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MICROALGAL CULTURE WITH DIGESTATE FROM METHANE FERMENTATION - CONCENTRATION OF DIGESTATE ON GROWTH OF THREE MICROALGAL SPECIES

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ABSTRACT

In order to design a culture system for microalgal biomass production with a low cost and convenient cell collection, growth performance of mixtures of microalgal cells, including *Euglena gracilis*, *Chlorella vulgaris*, and *Dunaliella tertiolecta* cultured in a volume of 1 L were investigated at a PPFD of 300 µmol m^{-2} s^{-1} at the surface of the solution with continuous illumination at 30 °C. Each culture container contained diluted digestate at concentrations of 5, 10, 15, 20, and 50 %. Sample cells for counting cell number were collected daily. Pseudo-specific growth rates (μ_s) of each species at each depth were calculated as cellular multiplication rates using number of cells per time. The average μ_s of each species was highest in 5 % digestate. The average μ_s of all three microalgal species (0.035 h^{-1}) was observed in all layers in 5 % digestate solution. The μ_s of each species was highest in 5 % digestate (0.048 h^{-1}, 0.041 h^{-1}, and 0.022 h^{-1}, respectively for *C. vulgaris*, *E. gracilis*, and *D. tertiolecta*). In conclusion, *E. gracilis*, *C. vulgaris*, and *D. tertiolecta* showed the highest specific growth rate in 5 % digestate.

Keywords: Chlorella, Dunaliella, digestate, Euglena, microalgae.

1. INTRODUCTION

Microalgae have been used as sources of human foods, animal feeds, and pharmaceutical products, because of their marked ability to convert CO_2 to biomass and their unique capacity to transform photosynthates to other useful compounds. Microalgae have also recently been used as a renewable biofuel source [1 – 3].

Euglena sp. are a group of freshwater motile microalgae. *Euglena* is able to grow in a highly acidic medium (pH 3) in which most other microorganisms can barely survive [4]. This characteristic offers an exceptional advantage, as it allows for the culture of *Euglena* under unsterilized conditions at low pH levels without the risk of contamination by other microorganisms.

The optimum pH for *Chlorella* was found to be in a range between 7.0–7.5 [5] Therefore, it has been used to remove nutrient loads, as well as a heavy metal detoxifier, for various types of

wastewater treatments, including industrial, municipal, and agricultural wastewater [6].

Dunaliella sp. is a group of halotolerant, motile microalgae that survive a wide range of stress factors. *D. tertiolecta*, for example, can survive in a wide range of NaCl concentrations, from 0.05 M to 5.5 M, and of pH values, from 1 to 11, even under intense light and high temperature conditions [7, 8].

Methane fermentation with organic residues and wastes is one of the most attractive renewable energy production technologies for reducing greenhouse gas emissions as well as for reducing the load of organic waste. The resultant digestate contains nutrients for plants and can be utilized as valuable fertilizer, particularly due to its high nitrogen concentration [9 - 11].

In this study, the effects of the depth and concentration of digestate from methane fermentation on the growth of three mixed microalgae *C. vulgaris*, *E. gracilis*, and *D. tertiolecta* cultured in diluted digestate was investigated to identify suitable culture conditions.

2. MATERIALS AND METHODS

Chlorella vulgaris and *D. tertiolecta* were obtained from the Walne culture collection of the Faculty of Fisheries, Can Tho University, Vietnam, and *E. gracilis* (strain name: Z) was obtained from Osaka Prefecture University, Japan. *C. vulgaris* and *D. tertiolecta* were subcultured in Cramer–Myers (CM) medium [12]. *E. gracilis* was subcultured CM at a modified pH of 3.5.

Chlorella vulgaris, *E. gracilis* and *D. tertiolecta* were cultured in open plastic boxes with a volume of 1000 mL (222 mm × 150 mm × 30 mm). Different concentrations of digestate were prepared at 5, 10, 15, 20, 25, and 50 % with deionized water. The percent of the digestate concentration means the dilution rate. CM (Cramer–Myer) medium was used as the control solution. Samples were collected from different depths of the culture box: 0–5 mm (the surface layer), 10–15 mm (the middle layer), and 25–30 mm (the bottom layer). The digestate, which was collected from a methane fermentation facility in Yagi Biomass Town, Kyoto, Japan, mainly contained cattle manure. The original digestate was centrifuged at 2000 RPM for 10 min to remove large particles. No other sterilization was used in the preparation of the medium.

The components of the original digestate used in this experiment and the CM solution used as the control medium are listed in Table 1. Concentrations of ions in the diluted digestate were inversely proportional to dilution rates.

Table 1. Components of the original digestate used in this experiment and the Cramer–Myer (CM) solution used as the 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

The initial cell densities of *C. vulgaris*, *E. gracilis* and *D. tertiolecta* were: 7–14 × 10^3 cells mL^{-1}, 4–8 × 10^3 cells mL^{-1} and *C. vulgaris* were 4–14 × 10^3 cells mL^{-1}, respectively. Kitaya et al. (2005) applied a PPFD of 150 µmol m^{-2} s^{-1} and a droplet method for *E. gracilis*. The volume of each solution drop of the droplet method was 3 µl [13]. The volume of this experiment (1 L) was

larger than 3 μl. PPFD at the solution surface was 300 μmol m^{-2} s^{-1} (assessed with a quantum sensor; Li-190, LI-COR, USA) applied as continuous illumination. Fluorescent lamps (FPL55EX-N, Matsushita Electric Co., Osaka, Japan) were used as the light source. Temperature was maintained at 30°C (Figure 1).

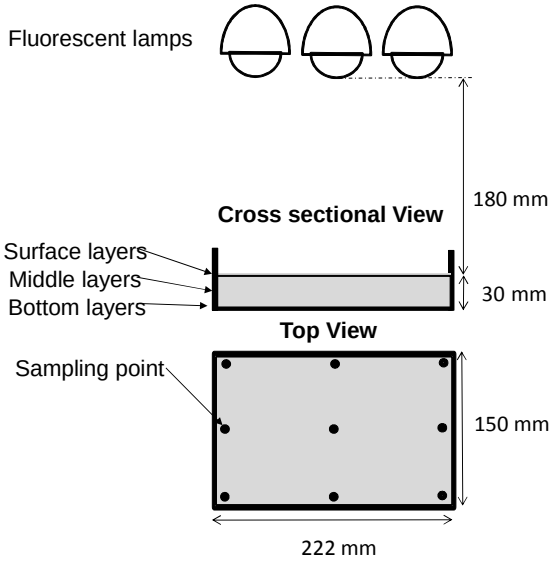

Figure 1. Experimental systems for evaluating the effects of environmental variables on the growth rates of microalgal cells.

The NH_4^+, K^+, Na^+, Cl^-, and SO_4^{2-} components of the original digestate were measured with an ion-chromatograph (pump, LC-10ADvp; cation column, Shim-pack IC-SC1; anion column, Shim-pack IC-A3; detector, ECD: Shimadzu Co., Kyoto, Japan). The pH was determined with a pH meter (D-52, Horiba Co., Japan).

The number of microalgal cells in 5, 10, 15, 20 and 25 % digestate solutions and CM were counted in three samples by using a counting chamber under a microscope of 70 × magnification.

The specific growth rate (μ) was determined in the logarithmic multiplication stage.

The cell number is theoretically given by Eq. (1)

$$N_t = N_0 \exp(\mu t) \quad (1)$$

where N_t is the cell number at time t, N_0 is the initial number of cells, and μ. Then:

$$\ln N_t = \ln N_0 + \mu t \quad (2)$$

μ, is then

$$\mu = (\ln (N_2) - \ln (N_1))/ (T_2 - T_1) \quad (3)$$

where N_1 and N_2 are the number of cells at times T_1 and T_2, respectively. In this experiment, T_1 and T_2 were 21 h and 72 h, respectively. The doubling time or the mean generation time (T_d) is given by the equation, $t_d = \ln 2/\mu$.

The μ values in each layer were variable because some microalgal cells moved in the box and the initial time (T_1) and the final time (T_2) of measurements were not counted for the same cell. Therefore the word “pseudo” was used in this experiment. The number of cells was

monitored daily and the pseudo specific growth rates (μ_s) in the surface, middle and bottom were calculated as cellular multiplication rates.

Statistical analyses were performed using Analysis of Variance (two way ANOVA) to examine the effects of digestate concentration and depth level on μ_s.

3. RESULTS AND DISCUSSION

In each layer, the average μ_s of each species was highest in 5 % digestate. The average μ of all the microalgal species was 0.035 h^{-1} (0.028 for *E. gracilis*, 0.046 for *C. vulgaris*, 0.019 for *D. tertiolecta*) at all layers in 5 % digestate solution. The maximum μ values of these species were smaller in appropriate concentrations of digestate than in CM medium (Figure 2).

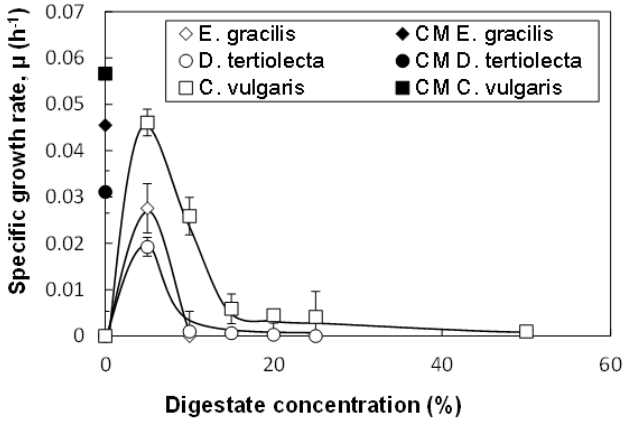

Figure 2 Effects of digestate concentration on average specific growth rates (μ) of *E. gracilis*, *C. vulgaris* and *D. tertiolecta* cells, at a PPFD of 300 μmol m^{-2} s^{-1} at the solution surface and a temperature of 26 °C. Each plot indicates mean ± standard error (n = 3−6).

In the analysis of variance (ANOVA), solution including 5 % digestate, depending on the depth, did not significantly affect the μ_s of the three microalgal species. However, the interactive effect of digestate concentrations and depth level on μ_s was significant for *E. gracilis* ($p < 0.05$), *C. vulgaris* ($p < 0.05$) and *D. tertiolecta* ($p < 0.05$).

This study confirmed that the highest values of □$_s$ for *E. gracilis*, *C. vulgaris*, and *D. tertiolecta* were 0.041, 0.048, and 0.022 h^{-1}, respectively (Figure 2), compared with that of previous estimates of 0.045, 0.024 and 0.023 h^{-1}, respectively [13 - 15]. The *C. vulgaris* □$_s$ value was two-fold higher than the previous study because this study applied a mixed culture of *E. gracilis*, *D. tertiolecta* and *C. vulgaris* in digestate solutions using digestate from methane fermentation. An earlier study concluded and this can be a good environment for *C. vulgaris* growth when added with other species [9] .

Moreover, pH also affects the growth of microalgae significantly. The growth of *Chlorella* and *Chaetoceros* sp. was reduced by 22 % when pH was increased from 8 to 9 [16 - 18]. *E. gracilis* survived in different digestate concentrations at pH 3.4 among the three microalgal (*Euglena gracilis, Chlorella* and *Dunaliella*) species tested. *Euglena gracilis* can be cultured at a high growth rate with diluted methane fermentation-derived digestate that was adjusted to a relatively low pH. The highest specific growth rate of *E. gracilis* cells was 0.053 h^{-1} and the

doubling time was 13 h with 25 % digestate at pH 3.4 [10].

In our culture method, pH 8.2 had no negative effect on the growth of three microalgal species. In terms of pH, these species could be suitably cultured in digestate from methane fermentation (Table 1). In general, pH significantly depends on the concentration of ammonia or the ammonium ion. The growth of some algal species may not be significantly inhibited by free ammonia at low pH while considerable inhibition may occur at a pH higher than 9. However, *Amphora* sp. and *Ankistrodesmus* sp. are able to grow well at a pH ranging between 9 and 10 [16, 17].

Nguyen et al. [9] applied a PPFD of 150 µmol m^{-2} s^{-1} and a droplet method for *E. gracilis.* The volume of each solution drop of the droplet method was 3 µl. In this study, The volume of this experiment (1 L) was larger than 3 µl. PPFD at the solution surface was 300 µmol m^{-2} s^{-1} applied as continuous illumination was affected the growth of microalgae cells (*C. vulgaris*, *E. gracilis* and *D. tertiolecta)* showed the highest specific growth rate (µ) at 5 % digestate.

4. CONCLUSION

C. vulgaris, *E. gracilis* and *D. tertiolecta* showed the highest specific growth rate (µ) at 5 % digestate when the PPFD at the solution surface was 300 µ mol m^{-2} s^{-1} and at 30 °C. However, under different conditions, such as less or more than 5 % digestate, µ decreased. The decrease in µ when digestate concentration is less than 5 % may be due to fewer nutrients. The decrease in µ at a digestate concentration greater than 5 % is due to lower light intensity. Therefore, the effects of PPFD on the growth of microalgae cultured with digestate from methane fermentation at different digestate concentrations were investigated in nology [9]. In addition, the effects of the light environment in culture solution with different digestate concentrations and microalgal densities will be considered.

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