Contribution of coral rubble associated microbial community to the dissolution of calcium carbonate under high $p\text{CO}_2$

&

Coral reefs perspective Bangladesh

Md. Nazrul ISLAM

Graduate School of Science and Technology,
Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan
Introduction

We all know about global warming, but carbon dioxide (CO₂) has been giving another impact, which is less known: Carbon dioxide is slowly acidifying our oceans, which is harming anything in the seas that is composed by calcium carbonate (CaCO₃) such as corals, mollusks and echinoderms.
The concentration of CO$_2$ in atmosphere is now **392 ppm** and during last decade rose by **2.0 ppm yr$^{-1}$** with a corresponding decrease in pH of **0.0015 yr$^{-1}$**

(Tans, 2011; NOAA/ESRL and Midorikawa et al. 2010).
Coral reefs are one of the first ecosystems to be recognized as vulnerable to ocean acidification. Until now many studies on ocean acidification have focused on the **physico-chemical** aspects. However, **biological processes** (respiration vs. photosynthesis) are more important in determining calcification and dissolution.

\[
\text{CH}_2\text{O} + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3 \quad \text{[Oxidation]} \quad [1] \\
\text{CaCO}_3 + \text{H}_2\text{CO}_3 = \text{Ca}^{2+} + 2\text{HCO}_3^- \quad \text{[Dissolution]} \quad [2]
\]
How CO₂ is making acidity of the Oceans?

Atmospheric Carbon Dioxide

CO₂

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

Increasing Hydrogen (H⁺) ions decreased pH and leads to CO₃²⁻ saturation

Definition: \( \text{pH} = -\log_{10}[\text{H}^+] \)

So, low pH = high [H⁺] \( \Rightarrow \) acidic
high pH = low [H⁺] \( \Rightarrow \) basic
Objectives

- to know the contribution of microbial communities (epilithic and endolithic) to the dissolution of calcium carbonate under high $p\text{CO}_2$;

- to determine the contribution of chemical vs. biological factors for the dissolution of carbonate;

- to determine the effect of organic matter and diurnal variation of carbonate dissolution.
Materials and Methods
Study area and Sample collection
Sesoko Island, Okinawa, Japan

Location
26° 38' 46" North latitude
127° 51' 53" East longitude

Sesoko Beach
Sample collection

Okinawa
Sesoko Island
Preparation of incubation experiment

1. Incubation experiments were carried out in natural illumination and dark condition under high $p$CO$_2$ levels;

2. Seawater was filtered using a cartridge filter (0.2 μm isopore membrane filter);

3. All incubation bottles were washed using neutral Extran and rinsed with Milli-Q before use;

4. Rubble biofilm scarped off by using tooth brush and water pick;

5. Two branches of similar sizes coral rubble was placed into each incubation bottle; [Length: 5.51±0.02 cm & Diameter 1.00±0.01 cm]

6. CO$_2$ saturated seawater was prepared by bubbling pure CO$_2$ gas;

7. Stock glucose solution (1.8 g l$^{-1}$) was prepared as source of organic matter.
### Table: Experimental design for short and long incubations

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Duration</th>
<th>Condition</th>
<th>pCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ambient</td>
</tr>
<tr>
<td>Short</td>
<td>24h</td>
<td>Natural illumination</td>
<td>WCSk 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TR 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR 1 2 3</td>
</tr>
<tr>
<td>Long</td>
<td>4 days</td>
<td>Dark</td>
<td>WCSk 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR+G 1 2 3</td>
</tr>
<tr>
<td>Long</td>
<td>4 days</td>
<td>Natural illumination</td>
<td>WCSk 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR 1 2 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR+G 1 2 3</td>
</tr>
</tbody>
</table>

**WCSk**: White coral skeleton; **NR**: Natural rubbles with associated epilithic and endolithic communities; **TR**: Treated rubble keeping only their endolithic communities; **NR+G**: Natural rubbles with addition of glucose; **Natural illumination**: outdoor
Measurement and analysis

1. Measurement of short incubation experiment was done 24 times per day (1h interval) and 4 times per day (6h interval) for long incubation experiments;

2. Temperature and light intensity was monitored using in situ sensor;

3. Net primary production (NPP) and Respiration (R) were calculated from changes in dissolve oxygen (DO) concentration; \( \text{GPP} = \text{NPP} + \text{R} \)

4. Heterotrophic bacterial abundance was assessed by counting bacteria cells under an epifluorescence microscope using a UV-filter;

5. Carbonate dissolution rates were analyzed using the alkalinity anomaly technique (Gattuso et al. 1997; Fujimura et al. 2001); the variation of \( A_T \) (\( A_{T\text{final}} - A_{T\text{initial}} \))

\[ \text{Dissolution rate} = \frac{\Delta A_T}{2} \]

6. Aragonite saturation state (\( \Omega_a \)) were calculated with the program CO2SYS (Lewis and Wallace 1998). \[ \Omega_a = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}} \]
Results and Discussion
Is coral rubble DEAD?

Desert of coral rubble in the lagoon
Epilithic communities in rubble

Figure: Epilithic microbial communities (a) coccoid green algae; (b) green algae; (c) red calcareous algae; (d) algal bloom; (e, f) diatom; (g) nematodes; (h, i) foraminifera; (j) cyanobacteria
Heterotrophic bacterial communities in rubble

Islam et al., 2012
Endolithic communities in rubble

**Figure:** Endolithic algae in coral rubble. A green band surrounding the rubble skeleton can be observed in a transverse section. This green layer mostly consists of endolithic algae.
### Table: Summary result of 24h Natural Illumination experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GPP [µgCm(^{-2})d(^{-1})]</th>
<th>NPP [µgCm(^{-2})d(^{-1})]</th>
<th>R [µgCm(^{-2})d(^{-1})]</th>
<th>Rd [µgCm(^{-2})d(^{-1})]</th>
<th>P/R</th>
<th>DR [µmol m(^{-2})d(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambient condition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR (endo)</td>
<td>3763 ± 0.2</td>
<td>1321 ± 1.4</td>
<td>2442 ± 2.8</td>
<td>1263 ± 1.6</td>
<td>1.54</td>
<td>90.8 ± 0.0</td>
</tr>
<tr>
<td>NR (epi + endo)</td>
<td>3909 ± 0.3</td>
<td>1373 ± 2.8</td>
<td>2536 ± 2.4</td>
<td>1293 ± 1.2</td>
<td>1.54</td>
<td>100.0 ± 0.1</td>
</tr>
<tr>
<td>Contribution of TR</td>
<td>96.25%</td>
<td>96.21%</td>
<td>96.27%</td>
<td>97.71%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High pCO(_2) (1120 ppm) condition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR (endo)</td>
<td>4269 ± 1.4</td>
<td>1499 ± 1.5</td>
<td>2768 ± 0.0</td>
<td>1433 ± 2.0</td>
<td>1.54</td>
<td>108.9 ± 0.1</td>
</tr>
<tr>
<td>NR (epi + endo)</td>
<td>5129 ± 1.4</td>
<td>1803 ± 1.5</td>
<td>3328 ± 0.0</td>
<td>1721 ± 1.1</td>
<td>1.54</td>
<td>127.6 ± 0.0</td>
</tr>
<tr>
<td>Contribution of TR</td>
<td>83.23%</td>
<td>83.09%</td>
<td>83.17%</td>
<td>83.26%</td>
<td></td>
<td>85.32%</td>
</tr>
</tbody>
</table>

\(r = +0.98; p = 0.014\)

**NR:** Natural rubbles with associated epilithic and endolithic communities;  
**TR:** Treated rubble keeping only their endolithic communities;  
**GPP:** Gross primary production rates;  
**NPP:** Net primary production rates;  
**R:** Total respiration;  
**Rd:** Dark respiration rates;  
**P/R:** ratio of gross primary production and respiration;  
**DR:** CaCO\(_3\) dissolution rates; Mean ± SD (n = 3)
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Net Respiration $[\Delta \text{CO}_2 \text{ ppm}]$</th>
<th>Bacterial Abundance $[\text{Cells} \times 10^6 \text{ ml}^{-1}]$</th>
<th>Dissolution rate $[\mu\text{mol} \text{ m}^{-2} \text{d}^{-1}]$</th>
<th>Saturation state $[\Omega_a]$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural Rubble (NR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td><strong>62 ± 0.2</strong></td>
<td>4.3 ± 3.5</td>
<td><strong>38.4 ± 2.4</strong></td>
<td>2.5</td>
</tr>
<tr>
<td>520 ppm</td>
<td>76 ± 0.5</td>
<td>4.9 ± 2.0</td>
<td>48.9 ± 1.7</td>
<td>2.1</td>
</tr>
<tr>
<td>720 ppm</td>
<td>102 ± 0.1</td>
<td>5.5 ± 3.5</td>
<td>66.3 ± 0.7</td>
<td>1.7</td>
</tr>
<tr>
<td>1120 ppm</td>
<td><strong>128 ± 1.5</strong></td>
<td>6.1 ± 2.5</td>
<td><strong>81.6 ± 0.2</strong></td>
<td>1.5</td>
</tr>
<tr>
<td><strong>NR with addition of glucose (4µM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td>243 ± 0.5</td>
<td>6.6 ± 5.5</td>
<td>49.5 ± 0.2</td>
<td>2.0</td>
</tr>
<tr>
<td>520 ppm</td>
<td>217 ± 1.1</td>
<td>7.1 ± 7.0</td>
<td>70.0 ± 0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>720 ppm</td>
<td>281 ± 1.3</td>
<td>8.1 ± 3.5</td>
<td>92.6 ± 0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>1120 ppm</td>
<td><strong>323 ± 1.7</strong></td>
<td><strong>9.8 ± 4.5</strong></td>
<td><strong>120.0 ± 0.1</strong></td>
<td>1.2</td>
</tr>
</tbody>
</table>

$r = +0.99; p = 0.008$  
$r = +0.96; p = 0.03$  
$r = +0.99; p = 0.003$  
$r = -0.99; p = 0.01$  

NR: Natural rubbles with associated epilithic and endolithic communities; Net respiration: $[\Delta \text{CO}_2 = \text{CO}_2_{\text{final}} - \text{CO}_2_{\text{initial}}]$, Mean ± SD (n = 3)
Figure: Aragonite saturation state ($\Omega_a$) vs. Carbonate dissolution

$$y = 152.84x^{-1.552}$$

$$R^2 = 0.9923$$
**Table: Summary results of 4 days long incubation under Natural Illumination**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Bacterial Abundance [Cells $\times 10^6$ ml$^{-1}$]</th>
<th>Net Respiration $[\Delta CO_2$ ppm]</th>
<th>Dissolution rate $[\mu$mol m$^{-2}$d$^{-1}$]</th>
<th>Saturation state $[\Omega_a]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at night</td>
<td>at day</td>
<td>at night</td>
<td>at day</td>
</tr>
<tr>
<td>Natural Rubble (NR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td>4.5 ± 4.0</td>
<td>70 ± 0.1</td>
<td>9 ± 0.3</td>
<td>23.7 ± 0.1</td>
</tr>
<tr>
<td>520 ppm</td>
<td>5.3 ± 1.5</td>
<td>119 ± 0.2</td>
<td>12 ± 0.8</td>
<td>29.5 ± 0.2</td>
</tr>
<tr>
<td>720 ppm</td>
<td>6.2 ± 3.5</td>
<td>180 ± 0.5</td>
<td>16 ± 1.1</td>
<td>39.5 ± 1.1</td>
</tr>
<tr>
<td>1120 ppm</td>
<td><strong>7.2 ± 3.0</strong></td>
<td><strong>252 ± 0.9</strong></td>
<td>19 ± 1.0</td>
<td><strong>50.5 ± 1.5</strong></td>
</tr>
<tr>
<td>NR with addition of glucose (4μM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td>7.3 ± 6.0</td>
<td>109 ± 0.8</td>
<td>11 ± 0.6</td>
<td>31.1 ± 1.3</td>
</tr>
<tr>
<td>520 ppm</td>
<td>9.4 ± 4.0</td>
<td>164 ± 0.9</td>
<td>16 ± 1.3</td>
<td>50.5 ± 0.5</td>
</tr>
<tr>
<td>720 ppm</td>
<td>12 ± 6.5</td>
<td>234 ± 2.1</td>
<td>22 ± 1.1</td>
<td>75.8 ± 1.4</td>
</tr>
<tr>
<td>1120 ppm</td>
<td><strong>14 ± 7.0</strong></td>
<td><strong>351 ± 2.7</strong></td>
<td>34 ± 2.1</td>
<td><strong>85.8 ± 1.2</strong></td>
</tr>
</tbody>
</table>

NR: Natural rubbles with associated epilithic and endolithic communities; Values are given for midnight (00:00) and noon (12:00) measurements as night and day respectively; Net respiration: $[\Delta CO_2 = CO_2_{final} - CO_2_{initial}];$ Mean ± SD (n = 3)
Figure: Diurnal variation of carbonate dissolution

- at night (without glucose)
- at night (with glucose)
- at day (without glucose)
- at day (with glucose)

Dissolution rate (µmol m⁻² d⁻¹)

$pCO_2$ levels (ppm)

10 times higher than day time
Experimental conditions

- High CO₂
- Addition of DOM (glucose)

Chemical process

- Saturation state (Ω)
- High CO₂ & Low pH

Biological process

- DOM
- Production of organic matter
- Photosynthesis
- Respiration
- Bacteria
- Consumption or Degradation

Epilithic and Endolithic Community

Increase of Primary Production

Epilithic and Endolithic Community

Domino effect:

- Enhance Bacteria
- Higher CO₂ & Lower pH

Dissolution by Chemical process

Dissolution by Biological process

Bio-Chemical Dissolution Processes (BCDP)

Islam et al., 2012
Summary

- Endolithic communities are one of the major primary producers (83~96%) in coral rubble;

- The net primary production [NPP] was enhanced under high $pCO_2$ conditions and microbial communities also increased;

- Addition of organic matter enhanced growth of heterotrophic bacteria and therefore more $CO_2$ is released from bacterial respiration;

- The higher respiration promoted $CaCO_3$ dissolution;

- $\Omega_a < 1$ promotes dissolution but below the super saturation threshold value [3~4 for aragonite] (Kleypas, et al. 1999) dissolution were observed.

- Finally, dissolution of calcium carbonate is not only governed by chemical parameters but also by biological processes demonstrated in the “Bio-Chemical Dissolution Processes (BCDP)”.
Coral reefs perspective Bangladesh
ST. MARTIN’S ISLAND
[CORAL ISLAND; NARIKEL JINJIRA]

Ship on the way to Saint Martin Island 29 km
Most coral reefs under threat

Threats from human activities include:

1. Sewage, fertilizer and chemical pollution
2. Sedimentation
3. Coastal construction and development
4. Sea grass and mangrove habitat loss
5. Ocean acidification, coral bleaching and rising carbon dioxide
6. Overfishing
7. Destructive fishing practices
8. Careless recreation
Culture vs. Environment?
1. Awareness and changing mind setup;
2. Thinking tank and research skill;
3. Improved institutional setup;
4. Habitat conservation and restoration;
5. Controlling and reducing pollution;
6. Strong legal backup;
7. Env. Research Project, Funding and Technological support;
8. Established a facilitated environmental research field laboratory.
Thank you for your attention