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CO_2 leaking from sub-seabed storage: Responses of two marine bacteria strains

A.R. Borrero-Santiago [a, *], M. Carbú [b], T.Á. DelValls [a], I. Riba [a]

[a] Departamento de Química-Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, UNESCO/UNITWIN WiCoP, Campus de Excelencia Internacional del Mar (CEIMAR), Polígono Río San Pedro s/n, Puerto Real, 11510 Cádiz, Spain
[b] Departamento de Biomedicina, Biotecnología y Salud Pública, Laboratorio de Microbiología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro s/n, Puerto Real, 11510 Cádiz, Spain

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ABSTRACT

Carbon capture and storage (CCS) in stable geological locations is one of the options to mitigate the negative effects of global warming produced by the increase in CO_2 concentrations in the atmosphere. A CO_2 leak is one of the risks associated with this strategy. Marine bacteria attached to the sediment may be affected by an acidification event. Responses of two marine strains (*Roseobacter* sp. CECT 7117 and *Pseudomonas litoralis* CECT 7670) were assessed under different scenarios using a range of pH values (7.8, 7, 6.5, 6, and 5.5) to mimic a CO_2 leak. A CO_2 injection system was used to simulate an escape from a stable sub-seabed. Growth rate (μ), cell number, inhibition of Relative Inhibitory Effect (RI CO_2) and inhibited population were analysed as endpoints. *P. litoralis* showed more sensitivity to high CO_2 concentrations than *Roseobacter* sp. Our results highlight the diversity and resistance in marine bacteria and their capacity to adapt under a stressful CO_2 leakage.

1. Introduction

Anthropogenic activities are generating an increasing concentration of CO_2 in the atmosphere, contributing to global climate change and the warming of Earth (Sabine et al., 2004). During the period between 2000 and 2006, atmospheric CO_2 increased to 1.93 ppm, which was significantly higher than the concentrations measured during the 1980s and 1990s (1.49 and 1.58 ppm, respectively) (Canadell et al., 2007). An increase in atmospheric CO_2 causes acidification of water (Caldeira, 2005), which results in negative effects on marine ecosystems (Ishimatsu et al., 2005; Nakayama et al., 2005). In this context, it is essential to reduce the greenhouse gas emissions and mitigate the effects. Currently, there are several options to reduce the CO_2 concentration in the atmosphere. Capture, transport and geological sequestration (CCS) is one of these options aimed to reducing CO_2 concentrations in the air.

Although this strategy is viable, a possible leakage of CO_2 from geological stores may be possible (Damen et al., 2006). It is essential to assess the consequences and evaluate the potential responses of marine ecosystems within the surrounding area (Reguera et al., 2009).

The effects of an acidification event from CO_2 fluxes from the atmosphere into the ocean (and vice versa) on the water column have been documented (Portner et al., 2004; Beardall et al., 2009). As a result, scientists have noted that variations in CO_2 concentrations in seawater generate changes in the carbon chemistry (e.g., total alkalinity $_{AT}$, Dissolved Inorganic Carbon $_{DIC}$) (Gruber et al., 2004).

A possible CO_2 leak may cause acidification of the seawater located around the sediment. The acidification of the sediments and the consequences on the benthic community are currently being studied by the scientific community (Almagro-Pastor et al., 2015; Basallote et al., 2014; 2012; De Orte et al., 2014a, b; Hale et al., 2011; Tamburri et al., 2000). Unfortunately, the effects on marine bacteria are still poorly understood (Labare et al., 2010). It is critical to fill this data gap because marine bacteria are the base of many food webs and play an important role in carbon chemistry (Pomeroy et al., 2007). All of these changes in the carbon chemistry may affect the marine bacterial community. This paper addresses the potential effects of an acidification event caused by CO_2 leaking from sub-seabed storage and its possible consequences on bacterial communities. As the bacterial population from the marine

* Corresponding author.
E-mail address: ana.borrero@uca.es (A.R. Borrero-Santiago).

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sediment is affected by acidification (De Beer et al., 2013; Raulf et al., 2015), two typical bacterial species from marine environments (*Roseobacter* sp. and *Pseudomonas litoralis)* were studied. *Pseudomonas* and *Roseobacter* genus are representative genera from different environmental locations, including sedimentary environments (Nair et al., 2008; Köchling et al., 2011; Pascual et al., 2012). In this study, toxicity tests were used to evaluate the effects caused by a CO_2 leakage. Growth rates (μ), the relative inhibitory effect (RI) of CO_2, total number of cells and the evolution of growth were estimated.

2. Materials and methods

2.1. Chamber and CO_2 system

A CO_2 injection system was integrated inside a non-pressurized chamber (300 × 150 × 240 cm) to evaluate the response of marine bacteria to acidification simulating a CO_2 leak (Fig. 1). This chamber was designed and previously described by Borrero-Santiago et al. (2016) to work under sterile conditions and a controlled temperature to avoid any variation or contamination of the results. The CO_2 injection system was adapted and characterized by membrane filters of 0.2 μm pore size to guarantee the sterile condition of the CO_2 prior to injection. The CO_2 system enables expose marine bacteria to treatments in a range of pH, which were controlled by electronic control system. If the pH sensors detected that the pH of the water deviated ±0.01 units from the desired value, the pH was adjusted by injections of CO_2. This injection was carried out by solenoid valves, which opened and allowed a flow of CO_2 when the actual pH was higher than the desired pH, and closed when the target pH was reached.

2.2. Bacterial cultures and experimental setup

Two Gram-negative strains were selected from the Spanish Collection Cultive Type (www.CECT.org) to carry out the toxicity tests: *Pseudomonas litoralis* (CECT 7670) and *Roseobacter* sp. (CECT 7117).

According to Borrero-Santiago et al. (2016), a preliminary step was used to determine adequate growth for use in a toxicity test with CO_2 under laboratory conditions. Three criteria were followed: 1) the duration of the exponential growth phase must be at least 4 days; 2) the growth medium must have a larger proportion of seawater than marine broth (nutrients) to simulate an oligotrophic environment similar to the ocean; and 3) sustains a high number of cells by the end of the test. Thus, seven dilutions of Difco Marine Broth 2216 (MB) in seawater (SW) were assessed (MB:SW dilution; V:V): MB not diluted, 1:2, 1:5, 1:10, 1:50, 1:100 and 1:1000. Pure culture with only Marine Broth was used as a control to confirm that the inoculated strains were viable. Dilution 1:2 (MB: SW) was discarded because this medium did not meet the criteria by containing half nutrients and half seawater. The 1:50 and 1:100 dilutions were removed from the study because the exponential growth phase of the chosen bacteria lasted two or fewer days. Dilution 1:1000 did not permit any growth for *P. litoralis* and also was removed for consideration in the study. Thus, our preliminary analysis found that Marine Broth in seawater at dilutions of 1:5 and 1:10 could be selected for the CO_2 toxicity test as an adequate growth medium. However, statistical analysis showed significant differences ($p \leq 0.05$) in the total number of cells using different diluted mediums for five pH treatments (7.8, 7, 6.5, 6, 5.5) in both strains. Therefore, dilution 1:10 was chosen as the best growth medium, as also suggested by Borrero-Santiago et al. (2016). All the criteria were met, and dilution 1:10 is a realistic growth medium that is similar to the true environment of marine bacteria.

2.3. pH selection

According to Taylor et al. (2014), a CO_2 dispersion along the sediment may result in a pH gradient. Thus, five pH treatments were selected to simulate different CO_2 leak scenarios. In this context, the lowest pH value corresponds to the area closest to source of the CO_2 release and the highest pH was used as a control (without CO_2), which meant it was located the farthest distance

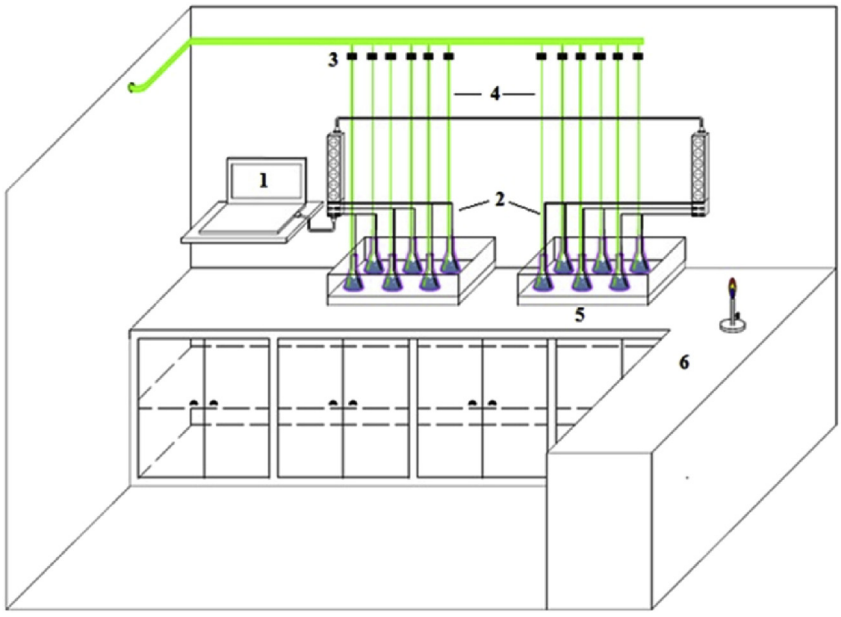

Fig. 1. Design of CO_2 injection system integrated in a chamber used in this work to carry out the experiments. The CO_2 injection system was comprised of: 1) a PC, which controls the CO_2 injection by software, 2) pH sensors, 3) solenoid valves, which permit the flow of the CO_2, 4) CO_2 injectors, 5) containers or aquariums for the bioassays, and 6) working desk.