Journal of Water Process Engineering 17 (2017) 229–236

Contents lists available at ScienceDirect

Journal of Water Process Engineering

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

Evaluation of *Lemna minor* and *Chlamydomonas* to treat palm oil mill effluent and fertilizer production

Hesam Kamyab[a,c,*], Shreeshivadasan Chelliapan[a,**], Mohd Fadhil Md Din[b], Reza Shahbazian-Yassar[c], Shahabaldin Rezania[a], Tayebeh Khademi[d], Ashok Kumar[e], Mohammadamin Azimi[f]

[a] Engineering Department, UTM Razak School of Engineering & Advanced, Universiti Teknologi Malaysia, Malaysia
[b] Centre for Environmental Sustainability and Water (IPASA), Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, 81310 Johor, Malaysia
[c] Department of Mechanical and Industrial Engineering, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607, USA
[d] Faculty of Management, Universiti Teknologi Malaysia, 81310 Johor, Malaysia
[e] Department of Biotechnology, Himachal Pradesh University, Shimla, India
[f] Institute for Smart Infrastructure and Innovative Construction (ISIIC), Faculty of Civil Engineering, Universiti Teknologi Malaysia, 81310 Johor, Malaysia

A R T I C L E I N F O

Keywords:
Nutrient removal
Bioremediation
Lemna minor
Chlamydomonas
Palm oil mill effluent (POME)

A B S T R A C T

Malaysia is considered as one of the major palm oil producers in the world. Therefore, it is important to develop an environmentally friendly and economic method to treat palm oil mill effluent (POME). The main aim of this study was to investigate the potential of *Lemna minor* (*L. minor*) and *Chlamydomonas incerta* (*C. incerta*) with catalog number (KR349061) for the bioremediation of POME in order to achieve higher water quality standard and further produce organic fertilizer. In this study, three different experiments were conducted by using *L. minor*, followed by addition of *C. incerta*, and further combination of *L. minor* and *C. incerta*. The concentration of nitrate (NO_3^-), ammoniacal nitrogen (NH_3-N), phosphate (PO_4-P), Electrical Conductivity (EC) and salinity in produced fertilizer were measured, and then they were compared with two current commercial fertilizers. Growth factors such as growth rates, average number of leaf and height of root of *L. minor* of plants were also determined. The results showed that the microalgae and macrophytes were capable of removing only 4.4% of chemical organic demand (COD) whereas the respective maximum removal rates for NO_3^-, NH_3-N, and PO_4-P were 12.5%, 11.3%, and 70.47%. Also, the average differences of NO_3^-, NH_3-N, and PO_4-P concentrations in produced fertilizer in comparison with two current commercial fertilizers were 95, 39.5, and 62.5 mg/g, respectively. The results of this study revealed that only *L. minor* was converted into fertilizer. This study elaborated that both *L. minor* and *C. incerta* are able to remove a part of organic pollutants and nutrients from POME.

1. Introduction

In recent decades, Malaysia has become well known as one of the world's leading producers and exporters of palm oil products [1]. Palm oil mill effluent (POME) is the wastewater generated by processing palm oil, which has various dissolved and suspended pollutants [2]. POME contains high concentration of nitrogen, phosphorus, and other nutrient contents [3,4]. The effluent produced from palm oil mills is dangerous to the environment due to its high concentration of pollutants [5]. Currently, POME is treated by conventional wastewater treatment methods such as activated sludge which is considered as an expensive treatment method [6,7]. In addition, several studies have been conducting using chemical experiments, for example, flocculation [8], ozonation [9], etc. and physical experiments, for example, membrane [10]. However, these techniques have their own advantages and disadvantages. The idea of using microalgae in wastewater treatment extends back to the 1950s [11]. Algae can help in the treatment of different types of wastewater such as municipal and industrial wastewater, while benefiting from using the nutrients present [12]. It is also reported that using algae is an economical method for removing heavy metals from wastewater, resulting in high quality water discharge and valuable algal biomass that could be useful for different purposes such

* Corresponding author at: Engineering Department, UTM Razak School of Engineering & Advanced, Universiti Teknologi Malaysia, Malaysia.
** Corresponding author.
E-mail addresses: hesam_kamyab@yahoo.com (H. Kamyab), shreeshivadasan.kl@utm.my (S. Chelliapan).

http://dx.doi.org/10.1016/j.jwpe.2017.04.007
Received 23 November 2016; Accepted 9 April 2017

Fig. 1. *L. minor* species on the surface of water (source by authors).

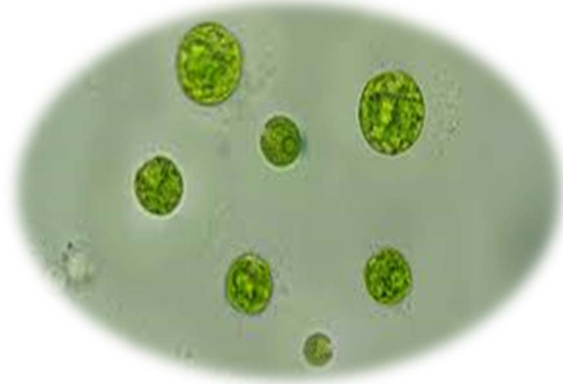

Fig. 2. A microscopic picture of *C. incerta*. Source by authors.

as production of fertilizer, animal feedstock and biogas [13,14]. Algal biomass contains high nutrient levels, and many studies have shown its effectiveness as a bio-fertilizer in increasing the level of plant growth [15,16].

Lemna minor is a species that can be found in Africa, Asia, Europe, and North America. *L. minor* can grow everywhere in freshwater ponds and slow-moving streams [17]. However, this species cannot grow in arctic and subarctic areas. This species is a floating aquatic plant which has around 1–3 leaves (see Fig. 1). Each leave of *L. minor* has a single root which will be hanging in the water. The size of the root of *L. minor* is between 1 and 2 cm. When more leaves grow, the plants divide and become separate individual plants. *L. minor* can grow in water with high concentration of nutrients such as nitrogen and phosphorus. The best pH for this species is from 6.5 to 7.5. Also, it has the best growth rate at a temperature between 6 and 33 °C [18].

Several researchers have reported the unique potential of *L. minor*, such as high protein content, phenomenal growth rate, and the ability to treat wastewater and thrive in fresh and brackish water [18,19]. Moreover, additional evaluation may help researchers to determine any possible toxic effects of plants, biochemical parameters such as growth rates, dry/fresh weight ratios and chlorophyll contents. Growth inhibition was measured as reduction in fresh and dry weight in industrial wastewater exposed *L. minor* plants. According to Uysal (2013), the amount of dry/fresh weight ratios of plants was determined to measure toxic effects of chromium [20].

Microorganisms are currently drawing a lot of attention as a potential organism to treat groundwater and wastewater [21,22]. Microalgae as a main feedstock has attracted much attention in recent years but is still not economically feasible due to high algal culture cost [23]. Microalgae can be cultivated under stress conditions such as nutrient starvation, high salinity and high temperature [24,25]. It also accumulates considerable amounts of lipids or carbohydrates (up to 60–65% of dry weight) [26]. Some microalgae species could convert molecular nitrogen into nitrate, ammonia, and urea (or combinations of them), which are the most common nitrogen sources for microalgae [27,28]. Many microalgae species are able to effectively grow in wastewater due to their ability to utilize abundant organic carbon, inorganic nitrogen (N), and phosphorus (P) [29]. However, the application of microalgae in the wastewater industry is still fairly limited [30]. An economically viable and ecologically sustainable approach to nutrient pollution control could involve the integration of retention ponds, wetlands and greenways into water management systems [31]. Algae are used throughout the world for wastewater treatment albeit on a relatively minor scale [16,32]. *Chlamydomonas* is a unicellular organism which belongs to green microalgae [33]. *Chlamydomonas* can be found in all stagnant water sources containing seawater, freshwater, even on damp soil, and snow [34]. The growth characteristics and the composition of microalgae such as *Chlamydomonas* are known to significantly depend on the cultivation conditions [35]. Fig. 2 shows a microscopic picture of *Chlamydomonas*.

The main purpose of the study was to investigate the potential of duckweed (*L. minor*) and microalgae (*C. incerta*) in order to achieve a higher standard quality of palm oil mill effluent discharge and to examine its potential as organic fertilizer. However, there has been no research on the fertilizer value of combination of duckweed and algal biomass (*L. minor* and *Chlamydomonas*) from treatment of agro-wastewater (POME). In addition, the amount of nutrients in the produced fertilizer in this study is compared to the common commercial fertilizer in Malaysia. Since *L. minor* and *Chlamydomonas* have high ability of nutrient uptake from POME, the procedure for synthetic fertilizer production can be considered as POME treatment method for pollutant removal. This process would simultaneously create algae feedstock for organic fertilizer, biofuel, and pollutant removal from wastewater. POME could supply the growth of *L. minor* and *Chlamydomonas* with its pollutants acting as the nutrients. *L. minor* and *Chlamydomonas* can also absorb the nutrients and at the same time treat the effluent of palm oil mill. The high nutrient contents in *L. minor* and *Chlamydomonas* make them a good fertilizer. Algae species were recommended to be used as bio-fertilizer as an alternative to mainstream synthetic fertilizers. This is due to the increased cost of chemical fertilizer that cause soil and water pollution. In comparison, algae are cheap source nutrients and minimize environmental pollution.

2. Materials and methods

2.1. Characteristics of POME

In this study, FELDA palm oil in Kulai and Kahang Johor, Malaysia were chosen as source of POME. The collected POME was preserved in refrigerator at a temperature of 4 °C. Several essential parameters of the collected POME were determined including chemical oxygen demand (COD), mixture liquid suspended solid (MLSS), mixture liquid volatile suspended solid (MLVSS), nitrate (NO_3^-), ammoniacal nitrogen (NH_3-N) phosphorus (PO_4-P), Electrical Conductivity (EC) and salinity. Electrical conductivity and salinity of the digested sample were quantified at 24 h intervals. The parameter assessments were performed in the water laboratory of Centre for Environmental Sustainability and Water (IPASA) at University Technology Malaysia (UTM).

2.2. Sample collection, preparation of L. minor and Chlamydomonas

The experiment was initially conducted using *L. minor*, followed by *C. incerta* (KR349061), in which both were isolated from the pond located in FELDA oil palm mill, Kulai and Kahang, Johor, Malaysia. The experiment was then conducted with the combination of *L. minor* and *Chlamydomonas* for treating POME [33]. A small amount (10%, v/v concentration) of *L. minor* and *Chlamydomonas* under aseptic conditions