

CHAPTER 7

Summary

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- ⇒ In this thesis, a study on the dynamics of three types of EPS viz. NaCl-soluble EPS, EDTA-soluble EPS and TEP was carried out at a shallow tropical station at the Dona Paula Bay, west coast of India during 1998-2000. During this study, factors like hydrography, nutrients, dry weight of particulate matter, Chl *a* (phytoplankton), POC and bacterial abundance were monitored to evaluate its influence on the seasonal variations of EPS.

- ⇒ The study site experienced three seasons- monsoon (June- September), post-monsoon (October to January) and pre-monsoon (February-May). Seasonal differences were observed in the concentrations of NaCl-soluble and EDTA-soluble forms of EPS in both suspended particulate matter and sinking macroaggregates. The EDTA-soluble EPS in the suspended matter was higher during monsoon. This coincided with an increase in suspended load, POC and nutrients but with a decreased phytoplankton (Chl *a*) biomass. On the other hand, NaCl-soluble EPS in the suspended matter was higher during post-monsoon months and coincided with higher Chl *a* concentrations. Unlike the two soluble forms of EPS, TEP concentrations at the study site remained unchanged during both monsoon and pre-monsoon months.

- ⇒ In the macroaggregate samples, NaCl-soluble EPS was higher during monsoon and coincided with low chl *a* concentration. On the other hand,

EDTA-soluble EPS was higher during pre-monsoon months and coincided with increased POC concentrations. However, Chl *a* values in the macroaggregates did not change much seasonally.

- ⇒ The concentrations of all the three types of EPS in the suspended matter and macroaggregates had no correlation with changes in phytoplankton biomass suggesting that phytoplankton was not the major source of EPS at the sampling station. Moreover, factors like nitrate concentrations that regulate EPS production in phytoplankton cells did not correlate with EPS and TEP concentrations in the suspended matter.
- ⇒ The contribution of EPS (both NaCl and EDTA-soluble) to POC was < 5% in both water column and sinking macroaggregate samples. Similarly, TEP-C contribution to POC in the suspended matter was 6.9% but only 2% in the macroaggregates.
- ⇒ Since EPS and TEP are rich in organic carbon, their influence on the variation of ecto-glucosidase (both α - and β -) activity and bacterial production was also evaluated between 1999-2000. The total α -glc and β -glc activity was exceptionally high in the month of September 1999. On the other hand, higher total α -glc and β -glc activity was also recorded during the month of May 2000 in the macroaggregate samples. The higher enzyme activity coincided with higher total bacterial production, Chl *a* and EDTA-soluble EPS.

- ⇒ Particle associated bacteria accounted for 49% and 61% of the total bacterial population in the suspended matter and macroaggregate samples, respectively. Similarly, a substantial fraction of total enzyme activity (85 to 95%) and bacterial production (75%) was associated with particles, which had significant linear correlation ($r= 0.663$, $p<0.01$) with EDTA-soluble EPS of the suspended matter. Such correlation highlights the significance of particle associated bacterial population in the turnover of EPS in the water column at the study site.
- ⇒ The total bacterial production and the glucosidase activity (both α - and β -forms) had positive linear trends with Chl *a* and EPS (both types) in both suspended matter and macroaggregates. Moreover, microbial activity of the macroaggregates showed significant correlations with Chl *a* and EPS of suspended matter. This indicates that the composition of suspended matter had a significant influence on the microbial activity in the macroaggregates.
- ⇒ TEP had no correlation with microbial activity in both suspended matter and macroaggregate samples, suggesting that TEP was not the preferred carbon source for bacterioplankton and probably served as a carbon sink at the study area.
- ⇒ The various aspects of microbial EPS were studied using a marine bacterium *Marinobacter* sp. Growth conditions for production of EPS by the bacterium were standardized. The EPS was isolated, partially purified

and chemically characterized. *Marinobacter* EPS was richer in proteins compared to carbohydrates and also showed the presence of substantial amounts of residues like uronic acid and sulphates. Glucose was the dominant monosaccharide whereas aspartate was the major amino acid in the EPS.

⇒ EPS from *Marinobacter* sp. and a diatom *Skeletonema costatum* were used to evaluate its ability to produce large visible macroaggregates. Large macroaggregates (>15 mm² size) were produced at a faster rate in presence of bacterial EPS than diatom EPS. Significant differences were observed in the abundance (Fs= 28.07; p<0.001) and size (Fs= 9.15; p<0.01) of aggregates produced using EPS derived from two different sources. Interestingly, macroaggregate formation was not influenced by abundance of TEP or Coomassie-stained particles (CSP). Moreover, presence of killed bacteria decreased the formation of macroaggregates in both the experiments. Thus, it was clear that EPS was essential for the abiotic production of macroaggregates and could form particles even in the absence of TEP. Although macroaggregate production involves collision of particles, some organic particles- like killed bacterial cells in this study, might depress the aggregation process.

⇒ EPS from *Marinobacter* sp. was used to assess its suitability as a carbon source for heterotrophs. ¹⁴C-glucose labeled *Marinobacter* EPS was coated on to sediments and fed to a benthic polychaete *Nereis (Hediste) diversicolor*. At the end of the feeding period, the animals ingested 27.85

dpm to 114.9 dpm of the labeled EPS. On an average, the absorption efficiency of the animal was 65.6%. However, substantial amount (51%) of the ingested EPS was respired as CO₂ and only 26% of the label was retained in the tissues after depuration. From this study, it was clear that the bacterial EPS served more as an energy source to these benthic animals and the bacterial EPS was not efficiently incorporated into the tissues of the animal.

⇒ Natural bacterioplankton population collected from Dona Paula Bay was used to degrade the ¹⁴C-glucose labeled *Marinobacter* EPS to evaluate its incorporation into the natural microbial population over a period of 11 days. During this experiment, the microbial cells were grown in aged seawater supplemented with labeled *Marinobacter* EPS as a source of organic carbon. A significant correlation ($r=0.993$, $p<0.001$) between the bacterial production and the incorporation of labeled EPS was observed during the course of the experiment. Both ¹⁴C-incorporation and bacterial production were maximal in the first 24 h. A decline in ¹⁴C-incorporation and bacterial production with time matched with the decline in monosaccharide (MCHO) concentrations and higher ecto-glucosidase activity. This suggests the utilization of MCHO by the natural marine bacterioplankton.

⇒ Metal binding properties of the bacterial EPS were evaluated using copper and lead as model substrates. The EPS bound both copper and lead to varying amounts. The polymer had a better binding capacity for copper

than lead, which might be due to differences in the ionic radii of the metal ions, electro negativity of their complexes and accessibility of the binding sites. The binding was greater at near neutral pH for both copper and lead suggesting that the presence of carboxyl ions and/or CONH⁺ groups might be responsible for the observed binding of copper and lead to the EPS. A substantial reduction in the amount of Cu or Pb ions bound by the EPS was observed in presence of sodium chloride, which might be due to competition for the binding sites between Na⁺ ions with Cu²⁺ and Pb³⁺.

Although the significance of microbial EPS in the biogeochemical cycling of carbon and food-web is well-acknowledged, the chemical characteristics, properties, behavior and distribution of EPS may vary for several reasons. In this regard, the following could be concluded from the research work presented here:

- i) The three forms of particulate EPS studied in a near shore environment did not contribute substantially to the organic carbon pool and all the forms of EPS did not always support microbial production and enzyme activity.
- ii) Although bacterial EPS possessed gelling and adsorptive properties, its ability to form particulate matter and bind metals varied considerably.
- iii) EPS derived from *Marinobacter* sp. served as an energy source for heterotrophs.

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