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Isolate New Microalgal Strain for Biodiesel Production and Using FTIR Spectroscopy for Assessment of Pollutant Removal from Palm Oil Mill Effluent (POME)

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In tropical countries, the palm oil industry discharges a large amount of wastewater. The wastewater can serve as an economical nutrient source or substrate that can support the cultivation of microalgae. This study aimed to identify the local species of microalgae potentially existing in the industrial wastewater of palm oil mill effluent (POME). POME was selected as the key source of waste due to its higher potential in producing lipids from microalgae as biofuel substrate. A novel green microalgal strain was isolated from POME of Kahang-Johor west palm oil mill in Malaysia and was identified as Chlamydomonas sp. and subsequently named UTM 98 with Catalogue No. of KR349061. This study emphasised the effectiveness of POME as the main carbon source to maintain the growth of microalgae and simultaneously to increase the lipid content. In this study, Fourier Transform Infrared spectroscopy (FTIR) and Gas Chromatography (GC-FID) were used to identify and quantify lipids in the freshwater microalgae. Cultivation of microalgae were initially carried out in 250 mL Erlenmeyer flask containing 100 mL medium at ± 30 °C with continuous illumination (± 14 μmol^{-1} m^{-2} s^{-1}) and up to 20 d of cultivations. Results demonstrated that on the chromatogram, the highest retention achieved is belong to palmitic acid (C16:0). Chlamydomonas incerta (C. incerta) species is found to contain shorter chain fatty acids, mainly 16 - 18 carbon length, which is ideal for biodiesel production. FTIR spectrum of POME treated biomass displayed the shifting of peak at 591 cm^{-1} and also removal of C-Cl stretching. The spectrum of POME effluent treated biomass revealed broad peak at 3,430 cm^{-1}. The results of SEM micrographs showed that, after treating POME with C. incerta, the cells became slightly rough and corrugated textures and some particles were found on the surface of the cell wall. Using POME as a rich carbon and nutrient source is also a promising approach either as natural environment treatment or as high-lipid-content raw material for production of biofuel.

1. Introduction

Palm oil is one of the world's most rapidly expanding equatorial crops. Malaysia is one of the major palm oil producers in the world (Lam et al., 2009). Palm oil mill effluent (POME) is the wastewater generated by processing oil palm and consists of various suspended materials. POME has a very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which is 100 times more than the municipal sewage (Hadiyanto and Hartanto, 2012). The effluent also contains higher concentration of organic nitrogen,

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phosphorus, and other nutrient contents (Kamyab et al., 2014). POME is a non-toxic waste but will pose environmental issue due to large oxygen depleting capability in aquatic system due to organic and nutrient contents. It is also known to be a good source of nutrients (Kamyab et al., 2016). It was estimated that for each t of crude palm oil produced, 5 - 7.5 t of water are required, and more than 50 % of the water will end up as palm oil mill effluent (POME) (Wu et al., 2007).

Freshwater microalgae are globally ubiquitous and highly deserve, with tens or perhaps hundreds of thousands of species, in a myriad of forms and sizes (Guiry and Guiry, 2014). Microalgal culture has received much attention, given its prospects as a source of bioenergy and its potential for wastewater treatment. Microalgae have been used in the past to recycle some of the nutrients present in the wastewater sources and as an aid for wastewater treatment (Pizarro et al., 2002). The integration of POME treatment using microalgal culture will potentially reduce the wastewater treatment retention time and eliminate toxic elements, which serve as nutrients for the growth of microalgae (Kamyab et al., 2013). For several years, it has been studied that microalgae have the potential to remove organic and inorganic matters present in the polluted water. It is also concluded that this method is an economic method for removing inorganic and organic materials from the wastewater, resulting in better quality water discharge and obtaining valuable algal biomass which could be useful for different purposes such as the production of biofuel, fertiliser, animal feedstock, biogas etc., (Gonçalves et al., 2016). POME contains high content of degradable organic matter, which could become one of the promising sources for renewable energy in Malaysia (Chin et al., 2013). The discharge of improperly treated POME creates adverse impact to the environment. However, the substances in POME are able to support the growth of microalgae. Transform Infrared Spectrometry (FTIR) has been successfully applied for the study of the evolution with time of the volatile products evolved in the thermal and catalytic pyrolysis of polymers and for the characterisation of the different decomposition steps of this microalgal specie (Dean et al., 2010). The aim of this research was to obtain more efficient strains for the production of microalgae-based biofuel in tropical country such as Malaysia, this study was conducted in order to investigate lipid producing and characteristic features of the newly isolated microalgal strain from the palm oil mill located at Kulai, Johor, Malaysia.

2. Material and Methods

2.1 Sample preparation

The source of POME and mixed consortia of microalgae was obtained from the facultative ponds of FELDA palm oil mill in Kulai, Johor, Malaysia (geographical location, latitude 9° 17" N and longitude 5° 18" E). The sample was stored in 5 L plastic containers with proper labelling and refrigerated at about 4 °C, in order to prevent contamination and to limit the activity of biodegradation process. The frequency of sampling was done once in three months, and then before starting of the experiment, collected POME was left for few hours at the room temperature for it to settle. The cultivation of microalgae was initially carried out in the 250 mL Erlenmeyer flask containing 100 mL of liquid growth medium. The POME used in the batch experiments was previously settled for 1 h and further diluted with Bold Basal Medium (BBM) at the different COD concentrations was prepared at the concentration of 250 mg COD L^{-1}, in synthetic medium, 10 vol% of microalgae was added in to the flask bottle and placed at ambient room temperature of ± 30 °C under continuous illumination at intensity ± 14 µmol m^{-2} s^{-1} for 20 d (Kamyab et al., 2016).

2.2 Lipid Measurement using Gas chromatography

The lipid content present in microalgae was measured by Gas chromatography using a 30 m VF-5MS column with 0.25 mm film thickness and 10 m Integra guard column (Agilent Technologies, Santa Clara, CA). The injection temperature was set to 250 °C, the MS transfer line at 280 °C, the ion source adjusted to 250 °C and the quadrupole at 150 °C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. According to (Hossain et al., 2008) the samples were prepared and derivatived using a Gerstel 2.5.2 Auto sampler with an incubation period of 30 min at a temperature of 37 °C using an agitator speed of 500 rpm (Guihéneuf et al., 2015). The corresponding GC–FID method was performed using the following temperature program; injection at 50 °C, hold for 1 min, followed by a 15 °C/min oven temperature ramp to 230 °C; hold for 3 min, followed by a 10 °C/min ramp to 300 °C.

2.3 FTIR-Spectroscopy

In the additional approach to measure lipid content Fourier transform infrared (FTIR) micro spectroscopy was used. This is a method which involves the measurement of infrared absorption in relation to a range of molecular vibrational modes (Murdock and Wetzel, 2009). Specific molecular groups can be identified by their absorption bands, allowing macromolecules (including proteins, lipids, carbohydrates, and nucleic acids) to be quantified. A few studies have begun to demonstrate the potential of FTIR as a tool to identify the changes in

cellular components, including lipids, in response to a nutrient stress, such as at low-N (Stehfest et al., 2005) and low-P (Sigee et al., 2007). The dried samples of microalgae was directly placed on specific cab and scanned in Fourier Transform Infrared Spectroscopy (Nicolet iS5, Thermo Fisher Scientific, Inc., USA) to obtain the FTIR spectra. The spectrum was obtained in the transmission mode and the range of 600 - 4,000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per each sample. Each sample's spectrum was recorded in the transmittance mode and corrected using OMNIC Specta software (Dean et al., 2010).

2.4 Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) is generally used to observe a variety of specimen's surface. It produces superior resolution at the analytical working distance of 10 mm, as resolution is guaranteed at 8 mm and the operate up to 30 kV. The surface morphology and cell wall composition of microalgae was observed using Scanning Electron Microscope (SEM). After drying, the sample (0.71 to 1.0 mm) was covered with a thin layer of gold (10 nm) using a sputter coater. The coated samples were placed in to a JSM - 6390 SEM / EDS unit, and different sections in the samples were examined under the voltage of 20 kV (Ajayan et al., 2015).

3. Results and Discussions

3.1 Preliminary Study for the growth of C. incerta in POME

The isolated strain proved to be a new microalgal strain, identified as Chlamydomonas incerta (C. incerta) based on the 18srRNA sequencing method were shown in Figure 1. The results have shown that this new strain (Gen Bank KR349061) has a suitable potential for biodiesel production due to its faster growth and easier cultivation when compared to other strains (Kamyab et al., 2017). It also shows that, C. incerta can be a good candidate for accumulating biomass and lipids (Park et al., 2015). All sequences were compared with the GeneBank database using BLAST (Basic Local Alignment Search Tool) and were manually aligned with the representative sequences from microalgae strains and related taxa, according to the similarities in secondary structure identified by the Clustal X program. C. incerta can grow in autotrophic, heterotrophic, or mixotrophic conditions (Janssen et al., 2000).

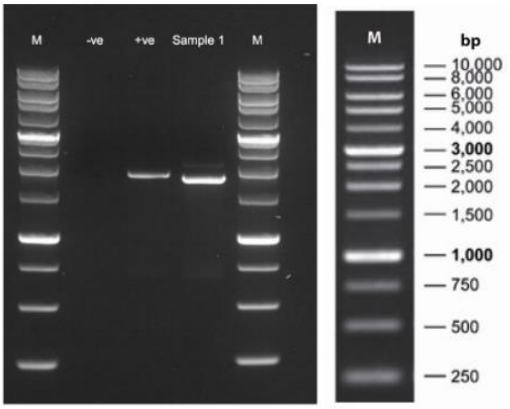


Figure 1: 18 srRNA gene sequence of C. incerta

3.2 Lipid Content Analysis by GC-FID for the newly strain of C. incerta

According to Knothe (2008), palmitic, stearic, oleic and linoleic acid are recognised to be as the commonest fatty acids contained in biodiesel. For this experiment, five different standards of fatty acids were used, which are heptadecanoic acid (C17:0), heneicosanoic acid (C21:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). Using gas chromatography analysis, the identification of fatty acids was performed by comparison with the retention time of standards (D'Oca, et al., 2011). Figure 2 shows the chromatogram of methyl ester from the optimised sample and highest retention achieved was at 16.5 min, which had shown the presence of another type of FAME, which is palmitic acid (C16:0). C. incerta species is found to contain shorter chain fatty acids, mainly 16 - 18 carbon length, which is ideal for biodiesel production and, in C. incerta, palmitic acid was commonly found to be more. Palmitic acid and stearic acid content which are known as the most common fatty acids contained in biodiesel (Li et al., 2011), are present in this strain.

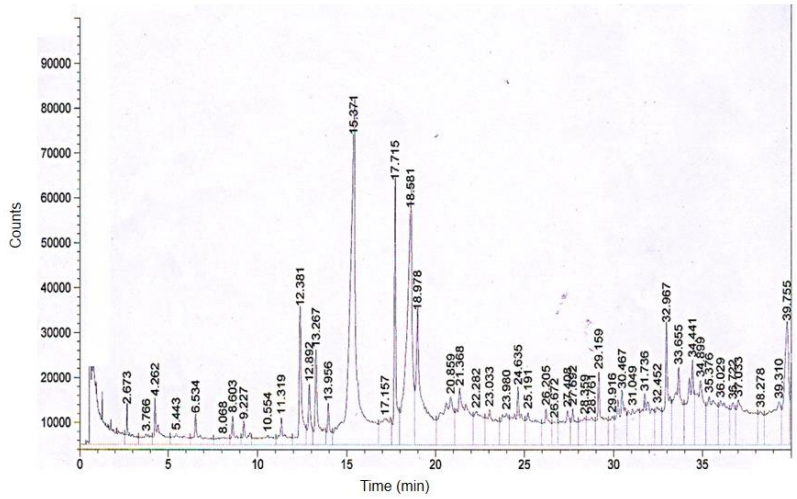


Figure 2: Analysis of fatty acid content present in C. incerta

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The IR spectrum of dried algal biomass was recorded on Nicolet IR spectrometer at room temperature. The dried algal powder was prepared. A region of 4,000 - 1,000 cm^{-1} was used for scanning. The FTIR spectra showed five important peaks explaining the stretching, bending and double bond absorption of the sample. It was observed that the absorption peaks are the same for sample, where the C-H stretching absorption occurs at wavelength 2,971 cm^{-1}. This peak appears strong in microalgae sample as shown in Figures 3 and 4. Two alkanes peaks which is attributed to the bending absorption of methylene (CH_2) and methyl (CH_3) groups that appears at 1,464 and 1,375 cm^{-1}. Two peaks observed at 1,764 and 1,159 cm^{-1} are due to stretching absorption of aldehyde (C=O) and esters (C-O). All these peaks appeared in the C. incerta sample. However, the stretching absorption of (O-H) at 3,346 cm^{-1} is strong in sample. This stretching (O-H) absorption is intermolecular hydrogen bonding for water. These results are in agreement as reported in the literature (Bradley, 2007).

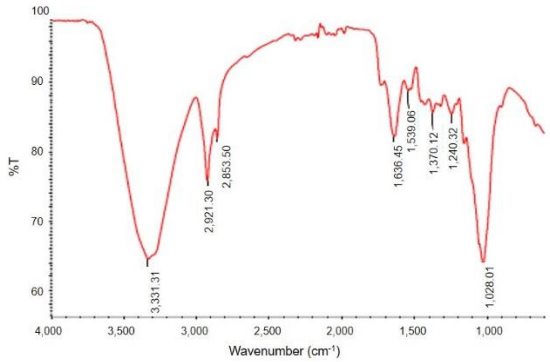


Figure 3: FTIR spectrum of C. incerta from control using BBM media

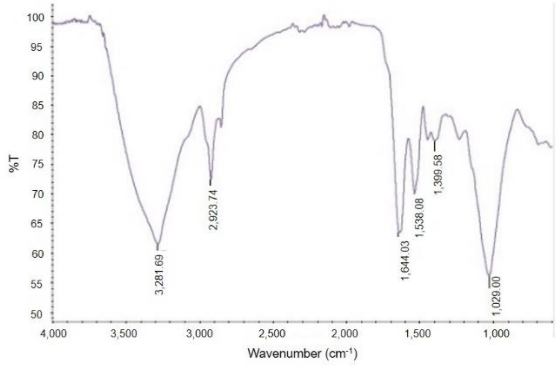


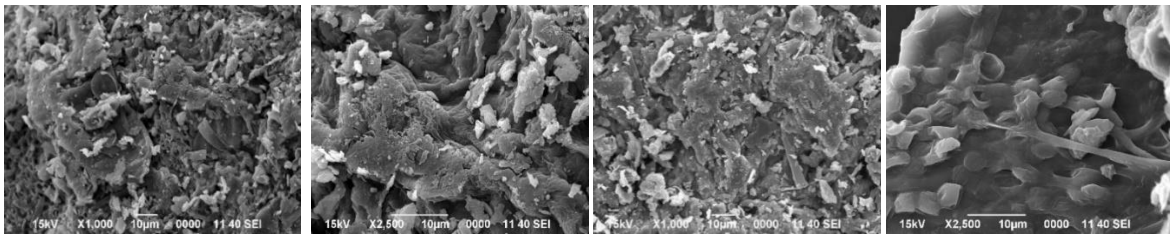
Figure 4: FTIR spectrum of C. incerta biomass from control culture using POME

The region between 2,800 to 3,000 cm^{-1} exhibited the stretching of C-H groups. The control culture biomass of microalgae showed the peaks at 2,922 cm^{-1} of C-H asymmetric stretching vibration of CH_2 functional groups. The wave number 1,626 cm^{-1} became sharper indicating the involvement of C=O stretching indicating carbonyl groups. 1,100 - 1,058 cm^{-1} were P=O or C-O stretching of polysaccharides respectively (Figure 3). FTIR spectrum of control culture biomass and their respected peaks was exhibited at 603 cm^{-1} wave numbers indicating the presence of C-Cl stretching (Figure 4) Spectrum of POME treated biomass exhibited the shifting

of peak at 591 cm^{-1} and also removal of C-Cl stretching. The spectrum of POME effluent treated biomass revealed broad peak at 3,430 cm^{-1} (Figure 4).

3.4 Scanning Electron Microscopic (SEM)

The results of SEM micrographs showed that, the algal cell before POME treatment had normal shape with smooth and transparent external layer outside cell surface. After treating POME with C. incerta, the cells became slightly rough and corrugated textures and some particles were found on the surface of the cell wall. Therefore, the microalgae after solvent extraction may have had some structural changes that were not visually significant. It could be assumed that solvent extraction caused hollower structures in microalgae (Bi and He, 2013). Figure 5 is the surface texture of algal biomass before and after treatment of POME.



(a) (b) (c) (d)

Figure 5: SEM of C. incerta in BBM media under: (a) 1000x and (b) 2500x magnification before POME treatment, and (c) 1000x and (d) 2500x after POME treatment

The difference in the surface of the biomass became slightly rough and corrugated textures on the surface after nutrients uptake may be due to the accumulation of different elements quantities on the cell wall. After POME wastewater treatment, C. incerta appears to bring about an increase in surface roughness in comparison to the normal cell surface with BBM media. The salt crystalloid deposition on the biomass surface might be the result of the surface protuberance on the biomass after treating POME by C. incerta.

4. Conclusions

The results revealed that new strain (Gen Bank KR349061) has a suitable potential for biodiesel production due to its faster growth and easier cultivation. Based on the chromatogram of methyl ester from the optimised sample and highest retention achieved was at 16.5 min, which is correlate with palmitic acid (C16:0). C. incerta species is found to contain shorter chain fatty acids, mainly 16 - 18 carbon length, which is ideal for biodiesel production. FTIR spectrum of POME treated biomass exhibited the shifting of peak at 591 cm^{-1} and also removal of C-Cl stretching. The spectrum of POME effluent treated biomass revealed broad peak at 3,430 cm^{-1}. The results of SEM micrographs showed that, after treating POME with C. incerta, the cells became slightly rough and corrugated textures and some particles were found on the surface of the cell wall. As an overall conclusion it can be mentioned that C. incerta has a greater potential for the production of biodiesel due to its faster growth and higher lipid production.

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