JSR Publications

Available Online

J. Sci. Res. **8** (3), 427-438 (2016)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Eutrophication Accelerates Carbonate Dissolution under High $_pCO_2$ Condition in Coral Reef Ecosystem

M. N. Islam[1,2*], B. E. Casareto[2], Y. Suzuki[2]

[1]JICA Project Office in Bangladesh, The Project for Capacity Building on Human Development Television (HDTV) Programmes in Bangladesh, HDTV Project Office, Television Bhaban, Rampura, Dhaka-1219, Bangladesh

[2]Graduate School of Science and Technology, Biogeochemistry Laboratory, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

Received 29 May 2016, accepted in final revised form 28 June 2016

Abstract

Incubation experiments were carried out to determine the effect of eutrophication on carbonate dissolution under high pCO_2 (partial pressure of carbon dioxide) condition in coral reef ecosystem at Sesoko Island, Okinawa, Japan. Short incubation (24 h under natural illumination) and long incubations (4 days under dark condition) were carried out using white coral skeleton (without attachment of living organism, control); natural rubble (with associated epilithic and endolithic communities) and natural rubble with addition of dissolve organic matter (glucose and coral mucus). Addition of DOM significantly enhanced bacterial abundance (*t*-test; p=0.01) and net respiration (*t*-test; p=0.0001) with increasing pCO_2 levels ($p < 0.05$) under natural illumination. Consistent with increase in respiration, dissolution rates also increased from 136.22±2.04 to 652.38±4.51 $\mu molm^{-2}d^{-1}$. Under dark condition, where photosynthesis was inhibited, dissolution of calcium carbonate further increased with addition of different level of DOM. In addition of DOM incubation bottles, bacterial abundance increased by 3~4 orders of magnitude and the dissolution rates increased by 2.5~10 times more than the control. The results suggest that availability of organic matter in the reefs will enhance metabolic activities (respiration) of microbial communities associated with coral rubble which ultimately increase dissolution of calcium carbonate.

Keywords: Eutrophication; Organic matter; Bacterial activity; Carbonate dissolution.

doi: http://dx.doi.org/10.3329/jsr.v8i3.27937 J. Sci. Res. 8 (3), 427-438 (2016)

1. Introduction

Eutrophication is the enrichment of water bodies and associated sediments by inorganic plant nutrients (e.g. nitrate, phosphate) that occurs naturally or human activity (cultural eutrophication from fertilizer runoff and sewage discharge) and is particularly evident in slow-moving rivers and shallow lakes where high nutrient concentrations stimulate

* *Corresponding author:* drislamsujp@gmail.com

blooms of algae [1]. On the other hand, organic matter in aquatic environment occurs in form of living organisms, organic detritus and dissolved substances. In case of coral reef ecosystems, habitually coral colonies release organic matter to the seawater as dissolved (DOM) and particulate organic matter (POM) [2]. The coral-derived organic matter (DOC) is often collectively referred to as mucus, and that is rapidly utilized by bacteria for their enhanced growth and abundance. The coral mucus has been regarded as ecologically important, bacterial aggregation was found in coral mucus [3] and coral exudates actually enhanced the growth of pico- and nanoplankton [4]. Some part of DOC in coral mucus was rapidly mineralized by bacteria into CO_2 in the reef sediment [5] and conversely, remain labile DOC contributes to long term C fixation as refractory organic matter [2].

Glucose, the most common monosaccharide (MCHO) in the seawater [6], was used as the MCHO supplement because seawater concentrations remain consistently low [7,8], possibly indicating rapid uptake to a threshold level by the indigenous bacterial populations. Nevertheless, the marine environment is extremely oligotrophic [9-11], which implies that microorganisms must face significant periods of shortage of carbon and energy [12,13].

The purpose of the study is to determine the effect of eutrophication accelerates carbonate dissolution by examining the response of coral rubble associated microbial community under high pCO_2 condition in coral reef ecosystem. We wanted to determine the level of DOM and pCO_2 has really enhanced carbonate dissolution by influencing bacterial activity.

2. Materials and Methods

2.1. *Study area and collection of samples*

The study area is located in a shallow fringing coral reef at Sesoko Island, Ryukyu Archipelago, Okinawa, Japan between 26° 38′ N and 127° 51′ E (Fig. 1). Coral rubble samples were collected in the middle of the lagoon at about 1~2 m depth, and coral mucus was collected from *Acropora digitifera* species using air exposure method [5] (Fig. 2) into cleaned syringe and transferred into washed clean beaker. Seawater was collected by using 10 L Nalgene bottle. After collection, seawater was filtered using a cartridge filter (0.2 μm isopore membrane filter) and dispensed into 1 L Nalgene bottles for incubation. All the bottles were washed using neutral Extran (MA02; MERCK) detergent and rinsed with Milli-Q water before use.

2.2. *Experimental design*

Incubation experiment were carried out in natural illumination (24 h short) and dark (4 day's long) under different level of pCO_2 conditions using natural rubble (NR: with associated epilithic and endolithic communities); natural rubble with addition of different

level of glucose (NR+G) and natural rubble with coral mucus (NR+M) as source of organic matter to assess the role of organic maters on carbonate dissolution. As control, white coral skeleton (WCSk) was incubated in the same conditions as mentioned previously (Table 1).

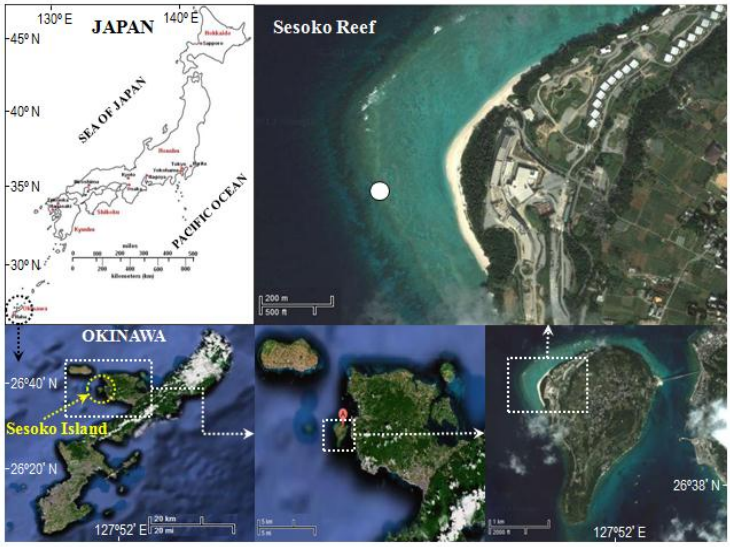

Fig. 1. Map showing the study area and the location of sample collection (○) at Sesoko Island, Okinawa, Japan (Fig. developed from Google map).

Fig. 2. Collection of coral mucus from *Acropora digitifera* species.

Table 1. Experimental design for short and long incubations.

Incubation	Duration	Condition	pCO_2			
			Ambient	520 ppm	720 ppm	1120 ppm
i. Short	24 h	Natural	NR	-	-	NR
		illumination	NR+G	-	-	NR+G
			NR+M	-	-	NR+M
ii. Long	4 day's	Dark	WCSk	WCSk	WCSk	WCSk
			NR	NR	NR	NR
			NR+G1	NR+G1	NR+G1	NR+G1
			NR+G2	NR+G2	NR+G2	NR+G2
			NR+G4	NR+G4	NR+G4	NR+G4

WCSk: White coral skeleton; TR: Treated rubble keeping only their endolithic communities; NR: Natural rubbles with associated epilithic and endolithic communities; NR+G: Natural rubbles with addition of organic matter (10 µM glucose); NR+M: Natural rubbles with addition of organic matter (10% coral mucus); G1, G2 and G4: Level of organic matter addition (1 µM, 2 µM and 4 µM of glucose); Natural illumination: Out door

2.3. *Preparation of incubation experiment*

Two small branches of natural coral rubbles of similar sizes were placed into 1 L Nalgene bottles which were filled with filtered seawater. Three (03) replicates were used for each incubation experiment. The different levels of pCO_2 in the incubation bottles were adjusted by injecting CO_2 saturated seawater into the bottles until pH values equivalent to the desired pCO_2 levels were obtained. CO_2 saturated seawater was prepared by bubbling pure CO_2 gas into natural seawater until pH was stable. Glucose stock solution was prepared as 1.8 g L^{-1}, and 100 µL, 200 µL, 400 µL and 1000 µL of this solution were added to the incubation bottles (1 L) to make final concentration of 1 µM, 2 µM, 4 µM and 10 µM. In case of coral mucus, 10% mucus solution was added to the incubation bottles. Incubations were conducted in natural temperature varied at 25°C to 33°C. Temperature and light intensity were monitored during the experiments using *in situ* sensor (MDS-MkV/T and MDS-MkV/L, Alec electronics).

2.4. *Laboratory measurement and analysis*

Measurement of short incubation experiment was done 24 times per day (1 h interval) and 4 times per day (6 h interval) for long incubation experiments. pH and DO were measured using a pH meter (with 3 sensors; temperature, pH and DO) ORION 4 STAR calibrated with NIST (NBS) scaled buffer solutions (Mettler pH 9.228 and 6.880 buffers at 20°C). Alkalinity (A_T) was measured by the total alkalinity titrator (KIMOTO ATT-05). Reproducibility of the A_T measurement was ± 2 µmol kg^{-1} (1σ; n = 10).

Heterotrophic bacteria were collected on 0.2 µm black polycarbonate filters by filtering 10 to 15 mL aliquots that were previously stained with DAPI (4',6-diamidino-2-phenylindole) at 1 µg mL^{-1} concentration. Abundance was assessed by counting bacteria cells under an epifluorescence microscope (Nikon; ECLIPSE/E600), using a UV-filter. Net respiration of the rubble associated communities was calculated from changes in dissolve oxygen concentration and converted to carbon (C) using "Redfield-Richards

Ratio" empirical formula [14]. Statistical data were analyzed and the variances with 0.05 level of significance by using Microsoft Office Excel 2007.

Different levels of pCO_2 (ambient, 520, 720 and 1120 ppm) was calculated using pH and A_T according to the carbonate equilibrium in seawater described by Millero and Fujimura et al. [15,16]. Carbonate dissolution rates (µmol $L^{-1}d^{-1}$) were analyzed using the alkalinity anomaly technique [16-18] following the equation: Dissolution rate = $\Delta A_T/2$, where ΔA_T is the variation of A_T ($A_{T\ final} - A_{T\ initial}$) during the incubation period at the start and end of each incubation. The alkalinity anomaly method actually yields an estimate of the net value of the $CaCO_3$ precipitation/dissolution balance [19,20].

The carbonate system parameters in seawater and saturation state of aragonite (Ω_a) were calculated with the program CO2SYS [21] using the apparent equilibrium constants K'_0 from Weiss [22], K'_1 and K'_2 from Mehrbach *et al.* [23] as described by Dickson and Millero [24] and the HSO_4^- constant according to Dickson [25]. Saturation degree of aragonite (Ω_a) was calculated from pH, alkalinity, salinity and temperature data sets. The degree of saturation is defined as: $\Omega_a = [Ca^{2+}]\ [CO_3^{2-}] / K'_{sp}$, where K'_{sp} is the stoichiometric solubility product of aragonite derived from a function of salinity and temperature [26].

3. Results

3.1. *Addition of bioavailable organic matter*

Bioavailable organic matter (glucose and coral mucus), was added to the incubation bottles to revitalize bacterial activity and their physiological activities accelerates carbonate dissolution. Addition of bioavailable organic matter enhanced bacterial abundance and dissolution rates were observed under natural rubble (NR), natural rubble with addition of glucose (NR+G) and with addition of coral mucus (NR+M) in ambient and high pCO_2 condition (Fig. 3 and Table 2). During 24 h natural illumination experiment, the bacterial abundance (*t*-test; p=0.01) and net respiration (*t*-test; p=0.0001) increased significantly with addition of bioavailable organic matter and also with increasing pCO_2 levels ($p < 0.05$). Consistent with increase in respiration, dissolution rates also increased. In the organic matter addition sample, bacterial abundance and dissolution rates both increased 5~6 times more than the natural rubble (NR) (Table 2).

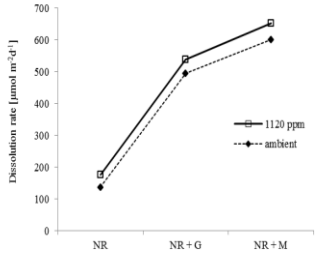

Fig. 3. Organic matter increased carbonate dissolution.

Table 2. Summary result of 24 h natural illumination short incubation experiment.

	Labile DOC (Initial – Final) [µmol C L^{-1}]	Bacterial abundance [Cells×10^5 mL^{-1}]	Net Respiration [×10^5 µgC$m^{-2}d^{-1}$]	Dissolution rate [µmol $m^{-2}d^{-1}$]	Saturation state [Ω_{arg}]
Ambient condition					
NR	29.10 ± 0.12	4.5 ± 0.01	3.2 ± 0.02	136.22 ± 2.04	2.87
NR + G	61.91 ± 0.32	16.4 ± 0.01	5.3 ± 0.04	495.34 ± 4.51	1.54
NR + M	74.01 ± 0.66	22.3 ± 0.02	6.1 ± 0.03	600.12 ± 3.01	1.22
High pCO_2 (1120 ppm) condition					
NR	31.83 ± 0.23	5.2 ± 0.02	3.5 ± 0.01	176.36 ± 1.52	2.42
NR + G	65.65 ± 0.46	18.2 ± 0.00	5.6 ± 0.03	537.18 ± 2.02	1.32
NR + M	80.38 ± 0.15	25.3 ± 0.01	6.8 ± 0.02	652.38 ± 4.51	1.13

DOC: Dissolved Organic Carbon; NR: Natural rubbles with associated epilithic and endolithic communities; NR + G: Natural rubbles with addition of organic matter (10 µM glucose); NR + M: Natural rubbles with addition of organic matter (10% coral mucus); Natural illumination: Out door; Mean ± SD (n = 3).

3.2. *Effect of glucose addition*

After addition of glucose; bacterial abundance, net respiration rates as well as dissolution rates were increased. The bacterial abundance, net respiration and dissolution rates were 16.4±0.01×10^5 cells mL^{-1}, 5.3±0.04 µgC$m^{-2}d^{-1}$ and 495.34±4.51 µmol $m^{-2}d^{-1}$ in the ambient condition, whereas in high pCO_2 (1120 ppm) condition were 18.2±0.00×10^5 cells mL^{-1}, 5.6±0.03 µgC$m^{-2}d^{-1}$ and 537.18±2.02 µmol $m^{-2}d^{-1}$ respectively (Table 2). Glucose addition had a significant effect on bacterial abundance (*t*-test; p=0.01); and strong positive correlation between bacterial abundance vs. respiration (r=+0.99; p=0.0001) as well as carbonate dissolution (r=+0.99; p=0.0001) was found.

3.3. *Effect of coral mucus addition*

On the other hand, with addition of coral mucus (10%) as source of natural organic matter, bacterial abundance, net respiration rates as well as dissolution rates also increased higher than the glucose addition (Table 2). The bacterial abundance, net respiration and dissolution rates were 22.3±0.02×10^5 cells mL^{-1}, 6.1±0.03 µgC$m^{-2}d^{-1}$ and 600.12±3.01 µmol $m^{-2}d^{-1}$ in the ambient condition, whereas in high pCO_2 (1120 ppm) condition were 25.3±0.01×10^5 cells mL^{-1}, 6.8±0.02 µgC$m^{-2}d^{-1}$ and 652.38±4.51 µmol $m^{-2}d^{-1}$ respectively (Table 2). Addition of coral mucus also had a significant effect on bacterial abundance (*t*-test; p=0.01); and strong positive correlation between bacterial abundance vs. respiration (r=+0.99; p=0.0001) as well as carbonate dissolution (r=+0.99; p=0.0001) was found.

3.4. *Level of organic matter addition*

During 4 day's long incubation experiment under dark condition, where photosynthesis was inhibited, dissolution of calcium carbonate increased with increasing pCO_2 levels and

different levels (1 µM, 2 µM and 4 µM) of organic matter addition (glucose). After addition of different level of organic matter, the bacterial abundance and net respiration as well as dissolution rates were increased significantly ($p < 0.05$). During incubation experiment of without addition of organic matter, the bacterial abundance were $1.82\pm0.001\times10^6$ cells mL^{-1} of sample water and dissolution rate were 70.67 ± 1.22 µmol $m^{-2}d^{-1}$ in the ambient condition, whereas in high pCO_2 (1120 ppm) condition were $2.39\pm0.001\times10^6$ cells mL^{-1} and 84.32 ± 0.97 µmol $m^{-2}d^{-1}$ respectively. But in case of different levels of organic matter addition, the bacterial abundance was increased $8.24\pm0.002\times10^6$ ~ $14.62\pm0.002\times10^6$ cells mL^{-1} and dissolution rates were increased 152.22 ± 1.11 ~ 318.93 ± 1.13 µmol $m^{-2}d^{-1}$ with increasing both of glucose levels and pCO_2 levels ($p < 0.05$) (Table 3). There was strong positive correlation between different level of organic matter addition and calcium carbonate dissolution ($r=+0.99$; $p=0.0001$).

3.5. *Carbonate dissolution vs. Saturation state*

During 24 h natural illumination experiment, with increasing carbonate dissolution rates (from 136.22 ± 2.04 to 652.38 ± 4.51 µmol $m^{-2}d^{-1}$), aragonite saturation state (Ω_{arg}) were decreased (from 2.87 to 1.13) in ambient and high pCO_2 condition respectively. Coral mucus addition had more significant effect than glucose addition for carbonate dissolution as well as CO_3^{2-} saturation state. In case of 4 day's long incubation experiment (under dark condition), carbonate dissolution rates were increased from 70.67 ± 1.22 to 318.93 ± 1.13 µmol $m^{-2}d^{-1}$ and aragonite saturation state (Ω_{arg}) were decreased from 1.82 to 0.96 under different level of organic matter (glucose) addition and different pCO_2 levels. There were strong negative correlation between carbonate dissolution rate and saturation state ($r=-0.99$; $p=0.0002$) (Fig. 4).

Table 3. Summary results of 4 day's long incubation under dark condition with different level of organic matter addition.

	Bacterial abundance [Cells $\times10^3$ mL^{-1}]	Net Respiration [ΔCO_2 ppm]	Dissolution rate [µmol $m^{-2}d^{-1}$]	Saturation state [Ω_{arg}]
WCSk				
Ambient	1.08 ± 0.014	03 ± 0.01	00.02 ± 0.01	1.53
520 ppm	1.17 ± 0.011	07 ± 0.02	12.08 ± 0.48	1.28
720 ppm	1.25 ± 0.015	12 ± 1.01	16.36 ± 0.95	1.06
1120 ppm	1.37 ± 0.010	16 ± 1.03	30.38 ± 0.84	0.88
NR	[Cells $\times10^6$ mL^{-1}]			
Ambient	1.82 ± 0.001	56 ± 1.53	70.67 ± 1.22	1.82
520 ppm	2.09 ± 0.002	80 ± 2.00	73.32 ± 0.82	1.53
720 ppm	2.19 ± 0.001	84 ± 1.00	78.26 ± 0.66	1.34
1120 ppm	2.39 ± 0.001	95 ± 1.00	84.32 ± 0.97	1.12
NR + G 1 µM				
Ambient	8.24 ± 0.002	272 ± 2.00	152.22 ± 1.11	1.42
520 ppm	8.40 ± 0.001	284 ± 1.53	155.34 ± 1.12	1.28
720 ppm	8.60 ± 0.001	296 ± 2.52	166.53 ± 0.96	1.15

	Bacterial abundance [Cells $\times 10^3$ mL^{-1}]	Net Respiration [ΔCO_2 ppm]	Dissolution rate [μmol $m^{-2}d^{-1}$]	Saturation state [Ω_{arg}]
1120 ppm	8.94 ± 0.002	301 ± 1.00	168.11 ± 0.86	0.99
NR + G 2 μM				
Ambient	9.00 ± 0.001	315 ± 1.00	173.04 ± 1.03	1.37
520 ppm	9.21 ± 0.002	326 ± 2.06	175.42 ± 0.53	1.24
720 ppm	9.33 ± 0.001	342 ± 2.58	188.82 ± 0.64	1.11
1120 ppm	9.62 ± 0.002	357 ± 1.53	201.96 ± 0.98	0.98
NR + G 4 μM				
Ambient	11.95 ± 0.001	458 ± 3.33	282.91 ± 0.86	1.28
520 ppm	12.54 ± 0.001	472 ± 2.10	287.71 ± 0.78	1.17
720 ppm	13.57 ± 0.001	484 ± 2.50	300.48 ± 0.99	1.07
1120 ppm	14.62 ± 0.002	496 ± 2.51	318.93 ± 1.13	0.96

WCSk: White coral skeleton (no attachment of living organism; NR: Natural rubbles with associated epilithic and endolithic communities; NR + G: Natural rubbles with addition of different level of organic matter (1 μM, 2 μM & 4 μM of Glucose); Net respiration: [$\Delta CO_2 = CO_2$ final – CO_2 initial]; Mean ± SD (n = 3).

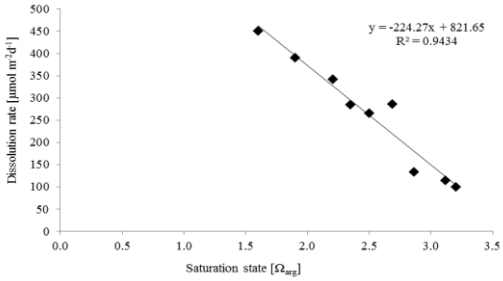

Fig. 4. Relationship between aragonite saturation state (Ω_{arg}) and carbonate dissolution rates.

4. Discussion

4.1. *Addition of bioavailable organic matter*

Natural seawater normally contains 10^5 to 10^6 bacterial cells mL^{-1} [27]. The bacterial composition of natural seawater is regulated by such factors as grazing pressure [28, 29], nutrient limitation or starvation [12, 30], and various other physicochemical stresses (e.g., temperature variation, oxidative stress, etc.). Addition of bioavailable organic matter (glucose and coral mucus) to the samples was influencing bacterial activity [31, 32], growth and their abundance (3~4 orders of magnitude); and caused subsequent increase of calcium carbonate dissolution (2.5~10 times more than in control) in the dissolution experiments (Table 2). In coral reef ecosystem, endolithic communities of coral rubble play a crucial role as primary producers and control calcification and dissolution of calcium carbonate by "Bio-Chemical Dissolution Processes (BCDP)" [31]. The results suggest that availability of organic matter (eutrophication) accelerates carbonate

dissolution with enhancing metabolic activities (respiration) of microbial communities associated with coral rubble which ultimately increase dissolution of calcium carbonate.

4.2. *Level of organic matter addition*

Natural rubble (NR) is colonized by epilithic and endolithic communities with other organisms of Heterotrophs as foraminifera, nematodes and copepods. When photosynthesis is inhibited under dark condition, calcium carbonate dissolution was increased with increasing pCO_2 and addition of glucose levels. On the other hand, without any attachment of living organism, very small amount (00.02~30.38±0.84 µmol $m^{-2}d^{-1}$) of dissolution were observed from white coral skeleton (control) by the pressure of CO_2 only. This suggests that dissolution increased with enhance bacterial abundance and their physiological activities, and availability of organic matter also accelerates carbonate dissolution. Different level of organic matter (glucose) addition regulates bacterial activity as well as dissolution rates. The effect of adding glucose (3 mM) was examined by Eguchi et al. [33]; bacterial cells grow instantaneously and increased cell density after glucose addition, probably because of rapid initial intracellular poly glucose accumulation [34]. Church et al. [35] found that biomass production of heterotrophic bacteria in the Southern Ocean was stimulated by addition of organic carbon. Organic carbon additions also consistently stimulated bacterial growth rates in the subarctic Pacific [12] and the equatorial Pacific [36]. Moulin *et al.* [37] mentioned the bacterial respiration of organic matter can induce rapid dissolution of a significant amount of carbonate in the sediments. However, during 4 day's long incubation experiment under dark condition, we found 14 times higher bacterial abundance than the normal seawater condition with addition of different level of organic matter (1 µM, 2 µM and 4 µM glucose), and also found highly significant effect (r=+0.999; p=0.0005) on different level of organic matter addition. Consequently, we suggest that level of organic matter addition increased bacterial abundance and their physiological activity (especially respiration) regulates carbonate dissolution in seawater.

4.3. *Carbonate dissolution vs. Saturation state*

Calcium carbonate dissolution occurs due to reduction in CO_3^{2-} saturation state at elevated pCO_2 [38-39] and the value of $\Omega_{arg} < 1$ promotes dissolution [40-44]. However, in coral reefs shows large variation in CO_2 concentration and Ω occur due to their high diversity and productivity. Therefore, below the super saturation threshold value 3~4 for aragonite [40-41] dissolution was observed. Previous studies reported that calcium carbonate dissolution occurred when Ω_{arg} ranged from 4.38 to 2.84 [45]; 3.06 to 1.83 [46] and 3.7 to 1.3 [47]. In our experiment dissolution of calcium carbonate occurred when the saturation state (Ω_{arg}) ranged from 2.87 to 1.13 in natural illumination experiment and from 1.82 to 0.96 in dark incubation experiment under different level of organic matter addition with

increasing pCO_2 levels. We found strong negative correlation between carbonate dissolution rate and saturation state (r=–0.99; p=0.0002) (Fig. 4).

5. Conclusions

Eutrophication has a potentially important role for calcium carbonate dissolution. In coral reef ecosystems, coral reef itself release mucus as organic matter (DOM, POM) that enhancing growth of bacteria and their abundance. Thus, the levels of organic matter in the coral reefs also accelerate carbonate dissolution by enhancing bacterial activities (especially respiration) under different levels of pCO_2.

Acknowledgments

We would like to thank to Environmental Leader Program of Shizuoka University supported by a Grant-in-Aid for Scientific Research on Innovative Areas for "Coral reef science for symbiosis and coexistence of human and ecosystem under combined stresses" (20121003) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan and by the Global Coral Reef Conservation Project of Mitsubishi Corporation, Japan. We are also thankful to Tropical Biosphere Research Center of the University of Ryukyus at Sesoko Island, Okinawa, Japan for providing laboratory facilities.

References

1. E. Lawrence, A. R. W. Jackson, and J. M. Jackson, Eutrophication, *in* Longman Dictionary of Environmental Science (Addison Wesley Longman Limited, London, 1998) pp. 144.
2. Y. Tanaka, T. Miyajima, I. Koike, T. Hayashibara, and H. Ogawa, Bull. Mar. Sci. **82**, 237 (2008).
3. H. W. Ducklow and R. Mitchell, Limnol. Oceanogr. **24**, 715 (1979). http://dx.doi.org/10.4319/lo.1979.24.4.0715
4. C. Ferrier-Pagès, N. Leclercq, J. Jaubert, and S. P. Pelegrí, Aquatic Microbial Ecol. **21**, 203 (2000). http://dx.doi.org/10.3354/ame021203
5. C. Wild, M. Huettel, A. Klueter, S. G. Kremb, M. Y. M. Rasheed, and B. B. Jørgensen, Nature **428**, 66 (2004). http://dx.doi.org/10.1038/nature02344
6. K. Mopper, R. Dawson, G. Liebezeit, and V. Ittekot, Mar. Chem. **10**, 55 (1980). http://dx.doi.org/10.1016/0304-4203(80)90058-4
7. C. M. Burney, K. M. Johnson, and J. M. Sieburth, Mar. Biol. **63**, 175 (1981). http://dx.doi.org/10.1007/BF00406826
8. K. M. Johnson and J. M. Sieburth, Mar. Chem. **5**, 1 (1977). http://dx.doi.org/10.1016/0304-4203(77)90011-1
9. R. Y. Morita, Can. J. Microbiol. **34**, 436 (1988). http://dx.doi.org/10.1139/m88-076
10. R. Y. Morita, Experientia **46**, 813 (1990). http://dx.doi.org/10.1007/BF01935530
11. U. Munster, Antonie van Leeuwenhoek **63**, 243 (1993). http://dx.doi.org/10.1007/BF00871222
12. D. L. Kirchman, Mar. Ecol-Prog. Ser. **62**, 47 (1990). http://dx.doi.org/10.3354/meps062047
13. S. Kjelleberg, M. Hermansson, P. Marden, and G. W. Jones, Annual Rev. microbial. **41**, 25 (1987). http://dx.doi.org/10.1146/annurev.mi.41.100187.000325

14. S. M. Libes, Organic Matter: Production and Destruction: An Introduction to Marine Biogeochemistry (John Wiley & Sons, Inc., USA, 1992) pp. 129-141.
15. F. J. Millero, Geochimica et Cosmochimica Acta **43**, 1651 (1979). http://dx.doi.org/10.1016/0016-7037(79)90184-4
16. H. Fujimura, T. Oomori, and K. M. Maehira, Galaxea **3**, 41 (2001). http://dx.doi.org/10.3755/jcrs.2001.41
17. S. V. Smith, Limnology and Oceanography **20**, 243 (1973). http://dx.doi.org/10.4319/lo.1973.18.1.0106
18. J-P. Gattuso, C. E. Payri, M. Pichon, B. Delesalle, and M. Frankignoulle, J. Phycol. **33**, 729 (1997). http://dx.doi.org/10.1111/j.0022-3646.1997.00729.x
19. S. V. Smith, In: Coral Reefs: Research Methods, Monographs on Oceanographic Methodology, ed. D. R. Stoddart et al. (UNESCO, Paris, 1978).
20. N. Bensoussan and J. P. Gattuso, Mar. Ecol-Prog. Ser. **334**, 37 (2007). http://dx.doi.org/10.3354/meps334037
21. E. Lewis and D. W. R. Wallace, CO2SYS–Program Developed for the CO_2 System Calculations (Carbon Dioxide Information Analysis Center, Report ORNL/CDIAC-105, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1998).
22. R. Weiss, Mar. Chem. **2**, 203 (1974). http://dx.doi.org/10.1016/0304-4203(74)90015-2
23. C. Mehrbach, C. H. Culberson, J. E. Hawley, and R. M. Pytkowicz, Limnol. Oceanogr. **18**, 897 (1973). http://dx.doi.org/10.4319/lo.1973.18.6.0897
24. A. G. Dickson and F. J. Millero, Deep Sea Res. **34**, 1733 (1987). http://dx.doi.org/10.1016/0198-0149(87)90021-5
25. A. G. Dickson, Deep Sea Res. **40**, 107 (1993). http://dx.doi.org/10.1016/0967-0637(93)90055-8
26. A. Mucci, Am. J. Sci. **283**, 780 (1983). http://dx.doi.org/10.2475/ajs.283.7.780
27. F. B. Van Es and L. A. Meyer-Reil, Adv. Microb. Ecol. **6**, 111 (1982). http://dx.doi.org/10.1007/978-1-4615-8318-9_4
28. K, Simek and T. H. Chrzanowski, Appl. Environ. Microbiol. **58**, 3715 (1992).
29. R. T. Wright, Hydrobiologia **159**, 111 (1988). http://dx.doi.org/10.1007/BF00007372
30. S. Kjelleberg, K. Flärdh, T. Nyström, and J. W. Moriarty, Growth Limitation and Starvation of Bacteria, In: Aquatic Microbiology: An Ecological Approach, ed. T. E. Ford (Blackwell Scientific Publications, Boston, USA, 1993), pp. 289–320.
31. M. N. Islam, B. E. Casareto, T. Higuchi, M. P. Niraula, and Y. Suzuki, Galaxea J. Coral Reef Studies **14**, 119 (2012). http://dx.doi.org/10.3755/galaxea.14.119
32. Y. Natori, A. Haneda, and Y. Suzuki, Mar. Chem. **102**, 230 (2006). http://dx.doi.org/10.1016/j.marchem.2006.04.007
33. M. Eguchi, T. Nishikawa, K. MacDonald, R. Cavicchioli, J. C. Gottschal, and S. Kjelleberg, Appl. Environ. Microbiol. **62**, 1287 (1996).
34. J. M. Preyer and J. D. Oliver, Appl. Environ. Microbiol. **59**, 2653 (1993).
35. M. J. Church, D. A. Hutchins, and H. W. Ducklow, Appl. Environ. Microbiol. **66**, 455 (2000). http://dx.doi.org/10.1128/AEM.66.2.455-466.2000
36. D. L. Kirchman and J. H. Rich, Microb. Ecol. **33**, 11 (1997). http://dx.doi.org/10.1007/s002489900003
37. E. Moulin, A. Jordens, and R. Wollast, "Influence of the Aerobic Bacterial Respiration on the Early Dissolution of Carbonates in Coastal Sediments - *Proc. Prog Belgian Oceanographic Research* (Brussels, 1985) pp. 196-208.
38. S. Comeau, R. C. Carpenter, C. A. Lantz, and P. J. Edmunds, Biogeosci. **12**, 365 (2015). http://dx.doi.org/10.5194/bg-12-365-2015
39. A. D. Stubler and B. J. Peterson, Coral Reefs **1** (2016). http://dx.doi.org/10.1007/s00338-016-1436-x
40. J-P. Gattuso, D. Allemand, and M. Frankignoulle, Am. Zool. **39**, 160 (1999). http://dx.doi.org/10.1093/icb/39.1.160
41. J. A. Kleypas, R. W. Buddemeier, D. Archer, J-P. Gattuso, C. Langdon, and B. N. Opdyke,

Science **84**, 118 (1999). http://dx.doi.org/10.1126/scie nce.284.5411.118
42. D. J. Barnes and C. Cuff, Solution of Reef Rock Buffers Seawater against Rising Atmospheric CO_2 – *Proc. of 9th International Coral Reef Symposium* (Abstracts: Indonesia, State Ministry for the Environment, 2000) pp. 248.
43. R. B. Halley and K. K. Yates, Will Reef Sediments Buffer Corals from Increased Global CO_2 – *Proc. of 9th International Coral Reef Symposium* (Abstracts: Indonesia, State Ministry for the Environment, 2000).
44. A. J. Andersson, F.T. Mackenzie, and L. M. Ver, Geology **31**, 513 (2003). http://dx.doi.org/10.1130/0091-7613(2003)031<0513:SOSCAI>2.0.CO;2
45. K. K. Yates and R. B. Halley, Biogeosci. **3**, 357 (2006). http://dx.doi.org/10.5194/bg-3-357-2006
46 S. Ohde and R. van Woesik, Bull. Mar. Sci. **65**, 559 (1999).
47. S. Yamamoto, H. Kayanne, M. Terai, A. Watanabe, K. Kato, A. Negishi, and K. Nozaki, Biogeosci. **9**, 1441 (2012). http://dx.doi.org/10.5194/bg-9-1441-2012