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Research article

Effects of light intensity on biomass, carbohydrate and fatty acid compositions of three different mixed consortia from natural ecological water bodies

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ABSTRACT

This study investigated the effect of light intensity on three various microalga consortia collected from natural ecological water bodies (named A, B and C) towards their fatty acid profiling and fractions, carbohydrate and protein production at different light intensities of 100, 200 and 300 μmol $m^{-2} s^{-1}$. The results indicating that increasing light intensity positively correlated with the lipid production than carbohydrate and protein. Irrespective to the solids (Total and Volatile Solid) content, lipids and carbohydrate has varied significantly. Consortia C showed higher productivity toward lipids, whereas consortia A and B accumulated more carbohydrate and protein, respectively. The microscopic images revealed the breakdown of cells during the increase in light intensity, in spite, the similar algal species were observed in all consortia experimented. Principal component analysis (PCA) revealed that low light intensity aid relatively in high protein, Total Nitrogen and Total Phosphorus, meanwhile high intensity attributed carbohydrates and unsaturated fatty acids (USFA) contents.

1. Introduction

In this modern and industrialized 20th century, environmental pollution and energy demand are the serious issues to be solved to make the sustainable community for the better future (Kumar et al., 2017; Sivagurunathan et al., 2016). Over the past decades, world energy consumption has been grown rapidly as mentioned in global statistical energy consumption report. Fossil fuels have been marked to be the popular fuel resources. Therefore, fossil fuel depletion has given a widely concern towards the prospecting of renewable and clean energy sources that are sustainable (BP, 2017). In order to cope up with the climate change and CO_2 emission issues, algal biotechnology has been taken into account. Due to high CO_2 fixation efficiency and biomass production, algae biofuel has been put forth as propitious green fuel for future prospective (Arvindnarayan et al., 2017; Cea-Barcia et al., 2014; Ghimire et al., 2017).

Microalgae biofuel technology has been gaining attention to the researchers, investors and business venture peoples as it promises to provide various valued added intermediates as well as noteworthy energy carriers for future biorefinery (Abdel-Raouf et al., 2012; Sarpal et al., 2016; Hannon et al., 2010). Additionally, algae could be grown in various systems such as autotrophic mode which utilize CO_2, salts, and a light energy source, whereas, heterotroph require external source such as organic compounds for its biomass productivity (Brennan and Owende, 2010). Hyper accumulation of carbohydrate, lipid and protein in a single cell (microalgae) overcome the issue of food versus fuel,

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which is a controversial problem for lignocellulose based plant biomass.

In addition, cultivation of microalgae in wastewater/waste as cheaper nutrient sources adds more credits towards algae biofuels towards greener economy (Mata et al., 2010) Furthermore, in order to efficiently cultivate microalgae few parameters such as pH, temperature, dissolved oxygen concentration and light supply should be carefully considered to design efficient photo-bioreactor (Munoz and Guieysse, 2006). More importantly, light source is one of the major criteria for the microalgae growth because it can affect the growth and metabolism of microalgae (Show et al., 2017).

Moreover, algal growth is affected by different types of shading light as light is a fundamental variable for benthic region (Singh and Singh, 2015). Proper light levels and intensity should be investigated to enhance the growth rate to achieve the optimum biomass productivity. The previous study of Yoshimura et al. (2013) reported that different kinds of light irradiance over the cultivation of *Botryococcus braunii* show the highest growth rate at 0.5 d^{-1} with 850 μmol $m^{-2} s^{-1}$ of light irradiance (Yoshimura et al., 2013). One other study reported that cultivated *Chattonella marina* under 150 μmol $m^{-2} s^{-1}$ of light irradiance with the optimal growth over 0.5 d^{-1} and grow much faster compared to 1.08 d^{-1} at an irradiance of 450 μmol $m^{-2} s^{-1}$ (Marshall and Hallegraeff, 1999; Yoshimura et al., 2013).

Natural ecological systems consist of multi species of algae biomass that are able to produce higher biomass compared to the specific indoor/outdoor photo bioreactors that are used for mono culture of microalgae species and accumulation of particular components (Smith et al., 2009). There forward, all of microalgae strains have different oil content present in its cell constituents. Previous studies by Araujo et al. (2011) and Zhou et al., 2011 suggested that *Chorella* sp. and *Scendesmus species* yield better biomass and oil productivity (Araujo et al., 2011; Zhou et al., 2011). To make biofuel, especially biodiesel as reality for the suitable transport fuel in future, enhancing the lipid content and productivity of the microalgae biomass is very essential (Ho et al., 2015). The excess of carbohydrates usually stored as lipids in microalgae biomass in the presence of enzyme called CoA carboxylase. These lipid components will be further undergoing transesterification process to produce biodiesel (Venkata Mohan et al., 2011).

Therefore, this study seeks to investigate the effect of light intensity on three different microalgae consortia collected from natural ecological freshwater bodies. Besides, fatty acids profiling and other components accumulations were analyzed. Moreover, principal component analysis applied to evaluate the better use of light intensity for different uses such as biofuel production or food materials. This study would provide new insights to the readers concerning the direction of efficient use of light intensity for sustainable and economical cultivation of microalgae for biofuel production and various other purposes.

2. Materials and methods

2.1. Sampling of microalgae consortia and cultivation

Microalgae consortia from three different water-bodies with ecologically diverse functional properties, characteristics were collected from nearby Kasumigaura Lakeside and also Bio-eco Engineering Laboratory of National Institute of Environmental Studies (NIES, Japan) in Tsukuba, Japan. Lentic and lotic systems were also considered for the selection. Sampling of microalgae consortia was carried out in summer session during the month of August–September 2014. The consortia was collected by a mesh net (10–20 μm) in a plastic container to avoid the major particles and other unwanted materials associated with them and then stored in plastic vials prior to inoculation. In the sampling site the environmental conditions were measured using a portable multi probe system pH, dissolved oxygen (DO) and TS was measured later in the lab. The values are mentioned in Table 1. Bedsides, their parameters during the collection time also added in Table 1. Collected consortia were again filtered for removal of the impurities such as dust particles, sand and small insect larvae. Later, the consortia was grown in Bold's basal medium which is a widely used nutrient medium for freshwater microalgae of the classes *chlorophyceae*, *xanthophyceae*, *chrysophyceae*, and *cyanophyceae* (Batista et al., 2014; Bold, 1963). The mixed microalgae consortia has been cultivated in plastic bags (25 cm in height and 5 cm in diameter) using bolds mineral medium. The light intensity of 7000 lux (140 μmol $m^{-2} s^{-1}$) was applied and the incubation was followed by 12 h dark and 12 h photo period using a controller at room temperature of 23 °C. The reactors were aerated at a flow rate of 2 L/min (air contains 0.035% CO_2 in the inlet) using air spargers (stone diffusers) and keeping the same culture conditions as mentioned in our earlier study (Kumar et al., 2016). From these mother cultures, 10% v/v of inoculum was further transferred to study the effect of light intensities. Biomass growth was measured by following the optical density (OD) values at 680 and 750 nm (UV/Visible spectrophotometer Shimadzu). The light microscopic images of the consortia A, B and C (predominantly composed of *chlorella* and *scenedesmus)* are provided in Fig. 1.

2.2. Effect of light intensity

Photoautotrophic growth of microalgae is heavily rely on the irradiation intensity and also the source of the light (Ho et al., 2012; Liu et al., 2012). Considering this factor, 3 different light intensities of 100, 200 and 300 μmol $m^{-2} s^{-1}$ which represent 5000, 10000 and 15000 lux were used in this study by employing lab scale (plastic bags 5 L) photo bioreactors fixed with an incandescent light (external light source) for 3 different microalgae consortia (named as A, B and C). Different intensities were set based on the distance between the culture and also the light source (measured using photometer). Photo bioreactors (plastic bags) were used as mentioned in previous section and are cultivated using bolds basal medium and the initial pH values were not altered and they were ranging between 7.5 and 9.0. Liquid samples were taken periodically in the sealed glass vials (20–30 ml) for the analysis of various components and also OD values.

Light source was provided with HITACHI lamp (FH32EN, GY10q-9, HITACHI, Japan). Light intensity was measured using photo meter and converted to μmol $m^{-2} s^{-1}$ following the equation (1) (Thimijan and Heins, 1983)

$$\frac{\text{Lx}}{\text{constant}} = \mu\text{mol. m}^{-2}\text{. s}^{-1} \quad (1)$$

where Lx = light intensity obtained in Lux (Lumen per square meter)

Constant = light source converted constant (lux to μmol $m^{-2} s^{-1}$)

2.3. Analytical procedure and principal component analysis (PCA)

Total Nitrogen (T-N) and Total (T-P), NH_4 and PO_3 were measured using an auto-analyzer (QuAAtro, BL-Tech, Osaka, Japan), for (T-N) and Total (T-P) autoclave pretreatment (with potassium persulfate) has been applied. Long-Chain Fatty Acids (LCFAs), including caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), cis-11-Eicosenoic acid (C20:1), behenic acid (C22:0), and erucic acid (C22:1) were measured as fatty acid methyl esters using a gas chromatograph (6890 series GC system, Agilent) equipped with a flame ionization detector (FID) and a 30 m DB-WAX capillary column (0.25 mm i. d., 0.75 mm o. d.) (Agilent J&W). The carrier gas was helium. The injector and detector temperatures were both 250 °C. The column temperature was maintained at 50 °C for 1 min and then increased to 200 °C at a rate of 25 °C/min before being increased again to 230 °C at a rate of 3 °C/min. Methylation before the measurements were performed using a fatty acid methylation kit (Nakarai Tesque). Lipids were extracted according