol. Biotechnol. 80, 1306–1318. https://doi.org/10.1002/jctb.1331.

Dutt, V., Srivastava, S., 2018. Novel quantitative insights into carbon sources for synthesis of poly hydroxybutyrate in *Synechocystis* PCC 6803. Photosynth. Res. 136, 303–314. https://doi.org/10.1007/s11120-017-0464-x.

Eroğlu, E., Gündüz, U., Yücel, M., Türker, L., Eroğlu, I., 2004. Photobiological hydrogen production by using olive mill wastewater as a sole substrate source. Int. J. Hydrog. Energy 29, 163–171. https://doi.org/10.1016/S0360-3199(03)00110-1.

Fasaei, F., Bitter, J.H., Slegers, P.M., van Boxtel, A.J.B., 2018. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res. 31, 347–362. https://doi.org/10.1016/j.algal.2017.11.038.

Fei, T., Cazeneuve, S., Wen, Z., Wu, L., Wang, T., 2016. Effective recovery of poly-β-hydroxybutyrate (PHB) biopolymer from Cupriavidus necator using a novel and environmentally friendly solvent system. Biotechnology Progress 32 (3), 678–685. https://doi.org/10.1002/btpr.2247.

Fradinho, J.C., Oehmen, A., Reis, M.A.M., 2014. Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): substrate preferences and co-substrate uptake. J. Biotechnol. 185, 19–27. https://doi.org/10.1016/j.jbiotec.2014.05.035.

Fradinho, J.C., Oehmen, A., Reis, M.A.M., 2019. Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int. J. Biol. Macromol. 126, 1085–1092. https://doi.org/10.1016/j.ijbiomac.2018.12.270.

Gabriel, F., Fernández, A., Gómez-serrano, C., 2018. Recovery of nutrients from wastewaters using microalgae. 2, 1–13. https://doi.org/10.3389/fsufs.2018.00059.

Garcia-Gonzalez, L., Mozumder, M.S.I., Dubreuil, M., Volcke, E.I.P., De Wever, H., 2015. Sustainable autotrophic production of polyhydroxybutyrate (PHB) from CO2 using a two-stage cultivation system. Catal. Today 257 (Part 2), 237–245. https://doi.org/10.1016/j.cattod.2014.05.025.

Gasser, I., Müller, H., Berg, G., 2009. Ecology and characterization of polyhydroxyalkanoate-producing microorganisms on and in plants. FEMS Microbiol. Ecol. 70, 142–150. https://doi.org/10.1111/j.1574-6941.2009.00734.x.

Ge, S., Champagne, P., 2016. Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Res. 88, 604–612. https://doi.org/10.1016/j.watres.2015.10.054.

Ge, S., Champagne, P., 2017. Cultivation of the marine macroalgae *Chaetomorpha linum* in municipal wastewater for nutrient recovery and biomass production. Environ. Sci. Technol. 51, 3558–3566. https://doi.org/10.1021/acs.est.6b06039.

Ge, S., Peng, Y., Qiu, S., Zhu, A., Ren, N., 2014. Complete nitrogen removal from municipal wastewater via partial nitrification by appropriately alternating anoxic/aerobic conditions in a continuous plug-flow step feed process. Water Res. 55, 95–105. https://doi.org/10.1016/j.watres.2014.01.058.

Ge, S., Wang, S., Yang, X., Qiu, S., Li, B., Peng, Y., 2015. Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: a review. Chemosphere 140, 85–98. https://doi.org/10.1016/j.chemosphere.2015.02.004.

Ge, S., Champagne, P., Plaxton, W.C., Leite, G.B., Marazzi, F., 2017. Microalgal cultivation with waste streams and metabolic constraints to triacylglycerides accumulation for biofuel production. Biofuels, Bioprod. Biorefining 11, 325–343. https://doi.org/10.1002/bbb.

Ge, S., Madill, M., Champagne, P., 2018a. Use of freshwater macroalgae *Spirogyra* sp. for the treatment of municipal wastewaters and biomass production for biofuel applications. Biomass Bioenergy 111, 213–223. https://doi.org/10.1016/j.biombioe.2017.03.014.

Ge, S., Qiu, S., Tremblay, D., Viner, K., Champagne, P., Jessop, P.G., 2018b. Centrate wastewater treatment with *Chlorella vulgaris*: simultaneous enhancement of nutrient removal, biomass and lipid production. Chem. Eng. J. 342, 310–320. https://doi.org/10.1016/j.cej.2018.02.058.

Getachew, A., Woldesenbet, F., 2016. Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. BMC Res. Notes 9, 1–9. https://doi.org/10.1186/s13104-016-2321-y.

Ghimire, A., Valentino, S., Frunzo, L., Pirozzi, F., Lens, P.N.L., Esposito, G., 2016. Concomitant biohydrogen and poly-β-hydroxybutyrate production from dark fermentation effluents by adapted *Rhodobacter sphaeroides* and mixed photofermentative cultures. Bioresour. Technol. 217, 157–164. https://doi.org/10.1016/j.biortech.2016.03.017.

Goffredo, V., Falk, M.W., Schroeder, E.D., Irvine, R.L., Ranieri, E., 2009. Biostorage Polymers Phenomena in Cheese Wastewater Treatment by a Sequencing Batch Reactor. Journal of Environmental Engineering 135 (2), 101–104. https://doi.org/10.1061/(asce)0733-9372(2009)135:2(101).

Goh, Y.S., Tan, I.K.P., 2012. Polyhydroxyalkanoate production by antarctic soil bacteria isolated from Casey Station and Signy Island. Microbiol. Res. 167, 211–219. https://doi.org/10.1016/j.micres.2011.08.002.

Haddadi, M.H., Asadolahi, R., Negahdari, B., 2019. The bioextraction of bioplastics with focus on polyhydroxybutyrate: a review. Int. J. Environ. Sci. Technol. 16, 3935–3948. https://doi.org/10.1007/s13762-019-02352-0.

Hahn, J.J., Eschenlauer, A.C., Sleytr, U.B., Somers, D.A., Srienc, F., 1999. Peroxisomes as sites for synthesis of polyhydroxyalkanoates in transgenic plants. Biotechnol. Prog. 15, 1053–1057. https://doi.org/10.1021/bp990118n.

Hand, S., Gill, J., Chu, K.H., 2016. Phage-based extraction of polyhydroxybutyrate (PHB) produced from synthetic crude glycerol. Science of the Total Environment 557–558, 317–321. https://doi.org/10.1016/j.scitotenv.2016.03.089.

Handrick, R., Reinhardt, S., Jendrossek, D., 2000. Mobilization of poly(3-hydroxybutyrate) in *Ralstonia eutropha*. J. Bacteriol. 182, 5916–5918. https://doi.org/10.1128/JB.182.20.5916-5918.2000.

Hankermeyer, C.R., Tjeerdema, R.S., 1999. Polyhydroxybutyrate: plastic made and degraded by microorganisms. Rev. Environ. Contam. Toxicol. 159, 1–24.

Hempel, F., Bozarth, A.S., Lindenkamp, N., Klingl, A., Zauner, S., Linne, U., 2011. Microalgae as bioreactors for bioplastic production. Microb. Cell Factories 10, 81. https://doi.org/10.1186/1475-2859-10-81.

Hoffmann, J.P., 1998. Wastewater treatment with suspended and nonsuspended algae. J. Phycol. 34, 757–763. https://doi.org/10.1046/j.1529-8817.1998.340757.x.

Hollinshead, W.D., Varman, A.M., You, L., Hembree, Z., Tang, Y.J., 2014. Boosting d-lactate production in engineered cyanobacteria using sterilized anaerobic digestion effluents. Bioresour. Technol. 169, 462–467. https://doi.org/10.1016/j.biortech.2014.07.003.

Horowitz, D.M., Brennan, E.M., 2005. Method of decolourizing or deodourizing polyhydroxyalkanoates from biomass with ozone. United States Pat. 2, 2–8.

Ishak, K.A., Annuar, M.S.M., Heidelberg, T., Gumel, A.M., 2015. Ultrasound-Assisted Rapid Extraction of Bacterial Intracellular in Medium Mixture of Solvent / Marginal Non-solvent. Arab J Sci Eng. https://doi.org/10.1007/s13369-015-1833-4.

Jacquel, N., Lo, C.W., Wei, Y.H., Wu, H.S., Wang, S.S., 2008. Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem. Eng. J. 39, 15–27. https://doi.org/10.1016/j.bej.2007.11.029.

Jendrossek, D., 2009. Polyhydroxyalkanoate granules are complex subcellular organelles (carbonosomes). J. Bacteriol. 191, 3195–3202. https://doi.org/10.1128/JB.01723-08.

Jiang, X., Chen, G., 2015. Morphology engineering of bacteria for bio-production. Biotechnol. Adv. 34, 435–440. https://doi.org/10.1016/j.biotechadv.2015.12.007.

Jiang, Y., Marang, L., Kleerebezem, R., Muyzer, G., van Loosdrecht, M.C.M., 2011a. Polyhydroxybutyrate production from lactate using a mixed microbial culture. Biotechnol. Bioeng. 108, 2022–2035. https://doi.org/10.1002/bit.23148.

Jiang, Y., Marang, L., Kleerebezem, R., Muyzer, G., Van Loosdrecht, M.C.M., 2011b. Effect of temperature and cycle length on microbial competition in PHB-producing sequencing batch reactor. ISME J 5, 896–907. https://doi.org/10.1038/ismej.2010.174.

Jiang, Y., Marang, L., Tamis, J., van Loosdrecht, M.C.M., Dijkman, H., Kleerebezem, R., 2012. Waste to resource: converting paper mill wastewater to bioplastic. Water Res. 46, 5517–5530. https://doi.org/10.1016/j.watres.2012.07.028.

Jiang, X.R., Wang, H., Shen, R., Chen, G.Q., 2015. Engineering the bacterial shapes for enhanced inclusion bodies accumulation. Metab. Eng. 29, 227–237. https://doi.org/10.1016/j.ymben.2015.03.017.

Jiang, G., Johnston, B., Townrow, D.E., Radecka, I., Koller, M., Chaber, P., Adamus, G., Kowalczuk, M., 2018. Biomass extraction using non-chlorinated solvents for biocompatibility improvement of polyhydroxyalkanoates. Polymers 10 (7). https://doi.org/10.3390/polym10070731.

Jochum, M., Moncayo, L.P., Jo, Y., 2018. Microalgal cultivation for biofertilization in rice plants using a vertical semi-closed airlift photobioreactor. PLoS One 13, 1–13.

Kaewbai-ngam, A., Incharoensakdi, A., Monshupanee, T., 2016. Increased accumulation of polyhydroxybutyrate in divergent cyanobacteria under nutrient-deprived photoautotrophy: an efficient conversion of solar energy and carbon dioxide to polyhydroxybutyrate by *Calothrix scytonemicola* TISTR 80. Bioresour. Technol. 212, 342–347. https://doi.org/10.1016/j.biortech.2016.04.035.

Kamravamanesh, D., Pflügl, S., Nischkauer, W., Limbeck, A., Lackner, M., Herwig, C., 2017. Photosynthetic poly-β-hydroxybutyrate accumulation in unicellular cyanobacterium *Synechocystis* sp. PCC 6714. AMB Express 7, 1–12. https://doi.org/10.1186/s13568-017-0443-9.

Kamravamanesh, D., Lackner, M., Herwig, C., 2018. Bioprocess engineering aspects of sustainable polyhydroxyalkanoate production in cyanobacteria. Bioengineering 5, 111–129. https://doi.org/10.3390/bioengineering5040111.

Kanesaki, Y., Shiwa, Y., Tajima, N., Suzuki, M., Watanabe, S., Sato, N., Ikeuchi, M., Yoshikawa, H., 2012. Identification of substrain-specific mutations by massively parallel whole-genome resequencing of *Synechocystis* sp. PCC 6803. DNA Res. 19, 67–79. https://doi.org/10.1093/dnares/dsr042.

Kang, Z., Gao, C., Wang, Q., Liu, H., Qi, Q., 2010. A novel strategy for succinate and polyhydroxybutyrate co-production in *Escherichia coli*. Bioresour. Technol. 101, 7675–7678. https://doi.org/10.1016/j.biortech.2010.04.084.

Kassim, M.A., Meng, T.K., 2017. Carbon dioxide (CO2) biofixation by microalgae and its potential for biorefinery and biofuel production. Sci. Total Envir on. 585, 1121–1129. https://doi.org/10.1016/j.scitotenv.2017.01.172.

Kavitha, G., Kurinjimalar, C., Sivakumar, K., Kaarthik, M., Aravind, R., Palani, P., Rengasamy, R., 2016a. Optimization of polyhydroxybutyrate production utilizing waste water as nutrient source by *Botryococcus braunii* Kütz using response surface methodology. Int. J. Biol. Macromol. 93, 534–542. https://doi.org/10.1016/j.ijbiomac.2016.09.019.

Kavitha, G., Kurinjimalar, C., Sivakumar, K., Palani, P., Rengasamy, R., 2016b. Biosynthesis, purification and characterization of polyhydroxybutyrate from *Botryococcus braunii* kütz. Int. J. Biol. Macromol. 89, 700–706. https://doi.org/10.1016/j.ijbiomac.2016.04.086.

Khanna, S., Srivastava, A.K., 2005. Recent advances in microbial polyhydroxyalkanoates. Process Biochem. 40, 607–619. https://doi.org/10.1016/j.procbio.2004.01.053.

Khardenavis, A.A., Suresh Kumar, M., Mudliar, S.N., Chakrabarti, T., 2007. Biotechnological conversion of agro-industrial wastewaters into biodegradable plastic, poly β-hydroxybutyrate. Bioresour. Technol. 98, 3579–3584. https://doi.org/10.1016/j.biortech.2006.11.024.

Khosravi-Darani, K., 2015. Application of poly(hydroxyalkanoate) in food packaging: improvements by nanotechnology. Chem. Biochem. Eng. Q. 29 (2), 275–285. https://doi.org/10.15255/cabeq.2014.2260.

Klasener, C., Alberto, J., Costa, V., De Morais, M.G., 2018. Polyhydroxybutyrate (PHB) synthesis by *Spirulina* sp. LEB 18 using biopolymer extraction waste. Appl. Biochem. Biotechnol. 185, 822–833.

Koch, M., Berendzen, K.W., Forchhammer, K., 2020. On the role and production of polyhydroxybutyrate (Phb) in the cyanobacterium *Synechocystis* sp. pcc 6803. Life 10 (4). https://doi.org/10.3390/life10040047.

Koskimäki, J.J., Kajula, M., Hokkanen, J., Ihantola, E.L., Kim, J.H., Hautajärvi, H., Hankala, E., Suokas, M., Pohjanen, J., Podolich, O., Kozyrovska, N., Turpeinen, A., Pääkkönen, M., Mattila, S., Campbell, B.C., Pirttilä, A.M., 2016. Methyl-esterified 3-hydroxybutyrate oligomers protect bacteria from hydroxyl radicals. Nat. Chem. Biol. 12, 332–338. https://doi.org/10.1038/nchembio.2043.

Kosseva, M.R., Rusbandi, E., 2018. Trends in the biomanufacture of polyhydroxyalkanoates with focus on downstream processing. Int. J. Biol. Macromol. 107, 762–778. https://doi.org/10.1016/j.ijbiomac.2017.09.054.

Kourmentza, C., Ntaikou, I., Kornaros, M., Lyberatos, G., 2009. Production of PHAs from mixed and pure cultures of *Pseudomonas* sp. using short-chain fatty acids as carbon source under nitrogen limitation. Desalination 248, 723–732. https://doi.org/10.1016/j.desal.2009.01.010.

Kovalcik, A., Meixner, K., Mihalic, M., Zeilinger, W., Fritz, I., Fuchs, W., Kucharczyk, P., Stelzer, F., Drosg, B., 2017. Characterization of polyhydroxyalkanoates produced by *Synechocystis salina* from digestate supernatant. Int. J. Biol. Macromol. 102, 497–504. https://doi.org/10.1016/j.ijbiomac.2017.04.054.

Kumar, P., Kim, B.S., 2018. Valorization of polyhydroxyalkanoates production process by co-synthesis of value-added products. Bioresour. Technol. 269, 544–556. https://doi.org/10.1016/j.biortech.2018.08.120.

Kumar, M., Mudliar, S.N., Reddy, K.M.K., Chakrabarti, T., 2004. Production of biodegradable plastics from activated sludge generated from a food processing industrial wastewater treatment plant. Bioresource Technology 95 (3), 327–330. https://doi.org/10.1016/j.biortech.2004.02.019.

Lathwal, P., Nehra, K., Singh, M., Jamdagni, P., Rana, J.S., 2015. Optimization of culture parameters for maximum polyhydroxybutyrate production by selected bacterial strains isolated from rhizospheric soils. Polish J. Microbiol. 64, 227–239.

Lee, S.Y.U.P., Choi, J., Han, K., Song, J.I.Y., 1999. Removal of endotoxin during purification of poly (3-hydroxybutyrate) from Gram-negative bacteria. 65, 2762–2764.

Li, Y., Strathmann, T.J., 2019. Kinetics and mechanism for hydrothermal conversion of polyhydroxybutyrate (PHB) for wastewater valorization. Green Chem. 21, 5586–5597. https://doi.org/10.1039/c9gc02507c.

Liu, Q., Luo, G., Rong, X., Chen, G., 2011. Biosynthesis of poly(3-hydroxydecanoate) and 3-hydroxydodecanoate dominating polyhydroxyalkanoates by b-oxidation pathway inhibited *Pseudomonas putida*. Metab. Eng. 13, 11–17. https://doi.org/10.1016/j.ymben.2010.10.004.

Löwe, H., Hobmeier, K., Moos, M., Kremling, A., Pflüger-Grau, K., 2017. Photoautotrophic production of polyhydroxyalkanoates in a synthetic mixed culture of *Synechococcus elongatus* cscB and *Pseudomonas putida* cscAB. Biotechnol. Biofuels 10 (2017), 190. https://doi.org/10.1186/s13068-017-0875-0.

Mango Materials, n.d. Innovation_Mango Materials [Assessed Online 09/05/2020]. 2020. URL https://www.mangomaterials.com/innovation/.

Manna, A., Banerjee, R., Paul, A.K., 1999. Accumulation of poly (3-hydroxybutyric acid) by some soil Streptomyces. Curr. Microbiol. 39, 153–158. https://doi.org/10.1007/s002849900437.

Marang, L., Jiang, Y., van Loosdrecht, M.C.M., Kleerebezem, R., 2014. Impact of non-storing biomass on PHA production: an enrichment culture on acetate and methanol. Int. J. Biol. Macromol. 71, 74–80. https://doi.org/10.1016/j.ijbiomac.2014.04.051.

Markl, E., Hannes, G., Maximilian, L., 2018. PHB - bio based and biodegradable replacement for PP: a review. Novel Techniques in Nutrition & Food Science 2 (5), 206–209. https://doi.org/10.31031/ntnf.2018.02.000546.

Martínez, V., Herencias, C., Jurkevitch, E., Prieto, M.A., 2016. Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: The case of the polyhydroxyalkanoates. Scientific Reports 6 (April), 1–12. https://doi.org/10.1038/srep24381.

Mathuriya, A.S., Yakhmi, J.V., 2019. Polyhydroxyalkanoates: biodegradable plastics and their applications. Handb. Ecomater. 4, 2873–2900. https://doi.org/10.1007/978-3-319-68255-6_84.

Meixner, K., Fritz, I., Daffert, C., Markl, K., Fuchs, W., Drosg, B., 2016. Processing recommendations for using low-solids digestate as nutrient solution for poly-ß-hydroxybutyrate production with *Synechocystis salina*. J. Biotechnol. 240, 61–67. https://doi.org/10.1016/j.jbiotec.2016.10.023.

Meixner, K., Troschl, C., Fritz, I., Drosg, B., 2017. Cyanobacteria for bioplastic production. Univ. Nat. Resour. Life Sci. Vienna Dep. Agrobiotechnology. IFA-Tulln Inst. Environ. Biotechnol. https://doi.org/10.13140/RG.2.2.32919.27049.

Mendhulkar, V.D., Shetye, L.A., 2017. Synthesis of biodegradable polymer polyhydroxyalkanoate (PHA) in cyanobacteria *Synechococcus elongates* under mixotrophic nitrogen- and phosphate-mediated stress conditions. Ind. Biotechnol. 13, 85–93. https://doi.org/10.1089/ind.2016.0021.

Mezzina, M.P., Pettinari, M.J., 2016. Phasins, multifaceted polyhydroxyalkanoate granule-associated proteins. Appl. Environ. Microbiol. 82, 5060–5067. https://doi.org/10.1128/AEM.01161-16.

Moita, R., Lemos, P.C., 2012. Biopolymers production from mixed cultures and pyrolysis by-products. J. Biotechnol. 157, 578–583. https://doi.org/10.1016/j.jbiotec.2011.09.021.

Monshupanee, T., Incharoensakdi, A., 2014. Enhanced accumulation of glycogen, lipids and polyhydroxybutyrate under optimal nutrients and light intensities in the cyanobacterium *Synechocystis* sp. PCC 6803. J. Appl. Microbiol. 116, 830–838. https://doi.org/10.1111/jam.12409.

Monshupanee, T., Nimdach, P., Incharoensakdi, A., 2016. Two-stage (photoautotrophy and heterotrophy) cultivation enables efficient production of bioplastic poly-3-hydroxybutyrate in auto-sedimenting cyanobacterium. Sci. Rep. 6, 1–9. https://doi.org/10.1038/srep37121.

Montiel Corona, V., Le Borgne, S., Revah, S., Morales, M., 2017. Effect of light-dark cycles on hydrogen and poly-β-hydroxybutyrate production by a photoheterotrophic culture and *Rhodobacter capsulatus* using a dark fermentation effluent as substrate. Bioresour. Technol. 226, 238–246. https://doi.org/10.1016/j.biortech.2016.12.021.

Montiel-Jarillo, G., Carrera, J., Suárez-Ojeda, M.E., 2017. Enrichment of a mixed microbial culture for polyhydroxyalkanoates production: effect of pH and N and P concentrations. Sci. Total Environ. (2017) https://doi.org/10.1016/j.scitotenv.2017.01.069.

Moralejo-Gárate, H., Palmeiro-Sánchez, T., Kleerebezem, R., Mosquera-Corral, A., Campos, J.L., van Loosdrecht, M.C.M., 2013. Influence of the cycle length on the production of PHA and polyglucose from glycerol by bacterial enrichments in sequencing batch reactors. Biotechnol. Bioeng. 110, 3148–3155. https://doi.org/10.1002/bit.24991.

Morgan-Sagastume, F., Valentino, F., Hjort, M., Cirne, D., Karabegovic, L., Gerardin, F., Johansson, P., Karlsson, A., Magnusson, P., Alexandersson, T., Bengtsson, S., Majone, M., Werker, A., 2014. Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. Water Sci. Technol. 69, 177–184. https://doi.org/10.2166/wst.2013.643.

Morgan-Sagastume, F., Heimersson, S., Laera, G., Werker, A., Svanström, M., 2016. Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment. J. Clean. Prod. 137, 1368–1381. https://doi.org/10.1016/j.jclepro.2016.08.008.

Mostafa, Y.S., Alrumman, S.A., Alamri, S.A., Otaif, K.A., Mostafa, M.S., Alfaify, A.M., 2020. Bioplastic (poly-3-hydroxybutyrate) production by the marine bacterium *Pseudodonghicola xiamenensis* through date syrup valorization and structural assessment of the biopolymer. Sci. Rep. 10 (1), 1–13. https://doi.org/10.1038/s41598-020-65858-5.

Mozumder, M.S.I., Goormachtigh, L., Garcia-Gonzalez, L., De Wever, H., Volcke, E.I.P., 2014. Modeling pure culture heterotrophic production of polyhydroxybutyrate (PHB). Bioresour. Technol. 155, 272–280. https://doi.org/10.1016/j.biortech.2013.12.103.

Muhammadi, Shabina, Afzal, M., Hameed, S., 2015. Bacterial polyhydroxyalkanoates-eco-friendly next generation plastic: production, biocompatibility, biodegradation, physical properties and applications. Green Chem. Lett. Rev. 8, 56–77. https://doi.org/10.1080/17518253.2015.1109715.

Nakaya, Y., Iijima, H., Takanobu, J., Watanabe, A., Hirai, M.Y., Osanai, T., 2015. One day of nitrogen starvation reveals the effect of sigE and rre37 overexpression on the expression of genes related to carbon and nitrogen metabolism in *Synechocystis* sp. PCC 6803. J. Biosci. Bioeng. 120, 128–134. https://doi.org/10.1016/j.jbiosc.2014.12.020.

Narancic, T., Scollica, E., Kenny, S.T., Gibbons, H., Carr, E., Brennan, L., Cagney, G., Wynne, K., Murphy, C., Raberg, M., Heinrich, D., Steinbüchel, A., O'Connor, K.E., 2016. Understanding the physiological roles of polyhydroxybutyrate (PHB) in *Rhodospirillum rubrum* S1 under aerobic chemoheterotrophic conditions. Appl. Microbiol. Biotechnol. 100 (20), 8901–8912. https://doi.org/10.1007/s00253-016-7711-5.

Nath, A., Dixit, M., Bandiya, A., Chavda, S., Desai, A.J., 2008. Enhanced PHB production and scale up studies using cheese whey in fed batch culture of *Methylobacterium* sp. ZP24. Bioresour. Technol. 99, 5749–5755. https://doi.org/10.1016/j.biortech.2007.10.017.

Neves, A., Müller, J., 2012. Use of enzymes in extraction of polyhydroxyalkanoates produced by Cupriavidus necator. Biotechnology Progress 28 (6), 1575–1580. https://doi.org/10.1002/btpr.1624.

Obruca, S., Marova, I., Melusova, S., Mravcova, L., 2011. Production of polyhydroxyalkanoates from cheese whey employing *Bacillus megaterium* CCM 2037. Ann. Microbiol. 61, 947–953. https://doi.org/10.1007/s13213-011-0218-5.

Ong, S.Y., Kho, H.P., Riedel, S.L., Kim, S.W., Gan, C.Y., Taylor, T.D., Sudesh, K., 2018. An integrative study on biologically recovered polyhydroxyalkanoates (PHAs) and simultaneous assessment of gut microbiome in yellow mealworm. Journal of Biotechnology 265, 31–39. https://doi.org/10.1016/j.jbiotec.2017.10.017.

Oswald, A.W.J., Gotaas, H.B., Golueke, C.G., Kellen, W.R., Gloyna, E.F., Sewage, S., Wastes, I., Apr, N., Oswald, J., Kellen, R., 1957. Algae in waste treatment [with discussion] all use subject to JSTOR terms and conditions in waste. Sewage Ind. Waste 29, 437–457.

Ozdemir, S., Akman, D., Cirik, K., 2014. Effect of cycle time on polyhydroxybutyrate (PHB) production in aerobic mixed cultures. Appl. Biochem. Biotechnol. 172, 2390–2399. https://doi.org/10.1007/s12010-013-0676-2.

Pakalapati, H., Chang, C.K., Show, P.L., Arumugasamy, S.K., Lan, J.C.W., 2018. Development of polyhydroxyalkanoates production from waste feedstocks and applications. J. Biosci. Bioeng. 126, 282–292. https://doi.org/10.1016/j.jbiosc.2018.03.016.

Patel, V.K., Sundaram, S., Kumar, A., Alok, P., 2018. Characterization of seven species of cyanobacteria for high-quality biomass production. Arab. J. Sci. Eng. 43, 109–121. https://doi.org/10.1007/s13369-017-2666-0.

Philip, S., Sengupta, S., Keshavarz, T., Roy, I., 2009. Effect of impeller speed and pH on the production of poly(3- hydroxybutyrate) using *Bacillus cereus* SPV. Biomacromolecules 10, 691–699. https://doi.org/10.1021/bm801395p.

Pisco, A.R., Bengtsson, S., Werker, A., Reis, M.A.M., Lemos, P.C., 2009. Community structure evolution and enrichment of glycogen-accumulating organisms producing polyhydroxyalkanoates from fermented molasses. Appl. Environ. Microbiol. 75, 4676–4686. https://doi.org/10.1128/AEM.02486-08.

Poirier, Y., Somerville, C., Schechtman, L.A., Satkowski, M.M., Noda, I., 1995. Synthesis of high-molecular-weight transgenic *Arabidopsis thaliana* plant cells. Int. J. Biol. Macromol. 17, 7–12.

Policastro, G., Luongo, V., Fabbricino, M., 2020. Biohydrogen and poly-β-hydroxybutyrate production by winery wastewater photofermentation: Effect of substrate concentration and nitrogen source. Journal of Environmental Management 271 (May), 111006. https://doi.org/10.1016/j.jenvman.2020.111006.

Qiu, S., Wang, L., Champagne, P., Cao, G., Chen, Z., Wang, S., Ge, S., 2019a. Effects of crystalline nanocellulose on wastewater-cultivated microalgal separation and biomass composition. Appl. Energy 239, 207–217. https://doi.org/10.1016/j.apenergy.2019.01.212.

Qiu, S., Wang, S., Xiao, C., Ge, S., 2019b. Assessment of microalgae as a new feeding additive for fruit fly *Drosophila melanogaster*. Sci. Total Environ. 667, 455–463. https://doi.org/10.1016/j.scitotenv.2019.02.414.

Qiu, S., Shen, Y., Zhang, L., Ma, B., Amadu, A.A., Ge, S., 2020a. Antioxidant assessment of wastewater-cultivated *Chlorella sorokiniana* in *Drosophila melanogaster*. Algal Res. 46, 101795. https://doi.org/10.1016/j.algal.2020.101795.

Qiu, S., Wang, L., Chen, Z., Yang, M., Yu, Z., Ge, S., 2020b. An integrated mainstream and sidestream strategy for overcoming nitrite oxidizing bacteria adaptation in a continuous plug-flow nutrient removal process. Bioresour. Technol. 319, 124133. https://doi.org/10.1016/j.biortech.2020.124133.

Quagliano, J.C., Miyazaki, S.S., 1999. Biosynthesis of poly-β-hydroxybutyrate and exopolysaccharides on *Azotobacter chroococcum* strain 6B utilizing simple. Appl. Biochem. Biotechnol. 82, 199–208.

Rahman, A., Putman, R.J., Inan, K., Sal, F.A., Sathish, A., Smith, T., Nielsen, C., Sims, R.C., Miller, C.D., 2015. Polyhydroxybutyrate production using a wastewater microalgae based media. Algal Res. 8, 95–98. https://doi.org/10.1016/j.algal.2015.01.009.

Ranade, V.V., Bhandari, V.M., 2014. Industrial Wastewater Treatment, Recycling, and Reuse-Past, Present and Future. Elsevier Inc., pp. 521–535 https://doi.org/10.1016/B978-0-08-099968-5.00014-3.

Ranaivoarisoa, T.O., Singh, R., Rengasamy, K., Guzman, M.S., Bose, A., 2019. Towards sustainable bioplastic production using the photoautotrophic bacterium *Rhodopseudomonas palustris* TIE-1. J. Ind. Microbiol. Biotechnol. 46 (9–10), 1401–1417. https://doi.org/10.1007/s10295-019-02165-7.

Raza, Z.A., Abid, S., Banat, I.M., 2018. Polyhydroxyalkanoates: characteristics, production, recent developments and applications. Int. Biodeterior. Biodegrad. 126, 45–56. https://doi.org/10.1016/j.ibiod.2017.10.001.

Rodgers, M., Wu, G., 2010. Production of polyhydroxybutyrate by activated sludge performing enhanced biological phosphorus removal. Bioresource Technology 101 (3), 1049–1053. https://doi.org/10.1016/j.biortech.2009.08.107.

Rodriguez-Perez, S., Serrano, A., Pantión, A.A., Alonso-Fariñas, B., 2018. Challenges of scaling-up PHA production from waste streams. A review. J. Environ. Manag. 205, 215–230. https://doi.org/10.1016/j.jenvman.2017.09.083.

Roja, K., Ruben Sudhakar, D., Anto, S., Mathimani, T., 2019. Extraction and characterization of polyhydroxyalkanoates from marine green alga and cyanobacteria. Biocatal. Agric. Biotechnol. 22, 101358. https://doi.org/10.1016/j.bcab.2019.101358.

Roland-holst, D., Heft-neal, S., 2013. Bioplastics in California Economic Assessment of Market Conditions for PHA/PHB Bioplastics Produced From Waste Methane.

Rosengart, A., Cesário, M.T., De Almeida, M.C.M.D., Raposo, R.S., Espert, A., Díaz, E., Apodaca, D., Manuela, M.R., 2015. Efficient P (3HB) extraction from *Burkholderia sacchari* cells using non-chlorinated solvents. Biochem. Eng. J. 103, 39–46. https://doi.org/10.1016/j.bej.2015.06.013.

Ruiz, J.A., López, N.I., Fernández, R.O., Méndez, B.S., 2001. Polyhydroxyalkanoate degradation is associated with nucleotide accumulation and enhances stress resistance and survival of *Pseudomonas oleovorans* in natural water microcosms. Appl. Environ. Microbiol. 67, 225–230. https://doi.org/10.1128/AEM.67.1.225-230.2001.

Sabarinathan, D., Chandrika, S.P., Venkatraman, P., Easwaran, M., Sureka, C.S., Preethi, K., 2018. Production of polyhydroxybutyrate (PHB) from *Pseudomonas plecoglossicida* and its application towards cancer detection. Informatics in Medicine Unlocked 11 (March), 61–67. https://doi.org/10.1016/j.imu.2018.04.009.

Salgaonkar, B.B., Bragança, J.M., 2017. Utilization of Sugarcane Bagasse by *Halogeometricum borinquense* Strain E3 for Biosynthesis of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate). https://doi.org/10.3390/bioengineering4020050.

Samantaray, S., Nayak, J.K., Mallick, N., 2011. Wastewater utilization for poly-β-hydroxybutyrate production by the cyanobacterium Aulosira fertilissima in a recirculatory aquaculture system. Applied and Environmental Microbiology 77 (24), 8735–8743. https://doi.org/10.1128/AEM.05275-11.

Sangyoka, S., Poomipuk, N., Reungsang, A., 2012. Optimum conditions for the production of polyhydroxybutyrate from cassava wastewater by the newly isolated *Cupriavidus* sp. KKU38. Sains Malaysiana 41, 1211–1216.

Sathya, A.B., Sivashankar, R., Sivasubramanian, V., Santhiagu, A., 2018. Green algae as source for polyhydroxyalkanoate synthesis from food processing wastewater. Res. J. Chem. Environ. 22, 66–74.

Sauer, Jörg, Schreiber, U., Schmid, R., Völker, U., Physiology, S.P., May, N., Sauer, Jorg, Schreiber, U., Schmid, R., Volker, U., Forchhammer, K., 2001. Nitrogen starvation-induced chlorosis in *Synechococcus* PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Am. Soc. Plant Biol. 126, 233–243.

Sedlacek, P., Slaninova, E., Koller, M., Nebesarova, J., Marova, I., Krzyzanek, V., Obruca, S., 2019. PHA granules help bacterial cells to preserve cell integrity when exposed to sudden osmotic imbalances. New Biotechnol. 49, 129–136. https://doi.org/10.1016/j.nbt.2018.10.005.

Sen, K.Y., Hussin, M.H., Baidurah, S., 2019. Biosynthesis of poly(3-hydroxybutyrate) (PHB) by Cupriavidus necator from various pretreated molasses as carbon source. Biocatalysis and Agricultural Biotechnology 17 (August 2018), 51–59. https://doi.org/10.1016/j.bcab.2018.11.006.

Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M., 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnol. Bioeng. 87, 145–160. https://doi.org/10.1002/bit.20085.

Serafim, L.S., Lemos, P.C., Albuquerque, M.G.E., Reis, M.A.M., 2008. Strategies for PHA production by mixed cultures and renewable waste materials. Appl. Microbiol. Biotechnol. 81, 615–628. https://doi.org/10.1007/s00253-008-1757-y.

Shalin, T., Sindhu, R., Binod, P., Soccol, C.R., Pandey, A., 2014. Mixed cultures fermentation for the production of poly-β-hydroxybutyrate. Brazilian. Arch. Biol. Technol. 57, 644–652. https://doi.org/10.1590/S1516-89132013005000016.

Sharifzadeh, M., Branch, A., Najafpour, G., Younesi, H., 2010. Optimization PHAs Production from Dairy Industry Wastewater (Cheese Whey) by Azohydromonas lata DSMZ 1123. Iranica Journal of Energy and Environment 1 (2), 132–136.

Shen, Y., Qiu, S., Chen, Z., Zhang, Y., Trent, J., Ge, S., 2020. Free ammonia is the primary stress factor rather than total ammonium to *Chlorella sorokiniana* in simulated sludge fermentation liquor. Chem. Eng. J. 397, 125490. https://doi.org/10.1016/j.cej.2020.125490.

Shweta, S., Samuel, K., 2015. Study of Cyanobacteria as biofertilizer from the rice field. world J. Pharm. Res 4, 1696–1706.

Silva-queiroz, S.R., Silva, L.F., Pradella, J.G.C., Pereira, E.M., Gomez, J.G.C., 2009. PHA MCL biosynthesis systems in *Pseudomonas aeruginosa* and *Pseudomonas putida* strains show differences on monomer specificities. J. Biotechnol. 143, 111–118. https://doi.org/10.1016/j.jbiotec.2009.06.014.

Singh, G., Kumari, A., Mittal, A., Goel, V., Yadav, A., Aggarwal, N.K., 2013. Cost Effective Production of Poly-β-Hydroxybutyrate by Bacillus subtilis NG05 Using Sugar Industry Waste Water. Journal of Polymers and the Environment 21 (2), 441–449. https://doi.org/10.1007/s10924-012-0533-3.

Singh, A.K., Srivastava, J.K., Chandel, A.K., Sharma, L., Mallick, N., Singh, S.P., 2019. Biomedical applications of microbially engineered polyhydroxyalkanoates: an insight into recent advances, bottlenecks, and solutions. Appl. Microbiol. Biotechnol. 103, 2007–2032. https://doi.org/10.1007/s00253-018-09604-y.

Soni, R.A., Sudhakar, K., Rana, R.S., 2017. *Spirulina* – from growth to nutritional product: a review. Trends Food Sci. Technol. 69, 157–171. https://doi.org/10.1016/j.tifs.2017.09.010.

Steinbüchel, A., Füchtenbusch, B., 1998. Bacterial and other biological systems for polyester production. Trends Biotechnol. 16, 419–427. https://doi.org/10.1016/S0167-7799(98)01194-9.

Sung, Y.J., Kim, J.Y.H., Bong, K.W., Sim, S.J., 2016. Microdroplet photobioreactor for the photoautotrophic culture of microalgal cells. Analyst 141 (3), 989–998. https://doi.org/10.1039/c5an02211h.

Suriyamongkol, P., Weselake, R., Narine, S., Moloney, M., Shah, S., 2007. Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants - a review. Biotechnol. Adv. 25, 148–175. https://doi.org/10.1016/j.biotechadv.2006.11.007.

Sutherland, D.L., Howard-Williams, C., Turnbull, M.H., Broady, P.A., Craggs, R.J., 2015. Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol. 184, 222–229. https://doi.org/10.1016/j.biortech.2014.10.074.

Takeshita, T., Ota, S., Yamazaki, T., Hirata, A., Zachleder, V., Kawano, S., 2014. Starch and lipid accumulation in eight strains of six *Chlorella* species under comparatively high light intensity and aeration culture conditions. Bioresour. Technol. 158, 127–134. https://doi.org/10.1016/j.biortech.2014.01.135.

Tan, S.M.A., Govindasamy, S., Tamothran, A.M., Vigneswari, S., Bhubalan, K., 2019. Applications of PHA in agriculture. Biotechnological Applications of Polyhydroxyalkanoates, 1–420 https://doi.org/10.1007/978-981-13-3759-8.

Tavernier, P., Portais, J.C., Nava Saucedo, J.E., Courtois, J., Courtois, B., Barbotin, J.N., 1997. Exopolysaccharide and poly-β-hydroxybutyrate coproduction in two R*hizobium meliloti* strains. Appl. Environ. Microbiol. 63, 21–26.

Technavio, 2020. Polyhydroxyalkanoate Market, Size, Growth, Trends, Industry Analysis & Forecast.

Teixeira, C.S., Holthman, F., Pedrosa, D.O., De Souza, M., 2019. Importance of poly-3-hydroxybutyrate metabolism to the ability of *Herbaspirillum seropedicae* to promote plant. Appl. Environ. Microbiol. 1–14.

Thapa, C., Shakya, P., Shrestha, R., Pal, S., Manandhar, P., 2019. Isolation of polyhydroxybutyrate (PHB) producing bacteria, optimization of culture conditions for PHB production, extraction and characterization of PHB. Nepal Journal of Biotechnology 6 (1), 62–68. https://doi.org/10.3126/njb.v6i1.22339.

Timm, A., Byrom, D., Steinbüchel, A., 2004. Formation of blends of various poly(3-hydroxyalkanoic acids) by a recombinant strain of *Pseudomonas oleovorans*. Appl. Microbiol. Biotechnol. 33, 296–301. https://doi.org/10.1007/BF00164525.

Tohme, S., Hacıosmanoğlu, G.G., Eroğlu, M.S., Kasavi, C., Genç, S., Can, Z.S., Toksoy Oner, E., 2018. *Halomonas smyrnensis* as a cell factory for co-production of PHB and levan. Int. J. Biol. Macromol. 118, 1238–1246. https://doi.org/10.1016/j.ijbiomac.2018.06.197.

Touloupakis, E., Cicchi, B., Margarita, A., Benavides, S., Torzillo, G., 2016. Effect of high pH on growth of Synechocystis sp. PCC 6803 cultures and their contamination by golden algae (*Poterioochromonas* sp.). Appl. Microbiol. Biotechnol. 100, 1333–1341. https://doi.org/10.1007/s00253-015-7024-0.

Trainer, M.A., Charles, T.C., 2006. The role of PHB metabolism in the symbiosis of rhizobia with legumes. Appl. Microbiol. Biotechnol. 71, 377–386. https://doi.org/10.1007/s00253-006-0354-1.

Tripathi, A.D., Yadav, A., Jha, A., Srivastava, S.K., 2012. Utilizing of Sugar Refinery Waste (Cane Molasses) for Production of Bio-Plastic Under Submerged Fermentation Process. Journal of Polymers and the Environment 20 (2), 446–453. https://doi.org/10.1007/s10924-011-0394-1.

Tripathi, L., Wu, L., Dechuan, M., Chen, J., Wu, Q., Chen, G., 2013. *Pseudomonas putida* KT2442 as a platform for the biosynthesis of polyhydroxyalkanoates with adjustable monomer contents and compositions. Bioresour. Technol. 142, 225–231. https://doi.org/10.1016/j.biortech.2013.05.027.

Troschl, C., Meixner, K., Fritz, I., Leitner, K., Palacios, A., Kovalcik, A., Sedlacek, P., Drosg, B., 2018. Pilot-scale production of poly- β -hydroxybutyrate with the cyanobacterium *Synechocytis* sp. CCALA192 in a non-sterile tubular photobioreactor. Algal Res. 34, 116–125. https://doi.org/10.1016/j.algal.2018.07.011.

Uchino, K., Saito, T., Jendrossek, D., 2008. Poly(3-hydroxybutyrate) (PHB) depolymerase PhaZa1 is involved in mobilization of accumulated PHB in *Ralstonia eutropha* H16. Appl. Environ. Microbiol. 74, 1057–1063. https://doi.org/10.1128/AEM.02342-07.

Urtuvia, V., Villegas, P., González, M., Seeger, M., 2014. Bacterial production of the biodegradable plastics polyhydroxyalkanoates. Int. J. Biol. Macromol. 70, 208–213. https://doi.org/10.1016/j.ijbiomac.2014.06.001.

Wang, Y., Wu, H., Jiang, X., Chen, G., 2014. Engineering Escherichia coli for enhanced production of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) in larger cellular space. Metab. Eng. 25, 183–193. https://doi.org/10.1016/j.ymben.2014.07.010.

Wang, X., He, Q., Yang, Y., Wang, J., Haning, K., Hu, Y., Wu, B., He, M., Zhang, Y., Bao, J., Contreras, L.M., Yang, S., 2018. Advances and prospects in metabolic engineering of *Zymomonas mobilis*. Metab. Eng. 50, 57–73. https://doi.org/10.1016/j.ymben.2018.04.001.

Wu, H., Fan, Z., Jiang, X., Chen, J., Chen, G.Q., 2016. Enhanced production of polyhydroxybutyrate by multiple dividing *E. coli*. Microb. Cell Fact 15, 1–13. https://doi.org/10.1186/s12934-016-0531-6.

Xu, M., Qin, J., Rao, Z., You, H., Zhang, X., Yang, T., Wang, X., 2016. Effect of polyhydroxybutyrate (PHB) storage on l-arginine production in recombinant Corynebacterium crenatum using coenzyme regulation. Microb. Cell Factories 15, 1–12. https://doi.org/10.1186/s12934-016-0414-x.

Xu, X., Ma, B., Lu, W., Feng, D., Wei, Y., Ge, C., Peng, Y., 2020. Effective nitrogen removal in a granule-based partial-denitrification/anammox reactor treating low C/N sewage. Bioresour. Technol. 297, 122467. https://doi.org/10.1016/j.biortech.2019.122467.

Xu, M., Tremblay, P.L., Ding, R., Xiao, J., Wang, J., Kang, Y., Zhang, T., 2020. Photo-augmented PHB production from CO2 or fructose by *Cupriavidus necator* and shape-optimized CdS nanorods. Sci. Total Environ. 753, 142050. https://doi.org/10.1016/j.scitotenv.2020.142050.

Yates, M.R., Barlow, C.Y., 2013. Life cycle assessments of biodegradable, commercial biopolymers - a critical review. Resour. Conserv. Recycl. 78, 54–66. https://doi.org/10.1016/j.resconrec.2013.06.010.

Yuan, Q., Sparling, R., Oleszkiewicz, J., 2015. Polyhydroxybutyrate production from municipal wastewater activated sludge with different carbon sources. Air, Soil Water Res 8, 53–58. https://doi.org/10.4137/ASWR.S27218.

Zinn, M., Witholt, B., Egli, T., 2001. Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. Pulp Pap. Week. 53, 5–21. https://doi.org/10.1016/S0169-409X(01)00218-6.