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REVIEW PAPER

Review on the use of Microalgae Biomass for Bioplastics Synthesis: A Sustainable and Green approach to control Plastic Pollution

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Abstract
Worldwide there is an immense demand for plastic material that results in "white pollution". Petrochemical-based plastic is used worldwide, which leads to adverse impacts on every sphere of the earth. However, many steps had been taken to control this plastic pollution globally, such as chemical treatments, plastic waste incineration, sanitary landfilling, and 7 R programs. Still, plastic pollution is one of the major international problems. Non-biodegradable plastic would not eradicate from our environment until we have an economically feasible and more biodegradable substitute. Algae in recent years, especially microalgae, have got attention worldwide, owing to their various applications. Microalgae is one of the sustainable ways of bioplastic synthesis as during cultivation, it also purifies wastewater. This review paper has summarized different microalgae species used to synthesize bioplastic, their cultivation system, and methods for bioplastic production by using microalgae biomass, followed by multiple challenges, solutions, and future prospects.

Keywords: Microalgae, Biomass, Bioplastic, Biodegradable, Pollution

INTRODUCTION

What is plastic pollution?

According to recent reports, it was estimated that about 400 million plastic being produced yearly. Although the percentage varies from country to country, on average, 14-18% of plastic waste is recycled, 25% is incinerated, and 56% is dumped into landfills and oceans (WEF, 2020). Plastic is a hydrophobic synthetic organic polymer with long monomer chains joined by covalent bonds (Gandini and Lacerda, 2015). It is known for its flexibility, durability, stability, and affordability. The consumption of plastic has been increased day by day as it is widely used in water bottles, food packaging, electronic goods, medical supplies, clothing, construction purpose, and many more applications (Cleetus et al., 2013). Solar UV radiation or any other natural factor breaks plastic into smaller fragments called micro-plastic or nano-plastic (De Stephanis et al., 2013). This plastic debris persists for millions of years due to its non-biodegradable nature. The most harmful and visible impacts of plastic waste (micro-plastic or nano-plastic) on marine species are ingestion, entanglement, suffocation, and reduced ability to flow. The world wildlife fund has estimated that about 1000 whales, seals, turtles, and marine mammals die, and many more get injured every year due to eating or being trapped by plastic junk. The floating debris of plastics also contributes to the spread of invasive organisms like bacteria and algae, disrupting

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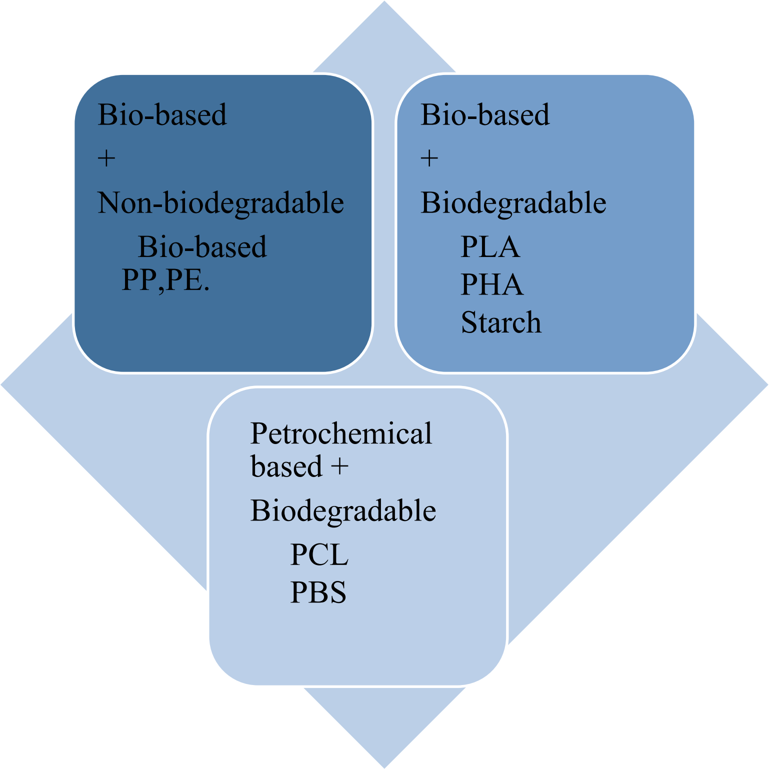

Fig. 1: Types of bio-plastic.

the marine ecosystem. Even human life suffers as this plastic debris gets biomagnified in the food chain from marine life. It is also responsible for climate change as the burning of plastic leads to the release of harmful greenhouse gases like carbon dioxide, methane, nitrous oxide (Casabianca et al., 2020).

Bioplastic- an alternative to conventional plastic

Bioplastic has almost similar properties like flexibility, durability, stability to ordinary plastic. Algal-based bioplastic may have a vital role as an eco-friendly and biodegradable plastic that has less potent to damage our environment than conventional plastic (Hempel et al., 2011). The term 'bioplastic" includes two separate things: bio-based and biodegradable. The term Bio-based means that the material used for making plastic is derived fully or partially from natural biomass like corn, sugarcane, bacteria, or algae. While Biodegradable term means the plastic can be completely degraded by microorganisms and substances like water, carbon dioxide, and compost under specific conditions and time frames. Not all Bio-plastic are bio-based and biodegradable. They can be either of them or both (Reddy et al., 2013; EB, 2018).

Bioplastic is divided mainly into three groups (Fig 1):

1- Bio-based (fully or partially) and non-biodegradable – Bio-based PP, PE.
2- Bio-based and biodegradable- PLA, PHA, starch.
3- Petroleum-based but biodegradable- PCL, PBS.

Use of microalgae in bioplastic: an emerging field

There is various form of bioplastic, but not all are eco-friendly. Only biodegradable bioplastics are good for our environment. Many research papers have been published on biodegradable bioplastic, and it was reported that bioplastics can be obtained from natural resources like Polylactic acid, a biodegradable polymer obtained from corn starch, potato starch, and banana peel (Marichelvam et al., 2019; Moshood et al., 2022). Even various heterotrophic bacteria are also fermented to produce biodegradable polymers like PHA and PHB (Ross et al., 2017).

Nowadays, researchers have focused on using microalgae as a source of bioplastic, as algal biomass can be cultured easily and less expensive than bacteria because bacterial cultivation requires nutrients media like carbon (glucose), salts, and minerals. In contrast, microalgae require sunlight, carbon dioxide, and they can grow in wastewater also (Ross et al., 2017). Microalgae is an exciting source to improve the sustainability of bioplastic production and improve water supplies during its production. The PHB content of algal biomass enhances the recycling property of plastic compared to bacteria-based bioplastic (Cinar et al., 2020).

Companies like Algix use microalgae to produce foam polymer, which may have displaced a significant percentage of the fossil fuel-based polymer and act as sustainable polymer. Algix first harvested microalgae through the wildsafe water filtration method, and then after drying and grounding it, they have compounded it into plastic composite pellets. In 2015, Aglix entered into the footwear business, and now it supplies Bloom Foam (which is algal-based) to leading brands like Adidas, Tomas, and Billabong, which expand it into a flexible foam to use in footwear. The use of microalgae in bioplastic formation got attention in 2011, but in later years number of publications decreased, and in 2016 it again increased, and a spike occurred in 2019 that shows the recent increase in interest in this field.

The research activities used two approaches for bioplastic synthesis using microalgae biomass-direct and indirect (Rahman & Miller, 2017). The direct approach methods include:

(1) Blending of microalgae with conventional plastic.

(2) Blending microalgae biomass with other bioplastics.

While indirect approach include:

(1) Harvesting microalgae for biopolymer production such as poly hydroxyl butyrate (PHB). (2) Bio-refinery approach of using wastewater microalgae for multi-products includes biopolymer production.

(3) Genetic engineering of microbial strains to enhance biopolymer production like PHB.

MICROALGAE

Algae is an aquatic, either prokaryotic or eukaryotic organism able to perform photosynthesis. More than 25000 species of algae have been identified, which are categorized into green algae, blue algae, and brown algae (Mathimani and Pugazhendhi, 2019). Microalgae are microscopic algae found in both marine water and fresh water. These are also known as microphytes, planktonic algae, or phytoplankton. Macro components of microalgae are proteins (6-52%), lipids (7-23%), and carbohydrates (5-23%) while micro components consists of Calcium (0.1-3%), Magnesium(0.3-0.7%), Sodium(0.-2.7%), Potassium (0.7-1.5%), Iron (1395-11,101 mg/kg), Selenium(0-0.5mg/kg), Zinc(28-64mg/kg) and Manganese(45-454 mg/kg) (Tibbetts et al., 2015; Kusmayadi et al., 2021).

Use of microalgae in various fields

Microalgae with a high content of proteins and lipids are used to synthesize biopolymer and various products like supplements (vitamins, antibiotics), recently used for bioremediation to remediate toxic elements present in wastewater. Microalgae have also been used to produce biofuel and fatty acids (Li et al., 2021: Musa et al., 2019), as shown through Fig 2.

Commonly used Microalgae species for Bioplastic formation-

Most of the studies in this field have used *Chlorella* and *Spirulina* species, as they have a high concentration of polysaccharides.

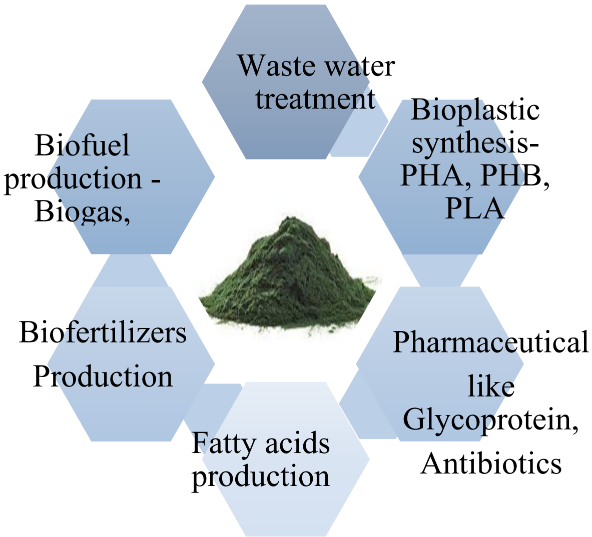

Fig. 2. Microalgae in various fields.

Table. 1. *Chlorella* Species used in Bioplastic formation with their Characteristics

Microalgae species	Type of Product	Characteristics	References
C. vulgaris	100% algae-based plastic	57% protein	Zeller et al., 2013
C. vulgaris	Chlorella/PVA Composites	58.5% protein	Dianursanti et al., 2018
Chlorella	Chlorella/PP Composites	-	Zhang et al., 2000
Chlorella	Chlorella/PVC Composites	-	Zhang et al., 2000
Chlorella	Chlorella/PVA blend films	-	Sabathini et al., 2018
Chlorella	Chlorella/PE Composites	38%starch	Otsuki et al., 2004

Chlorella

It is a green alga that is single-celled and found in freshwater. Their size varies from 2-10 mm with spherical cells. Due to its small size is commonly used to make biomass-polymer bends (Musa et al., 2019). There are about 30 different species of *Chlorella*, but only two are widely used in research – *Chlorella Vulgaris* and *Chlorella Pyrenoidosa*. Different *Chlorella* species used in bioplastic production and their characteristics have been compiled in Table 1.

Spirulina

It is blue-green microalgae. It is commercially important and has been consumed for centuries for its high nutrient value (55-75% protein by dry weight, vitamin B, vitamin E, beta–carotene, antioxidants, lipids, and many minerals). It grows naturally in both freshwaters and marine water. It is easy to harvest compared to *Chlorella*, so researchers are more interested in how to use it in various product formations. Many research papers have been published showing how the usage of *Spirulina* for biodegradable plastic synthesis is beneficial as its high protein content enhances tensile strength and improves overall functioning. It is also used to make hybrid plastic by blending with bioplastic or conventional petroleum-based plastic-like Polyethylene

(PE). Different species of *Spirulina* used for bioplastic production and their characteristics have been compiled in Table 2.

Other species of microalgae used for bioplastic synthesis

Besides *Chlorella* and *Spirulina,* other microalgae species are also used for bioplastic synthesis compiled in Table 3.

Microalgae cultivation

Various microalgae cultivation methods are mentioned in the literature to produce algal biomass, but two commonly used cultivation systems are open and closed culture systems, as discussed in table 4. Open culture systems are similar to ponds and lakes. They are less expensive to build and maintain, allow for large-scale production and use less energy, but they have a high risk of contamination and poor control over growth and culture parameters such as mixing, pH, temperature, and nutrients (Suparmaniam et al., 2019). The most common microalgae cultivated in open pond systems are Scenedesmus, Spirulina, and Chlorella (Siddiki et al., 2022). Raceway and Circular are two common open pond system configurations. The closed Culture system includes Photobioreactor, which is an enclosed cultivation vessel with a CO_2 and light source. It takes up less space, has better control over cultivation parameters, poses a low risk of contamination, has high productivity, but is extremely difficult to scale up production, costs a lot of money to build, and uses a lot of energy (Zhu, 2015). Cultivation of axenic single-species microalgae can be done in a close culture system. A photobioreactor is a type of closed pond system that uses a photobiological reaction to control the growth of microalgae, allowing the

Table. 2. Spirulina Species used in bioplastic synthesis with their Characteristics.

Microalgae Species	**Product**	**Characteristics**	**References**
S. platensis	Blend with PE	57% protein	Otsuki et al., 2004
S. platensis	Bioplastic biofilm	60% protein	Zeller et al., 2013
S. platensis	Bio filler	-	Dianursanti et al., 2019
Spirulina	Blend with PE	-	Ciapponi et al., 2019
Spirulina	Compatibilized Bioplastic	58% protein	Wang, 2014
Spirulina	PBS/Spirulina Composites	60% protein	Zhu et al., 2017

Table. 3. Other species of microalgae used for bioplastic synthesis

Microalgae Species	Product	Characteristics	References
Chlorogloea fritschii	Bioplastic –PHBs	PHBs levels: 14-17%	Monshupanee et al., 2016
Phaeodactylum tricornutum	Bioplastic-PHBs	PHBs level: 10.60%	Hempel et al., 2011
Calothrix scytonemicola	Bio-based plastic film	-	Johnsson and Steuer, 2018
Calothrix scytonemicola	PHAs	PHAs levels: 10-13%	Johnsson and Steuer, 2018
Nannocloropsis gaditana	PBAT	PBAT level: 17-19%	Torres et al., 2015

Table. 4. Microalgae Cultivation Systems (Debowski et al., 2012; Banerjee and Ramaswamy, 2017; Onen et al., 2020).

Microalgae Cultivation System	Descriptions
Open Culture System	It is an open air culturing system that includes ponds, tanks and raceway ponds. Here cultured microalgae get influenced by exterior surroundings. Advantages ● Easy to clean up ● Durable ● Low cost Disadvantages ● Difficult to control cultured media ● High contamination risks
Close Culture System	It is commonly known as Bio-reactor. No interaction occurred between cultured microalgae and exterior surroundings. Advantages ● More cell growth ● Higher productivity ● Fewer contaminations Disadvantages ● Expensive ● Difficult to scale up

Table. 5. Limting factors for microalgae cultivation (Knuckey et al., 2006; Mathimani and Pugazhendhi, 2019; Madadi et al., 2021)

S.No.	Limiting Factors	Remarks
1.	Space Constraints	Microalgae require proper space for their growth
2.	Deficit of resources	Microalgae growth depend on various resources like carbon, oxygen, Nitrogen etc
3.	Water scarcity	water is essential for sustain of microalgae
4.	Inadequate financial aid	Some industries don't have good financial support, which influences the growth of microalgae
5.	Knowledge gap	Adequate knowledge require for handling various equipment and resourrces
6.	Light	For synthesize of essential molecules and to produce ATP and NADPH microalgae requires light
7	pH	The optimum range of pH varies between 7.5 and 8.5 for microalgae growth which varies with different strain

cultivation of species that would otherwise be impossible to grow (Singh and Sharma 2012; Siddiki et al., 2022).

Light intensity is the most important factor in both systems. The sun is the light source in open cultivated systems, while optical fibres and light emitting devices are used in closed cultivated systems (LED). If the photosynthetically active radiation (PAR) level in both cultivation systems exceeds the saturation level, it may become a limiting factor, inhibiting cell growth and lowering productivity (Hempel et al., 2011). Yeh et al. (2010) investigated the effects of three key variables on the growth of an indigenous microalgae, C. vulgaris: carbon content, light intensity, and light source. The carbon content and light sources for microalgae growth were bicarbonate and fluorescent lamps. The optimal light intensity for maximum power conversion and overall biomass productivity was 9 W/m^2. When growing microalgae, pH is a crucial factor to consider because it affects a variety of cellular processes (energy metabolism, proteins, structure and function of the cell organelles, etc.). The rate of growth of microalgae is influenced by the pH of the medium. The optimal pH and salinity for various microalgae species vary (Chhandama et al., 2021). Phosphorus and nitrogen are essential macronutrients for algal development, and they regulate metabolic activity if provided in the right form (Enamala et al., 2018). Microalgae cultivation's expansion necessitated Calcium, iron, magnesium, potassium, phosphorous, nitrogen, carbon, and other nutrients.The limting factors affectng the large scale cultivation of microalgae directly or indirectly are shown through Table 5.

METHODS

Microalgae biomass can be used directly or indirectly for bioplastic synthesis, as shown through flowchart (Fig.3).

Direct use

In this, the whole microalgae biomass such as lipids, proteins, carbohydrates, fats, and polymers are processed and this algal biomass is blended either with petrochemical-based plastic or bioplastic. Then they passed through different mechanical and thermo-chemical polymerization processes to form hybrid bioplastic with better tensile strength.

The blending of microalgae biomass with conventional plastics

Microalgae biomass can blend with petro-chemical based plastic during bioplastic production to enhance and improve their properties, functioning, and lifespan.

Production Technology:

Molding and compression

One of the most common ways to produce microalgae biomass is polymer blends. In this, bio-

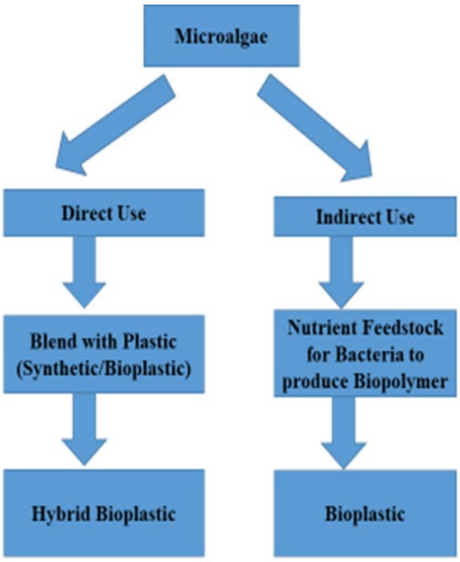

Fig. 3. Flowchart showing the use of microalgae biomass for bioplastic synthesis

composites are formed by compression (at elevated temperature and pressure) of the mixture containing algal biomass, additives, and plastic polymer by placing in mold. The pressure, temperature, and time for compression differ from literature to literature. The most standard ranges reported are 130 to 160 °C, pressure ranges 20 kilopascals to 10 megapascals, and time ranges from 3 to 20 minutes (Fabra et al., 2018).

Otsuki et al. (2004) performed melt mixing with the help of a roller mixer at 160 °C for 7.5 min. On the other hand, Fabra et al. (2018) performed a mix melting at 130 °C for 4 min by utilizing an internal mixer before compression molding. Dianursanti et al. (2018) excluded the compression by pressure and heated the melt mixed mixture in the oven to form hybrid bioplastic.

The size of the produce hybrid bioplastic prototype depends on the dimensions used for molding. The possible size of a prototype can be rectangular flex bars, films or slabs.

Solvent casting

It is another way for the production of hybrid bioplastic films. In this, microalgae biomass additives and conventional polymer mix in a solvent, cast on the surface, and then dried by air to form films. Here also, the criteria differ from study to study. Sabathini et al. (2018) mixed the components (algal biomass, petrochemical plastic, and additives) in water as a solvent, then cast the mixture on glass plates and air-dried to form microalgae-PVA films. However, Zhang et al. (2000) performed an extra homogenization step on algal biomass aqueous mixture before mixing polymers and additives to ensure proper dissolving to the microalgae biomass into the suspension.

Compression molding and solvent casting are the two most common methods for making bioplastic by blending microalgae biomass and conventional plastics. However, apart from these, twin-screw extrusion and Injection molding are also used in the market. Torres et al. (2015) used a combination of both Injection molding (at 30 °C) and twin-screw extrusion at 140 °C to form microalgae –PBAT hybrid bioplastic.

Table. 6. shows microalgae blend with conventional plastics in a specific ratio. It can be concluded from the table that, in general higher the Microalgae biomass/plastic ratio microalgae higher will be the tensile strength of plastic and make it more compactable and long-lasting.

Microalgae biomass blended with Other Bioplastics

Microalgae biomass can blend with bioplastic (derived from a natural polymer like PLA, starch, cellulose) to manufacture a hybrid bioplastic with a better life span and other properties.

Polylactic acid (PLA) is commonly used bioplastic to blend with microalgae. It is a bio-based biodegradable polymer. It is synthesized by anaerobic batch fermentation of sucrose or glucose at

Table. 6. Microalgae blend with Conventional plastics and their Resulting Mechanical properties

Microalgae Sources	Plastic Blend	Ratio (Microalgae biomass/plastic)	Tensile Strength (MPa)	References
Chlorella	PE	10/90	22	Zhang et al., 1999
Chlorella	PP	10/90	32	Zhang et al., 2000a
Chlorella	PVC	20/80	30	Zhang et al., 2000b
Botryococcus Braunii	PBS	20/80	21.6	Toro et al., 2013
Nannochloropsis gaditana	PBAT	20/80	10	Torres et al., 2015

Table. 7. Comparison of PHB with Polyethylene (Khanna and Srivastava, 2005)

Properties	PHB	PP
Crystalline melting Point (oC)	175	176
Crystallinity (%)	80	70
Molecular weight (KDa)	179	200
Density(g/cm)	1.25	0.905
Tensile strength (MPa)	40	38
Flexural modulus (GPa)	4	1.7
UV resistance	Good	Poor

higher temperatures (313 K) and pH 5-6 range, followed by purification and polycondensation. It is approved by Food and Drug Administration (FDA). Bulota and Budtova (2015) blended microalgae (0-40 weight% fractions) with PLA and observed that tensile strength had started decreasing if microalgae biomass increased. This showed that algal biomass should blend with bioplastic in a suitable ratio, and excess or least ratio degrades tensile strength. They dried PLA and flaked algal at 80 °C and blended at different compositions (40g final mass) at 180 °C. This ratio was used to make ISO 527 standard “Dumbbell” then Young’s modulus and tensile strength was measured. By mixing 80% PLA with 20% (by weight) of green algae, tensile strength value near about 45 MPa while mixing 60% PLA with 40% (by weight) of green algae resulted in higher Young’s modulus and this hybrid bioplastic with increased Young’s modulus has greater implication in the market.

Indirect method

In this one algal biomass is used as nutrient feedstock for the growth of other microbes, especially bacteria, for the production of different kinds of bioplastics like polyhydroxy acetate (PAH), poly-lactic acid (PLC), poly-hydroxy butyrate (PHB) inside the bacterial cells (Castro et al., 2015).

Microalgae Biomass as a feedstock for the production of PHAs

PHA is a bio-based and biodegradable polymer produced by nearly 75 genera of bacteria, including gram-positive and gram-negative bacteria such as Cyanobacteria, Methylocystis parvus, and *Pseudomonas aeruginosa and Azotobacter vinelandi.* Its molecular size range between 50000- 100000 Da. It was estimated that PHA contributes 5% of the world’s bioplastic and 0.05% to overall plastic production. There are about 155 unique PHA monomers, but the most commercially important one is Polyhdydroxybutyrates (PHBs) due to their similarity with the properties of Polyethylene (PP) (Khanna and Srivastava et al., 2005; Zhou et al., 2011).

Table 7. shows the comparison between microalgae-based Polyhdydroxybutyrates (PHB) and conventional polymer Polyethylene (PP). The value of different properties like crystalline melting point (in °C), crystallinity, molecular weight, density, tensile strength, flexural modulus, and UV resistance have been discussed in this table. It has been observed that the properties mentioned in the table for both the polymer are almost similar. The demand for the production of PHA is enhancing day by day because it has wide implications in markets, such as being used for making rubbers, tires. Currently, the focus is on reducing the production cost of PHAs and their other monomers like PHBs. The most common production process is the fermentation of bacterial colonies, but it is economically less feasible. To reduce the production cost, the

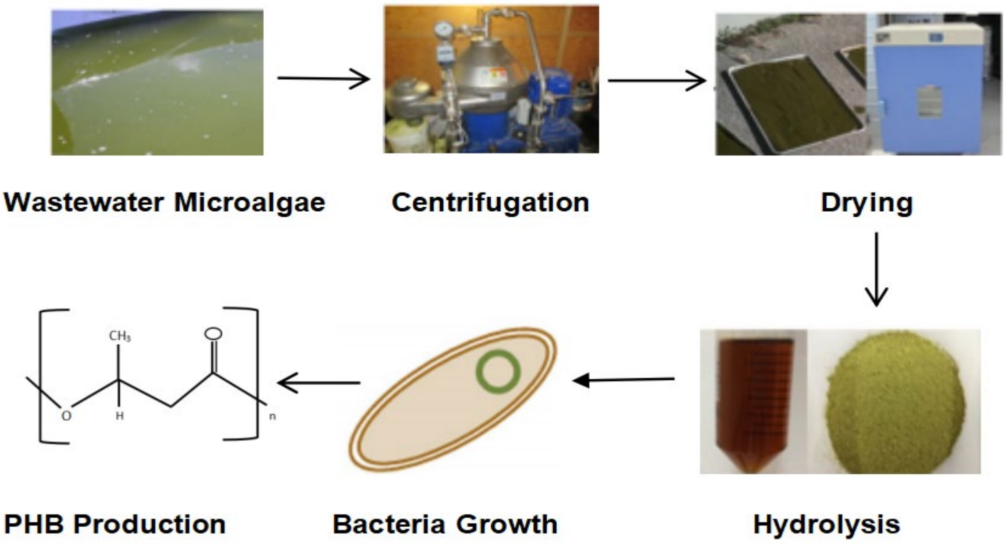

Fig. 4. Process of Polyhydroxybutyrate (PHB) production in E.coli using harvesting microalgae from a wastewater treatment plant (Rahman et al., 2015).

researchers started to use microalgae biomass as a feedstock to cultivate bacterial colonies to produce PHAs.

Microalgae Biomass Hydrolysis for production of PHBs

The wastewater microalgae could be hydrolyzed to reduce the cost of PHAs production. Many studies have concluded that algal biomass act as a good media supplement for the growth of bacteria colonies. Castro et al. (2015) used hydrolyzed microalgae biomass to cultivate *Clostridium* to form butanol, acetone, and ethanol. Zohu et al. (2011) used acid hydrolysis of *Chlorella* biomass as a substrate for the growth of *Saccharomyces cerevisiae* for bioethanol production. Moreover, it has been observed that protein is extracted from microalgae for E.coli growth to produce biofuels.

Similarly, microalgae biomass is used to cultivate E.coli to produce PHBs. Ellis et al. (2012) find out that weak domestic wastewater having about 20mg/L Nitrogen and 4mg/L Phosphorus is enough for microalgae biomass growth. Rosano and Ceccarelli (2014) demonstrated that using hydrolyzed wastewater microalgae biomass as a growth media for recombinant E.coli strain that expresses the phaCAB operon produces a good amount of PHBs. The microalgae biomass was collected from the wastewater treatment plant in Logan, UT, United States. Then this algal biomass undergoes continuous flow centrifugation with speed 9000 l/h to scalable the harvesting process. The harvested microalgae were dried and then hydrolyzed at 90 °C with 0.5M sulphuric acid. After that, the biomass was neutralized and centrifuged and the supernatant was used as growth media for recombinant E.coli. The hydrolyzed microalgae biomass was used without any intermediate sterilization to reduce the cost of production of PHBs.

Rahman et al. (2015) harvested the microalgae from the effluent of a wastewater treatment facility via centrifugation and hydrolyzed them to create a liquid medium for recombinant E. coli growth and PHB production (Fig. 4). A maximum of 31% PHB (dry cell weight) of E. coli was produced by using different concentrations of hydrolyzed microalgae biomass to supplement the growth of E.coli. This yield of PHB is less than standard supplement glucose media, which provides a yield near 50%, but if we compare cost, then PHB accumulation by using wastewater microalgae as nutrient media is very low over glucose media (Kesaano et

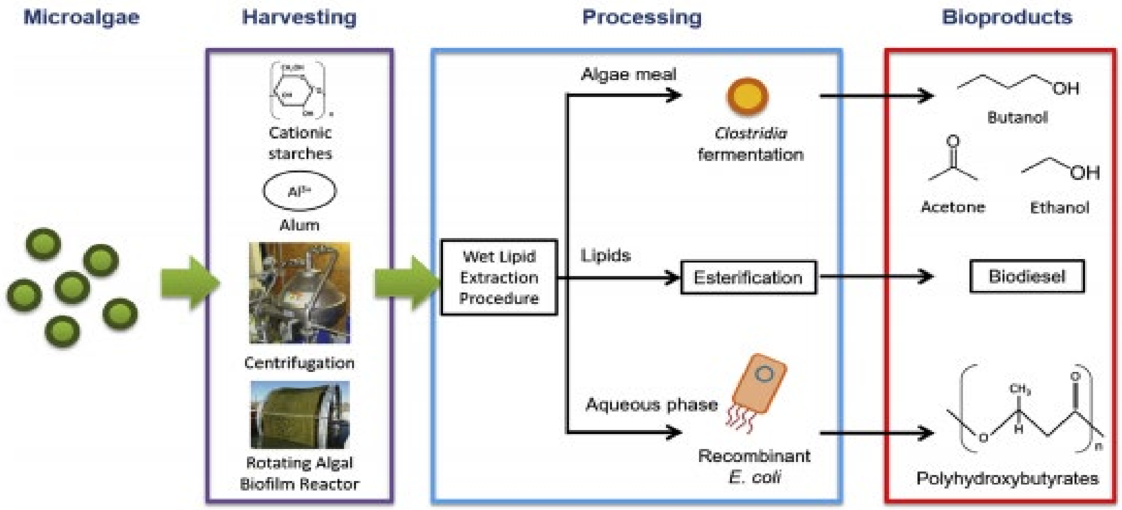

Fig. 5. Wet Lipid Extraction Procedure in bio-refinery to produce various products including the bio-polymer Polyhydroxybutyrate (PHB) (Rahman and Miller, 2017).

al., 2014). Moreover, the recombinant bacteria used for bioplastic synthesis using microalgae biomass could be used again and again by just changing the genetic makeup of bacteria. So this also makes bioplastic production a more economically feasible process with the help of algal biomass.

Bio-refinery approach for PHBs production by using Microalgae biomass

The wastewater microalgae acts as a feedstock to produce multiple products in this approach. This microalgae species (Scenedesmus obliquus), which occurs naturally in wastewater, was cultivated in Solar Simulated Bioreactors and then harvested to obtain various products. Wet Lipid Extraction Procedure (WLEP) is a bio-refinery model that is commonly used. Basically, WLEP generates three products: the first stage produce acetone, butanol and ethanol by anaerobic fermentation of Clostridium saccharoperbutylacetonicum N1-4 using hydrolyzed algal biomass, the second stage produce biodiesel by transesterification of lipids using algal biomass and in the third stage, biopolymer (PHBs) are formed by recombinant E.coli using algal biomass as growth media (Brennan and Owende, 2010; Huo et al., 2011; Gerardo et al., 2015) as shown through Fig.5.

Anthony and Sims (2013) focused on multiple products formed through bio-refinery approach by cultivated S. obliquus, single strain microalgae in photo-bioreactor. But it is economically less feasible due to the high cost of photo-bioreactor and using single strain microalgae lead to biased results due to psychological biomass makeup. So mixed cultured microalgae source is better for wastewater remediation and biopolymer synthesis like PHB.

Rahman and Miller (2017) demonstrated the harvesting technique and WLEP bio-refinery method. In this study, they first harvested the wastewater microalgae then an aqueous solution was formed via WELP. To prepare E.coli culture, the aqueous solution was neutralized followed by sterilization. The four different growth media were used to grow recombinant E.coli. The highest PHB yield of 7.8% (percentage of dry cell weight) was observed in centrifuged microalgae using aqueous phase media and other aqueous phase media from corn starch, potato starch and alum-harvested microalgae yield was approximately 1%, 2%, and 0%, respectively. The high yield PHB was produced using WELP in the above two studies, but the high economic cost was still a limitation.

Genetic Engineering of microalgae strains to produce PHA

In biofuel production, using genetically recombinant microalgae is common, but the usage is restricted to bioplastic synthesis. Little research has been done on how genetically engineered microalgae strain increased biopolymer production (Rahman et al., 2013). Hempel et al. (2011) studied the complete PHB formation pathway by genetically engineered *R. eutropha* H16 into *P. tricornutum*. The result of the study was quite impressive as after seven days of culturing PHB was about 10.6% (% dry cell weight). Castro et al. (2015) concluded that PHB production by E.coli using genetically engineered wastewater microalgae produces more PHB approximately 31% and potentially more economically viable. By comparing different production technology for PHBs production, it was concluded that the genetically recombinant microalgae were unable to give good yield than genetically recombinant *E.coli* produce using glucose as a source of energy and food. In addition, the cost of PHB polymer by genetically engineered microalgae is significantly more than PHB produced by natural resources like corn, sugarcane or potato. However, more genetically advanced technologies may come to enhance the overall production of PHB by using microalgae.

LCA OF BIOPLASTIC PRODUCED BY USING MICROALGAE BIOMASS

There are few studies available, which describe the Life Cycle Assessment (LCA) of bioplastic from microalgae (Atiwesh et al;, 2021; Chen and Quinn, 2021). Beckstrom (2019) had compared the greenhouse gas contribution of various microalgae cultivation systems used for bioplastic synthesis . Authors found that cyclic flow photo-bioreactors showed better impact than open cultivation ponds. Bussa et al. (2019) compared the environmental impact of PLA, derived using microalgae and plant-based source and found that microalgae route contribute less terrestrial eco-toxicity compared to plant-based PLA. There is a need for more LCA studies, to understand the comparison in better way and rectify the limitations.

CHALLENGES AND **SOLUTIONS**

Microalgae biomass is one of the sustainable steps toward the green environment as bioplastic formed from algal biomass is more eco-friendly than conventional fossils fuel-based plastic. But there are some issues which need to be resolved. As microalgae are diverse, there are many classes and species of microalgae which are yet to be identified. So the selection of species for the experiment is complex, and more research is required to explore the properties and efficiency of each species. Moreover, algal genetic engineering experiments are limited to laboratory study. Bio-plastic synthesis using miroalgae also result in the emission of harmful greenhouses gases such as carbon dioxide, methane, nitrous oxide during bioplastic degradation (Reddy et al., 2013; Atiwesh et al;, 2021). This problem can be reduced by using methods like incineration, mechanical and chemical recycling. Wang et al. (2016) pointed towards the odor issue during blending of microalgae biomass with Polyethylene (PE), which can be reduced by using absorbent like zeolite or activated charcoal (Thakur et al., 2018). Lack of awareness is also one of the major challenges as most consumers are unfamiliar with the term “bioplastic” and assume that it would be more expensive than conventional plastic (Rasul et al., 2017). So by creating advertisement and global marketing, this problem can also be resolved.

FUTURE PROSPECT AND CONCLUSION

A study by Nova Institute has estimated that at the global level bioplastic was produced near about 2.41 million tonnes in 2021 and it was expected that this could reach approximately 7.59 million in 2026 (EB., 2021). Undoubtfully, it can be concluded that the global production of bioplastic will increase in the incoming years and microalgae will play an important role in this.

In this review paper, the various species of microalgae used for the production of bioplastic, various microalgae cultivation methods, and different techniques used for the synthesis microalgae-based bioplastic were discussed. The most common microalgae species used were *Chlorella* and *Spirulina*. It was found that both open and closed microalgae cultivation methods had their pros and cons. PHBs production using hydrolysed wastewater microalgae biomass is a less costly technique for bioplastic synthesis. However, further research is needed to explore various methods of using microalgae biomass for bioplastic production at a low cost with a higher lifespan, less moisture content, better mechanical, and quicker biodegradability .

ABBREVIATIONS

PHAs-Polyhdyroxyakonoates	**PHBs- Polyhdroxybutyronates**	**PP-Polypropylene**
PE -Polyethylene	**PLA-Polylactic acid**	
PCL-Polycaprolactone	**PBS-Polybutylene succinate.**	

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