Chemical Science Review and Letters ISSN 2278-6783

Research Article

Study on Carbon Sequestration Potential of Cyanobacteria (Blue Green Algae) in Rice Cultivation Ecosystems

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Abstract

Employment of cyanobacteria in biomineralization of atmospheric carbon dioxide by calcium carbonate precipitation offers novel and self-sustaining strategy for point-source carbon capture and sequestration. In our study high calcite content was registered in Blue green algae (BGA) applied plots and cyanobacterial calcite precipitation increased after 30-35 days of inoculating into the field. Scanning Electron Microscopic (SEM) images and energy dispersed Analysis of X-rays (EDAX) results confirmed the calcite crystals formation by cyanobacterial strains and the size and shape of the crystals varied depending on cyanobacterial species.

Keywords: Cyanobacteria, Blue green algae, calcite, sequestration

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Introduction

Soil carbon sequestration in agricultural ecosystems is an immediate and significant option to mitigate the increase in the atmospheric CO_2 concentration. Scientists propose terrestrial carbon sequestration as an effective mitigation option because it combines mitigation with positive effects on environmental conservation and soil fertility [1]. Carbon sequestration involves the capturing and storing of carbon, and its subsequent removal from the global carbon cycle [2]. Employment of cyanobacteria in biomineralization of carbon dioxide by calcium carbonate ($CaCO_3$) precipitation offers novel and self-sustaining strategies for point source carbon capture and sequestration [3].

Microbial based technologies, specifically those utilizing photoautotroph, represent a promising solution once methods of carbon uptake and disposition by the cell are determined [4]. The influence of photosynthesis on $CaCO_3$ precipitation in general is based on the uptake mechanism of inorganic carbon. Factors important in $CaCO_3$ precipitation are calcium concentration, dissolved inorganic carbon (DIC) concentration, the pH of the growth environment and the availability of nucleation sites for the formation of $CaCO_3$ [5]. Obst *et al.* (2009) [6] reported that under low nutrient concentrations and permanent CO_2 supply, photosynthetic uptake of inorganic carbon predominantly uses CO_2. Keeping in view the above facts, the present study was carried out to assess the carbon sequestration potential of cyanobacterial systems in rice ecosystem.

Materials and Methods

Collection of sample

Blue green algae were applied as a nitrogen source to rice crop. These organisms are floating in nature and it can be easily separated from the soil. The samples were collected in the field directly by using sterilized conical flask and the samples were rinsed with double distilled sterile water. Then the samples were directly observed under the SEM.

Microscopic analysis of cyanobacterial cultures obtained from experimental plots

The cyanobacterial biomass from the rice fields were aseptically transferred to the laboratory in conical flasks and examined under Scanning Electron Microscopic (SEM) to check the formation of calcite crystals and confirmation of calcite precipitation through Energy Dispersed Analysis of X-rays (EDAX). The methodology followed to observe the cyanobacterial cultures under SEM and EDAX is described below.

Scanning electron microscopy

Samples for SEM were taken with syringes from the conical flasks. Ten milliliter aliquots were vacuum filtered onto Nucleopore polycarbonate filters with a pore diameter of 0.2 µm. The samples were rinsed with a few drops of Nanopure water in order to remove the remaining $NaHCO_3/CaCl_2$ solution, but prevent dissolution of the precipitated

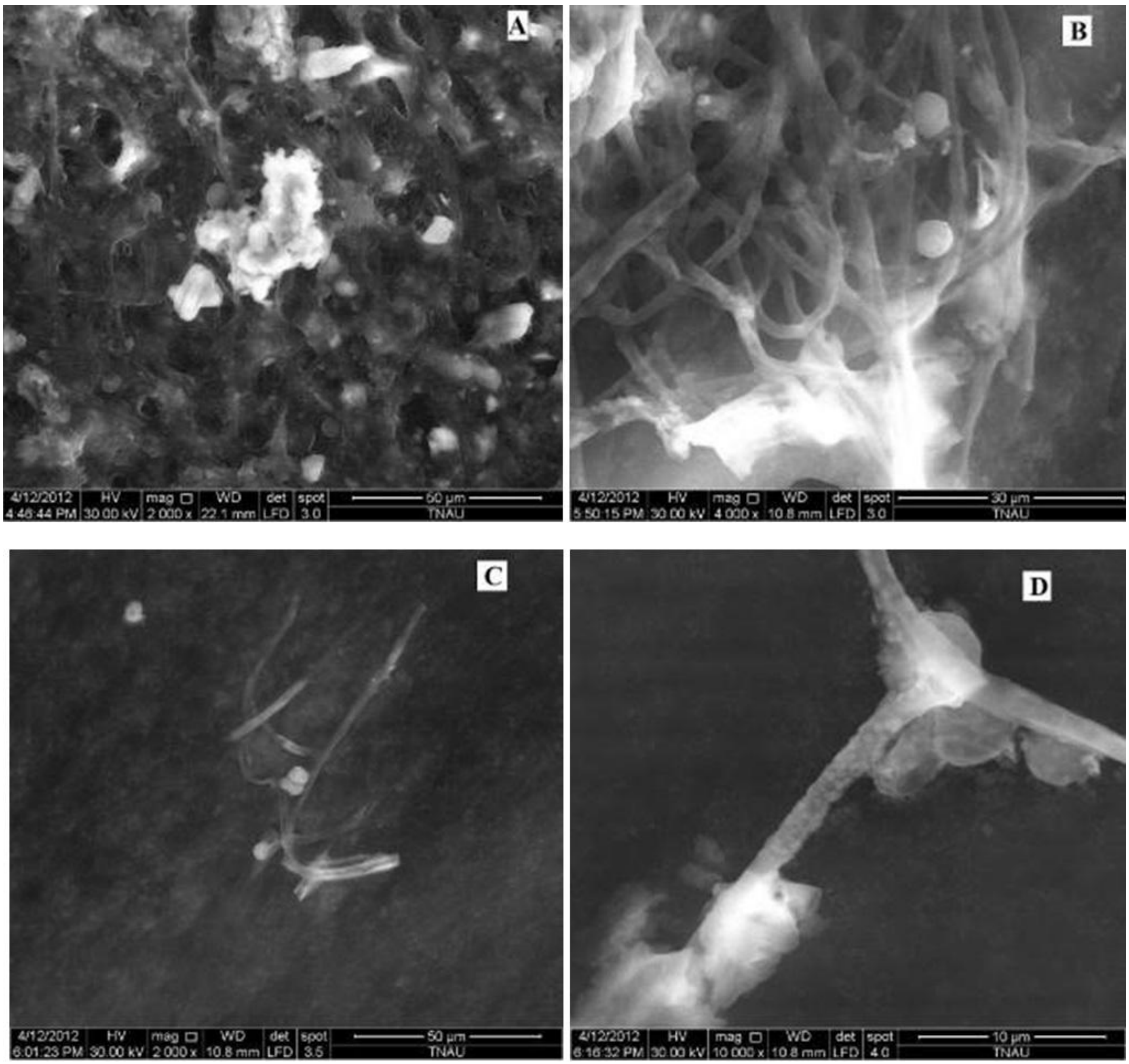
$CaCO_3$ crystals of cyanobacterial strains. The filters were then dried immediately on a vacuum filtering apparatus and stored at 4^{o}C for scanning electron microscopy analysis.

Energy Dispersed Analysis of X-rays

Acceleration voltages between 5 and 20 kV were applied. The element composition of the crystals was determined qualitatively with an EDAX EDS detector using FEI quanta 200 EDAX to exclude artifacts from particles containing elements other than carbon, oxygen and calcium. The size distribution of the precipitated crystals was determined by image analysis of the SEM images. On each filter, 15 images were randomly selected at a pixel size of 95 x 95 nm^{-2}. The images were analyzed semi-automatically using the Image Analysis Toolkit [6].

Results and Discussion

The presence of carbon, oxygen, magnesium, silicon and calcium was noticed on the cell surface of cyanobacterial strains obtained from experimental plots. As per SEM image (**Figures 1A-1F**), it is evident that cyanobacteria are able to precipitate calcite with varying sizes and shapes.



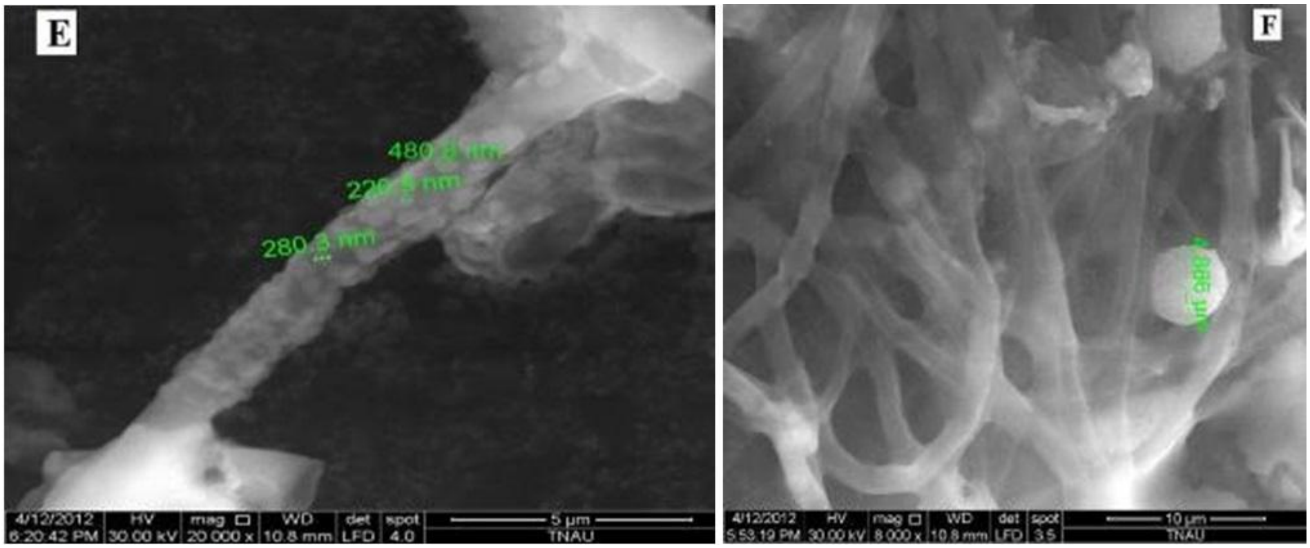


Figure 1 SEM image showing (A) cyanobacterial induced precipitates of calcite, (B) calcite crystals attached to cyanobacterial filaments, (C) intracellular calcite precipitation by cyanobacterial strains (2000x), (D) intracellular calcite precipitation by cyanobacterial strains (10000x), (E) calcite with size ranging from 200-500 nm (10000x), (F) calcite crystals embedded in algal matrix under higher magnification (8000x)

Role of cyanobacterial (blue green algal) systems on carbon sequestration/ calcite precipitation

In the present investigation, calcite precipitation was reported in blue green alga which was applied as biofertilizers in rice cultivation. Soil temperature of the rice ecosystem favoured calcite precipitation. The similar results were also obtained by Paola *et al*. (2003) [7], who reported that when different strains from loamy soil and limestone cave soil were tested for their ability to calcify, results showed that the capability to precipitate calcite was present to a microbial community level, not a single species level. The experiments conducted by Paola *et al*. (2003) [7] states that after 25 days, all calcifying strains (irrespective of their origin) were capable of forming carbonate crystals under different temperatures.

Confirmation of calcite precipitation by SEM with EDAX studies:

The carbon, oxygen, magnesium, silicon and calcium exhibited weight percentage of 31.31, 37.55, 1.01, 1.22 and 28.90 respectively (**Figure 2**). The chemical composition of calcite crystals precipitated by cyanobacterial strains was confirmed by the Energy Dispersive X-ray Spectroscopy (EDAX) values in SEM.

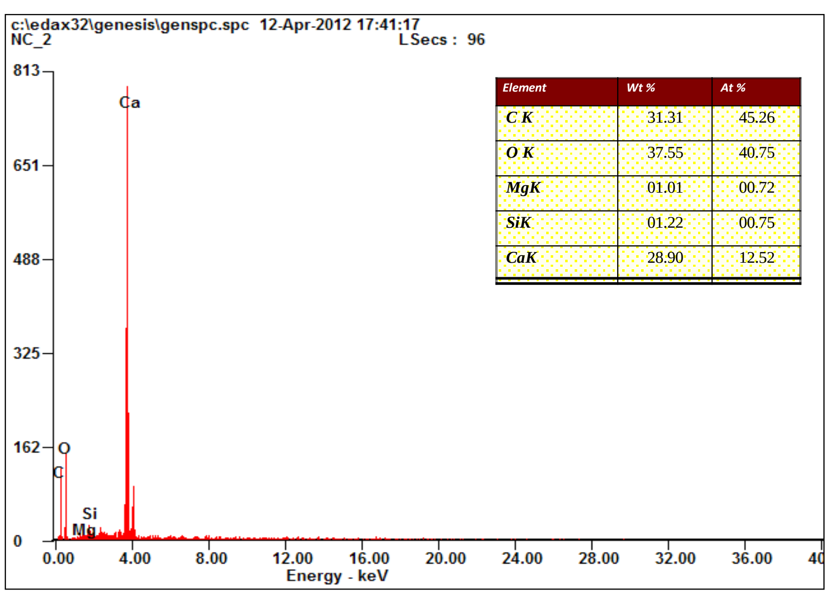


Figure 2 Graphical representation of EDAX values of SEM image of calcite precipitation by cyanobacterial strains

Most of the carbon presented in the global carbon cycle is sequestered primarily as calcium and calcium-magnesium carbonates. In many cases, the carbonates are of biogenic origin, some precipitated by bacteria, cyanobacteria, and fungi. The microalgae are capable of using free CO_2 and bicarbonate ions as a source of inorganic carbon during photosynthesis, transporting them across the fine plasmatic membrane where they accumulate in the cell as an inorganic carbon reservoir for photosynthesis [8]. In the present study the SEM images confirmed the calcite crystals formation by cyanobacterial strains and the size and shape of the crystals varied depending on cyanobacterial species and more amount of calcium. This is in confirmation with earlier reports [7] that the size and shape of the newly formed crystals depend on the bacterial strains.

Conclusion

The higher biomass generation of cyanobacterial strains might have also contributed to higher calcite precipitation. The study also has brought out the point-source carbon capture and sequestration potential of blue green algal systems as a climate change mitigation strategy.

Acknowledgements

We wish to express our appreciation to Royal Norwegian Embassy, New Delhi for providing financial assistance to carry out this study under the ClimaAdapt research programme.

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Publication History

Received	09th Nov 2017
Revised	28th Nov 2017
Accepted	07th Dec 2017
Online	30th Dec 2017