Distribution and ecology of methanogenic bacteria in mangrove sediments of Pitchavaram, east coast of India

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Distribution and ecology of methanogenic bacteria were studied in sediment samples of Pitchavaram mangrove (11° 27'N; 79° 47'E, Tamilnadu coast) during September 1986-February 1987. For estimation of anaerobic heterotrophs, sulphate reducing and methanogenic bacteria in sediment, core samples were taken up to a depth of 105 cm at 4 different sites. Most probable number (MPN) of methanogenic bacteria was maximum, at a depth of 90 cm, in sites 2 and 4 during postmonsoon season. While ecological parameters like hydrogen-ion-concentration, Eh, texture and organic carbon content of the sediment seem to play a positive role on the distribution of methanogens, the presence of sulphate reducing bacteria acts as a limiting factor.

Methanogenesis in mangrove sediments has not been studied so far world over, and in mangrove environments microbial methanogenesis is associated with decomposition of organic matter derived mainly from the leaf litter. In the present communication influence of various factors on the vertical distribution of methanogenic bacteria in the Pitchavaram mangrove sediments is reported.

Materials and Methods

Sediment samples were collected during September 1986-February 1987 from 4 different sites of Pitchavaram mangrove (Fig. 1) at different depths (45, 90 and 105 cm). Observation on sediment texture showed clay type of sediment at sites 2 and 4 and sandy-clay at sites 1 and 3, at all depths examined. Samples were analysed in the laboratory within 4 h of collection. The core was subsampled, parallel to the core liner, by pushing a 5 ml syringe with the hub end cut off into the sediment; 3 ml aliquot of sediment was withdrawn and dispensed into bottles containing 99 ml of sterile 50% seawater with Rasazurin (0.001%). The sediment was pushed out of the syringe with the plunger, while being gassed with N₂ and sealed with butyl rubber stoppers and stored at in situ temperature. Appropriate decimal dilutions were made to determine total anaerobic heterotrophic bacteria (AHB), sulphate reducing bacteria (SRB) and methanogenic bacteria (MB). AHB were enumerated on 2216 E Marine agar using roll tube technique. SRB were enumerated on Postgate's medium as modified by Jacq. Tubes were incubated at 35°C up to 15 days and colonies of SRB identified by a black halo. MB were quantified by 5 vial, 3 dilution most probable number (MPN) method using the medium recommended by Mathr-
ani and Boone with 0.01 mg L⁻¹ Na₂MoO₄ and incubating 10, 1 and 0.1 ml of decimally diluted sediment samples. Production of methane was analysed after 15 days of incubation at 35°C using a gas chromatograph. pH and Eh were determined immediately on return to the laboratory using a pH/Eh meter (Industrial Electronics Corporation) under anaerobic conditions, by flushing with oxygen free nitrogen. Sediment organic carbon (OC) was estimated by the method of E1-Wakeel and Riley.

Results

The pH (Fig. 2) of sediment samples remained almost neutral (7-7.5) at all the sites. OC in sediment (Fig. 2) fluctuated from 1 to 7 mg g⁻¹ and the carbon minimum layer was observed at 105 cm depth in all sampling sites. Maximum OC was found in surface sediments (45-90 cm depth) at sites 2 and 4. Eh values showed minimum of −94 mV in surface sediments and maximum of −190 mV in 105 cm depth sediment at all sites.

Counts of total AHB, SRB and MB are given in Fig. 3. Among different sites examined, all bacterial fractions showed maximal occurrence at sites 2 and 4 (clayey sediment), indicating the specific affinity to clayey soil. The population density of AHB and SRB decreased with increase in depth of the sample. Vertical distribution of MB was maximum in 90 cm depth sediment at all sampling sites and the counts fluctuated from 0 to 80 MPN.100 g⁻¹. At 105 cm depth SRB were very much reduced. However AHB density remained high at all depths observed. AHB and SRB occurred both during monsoon (October-December) and post-monsoon (January and February) with a maximum of 1.6 × 10⁴ for SRB and 1.1 × 10⁵ for AHB during the later season. Similarly MB showed maximum during post-monsoon season at all sites. At 45 cm depth MB were scanty and remained below detectable levels during first phase of monsoon season at all sampling sites and were completely absent during the entire period of monsoon at site 3. At 105 cm depth, even though the occurrence of MB was recorded, maximal density was found in 90 cm depth at all sites.

Correlation coefficient values calculated between different parameters are given in Table 1. MB and SRB generally increased with an increase in Eh. While SRB and total AHB increased with increase in OC, total AHB also showed an increase when SRB decreased.

MB showed significant positive correlation with depth in all the 4 sites (r = 0.46 to 9.6) while SRB showed significant negative correlation with depth at all

![Fig. 2 — Variation of pH and organic carbon at different depths](image-url)
Fig. 3—Distribution of methanogenic bacteria (MB), sulphate reducing bacteria (SRB) and anaerobic heterotrophic bacteria (AHB) at different depths

Discussion

The present study revealed that the upper sediment strata (45 cm) served as a good niche for the proliferation of SRB due to favourable conditions such as high OC and neutral pH. At depths beyond 45 cm in the present study, SRB decreased gradually, so also OC as reported in Bay Tree Creek salt marsh previously. The density variations in MB and other bacterial fractions in mangrove sediments at different sampling sites is due to variations in environmental factors. Generally, under culture conditions, MB metabolize best at pH range 6-8. In the present investigation also, throughout the study period pH of the mangrove sediment remained almost neutral (7.5) favouring methanogenic activity. High MB counts were observed whenever the Eh value was high especially during postmonsoon season. The redox potential in sediment cores increased with depth while the oxygen level decreased.

Vertical distribution of MB in the mangrove sediments is characterized by relatively high density at 90 cm depth and less density towards surface and at increasing depths. Methanogenic substrates are the products of organic matter decomposition and the low rates of methanogenesis with depth are likely due to low amounts of readily degradable organic matter. Differences in sediment pH, Eh and OC appear to be major factors controlling the depth of maximal density of MB at all the sites. Similar phenomenon was also observed in the Virginia salt marsh. Williams and Crawford observed significant population (10⁴ ml⁻¹) of methanogen even at a depth of 210 cm and attributed it to the increased nutrient availability even at that depth.

The amount of OC in sediments explained to a greater extent the variability in MB density and also the Eh. Winfrey and Zeikus observed that methanogenesis was not inhibited when either acetate or hydrogen was added to sulphate inhibited fresh-water sediments. They also suggested that increased amounts of
these substrates in the presence of sulphate decrease the competition for them between MB and SRB, allowing methanogenesis to occur. In clayey sediments (sites 2 and 4) OC was more than in sandy clay sediments (sites 1 and 3) and this very well explains the high density of MB at sites 2 and 4. Pedersen and Sayler observed maximum methanogenesis in Melton Hill reservoir sediments and attributed this to organic matter. Even though OC was maximum in surface sediments, MB density was very low due to other factors such as low Eh, higher density of SRB, etc. Vertical distribution of SRB was low at 90 cm wherein maximum MPN count of MB was recorded. This corroborates the findings of Winfrey and Ward. Maximum concentrations of methane were below the zone of high sulphate and a decrease in methane level corresponds with an increase in sulphate concentration as observed by Winfrey et al. It has been shown that high levels of methane in anoxic marine sediments were found in deeper layers, where the sulphate concentration was low.

Distribution of methanogens in 45 cm layer was very low/ absent in the present study. Specific growth substrate, fulfilling growth requirements for MB, was used with the addition of trimethylamine and Na2MoO4 to reduce the SRB populations. Reduction in MB counts in the upper layers might be due to toxicity of sulphate to MB and/or competition between MB and SRB for essential nutrients.

It can be concluded that methanogenesis and sulphate reduction can occur simultaneously in the same region provided there is an adequate supply of noncompetitive substrate (e.g. methanol, methylamine) or an abundance of competitive substrates (e.g. H2 plus CO2 or acetate) for the methanogenic bacteria. This phenomenon also explains why a spatial isolation is often observed in many sediments between the zone of sulphate reduction, overlying a sulphate depleted zone of methane production.

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**References**

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