Effects of Environmental Factors on the Extracellular Release of Photosynthetic Products by *Scenedesmus quadricauda*

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*Scenedesmus quadricauda*에 의한 광합성 산물의 세포 외 배출에 미치는 환경요인의 영향

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ABSTRCT: The effects of environmental factors on the extracellular release of organic carbon by *Scenedesmus qudricauda* were studied. The PER (percentage extracellular release) was greater at high temperature and at high concentration of nitrogen and phosphate. The PER variation according to the change of N/P ratio showed high values at each extreme N/P ratio. This result suggested that the limitation of nitrogen or phosphorous resulted in the accumulation of carbohydrates as photosynthetic products, and the products in high concentration were excreted through algal cell membrane.

KEY WORDS □ ***Scenedesmus quadricauda*, photosynthetic products, extracellular release environmental factors.**

The influx of organic matter in aquatic ecosystems can be divided into two groups. These are influx of allochthonous organic matters from terrestrial ecosystems and human activities, and that of autochthonous organic matters produced by primary producers in water column. Natural phytoplankton communities play an important role in supplying the carbon and energy source to the food chain, and determine, thus, the structure and function of the ecosystems. The organic carbon released by phytoplankton can be used by bacterioplankton, and thus the carbon and energy flow in natural ecosystems can integrated into "microbial loop" (Azam *et al.*, 1983).

After the introduction of C^{14} method in measuring of primary production of phytoplankton (Steeman Nielsen, 1952), it has been reported that healthy algal cells release organic matters extracellularly during photosynthesis. But it was accepted as a real phenomenon in natural ecosystems after the long controversy and many experiments (Sharp, 1977; Goldman and Dennett, 1985; Jensen and Sondergarrd, 1985).

The excretion of organic matters was studied in natural ecosystems and through the laboratory experiments, and the results were reported as PER (percentage extracellular release, i.e. the released organic matter as a percentage of the total pri-

mary production). *Skeletonema costatum* release 7% (Bell *et al.*, 1974), 8-10% (Bell and Sakshaug, 1980), or 2.6-5.5% (Williams and Yentsch 1976) of total primary production. It has been measured that phytoplankton release organic matters ~ 70% in freshwater ecosystems and ~ 45% in marine ecosystems (Sharp 1977; Jensen and Sφndergaard, 1985; Sφndergard and Schierup, 1982; Blaauboer *et al.*, 1982; Conveney, 1982).

In natural ecosystems, heterotrophic bacteria can uptake and consume the release organic matters, and the PER may be understimated. Thus the inhibition of heterotrophic activity of bacteria need to measure the correct PER. Jensen and Sφndergaard (1985) used antibiotics to inhibit heterotrophic uptake of EOC (Extracellular organic carbon) by bacteria, and observed that the PER was about 50%.

These results suggest that the PER and quantity of exudates are highly affected by environmental factors (Lancelot 1983). The PER is great in high light intensity and in low cell density (Sharp, 1977), and at low temperature and in the deficiency of inorganic nutrients (Fogg 1983). In this study, the effects of environmental factors on the release of organic matters of phytoplankton *Scenedesmus quadricauda* was measured as the variation of PER. As environmental factors, temperature, nitrogen, and phosphorous were varied.

MATERIALS AND METHODS

Axenic culture of *Scenedesmus quadricauda* isolated from Lake Soyang (the largest man-made lake in Korea) was obtained from Prof. Young-Nam Hong Department of Botany, Seoul National University, and was grown in Chu 10 medium (Chu, 1942) at 140 μE/m^2/s constant fluorescent illumination and 25°C. Routine tests for bacterial contamination were done with acridine orange direct count (AODC) method (Watson *et al.*, 1977).

20 μCi of $NaH^{14}CO_3$ (NEN, 55.5 mCi/mmole) was added to 250 m*l* algal cultures in 500 m*l* flask. To measure the time-dependent variation of the PER and total primary production 10 m*l* aliquot of algal cultures were removed and filtered through 0.45 μm pore size filter (Gelman membrane) under low vacuume pressure (< 100 mmHg) at each time. Filters were fumed with HCl in dessicator for 3 min (Parsons *et al.*, 1984) to remove precipitated inorganic carbon and transferred to scintillation vials containing 10 m*l* of scintillation cocktail.

The composition of scintillation cocktail was PPO (Sigma) 5g, POPOP (Sigma) 0.25g and Naphthalene (Junsei, Japan) 100g in 1000 m*l* of 1, 4-dioxane (Junsei). The radioactivity in the samples was determined with Packard Tri-Cab liquid scintillation spectrometer model 1500 (Packard Instrument Co.). Filtrates (5 m*l*) were recovered in glass test tubes, acidified to pH 2 by addition of 1 N HCl, and bubbled with air for 20 min to remove all inorganic carbon (McFeters *et al.*, 1978). Then, 1 m*l* of the filtrates were transferred to scintillation vial with 10 m*l* of cocktail, and measured the radioactivity.

Percent release of photosynthetic products was calculated as follows.

$$\text{PER} = \frac{\text{radioactivity in filtrate (dpm/10 ml)} \times 100}{\text{radioactivity in filter (dpm)} + \text{filtrate (dpm/10 ml)}}$$

The effects of environmental factors on extracellular release of organic carbon by *Scenedesmus quadricauda* were examined through the change of temperature and concentrations of nitrogen and phosphorous in the culture medium. For the study of the effect of temperature, the cultivations of algae were done under 20 and 25°C for 7days and the variation of PER and total carbon incorporation were measured in 24 h intervals. The concentration of nitrogen adjusted with urea were 35, 70, 140, 280 mg/*l* and the concentration of phosphorus adjusted with phosphate were 0.93, 9.30, 93.0, 930, and 9300 mg/*l*.

RESULTS

Effect of temperature

The percenet release of organic carbon by *Scenedesmus quadricauda* at 20 and 25°C are shown in Fig. 1. The total incorporation and PER were higher at 25°C at than 20°C. At the early stage the values of PER from the two cultures were very similars, but the difference enlarged after 2nd day. After 7days the PER at 20 and 25°C were 11.7 and 13.5%, respectively.

Effect of Nitrogen

Concentrations of nitrogen in culture media were 35, 70, 140, and 280 mg-N/*l*, and correspon-

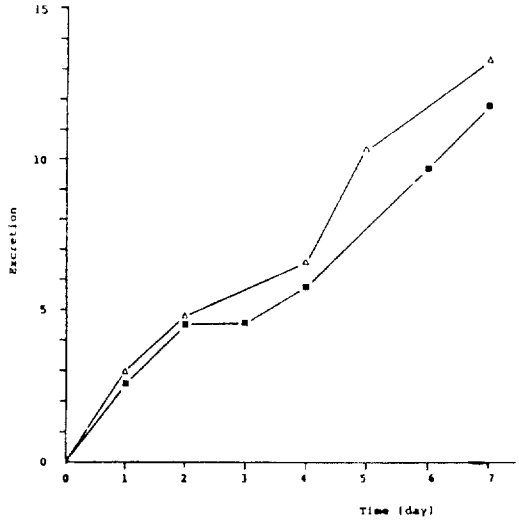

Fig. 1. *Effect of temperature on percent excretion of organic matter in Scenedesmus quadricauda.*

ding N/P ratio were 0.38, 0.75, 1.51, and 3.01, respectively. Until 120 h the difference among each concentration was not significant but in the case of concentration at 35 mg/*l* the PER was 22.2% in 7day which was 10% higher than others. The PER of *S. qadricauda* at 70, 140, 280 mg/*l* on 7th day were 10, 14 12% respectively.

Effect of Phosphorus

Concentrations of phosphate in culture media were 0.93, 9.30, 93.0, 930, and 9300 μg-P/*l*, and corresponding N/P ratio were 150537, 15054, 1505.4, 150.5, and 15.1, respectively. When N/P ratio was higher than 150, the growth rate was low and PER was great. As a distinctive feature very rapid increases in PER were observed at low phosphate concentrations and followed by gradual decrease. But at high concentrations the increases of PER were not rapid and the decrease were moderate. After 6 days the patterns of extracellular release of organic carbon appeared in three kinds. First, the PER at the concentration of 0.93 μg-P/*l* was very great and more than 66%. Second, the PER was in the range of 31-46% at the cocnentration of 9.30-930 μg-P/*l*. Finally, the PER was not exceed

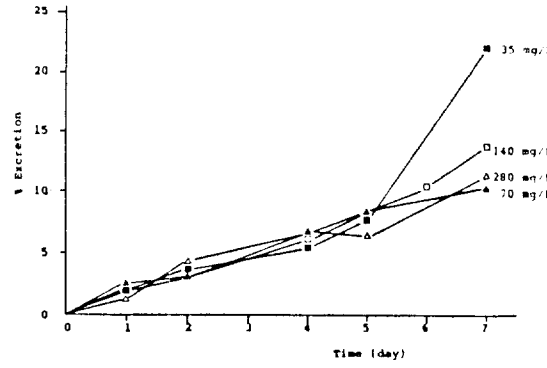

Fig. 2. *Effect of concentration of nitrogen on percent excretion of organic matter in Scenedesmus quadricauda.*

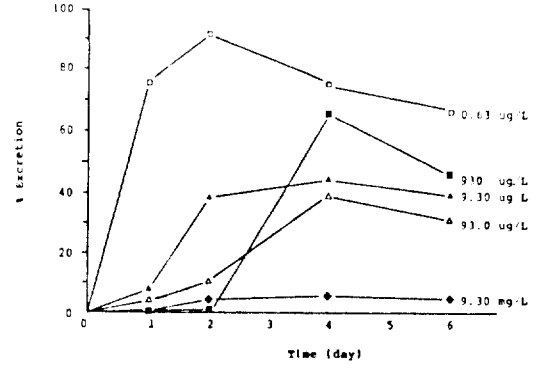

Fig. 3. *Effect of the concentration of phosphate on the percent excretion of Scenedesmus quadricauda.*

6% at 9300 μg-P/*l*.

Effect of N/P Ratio

The variation of PER according to the change of N/P ratio showed maximum at either extremes and minium at mid point 15.1. Either high phosphate or high nitrogen concentration made the algal nutrition unbalanced, and made the algal cells excrete much photosynthetic products.

DISCUSSION

The excretion of organic carbon by phytoplankton has been extensively studied, but due

Table 1. *The variation of PER based on the change of N/P ratio*

N/P	0.38	0.75	1.51	3.01	15.1	150.5	1505.4	15054	150537
PER(%)	22.2	10	14	12	6	46	31	41	66

to methodological problems the physiology and mechanism of exudation are not fully understood. The PER as a percentage of total photoassimilated carbon is variable. The PER reported distributed in the range of 0-70%. This great variation may be partially due to the algal cell breakage during filtration (Goldman and Dennet, 1985), but the possibility of effects of environmental factors on the extracellular release of organic carbon by phytoplankton cannot be ignored.

It has reported that PER was increased in high light intensity and low cell density (Sharp, 1977; Fogg, 1983) and in the depletion of inorganic nutrients (Fogg 1983). In this studies, the PER was higher at high temperature and at high and low N/P ratio. Compared with the results reported by Watanabe (1980) that higher PER at low temperature in lake water phytoplankton, the results of effect of temperature on PER from our experiment were contrary to it. But in laboratory experiments with batch culture, due to the fast growth of algae the depletion of inorganic nutrients could occur at higher temperature. The time-course experiments for 7 days, first 2 day cultures showed no severe difference between 20 and 25°C, but the difference in PER increased after 4 day.

The effect of inorganic nutrients on the extracellular release of organic carbon by *S. quadricauda* showed that the PER measured was higher at low concentration of either nitrogen or phosphorous. This increased PER by the depletion of nitrogen (Joris *et al.*, 1982; Goldman *et al.*, 1979) and phosphorous (Myklestad, 1977) can be interpreted as a metabolic feedback between algae and bacteria (Smith and Higgens, 1978). Azam and Cho (1987) suggested that the organic carbon released by phosphrous- or nitrogen-limited phytoplankton may be used as attractants for heterotrophic bacterial chemotaxis, and the increased abundance of bacteria results in a high rate of N and P mineralization.

Since it is possible that the concentration of nitrogen and/or phosphorous in culture medium can altered the C/N/P ratio of algal biomass (Lancelot and Billen, 1985), the variation of N/P ratio in culture medium may change the phytoplankton physiology, and the PER, thus, may increase. The extremely low or high N/P ratio in medium may result in unbalanced growth that is high C/N or C/P ratio in algal biomass. Thus the high PER with the depletion of inorganic nutrients can be explained by the hypothesis that phytoplankton in N- or P-limited condition excrete organic carbon with the purpose of increased mineralization of N and P by attracting bacteria. And if most of the immediate photosynthetic products are carbohydrates, the limitation in one of the inorganic nutients may prevent the incorporation of the photosynthetic products into macromolecules and algal biomass. By this process the accumulation of simple carbohydrates may occur, and high concentration of simple carbohydrates in cytoplasm of algal cells will be secreted through the cell membrane (Bjϕrnsen, 1988). Thus it can explain the relative high C/N ratio of exudate in comparison with that of particulate production (Lancelot and Billen, 1985).

적 요

*Scenedesmus quadricauda*에 의한 광합성 산물의 세포 외 배출에 미치는 환경요인의 영향을 조사하였다. PER(총 광합성량에 대한 세포 외 배출의 백분율)은 온도가 높을 때와 질소와 인의 농도가 높을 때 높게 나타났다. 질소와 인의 비율에 따른 PER의 변화는 비율이 아주 낮거나 아주 높을 때 높게 나타났다. 이러한 결과는 식물성 플랑크톤의 세포 내에 광합성 산물로서의 탄수화물의 축적이 일어나고, 높은 농도의 유기물이 세포막을 통해 세포 밖으로 배출됨을 시사한다.

ACKNOWLEDEGMENTS

This work was partly supported by the Basic Science Research Institute Program, Ministry of Eduction, 1988.

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(Received, November 2, 1989)