Energy Conversion and Management 171 (2018) 1416–1429

Contents lists available at ScienceDirect

Energy Conversion and Management

journal homepage: www.elsevier.com/locate/enconman

Review

Harvesting and pre-treatment of microalgae cultivated in wastewater for biodiesel production: A review

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ARTICLE INFO

Keywords:
Microalgae
Biodiesel
Wastewater
Pre-treatment
Lipid

ABSTRACT

Cultivation of microalgae using wastewater has received considerable attention around the globe as a platform to remove inorganic nutrients from the wastewater and producing microalgae biomass for biodiesel production. This can be done by converting freely available nutrients from the wastewater (particularly nitrogen, N and phosphorus, P) into microalgae biomass with concomitant carbon dioxide (CO_2) sequestration via photosynthesis process. In fact, microalgae biodiesel could help to reduce greenhouse gas (GHG) emissions to the atmosphere through the replacement of fossil diesel as it is made from renewable resources. Despite the various benefits to produce microalgae biodiesel, a number of technical hurdles such as complex processes, requirement of high energy input during cultivation and dewatering (harvesting steps) need to be addressed for commercialization purpose. Besides, additional pre-treatment step is necessary to disrupt the microalgae cell wall to enhance lipid extraction efficiency. Hence, this paper is aims to reveal in-depth analysis and discussions on the process of microalgae-based wastewater treatment as well as microalgae cells disruption technology for lipid extraction. The paper also includes several new directions for technological improvements to improve commercialization potential of microalgae biodiesel.

1. Introduction

As urbanization and industrial activities have spread around the globe, water pollution has become a growing concern over the last century. Nearly 71% of the Earth's surface is covered by water and currently they are being polluted by human activities as more waste are being disposed into rivers and coasts without any prior treatment [1]. Once water is contaminated, it is unsafe for human and animal consumption due to toxic or harmful compounds that are contained in the water [1]. Eventually, this causes water-related diseases to occur, which responsible for 80% of all illnesses and deaths in developing countries. Hence, proper wastewater treatment need to be intensively encouraged to reduce the contaminants to acceptable levels or meeting the recommended microbiological and chemical quality before it is being discharged to water bodies. Wastewater treatment is one of the most important environmental conservation processes to improve the quality of the discharged wastewater by making it more appropriate for reuse and to prevent water pollution.

Currently, coupling microalgae-based treatment processes focuses on the production of microalgae biomass and the removal of inorganic nutrients from various type of wastewaters have been studied widely as it is considered to be a feasible method for saving large amount of nutrients and water required for microalgae cultivation. Oswald and Golueke proposed to conduct large-scale production of microalgae biomass by using existing wastewater treatment ponds for treatment of municipal, agriculture and industrial wastewaters [2–4]. Also, several studies have been performed to evaluate the applicability of high rate algal ponds (HRAPs) systems in wastewater treatment process [2,3]. The microalgae have the capability to remove inorganic nutrient in symbiosis with heterotrophic bacteria, in which bacteria degrade organic matter by consuming oxygen (O_2) generated by microalgae through photosynthesis process (Fig. 1) [5].

Nowadays, the search for sustainable and renewable fuels is increasingly important due to depletion of fossil fuels, progressing climate change confronted the world's population and also development in various industrial activities globally. Hence, renewable liquid biofuels

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https://doi.org/10.1016/j.enconman.2018.06.074
Received 23 March 2018; Received in revised form 26 May 2018; Accepted 20 June 2018

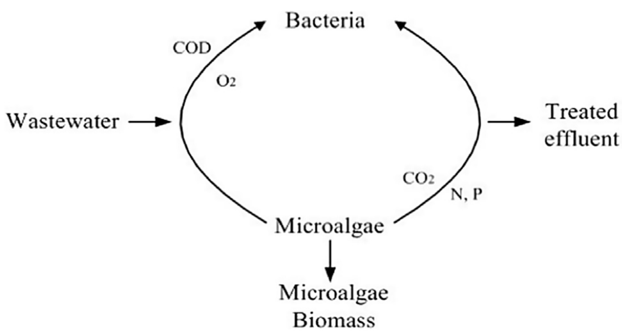

Fig. 1. Symbiosis between microalgae and bacteria in HRAP for wastewater treatment [5].

(e.g. biodiesel, bioethanol, biobutanol and bio-oil) have received considerable high attention with growing concerns surrounding the heavy dependence of fossil fuels as well as to meet the global energy demand. Biodiesel has attracted public attention in many European countries, particularly for the transportation application as it has close similarity with conventional fossil diesel in terms of physical properties, energy content and chemical structure [6]. Apart from that, biodiesel has been considered as a clean burning fuel with low pour point and viscosity, nontoxic, biodegradable and eco-friendly due to its relatively low emissions of sulphur dioxide (SO_2) [7]. Biodiesel has been commercially blended with petro-diesel in all ratios and can be used in any diesel engine without modification.

Microalgae are currently considered as a promising feedstock for biodiesel production due to their GHG fixation ability, rapid growth rate and high production rate of lipid [8]. Besides, the biodiesel production from microalgae is expected to be 15 to 300 times higher compared to conventional crop plants which are usually harvested once or twice a year while microalgae possess a very short harvesting cycle (around 12 days, depending on the species and cultivation method) which allows continuous harvesting throughout the year. Despite the various benefits associated with the production of biodiesel using microalgae, economic feasibility for large-scale production of microalgae biodiesel is yet to be realized as it faces a number of technical hurdles, such as complex processes, requirement of high production costs and energy input [9].

Therefore, a number of researches have been carried out to develop cost-effective technologies for economically viable microalgae biodiesel production by performing bioprospecting of high-lipid containing strains as well as by inducing higher lipid production through various methods [10]. On the other hand, one of the major obstacles for the development of microalgae-based wastewater treatment system is the dewatering step (harvesting process) of the biomass. This is because, harvesting process usually accounted to more than 30% of the total production cost and varies based on the type of harvesting technology used and the characteristics of the microalgae chosen viz. the size and density, as well as the specifications of the desired product [11,12]. In addition, effective harvesting process plays an important role in microalgae-based biodiesel production for recovery of high quantity biomass as well as to enable recycle use of nutrients. For wastewater treatment pond system that does not harvest the microalgae, the microalgae are remained in the pond and decompose on the pond floor. As a result, methane will be slowly released to the atmosphere as greenhouse gas if no proper capture and treatment system are constructed [13].

Lardon et al. [14] also stated that microalgae recovery is energy intensive when these processes were combined with lipid extraction as it may contributes to 90% of the total energy input required, which often resulted to negative energy balance in producing microalgae biodiesel at large scale. This is because microalgae possess a thick and rigid cell wall composed of glycoproteins and complex carbohydrates with high chemical resistance and mechanical strength. Thus, additional pre-treatment step is needed to disrupt the microalgae cell wall and to enhance the efficiency of lipid recovery.

Cell disruption and pretreatment are particularly important as it enhances the release of intracellular lipid embedded within the microalgae cells by improving the access of chemical solvent to fatty acids [15,16]. Besides, Dong et al. [17] reported that pretreatment is a necessary step to disrupt microalgae cell for subsequent biofuel production. Pretreatment of microalgae can be broadly divided into four (4) categories depending on the desired biomass alteration, namely: (i) thermal; (ii) mechanical; (iii) chemical and (iv) biological methods [18]. In addition, the effectiveness of pretreatment methods on microalgae-based biofuel production depends on cell's characteristics, such as the structure and toughness of the cell wall, and also the macromolecular composition of cells [5]. Apparently, variety of cell disruption methods have been evaluated and new approaches are being explored in parallel. It should be noted that cell disruption technologies need to be energy efficient and cost effective in order to attain high product quality. Hence, this paper is aims to give an in-depth analysis and discussion on the current trend of pretreatment technology on wastewater treatment as well as disruption of microalgae cells for lipid extraction. The paper also includes several new directions for technological improvements that will enable the commercialization of microalgae-base biofuels.

2. Microalgae and wastewater

Microalgae have been proposed for a long time as a potential renewable fuel source due to its potential to generate significant quantities of biomass and lipid. The triacylglycerol (TAG) lipid which can be separated and isolated from harvested microalgae biomass is suitable for conversion to biodiesel (fatty acids methyl esters, FAME) via transesterification reaction [19]. In fact, microalgae biodiesel is predicted to have the potential to reduce GHG emissions through the replacement of fossil diesel. In addition, the growth rate of microalgae is extremely fast as compared to other terrestrial plants such as corn or soybean, which indicated higher biomass productivity with less land area requirement for cultivation (Table 1) [20]. Besides, the use of microalgae for biodiesel production has attracted a significant interest from researchers as the lipid contents in several microalgae species can produce approximately 80% by weight of dry biomass under stress conditions [21]. Lipid content in microalgae can reach 75% by weight of dry biomass but associated with slower growth rate compare to microalgae which have low content of lipid (e.g. for *Dunaliella salina*) as shown in Table 2 [12]. *Chlorella protothecoides, Chlorella vulgaris, Nannochloropsis* sp., and *Neochloris oleabundans* are identified as potential candidates for biodiesel production due to their high lipid content and high lipid productivity (Table 2) [7]. Even though these facts have been a driving force in commercialization of microalgae biodiesel, large scale of microalgae biomass production is not economically feasible due to high production cost.

Therefore, a number of researches have been carried out to reduce the production cost of microalgae by implementing dual application of microalgae for phytoremediation and biomass production. This can be done by converting freely available nutrient from wastewater (particularly nitrogen, N and phosphate, P) into microalgae biomass with concomitant carbon dioxide (CO_2) sequestration via photosynthesis process [24,25]. Besides, most of the microalgae species are able to grow under nutrient-rich environments by absorbing the nutrients and metal from the wastewater, which make them an extremely attractive means for sustainable and low-cost wastewater treatment [26–28]. However, the N:P ratio in wastewater should be in an optimum range as it plays an important role for successful microalgae cultivation [29]. Green microalgae especially *Chlorella* are ideally suited to play a dual role of treating wastewater and biomass production by converting wastewater nutrients to biomass and lipid [30]. The release of