MICROALGAE BIOPOLYMERS: A REVIEW

P. H. S. Zatta[a],
B. Jacob-Furlan[b],
R. C. Mirabile[c],
R. S. R. Gonçalves[d],
P. A. S. da Silva[d],
D. M. Taher[d],
G. S. Lira[e],
L. S. Martins[d]
and J. C. Ordonez[f]

[a] Universidade Federal do Paraná
Departamento de Engenharia de Bioprocessos e
Biotecnologia - Curitiba, Paraná, Brasil
pedroh.zatta@ufpr.br

[b] Universidade Federal do Paraná
Departamento de Química
Curitiba, Paraná, Brasil
beatrizfurlan@ufpr.br

[c] Universidade Federal do Paraná
Departamento de Engenharia Ambiental
Curitiba, Paraná, Brasil
rafaelamirabile@ufpr.br

[d] Universidade Federal do Paraná
Departamento de Engenharia Mecânica
Curitiba, Paraná, Brasil
rafael.silva@ufpr.br
paulo.alexandre@ufpr.br
dhyogomt@gmail.com
lauber@ufpr.br

[e] Universidade Federal do Paraná
Programa de Pós-graduação em Engenharia e
Ciência dos Materiais - PIPE
Curitiba, Paraná, Brasil
gilvana@ufpr.br

[f] Florida State University
Department of Mechanical Engineering -
FAMU-FSU College of Engineering
ordonez@eng.famu.fsu.edu

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ABSTRACT

Algae are ubiquitous organisms whose capabilities have drawn much attention as of late in the bioengineering field due to their potential to enable a wide range of bioproducts. Microalgae are ideal organisms for the application of the biorefinery concept since they can be grown in wastewater and, at the same time, produce many products of commercial interest. These microorganisms are also known for their resilience to extreme environmental conditions and suitable cell growth rates. Beyond the known potential for biofuel production, these microorganisms can still produce other compounds, being lipids, pigments, vitamins, proteins, and polysaccharides, whose applications go from pharmaceutical to agricultural industries. Recently, the research focus has been directed to the biopolymer-producing ability of both micro- and macroalgae, as they can be rather varied and useful to many applications. However, this is still an ongoing research field, and new data are frequently added in the literature, notably on biomass processing, which can be done with the intent of use into dyes, bioplastics, paints, and even as biochar in solid fuel cells. Microalgae-based biopolymers can be used in a wide range of products, nevertheless, the resulting process efficiency and yields depend on the extraction process utilized, as well as on the microalgae species used and the culture conditions. Furthermore, the polymer extraction can be done directly with common solvents at atmospheric pressure or with other fluids, such as supercritical CO_2 or subcritical solvents, and assisted by specific treatments, e.g., ultrasound and microwave. The residual biomass can still be used to produce other less valuable products, such as feedstock, and energy via combustion. In this sense, the present work aims to provide a state-of-the-art review on microalgae biopolymers. Issues related to the efficiency of current treatment methods, industrial applications, and environmental performance are presented and discussed. Besides, the perspectives in this area of knowledge are also a contribution of the present work, the extent to which scientific research is still under development.

Keywords: Cyanobacteria, biomass, polysaccharides, bioengineering, sustainability

INTRODUCTION

As human populations grow and rely heavier on plastics, greater becomes the environmental, economic, and social need for alternatives to traditional oil-based polymers. Current plastic technology is mostly fossil-fuel derived, and although varied in option, relies heavily on just a few plastics. In recent years, 70% of all non-fibre plastic production were of just polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC) (Geyer et al., 2017) . Even though there are renewable options, these are used in around 1% of available polymers, and amidst those, nearly 45% behave similarly to fossil-fuel derived plastic, therefore being "non-recyclable (Bioplastics market data 2018, 2018).

Microalgae represent an eco-friendly alternative for the production of biopolymers (Kartik et al., 2021). These microorganisms are already used extensively in phycoremediation, through which algae metabolise pollutants and hinder their effects (Olguín, 2003), both micro and macroalgae are thus suitable. Macroalgae can be more easily harvested, hence the great interest in their bioabsorption capabilities of pesticides (Contarini & Dromard, 2021) and metals (Ociński et al., 2021) which may be subject to bioaccumulation. Macroalgae can also produce biopolymers of economic interest, such as bioplastics and biosolvents (Casoni et al., 2020) .

Microalgae are photosynthetic organisms, so they are able to grow on autotrophic media, however, the main focus of algae research is on heterotrophic or mixotrophic media, because these allow for wastewater treatment (Chisti, 2008) . Algae, in general, are capable of carbon capture through photosynthesis, thus absorbing carbon (C) while producing oxygen (O2), and while growing they also need nitrogen (N) and phosphorus (P), which are found in great quantities in wastewaters (Doran, 2013) and throughout their growth, they may metabolize complex compounds (Lutzu et al., 2021) while treating the water medium.

Microalgae have attracted attention due to their ability to grow on waste media and also produce interesting molecules, consequently opening the possibility for the concept of biorefinery to be put to use (Katiyar et al., 2021) . These products, which are highly dependent on species and media conditions, may be applied from agriculture to health and chemical industries (Parsons et al., 2020). Due to the fact that microalgae are highly adaptable and can grow in a wide range of media, their use for the production of biopolymers (extensively used in packaging, medicine, cosmetics, and as feedstock) has drawn much attention (Kartik et al., 2021).

CURRENT MICROALGAE TECHNOLOGIES

System geometry

In regards to system geometry, four main factors limit the growth of microalgae: light level, agitation, aeration and the choice between closed and open systems (Kirnev *et al*., 2020) .

According to Molina Grima *et al.*, 2003, the first step in microalgal culture is to select the type of system, either open or closed, in which the process will be carried out . Open systems are cheaper to build and easier to maintain (Lutzu *et al.*, 2021), as they do not require constant control of some variables. There are also open raceway systems in which some variables are controlled, such as agitation, aeration, culture depth, and nutrient concentration. In these systems, greater yields require increasingly larger areas, the surface to volume ratio has to be small (to allow the light to reach the whole cultive) and aeration plays also a limiting factor (Jankowska *et al*., 2017) .

Closed systems, on the contrary, represent a more investment-heavy option on the premise of achieving higher control levels. The cultive remains isolated from the exterior world and any inputs are closely controlled to minimize contamination (Chisti, 2007) , thus allowing for single species culture. Most frequently, they have tubular geometry made of transparent plastic or glass, these tubes have limited diameter due to sunlight penetration limitations.

Moreover, tubular bioreactors are not the only type of closed systems, there are also bubble column, airlift and flat panel bioreactors. The two first are rather similar in design, they are water columns through which air is bubbled from the base of the bioreactor, the difference is that an airlift has an internal draft tube through which all the air is pumped, creating an internal upwards current that promotes better mass-transfer and mixing (Duan and Shi, 2014). The bubble column design is simpler, and all the air is bubbled from the sparger on the base of the column, agitating the medium only (Williams and Laurens, 2010) .

At last, flat panel bioreactors try to minimize light pathing and surface area, the reactor consists of a thin cuboid-shaped tank or membrane in which the biomass is produced, stirring is conducted through aeration or by agitating whole-system (Duan and Shi, 2014).

Biomass harvesting

There are two major harvesting classifications: dewatering, which consists of separating the biomass from all of the solution, here are included centrifugation and filtration; and thickening, which aims to separate a more concentrated solution from the bulk cultivation media.

Generally speaking, dewatering methods do not require any chemicals added to the medium, offer satisfactory recovery rates and can be applied to a wide range of species (Milledge and Heaven, 2011). Filtration is usually conducted under a vacuum with aid of gravitational forces (Ananthi *et al*., 2021). The yielded biomass has good quality. The mostly used membranes used for filtration are made of polyvinyl chloride (PVC), polyvinylidene fluoride (PVDF), polyethersulfone (PES), polyacrylonitrile (PAN), polytetrafluoroethylene (PTFE), polyethersulfone polyvinyl-pyrrolidone (PES-PVP), (Baerdemaeker *et al*., 2013; Drexler and Yeh, 2014). The effectiveness of ceramic filters and supports was assessed by Mo *et al*. (2015) yielding good results. Furthermore, ultrafiltration could become a viable option in the future (Baerdemaeker *et al*., 2013; Drexler and Yeh, 2014; Mo *et al*., 2015).

Centrifugation relies on the application of a centripetal force which, according to density, separates the sample. More dense particles move outwards while less dense remain inwards. Centrifugation has been used extensively in industry (Molina Grima et al., 2003) even though its energy consumption is rather high.

Thickening methods rely on the addition or production *in situ* of chemicals that increase biomass aggregation. Microalgae cells are negatively charged in their outwards, and thus, usually repel each other (Uduman *et al*., 2010). Flocculants are able to neutralise or patch this charge and allow cells to form clusters which sink more easily. The flocculant source might be chemically induced or natural. In the first case, according to Ananthi *et al*. (2021), a compound is added to the medium to start flocculation. Bioflocculation may happen naturally or induced via a change in the medium once the microorganisms become stressed (Larkum *et al*., 2012) . Another possible flocculation method, whose greatest advantage is that no chemical agents are added, is electrolytic flocculation (Uduman *et al*., 2010). In this method, an electrical current is applied to the culture and the algae cells (negatively charged) tend to move to the anode or precipitate on the bottom (Kim *et al*., 2012).

Contrary to flocculation, where biomass sinks to the bottom, flotation is the process through which biomass’s tendency to float is taken into advantage (M. R. Teixeira and Rosa, 2006). It is commonly used for sludge removal and has been proven to be effective at large scales. In this process, which may be aided by flocculants, particles are separated from the solution by the lifting force of air bubbles and then are removed from the top as liquid sludge (M. R. Teixeira and Rosa, 2006).

Polymer extraction

Current extraction techniques consist mainly of solvent, microwave-assisted and ultrasound extraction (Kartik et al., 2021). Solvent extraction is carried out with a mix of chemical compounds aiming to extract and precipitate the polymers. Usually the biomass is washed with a clean solvent and then comes the need to break down hard cell walls (El-malek et al., 2021). This particular step can be carried out in many ways. Pez Jaeschke et al. (2021) compiled data of the extraction of phycocyanin from spirulina and evaluated the results of many methods such as freezing and thawing, homogenization, mixing, mixing with glass beads, bead milling, high pressure homogenization and processing, ultrasound (with horn, in bath and without aid), microwaving, pulse electric field, and even combinations of those aforementioned. This is seen as a key step in obtaining high-quality, high-purity polymers.

Microwave assisted extractions present a greener approach to biomass extraction (Kartik et al., 2021). It consists of applying electromagnetic waves in the range of 0.3 to 300 GHz, these produce changes in the electromagnetic field that lead to the movement of charged particles and the alignment of dipoles. The medium poses resistance to those changes in the form of friction, furthermore, the collision between moving particles, among themselves and with static particles, both generate heat, thus increasing the temperature (Soria et al., 2014). Ultrasound processing consists of applying sound waves in frequencies above human hearing (in average 20 kHz) up to several GHz (Picó, 2013), these sound waves produce local cavitation bubbles which implode locally. They transfer kinetic energy to the microparticles in the cell wall and lead to their disruption (Kartik et al., 2021). Ultrasound-assisted extraction produces reduced extraction times, prevents the unnecessary use of solvents, and is environmentally friendly (Soria et al., 2014).

Another interesting extraction method is with subcritical water, which is pressurised to just under its critical conditions (the subcritical region). Temperatures from 100 to 375 °C and pressures of 22,1 MPa are attained (Thiruvenkadam et al., 2015). At this stage, water becomes less dense and its dielectric constant increases significantly, thus increasing hydrocarbon solubility (2013; Lindström, 2007) . Ghosh et al. (2021) investigated the yield of subcritical polyhydroxyalkanoate extraction from Ulva sp. to rank the impact of temperature, solid load, residence time and salinity in the process; temperature was found to be the most important factor followed by salinity and residence time.

POLYMERS OF INTEREST

Since microalgae has high growth rate, elevated photosynthetic efficiency and great potential for carbon dioxide fixation, they are a great source of biopolymer production. Essentially biopolymers are polymers which are biodegradable and produced from biobased materials. Basically, there are three methods to obtain polymers from algae biomass: (1) Polymers extracted from biomass (Natural occurring polymers), (2) Polymers produced by microorganisms, (3) Polymer synthesized by bio-derivative monomers (Niaounakis M., 2014).

Algal Polysaccharides

Being water soluble, biodegradable and functionally active, polysaccharides have been used in biomedical applications. Microalgae can be used to produce a variety of polysaccharides such as alginates, fucoidans and carrageenan which are structurally and morphologically different (Priyan Shanura Fernando *et al*., 2019).

PHA (POLYHYDROXYALKANOATES)

Polyhydroxyalkanoates present high molecular weight, thermoplastic processability and hydrophobicity similar to synthetic polymers. Since it is biodegradable, PHA is a great substitute for synthetic polymers. However, the production cost of PHA is higher than that of synthetic ones and their applications are limited (Tan *et al*., 2017). PHA can be synthesized and accumulated inside the cells as granules for energy and carbon storage by many organisms, such as Chemoautotrophic bacteria and cyanobacteria. The usage of cyanobacteria as a PHA-producing host has many advantages over bacteria since cyanobacteria use CO_2 and sunlight as carbon and energy source.

Composition of PHA biopolymers depends on microbial strains and cultural strains. Up to date, 150 different structures were identified, most well-known monomers are:

3-hydroxypropionate, 3-hydroxybutyrate, 3-hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxy tetradecanoate and 4-hydroxybutyrate (Balaji *et al*., 2013).

PHB (Poly-Hydroxybutyrate)

PHB is the most abundant PHA present in various cyanobacteria such as *Chlorogloea fritschii, Spirulina* spp., *Aphanothece* spp., *Gloeothece* spp., *and Synechococcus* spp.*, Synechocystis* sp.*, Botryococcus braunii*, *Gloeocapsa* sp., *Spirulina platensis, Phormidium sp.,* and many others (Balaji *et al*., 2013). Large amounts of lipids are required for the production of PHB (Cassuriaga *et al*., 2018). An analysis of the production of PHB done by Kavitha *et al*. (2016) demonstrates the highest amount of PHB production (17,4%) was obtained by *Botryococcus braunii*.

POSSIBLE CO-PRODUCTS OF INTEREST

Microalgae have the potential for co-production of valuable products like carbohydrates, lipids, and proteins, starch, cellulose and polyunsaturated FAs (PUFAs), pigments, antioxidants, pharmaceuticals, fertilizer, energy crops, natural colorants and also as biomass that can be used as animal feed after oil extraction.

Proteins are highly important in human nutrition and the deficiency thereof can cause malnutrition. They are part of the main constituents of microalgae, comprising of 50–70% of the microalgae composition (Wayne *et al*., 2017) and it can be used for human or animal nutrition as well as care products, emollients (as an anti-irritant in peelers and sunscreens) and hair care products. Microalgae also represent a valuable source of almost all essential vitamins, such as A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, and pantothenic acid (Richmond, 2003).

Microalgae contain pigments that are associated with light incidence. The main role of pigments in microalgae is to provide photoprotection against high light intensity. Phycobiliproteins are helpful in improving the efficiency of light energy utilization and carotenoids serve as photo-protectors against the photo-oxidant damage resulting from excess light capture. The most common application of natural pigments relies on the food industry due to the benefits for human health related to their antioxidant and pro vitamin A properties. The global market of carotenoid is covered about 50% by astaxanthin and β-carotenoid (Giraldo-Calderón *et al.* 2018). Astaxanthin is known for its powerful antioxidant properties, showing benefits in the prevention and treatment of various conditions. Likewise, phycobiliproteins, phycocyanin and phycoerythrin are already being used for food and cosmetics applications.

Microalgae normally have high carbohydrate content which is primarily composed of glucose, starch, cellulose and various kinds of polysaccharides (Yen *et al*., 2013). Algal glucose or starch are used for bioethanol and hydrogen production. Microalgae polysaccharides are capable of modulating the immune system and inflammatory reactions, making them sources of cosmetics additives,food ingredients and natural therapeutic agents (Yen *et al*., 2013).

The use of microalgae for agricultural applications is focused on the use of cyanobacteria, which have a high ability to fix atmospheric nitrogen (N_2) and dispose of it in the form of NH_3 for the direct assimilation by plants. They have the capability to release grown-inducing substances such as auxins and cytokinins, while also solubilizing phosphates; the inactive biomass provides nutritious organic matter that improves soil fertility and crop quality (Wang *et al*., 2015).

SPECIES OF INTEREST

Arthrospira and Spirulina

Arthrospira and *Spirulina* are a type of filamentous cyanobacteria commercially called namely as *Spirulina*, however, scientific nomenclature claims that this name is inappropriate for strains used for food supplement and recommends the use of *Arthrospira* (Fujisawa *et al*., 2010). In the bioplastic medium, *Spirulina* spp. are commonly used for feedstock due to its high protein content (Pez Jaeschke *et al*., 2021). They also present a highly adaptable metabolism, easiness to harvest and capability to produce many different products of interest, such as phycobiliproteins of which phycocyanins are the best known example (Tavanandi and Raghavarao, 2020), carotenoids and antioxidants (Moradi *et al*., 2021), and essential fatty acids (Matos, 2020).

A. platensis cultivated under mixotrophic conditions may yield up to 8.14% ± 0,30 of its dry weight in PHA (Costa, 2018). *A. platensis* grown with Direct Green 6 dye had a positive increase in the production of carotenoids and astaxanthin correlated with higher concentrations of the dye (Moradi *et al*., 2021). Dos Santos *et al*. (2019) investigated the production of C-phycocyanin (C-PC) and PHA by *A. platensis* in N-deficient and N-free media; the results show an important correlation of N-deficiency with C-PC production and N-absence with PHA production.

Another biopolymer of interest produced by *A. platensis* are extracellular polymeric substances (EPS). Chentir *et al*. (2017) obtained good results with a two-step approach; the first step aimed for biomass production under optimal growth conditions and the second step addresses EP induction by NaCl in increasing quantity to access the optimal concentration; their results show that optimal conditions require high NaCl concentrations (40 $g \cdot l^{-1}$) coupled with low light intensities (100 to 650 µmol $photons \cdot m^{-2} \cdot s^{-1}$), yielding 0,97 to 0,98 $g \cdot g^{-1}$ (DW). Production of EPS coupled with C-PC was also investigated in lab scale for 14 days of culture; higher salt and lower light levels (under 70 µmol $photons \cdot m^{-2} \cdot s^{-1}$) correlated to better C-PC yields, whilst the contrary was observed for EPS concentration (Dejsungkranont *et al*., 2017).

Chlorella

Chlorella are fast-growing green algae with the potential to be used in biofuel and bioplastic production (Zainan *et al*., 2018). It is a highly proteic alga (over 50% dry weight) and whose biomass may be plasticized with glycerol as demonstrated by Zeller *et al*. (2013). *C. pyrenoidosa* was shown to produce up to 27% (dry weight) PHB after 14 days of growth, the produced polymer was highly biodegradable and environmental friendly in addition to the fact that *C. pyrenoidosa* was grown while absorbing Ni and Cr from the medium (Das et al., 2018).

Botryococcus braunii

Botryococcus braunii has been widely studied due to its potential as hydrocarbon-rich microalgae, for it is able to produce liquid hydrocarbons in the form of alkenes and isoprenoids, which are stored in the extracellular matrix (Metzger and Largeau, 2005) . These hydrocarbons are of special interest because they can be converted into gasoline (Hillen *et al*., 1982). Concentrated extracts of this microalgae have been used as a biopolymer for the development of ultrafine fiber through electrospinning (Verdugo *et al*., 2014). *B. braunii* does also produce extracellular proteins and polysaccharides whose structure and perspectives of use are still being studied. Increases in hydrocarbon quantities correlated to an increase in biomass and polymer-matrix production (Tasić *et al*., 2016). Furthermore, the structure of the fibrillar sheath of *B. braunii* colonies was found to be rich in a novel polysaccharide rich in galactose and arabinose with 4-O-methylglucuronic acid and 6-deoxyaltrose features (Heiss *et al*., 2021).

Stigeoclonium

Stigeoclonium sp. is a rather unknown genus to biopolymer production. Recently, there have been experimental data by Mourão *et al*. (2020) which showed for the first time that *Stigeoclonium* sp. B23 produces PHB, but its production rate is over 6 g l^{-1} of PHA, which was above the standard set by *Bacillus* sp. (5,2 g l^{-1}). Mourão *et al*. (2021) also showed that *Stigeoclonium* sp. B23 could still produce PHB when cassava peel was used as a carbon source, moreover the productivity actually increased to 12,16 ± 1,28% (dry weight); however, the obtained PHB revealed the induction of some mortality and lethality indicators in zebrafish embryos, but other characterization data were satisfactory.

Scenedesmus

Scenedesmus sp. is widely known due to its potential for oil and starch accumulation, however, there is little to no data on the potential for biopolymer production. Garcia *et al.* (2020) were the first to document the presence of PHA in *Scenedesmus* sp. with very promising results; the normal metabolism production of PHA was found to be 8,61%, which is already higher than previously induced species, while induced production reached 29,92 50 (dry weight). *Scenedesmus* sp. is well known to be a robust fresh-water algae which may facilitate its cultivation and wide-scale production of microalgae PHA.

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REFERENCES

Abdo, S.M., Ali, G.H. “Analysis of polyhydroxybutrate and bioplastic production from microalgae”. 2019.

Ananthi, V., Balaji, P., Sindhu, R., Kim, S. H., Pugazhendhi, A., and Arun, A., 2021."A critical review on different harvesting techniques for algal based biodiesel production".In Science of the Total Environment (Vol. 780, p. 146467).Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2021.146467

Apurav Krishna Koyande,Pau-Loke Show,Ruixin Guo,Bencan Tang,Chiaki Ogino and Jo-Shu Chang.,2019.“Bio-processing of algal bio-refinery: a review on current advances and future perspectives”.https://doi.org/10.1080/21655979.2019.1679697

Baerdemaeker, T. De, Lemmens, B., Dotremont, C., Fret, J., Roef, L., Goiris, K., and Diels, L., 2013."Benchmark study on algae harvesting with backwashable submerged flat panel membranes".Bioresource Technology, Vol. 129, , pp. 582–591. https://doi.org/10.1016/j.biortech.2012.10.153

Balaji, S.; Gopi, K.; Muthuvelan,B.,2013. “ A review on production of poly β hydrobybutyrates from cyanobacteria for production of bioplastic.” Algal research(v.2, p. 278-285).

Bioplastics market data 2018., 2018. https://environmentalmicrobiome.biomedcentral.com/track/pdf/10.1186/s40793-020-00371-w.pdf#page=15&zoom=100,0,0

Casoni, A. I., Ramos, F. D., Estrada, V., and Diaz, M. S., 2020."Sustainable and economic analysis of marine macroalgae based chemicals production - Process design and optimization".Journal of Cleaner Production, Vol. 276, . https://doi.org/10.1016/j.jclepro.2020.122792

Cassuriaga, Ana Paula Aguiar.,2018 . “Produção de PHB por Chlorella fusca cultivada com pentoses como fonte de carbono”. Tese (master's degree). Universidade Federal do Rio Grande - FURG.

Chentir, I., Hamdi, M., Doumandji, A., HadjSadok, A., Ouada, H. Ben, Nasri, M., and Jridi, M., 2017."Enhancement of extracellular polymeric substances (EPS) production in Spirulina (Arthrospira sp.) by two-step cultivation process and partial characterization of their polysaccharidic moiety".International Journal of Biological Macromolecules, Vol. 105, , pp. 1412–1420. https://doi.org/10.1016/j.ijbiomac.2017.07.009

Chew Kit Wayne, Pau-Loke Show,Yap Jing Ying and Hui Suan Ng. “Microalgae biorefinery : High value products perspectives”. 2017.

Chisti, Y., 2007."Biodiesel from microalgae".In Biotechnology Advances (Vol. 25, Issue 3, pp. 294–306).Elsevier. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chisti, Y., 2008."Biodiesel from microalgae beats bioethanol".Trends in Biotechnology, Vol. 26, No. 3, pp. 126–131. https://doi.org/10.1016/j.tibtech.2007.12.002

Contarini, P. E., and Dromard, C. R., 2021."Biosorption capacity of genus Dictyota facing organochlorine pesticide pollutions in coastal areas of the Lesser Antilles".Aquatic Botany, Vol. 169, , pp. 103346. https://doi.org/10.1016/j.aquabot.2020.103346

Corrêa, P. S., and Teixeira, C. M. L. L., 2021."Polyhydroxyalkanoates and pigments coproduction by Arthrospira (Spirulina) platensis cultivated in crude glycerol".Journal of Applied Phycology, Vol. 33, No. 3, pp. 1487–1500. https://doi.org/10.1007/s10811-021-02373-8

Das, S. K., Sathish, A., and Stanley, J., 2018."Production of Biofuel and Bioplastic from Chlorella Pyrenoidosa".Materials Today: Proceedings, Vol. 5, No. 8, pp. 16774–16781. https://doi.org/10.1016/j.matpr.2018.06.020

Da Silva, T.L., Gouveia, L. and Reis, A.,2014. “Integrated microbial processes for biofuels and high value-added products: the way to improve the cost effectiveness of biofuel production”. Appl. Microbiol. Biotechnol.

Demirbas,A.,2009. “Biorefineries: Current activities and future developments”

Dejsungkranont, M., Chisti, Y., and Sirisansaneeyakul, S., 2017."Optimization of production of C-phycocyanin and extracellular polymeric substances by Arthrospira sp".Bioprocess and Biosystems Engineering, Vol. 40, No. 8, pp. 1173–1188. https://doi.org/10.1007/s00449-017-1778-y

Doran, P. M., 2013."Bioprocess engineering principles".In Academic Press (1st ed., Vol. 1, Issue 1). https://doi.org/10.1016/S0892-6875(96)90075-8

Drexler, I. L. C., and Yeh, D. H., 2014."Membrane applications for microalgae cultivation and harvesting: a review".Reviews in Environmental Science and Bio/Technology, Vol. 13, No. 4, pp. 487–504. https://doi.org/10.1007/s11157-014-9350-6

Duan, Y., and Shi, F., 2014."Bioreactor design for algal growth as a sustainable energy source".In Reactor and Process Design in Sustainable Energy

Technology (pp. 27–60).Elsevier Inc. https://doi.org/10.1016/B978-0-444-59566-9.00002-8

El-malek, F. A., Rofeal, M., Farag, A., Omar, S., and Khairy, H., 2021."Polyhydroxyalkanoate nanoparticles produced by marine bacteria cultivated on cost effective Mediterranean algal hydrolysate media".Journal of Biotechnology, Vol. 328, , pp. 95–105. https://doi.org/10.1016/j.jbiotec.2021.01.008

Fujisawa, T., Narikawa, R., Okamoto, S., Ehira, S., Yoshimura, H., Suzuki, I., Masuda, T., Mochimaru, M., Takaichi, S., Awai, K., Sekine, M., Horikawa, H., Yashiro, I., Omata, S., Takarada, H., Katano, Y., Kosugi, H., Tanikawa, S., Ohmori, K., … Ohmori, M., 2010."Genomic structure of an economically important cyanobacterium, arthrospira (Spirulina) platensis NIES-39".DNA Research, Vol. 17, No. 2, pp. 85–103. https://doi.org/10.1093/dnares/dsq004

García, G., Sosa-Hernández, J. E., Rodas-Zuluaga, L. I., Castillo-Zacarías, C., Iqbal, H., and Parra-Saldívar, R., 2020."Accumulation of PHA in the Microalgae Scenedesmus sp. under Nutrient-Deficient Conditions".Polymers, Vol. 13, No. 1, pp. 131. https://doi.org/10.3390/polym13010131

Geyer, R., 2020."Production, use, and fate of synthetic polymers".In Plastic Waste and Recycling (pp. 13–32).Elsevier. https://doi.org/10.1016/B978-0-12-817880-5.00002-5

Geyer, R., Jambeck, J. R., and Law, K. L., 2017."Production, use, and fate of all plastics ever made.".Science Advances, Vol. 3, No. 7, pp. e1700782. https://doi.org/10.1126/sciadv.1700782

Giraldo-Calderón, N. D., Romo-Buchelly, R. J., Arbeláez-Pérez, A. A., Echeverri-Hincapié, D., & Atehortúa-Garcés, L. 2018. "Microalgae biorefineries: applications and emerging technologies." https://doi.org/10.15446/dyna.v85n205.68780

Ghosh, S., Greiserman, S., Chemodanov, A., Slegers, P. M., Belgorodsky, B., Epstein, M., Kribus, A., Gozin, M., Chen, G. Q., and Golberg, A., 2021."Polyhydroxyalkanoates and biochar from green macroalgal Ulva sp. biomass subcritical hydrolysates: Process optimization and a priori economic and greenhouse emissions break-even analysis".Science of the Total Environment, Vol. 770, , pp. 145281. https://doi.org/10.1016/j.scitotenv.2021.145281

Heiss, C., Black, I., Ishihara, M., Tatli, M., Devarenne, T. P., and Azadi, P., 2021."Structure of the polysaccharide sheath from the B race of the green microalga Botryococcus braunii".Algal Research, Vol. 55, , pp. 102252. https://doi.org/10.1016/j.algal.2021.102252

Hillen, L. W., Pollard, G., Wake, L. V., and White, N., 1982."Hydrocracking of the oils of Botryococcus braunii to transport fuels".Biotechnology and Bioengineering, Vol. 24, No. 1, pp. 193–205.

J Matos, C. C. P. F. C. A. N. B., 2020."Investigation of nutraceutical potential of the microalgae Chlorella vulgaris and Arthrospira platensis".Food Sci Technol, Vol. 55, , pp. 303–312.

Jankowska, E., Sahu, A. K., and Oleskowicz-Popiel, P., 2017."Biogas from microalgae: Review on microalgae’s cultivation, harvesting and pretreatment for anaerobic digestion".In Renewable and Sustainable Energy Reviews (Vol. 75, pp. 692–709).Elsevier Ltd. https://doi.org/10.1016/j.rser.2016.11.045

Kartik, A., Akhil, D., Lakshmi, D., Panchamoorthy Gopinath, K., Arun, J., Sivaramakrishnan, R., and Pugazhendhi, A., 2021."A critical review on production of biopolymers from algae biomass and their applications".Bioresource Technology, Vol. 329, , pp. 124868. https://doi.org/10.1016/j.biortech.2021.124868

Kavitha, G., Kurinjimalar, C., Sivakumar, K., Palani, P, Rengasamy, R. “Biosynthesis, purification and characterization of polyhydroxybutyrate from Botryococcus braunii kütz”. 2016.

Katiyar, R., Banerjee, S., and Arora, A., 2021."Recent advances in the integrated biorefinery concept for the valorization of algal biomass through sustainable routes".In Biofuels, Bioproducts and Biorefining (Vol. 15, Issue 3, pp. 879–898).John Wiley and Sons Ltd. https://doi.org/10.1002/bbb.2187

Khanra, S., Mondal, M., Halder, G., Tiwari, O. N., Gayen, K., and Bhowmick, T. K., 2018."Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: A review".In Food and Bioproducts Processing (Vol. 110, pp. 60–84).Institution of Chemical Engineers. https://doi.org/10.1016/j.fbp.2018.02.002

Kim, J., Ryu, B.-G., Kim, B.-K., Han, J.-I., and Yang, J.-W., 2012."Continuous microalgae recovery using electrolysis with polarity exchange".Bioresource Technology, Vol. 111, , pp. 268–275. https://doi.org/10.1016/j.biortech.2012.01.104

Kirnev, P. C. S., Carvalho, · J C, Vandenberghe, · L P S, Karp, · S G, and Soccol, · C R., 2020."Technological mapping and trends in photobioreactors for the production of microalgae".World Journal of Microbiology and Biotechnology, Vol. 36, No. 3, pp. 42. https://doi.org/10.1007/s11274-020-02819-0

Larkum, A. W. D., Ross, I. L., Kruse, O., and Hankamer, B., 2012."Selection, breeding and engineering of microalgae for bioenergy and biofuel production".In Trends in Biotechnology (Vol. 30, Issue 4, pp. 198–205).Elsevier Ltd. https://doi.org/10.1016/j.tibtech.2011.11.003

L. Gouveia, A.P. Batista, I. Sousa, A. Raymundo and N.M. Bandarra. “Microalgae in Novel Food Products”. 2008.

Lindström, U. M., 2007."Organic Reactions in Water: Principles, Strategies and Applications".In Organic Reactions in Water: Principles, Strategies and Applications.John Wiley and Sons. https://doi.org/10.1002/9780470988817

López Rocha, C. J., Álvarez-Castillo, E., Estrada Yáñez, M. R., Bengoechea, C., Guerrero, A., and Orta Ledesma, M. T., 2020."Development of bioplastics from a microalgae consortium from wastewater".Journal of Environmental Management, Vol. 263, , pp. 110353. https://doi.org/10.1016/j.jenvman.2020.110353

Lutzu, G. A., Ciurli, A., Chiellini, C., Di Caprio, F., Concas, A., and Dunford, N. T., 2021."Latest developments in wastewater treatment and biopolymer production by microalgae".Journal of Environmental Chemical Engineering, Vol. 9, No. 1. https://doi.org/10.1016/j.jece.2020.104926

Metzger, P., and Largeau, C., 2005."Botryococcus braunii: A rich source for hydrocarbons and related ether lipids".In Applied Microbiology and Biotechnology (Vol. 66, Issue 5, pp. 486–496). https://doi.org/10.1007/s00253-004-1779-z

Milledge, J. J., and Heaven, S., 2011."Disc Stack Centrifugation Separation and Cell Disruption of Microalgae: A Technical Note".Environment and Natural Resources Research, Vol. 1, No. 1. https://doi.org/10.5539/enrr.v1n1p17

Mo, W., Soh, L., Werber, J. R., Elimelech, M., and Zimmerman, J. B., 2015."Application of membrane dewatering for algal biofuel".In Algal Research (Vol. 11, pp. 1–12).Elsevier. https://doi.org/10.1016/j.algal.2015.05.018

Molina Grima, E., Belarbi, E. H., Acién Fernández, F. G., Robles Medina, A., and Chisti, Y., 2003."Recovery of microalgal biomass and metabolites: Process options and economics".Biotechnology Advances, Vol. 20, No. 7–8, pp. 491–515. https://doi.org/10.1016/S0734-9750(02)00050-2

Moradi, Z., Madadkar Haghjou, M., Zarei, M., Colville, L., and Raza, A., 2021."Synergy of production of value-added bioplastic, astaxanthin and phycobilin co-products and Direct Green 6 textile dye remediation in Spirulina platensis".Chemosphere, Vol. 280, , pp. 130920. https://doi.org/10.1016/j.chemosphere.2021.130920

Mourão, M. M., Gradíssimo, D. G., Santos, A. V., Schneider, M. P. C., Faustino, S. M. M., Vasconcelos, V., and Xavier, L. P., 2020."Optimization of polyhydroxybutyrate production by amazonian microalga Stigeoclonium sp. B23".Biomolecules, Vol. 10, No. 12, pp. 1–15. https://doi.org/10.3390/biom10121628

Mourão, M. M., Xavier, L. P., Urbatzka, R., Figueiroa, L. B., da Costa, C. E. F., Dias, C. G. B. T., Schneider, M. P. C., Vasconcelos, V., and Santos, A. V., 2021."Characterization and biotechnological potential of intracellular polyhydroxybutyrate by stigeoclonium sp. B23 using cassava peel as carbon source".Polymers, Vol. 13, No. 5, pp. 1–21. https://doi.org/10.3390/polym13050687

Niaounakis M. Biopolymers: Processing and Products”. Elsevier Science, William Andrew (PDL handbook series), 2014.

Ociński, D., Augustynowicz, J., Wołowski, K., Mazur, P., Sitek, E., and Raczyk, J., 2021."Natural community of macroalgae from chromium-contaminated site for effective remediation of Cr(VI)-containing leachates".Science of the Total Environment, Vol. 786, , pp. 147501. https://doi.org/10.1016/j.scitotenv.2021.147501

Olguín, E. J., 2003."Phycoremediation: Key issues for cost-effective nutrient removal processes".Biotechnology Advances, Vol. 22, No. 1–2, pp. 81–91. https://doi.org/10.1016/S0734-9750(03)00130-7

Parsons, S., Allen, M. J., and Chuck, C. J., 2020."Coproducts of algae and yeast-derived single cell oils: A critical review of their role in improving biorefinery sustainability".In Bioresource Technology (Vol. 303).Elsevier Ltd. https://doi.org/10.1016/j.biortech.2020.122862

Pez Jaeschke, D., Rocha Teixeira, I., Damasceno Ferreira Marczak, L., and Domeneghini Mercali, G., 2021."Phycocyanin from Spirulina: A review of extraction methods and stability".In Food Research International (Vol. 143, p. 110314).Elsevier Ltd. https://doi.org/10.1016/j.foodres.2021.110314

Picó, Y., 2013."Ultrasound-assisted extraction for food and environmental samples".TrAC Trends in Analytical Chemistry, Vol. 43, , pp. 84–99. https://doi.org/10.1016/j.trac.2012.12.005

Priyan Shanura Fernando, I., Kim, K.-N., Kim, D., Jeon, Y.-J., 2019. “Algal polysaccharides: potential bioactive substances for cosmeceutical applications”. 2015.

Richmond A. “Handbook of microalgal culture: biotechnology and applied phycology”. 2004.

Soria, A. C., Ruiz-Aceituno, L., Ramos, L., and Sanz, L. M., 2014."Microwave-Assisted Extraction of Polysaccharides".In Polysaccharides (pp. 1–18).Springer International Publishing. https://doi.org/10.1007/978-3-319-03751-6_43-1

SS Costa, A. M. D. A. C. S. M. M. J. C. J. D., 2018."Efficacy of Spirulina sp. polyhydroxyalkanoates extraction methods and influence on polymer properties and composition".Algal Res, Vol. 33, , pp. 231–238.

Tasić, M. B., Pinto, L. F. R., Klein, B. C., Veljković, V. B., and Filho, R. M., 2016."Botryococcus braunii for biodiesel production".In Renewable and Sustainable Energy Reviews (Vol. 64, pp. 260–270).Elsevier Ltd. https://doi.org/10.1016/j.rser.2016.06.009

Tan D, Yin J. and Chen G. Q. “Production of Polyhydroxyalkanoates. In Pandey A, Negi S, Soccol CR. Eds. Current Developments in Biotechnology and Bioengineering Production. Isolation and Purification of Industrial Product”. 2017.

Tavanandi, H. A., and Raghavarao, K. S. M. S., 2020."Ultrasound-assisted enzymatic extraction of natural food colorant C-Phycocyanin from dry biomass of Arthrospira platensis".LWT, Vol. 118, , pp. 108802. https://doi.org/10.1016/j.lwt.2019.108802

Teixeira, M. R., and Rosa, M. J., 2006. "Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of Microcystis aeruginosa. Part I: The key operating conditions".Separation and Purification Technology, Vol. 52, No. 1, pp. 84–94. https://doi.org/10.1016/j.seppur.2006.03.017

Thiruvenkadam, S., Izhar, S., Yoshida, H., Danquah, M. K., and Harun, R., 2015. "Process application of Subcritical Water Extraction (SWE) for algal bio-products and biofuels production".In Applied Energy (Vol. 154, pp. 815–828).Elsevier Ltd. https://doi.org/10.1016/j.apenergy.2015.05.076

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., and Hoadley, A., 2010.Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. https://doi.org/10.1063/1.3294480

Ugwu, C. U., Aoyagi, H., and Uchiyama, H., 2008. "Photobioreactors for mass cultivation of algae".In Bioresource Technology (Vol. 99, Issue 10, pp. 4021–4028).Elsevier. https://doi.org/10.1016/j.biortech.2007.01.046

Verdugo, M., Lim, L. T., and Rubilar, M., 2014. "Electrospun Protein Concentrate Fibers from Microalgae Residual Biomass".Journal of Polymers and the Environment, Vol. 22, No. 3, pp. 373–383. https://doi.org/10.1007/s10924-014-0678-3

Wang, R., Peng, B. and Huang, K., “The research progress of CO2 sequestration by algal bio-fertilizer in China”. 2015.

Wicaksana, F., Fane, A. G., Pongpairoj, P., and Field, R., 2012."Microfiltration of algae (Chlorella sorokiniana): Critical flux, fouling and transmission".Journal of Membrane Science, Vol. 387–388, No. 1, pp. 83–92. https://doi.org/10.1016/j.memsci.2011.10.013

Williams, P. J. le B., and Laurens, L. M. L., 2010. "Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics".Energy & Environmental Science, Vol. 3, No. 5, pp. 554. https://doi.org/10.1039/b924978h

Yen, H.W., Hu, I.C., Chen, C.Y., Ho, S.H., Lee, D.J., Chang, J.S. “Microalgae-based biorefinery–from biofuels to natural products”. 2013.

Yirgu, Z., Leta, S., Hussen, A., Khan, M. M., and Aragaw, T., 2021. "Optimization of microwave-assisted carbohydrate extraction from indigenous Scenedesmus sp. grown in brewery effluent using response surface methodology".Heliyon, Vol. 7, No. 5, pp. e07115. https://doi.org/10.1016/j.heliyon.2021.e07115

Zeller, M. A., Hunt, R., Jones, A., and Sharma, S., 2013. "Bioplastics and their thermoplastic blends from Spirulina and Chlorella microalgae".Journal of Applied Polymer Science, Vol. 130, No. 5, pp. 3263–3275. https://doi.org/10.1002/app.39559

Zenouzi, A., Ghobadian, B., Hejazi, M. A., and Rahnemoon, P., 2013. "Harvesting of microalgae Dunaliella salina using electro flocculation”. Journal of Agricultural Science and Technology, Vol. 15, No. 5, pp. 879–888.