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Growth and some metabolic activities of *Scenedesmus obliquus* cultivated under different NaCl concentrations

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Abstract

The physiological response of *Scenedesmus obliquus* to salinity (NaCl concentration of 40, 80, 120, 60 and 200 mM) for 7 d (long-term experiments) or 2 h (short-term experiments) was followed. Cell number, dry matter and the content of photosynthetic pigments decreased with the rise of NaCl concentration. However, the photosynthetic O_2 evolution mostly increased with the increase of NaCl concentration up to 80 mM, and respiration (dark O_2 uptake) was markedly promoted. Photosynthesis/respiration ratio went in concomitance with the cell number, dry matter or chlorophyll content. Contents of soluble saccharides and soluble proteins increased with the rise of salinization, while the content of insoluble and total saccharides or proteins decreased. Proline content increased greatly with salinization, whereas of other free amino acids were mostly reduced, especially at higher salinities. Similarly, the lipoid contents of salinized *Scenedesmus obliquus* were obviously higher than those of the control cultures.

Introduction

The response of micro-algae to salinity stress differs from marine to freshwater algae and from one type to another. In this respect, the growth and metabolism of the freshwater algae *Chlorella emersonii* (Setter and Greenway 1979), *Anacystis nidulans* (Batterton and Van Ballen 1971), *Chlorella vulgaris* and *Ankistrodesmus falcatus* (Abdel-Basset 1986) were variably reduced in response to salinization treatments. Similarly, the contents of photosynthetic pigments and the photosynthetic activities of algal cells were variously affected (Brüggemann *et al.* 1978). Respiration responded to salinity by either a stimulation or a repression in variously salinized plants (Frank and Wegmann 1974, Kaplan *et al.* 1980, Kessly and Brown 1981, Loeblich 1982, Gilmour *et al.* 1984a, Abdel-Basset 1986).

As a result of these alterations in photosynthesis and respiration, the soluble and insoluble organic constituents are variously consumed or accumulated. This deviat-

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ion in metabolic pathways depend on the plant species and on the degree of stress exerted. Therefore, some soluble metabolites accumulate in stressed plants at the expense of some others (Ahmed *et al.* 1985). Similarly the lipid content correlates with salinity in various plant species (Wintermans *et al.* 1969, Ben-Amotz and Avron 1973, Frank and Wegmann 1974, Ahmed *et al.* 1979, De Gier *et al.* 1982). The amino acid proline gained prominance in view of its accumulation in higher plant or in eukaryotic algae (Kirst 1975, Stewart *et al.* 1977, Schrobert 1980, Daines and Gould 1895, Imamul-Hug and Larher 1985, Abdel-Basset 1986).

The aim of the present work was to study the effect of short or long salinization treatments on growth and some metabolic activities of *Scenedesmus obliquus*. This alga is one of the dominant green algae in freshwater habitats in Egypt, regardless of the physico-chemical characteristics of the water area.

Material and methods

The green alga, *Scenedesmus obliquus* (Turp.) Kuetz., which was repeatedly recovered from the Nile system in Egypt, was grown in a modified Beijerinck medium (Stein 1966). Aeration was performed by filtered dry air to provide CO_2 and to prevent settling of algal cells. Illumination was provided by fluorescent tubes (3500 lux), temperature was 25 ± 1 °C.

In the short-term experiments the algae inoculated in the above mentioned nutrient medium were subjected to various salinization levels (40, 80, 120, 160 and 200 mM NaCl) for only two hours. Afterwards the cells were harvested for assay and analysis. The above mentioned NaCl levels were chosen according to preliminary experiments that showed these concentrations of NaCl as tolerated by *S. obliquus.* In the long-term experiments, algae were grown at the above mentioned levels of salinization for 7 d prior to analyses.

Haemocytometer, 0.1 mm deep, having improved Neubauer rulling (*A.O. Spencer "Bright line"*), was used for the determination of the cell number. A spectrocolorimeter method was used for the determination of pigment contents (Metzner *et al.* 1965). Light O_2 evolution and dark O_2 uptake were determined manometrically (Ummreit *et al.* 1959). Photosynthesis was measured under growing irradiance and temperature; constant CO_2 concentration of 33×10^{-2} mol m^{-3} was provided to the algal cells. The oxygen evolved or taken up was calculated according to Halliwell (1984).

The saccharide fractions were colorimetrically estimated by the anthrone sulphuric acid method (Fales 1951). Using the mass method given by Meara (1955), the lipoids contents were quantitatively determine. Protein fractions were estimated according to Lowry *et al.* (1951). Free proline was colorimetrically estimated according to Bates *et al.* (1973). Other free amino acids were colorimetrically determined using a ninhydrin reagent (Moore and Stein 1948). The results were mostly given as percentages of those of the control culture.

Results and Discussion

After 7 d a slight increase in cell number (103.9 % of the control) was recorded at 40 mM NaCl, yet the cell number than decreased considerably with the rise of salinity up to the lowest value (43.1 % that of the control) at 200 mM NaCl (Fig. 1A). Dry matter was somewhat elevated giving 117.2 and 104.4 % of the control value at 40 and 80 mM NaCl, respectively. Thereabove, the dry matter decreased gradually with the elevation of salinization, so that it reached 75.6 % of control at 200 mM NaCl. Such a retardation in cell number and dry matter was also observed in the works of Czernas (1978), Ahmed *et al.* (1985) and Abdel-Basset (1986). On the other hand, Hsiao (1973) reported that many microalgae can survive large increases in external osmotic pressure showing nothing more serious than a delay in cell division.

The content of photosynthetic pigments was greatly lowered with the rise of the salinization level, whatever the duration of stress (Fig. 1A). Similar results were found by Ashour and Thalooth (1971), Weimberg (1975), Heikal (1976), Ahmed *et al.* (1979), Joshi and Naik (1980) in various plant types.

Photosynthetic O_2 evolution of *S. obliquus* cells was promoted in cultures subjected to low and moderate salinization treatments (Fig. 1B), but it was retarded at 120 - 200 mM NaCl. Thus the available chlorophylls were satisfactory to sustain the photosynthetic activity under 40 - 80 mM NaCl. The stimulation of photosynthetic O_2-evolution at relatively low salinization was also found by Ben-Amotz and Avron (1972), Chimiklis and Karlander (1973), Frank and Wegmann (1974), Loeblich (1982), Apte and Thomas (1983), Rees (1984), Thomasset *et al.* (1984), *etc.* On the other hand, photosynthetic rate may retard owing to ionic stress (Plaut 1971, Plaut and Bravdo 1973, Potter and Boyer 1973, Yeo *et al.* 1985).

In short-term experiments the photosynthetic O_2-evolution of *S. obliquus* was greatly reduced with the rise of NaCl level in the culture medium (Fig. 1C). This could be attributed to the osmotic shock exerted in this short period when the cells could not yet adapt themselves to this treatment.

Table 1.Photosynthesis/respiration ratio of *Scenedesmus obliquus* cultures after being subjected to various salinization levels for 7 d (long-term experiment) or for only 2 h (short-term experiment)

	NaCl concentration [mM]					
	control	40	80	120	160	200
7 d	3.7	3.8	3.1	2.7	2.3	2.3
2 h	5.4	4.9	3.3	2.5	2.3	2.3

Respiration of NaCl salinized *S. obliquus* cultures was markedly promoted (Fig. 1B) with the rise of salinization. Such stimulation in respiration coincide with the relatively low dry matter content obtained. In short-term experiments respiration of algae was also enhanced with the elevation of salinization and reached the maximum value at 200 mM NaCl (210.4 % of the control - Fig. 1C). This could be due to the need for a higher energy allocation for maintenance of osmotic adjustment, ion concentration gradient (Penning de Vries 1975), for the repair of tissues, and for active transport processes (Schwarz and Gale 1981). The increase in maintenance respiration under salt stress may be a characteristic of salt tolerance. This enhancement of respiration in variously stressed plants were reported by Bear *et al.* (1980), Kaplan *et al.* (1980), Lambers (1980), Loeblich (1982), Gilmour *et al.* (1984 a, b), Abdel-Basset (1986), *etc.*

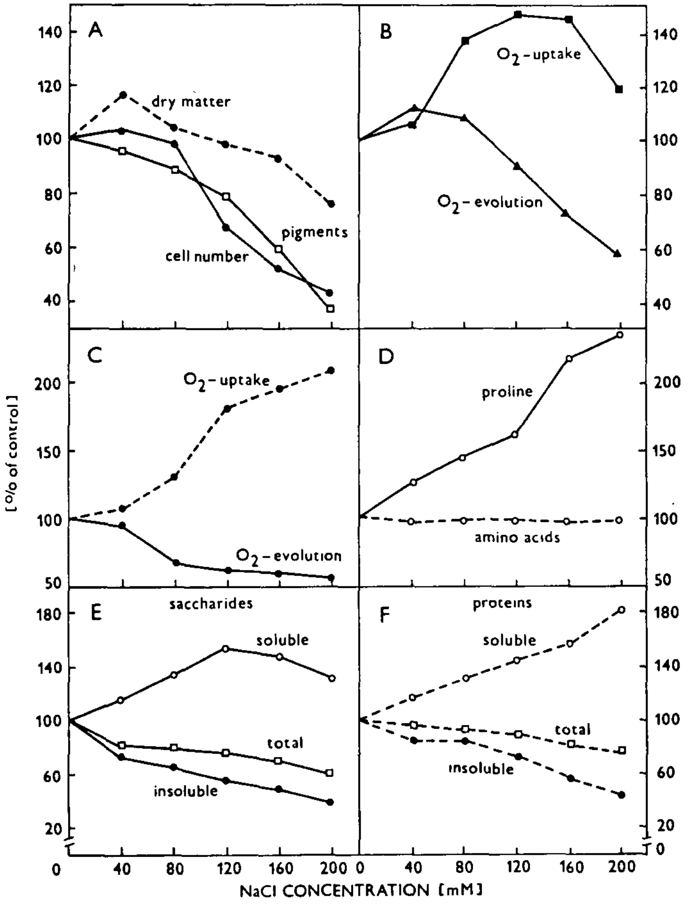

Fig. 1. Dry matter, total pigments, cell number, photosynthetic O_2 evolution, dark O_2 uptake, free proline content, other free amino acids content, soluble, insoluble and total saccharides or proteins of *Scenedesmus obliquus* cultures subjected to different NaCl concentrations for 2 h (C,D) or 7d (A,B,E,F).

As a result of decreased photosynthesis and increased respiration, photosynthesis/respiration ratio was considerably lowered with the rise of salinization (Table 1), but the maximum photosynthesis/respiration ratio remained always in coincidence with the maximum growth of *S. obliquus*.

The soluble fractions of saccharides and proteins of variously salinized *S. obliquus* cultures exhibited higher values than those of the control (Fig. 1E): soluble saccharids reached a maximum of 153.7 % of the control at 120 mM NaCl and for soluble proteins, the maximum was 182 % at 200 mM NaCl. Hence the soluble fractions were accumulated at the expense of the insoluble ones. Wegmann (1968, 1969) reported that such sucrose accumulation originated partially from photosynthates and partially from hydrolysis of stored saccharides.

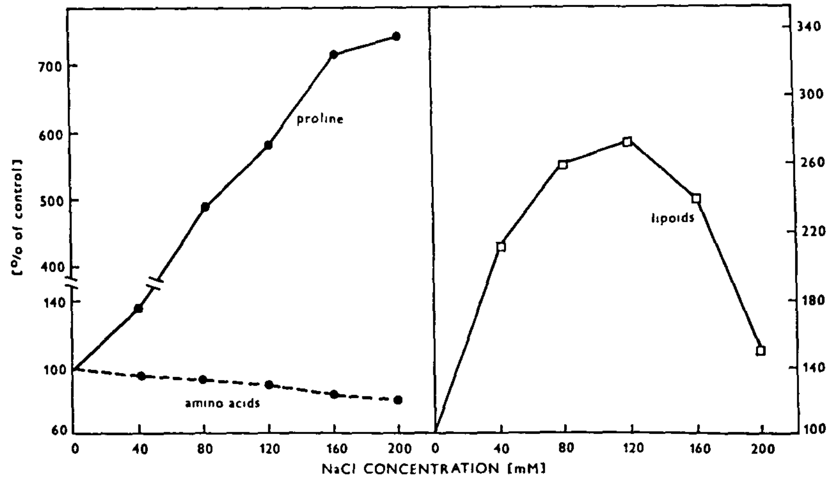

Fig. 2. Free proline, other free amino acids and lipoids content of *Scenedesmus obliquus* cultures subjected to different NaCl concentrations for 7 d.

The lipoid contents increased sharply with salinization. It reached the highest value (272.9 %) of control at 120 mM NaCl and than decreased again (Fig. 2). The increase in lipoids content in relation to salinity was also recorded in higher plants (*e.g.* Ferguson 1966, Ahmed *et al.* 1978, 1985, Heikal *et al.* 1980, Gopal and Rao 1982). This increase belongs to the adaptation to environmental stress (*e.g.* Kuiper 1968, 1980, Twersky and Felhender 1973, Stuiver *et al.* 1978, Ferrari-Iliou *et al.* 1984) which may lead to maintenance of structure and function of the membrane system (Wintermans *et al.* 1969, De Gier *et al.* 1982). Moreover, Ben-Amotz and Avron (1973) reported that lipoids may act as osmoregulators.

The contents of free proline (Fig. 2) were greatly elevated with the rise of salinization, whatever the duration of the experiments used. In long-term experiments, it reached 739.5 % of the control culture at the highest tolerable level used (200 mM NaCl). On the other hand, the contents of other free amino acids were mildly lowered with salinization. In short-term experiments, the content of free proline was also greatly elevated with salinization (up to the 236.3 % of the control - Fig. 1D), while the changes in contents of other amino acids were insignificant. For similar results see Mukherjee (1974), Stewart and Lee (1974), Setter and Greenway

(1979), Tal *et al.* (1979), Schobert (1980), Daines and Gould (1985), Abdel-Basset (1986).

The comparison of results from short- and long-term treatments indicate that the cells began to adapt early after being subjected to salinization treatments. The main response of *S. obliquus* to salinity was the accumulation of lipoids and proline, in a lesser degree accumulation of soluble sugars and proteins. The synthesis and accumulation of these components consume energy, which is mostly supplied from respiratory substrates, resulting in adverse effects on growth.

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