Microalgae Cultivation Techniques and Growth Conditions

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Microalgae, constituting a wide range of photosynthetic organisms, span from fundamental blue-green algae (cyanobacteria/prokaryotes) to complex seaweeds, rendering them one of the most diverse groups in the biological kingdom.

Keywords: biosorption ; bioaccumulation ; decentralized water systems

1. Introduction

Microalgae, constituting a wide range of photosynthetic organisms, span from fundamental blue-green algae (cyanobacteria/prokaryotes) to complex seaweeds, rendering them one of the most diverse groups in the biological kingdom. The microalgal kingdom comprises more than 350,000 identified species as of 2017 [1]. These organisms possess a notable biochemical composition: approximately 70% lipids, 60% carbohydrates, and 65% proteins. Additionally, they contain crucial amino acids [2]. Jamshaid et al. [3] argue that microalgae offer a compelling alternative to conventional feedstocks. This preference arises from their short growth cycle, high biomass productivity, impressive harvesting efficiency, and unparalleled carbon fixation rates. Furthermore, microalgae thrive without the need for extensive arable land, instead flourishing in marginal regions using seawater or effluent as their growth medium [4].

The ecological importance of microalgae is emphasized by their extraordinary photosynthetic efficiency, surpassing terrestrial plants by 40–50% [5]. Their notable carbon sequestration capacity significantly contributes to global CO_2 sequestration, with 1 kg of microalgae absorbing 1.83 kg of CO_2 [6]. Additionally, they play a vital role in producing around 50% of Earth's atmospheric oxygen. Despite the estimated diversity of microalgae species ranging from 30,000 to over 1,000,000, only 15 are feasible for large-scale commercial cultivation [7]. Characterized by their rapid growth rate and brief life cycle, typically spanning just a few days, microalgae exhibit an exceptional capacity for biomass production [8]. This unique attribute has spurred research into various applications, including bioremediation and transforming microalgal biomass into value-added commodities, such as bio-oil and syngas [9].

Microalgae find extensive application in the remediation of diverse wastewater categories, constituting industrial, agronomic, and mining discharges. Their proficiency in nutrient removal, potent adsorption capabilities, and environmentally sustainable attributes confer them an advantage over conventional treatment methodologies [10]. Their effectiveness spans various wastewater applications, demonstrating their versatility and efficiency. Research by Ferreira et al. [11] has illustrated their capability in treating brewery wastewater, while Calicioglu et al. [12] have observed their effectiveness in managing everyday domestic wastewater. They excel in mitigating the vivid tones of textile wastewater and handling industrial and pharmaceutical effluents [13]. Additionally, they exhibit determination in addressing waters laden with heavy metals, offering a chance at redemption [14]. Hariz et al. [15] have conducted studies affirming the efficacy of microalgae in treating oil effluents and starch-containing textile wastewater. In agro-industrial settings, microalgae emerge as a steadfast wastewater quality solution, as Jayakumar et al. [16] have highlighted. Their wide-ranging applications emphasize their significance in wastewater treatment across diverse sectors.

Microalgae's genetic reservoir includes essential genes pivotal in degrading a broad spectrum of impurities [17][18]. Consequently, selecting suitable microalgal strains is paramount in determining effective strategies for remediating contaminated wastewater [19]. Specific microalgae strains hold versatile applications across various industries, including face painting, poultry, fertilizers, and medication, as well as in the production of green fuels like bio alcohols, biogas, and biodiesel [2]. Ng et al. [6] emphasize that to achieve the cost-competitiveness of algal products with fossil fuels, it is crucial to concentrate efforts on strain selection, cost-effective media, optimized conditions for augmented biomass production, and the feasibility of commercialization. Notably, integrating microalgae into wastewater treatment processes can lead to reduced production costs and a lower overall carbon footprint, owing to the nutrient-rich composition of wastewater.

Microalgae have demonstrated their efficacy in treating domestic wastewater, as shown in **Table 1**. These microalgae-based wastewater treatment technologies stand out because they can achieve bioremediation in a single step [20]. The harvested biomass of microalgae holds the potential for conversion into valuable biobased compounds, such as biohydrogen, biohydrocarbons, bioalcohols, and health enhancements [21]. Innovative methods have paved the way for annual biomass production of up to 70,000 Kg and the generation of 15,000 L of oil per hectare. These advancements present promising opportunities for commercial applications in aviation and vehicular biofuels [22].

Furthermore, the contribution of microalgae in mitigating eutrophication, ozone depletion, and global warming emphasizes their potential as a sustainable approach for biofuel production in conjunction with wastewater treatment [23]. In addition to biofuels, microalgal biomass is a valuable resource of carbohydrates, proteins, vitamins, lipids, and a range of low- and high-value by-products, achieved through biorefinery processes. These processes encompass the production of microalgal plastics, fertilizers, fibres, and protein-rich animal feed [20].

Table 1. Microalgae biomass production from wastewater cultivation.

Wastewater Type	Microalgae	Biomass Cultivation	Reference
Municipal effluent	***Scenedesmus obliquus***	**0.22 g/L**	[24]
Household effluent	***Chlorella* sp.**	**0.73–1.38 mg/L/d**	[25]
Municipal effluent	***Chlorella sorokiniana***	**1 g/L**	[26]
Municipal effluent	***Scenedesmus* sp.**	**1.1 g/L**	[27]
Household effluent	***Chlorella vaiabilis***	**1.72 g/L**	[28]
Municipal effluent	***Scenedesmus* sp.**	**1.81 g/L**	[29]
Household effluent	***Scenedesmus obliquus***	**3.55 g/L**	[30]

While there are various methods for remediating pollutants, including physical, chemical, and biological approaches, microbial approaches have become favored for their environmentally friendly characteristics [31]. While bacteria and fungi have been extensively researched for their pollutant removal capabilities, microalgae-based remediation has received comparatively less attention [32]. Hence, there is a pressing need to explore the potential of microalgae in xenobiotic remediation from the environment. The technology of wastewater treatment using microalgae presents several benefits, including solar energy generation, adequate CO_2 fixation, and sustainable biomass production with low environmental impact. However, their growth rate, nutrient utilization, and efficiency in removing pollutants are limited under conditions where CO_2 is deficient [33].

2. Characteristics and Classification of Microalgae

The characteristics and classification of microalgae incorporate a range of defining features that collectively shape their diversity and ecological significance. These minute organisms typically exhibit microscopic dimensions ranging from 3 to 25 µm and are primarily unicellular, although some can form colonies or simple multicellular structures [34]. One of their most remarkable attributes is their ability to harness solar radiation for photosynthesis, which drives their growth and biomass production [35].

Microalgae comprise two main groups: prokaryotic cyanobacteria and eukaryotic protists [36]. These organisms thrive in various aquatic environments, including freshwater, marine, and brackish water habitats [35]. Their ability to thrive in varied environments showcases their adaptability and ecological flexibility [37]. The vibrant pigments found in microalgae, including chlorophyll and others, play a pivotal role in their photosynthetic capabilities [38]. This process forms the basis of their energy acquisition and is central to their ecological importance. Based on a combination of morphological, physiological, genetic, and metabolic features, microalgae are classified into distinct categories [39].

The morphological classification is a foundational system based on critical features such as cell shape, size, and the existence of flagella. This method categorizes microalgae into specific groups, including prokaryotic cyanobacteria, which display a range of blue-green and green varieties [40]. According to the research by Nitsos et al. [41], eukaryotic microalgae are classified within this framework, featuring green *Chlorophyta*, brown *Phaecophyta*, and golden *Chrysophyceae*. This method enables categorizing microalgae based on their visible physical traits, providing a valuable initial understanding of their diversity and evolutionary relationships [41].

Phylogenetic classification represents a deeper exploration of the evolutionary relationships among various microalgae species [42]. This classification method harnesses cutting-edge DNA sequencing techniques to unravel the genetic relatedness among these organisms, providing profound insights into their evolutionary history and intricate interconnections [43]. The ancestral lineages and evolutionary trajectories that have shaped these microscopic life forms over vast stretches of time can be uncovered by deciphering the genetic blueprints of microalgae. This approach offers a comprehensive understanding of their evolutionary heritage and how they are biologically interconnected [42].

Ecological classification finds its foundation in the habitat preferences of microalgae, as noted by Verdelho et al. [44]. Through a meticulous examination of their distribution patterns and thriving conditions, microalgae can be differentiated based on their predominance in specific environments, whether it be freshwater, marine, or brackish water settings, as also highlighted by Khoironi et al. [45]. This approach yields invaluable insights into the ecological niches these organisms occupy and the adaptations they have developed to thrive in their respective habitats [44]. It serves as a crucial tool for understanding how microalgae interact with and respond to their surrounding environment, shedding light on their ecological significance and contributions to various ecosystems.

Metabolic modes represent another critical aspect of microalgae classification [46]. Their energy and nutrient acquisition strategies characterize these modes. Microalgae can be classified into distinct groups based on their metabolic preferences, which include photoautotrophic (relying solely on photosynthesis), heterotrophic (acquiring organic carbon from external sources), mixotrophic (combining both autotrophic and heterotrophic strategies), and photoheterotrophic (utilizing light as an energy source while obtaining carbon from external sources) modes [47]. Their ability to thrive in diverse environmental conditions highlights their ecological resilience and survival tactics.

3. Cultivation Techniques and Growth Conditions

Microalgae find diverse applications across industries, including textiles, poultry, biofertilisers, pharmaceuticals, and green fuels like bioalcohols, biogas, and biodiesel [2]. However, attaining cost-competitive algal products remains a challenge, as current prices cannot match those of fossil fuels [48]. Therefore, critical aspects of algal research encompass strain selection [49], the use of economically viable growth media, optimization of conditions for increased biomass production, aligning cell stoichiometry with the desired product, ensuring effective commercialization, and minimizing operational costs, particularly in the cultivation and harvesting phases [6]. Wastewater proves to be an optimal resource for algal biomass production for several compelling reasons. It serves as a cost-effective growth medium, facilitating extensive biomass and biofuel generation and providing ample nutrients. Moreover, it holds the potential for seamless integration of algal cultivation with pre-existing wastewater treatment infrastructure [50].

Photobioreactors are specialized systems that cultivate microorganisms, particularly microalgae, under controlled environmental conditions. They are enclosed systems that meticulously regulate environmental parameters such as light intensity, temperature, pH, and nutrient availability [51]. These reactors can be categorized into two main types: aerobic and anaerobic [52]. Aerobic photobioreactors provide a controlled environment with an adequate oxygen supply to support aerobic metabolism. They are equipped with mechanisms to ensure sufficient aeration, which is crucial for the growth of oxygen-dependent microorganisms like microalgae [53][54]. Oxygen is continuously supplied to the culture to meet the metabolic demands of the microorganisms. This is typically achieved through the introduction of air or pure oxygen. The circulation of the culture ensures that oxygen is distributed evenly, promoting healthy cell growth [54]. It enables the cultivation of oxygen-dependent microorganisms and facilitates higher growth rates and biomass production due to the ample oxygen supply [53]. Unlike aerobic photobioreactors, anaerobic systems operate in the absence of oxygen. These reactors create an environment conducive to the growth of anaerobic microorganisms [55]. This is achieved by carefully controlling the ingress of air and by utilizing specialized equipment that prevents oxygen from entering the system [56]. This environment supports the growth of anaerobic microorganisms, which can thrive in the absence of oxygen, and those that may have unique properties or produce specific products.

Aerobic and anaerobic photobioreactors are crucial in various fields, including biotechnology, environmental science, and bioenergy production. They come in various designs, such as tubular, flat-panel, or bubble-column reactors, while open ponds are large, shallow basins or raceways where microalgae are cultivated under natural sunlight [57]. The choice between the two types depends on the specific microorganisms being cultured and the intended applications [56]. Some systems are designed to be versatile, allowing for aerobic and anaerobic conditions to be achieved as needed. These reactors are valuable tools in research and industry for exploring and harnessing the potential of microorganisms, particularly microalgae, for various applications [53].

Nutrient availability is crucial for microalgae growth, with essential nutrients like nitrogen, phosphorus, and trace elements being provided from sources such as synthetic fertilizers, wastewater, or agricultural runoff [58]. The nutrient concentration and ratio in the growth medium are pivotal factors influencing microalgae growth and biomass production [59]. Sufficient light exposure is essential for photosynthesis [60]. Light exposure's intensity and duration directly impact microalgae's growth rate and lipid content. The ideal light conditions differ based on the microalgal species and can be regulated using artificial lighting in indoor cultivation systems [61]. Microalgae thrive within specific temperature and pH ranges, varying depending on the species. Maintaining suitable temperature and pH levels is crucial for maximizing microalgal growth and productivity [62]. According to the study by Song et al. [63], a significant issue arises with generating a strong, unpleasant odor during the microalgae cultivation process in wastewater. As the cultivation period extends, the microalgae tend to adhere to the walls of the apparatus, thereby impeding light penetration and adversely affecting the photosynthetic activity of the algae cells. It is imperative to address both the malodor concern and the adhesion of algal cells. Given that various microalgae respond differently to distinct conditions, optimizing growth parameters that are easily modifiable becomes crucial to enhance production efficiency.

Strain selection involves considering factors like biomass productivity, nutrient requirements, and environmental stress tolerance [47]. Strains like *Chlorella vulgaris* and *Spirulina platensis* are known for high biomass productivity and nutrient content, making them suitable for biochar production [64]. Other strains, like *Nannochloropsis* sp. and *Scenedesmus* sp., are valued for their high lipid content and potential for biofuel production [65].

Cultivation optimization is pivotal for maximizing biomass productivity and quality [66]. Light intensity, temperature, pH, and nutrient availability impact microalgal growth and biomass production. For instance, optimizing light conditions and nutrient availability can significantly enhance biomass productivity [67]. Utilizing photobioreactors and closed cultivation systems offers better control over environmental factors, further improving biomass productivity [51]. Recent research has shown that, with a few exceptions, the most favorable temperature range for the growth of the majority of algae species lies between 15 °C and 35 °C. An ideal light intensity typically falls between 1850 and 14,800 lux for the optimal proliferation of microalgal species. While some algae species can withstand both acidic and basic environments, the majority thrive within a pH range of 7.0–9.0.

Furthermore, the availability of essential nutrients in the medium significantly influences the development of microalgae [68]. Effective harvesting methods like centrifugation, flocculation, and filtration are employed to separate microalgal biomass from the growth medium. The resulting biomass can then undergo processing techniques such as pyrolysis, hydrothermal carbonization, or torrefaction to yield microalgae-based biochar [69].

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