Cultivation process of microalgae using wastewater for biodiesel production and wastewater treatment: a review

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Abstract. Combining microalgae cultivation with nutrient removal is a promising technique as it enables renewable energy generation with the additional potential removal of wastewater contaminants in a single process. Performance and total yield of this process are still below the standard for industrialization. Thus, optimization is needed to reach the feasibility and actualize the concept. Cultivation conditions and reactor design play essential roles in the application and feasibility of this process. Both aspects have been developed through the years to enable the industrial application of this concept. Cultivation conditions are usually categorized into trophic conditions in which each situation has its specific function and target of removal. These conditions, however, are also applied in various reactor systems. Closed photobioreactor and open pond are two central systems for the reactor. Two of the most applied reactor models in wastewater are reviewed here to create a broad picture of the algae cultivation process by emphasizing biomass production and considering different aspects.

1. Introduction

Microalgae culture is considered as the future generation of biofuel source with many additional advantages. Among the advantages, nutrient removal and carbon sequestration are on top of the priority list; hence, this technology's benefit in overcoming environmental issues is very favorable [1-3]. Lately, more significant scale applications with numerous technologies vary the possibility of applying many wastewater sources and characteristics.

The microalgae cultivation process with a specific bioreactor design shows essential roles in the application and feasibility of coupling biomass generation with a wastewater treatment system [4]. Among factors that determine the coupling feasibility, light penetration and agitation process are commonly mentioned in this system. Both of operational parameters are mostly affected by the design of the reactor in which the generation of algae biomass is conducted [5]. The agitation and light penetration are essential to ensure high biomass productivity and wastewater recovery [4,6]. Similarly, the trophic condition must count as the first consideration since algae can cope with many carbon and energy, including the one in the system of wastewater treatments [7].

Nonetheless, many wastewater applications as sources of nutrients for microalgal growth failed to reach high biomass yield. Some of the applications focused on the strains and co-cultivation microorganisms while the operational conditions were less considered. Failure to identify and construct
the proper system and shape of microalgal cultivation can lead to the low removal of nutrients with low biomass yield.

Understanding cultivation conditions leads to the feasible decision by which the desired nutrient and contaminant removal from the wastewater and sufficient biomass production can be achieved [4]. In this review, the culture's trophic conditions are separately discussed and compared to each other based on wastewater nutrition. Furthermore, the development of the cultivation process in terms of reactor design that focus on the benefit and drawback were discussed for better treatment and biomass harvesting.

2. Microalgae cultivation

Microalgae have several cultivation conditions regarding the source of energy and carbon [7]. Energy and carbon sources are essential factors for synthesizing the biomass and supporting other algae's metabolic activities [8]. Phototrophic, heterotrophic, mixotrophic, and photoheterotrophic are algae conditions to grow based on algae carbon and energy source [9]. The cultivation condition is vital to achieving high removal efficiency in wastewater and biomass production [10].

2.1. Phototrophic cultivation

Most of the microalgae are photoautotrophic by which characteristic the algae can produce energy using renewable and inexpensive resources as sunlight, inorganic salts, water, and CO₂ [11]. Some showed a higher growth rate under phototrophic cultivation than other conditions such as Chlorella sp. and Scenedesmus sp. [12]. Cultivation by phototrophic could also consume a significant amount of heavy metals [13]. When the photoautotrophic algae conduct the photosynthesis process, most of the nutrients for this process are required in inorganic forms [14,15]. Thus, the application of sole photoautotroph is emphasized on the secondary treatment effluent as it has been done by [16] using wastewater treatment effluent of food wastewater. Usually, this application has co-culture with other microbial for organic contaminants removal [14,17].

2.2. Heterotrophic cultivation

Heterotrophic cultivation is a term used when microalgae use organic carbon for energy and carbon sources [18]. The advantage of heterotrophic algae culture is a fast growth, high production rate, and convenient harvesting. The heterotrophic of microalgae cultivation had shown better biomass and metabolites productivities than the phototrophic mode 25-fold [19]. Heterotrophic cultivation on aquaculture wastewater, organic waste, and municipal wastewater could act as a nutrient substrate to cultivate microalgae [20]. The biorefinery concept of integration of aquaculture and the microalgae industry can be applied in the future [21].

2.3. Mixotrophic cultivation

Mixotrophy means growth whereby organic carbon is simultaneously assimilated with light and CO₂. The energy yields of mixotrophic cultures were 4–10 times higher than the photoautotrophic cultures because it combines the advantages of autotrophic and heterotrophic [20,22]. Genus of Chlorella and Isochrysis were reported to grow successfully in mixotrophic than heterotrophic conditions [23,24]. Removal of some nutrients such as phosphate, on the other hand, is affected by this condition [25]. Another concern to apply this is that mixotrophic is sensitive to the light intensity change [26]. Here, most of the wastewaters with a combination of organic and inorganic components can treat in this condition.

2.4. Photoheterotrophic cultivation

Organisms that use light for energy but cannot use CO₂ as a carbon source is called photoheterotrophs. As a result, they use organic compounds from the environment as carbon sources, including carbohydrates and fatty acids. One of the studies in this condition was C. Vulgaris ESP-31, and Ettlia texensis were found to have this kind of trophic state [27,28]. Poultry Litter, Industrial, agricultural, and
other wastes are often used as the substrates of this bioenergy [29-31]. For solid waste, the target is substrate fermentation for further bioenergy production [32].

3. Reactors
Apart from cultivation, reactor design and concepts play an essential role in the successful cultivation and nutrient removal in the thi cultivation process. Generally, the two most common reactors are Open pond reactor and Photobioreactor (PBR) systems. Both reactors are designed for specific purposes with advantages and disadvantages (table 5).

| Parameters                  | Open Pond                                                                 | PBR                                                                 |
|-----------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------|
| Location                    | Location is crucial since it affects temperature, medium procurement cost,  | Abiotic factors such as temperature still play an important role in  |
|                             | transportation, rainfall precipitation [33]                               | outdoor cultivation but are less affected. However, climate affected |
| Dimension                   | Depth is the most crucial dimension scale to ease the light penetration,  | Based on the mixing and agitation process, mostly cylinder shape is  |
|                             | agitation, and mixing concerning power consumption [33]                  | preferable since it has complete circulation [33]                   |
| Power consumption           | Mainly to operate the paddles for circulation and agitation [34]         | Including paddling, aeration, light generation, and materials pumping, thus it consumes higher power [34] |
| Mixing rate                 | Crucial for even exposure to the ambient air and light [33]              | Shear mixing, in some cases, can cause damage in the cells. However, bubbling and paddle are preferably [33]. |
| CO₂ delivery and intake     | Important but not essential for cultivation since diffusive CO₂ is        | Injection of CO₂ is usually occupied in this system to provide carbon source to the system where the CO₂ virtually [36] |
|                             | supplementary for cultivation enhance the production [35]                | Moderate, some models occupy sunlight where the others use bulbs or illumination from other sources. However, light penetration in this model of the reactor can be adjusted [33] or combined [37] |
| Light                       | Formed from sunlight. Thus the shallow pond is favorable for optimum light penetration [33] | Less fluctuate and completely controlled since the evaporation is negligible; however, for some of the saline algae, a minor change could still affect the growth [39] |
| Salinity                    | Fluctuate due to high evaporation. Thus the fluctuation of salinity on   | Highly accumulated since the closed system makes and collect more O₂ at a higher rate [40] |
|                             | the growth of several species would be more significant in this model of  | closed photobioreactors have been proven to have a lower risk of contamination than open ponds. Secure system cause material exchange is only from the gasses injection and feeds water or medium yet the possibility to be contaminated still occur [43] |
|                             | the reactor [38]                                                         |                                                     |
| Oxygen accumulation         | Optional to remove since the low density of algae produces less oxygen  |                                                     |
|                             | that inhibitory in excess level [38]                                      |                                                     |
| Contamination               | Highly risk for contamination since the airflow can bring the contaminants as well as the predatory such as protozoa and other organisms [38,41], Yet some designs are almost closed to avoid massive contamination [42] |                                                     |

3.1. Open ponds
Open pond system refers to a pond in an open area with sunlight harvesting as the primary source of light using natural or additional agitation system from a mixture of the algae and the medium [44]. Open pond systems usually use phototrophic conditions since microalgae can harvest energy from the sunlight directly and yield inorganic carbon sources in the form of carbon dioxide [45,46]. The Raceway pond is often recognized as the most used model for open pond systems [38]. The natural depending factors thus become challenging factors that cannot be controlled in the open pond system. On the other hand,
advantages can come from a genuine factor such as light. A study of Dahmani et al. (2016) showed that the desert area has the potential of high productivity algae culture because of the abundance of sunlight in the optimum radiation range despite the temperature fluctuation [47].

3.2. Closed photobioreactors

The photobioreactor system is a closed system where all the components are closed and isolated from the environments. However, some of the structures of photobioreactors are still exposed. Thus, most of the operational parameters are controlled with specific agitation with-or-without additional light apart from the sun. In the situation where sunlight is the primary source of light, the reactor's geometry is usually flat and panel shape because the source of light only comes from one direction. However, the tubular constellation is still applicable when the conformation is adjusted to be in a single row with optimum expose to the sunlight [48]. Harvesting the light is vital in microalgal reactors with a certain amount and duration. Iluz and Abu-Ghosh (2016) have shown that direct periodical light with dim continuous dark light has a higher biomass yield due to the appropriate light distribution and increase of photon flux [49].

Conversely, González-Camejo and Viruela (2019) (found that optimum photon flux can increase the biomass [37]. It was also proven that the light distribution and regime are not related to the increase of algae's biomass. For the artificial light source, a lamp or other fluorescence tools could be placed either inside the reactor or outside with single exposure. The tube model is preferable since it can be modeled to be surrounded by the light source in the single tube [50,51] or helical shape where the tube cycle around the light [52-54].

4. Conclusion

To yield a high amount of biomass from algae using wastewater as the source of nutrients, the reactor's cultivation condition and design play a crucial production effect to determine the yield's final amount. Cultivation conditions with the head of carbon and energy for the biomass generation are crucially affected by water constituent composition. Moreover, the type of reactor and its operational requirements are crucial to obtain such a high yield with reasonable capital and operating costs. Many comprehensive studies and development of this topic have been conducted for many years. However, mass applications are still limited and exclusive for a particular geographical situation or high capital cost. Combining advantages features from closed photobioreactor and open pond, such as effective light utilization with less contamination risk, is needed for broader application of algae biomass production and wastewater reclamation.

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