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Continuous high-pressure disruption of marine diatom *Haslea ostrearia*. Assessment by laser diffraction particle sizer

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Abstract

In order to recover the intracellular blue-green pigment, marennine, synthesized by the microalga *Haslea ostrearia*, a continuous flow high-pressure disrupter was evaluated in the range: 30–270 MPa. Cells were partly broken from 30 MPa, but a pressure of 100 MPa (1 cycle) was required to obtain optimal pigment release. The latter was directly linked to the physical cell breakage dependent upon the applied pressure and the number of disintegration cycles. Granulometric analysis by laser diffraction technology (0.04–2000 μm) revealed a size reduction of cell fragments when increasing these two operating parameters.

Introduction

The marine diatom *Haslea ostrearia* has the unique peculiarity among diatoms to synthesize at its extremities a blue-green hydrosoluble pigment named 'marennine'. This microalga is known, in aquaculture, to be responsible for the greening of oysters (Robert 1983) and bears promising applications in the cosmetic field and anticancer research (Carbonnelle *et al.* 1999). This pigment is released in the medium under specific environmental stress conditions. Recently, membrane photobioreactors, with free and immobilized cells, have been successfully applied for the optimization of exocellular pigment recovery (Vandanjon *et al.* 1999a). The present study is dedicated to the recovery (through cell disruption) of the intracellular form of the pigment.

A wide range of cell disruption techniques for the release of intracellular metabolites are available, including mechanical, chemical methods or enzymatic treatment. The choice of the process is determined by the resistance to disruption of micro-organisms and the intracellular product type and localization in the cell (cytoplasm, vacuole, etc.). High-pressure technique is known to break successfully a variety of cells (maximum pressures reached up to 120 MPa until the 1990s) and shows potential for scale-up. The cell debris produced after breakage often consist of small fragments. So, a precise identification method is required to optimize both disruption and subsequent clarification and partial purification steps (Asenjo 1990).

The high-pressure disrupter used in this experimental study presents a new design because it allows continuous disruption up to 6 l h^{-1}, irrespective of cell concentration with potentially a single pass, with the benefit of minimal temperature changes (Foster 1995). Afterwards, particle sizing of disrupted suspension is assessed by a laser-based optical analysis that allows to identify fragments as small as 0.04 μm in diameter.

Materials and methods

Biological material

Haslea ostrearia strain was isolated from oyster-ponds in the Bay of Bourgneuf (Vendée, France). Cells were grown in standardized batch mode in the Laboratory of Marine Biology (ISOMer, Nantes University).

High-pressure disruption

The 'Z-Plus, 2.2 kW' disrupter is from Constant Systems Ltd, Warwick, UK. A piston compresses the sample, forcing it through a small orifice at high speed. The rapid transfer of the sample from a region of high pressure (30–270 MPa) to one of low pressure (0.1 MPa) causes cell breakage. When the jet containing disrupted cells hits the target, it is spread radially, then vertically, across a cooled heat exchange surface. Temperature in outflow of disrupter is 17 ± 0.5 °C. Then, suspension flows from the disruption chamber for collection. A peristaltic pump allows the culture of *Haslea ostrearia* (40×10^6 cells l^{-1}, at stationary phase of growth) to feed a reservoir connected with a float. When the reservoir is filled (250 ml), the disruption cycle is allowed to start.

Analysis

Cell breakage was observed using a scanning electronic microscope (SEM), JEOL 6400 at Nantes University (France). Biomass concentration was estimated using a Nageotte cell with optical microscope. The percentage of cell damage is calculated as follows:

$$\text{Cell damage (\%)} = 100(1 - N_1/N_0),$$

N_0 and N_1, are, respectively, the concentrations of non-disrupted cells before and after disruption cycle.

Marennine concentrations were assessed by measuring the optical density (OD) at 663 nm of filtered samples on Whatman GF/C filters referring the OD values of a calibration curve (extinction coefficient: 2.4 g l^{-1} cm^{-1}).

The particle size distributions were evaluated with a laser diffraction granulometer LS 230 (Coultronics-Beckman, Margency, France). This technique uses optical analysis of particles and determines the relative volume of particles (assumed spherical) based on the Fraunhofer and Mie models of light scattering. The size range is from 0.04 to 2000 μm.

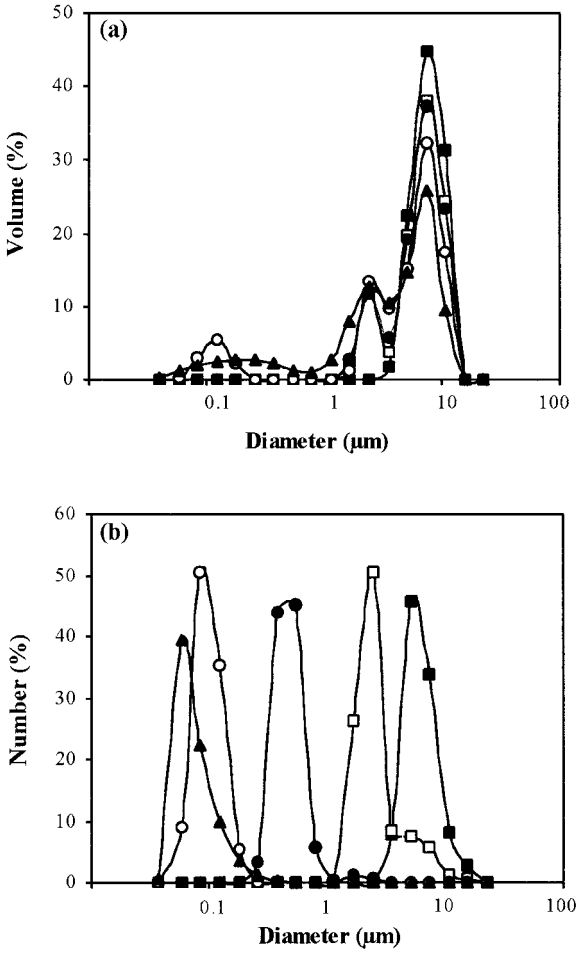

Fig. 1. Influence of disrupting pressure (1 cycle) on particle size distribution according to (a) % volume or (b) % number. (■) initial culture, (□) 30 MPa, (●) 100 MPa, (○) 150 MPa, (▲) 270 MPa.

Results and discussion

In a first step, high pressure disruption of *Haslea ostrearia* cells was achieved by a single pass. The size repartitions of cultures, before and after treatment with four operating pressures, are given in Figure 1.

After treatment, whatever the pressure considered, two main peaks are present on the distribution curves expressed in % volume (Figure 1a): the one at mean diameter of 7.5 μm (whole cells), the other at 2.5 μm (debris and cell fragments), which was undetectable in initial suspension. The increase of disrupting pressure leads to a height reduction of the peak corresponding to whole cells. The debris bear such small dimensions that relative volume percentages are almost negligible except for high pressure levels (150