Mineralogical Magazine, February 2008, Vol. 72(1), pp. 251–256

Biocalcification by *Emiliania huxleyi* in batch culture experiments

C. De Bodt, J. Harlay and L. Chou*

Laboratoire d'Océanographie Chimique et Géochimie des Eaux, Faculté des Sciences, Université Libre de Bruxelles, Campus de la Plaine – CP 208, B-1050 Brussels, Belgium

ABSTRACT

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 CO_2 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 ($CaCO_3$) 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, Ω_{cal}. 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 $CaCO_3$ 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 CO_2 in the surface ocean, via photosynthesis in the photic zone and vertical export of organic matter to deep waters:

$$106CO_2 + 16NO_3^- + H_2PO_4^- + 17H^+ + 122H_2O \rightleftharpoons (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \quad (1)$$

In contrast, the reaction of calcification is a source of CO_2:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \quad (2)$$

Whether the overall process is a net sink or a source of CO_2 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 CO_2. Moreover, polysaccharide aggregation, via transparent exopolymer particles (TEP) formation, together with the $CaCO_3$ 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 polyssacharides 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

* E-mail: lei.chou@ulb.ac.be
DOI: 10.1180/minmag.2008.072.1.251

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 pCO_2 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 d_x 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 (pH_{sws}) 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 pCO_2 and saturation state with respect to calcite (Ω_{cal}). We also measured chlorophyll a (Chl-a) by fluorimetry, dissolved nutrients (NO_3 and PO_4) 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 $CaCO_3$ 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,

$$CaCO_3 \text{ accumulation} = -\tfrac{1}{2} \times \Delta TA \qquad (3)$$

The TA was corrected for the drawdown of nitrate and phosphate due to photosynthesis (equation 1). When Ω_{cal} is far greater than 1, TA consumption by calcification is greater than its release from the dissolution of $CaCO_3$. Thus, $CaCO_3$ 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*:

$$[\text{TEP} - \text{C}] = 0.033 \times [\text{TEP}] \qquad (4)$$

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 d_{16} and induced an increase in Chl-a concentrations up to 28.52±4.17 μg l^{-1}. The PO_4 (data not shown) and NO_3 (Fig. 1) were consumed during the growth of *E. huxleyi*. Consumption was slow at the beginning, and became more intense from d_{20}

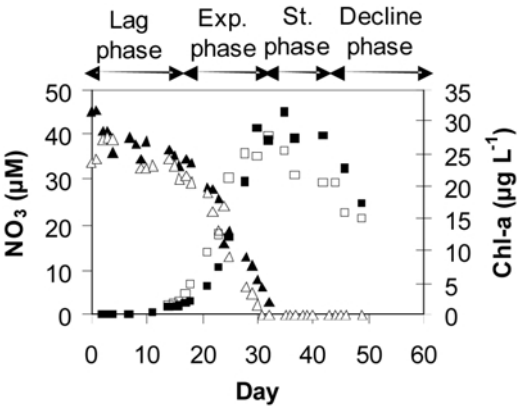

FIG. 1. Evolution of NO_3 (triangles) and Chl-a (squares) concentrations during the experiment. Exp. phase = exponential growth phase; and St. phase = stationary phase. Black and open symbols represent results of the duplicate experiments.

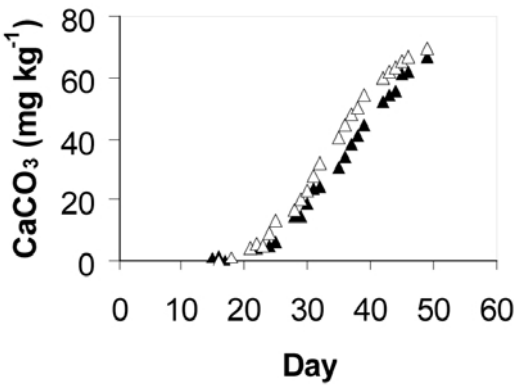

FIG. 3. Net accumulation of calcite during the experiment.

until approximately d_{30}, 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

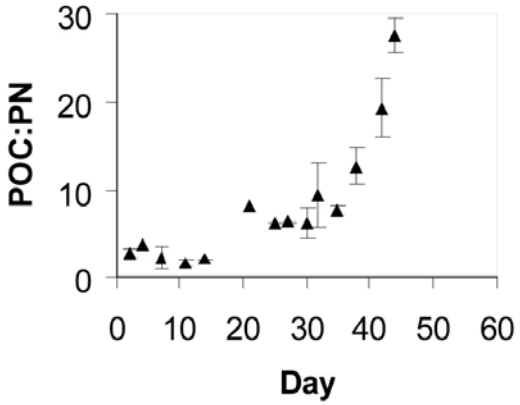

FIG. 2. Evolution of the POC:PN molar ratios (average of the duplicate samples).

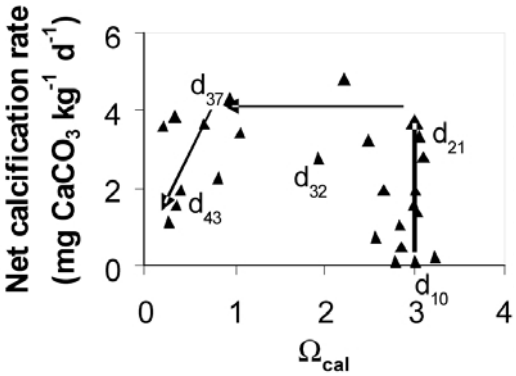

FIG. 4. Evolution of the net calcification rate as a function of Ω_{cal} (average of the duplicate sample). Below a Ω_{cal} of 1, the net calcification rate decreased.

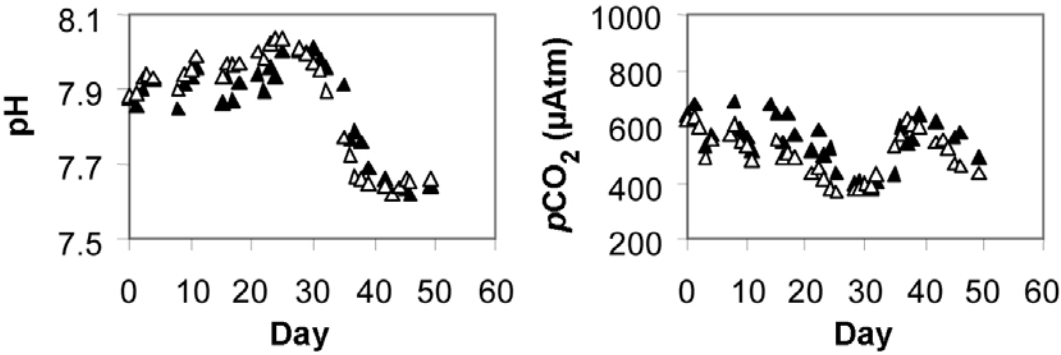

FIG. 5. Evolution of (*a*) pH; and (*b*) pCO_2 in seawater during the experiment for the duplicate samples.

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 d_{21} (data not shown). The decrease of alkalinity by 1406±71.1 µmol kg^{-1} corresponds to the precipitation of 67.9±1.8 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 Ω_{calc} during the experiment, from its initial value (~3) after d_{25} to <1 on d_{37}. The maximum rates of net calcification were observed at a Ω_{calc} close to 3 and decreased with decreasing Ω_{calc} towards saturation (Ω_{calc} = 1) (Fig. 4).

Evolution of pH and pCO_2

The initial pH_{SWS} was 7.879±0.005. It increased slowly until d_{25}, then decreased until d_{40} and remained constant afterwards (Fig. 5*a*). 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 d_{28} to a final pCO_2 of 617.4±27.1 µAtm on d_{39} (Fig. 5*b*). 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 d_{21} to d_{30}, 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 d_{30} 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.

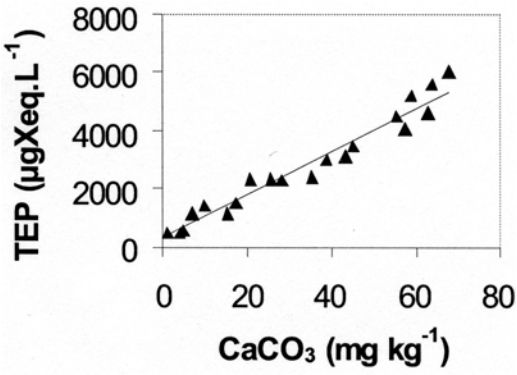

FIG. 6. TEP concentrations as a function of $CaCO_3$ concentrations (average of the duplicate samples).

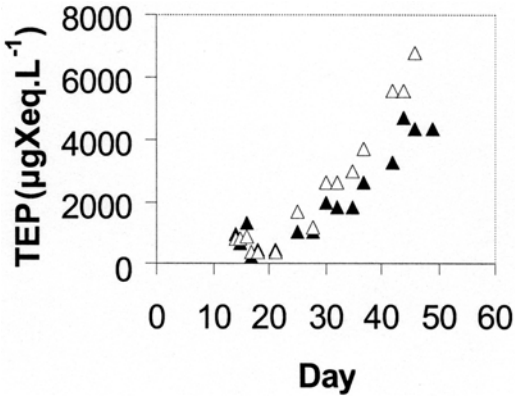

FIG. 7. Evolution of TEP concentrations.

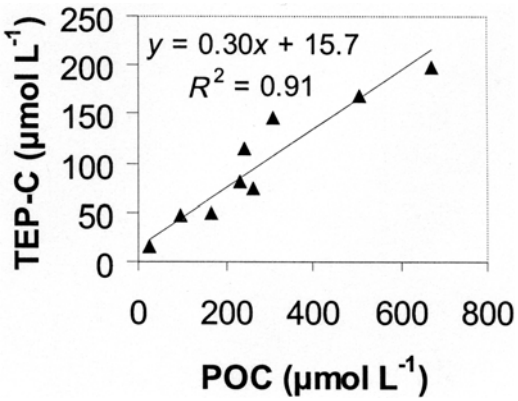

FIG. 8. TEP-C as a function of POC (average of the duplicate samples).

Production of TEP

Link between TEP and calcification

By plotting the concentration of TEP against that of $CaCO_3$, we obtained a linear relationship ($n = 21$, $R^2 = 0.96$, 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 ($n = 9$, $R^2 = 0.91$, 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 PO_4 or NO_3 than cell division. *E. huxleyi* could be great producers of TEP that contribute to particle aggregation. Combined with the $CaCO_3$ ballast effect, large-scale coccolithophore blooms have the potential to promote deep export of organic carbon on relatively short time scales.

Acknowledgements

This work was supported by the EU FP6 IP CarboOcean project (contract no. 511176-2). Additional funding was provided by the Belgian Federal Science Policy Office in the framework of the PEACE project (contract no. SD/CS/03A). This study is a contribution to the EU FP6 European NoE EUR-OCEANS (contract no. 511106-2). It is also a Belgian input to the SOLAS international research initiative.

References

Banse, K. (1994) Uptake of inorganic carbon and nitrate by marine plankton and the Redfield ratio. *Global Biogeochemical Cycles*, **8**, 81–84.

Brown, C.W. and Yoder, J.A. (1994) Coccolithophorid blooms in the global ocean. *Journal of Geophysical Research*, **99**, 7467–7482.

Chisholm, J.R.M. and Gattuso, J.-P. (1991) Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral reef communities. *Limnology and Oceanography*, **36**, 1232–1239.

Dickson, A.G., Sabine, C.L. and Christian, J.R. (2007) Guide to best practices for ocean CO_2 measurements. *PICES Special Publication*, **3**, 191 pp.

Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbrüggen, A. and Zondervan, I. (2004) Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO_2 concentrations: a mesocosm experiment. *Aquatic Microbial Ecology*, **34**, 93–104.

Gran, G. (1952) Determination of the equivalence point in potentiometric titrations – Part II. *The Analyst*, **77**, 661–671.

Klaas, C. and Archer, D.E. (2002) Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the Rain Ratio. *Global Biogeochemical cycles*, **16**, 63-1–63-14.

Passow, U. (2002) Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography*, **55**, 287–333.

Passow, U. and Alldredge, A.L. (1995) A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnology and Oceanography*, **40**, 1326–1335.

Schartau, M., Engel, A., Schröter, J., Thoms, S., Völker, C. and Wolf-Gladrow, D. (2007) Modelling carbon overconsumption and the formation of extracellular particulate organic carbon. *Biogeosciences*, **4**, 433–454.

Toggweiler, J. (1993) Carbon overconsumption. Nature, **363**, 210–211.

Van Emburg, P.R., de Jong, E.W. and Daems, W.T. (1986) Immunochemical localization of a polysaccharide from biomineral structures (coccoliths) of *Emiliania huxleyi*. *Journal of Ultrastructure and Molecular Structure Research*, **94**, 246–259.

Zondervan, I., Zeebe, R.E., Rost, B. and Riebesell, U. (2001) Decreasing marine biogenic calcification: A negative feedback on rising atmospheric pCO_2. *Global Biogeochemical Cycles*, **15**, 507–516.