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## Producing liquid transportation fuels from heterotrophic microalgae

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#### HIGHLIGHTS

- ► Challenges in cultivating heterotrophic microalgae in large scale are reviewed.
- ► Alternative carbon sources for growing heterotrophic microalgae are discussed.
- ► Thermochemical processes can be used to produce oils from algal biomass.

#### ARTICLE INFO

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#### ABSTRACT

Compared to autotrophic microalgae, heterotrophic microalgae have the potential for providing higher yield of biomass and lipids for biofuel production. But this cultivation mode does possess several challenges, among which cheap carbon sources, bioreactor design, and downstream processing are the major bottlenecks impeding large scale cultivations for producing biofuels in a cost-effective way. This paper reviews the most recent research and development in heterotrophic microalgae covering different carbon sources (wastewater, non-sugar materials, and lignocellulosic feedstocks), design of bioreactor, and production of liquid transportation fuels, in particular, biodiesel and bio-oils from algal biomass through biochemical or thermochemical pathways. Besides summarizing promising technologies currently available, this review also recommends future research directions that can really benefit the biofuel research community.

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#### 1. Introduction

Numerous research and review articles have focused on growing microalgae autotrophically or photosynthetically where  $CO_2$ and sun light serve as the carbon and energy sources for cell proliferation. This mode of cultivation does contribute to global  $CO_2$ reduction. But, it is difficult to reach a high density of microalgae biomass due to: (1) light penetration is inversely proportional to cell concentration [1] and (2) mutual shading of cells can cause light insufficiency, which leads to a very low algal biomass and hence very low yield of products [2]. Low biomass concentration also increases the biomass harvesting cost [3,4]. As a result, producing biofuels from autotrophically-grown algal cells will need a long development time and huge investments before it becomes commercially viable. As such, for example, an insignificant amount of biodiesel is currently being made from microalgae grown under autotrophic conditions.

To abundantly produce valuable products from microalgae, in particular, biofuels, microalgae must be cultured in a heterotrophic mode where organic carbons, such as sugars or organic acids, serve as carbon and energy sources. This culture condition eliminates the requirement for light and therefore, offers the possibility of greatly increased cell density and productivity. However, impediments to commercial scale culture of heterotrophic microalgae are still economic. To overcome the cost hurdle and to make biofuels from microalgae economically feasible, at least three areas need to be explored: (1) finding of low- or zero-value carbon sources to support heterotrophic microalgal growth; (2) design of bioreactors appropriate for industrial scale heterotrophic cultivation; and (3) identification of suitable pathways for converting algal biomass to biofuels.

During recent years, rapid progresses have been made on research and development regarding the three aforementioned strategies. This review paper aims to: (1) provide up-to-date information related to heterotrophic microalgal cultivation; (2) reveal the most promising directions for future research and development; and (3) identify problems that still need to be resolved.

#### 2. Carbon and energy sources for heterotrophic microalgae

#### 2.1. Wastewater

Except certain types of industrial wastewater, most domestic wastewater contains organic carbon, nitrogen, phosphorous, and other minor compounds. This composition makes wastewater suitable for growing microalgae. Besides growing algal biomass for biofuel use, the wastewater can be treated simultaneously. Thus, the double benefits have attracted extensive attention over the years. But this approach does have its drawbacks: (1) some wastewater may be too toxic to support algal growth; (2) due to the outdoor nature, algal growth and wastewater treatment efficiency can be significantly affected by seasonal alterations; and (3) competition among the microbial community in the wastewater may make algal growth very slow. Hence, using microalgae to treat wastewater while expecting a high biomass productivity can be problematic.

To overcome these problems, several research groups have attempted to screen microalgal species that can be dedicated for this dual-process. One example is to select algal strains that are facultative heterotrophic, adaptable to northern climate, able to consume organic carbon, nitrogen, and phosphorous in wastewater, and capable of high yield of biomass and lipid [5]. From five types of water bodies, such as lakes, rivers, creeks, ponds, and wastewater in Minnesota, USA, water samples were collected. Following multi-step purification and isolation, five top strains out of 17 that were tolerant to concentrated municipal wastewater (CMW) were further evaluated. Compared with results from other algal strains that grew on various wastewater [6,7], the biomass productivity between 231 and 275 mg/l-day and lipid productivity between 74.5 and 77.8 mg/l-day was impressive. However, the algal growth could only be sustained for 3 days due to rapid consumption of organic carbon. For nitrogen and phosphorus, however, the utilization rates were slower. After 3 days, concentrations of these two were still high. Thus, though it may be possible to use the top strains to achieve two purposes: wastewater treatment and lipid production, there are three potential problems. First, the treatment system needs to be optimized to accomplish the goal of removing nitrogen and phosphorus completely from CMW. Second, the studied strains were evaluated individually in a controlled laboratory environment. Whether these strains can out-compete those originally in wastewater and still accumulate lipids are unknown. Third, if these strains are ever applied for wastewater treatment in an outdoor environment, how they perform during winter or how this performance will affect the treatment efficiency still awaits further evaluation.

Instead of using isolated algal strains for wastewater treatment and lipid production as described above, a culture of mixed algae was tested for the same purpose [8]. This culture from a lake was used to treat domestic wastewater either with no nutrient addition or with addition of glucose, nitrogen, phosphorus, carbon plus nitrogen, carbon plus phosphorus, carbon plus nitrogen plus phosphorus, nitrogen plus phosphorus, or nitrogen plus phosphorus plus potassium. The total growth was divided into two stages: growth phase (GP) and starvation phase (SP). At the end of 8-day GP, the biomass concentration was 0.98 g/l for the control sample without any nutrient supplementation. Among samples supplemented with different nutrient, phosphorus addition only resulted in the maximum cell density of 1.64 g/l. At the end of 8-day SP, the highest lipid content of 28.2% was observed for sample with glucose addition. Removal efficiencies for COD, nitrogen, and phosphorus were 18.3%, 54.2%, and 32.8%, respectively. Maximum removal of COD, nitrogen and phosphorus took place in samples supplemented with glucose and nitrogen (98.4%), with nitrogen (66.6%), and with phosphorus (65.2%), respectively.

Percentage wise, nitrogen and phosphorus addition seemed to result in higher removal efficiencies of these two. But, a further calculation of the final nitrogen and phosphorus concentration in the sample showed contradictory results. For example, based on nitrogen concentration in the original wastewater (115 mg/l) and the nitrogen removal efficiency of 54.2%, the final nitrogen concentration in the control sample was 72.7 mg/l. For samples supplemented with 500 mg/l of NaNO<sub>3</sub>, considering the removal rate of 66.6%, the final nitrogen concentration was 205.4 mg/l which was much higher than the original nitrogen concentration. The same conclusion can be drawn for phosphorus. Thus, addition of extra nutrient for the purpose of increasing biomass production led to a wastewater even worse than the original one. In addition, this study only revealed the cellular lipid contents after the SP phase. Since the common knowledge is that during SP phase, the cell den-

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