Journal of Environmental Chemical Engineering 4 (2016) 3426–3432

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

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

Enhanced growth and nutrients removal efficiency of *Characium* sp. cultured in agricultural wastewater via acclimatized inoculum and effluent recycling

Azianabiha A Halip Khalid[a,b,*], Zahira Yaakob[a], Siti Rozaimah Sheikh Abdullah[a], Mohd Sobri Takriff[c]

[a] *Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment,Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia*
[b] *Faculty of Civil Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[c] *Research Center for Sustainable Process Technology, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 30 June 2016
Accepted 21 July 2016
Available online 22 July 2016

Keywords:
Microalgae growth rate
Acclimatization
Wastewater recycling
Nutrients removal
Palm Oil Mills Effluent (POME)
Characium sp

ABSTRACT

Effective wastewater treatment by microalgae depends on its ability to grow well in the wastewater. This is particularly challenging in concentrated agricultural wastewater such as Palm oil Mill Effluent (POME) where light is limited. This study assessed the effect of acclimatized inoculum in combination with POME dilution using final effluent discharge to improve microalgae growth. A native microalgae, *Characium* sp. that exhibited superior growth rate from preliminary study was used. Firstly, the effect of inoculum density and POME concentration on the maximum specific growth rate (μ_{max}) was investigated. From the result, the highest μ_{max} of 0.57 was achieved by 0.5 g L^{-1} inoculum in 50% POME concentration. In the second stage, analysis was continued using acclimatized inoculum cultured into 50% POME diluted with final effluent and distilled water respectively. Findings showed that dilution using final effluent produced remarkably higher μ_{max} of 1.87 compared to 1.06 when diluted with distilled water. It also successfully removed 21.5% of Chemical Oxygen Demand (COD), 80.0% of Total Nitrogen (TN) and 89.9% of Total Phosphorus (TP), within five (5) days of cultivation. Outcome of this study suggested that microalgae growth in wastewater can be stimulate, via feasible acclimatization and recycling of final effluent as dilution water. This improved growth will imply better performance in the bioremediation of the wastewater.

1. Introduction

Global demand for palm oil continues to expand sharply over the last few years. It is the most widely produced vegetable oil that has been used as the base in wide range of products. Although its primary use remains for food, palm oil is increasingly being used as feedstock in the production of biodiesel. Malaysia is the second largest producer of palm oil in the world, contributing more than 40 percent of world's palm oil production in 2014 [1]. Currently the fourth largest contributor to Malaysia's economy, the industry is forecasted to contribute up to RM 178 billion of gross national income by year 2020 [2]. While this situation is economically beneficial, the consequence of escalating production will inevitably degrade the environment due to large amount of wastewater being generated. For instant, 0.2 ton of crude palm oil produced from one ton fresh fruit bunches would discharge about 600–700 kg of highly polluting palm oil mill effluent (POME) [3]. This merit a huge concern as typical characteristic of POME contains high pollutants load such as Chemical oxygen Demand (COD), Nitrogen (N) and Phosphorus (P). Excessive N and P has resulted in eutrophication to the receiving water bodies [4]. For that reason, further treatment is required and potential solution is by using microalgae. Microalgae are able to naturally remove these remaining pollutants in POME by utilizing it as nutrient source.

Cultivation of microalgae in POME for subsequent nutrients removal or biomass production has been explored using few techniques and strains [5–7]. Researchers employed different

* Corresponding author at: Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.
E-mail addresses: azianabiha@gmail.com (A.A.H. Khalid), zahira@ukm.edu.my (Z. Yaakob).

http://dx.doi.org/10.1016/j.jece.2016.07.020
2213-3437/

cultivation approaches to find most promising result. Study by Rajkumara and Takriff [8] used outdoor raceway reactor using field relevant designs to increased nutrients removal efficiency in POME, whereas Vairappan and Yen [9] compared pollutants removal between outdoor and photo-bioreactor systems. Modification of factors such as POME initial pH, nutrient sources and retention time did also resulted in improvement of microalgae growth and nutrient removal capability in POME [10–12]. Besides, Hadiyanto and Soetrisnanto [13] reported on potential POME bioremediation by combining microalgae and aquatic plant in a range of culture heights and remediation time. While these efforts are important, another key factor is to primarily enhance the ability of microalgae to grow faster in POME. Majority of POME treatment are open pond system that vastly increased the risk for contamination. Thus, fast growing microalgae that is capable to outgrown any competitor is necessary for effective nutrients removal. In addition, rapid growth will also lessen the treatment period due to faster nutrient assimilation undertaken by microalgae. However, growth enhancement technique using acclimatized inoculum was limitedly explored in earlier POME researches. As highlighted by Osundeko et al. [14], acclimatization of microalgae to wastewater culture condition has exhibit better growth that resulted in more efficient nutrient removal. Report from Wahal [15] observed 4.8 fold increase of growth rates via adaption of microalgae strains to the wastewater effluent.

On the other hand, light availability in POME is also essential to support faster microalgae growth. Without optimum light penetration, most microalgae are unable to grow well resulted in reduced nutrients uptake from the wastewater. Therefore, dilution is required since POME has very low light penetration due to its dark color and high turbidity. Although previous studies demonstrated microalgae ability to thrive in POME, but mostly was conducted using POME diluted with either freshwater [4,16] or synthetic media [17–19] to achieve better growth rate. However this approach is undesirable for industrial scale application because it will incurs additional cost. Therefore, to ensure feasible application of microalgae in POME treatment, it is important to improve growth using light in its natural form. Otherwise, more economical and sustainable dilution method is necessary to satisfy the light requirement for proper microalgae growth in POME.

In view of these limitations, this study aims to enhance growth of microalgae by inoculum acclimatization. Physiological acclimatized inoculum will not undergo lag phase so C, N and P uptake will began almost instantly. To further improve the growth, light penetration was also increased by diluting the POME using final effluent from the treatment plant itself. This potential effluent recycling is not only economical but sustainable in achieving zero waste concepts in palm oil mill life cycle. By implementing these dual strategies, removal of COD, N and P is expected to accelerate as microalgae assimilated more nutrients during rapid multiplication. The performances of the systems were evaluated in term of μ_{max} and percentage of pollutants successfully removed.

2. Experimental method

2.1. Collection and pre-treatment of wastewater

POME used in this study was collected from facultative pond and final effluent from Seri Ulu Langat palm oil mill in Sepang, Malaysia. The facultative pond is the third treatment process in the stages of treatment at the plant; after anaerobic and aerobic ponds. After facultative pond, the wastewater will be discharged as final effluent to the environment. The POME collected were transported in 20 L labeled containers and allowed to settle in 4 °C storage room to preserve the condition. Prior to usage, the POME was filtered using filter cloth and further centrifuged to remove any visible solid particle. The supernatant was autoclaved for sterilization at 121 °C for 20 min. Then POME initial pH 9.2 ± 0.05 was adjusted to pH 7.0 ± 0.05 using 3 M HCl before it being used as culture medium.

2.2. Microalgae strain and culture condition

A native microalga, *Characium* sp. (UKM1), locally isolated from POME was used in this study as it recorded highest growth rate (μ_{max} = 0.45 in synthetic medium) and efficient nutrient removal capability in preliminary test (TN and TP removal were more than 88%). Pure microalgae was continuously maintained in BG11 medium [20] in flask covered with cotton stopper and keep suspended at 120 rpm using orbital shaker. 1 L of BG11 medium composition containing the following ingredients: 100 mL of 15 g L^{-1} $NaNO_3$; 10 mL each of 7.5 g L^{-1} $MgSO_4{\cdot}7H_2O$, 4.0 g L^{-1} K_2HPO_4, 3.6 g L^{-1} $CaCl_2{\cdot}2H_2O$, 2 g L^{-1} Na_2CO_3, 0.6 g L^{-1} ferric ammonium citrate, 0.6 g L^{-1} citric acid and 0.1 g L^{-1} Na_2EDTA. Finally, 1 mL L^{-1} trace elements solution was added which contained: 2.86 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2{\cdot}4H_2O$, 0.22 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.287; 0.08 g L^{-1} $CuSO_4{\cdot}5H_2O$, 0.05 g L^{-1} Co $(NO_3){\cdot}2{\cdot}6H_2O$ and 0.39 g L^{-1} (NH_2) $MoO_4{\cdot}2H_2O$.

The strain was monitored until it reached designated density before used as inoculum in the experiments. For acclimatization analysis, the microalga was cultivated in diluted POME before inoculated in second stage experiments. Inoculations were done in sterile condition to prevent any contamination to the culture.

2.3. Experimental layout

This study was carried out in two consecutive stages. The first stage was to determine the effect of inoculum density and POME dilution towards the growth rate of the microalgae. In this stage, each of 0.1 g L^{-1} and 0.5 g L^{-1} inoculum density was cultured respectively in 100% and 50% POME concentration diluted with distilled water.

The second stage was aimed to improve the growth rate using acclimatized inoculum. For this purpose, inoculum was cultured in 20% POME diluted with BG-11 prior to cultivation in actual experiment. It was used as inoculum when it reached 0.5 g L^{-1} after the biomass washed twice with distilled water to remove effect of POME residual. Cultivation was done in the best culture condition chosen based on result from the first stage. In addition, the potential of final effluent used for dilution of POME as compared to distilled water was also assessed. Summary of the experimental setups was presented in Table 1. All experiments were carried out in batch mode incubated at 25 ± 2 °C and 7000 Lux 12:12 light/dark cycle. Each flask was aerated for 24 h continuously with 5% v/v of CO_2.

2.4. Microalgae growth measurement in POME

Microalgae growth in POME was monitored for 14 days or until it reached stationary phase. Biomass was obtained by harvesting the microalgae and centrifuged it at 8000 rpm for 10 min. Prior to growth measurement, the biomass pallet was washed twice with distilled water to remove suspended solid from POME. The dry cell weight (DCW) was measured by gravimetric method using pre-weighted blank Whatman GF/C, 47 mm glass microfiber filter paper dried at 100 °C for 24 h 10 mL of microalgae culture was filtered then the particulate was dried at 100 °C for 24 h before being weighted again. The DCW was then determined by Eq. (1) [21]:

$$^{D}CW\left(gL^{-1}\right) = (a-b) \times 1000 \Big/ {}_{s}ample\ volume(L) \quad (1)$$