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MICROALGAL CULTURE WITH DIGESTATE FROM METHANE FERMENTATION - LIGHT ENVIRONMENT IN THE CULTURE SOLUTION WITH DIFFERENT DIGESTATE CONCENTRATIONS AND MICROALGAL CELL DENSITIES

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ABSTRACT

The light intensity decreases logarithmically in the solution according to Lambert- Beer's law that is given by equation $P_2 = P_1 \exp(-\beta(Z_2-Z_1))$. Absorption coefficient is expressed as $(\beta) = (\ln (P_1)-\ln (P_2))/(Z_2-Z_1)$, where P_1 and P_2 are photosynthetic photon flux densities (PPFDs) at depth Z_1 and Z_2, respectively, in the culture solution. The light absorption coefficients of culture solutions with different digestate concentrations and different microalgal densities were determined by spectrophotometer in the wavelength range of photosynthetic active radiation (400-700 nm). A linear regression was obtained between the absorption coefficient (cm^{-1}) and digestate concentration (%) expressed as $\beta_{digestate} = 0.0546 \times$ "digestate concentration" + 0.005. A linear regression was also obtained between the absorption coefficient and the microalgal density (cells ml^{-1}) expressed as $\beta_{microalgae} = 0.0655 \times$ "microalgal density" + 0.0402. In simulation experiment conducted with microalgal density of 30×10^5 cells ml^{-1}, more than 10% of light was transmitted at the depths shallower than 15 mm, using 20% diluted digestate.

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1 INTRODUCTION

Microalgae have been used as sources of food or feed supplements, nutriceuticals, cosmetics and pharmaceutical products. Microalgal cell growth rates are affected by combinations of environmental parameters such as light intensity, temperature, pH, and nutrients in the culture solutions *(Kitaya et al., 2005*; Chisti, 2007; Kitaya *et al*., 2008; Parmar *et al*., 2011; Nguyen *et al*., 2013; Nguyen *et al*., 2015). The maximum specific growth rate (μ) values were 0.047 h−1 in 10% digestate for Euglena gracilis, 0.065 h−1 in 20% digestate for Chlorella vulgaris, and 0.052 h−1 in 50% digestate for Dunaliella tertiolecta at a PPFD of 150 μmol m−2 s−1. The μ values of Dunaliella tertiolecta were 2.5 and 1.1 times higher than those of Euglena gracilis and Chlorella vulgaris, respectively, in 50% digestate (Nguyen *et al*., 2013).

In current years, the advancement of renewable energy production technologies has resulted in an important increase of agricultural biogas production (Weiland, 2010). Digestate is a by-product of

microbial anaerobic digestion (Bauer *et al*., 2009) from biogas. The large amounts of digestate are now being used more widely as valuable fertilizer, particularly due to its high nitrogen concentration in agriculture.

Light intensity and wavelength are essential parameters for microalgal growth. However, varying illumination intensities in outdoor conditions are likely to inhibit the growth of microalgae because of the shortage of light energy, for example very low light intensity during rainy days or photoinhibition caused by excessive irradiation, or very high light intensity at noon during summer time (Ugwu et al., 2007).

2 MATERIALS AND METHODS

Euglena gracilis (strain name: Z) obtained from Osaka Prefecture University, Japan was subcultured in Cramer–Myers (CM) medium (1000 mL) (Cramer and Myers, 1952) in a translucent plastic vessel (3000 mL) at room temperature 28°C and at a photosynthetic photon flux density (PPFDs) of 300 μmol m^{-2} s^{-1}. The vessel had sufficient air volume to maintain CO_2 and O_2 inside the vessel at 0.04% and 21%, respectively, throughout the experimental period. However, the air used was normal atmospheric air because we intended to extend knowledge derived from this study to actual microalgal culture in an open pond system.

The light intensity expressed by photosynthetic photon flux density (PPFDs) decreases logarithmically in the solution according to Lambert-Beer's law which is given by equation (1). Absorption coefficient (β) is given by equation (2) where P_1 and P_2 are the distribution of PPFDs at depth Z_1 and Z_2, respectively, in the culture solution.

$$P_2 = P_1 \exp(-\beta(Z_2-Z_1)) \quad (1)$$

$$\beta = (\ln (P_1)-\ln (P_2))/ (Z_2-Z_1) \quad (2)$$

2.1 Effects of digestate concentrations to light environment in the culture solution

The simulation is based on using UV light. The light spectral properties of microalgae at different densities (cells ml^{-1}) were determined by spectrophotometer. Digestate was diluted to 5%, 15%, and 25% with deionized water. No aeration was used. The original digestate was centrifuged 2000 rpm for 10 min to remove large particles (Table 1). The light spectral properties of 5% 15%, and 25% digestate were determined by spectrophotometer (UV1240, Shimadzu Co., Kyoto, Japan).

Table 1: Components of the original digestate used, the Cramer–Myer (CM) solution used as control medium

Solution medium	pH	NH_4^+ (mg l^{-1})	K^+ (mg l^{-1})	Na^+ (mg l^{-1})	SO_4^{2-} (mg l^{-1})
CM	3.5	281	299	244	123
Digestate	8.4	973	1202	328	60

Light environment in the culture solution with different digestate concentrations (%) was simulated according to the equation $P_3 = P_1 \exp(-(\beta_{digestate})(Z_2-Z_1))$ where $\beta_{digestate}$ is the absorption coefficient of the digestate at depth 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mm.

2.2 Effects of microalgal densities to light environment in the culture solution

Euglena gracilis (E. gracilis) was used as the green microalgae in this experiment. Microalgae cultured at 162×10^5, 325×10^5, and 486×10^5 cells mL^{-1} were diluted at a ratio of 1:2:3 with CM solution. Absorbance of the CM solution was approximately zero. The light spectral properties of microalgae at different densities (cells mL^{-1}) were determined by spectrophotometer.

2.3 Effects of microalgal densities and digestate concentrations to light environment in the culture solution

Light environment in the culture solution with different digestate concentrations (%) and microalgal densities (cells mL^{-1}) was simulated according to the equation $P_5 = P_1 \exp(-(\beta_{digestate}+ \beta_{microalgae})(Z_2-Z_1))$ where $\beta_{digestate}$ is the absorption coefficient of digestate; $\beta_{microalgae}$ is the absorption coefficient of microalgae.

In the preliminary experiment, a microalgal species, *Dunaliella tertiolecta,* showed the highest specific growth rate at a cell density of 30×10^5 cells mL^{-1}. This microalgal density was selected for the experiment (Nguyen *et al*., 2013).

3 RESULTS AND DISCUSSION

3.1 Effects of digestate concentrations to light environment in the culture solution

Figure 1 showed that the light absorbance of 5%, 10%, and 15% digestate concentration was higher at the longer wavelength region of the range of PPFDs. All digestates absorbed across the entire visible spectrum, with a stronger absorption intensity over the lower half in the lower wavelength region (Marcilhac et al., 2014). A linear regression was obtained between the absorption coefficient and the digestate concentration, and expressed as followed:

Absorption coefficient (cm-1) = 0.0546 × digestate concentration (%) + 0.005

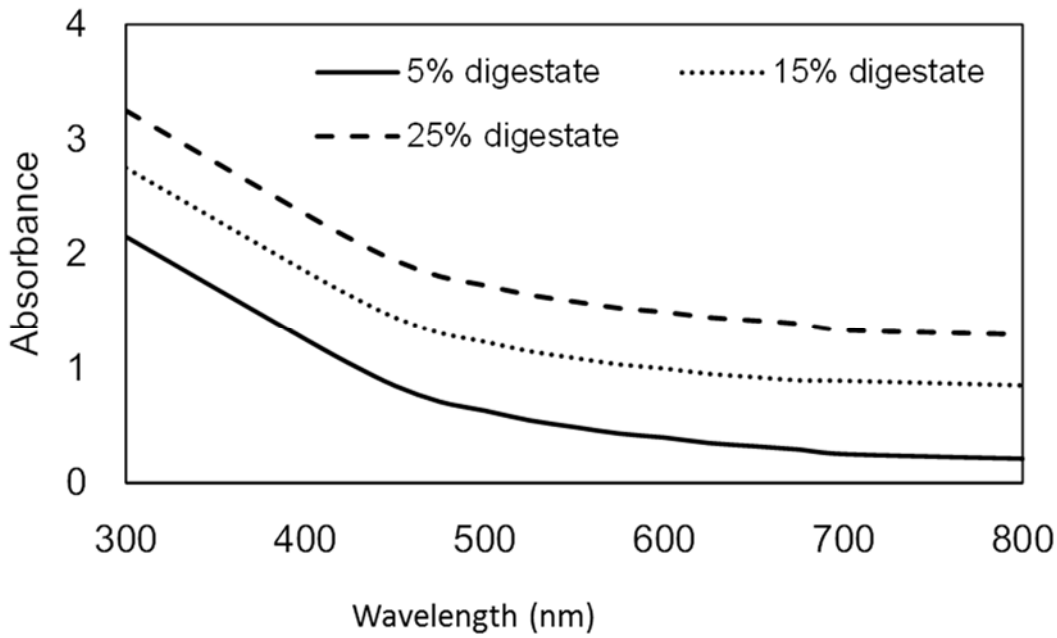

Fig. 1: Light absorbance of 5%, 15%, and 25% digestate

As shown in Figure 2, the light intensity was affected by depth and digestate concentration of the solution. Higher PPFDs can be obtained at shallower depths and lower digestate concentrations. At 20% digestate, the depth of the solution must be less than 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mm to sustain the preferred PPFD penetration at more than 58, 34, 19, 11, 7, 4, 2, 1, 1, and 0%, respectively, in the digestate solution. A logarithmic relationship between intensity and distance would suggest a more rapid decline in intensity with initially increasing distance, resulting in an increased potential for a relatively shallower depth of cure at shorter distances (Pires *et al*., 1993; Prati *et al*., 1999; Meyer *et al*., 2002; Felix and Price, 2003; Aravamudhan *et al*., 2006).

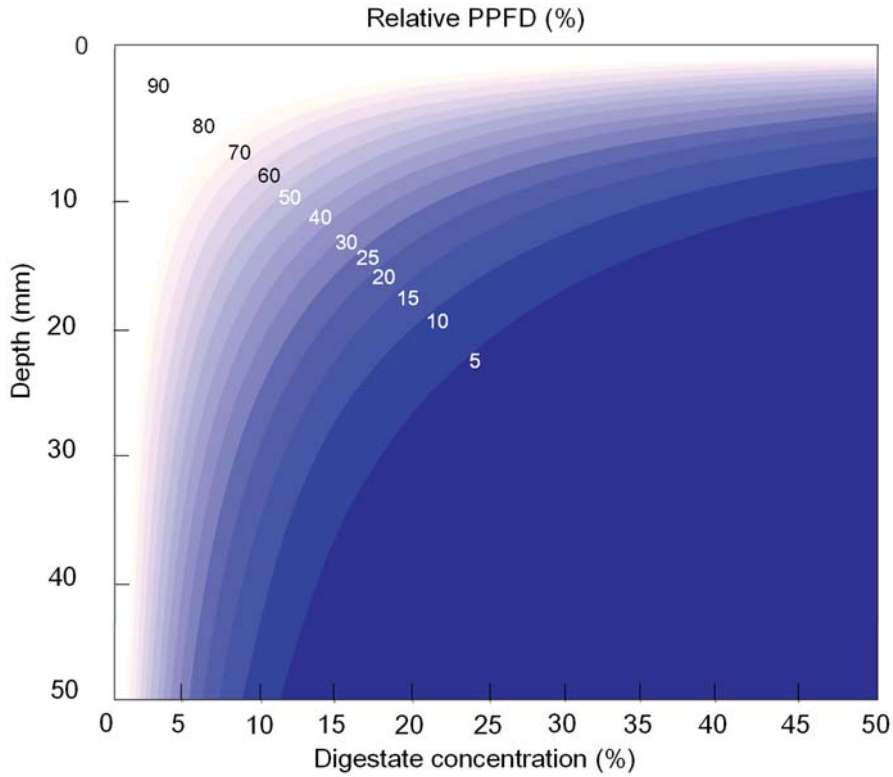

Fig. 2: Light intensity affected by depth and digestate concentration

3.2 Effects of microalgal density to light environment in the culture solution

The absorbance of microalgal solutions at 162×10^5, 325×10^5, and 488×10^5 cells mL^{-1} are shown in Figure 3. A linear regression was obtained between the absorption coefficient and microalgal density, expressed as followed:

Absorption coefficient (cm^{-1}) = 0.0655 × microalgal concentration (%) + 0.0402.

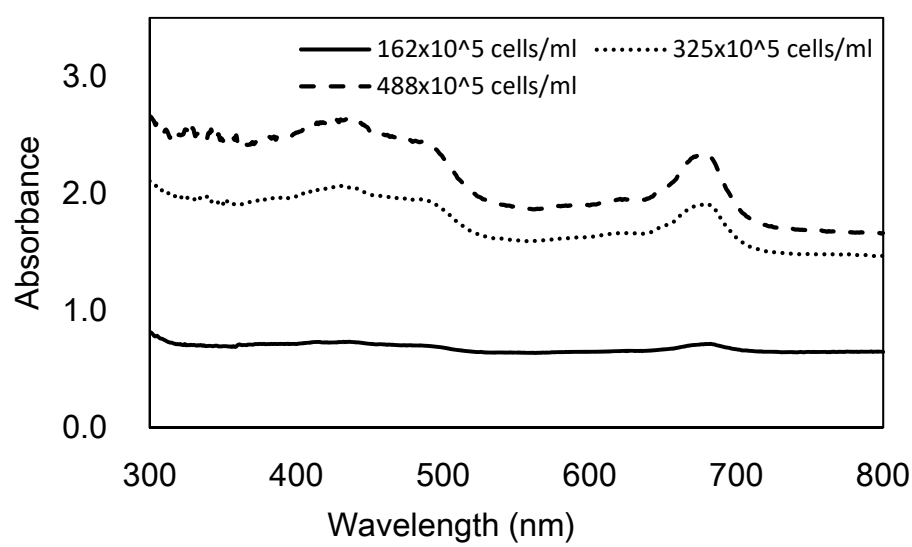

Fig. 3: Light absorbance of solutions with different microalgal densities

Light intensity was affected by solution depth and microalgal density (cells mL-1) is shown in Figure 4. More than 81, 65, 52, 42, 34, 27, 22, 18, 14, and 12% of light transmission was obtained at depths shallower than 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mm, respectively, in CM solution with a microalgal density of 30×105 cells mL-1. To estimate and predict algal growth under various levels of light intensity, it is also essential to formulate the relationship between growth and light intensity. In several harmful algae, such as Chattonella marina/C. ovate (Yamaguchi *et al*., 1991; Yamaguchi *et al*., 2010), Kareniamikimotoi (Yamaguchi and Honjo, 1989), and Gambierdiscus species (Kibler *et al*., 2012), such relationships were established using Michaelis–Menten (MM) (Yamaguchi and Honjo, 1989), modified MM (mMM) (Yamaguchi *et al*., 1991; Yamaguchi *et al*., 2010) or Gaussian/Lorentzian model equation (Kibler *et al*., 2012). Among them, the MM and mMM model equations are incapable of displaying algal growth inhibition at intense levels of light intensity due to the appearance of a saturated growth rate at infinite light intensity. In contrast, the Gaussian/Lorentzian model equation is capable of displaying algal growth inhibition as well as promotion as light intensity increases when the growth-light intensity curve resembles a normal distribution; however, such curve-forms are infrequently observed in dinoflagellates (Morton *et al*., 1992; Kibler *et al*., 2012). Growth-light intensity relationships can be estimated quantitatively by formulae. Importantly, none of these model equations are capable of displaying algal growth inhibition and promotion with varying light intensity or determining the threshold of light intensity required for algal growth.

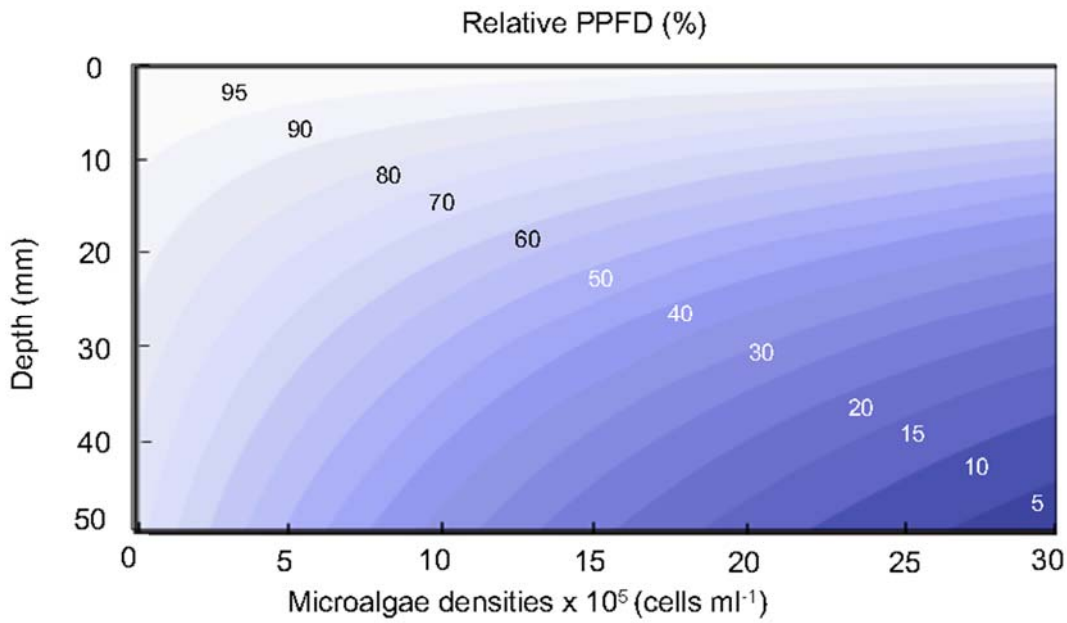

Fig. 4: Light intensity affected by depth and microalgal density

3.3 Effects of microalgal density and digestate concentration to light environment in the culture solution

The light environment in the culture solution with difference digestate concentrations at a microalgal density of 30×10^5 cells mL^{-1} is shown in Figure 5. More than 20% of light transmission was obtained at a depth shallower than 5, 10, 15, and 20 mm at a microalgal density of 30×10^5 cells mL^{-1} at 47, 22, 10, and 5% digestate concentration, respectively. Result obtained by simulation conducted with microalgal density of 30×10^5 cells mL^{-1} indicating that more than 10% of light was transmitted at the depths shallower than 15 mm, using 20% diluted digestate. To establish a culture system that can accommodate the digestate solution, the depth of the solution must be designed to maintain more light penetration. A culture system consisting of a thin layer of solution under natural light condition is proposed. The depth of solution will be controlled to maintain optimal PPFD penetration, depending on solar radiation.

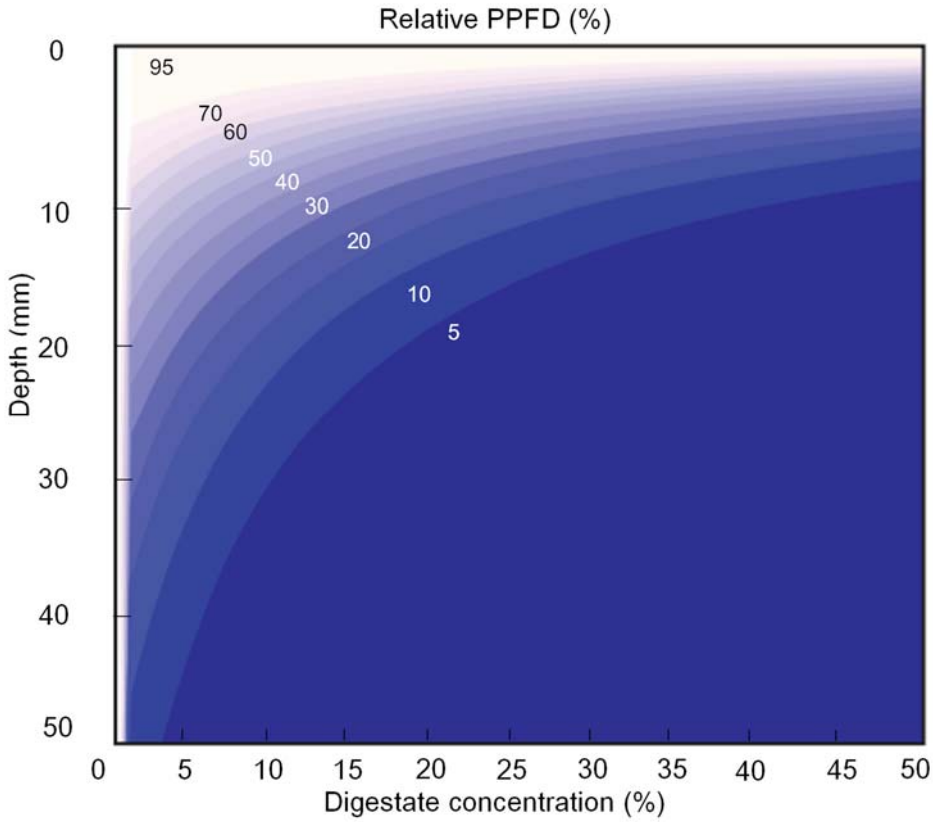

Fig. 5: Light intensity affected by depth and digestate concentration at a microalgal density of 30 × 10^5 cells mL^{-1}

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