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Carbon Capture and Storage (CCS): Risk assessment focused on marine bacteria

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ABSTRACT

Carbon capture and storage (CCS) is one of the options to mitigate the negative effects of the climate change. However, this strategy may have associated some risks such as CO_2 leakages due to an escape from the reservoir. In this context, marine bacteria have been underestimated. In order to figure out the gaps and the lack of knowledge, this work summarizes different studies related to the potential effects on the marine bacteria associated with an acidification caused by a CO_2 leak from CSS. An improved integrated model for risk assessment is suggested as a tool based on the rapid responses of bacterial community. Moreover, this contribution proposes a strategy for laboratory protocols using *Pseudomona stanieri* (CECT7202) as a case of study and analyzes the response of the strain under different CO_2 conditions. Results showed significant differences ($p \leq 0.05$) under six diluted enriched medium and differences about the days in the exponential growth phase. Dilution 1:10 (Marine Broth 2216 with seawater) was selected as an appropriate growth medium for CO_2 toxicity test in batch cultures. This work provide an essential and a complete tool to understand and develop a management strategy to improve future works related to possible effects produced by potential CO_2 leaks.

1. Introduction

Since the 1950s, great efforts to understand and assess the possible consequences associated with the climate change have been developing (Doney et al., 2009). According to the last Intergovernmental Panel on Climate Change Report (2013), human activities using fossil fuel, electric power plants, automobiles, and also, land uses are causing an increase of CO_2 into the atmosphere. There is a natural CO_2 equilibrium in the ocean which regulates CO_2 gas between the ocean and the atmosphere. When there is an excess of atmospheric CO_2 concentrations into the atmosphere due to the anthropogenic activities, the gas is absorbed by the ocean as a sink (Sabine et al., 2004), reacting with the seawater and consequently the pH decrease (Millero, 1995) and the natural equilibrium mentioned is altered. This process is called "ocean acidification" (Zeebe et al., 2008). Scientific and politics communities are working in some mitigating strategies to face the high concentrations of CO_2 on the earth. The main goal of mitigation is to prevent negative effects on planet due to an increase of CO_2 in to the atmosphere.

According to IPCC (2013), one of the options to mitigate the possible effects due to the high concentration of carbon dioxide is to capture and subsequent storage in stable sub-seabed geological formations (CSS). The storage could be either in sub-seabed or in terrestrial geological formations such as saline aquifers, deep saline formations, oil and gas reservoirs, etc. (Hofmann and Schellnhuber, 2010). However, Damen et al. (2006) suggested that this technique has associated several risks. These risks could be classified in five categories depending on the stage of the CCS technique:

1. CO_2 leakages due to an escape from the reservoir
2. CH_4 leakages due to the injection
3. Seismicity due to the injection also, could generate micro earth tremors
4. Ground movement after a subsidence due to pressure changes
5. Displacement of brine as a consequence of the CO_2 injection.

All these potential risks may provoke negative effects on the marine ecosystem, marine resources and, therefore, human health (Strong et al., 2014). Thus, a perfect location for CO_2 storage and the biogeochemical characterization are essential tools to establish a baseline for the management assessment and to be used during the monitoring process (Carroll et al., 2014; Reguera et al., 2013).

Potential impacts deriving from possible CO_2 leakages on sediment fauna are getting better understanding by the scientific community (Almagro-Pastor et al., 2015). Nevertheless, specifically in marine bacteria research, there is a lack of knowledge. This issue

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has to be considered for future studies because marine bacteria play an essential role in remineralization process in the sediments as well as in the water column (Pomeroy et al., 2007). They are considered as "ecosystem engineer". Possible changes in bacterial community, either in the benthic community or in the community from the water column, may directly or indirectly affect the ocean food chain as it occurs with marine plankton (Hays et al., 2005). Therefore dramatic consequences may occur in marine environments. Marine bacteria assessment related to CCS is increasing due to the topic relevance. However, it is necessary to increase knowledge on this issue, analyzing the current situation and identify what needs to be done. This article was dived in three stages depending on the objectives: 1) reviews a global vision of the state of the art in this field of science, 2) proposes an improved integrated model for future projects and studies related to marine bacteria assessment and 3) provides a new growth medium to carry out future research related to marine bacteria population under laboratory conditions.

2. Materials and methods

2.1. Experimental set up

There is not still a common protocol for the growth medium to carry out toxicity test under laboratory conditions with bacteria populations. To date, this issue depends on the author (Takeuchi et al., 1997; Labare et al., 2010). For this reason it is essential to find out an adequate medium of growth to use in toxicity test. As marine bacteria growth curve is characterized by the same type of growth phases of microalgae with four stages, where the exponential phase is the most important, a similar experimental bioassay was designed to solve the controversial medium growth issue. According to Riebesell et al., (2010) a batch culture bioassay was selected, which means a closed system without any input and output flows in the system. A batch culture allows for estimating the numbers of cells growing in the culture along the time, the growth rate (μ) and the relative inhibitory effect (RI) of CO_2. All these parameters may give an important source of information about the responses of a bacterial population. Moreover, this culture does not need such efforts to be maintained during the bioassays, it is inexpensive and the data are reliable.

Diluted batch culture to avoid the excess of nutrients, is as an appropriate strategy for ocean acidification proposed by Riebesell et al. (2010). Seven dilution of Difco Marine Broth 2216 (MB) with seawater were evaluated in order to assess the adequacy of a growth medium to stablish a toxicity test for bacterial population assessment (Table 1). *Pseudomona stanieri* (CECT 7202T) was selected from the Spanish Collection Cultive Type (www.CECT.org). This strain is a very representative population in different marine environmental locations (Urakawa et al., 2000).

Table 1
In this table all the different diluted medium are written with their proportions of seawater (SW) and Marine Broth (MB). Example: MB (1:2) means that per 1 ml of SW, 1 ml of MB was added. * Growth Medium used by Labare et al. (2010), **Growth Medium used by Takeuchi et al. (1997),*** Growth Medium used by Teira et al. (2012) in a semi-continuous culture.

Growth medium	**SW (volume)** ml	**MB (volume)** ml
MB no diluted*	No	Yes
MB diluted in seawater (1:2)	1	1
MB diluted in seawater (1:5)**	1	4
MB diluted in seawater (1:10)	1	9
MB diluted in seawater (1:50)	1	49
MB diluted in seawater (1:100)	1	99
MB diluted in seawater (1:1000)***	1	999

The growth experiments for the test were conducted in 50 ml falcon tube in an orbital shaker. Triplicates were prepared per dilution. The total volume prepared per tube was 45 ml. Each tube was inoculated with the same number of cells (10^8 cells ml^{-1}). The temperature was controlled during the growth period (22 ± 1 °C). The seawater used for the dilutions, was filtered by a pore size of 0.2 μm and later autoclaved during 20 min at 121 °C to ensure sterile conditions (Schut et al., 1993). Negative growth controls for the seawater, once filtered and autoclaved, were sowed in a solid MB with agar-agar during all the assays to test out that the seawater did not contain any cell which could growth and alter the data.

Total cells per ml were obtained using a calibration curve for the all the different mediums. Calibration curves were obtained by Optical density (OD) measured using a spectrophotometer (Zuzi Spectrophotometer Model 4201/50) and cell count by DAPI (4′,6-diamidino-2-phenylindole) (Porter and Feig, 1980). Relative inhibitory effect (RI) of CO_2 was determined according to Enfors and Molin (1981) and growth rate (μ) according to (Widdel, 2007).

According to the *Guide to best practices for ocean acidification research and data reporting* of the European Commission (Riebesell et al., 2010) , a CO_2 injection system is an adequate strategy for studies related to ocean acidification under laboratory conditions. In this work, an adapted CO_2 injection system in a sterile chamber for microorganisms was used in order to mimic CO_2 leakages (Fig. 1). CO_2 and pH measures were controlled by software installed in a PC. When pH sensor measure a pH lower than is programmed, solenoid valve is opened and CO_2 is injected in the containers. CO_2 toxicity tests were carried out during 3 days at different pH treatment (7, 6.5, 6 and 5.5) to be compared with the control (7.8 ± 0.2) without CO_2 injection to evaluate the differences between growth mediums.

2.2. Statistical analysis

T-test statistical analysis (SPSS 15.0 statistical software) was used to evaluate the differences depending on the growth medium comparing growth rate (μ), cells per ml and the relative inhibitory (RI) effects of CO_2 between pH treatments. Levene's test was also used to analyze the homogeneity of variances and Kolmorov-Smirnov was applied to study the normal distribution.

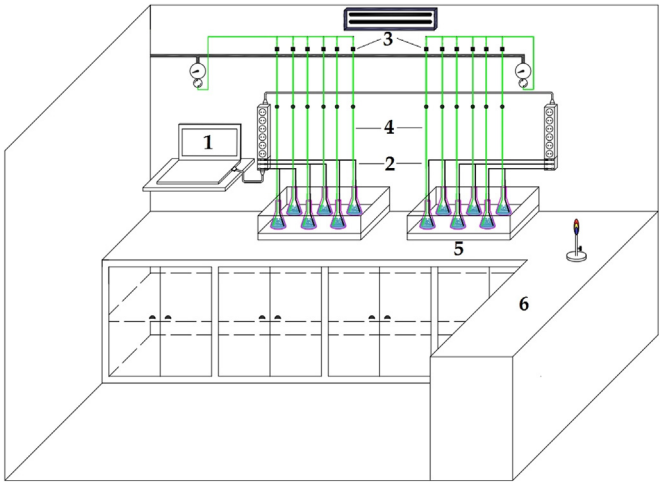

Fig. 1. This figure shows 1) PC which controls the CO_2 injection. 2) pH sensors which measure the pH, 3) solenoid valves which permit the flow of the CO_2, 4) CO_2 injectors, 5) containers for the bioassays, 6) working desk with Bunsen burner flamer. This chamber and an integrated CO_2 system were designed by Autocad program.

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