0273-2289/98/70–72—0739$10.00

The Production of Hydrocarbons from Photoautotrophic Growth of *Dunaliella salina* 1650

DON-HEE PARK,* HWA-WON RUY,[1] KI-YOUNG LEE,[1] CHOON-HYOUNG KANG,[2] TAE-HO KIM,[2] AND HYEON-YONG LEE[3]

[1]*Department of Biochemical Engineering,* [2]*Department of Chemical Engineering, Chonnam National University, Kwangju 500-757, Korea, and* [3]*Division of Food and Biotechnology Kangwon National University, Chuncheon 200-701, Korea*

ABSTRACT

Microalga, *Dunaliella salina* 1650 was selected to produce hydrocarbons that may possibly substitute for fossil fuels in the near future. It can produce 0.22 (mg/L) of hydrocarbons over 20 d batch cultivation, maintaining 1.32 (g-dry wt./L) of cell density. Its productivity was similar to that from *Botryococcus braunii*, which was known to economically produce liquid fuels. Optimal growth conditions for the alga were also determined as pH 7.2, 28°C, and 0.00034 (Kcal/cm^2/h) of light intensity. It was shown that the hydrocarbon production from the alga was closely related to cell growth, except for the later periods of batch cultivation. Better hydrocarbon production was observed during light periods in light/dark cycle cultivation. Under chemostat conditions, maximum steady cell concentration was maintained as 1.1 (g-dry wt./L) at 0.12 (1/d) of dilution rate. The system reached to the steady state after 30 d of the cultivation. The maximum specific hydrocarbon production rate, 0.024 (mg/cell/d) was also obtained under this condition. It proves that the hydrocarbon production from *D. salina* 1650 can compete with that from *B. braunii*.

Index Entries: Algal hydrocarbon production; photoautotrophic growth; *Dunaliella salina*.

INTRODUCTION

In algal biotechnology, one of the current research interests in developing new energy resources is to produce usable liquid fuels that can

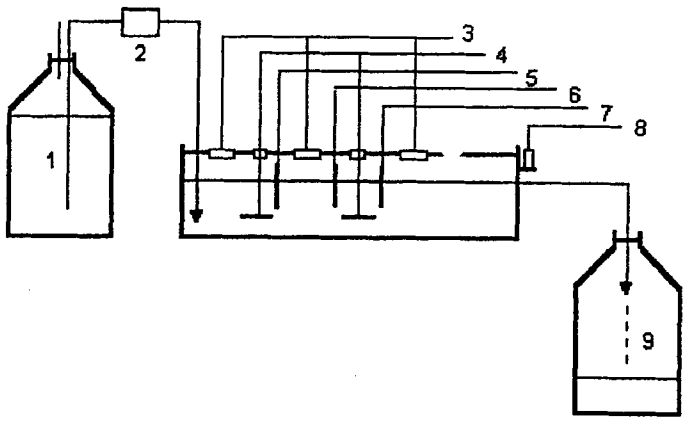
* Author to whom all correspondence and reprint requests should be addressed. E-mail: dhpark@orion.chonnam.ac.kr

substitute fossil fuels from photosynthetic algae through biological conversion of solar energy *(1–3)*. The algae can produce saturated and unsaturated hydrocarbons by assimilating carbon dioxide. The algal hydrocarbons can also be used directly or indirectly for substituting gasoline by several cracking processes *(4,5)*. It has been reported that the green alga, *Botryococcus braunii* is most promising photosynthetic organism since it can produce economic quantities of hydrocarbons by utilizing artificial or natural light *(6–8)*. It has been intensively investigated to develop a process of cultivating mass amounts of biomass in indoor and outdoor photobioreactors by using various sources of light energy. The development of mass-cultivation technologies is most economic methodology in producing biomass and the products of interest from photosynthetic algae *(9–11)*. However, the outdoor cultivation of photosynthetic algae has been less studied to produce hydrocarbons because an open-pond cultivation requires delicate elaboration of strong light intensity of solar energy, effective pH and temperature controls for long periods of the cultivation, and continuous supply of culture media with limited contamination of other species *(12)*. Therefore, in this work, we investigated the kinetics of hydrocarbon production from green alga, *Dunaliella salina* 1650, using an outdoor cultivation process *(13)*.

MATERIALS AND METHODS

The green alga, *Dunaliella salina* 1650 was originally obtained from the Algal Culture Center (UTEX, USA), and adapted by growing cells in hydrocarbon-producing medium *(13)* (pH 7.58 and 6.8% (w/v) NaCl) at 25°C with 2.3×10^{-4} (Kcal/cm^2/h) of light intensity. An open-culture system was designed for outdoor batch and continuous cultivations as shown in Fig. 1 (150 × 150 × 25-cm, total working volume was 500 L). To simulate outdoor environment, 12:12 hour light and dark cycle was used by eight 20 W white cool fluorescent lamps illuminated the pond in a dark room. The pH and temperature were not controlled for this experiment. For continuous cultivations, a peristaltic pump was used for feeding fresh medium and the effluent was collected out of the drain at the top of the pond. Two top-driven paddle mixers were also used for the agitation of the media in the pond as shown in Fig. 1. The outdoor cultivation was carried out when the change of seasonal temperature was minimum.

The light intensity was measured by a quantum sensor (Li-Cor LB-125, Yellowsprings, USA) every day at same time (noon). Cell density was measured by filtering 10 mL of the sample through 0.45-m pore-size filter paper and drying it at 105°C for 24 h. To determine the concentration of hydrocarbons within the algae, filtered medium and algae disrupted by a sonicator (Far M-150, Madison, WI) were centrifuged at 1300*g* for 20 min, then they were extracted by adding two volumes of hexane into the supernatant for 1 h at room temperature *(14)*. The extracts were dried in



1. Substrate reservoir
2. Peristaltic pump
3. Cool-white flourescent 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.

a rotary-vacuum evaporator and measured for total crude hydrocarbons. The crude hydrocarbons were purified by 110°C activated silica gel (60 GF-2.5, Merk) chromatography *(15)*, then identified by a thin-layer chromatography (TLC) with diluted commercial gasoline. The energy content of algal hydrocarbons was also estimated by differential scanning calorimeter (DSC) (DuPont 2100, Boston, MA) at 10°C/min of the heating rate up to 400°C.

RESULTS AND DISCUSSION

Figure 2 shows the effect of light intensity on the growth of *D. salina* 1650. An optimal light intensity was observed as 3.4×10^{-4} (Kcal/cm^2/h) when 0.155 (1/d) of specific growth rate was maintained at 1.32 (g-dry wt/L) of maximum cell density. At higher light intensity, cell growth rate was hampered, compared to that at lower light intensity, with 0.063 (1/d) vs 0.104 (1/d) of specific growth rate, respectively. The cell density gradually increased during 17 d of cultivation at relatively low-light intensity. Figure 3 shows the kinetics of cell growth and hydrocarbon production at 3.4×10^{-4} (Kcal/cm^2/h) of constant light intensity in batch cultivation. The hydrocarbon production seems to be closely related to cell growth except for the later periods of cultivation. The concentration of crude hydrocarbons within the cells remained constant even though overall cell density dropped. However, the hydrocarbon yield per g-dry cell was gradually increased according to the cultivation time, which implies that the produc-

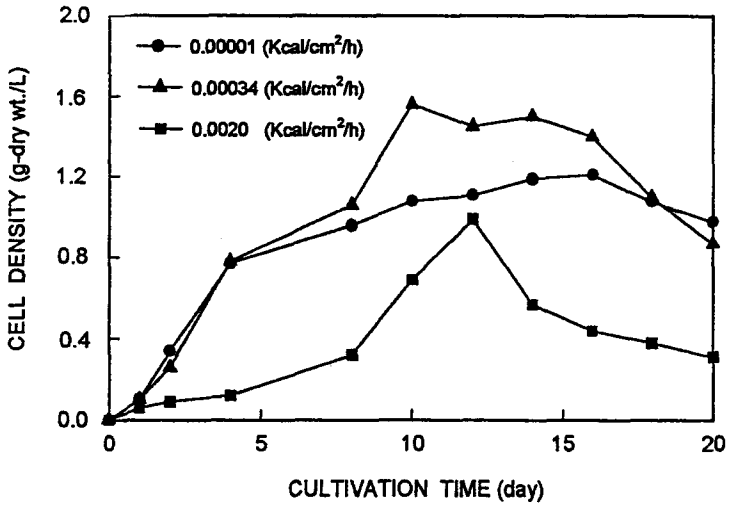


Fig. 2. The effect of light intensity on the growth of *D. salina* 1650 as a function of light intensity for batch cutivation.

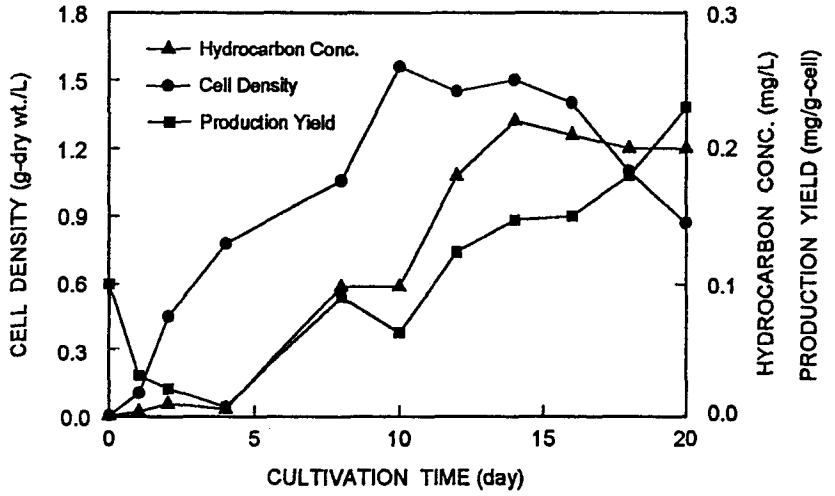


Fig. 3. The cell growth and hydrocarbon production for batch cultivation of *D. salina* 1650 at 3.4×10^{-4} (Kcal/cm^2/h) of the incidence light intensity.

tion of hydrocarbons in the cell can be continued to the last period of cultivation. The maximum specific hydrocarbon production rate was estimated as 0.029 (mg crude hydrocarbon/g-cell/d) at 0.22 (mg/g-cell) of maximum specific hydrocarbon production. 0.22 (mg/g-cell) of hydrocarbon production yield from *D. salina* 1650 in this process is close to the reported value of 0.345 (mg/g-cell) for *Botryococcus braunii (16).*

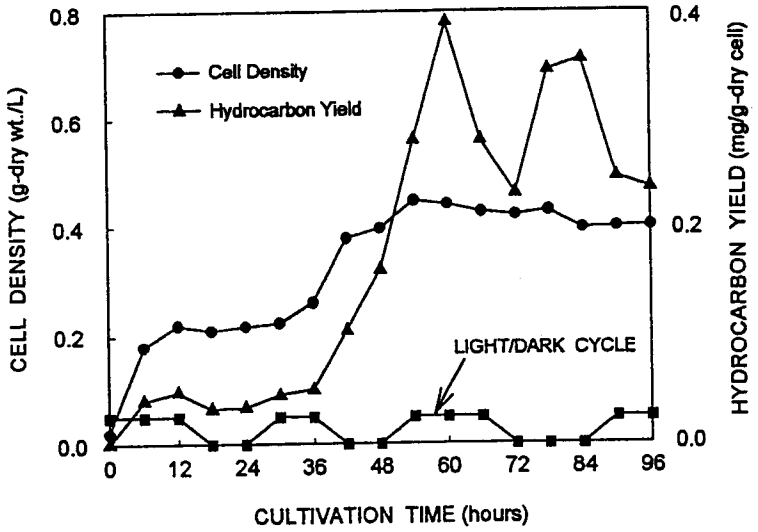


Fig. 4. The kinetics of cell growth and hydrocarbon yield for 12:12 light/dark diurnal cultivation at the light intensity of 3.4×10^{-4} (Kcal/cm^2/h).

Figure 4 is the result of cultivating the cells by a 12:12 hour light/dark-cycle process. The cell growth seems to be affected by light/dark-cycle cultivation since better cell growth was observed in light periods. However, hydrocarbon yield increased during the light phases and reached maximum value during the third light phase, then it fell. This implies that the metabolism of producing hydrocarbon within the cells lags behind the overall cell growth. This might be the reason that the hydrocarbon yield slowly responds to light/dark cycle.

Figure 5 shows the kinetics of cell growth and hydrocarbon production as well as the changes of pH at 0.12 (1/d) of dilution rate under chemostat condition. The system reached to the steady state after 40 d cultivation, based on relatively constant cell concentration and pH change, whose data well fits to chemostat theory. The hydrocarbon production could also be correlated to cell growth during the steady state. pH of the medium was dropped in later periods of the cultivation because of the decrease of the cell growth. Figure 6 illustrates the relationship between cell growth and specific hydrocarbon-production rate according to dilution rate by 12:12 light/dark-cycle process. The cell growth remained stable at relatively wide ranges of dilution rates. The wash-out dilution rate was observed 0.3 (1/d). The maximum specific hydrocarbon production rate was calculated as 0.024 (mg/g-cell/d) at 0.12 (1/d) of dilution rate. Therefore, for maximum hydrocarbon production an optimal operating condition can be determined as 0.12 (1/d) of dilution rate, having 1.11 (g-dry wt./L) of cell density. It also proves that long-term outdoor cultivation is possible for green alga, *D. salina* 1650 at relatively wide ranges of dilution rate (0.05–0.25 1/d of dilution rate).

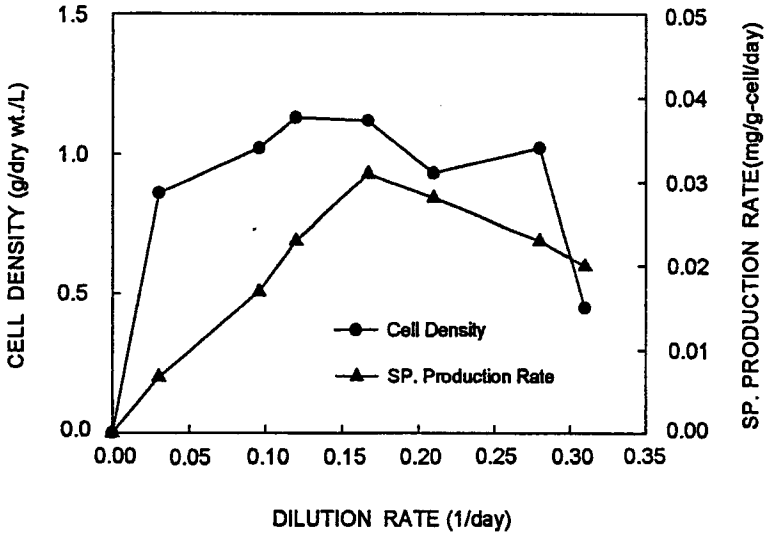


Fig. 5. The changes of cell density, hydrocarbon concentration, and pH at 0.12 (1/d) of dilution rate in 12:12 light/dark cycle continuous cultivation.

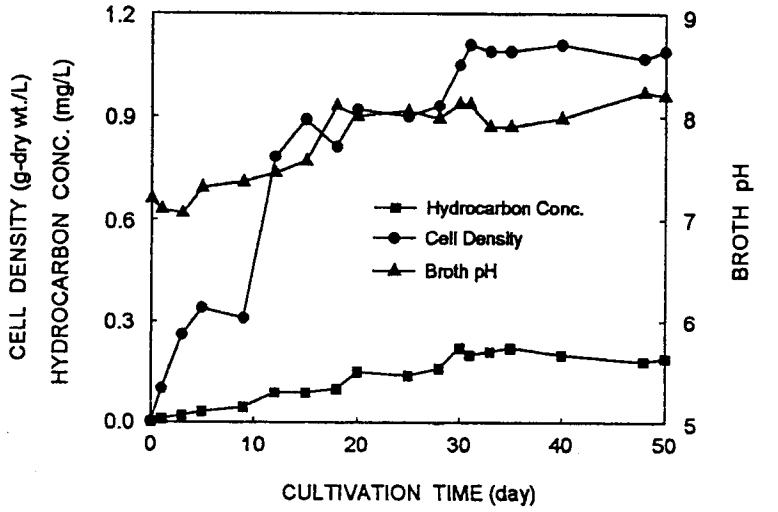


Fig. 6. The relationship between cell density and specific-hydrocarbon production rate as a function of dilution rate.

Table 1 is to compare the elemental analysis of high-hydrocarbon-producing microalgae since the carbon content in a cell can represent the hydrocarbon productivity during the cultivation. *D. salina* 1650 has relatively high carbon fraction among other algae, even the well-known high-hydrocarbon-producing algae, *B. braunii*. Figure 7 is the result of DSC analysis of hexane extracts for evaluating algal hydrocarbons from *D. salina* as an alternative energy source. Energy contents of the extracts was com-

Table 1
The Results of Elemental Analysis of Hexane-Extracted Hydrocarbons from Several 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	(7)
Spirulina sp.	66.86	10.37	10.05	0.43	(10)

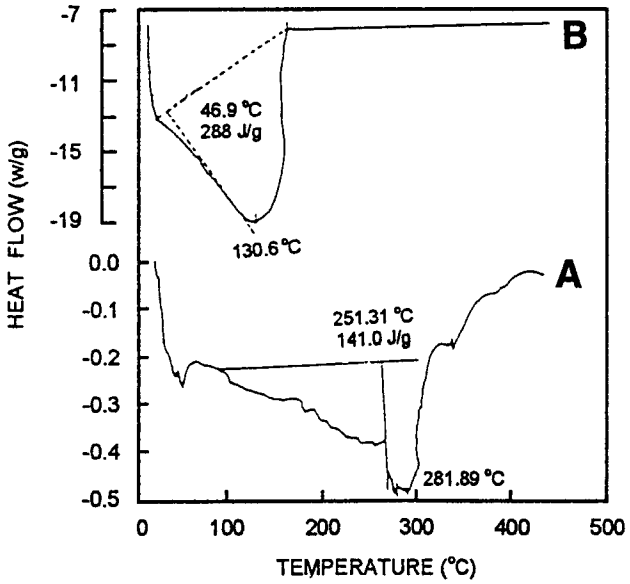


Fig. 7. The thermogram of DSC analysis for algal hydrocarbons from *D. salina* 1650 **(A)** and petroleum **(B)**.

pared to that of currently used petroleum. Algal hydrocarbons have good energy level as 141 (J/g) and high heating temperature at 251.3°C. It tells that the hydrocarbons from *D. salina* can be used as a substitute energy directly or through cracking processes.

ACKNOWLEDGMENT

This paper was supported by Special Fund (on the studies of biofuel production) for University Research Institute, Korea Research Foundation. The authors deeply appreciate their financial support.

REFERENCES

1. Bailliez, C., Largeau, C., Berkaloff, C., and Casadevall, E. (1986), *Appl. Microbiol. Biotechnol.* **23,** 361–366.

2. Bailliez, C., Largeau, C., Casadevall, E., Yang. L., and Berkaloff, C. (1988), *Appl. Microbiol. Biotechnol.* **29,** 141–147.
3. Barclay, W. and McIntosch, R, eds., (1986), *Algal Biomass Technologies: An Interdisciplinary Perspective,* Berlin-Stuttgart, Berlin, pp. 126.
4. Benemann, J. R. (1987), *Microalgae Biotechnology: Products, Processes and Opportunities,* vol. 2, OMEC, Washington, DC.
5. Brown, A. C. and Knights, B. A. (1969), *J. Phytochem.* **8,** 543–549.
6. Cane, R. and Albion, P. R. (1973), *Geochim. Cosmochim. Acta.* **37,** 1543–1550.
7. Casadevell, E., Largeau, D. T., Guidin, C., Chaumount, D., and Desanti, O. (1985), *Biotechnol. Bioeng.* **27,** 286–295.
8. Freeman, C. P. and West, D. (1966), *J. Lipid Res.* **7,** 324–329.
9. Frenz, J., Largeau, C., Casadevall, E., Kollerup, F., and Dougulis, A. (1989), *Biotechnol. Bioeng.* **34,** 755–762.
10. Lee, H. Y., Erickson, L. E., and Yang, S. S. (1987), *Biotechnol. Bioeng.* **29,** 832–841.
11. Lee, Y. K. and Low, C. S. (1991), *Biotechnol. Bioeng.* **38,** 995–1000.
12. Meier, R. L. Solar Energy Research. F, Daniels, and J. A. Duffies, eds. (1986), *Microalgal Culture, Crit. Rev. Biotechnol.* **4,** 369–438.
13. Pak, J. H., Lee, S. Y., Shin, W. C., and Lee, H. Y. (1991), *J. Microbiol. Biotechnol.* **1,** 111–115.
14. Schelef, G. and Soeder, C. J., eds., (1980), *Algal Biomass and Production and Use,* Elsevier/North-Holland Biomedical Press, Amsterdam, p. 852.
15. Wolf, F. R., Nonomura, A. M., and Basshan, J. A. (1985), *J. Phycol.* **21,** 388–394.