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Growth Responses of *Spirulina platensis* to Some Physico-Chemical Factors and the Kinetics of Phosphorus Utilization

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The growth responses of *Spirulina platensis* NIES-46, a brackishwater strain originally isolated from Lake Texcoco Mexico, to some physico-chemical factors and nutrients were investigated. The optimum conditions for growth were the following: light intensity of 160 μE m^{-2} sec^{-1}, temperature of 30°C, pH 10, and chlorinity of 0.55‰. NIES-46 strain could utilize both inorganic and organic phosphorus sources. Values on the different growth parameters for orthophosphate and other organic phosphorus sources were as followings: half-saturation constant of 0.02–0.07 mg-P/*l*; maximum growth rate of 0.8–1.0/d; minimum cell quota of 0.08–0.32 pg-P/cell, and level for saturated growth yield of 0.3–1.0 mg-P/*l*. The result that this species utilized effectively a rather wide range of both inorganic and organic phosphorus and showed a high growth rate suggests that mass production of this species is possible by recycling organic waste.

Key words: *Spirulina platensis*, growth kinetics, physico-chemical factors, organic phosphorus, waste water

Spirulina, a blue-green alga known for its high nutritive value, exists in freshwater, brackishwater, and seawater habitats. It grows in warm temperature and tropical regions and is reported to grow abundantly in alkaline waters in salty lakes.[1,2]

Previous work by Watanabe and Ichimura[3] have assessed morphologically the differences between freshwater and saltwater forms of *Spirulina*. Recently, *Spirulina* strains were characterized from its growth physiology and biochemical composition.[4] Using growth kinetics studies approach, the physiological growth characteristics of a freshwater strain of *Spirulina platensis* were determined.[5,6] In this paper, the growth responses of *Spirulina platensis* NIES-46, a brackishwater strain, to some physico-chemical factors and the kinetics of phosphorus utilization were described. This study is important in providing baseline information for the mass culture of *Spirulina*.

Materials and Methods

Culture Organisms and Incubation

The axenic stock culture of *Spirulina platensis* NIES-46 was obtained from the Microbial Culture Collection of the National Institute for Environmental Studies. This was initially incubated at 25°C under the light of 80 μE m^{-2} sec^{-1}, 14:10 LD cycle, in SP medium.[7]

The growth of *S. platensis* NIES-46 strain in various incubation conditions was determined by either chlorophyll *a* (chl. *a*) concentration or cell density.

Effects of Light Intensity, Temperature, pH, and Chlorinity

The stock culture of NIES-46 strain in its exponential growth phase was used for the different experiments. About 5 m*l* of the stock culture was inoculated into Erlenmeyer flasks containing 100 m*l* of SP medium. Cultures with an initial chl. *a* content of 38.1 μg/*l* were grown at a temperature of 25°C in various light intensities: 8, 24, 40, 80, 120, and 160 μE m^{-2} sec^{-1} at 14:10 LD cycle. The strain was also incubated at different temperatures: 15, 20, 25, 30, 35, and 40°C. The cultures were illuminated at 96 μE m^{-2} sec^{-1}, 14:10 LD cycle. The effects of varying pH ranged between 7 to 12 was investigated. The salinity tolerance was also tested using NaCl of up to 16.61‰ chlorinity. In both experiments, the cultures were incubated at 30°C under a light intensity of 160 μE m^{-2} sec^{-1}, LD cycle of 14:10, because these conditions gave the maximum growth that was obtained in the first two experiments.

Utilization of Various Phosphorus Sources and Kinetics Studies

S. platensis NIES-46 strain in axenic condition was incubated in SP medium with the following as a sole phosphorus source: K_2HPO_4 (PO_4-P), β-glycerophosphate disodium salt (glycero-P), D-fructose-1,6 diphosphate tetrasodium salt (fructose-P), cytidine-2′3-monophosphate (cytidine-P), guanosine 5′-phosphate sodium salt (GMP), adenosine-5′-diphosphate sodium salt (ADP-P), adenosine-5′-triphosphate (ATP-P), and phosphocreatine disodium salt (creatine-P). These compounds were tested for utilization at concentration of 0.1 to 100 mg-P/*l*. The culture grown in SP medium without any phosphorus source was used as the control.

Prior to kinetic study experiments, axenic cultures of *Spirulina* were prepared for complete starvation. About 10 m*l* of *Spirulina* culture in its exponential growth phase

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was centrifuged and washed three times with phosphorus-free medium. After the last washing, the cells were resuspended into 100 m*l* phosphorus-free SP medium, afterwhich about 1 m*l* of the suspension was inoculated into 100 m*l* phosphorus-free SP medium. The culture was allowed to grow for one week, and then 1 m*l* of the culture was transferred to fresh phosphorus-free SP medium. After 9 days of incubation, when the cell density was constant or slightly decreasing, the cells were supposedly completely starved with phosphorus. Four phosphorus compounds (PO_4-P, glycero-P, ADP-P, and creatine-P), which were found to support growth of the NIES-46 strain at a wide range of concentration, were used for the study of growth kinetic analysis. Phosphorus-starved cells were inoculated into 100 m*l* SP medium containing the different P-sources at concentrations of 0.001 to 10 mg-P/*l*. The initial density of NIES-46 was 6.2×10^2 cells/m*l*. All experiments were conducted under a light intensity of 160 μE m^{-2} sec^{-1}, 14:10 LD cycle, at 30°C. The pH was maintained between 9.2 to 9.6 during the incubation. The growth kinetic parameters, half-saturation constant (K_s) and maximum growth rate (μ_{max}) were determined by using the Woolf's equation, and the minimum cellular quota (Q_0) from the relationship between cell yields and the initial concentration of the limited nutrient as previously described.[5]

Results

Effects of Light Intensity, Temperature, pH, and Chlorinity

Growth of *Spirulina platensis* NIES-46 on various light intensities was illustrated in Fig. 1. The growth was comparable at 8 and 24 μE m^{-2} sec^{-1}, and slightly increased at 40 μE m^{-2} sec^{-1}. The optimal growth was obtained at 160 μE m^{-2} sec^{-1}, however, good growth was achieved between 80 to 160 μE m^{-2} sec^{-1}.

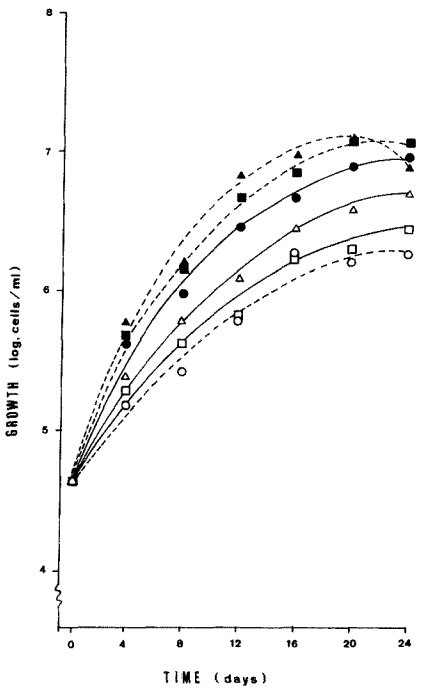

Fig. 1. Growth of *Spirulina platensis* NIES-46 strain in various light intensities at 25°C.
○: 8 μE m^{-2} sec^{-1}, □: 24, △: 40, ●: 80, ■: 120, ▲: 160.

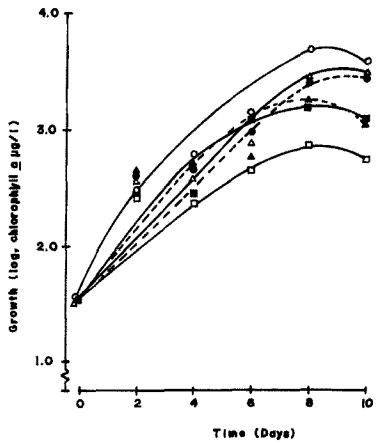

Fig. 2. Growth of *Spirulina platensis* NIES-46 strain at various temperatures in the light intensity of 96 μE m^{-2} sec^{-1}.
▲: 15°C, △: 20°C, ●: 25°C, ○: 30°C, ■: 35°C, □: 40°C.

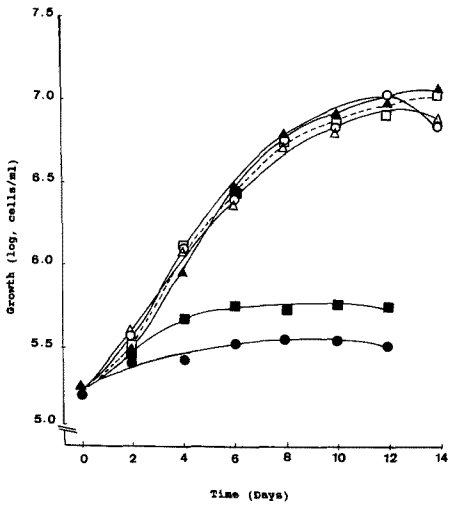

Fig. 3. Growth of *Spirulina platensis* NIES-46 strain at various pH levels at 30°C, 160 μE m^{-2} sec^{-1}.
△: pH 7.0, ○: 8.0, □: 9.0, ▲: 10.0, ■: 11.0, ●: 12.0.

Among the different temperature levels tested, growth was optimal at 30°C, whereas comparably good growth was obtained between 20 to 30°C (Fig. 2). At 35°C no further increase in chl. *a* was observed after one week of incubation, and remarkably worse growth was observed at 40°C, in which temperature lysis of cells was evident in the culture.

In Fig. 3 was shown the growth of NIES-46 strain at various pH levels. Growth was comparably good between pH 7 to 10. However, poor growth was exhibited at higher pH level of 11 and 12. Unfortunately, growth at pH under 7 was not investigated, it is suggested that *S. platensis* NIES-46 grows preferably in a slightly alkaline environment.

NIES-46 strain could tolerate a wide range of chlorinity, that is, from 0 up to 16.61‰ (Fig. 4). Better growth was obtained between 0.55 to 9.41‰, with the best growth at chlo-

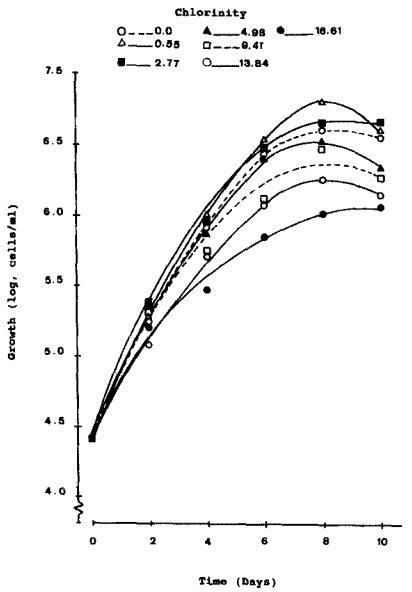

Fig. 4. Growth of *Spirulina platensis* NIES-46 strain at various chlorinity levels at 30°C, 160 μE m^{-2} sec^{-1}.
○: 0.0‰, △: 0.55, ■: 2.77, ▲: 4.98, □: 9.41, ○: 13.84, ●: 16.61.

rinity of 0.55‰.

Utilization of Phosphorus Sources and Their Kinetic Analysis

Table 1 shows the utilization of various phosphorus sources by *S. platensis* NIES-46. All phosphorus compounds were utilized except at high concentration (100 mg-P/*l*) of GMP-, ADP-, ATP-, and creatine-P. The maximum cell yields of PO_4- and fructose-P (100 mg-P/*l*), and glycero- and CMP-P (10 mg-P/*l*) were more or less comparable.

The growth parameters of NIES-46 strain for the different phosphorus sources are presented in Table 2. Among the phosphorus sources utilized, nearly similar K_s and μ_{max} values were observed for glycero-, ADP-, and creatine-P, whereas slightly high K_s and lower μ_{max} were obtained for PO_4-P.

To compare the response of NIES-46 strain for different P-concentrations of organic (glycero-P) and inorganic (PO_4-P) sources, the specific growth rates, mean generation times, and maximum cell yields are shown in Table 3. The specific growth rate and the maximum cell yield were highest at 5 mg-P/*l* of both glycero- and PO_4-P. The saturated concentration of glycero-P for the growth yield was some 0.3 mg-P/*l* and that of PO_4-P was 1.04 mg-P/*l* (Fig. 5). The minimum cell quota (Q_0) of phosphorus as derived from these figures was much lower in glycero-P than in PO_4-P (Table 2).

Table 1. Utilization of various phosphorus compounds by *S. platensis* NIES-46

Source	Range with observed growth*1 (mg-P/*l*)	Maximum growth yield ($\times 10^6$ cells/m*l*)
K_2HPO_4	0.1–100	3.64 (100)*2
β-Glycerophosphoric acid disodium salt	0.1–100	3.50 (10)
D-Fructose-1,6-diphosphate tetrasodium salt	0.1–100	3.52 (100)
Cytidine-2′3-monophosphate	0.1–100	3.20 (10)
Guanosine-5′-phosphate sodium salt	0.1–10	1.17 (1.0)
Adenosine-5′-diphosphate sodium salt	0.1–10	2.67 (10)
Adenosine-5′-triphosphate disodium salt	0.1–10	3.08 (10)
Phosphocreatine disodium salt	0.1–10	1.55 (10)

*1 Concentration range is from 0–100.
*2 The value in parentheses represents the concentration (mg-P/*l*) with the maximum growth yield.

Table 2. Growth parameters of *Spirulina platensis* NIES-46 for phosphorus

Phosphorus source	K_s (mg P/*l*)	μ_{max} (/day)	Q_0 (pg P/cell)	Phosphorus level for saturated growth yield (mg P/*l*)	Maximum cell yield (log, cells/m*l*)
Orthophosphate	0.07	0.80	0.26	1.04	6.6
Glycerophosphate	0.04	0.84	0.08	0.30	6.5
Adenosine diphosphate (ADP)	0.02	0.98	0.32	0.46	6.2
Phosphocreatine	0.03	1.0	0.14	0.60	6.6

Table 3. Specific growth rates, mean generation times, and maximum cell yields of *Spirulina platensis* NIES-46 in different concentrations of β-glycerophosphate (glycero-P) and K_2HPO_4 (PO_4-P)

P conc. (mg-P/*l*)	Specific growth rate (day^{-1})		Mean generation time (days)		Maximum cell yield (log, cells/m*l*)	
	glycero-P	PO_4-P	glycero-P	PO_4-P	glycero-P	PO_4-P
10	0.822	0.789	0.84	0.88	6.6	6.7
5	0.956	0.836	0.72	0.83	6.8	6.7
1	0.866	0.716	0.80	0.97	6.4	6.3
0.5	0.845	0.469	0.82	1.48	6.5	6.2
0.1	0.737	0.463	0.94	1.50	6.1	5.1
0.05	0.638	0.391	1.09	1.77	5.7	5.0
0.01	0.603	0.375	1.15	1.85	5.3	4.5
0.005	0.518	0.230	1.34	3.01	4.7	4.0
0.001	0.449	0.219	1.54	3.16	4.2	3.8

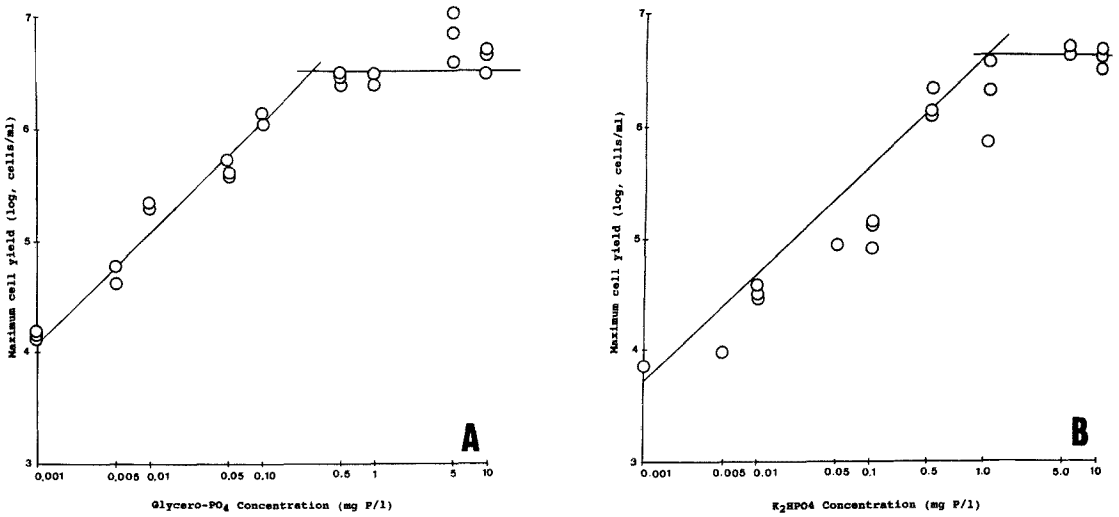

Fig. 5. Maximum cell yields of *Spirulina platensis* NIES-46 strain at different concentrations of glycerophosphate (A) and K_2HPO_4 (B).

Discussion

Within the range of light intensity tested, *Spirulina platensis* NIES-46 strain exhibited good growth at between 80 to 160 μE m^{-2} sec^{-1} with the best growth at 160 μE m^{-2} sec^{-1} (Fig. 1). This indicates that NIES-46 strain has high light saturation capacity. Vonshak[4] mentioned that *Spirulina* with high light saturation values showed remarkable production rates when grown outdoors. Thus, the better response of NIES-46 for the higher light intensity could be one of the most suitable characteristics of the strain for outdoor cultivation.

Considering that the optimal temperature for growth of NIES-46 was between 20 to 30°C (Fig. 2), this alga can be classified as mesotrophic. According to Richmond,[8] the minimum temperature which permitted some growth in *Spirulina* sp. was about 18°C, although the optimal temperature was between 35 and 37°C. It was also reported that a thermophilic strain of *Spirulina maxima* had the optimum growth temperature of between 30 to 40°C in a synthetic medium and between 25 to 35°C in the Greenway effluent.[9] In the present study, the optimal growth temperature of NIES-46 strain did not differ so much from these findings of other *Spirulina* strain.

NIES-46 strain can grow at a wider pH range of 7 to 10 with the best growth at pH 10, indicating that this strain has a preference to a more alkaline condition. According to Rippka *et al.*,[10] the great majority of cyanobacteria favored a neutral to alkaline pH (7 to 10), however, drastic growth inhibition was observed at pH 11 in *S. maxima*.[9] In the present study, the growth of NIES-46 strain was also inhibited at pH 11 (Fig. 3).

S. platensis NIES-46 showed wider tolerance for salinity (0.55–16.61‰ as chlorinity) (Fig. 4), which had been observed in *S. platensis* M-185 (0.61–16.61‰ as chlorinity).[3] Since NIES-46 strain was originally isolated from the brackishwater environment, the ability to adapt to increasing salt concentration appeares to be one of its better advantages over other strains. This would suggest NIES-46 strain's better chance to survive and grow in waters with elevated salinity when cultivated outdoors.

NIES-46 strain could utilize efficiently both inorganic and various types of organic phosphorus sources for growth at a concentration of up to 10 mg-P/*l* (Table 1). These results were nearly similar to those reported by Venkataraman,[11] that is, the range of phosphorus concentration for the growth of different species varies from 8.9 to 17.8 mg-P/*l* and higher concentrations inhibited the growth. In fact, very low concentration of phosphorus is sufficient for algal growth. A previous report[12] mentioned that 1 μg-P/*l* would support about 1.6×10^7 cells/*l* of *Asterionella formosa*.

The kinetic parameters for phosphorus utilization indicated that organic phosphorus is equally effective or better than the inorganic for the growth of NIES-46. The lower K_s and higher μ_{max} for organic phosphorus suggest that organic-P can be utilized more efficiently and gives an advantage for mass cultivation by using organic waste water. Consequently, this would promote recycling of organic wastes and minimize the cost of algal production; such that *Spirulina* is cultivated in a pond fertilized by animal manures and is harvested and fed to fish.

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