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Effect of gaseous cement industry effluents on four species of microalgae

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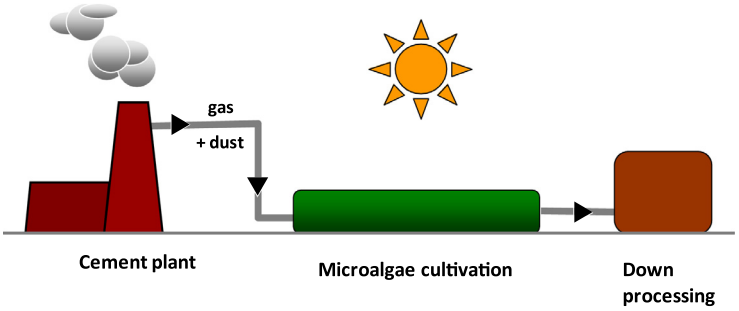
HIGHLIGHTS

- Cement flue gas can sustain microalgae growth.
- Cement flue gas harmlessness have been demonstrated for four different species.
- Cement flue gas does not modify algal biochemical composition.
- Dust can release inhibiting compounds at higher concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Experiments were performed at lab scale in order to test the possibility to grow microalgae with CO_2 from gaseous effluent of cement industry. Four microalgal species (*Dunaliella tertiolecta*, *Chlorella vulgaris*, *Thalassiosira weissflogii*, and *Isochrysis galbana*), representing four different phyla were grown with CO_2 enriched air or with a mixture of gasses mimicking the composition of a typical cement flue gas (CFG). In a second stage, the culture submitted to the CFG received an increasing concentration of dust characteristic of cement industry. Results show that growth for the four species is not affected by the CFG. Dust added at realistic concentrations do not have any impact on growth. For dust concentrations in two ranges of magnitude higher, microalgae growth was inhibited.

1. Introduction

Marine and freshwater photosynthetic microalgae have the ability to assimilate inorganic dissolved carbon into organic matter. These processes represent massive fluxes on earth where one third of the atmospheric CO_2 emitted by human activities is absorbed by phytoplankton (Sabine et al., 2004). The idea to reproduce and intensify this natural process to mitigate industrial emissions of CO_2 is more and more considered, with potential biomass valorization through lipid or carbohydrate storage (Abd El Baky et al., 2012). As phytoplankton is diluted in natural biotopes, the dissolved inorganic carbon generally does not limit primary production, which is mostly controlled by photon and nutrient fluxes. This is not the case for intensive microalgae cultivation devices such as high rate ponds or photobioreactors, where the high biomass concentrations require the supply of large amounts of inorganic carbon, as gaseous CO_2 or bicarbonate.

The overall objective of this study is to evaluate the feasibility of using cement-plant flue gas as a source of CO_2 for microalgae cultivation. The cement process can be represented as a counter-current

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flow of matter and gas: the raw materials enter the process at ambient temperature and are continuously heated by the counter-current flow of hot gasses produced by the burner at the end of the furnace. The gasses then pass through filters before being exhaust at the chimney around 120 °C. The dust collected at this stage proceeds from the whole process, and is composed of both un-reacted and partially reacted raw matter, clinker and dust of combustion. It follows that the cement flue gas (CFG) comprises two gaseous and solid components, also known as cement kiln dust. Potentially toxic compounds such as carbon monoxide, nitrogen and sulfur oxides are often present in the gaseous part of the CFG. It is therefore necessary to evaluate their effects on algal physiology, and the corresponding tolerance threshold for phytoplankton. Carbon monoxide, at 3 ppm, was proven to be without effect on microalgae (Doucha et al., 2005). Moderate SO_x and NO_x contents (a few tens of ppm) can be tolerated by microalgae (Brown, 1996; Lee et al., 2000, 2002). But higher concentrations have been shown to have moderate (Negoro et al., 1993) to strong inhibiting effects (Yanagi et al., 1995) on microalgae production, depending on culture conditions and species. The causes of toxicity are not always easy to highlight, as these molecules can act directly on the organism physiology, or indirectly by altering the properties of their medium. For example, the deleterious effects of SO_x (Matsumoto et al., 1997) and NO_x (Jin et al., 2005; Lee et al., 2002) can be significantly attenuated if the pH of the media is regulated within physiological acceptable ranges. Besides the gas itself, dust can contain potentially detrimental compounds, such as soot (Matsumoto et al., 1997) or trace metals (Borkenstein et al., 2011).

This study is aiming at evaluating the effects of CFG, including dust, on both the growing potential and biochemical composition of photosynthetic microorganisms. A fully controlled culturing device was used to regulate the gaseous flow rate in the culture, through pH adjustment (Sciandra et al., 2003). Four phytoplankton species were used for the experiments: *Dunaliella tertiolecta*, *Chlorella vulgaris*, *Thalassiosira weissflogii*, and *Isochrysis galbana*, in order to assess the CFG impact on different phyla.

2. Methods

2.1. Culture devices

Four autotrophic microalgae were selected (Table 1). The choice was driven first by the desire of using a panel of species belonging to: (1) different phyla, (2) different biotopes (seawater vs. fresh water), (3) different industrial applications, and second, by the suitability of these species to be to grown properly in photobioreactors within long term experiments. The culture vessels consisted of water-jacketed 2-liter cylinders connected to a circulating water bath that maintain constant temperature of 25 ± 0.1 °C. Plexiglas lid, fitted with a toric seal, insured a hermetic closure of bioreactors. Glass tubes, passing through the lid, allow exchanges of fluids and gas, and sampling for analysis. Cultures were subject to continuous gentle stirring and bubbling with sterile-filtered air, which passed through a 0.2-μm Whatman filter and activated charcoal. The entire culture system and its analytical resources are detailed in Sciandra et al. (2000) and Stramski et al. (2002). For marine species, the growth medium was prepared using 0.2-μm Millipore filtered and autoclaved (110 °C for 20 min) seawater and nutrient enrichment according to f/2 formulation (Guillard and Ryther, 1962). For freshwater species, the culture medium was prepared from autoclaved (110 °C for 20 min) demineralized water, enriched with BG11 broth, trace metals, and vitamins according to Rippka et al. (1979). After cooling and sterile addition of nutrients, the medium was transferred to the culture vessels through a 0.22-μm sterile filter (Sartobran-300, Sartorius Stedim), using a peristaltic pump (Gilson 3). Flow rate was regularly controlled by weighing the inflowing medium. The cultures were grown under continuous illumination from two clusters of neon tubes, which provide a high output of radiations in the whole visible spectra (Bruyant et al., 2001). Photosynthetically active radiation (PAR) was measured with a quantum scalar irradiance meter (QSL-100, Biospherical Instruments) by immersing the spherical collector in the cultures. Measurements of PAR before and after additions of dust solution into the cultures (see below) showed that light intensity and presumably light spectrum were not modified by these additions. Inorganic carbon was provided to the cultures by controlled injections of CO_2, using a pH-stat approach (Sciandra et al., 2003). When the uptake of dissolved inorganic carbon (DIC) leads the pH to exceed a fixed value, pure CO_2 was bubbled into the chemostats to lower the pH, using solenoid valves. The pH was measured at 0.5 s intervals using electrodes (Fisherbrand, gelled electrolyte) regularly calibrated. This regulation system enabled to keep a constant pH during the whole experiment. For the marine species, pH was maintained at 8.0 ± 0.02, for which the DIC concentration is not limiting for photosynthesis. For the freshwater species, pH was maintained at 7.5 ± 0.04 as advised for BG-11 growth medium preparation (Rippka et al., 1979).

2.2. Culture and biochemical analyses

The mean diameter, number and total biovolume of microalgae were estimated from their size distribution measured daily with two particle counters (Hiac Royco – Pacific Scientific and Multisizer III – Beckman). All biochemical analyses were made in triplicates. For each replicate, 5 ml of culture were filtered onto glass fiber filters (Whatman GF/C, porosity 1.2 μm) previously burned 4 h at 450 °C. All samples were then stored at −20 °C until analyses, except the filtered samples for elemental C and N analyses that were dried at 60 °C. Particulate organic carbon (POC) and nitrogen (PON) were analyzed with a Perkin-Elmer CHN analyzer. Chlorophyll, extracted with a mix of 90% acetone/dimethyl sulfoxide in the proportion 3:2 (v/v) (Shoaf and Lium, 1976) was measured with a Turner 10-AU fluorimeter (exc. 436 nm; em. 680 nm), following Welschmeyer (1994). Proteins were extracted and solubilized by filter sonication in a detergent buffer. The bulk proteins were measured from the absorbance measured at 750 nm with a spectrophotometer (Perkin-Elmer UV/VIS Lambda2), after a colorimetric reaction derived from Lowry et al. (1951), using the "Bio-Rad" detection kit (DC Protein Assay Kit II, ref 500-0112-MSDS). As the algae proteins do not give exactly the same signal intensity as the standard (protein BSA bovine serum albumin), data are given in BSA equivalents. Carbohydrates were measured using the

Table 1
Autotrophic phytoplankton species tested.

Species	Taxonomic group	Original biotope	Potential application	Reference
Dunaliella tertiolecta	Chlorophyta	Marine, estuarine	Mariculture lipid production	Jimmy et al. (2003); Minowa et al. (1995)
Chlorella vulgaris	Chlorophyta	Freshwater	Freshwater aquaculture lipid production	Ashraf et al. (2010); Sasi et al. (2011)
Thalassiosira weissflogii	Bacillariophyta (Diatom)	Marine	Mariculture	Isari and Saiz (2011)
Isochrysis galbana	Haptophyta	Marine	Mariculture lipid production	Ferreira et al. (2008); Lee et al. (2011)