Photosynthetic microorganisms producing polyhydroxyalkanoates: Production, extraction, biosynthesis and alternative application in active packaging incorporated with essential oils

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ABSTRACT

Biopolymers have vast applicability, besides being biodegradable sources and presenting relatively shorter life cycles when compared to fossil energy sources. Some of these biopolymers are polyhydroxyalkanoates (PHAs), a class of polymers with the ability to form plastic membranes, similar to petrochemical plastics. Several studies suggest that microalgae/cyanobacteria are types of photosynthetic microorganisms that can be used to obtain PHAs at a lower cost because they have minimal nutritional requirements for growth and are naturally photoautotrophic, meaning they use light and CO 2 as their main energy sources. Furthermore, microalgae have potential for high productivity, are tolerant to changes in environmental conditions, and can be cultivated in areas unsuitable for agriculture. These PHA plastic membranes produced by these photosynthetic microorganisms can be an alternative for constructing a functional film with great antimicrobial characteristics when incorporated with essential oils, the famous active packaging, the future of packaging industries. This work demonstrates the production, extraction, biosynthesis, and application perspectives of these biopolymers in packaging industries, such as films incorporated with essential oils.

Keywords: Microalgae, Cyanobacteria, Bioplastic, Biopolymer, Polyhydroxyalkanoate, Essential oils.

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INTRODUCTION

Polymers have a variety of applications, which make them a necessary tool in society. Lowering production costs, minimizing energy consumption and environmental pollution, as well as reducing the generation of gases harmful to the environment. In addition to being versatile, with protective and preservative properties (electrical, acoustic and thermal) (Jamnongkan et al., 2022; Rosenboom et al., 2022). According to Wu et al. (2017), some polymers have the ability to form very thin films and films, which are attractive in production and application in food packaging.

These polymers, called polyhydroxyalkanoates (PHAs), are a class of polyesters that can be produced by a diversity of microorganisms, including photosynthetic microorganisms (Silva and Houllou, 2022), as alternative, ecological, important and sustainable sources of monomers for the production of bioplastics (Coppola et al., 2021; Nandal et al., 2022). The biosynthesis of these bioplastics is generally carried out under nutrient-limiting conditions, such as nitrogen, phosphorus, sulfur and excess carbon. However, the most interesting thing is that under aerobic conditions, several microorganisms can degrade these PHA films and generate carbon dioxide and water, while under anaerobic conditions they can generate methane and water (Surendran et al., 2020).

In the biosynthesis of PHAs, the main enzymes involved are PHA synthase (phaC) and are divided into gene classes I, II, III and IV. Class II enzymes are responsible for the synthesis of Mcl-PHA (medium chain length), while the rest (I, III and IV) synthesize Scl-PHA (short chain length) (Jia et al., 2016) . The yield of PHAs in photosynthetic microorganisms can vary between 1.0-70% (w/w) and the main tools for obtaining the polymer currently are still the use of sodium hypochlorite/chloroform/methanol (García et al., 2021; Panda et al., 2005;Sharma and Mallick, 2005; Bhati and Mallick, 2015; Ansari and Fatma, 2016). However, the prospect of applying these polymers in the formation of PHA films incorporated with essential oils (OE) to form active packaging (EA) is a major innovation in the packaging industry (Giaquinto et al., 2017).

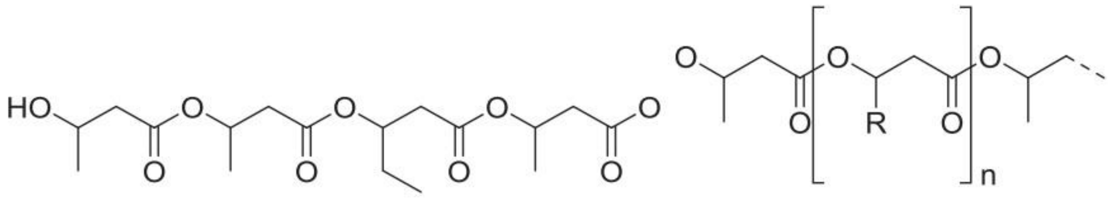
The purpose of this work is to demonstrate that photosynthetic microorganisms can be a low-cost and promising tool in the production of polyhydroxyalkanoates, showing extraction methods, polymer biosynthesis and future perspectives for the application of these PHAs in the formation of thin films incorporated with essential oils, the famous active packaging, the future of the packaging industry.

POLYHYDROXYALKANOATE (PHA)

Large quantities of non-degradable plastics are among the main global problems currently known. Biodegradable biopolymers produced by microorganisms are potential substitutes for plastics of petrochemical origin (Amadu et al., 2021). Polyhydroxyalkanoates (PHAs) are a class of natural esters secreted by microorganisms as intracellular granules under nitrogen-limiting conditions along with excess carbon source. This class of natural esters presents high variability as it covers more than 150 types of monomers and weighs up to 100,000 Da (Dalton). The polymer remains amorphous inside cells due to its stability contributed by phospholipids while it appears crystalline after extraction. Its composition was enunciated by Lemoigne in 1925, although Beijerinck reported its occurrence in 1888 (De Koning, 1993; Pal et al., 1999).

The basic structure of PHAs contains repeating units of aliphatic polyesters (Figure 1) ranging from 600 to 35,000 (Albuquerque and Malafaia, 2018). PHA with n=1 represents a class of poly(3-hydroxyalconates) while n=2 represents poly(4-hydroxyalconates). The type of derivative attached to the side chain (R) of the unit determines the functionality of the monomer. Depending on the number of carbon atoms in a monomeric chain unit, PHAs are classified into three types: short-chain monomers (3 to 5 carbon atoms), medium-chain monomers (6 to 15 carbon atoms), and long chain (more than 15). The type of PHA produced depends on the biochemical pathways inherently (or genetically modified) present in microorganisms (Arumugam, 2020).

Figure 1 - Repeating units of the Polyhydroxyalkanoate monomer.



Source: Reproduced from Koller et al., 2017.

PHA-PRODUCING PHOTOSYNTHETIC MICROORGANISMS

Several photosynthetic microorganisms are producers of PHAs, however, several modeling techniques in the cultivation medium are applied to accumulate this biopolymer inside the cells, among which we can highlight the reduction in the levels of nitrogen, phosphorus , iron and the increase in carbon source demand. Costa et al. (2018A) evaluating the production of PHA from the microalgae *Chlorella minutíssima* and the cyanobacteria *Synechococcus subsalsus* and *Spirulina* sp. LEB-18, in standard media using only salts and with 70% limitation of the inorganic source of nitrogen ($NANO_3$), found that *C. minutíssima* did not produce the biopolymer, however, cyanobacteria managed to produce PHA, and with greater intensity when the nitrogen source was limited. The results show that nitrogen reduction causes notable changes in the biochemical composition of cells, degrading proteins and photosynthetic pigments, but can favor the accumulation of other biomolecules such as lipids and polyhydroxyalkanoates. Costa et al. (2018B) also found that the cyanobacterium *Spirulina* sp. LEB-18 in open raceway culture using Zarrouk medium was also able to produce PHA. Roja et al. (2019) also managed to produce PHA in four different photosynthetic microorganisms: (i) *Chlorella* sp., (ii) *Oscillatoria salina* , (iii) *Leptolyngbya* *valderiana* and (iv) *Synechococcus elongatus* . García et al. (2021) evaluated the production of PHA from the microalgae *Scenedesmus* sp. under conditions of nutrient deficiency. Sixteen different types of modified culture media were prepared by varying the concentrations of nitrogen, phosphorus, iron, salinity and added carbon source (glucose). All media produced the biopolymer, with emphasis on the culture medium composed of: glucose (1 g L^{-1}), nitrogen (17.6 mM), phosphorus (0 mM), iron (0.021 mM) and salinity (0. 5 g L^{-1}). Table 1 shows the production of PHA from various photosynthetic microorganisms. Mourão et al. (2020) also verified the production of PHA from the microalgae *Stigeoclonium* sp. B23 in BG-11 medium supplemented with sodium acetate and sodium bicarbonate as source and carbon, or with carbon/nitrogen deficiency. Silva and Houllou (2022) also managed to obtain the production of PHA using the microalgae *Chlorella vulgaris* and *Tetradesmus obliquus* using Bold's Basal medium supplemented with agro-industrial waste corn.

Table 1 - PHA yield in different types of algae

Seaweed	Performance (%)	References
Anabaena sp.	2.3%	Lama et al., (1996)
Arthrospira subsalsa	14.7%	Shrivastav et al., (2010)
Very fertile aulosira	10%	Samantaray and Mallick (2012)
Botryococcus braunii	16.4%	Kavitha et al. (2016)
Calothrix scytonemicola TISTR 8095	25.2%	Kaewbai-ngam et al., (2016)
Calothrix sp.	6.4%	Bhati et al., (2010)
Microalgae consortium	31%	Rahman et al. (2015)
Nostoc muscorum	8.7 - 69%	(Panda et al., 2005; Sharma and Mallick, 2005; Bhati and Mallick, 2015; Ansari and Fatma, 2016)
Oscillatoria jasorvensis TITR 8980	15.7%	Kaewbai-ngam et al., (2016)
Phaeodactylum tricornutum	10.06%	Hempel et al. (2011)
Phormidium sp. TIST 8462	14.8%	Kaewbai-ngam et al., (2016)
Scenedesmus sp.	0.831 – 29.92%	García et al. (2021)
Scytonema sp.	7.4%	Bhati et al., (2010)
Spirulina sp. LEB-18	12 – 30.7%	(Costa et al., 2018A; Coelho et al., 2015)
Synechococcus elongates	7.02 – 17.15%	Mendhulkar and Shetye (2017)
Synechococcus MA19	55%	Nishioka et al., (2001)
Synechococcus subsalsus	16%	Costa et al. (2018C)
Synechocystis PCC6803	4.1 – 26%	(Khetkorn et al., 2016; Panda and Mallick, 2007; Wu et al., 2001)
Synechocystis saline	5.5 – 6.6%	Kovalcik et al. (2017)
Chlorella sorokiniana SVMIICT8	29.5%	Kumari et al. (2022)

Source: Author.

METHODS FOR EXTRACTING POLYHYDROXYALKANOATES FROM PHOTOSYNTHETIC MICROORGANISMS

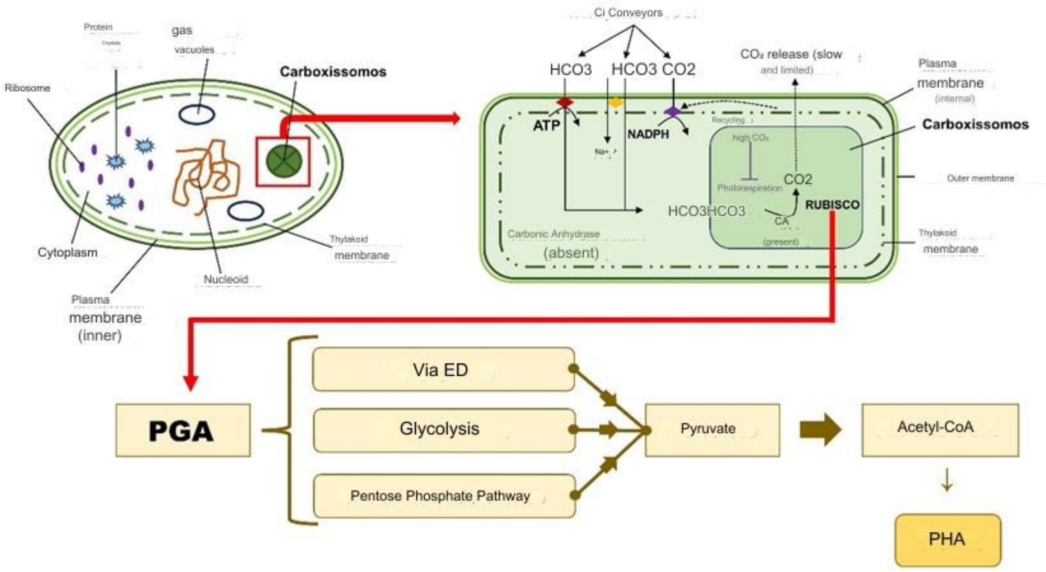
Several extraction methods are applied to obtain PHA polymers. Costa et al. (2018) obtains the biopolymer from an extraction using sodium hypochlorite at a final concentration of 4%, the sample is then incubated at 45 °C for 20 minutes, followed by centrifugation, extraction of the polymer using chloroform and followed by precipitation with methanol. Roja et al. (2019) uses the same extraction method mentioned above to extract the polymer from four species of algae, only modifying the extraction time (30 minutes). In turn, Silva and Houllou (2022) obtained two extracts from two microalgae using simultaneous extraction with 4% hypochlorite + chloroform, followed by centrifugation, collection of the organic phase, evaporation and degreasing with hexane. In studies by García et al. (2021) the dry biomass was first washed with distilled water and ethanol, then the centrifuged pellet was subjected to polymeric extraction with chloroform, and the solution was passed through a glass fiber filter (0.45μm) and the chloroform it is then evaporated in a rotoevaporator, ultimately obtaining the polymer. Kovalcik et al. (2017) obtained the biopolymer from the cyanobacterium *Synechocystis salina* , first using an ultrasonic bath of the biomass in ethanol and acetone, to extract the pigments. Then, PHA was recovered by Soxhlet extraction using hot chloroform, and subsequently precipitated with ice-cold ethanol. Finally, Morais et al. (2015B) evaluated three extraction methods: i) 4% sodium hypochlorite; ii) trichloromethane and methanol precipitation; iii) trichloromethane with pre-treatment with 4% hypochlorite. In general, extractions of polyhydroxyalkanoates from photosynthetic microorganisms are carried out with sodium hypochlorite, chloroform and methanol precipitation.

BIOSYNTHESIS OF POLYHYDROXYALKANOATES

Photosynthetic microorganisms are photoautotrophic organisms that produce primary biomass. These microorganisms have the ability to use minimal inorganic nutrients (CO_2) that are readily available, as well as sunlight and water, demonstrating high photosynthetic efficiency, which gives them the title of "microbial factory", and can generate several products of industrial interest (Rahman et al., 2013, 2014; Singh and Mallick, 2017), such as PHA. Several studies suggest that photosynthetic microorganisms produce several basic materials that can generate plastic biofilms. Poly-3-hydroxybutyrate (PHB) is a type of PHA that can be widely produced by microalgae and cyanobacteria, has a wide application and can be a sustainable alternative for the plastic industry, as it is a bioderived and biodegradable polymer (Costa et al., 2019; Mendhulkar and Shetye 2017;

Under autotrophic conditions, cyanobacteria fix the carbon source in the Calvin-Benson-Bassham (CBB) cycle through ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO). RuBisCO is responsible for assimilating most of the carbon available on Earth, due to its high efficiency in capturing CO_2. Inorganic carbon transporters (Ci) present in the cell wall of photosynthetic microorganisms transport atmospheric CO_2 and help maintain the local carbon concentration for RuBisCO. The Calvin cycle output, glyceraldehyde-3-phosphate after its conversion to 3-phosphoglycerate (PGA), can then enter any of the three pathways for sugar metabolism, i.e. Entner-Doudoroff (ED) pathway, glycolysis , via pentose phosphate and be finally converted into acetyl-CoA to be used in the PHA synthetic pathway (Singh and Mallick, 2017; Figure 2).

Figure 2 - PHA conversion from carbon accumulation and CO $_{2\ \text{fixation}}$ in cyanobacteria



Source: adapted from Afreen et al. 2021.

ESSENCIAL OILS

Essential oils (EOs) consist of secondary metabolites that can protect plants against environmental threats, pathogenic microorganisms, among others, being mixtures of phenols, monoterpenes, sesquiterpenes and other aromatic plant compounds (Ballester-Costa et al., 2017; Sangha et al., 2017). Regarding the physical aspect, EOs are aromatic oily liquids derived from plants and can be extracted from different matrices such as leaves, buds, flowers, seeds, bark, roots, twigs, wood and fruits (Ghabraie et al., 2016; Lee et al. , 2018). They have traditionally been used in natural therapy, food preservation, as complementary medicines in various treatments and as culinary flavorings, given their organoleptic characteristics with great acceptability among consumers (Ballester-Costa et al., 2017; Fratianni et al., 2010). Currently, approximately 3,000 EOs are known and some of them are commercially important, being used in the agricultural, cosmetics - especially perfumery -, chemical, pharmaceutical and food industries due to their bioactive potential, highlighting their antimicrobial potential against different strains bacterial and fungal (Cutillas et al., 2018; Ghabraie et al., 2016; Lagha et al., 2019).

These compounds are generally recognized as safe (GRAS) (Ballester-Costa et al., 2016), and some are approved by the *Food & Drug Administration* (FDA) for use as food additives, such as lemon balm, EOs of basil, coriander, cloves, thyme and vanilla (FDA | US *Food & Drug*

Administration). Therefore, they are gaining interest for their potential as natural preservatives (Ballester-Costa et al., 2016), while their potential as antimicrobial and antioxidant agents provide the basis for many applications in the preservation of processed and raw foods (Ballester-Costa et al., 2013).

APPLICATION OF ACTIVE PACKAGING INCORPORATED WITH ESSENTIAL OILS

Polyhydroxyalkanoates (PHAs) are attractive sources for the polymer industry due to their great properties, such as their high biodegradability and processing versatility, and their potential to replace petrochemical plastics (Bugnicourt et al., 2014) . The use of packaging in the food processing industries is essential to maintain food quality and is being increasingly improved nowadays. Alternatives for the application of PHA packaging incorporated with essential oils (OE), the so-called active packaging (EA), are the most recent biotechnological innovations, and these packagings with antimicrobial characteristics provide great technological application, in addition to improving the mechanical characteristics of polymer matrices (Wani et al., 2014; Muppalla et al., 2014; Giaquinto et al., 2017).

In studies by Basnett et al. (2020) an msc-PHA was produced from the bacteria *Pseudomonas mendocina* using a cheap carbon substrate, sugar cane molasses. Characterization analyzes confirmed that it was a copolymer called P(3HO-co-3HD), which was incorporated with lemon essential oil (LEO) and its antimicrobial action capacity was evaluated. The antimicrobial properties of the film manufactured and incorporated with LEO against *Staphylococcus aureus* and *Escherichia coli* showed high activity against gram-positive bacteria. Storage studies also demonstrated that after one year the films showed a reduction in LEO content, however, they still showed activity against *S. aureus* . These so-called packaging guarantee quality, hygiene, safety and increase the shelf life of food, protecting food from internal and external environmental factors (Gouvêa et al., 2015, Wrona et al., 2015). The main components present in EOs that promote these antimicrobial properties in polymeric matrices are aldehydes, phenols and oxygenated terpenoids. Furthermore, the hydrophobicity of EOs allows lipids found in the membrane of bacterial cells to interact with each other, making the microbial cell wall less stable and permeable, allowing cellular components and ions to escape, which can cause cell death (Ju et al. al., 2017; Khaneghah et al., 2018).

In the analyzes by Silva and collaborators (2020), new antimicrobial films made of polyhydroxybutyrate (PHB) added with polyethylene glycol (PEG) and clove essential oil (CEO), with antimicrobial activity against three bacteria (*E. coli* , *E. aerogenes* and *S. aureus*) were evaluated. The main component found in CEO was eugenol (72.96%), a phenolic molecule found in several aromatic plants. The addition of EO provided greater flexibility and reduced intermolecular interactions between the polymer matrix structures, obtaining less crystalline and consequently more elastic films. The influence of orange essential oil (OEO) on PHB/PEG blends with additives was also evaluated against the bacteria *S. aureus* and *E. coli* , showing that the main component of EO (d-limonene) makes the films more resistant and flexible. mechanically and also has antimicrobial activity (Alves et al., 2021).

In turn, Giaquinto et al. (2017) also evaluated the antimicrobial activity of PHB films, however, incorporated with canola essential oil (CAOE). Mechanical analyzes also showed that films containing CAOE presented greater flexibility, and thermal analyzes showed that the addition of oil also changes the thermal properties of PHB, such as melting and crystallization temperatures, relative crystallinity and maximum crystallization rate. According to Basnett et al. (2020), the feasibility of EOs conferring antimicrobial activity to PHA films may lead to an expansion of the application of these biofilms in various industrial sectors, including implantable materials, allowing these antimicrobial materials to be used in the regeneration of soft tissues (skin).

CONCLUSION

This work demonstrates that photosynthetic microorganisms can be a viable and low-cost alternative for the production of biopolymers (PHAs) with great industrial application, with production similar to those found in the literature by bacteria, with biosynthetic pathways still to be better studied and elucidated, and with great prospects for the application of these biopolymers in the formation of thin films incorporated into essential oils, active packaging, with antimicrobial characteristics and great applicability in the packaging industry.

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REFERENCES

1. Abdo, S. M., & Ali, G. H. (2019). Analysis of polyhydroxybutyrate and bioplastic production from microalgae. *Bulletin of the National Research Centre, 43*(1–4).

2. Albuquerque, P. B. S., & Malafaia, C. B. (2018). Perspectives on the production, structural characteristics and potential applications of bioplastics derived from polyhydroxyalkanoates. *International Journal of Biological Macromolecules, 107*, 615–625.

3. Alves, J. C. A., Silva, G. S., Souza, K. C., Silva, I. D. L., Silva, C. F., Caetano, V. F., & Vinhas GM. (2021). Evaluation of the influence of orange essential oil on PHB/PEG films. *Research, Society and Development, 10*(2), e30810212547.

4. Amadu, A. A., Qiu, S., Ge, S., Addico, G. N. D., Ameka, G. K., Yu, Z., Xia, W., Abbew, A-W., Shao, D., Champagne, P., & Wang, S. (2021). A review of biopolymer (Poly-β-hydroxybutyrate) synthesis in microbes cultivated on wastewater. *Science of The Total Environment, 756*, 143729.

5. Ansari, S., & Fatma, T. (2016). Cyanobacterial polyhydroxybutyrate (PHB): Screening, optimization and characterization. *PLoS ONE, 11*(1), 20.

6. Arumugam, A. (2020). Polyhydroxyalkanoates (PHA) Production. *Encyclopedia of Renewable and Sustainable Materials, 2*, 236-252.

7. Ballester-Costa, C., Sendra, E., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2013). Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth. *Industrial Crops and Products, 50*, 304–311.

8. Ballester-Costa, C., Sendra, E., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2017). Assessment of antioxidant and antibacterial properties on meat homogenates of essential oils obtained from four Thymus species achieved from organic growth. *Foods, 6*(8), 59.

9. Basnett, P., Marcello, E., Lukasiewicz, B., Nigmatullin, R., Paxinou, A., Ahmad, M. H., Gurumayum, B., & Roy, I. (2020). Antimicrobial Materials with Lime Oil and a Poly(3-hydroxyalkanoate) Produced via Valorisation of Sugar Cane Molasses. *Journal of Functional Biomaterials, 10*(2), 24.

10. Bhati, R., & Mallick, N. (2015). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production by the diazotrophic cyanobacterium Nostoc muscorum Agardh: process optimization and polymer characterization. *Algal Research, 7*, 78–85.

11. Bhati, R., Samantaray, S., Sharma, L., & Mallick, N. (2010). Poly-β hydroxybutyrate accumulation in cyanobacteria under photoautotrophy. *Biotechnology Journal, 5*, 1181–1185.

12. Bugnicourt, E., Cinelli, P., Alvarez, V. A., & Lazzeri, A. (2014). Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging. *Express Polym Lett, 8*(11), 791–808.

13. Coelho, V. C., Silva, C. K., Costa, J. A. V., & Morais, M. G. (2015). Polyhydroxybutyrate production by Spirulina sp. LEB 18 grown under different nutrient concentrations. *African Journal of Microbiology Research, 9*, 1586–1594.

14. Coppola, G., Gaudio, M. T., Lopresto, C. G., Calabro, V., Curcio, S., & Chakraborty, S. (2021). Bioplastic from renewable biomass: a facile solution for a greener environment. *Earth SysEnviron, 5*(2), 231–251.

15. Costa, S. S., Miranda, A. L., Andrade, B. B., Assis, D. J., Souza, C. O., Morais, M. G., Costa, J. A. V., & Druzian, I. (2018A). Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae. *International Journal of Biological Macromolecules, 116*, 552-562.

16. Costa, S. S., Miranda, A. L., Andrade, B. B., Assis, D. J., Souza, C. O., Morais, M. G., Costa, J. A. V., & Druzian, I. (2018B). Efficacy of Spirulina sp. polyhydroxyalkanoates extraction methods and influence on polymer properties and composition. *Algal Research, 33*, 231–238.

17. Costa, S. S., Miranda, A. L., Andrade, B. B., Assis, D. J., Souza, C. O., Morais, M. G., Costa, J. A. V., & Druzian, I. (2018C). Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae. *International Journal of Biological Macromolecules, 116*, 552–562.

18. Costa, S. S., Miranda, A. L., Morais, M. G., Costa, J. A. V., & Druzian, J. I. (2019). Microalgae as source of polyhydroxyalkanoates (PHAs) — A review. *International Journal of Biological Macromolecules, 131*, 536-547.

19. Cutillas, A. B., Carrasco, A., Martinez-Gutierrez, R., Tomas, V., & Tudela, J. (2018). Thyme essential oils from Spain: Aromatic profile ascertained by GC–MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities. *Journal of Food and Drug Analysis, 26*(2), 529–544.

20. De Koning, G. J. (1993). *Prospects of Bacterial Poly[(R)-3-(Hydroxyalkanoates)].* Eindhoven: Technische Universiteit Eindhoven, 145.

21. FDA | U.S. Food & Drug Administration. (n.d.). Retrieved May 25, 2021, from https://www.fda.gov/.

22. Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science, 75*(8), M528–M535.

23. García, G., Sosa-Hernández, J. E., Rodas-Zuluaga, L. I., Castillo-Zacarías, C., & Parra-Saldívar, R. (2021). Accumulation of PHA in the Microalgae Scenedesmus sp. under Nutrient-Deficient Conditions. *Polymers, 13*(1), 131.

24. Ghabraie, M., Vu, K. D., Tata, L., Salmieri, S., & Lacroix, M. (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT – Food Science and Technology, 66*, 332–339.

25. Giaquinto, C. D. M., Souza, G. K. M., Caetano, V. F., & Vinhas, G M. (2017). Evaluation of the mechanical and thermal properties of PHB/canola oil films. *Polímeros, 27*(3), 201-207.

26. Gouvêa, D. M., Mendonça, R. C. S., Soto, M. L., & Cruz, R. S. (2015). Acetate cellulose film with bacteriophages for potential antimicrobial use in food packaging. *LWT - Food Science and Technology, 63*(1), 85-91.

27. Hempel, F., Bozarth, A. S., Lindenkamp, N., Klingl, A., Zauner, S., Linne, U., Steinbüchel, A., & Maier, U. G. (2011). Microalgae as bioreactors for bioplastic production. *Microbial Cell Factories, 10*, 81.

28. Jamnongkan, T., Intraramongkol, N., Samoechip, W., Potiyaraj, P., Mongkholrattanasit, R., Jamnongkan, P., Wongwachirakorn, P., Sugimoto, M., Ito, H., & Huang, C-F. (2022). Towards a circular economy: study of the mechanical, thermal, and electrical properties of recycled polypropylene and their composite materials. *Polymers, 14*(24), 5482.

29. Jia, K., Cao, R., Hua, D.H., & Li, P. (2016). Study of Class I and Class III polyhydroxyalkanoate (PHA) synthases with substrates containing a modified side chain. *Biomacromolecules, 17*, 1477–1485.

30. Ju, J., Xu, X., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2017). Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chemistry, 240*, 850–855.

31. 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 8095. *Bioresource Technology, 212*, 342–347.

32. Kavitha, G., Kurinjimalar, C., Sivakumar, K., Kaarthik, M., Aravind, R., Palani, P., & Rengasamy, R. (2016). Optimization of polyhydroxybutyrate production utilizing wastewater as nutrient source by Botryococcus braunii Kütz using response surface methodology. *International Journal of Biological Macromolecules, 93*, 534–542.

33. Khaneghah, A. M., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. *Food and Bioproducts Processing, 111*, 1–19.

34. Khetkorn, W., Incharoensakdi, A., Lindblad. P., & Jantaro, S. (2016). Enhancement of poly-3-hydroxybutyrate production in Synechocystis sp. PCC 6803 by overexpression of its native biosynthetic genes. *Bioresource Technology, 214*, 761–768.

35. Koller, M., Vadlja, D., Braunegg, G., & Atlic, A. (2017). Formaland high-structured kinetic process modelling and footprint area analysis of binary imaged cells: Tools to understand and optimize multistagecontinuous PHA biosynthesis. *The EuroBiotech Journal, 1*(3), 203–211.

36. 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. *International Journal of Biological Macromolecules, 102*, 497-504.

37. Kumari, P., Kiran, B. R., & Mohan, S. V. (2022). Polyhydroxybutyrate production by Chlorella sorokiniana SVMIICT8 under Nutrient-deprived mixotrophy. *Bioresource Technology, 354*, 127135.

38. Lagha, R., Abdallah, F. B., AL-Sarhan, B. O., & Al-Sodany, Y. (2019). Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against Escherichia coli isolated from UTI patients. *Molecules, 24*(6), 1161.

39. Lama, L., Nicolaus, B., Calandrelli, V., Manca, M.C., Romano, I., & Gambacorta, A. (1996). Effect of growth conditions on endo-and exopolymer biosynthesis in Anabaena cylindrica 10 C. *Phytochemistry, 42*, 655–659.

40. Lee, G., Kim, Y., Kim, H., Beuchat, L. R., & Ryu, J. H. (2018). Antimicrobial activities of gaseous essential oils against Listeria monocytogenes on a laboratory medium and radish sprouts. *International Journal of Food Microbiology, 265*, 49–54.

41. Mendhulkar VD, & Shetye L. (2017). Synthesis of biodegradable polymer polyhydroxyalkanoate (PHA) in cyanobacteria Synechococcus elongates under Mixotrophic nitrogen- and phosphate-mediated stress conditions. *Industrial Biotechnology, 13*, 85–88.

42. Mourão, M. M., Gradíssimo, D.G., Santos, A.V., Schneider, P.C., Faustino, S.M.M., Vasconcelos, V., & Xavier, L.P. (2020). Optimization of Polyhydroxybutyrate Production by Amazonian Microalga Stigeoclonium sp. B23. *Biomolecules, 10*(12), 1628.

43. Muppalla, S. R., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Carboxymethyl cellulose–polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packaging and Shelf Life, 2*(2), 51-58.

44. Nandal, M., Khyalia, p., Ghalawat, A., Jugiani, H., Kaur, M., Laura, J.S. (2022). Review on the use of microalgae biomass for bioplastics synthesis: a sustainable and green approach to control plastic pollution. *Pollution, 8*(3), 844–859.

45. Nishioka, M., Nakai, K. Miyake, M., Asada, Y., & Taya, M. (2001). Production of poly-β-hydroxybutyrate by thermophilic cyanobacterium, Synechococcus sp. MA19, under phosphate limited conditions. *Biotechnology Letters, 23*, 1095–1099.

46. Pal, S., Manna, A., Paul, A.K. (1999). Production of poly(b-hydroxybutyric acid) and exopolysaccharide by Azotobacter beijerinckii WDN-01. *World Journal of Microbiology and Biotechnology, 15*, 11–16.

47. Panda, B. & Mallick, N. (2017). Enhanced poly-β-hydroxybutyrate accumulation in a unicellular cyanobacterium, Synechocystis sp. PCC 6803. *Letters in Applied Microbiology, 44*, 194–198.

48. Panda, B., Sharma, L., Mallick, N. (2005). Poly-β-hydroxybutyrate accumulation in Nostoc muscorum and Spirulina platensis under phosphate limitation. *Journal of Plant Physiology, 162*, 1376–1379.

49. Rahman, A., Anthony, R. J., Sathish, A., Sims, R. C., & Miller, C. D. (2014). Effects of wastewater microalgae harvesting methods on polyhydroxybutyrate production. *Bioresource Technology, 156*, 364–367.

50. Rahman, A., Linton, E., Hatch, A. D., Sims, R. C., & Miller, C. D. (2013). Secretion of polyhydroxybutyrate in Escherichia coli using a synthetic biological engineering approach. *Journal of Biological Engineering, 7*:24.

51. 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 Research, 8*, 95–98.

52. Rosenboom, J.-G., Langer, R., & Traverso, G. (2022). Bioplastics for a circular economy. *Nature Reviews Materials, 7*(2), 117–137.

53. Samantaray, S. & Mallick, N. (2012). Production and characterization of polyhydroxybutyrate (PHB) polymer from Aulosira fertilissima. *Journal Applied Phycology, 24*, 803–814.

54. Sangha, J. S., Astatkie, T., & Cutler, G. C. (2017). Ovicidal, larvicidal, and behavioural effects of some plant essential oils on diamondback moth (Lepidoptera: Plutellidae). *Canadian Entomologist, 149*(5), 639–648.

55. Sharma, L. & Mallick, N. (2005). Accumulation of poly-β hydroxybutyrate in Nostoc muscorum: regulation by pH, light–dark cycles, N and P status and carbon sources. *Bioresource Technology, 96*, 1304–1310.

56. Shrivastav, A., Mishra, S.K., Mishra, S. (2010). Polyhydroxyalkanoate (PHA) synthesis by Spirulina subsalsa from Gujarat coast of India. *International Journal of Biological Macromolecules, 46*, 255–260.

57. Silva, P.E.C., & Houllou, L.M. (2022). Obtainment of Polyhydroxyalkanoates (PHAs) from Microalgae Supplemented with Agro-Industry Residue Corn Steep Liquor. *Journal of Botany Research, 5*(1), 138-140.

58. Singh, A. K., & Mallick, N. (2017). Advances in cyanobacterial polyhydroxyalkanoates production. *FEMS Microbiology Letters, 364*:fnx189.

59. Surendran, A., Lakshmanan, M., Chee, J.Y., Sulaiman, A.M., Thuoc, D.V., & Sudesh, K. (2020). Can polyhydroxyalkanoates be produced efficiently from waste plant and animal oils? *Frontiers in Bioengineering and Biotechnology, 8*, 169.

60. Wani, A. A., Singh, P., & Langowski, H. C. (2014). Packaging. In Y. Motarjemi & L. Gorris (Eds.), *Encyclopedia of food safety*. UK: Elsevier Science, 211-218.

61. Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms (Agaricus bisporus). *Food Control, 54*, 200-207.

62. Wu, H.Y., Liu, T.X., Hsu, C.H., Cho, Y.S., Xu, Z.J., Liao, S.C., Zeng, B.H., Jiang, Y.L., & Lien, S.Y. (2017). Thin-film coated plastic wrap for food packaging. *Materials 10*(7).