Coccolithophores, among which *Emiliania huxleyi* is the most abundant and widespread species, are considered the most productive calcifying organism on earth. The export of organic carbon and calcification are the main drivers of the biological CO₂ pump and are expected to change with oceanic acidification. Coccolithophores are further known to produce transparent exopolymer particles (TEP) that promote particle aggregation. As a result, the TEP and biogenic calcium carbonate (CaCO₃) contribute to the export of carbon from the surface ocean to deep waters. In this context, we followed the development and the decline of *E. huxleyi* using batch experiments with monospecific cultures. We studied the link between different processes such as photosynthesis, calcification and the production of TEP. The onset of calcification was delayed in relation to photosynthesis. The timing and the general feature of the dynamics of calcification were closely related to the saturation state of seawater with respect to calcite, $\Omega_{\text{calc}}$. The production of TEP was enhanced after the decline of phytoplankton growth. After nutrient exhaustion, particulate organic carbon (POC) concentration increased linearly with increasing TEP concentration, suggesting that TEP contributes to the POC increase. The production of CaCO₃ is also strongly correlated with that of TEP, suggesting that calcification may be considered as a source of TEP precursors.

Introduction

Coccolithophores, especially *Emiliania huxleyi*, produce large blooms in oceans, in particular at continental margins and shelf seas. They form tests of calcium carbonate platelets (coccoliths) and are major contributors to marine calcification in temperate and subpolar latitudes (Brown and Yoder, 1994). The coccolithophores can cause a draw down of CO₂ in the surface ocean, via photosynthesis in the photic zone and vertical export of organic matter to deep waters:

$$106\text{CO}_2 + 16\text{NO}_3^- + 2\text{H}_2\text{PO}_4^- + 17\text{H}^+ + 122\text{H}_2\text{O} \rightleftharpoons (\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 138\text{O}_2$$ (1)

In contrast, the reaction of calcification is a source of CO₂:

$$\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$$ (2)

Whether the overall process is a net sink or a source of CO₂ for the atmosphere depends on the ratio of particulate inorganic carbon (PIC) and organic carbon (POC) produced, the so-called ‘rain ratio’. Calcium carbonate may act as ballast due to its large density and this increases the organic carbon flux to the seafloor (Klaas and Archer, 2002). As a result, calcifying organisms could contribute efficiently to the export of organic matter and enhance the oceanic biological pump for the drawdown of atmospheric CO₂. Moreover, polysaccharide aggregation, via transparent exopolymer particles (TEP) formation, together with the CaCO₃ ballast effect in *E. huxleyi* blooms, have the potential to promote the deep export of carbon on a relatively short time scale (Engel et al., 2004).

The TEP are defined as transparent particles that are formed from acidic polysaccharides and are stainable with Alcian Blue (Passow, 2002). They play an important role in aggregation processes and are formed from phytoplankton excretion products (released as dissolved organic matter

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during the exponential growth or the senescence of phytoplankton bloom) and therefore have a large content of organic carbon (Passow, 2002).

The aim of the batch culture experiments is to study the dynamics of the different processes (growth, calcification and TEP production) following the development and the decline of *E. huxleyi*. To describe the phytoplankton compartment, we followed chlorophyll a, dissolved nutrients (nitrates and phosphates), cell density in addition to POC, particulate nitrogen and TEP. Parameters of the inorganic carbonate system were also monitored to quantify the rate of calcification.

**Materials and methods**

**Description of the experiment**

Duplicate laboratory batch experiments were conducted on monospecific cultures of *E. huxleyi* (strain AC481 from Normandy, France). The strain was maintained in flasks of 250 ml before starting the experiments. Culture medium consisted of filtered (0.2 µm) and autoclaved surface post-bloom seawater sampled in the northern Atlantic ocean (47º45'N, 7º00'W), enriched with nitrates and phosphates. Cultures were not axenic. Experiments were carried out at an initial pCO2 of 600 µatm in an incubator at 13ºC, incident photon flux density was 150 µmol m⁻² s⁻¹ and the light/dark cycle was 14 h/10 h. Cultures were carried out in Nalgene polycarbonate carboys (10 l) filled to 8 l. Experiments were monitored for a period of 49 days. Time is referred to as dₓ with x as the number of days after strain addition. Samples were taken with a sterile syringe. The pH was measured on a seawater scale (pHsw) according to Dickson *et al.* (2007) and the total alkalinity (TA) was determined by the Gran titration method (Gran, 1952); these two parameters allowed the calculation of pCO2 and saturation state with respect to calcite (Ωcal). We also measured chlorophyll a (Chl-a) by fluorimetry, dissolved nutrients (NO₃ and PO₄) by colorimetry, cell density by microscopy, POC and particulate nitrogen (PN) by CHN analyser, and TEP by colorimetry.

**Measurements of calcification**

The TA of the seawater is affected by calcification (or dissolution) because the precipitation (or dissolution) of 1 mol of CaCO₃ reduces (or increases) the TA by 2 molar equivalents (equation 2). Calcium carbonate accumulation and calcification rates were calculated using the alkalinity anomaly technique (Chisholm and Gattuso, 1991). Calcification can be estimated directly from changes in total alkalinity using the relation,

\[
\text{CaCO}_3 \text{ accumulation} = -\frac{1}{2} \times \Delta TA 
\]

The TA was corrected for the drawdown of nitrate and phosphate due to photosynthesis (equation 1). When Ωcal is far greater than 1, TA consumption by calcification is greater than its release from the dissolution of CaCO₃. Thus, CaCO₃ accumulation, corresponding to the net concentration, is close to its gross value. On the contrary, when seawater is close to calcite saturation or slightly under-saturated with respect to calcite, dissolution becomes non-negligible and the variations of TA represent the net result of dissolution and calcification.

**Measurement of TEP and estimation of the carbon content of TEP**

The TEP concentrations were measured colorimetrically according to Passow and Alldredge (1995). To evaluate the carbon content of TEP (TEP-C), we adopted the following linear relationship used by Engel *et al.* (2004) to estimate the TEP-C produced during a mesocosm experiment with *E. huxleyi*:

\[
[\text{TEP} - \text{C}] = 0.033 \times [\text{TEP}] 
\]

where [TEP - C] is expressed in µmol l⁻¹ and [TEP] in µg X eq. l⁻¹.

**Results and discussion**

**The growth phase of *E. huxleyi***

The growth of *E. huxleyi* led to an increase in Chl-a concentrations. Its development can be characterized by 4 phases: (1) a lag phase; (2) an exponential growth phase during which cell division occurs under nutrients-replete conditions; (3) a stationary phase where cell division is reduced and nutrients depleted while cells continuously produce large amounts of coccoliths followed by (4) the decline phase (Fig. 1). Growth of *E. huxleyi* was detected after d₁₆ and induced an increase in Chl-a concentrations up to 28.52±4.17 µg l⁻¹. The PO₄ (data not shown) and NO₃ (Fig. 1) were consumed during the growth of *E. huxleyi*. Consumption was slow at the beginning, and became more intense from d₂₀
until approximately d30, when the nutrient was completely depleted. The onset of the exponential growth phase was concomitant with that of enhanced nutrient consumption. The production of Chl-a stopped when nutrients were depleted (Fig. 1).

POC concentrations increased at the same time as Chl-a but continued to increase after nutrient exhaustion during the stationary and decline phases (data not shown). The largest concentration of POC yielded 8 mg L\(^{-1}\) at the end of the experiment. The PN concentrations also increased at the onset of cell development, but decreased after nutrient depletion, suggesting the remineralization of particulate N. During the exponential growth phase, POC concentrations were closely related to changes in PN, yielding a POC:PN molar ratio close to 6.4, slightly below the Redfield ratio of 6.6. The POC:PN increased rapidly after nutrient exhaustion until the end of the experiment, indicating the carbon uptake was continuous (Fig. 2). Banse (1994) suggested that when nitrate or phosphate became depleted, photosynthesis would still proceed. This process was termed ‘carbon overconsumption’ by Toggweiler (1993). A pathway for the excess of DIC uptake by phytoplankton is the release of dissolved organic matter (DOM) followed by the formation of particulate organic carbon (POM) (Schartau et al., 2007). This release of DOM (for example, polysaccharides) leads to the formation of particulate organic carbon (POM).
of larger colloidal particles that will be observed in the TEP formation.

**Biocalcification**

The TA was constant at the beginning of the experiment, but decreased sharply from d21 (data not shown). The decrease of alkalinity by $1406\pm71.1$ μmol kg$^{-1}$ corresponds to the precipitation of $67.9\pm1.8$ mg kg$^{-1}$ of calcite, according to the TA anomaly calculation (Fig. 3). The consumption of carbonate ions by calcification (equation 2) leads to a decline in $\Omega_{\text{calc}}$ during the experiment, from its initial value (~3) after d25 to <1 on d37. The maximum rates of net calcification were observed at a $\Omega_{\text{calc}}$ close to 3 and decreased with decreasing $\Omega_{\text{calc}}$ towards saturation ($\Omega_{\text{calc}} = 1$) (Fig. 4).

**Evolution of pH and pCO$_2$**

The initial pH$_{\text{SWS}}$ was 7.879±0.005. It increased slowly until d25, then decreased until d40 and remained constant afterwards (Fig. 5a). The calculated initial pCO$_2$ averaged 627.8±10.9 μAtm and reached a minimum of 385.6±14.6 μAtm before increasing again on d28 to a final pCO$_2$ of 617.4±27.1 μAtm on d39 (Fig. 5b). The pCO$_2$ decreased again until the end of the experiment. The decrease of pCO$_2$ depends on the buffer capacity of the system but also on the intensity of the biological sink of CO$_2$ (i.e. photosynthesis) during the exponential phase. From d21 to d30, calcification occurs concomitantly with Chl-a production. At this moment, a part of the CO$_2$ released by calcification could be taken up by photosynthesis. From d30 onward, calcification became the dominant process and the subsequent increase of pCO$_2$, in the second part of the experiment was related to the intense calcification (equation 2). The decrease in pCO$_2$ during the last 10 days of the experiment was related to the decreasing calcification and possibly to the overconsumption of carbon.

**Fig. 5.** Evolution of (a) pH; and (b) pCO$_2$ in seawater during the experiment for the duplicate samples.

**Fig. 6.** TEP concentrations as a function of CaCO$_3$ concentrations (average of the duplicate samples).

**Fig. 7.** Evolution of TEP concentrations.
By plotting the concentration of TEP against that of CaCO₃, we obtained a linear relationship \((n = 21, R^2 = 0.96, \text{Fig. 6})\). This suggests that the production of TEP and that of inorganic carbon are closed linked and calcification is a source of TEP precursors.

**Evolution of TEP concentration and estimation of the carbon content of TEP (TEP-C)**

The TEP concentration increased rapidly after nutrient decline, reaching a maximum value of 6754 μg X eq. L⁻¹ at the end of the experiment (Fig. 7). The linear relationship between TEP-C and POC \((n = 9, R^2 = 0.91, \text{Fig. 8})\) indicates that TEP was responsible for 30% of the POC increase after nutrient depletion.

**Conclusions**

The production of biogenic calcite was detected on \(d_{21}\), five days after the onset of \(E. huxleyi\) growth, and slowed down on \(d_{43}\). Calcification continued when growth became limited by nutrients. Our results are in accordance with Zondervan et al. (2001), who suggests that coccolith formation is less dependant on PO₄ or NO₃ than cell division. \(E. huxleyi\) could be great producers of TEP that contribute to particle aggregation. Combined with the CaCO₃ ballast effect, large-scale coccolithophore blooms have the potential to promote deep export of organic carbon on relatively short time scales.

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