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The phage-host interaction as a model for studying carbon regulation in aquatic system

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Introduction:

✓The understanding about the interconversion of carbon in nature is still beyond the grasp of present environmentalists.

✓Considering the present status of global warming there is a necessity for studying carbon regulation in aquatic systems.

✓The bacteria and their phages being the smallest and most abundant constituents of the aquatic environment, represents an ideal model for studying carbon regulation in aquatic system as discussed in this presentation.

- The regulation of carbon in aquatic system is one of the major processes among biogeochemical cycles.
- The inedible or refractory dissolved organic carbon is a recently coined terminology from the microbe-driven conversion of bioavailable organic carbon into difficult-to-digest refractory DOC by microbial carbon pump (MCP).
- This concept is suggested by some workers that have potential to revolutionize our view of carbon sequestration.
- It is also said that the ocean surface take up about 2% more CO_2 gas than they release of which some amount of CO_2 dissolves into the water, forming carbonic acid.

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- The increase in level of CO_2 in oceans decreases the pH resulting in acidification which affects the aquatic ecosystem.
- However, much more amounts of carbon are in the water as DOC.

- It is estimated by some workers that about 95% of organic carbon is in the form of refractory DOC which is the largest pool of organic matter in the ocean.

- The refractory DOC is supposed to be the major factor in the global carbon cycle whose source is not yet well understood **(Ogawa et al., 2001; Stone 2010; Zbigniew et al., 2001).**

- A key element of the carbon cycle is the microbial conversion of dissolved organic carbon into inedible forms.

- Microbes play a dominant role in "pumping" bioavailable carbon into a pool of relatively inert compounds.
- The MCP "may act as one of the conveyor belts that transport and store carbon in the deep oceans."
- The MCP also appears to function in deep waters, where bacteria adapted to the high-pressure environment may have "a special capacity" to degrade refractory DOC.
- It is known that marine microbes are able to convert bioavailable DOC to refractory DOC **(Ogawa et al., 2001; Stone 2010; Zbigniew et al., 2001).**

Methodology:

- The present communication represents the time studies of phage-host interaction under control conditions, to analyze their impact on the total carbon content of the source (Nutrient broth) and their interconversion among organic, inorganic and other forms of carbon with respect to control.
- The data generated is based on the results obtained from TOC- analyzer.

- We used sterilized Nutrient broth media for inoculation of *E. coli* (ATCC 13706) strain and incubate it at 37^0 C for 18 hours in 4 conical flasks.
- The 2 flasks of control broth were not inoculated with bacterium and were preserved in refrigerator at 4^0 C after autoclaving till the experiment begins.
- The initial reading of all 6 cultures were analysed by TOC (Total Organic Carbon) analyzer after 18 hours of incubation.

- The phage phi X174 ATCC 13706 B1 were added to the 2 conical flasks containing *E.coli* (ATCC 13706) strain after taking samples for initial reading.
- The analysis was further carried out after every hour till the stationary state is achieved in the results.
- The experiment was carried out in duplicates so as to avoid the effect of time factor and manual error on the results obtained.

- The experiment was designed to measure the inorganic carbon from three sets viz.

a) Control sample,
b) Sample with bacteria and
c) Sample with bacteria and its specific phage

- The nutrient broth was used for all three set of experiment **(Clescerl et al., 1999).**

- The study the effect of phage–host interaction on the carbon regulation we prepared three sets of samples namely;

Control sample:

➢The control sample prepared was of nutrient broth (Standard methods, 1999) devoid of any bacterial or viral inoculation and was stored at 4^0C throughout the experimentation process.

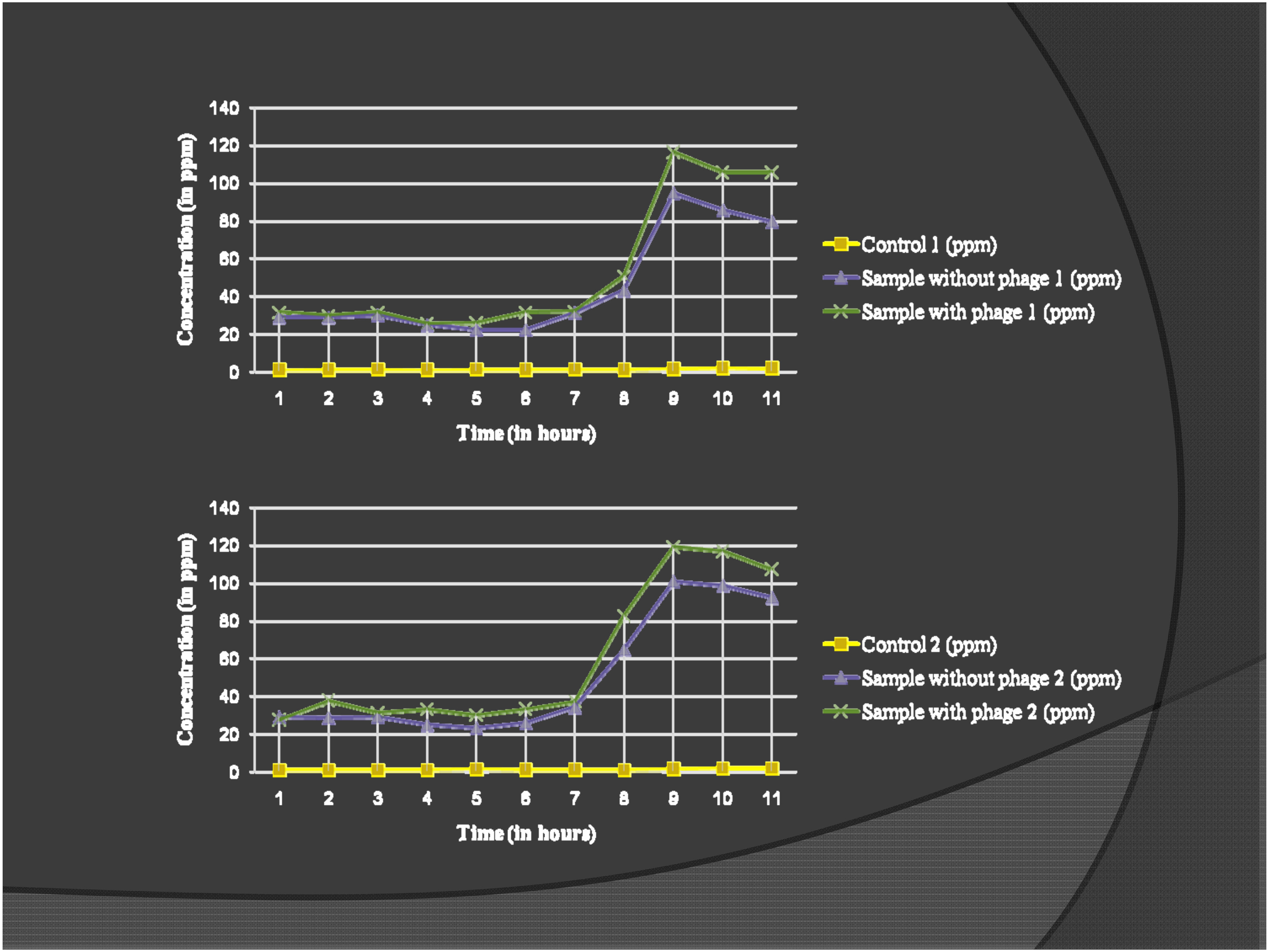
➢It was kept in duplicate so as to minimize the manual or instrumental error (if any).

Sample with bacteria:

➢The Sample of host *E. coli* strain (ATCC-13706) was prepared in nutrient broth (Standard methods, 1999). which was inoculated and incubated at 37^0C throughout the experimentation process.

Sample with bacteria and its specific phage:

➢The Sample of host *E. coli* strain (ATCC-13706) and phage phi X174 (ATCC 13706 B1) was prepared in nutrient broth (Standard methods, 1999). which was inoculated with its specific phage and incubated at 37^0C throughout the experimentation process


Concentration (in ppm)
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11
Time (in hours)
Control 1 (ppm)
Sample without phage 1 (ppm)
Sample with phage 1 (ppm)
Concentration (in ppm)
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11
Time (in hours)
Control 2 (ppm)
Sample without phage 2 (ppm)
Sample with phage 2 (ppm)

- The analysis of experimental samples for determining the inorganic carbon, organic carbon and total carbon content was carried out by using PC controlled highly sensitive instrument TOC-V_{CPH} (Shimadzu Total Organic Carbon Analyzer) which applies combustion catalytic oxidation/NDRI method for detection of carbon and carbon compounds (especially Carbon dioxide).

- It is clear from the results that the nutrient broth sample showing low inorganic carbon content was increased by 15-25 percent at the end of the experiment in the sample with phage as compared to sample without phage.

- Overall, the analysed results showed 60-70 fold increase in inorganic carbon content of the sample with phage-host interaction as compared with control (whereas, in the case of sample without phages it was 50-55 fold increase).

- This increase in inorganic carbon content may be due to lysis of the host cell releasing its cellular constituents and utilization of carbon constituent for phage assembly and development.

- It also proves the role of phages in regulating the carbon flow in the aquatic systems like oceans where their concentration outnumbered other species.

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Thank you