Pak. J. Bot., 8 (2): 103—110, 1976.

CHANGES IN THE CELLULAR MORPHOLOGY OF *BRYOPSIS PLUMOSA* (BRYOPSIDOPHYCEAE) UNDER HIGH HYDROSTATIC PRESSURE AND TEMPERATURE.

MUSTAFA SHAMEEL

*Institut für Meereskunde an der Universitat Kiel, F. R. Germany**

Abstract

Influences of hydrostratic pressure (200-800 atm) and temperature (5°-15°C) applied for 5 hours were studied on the assimilating filaments of *B. plumosa* (Hunds.) C. A. Ag.; the after-effects were observed upto 3 weeks. The cell morphology of the filaments was found to be increasingly affected by the rising pressure. The physiological limits of tolerance of the alga were much lower than other observed seaweeds, it seems to be an extreme barophobe. Pressures upto 400 atm produced reversible changes, but the higher ones rendered irreversible effects leading to the ultimate death of the thalli either immediately or after sometimes depending on the temperature. Increasing temperature decreased the pressure effects.

Introduction

High hydrostatic pressure and low temperature are the two environmental parameters which directly affect the life of deep sea organisms. It is a unique combination which acts at the molecular level in ways which are poorly understood. Brunn (1957) considered temperature to have a more significant effect on deep sea life than pressure, and Ponant (1967) laid down more emphasis on the latter, but recent studies have shown that the relationship between hydrostatic pressure and temperature is quite complicated in the case of marine invertebrates (Menzies & George, 1972; Ponant and Theede, 1973; Neuhoff and Theede, 1975) as well as marine algae (Shameel, 1973a, 1975a, 1977). Despite a considerable amount of work done in the fields of deep-sea biology and biochemistry (Macdonald, 1975; Hochachka, 1976) there appears no such report that the microalgae, which have been collected from oceanic depths of 1000 - 4000 m (Bernard, 1967; Wiebe *et al.*, 1974), differ in their cell morphology from those occurring at sea surface. Due to this reason it appeared interesting to investigate, how far algae occuring in shallow water show influence of pressure and temperature on their cellular morphology, in the hope that this will guide our thinking about adaptation to high hydrostatic pressure. *Bryopsis plumosa* was selected for this purpose, because it is sensitive to pressures even lower than 100 atm (Shameel, 1974a), its nuclei and chloroplasts can be easily observed under microscope, and it exhibits a simple cytology with no partition walls being a siphonous coenocyte.

Materials and Methods

Bryopsis plumosa (Huds.) C.A. Agardh was collected from the stony ledge near light house at Kieler Fôrde, western Baltic Sea, and was cultured as described elsewhere (Shameel, 1977). After keeping the algae for 2 weeks' adaptation to 5°C,

*Permanent address: Institute of marine biology, University of Karachi, Karachi-32, Pakistan.

non-reproductive, free from epiphytes and uninjured thalli were selected and placed together with culture medium in plastic tubes of 25 ml capacity. Three such tubes could be placed in each of the cylindrical chambers (C, fig. 1). The pressure apparatus used for this study was different as devised for previous studies (Shameel, 1973a). It has the advantage that during pressure treatment the control specimens can also be placed in a chamber (C_1), which is exactly similar with the pressure chamber (C_2), and the position of the chambers may be changed during pressure action due to the use of a connecting thin and spiral wire (W). The apparatus was constructed for the laboratory of Prof. H. Theede, who kindly allowed me to use it for this investigation.

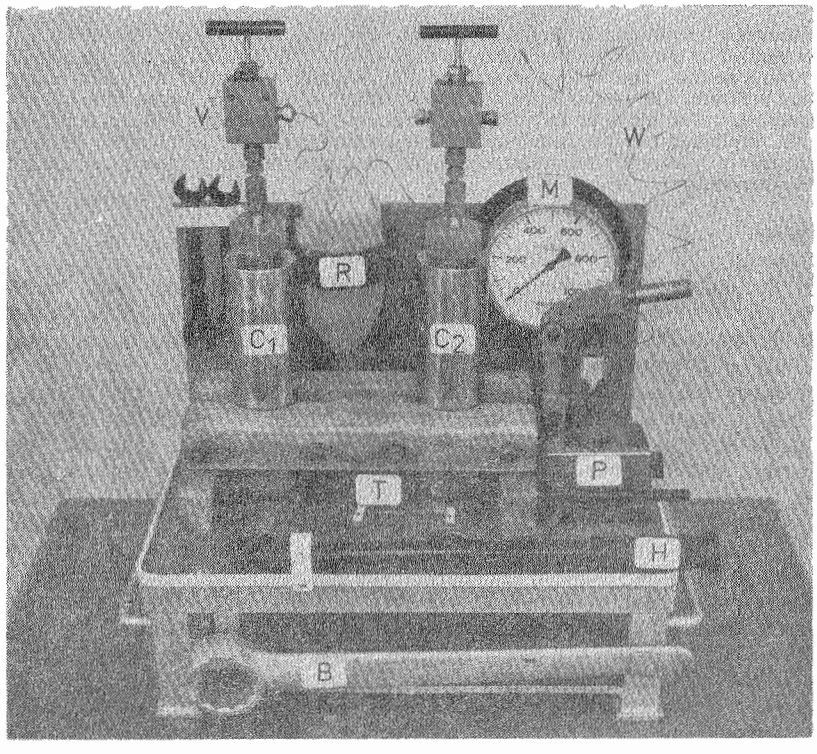

Fig. 1. Hydrostatic pressure apparatus (B = big winch for opening the chambers, C_1 = control chamber, C_2 = pressure chamber, H = handle for the pump, M = manometer, P = hydraulic pump, R = reservoir for hydraulic fluid, T = rubber tubing, V = needle valve, W = hollow wire).

The pressure apparatus consists of a hydraulic hand pump (P, fig. 1), which is connected on one hand with a tap-water reservoir (R) through a rubber tubing (T)

and on the other side with a manometer (M) and via a three way simple needle valve (V) of Messers Aminco (USA) with a pressure chamber (C_2) through pressure resistant, spiral and hollow wire (W). The pressure chamber may or may not be connected with the control chamber (C_1). The chambers are stainless steel cylinders, 38 cm high, 7.6 cm in diameter, 2 cm in wall thickness, have about 300 ml inner volume, and were filled with tap water, which acted as hydraulic fluid. During experimentation the whole apparatus was kept in the constant temperature room. A reference may be made to Theede (1972) for technical details of the apparatus and the techniques employed during pressure treatment.

Three experimental series were set up at 5° 10° and 15°C, the pressure levels used were 200, 300, 400, 500, 600, 700 and 800 atm, and the duration of pressure action was 5 hours in all the cases. After pressure release the algae were brought back to 5°C and immediately examined in culture medium under Zeiss Winkel microscope employing phase contrast assembly. The cell morphology of pressure treated and control algae were regularly observed under microscope upto 3 weeks. The microscopy was done in temperature constant room in order to avoid a temperature shock. After every microscopic observation the rate of respiration of the treated algae was measured by Winkler's method as described earlier (Shameel, 1973a). The purpose of this measurement was simply to determine, whether the injured algae were still alive.

Results and Discussion

As was initially thought *Bryopsis plumosa* appeared to be highly sensitive to hydrostatic pressure. When examined under microscope immediately after the removal of pressure it showed a variety of changes produced in its cellular morphology (fig. 2). The cytoplasm lost its gel structures and was converted towards the sol condition (2a). The cell organelles were irregularly distributed, and the primordial utricle lost its continuity here and there (2b). At certain places the chloroplasts, nuclei and other cell organelles were seen accumulated either in the centre or on one side of the filament (2c, d). It is due to the liquefying effect of high pressure on cytoplasm, during which the inter-or intramolecular bonds and particularly hydrophobic bonds are dissociated. It is similar to the pressure effect on methyl cellulose in which it dissociates hydrophobic bonds (Macdonald, 1975). These bonds are important in determining viscosity of the cytoplasm. The significance of high hydrostatic pressure at the structural level of cytoplasmic organisation, however, may not be overestimated regarding adaptation to the deep sea.

The young branchlets and the old main axes were equally affected after an exposure to the pressures higher than 200 atm (fig. 2). However after subjection to 200 atm no visible change in the cell morphology was produced and it also showed no after-effect even upto 3 weeks after pressure treatment. This is probably the low st physiological limit of tolerance for the alga. The other algae studied previously behaved differently. When subjected to 400 atm for 5 hours for example, there was no observable change in the cytoplasm and cell walls of the filaments of *Callithamnion corymbosum*, *Cladophora glomerata*, *Polysiphonia nigrescens* and *P. urceolata* (Shameel, 1973 b, c, 1974 b). The lower physiological limit of tolerance, therefore, appears to be variable in different algae. *B. plumosa* has a very low value for that and seems to be an extreme barophobe among all of the seaweeds studied so far.

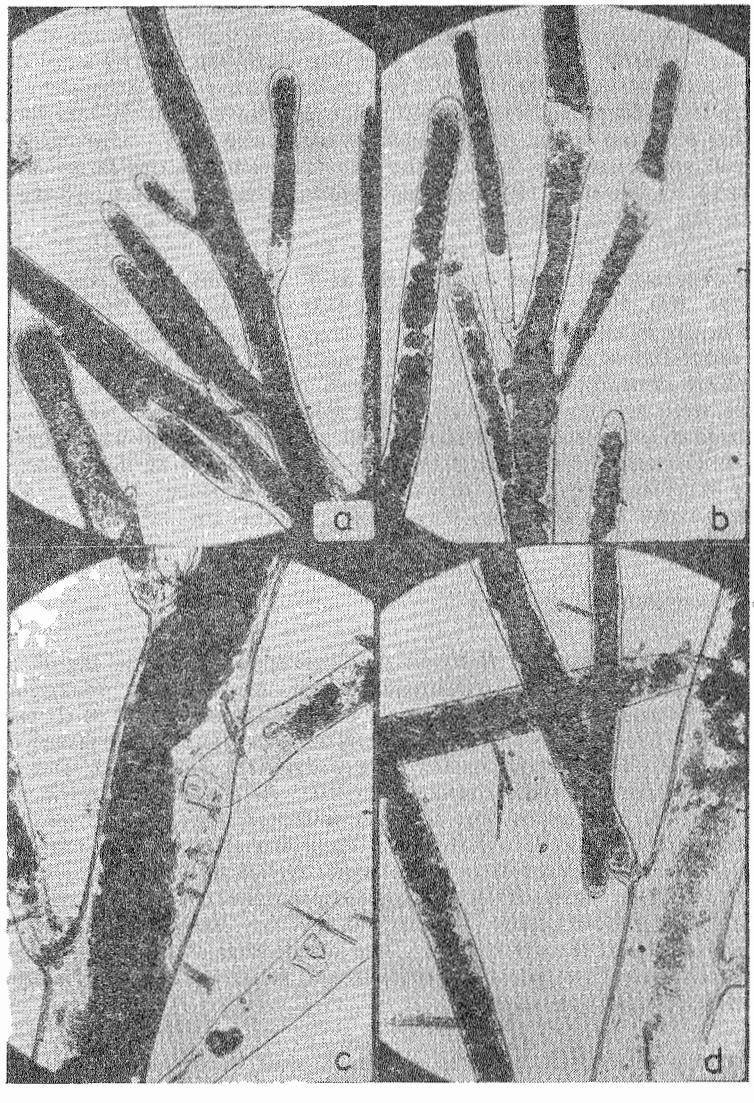

Fig. 2. Parts of *Bryopsis plumosa* thalli after subjection to 400 atm hydrostatic pressure for 5 hours at 5°C (a & b=young branchlets, c & d=older main axes).

The protoplasm morphology of *B. plumosa* was found to be increasingly affected by pressure levels ranging from 300 to 800 atm (Table 1). The pressure action is composed of 2 components, one of them depends on the product of pressure level and the duration of pressure action, and the other one is determined mainly by the quantity of pressure applied and causes a reversible injury to the cell morphology of the filaments. The pressure levels of 300 and 400 atm produced a change in the cellular mor-

phology which was reversible, but the higher pressure levels caused an irreversible change depending on the temperature. This appears to indicate the upper physiological limit of tolerance of the alga, which is also temperature dependent and is much lower than those of other algae. *Ulva lactuca* died within a few days after an exposure to 800 atm for an hour (Fontaine, 1929), whereas the changes produced in the cytoplasm of *Cladophora glomerata* after a pressure exposure of 800 atm for 5 hours were reversible (Shameel, 1973b). Hence the upper limit also varies in the case of different seaweeds.

Table 1. Changes produced in the cell morphology of *Bryopsis plumosa* due to hydrostatic pressure applied for 5 hours at different temperatures as observed immediately after pressure release (— =no change, + =25% changes + + =50% changes, + + + =75% changes and + + + + =100% changes).

Hydrostatic pressure in atm	Temperature in°C		
	5	10	15
200	-	-	-
300	+	+	-
400	+ +	+	+
500	+ + +	+ +	-
600	+ + + +	+ + +	+
700	+ + + +	+ + + +	+ + +
800	+ + + +	+ + + +	+ + + +

The pressure levels of 500 atm and above appeared to be lethal and the affected algae died within a few weeks or days depending on the pressure intensity and temperature (table 2). The algae subjected to 800 atm for example were found dead immediately after pressure release at 5°C or died after 1-3 days at 10°- 15°C. Their death was confirmed by their zero rate of respiration. The lethality is due to a disruption in the inner and outer plasma membranes. Pressure application can affect regulatory function of the plasma membranes (Murakami, 1963), and it can also cause a disruption of the plastid membrane in certain seaweeds (Shameel & Ohno, 1972; Vidaver, 1972). High hydrostatic pressure could cause a solation of the cytoplasmic membrane and in these circumstances the cell viability becomes doubtful. Sustained application of high hydrostatic pressure thus appears to be lethal for algal filaments.

Table 2. Changes left in the cellular morphology of *Bryopsis plumosa* after subjection to hydrostatic pressures applied for 5 hours at different temperatures (. . =dead, further description same as in table 1).

Temperature in°C	Hydrostatic pressure in atm	Days after subjection to pressure						
		0	1	3	5	7	14	21
5	300	+	+	+	+	–	—	–
	400	++	++	++	+ +	+	—	—
	500	+++	+++	+ + +	+ ++	+ ++	..	..
	600	+ +++	+++++	..	..	..	..	..
	700	+++++	..	.	.	..	..	..
	800	.	..	..	..	.	..	..
10	300	+	+	–	—	—	—	—
	400	+	+	+	+	–	—	—
	500	++	+ +	+ +	+ +	+ +	+	—
	600	+ + +	+ + +	+ ++	+ + +	+ + +	..	..
	700	+ ++ +	+ + ++			..	..	..
	800	+ + + +	..	..	..		..	.
15	400	+	+	—	—	—	–	—
	500	+	+	+	+	–	–	—
	600	+ +	+ +	+ +	+ +	+	+	–
	700	+ + +	+ + +	+ + +	+ + +	· + +		..
	800	+ +++	+ + + +	..	..	..	..	..

The observations on the cell morphology and lethality of the filaments demonstrate that the pressure effects are strongly dependent on the testing temperature (tables 1 & 2). Increasing temperature appears to decrease the pressure effects. The pressure level of 500 atm was lethal at 5°C and the subjected algae died in 2 weeks, but at higher temperatures the same pressure level simply produced reversible effects and the algae became normal after 3 weeks when subjected to pressure at 10° C or 1 week only when treated at 15°C. It seems that the increasing temperature also moves the upper physiological limit of tolerance still upwards. At 5°C the algae died after a pressure treatment of 500 atm or above, at 10°C they died after 600 atm or above and at 15°C only the levels of 700 atm and above were lethal. Even the period required for the removal of pressure effects was temperature dependent. The effects produced in the cellular morphology of the filaments after subjection to 400 atm were removed after 2 weeks when the experimental temperature was 5°C, 1 week when the temperature was 10°C and only 3 days at a temperature of 15°C. It is probably due to sol-gel equilibrium which stabilizes at higher temperatures and therefore counteracts the deleterious effects of hydrostatic pressure.

A cell obtains mechanical energy when its gel structures contract and gives up metabolic energy in the formation of gels (Marsland, 1970). In the course of contraction gel structures revert towards the sol condition, therefore sustaining the source of mechanical energy involves gel rebuilding. The gelations are progressively weakened by increasing hydrostatic pressure and strengthened by increasing temperature, which acts antagonistically to the effects of pressure. Similar observations have also been made in the case of rates of respiration, photosynthesis, growth of young leaflets and activity of alkaline phosphates in different seaweeds (Shameel, 1973a, 1975a, c, 1977). The pressure uncouples the mechanically active units in cytoplasm during the dissociation of gel structures (Macdonald, 1975). The direct effect of pressure on more distant energy providing and regulating processes may also be taken into consideration. The use of high pressure research in the laboratory, therefore, provides us a clue for the understanding of certain physiological and biochemical problems not only at the cellular but also at the sub-cellular level just like other physical parameters affecting our biotope.

It is interesting to mention that under these sets of experiments no such change was observed in the cell wall of *Bryopsis plumosa* as have been studied in the case of *Callithamnion corymbosum*, *Polysiphonia nigrescens* and *P. urceolata* (Shameel, 1973c, 1974b). It is not at all surprising because the last 3 mentioned algae belong to Rhodophyceae and differ in the constituent polymers and the arrangement of polysaccharide microfibrills in their cell walls than *B. plumosa*. The cell wall of Rhodophyceae usually contains cellulose, chitin, gulose and polygalactose sulphate esters, while *B. plumosa* belongs to Bryopsidophyceae and apart from cellulose also contains β 1, 3-linked xylan in its cell wall (Shameel, 1975b). For a real picture we have to increase our knowledge about the pressure effects on the biological polymers. Agar-agar, cellulose, chitin and starches are the most common polymers produced in the marine environment by different groups of seaweeds. In our present state of knowledge the study of pressure effects on the properties of cell membranes would be of great interest. It is one of the most difficult areas of barobiology to be investigated.

Acknowledgements

I wish to record my sincere gratitude to Profs. H. Schwenke, H. Theede and Dr. W. Schramm for their kind help and suggestions, and to Alexander von Humboldt Foundation, F. R. Germany for financial support in the form of a Senior Research Fellowship.

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