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INFLUENCE OF HYDROSTATIC PRESSURE ON THE RELEASE OF DISSOLVED ORGANIC SUBSTANCES FROM *FUCUS VESICULOSUS* (PHAEOPHYTA).

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Abstract

Under the influence of high hydrostatic pressure the thalli of *Fucus vesiculosus* L., release macromolecules, which undergo a slow disintegration even after the removal of pressure. The amount of exudation is directly proportional to the length of incision given in the thallus. The excretion of organic substances increases with the rising pressure intensity (200 - 1000 atm) as well as the duration of its action (1 - 10 hours). Temperature acts antagonistically to the pressure effects, the quantity of released macromolecules decreases with the increase in temperature (5° - 30°C). High temperatures (40°C), however, intensify the pressure effects.

Introduction

The occurrence of procaryotic as well as eucaryotic microalgae in the aphotic zone is a well documented phenomenon. The growth of a coccoid chlorophyte was observed by Amos *et al.* (1972) in the sediments and bottom water from 800 m. A luxuriant growth of certain coccolithophores, chrysomonads, cyanophytes, dinoflagellates and volvocaleans has been reported at depths of 1000 - 4000 m in the Mediterranean Sea (Bernard, 1967). Viable diatoms and flagellates have been collected from oceanic depths as great as 4300 m (Kimball *et al.*, 1963). The abundance of pigmented microorganisms had occasionally been reported from depths upto 5000 m in the Atlantic as well as Pacific Ocean (Hamilton, *et al.*, 1968; Fournier, 1971; Malone *et al.*, 1973). This indicates that marine algae can withstand high hydrostatic pressure, which is one of the most important ecological parametrs in the deep sea.

Seaweeds exhibit biochemical and physiological modifications when subjected to different conditions of high hydrostatic pressure (Shameel, 1975 c, 1977 a). During investigation of pressure influence on the photosynthetic and respiratory rates of certain marine macroalgae it was found that dissolved organic substances were excreted by *Fucus vesiculosus* (Shameel, 1973 a). In the present study the extent to which hydrostatic pressure influences the release of such substances is reported.

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Materials and Methods

Fucus vesiculous L., occurs in abundance during summer in the Kiel Bight (Western Baltic Sea) attached with the hard substratum adjacent to coast line at a depth of 0 – 8 m. Experimental material was collected near Moeltenort, Kiel. Equally long pieces of the thallus were cut and kept at 15°C in double filtered sea water of 15°/oo S as mentioned previously (Shameel, 1977 b). After one week's adaptation to the experimental conditions healthy and strong pieces of thalli were selected and subjected to high hydrostatic pressure as described earlier (Shameel, 1973 a). The sea water in which thallus pieces of *F. vesiculosus* were subjected to hydrostatic pressure, hereafter called as *experimental water* after the removal of algae, was used to determine the amount of dissolved oxygen according to Winkler's titration as given by Grasshoff (1976).

To study the amount of organic substances released, the experimental water was filled upto the mark M_2 in a capillary viscosimeter (Fig. 1), which was hanged in a temperature constant water bath of ± 0.02°C accuracy, because viscosity is strongly temperature dependent. The viscosimeter has 3 arms A_1, A_2 and A_3; and one of them A_3 possesses a bulb B at the top, containing experimental water before falling down. Through the arm A_3 the experimental water containing macromolecules was sucked through a tube T by the pump P and the time taken by the water to move from M_1 to M_2 through a very thin capillary tube C was noted and compared with that of the control water. The

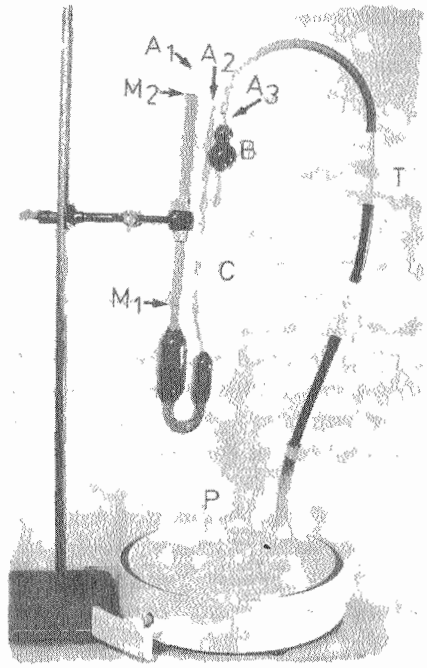

Fig. 1. Capillary viscosimeter. A_1, A_2 & A_3 = three arms, B = bulb, C = capillary tube, M_1 & M_2 = two markings, P = sucking pump, T = tube which connects or disconnects the pump.

temperature at which measurements were made was 15°C. For each experiment 8 – 12 samples were studied and arithmetic mean was calculated. Those experiments in which standard deviation was more than 5 % were repeated several times. In order to obtain a better way of comparison the values of viscosity measurements are expressed in the term of control percentage.

Results and Discussion

When experimental water samples were titrated against $Na_2S_2O_3$ upto the null point, the blue colour of the indicator (starch) was found to reappear after some time. It was retitrated but shortly after that the blue colour appeared again, and this was found to be repeated upto 20 – 30 hours. The reappearance of blue colour was ascertained by saturation test, in which some water samples were shaked to saturate them with O_2 (Table 1). It would, therefore, appear that the thallus pieces of F. *vesiculosus* released certain organic substances under pressure, these were macromolecules which underwent a slow disintegration.

The I_2 liberated by the addition of H_2SO_4 was attached with the excreted macromolecules by week bonds. The remaining I_2 was reduced by $Na_2S_2O_3$ to I' and so the apparent null point was obtained. In due course of time these macromolecules started breaking down and the attached I_2 molecules were again set free. They were attached with free starch molecules, which appeared blue due to the polarisation of electron orbitals of I_2. The act of disintegration of macromolecules was very slow and therefore the titrated and retitrated samples of experimental water developed blue colour.

TABLE 1. Saturation test of water samples kept in dark for 5 hours at 15°C with or without *Fucus vesiculosus* at atmospheric pressure.

Water sample	$Na_2S_2O_3$ used/ ml				Colour
	at O hour	after 1/2 hour	after 1 hour	after 2 hours	after 4 hours
Water unshaked:					
without alga	0.0980	0.0980	0.0980	0.0980	colourless
with pressurized alga	0.0637	0.0843	0.0894	0.0951	blue
with control alga	0.0545	0.0545	0.0545	0.0545	colourless
Water shaked:					
without alga	0.1092	0.1092	0.1092	0.1092	colourless
with pressurized alga	0.1101	0.1392	0.1456	0.1513	blue
with control alga	0.1010	0.1010	0.1010	0.1010	colourless

1. Effect of length of incision in the thallus

To investigate whether the organic substances are released from the entire thallus surface or only from the cut edges, pieces of *F. vesiculosus* thallus, 60 – 65 mm long, 5 g in weight and similar in thickness and morphology were selected. Four series of experiments were conducted: i) the whole piece was employed so that it contained only one cut edge, ii) it was cut into 2 pieces, iii) it was cut into 8 pieces and iv) to increase the length of incision it was cut into 10 pieces. The thallus pieces along with sea water were subjected to hydrostatic pressure of 600 atm for 5 hours at 15°C. Control pieces were similarly treated and kept at atmospheric pressure. After the release of pressure, algal pieces were taken out and the experimmental water was treated according to Winkler's method. The null point of titration was designated as O h, the experimental water was retitrated several times upto 16 hours' period.

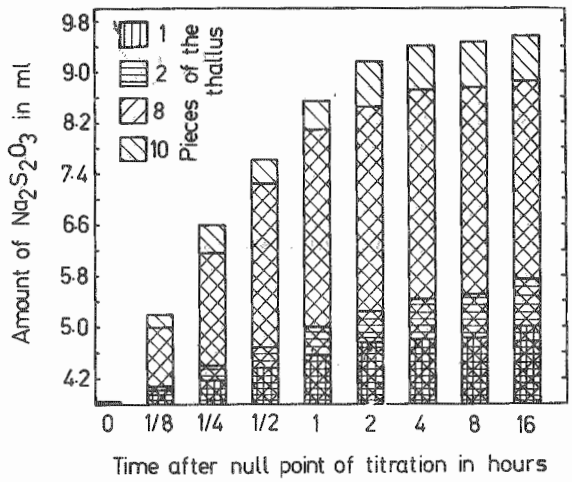

Fig. 2. Amount of sodium thiosulphate used in titration by the sea water in which thallus pieces (1 – 10) of of *Fucus vesiculosus* were subjected to hydrostatic pressure of 600 atm for 5 hours at 15°C.

Amount of $Na_2S_2O_3$ used by all the four series has been graphically represented in Fig. 2. The experimental water in which thallus with a single cut was kept, utilized the least amount of $Na_2S_2O_3$ as compared to greatest number of pieces. Greater the amount of $Na_2S_2O_3$ consumed higher would be the quantity of macromolecules released during pressure treatment. A big amount of organic substances would have attached a high amount of I_2 molecules with them which were gradually liberated in due course of time corresponding with high amount of $Na_2S_2O_3$. The sea water from the control set did not show any blue colouration after the null point of titration irrespective of the number of thallus pieces used suggesting that the organic substances are mainly released from cut surface of the thallus under pressure influence.

The thallus of *F. vesiculosus* is thick and composed of several layers of elongate parenchymatous cells, but a similar observation was also made in the thin, siphonous and coenocytic thallus of *Caulerpa prolifera* (Shameel, 1973 a). The pressure resistance of this alga was directly proportional to the length of incision given in its delicate assimilator. This may be due to the influence of hydrostatic pressure on the sol-gel interconversion of cytoplasm.

2. Effect of pressure intensity

As the organic substances are released under pressure influence, the experimental water must increase its viscosity. Water passed through a capillary viscosimeter causes an increase in the viscosity, while the sea water in which control thalli were kept at atmospheric pressure remained unaltered.

To study the relationship between pressure intensity and amount of the substances released, equally long, broad and thick pieces of about 2 g in weight were cut from central part of the thallus of *F. vesiculosus,* so that they may have two cut edges

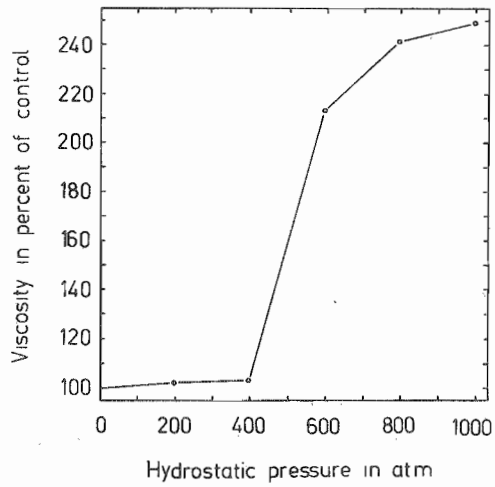

Fig. 3. Relative viscosity of the sea water in which thallus pieces of *Fucus vesiculosus* were kept under different levels of hydrostatic pressure (200 – 1000 atm) for 4 hours at 15°C (measurements immediately after the removal of pressure at 15°C).

of equal length. The pieces were kept for 4 hours at 15°C under 200, 400, 600, 800 and 1000 atm. The control thalli were similarly treated and kept at normal atmospheric pressure. At the end of pressure the thallus pieces were removed from water and viscosity measurements made (Fig. 3). The pressures upto 400 atm were well tolerated by the thallus pieces and caused a negligible release of organic substances. At a pressure of 600 atm, a high rate of release was exhibited. Increasing pressure levels accelerated the rate of release, but the intensity of this increase gradually slowed down. It becomes evident that the release of macromolecules depends on the pressure intensity.

Several marine benthic algae at first strongly increase their rates of respiration and photosynthesis and then slowly decrease it with rising pressure intensity (Shameel, 1973 a). The increasing pressure also retards the growth of young prolifications of *Delesseria sanguinea* (Shameel, 1975 a). The causes of such retarding influences of pressure may be looked in the depolymeration of macromolecules.

3. Effect of pressure duration

Assuming that the pressure intensity was directly proportional to the release of macromolecules, the duration of pressure action should also have a bearing on it. To study this thallus pieces of *F. vesiculosus,* as described in Sect. 2, were subjected to 800

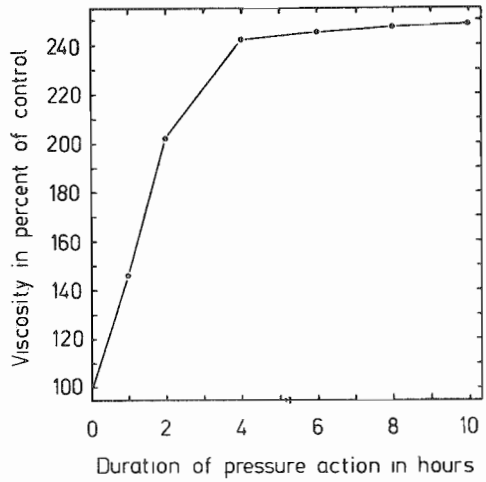

Fig. 4. Relative viscosity of the sea water in which thallus pieces of *Fucus vesiculosus* were subjected to hydrostatic pressure of 800 atm for different durations (1 – 10 hours) at 15°C (measurements immediately after the removal of pressure at 15°C).

atm at 15°C for 1, 2, 4, 6, 8 and 10 hours. The results of viscosity measurements indicate that the duration of pressure has an influence on the release of macromolecules only upto a particular point, beyond which it plays no important role (Fig. 4). Under the pressure level of 800 atm the release of macromolecules increased very rapidly with the rising duration of pressure action upto 4 hours, but after that the increase of releasing intensity was extremely slow. The increase of released macromolecules with the duration of pressure action indicates that hydrostatic pressure does not affect instantaneously but influences gradually till all the macromolecules have been unfolded.

A decrease in the rate of oxygen exchange with the increasing duration of pressure was observed in several marine benthic algae (Shameel, 1973 a). Similar observations were also made on the pressure influences on cellular morphology of different marine filamentous algae (Shameel, 1973 b, c, 1974 a, b, 1976). Pressure intensity and the duration of its action must be considered together, and both of them constitute a stimulus which may promote or have a retarding effect depending on its strength.

4. After-effects of pressure

Whether the increased viscosity of the experimental water remains constant was examined. Thallus pieces of *F. vesiculosus* were subjected to hydrostatic pressure of 600, 800 and 1000 atm for 4 hours at 15°C as described in Sect. 2. The viscosity measurements of water samples were made at different intervals upto 5 hours (Fig. 5).

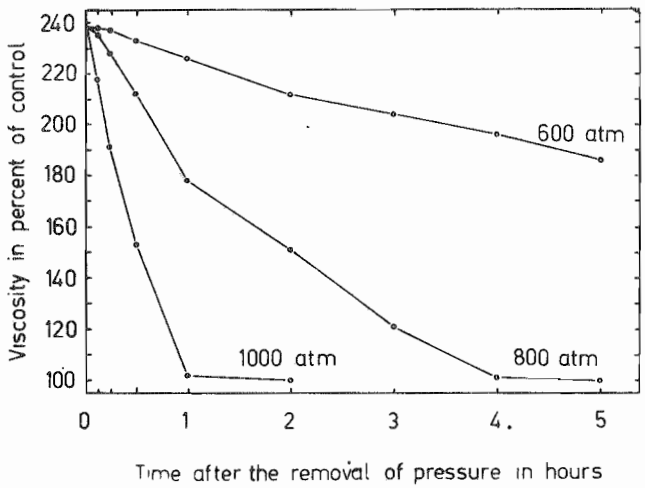

Fig. 5. Relative viscosity of the sea water in which thallus pieces of *Fucus vesiculosus* were treated with high hydrostatic pressure (600, 800 and 1000 atm) for 4 hours at 15°C (measurements after the removal of pressure at different time intervals from 0 – 5 hours at 15°C).

The viscosity of water samples slowly decreased and after some time equalled that of control water. The water samples kept at 600 atm decreased their viscosity very slowly. Only 22 % declined after 5 hours and it required a long time to equalize the value of control samples. The water samples subjected to 800 atm needed 4 hours and those treated to 1000 atm took only 1 hour to come to this point. This indicates that the released substances are macromolecules which are unfolded under pressure influence. Then unfloding increases with the pressure intensity, finally leading to depolymeration whereby elongated macromolecular complexes are converted to a number of protein subunits or monomeres which after removal of pressure gradually undergo disintegration.

5. Effect of temperature

The pressure effects are strongly influenced by other ecofactors acting simultaneously, in which temperature plays an important role. Although hydrostatic pressure and temperature generally counteract one another, there are variable observations on the modifying influences of temperature on pressure effects. For the study of temperature-pressure relationship the viscosity experiment was also used. At 15°C adapted thallus-pieces of *F. vesiculosus* were subjected to hydrostatic pressure of 800 atm for 4 hours at 5°, 10°, 15°, 20°, 30° and 40°C. At the end of pressure treatment the viscosity measurements were made at 15°C.

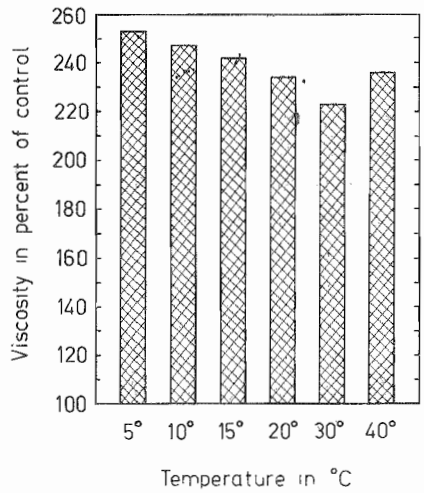

Fig. 6. Relative viscosity of the sea water in which thallus pieces of *Fucus vesiculosus* were kept under hydrostatic pressure of 800 atm at different temperatures (5° - 40°C) for 4 hours (measurements immediately after the removal of pressure at 15°C).

The results confirm the previous observation that hydrostatic pressure and temperature act adversely to one another (Fig. 6). At constant hydrostatic pressure the relative viscosity of the experimental water decreased with temperature rising from 5° to 30°C indicating a deceleration in the release of macromolecules. It was observed that higher the temperature lesser was the amount of released substances, but this was true only upto a certain limit, the physiological limit. After this limit temperature does not reduce the effect of pressure but acts independently and more effectively, since thallus pieces subjected to pressure at 40°C released more macromolecules than those at 30° and 20°C. This high amount of release was not due to pressure-temperature relationship but due to detrimental effect of temperature.

Certain marine bacteria behave differently under the combined effects of pressure and temperature (Brown *et al.*, 1942; ZoBell & Cobet, 1964). With the increase of temperature the pressure resistance of marine invertebrates generally increases (Ponat, 1967; Schlieper, 1968), but other results indicate that certain invertebrates decrease their pressure resistance with the rise of temperature (Naroska, 1968; Menzies & George, 1972). In contrast algae show a unifrom effect of pressure and temperature.

Sturm (1957) found that the growth retarding effect of hydrostatic pressure on *Hydrodictyon reticulatum* is rectified by high temperature. Vidaver (1969) observed that the retarding effect of pressure on the thallus growth of *Ulva lobata* and *Porphyra perforata* is lesser at 30°C than at lower temperatures. The temperature was also found to reduce the influences of hydrostatic pressure on the rate of 0_2 exchange in *Delesseria sanguinea, Fucus vesiculosus* and *Porphyra umbilicalis,* growth of thallus prolifications in *D. sanguinea,* cellular morphology of *Bryopsis plumosa,* activity of alkaline phosphatases in *D. sanguinea* and *F. vesiculosus* and the rate of reproduction in *Cladophora vagabunda*

(Shameel, 1973 a, 1975 a, b, 1976, 1977 b, 1978 a, b). In the barobiology of marine algae the antagonistic effects of hydrostatic pressure and temperature appear to be a general phenomenon.

6. General remarks

The mechanical work is done by homogenous cells as well as those with specialized structures, both of them are sensitive to high hydrostatic pressure. A mean to measure the coherence of those cytoplasmic structures which are essential for the accomplishment of mechanical work, is to determine their viscosity. This determination has shown that under high hydrostatic pressure cytoplasm exhibits solation (Brown, 1934). The mechanical activity of incompletely solated cytoplasm as well as its streaming in *Elodea canadensis* is decreased by increasing hydrostatic pressure (Marsland, 1939). The solationing effect of pressure lies in the break down of hydrophobic bonds. The present observations may be explained as weakening of the more rigid cortical portions of the cell and breakdown of inter- and intracellular bonds, especially hydrophobic ones of cytoplasmic structures. Swelling of cell walls under high hydrostatic pressure has already been observed in certain marine filamentous algae (Shameel, 1973 c, 1974 b), in which the openings between constituent polysaccharide microfibrills expand. Most probably through these expanded pores the macromolecules are released under pressure.

The fact that low temperatures strengthen the pressure influences suggests that hydrophobic bonds are important to increase the cytoplasmic viscosity. A simple explanation of the present observations lies in that hydrostatic pressure loosens the mechanically active units of cytoplasm during breakdown of gel structures. It is highly probable that hydrostatic pressure directly influences the energy supplying and directing biochemical reactions of the cell. Detailed biochemical studies are needed to elucidate the actual mechanism involved.

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