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Current application of algae derivatives for bioplastic production: A review

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Abstract

Improper use of conventional plastics poses challenges for sustainable energy and environmental protection. Algal derivatives have been considered as a potential renewable biomass source for bioplastic production. Algae derivatives include a multitude of valuable substances, especially starch from microalgae, short-chain length polyhydroxyalkanoates (PHAs) from cyanobacteria, polysaccharides from marine and freshwater macroalgae. The algae derivatives have the potential to be used as key ingredients for bioplastic production, such as starch and PHAs or only as an additive such as sulfated polysaccharides. The presence of distinctive functional groups in algae, such as carboxyl, hydroxyl, and sulfate, can be manipulated or tailored to provide desirable bioplastic quality, especially for food, pharmaceutical, and medical packaging. Standardizing strains, growing conditions, harvesting and extracting algae in an environmentally friendly manner would be a promising strategy for pollution control and bioplastic production.

Keywords: Bioplastic; Algae; Cyanobacteria; Polyhydroxyalkanoates (PHAs); Seaweed; Starc

1. Introduction

Plastic is an essential artificial product covering modern society. As known, plastic is popular because it possesses unique properties such as being lightweight, flexible, resistant to water, heat, electricity, and ease in production. Plastics can be constituted from petroleum-based polymers where the successful downstream processing has subsequently given rise to the term fossil-based plastics (i.e., derived from crude oil and natural gas). It includes several familiar plastics such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and others. The current total plastic production is mainly distributed in South America (10%), North America (17%), Europe (26%), and Asia (46%). Common fossil-based plastics are mostly considered chemically stable, biodegradable but extremely slow rate, and are principally fallen into a component of non-biodegradable (Fig.1a). Consequently, plastic waste has aroused increased attention as a hotspot regarding its potential impact on the terrestrial ecosystems, atmosphere (Brahney et al., 2020), freshwater (Winton et al., 2020), and the marine environment (González-Fernández et al., 2021). According to González-Fernández et al. (2021), between 307 and 925 million liters of items are discharged into the ocean each year in Europe, of which plastic products account for about 82%. It is mainly fragments and single-use items like bottles, packaging, and bags. Moreover, it is predicted that 11 billion tons of plastics will be accumulated into the environment by 2025 (Brahney et al., 2020). With such an enormous quantity of "white pollution", the subsequently degraded macro-, meso-, micro-, and nano-plastic could pose severe threats to human health through the food chain.

Recently, research has focused on bioplastics as a potential direction towards sustainable products and reduced environmental impact (Talan et al., 2022). Synthetic plastics made from renewable resources are bioplastics, such as agro-polymers (terrestrial crops), algal-polymers, and bacterial polymer (Nandakumar et al., 2021; Devadas et al., 2021). Currently, bioplastics account for about one percent of the more than 368 million tons of plastics produced annually (European Bioplastics, 2020). These results suggest that the application of bioplastics is still in its infancy, as evidenced by a

global production capacity of 2.11 million tons, of which only 55.5% is derived from biodegradable materials (Fig. 1b). A major factor limiting the production of biodegradable materials is their hygroscopic nature, which causes them to absorb water, reducing structural integrity. Therefore, bioplastics need to be improved its properties such as toughness, stiffness, brittleness, thermal stability, and reduced production cost (Kato, 2019; Sangroniz et al., 2019). According to European Bioplastics (2020), the application of bioplastics to manufacture rigid and flexible packaging accounts for 52% of the market share (Fig.2a). Recent forecasts suggested that the biodegradable component could gradually increase to compete with fossil-based plastics thanks to the increasing production capacity from 1.051 million tons (2019) to 1.800 million tons (2025) (Fig.2b).

Bioplastics could be made from agro-polymers due to the availability of renewable biomass such as corn starch, straw, woodchips, sawdust, recycled food waste, and vegetable oils. Although the material is environmentally friendly, the structure of terrestrial plants such as bamboo (21-31% lignin, 26-43% cellulose, and 30% hemicelluloses) certainly requires additional activation energy to biomass conversion (Noreen et al., 2016; Yang et al., 2019). The cultivation of terrestrial plants takes time and may be unstable due to population growth, social influence, extreme climate, and other environmental consequences (Niaounakis, 2015; Noreen et al., 2016). However, biomass is a renewable resource only if its consumption rate does not exceed the rate of replenishment. Therefore, it may be a sound strategy to select biomass sources with a fast growth rate, easy to exploit and not disturb agricultural activities. As the bioplastics market continues to grow and diversify to a large extent (Fig.2a,b), a sustainable biomass supply strategy is undoubtedly required.

Algae-based bioeconomy has recently gained interest in bioplastic production. Algae biomass possesses a low percentage of lignin while rich in long-chain hydrocarbons; therefore, high purity cellulose can be extracted in economical ways to produce bioplastics (Zanchetta et al., 2021). Algae, especially microalgae, have great potential as a renewable resource because doubling time can be obtained within a day, diverse cultivation environment, its growth does not require arable land,

and unexploited resource. They can grow under polluted conditions such as CO_2-rich gases or nitrogen and phosphorus-containing wastewater (Zerrouki and Henni, 2019), a vital feature to balance the water-food-energy nexus. There are many holistic overviews and discussions on the general applications of algae biopolymer towards a sustainable circular economy (Devadas et al., 2021). Scientific is also focused on the potential algae strains for bioplastic production (Kartik et al., 2021) or particular functionality of compounds such as polyhydroxyalkanoates (PHAs) (Larsson et al., 2016; Troschl et al., 2017). It is highlighted that the biopolymers from algal biomass have an essential role in industrial fields such as cosmetics, pharmaceuticals, food packaging and medicine (Mehta et al., 2018; Kartik et al., 2021).

Although algae are considered a low-cost source, algae derivatives are often expensive. This fact is because other related processes such as cultivation, harvesting, pretreatment, and physiochemical extraction of polysaccharides could add up to hundreds of dollars per unit product, preventing the competition of bioplastics on a commercial basis. As a result, each type of algal polysaccharide can be used as the main ingredient or an additive of bioplastics, based entirely on polymer compatibility, yield biomass, and cost production. Therefore, it is necessary to comprehend the characterization and production of algal derivatives, including classification, bio-polymer properties, and application strategies for bioplastic production. This review focused on valuable substances for bioplastic production, namely starch of microalgae, PHAs derived from Cyanobacteria, and others like Agar/Agarose, Alginates, Carrageenan, Fucoidan, and Ulvan derived from seaweeds. In addition, the cultivation processes (open and closed systems, nutrient starvation strategies) and extraction methods (chemical, thermal, enzymatic treatments) will also be discussed. This information is considered beneficial to support the standardization of algae production and increase the commercial competitiveness of bioplastics.

2. Algae starch-based bioplastics

2.1. Algae sources for starch production

Algae are divided into microalgae and macroalgae based on their size and morphology. In addition, different habitats such as freshwater or marine evironment contribute to their diversity, namely freshwater microalgae or marine macroalgae. Generally, microalgae are unicellular organisms that are mostly less than 1000 µm in their largest dimension, with rapid growth rate and high productivity (Chisti, 2007; Hannon et al., 2010). It doubles itself with an average time of 26 h (8-70 h) (Liu et al., 2011), and some strains of the phylum *Chlorophyta* (genus *Chlamydomonas*) even reproduce within 6-8 h (Griffiths and Harrison, 2009; John et al., 2011). Starch is naturally accumulated in microalgae as granules with a size distribution range of about 0.5-2.1 µm. Starch is a predominant component of microalgae biomass produced by many strains such as *Chlorella*, *Chlamydomonas*, and *Scenedesmus* (Gifuni et al., 2017; Mathiot et al., 2019). Table 1 summarizes the studies on culturing and promoting starch yield from different groups of algae. Among them, freshwater microalgae attract the most attention for research. In contrast, the number of studies on large-sized algae for starch production is limited, except for the green seaweed group *Ulva ohnoi* (Prabhu et al., 2019). Microalgae species usually grow faster than freshwater/marine macroalgae species thus often being the most research target for starch production in recent years.

Starch is a natural polymer that acts as the energy storage unit in plants and algae. It consists of two types of α-D-glucose polymers, namely amylose (20-30%), a substantially linear polymer with a molecular weight of about 10^5 –10^6 g mol^{-1}, and amylopectin (70-80%) with a molecular weight of about 10^7–10^8 g mol^{-1} (Niaounakis, 2015). It has been identified that algae-derived starch possesses similar characteristics in terms of crystalline, molecular weight, and thermal properties compared to the starch derived from vascular plants such as corn, wheat, rice, oats, and amaranth. Therefore, starch from algae can be considered as a valid alternative compound in several fields, such as producing bioplastics (Gifuni et al., 2017).

2.2. Bioplastic production from algae starch

The conformational changes of polysaccharides are a function of their monosaccharides and their order, their glycosidic bonds, degree of branching, molecular weight, and functional groups, such as sulfate esters and hydroxyl groups. Natural starch possesses a high number of hydroxyl groups on the polymers amylose and amylopectin. This makes starch naturally hydrophilic, which cannot be directly used to produce plastic due to intermolecular forces and hydrogen bonding effects. Starch itself has poor mechanical properties, low moisture resistance, and is unworkable as a thermoplastic material. To improve the mechanical properties, starch can be mixed with other substances derived from renewable, synthetic, and mixed sources. Starch has been subjected to integrating with a plasticizer such as glycerol, sorbitol, xylitol, tri-, di-ethanolamine, or glucose to make a deformable thermoplastic material (thermoplastic starch) (Özeren et al., 2020).

Among these, waste glycerol is generated in large quantities as a major by-product of biodiesel production. With this in mind, a combination of starch from microalgae and glycerol from biodiesel production could be a viable option. Adding 30-40% (wt) glycerol can effectively reduce the formation of a hydrogen bond network of starch, expanding the free volume of starch and reducing the intermolecular forces (Hashemi Tabatabaei et al., 2018; Özeren et al., 2020). From Fig 3a, the added glycerol can either serve the hydrogen bonds between the hydroxyl groups of the starch or establish hydrogen bonds between the starch and the plastic. This combination almost changes the initial structure of starch and improves the physicochemical stability of polymers (Nandakumar et al., 2021). Starch from microalgae is highly attractive to produce bioplastics under high processing temperature with the proper dosage of glycerol. For example, starch can synthesize with 30% w/w glycerol and then mixed in a twin-screw extruder up to 120^0C to obtain a homogeneous plasticized structure with no visible cell fragments (Gifuni et al., 2017). It is inevitable to combine starch with additives or reinforcements ingredients to enhance quality for production standards. However, the excessive addition of non-biodegradable substances can reduce the biodegradability of bioplastic.

2.3. Factors affecting starch production

2.3.1. Cultivation conditions

Cultivation conditions are an important factor affecting starch production. To enhance the richness of single microalgae species, a bubble column photobioreactor or a closed system can be an ideal configuration for culture (Table 1). The bubble column photobioreactor allows the production of high starchy algae because it can shock algae metabolite and easily control other operating parameters. It is proved that the target of rapamycin (TOR) in plants or algae will be inactivated by lack of energy, starvation, and stress, thus leading to starch accumulation to a large extent (Pancha et al., 2019; McCready et al., 2020). Under stress response conditions such as extreme pH, CO_2, nitrogen starvation, sulfur-deprived medium, high salinity, light to dark transitions, or genes mutation, the green algae are capable of triggering the accumulation of starch granules (Cheng et al., 2017). Table 1 shows that the genus Chlorella is currently favored for culture (light intensity 100-1300 µmol $m^{-2}s^{-1}$ and CO_2 1-2%). Besides, screening for microalgae species and other optimal culture conditions is still of interest, for example:

- *Chlamydomonas reinhardtii* was found to accumulate 49% starch after being transferred to a sulfur-deficient condition for 460 h (Mathiot et al. 2019).
- 25% starch could be achieved for *Scenedesmus obliquus* in 4 days (Li et al., 2015) or 40% (w/w) in 1 day for *Chlorella sorokiniana* with light irradiance of 300 µmol m^{-2} s^{-1} and CO_2 of 2% (De Jaeger et al., 2014).
- The growth of many *Chlamydomonas* species in bubble column photobioreactors was increased in starch productivity (i.e., *C. pitschmannii* (30%), *C. oblonga* (44%), *C. applanata* (30%), *C. moewus* (36%) (Gifuni et al., 2017). Moreover, reactor could control adequate light (220 µ mol photons m^{-2} s^{-1}) and CO_2 supply (2%) show the highest starch productivity for *C. oblonga* and

C.moewusii (0.053 and 0.046 g L^{-1} d^{-1}, respectively) (Gifuni et al., 2017). The *Chlamydomonas moewusii* was characterized by high starch content (45% dry weight).

- High concentrations of 10% CO_2, 1000 µmol m^{-2} s^{-1} of high light intensity, 0.375 g L^{-1} $NaNO_3$, and limited nitrogen concentration were critical for carbohydrate and starch accumulation. At five days, the total starch content was 60.3% (w/w) (Cheng et al. 2017).

2.3.2. Extraction and quantification

For extraction, the cell disruption method can be used for extracting starch. It is involved ultrasonication (sonicator using a titanium probe, 20 kHz and 30 W), bead milling (glass beads), and physicochemical method (NaOH 1M, 90 °C for 30 min) (Wong et al., 2019). It revealed that the bead-beating method (950 mg, 5 min) achieved the highest starch recovery (96.6 ± 2.73%), followed by physicochemical (95.1 ± 6.74%) and ultrasonication (70.40 ± 4.48%). The bead milling is regarded as a convenient method that induces compaction and leads to a high energy transfer from the bead to the microalgae, disrupting the cells and thus facilitating starch extraction.

Starch content in *Chlorella sp.* can be quantitatively determined by measuring the amount of glucose released by enzymatic hydrolyses such as α-amylase and amyloglucosidase, as quantified using the D-Glucose Assay Kit or high-performance liquid chromatography (HPLC) (Laurens et al., 2012; Warren et al., 2015). Roundly 10% starch can be found in a *Chlorella sp*. in wild conditions; other components could be found such as Carbohydrates (22%), Lipids (17%), Protein (44%), and Ash (4%) (Laurens et al., 2012). The presence of carbohydrates in microalgae serves two main purposes: (1) as a structural component in the cell wall; (2) as an energy storage component inside the cell, such as starch. The most common monosaccharides of microalgae carbohydrates are glucose, rhamnose, xylose, mannose and galactose. The distribution of these monosaccharide ratios is influenced by strain, cultivation, and environmental conditions. For example, the high glucose content was found in *Dunaliella tertiolecta* (85%), *Chlamydomonas rehardtii* (74.9%), *Spirulina*

platensis (54.4%), *Chloroccum sp.* (47%) (Markou et al., 2012). *Chloroccum sp.* and *Spirulina platensis* also possess a remarkably high xylose content (27%) and rhamnose (22.3%), respectively. Meanwhile, mannose and galactose usually account for a high percentage in *Phaeodactylum tnicornutum* (45.9%) and *Nitzchia ciosterium* (18.4%), respectively. Some microalgae are innately higher in carbohydrates than others, which provides an advantage for making higher-value products. The polymeric derivatives from microalgae are more easily extracted and pretreated due to their low lignin content, making them a good choice for biomass conversion technology than the conventional lignocellulosic feedstock (John et al., 2011). In short, the production of bioplastic from starch is currently affected by several factors such as algae strain, culture technology, extraction method, and associated additives. The cultivation process should also be paid attention to the suitability and stability of the microalgae strain, which is expected to be done widely for different species and cultural conditions.

3. Polyhydroxyalkanoates from Cyanobacteria

3.1. Strategies for polyhydroxyalkanoates production from cyanobacteria

During the COVID-19 pandemic, the proliferation of retail channels increased the consumption of plastic packaging due to the restaurant business being shut down (Jia, 2020; Oliveira et al., 2020). This fact has highlighted the role of plastic packaging as being too convenient for daily life. Plastic packaging can be made of fossil-derived plastic or polyhydroxyalkanoates (PHA)-derived plastic. PHA is aliphatic polyesters, which can be accumulated as intracellular granules by heterotrophic bacteria, mainly through the natural fermentation process or from recombinant *E. coli* as genetically engineered pathways. According to the number of carbons in the PHA structure, the monomer precursors can be classified into short-chain length (scl-PHA, ≤ 5 carbons) and medium-chain length PHAs (mcl-PHA, 6–14 carbons) and long-chain PHA (lcl-PHA, >15 carbons) (McAdam et al., 2020; Suzuki et al., 2021). Scl-PHA can be used in food packaging and disposable products, while lcl-PHA

is rare in nature and of little interest to develop bioplastics (Muneer et al., 2020). The first compound of the scl-PHA group is poly(3-hydroxybutyrate), denoted P(3HB) or PHB. The P(3HB) was discovered during research on *B. Megaterium* by Francois Lemoigne in 1926 (Yadav et al., 2020). However, bioplastics from scl-PHA such as PHB produced from microorganisms is almost 5 times more expensive than polypropylene due to the cost invested for substrate sources such as sucrose, lactose, starch for biopolymer production (Costa et al., 2019). For example, a large substrate is usually required before PHB extraction until the bacteria grow into the stationary phase. As a result, the market price of PHB bioplastics is typically between 2.4 and 5.5 US$ kg^{-1} vs. 1.2 US$ kg^{-1} (PHAs petroleum-based plastics), the carbon source attributed to 30–50% of production costs (Costa et al., 2019). Furthermore, the circular economy requires production bioplastics at a sustainable level, such as reducing production costs, reusing waste, reducing CO_2 and greenhouse gases, promoting bioremediation, and so on. This fact underscores the importance of finding an inexpensive source of PHB for downstream processes, such as *Cyanobacteria*. Unlike prokaryotes, Cyanobacteria can produce polyhydroxyalkanoates (PHAs) as intracellular carbon and energy storage compounds while they supply O_2 and consume CO_2 through algae-like photosynthesis (Troschl et al., 2017). *Cyanobacteria* do not require as much sugar as heterotrophic microorganisms (e.g., sugar cane molasses) for PHB production, resulting in less impact on agricultural activity. The use of cyanobacteria to produce bioplastics may not be cost-competitive in the early stages of application (*see in next section*), but it can warrant a strategy to produce environmentally friendly plastic products.

3.2. Bioplastic production from polyhydroxyalkanoates

The scl-PHA such as P(3HB) displays thermoplastic properties that are similar to commercial polypropylene (PP). The melting temperature was 175°C and 176°C while tensile strength was 40 MPa and 38 MPa, respectively (Bugnicourt et al., 2014). Besides, the pure P(3HB) structure is known for its biodegradability, absolute water resistance, and reduced permeability to atmospheric

gaseous components and water vapor (McAdam et al., 2020). Thus, it is widely reported as a candidate alternative to synthetic polymers such as PP. However, the processing of P(3HB) for bioplastic is tricky due to its melting temperature (~180°C) nearly to its degradation temperature (~200°C). The elongation at break of P(3HB) is extremely low than PP (5% vs. 400%). In contrast, other members of scl-PHA can overcome this drawback, such as poly 4-hydroxybutyrate, P (4HB) and poly 3-hydroxyvalerate, P (3HV) (Larsson et al., 2016). For example, the P(4HB) has the potential to be stretched up to 1000% and significantly low melting temperature at ~ 60°C (Utsunomia et al., 2020). Thus, copolymerization of 3-HB with other monomers such as 4-HB to yield P(3HB-co-4HB) can increase flexibility, decrease melting point and crystallinity compared with P (3HB) (Larsson et al., 2016). The member of scl-PHA and its copolymer are suitable for cosmetic, medical, packaging, molded goods, paper coatings, non-woven fabrics, adhesives, and films (Table 2).

Many industrial sectors deal with scl-PHA and its copolymer production because biodegradability is considered a substitute power for petroleum-based plastics. Environmental considerations, not all biodegradable plastics can be decomposed anywhere on our planet, and the truth is that marine environments will inevitably reduce biodegradation rates than terrestrial environments. In this light, the use of scl-PHA and its copolymer allows bioplastics to biodegrade in many environments within a reasonable timescale. The biodegradability of a substance is defined as the degree of biodegradation (≥ 90%) that must be reached in less than 6 months (Ashter, 2016). Using P(3HB-co-3HV), microorganisms can metabolize 65% initial weight of P (3HB-co-14% 3HV) in seawater after 8 weeks, and fiber's strain and stress rapidly decrease to zero. The rate of surface erosion (weight loss) was almost independent of the copolymer compositions (ratios) but noticeably dependent upon the temperature of the seawater (Doi et al., 1992). Results indicated that P(3HB-co-3HV) could be a good choice for rapidly degraded microbial enzymes from different environments such as coastal, shallow water, and deep-sea environments (Doi et al., 1992; Suzuki et al., 2021).

Besides, the P(3HB-co-3HV) does not float but will sink in aquatic systems due to its high density and rapid biodegradation.

3.3. Factors affecting starch production

3.3.1. Cultivation conditions

Typically, the PHB content in *Cyanobacteria* is < 10%, one order of magnitude lower than that of heterotrophic bacteria (up to 87%) (Lane and Benton, 2015). However, both phototrophic and heterotrophic conditions can stimulate PHB accumulation, depending on the *Cyanobacteria* strains, such as genera: *Synechocystis, Synechococcus, Arthrospira (Spirulina), Nostoc*, and others. *Cyanobacteria* culture can be performed using an open thin-layer cascading system (TLS) and a closed tubular photobioreactor (PBR) (Panuschka et al., 2019). Some potential species for outstanding PHB production have been selected as an example:

- Using thermophilic *Cyanobacterium*, *Synechococcus sp. MA19PHB* can spike up to 55% (w/w) of PHB under phosphate-limited culturing conditions (Nishioka et al., 2001)
- Using *synechocystis PCC6803* under heterotrophic conditions (0.4% of acetate + 0.4% of fructose + P-deficiency + gas-exchange limitation) can get 38% (w/w) of PHB in 10 days (Panda and Mallick, 2007).
- *Nostoc muscorum sp.* was obtained 35% (w/w) of dry cells when cells supplemented with 0.2% acetate were subjected to dark incubation for 7 days (Sharma and Mallick, 2005).
- *Nostoc muscorum sp.* can directly accumulate the copolymer P(3HB-co-3HV) up to 78% (w/w) under P and N-deficiency (Bhati and Mallick, 2015).

Such results have suggested that stimulation through N, P-deficiency may be an essential factor for achieving high yields of PHB. However, the culture of *Cyanobacteria* under heterotrophic conditions often entails the proliferation of *Ciliated protozoa, Bacterial and Fungal* contaminations

in the cultivation process (Troschl et al., 2017). Beside, the profitability of *Cyanobacterial* is controlled by naturally low PHB yields, harboring potential toxicity, and expensive growing, harvesting, and dewatering equipment due to its small size (0.5-40 µm). Therefore, a strategy to improve the quality of cultivation is needed. The stable culture of *Cyanobacteria* is the most crucial part and can be challenging to achieve for different strains (Troschl et al., 2017).

3.3.2. Extraction and quantification

PHB extraction from *Cyanobacteria* can be done by using sodium hypochlorite, methanol, and hot chloroform (Table 2). Dry biomass is added 4% sodium hypochlorite solution for 30 min at 45°C; then the sample was centrifuged at 6000 rpm in 30 minutes. Hot chloroform was added to the precipitated product overnight and then precipitated with methanol. The precipitation product was centrifuged at 6000 rpm for 30 min, dissolved in hot chloroform, and finally dried at 60°C (Roja et al., 2019).

Phenotypic detection methods for detecting intracellular PHB granules can be done by staining of cells with Sudan black B (Murray et al.,1994), using basic oxazine/oxazone dyes such as Nile blue A (Ostle and Holt, 1982) or the Nile red (Spiekermann et al., 1999). Although it is considerably time-consuming, the process could be successful when dark green or fluorescent granular PHA appears. The PHA synthase protein family is divided into four major types (type I, II, II, and IV) that are responsible for the polymerization of monomeric for a variety of microorganisms. However, only type-III PHA synthases were found in *Cyanobacteria* (Lane and Benton, 2015). The type-III synthases have two subunits typically coded in a single operon, PhaE (~40 kDa encoded by *phaE* genes) and PhaC (~40 kDa, encoded by *pha*C genes). Especially, the PhaC subunit exhibits a higher degree of conservation, making it an ideal target for PCR-based PHA genetic characterization. Using a colony-based PCR assay it is possible to rapidly determine whether the *Cyanobacteria* of interest contain the PHA synthase subunit PhaC (Lane and Benton, 2015).

3.3.3. Problems exist related to the cultivation

Although PHB from cyanobacteria is expected to help develop bioplastics in a more environmentally friendly direction. However, recent reports have shown that if *Cyanobacteria* are cultured from PBR (PHB yield of 15%), the final PHB production price could be 353 US$ kg^{-1} which is 100 times higher than the lowest heterotrophic market price. At PHB yield of 60%, the lowest price was about 28 US$ kg^{-1} when cultured from TLS (Panuschka et al., 2019). In both situations, more than 62% of total costs come from the cultivation and harvesting of *Cyanobacteria*. It is, therefore, necessary to expand research on strain screening, gene editing, optimization cultivation, and downstream processing.

So far, an idea that integrates wastewater treatment to culture *Cyanobacteria* has been reported as a sustainable strategy. However, when combined with wastewater treatment, *Cyanobacteria* can produce toxic microcystins (MCs) that reduce wastewater treatment function by increasing the toxicity of the treated water and reducing the potential for reuse (Romanis et al., 2021; Aye et al., 2021). Significantly, *Cyanobacteria* species can produce toxins such as microcystin-leucine arginine (MC-LR), which causes co-stress in marine ecosystems and freshwater (Griffith and Gobler, 2020). The treated water containing MC-LR can cause muscle tremors, bleeding in the liver, and coma of livestock. The selection of a non-toxic cyanobacterial strain for PHB production is critical (Fig. 3b). As reported, the addition of membrane technology such as micro, ultrafiltration, or forward osmosis could support the removal of toxicity under fresh and saline conditions (Dixon et al., 2020). Connecting recent studies has implied that PHB is the leading source of materials to compete with conventional plastics if its cost is feasible. In-depth studies should be prioritized to optimize cultivation and harvesting techniques to reduce costs.

4. Marine macroalgae

Marine macroalgae or seaweeds can be classified into three major groups: red algae (phylum: *Rhodophyta*), brown algae (phylum: *Ochrophyta*), and green algae (phylum: *Chlorophyta*). It grows in seawater instead of farmland with sizes ranging from a few millimeters to 50 meters. Seaweed produces high yields in the range of 30-83 dry tons ha^{-1} $year^{-1}$ compared with 3–30 dry tons ha^{-1} $year^{-1}$ for crops such as corn, sugarcane, corn silk, or poplar (Konda et al., 2015). Ecologically and commercially consideration, seaweed plays a pivotal role in the aquatic food chain, producing up to 50% of Earth's oxygen and is a raw material for humans to develop pharmaceuticals, cosmetics, and food (Kılınç et al., 2013). Seaweed feed ingredients in livestock diets that can reduce greenhouse gases attracted significant attention in the livestock industry sectors (Vijn et al., 2020).

The world's seaweeds production comes from China (47.9%), Indonesia (38.7%), the Philippines (4.7%), the Republic of Korea (4.5%), the Democratic People's Republic of Korea (1.6%), Japan (1.3%), and Malaysia (0.7%) (Mensi et al., 2020). It can be expected that the extending production of seaweed as a feedstock for biodegradable bioplastics has significant global advantages due to no freshwater consumption, reduced CO_2 emissions, and no fertilizers or pesticides used. Besides, the incorporation of seaweed into natural polymers shows an excellent potential for food packaging uses (Carina et al., 2021) due to increasing consumer awareness of product sustainability and polymer science. In Europe, seaweeds production has still relied on wild capture (68%), while the remaining portion (32%) is from macroalgae aquaculture (on land and at sea) (Araújo et al., 2021). For bioplastic production, the raw seaweed is quite expensive (21–112 US \$ MT^{-1}) depending on species and production method (Konda et al., 2015). Therefore, it is essential to locate some valuable compounds concerning sustainable exploitation.

4.1. Agar/Agarose

The valuable compound from red marine seaweed was agar. Agar is a polysaccharide composed of agarose (70%) and agaropectin (30%) extracted from membranal components of macroalgae.

Agarose is present as the gelling fraction, a neutral polysaccharide and a linear molecule essentially free of sulfates (D-galactose and 3,6-anhydro-L-galactopyranose). By contrast, agaropectin is a non-gelling fraction, an acid polysaccharide consisting of 3% to 10% sulfate. This makes agar possess gel or liquid properties and can alternate between states by heating or cooling (Şahin, 2021).

The most studied agars included genus *Gracilaria, Gelidium, Pterocladia, Acanthopeltis, Ahnfeltia, and Sesquipedale*. The *Gracilaria* is common in the tropics and has a high potential for cultivation. However, price escalation occurs when *Gracilaria* is required for careful storage and pre-treatment, such as dehydration, to avoid hydrolysis of agar caused by fermentation. The quality of the gel obtained from *Gracilaria* is often low due to its high sulfate content which acts as kinks in an agar helix formation, hindering gel network formation (Yarnpakdee et al., 2015). The additional step, such as alkaline pretreatments, is often requested to transform L-galactose 6-sulfate into 3,6-anhydro-L-galactose (i.e., desulphation).

Hii et al. (2016) compared the extraction method using alkali and photobleaching agar for bioplastic film production from *Gracilaria*. The bioplastic from alkali extracted agar exhibited excellent biodegradability within 30 days (99.29% weight loss) in comparison to photo bleached agar (43.27 % weight loss). Moreover, the thermal stability of bioplastic via alkali pre-treatment is better. However, alkaline pretreatment contributes to the increased cost of bioplastics production. The extractions that yield 0.5 kg of nontreated (120°C and 1 h) and treated (30% NaOH; 2h and 120°C) agars cost about 38.9 US$ and 116.4 US$, respectively (Mpatani and Vuai, 2019). This result is not favorable for mass production as its production price is considered to be higher than that of PHB produced from microorganisms (1.2 - 5.5 US$ kg^{-1}). To increase economic efficiency for food packaging film, the combination of heat and sonication for *Gelidium sesquipedale* extraction (no pre-treatment) was reduced 4-fold for the extraction time with optimal mechanical and water barrier performance, improved resistance for bioplastic (Martínez-Sanz et al., 2019).

418 *4.2. Carrageenan*

419 Carrageenan is anionic linear sulfated polysaccharides derived from class *Rhodophyceae* of red
420 seaweed. The level of sulfate esters accounts for about 15-40% of the structure resulting in a high
421 degree of solubility and low gel strength. Commercial carrageenan is extracted from *genera*
422 *Kappaphycus, Gigartina, Eucheuma, Chondrus*, and *Hypnea*, which is widely used in food
423 preparation for its gelling, thickening, and emulsifying properties, although it has little nutritional
424 value (Table 3). Based on the potential solubility in potassium chloride (KCl) and degree of sulfation,
425 carrageenan was commonly classified into lambda (l), kappa (k), and iota (i). Among them, k- and i-
426 carrageenan demonstrated a thermo-reversible sol-gel transition. The k-carrageenan accounted for
427 70% of the market share. Besides, l-carrageenan only creates a viscous solution, not a gel-like the
428 other two forms (Sedayu et al., 2019).

429 Carrageenophyte seaweed is extracted by hot water or alkaline solution (NaOH, KOH) or using
430 enzymes such as cellulose (Tarman et al., 2020). Carrageenan can be extracted by NaOH solution
431 (6% NaOH for 3.5 h at 70^0C) (Al-Nahdi et al., 2019) or hot water temperature of 74^0C in 4 h
432 (Martiny et al., 2020), and such optimum temperature that needs to be done correctly. Alkali is used
433 because it can increase gel strength in the final product by removing some of the sulfate groups, very
434 similar to agar extraction. Carrageenan extraction can be done by using enzymes such as cellulase.
435 The use of enzymes that help break down the cell walls to release carriganna in which the cellulase-
436 treated extracts obtained 45% compared to traditional boiling of 37.5% (Tarman et al., 2020).

437 Semi-refined and refined carrageenan can be further isolated by precipitating the extract with
438 alcohol to obtain refined carrageenan, although it can increase the final cost product (e. g., 88-95
439 US$/kg k-carrageenan). Economic and technical considerations, carrageenan can not act as the main
440 ingredient for bioplastic production but instead is an additive to combine with a variety of
441 compounds:

- For food packaging, the combination of carrageenan 1% (w/v) with olive leaf extract and glycerol can produce a biodegradable film with additional antimicrobial effects *(E.coli).* (Martiny et al., 2020). The olive leaf extract has a significant amount of phenolic compounds that contribute to antibacterial properties for biopolymer. Besides, the thickness of the biodegradable film was 28% higher than the control condition, 167-fold reduction in the initial count of aerobic mesophiles bacteria, and 54% reduction of vapor permeability.
- For bionanocomposites, Hashemi Tabatabaei et al. (2018) prepared by combination of gelatin (10% w/v), k- carrageenan (0.5%) and nano-SiO_2 (1, 3 or 5%). Mechanical and gas characteristics of gelatin films were improved, which is a favorable feature in the packaging industry of food products. Besides, their resistance against high humidity (water solubility) was reduced from 100% to 68 and 50% after the addition of k-carrageenan and nano-SiO_2 (5%), respectively (Hashemi Tabatabaei et al., 2018). Semi-refined carrageenan shows good compatibility with many substances such as nanoclay (Cloisite® 30B), SiO_2–ZnO nanoparticles, and other materials (Praseptiangga et al., 2021).
- For bioplastic production, the production based on cassava and glycerol under the effect of adding carrageenan from 0 to 10% (interval of 2.5%) was conducted by Suryanto et al. (2019a). As reported, 5% (w/w) carrageenan successfully reacted with the cassava starch and glycerol to enhance moisture resistance, brittle, and improve tensile properties of the polymer. The added carrageenan is proportional to the mechanical strength, such as tensile strength from 1.1 to 2.87 MPa and reduced elongation from 28.69 to 14.78% (Suryanto et al., 2019a,b). It emphasizes that glycerol has good compatibility with carrageenan by producing a strong hydrogen bond (Sedayu et al., 2020).

The overuse of carrageenans may reduce water vapor permeability and water resistance of packaging films due to their hydrophilicity. Mixing carrageenans with hydrophobic and

nanomaterial-reinforced compounds has resulted in improving physical properties and cost-effectiveness. The review results highlighted the potential use of carrageenan for edible food packaging and pharmacological, biomedical, and electrical applications.

4.3. Ulvan

Ulvan is an anionic sulfated polysaccharide extracted from the cell wall of green seaweeds (Glasson et al. (2019). Ulvan backbone is constituted by sulfated disaccharide repeating units, mainly composed of monosaccharides such as rhamnose, xylose, glucuronic acid, and iduronic acid as the main building blocks. The presence of sulfated polysaccharides is related to the physiological adaptation of organisms to environmental ions such as high salinity. The ulvan can be found in Ulvaceae, a family of green algae (genera *Ulva* and *Enteromorpha sp).* Genera *Ulva* and *Enteromorpha* are known for causing "blue tides" that lead to hypoxia and death of most aquatic organisms due to the rapid biomass proliferation in eutrophic coastal waters. Economic considerations, such as high growth rates and exploitable biochemical profiles, could target the biorefinery perspective.

Ulvan accounts for 9-36% of Ulva's dry weight (Kidgell et al., 2019). Extraction methods for *Ulvan* are diverse, depending on the type of algae, its derivatives, ecophysiology, and seasonality. Adopting Yaich et al. (2014), the acid extraction method was carried out by heating algal powder in HCl solution, 80°C, pH 2, and stirring for 1h. Others, the combined enzymatic extraction (cellulase for 2 h, and protease for 2 h) by using hot water solution (50°C) can improve ulvan yield (Yaich et al., 2014). The solubility of ulvan and its intermolecular interactions are pH-dependent, where the pH of the medium > pKa of both uronic acid (~3.28) and sulfate ester (~2.0) will promote its solubility during extraction (Kidgell et al., 2019). To optimize acid extraction, the response surface methodology analysis has been conducted by Glasson et al. (2019). The results found that the extraction condition could be achieved at pH 2.92, 90 min, and 90°C, which in addition help to

minimize the requirement for downstream purification and are suitable for upscaling the extraction of a high-quality ulvan product. Sulfated polysaccharides such as ulvan possess various biological activities such as antiviral, anticoagulant, antioxidant, anticancer, antiallergy, and anti-inflammation (Table 3). From Elicityl biotech company, the cost of Ulvan polysaccharides from *Enteromorpha sp*. is about 233 US$ kg^{-1} for a native grade, while it will be 10 times more expensive for a fine grade (2,475 US $ kg^{-1}). Many potential applications of Ulvan were known as gelling agents and a source of sugars to synthesize fine chemicals. The application of Ulvan for bioplastics is currently under-researched, while other medical-related applications are receiving interest.

4.4. Alginates

Alginates are anionic polymers extracted from brown seaweed that account for 20-60% (average 40%) of dry weight (Rashedy et al., 2021). Alginates structure is a linear polysaccharide derivative of alginic acid comprised of 1,4-β-d-mannuronic (M) and α-l-guluronic (G) acids. This structure is organized as homopolymeric regions of G units (G blocks) and M units (M blocks) and random combinations of M and G monomers (MG blocks) (Lee and Mooney, 2012).

Following the model of an egg-box (Kohn, 1975), in the presence of divalent cations, for example, Ca^{2+} (calcium chloride), cooperative cross-linking occurs between calcium ions and the G blocks. This cross-linking occurs at the negative charge of carboxylate groups in G units which conformational change alginates structure into a hydrogel (Beaumont et al., 2021). Alginate-based hydrogels could be used as drug delivery vehicles to protect drugs from degradation and improve plasma half time to ensure the transport and release of drugs. The properties of the gel formed heavily depend on the M/G ratio and the G block length, molecular weight, and Ca^{2+} content (Lee and Mooney, 2012). The alginates with a high proportion of M blocks have a higher viscosity. In contrast, those with a high proportion of G blocks possess higher gelling properties, an outstanding feature for physicochemical manipulation to produce bioplastics.

Laminaria spp., *Macrocystis* spp., *Ascophyllum* spp., *Sargassum* spp., and *Fucales* spp. are the main species used to extract alginate (Rhein-Knudsen et al., 2015). There are some methods to extract alginate from brown seaweed, such as using conventional alkaline extraction, microwave, and ultrasound-assisted extractions (Łabowska et al., 2019). For traditional alkaline extraction, the biomass was dried, cut into 0.1–0.5 cm lengths, and dried to constant weight. Then, soak for one night in 2% formaldehyde solution to remove the pigment and soften the seaweed tissue, rinse again with distilled water and add 0.2 M HCl solution. Samples were rinsed again with distilled water before adding 2% sodium carbonate solution overnight. Collecting the supernatant by centrifugation, the extracted sodium alginate was precipitated from the solution with ethanol, the product was washed with acetone, then dried overnight at 60°C (Rashedy et al., 2021). For industrial extraction, the refined sodium alginate is carried out with a few more purification steps using HCl and Na_2CO_3 (Tiwari et al., 2020). The obtained alginate solutions could be increased viscosity as pH decreases due to carboxylate groups in the alginate backbone being protonated and forming hydrogen bonds. This is the primary method for alginate extraction; however, times (more than 2.5 h for each step) and many chemicals were identified as an unsustainable factor. Therefore, to minimize the chemicals used, ultrasound was used for alginate extraction (Flórez-Fernández et al., 2019). This method was conducted under 25°C in 5-30 minutes, which reduces the reaction time and temperature. The commercial alginates are applied in frozen food, pharmaceutical fields, and wastewater treatment (Table 3).

The efficiency of biodegradable films using alginates was studied by Brandelero et al. (2016). The produced polymers consist of 80% mass starch, 8.6% mass polyvinyl alcohol, and 11.4% alginate. The biodegradable films have tensile strength and elongation, and water vapor permeability is suitable for biodegradable packaging (Brandelero et al., 2016). Paixão et al. (2019) compared alginate biofilms plasticized with hydrophilic and hydrophobic plasticizers for application in food packaging. Sodium alginate was dissolved in distilled water and added the plasticizers glycerol,

tributyl citrate (TC), and mixtures of TC with glycerol. As a result, films plasticized with pure TC were least soluble in water and had higher tensile stress, exhibiting better compatibility than glycerol or blends. This implied a more significant interaction between the secondary bonds of TC and alginate, thus increasing the mechanical resistance. By contrast, the elongation at break can be increased by adjusting the concentration of hydrophilic plasticizers (i.e., glycerol) (Paixão et al., 2019). Refining sodium alginate for food use currently costs around 92 US$ kg^{-1}, within the k-carrageenan cost range, and can be used as an additive for producing biodegradable films (Azucena Castro-Yobal et al., 2021).

4.5. *Fucan (Fucoidan)*

Fucoidan is a family of sulfated polysaccharides mainly composed of L-fucose and sulfate found in the cell wall matrix of brown algae (Etman et al., 2020). The structure mainly contains fucose, sulfate, uronic acids, and a small number of other monosaccharides such as galactose, xylose, arabinose, and/or mannose, glucose, and sometimes even proteins. Fucoidan has many uses due to pharmacological activities and potential safety for antibacterial, antiviral, and anti-inflammatory. In particular, it has attracted increasing interest in research to fight cancer (Etman et al., 2020). Fucoidan exists in the mucilage of brown seaweeds species such as *Undaria pinnatifida*, *Laminaria digitata, Ascophyllum nodosumand*, *Fucus vesiculosus*, and *Kjellmaniella crassifolia*. However, because of the often high heavy metal content in marine algae, only *Fucus vesiculosus* and *Undaria pinnatifida*, are approved for fucoidan extracts to be used in foods and supplements (Lähteenmäki-Uutela et al., 2021). The classical fucoidan extraction can be done by using hot water, hydrochloric acid (HCl) or sulphuric acid (H_2SO_4), and salt ($CaCl_2$) to promote alginate precipitation during purification. These methods are often considered traditional, do not optimize the extraction yield, and can cause structural deformation that reduces the biological activity of fucoidan (Ale and Meyer,

2013). Therefore, the enzymolysis procedure has been proposed as "green techniques" for extraction by using cellulase and pectinase or cellulase and alginate lyase (Nguyen et al., 2020).

The refined fucoidan is an expensive product. Each gram of fucoidan from *Fucus vesiculosus* costs more than 600 euros from Sigma-Aldrich (Etman et al., 2020), which is attributed to the high medicinal value. Besides, the instability and inconsistency in the composition of fucoidan affect cost production. The extraction of fucoidan is currently targeted for the preparation of microencapsulation, nanoparticles, or coating material for their prepared nanosystems, while its use as an additive for bioplastics production such as plastic packaging is rarely reported (Table 3).

5. A multidimensional approach to bioplastic production from algae

5.1. Other seaweed derivatives

Seaweed can be a valuable feedstock for biorefinery governed by seaweed type and species (Balina et al., 2017). Besides the polysaccharides as mentioned earlier, other compounds can also be applied in the production of bioplastics, such as water-soluble sulfated Galactans in red and green algae (Pierre et al., 2015); Laminarin (β-1,3-glucan) and Sargassan in brown algae (Ale and Meyer, 2013); Floridean starch (amylopectin-like α-D-glucan) and Porphyran in red algae (Beaumont et al., 2021). Consideration should be given to reducing costs through improved extraction, harvesting methods, increasing yields by cultivation. An increase in the cultivated area can create favorable conditions for CO_2 reduction.

5.2. Freshwater macroalgae

In addition to seaweed, freshwater macroalgae are also the potential raw materials. Freshwater macroalgae such as *Cladophora sp, Cystophaera sp, Ulva sp, and Rhizoclonium sp* have been reported to produce rich biopolymers for bioplastic production such as *cellulose, starch and alginate*. Currently, available information of freshwater macroalgae applications study is also reported (see E-supplementary). Large-scale cultivation of freshwater macroalgae is also feasible at a relatively low

cost using currently available technologies. Freshwater macroalgae tend to form dense floating mats on the water surface, improving the cost-efficiency compared to harvesting suspended marine microalgae (Rybak, 2021).

5.3. Algae biomass for bioplastic production through integrated wastewater treatment

The cultivation process comes from two groups: traditional such as open ponds or photobioreactors (Fig. 4a, b), hybrid systems such as membrane photobioreactor (Fig. 4c) and microalgae biofilm reactor. Among those, the high-rate algae pond is an example of a conventional approach because of its design, construction, and operation convenience for integrating wastewater treatment and biomass recovery (Fig. 4a). The open pond configuration achieved a high nutrient removal rate (82–99%) while the organic matter removal efficiency was low (46–76%) (Godos et al., 2009). Moreover, open ponds have some limits, such as water evaporation, large footprint requirement, low biomass productivity, less nutrient removal efficiency, and several difficulties in operating parameter adjustment (Li et al., 2019). Membrane filtration has emerged as a promising platform for microalgae harvesting because of easy operational maintenance and low operating temperature. The combination of membrane filtration with PBR as the membrane photobioreactor (MPBR) has revealed numerous benefits in recent years (Gao et al., 2016; Sheng et al., 2017). Further, Gao et al. (2016) found that MBPR got better removal efficiencies of total nitrogen and phosphorus (86.1% and 82.7%) and contributed to high biomass productivity of 42.6 mg L^{-1} d^{-1}. The operation costs of MPBRs were calculated to be 0.113 US \$ m^{-3} based on a design treatment capacity of 5520 m^3 d^{-1}, which showed apparent advantages compared to other conventional PBRs (usually 0.65–0.96 US \$ m^{-3}) (Sheng et al., 2017).

5.4. Blending raw algae biomass for bioplastic production

Raw algal biomass obtained from wastewater treatment could be used to produce bioplastics from including: (1) mixing raw algal biomass and petroleum plastics; (2) blending algal biomass with bioplastics (such as polylactic acid, PLA); or (3) hydrolysis of algal biomass as a feedstock for PHAs

615 production (Guedes et al., 2019; Rahman and Miller, 2017). This strategy is considered when
616 producing bioplastics from algae derivatives is no cost-benefit. Both *Cyanobacteria* and *Chlorella*
617 are small in size and have high protein composition, which could allow them to be suitable for
618 bioplastic production (Rahman and Miller, 2017). According to the author, microalgal biomass was
619 determined to be most effectively accomplished at a 4:1 ratio of biomass to glycerol.
620
621 **6. Research needs and future prospects**
622 Cultivation of fast growing freshwater microalgae has been shown to have a high starch yield.
623 However, starch has poor mechanical properties; combining starch with plasticizers such as glycerol
624 to improve the quality of bioplastics is inevitable. PHB-producing cyanobacteria can ensure an eco-
625 friendly plastic production process compared to heterotrophic microorganisms. However,
626 cyanobacteria for PHB production is currently inappropriate due to low natural PHB content. In
627 addition, this strategy is affected by technical problems arising from the cultivation and harvesting
628 processes. Wastewater treatment in conjunction with algae/cyanobacteria cultivation using membrane
629 photobioreactor could be a strategy towards sustainability. The nutrient starvation method effectively
630 increased both starch yield from marine/freshwater microalgae and the PHB from cyanobacteria. In
631 addition, waste algae obtained from cultivation or wastewater treatment can be considered as a source
632 of substrates for PHA-producing bacteria as well as blending methods for bioplastic production.
633 Complexity and inconsistency of polysaccharide and sulfate content in seaweed affect production
634 cost. Compared with chemical extraction methods, non-chemical methods such as enzyme- or
635 ultrasound- assisted extraction help improve yield of polysaccharide and are environmentally
636 friendly. However, the recovery yield only ranged from 12-56% depending on polysaccharide type
637 and extraction conditions (See E-supplementary). Therefore, a prerequisite is to optimize extraction
638 methods and increase global culture capacity. Alginate and carrageenan are suitable additives that
639 actively interact with plasticizers to produce biodegradable and edible food packaging. The increase

in macroalgae cultivation contributes to increased biomass for bioplastics as well as other related applications.

7. Conclusion

Increasing environmental issues surrounding fossil fuels have stimulated CO_2 emission control policies to be increasingly tightened. Thus, algae cultivation could help balance the water-food-energy nexus, and protecting the environment's health. The microalgae possess a more homogeneous monosaccharide composition than seaweed. The extraction of microalgae derivatives for bioplastic production is advantageous. The yield of starch (microalgae) and PHA *(cyanobacteria)* could be increased through proper cultivation practices in conjunction with nutrient starvation. Inconsistencies in polysaccharides and sulfate content in seaweed affect the extraction process and polymer's properties. Seaweed derivatives can be used as additives to save costs and enhance the bioplastics functionality.

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Table 1. Cultivation of algae for starch production

Types	Strains	Culture condition	Extraction/ Quantification	Starch content	Application	Refs.
Freshwater Microalgae	*Chlorella vulgaris*	Medium: Modified M8a Reactor: Tubular-PBR (V = 25 L) L: 1300 µmol m^{-2} s^{-1} T: 25°C, CO_2: 1%; pH: 7.5 N-starvation (Initial = 400 mg L^{-1})	Centrifuge sample & freeze-dry Wash: ethanol (80% v/v) Cell disruption: Bead beater (0.5 mm glass beads) Starch quantification: Starch kit by Megazyme (Wicklow, Ireland)	Maximum: 40% Concentration: 1 g L^{-1} Time: N = 0 + 0.5 d (12h)	Bioplastics biofuels & biorefining	(Carnovale et al., 2021)
	Chlorella sorokinana	Medium: BBM Reactor: PBR L: 300 µmol m^{-2} s^{-1} T: 25°C; CO_2: 2%, N-starvation	Wash: Ethanol Cell disruption: Bead beater Centrifugation: 10,000 g for 20 min Starch quantification: Starch kit by Megazyme	Maximum: 38% Productivity: 0.17 kg m^{-3} d^{-1} Time: N = 0 +1 d	Chemical additives Bioplastics productions	(Gifuni et al., 2017a)
	Chlorella sorokiniana	Medium: BBM Reactor: BC-PBR L: 300 µmol m^{-2} s^{-1} T: 25°C; CO_2: 2% N-starvation (N initial 0.25 g L^{-1})	Lysis buffer (60 mM Tris, 2% SDS) Cell disruption: Bead beater Centrifugation: 2500 g for 10 min Starch quantification: Starch kit by Megazyme	Maximum: 39.2% Time: N = 0 + 2 d	Food & bioplastics, textiles, paper preservation	(Petruk et al., 2018)
	Chlorella sorokiniana	Medium: synthetic medium L: 6000 Lux T: 28±2 °C; CO_2: 1% N-starvation	Wash: Ethanol (80%) Hydrolysis: 3 mL perchloric acid (30%); Stirred 15 min Starch quantification: Colorimetric method	Maximum: 34.06% N = 0 + 4 d	Biofuels	(Kaur et al., 2021)
	Chlorella zofingiensis	Medium: nitrogen-depleted BG11-N Reactor: airlift -PBR (V = 1 L) L : 150 µmol m^{-2} s^{-1} T: 25°C; CO_2: 1% N-starvation ($NaNO_3$ = 1.1 g L^{-1})	Lyophilized microalgal biomass Cell disruption: Bead beater (0.5mm glass beads for 4 min (2,700 rpm). Hydrolysis with $HClO_4$ (30%) Quantification: Sample + H_2SO_4 + phenol (6%), spectrophotometer (490 nm)	Maximum: 43.4% Time: N = 0 + 1 d	Feedstock for production of biofuels	(Zhu et al., 2014)
	Chlorella sp. AE10	Medium: BG11 medium Reactor: Tube-PBR (V = 0.35 L) L: 1000 µmol m^{-2} s^{-1} T: 28°C, CO_2: 10%; pH: 7.5 N-starvation ($NaNO_3$= 375 mg L^{-1})	Centrifugation: 3000 rpm for 10 min Wash: ethanol (80% v/v) Cell disruption: Ultrasonication Starch quantification: Megazyme total Starch kits (K-TSTA, Ireland)	Maximum: 60.5% Productivity: 0.311 kg m^{-3} d^{-1} Time: N = 0 + 5 d	Chemical or biochemical conversions	(Cheng et al., 2017)

	Chlorella emersonii	Medium: S-TAP T: 25 °C L: 7338 Lux	Centrifugation: 5000 rpm, 10 min Freeze-dried, stored at −25 °C in the dark & hydrolyzed using H_2SO_4 Starch quantification: hemocytometer by Bacteria counter A161	Maximum: 23.6%	Polymer	(Htet et al., 2018)
Filamentous green algae	*Zygnema extenue*	Medium: BBM Reactor: PBR L: 100 µmol $m^{-2}s^{-1}$ T: 25°C; CO_2: 1%, N-starvation	Centrifuge the sample, freeze-dry Wash: ethanol (80% v/v) at 75 °C Hydrolysis: sodium acetate + amyloglucosidase + amylase Starch quantification: Dinitro salicylic acid	Maximum: 24.3%	Feedstock for production of biofuels	(Zhang et al., 2016)
	Oedogonium nodulosum			Maximum: 30.9%		
	Stigeocloniu m sp.			Maximum:17.9%		
Marine Microalgae	*Chlorella salina*	Medium: Synthetic medium Reactor: V = 1 L L: red light 2000 Lux, 24h T: 30°C, CO_2: none; N and S-starvation	Centrifuge the sample 4500 rpm at 4 °C Wash: ethanol (80% v/v) Starch quantification: Starch kit by Megazyme (Wicklow, Ireland)	Maximum: 9% Concentration: 146 mg L^{-1}	Bioplastics production	(Chong et al., 2019)
	Tetraselmis chuii	Medium: BBM Reactor: BC-PBR (V =1.5L) L: 300 µmol m^{-2} s^{-1} T: 25 °C; CO_2: 2% N-starvation (Initial N= 32 mg L^{-1})	Lysis buffer: 60 mM Tris, 2% SDS Cell disruption: Bead beater (0.5mm glass beads, 10g biomass) Centrifugation: 2000 rpm for 10 min Starch quantification: Starch kit by Megazyme (Wicklow, Ireland)	Maximum: 53% Productivity: 0.08 kg m^{-3} d^{-1} Time: N = 0 + 1 d	Starch products in food and non-food industries	(Gifuni et al., 2018)
Marine Macroalgae	*Green marine seaweed Ulva ohnoi*	Medium: Artificial seawater 37‰ Reactor: MPBR L: natural irradiance T: 11-21°C; CO_2: (2–4 L min^{-1}); pH: 8.2 N & P-starvation (Initial = NH_4NO_3 (6.4 g m^{-3}) & H_3PO_4 (0.97 g m^{-3})	Wash: ethanol (80% v/v) Cell disruption: 2 M potassium hydroxide Starch quantification: Starch kit by Megazyme ((K-TSTA-100A, Ireland) Centrifugation: 1800 rpm for 10 min Spectrophotometer (510 nm)	Maximum: 21.44 % Productivity: 3.43 (t ha^{-1} y^{-1})	Starch production	(Prabhu et al., 2019)
	Green marine seaweed Ulva sp.	Medium: Mediterranean seawater Reactor: outdoor tanks (V = 40 L) Nutrient: 0.06 mM NaH_2PO_4 & 0.59 mM NH_4Cl) Cultivation time: 4 weeks		Maximum: 5.7 ± 0.32 (%w/DW)	Starch production	(Steinbruch et al., 2020)

Remarks: *PBR: Photobioreactors, MPBR: Membrane photobioreactors, BC-PBR: Bubble column photobioreactors, BBM: Bold basal medium, S-TAP: tris-acetate phosphate medium without sulfur, T: Temperature, L: Light*.

Table 2. Types of PHA & PHB from different sources and their applications

Compound	Source	Culture condition	Extraction	Application	References
Polyhydroxyalkanoates (PHA)	- *Synechococcus elongates*	Nitrogen & phosphate deficient conditions	- Methanol extraction - Hot chloroform extraction	- Manufacturing of bags, cloth - Biomedical science (disposable items, artificial bones, blood)	Mendhulkar and Shetye, 2017
	- *Chlorella minutissima* - *Synechococcus subsalsus* - *Spirulina* sp. *LEB 18*	Nitrogen-deficient condition	- Dried biomass added with NaOCl 4 % and incubated - Hot chloroform extraction and precipitated with cold methanol - Dissolved again in hot chloroform and dried	- Food industry - Agriculture - Pharmaceuticals - Paint industry - Materials for paint industry	Costa et al., 2018
	- *Chlorella sp.* - *Oscillatoria salina* - *Leptolyngbya valderiana* - *Synechococcus elongatus*	ASN III medium		- Packaging components - Biodegradable printing inks - Coatings and lamination	Roja et al., 2019
Polyhydroxybutyrate (PHB)	- *Spirulina* sp. *LEB 18*	Different nutritional conditions	- Biomass added with NaOCl 4 % - Acetone extraction	- Food industry - Pharmaceutical - Medical areas	Vanessa et al., 2015
	- *Aulosira fertilissima*	- Supplementation of 0.5% acetate - Gas exchange limitation	- Dried biomass added with NaOCl 4 % and incubated - Hot chloroform extraction & precipitated with cold methanol - Dissolved again in hot chloroform and dried	- Health industry	Samantaray and Mallick, 2015
	- *S. geitleri*	- Varying environmental conditions (pH, temperature and carbon sources)	- Lyophilized biomass added with NaOCl 4 % & incubated - Washed thrice with 10 mL of water, acetone, ethanol, ether - Chloroform extraction and filtered - Ice-cold methanol and centrifuged	- Agricultural - Biomedical fields	Singh et al., 2019

Poly (3-hydroxybutyrate) (P3HB)	- *Synechocystis salina*	BG-11 medium	- Ethanol and acetone (ultrasonic) - Hot chloroform extraction - Precipitated in ice-cold ethanol - Centrifuged, washed in ethanol & dried	- Packaging materials - Biomedical implant materials - Drug delivery carriers - Printing - Photographic materials	Kovalcik et al., 2017
3 Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	- *Nostoc muscorum Agardh*	Aetate and phosphate deficiency with 0.4% acetate + 0.4% valerate supplementation	- Biomass added with methanol at 4◦C (overnight) - Dried at 60 ◦C - Hot chloroform extraction - Precipitation with cold diethylether. - Centrifuged & washed with acetone	- Medical applications (orthopedic engineering, dental, wound management, urological stents)	Bhati and Mallick, 2012
Poly-β-hydroxybutyrate	- *Nostoc muscorum*	Phosphorus deficiency and addition of exogenous carbon sources	- Hot chloroform extraction	- Biodegradable plastic materials	Sharma and Mallick, 2005
		Phosphate starved medium with carbon stress	- Sulfuric acid extraction	- Packing, bag industries - Production of toiletries - Medicine & pharmacy	Haase et al., 2012

Remark: ASN: *Artificial Seawater Nutrient Medium (III)*, BG-11: *Blue-green medium*

Table 3. Different types of macroalgae-derived bioplastics from various sources and applications

Macroalgae	Major seaweed sources	Extraction	Application	References
Agar (Red seaweed)	*Gelidiella* spp. *Gelidium* spp. *Gracilaria* spp.	Hot-water extraction Alkali treatment	Gelling agent, thickener in creams, excipient in pills, bacteria culture	Rhein-Knudsen et al., 2015
			Food and pharmaceuticals	Hii et al., 2016
			Cosmetic, biotechnology industries	Lee et al., 2017
Agarose	*Gelidium* spp. *Gracilaria* spp. *Acanthopeltis* spp. *Ceramium* spp., *Pterocladia* spp. *Campylaephora* spp.	Alkali-treated method, Surfactant treatment	Molecular biology, electrophoresis, cell culture	Meena et al., 2014
			Materials design, extraction of polysaccharides and biopolymers	Sharma et al., 2015
Alginates (Brown seaweeds)	*Laminaria* spp. *Macrocystis* spp. *Ascophyllum* spp. *Sargassum* spp. *Fucales* spp.	Convert the insoluble calcium- (magnesium-) alginates to soluble sodium alginates	Stabilizers & thickeners, wound dressings & matrices, heavy-metal adsorption	Rhein-Knudsen et al., 2015
			Wastewater treatment	Flórez-Fernández et al., 2019
			Frozen food	Hu et al., 2014
Carrageenan (Red seaweed)	*C. Crispus* *G. stellata, G. radula, G. acicularis, G. pistillata, E. spinosum, P. rotundus*	Dried by propyl alcohol or ethyl alcohol – dehydrating agents	Food industry	Necas & Bartosikova, 2013
			Pharmaceutical applications	Agostinho et al., 2020
			Energy-saving battery	Nithya et al., 2020
Fucan (Fucoidan) (Brown seaweed)	*E. cava*	Hot water, HCl acid or H_2SO_4 acid, or $CaCl_2$ salt	Anticoagulant, antithrombotic activity	Jung et al., 2007; Etman et al., 2020
			Anti-inflammatory	Kang et al., 2011
	F. vesiculosus, *C. okamuranus* *K. crassifolia*		Anti-proliferative	Ale and Meyer, 2013 Song et al., 2018
Ulvan (Green seaweed)	*Ulva* spp. (*Ulva ohnoi; Ulvan conglobate; Ulvan lactuca; Ulvan rigida.);* *Enteromorpha* spp.	Acid extraction; Hot water extraction	Tissue engineering	Dash et al., 2014; Alves et al., 2012
			Therapeutics and health products	Alves et al., 2012
			Cosmetics	Adrien et al., 2017
			Drug delivery	Kidgell et al., 2019
Galactan (Red and green seaweed)	*Rhodophyta* (red algae); *Codium* (green algae)	Hot water extraction, NaOH extraction	Gelling, stabilizing, thickening agents in food products	Sousa et al., 2012
			Pharmaceutical applications	Pierre et al., 2015
			Cosmetics	Stengel & Connan, 2015
Porphyran (Red seaweed)	*Palmaria palmate*; *P. umbilicalis*	Hot water extraction with solvent (ethanol, methanol)	Cosmetics	Stengel and Connan, 2015

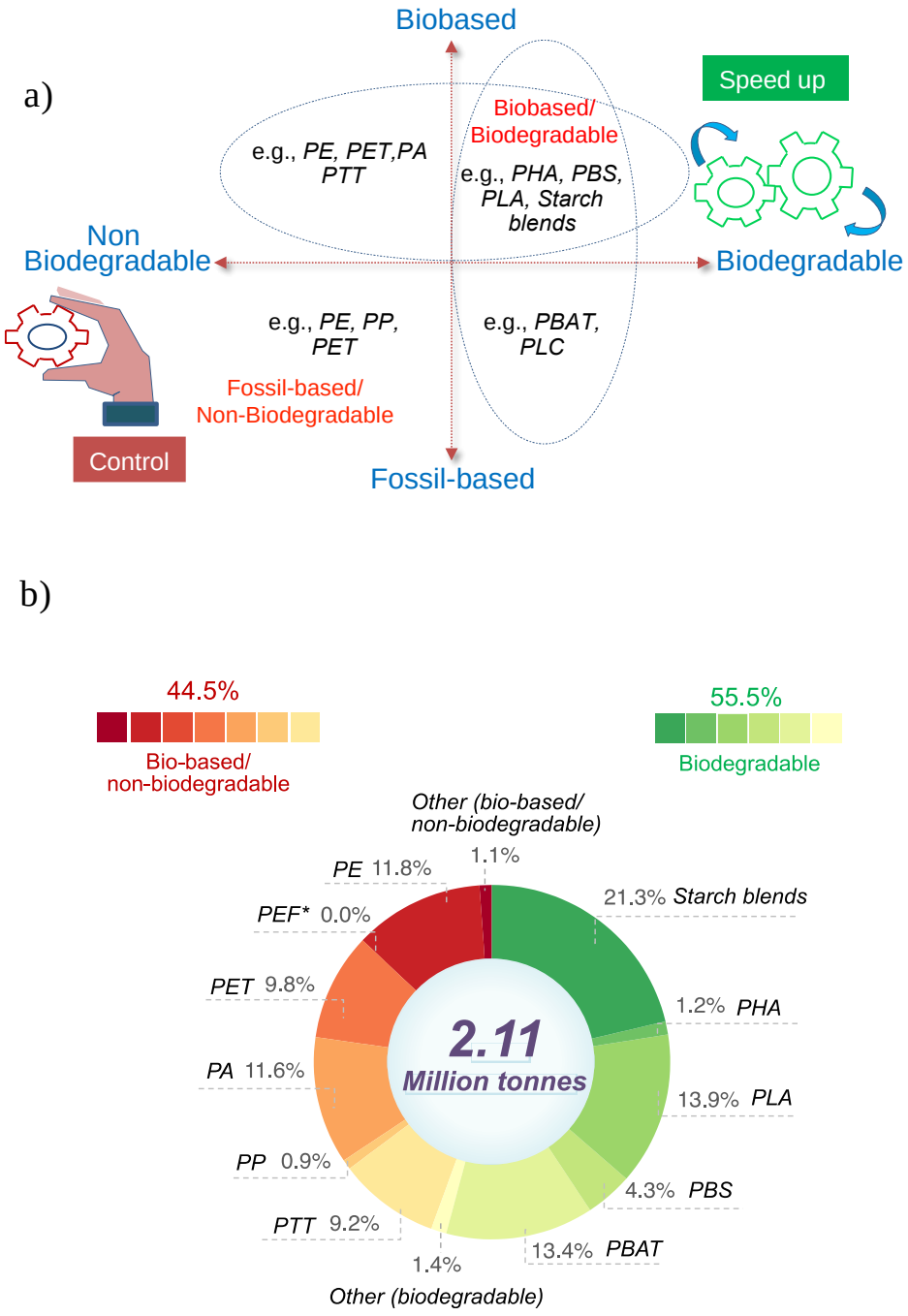


Fig 1. a) Classification of plastic, b) Bioplastic production capacity by material type worldwide (2020), adapted with modification from European Bioplastics (2020).

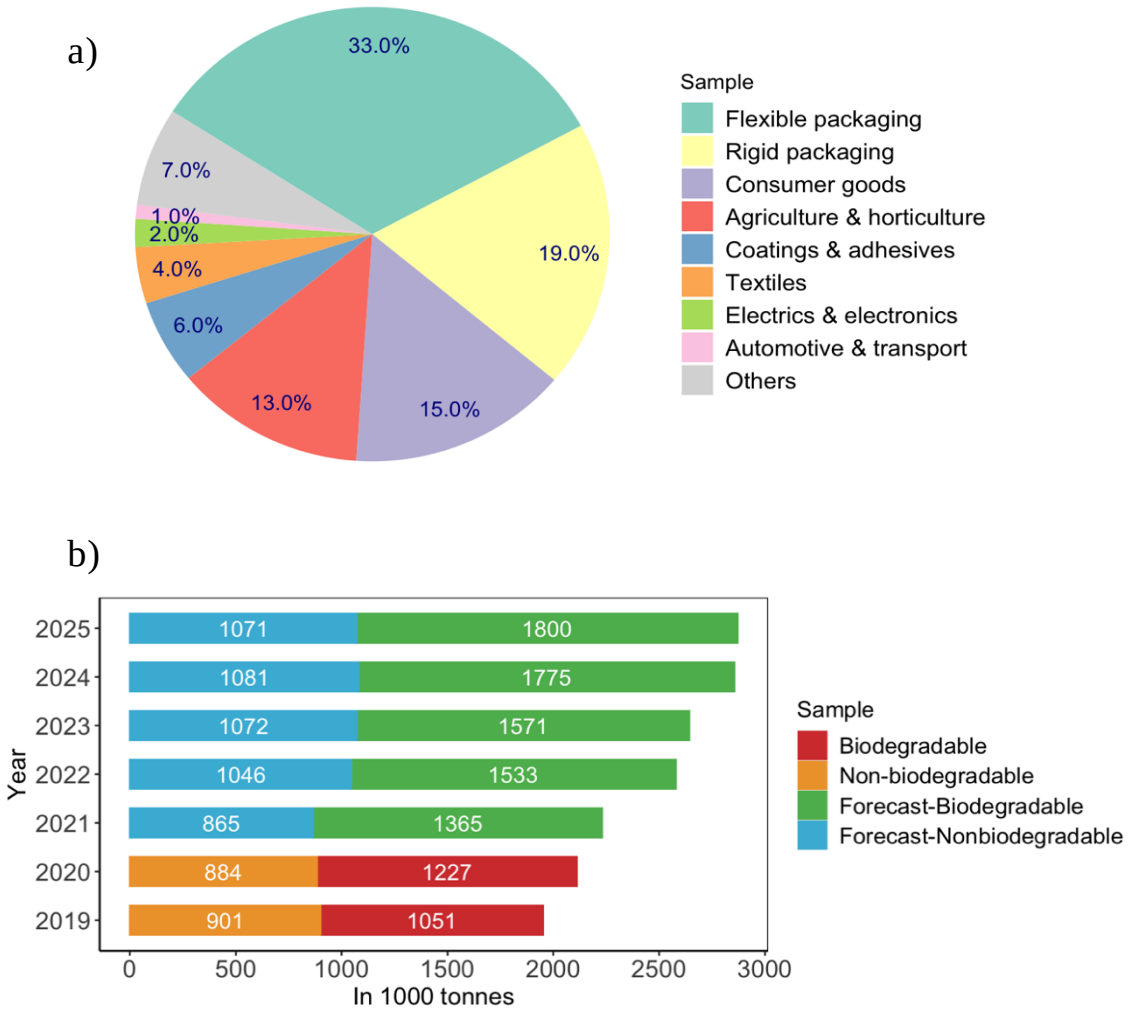
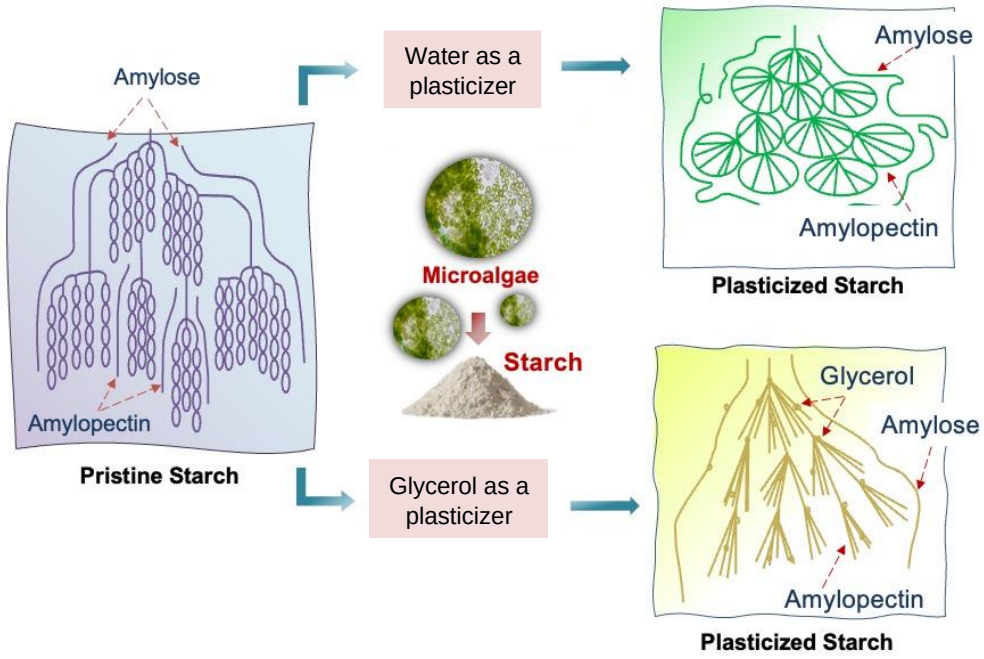


Fig 2. a) Application fields of biodegradable plastic, b) Forecast of global bioplastic production capacity from 2019 to 2025, adapted with modification from European Bioplastics (2020).

a)



b)

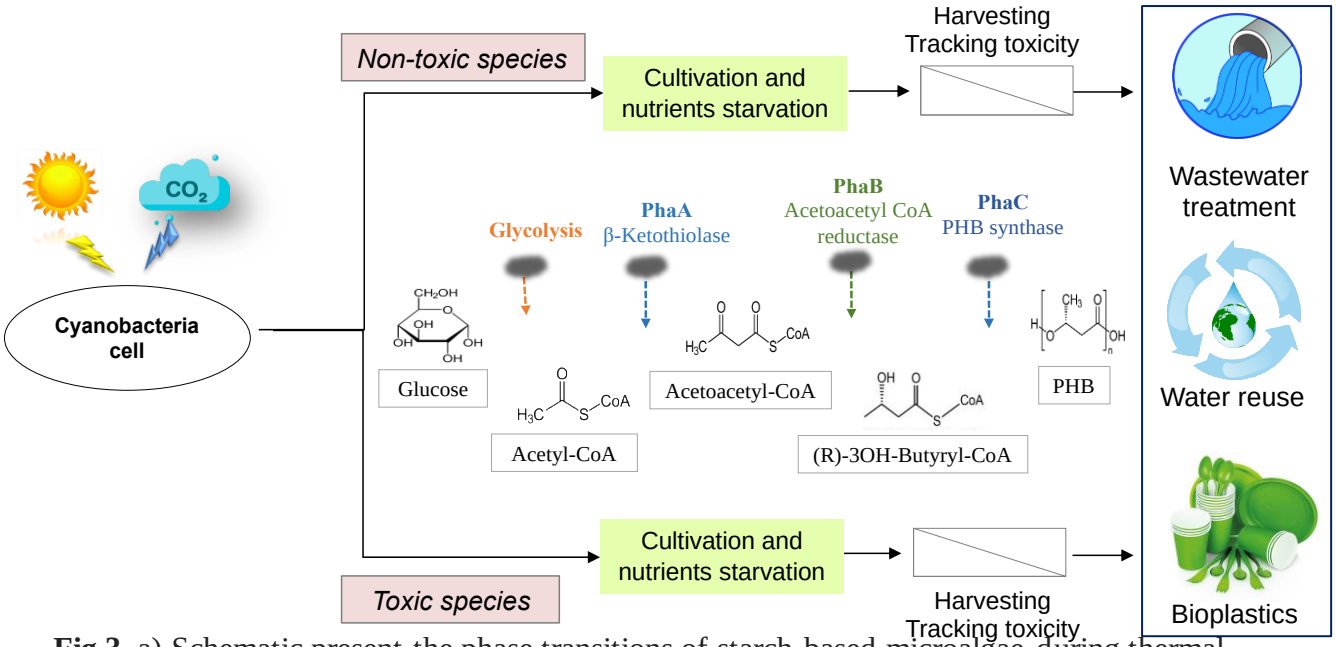


Fig 3. a) Schematic present the phase transitions of starch-based microalgae during thermal processing; b) Proposed strategies to culture-related toxic or non-toxic cyanobacteria. Toxin reduction can be effectively controlled by removing intracellular (microfiltration and ultrafiltration) and extracellular (nanofiltration and reverse osmosis) cyanotoxins

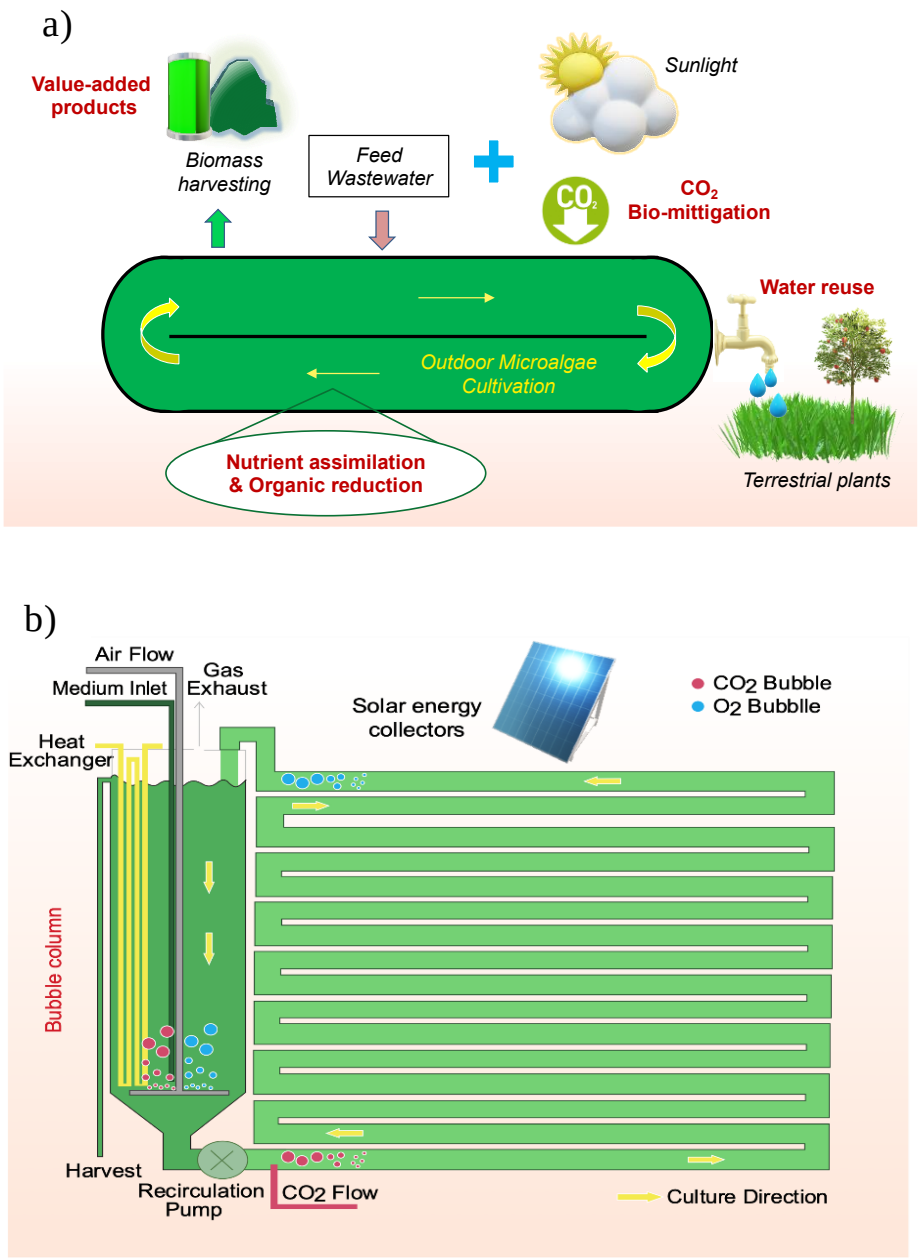


Fig 4. a) High-rate algal pond treating waste wastewater; b) Closed horizontal tubular photobioreactor scheme, adapted with modification from Fernández et al. (2014) and De Andrade et al. (2016); c) Lab-scale membrane photobioreactor.