CHAPTER III

RESULTS

Growth of *Aphanothece halophytica*

Aphanothece halophytica is a short cylindrlical shape cyanobacterium covered with mucous membrane as shown in Figure 4. The growth of *A. halophytica* did not exhibit a lag phase and had a specific growth rate of 0.425 day^{-1} (Figure 5). The relatonship between optical density at 650 nm (OD_{650}) and dry weight of *A. halophytica* was found to be linear (Figure 6).

Method employed for the extraction of phycocyanin from *Aphanothece halophytica*

Many preliminary experiments were done to obtain the best method of extracting phycocyanin from *A. halophytica*. The methods used were lysozyme digestion and freeze thaw (Boussiba and Richmond , 1979). The results from Figure 7 indicate that phycocyanin extraction by lysozyme digestion gave higher yield than that by freeze thaw.Phycocyanin content yielded 95.93 and 27.54 mg/g dry weight at day 10 when extracted by lysozyme digestion and freeze thaw, respectively (Figure 7). Therefore , in later experiments lysozyme digestion was selected as a method for phycocyanin extraction.

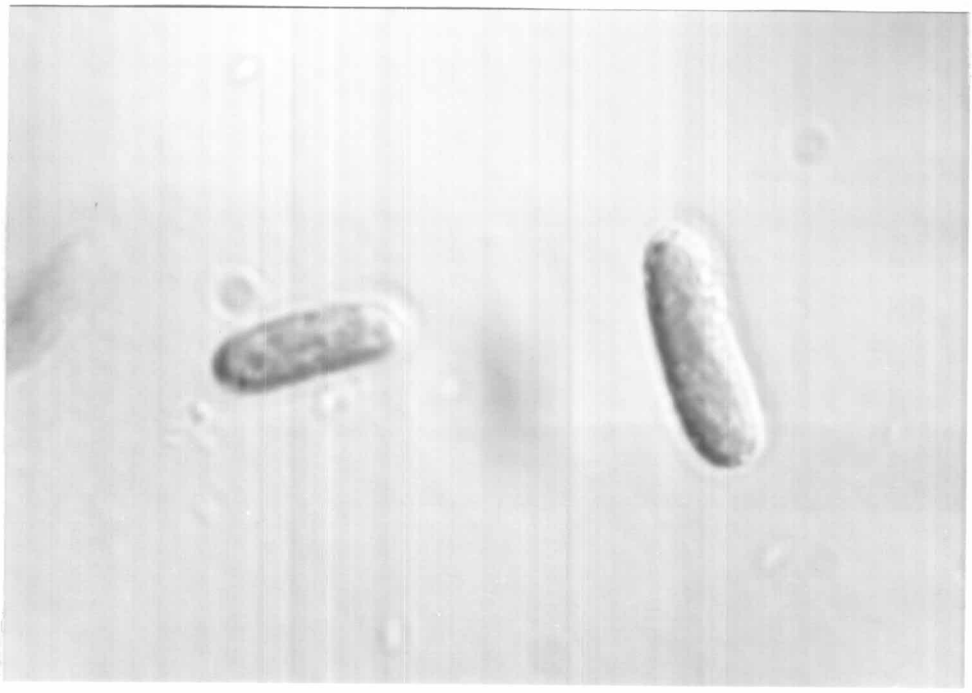

Figure 4 *Aphanothece halophytica* was grown in Turk Island Salt Solution + modified BG_{11} medium at day 14 (X2250 from microscope)

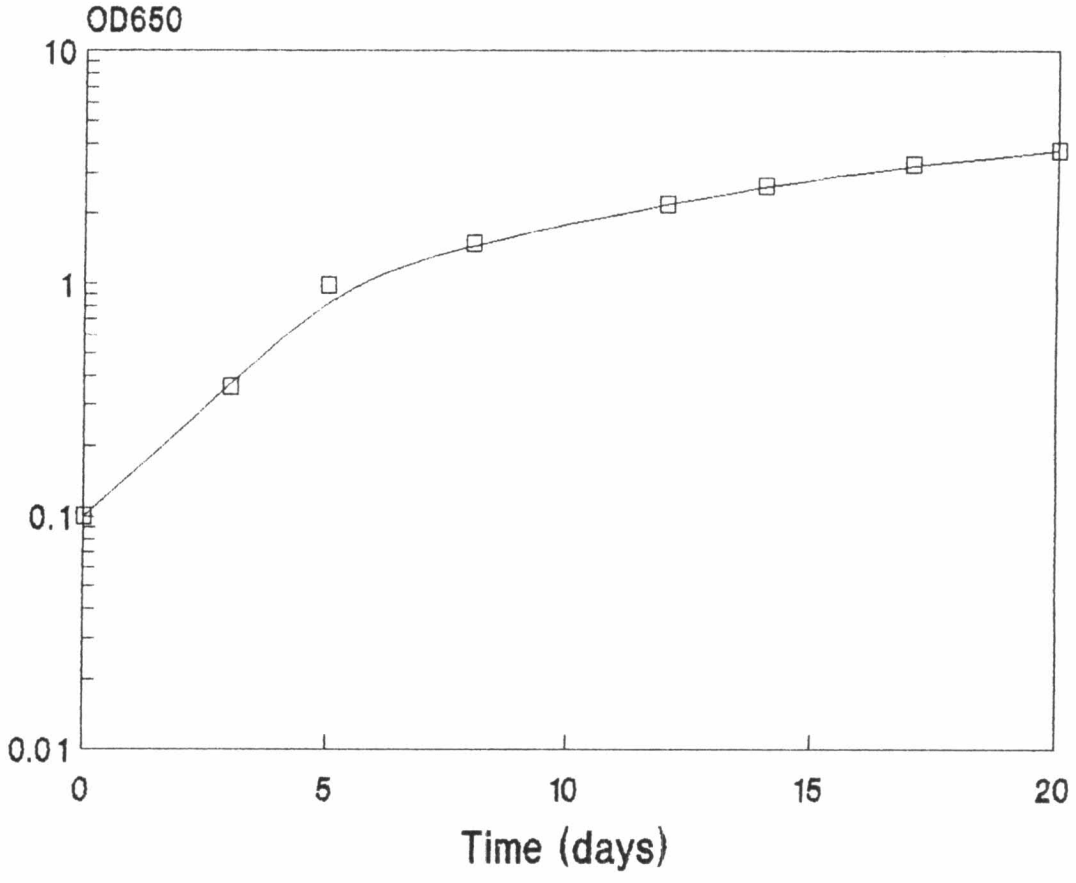

Figure 5 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium

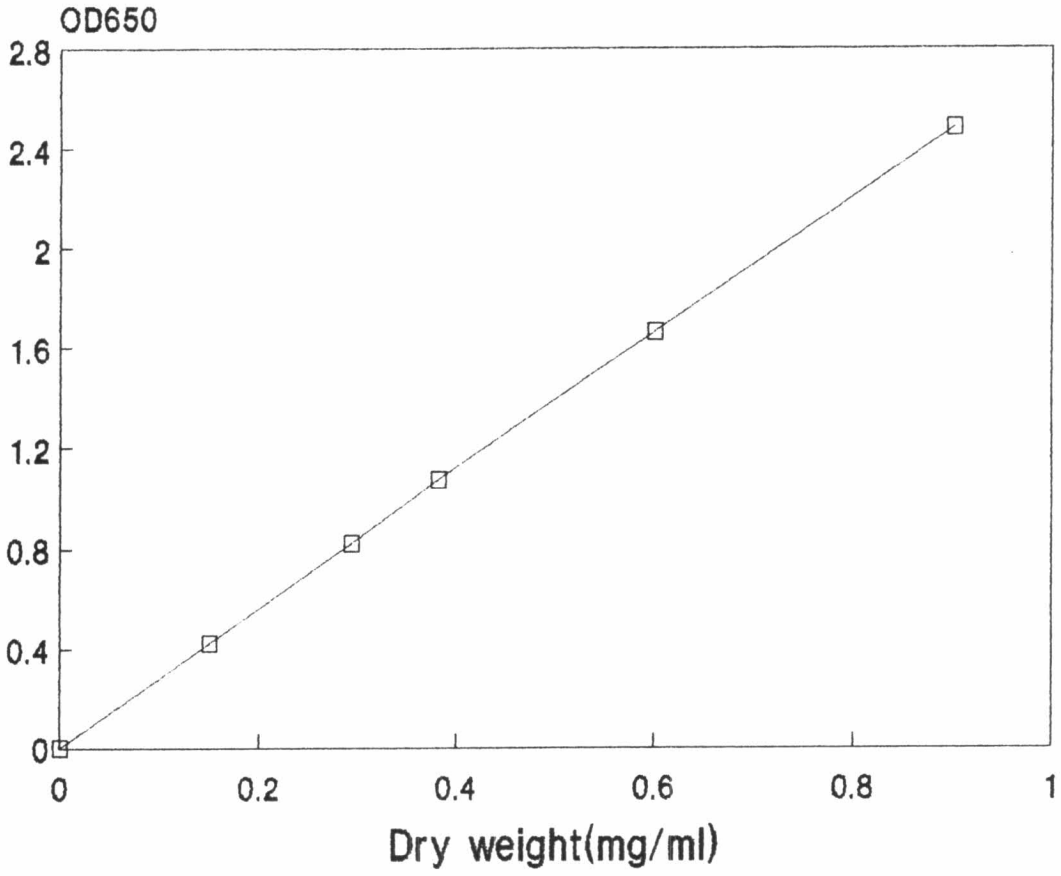

Figure 6 Relationship between OD_{650} and dry weight of *Aphanothece halophytica*

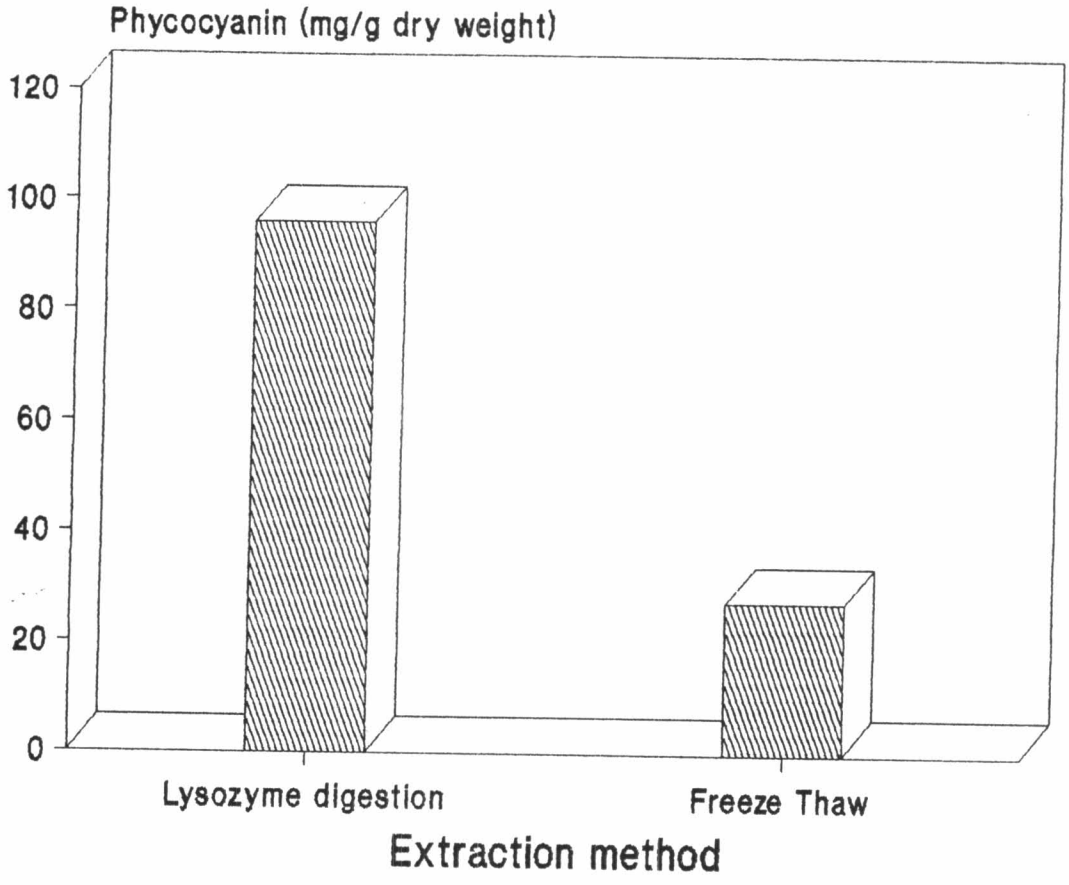

Figure 7 Phycocyanin content after lysozyme digestion and freeze thaw extraction from *Aphanothece halophytica*

Effect of Environmental Factors on *Aphanothece halophytica* Cultivation for High Phycocyanin Production

1. Effect of NaCl Concentration on Growth and Phycocyanin Content

Aphanothece halophytica was grown in a 250 ml flask containing 100 ml of Turk Island Salt Solution + modified BG_{11} medium as described in section 1 for 15 days. Figure 8 shows that 9 days culture gave the hightest yield of phycocyanin for *A. halophytica*. Consequently in later experiments we monitored the content of phycocyanin after 9 days cultivation. Cells were grown in Turk Island Salt Solution + modified BG_{11} medium with the final NaCl concentration of either 0.125 , 0.25 , 0.5 , 1 , 1.5 or 2 M for 15 days. Figure 9 shows that the lowest growth occurred at 0.125 M NaCl. After 9 days cultivation, the cells growth were slightly different when grown in the range of NaCl concentrations between 0.25 to 1.5 M. The cell growth was gradually increased when grown under 2 M NaCl but it was lower than that grown under 0.25-1.5 M NaCl. The highest phycocyanin content at about 100 mg/g dry weight was obtained at day 9 when cells were grown under 0.25 M and 0.5 M NaCl. At lower than 0.25 M or higher than 0.5 M NaCl , phycocyanin content decreased (Figure 10).

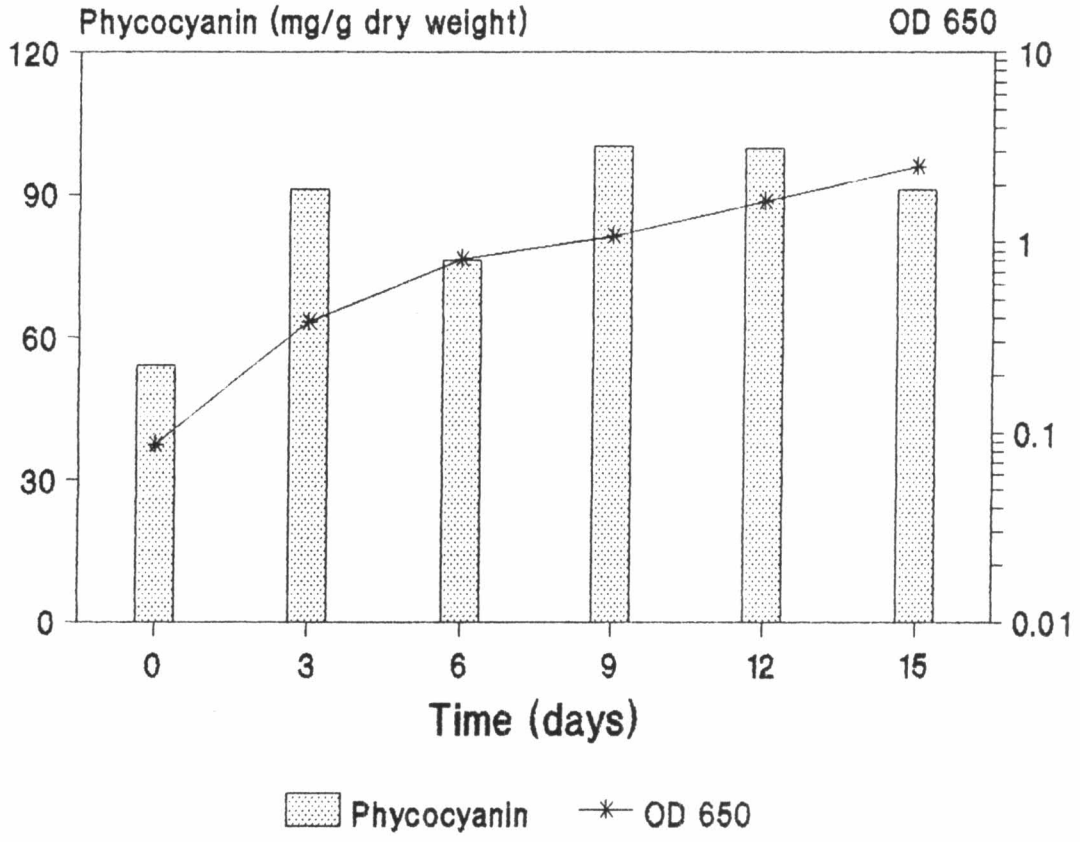

Figure 8 Growth and phycocyanin content of *Aphanothece halophytica* at various time intervals

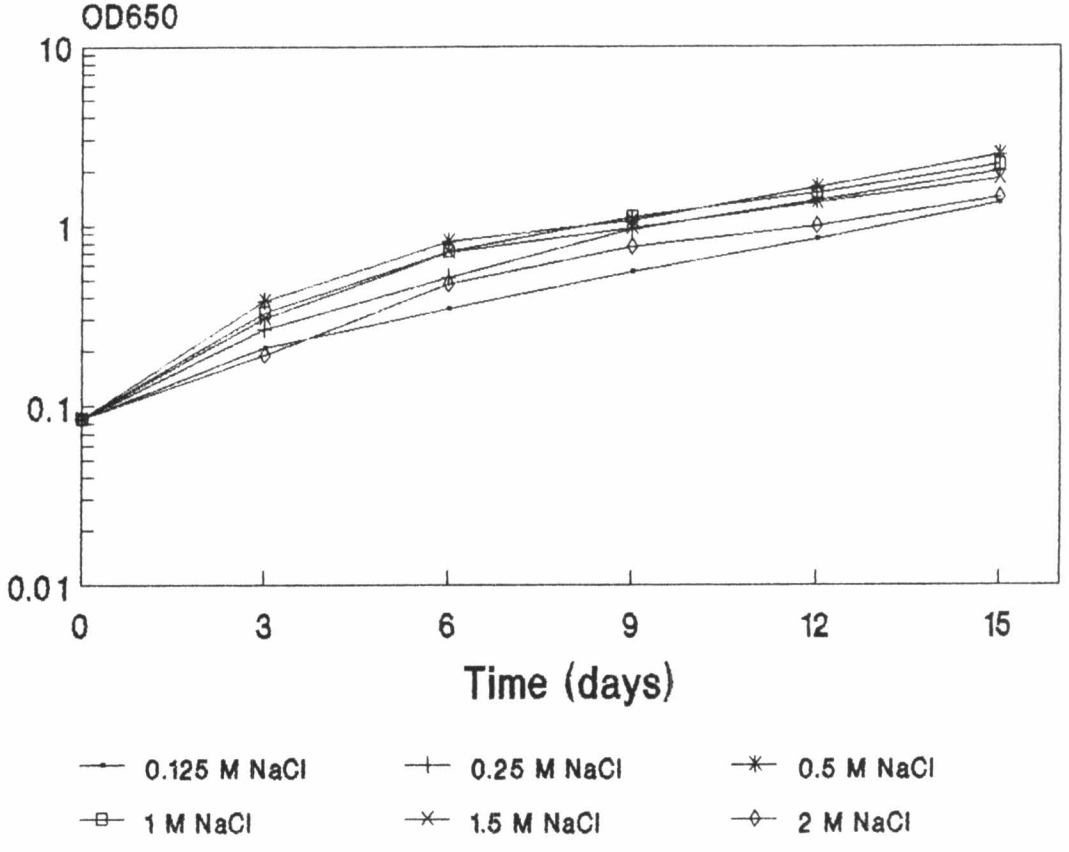

Figure 9 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium containing various NaCl concentrations

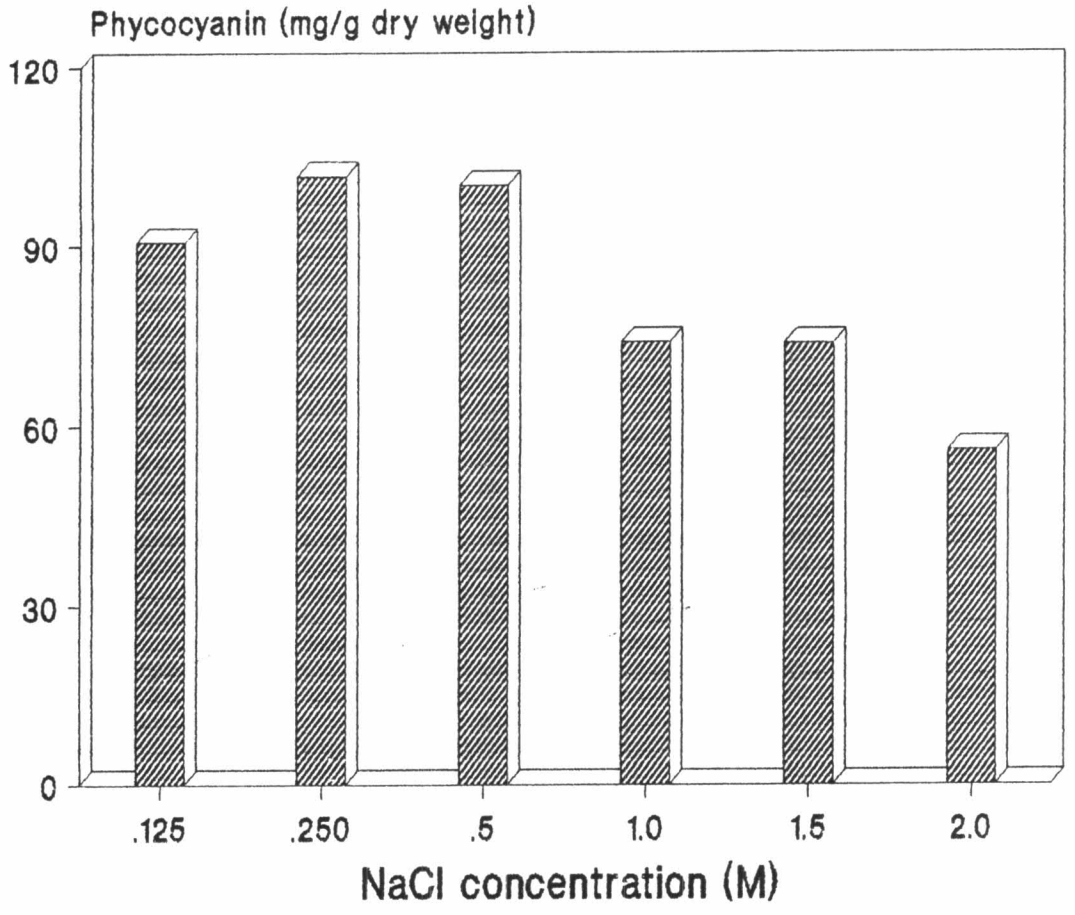

Figure 10 Effect of NaCl concentration on phycocyanin content of *Aphanothece halophytica* at day 9

2. Effect of various N-sources on Growth and Phycocyanin Content

Cells grown in Turk Island Salt Solution + modified BG_{11} medium containing various N-source such as sodium nitrate , ammonium acetate , ammonium nitrate or urea for 12 days were also performed in the same manner. Figure 11 shows that after 3 days cultivation , the growth of *A. halophytica* was decreased when grown under ammonium acetate and was no change when grown under ammonium nitrate as a N-source. The cell growth was increased when grown under sodium nitrate and urea as a N-source. However , sodium nitrate appeared to be the best source for growth and phycocyanin production yielding 94.87 mg/g dry weight at day 9 (Figure12). Therefore, sodium nitrate was used as a N-source for later experiments.

3. Effect of $NaNO_3$ Concentration on Growth and Phycocyanin Content

Cells grown in Turk Island Salt Solution + modified BG_{11} medium with the final $NaNO_3$ concentation of either 0 , 0.1 , 0.25 , 0.5 , 1.0 , 1.5 , 2.0 or 2.5 g/l for 15 days were also performed in the same manner.Figure 13 shows that the cell growth was obviously decreased when grown in the absence of $NaNO_3$. The cell growth ceased to grow after 6 days of cultivation at 0.1 g/l of $NaNO_3$. In the range of $NaNO_3$ concentration between 0.25 g/l and 2.5 g/l , similar growth pattern was observed (Figure 13).

From Figure 14 , the data indicate that in the range of $NaNO_3$ concentration between 0.25 and 2.5 g/l $NaNO_3$ except 1.5 g/l $NaNO_3$, phycocyanin content were not different. However , cells grown under 1.5 g/l of $NaNO_3$ gave the highest yield of phycocyanin content. The $NaNO_3$ deprivation resulted in the lowest phycocyanin content yielding only 9.13 mg/g dry weight and the highest at 1.5 g/l of $NaNO_3$ yielding 97.63 mg/g dry weight at day 9 (Figure 14).

4. Effect of $NaNO_3$ starvation on Growth and Phycocyanin Content

A. halophytica grown in Turk Island Salt Solution + modified BG_{11} medium with the final $NaNO_3$ content of 0 or 1.5 g/l were also performed in the same manner. Figure 15 shows that growth was slightly increased when the medium contained no $NaNO_3$ and gradually increased when grown under 1.5 g/l of $NaNO_3$ at 68 hours. At 68 hours $NaNO_3$ was added to $NaNO_3$-free mediumn until final concentration was 1.5 g/l of $NaNO_3$, cells could resume growth and at day 12 appeared to attain the same level of growth as the control. The phycocyanin content was sharply decreased when grown under $NaNO_3$-free medium (Figure 16). However, phycocyanin content was shapely increased after the addition of $NaNO_3$ to a final concentration of 1.5 g/l at 68 hours and slightly increased after 8 days. At day 12 , phycocyanin content of cell grown under constant level of $NaNO_3$ medium (1.5 g/l) yielded 99.32 mg/g dry weight whereas

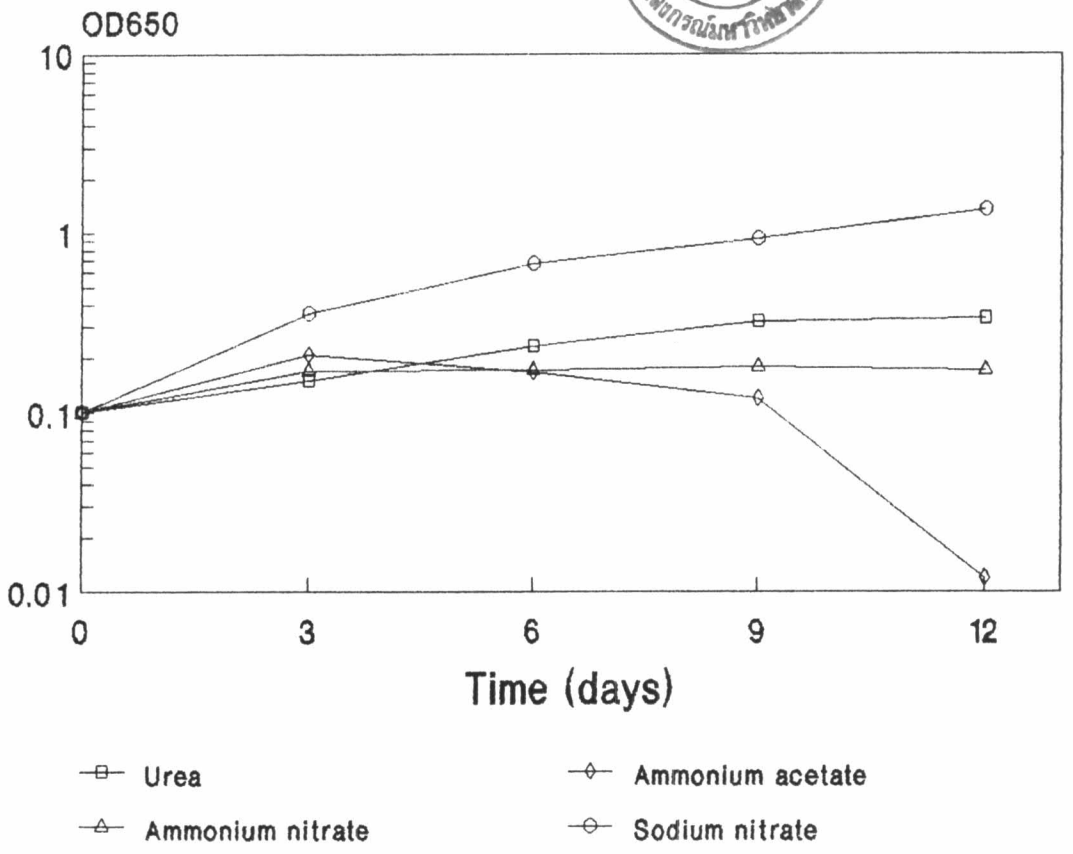

Figure 11 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium containing various N-sources

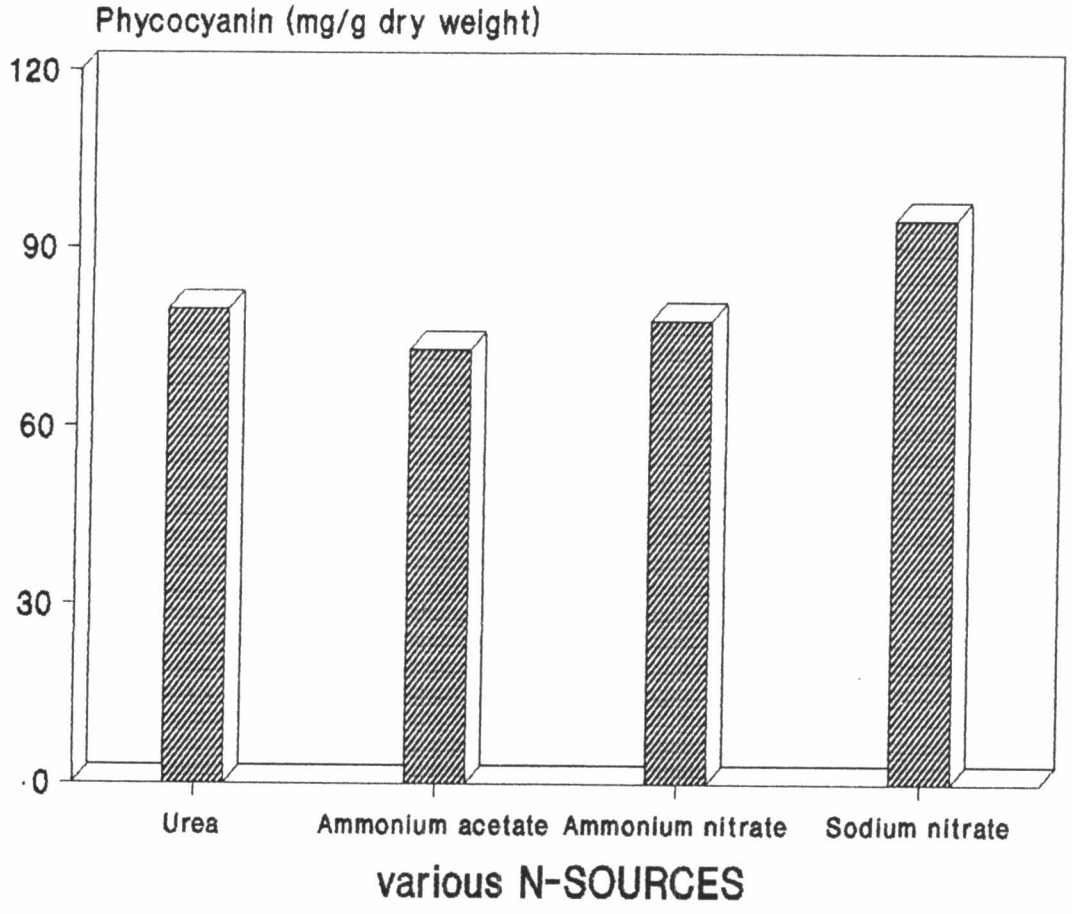

Figure 12 Effect of type of N-source on phycocyanin content of *Aphanothece halophytica* at day 9

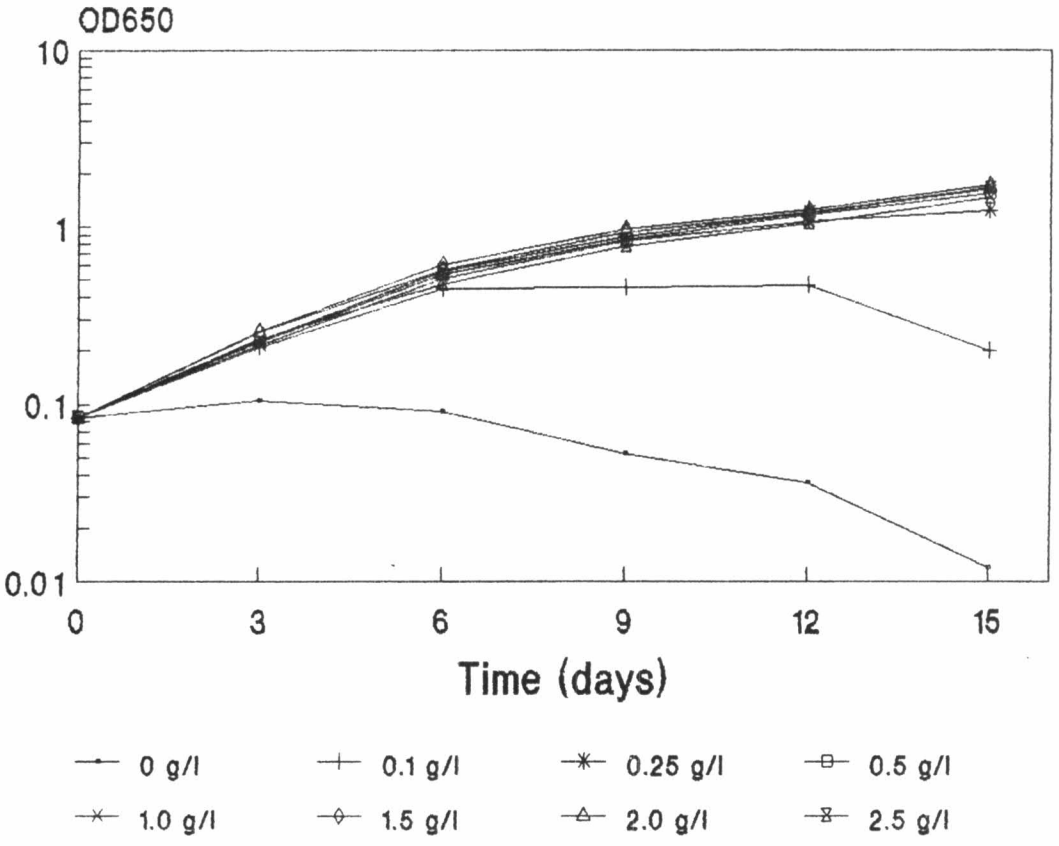

Figure 13 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium containing various $NaNO_3$ concentrations

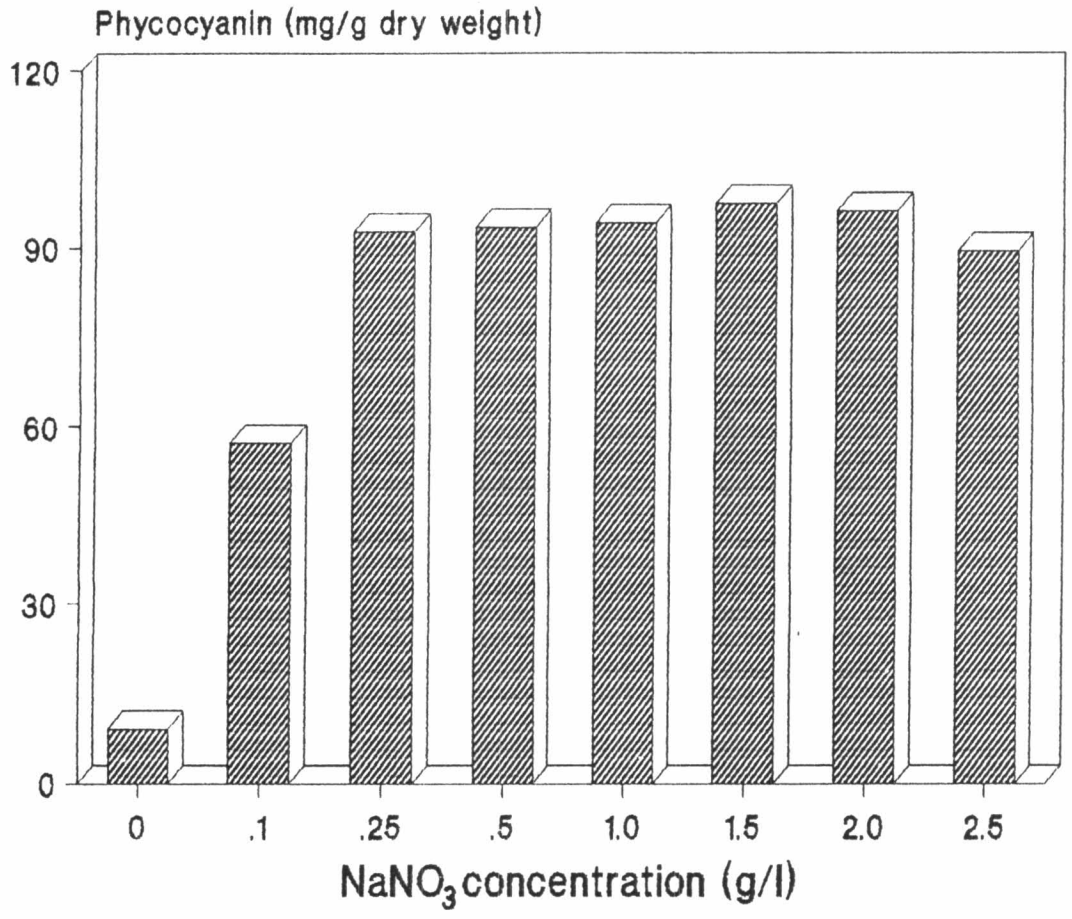

Figure 14 Effect of $NaNO_3$ concentration on phycocyanin content of *Aphanothece halophytica* at day 9

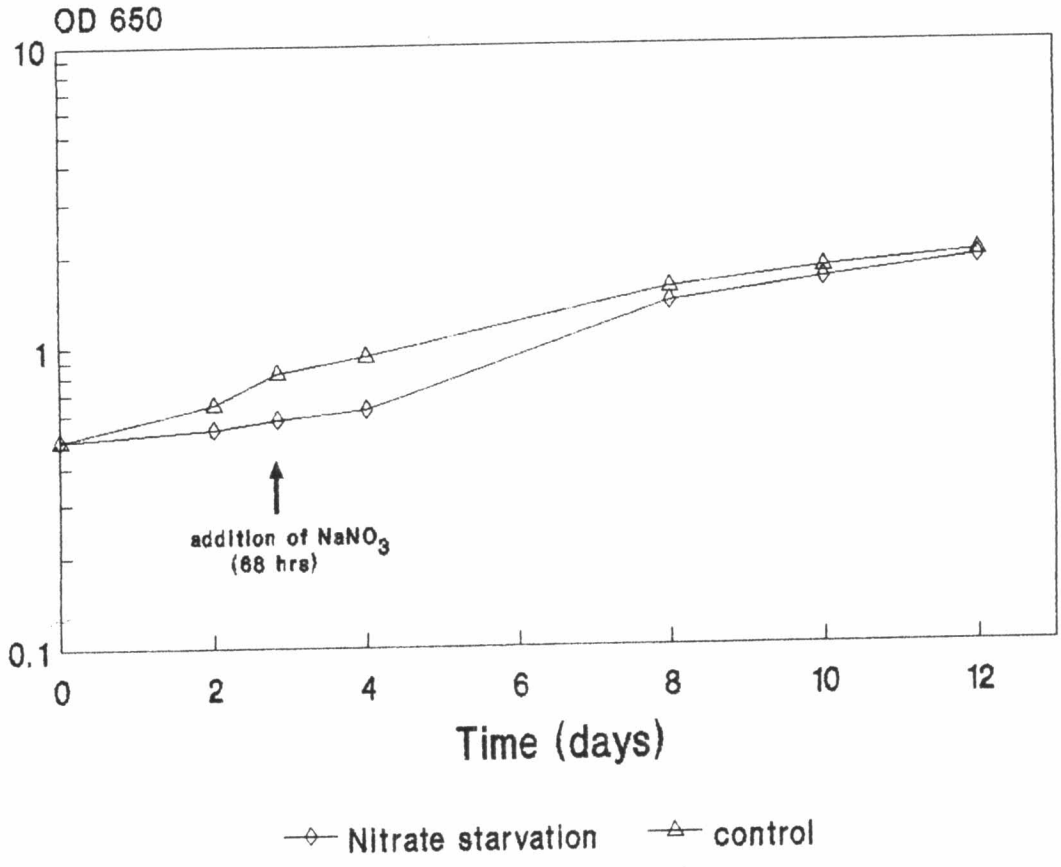

Figure 15 Growth of *Aphanothece halophytica* under $NaNO_3$ starvation

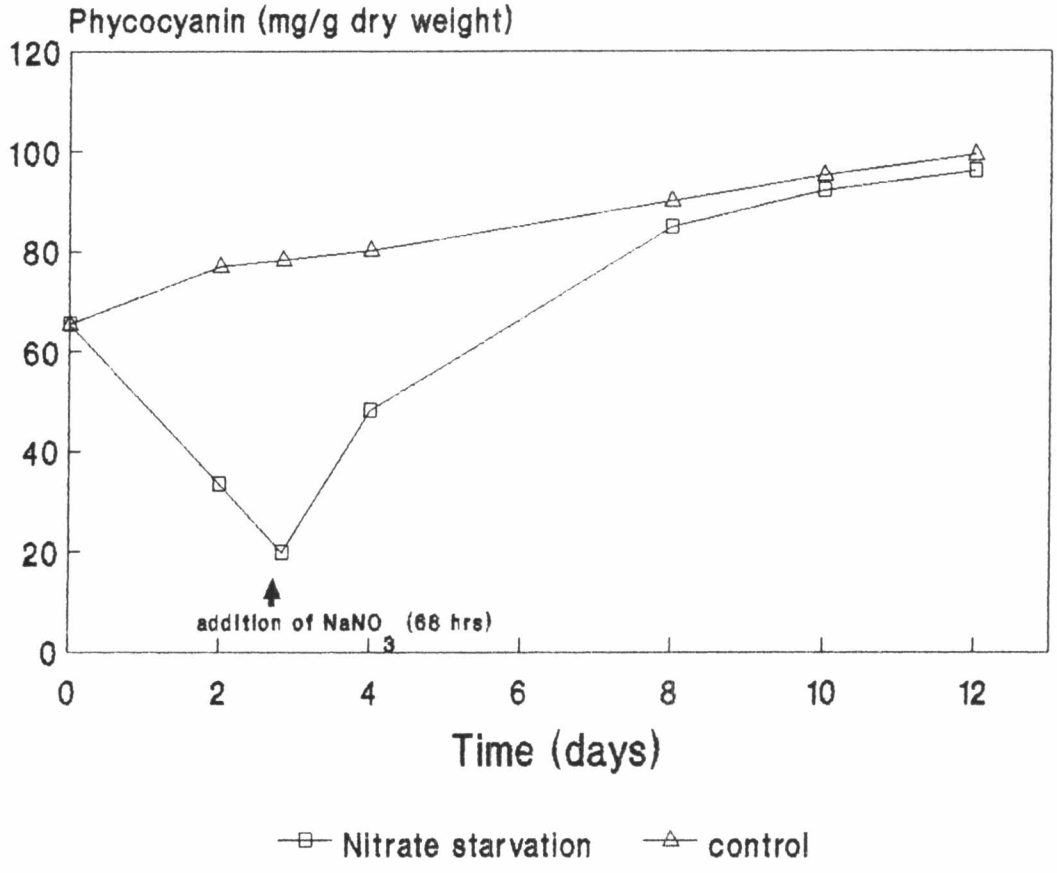

Figure 16 Effect of $NaNO_3$ starvation on phycocyanin production in *Aphanothece halophytica*

that grown under $NaNO_3$ depletion and restoration yielded 96.06 mg/g dry weight.

5. Effect of Light Intensity on Growth and Phycocyanin Content

A. halophytica was grown in Turk Island Salt Solution + modified BG_{11} medium under different light intensities at 1,500 , 3,000 ,5,000 or 8,000 lux for 8 days. Figure 17 shows that there were no differences on growth of *A. halophytica* when grown under these 4 light intensities. Phycocyanin content was the highest at light intensity of 1,500 lux yielding 87.93 mg/g dry weight at day 8 and phycocyanin content decreased with increasing light intensity (Figure 18). It appeared that the higher light intensity , the lower phycocyanin content.

6. Effect of Light Quality on Growth and Phycocyanin Content

A. halophytica was grown in Turk Island Salt Solution + modified BG_{11} medium under white , red and green light. The light source was a fluorescence lamp and specific chromatic illumination was provided by the interposition of plastic filters. Optical characteristics of the red and green light are shown in Figure 19. Growth was the highest when grown under white light followed by those under red and green light , respectively (Figure 20). Phycocyanin content was the highest when grown under red light and the lowest when grown under green light (Figure 21). Phycocyanin

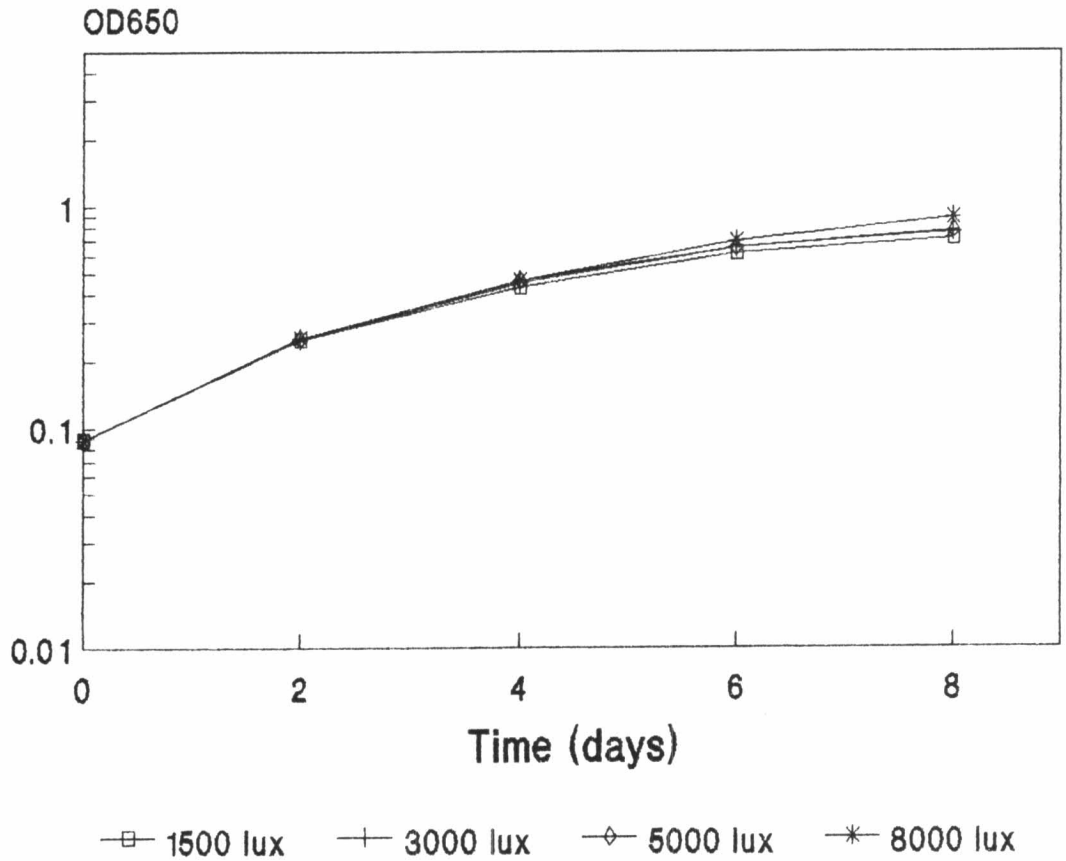

Figure 17 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium under various light intensities

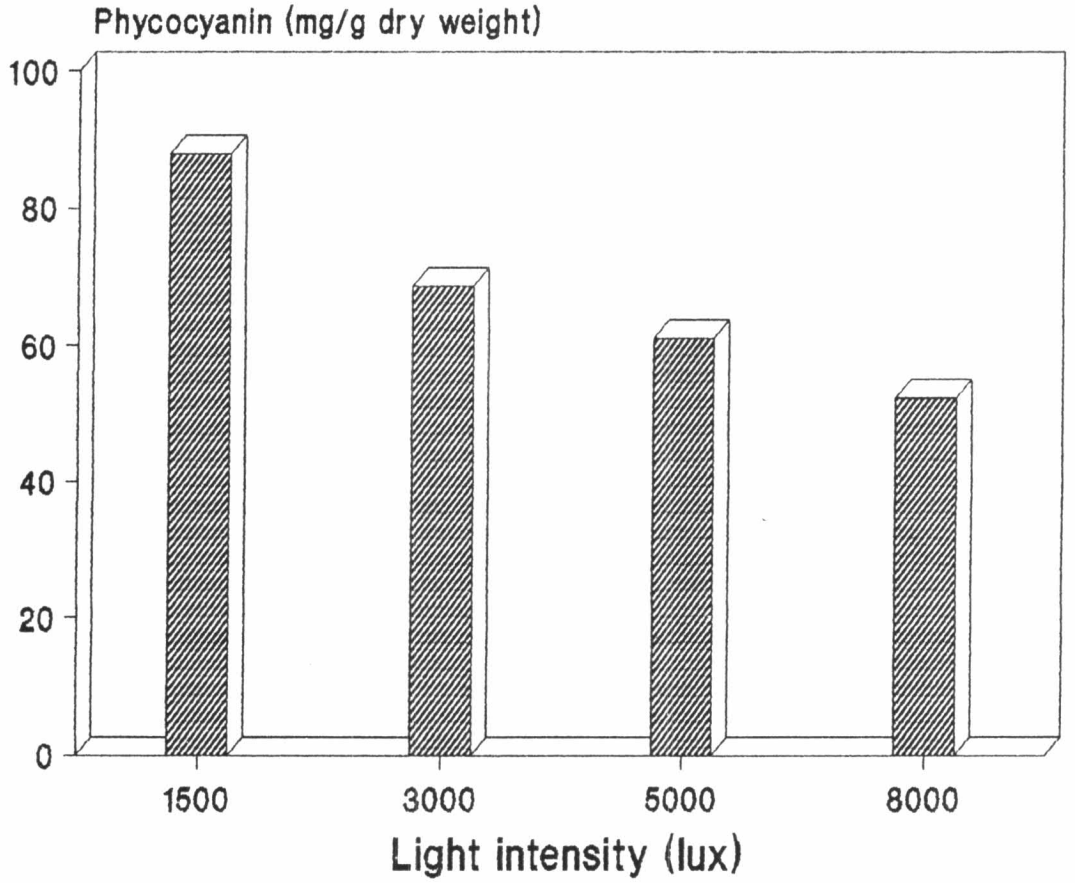

Figure 18 Effect of light intensity on phycocyanin content of *Aphanothece halophytica* at day 8

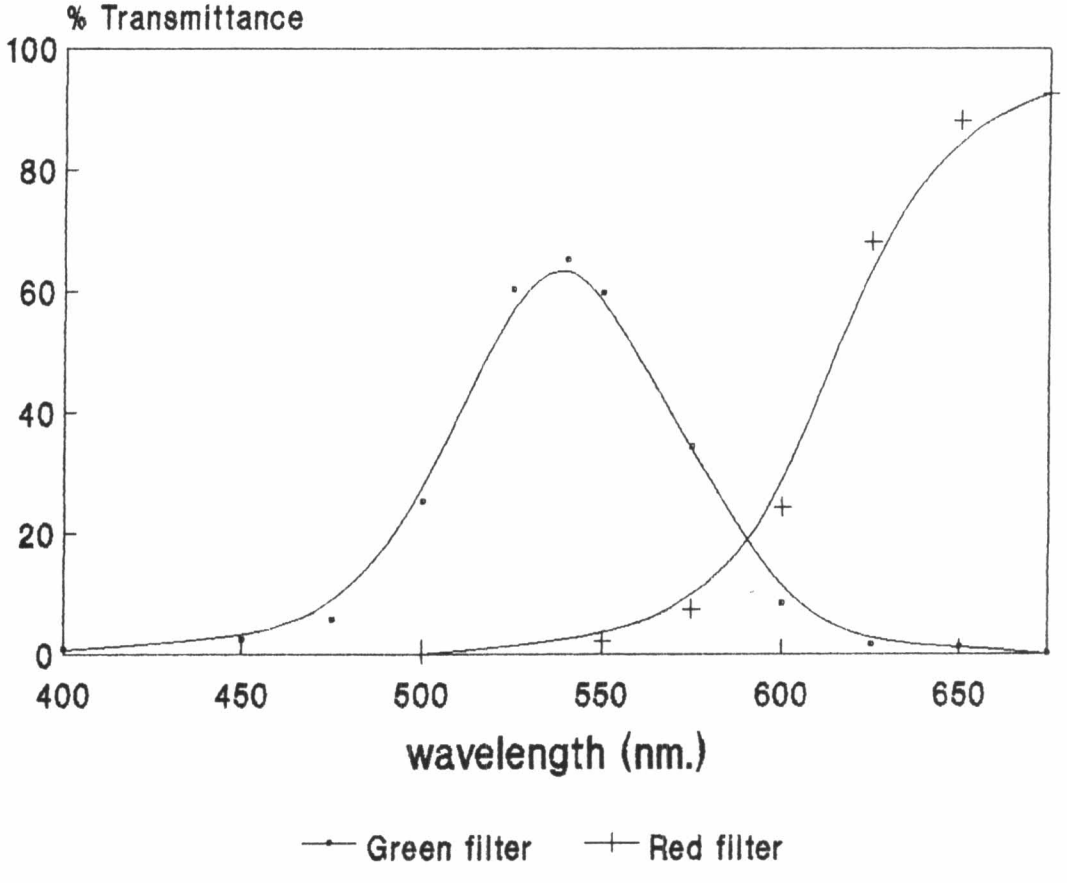

Figure 19 Optical characteristics of the red and green filters

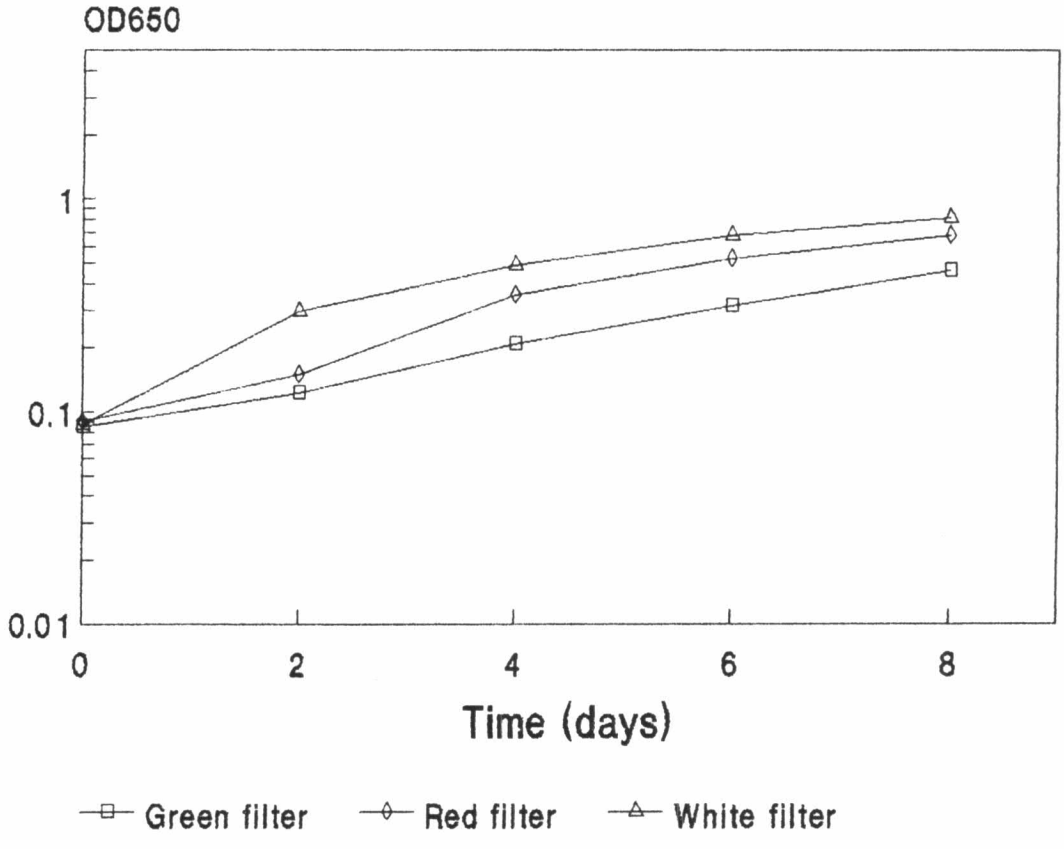

Figure 20 Growth of *Aphanothece halophytica* under red, white and green light

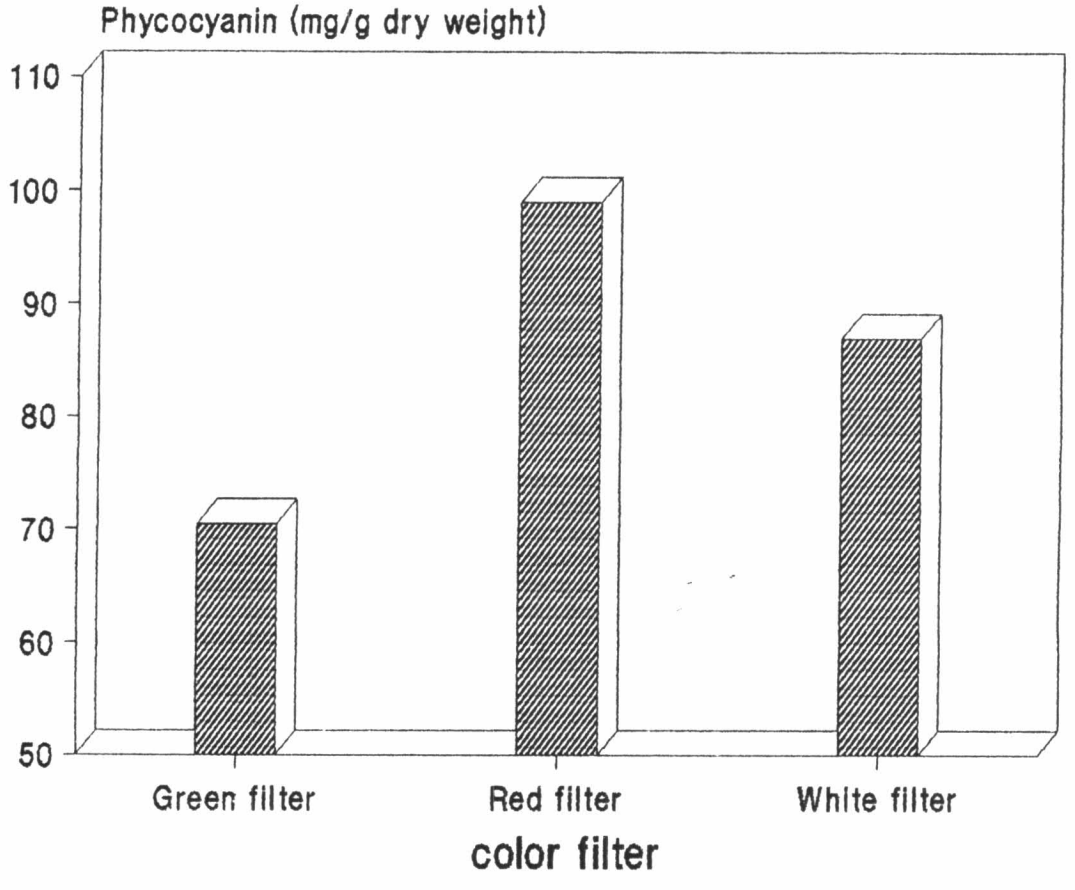

Figure 21 Effect of light quality on phycocyanin content of *Aphanothece halophytica* at day 8

content yielded 99.01 , 86.96 and 70.45 mg/g dry weight at day 8 when grown under red , white and green light , respectively.

7. Effect of Initial Chlorophyll Concentration on Growth and Phycocyanin Content

A. halophytica was grown in Turk Island Salt Solution + modified BG_{11} medium with different initial chlorophyll concentation of 50 , 100 , 250 and 500 μg/ml for 12 days. Figure 22 shows that the growth rate of *A. halophytica* was the highest when grown under initial chlorophyll concentration of 50 μg/ml and decreased with increasing initial chlorophyll concentration. From Figure 23,the data indicate that phycocyanin content was the highest when grown under 100 μg/ml of initial chlorophyll concentration yielding 91.53 mg/g dry weight at day 9 and phycocyanin content decreased when grown at above or below this initial chlorophyll concentration.

8. Effect of Cultivation Temperature on Growth and Phycocyanin Content

A. halophytica was grown in Turk Island Salt Solution + modified BG_{11} medium at different cultivation temperature of 25 , 30 , 35 and 40 $^{\circ}C$ for 5 days. A 100 ml of culture in a 250 ml flask was shaken on a Psycrotherm at 160 rpm with illumination at 1,900 lux. Figure 24 shows that during 5 days cultivation growth was retarded at 25 $^{\circ}C$. On the other hand growth appeared to be similar at 30 ,

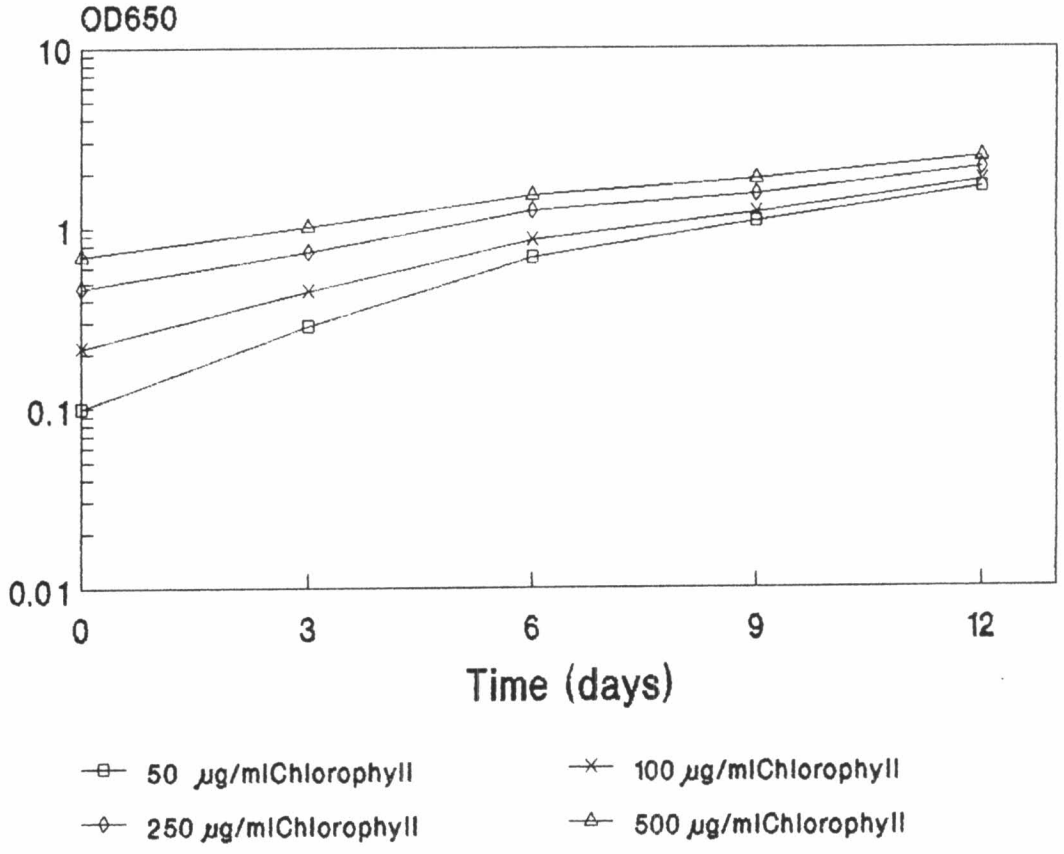

Figure 22 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium under various initial chlorophyll concentrations

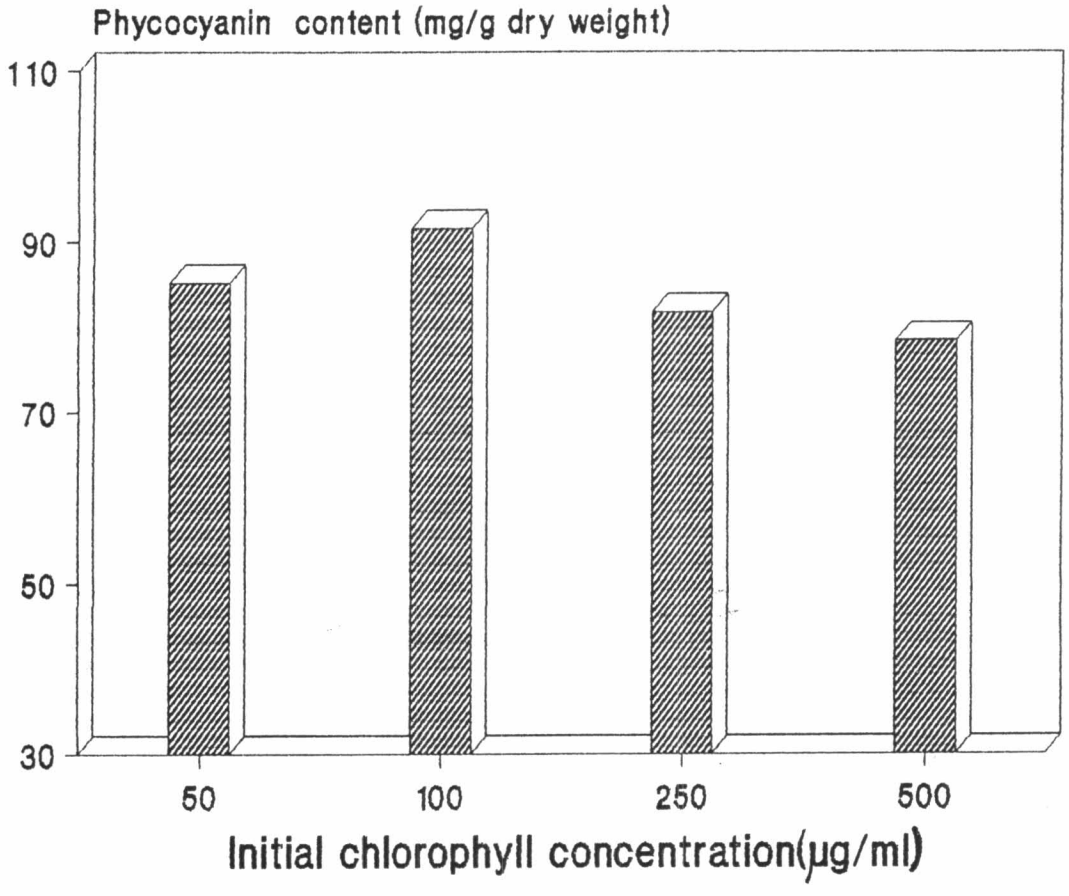

Figure 23 Effect of initial chlorophyll concentration on phycocyanin content of *Aphanothece halophytica* at day 9

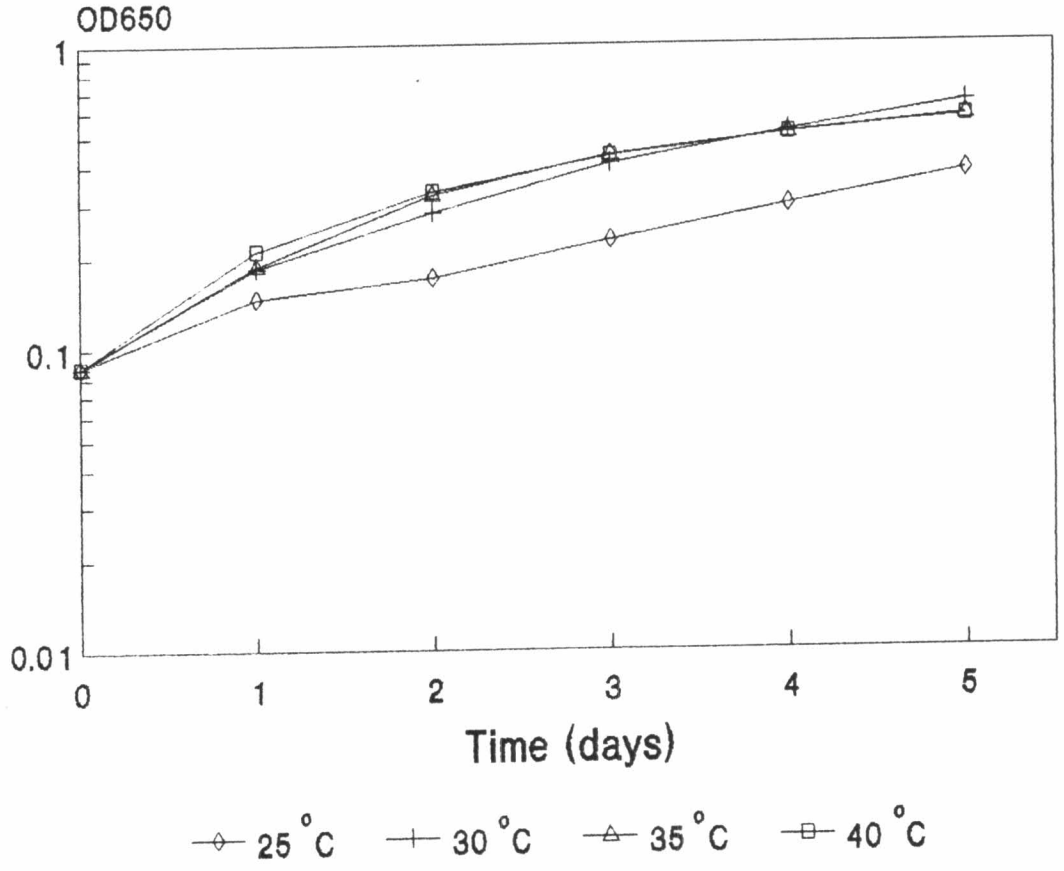

Figure 24 Growth of *Aphanothece halophytica* in Turk Island Salt Solution + modified BG_{11} medium at various cultivation temperatures

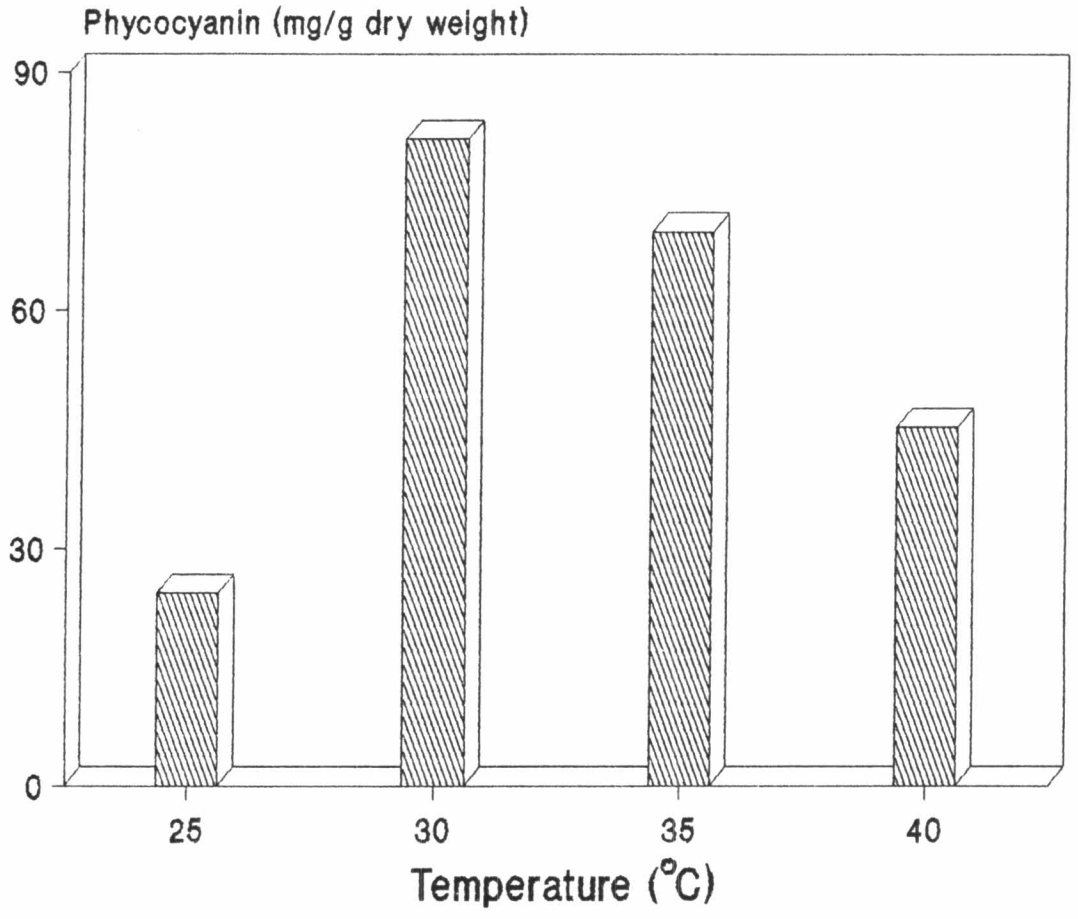

Figure 25 Effect of cultivation temperature on phycocyanin content of *Aphanothece halophytica* at day 5

35 and 40 °C. As shown in Figure 25, the highest phycocyanin content was obtained at 30 °C yielding 81.62 mg/g dry weight and at 25 °C , phycocyanin content was the lowest yielding 24.79 mg/g dry weight.

Comparison of phycocyanin content between *A. halophytica* and *S. platensis* (BP) in optimal conditions as shown in Table 1. The results in Table 1 shows that Phycocyanin content of *A. halophytica* in the optimal NaCl and $NaNO_3$ concentration were lower than *S. platensis*(BP). In addition, phycocyanin content of *A. halophytica* was lower than *S. platensis*(BP) when grown under red light.However, the phycocyanin content of *A. halophytica* in the optimal light intensity was higher than *S. platensis*(BP).

Effect of Mutagenesis on *Aphanothece halophytica* Cultivation for High Phycocyanin Production

1. Mutagenesis of *A. halophytica* by Irradiation with Ultraviolet Light

1.1 Survival Curve of *A. halophytica* Mutated by Irradiation with Ultraviolet Light

A 10 ml of *A. halophytica* in a 100-mm petridish was irradiated at a distance of 30 cm from 30 W germicidal UV-lamp and 1 ml portions were removed at 5-second intervals. The mutagenized cells and the normal were incubated under yellow light at the same intensity used fornormal growth under white light for at least 36 hr.

Table 1 Phycocyanin content at optimal conditions of *A. halophytica*

Factors	Phycocyanin content (mg/g DW)	
	A. halophytica	*S. platensis* (BP)*
[NaCl] (0.5 M)	107.22	(250)+
[$NaNO_3$] 1.5 g/l)	97.63	(280)+
[I] (1,500 lux)	87.93	(38)+
Light quality (red light)	99.01	(240)+
[Chl.] (100 μg/ml)	91.53	
Cultivation Temp. (25 °C)	81.62	

**S.platensis* was isolated from Wat Benjamaborpit pond

+Phycocyanin content at optimal conditions of *S. platensis* (BP) was studied by Miss Duangrat Inthorn (ISOLATION AND OPTIMIZATION SPIRULINA CULTURES FOR PHYCOCYANIN PRODUCTION ; 1991)

A 0.5 ml aliquot of each culture sample was spread on 100-mm plates containing 30 ml of Turk Island Salt Solution+ modified BG_{11} medium solidified with 1.5% agar. The plates were incubated under standard growth conditions until colonies formed. Figure 26 shows that the cell number was clearly declined with increasing irradiation time. In later experiments we chose 20 seconds as the irradiation time because this was a suitable period of time resulting in 99% killing.

1.2 Selection for Mutants of *A. halophytica*

A 0.5 ml aliquot of the cells was irradiated with UV-light for 20 seconds. The irradiated cells and the control sample (normal cells) were spread on agar plates and incubated under normal growth condition. On the other hand , 0.5 ml aliquots of the irradiated cells and the normal cells were added 150 μg/ml ofa selective agent, either ampicillin or cycloserine and incubated under normal growth conditions for 24 hr. The selective agent was then removed by two centrifugal washes and the resuspened cells were plated and incubated under normal growth conditions until colonies formed (10 days). Figure 27A shows that the normal cells without selective agent could grow in agar plate but the normal cells which was incubated in a selective agent , cycloserine , couldnot grow on agar plate (Figure 27C). When the cultures were irradiated with UV-light for 20 seconds and incubated in

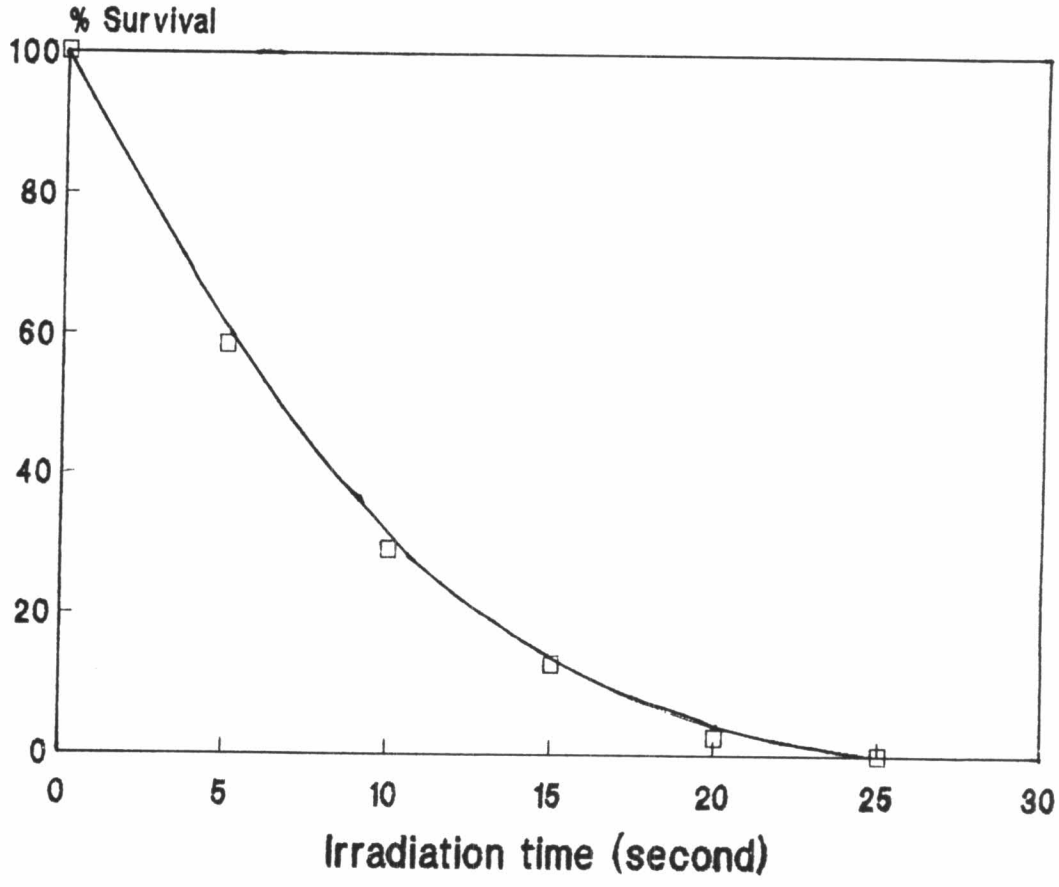

Figure 26 Survival Curve of *Aphanothece halophytica* after irradiation with UV-Light in Turk Island Salt Solution + modified BG_{11} medium

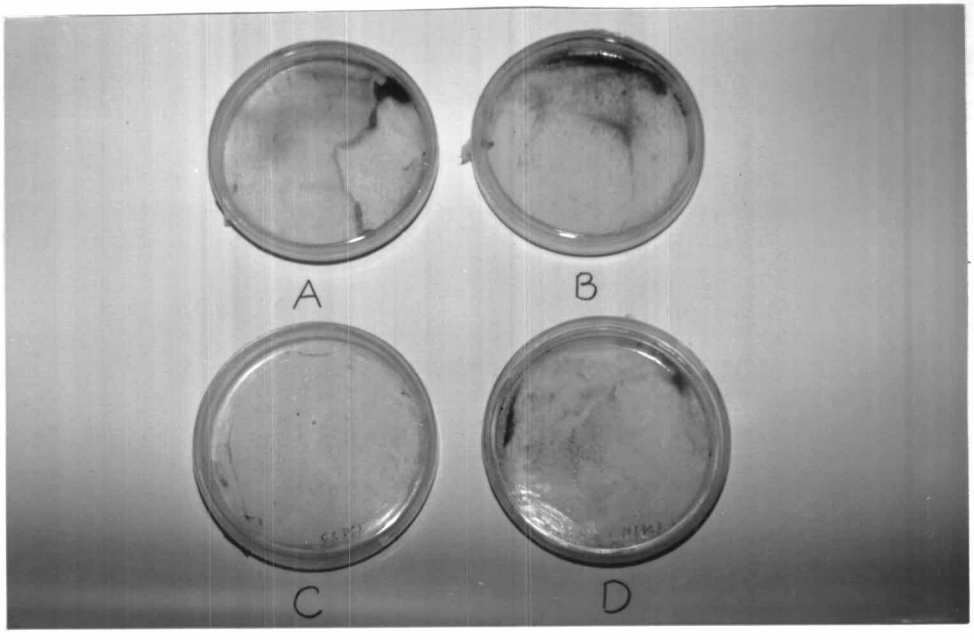

Figure 27 Growth of normal and UV-Light mutated *Aphanothece halophytica* after subjecting to incubation with or without cycloserine on plate of Turk Island Salt Solution + modified BG_{11} medium solidified with 1.5 % agar

A. normal without cycloserine

B. mutated cells without cycloserine

C. normal with cycloserine

D. mutated cells with cycloserine

the absence and presence of cycloserine , both cultures could grow on agar plate (Figure 27B,D). These results indicated that the cells in Figure 27D were the mutants caused by UV-light irradiation and selected by cycloserine.

1.3 Effect of *A. halophytica* Mutants on Growth and Phycocyanin Content

A. halophytica mutants were grown in a 250 ml flask containing 100 ml of Turk Island salt Solution + modified BG_{11} medium. Normal *A. halophytica* was also grown in Turk Island Salt Solution + modified BG_{11} medium. The relationships between optical densities at 650 nm(OD_{650}) and dry weight of normal and mutated cells of *A. halophytica* were found to be linear (Figure 28). From Figure 29 , the data indicate that there were no differences on growth of the mutated cells and normal sample of *A. halophytica*. Phycocyanin content of normal sample was higher than the mutated cells of *A. halophytica* (Figure 30). Furthermore , normal sample gave the highest yield of phycocyanin content at 97.88 mg/g dry weight at day 9 and slightly decreased when grown after day 9. However , phycocyanin content of *A. halophytica* mutants gave the highest yield of phycocyanin content at 90.99 mg/g dry weight at day 12 and slightly decreased when grown after day 12.

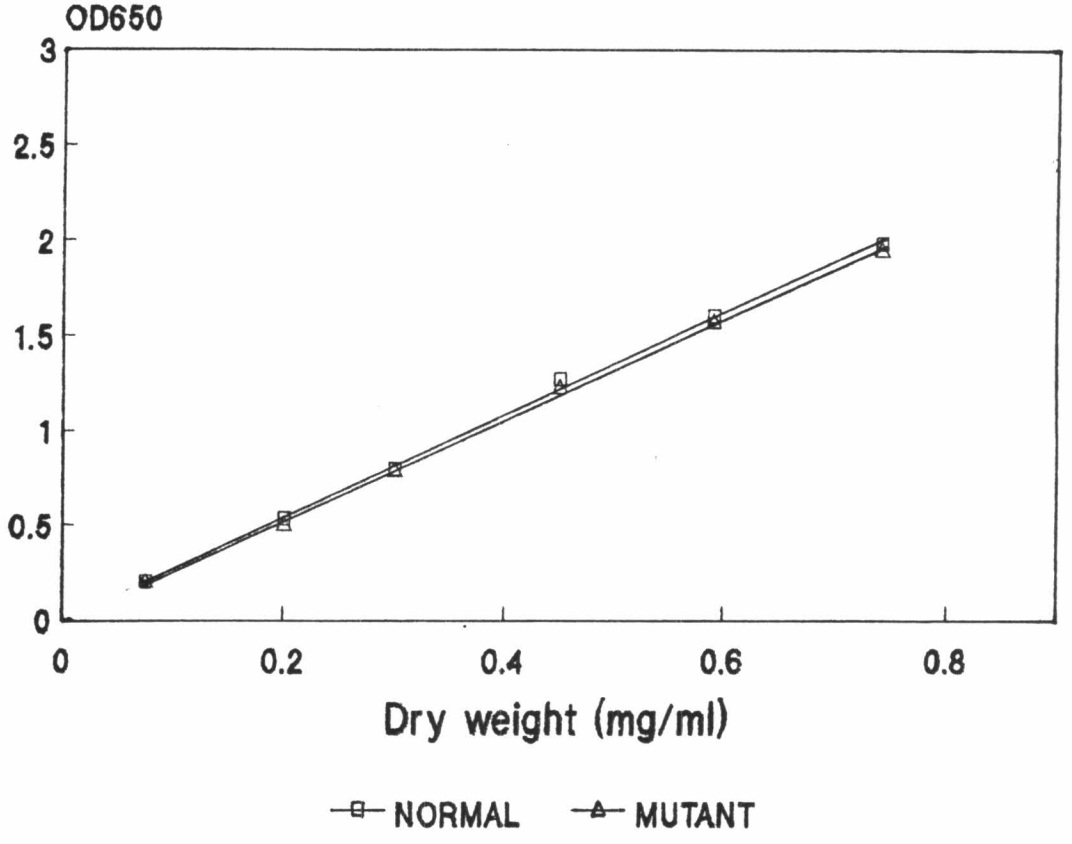

Figure 28 Relationships between OD_{650} and dry weight of normal and UV-Light mutated *Aphanothece halophytica*

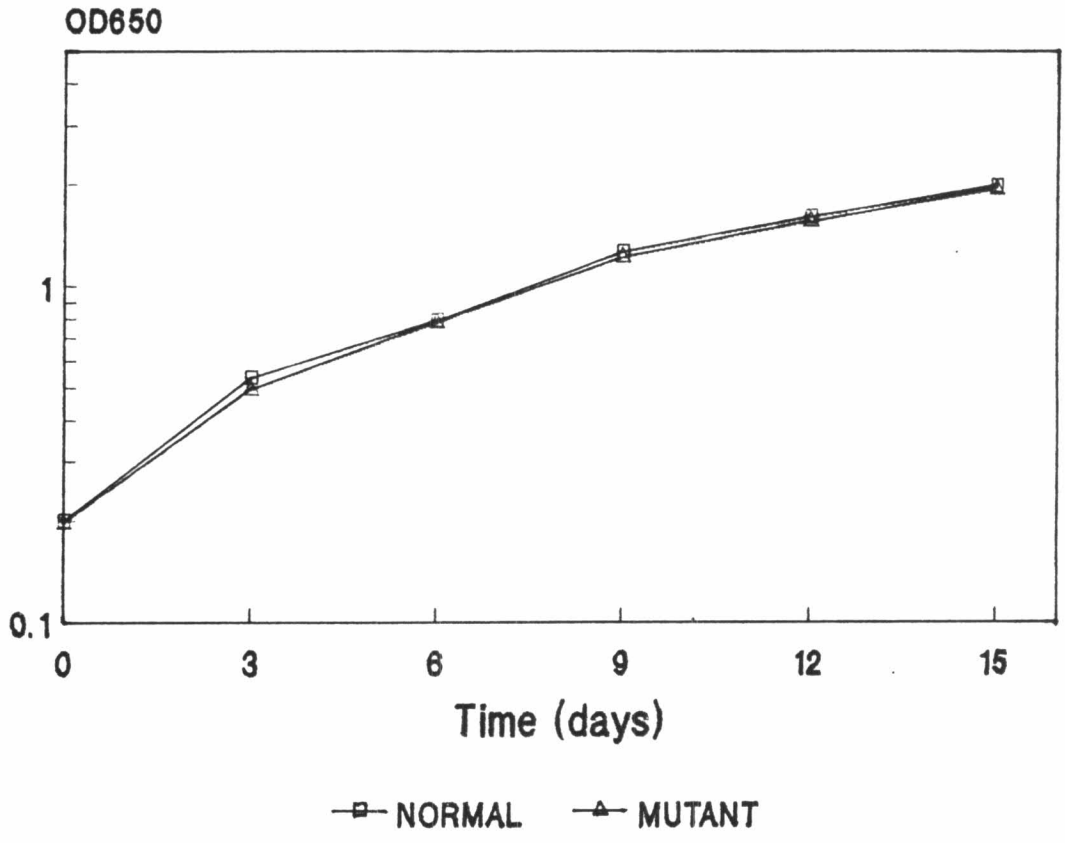

Figure 29 Comparison of growth between normal and UV-Light mutated *Aphanothece halophytica*

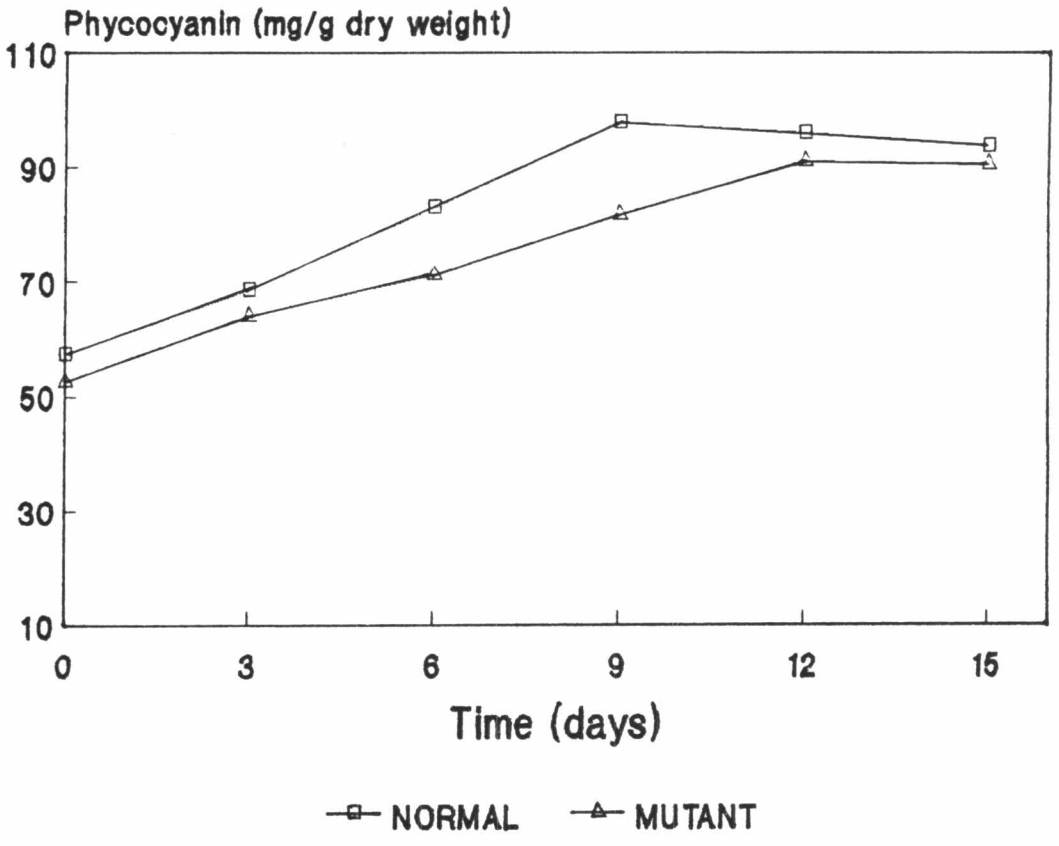

Figure 30 Comparison of phycocyanin content between normal and UV-Light mutated *Aphanothece halophytica*

2. Mutagenesis of *Aphanothece halophytica* by Treated with NTG

2.1 Survival Curve of Mutagenesis of *A. halophytica* by Treatedt with NTG

A 10 ml of *A. halophytica* were added with various NTG concentrations ; NTG was dissolved in 0.2 M Tris-malate, pH 7.6. The final concentration of NTG treatment in each culture sample were 0 , 10 , 20 , 30 , 40 , 60 , 80 or 100 μg/ml. The culture samples with NTG treatment and the normal sample were incubated under normal growth condition for 5 min and NTG was removed by three centrifugal washes. Growth of the cells was monitored as described in Materials and Methods. Figure 31 shows that the cell growth was clearly declined with increasing NTG concentration. Cells dried when treated with NTG concentration over 30 μg/ml. In later experiments we chose NTG at 10 and 20 μg/ml to mutagenized the cells.

2.2 Selection for Mutants of *A. halophytica*

A 0.5 ml aliquot of the cells was treated with 10 or 20 μg/ml NTG. The mutagenized and the normal sample were spread on agar plates and incubated under normal growth condition. On the other hand , 0.5 ml aliquots of the mutagenized cells and the normal sample were added with 150 μg/ml of ampicillin or cycloserine and incubated under normal growth condition for 24 hr. The selective agent was then removed by two centrifugal washes

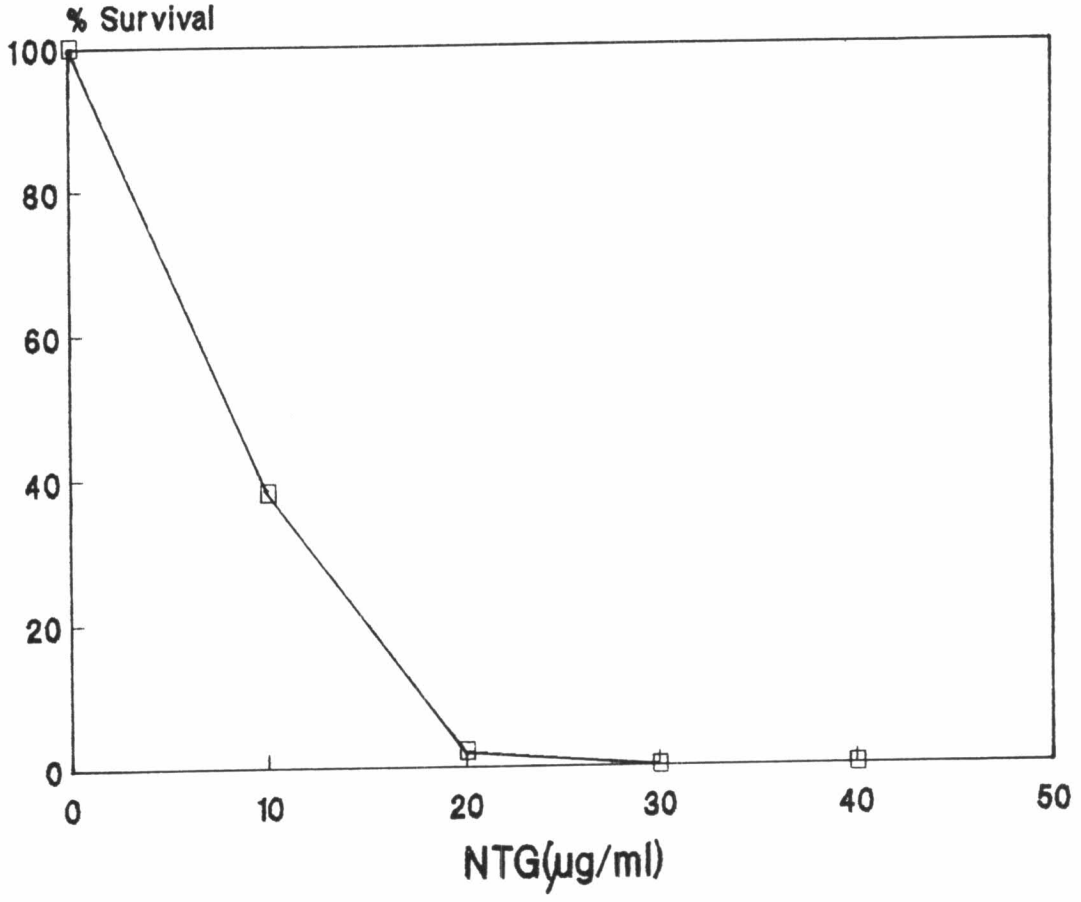

Figure 31 Survival Curve of *Aphanothece halophytica* mutant after treatment by various NTG concentrations

and the resuspended cells were plated and incubated under normal growth condition for 10 days. From Figure 32A , the data indicate that the normal sample without selective agent could grow on agar plate but the control sample which was incubated in a selective agent , cycloserine , couldn't grow on agar plate (Figure 32D). When the culture were treated with 10,20 μg/ml NTG and incubated in the absence aresence of cycloserine , both cultures could grow on agar plates (Figure 32 B,C,E,F). These results indicated that the cells in Figure 32 E,F were the mutants caused by treatment with NTG and selected by cycloserine.

2.3 Effect of *A. halophytica* Mutants by Treated with NTG on Growth and Phycocyanin Content

A. halophytica mutants were grown in 250 ml flask containing 100 ml of Turk Island Salt Solution + modified BG_{11} medium. Normal *A. halophytica* was also grown in the Turk Island Salt Solution + modified BG_{11} medium. The relationships between the optical density at 650 nm (OD_{650}) and dry weight of the mutated cells with NTG and normal sample of *A. halophytica* were found to be linear (Figure 33). There were no differences on growth of the mutated cells by treatment with 10 , 20 μg/ml NTG and normal sample of *A. halophytica* (Figure 34). Figure 35 shows that normal sample of *A. halophytica* gave the highest phycocyanin content yielding 98.76 mg/g dry weight and phycocyanin content decreased when treated culture with

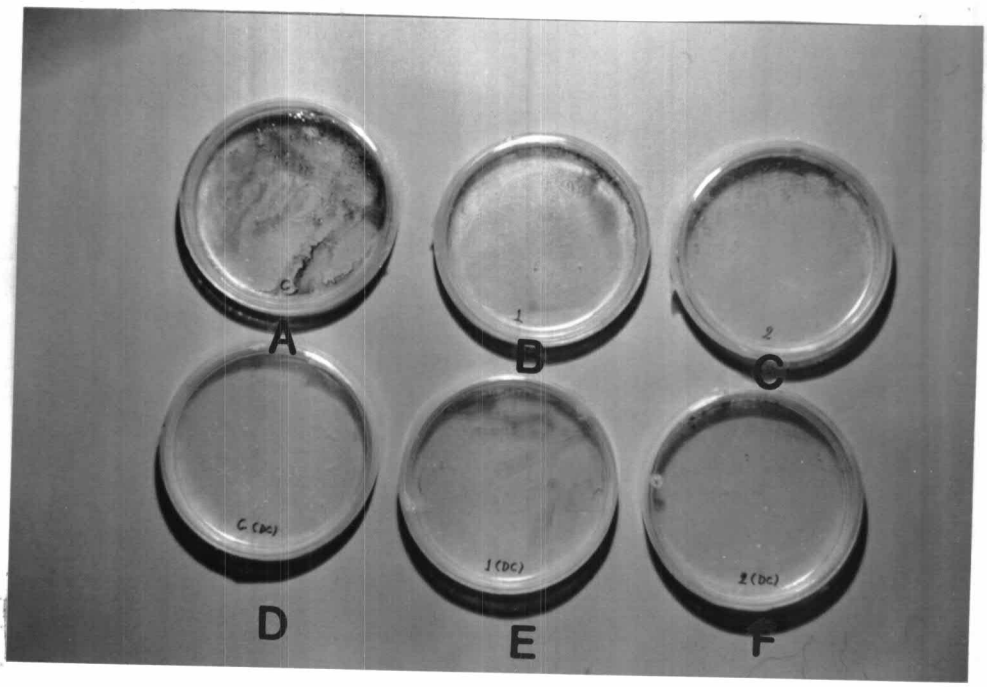

Figure 32 Growth of normal and NTG treatment mutated *Aphanothece halophytica* after subjecting to incubation with or without cycloserine on plate of Turk Island Salt Solution + modified BG_{11} medium solidified with 1.5 % agar

A,D. normal without, with cycloserine

B,E. mutated cells (10 μg/ml NTG) without, with cycloserine

C,F. mutated cells (20 μg/ml NTG) without, with cycloserine

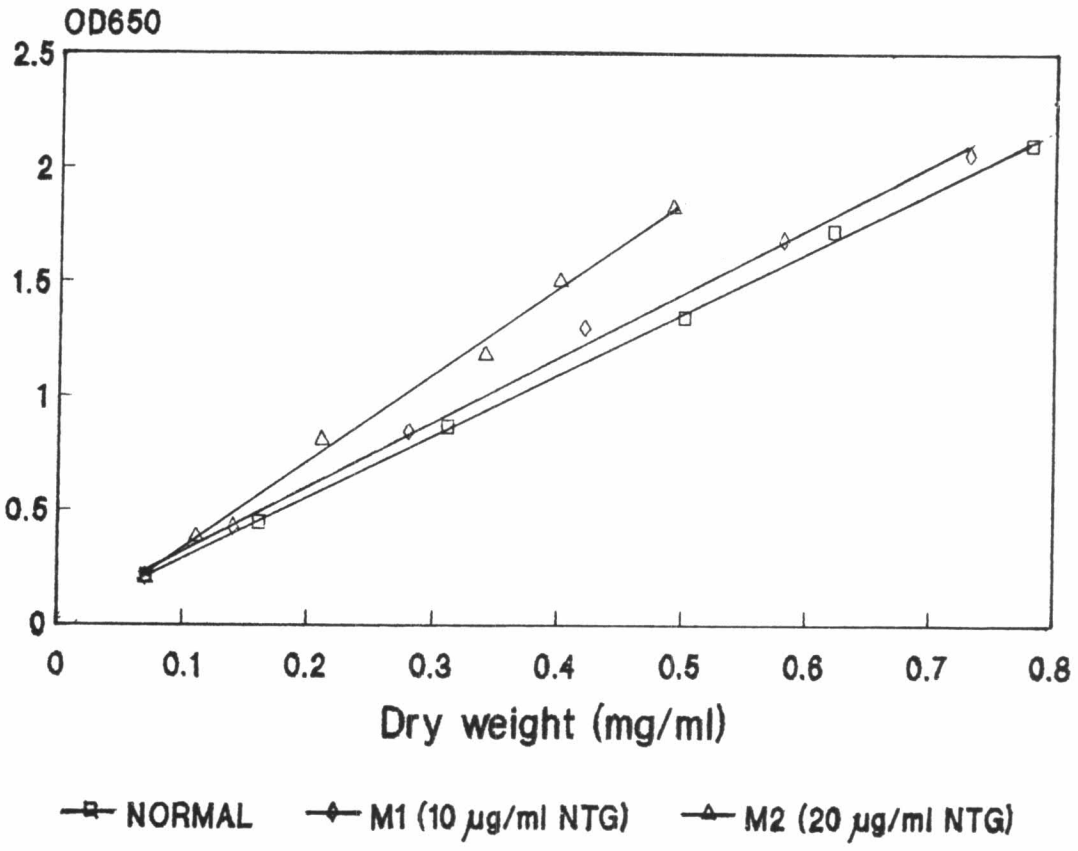

Figure 33 Relationships between OD_{650} and dry weight of normal and NTG treatment mutated *Aphanothece halophytica*

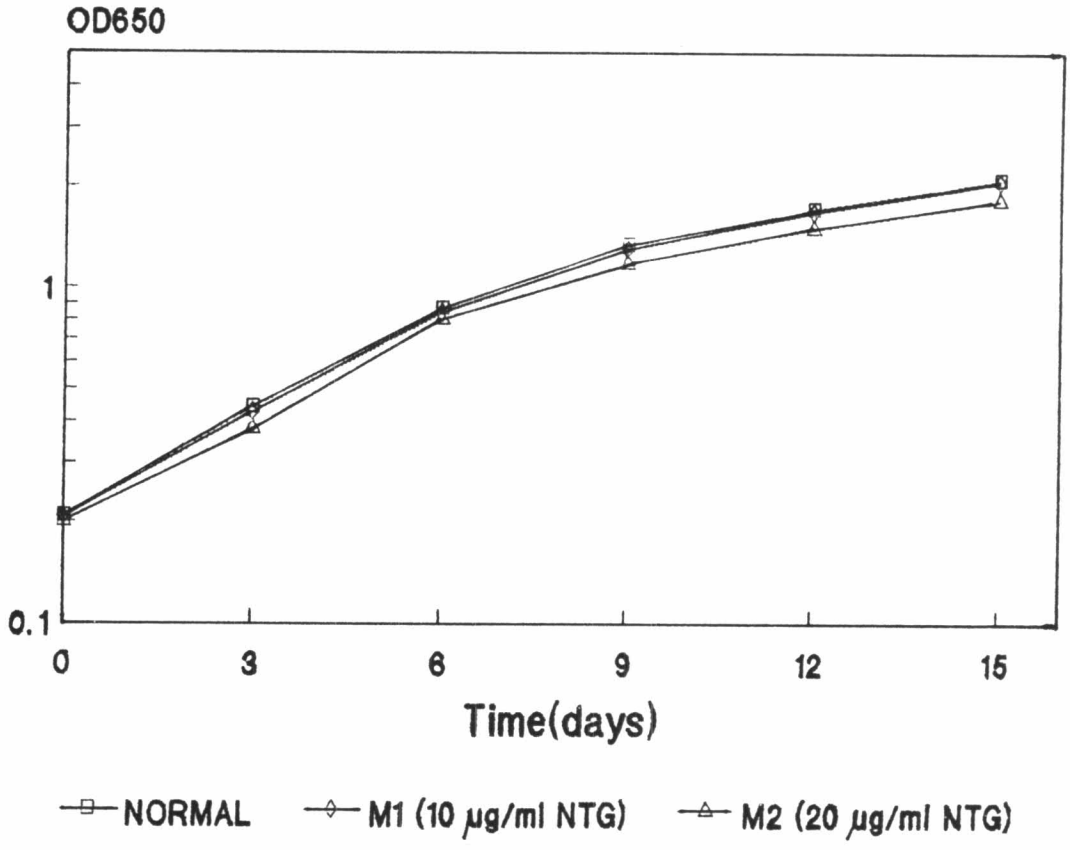

Figure 34 Comparison of growth between normal and NTG treatment mutated *Aphanothece halophytica*

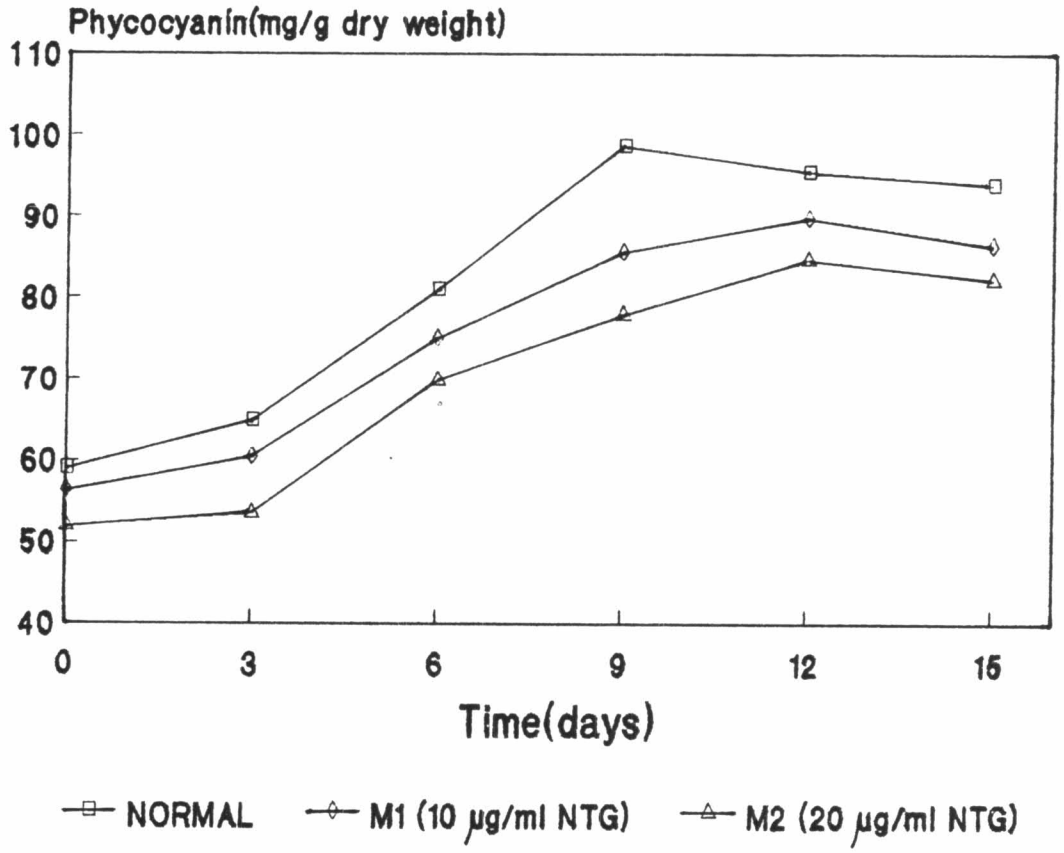

Figure 35 Comparison of phycocyanin content between normal and NTG treatment mutated *Aphanothece halophytica*

either 10 , 20 μg/ml NTG. Phycocyanin content yielded 85.73 and 77.91 mg/g dry weight at day 9 , respectively.

Partial Purification of Phycocyanin from *Aphanothece halophytica*

1. Ammonium sulfate precipitation

About 5.0 g wet weight of *A. halophytica* were suspended in 200 ml of 2 mg/ml lysozyme in 10 mM EDTA in 0.1 M sodium phosphate buffer, pH 7 and incubated at 37°C for 1 hr. The suspension was centrifuged at 3,500 rpm , 15 min. The supernatant was precipitated with 0-50 % , 20-45% , 20-65% and 20-75% ammonium sulfate. The suspension was centrifuged at 5,000 rpm , 30 min. The pellet was resuspended in a small volume of 0.02 M sodium phosphate buffer , pH 7.5. The adsorbance was measured at 620 nm and phycocyanin content was culculated by using $E^{1\%}_{1cm} = 73$ (Boussiba and Richmond , 1979). Table 2 shows at that 20-65% ammonium sulfate, a optimum condition for phycocyanin precipitation because this condition gave the highest yield of phycocyanin and the discarded supernatant was clear (no blue color of non precipitated phycocyanin) indicating the phycocyanin was completely precipitated.

2. Partial purification of phycocyanin

A. halophytica weight of 2.41 g were extracted phycocyanin with 100 ml of 2 mg/ml lysozyme in 10 mM EDTA in 0.1 M sodium phosphate buffer, pH 7 and incubated at 37°C for 1 hr. The fraction with 20-65 % ammonium sulfate

Table 2 Result of ammonium sulfate precipitation

% Ammonium sulfate	Phycocyanin (mg/50 ml extract)
0-50	1.97
20-45	1.94
20-65	2.07
20-75	1.98

precipitation was centrifuged at 5,000 rpm , 30 min. The pellet was resuspended in 20 ml of 0.02 M sodium phosphate buffer, pH 7.5 and dialyzed overnight in the same buffer.

2.1 DEAE-cellulose column chromatography

The elution profile after DEAE-cellulose column (I) (Figure 36) shows a single peak. Phycocyanin (OD_{620}) was eluted between NaCl concentration of 0-0.3 M. Absorption in the ultraviolet region (OD_{280}) coincided with the elution profile of the colored fraction (OD_{620}). The protein peak fractions (43-60) were pooled and precipitated by 20-65 % ammonium sulfate before rechromatography on DEAE-cellulose column. The elution profile in Figure 37 shows a single peak of protein coincident with phycocyanin peak which was eluted at 0-0.3 M NaCl. The fractions (29-36) were pooled and precipitated by 20-65 % ammonium sulfate before checking the purity by gel electrophoresis. Results of purification were summarized in Table 3.

2.2 Polyacrlamide gel electrophoresis

Fractions from DEAE-cellulose column (I) and (II) were dialyzed overnight and the purities of phycocyanin from *A. halophytica* were checked by polyacrylamide gel electrophoresis. Figure 38 shows that only one single band was present when the amount of the sample loaded was 25 μg of protein.

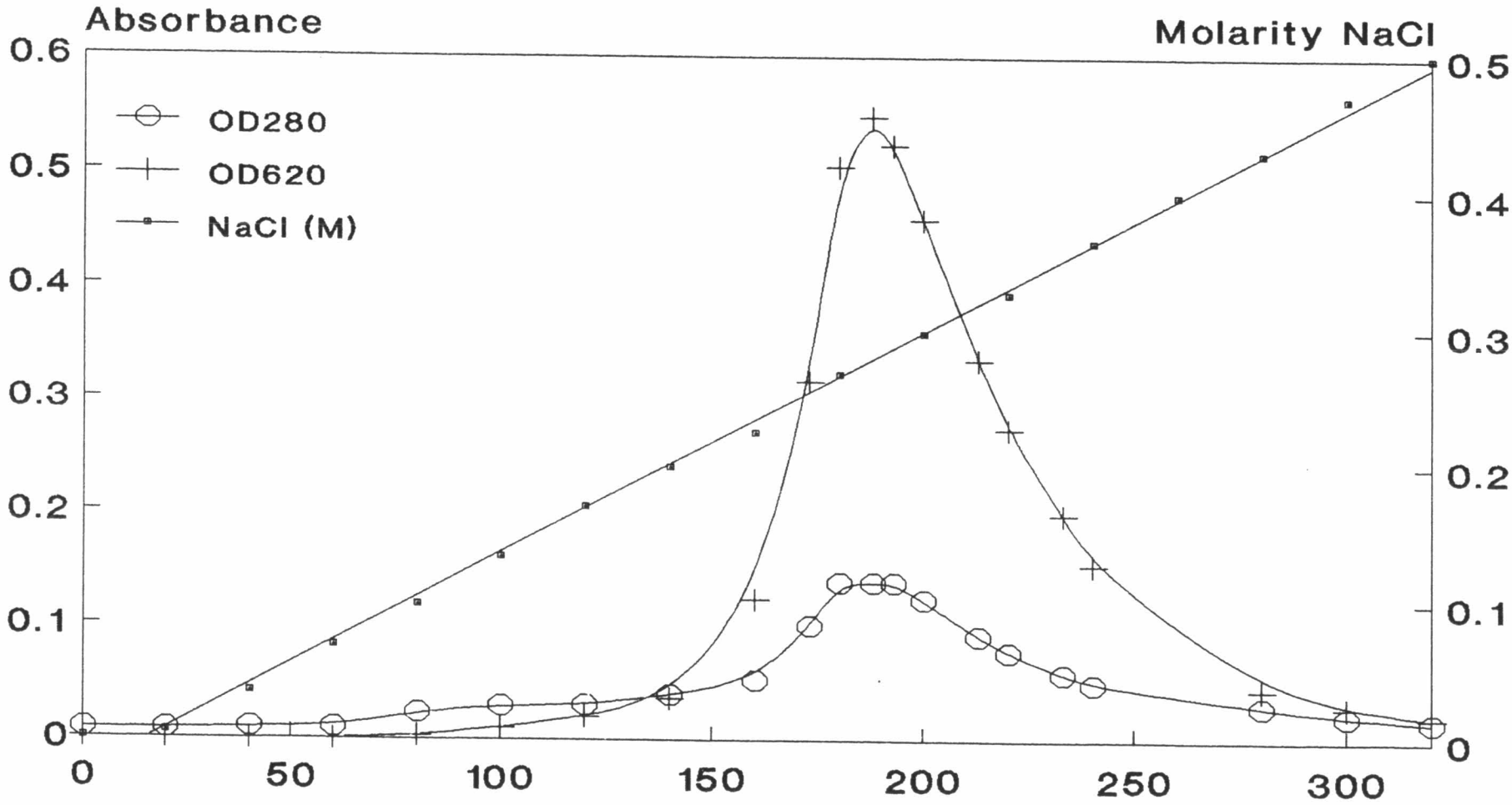
Absorbance
Molarity NaCl
OD280
OD620
NaCl (M)
0.6
0.5
0.4
0.3
0.2
0.1
0
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
Elution volume (ml)

Figure 36 Chromatographic profile of 65% ammonium sulfate fraction from *Aphanothece halophytica* on DEAE-cellulose column I. A 1.5 X 10 cm column and linear gradient of 0-0.5 M NaCl in 0.02 M sodium phosphate buffer, pH 7.5 was used. The flow rate was maintained at 30 ml/hr. The 5 ml fractions were collected and the absorbances were measured at 620 and 280 nm.

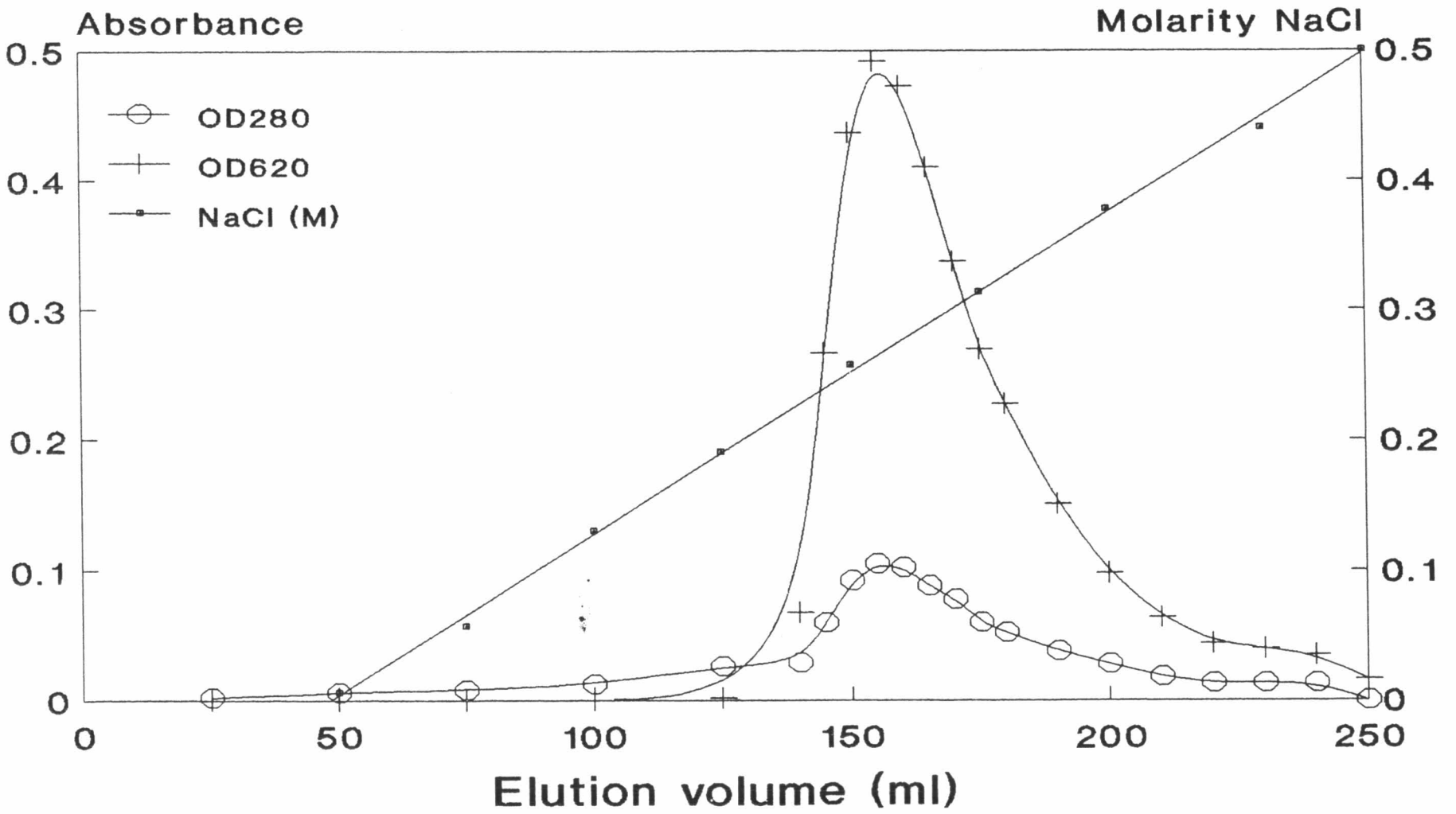
Absorbance
Molarity NaCl
OD280
OD620
NaCl (M)
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
Elution volume (ml)

Figure 37 Rechromatography of the first DEAE-cellulose fraction from *Aphanothece halophytica* on another DEAE-cellulose column. The protocol was the same as that Figure 36.

Table 3 Partial Purification of Phycocyanin from *A. halophytica*

Fraction	*A. halophytica*		
	Total phycocyanin (mg)	% Yield of phycocyanin	Absorbance ratio A620/A280
20-65 % ammonium sulfate fraction	5.31	100	1.59
DEAE-cellulose (I)	1.50	28	2.56
DEAE-cellulose (II)	0.56	11	4.03

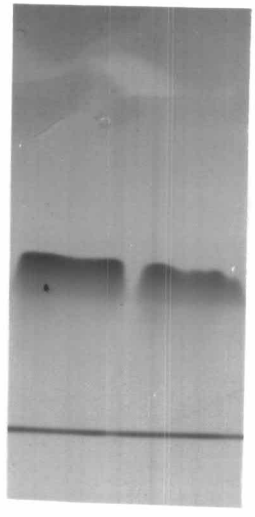

Figure 38 Polyacylamide gel electrophoresis of fraction from DEAE-cellulose column (I) and (II) from *Aphanothece halophytica*

A. 25 μg DEAE-cellulose (I) fraction

B. 25 μg DEAE-cellulose (II) fraction

2.3 Determination of Molecular Weight of Phycocyanin from *A. halophytica* by SDS-Polyacrylamige Gel Electrophoresis

The fractions having phycocyanin content from 20-65% ammonium sulfate fraction , DEAE-cellulose column (I) and (II) were analyzed by SDS-PAGE.The separation pattern is shown in Figure 39. The band pattern also indicated that rechromatography on the second DEAE-cellulose column effectively removed contaminating proteins. The purified protein of *A. halophytica* was likely to be phycocyanin as it corresponded to the intense band of standard phycocyanin (Linablue).Its molecular weight was found to be 17,000 daltons (Figure 40).

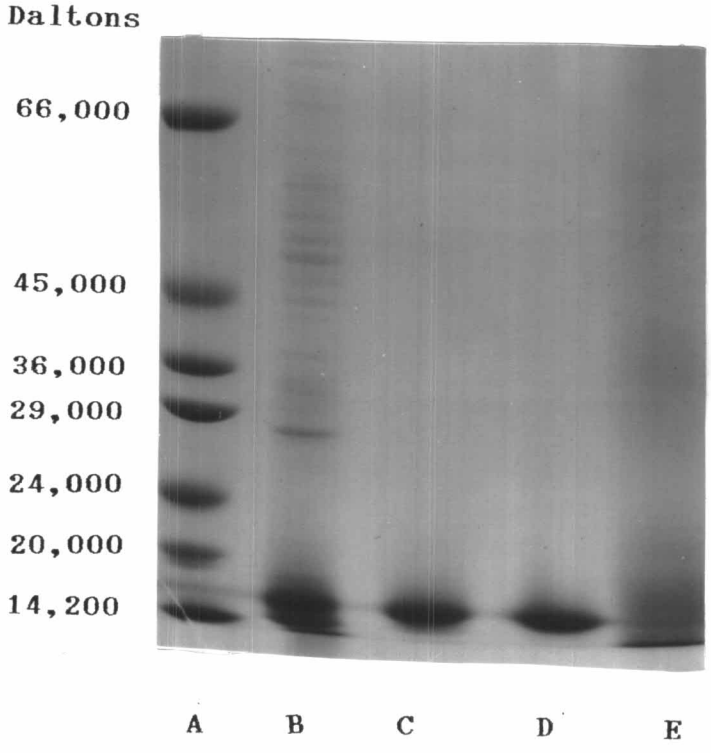

Figure 39 SDS-polyacrylamide gel electrophoresis of fractions from crude phycocyanin , DEAE-cellulose column (I) and (II) from *Aphanothece halophytica*

A. 25μg standard molecular weight marker proteins

B. 25μg 20-65% ammonium sulfate fraction

C. 25μg DEAE-cellulose (I) fraction

D. 25μg DEAE-cellulose (II) fraction

E. 25μg standard phycocyanin (Linablue)

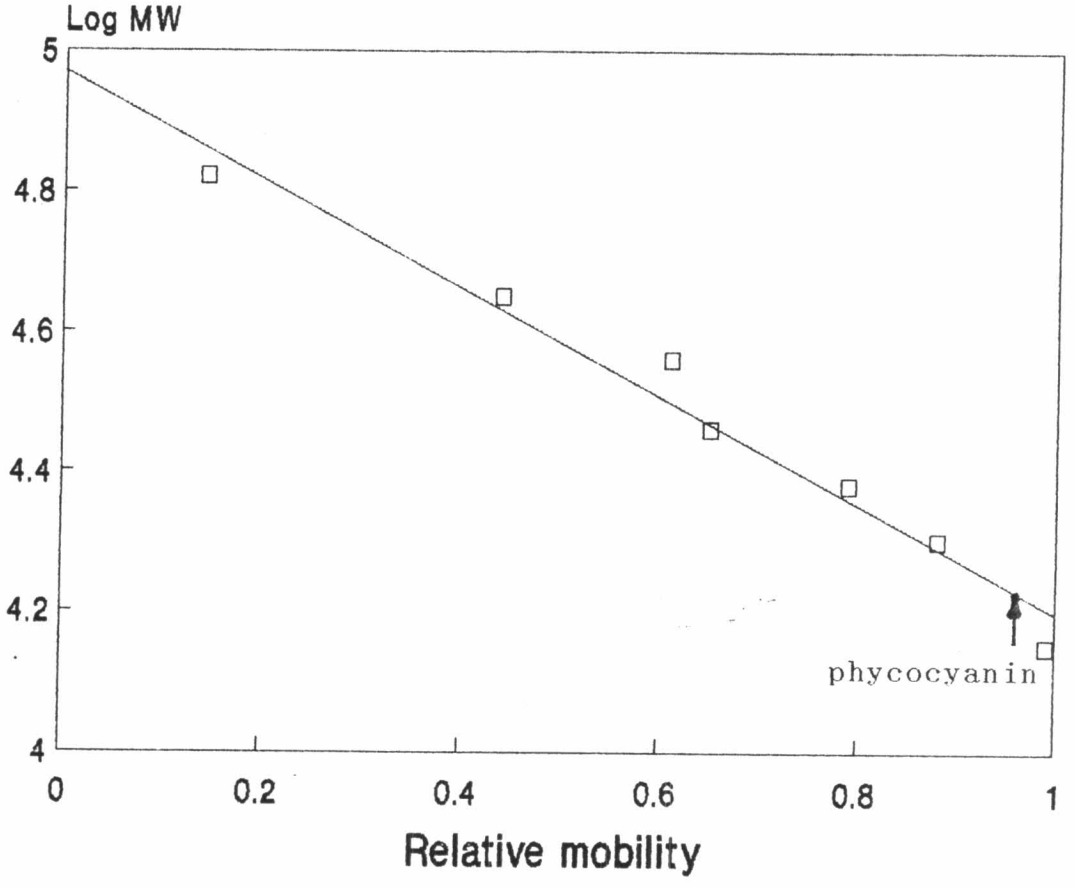

Figure 40 Determination of the molecular weight of phycocyanin from *Aphanothece halophytica* Standard proteins were (1) bovine serum albumin (66,000),(2) ovalbumin (45,000),(3) glyceraldehyde-3-p-dehydrogenase (36,000),(4) carbonic anhydrase (29,000),(5) trypsinogen (24,000),(6) trypsin inhibitor (20,000)and(7) α-lactabumin (14,200)