CULTURES AND THERMAL ANALYSIS OF THE MARINE MICROALGA TETRASELMIS SUECICA KYLIN (BUTCH)

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INTRODUCTION

Thermogravimetry (TG) and differential thermal analysis (DTA) are widely used to characterize several biological materials such as biomolecules, cells and tissues (1,2). In particular, while the former measures the variation of mass from a sample as a function of temperature, the latter records the enthalpy accompanying physico-chemical changes of substances subjected to temperature variations. Besides, these techniques can bring structural and thermodynamic information not only on the starting samples, but also on substances that originate in the thermal cycle. However, there are few ecological studies which involve thermal analysis. Some authors, for example, have used thermoanalytical techniques to characterize humic substances from several environments (3-5). Thermal analysis has also been used successfully for the in vitro estimation of age-related changes in biological materials such as human erythrocytes, spermatozoa, bacteria and porcine scleral tissue cells (6,7).

The aim of the present work was to study the growth of the marine microalga Tetraselmis suecica Kylin (Butch), commonly utilized in aquaculture systems (8-10) as food for the first larval stages of fish, crustacea and mollusca (11-13), using TG and DTA.

MATERIALS AND METHODS

As previously reported (12,14), microalgae were maintained in sterile sea water at a salinity of 19‰. Briefly, one litre of culture medium was autoclaved and then 1 ml of Walne medium (15) and 0.1 ml of vitamin solution (vitamin B12 0.005%, vitamin B1 0.1%) were added. Cultures were kept in a thermostatic chamber (Haeraeus BK 6160) and bubbled with filtered (Sartorius Ministart - HY, 0.2 μm porosity) air at 18°C constant temperature. Algae were illuminated by four cool-white fluorescent tube and a 12:12 h light:dark cycle was utilized. An optimal algal growth rate was obtained inoculating into flasks exponentially growing cells at an initial concentration of 100.000 cells ml^{-1}; algal growth, during exponential, stationary and senescence phases, was valued by counting microalgae in a Thoma haemocytometric counting cell.

For TG and DTA analysis, exponentially growing cells were prepared by centrifuging them repeatedly at 4000 rpm in order to obtain a final concentration of 108 cells ml^{-1}. Then, the algae were washed quickly in 1% ammonium formate, sterile water and finally resuspended in 0.5 ml of sterile water. This procedure was repeated for stationary and senescence phases. Afterwards, about 0.3 ml of each sample, previously dried at 110°C for 90', were heated continuously from 20°C to 1000°C in an atmosphere of air (gas flow 100 ml min^{-1}) with a heating rate of 10°C min^{-1}. Thermal analysis was performed using a Netzsch STA 409.

RESULTS

In relation to algal growth (fig. 1), the exponential phase began about two days after inoculation with a maximum value of $1.94 \cdot 10^6$ cells ml^{-1} at day 9. On the contrary, stationary phase was very short and during this phase the maximum abundance ($2.25 \cdot 10^6$ cells ml^{-1}) occurred at day 14; then, microalgae went into senescence phase quickly and density decreased to a minimum of $0.87 \cdot 10^6$ cells ml^{-1} at day 19.

With regard to thermal analysis, the thermograms of exponential, stationary and senescence phases (fig. 2a, 2b, 2c) revealed a loss of weight between 40°C and 180°C (5.2% - 5.7%) (tab. 1).

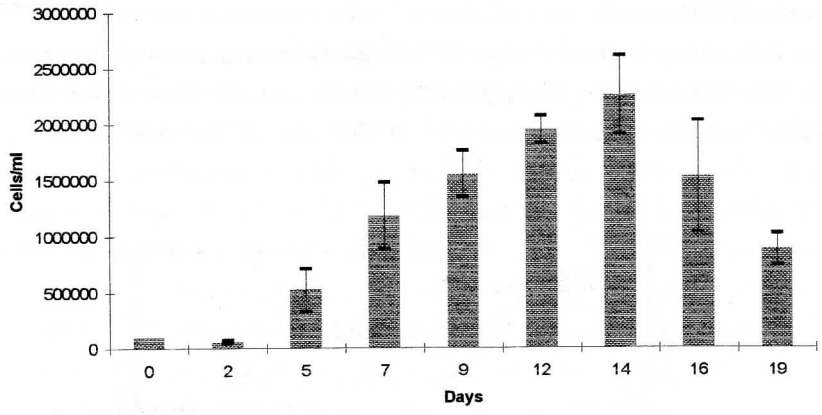

Fig. 1 - Growth curve of T. suecica cell cultures maintained at 18°C.

This small variation was almost steady during the algal growth and it was probably caused by the loss of water and higher thermolabile substances in the 40°C - 180°C range. From 180°C to 400°C, a high loss of weight has been observed which increased with the age of cultures (42.9% - 58.9%) (tab. 1). Finally, the organic matter completely oxidized when temperature exceeded 400°C and, between 400°C and 760°C, the weight loss was very high (42.3% - 50.6%) (tab. 1).

Tab. 1 - Percent averages of weight losses in different ranges of temperature and growth phases.

	40°C-180°C	180°C-400°C	400°C-760°C
Exponential phase	5.7%	42.9%	50.6%
Stationary phase	5.4%	56.2%	42.9%
Senescence phase	5.2%	58.9%	42.3%

The oxidation of substances such as proteins, polysaccharides and gas generation occured in this temperature range. Two wide peaks were also

detected: the first one around 300°C and the second around 500°C (fig. 2a, 2b, 2c). They shifted to the left of thermograms in relation to growth phase; this phenomenon was probably due to the increase of intracellular thermolabile biomolecules according to the age of cultures.

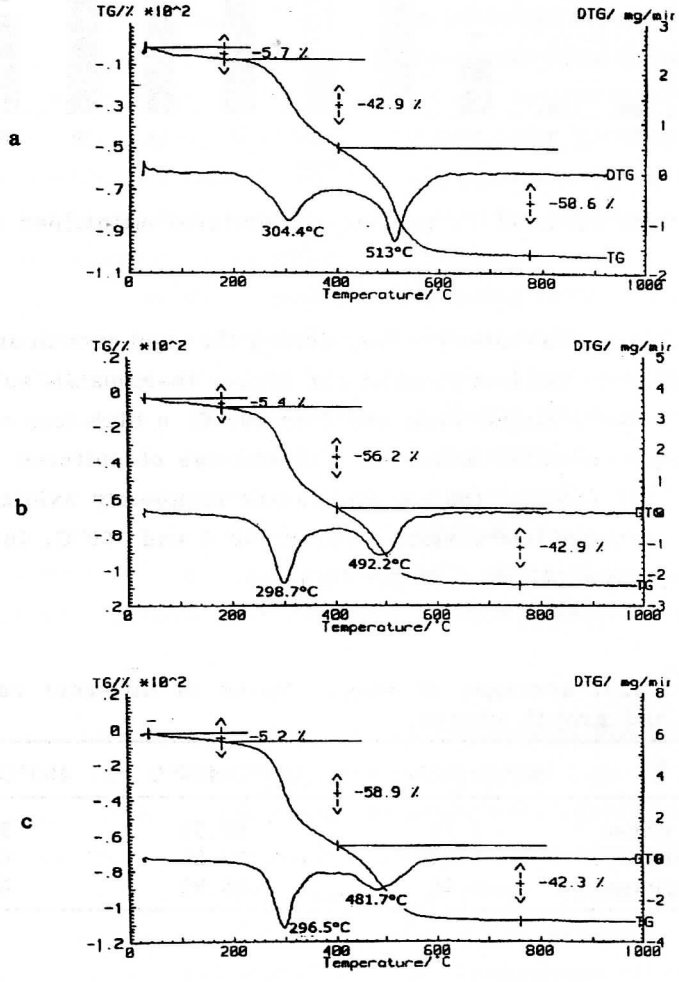

Fig. 2 - TG and DTG curves obtained from the analysis of exponential (a), stationary (b) and senescence (c) microalgae.

With regard to DTA (fig. 3a, 3b, 3c), the variation of enthalpy accompanying the decomposition of substances was very high when microalgae went into the exponential phase (ΔH = -64.398 KJ/g, temperature range: 99.76°C - 726.5°C) and decreased progressively in relation to algal growth (ΔH = -51.987 KJ/g, temperature range of stationary phase: 87.53°C - 707°C; (ΔH = -42.15 KJ/g, temperature range of senescence phase: 94.58°C - 700.8°C).

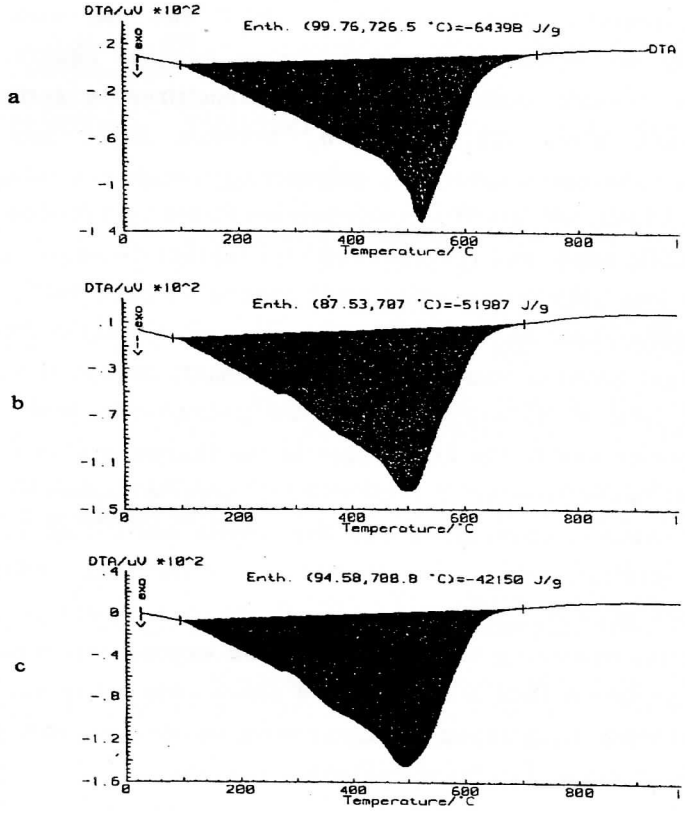

Fig. 3 - DTA of exponential (a), stationary (b) and senescence (c) microalgae.

DISCUSSION

The thermograms of T. suecica cultures maintained at 18°C show for each growth phase an oxidation pattern composed of three steps; initially, a loss of free water (it doesn't interact with biomolecules) and water loosely bound to biomolecules has been observed. This characteristic phenomenon of hydrophilic materials occurs, for example, during the decomposition of substances such as polysaccharides (16). With regard to this, the thermomechanical properties of biopolymers vary according to water content (17) and polymers with hydrophilic groups have various strengths of interaction with water (18).

In the temperature range of 40°C - 180°C the cell structure is progressively destroyed; in fact, phenomena such as denaturation of proteins and transformation of the lipid structures to a disordered structure take place (18,19). Then, between 180°C and 400°C, thermostable substances (proteins, polysaccharides, etc.) oxidize; with regard to this the pyrolysis of polysaccharides starts by a random scission of the glycosidic bonds and it is followed by a further decomposition (20). Finally, the last step occurs when temperature exceeds 400°C.

So marked differences have been observed in the thermal decomposition curves of algal growth; these results are probably due to the presence in the algal cells of different biomolecules produced or stored during growth processes and to the differences in the thermal stability of these biomolecules. In confirmation of these results, several studies have shown that the biochemical composition and the caloric content of T. suecica change in relation to growth phases and conditions under which phytoplankton grow (21,22). Moreover, it has been observed that the enthalpy varies according to algal growth and exponential cells contain higher energy levels than stationary and senescence cells; so, in these experimental conditions, exponentially growing microalgae could represent a suitable food for aquaculture systems.

In this paper, the growth of the marine microalga Tetraselmis suecica was

investigated using thermogravimetry (TG) and differential thermal analysis (DTA) to determine suitable diets for larval and juvenile development in aquaculture systems. Microalgae were maintained in synthetic sea water (19‰ salinity, 18°C constant temperature) and the algal growth was evaluated by cell abundance. Exponential, stationary and senescence cells were analyzed by TG and DTA. The results of thermal analysis pointed out marked differences between exponential, stationary and senescence phases and showed that exponentially growing microalgae could represent a suitable food in aquaculture systems.

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