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APPLICATIONS OF THERMAL ANALYSIS ON THE MARINE PHYTOPLANKTON, *TETRASELMIS SUECICA*

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Abstract

Marine microalgae represent an essential link in the planktonic food web and some of them are commonly utilised in aquaculture systems as food for larval and juvenile stages of fishes, crustacea and mollusca. However, the caloric content and the biochemical composition of these microrganisms vary in relation to ageing and to several environmental conditions; so if the parameters under which phytoplankton grow are not suitable, marine microalgae can supply small quantities of energy and essential nutrients.

The aim of this work was to study the effects of temperature on the marine planktonic alga *Tetraselmis suecica* using thermogravimetry (TG) and differential thermal analysis (DTA). Marked differences have been observed between exponentially, stationary and senescence phases probably due to both the presence of different biomolecules produced during algal growth and to the differences in the thermal properties of these intracellular molecules.

Keywords: cultures, phytoplankton, *Tetraselmis suecica*, thermal analysis

Introduction

In marine environments phytoplanktonic algae are responsible for a major portion of primary production. Some of these organisms are used in aquaculture systems successfully because they supply both energy and essential nutrients for larval stages and juvenile development of aquatic animals such as fishes, crustacea and bivalves [1–3]. Anyway, the biochemical composition and energy value of microalgae change quickly according to ageing and environmental conditions (temperature, salinity, pH, etc...) under which they grow [1, 3, 4–7]. Lourenço *et al.* [7], for example, showed that different culture media generate variable biochemical profiles in *Tetraselmis gracilis* cultures. In particular, their results indicated that nitrogen availability could affect the synthesis and accumulation of proteins, lipids and carbohydrates in *T. gracilis*; furthermore, low concentration of metals and boron could limit pigment biosynthesis and algal growth [7]. The effects of these parameters on algal growth are still valued using common calorimetric and biochemical techniques [1, 5, 8]. For example, calorific values are measured by burning phytoplankton in micro-bomb calo-

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rimeters. So, in order to obtain new biological data on the relationship between environmental factors and microalgal growth, we studied the effects of temperature on *Tetraselmis suecica*, a marine microalga commonly utilised in aquaculture systems as food for fishes, crustacea and mollusca, using thermogravimetry (TG) and differential thermal analysis (DTA).

Materials and methods

As previously described [3], microalgae were maintained in a culture medium composed of sterile sea water (salinity of 19‰), Walne medium [9] and vitamin solution (B_{12} 0.005%, B_1 0.1%). Furthermore, the cultures were kept in a thermostatic chamber (Heraeus BK 6160), bubbled with filtered (Sartorius Ministart – HY, 0.2 μm porosity) air at constant temperatures (18 and 14°C) and illuminated by four cool-white fluorescent tube (12:12 h light:dark cycle). An optimal algal growth rate was obtained inoculating into 250 mL flasks exponentially growing cells at an initial concentration of 10^5 cells mL^{-1}. Finally, algal growth was valued by counting microalgae in a Thoma haemocytometric counting cell. For biochemical and thermal analysis, exponentially, stationary and senescence suspended growing cells maintained at 14 and 18°C were concentrated by centrifugation at 4000 rpm (dead cells sedimentation was performed by means of cultures aeration with filtered air). Then, the algae (final concentration of 10^8 cells mL^{-1}) were washed quickly in 1% ammonium formate, sterile water and finally resuspended in sterile water. The biomass of *T. suecica*, expressed in terms of chlorophyll *a* (Chl*a*) and phaeopigments, was measured using spectrophotometric method [10]. The protein content was determined according to Lowry *et al.* [11]. For algae cells counts and biochemical analysis, 3 replicates per culture sample were carried out. TG and DTA analysis were performed using a Netzsch STA 409. About 0.3 mL of each sample, previously dried at 110°C for 90', were heated continuously from 20 to 1000°C in an atmosphere of air (gas flow 100 mL min^{-1}) with a heating rate of 10°C min^{-1}.

Results and discussion

The growth profiles of *T. suecica* cultures showed significant differences in relation to temperature conditions. At 14°C the exponential phase began about five days after inoculation with a maximum value of $2.43 \cdot 10^6$ cells mL^{-1} at day 21. The stationary phase approximately occurred from day 20 to day 22; finally, cells went into senescence phase (days 23–28) and density decreased to a mean of $0.7 \cdot 10^6$ cells mL^{-1} (Fig. 1).

At 18°C a shorter lag phase (about two days) was observed and microalgae went into exponential phase quickly. The maximum abundance ($1.94 \cdot 10^6$ cells mL^{-1}) occurred at day 9. Then, algal density reached a minimum of $0.87 \cdot 10^6$ cells mL^{-1} at day 19 (Fig. 2).

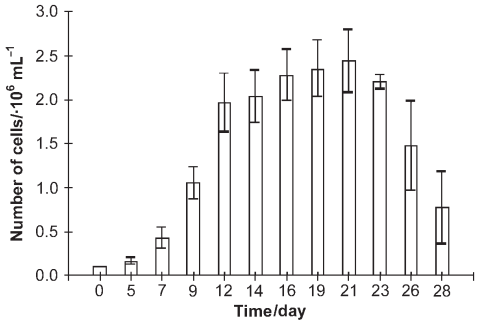

Fig. 1 Growth profile of algal cultures maintained at 14°C

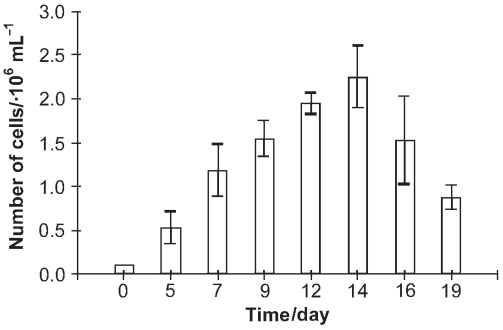

Fig. 2 Growth curve of *T. suecica* cultures maintained at 18°C

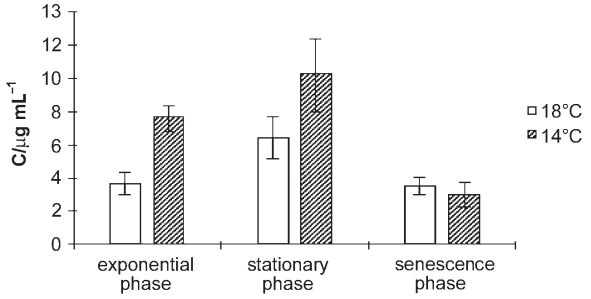

Fig. 3 Total chlorophyll *a* was more concentrated in the stationary phase of *T. suecica* cultures kept at 14 and 18°C. Furthermore, at 14°C algae contained a higher amount of pigment than cultures maintained at 18°C; on the contrary, Chl*a* content at senescence phase was similar for all cultures

These results are due to the dependence of algal metabolism on experimental conditions under which phytoplankton grow; in this regard, Pusceddu and Fabiano [5] showed a higher growth rate of *T. suecica* cultures maintained at 25°C and illuminated with a dark:light period of 14:10. In fact they observed $3.5 \cdot 10^6$ cells mL^{-1} at the end of the exponential phase (day 8).

In relation to biochemical profiles, it has been observed for Chl*a* (Fig. 3), phaeopigments (Fig. 4) and proteins (Fig. 5) similar increasing patterns. In fact, apart from temperature, cultures reached the highest values during stationary phase and a rapid decreased was observed when algae went into senescence phase. Besides, at 14°C Chl*a* and protein contents of microalgal cultures were constantly higher than cells maintained at 18°C.

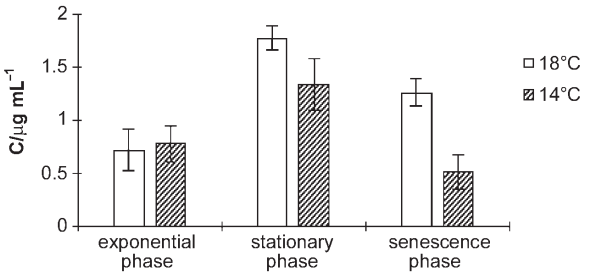

Fig. 4 Except exponential phase, total phaeopigments levels in algae kept at 18°C were higher than in algal cells maintained at 14°C

The values showed in this study were different from those observed in previous papers [3, 5]; it can be explained as being due to the relationship between environmental conditions and the biochemical composition of algae.

The curves of growth phases (Figs 6 and 7) revealed an initial mass loss in the 40–180°C range (Table 1: 5.2–5.7% for algae kept at 18°C; Table 2: 10.5–15.2% at 14°C) caused principally by the loss of free water and water loosely bound to biomolecules.

Table 1 Percent values of mass losses in different ranges of temperature and growth phases of algae maintained at 18°C

Temperature range/°C	Growth phases/%		
	Exponential phase	Stationary phase	Senescence phase
40–180	5.70	5.40	5.20
180–400	42.90	56.20	58.90
400–760	50.60	42.90	42.30

Table 2 Percent values of mass losses in different ranges of temperature and growth phases of algae maintained at 14°C

Temperature range/°C	Growth phases/%		
	Exponential phase	Stationary phase	Senescence phase
40–180	10.50	14.80	15.20
180–400	51.20	52.20	58.10
400–760	42.60	47.20	40.70

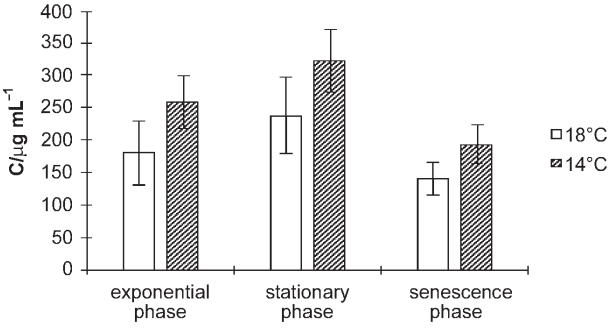

Fig. 5 At 14°C the protein content of microalgal cultures was constantly higher than cells maintained at 18°C. The maximum value was observed in the stationary phase

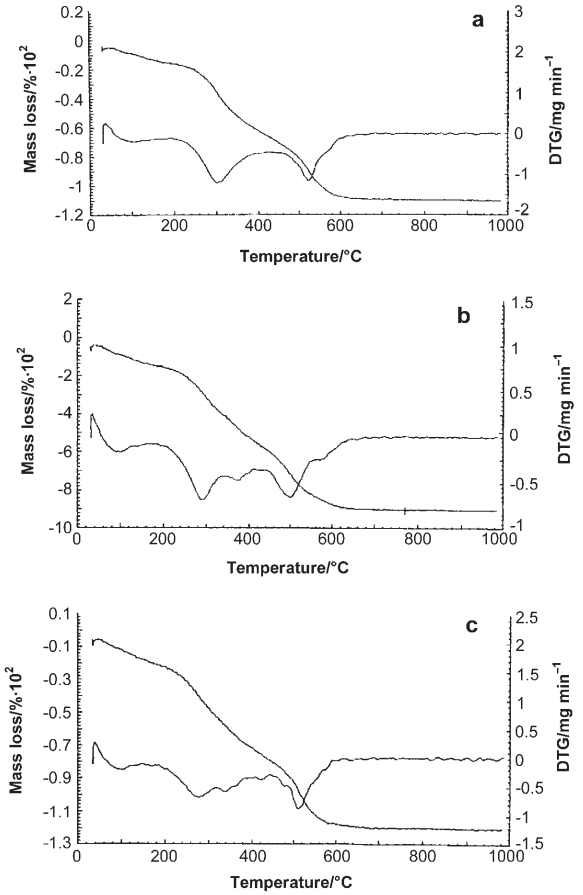

Fig. 6 TG and DTG curves obtained from the analysis of exponential (a), stationary (b) and senescence (c) microalgae maintained at 14°C

In this first temperature range the mass loss of microalgae maintained at 14°C was higher than cells kept at 18°C indicating a higher amount of these types of thermodynamic water in algae at 14°C [12]. In particular, while *T. suecica* at 18°C showed a slight decrease from the exponential to the senescence phase, at 14°C the mass loss tended to increase with the age of cultures. Besides, in 40–180°C range the cell structure is progressively destroyed and phenomena such as alterations of lipid structures [13, 14] and proteic thermal unfolding [15] occur. TG curves revealed a second mass loss at temperatures ranging from 180 to 400°C (Figs 6 and 7). The mass loss in this second step was very high and, apart from temperature, it tended to in-

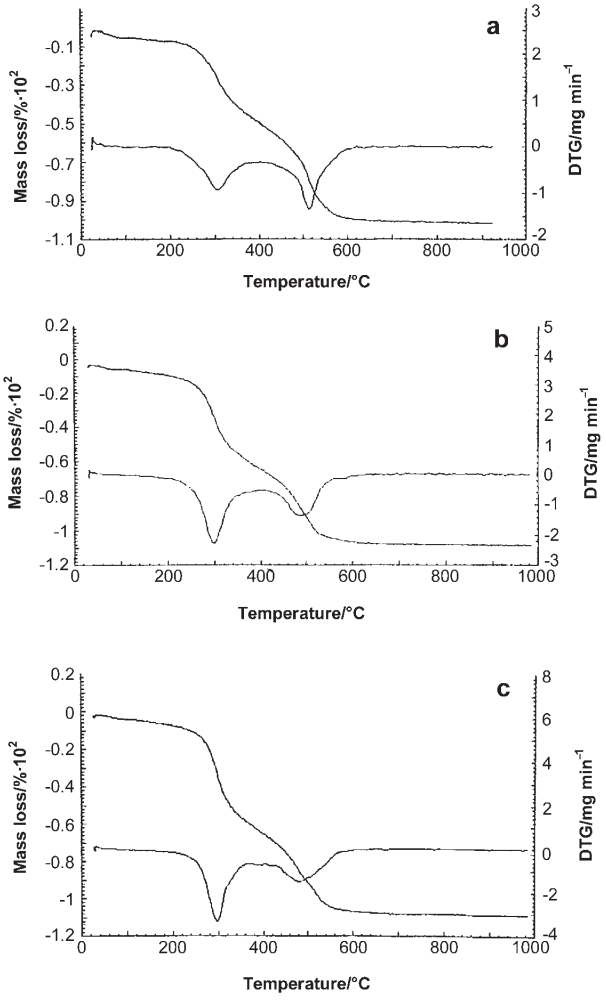

Fig. 7 TG and DTG curves of exponential (a), stationary (b) and senescence (c) algae kept at 18°C

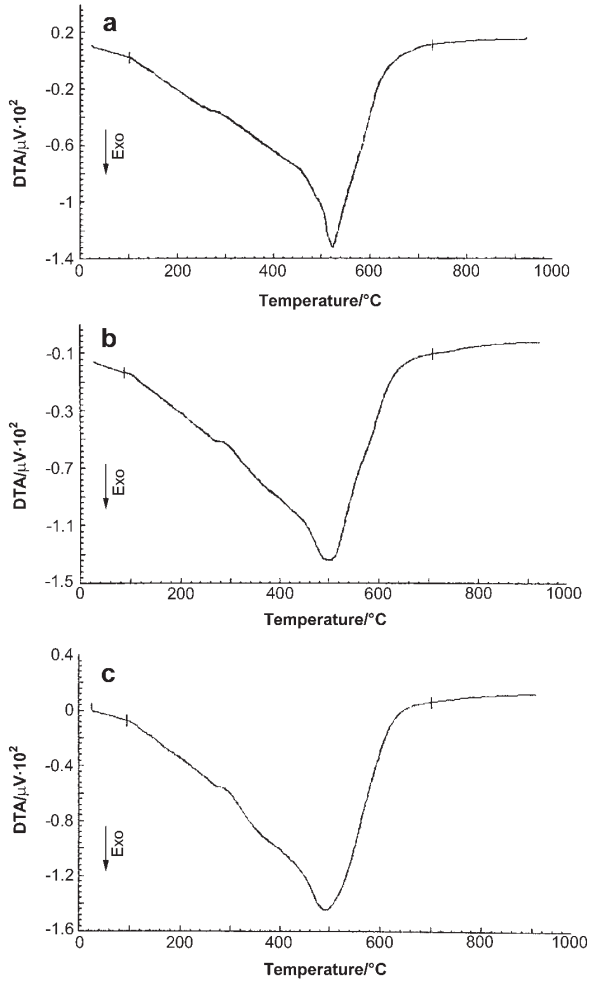

Fig. 8 DTA profiles of exponential (a), stationary (b) and senescence (c) microalgae maintained at 18°C

crease from the exponential to senescence phase. The highest values observed were 58.1% at 14°C and 58.9% at 18°C. Between 180 and 400°C decomposition of proteins and carbohydrates occur [16, 17]. Finally, the third step occurred between 400 and 760°C; in this temperature range the organic matter completely oxidize.

With regard to the cultures kept at 14°C, the maximum value was observed during stationary phase (Table 2); on the contrary at 18°C the highest mass loss occurred in the exponential phase (Table 1). DTG curves of *T. suecica* maintained at 18°C (Figs 6 and 7) showed two evident peaks for each growth phase: while the first one occurred between 180–400°C (about 300°C), the second was associated with the third step (about 495°C). At 14°C, instead, several peaks have been detected (Figs 6

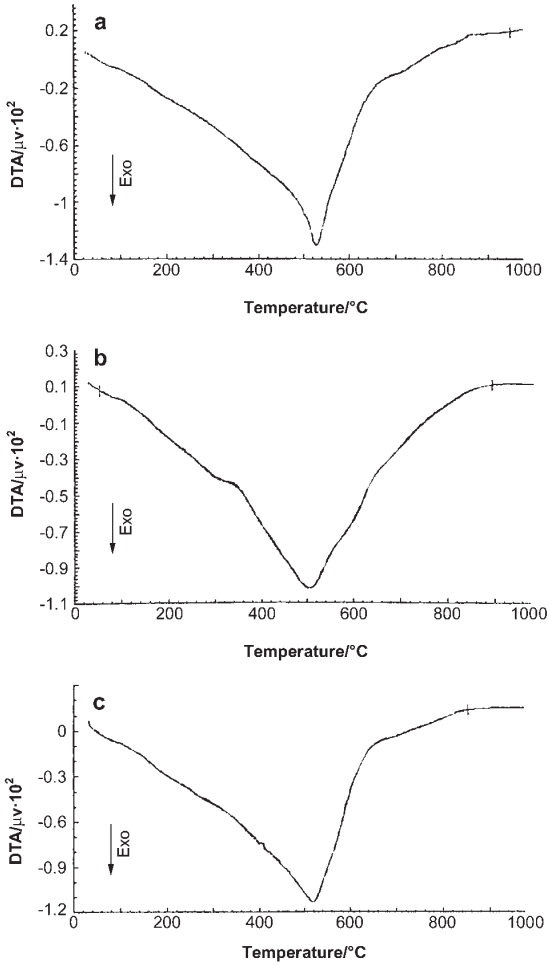

Fig. 9 DTA profiles of exponential (a), stationary (b) and senescence (c) microalgae maintained at 14°C

and 7): three peaks in the exponential phase and four at least in the stationary and senescence phase. Anyway, the main peaks were observed around 290 and 510°C. With regard to DTA curves of cultures maintained at 18°C (Fig. 8), the variation of enthalpy accompanying the decomposition of substances was very high when microalgae went into the exponential phase (ΔH= –64.398 kJ g^{-1}, temperature range: 99.76–726.5°C) and decreased progressively in relation to algal growth (ΔH= –51.987 kJ g^{-1}, temperature range of stationary phase: 87.53–707°C; (ΔH= –42.15 kJ g^{-1}, temperature range of senescence phase: 94.58–700.8°C). On the contrary, at 14°C (Fig. 9) the maximum value was observed for senescence cells (ΔH= –103.45 kJ g^{-1}, temperature range: 33.25–853,8°C) and enthalpy variation tended to decrease from stationary to exponential phase (ΔH= –99.338 kJ g^{-1}, temper-

ature range of stationary phase: 53.06–886.7°C; (ΔH= –72.563 kJ g^{-1}, temperature range of exponential phase: 38.0–949.8°C). These results confirm the biochemical data obtained in this study and they agree with the results of other workers [1, 5, 3]. In fact, differences of DTA curves are probably due to both the presence in algal cells of different biomolecules produced during growth processes and to the differences in the thermal stability of these biomolecules.

Conclusions

The effects of ageing and temperature on *Tetraselmis suecica* cultures were studied by TG and DTA. The curves and biochemical data show that strong differences exist between algae maintained at 18 and cells kept at 14°C. In particular, at 14°C biomolecules content of *T. suecica* cultures (Chl*a* and proteins) is higher than cells maintained at 18°C and the maximum value is reached in the stationary phase at 14°C. Thermodynamic differences give new important information; in fact, DTA and TG curves show that the energy levels and the thermostability of microalgae change according to ageing and temperature, too. These results can be explained as being due to both the relationship between temperature and algal metabolism and to the existence of different cellular hydration and different amount of biomolecules produced during algal growth. So, this paper shows that thermal analysis can be used as a novel approach to the study of the planktonic food web and will broaden our knowledge of aquatic ecosystems.

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