Impacts of ocean acidification on calcifying phytoplankton

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Abstract

Increased concentration of atmospheric carbon dioxide, primarily from human fossil fuel combustion, has caused a reduction of ocean pH and shifts in seawater carbonate chemistry. Coccolithophores are major calcium carbonate producers in the world’s oceans and thus are a key functional group in the marine carbon cycle. Among modern coccolithophorids, Emiliania huxleyi is the most successful species, exhibiting a worldwide distribution in open and coastal oceans. In this experiment we focused on the responses of Emiliania huxleyi to increased carbon dioxide, especially the photosynthesis and the growth rate.

In the experiment the pH and the alkalinity of the samples were measured in order to control the carbonate system in the growing environment of the algae. Measurements of the pigments and the cell number were used to evaluate the growth condition of the algae. Several parameters such as Fv/Fm, Fv'/Fm', NPQ and ETR were measured in order to compare the photosynthetic efficiency of the algae at different carbon dioxide concentrations.

According to the results of the laboratory experiments the photosynthesis and the growth rate of the coccolithophore Emiliania huxleyi are not significantly changed by higher CO₂ partial pressures.

Keywords: Ocean acidification, coccolithophore, photosynthesis, growth rate.
Project description

Introduction
The concentration of carbon dioxide in the atmosphere has increased rapidly since the middle of the 18th century and further increase is predicted as a result of anthropogenic activities, primarily combustion of fossil fuels. The ocean absorbs approximately 25 percent of the carbon dioxide emitted into the atmosphere each year. When the atmospheric carbon dioxide pressure increases more carbon dioxide is dissolved in the seawater which result in shifts of the seawater carbonate chemistry and reduction of seawater pH. This phenomenon, called ocean acidification, has caused a decline in surface ocean pH from a pH of 8.25 before the industrialisation to a present pH of 8.08 (Engel at al. 2008). Coccolithophores, calcifying algae such as *Emiliania huxleyi* are important calcium carbonate producers in the world’s ocean and changes in seawater carbonate chemistry is expected to have various impacts on the marine organism.

Purpose
The purpose of this project is to examine how the growth rate and the photosynthesis of the coccolithophore *Emiliania huxleyi* are affected by a higher carbon dioxide pressure.

Background
Carbonate chemistry
When carbon dioxide dissolves in surface ocean carbonic acid (H$_2$CO$_3$) is formed which causes the decline in pH. The carbonic acid combines with carbonate ions, (CO$_3^{2-}$) and water to form bicarbonate ions (HCO$_3^-$). A higher atmospheric carbon dioxide pressure therefore results in increased concentrations of carbonic acid and bicarbonate ions and decreased concentrations of carbonate ions in the world’s ocean (Iglesias-Rodriguez et al 2008). According to studied models the reduction of carbonate ions will cause saturated CaCO$_3$ states to become unsaturated which result in an increased CaCO$_3$ dissolution rate in the ocean (Findlay at al 2009).

Calcification
The process in which calcium is build up in soft tissue, causing it to harden is called calcification. The majority of marine biogenic calcification occurs in planktonic organisms but also in other organism such as molluscs, foraminifera, crustacean and corals. For the calcifying organisms calcification is important for processes such as growth, metabolism and regulation of internal pH (Findlay at al 2009). These organisms are therefore expected to be sensitive to ocean acidification and shifts in carbonate chemistry.

The calcification in coccolithophores can be described with following formula:

\[ \text{Ca}^{2+} + 2 \text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \]

Calcareous skeletons that are produced by calcifying planktonic organisms subsequent sink and supplies the deeper ocean and underlying sediments with calcium carbonate. This is an important
process that regulates the marine carbon cycling and the exchange of carbon dioxide between the atmosphere and the ocean (Riebesell et al 2000).

**Emiliania huxleyi**

*Emiliania huxleyi* is a calcifying unicellular alga with a global distribution. The cell is dominated by the chloroplast which contains photosynthetic pigments and is covered of calcite disks, coccoliths that are formed inside the cell via coccolithogenesis.

**Photosynthesis**

Carbon dioxide is crucial for the production of organic matter in the ocean through photosynthesis. Marine phytoplankton plays an essential role in the carbon cycle since it through photosynthesis removes dissolved inorganic carbon (DIC) from the upper ocean and transport it to deeper parts through sedimentation, the so called “biological pump” (Engel 2002). Although its importance, it is still purely understood how marine organism will be affected by a higher carbon dioxide pressure (Engel at al 2008).

**Materials and methods**

**Preparation of the cultures**

The artificial seawater in which the alga *Emiliania huxleyi* will bee cultivated is prepared through mixing distilled water with a number of chemical compounds in order to resemble the composition of the world’s seawaters. 10 l artificial seawater has following composition: 245.4g NaCl, 0.17g \( \text{SrCl}_2 \cdot 6\text{H}_2\text{O} \), 7g KCl, 11.1g CaCl\(_2\), 111g MgCl\(_2\) \( \cdot 6\text{H}_2\text{O} \), 0.03g H\(_3\)BO\(_3\), 40.9g Na\(_2\)SO\(_4\), 1g KBr, 0.03g NaF, 1.85g NaHCO\(_3\) and distilled water. The composition of the water is controlled by measuring the salinity. The artificial seawater is divided into two flasks with a volume of 3l each. To create good growth conditions for the algae 3ml metal solution and 3ml nutrient solution are added to each of the flasks before they are sterilized in an autoclave CL-32L at 105°C. The flasks are placed in an incubator, HP1000G, where the pH of the artificial seawater is adjusted through bubbling CO\(_2\) with different concentrations, 380 ppm and 750 ppm, into the flasks for approximately 20 hours. The CO\(_2\) concentrations correspond to the CO\(_2\) concentration in the atmosphere today and the future concentration in the atmosphere according to some future scenarios. 3 ml vitamin solution is after the incubation added to the flasks. The alga, *Emiliania huxley* purchased from Australia is subsequent added to the flasks and the substance in the two flasks is divided into 8 smaller vessels. The vessels are placed in an incubator, GXZ-300D, for three days at 20°C and with 12 hour exposure to light and 12 hour in darkness. The light density is 200 µmol photons/m\(^2\)s. The content in the 8 vessels, 4 of each CO\(_2\) concentration, represent the samples in the different measurements described below. If the number of cells in the cultures is too high it will cause a change in pH that is not desirable. The samples are therefore diluted every third day with new artificial seawater and the procedure describes above is repeated. The process is repeated three times, the first 6 days is the acclimatization phase when the algae adapt to its growth environment. The final measurements are then performed three days after the acclimatization phase.
**pH measurements**

It is important to control the pH of the samples during the experiment since a significant change with time could indicate that there is something wrong with the carbonate system in the water or that the concentration CO$_2$ in the water does not correspond to the CO$_2$-concentration bubbled into the flasks. The pH is therefore measured with an interval of three days. The equipment used to measure the pH is a Mettler Toledo DL15 Titrator (see Figure 2). The potential of the samples is measured instead of measuring the pH directly since this method is more precise. The pH-meter is at first calibrated and the results are used to create a standard curve. The equation of the standard curve is used to calculate the pH where x represent the measured potential and y the pH.

**Alkalinity measurements**

The alkalinity of the samples is measured three times during the experiment in order to control the carbonate system in the samples. Alkalinity samples were taken from the filtrate and measured by
potentiometric titration. Total alkalinity was calculated from linear gran plots (Dickson et al., 2003)

**Growth rate measurements**

The cells concentration in the samples is counted in a Beckman coulter; Z2 Coulter, Particle Count and size analyzer (see Figure 3). The amount of cells in the artificial seawater before the algae is added is first measured and correspond to a background value. The cell concentration in the samples containing algae is measured four times during the experiment. The first three values are performed in order to determine with how much artificial seawater the samples should be diluted so that the initial cell concentration is approximately 5000 cells/ml. The final cell concentration is used to calculate the growth rate of the algae with following formula:

\[
\mu = \frac{\ln x_2 - \ln x_1}{\Delta T}
\]

- \(x_1\) = initial cell concentration (5000 cells/ml)
- \(x_2\) = final cell concentration
- \(\Delta T\) = 3 days

![Z2 Coulter, Particle Count and size analyzer.](image)

**Pigments measurement**

Measurements of the pigments in the algae can give useful information both on the growth and the photosynthesis of the algae. The algae in 100ml samples are filtrated from the artificial seawater using a water-circulation multifunction vacuum pump. The filtrated algae are mixed with 3ml of methanol and placed in room temperature and darkness for six hours in order to extract the pigments, chlorophyll a, from the algae. The samples are then centrifuged. The absorbance of the samples is determined in a spectrophotometer at three different wavelengths 750\(\mu\)m, 665.2 \(\mu\)m and 652 \(\mu\)m. The absorbance at 750 \(\mu\) is measured in order to determine the amount of other particles in the samples while the two other wavelengths correspond to the absorbance maxima of chlorophyll a. The weight of Chl a in each sample is calculated with following formula:

\[
\text{Chl a} = 16.29 \times (E_{665.2} - E_{750}) - 8.54 \times (E_{652} - E_{750})
\]
E665.2, E750, E652=the absorbance at respective wavelength.

The weight of Chl a in pg/cell is calculated with following formula:

\[ \text{Chl a} \times \frac{3}{100} / C \times 10^6 \], \( C \)=cell concentration.

**Chlorophyll fluorescence measurements**

In order to determine if the photosynthetic efficiency of the algae differ between the samples with different pH the parameters ETR, Fm/Fv, Fm’/Fv’ and NPQ are measured using a XE-PAM Fluorometer. ETR stands for effective photosynthetic electron transport rate. Fm/Fv is the potential photochemical efficiency of open Photosystem II in darkness before sunrise which corresponds to maximum photosynthetic efficiency. Fm’/Fv’ is the transient photochemical efficiency of Photosystem II during exposure to light which correspond to the actual photosynthetic efficiency. NPQ, non-photochemical quenching, is a protective mechanism that convert and dissipate excess excitation energy to heat (Adams, W.W., Demming-Adams, B. 2004).

Before measuring Fm/Fv the samples are placed in darkness for 30 min while the samples are exposed to light before measuring Fm’/Fv’. In the measurements of NPQ the samples were exposed to different actinic lights, 72 and 385\( \mu \text{mol/m}^2\text{s} \).

**Results**

**pH, alkalinity, chl a and growth rate**

The results presented in Table 1 are mean values of the different measurements of samples with the two different CO\(_2\)-concentrations. The pH and the alkalinity presented in the table correspond to the first measured value after the adaption phase and the final value measure three days later. The pH has increased between the measurements while the alkalinity has slight decreased. The alkalinity does not differ significantly between the different CO\(_2\)-concentrations. The growth rate represents the calculated values with an initial concentration of 5000cells/ml and a final mean value of 143567 (380 ppm) and 142334 (750 ppm). The weight of the pigment chl a also represent calculated mean values. The growth rate and the pigments do not differ at all or very little between
the two different CO₂-concentrations.

Table 1: The results from the different measurements.

<table>
<thead>
<tr>
<th></th>
<th>380</th>
<th>750</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start point</td>
<td>Final point</td>
</tr>
<tr>
<td>pH</td>
<td>8.08 ± 0.01</td>
<td>8.16 ± 0.02</td>
</tr>
<tr>
<td>Alk (µmol/kg)</td>
<td>2142.42 ± 7.13</td>
<td>2047.87 ± 16.40</td>
</tr>
<tr>
<td>µ (/d)</td>
<td>1.12</td>
<td>1.12</td>
</tr>
<tr>
<td>chl a (pg/cell)</td>
<td>0.093 ± 0.01</td>
<td>0.099 ± 0.02</td>
</tr>
</tbody>
</table>

NPQ

Figure 5 show the NPQ of samples with the two different CO₂-concentrations and at the actinic light 385 µmol/m² s and 72 µmol/m² s. According to a statistic t-test there is no significant difference in the NPQ between the samples at the actinic light 385µmol/m² s. There are however a difference in NPQ between the samples at the actinic light 72µmol/m² s. The yield at the different actinic lights corresponds to Fv/Fm.

Figure 5: NPQ and yield (Fv/Fm) of samples with different CO₂-concentrations measured at actinic light of 385 µmol/m² s and 72 µmol/m² s.

Fm/Fv and Fm'/Fv'

The Fv/Fm of the algae is presented in Figure 6 which shows no considerable difference between the samples with different CO₂-concentrations.
Figure 6: Fv/Fm of the samples with different CO₂-concentrations.

The Fv'/Fm' of the algae is presented in Figure 7. The parameter does not differ significantly between the samples with different CO₂-concentrations.

Figure 7: Fv'/Fm' of the samples with different CO₂-concentrations.

**ETR**

The electron transport rate of samples at the different CO₂-concentrations is presented in Figure 8. The figure indicate that there are no significant difference between the samples.
Figure 8: Effective photosynthetic electron transport rate.

Discussion

According to Table 1 there is an increase in pH between the two measurements. This can be explained by the fact that during photosynthesis the algae fixate carbon. This carbon comes from the reaction where bicarbonate dissolves to carbon dioxide and a hydroxide ion. While the carbon is taken up by the algae the hydrogen ion is released to the water and causes an increase in pH. The alkalinity is on the other hand decreasing during the measurements due to the calcification process performed by the algae when they are growing. Calcification has a relatively strong effect on the alkalinity causing it to decline.

The growth rate of the algae are according to Table 1 not effected by increment of the CO$_2$-concentration. This assumption is reinforced by the fact that the amount of the pigments chl a are also more or less the same in the two samples. There are previous studies on cell division of *Emiliani huxleyi* that did not, in resemblance with the results of this experiment, show any significant effects due to elevated CO$_2$ concentrations (Ramos et al 2009).

The parameters measured by chlorophyll fluorescence ETR, Fm/Fv, Fm’/Fv’ and NPQ all show no or very little difference between the samples with different CO$_2$-concentration. This indicates that the higher carbon dioxide pressure do not have any significant effect on the photosynthesis of the algae. The same results were obtained by Igelsias-Rodriguez et al. (2008). In their research Fv/Fm did not changed over a range of pCO2 from 280 to 750 ppm. The only parameter that shows a significant difference between the samples is the NPQ measured during exposure to low actinic light intensity. One possible explanation to the difference could be that the primary production and therefore the photosynthesis of the algae is stimulated by a higher CO$_2$-pressure at low light intensity.
Conclusion

The results of the laboratory experiments indicate that the growth rate and photosynthesis of the algae E.huxleyi are not significantly affected of higher CO₂ partial pressures. Further research including a larger number of samples is however required in order to draw clear conclusions of the results.

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