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The Production of Algal Hydrocarbons in Outdoor Cultivations of *Dunaliella salina* 1650

PAK, JIN HONG[1], SHIN YOUNG LEE[1], YOUNG NAM KIM AND HYEON YONG LEE*

Department of Food Engineering
[1]Department of Fermentation Engineering,
Kangweon National University, Chuncheon 200-701, Korea

In 12:12 hour light/dark cycle cultivation of *D. salina* 1650, maximum specific growth rate of 0.59 (1/day) and 0.35 (g-crude hydrocarbons/l/day) were obtained. The cell growth was inhibited at above 15×10^{-4} (kcal/cm^2/h) of light intensity in an outdoor cultivation. It was also showed that temperature is one of the critical growth parameters in the outdoor cultivation. The hydrocarbon production from *D. salina* 1650 seems to be partially growth related production process, and these algal hydrocarbons can be used for subsituting petroleum directly or through cracking processes. The value of weight fraction carbon of *D. salina* 1650 was similar to that of *Botryococcus braunii* and so was the hydrocarbon productivity.

One of current research interests in developing new energy resources is producing usable hydrocarbons (liquid fuels) from photosynthetic algae through the biological conversion of solar energy (5, 9, 15). They can produce both saturated and unsaturated hydrocarbons by assimilating carbon dioxide and these kinds of algal hydrocarbons can also be used directly or indirectly for substituting gasoline (19, 20). It has been reported that the green alga, Botryococcus braunii is the most promising photosynthetic organism since it can produce economic quantities of hydrocarbons by utilizing artificial or natural light (2, 7, 8).

In algal biotechnology, the cultivation of mass amounts of biomass in indoor and outdoor cultivations by using various sources of light energy has been intensively investigated. The development of the mass cultivation technologies is the most economic methodology in producing biomass and the products of interest from photosynthetic algae (6, 13, 14). However, the outdoor cultivation of photosynthetic algae to produce hydrocarbons has been less studied because an open pond cultivation requires delicate elaboration of strong light intensity of solar energy, effective pH and temperature controls for long periods of cultivation, and continuous supply of culture media with less contamination of other species (17, 18). Therefore this work will be carried out to characterize growth and control parameters for an outdoor cultivation of newly selected hydrocarbon producing micro-algae, *Dunaliella salina* 1650 (ca. 0.17~0.22 g of crude hydrocarbons per g-dry algal biomass) (16).

*Corresponding author
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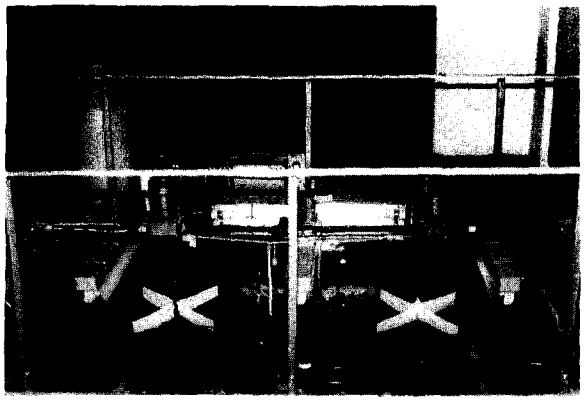
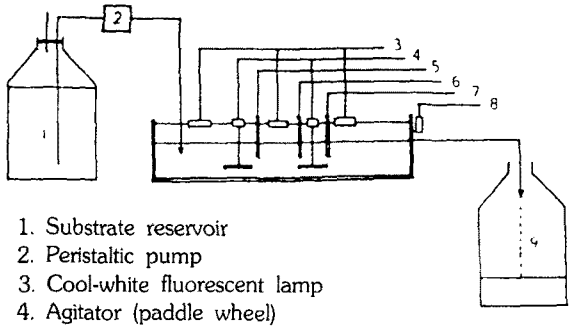
MATERIALS AND METHODS

The green algae, *Dunaliella salina* 1650 was obtained from Algal Culture Center (UTEX, USA) and adapted by growing the cells in a hydrocarbon producing medium (16) (pH 7.58 and 6.8% (w/v) NaCl) at 25℃ environmental chamber with 7.1×10^{-4} (kcal/cm^2/h) of light intensity. An open pond culture system was used for batch and continuous cultivations as shown in Fig.1 (150×150×25 cm, W×L×D, total working volume was 500 L). For 12:12 hour light and dark cycle cultivation, eight 20 W white cool flourescent lamps illuminated the pond in a dark room. pH and temperature in the system were not controlled in this experiment. For continuous cultivations, a peristaltic pump was used for feeding fresh medium andthe effluent was collected out of the drain at the top of the pond. Two top-driven paddle mixers (5×30 cm, H×W) were also used for the agitation of





1. Substrate reservoir
2. Peristaltic pump
3. Cool-white fluorescent lamp
4. Agitator (paddle wheel)
5. Thermometer
6. pH controller
7. Temperature controller
8. Radiometer
9. Effluent reservoir

Fig. 1. A schematic diagram of an open-pond culture system.

the media in the pond (Fig. 1). The outdoor cultivation was carried out when the change in seasonal temperature was at minimum.

The light intensity was measured by a quantum sensor (Licor LB-125, USA) every day at the same time (at noon). Fresh cell density was measured by filtering 10 m*l* of the sample through 0.45 μm pore size filter paper and dry cell density was also estimated by drying them at 105℃ for 24 hours in a drying oven. The samples from four different locations were taken to check the difference of cell concentrations caused by imperfect mixing within the reactor. For assaying algal hydrocarbons, filtered medium and algae disrupted by a sonicator (Far. M-150, USA) were centrifuged at 1300 g for 20 min, then they were extracted by adding two volumes of hexane into the supernatant for one hour at room temperature (11). The extracts were dried in a rotary vacuum evaporator and measured for total crude hydrocarbons. The crude hydrocarbons were purified by 110℃ activated silica gel (60 GF-2.5, MerK) chromatography (10), then identified by a thin layer chromatography (TLC) with eicosanpentaenoic acid (EPA, C20:5) as a standard because it was found to be that the hydrocarbons produced from microalgae were similar to EPA and squalene (7, 11). Chlorophylla concentration within the cell also was measured by a UV spectrometer at 660 nm after extracting the disrupted cells with 90 % acetone in the dark. The energy content of algal hydrocarbons was also estimated by a Differential Scanning Calorimeter (DSC) (DuPont 2100, USA). The samples were heated up to 400℃ at 10℃/min of the heating rate. The weight carbon fraction of *D. salina* 1650 and the composition of hexane extracted hydrocarbons were analyzed by an element analyzer (Perkin-Emler 240-C) to compare with the values of other microalgae.

RESULTS AND DISCUSSION

Fig. 2 shows the change in the ratios of dry to freshcell weight during light/dark cycle cultivation of *D. salina*. The light intensity of eight flourescent lamps was 9.04×10^{-4} (kcal/cm^2/h) for 12 hour light cycle cultivation. The significant change in the ratio occurred in the dark with 0.0067 as theaverage value for overall cultivations. Fig. 3 illustrates the kinetics of cell growth and the changes in pH and temperature during 24 hours, to find the effect of light/dark cycle cultivation on the batch mode. Cell density decreased during the dark period when the temperature dropped, while the pH remained relatively constant during cultivation.

24.5 (g-fresh cell wt./*l*) of maximum cell density was obtained at the end of light period, then it gradually decreased. This implies that the light intensity is a crucial factor in maintaining dense concentration of biomass in a diurnal cultivation. The concentrations of hydrocar-

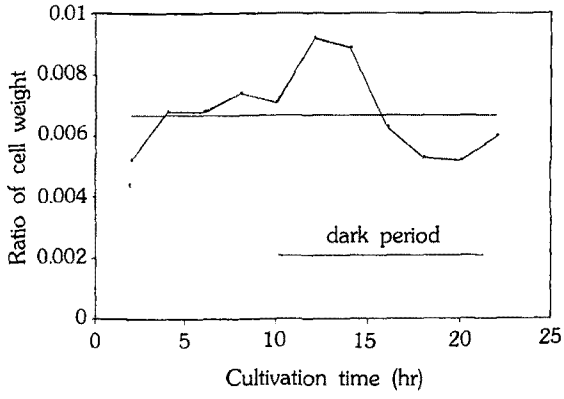


Fig. 2. The ratio of dry cell to fresh cell weight of *D. salina* 1650 in light/dark cycle cultivation (12:12 hours). The horizontal line is the average of data points.

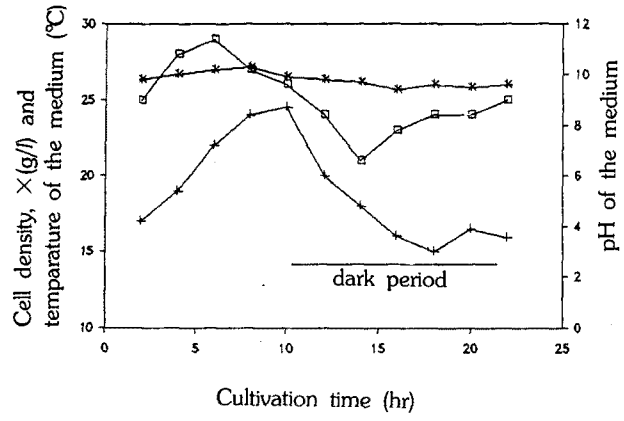


Fig. 3. The cell growth and the changes of pH and temperature according to the cultivation time for one cycle of diurnal cultivation at 0.94×10^{-4} (kcal/cm^2/h) of the incident light intensity.
+, fresh cell density (g/l); *, pH; □, temperature (℃).

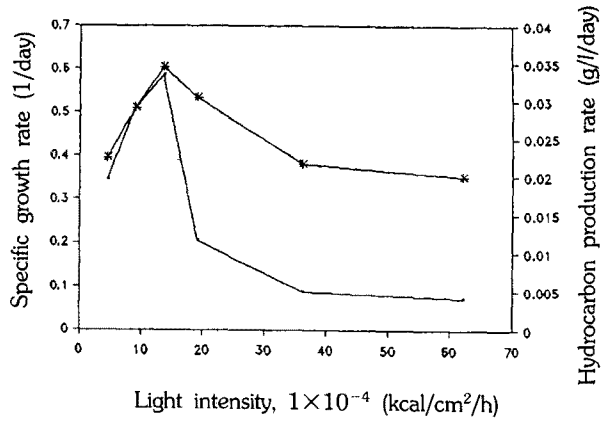


Fig. 5. The relationship between specific growth rate and hydrocarbon production rate as a function of the light intensity in light/dark cycle batch cultivation.
■, max.; specific growth rate (1/day); *, hydrocarbon production rate (g/l/day).

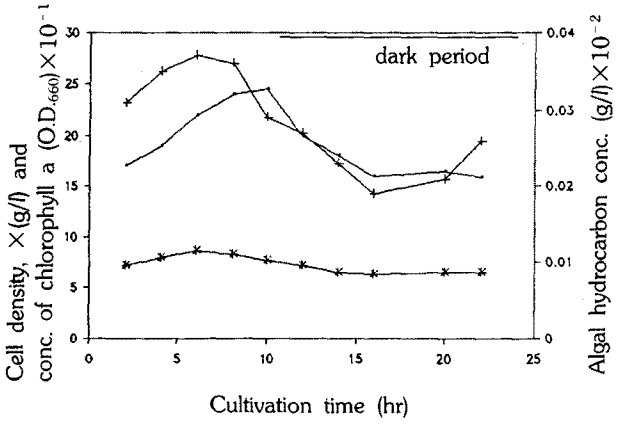


Fig. 4. Kinetics of cell growth, concentrations of the hydrocarbons and chlorophyll a for one cycle of light/dark diurnal cultivation at the light intensity of 9.04×10^{-4} (kcal/cm^2/h).
■, fresh cell density (g/l); +, hydrocarbon conc. (g/l); *, chlorophyll a conc. (O.D.$_{660}$).

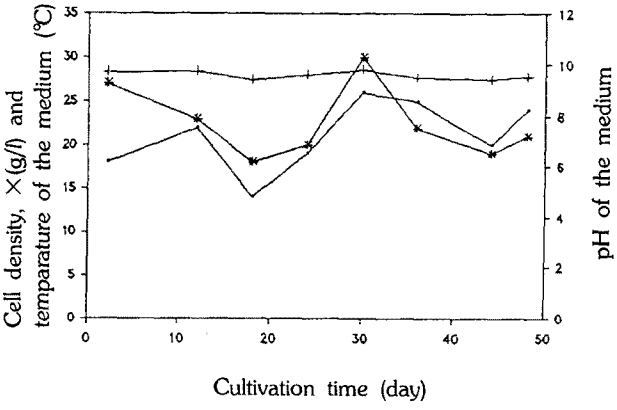


Fig. 6. The cell growth and the changes of pH and temperature in an outdoor cultivation of *D. salina* 1650.
■, fresh cell density (g/l); +, pH; *, temperature (℃). The depth of the pond was 7.5 cm.

bons and other competitive intracellualr component, such as chlorophyll a, were also measured during light/dark cycle cultivations as shown in Fig. 4. The concentration of chlorophyll a remained relatively constant even in the dark period of cultivation while the concentrations of hydrocarbons (from both medium and cells) decreased as the cell density dropped. It can tell that the hydrocarbon production is partially correlated to cell growth.

Fig. 5 is to show that maximum specific growth rate and hydrocarbon production rate are influenced by the light intensity in a light/dark cycle batch cultivation. The cell growth showed a photo-inhibition phenomenon in which maximum cell density was obtained at 15×10^{-4} (kcal/cm^2/h) of light intensity and gradually decreased. The result of this work is similar to other reported data applied to photo-inhibition models (1, 4, 12). However, the hydrocarbon production rate did not much decrease at the high light intensities, possibly due to the accumulation of unsaturated hydrocarbons in the culture broth (16). Maximum hydrocarbon production rate of 0.35 (g-crude hydrocarbon/l/day) was estimated at 15×10^{-4} (kcal/cm^2/h) of optimal light intensity with 0.59 (1/day) of specific growth rate.

Fig. 6 is the result of the outdoor cultivation of *D. salina* 1650 in an open-pond culture system (pond de-

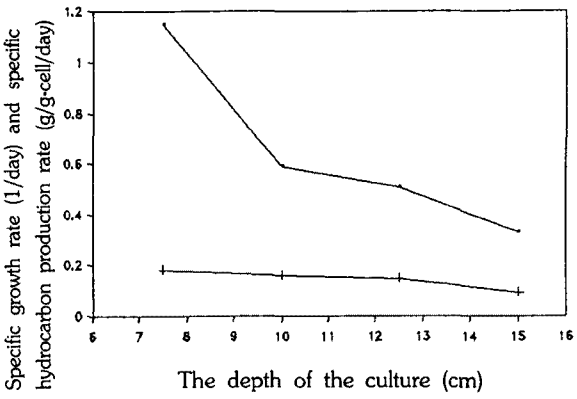


Fig. 7. Specific rates of cell growth and hydrocarbon production according to the depth of the pond in an outdoor cultivation.

■, specific growth rate (1/day); +, specific hydrocarbon production rate (g/g-cell/day).

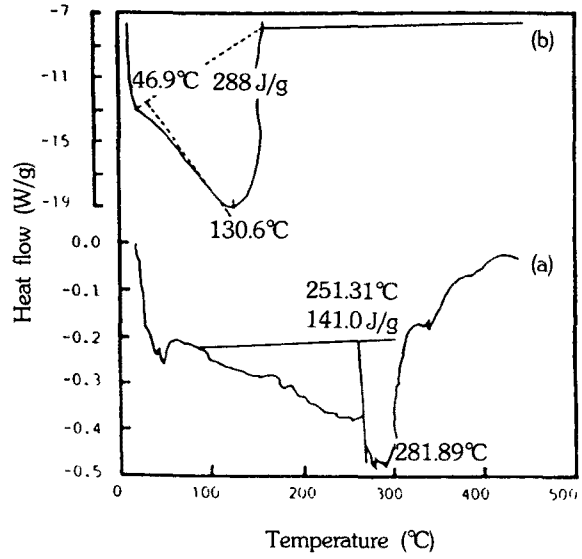


Fig. 8. The thermogram of DSC analysis for algal hydrocarbons from *D. salina* 1650 (a) and petroleum (b).

Table 1. The comparision of carbon fractions of several photosynthetic algae

Species	Weight fraction carbon*	References
Dunaliella salina 1650	0.598	
Botryococcus braunii	0.603	(21)
Chlorella pyrenoidosa	0.559	(22)
Spirulina plantensis	0.523	(23)
Stichococcus bacillaris	0.573	(24)
Scendesmus obliquus	0.549	(22)
Chlamydomonas sp.	0.475	(24)
Egregia menziesii	0.500	(24)
Nitzschia closterium	0.551	(22)

*Weight fraction carbon based on an ash-free basis.

Table 2. Results of elemental analysis of hexaneextracted hydrocarbons from microalgae

Species	Composition (Wt%)				References
	C	H	N	S	
D. salina	77.34	10.85	5.36	0.33	
B. braunii	83.38	11.96	0.17	<0.1	(21)
Spirulina pl.	66.86	10.37	10.05	0.43	(23)

pth was 7.5 cm) for two months from September to October, the most dry and fair season of the year in this region. The cell growth was also closely correlated to temperature changes as shown in Fig. 3, while the pH remained unchanged throughout cultivation. The maximum cell density was obtained as 28.5 (g/l) at high temperature of 30℃ in the middle of cultivation. Fig. 7 shows the relationship between cell growth and hydrocarbon production, according to the depth of the pond which is one of thecritical factors in an outdoor culture. It was interesting that the specific hydrocarbon production rate was not much affected by the pond depth, but that the specific growth rate greatly affected.

It proves that lower pond depth can maintain higher cell density because of the less shelf-shading of light intensity and the better mixing of the medium. However, specific hydrocarbon production was changed less in high depth of the pond. It implies that algal hydrocarbons can be much secreted in the dark period of cultivation.

Table 1 is to compare the weight fraction carbons of several photosynthetic microalgae (13) since the carbon content in the cells of a microalgae can represent it's hydrocarbon productivity during a cultivation. *D. salina* 1650 has the highest carbon fraction among other algae, even higher than the well-known high hydro-carbon-producing algae, *B. braunii*. The total hydrocarbon productivity from *D. salina* 1650 in an outdoor cultivation was calculated as ca. 0.20 (g/g-cell) from Fig. 4 and 7, and which is close to the reported value of 0.205 (g/g-cell) for *Botyrococcus braunii* (3). Fig. 8 is the result of DSC analysis of hexane extracts to evaluate the algal hydrocarbons from *D. salina* as an alternative energy source. Energy contents of the extracts were compared to that of the currently used petroleum. Algal hydrocarbons had a good energy level of 141 (J/g) and a high heating temperature of 251.3℃. This shows that the hydrocarbons from *D. salina*. can be used as a substitute energy directly or indirectly through the cracking processes. Table 2 also proves this point because the composi-

tion of hydrocarbons from *D. salina* was similar to that from *B. braunii*.

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