Energy Conversion and Management 164 (2018) 188–197

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation

Wai Yan Cheah[a,b], Pau Loke Show[c], Joon Ching Juan[d,e], Jo-Shu Chang[f,g], Tau Chuan Ling[a,*]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Department of Environmental Health, Faculty of Health & Sport Sciences, MAHSA University, 42610 Jenjarom, Selangor, Malaysia
[c] Bioseparation Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia
[d] Nanotechnology & Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia
[e] School of Science, Monash University, Sunway Campus, 46150 Sunway, Malaysia
[f] Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[g] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan

ARTICLE INFO

Keywords:
Microalgae
Mixotrophy
Pollutants remediation
Supplementation
Wastewater

ABSTRACT

Microalgae are a promising feedstock for biofuel generation. Economical and effective mass cultivation is essential for greater feasibility in microalgal-based biofuel full applications. The present study reported on cultivation of *Chlorella sorokiniana* CY-1 in palm oil mill effluent (POME) under photoautotrophic and mixotrophic cultivation. Enhancement of biomass and lipid productions were carried out by using glucose, urea and glycerol supplementations. Mixotrophic cultivation was more effective than photoautotrophic condition. Glycerol addition exhibited greater microalgae growth performance compared to supplementing glucose or urea. Biomass (1.68 g L^{-1}) and lipid (15.07%) production were highest in POME medium with combinations of 200 mg L^{-1} urea, glucose and glycerol supplementation. *Chlorella sorokiniana* CY-1 grown in POME with glucose and glycerol supplementation gave considerably comparable yields as in all supplements-added POME medium. Ideal fatty acids compositions shown in urea and glycerol supplemented-POME medium though lower biomass production obtained. The pollutant remediation efficiencies attained were 63.85% COD, 91.54% TN and 83.25% TP in all supplements-added medium. The estimated net energy ratio was 0.55 and nutrient cost could be reduced up to 76%. Cheap and effective carbon and nutrients supplementation is essential to minimize the economic impact and maximize yields in commercial scale microalgae cultivation for biofuel production and environmental sustainability.

1. Introduction

Microalgae are a promising feedstock for biofuel generation due to the increase in world population and therefore energy consumption [1,2]. Economical and mass scale microalgae cultivation is still in discussion aiming to improve biomass and lipid productions, subsequently allowing microalgal-based biofuels scale-up application [3]. The major challenge hindered towards effective application would be the cost incurred in its upstream cultivation and downstream processing [4]. Economic viability and commercial feasibility are urgently required to be improved [5,6]. Effort could be made to maximize biomass and lipid productions, using efficient and cost effective input to compensate the cultivation cost. Today, microalgae are cultured using photoautotrophic, mixotrophic and heterotrophic modes. Photoautotrophic cultivation rely on light energy for microalgae to perform photosynthesis, whereas heterotrophic cultivation uses organic carbons and nutrients for microalgae assimilation in the absence of light. Mixotrophic cultivation involves both autotrophic and heterotrophic cultivation conditions [7–9]. Photoautotrophic cultivation is most commonly used as it is simple and economical for outdoor cultivation [10]. Autotrophic cultivation is the only method which is technically and economically viable for commercial scale production [11]. However, the biomass growth is lower due to shading effects with insufficient light penetration through medium [10–12]. This is as well less feasible when applying photoautotrophic cultivation in wastewater treatment operation, as large volume of wastewater could delimit light penetration, especially dealing with dark color wastewater [7,13]. Heterotrophic cultivation can be performed in a simple bioreactor which could easily govern all cultivation parameters, at simple, low cost and allow large volume application for biomass and biofuel productions [8].

* Corresponding author.
E-mail address: tcling@um.edu.my (T.C. Ling).

https://doi.org/10.1016/j.enconman.2018.02.094
Received 27 September 2017; Received in revised form 26 February 2018; Accepted 27 February 2018

Light-independent heterotrophic cultures could reduce production cost compared to photoautotrophic culture, by enhancing biomass productivity significantly. *Chlorella protothecoides* exerted 55.2% and 15% of lipid content when cultivated under heterotrophic and autotrophic conditions, respectively [10]. *Monoraphidium* sp. exhibited 37.56% lipid content under heterotrophic condition [14]. *Chlorella protothecoides* shown 40% increase in lipid content after shifting photoautotrophic to heterotrophic cultivation [15]. Yet, the limitations of heterotrophic cultivation include species-specific as not all microalgae are grown heterotrophically, cultures are more susceptible to bacterial contamination due to organics additions into the medium, competition occurred between microalgae and bacteria for organics assimilation; and increase in expense due to organics and nutrients supplementation [16]. Mixotrophic cultivation system has been tested on its positive effects on microalgae species like *Chlorella* and *Botryococcus* species [7,12]. It assimilates organic carbons, nutrients and CO_2 simultaneously, performing both respiration and photosynthesis at the same time [8,17]. This lessen the drawbacks of photoautotrophic and heterotrophic cultivation, whereby mixotrophic cultivation can be performed in large volume pond, less dependency on light energy, allowing it to be performed even at locations without whole year adequate sunlight supply and non-species selective [16]. The growth of *Spirulina plantensis* and *C. reinhardtii* were improved by glucose supplementation through light and dark stages, indicated less dependency on light supply [11]. The organic carbon and nutrients supplementation which are commonly applied at present are glucose, xylose, acetate, urea and waste products [18,19]. Recently mentioned supplements involved xylose and carbohydrates from sugarcane bagasse pretreatment and hydrolysis, glucose from sugarcane juice, corn powder hydrolysate, nutrients derived from stillage, molasses, and industrial wastes, etc [9,15,20,21].Simple sugar, glucose is the most preferred carbon source used for microalgae growth [18]. It provides more energy content than other carbon source which is approximately 2.8 kJ mol^{-1} compared to 0.8 kJ mol^{-1} for acetate [16]. Glucose was found positive in increasing the cells size of *Chlorella vulgaris*, as well as enhancement in starch and lipid accumulations. Microalgae species are able to assimilate 85% of glucose, to form oligosaccharides and polysaccharides [16]. Glycerol is the economic carbon source which gave positive effects towards growth of *Phaeodactylum tricomutum*, *Nannochloropsis* sp., *Rhodomonas reticulate* and *Cyclotella cryptica* [16]. It is the waste product of biodiesel transesterification, could also serve as good carbon source for mixotrophic cultivation. Nitrogen source is essential towards microalgae growth and lipid regulation [14]. Nitrate and urea were found effective to improve growth and lipid accumulation of *Chlorella* sp. than ammonia [12]. Excessive ammonia nitrogen inhibits cell growth [22]. Urea was found to be more feasible for large application because it is more economical. 1.5 g L^{-1} of urea addition in domestic wastewater produced 0.218 g L^{-1} of biomass with 65% of lipid [12]. Mass cultivation of microalgae requires huge amount of nutrients. Wastewater could serve as the medium to provide organic carbons and nutrients for microalgae assimilation. Wastewater containing high organics, nitrogen and phosphorus sources shown high potential towards large scale microalgae cultivation and pollutants remediation [23–26]. Wastewater from pulp-paper industry is rich in xylose, could be used as cheap carbon source for microalgae cultivation and simultaneously microalgal wastewater treatment [17]. Microalgae strains were used to remediate piggery effluent due its simplicity to remove nutrients in single treatment step [27]. In our previous work, we have applied zero cost wastewater, palm oil mill effluent (POME) as culture medium for microalgae cultivation and wastewater remediation. Apart from using wastewater for nutrients and organic carbon supply, to further optimize the biomass and lipid production, supplements application is often required. Nevertheless, supplementation could impact the cost of medium, as it claimed to be constituting about 35% to 80% of medium cost depending on choice of nutrient source [17]. Cultivation using supplementations though could often be expensive but it can be justified for better growth [20]. Finding the cheap organic carbon or nutrients is essential to minimize the economic impact and maximize yields [28]. The supplements must be effective in maximizing yields, so as to enhance the feasibility of commercial scale microalgae cultivation for biofuel production. Till date, there are only little literature reported on effects of supplements addition to wastewater cultivation medium for microalgae biomass and lipid production, especially dealing with POME [12,29–32].Therefore, in the present study, the microalga *Chlorella sorokiniana* CY-1 was cultivated in POME under photoautotrophic and mixotrophic cultivation. Three types of supplements (glucose, urea and glycerol) were added into the cultivation medium at varied concentrations. To further enhance the biomass growth and lipid production of *Chlorella sorokiniana* CY-1, the best concentrations of each supplement were combined into cultivation medium to identify its effects towards biomass and lipid productivities. This provides information on the selection on prime supplement and the less significant ones could be eliminated. The information is important to support the economic microalgae cultivation in commercial scale, specifically POME wastewater. The pollutants remediation in POME, energy balance and cost reduction were being evaluated and discussed.

2. Materials and methods

2.1. Palm oil mill effluent (POME) and experimental conditions

POME was taken from Seri Ulu Langat Palm Oil Mill Malaysia. It was filtered using 0.45 μm membrane to remove suspended solids and bacteria. The culture medium was diluted to 30% (v/v) POME using distilled water and sterilised at 121 °C for 20 min. pH was adjusted to about pH 7.5 using NaOH. In our previous study, we have studied the optimal POME concentration to cultivate *Chlorella sorokiniana* CY-1 for biomass and lipid productions. The concentrations studied ranging from 5% (v/v) to 100% (v/v) POME. The best concentration selected was 30% (v/v) (data not shown). In the first part of this study, the *Chlorella sorokiniana* CY-1 was cultivated in the cultivation medium (mixotrophic mode) supplemented with glucose at concentrations of 100 mg L^{-1}, and compared with the cultivation medium (photoautotrophic mode) which was without any supplement addition. Both medium were sparged with CO_2 and illuminated continuously. This was to justify the differences in microalgae growth cultivated using photoautotrophic and mixotrophic system. The second part of experiment involved supplementation of POME using glucose, urea and glycerol at concentrations of 200 mg L^{-1}, 500 mg L^{-1} and 1000 mg L^{-1}. The best concentration of the most effective supplement was selected for the subsequent study. The third part of the experiment involved combination of best concentration of the supplements into culture medium, so as to determine the effectiveness in enhancing biomass growth and lipid yield. This was carried out to justify the effectiveness of prime supplement and less significant supplement can be eliminated.

2.2. Microalgae strains selection and medium compositions

High lipid-yielding microalga species was used in this study, namely *Chlorella sorokiniana* CY-1. BG11 medium were used to preculture the microalgae strains for five days with continuous supply of 2.5% CO_2. The BG11 medium used was with compositions of (g L^{-1}): $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4{\cdot}7H_2O$, 0.075; $CaCl_2{\cdot}2H_2O$, 0.036; citric acid, 0.006; Na_2CO_3,0.02; Ammonium ferric citrate, 0.006; EDTA·2Na, 0.001; H_3BO_3, 0.00286; $ZnSO_4{\cdot}7H_2O$, 0.000222; $MnCl_2{\cdot}4H_2O$, 0.00181; $Na_2MoO_4{\cdot}2H_2O$, 0.00039; $CuSO_4{\cdot}5H_2O$, 0.000079; $Co(NO_3)_2{\cdot}6H_2O$, 0.000049. The initial pre-cultured microalga inoculated into the photobioreactor (PBR) was with inoculums sizes of approximately 100 mg L^{-1}.