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Co-cultivation of activated sludge and microalgae for the simultaneous enhancements of nitrogen-rich wastewater bioremediation and lipid production

Wai-Hong Leong[a,c], Jun-Wei Lim[a,c,*], Man-Kee Lam[b,c], Yoshimitsu Uemura[b,c], Chii-Dong Ho[d], Yeek-Chia Ho[e]

[a] Department of Fundamental and Applied Sciences, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia
[b] Department of Chemical Engineering, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia
[c] Centre for Biofuel and Biochemical Research, Institute for Sustainable Living, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia
[d] Department of Chemical and Materials Engineering, Tamkang University, Tamsui, New Taipei City 251, Taiwan
[e] Department of Civil and Environmental Engineering, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia

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ABSTRACT

The symbiotic relationships between activated sludge and microalgae were explored for bio-remediating nitrogen-rich wastewater and producing lipid-based biofuel simultaneously. Various inoculation ratios of activated sludge to microalgae (AS:MA) biomasses were employed to unveil the mechanism of NH_4^+-N removal in relation to lipid production from algal-bacterial biomass. The presence of nitrifiers in converting NH_4^+-N into oxidized nitrogen (NO_2^--N and NO_3^--N) had greatly enhanced the total nitrogen removal in the co-cultivation bioreactors as compared with the individual culture of either activated sludge or microalgae. Accordingly, achieving near complete nitrogen removal (97–98%) when the bioreactors were inoculated with AS:MA ratios of 1:0.75 and beyond. The kinetic growths of co-cultivated activated sludge and microalgae biomasses were also investigated using Verhulst model; demonstrating a significant increase of specific biomass growth rate with increasing of initial microalgae inoculation ratio. The AS:MA ratio of 1:0.75 was considered optimum for the symbiotic algal-bacterial interactions, attaining the highest lipid yield of 130 mg/L and flocculation efficiency of 42% which would aid the biomass harvesting process.

1. Introduction

Sustainable energy production and development have remained as one of the main challenges addressed by the current energy industry with an effort to reduce the over-reliance on fossil fuels as the primary source for generating energy in meeting the global demands [1,2]. To date, oil is recognized as the world's leading fuel which accounting for 32.9% of the global energy consumption in year 2015 or approximately 95,008 thousand barrels (15,105 m^3) were being consumed everyday [3]. Accordingly, studies into developing technologies for generating energy in a renewable and sustainable manner, commonly known as biofuels, should be continuously forged in satisfying the ever-increasing demands for energy especially the oil resource. The production of biofuel oils from the renewable feedstock has been constantly explored since the past decades. However, the challenges of using crop-based biofuels have dampened the extension of such technologies mainly due to the food versus fuel crisis, massive lands utilization for establishment as well as high production and processing costs in maintaining high oil yields for long-term employment [1]. Worth noting, a new generation of biofuels termed as the third generation derived from oleaginous microorganisms has being extensively investigated as the potential feedstock for sustainable biofuels production.

Microalgae feedstock have garnered a tremendous attention from researchers by virtue of its advantages in accumulating high cell lipid content up to 20–77% [4] coupled with its high photosynthetic capability which results in high growth rate tendency and biomass productivity as opposed to the terrestrial plants [5,6]. Other advantage of microalgae is that its biomass can be further extracted in gaining high commercial value components through various biorefinery processes. In this case, microalgae fix atmospheric carbon dioxide as its carbon source for the conversions

* Corresponding author at: Department of Fundamental and Applied Sciences, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia.
E-mail address: junwei.lim@utp.edu.my (J.-W. Lim).

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into various value added products such as proteins, lipids, bioethanol, bio-hydrogen, etc. [2]. However, the commercialization of algal-based biofuels is still questionable and uncertain due to the high input requirements from upstream and downstream processes which virtually fail to attract further capitalism and commercialized production. In an attempt to reduce the energy input requirements particularly the nutrient sourcing for microalgae cultivation, algal wastewater treatment was initially proposed by Oswald et al. [7], exploiting the potential of microalgae cultivation in wastewater medium. Since the wastewaters contain elevated levels of organic nutrients especially nitrogen and phosphorus elements, it serves as an available nutrient source for microalgae growth while also treating wastewaters in the same manner. As a consequence, cultivating of microalgae in wastewaters is judged to have economic and environmental prospects in producing biomass for biofuel industries while also biologically abating the organic nutrients in wastewater [8–10].

Another alternative of low-cost, non-food feedstock for biofuels production is the sewage sludge. Sewage sludge is often regarded as a major solid waste generated after the primary and secondary treatment processes in wastewater treatment plants. The merit of utilizing sewage sludge as a potential feedstock for biofuels production is that sewage treatment is deemed as a continuous process; thus, making this feedstock readily available and easily accessible [11]. In general, the sewage sludge contains considerable amount of lipids, up to 26% of dry weight depending on the types of sewage sludge used [12,13]. Activated sludge is mainly composed of bacterial consortium responsible for wastewater treatment. The bacteria utilize the organic and inorganic compounds present in wastewaters for growing and storing the synthesized lipids such as triglycerides, diglycerides, monoglycerides, phospholipids and free fatty acids [14]. However, the main setback frequently reported in numerous studies was only small quantity of lipid (< 10% of dry weight) that could be harvested from the activated sludge feedstock [11,13]. Besides, the removal of nitrogen nutrients, typically ammonium-nitrogen (NH_4^+-N), by the conventional activated sludge process is costly due to the need of biodegradable carbon source supplementation [15]. In the absence of carbon source, the nitrite-nitrogen (NO_2^--N) and nitrate-nitrogen (NO_3^--N) produced from the nitrification of NH_4^+-N will remain in the mixed liquor. In other words, the biological nitrogen removal is incomplete because of the absence of carbon source to trigger the denitrification process that otherwise, reducing the oxidized nitrogen (NO_2^--N and NO_3^--N) to nitrogen gas. The discharge of wastewaters still bearing oxidized nitrogen will give rise to the eutrophication, afflicting the quality of receiving water bodies [16].

The algal-bacterial co-cultivation had been reported as an alternative to the nitrogen-rich wastewaters bioremediation, tapping into the symbiotic relationship between algae and bacteria [17–19]. The algal-bacterial symbiosis is also often utilized in promoting the algal biomass growth and production from photobioreactor system [19,20]. The surge of biomass growth is attributed to the simultaneous exchange of organic and inorganic nutrients between the photosynthesis and respiration processes performed by the mixed algal and bacterial consortium [17,21]. Nevertheless, there are still much to be learned in understanding the characteristics of nitrogen removal while employing the symbiotic relationship of microalgae and bacteria to bio-remediate nitrogen-rich wastewaters. The population dynamics in the mixed algal-bacterial consortiums may very well influence the characteristics of nitrogen removal and unveiling these characteristics would enable the process of nitrogen removal to be more predictable. This would further ease the bioremediation of nitrogen-rich wastewaters in concert with harvesting of algal-bacterial biomass feedstock for biofuels production. The characteristics of nitrogen removal were investigated in terms of the mechanisms and kinetics whilst exposing the algal-bacterial biomass to nitrogen-rich wastewater in this study. Subsequently, the optimum initial inoculation ratio of algal-bacterial biomass was then evaluated, prompted by the best biomass and lipid yields attained. The outcomes of this reported work are anticipated to aid the secondary wastewater treatment plants employing the activated sludge process. The solid waste of algal-bacterial biomass generated from the plants can eventually be a promising lipid-based feedstock that facilitates the green conversion of waste to energy.

2. Methods

2.1. Source of wastewater

The domestic wastewater was sampled from the primary clarifier at a sewage treatment plant located in Seri Iskandar, Perak, Malaysia. The concentrations of NH_4^+-N, chemical oxygen demand (COD) and 5-day biochemical oxygen demand (BOD_5) were measured at 48, 145 and 124 mg/L, respectively, following the Standard Methods [22].

2.2. Cultivation of activated sludge and Chlorella vulgaris using domestic wastewater

Sample of activated sludge collected from the same sewage treatment plant was inoculated in a sequencing batch reactor (SBR) with a working volume of 18 L. The activated sludge was then acclimated to the domestic wastewater. The SBR was operated with a cycle time of 24 h via the following sequencing periods: instantaneous FILL, 0 h; aerobic REACT, 10 h; SETTLE, 1.5 h; DRAW, 1 h and IDLE, 11.5 h. During each cycle, 14 L of feed medium was introduced into the SBR and the same volume of treated effluent was siphoned off during the DRAW period. The excess sludge biomass was removed from SBR to maintain the sludge age at 40 days.

Unicellular green algae belonging to the *Chlorella* sp. have been known to be able to colonise ponds naturally coupled with its robust growth and capability to tolerate high nutrient loadings in sewage effluents [23,24]. Therefore, the freshwater microalga species, i.e., *Chlorella vulgaris*, was chosen for this study. The microalgae sample was acquired from the culture collections belonged to the Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS. The microalgae was cultivated in a 5-L bottle containing 4.5 L of the domestic wastewater, aerated with compressed air and illuminated continuously with cool-white fluorescent light at the light intensity of 60–70 µmol/(m^2 s) with initial pH being adjusted to 7.1 ± 0.1.

2.3. Setup of co-cultivation bioreactors

The acclimated activated sludge and microalgae cultures to the domestic wastewater were used for the co-cultivation studies. The bioreactors inoculated with only activated sludge and microalgae separately were indicated as Control 1 and Control 2, respectively. The co-cultivation bioreactors inoculated with activated sludge to microalgae (AS:MA) ratios of 1:0.25, 1:0.50, 1:0.75 and 1:1 were designated as BR-25, BR-50, BR-75 and BR-100, respectively. The initial inoculation concentrations of activated sludge and microalgae in every bioreactor are shown in Table 1. Then, a pre-determined volume ratio of the domestic wastewater to total working volume (v/v) and inoculation culture to total working volume (v/ v) were introduced into the Erlenmeyer flask with a total working volume of 1 L. All bioreactors were continuously aerated using compressed air of 2 L/min (maintaining dissolved oxygen and carbon dioxide concentrations at above 4 and 12 mg/L, respectively) and illuminated with cool-white fluorescent light at

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