

## **4.1 Introduction**

Various external as well as internal factors affect the metabolic activities of a living cell. pH is a component of environmental conditions affecting the physiology of cyanobacteria. The pH value of the medium or surrounding water is one of the most important factors influencing metal separation, bioavailability and toxicity (Pawlik-Skowronska et al., 1997). Also the availability of certain nutrients, the water holding capacity and other conditions are influenced by high  $H^+$  concentrations (Uexkull and Mutert, 1995). Cyanobacteria are known to develop a certain electrical surface charge expressed as zeta potential depending upon the pH of the surrounding medium, the size of which affects the permeability of the cell wall (Hegewald, 1972). The pH value of the algal culture medium is one of the important factors in algal cultivation. It determines the solubility of carbon dioxide and minerals in the culture and directly or indirectly influences the metabolism of the cyanobacteria. The pH requirement varies from organism to organism. The pH of cultures is influenced by various factors such as composition and buffer capacity of the medium, amount of  $CO_2$  dissolved, temperature (which controls  $CO_2$  solubility) and metabolic activity of the algal cells.

The alkalization of the medium during photosynthesis is a common phenomenon and has been reported for intact cells of algae and cyanobacteria. Like other autotrophic plants, cyanobacteria also require an inorganic carbon source for photosynthesis in the form of carbon dioxide, which forms 0.03% of the air. Natural oligotrophic waters contain some supply of  $CO_2$  absorbed from the atmosphere. However, these concentrations are too low to achieve optimal growth in algal cultures. In a poorly buffered system like the nutrient medium, the assimilation of carbon dioxide and bicarbonate by rapidly growing algae

causes the equilibrium to shift, resulting in increased pH, due to a release of OH<sup>-</sup> ions (Becker and Venkataraman, 1982). According to studies conducted by many researchers, the blue green algae are not able to tolerate pH below 4.0 (Prescott, 1951; Lund, 1962; Rosa and Lhotsky, 1971). Although it is not known with certainty why acid is detrimental to cyanobacteria, one possibility is that it affects the photosynthetic apparatus, which in these organisms is usually at the periphery of the cell (Brock, 1973).

Thus pH may be playing a pivotal role in the photosynthetic activity of these autotrophs. The physiological adaptation of cyanobacteria, towards the H<sup>+</sup> therefore, needs thorough investigation. This chapter is an attempt at establishing a correlation between the pH of the surrounding environment and the pigment content of the organism, if there is any.

## **4.2 Materials and Methods**

### ***Materials:***

Standard protein molecular weight markers were obtained from Sigma-Aldrich Co., USA. All other reagents used were of analytical grade available from commercial sources and used without further purification.

### ***Methods:***

#### ***4.2.1 Quantification of Chl 'a' and Phycobiliproteins:***

Fresh water strain, *A. indica*, cultured in Zarrouk's medium and the two marine strains, *P. tenue* and *L. limnetica* cultured in ASN-III medium were grown at different pH (7.0, 8.0, 9.0, 10.0). The pH was regularly maintained with 0.1 M HCl and 0.1 M NaOH. All the experiments were carried out in duplicates and the graphical data represents the mean

values. Cultures were harvested every seven days for 5 weeks, by centrifugation at 10000 xg for 30 min at 4°C.

For PB extraction the harvested cell mass was suspended in 100 mM Na-Phosphate buffer (pH 7.0) and the cell mass was disrupted by sonication for 20 s. Repeated cycles of freezing at -195°C in liquid nitrogen and thawing at room temperature were carried out till complete extraction was done. This was then followed by centrifugation at 10000 xg for 30 min at 4°C and the clear supernatant containing PB was collected.

### **Estimation of PB**

Estimation of PC, APC and PE were done as mentioned in section 2.2.1.

For Chl 'a' extraction the cell mass was suspended in 90% methanol and the extraction was carried out twice at 4°C for an hour, in the dark, followed by centrifugation at 10000 xg for 10 min at 4°C. Cultures were kept under different pH conditions in duplicates.

### **Estimation of Chl 'a'**

Estimation of chl 'a' was done as mentioned in section 2.2.1

### **4.2.2 Spectral Analysis:**

#### **UV-VIS absorption spectroscopy**

To study the spectral nature of PB under different pH concentrations, the cells were cultured as mentioned in section 1.2.1 and were harvested in the log phase. Extraction was done as mentioned in section 2.2.1 and the spectra recorded in UV-Vis range with respect to phosphate buffer as blank. All UV-Vis absorption spectra were recorded on a CARY 500 Scan UV-Vis, NIR spectrophotometer with 1 cm path length.

## **FT-IR Spectra**

The PB extracted from the cells in the log phase was dialyzed against water at 4°C for 48 hrs and then freeze dried by Virtis Freeze mobile 8EL and the spectras were recorded on a Perkin Elmer Spectrum GX FT-IR spectrophotometer as KBr pellet.

### **4.2.3 Gel Electrophoresis:**

The PB extracted from cells grown under different pH conditions were electrophoresed by SDS-PAGE (15%) (Sambrook et al., 1989). Samples were heated for about 5 min at 95°C with 2% (w/v) SDS, 10% (v/v) glycerol, 4.5% (v/v)  $\beta$ -mercaptoethanol, 0.25% (w/v) bromophenol blue and 60 mM Tris (pH 6.8) for about 5 min at 95°C. Gels were run at 20 $\pm$ 2°C and visualized by silver staining (Wray et al., 1981) and photographed. The molecular weight of the separated linkers and subunits were determined by calibrating the gel with molecular weight markers.

## **4.3 Results**

### **4.3.1 Growth curves and PB content:**

The pH value of the culture medium is one of the most important factors in cultivation of aquatic flora. pH affects different factors like intake of essential mineral elements and solubility of CO<sub>2</sub> thus, directly or indirectly influencing the metabolism of cyanobacterial cells. Alterations in pigment metabolism due to pH changes are reported here.

*A. nidica* shows the lowest doubling time of ~7 hrs when grown under pH 10.0. As pH decreases, doubling time increases. The two marine strains *L. limnetica* and *P. tenue* grow well in neutral pH with a doubling time of ~10 hrs in pH 7.0 and pH 8.0. High alkaline conditions lead to lower growth rate and higher doubling time (Table 4.1).

**Table 4.1** Doubling time of the three strains under different pH conditions<sup>#</sup>.

<i>A. indicia</i>	<i>L. limnetica</i>	<i>P. tenue</i>
~12 hrs (7.0)	~15 hrs (7.0)	~19 hrs (7.0)
~9 hrs (8.0)	~12 hrs (8.0)	~13 hrs (8.0)
~11 hrs (9.0)	~14 hrs (9.0)	~15 hrs (9.0)
~7 hrs (10.0)	~11 hrs (10.0)	~11 hrs (10.0)

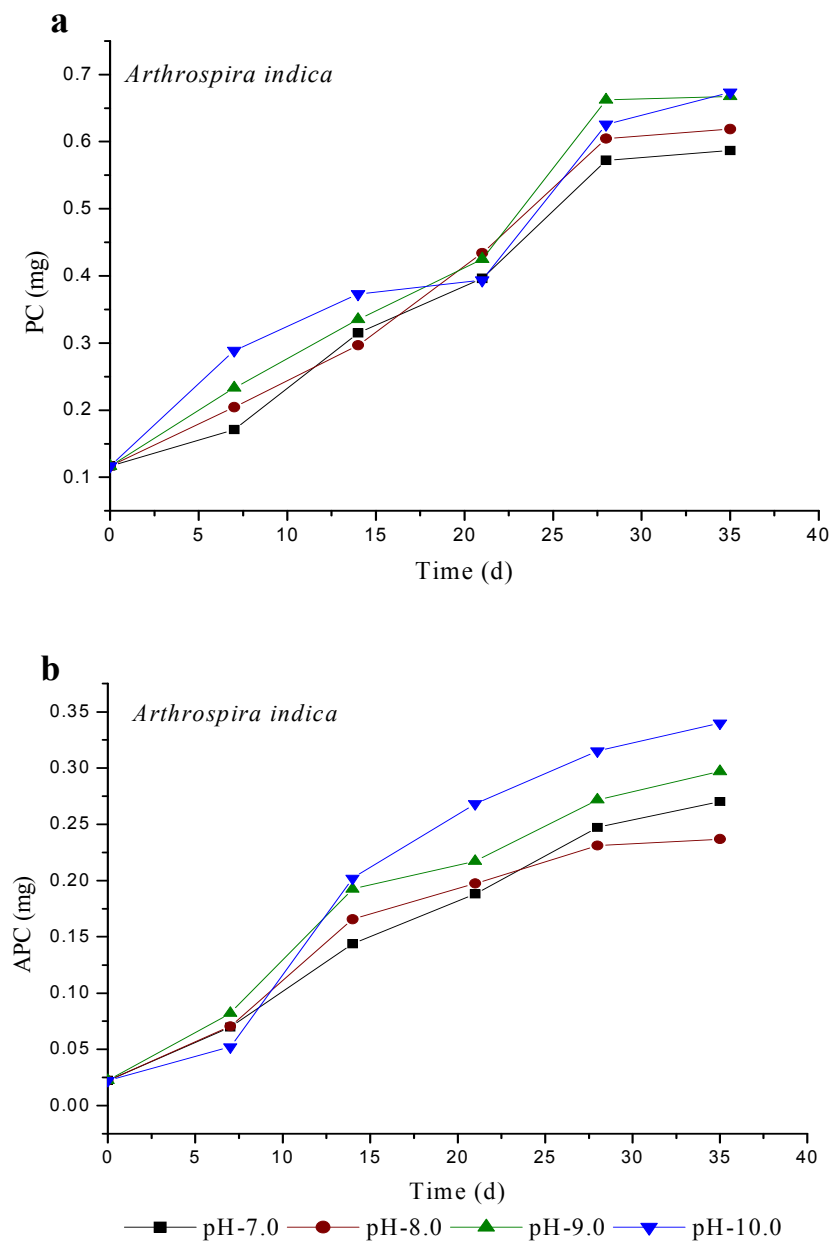
<sup>#</sup> pH values of the medium are shown in the bracket.

No striking variations were observed in the PC content in all the three strains. Though, a slightly high content of PC is observed in marine sp. at neutral pH and at alkaline pH in *A. indica* (Fig.4.1-4.3). APC content in *A. indica* does seem to show a considerable elevation when cultured under an alkaline pH of 10.0 (Fig.4.1b). In *L. limnetica* too a high APC content through out the log phase is observed in PE content (Fig. 4.2b).

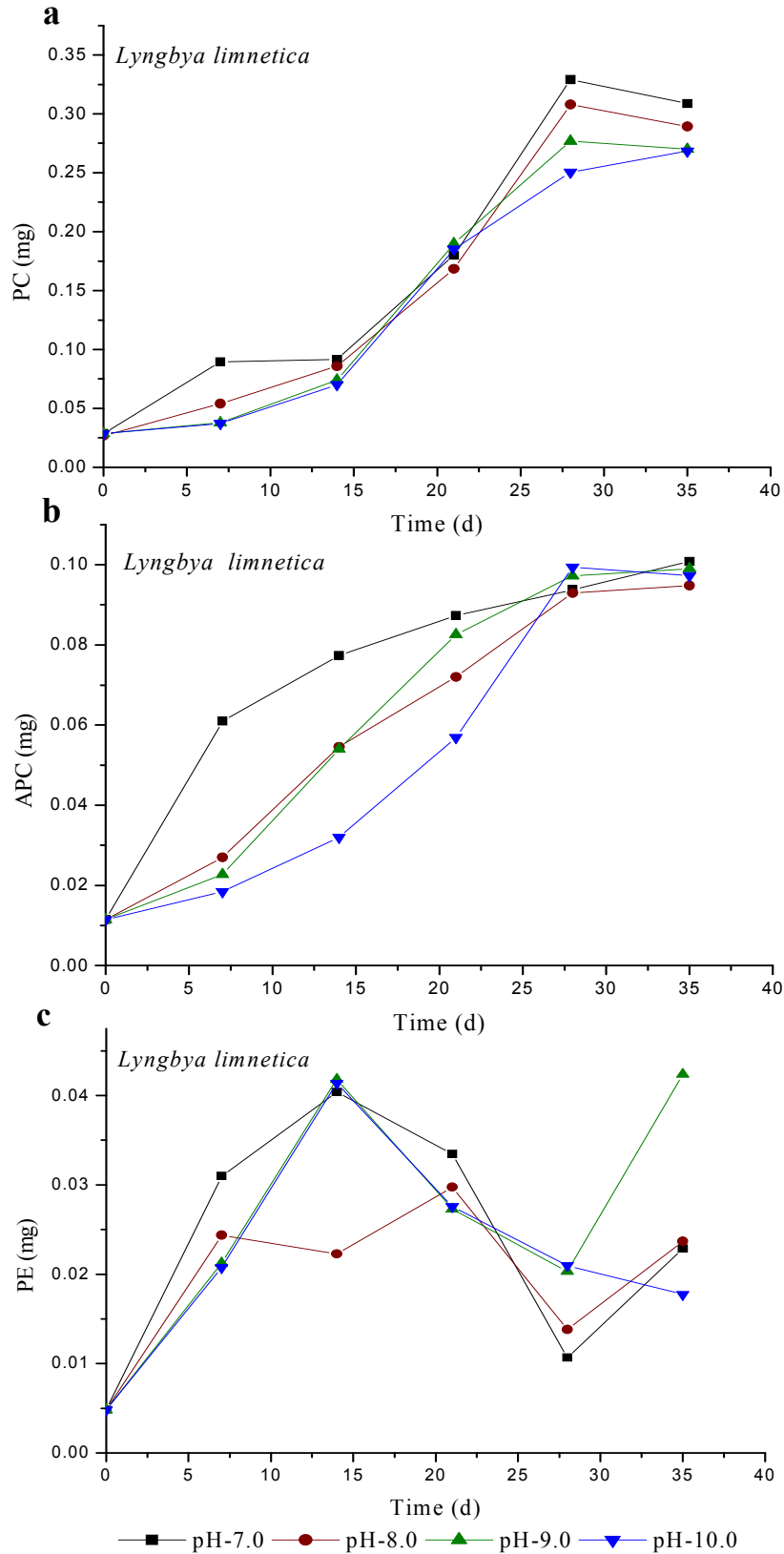
#### 4.3.2 Spectral studies:

Spectral analysis of PB indicates any change in the PB structure through shifts in  $\lambda_{\max}$ . Presented below is the analytical data inferred from the spectras of PB extract taken in mid log phase.

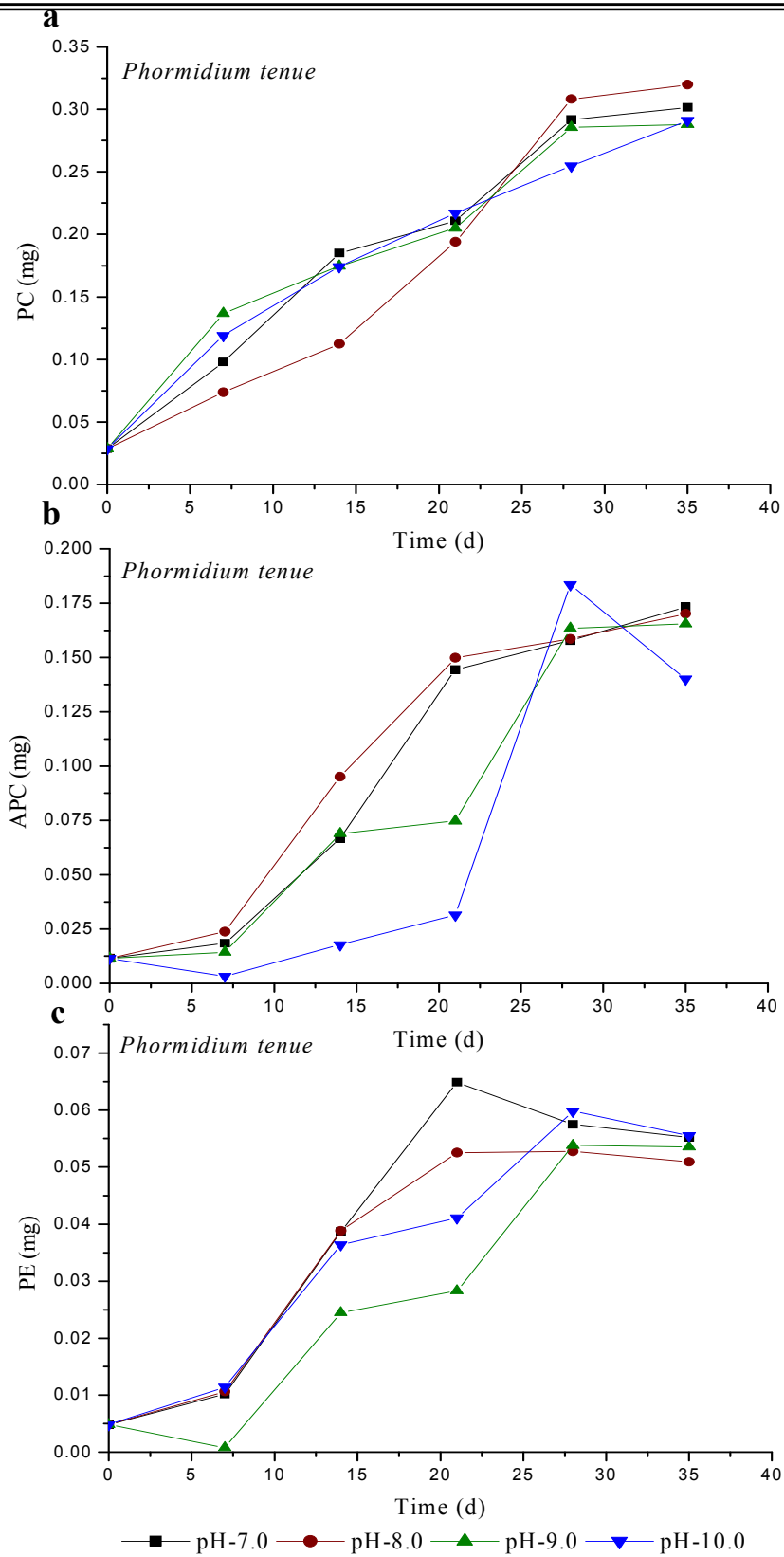
The spectral data shows a blue shift in PB extract of *A. indica* as the pH is changed from alkaline to neutral. The PB extract from cultures grown in medium of pH 7.0 shows a considerable shift of ~6 nm in the PC peak, which is the dominant pigment (Fig.4.4a).



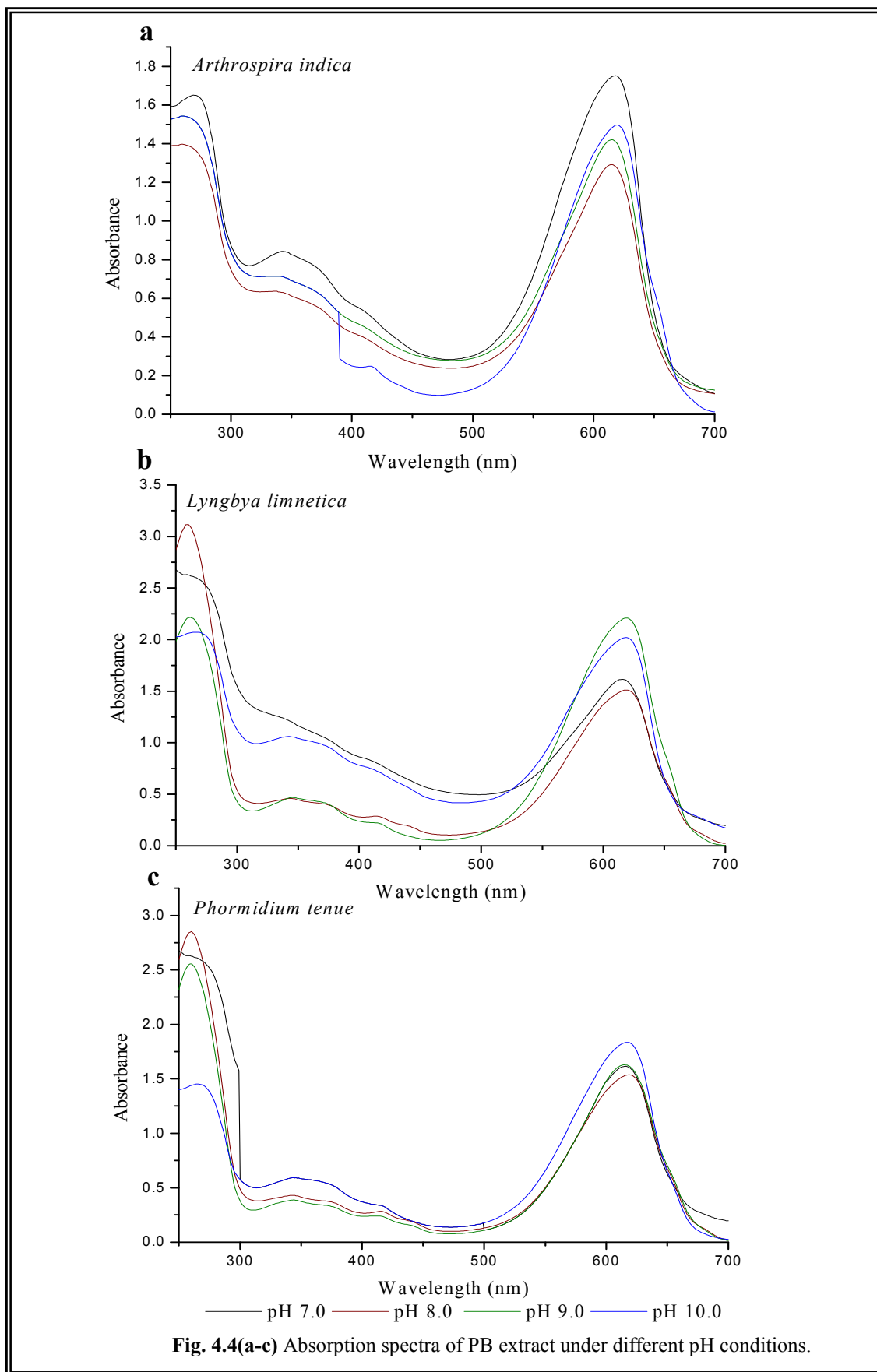
**Fig. 4.1(a-b)** Variation in PB content under different pH conditions in *A. indica*



**Fig. 4.2(a-c)** Variation in PB content under different pH conditions in *L. limnetica*



**Fig. 4.3(a-c)** Variation in PB content under different pH conditions in *P. tenue*.





**Table 4.2** Absorption maxima of PB extract in visible region under different pH conditions<sup>#</sup>.

<i>A. indica</i>	<i>L. limnetica</i>	<i>P. tenue</i>
613 nm (7.0)	620 nm (7.0)	620 nm (7.0)
617 nm (8.0)	620 nm (8.0)	620 nm (8.0)
619 nm (9.0)	619 nm (9.0)	619 nm (9.0)
620 nm (10.0)	617 nm (10.0)	618 nm (10.0)

# pH values of the medium are shown in the bracket.

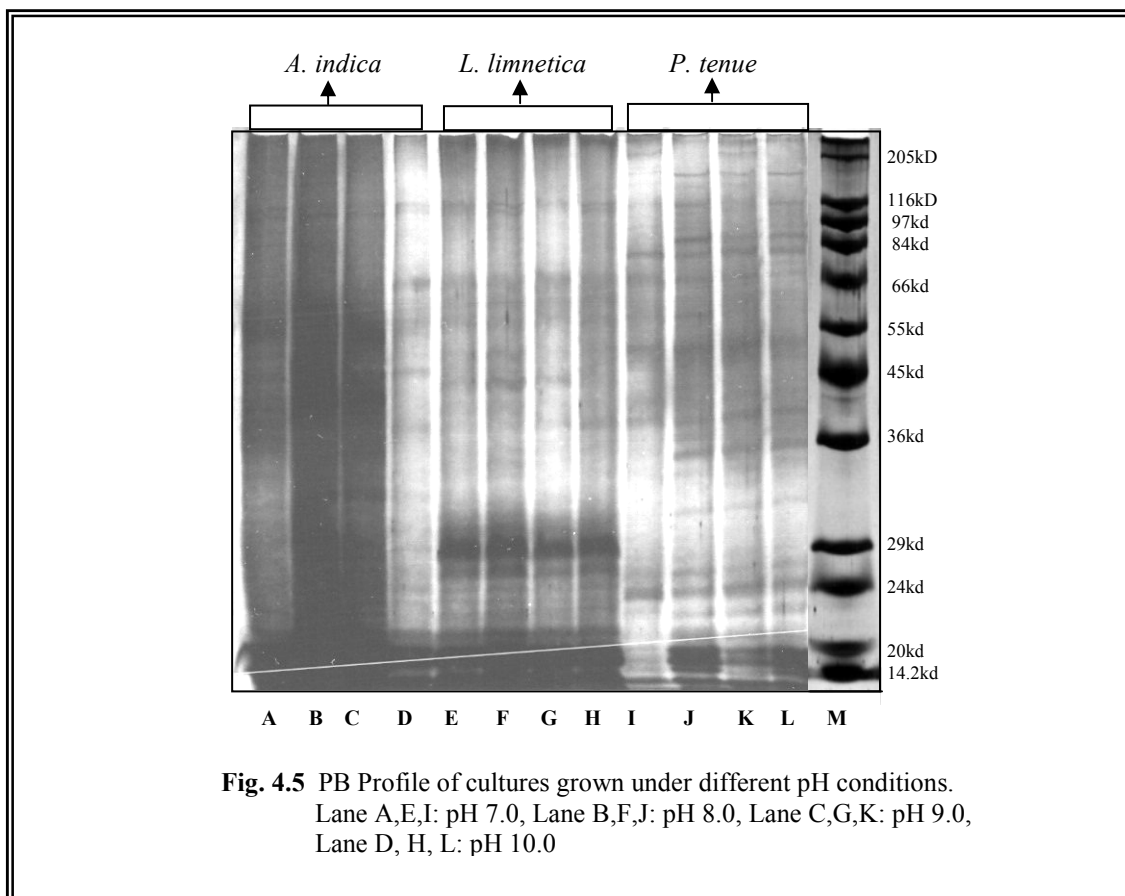
The marine strain, *L. limnetica* also shows a blue shift (~3 nm) but when the pH is alkaline (9.0 or 10.0). *P. tenue*, like *L. limnetica* also shows a blue shift in alkaline medium but the shift is not very considerable (Table 4.2).

FT-IR spectra of the different samples showed no major variation.

#### 4.3.3 Protein Profiling:

SDS polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used method for analyzing protein mixtures qualitatively. SDS PAGE reveals the electrophoretic pattern of the PB components.

No major differences in the protein profile of PB from *L. limnetica* was observed. But in *P. tenue* group III linkers seem to be affected. Protein profile shows a decrease in the number of group III linkers. The  $\alpha$  and  $\beta$  subunits, which belong to group I, are not clearly separated in any of the cultures. The PB samples of *A. indica* seem to be overloaded in the wells, due to which the separation is not good enough for any inferences (Fig.4.5).



#### 4.4 Discussion

pH is among the different environmental conditions which affect the biochemical and physiology aspects of cyanobacteria. Different strains thrive in different range of pH.

*A. indica* shows an affinity for alkaline medium whereas the marine *L. limnetica* and *P. tenue* prefer a neutral environment where they have a lower doubling time and thus a higher growth rate.

Considerable differences are not observed in the PB content in the three cultures. But the preference of the marine strains towards neutral pH and of the freshwater strain towards alkaline pH is very clear even in the PB components. Cells are known to develop an electrical charge on the surface, which depends on the pH of the medium around

(Hegewald, 1972). In cyanobacteria, PB localize on the periphery of the cell, adjacent to the plasma membrane. Thus it may be presumed that pH has a direct impact on the PBS assembly. Blue shift in spectral peaks of PB of *A. indica* under neutral conditions, and of the marine strains under alkaline conditions, indicate certain changes in the PB component or its environment. But there are many different factors affecting the spectral nature of as well as the PBS assembly (Rowan, 1989) so it is difficult on the basis of the studies carried out to pin point at one particular reason, but a blue shift does confirm a decrease in the energy transfer (Sidler, 1994). No conspicuous difference is observed in the polypeptide profile done through SDS-PAGE in *L. limnetica*. PBS in *P. tenue* are observed to be more sensitive towards pH changes, as linkers belonging to group III seem to decrease in number under alkaline pH.