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# Ecological stress stimulus to improve microalgae biofuel generation: a review

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## ABSTRACT

Microalgae-based biofuel generation is an encouraging alternative for the next generation renewable and sustainable biofuels. Microalgae biomass is a competent raw material for the generation of various biofuels including biodiesel, bioethanol, biomethane, and biohydrogen. Progress in the microalgae biofuel area desires abundant ecological conditions such as reasonable light, temperature, pH, and nutrients. Furthermore, the environmental conditions mentioned above are generally not noble with the beneficial conditions for generating biofuels. However, biochemical compositions, such as lipids and carbohydrates, can be influenced to increase the content under stress conditions of light, temperature, nutrients and salinity. In this paper, the impact of environmental stress factors is concise to improve microalgae biofuel generation by stimulating a significant increase in biochemical components.

**Keywords:***Microalgae; Ecological stress; Environmental conditions; Biochemical composition;Biofuels*

## 1. INTRODUCTION

Ecologically renewable and sustainable energy resources are the most important consideration worldwide (Owusu et al., 2016). Biofuels are fascinating as one of the most viable bottomless energy substitutes. The generation of microalgae biofuels has attracted a lot of attention recently. In this review, microalgae are explored as single-celled photosynthetic microorganisms with numerous attractive characteristics as a raw material for biofuel generation (Hannon et al., 2010). Microalgae grow rapidly and offer higher biomass productivity with a high content of biochemical cellular lipids compared to any other terrestrial plant used in the production of biodiesel. Microalgae refrain from competing with agricultural land. It sequesters greenhouse gases ($CO_2$) to mitigate the impacts of global warming and produces biomass (Khan et al., 2018).

Microalgae can use nutrient supplements (e.g. nitrates, phosphates, macro-, and micronutrients) from municipal, industrial, agricultural, etc. wastewater resources, which offer cost-effective bioremediation of wastewater (Arora et al., 2020; Kumar et al., 2018a; Fatima et al., 2020a; Jaiswal et al., 2016). It also has the potential to produce various value-added co-products for commercial applications. Microalgae have the ability to generate various types of biofuels, including biodiesel from lipid transesterification, bioethanol by fermentation of carbohydrates, biomethane through anaerobic assimilation of organic components, and biohydrogen from fermentation/photosynthesis (Kumar et al., 2018b; Jha et al., 2016; Jaiswal et al., 2014a; Dutta et al., 2019). In addition, the entire microalgae biomass can be transformed into bio-oils by hydrothermal liquefaction and pyrolysis, bio-chars by hydrothermal carbonization and synthesis gas by gasification (Vlaskin et al., 2018; Kumar et al., 2019).

Despite the fact that microalgae biofuels have incredible potential, the commercialization of biofuel production has a substantial drawback. Several further researches is being considered to make microalgae biofuel economically viable, such as selection and bioengineering of microalgae strains, optimization of culture conditions, development of the bioreactor for large-scale cultivation, improvement of the biomass harvesting efficiency and the reduction of costs and energy in the processing for the production of biofuels (Jagadevan et al., 2018; Jaiswal et al., 2014b; Kumar et al., 2017; Fatima et al., 2020b). The cultivation of microalgae for the generation of biofuels is influenced by several ecological factors, such as light, temperature, nutrients, salinity and pH (Musa et al., 2019). These ecological factors influence the growth of biomass productivity, as well as cellular biochemical compositions (Morales et al., 2018). This review presents the essential environmental factors for optimized microalgae cultivation and stress conditions to improve microalgae biofuel generation.

## 2. ECOLOGICAL FACTORS FOR THE GROWTH OF MICROALGAE BIOMASS

The cultivation of microalgae for the potential production of biomass is remarkably influenced by numerous ecological factors such as assessable light illumination, ambient temperature, nutrient availability, salt concentration, and pH of the culture media (Nanda et al., 2019; Kumar et al., 2018c; Kumar et al., 2018d). These factors significantly influenced photosynthesis and, subsequently, biosynthesis and the accumulation of biochemical molecules (Figure 1).

### Light illumination

Light is one of the key factors for the photoautotrophic existence of life. Light intensity, color, and duration (light/dark cycles) influence the utilization of photonic energy for photosynthetic performance. The growth rate of the microalgae biomass increased as the intensity of optimal light illumination increases (1000–2000 lx). Extensive light intensity above the optimal level leads to the formation of reactive oxygen species (ROS), which declines the biomass production efficiency due to photo-inhibition (Sforza et al., 2012; Singh et al., 2015).

### Ambient temperature

Microalgae are a ubiquitous organism that can survive in extreme conditions, even in environments with different temperatures. However, the ideal temperature for microalgae growth is restricted to narrow ranges, i.e., 20–30 °C. In general, increasing the optimal temperature improves the generation of microalgae biomass, while increasing the temperature above the optimal level, retards the growth and subsequent death of microalgae cells. Furthermore, the low temperature decreases the growth of biomass due to the interruption of the metabolic process. However, the increase in temperature during the morning and decreasing it at night to the optimum range increases the productivity of the biomass (Singh et al., 2015, Cho et al., 2007).

### Nutritional supplementation

The growth and survival of the microalgae depend on the availability of adequate macronutrient (such as N, P, C, S, etc.) and micronutrient (such as Fe, Zn, Cu, Co, etc.) supplements. Inadequate nutrient supplementation may cause physiological and morphological alterations of microalgae cells, resulting in a decrease in the growth rate and productivity of biomass (Wells et al., 2017). The ratio of most essential macronutrients of nitrogen and phosphorus (N: P) directly regulates cell expansion, since the N associated with the biosynthesis of nucleic acids, structural proteins and photosynthetic pigments and P is responsible for the various cellular metabolic processes (Merchant et al., 2012). Carbon dioxide is used in the photosynthesis process; however, it becomes harmful in excessive abundance. Sulfur is necessary for the synthesis of protein and lipid metabolism. Sulfur confinement has been considered to restrict the duplication of microalgae cells. Fe is responsible for the electron transport chain in photosynthesis; however, the excess can cause oxidative damage to microalgae cells (Shen et al., 2014; Show et al., 2017).

### Other factors

Several other factors such as pH and salinity also play a significant role in the accumulation of microalgae biomass. The optimal pH for microalgae growth varies between 7.5 and 8.5 and can vary from one strain to another. In microalgae cultures, the pH increases gradually during the day due to the consumption of CO2 in photosynthesis. It can be seen that pH influenced the accessibility and assimilation of nutrients such as Fe and C. Several species of microalgae can tolerate various ranges of salinity, while excessive concentrations of salt inhibit photosynthesis, resulting in decreased biomass yield (Usher et al., 2014; Cho et al., 2007).

## 3. ECOLOGICAL STRESS STIMULUS INFLUENCING MICROALGAE BIOFUEL GENERATION

Biochemical compositions of microalgae biomass (e.g., biofuel-related carbohydrates and lipids) fluctuate with ecological conditions. Ecological stresses have been reported to improve the efficiency of microalgae biofuel generation (Singh et al., 2011). Here, we are describing the influence of various ecological stress conditions;

### Photonic stress

Light photons are used in the photosynthesis process to synthesize biochemical components in the microalgae cells. Different light conditions radiate different amounts of light photons that support a notable alteration in the biochemical compositions of microalgae cells. In general, a higher intensity of light energy stimulates the accumulation of storage lipids, e.g. saturated and monounsaturated fatty acids (MUFA), while a lower intensity of light energy favors the accumulation of structural lipids, e.g. polyunsaturated fatty acids (PUFA) (Spoehr and Milner, 1949; Khotimchenko and Yakovleva, 2005). Since the saturated fatty acids and MUFA are preferably used in the synthesis of biodiesel; so, a relatively higher light intensity can be used to improve microalgae biodiesel production. In the literature, exposure to high light intensity to *Scenedesmusobliquus* has shown to increase carbohydrate content from 16.3 to 22.4% (Ho et al., 2012).

### Ambient temperature stress

The ambient temperature influences the accumulation of lipids in the microalgae biomass. A number of microalgae, e.g. *Ochromonasdanica* and *Nannochloropsis oculata* have been reported to improve cellular lipids (37% and 89%, respectively) with optimally increased temperatures. Moreover, the structural composition of lipids can also be altered by ambient temperature. Similar to the impact of light photons, the higher temperature favors fatty acid saturation, while the lower temperature tends to increase the degree of unsaturation in fatty acids (Liu et al., 2005). Therefore, a higher optimal ambient temperature stimulates the production of high quality and quantity biodiesel due to high total lipid yield and fatty acid saturation. The carbohydrate content of the microalgae biomass can also be influenced by temperature, e.g. the carbohydrate content of *Spirulina* spp. enhanced by 50% in the increased temperature of 25–40 °C (Ogbonda et al., 2007).

### Nutritional supplementation stress

Microalgae cells alter metabolic strategies and biochemical components under conditions of nutritional stress. Therefore, the desired improvement in biofuel production can be achieved by influencing nutrient supplementation.

### Nitrogen stress

Nitrogen is a key element for the biosynthesis of proteins. However, the nitrogen stress environment stimulates the fixation of most of the carbons in the photosynthesis process to manufacture cellular lipids or carbohydrates as a protein substitute. Nitrogen has been found to be the most imperative nutritional supplement influencing lipid metabolism in microalgae (Rodolfi et al., 2009). Numerous microalgae species have been reported to increase cellular lipid accumulation under N deprivation, e.g. *Neochloris oleoabundans* and *Nannochloropsis* spp. have increased its lipid content 1–2 times. Carbohydrate biosynthesis has also been improved ~ 4-fold under N-stress conditions in *Tetraselmis subcordiformis*. Furthermore, numerous cyanobacteria can generate biohydrogen as a by-product of nitrogen fixation under conditions of nitrogen stress (Ji et al., 2011; Ho et al., 2012).

### Phosphorous stress

Phosphorus is associated with numerous metabolic events in microalgae cells. Deprivation of phosphorus has been found to increase the accumulation of cellular lipids, e.g. ~ 53% increase in cellular lipids in *Scenedesmus* spp. under P stress condition. Furthermore, carbohydrate biosynthesis has been reported to be increased by 11-67% in Spirulina platensis under phosphorous limitation (Markou et al., 2012).

### Carbon stress

Carbon elements are anticipated to affect the activity of nitrogenase enzymes and, as a result, nitrogenase-dependent hydrogen generation. Furthermore, the variety of carbon sources and their concentration influence the structural composition of cellular lipids in microalgae (Dutta et al., 2005). The high concentration of $CO_2$ promotes the accumulation of saturated fatty acids, while the low concentration of $CO_2$ induces the accumulation of unsaturated fatty acids. Some of the microalgae species have the ability to use organic carbon sources as a substitute for $CO_2$, e.g. heterotrophic growth of microalgae. Heterotrophic growth of *Chlorella* spp. can synthesize up to 45% more carbohydrates and 280% more lipids compared to autotrophic growth (Miao and Wu, 2006).

### Sulfur stress

Sulfur elements are one of the noteworthy supplements that influence the generation of hydrogen in microalgae cells. Sulfur deprivation is responsible for creating an anaerobic environment within microalgae cells, which stimulates the action of hydrogenase enzymes and the discharge of biohydrogen. Therefore, the sulfur-deprivation environment has been applied to promote hydrogen generation in numerous microalgae species, such as *Chlamydomonase uyale*, *Gloeocapsa alpicola*, and *Chlamydomonas reinhardtii*. The total lipid content has increased 2 times higher in *C. reinhardtii* under conditions of sulfur stress. Furthermore, Chlorella vulgaris has been reported to synthesize ~ 50% more carbohydrates in a sulfur stress environment (Brányiková et al. 2011).

### Trace metal stress

Trace metal supplementation may influence carbohydrate and lipid accumulation in various microalgae cells. The Fe trace metal stress environment stimulated to increase glucose from 5 to 45% in *Agmenellum quadruplicatum*, while the increase in Fe trace metal-induced lipid composition by increasing 7-fold in C. vulgaris. The stress of the Si trace metal has the ability to increase the lipid composition in numerous diatom species (e.g. *Cyclotella cryptica*, *Naviculas eprophila* and *Chaetocero smuelleri*) (Griffiths and Harrison, 2009). Moreover, nutritional trace metals (e.g. Fe, Ni, Mg, Mo, and Zn) are essential for the nitrogen-catalyzed biohydrogen generation (Singh et al., 2016).

### Salinity stress

Salinity has shown a significant influence on numerous biofuel productions. Bioethanol production from *Spirulina maxima* has been enhanced by a high salinity environment (Carrieri et al. 2010). Several species of microalgae have also been reported to produce low molecular weight carbohydrates under salinity stress. Additionally, salinity stress has an impact on lipid accumulation in microalgae cells. In *Dunaliella tertiolecta*, increased salinity improved lipid composition by 60–70%. Similarly, the elevation of salinity tends to induce the synthesis of saturated fatty acids that contribute to increasing the profitability of biodiesel (Chen et al., 2008).

### Nanomaterials stress

Photochemical reactions during photosynthesis can be influenced by several other factors, such as magnetic nanomaterials and graphene oxides. Numerous investigations have been conducted for synthesis and characteristic properties of these nanomaterials (Jaiswal et al., 2018a; Jaiswal et al., 2018b; Sudhakar et al., 2017; Sudhakar et al., 2018). Magnetic nanoparticles, e.g. superparamagnetic $Fe_3O_4$ and graphene oxide have been studied to induce oxidative stress and inhibition of cell division rate in different microalgae. Nanomaterial triggers inhibition by the generation of reactive oxygen species and the drop in the photochemical activity of the photosystem II in microalgae cells (Barhoumi et al., 2013). Graphitic oxide nanosheets have been reported to improve lipid generation by 6-fold in *Chlorella pyrenoidosa* for biofuel frameworks (Khanra et al., 2018).

### Multiple stress condition

Different ecological stress factors have an additional impact on biofuel generations. Herewith, the combination of multiple stress factors may have a superior effect to improve the desired biofuel production compared to the single ecological stress factor (Pal et al. 2011). Currently, a number of investigations have focused on the co-impact of multiple ecological stress factors on the production of biofuels from microalgae. The productivity of cellular lipids in *Nannochloropsis* spp. has been maximized under the condition of simultaneous co-stress of high light intensity and high salinity. Combined high light illumination and nitrogen deprivation have resulted in increased triglycerides in *N. oleoabundans* (Sun et al. 2014).

### OUTLOOK ON MICROALGAE BIOFUEL PRODUCTION UNDER ECOLOGICAL STRESS

Reasonable biomass productivity is significant for good biofuel generation. However, ecological stress factors are used to increase the production of biofuels from microalgae in the outflow of inferior biomass yield. To achieve optimal environments for biofuel generation from microalgae, a two-stage strategy for microalgae cultivation can be adopted. In the first stage, the microalgae can be grown initially under typical conditions, and then in the second stage, they can be transferred to the ecological stress environment for the preferred biofuels (Dragone et al., 2011). With this strategy, microalgae can deliver biofuels to the maximum without a reduction in the biomass. Lipid content in microalgae species has been reported to increase by cultivating initially under adequate nutrient conditions and then in a nutrient-deficit medium. High cell growth has been recognized initially in a medium sufficient with N and Fe supplementation and then in a medium deprived of N and Fe to stimulate carbohydrate accumulation. Furthermore, genetic manipulation can confer stress tolerance to microalgae to lead the generation of biofuels (Singh et al., 2013). Another, stress-tolerant strains of microalgae have also been applied to avoid contamination and avoid the effect of biomass reduction for biofuel production.

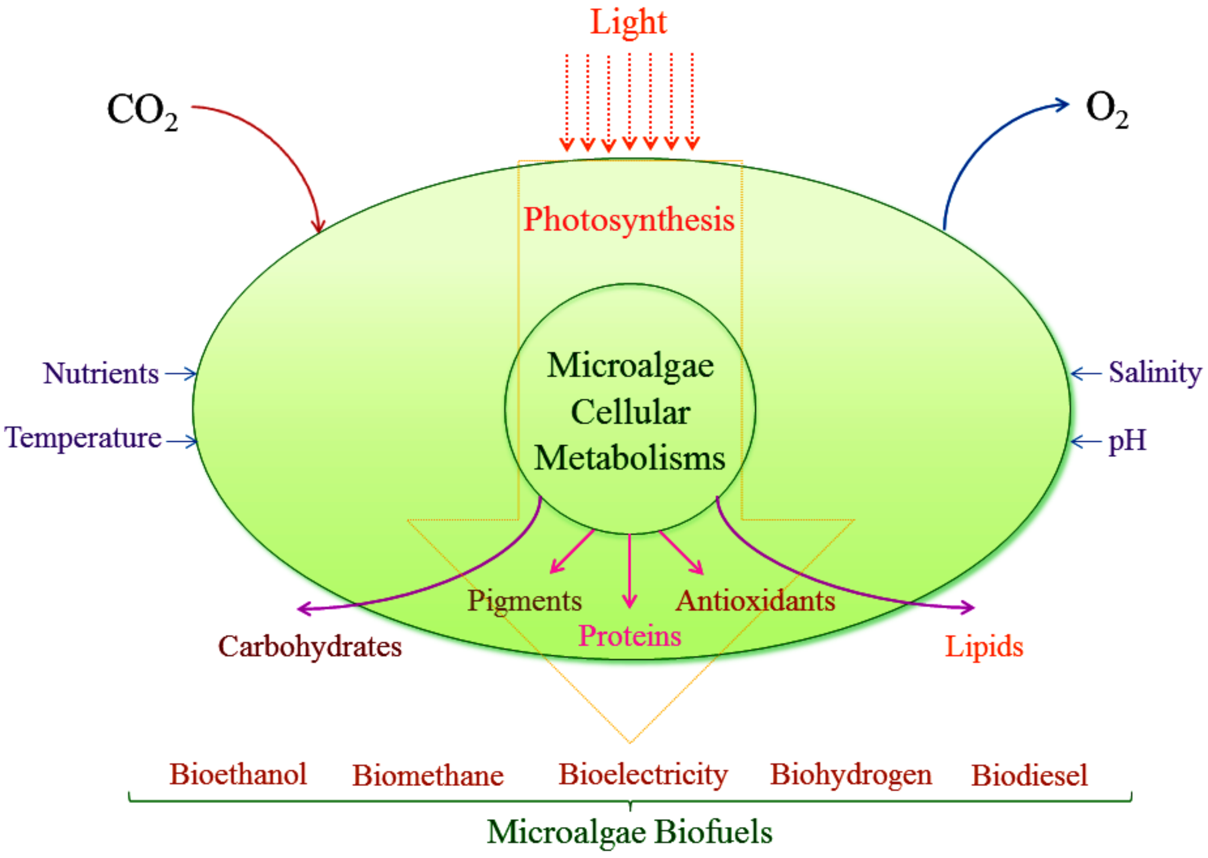

**Figure 1.** Ecological factors for the growth of microalgae and prospective biofuels.

## 4. CONCLUSIONS

Cultivation of microalgae biomass under optimized ecological stress factors (e.g. a combination of nutritional confinement and light stress) can reduce the cultivation budget, increase the accumulation of biofuel constituents, and avoid contamination of foreign organisms to offer a viable strategy to improve the production of microalgae biofuels. In the viewpoint, the life cycle assessment of the microalgae biomass production process and the appropriate underpins (e.g. research on ecological stress factors at pilot scale, promising use of stress environments, and advancing in biofuel improvement) fascinate industrial interest. An advance in key strategies to couple with ecological stress factors encourages the production of biofuels from microalgae commercially.

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