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Methods to Increase Microalgae Carbohydrates for Bioethanol Production

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ABSTRACT

Compared to traditional lignocellulose biomass, microalgae contain little or no lignin. Traditionally, bioethanol production from microalgae undergoes three major steps: (i) pretreatment; (ii) polysaccharides hydrolysis into simple sugars; and (iii) sugar conversion into bioethanol by fermentation. Microalgae convert sunlight, water, and CO_2 into algal biomass. Diatoms, green algae, blue-green algae, and golden algae are four main classes of microalgae, whereas the two main species of algae are filamentous and phytoplankton algae. Microalgae convert solar energy efficiently, producing an enormous number of various metabolites. Many studies have been conducted to convert microalgae into various biofuels, such as biodiesel, bioethanol, biohydrogen, and biogas. However, compared to biodiesel, bioethanol production from algae through fermentation consumes less energy with its simplified process. Considering these advantages, a number of potential applications for microalgae have been proposed and developed. Despite the promise of bioethanol from microalgae, it still has a number of obstacles, such as the low fermentable carbohydrate content of microalgae. This article intends to discuss the methods to increase microalgae carbohydrates thoroughly. To solve this problem, several nutritional starvations/limitations, like nitrogen and phosphorous starvation, are currently being considered in this paper.

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1. INTRODUCTION

The energy industries have contributed to global greenhouse gas (GHG) emissions. The research and applications of renewable energy have emerged. The application of biofuel production is one of the alternative policies to mitigate the climate change problem (Ihara, Zhao et al. 2020). Microalgae for biofuel has recently gotten a lot of attention and has a lot of potentials to replace fossil fuels (Milano, Ong et al. 2016).

Photosynthetically produced microalgae may be utilized as a variety of organic compounds, including feedstocks for a variety of biofuels (Srivastava, Shetti et al. 2020, Sarwer, Hamed et al. 2022, Thanigaivel, Priya et al. 2022). The energy inherent in these chemicals is photosynthesis-captured solar energy. Starch is made directly from glucose generated during photosynthesis, and algae may store huge amounts of it. Starch is easily hydrolyzed to glucose, which can then be fermented to produce bioethanol. The triglyceride oils accumulated by algae may be easily turned into biodiesel by transesterification with methanol, just like vegetable oils. Biogas, a mixture of flammable methane and carbon dioxide, can be produced by anaerobically digesting algae biomass. Additionally, the biomass could be fermented to produce high energy hydrogen. Therefore, microalgae can also be used to produce hydrogen.

All of this is scientifically possible, but there are so many uncertainties about the cost and environmental sustainability of microalgae for bioethanol, whether native or metabolically engineered, which seems doubtful for the near future. A biorefinery is able to produce a wide range of industrial chemicals from an algal biomass or other biomass, much as a petroleum refinery does from fossil feedstocks such as crude petroleum and coal. Even though commercial biorefineries exist, they primarily use lignocellulosic biomass rather than algal biomass as a feedstock. Algal biomass might theoretically be employed, but it is far too overpriced for this application.

Furthermore, as they grow, microalgae positively impact the environment by decreasing CO_2 emissions and alleviating water pollution issues, thus delivering the advantages of biomass-derived biofuels. Microalgae store a lot of triacylglycerol and starch, which are then turned into biodiesel and bioethanol, respectively (Nunes, Ansilago et al. 2021, Bharathiraja, Iyyappan et al. 2022, Khan, Naushad et al. 2022). Microalgae can be produced on non-agricultural land, neglected wastelands, as well as in seawater and wastewater, and they do not compete with agricultural land. The absence of non-photosynthetic structural supports such as roots and stems favours microalgae in aquaculture when compared to terrestrial plants. Algae are photosynthetic organisms that come in a variety of forms, from unicellular to multicellular.

Microalgae, or photosynthetic microorganisms, are a diverse and complex group of organisms that differ widely in the capacity of metabolic, environmental adaptations, and shape. They are small and autotrophic, which means they consume CO_2 to synthesize carbon molecules on their own for metabolic use in the sunshine). They may incorporate a variety of carbon compounds even in the absence of sunshine, since they are mixotrophic. Additionally, varying amounts of lipid oil in the form of diglycerides and triglycerides are produced by microalgae. Furthermore, certain algae can increase twofold their biomass in the laboratory for as little as 3.5 hours and in outdoor ponds for as little as 24 hours (De Bhowmick, Sarmah et al. 2019, Yadav, Dubey et al., 2020, Jacob, Ashok et al., 2021).

Oil-rich seeds, which make up a small portion of the oil seed plant's biomass, require an entire season to mature. Algae lack non-photosynthetic elements, and as microalgae are unicellular and float in the water column, they are not dependent on the large amounts of structural cellulose seen in terrestrial plants. Moreover, microalgae have a photosynthetic efficiency of up to 10%, whereas less than 0.5 percent of solar energy is converted into biomass by terrestrial plants in the middle latitudes (Barsanti and Gualtieri 2018, Chew, Khoo et al., 2021). They are tiny and autotrophic, meaning they consume CO_2 to produce their own carbon molecules for use in photosynthesis. They are also mixotrophic, which means they can take in a wide variety of carbon compounds even when there is no sunshine. Furthermore, microalgae create lipid oil in the form of diglycerides and triglycerides in varying proportions.

In contrast to other renewable energy sources, biomass may be directly transformed into liquid fuels known as "biofuels" to assist meet transportation fuel needs (Veza, Said et al. 2019, Veza, Roslan et al. 2020, Veza, Said et al. 2020, Veza, Muhammad et al. 2021, Veza, Roslan et al. 2021, Veza, Said et al. 2021, Veza, Said et al. 2021). Currently, the most prevalent biofuels are ethanol and biodiesel, which represent the first generation of biofuel technology (Roslan, Veza et al. 2020, Mohammed, Jaafar et al. 2021, Rusli, Said et al. 2021, Rahaju, Veza et al. 2022, Sule, Latiff et al. 2022, Veza, Djamari et al., 2022). Ethanol is made by fermenting sugar or starch-rich products like corn and sugar cane which undergo a distillation process to obtain pure ethanol (Hoang and Nghiem 2021, Tse, Wiens et al. 2021, Bhuyar, Shen et al., 2022). Yet, it was discovered that, while technologically possible, bioethanol from microalgae is not commercially viable compared to conventional fossil fuels or other renewable sources (Chowdhury, Loganathan et al. 2019, Hossain, Zaini et al. 2019, Siddiki, Mofijur et al. 2022). Research for microalgae has therefore shifted to applications in agriculture (fertilizer), nutraceuticals, functional foods, new high-value products, and wastewater treatment.

When compared to soy or corn, microalgae may produce 7–20 times more biomass per unit of land use, and many strains can thrive in saltwater or wastewater (Liu, Yu et al. 2021). When these factors are taken into account, the potential of algae-based biofuel is clear. This frequently debated topic deflects attention from the relevant figure, i.e., the overall cost of oil production. Some algae may produce biomass more quickly than terrestrial crops and store excess carbon as lipids rather than structural carbohydrates. Bioethanol production from microalgal biomass fermentation has several advantages: it can utilize residual microalgae from other methods (for example, oil extraction); and it takes place in an aqueous media, so energy drying is not required.

In 2011, the world's bioethanol production increased to 22 billion US gallons, with the USA and Brazil accounting for more than 65% of the total production (Halder, Azad et al., 2019). Although first-generation bioethanol (sugarcane, corn, etc) represents a minor contribution to the transportation sector, such early bioethanol generation has received harsh criticism as it competes with food production for arable soil, water, and nutrients. Also, land-based crops require months of growth to yield. For that reason, attention to algae-based bioethanol has been revived. Numerous attempts have been made to overcome a number of technical challenges related to economic and large-scale algal production.

Microalgae are submerged microorganisms with a similar photosynthetic mechanism to land-based plants. Microalgae can be found in marine and freshwater, thus having resourceful access to water, CO_2, and other essential nutrients to grow and convert solar energy into biomass. One major breakthrough in the development of a non-edible source of biodiesel is the introduction of microalgae. Studies on microalgae biodiesel were initiated in the middle of the 1980s (Gao, Xin et al. 2022, Vairaprakash and Arumugam 2022). Transesterification and catalytic cracking were predominant methods used back then to convert fat in microalgae's cell into fuel. Such methods were inadequate to produce high fat content to compromise economic performance.

Pyrogenation method was then proposed to develop a method that could attain high fat content from microalgae (Xu, Miao et al. 2006). In 1986, a German scientist deployed pyrolyzing method to convert fat into liquid fuel (Demirbas 2009). In the early 1990s, an Israeli Professor successfully used *Dunaliella salina* to produce high-quality microalgal oil with low nitrogen and sulphur levels. Income liquid oil had a concentration of 57–64 wt% and quality comparable to gasoline derived from petroleum (Flammini, Rauch et al. 2012, Sanderson 2018).

Each stage of microalgae-based bioethanol production consists of cultivation, harvesting, pre-treatment, hydrolysis, fermentation, and distillation. To become a competitive source for bioethanol, the production cost of microalgae should be low and highly efficient. Therefore, each of the above processes should be improved and optimised. At the end of this paper, future directions on bioethanol production from microalgae and macroalgae were highlighted. Yet, before discussing each step of the process, the selection of algae species is discussed first. In terms of investment, Exxon Mobil has put more than $600 million on studying and developing microalgae as a source of biofuel. They concluded that microalgae-based biofuels were not practical for the next 25 years in 2013 (Capodaglio and Bolognesi 2018, Capodaglio and Bolognesi 2019).

2. PRODUCTION OF MICROALGAE

From a historical point of view, research on microalgae has been conducted over the past 50 years, having the primary reason to explore the opportunities for them to be used in multi processes or to produce significant products. In the early 1960s, Nihon Chlorella in Japan began the pioneer of large-scale culture of microalgae (Spolaore, Joannis-Cassan et al. 2006, Khatoon and Pal 2015, Coaldrake 2021). When the awareness of using renewable energy rose during the first oil crisis in the 1970s, many were more inquisitive in using microalgae as an alternative fuel.

A specific R&D to find alternative renewable fuels was launched by the U.S National Renewable Energy Laboratory (NREL) in the 1970s. One of the programs was biodiesel from microalgae, aiming to study lipid production in oleaginous microalgae. The program lasted from 1978 to 1996. During that program, an "Outdoor Test Facility" was activated in Roswell, New Mexico, from 1987 to 1990 (Weissman, Tillett et al. 1989, Brown 1993, Mata, Martins et al. 2010). It was indicated that using microalgae as an alternative to skyrocketing petroleum-based oils was a viable option. Yet extensive R&D is needed for them to be produced in large numbers. By looking for genetic variability, the research was continued to produce improved algae strains that were higher in productivity. Before these experiments were conducted, the Department of Energy cut the budget to support the NREL R&D program in 1995. The program could not be continued further (Loiter and Norberg-Bohm 1999).

Microalgae that are grown on wastewater have great potential to produce bioethanol. The yield of bioethanol depends on fermentation environments. The most critical issue in the utilization of algae for biofuel production is its low sufficiency for large commercial applications due to increased production costs (Vassilev and Vassileva 2016). Massive investments are needed to grow algae with an abundant water supply. To reduce the operating cost, the use of waste such as municipal wastewater as a medium should be employed. Reyimu and Özçimen (Reyimu and Özçimen 2017) examined the effect of wastewater on a number of microalgae types and its productivity in bioethanol production. *N. oculata* and *T. suecica* were selected to be cultivated at various concentrations of seawater and municipal wastewater. The results showed that both microalgae could tolerate and use the wastewater. For *N. oculata*, the cultures specific growth rate was up to 0.5430 d−1 and 0.4778 d−1 for *T. suecica*. Different ratios have different outcomes owing to the influence of higher ratios on the growth stage fundamental sources and ionic composition change of the culture medium. It was found that *T. suecica* is far more suitable for bioethanol production with municipal wastewater as a medium due to its maximum carbohydrate content.

The characteristics of the microalgae, their cell density, and the prerequisite quality of the desired output influence the harvesting technique. The gathered biomass might go through more processes. As a result, the technique should not poison the biomass or release dangerous chemicals. The technique should enable the recycling of culture media and process wastewater for a cost and sustainably efficient operation.

It is crucial to keep in mind that depending on their strains, microalgae can be grown in a variety of environments. The fact that these microbes are photosynthetic means that they need light and CO_2. In addition, nitrogen and phosphorus, which make up 10% to 20% of microalgae biomass, are necessary for growth and cellular functions. Additionally, the production of microalgae requires the use of both macronutrients (Na, Ca, K, Mg) and micronutrients (B, Co, Fe, and Zn) (Markou, Vandamme et al. 2014, Procházková, Brányiková et al. 2014, Salama, Kurade et al. 2017, Paskuliakova, McGowan et al. 2018). Consequently, a variety of industrial wastewaters make good supplies for microalgae cultivation. In addition to recycling wastewater from the industrial sector, the cost of nutrients will be greatly reduced by using wastewater to produce microalgae. **Figure 1** shows a schematic of microalgae-mediated wastewater treatment with concurrent biomass production for biofuel generation. For advanced wastewater treatment with microalgae cultivation to create biomass and lipids for microalgae-based biofuel, it is crucial to choose the wastewater, robust microalgal species, and pre-treatment technology appropriately.

Chlorophyll is present in all algae and is classified into many taxonomic categories. Algae directly or indirectly produces organic molecules by using solar energy and fixing CO_2. Nevertheless, only a small number of species are considered appropriate for large-scale farming because of their high production in various environmental and cultivation conditions. Consequently, the main subject of this chapter is the development of microalgae production.

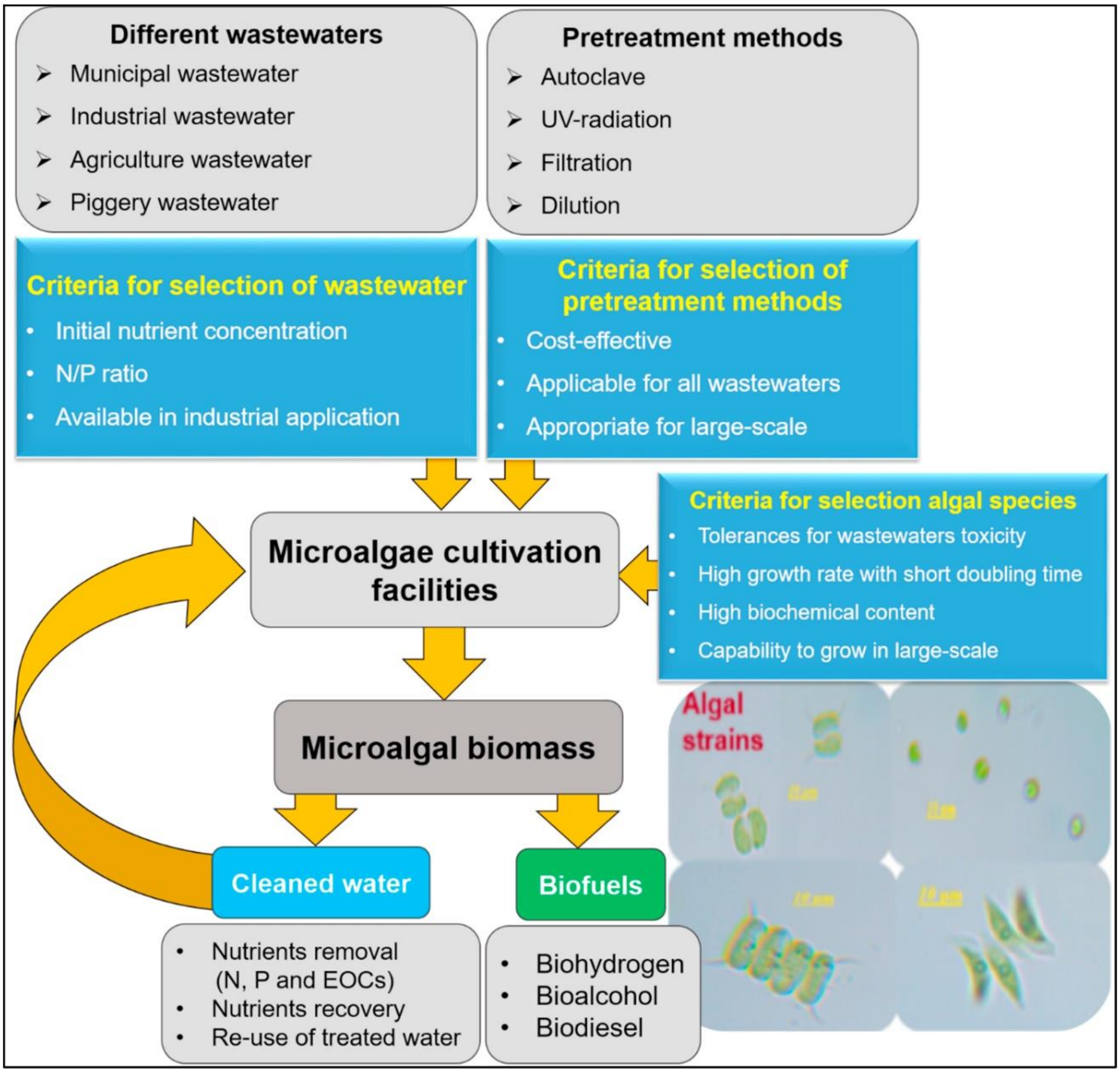

Figure 1. Schematic representation of wastewater treatment simulations with microalgal biomass cultivation for biofuel production, reproduced from (Salama, Kurade et al. 2017)

By further transforming the microalgae cells into a specific desired product, harvesting is the process of separating microalgae cells from the cultivation growing media. Three main stages of the harvesting process are performed in order: (1) biomass recovery, (2) dewatering, and (3) drying process. Due to the high level of energy inputs needed, harvesting microalgae from a diluted suspension with a concentration of less than 1 g/L is difficult.

Generally, harvesting is performed as a two-step process consisting of thickening and dewatering. A number of methods employed for thickening include chemical techniques (coagulation, flocculation), biological techniques (bioflocculation), and physical methods (gravity sedimentation, flotation, electrophoresis). Dewatering is carried out by applying physical techniques, predominantly by centrifugation and filtration.

Microalgae are harvested and dried before applying pre-treatments (physical, chemical, and biological) to disintegrate cell walls and release carbohydrates. Prior to being added to the fermenter, the carbohydrates are subsequently saccharified into smaller fermentable sugars. Although there are a lot of studies on this subject in literature, choosing the appropriate approach is still a challenge.

Besides chemical and enzymatic, other pre-treatment methods could be implemented in microalgae, including microwave, wet oxidation, electric pulses as well as hydrothermal. Production of bioethanol relies on raw material. It comprises eight major steps: (1) selection of microalgae species, (2) cultivation, (3) harvesting, (4) drying, (5) pre-treatment, (6) hydrolysis, (7) fermentation, and (8) distillation.

For sustainable production of bioethanol, the quantity of microalgae biomass should be capable of competing with other sources. This can be realized by utilizing large-scale production and optimized conditions. Provided that the production of microalgae becomes more affordable and plentiful, it can then be the preferred raw material choice for bioethanol production. The growing of raw materials in a photobioreactor is the first step in the synthesis of bioethanol from microalgae. Open ponds are an option for large-scale production. The biomass from the photobioreactor is then transferred to harvesting and drying processes. Also, carbohydrate production can be improved with calcium and magnesium owing to their significance for numerous enzymes and chlorophyll.

There are two types of microalgae cultivation systems: open and closed systems. Open ponds and lagoons are frequently employed in an open system. The open system is simple to construct, has little running and maintenance costs, and is appropriate for commercial-scale growth. The open system has certain drawbacks, though, such as the need for a lot of space, water loss from evaporation, CO_2 release into the atmosphere, decreased control of the environmental barrier, and significant contamination from outside sources. The open system is still the best option for the majority of commercial and large-scale bioethanol production from microalgae due to its cost-effectiveness.

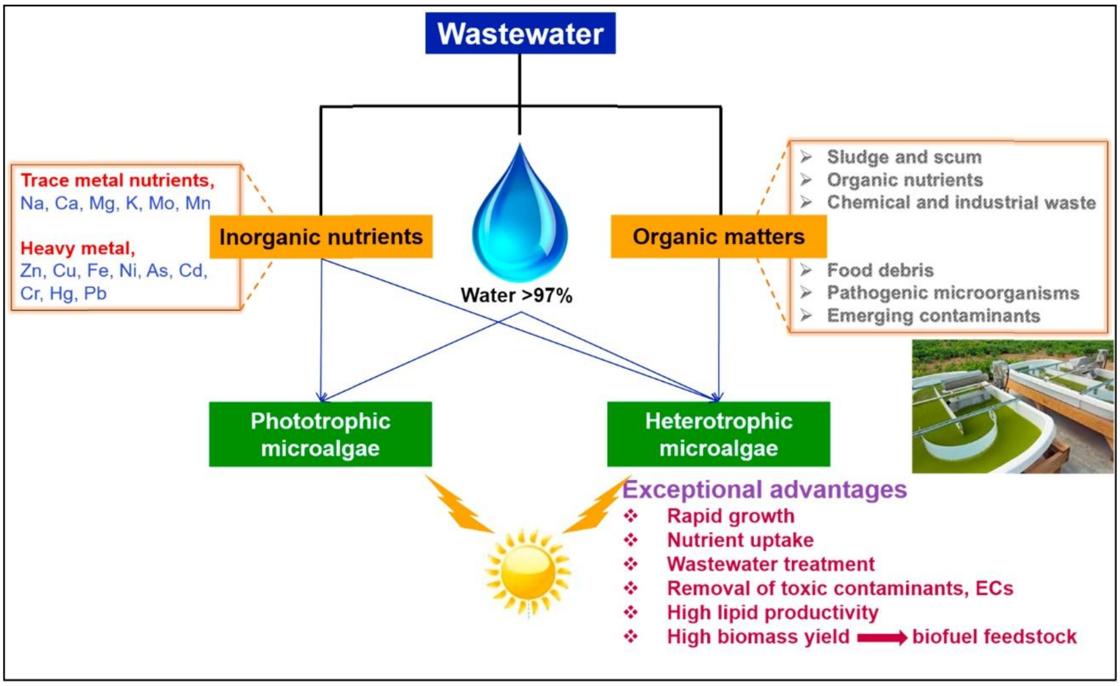

Figure 2. Wastewater resources for production of microalgae, reproduced from (Salama, Kurade et al. 2017)

The primary benefits of combining wastewater treatment with microalgae culture are the production of inexpensive biomass for biofuel production, recovering vital nutrients, and improving wastewater treatment (**Figure 2**).

There are other kinds of open systems, including raceway ponds, which are used the most frequently, unstirred ponds, circular ponds, and others. The mixing in the open system is done using impellers, paddle wheels, and rotating arms to maximize production by evenly distributing nutrients and preventing dead zones. Air that is high in CO_2 is used for mixing as well as a source of nutrients. To reduce the energy required for the mixing process, it is crucial to optimize the configuration of the raceway ponds.

Closed systems or closed photobioreactors (CPBRs) can be used to get around an open system's drawbacks. As the name implies, the culture is done in a closed system to keep the algae away from the environment. The closed system also offers controlled environmental factors and effective light use. Additionally, it can be used outside to use solar energy as a light source. Tubular, flat-plate, bubble-column, and vertical photobioreactors are the most popular closed systems.

A hybrid approach (two-stage cultivation) has recently been used to get around the drawbacks of open and closed systems. The conditions in the open raceway ponds, where the desired chemical accumulation occurs at a high pace, are combined with higher output in closed systems. As the biomass is kept in the open ponds for a few days, the pollution can be greatly decreased. Utilizing low-cost nutrients for microalgae cultivation, such as agro-industrial wastewater and flue gas, can further reduce the cost of cultivation. Such systems are advantageous for greenhouse gas fixation and wastewater bioremediation in addition to boosting biomass content.

An additional intriguing technique that is frequently used during the cultivation stage involves the manipulation of nutritional (nitrogen and phosphorus), environmental (e.g., temperature and light), and osmotic (salinity) conditions to create stress environments and to stimulate microalgae metabolic pathways, which results in the product accumulating in greater amounts. In recent years, introducing phytohormones and co-cultivating microalgae with yeasts and bacteria have both proven to be effective methods for increasing biomass production.

3. METHODS FOR INCREASING MICROALGAE CARBOHYDRATE CONTENT

The carbohydrate content depends on the species, cultivation, and environmental conditions. Microalgae cultivated under unobstructed and favorable conditions have typical carbohydrate content of around 10–30% (Harun, Danquah et al. 2010, Cea-Barcia, Buitrón et al. 2014, Suparmaniam, Lam et al. 2019).

Numerous studies investigated how to increase the microalgae carbohydrate content (Vieira Salla, Margarites et al. 2016, Samiee-Zafarghandi, Karimi-Sabet et al. 2018, Andreeva, Budenkova et al. 2021, Debnath, Bandyopadhyay et al. 2021). The carbohydrate content can be controlled under environmental stress conditions, such as nutrient starvation/limitation, high light intensities or salinity stress (Markou and Nerantzis 2013).

The nutrient starvation approach is considered to be an affordable strategy for increasing microalgae carbohydrate content (Dragone, Fernandes et al. 2011). Various investigations discussed nutrient starvation as an approach to increase the carbohydrate content. However, the exclusion of nutrients affects low growth rates and production. Hence, nutrient concentration optimization is a significant consideration (Hsieh and Wu 2009, Markou, Chatzipavlidis et al., 2012).

Several experiments investigated the optimum microalgae production. One of the common methods for optimizing the production of microalgae is Response Surface Method (RSM) by identifying the productivity and growth rate of microalgae (Dragone, Fernandes et al. 2011, Miranda, Lima et al. 2016, El-Mekkawi, Abdo et al. 2019, Mohd Apandi, Muhamad et al. 2021, Tazi, Jamai et al. 2022, Udayan, Sreekumar et al., 2022).

The most important variables affecting the composition of the microalgae biomass are explained below.

In addition to light and CO_2, microalgae cells' growth also depends on several other components, including nitrogen, phosphorus, potassium, and sulphur (Wang, Li et al., 2008, Uggetti, Sialve et al. 2014, Zhu, Li et al., 2016). Since the ratio of nutrients needed and their availability varies depending on the microalgae species, it impacts growth. Additionally, biochemical makeup may be significantly impacted by limiting a specific nutrient. The breakdown of protein-based pigments (phycocyanin and chlorophyll, which are both pigments) and either an increase in fats or carbs are the most significant impacts (Andreeva, Budenkova et al., 2021).

Nitrogen is the second most prevalent element in microalgae biomass (Chu, Cheng et al., 2019, Zarrinmehr, Farhadian et al., 2020). Proteins, pigments, and DNA are just a few of the vital macromolecules that require nitrogen for synthesis. Different types of nitrogen can be used by microalgae (urea, nitrate, ammonium or ammonia, and organic nitrogen like amino acids). The influence of nitrogen supply on the composition of biochemicals has been studied in a number of published studies. Few research focused on carbohydrate build-up, while the majority examined lipid accumulation. The flow of photosynthetically fixed carbon from the metabolic route of protein synthesis to the carbohydrate or lipid is altered by nitrogen starvation, resulting in their accumulation.

Phosphorus has a substantial role in the synthesis of vital biomolecules and contributes to critical metabolic processes. Under phosphorus starvation, microalgae have a tendency to accumulate carbohydrates (Yang, Xiang et al., 2018). It is important to note that lipid accumulation also tends to occur in a condition where phosphorus is limited.

Nitrogen and phosphorus are essential nutrients to increase microalgae growth and control metabolic activities. Both concentrations in microalgae cultivation may influence lipid and fatty acid yield (Xin, Hong-ying et al. 2010, Yang, Chen et al., 2018). Microalgae can take up high both nutrients concentrations for the accumulation of protein and nucleic acid synthesis (Muñoz and Guieysse, 2006).

Salla et al. experimented with increasing Spirulina's carbohydrate content by adding protein and nutrient starvation, and it led to high carbohydrate productivity (Vieira Salla, Margarites et al., 2016). Zafargandhi et al., (2018), by using RSM, examined and optimized the growth of Chlorella sp. under nutrient starvation and different light intensities to reach maximum carbohydrate content and biomass productivity. The carbohydrate content increased up to 4.63 times hydrogen production (Samiee-Zafarghandi, Karimi-Sabet et al., 2018).

Note that microalgae cultures grow at the other nutrients starvation for carbohydrate accumulation. These include sulphur, potassium, and manganese. It is important to remember that biohydrogen production also utilises carbohydrates, therefore, sulphur or potassium starvation have been recommended as promising approaches to produce biohydrogen from microalgae.

4. CONCLUSION

A novel replacement for fossil fuel sources has been reviewed: bioethanol production from microalgae biomass. Microalgae biomass does not include lignin, unlike other renewable sources (such as lignocellulosic materials), making the process of extracting carbohydrates simpler. Eventually, this should lead to the development of cleaner and safer bioethanol manufacturing methods. Microalgae contain carbohydrates, which can be found in numerous sections of the cells and in different forms (cellulose, starch, and/or glycogen; inner, inside, outside).

Conditions for cultivation and operation shall significantly impact the type, location, and concentration of carbohydrates, with concentrations ranging from 15% to 50%. In order to produce bioethanol from microalgae, many procedures must be followed. First, various techniques can be used, like physical-mechanical forces, chemicals, or a combination, to damage the cell wall before releasing the carbohydrates. The next step is enzymatic hydrolysis to turn the carbs into simple sugars. To convert these sugars into ethanol, a fermentation stage using yeast or bacteria is then carried out. Prior to industrial implementation, it is crucial to optimize the key variables of these many processes in the synthesis of bioethanol. Additionally, more economical as well as life cycle analysis research is required to guarantee the economic viability of the process. Also, the metabolic pathway of dark and photo fermentation should be examined thoroughly. Genetically engineered microorganisms should also be investigated in more detail.

The modulation of biomass biochemical composition and, by extension, carbohydrate accumulation has been hypothesized as a result of stress circumstances linked to other environmental/operational cultivation parameters. Another strategy that has been proposed is nutrient starvation, which might be the most effective way to promote carbohydrate accumulation. High salinity and high light intensity are conditions that could also cause carbohydrates to accumulate.

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