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Methanogenesis: Seasonal changes in human impacted regions of Ashtamudi estuary (Kerala, South India)

R.R. Reshmi [a], K. Deepa Nair [b], E.J. Zachariah [b], Salom Gnana Thanga Vincent [a, *]

[a] Department of Environmental Sciences, University of Kerala, Kariavattom, 695 581 Thiruvananthapuram, Kerala, India
[b] Atmospheric Sciences Division, Centre for Earth Science Studies, PB 7250, Thiruvananthapuram 695031, India

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ABSTRACT

Environmental variables as well as methanogenic abundance and activity were analysed in selected human impacted regions of Ashtamudi estuary. Sediment samples were collected during summer and monsoon of 2013. Each was analysed for environmental variables such as temperature, pH, electrical conductivity, sulphate, total kjeldahl nitrogen, organic carbon, organic matter and redox potential. Abundance and methanogenic potential of two distinct groups of methanogenic archaea (i.e. aceticlastic and methylotrophic methanogens) were quantified by incubating the sediment samples in basal media, added with acetate or methanol as substrate. Most of the environmental variables showed significant differences spatially and temporally. Among the environmental variables, temperature, pH, electrical conductivity and salinity were higher during summer, while total kjeldahl nitrogen, sulphate and organic carbon were higher during monsoon. Abundance of both aceticlastic and methylotrophic methanogens showed significant variations both spatially and temporally. Aceticlastic methanogens were abundant during monsoon, with a maximum value of 810 ± 13 CFU g^{-1}, and methylotrophic methanogens were abundant during summer, with a maximum value of 1770 ± 30 CFU g^{-1}. Results of methanogenic potential of sediment samples showed a range of 0.01 ± 0.00 to 12.03 ± 0.35 mol m^{-3}. Among the two substrates, methanol favoured the abundance of methylotrophic methanogens, while acetate induced the methanogenic activity. Methanogenic activity was higher during monsoon than summer that can be attributed to the favourable sedimentary conditions (like reduced redox potential and increased substrate availability). Aceticlastic methanogens were abundant at bottom layers and methylotrophic methanogens in top layers of the sediments. The results of canonical correspondence analysis revealed the existence of linear relationship between methanogenic archaea and environmental variables among the sampling stations. Higher values of salinity, electrical conductivity and total kjeldahl nitrogen favoured the distribution and abundance of aceticlastic methanogens. However, the abundance of methylotrophic methanogens was favoured by highly reduced conditions and high pH values.

1. Introduction

Methanogenesis is the terminal step in anaerobic organic matter degradation, resulting in the production of methane. Since methane formation requires strictly anaerobic conditions, methanogenesis occurs in anoxic environments, such as in sediments. Estuaries are considered to be an important link between land and the sea and function as natural sinks of organic matter that comes from marine, terrestrial and anthropogenic sources. Hence, the contribution of estuaries to marine methane emissions is important (Shalini et al., 2006; Torres-Alvarado et al., 2013). Methane is the most important greenhouse gas (next to carbon dioxide) and therefore, the microbial source of this gas is of considerable interest (Cicerone and Oremland, 1988). Wetlands are the largest natural source of methane, contributing to approximately 20% of the global emissions (Matthews, 2000). Although wetlands provide a potential sink for atmospheric carbon, if not managed properly, may become sources of greenhouse gases such as methane (Mitra et al., 2005). To understand the impacts of dissolved and particulate inputs on estuarine ecosystems, derived from anthropogenic sources is a major task for the future (Jennerjahn and Mitchell, 2013).

* Corresponding author.
E-mail addresses: ravirama6666@gmail.com (R.R. Reshmi), deepanairk@gmail.com (K. Deepa Nair), ejzach@gmail.com (E.J. Zachariah), salom@keralauniversity.ac.in (S.G.T. Vincent).

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Methane production may be aggravated in tropical wetlands due to high temperature and high rates of organic matter decomposition (Verma et al., 2002). In spite of its importance, studies pertaining to this concept especially in tropical conditions are scarce, although several studies on methanogenesis have been confined only to temperate regions (Torres-Alvarado et al., 2005).

Methane production is conducted by the metabolism of a large and diverse group of methanogenic bacteria (Knittel and Boetius, 2009). They are strict anaerobes and are phylogenetically placed exclusively as members of the domain Archaea (Woese, 1987; Woese et al., 1990). The magnitude and/or onset of methanogenic activity depends mainly on sediment redox conditions (Wang et al., 1993), the abundance of substrate (Boon and Mitchell, 1995; Kotsyurbenko, 2010) and sediment temperature (Zeikus and Winfrey, 1976). Physicochemical, nutritional and hydrochemical parameters controlling the distribution and abundance of methanogenic archaea (MA) have been explored in lacustrine sediments (ZeppFalz et al., 1999) as well as in coastal environments (Purdy et al., 2002; Wilms et al., 2006). Various types of anthropogenic pressures owing to dense human population in the catchment, added with strong seasonal precipitation patterns, results in significant fluctuations in dissolved and particulate substances transported by rivers (Jennerjahn et al., 2008). The associated hydrological conditions could affect the structure of microbial communities involved in the terminal phases of anaerobic organic matter mineralization (Verma et al., 2002).

Degradation of organic matter in anaerobic pockets of sediments is controlled by groups of closely interacting microorganisms, including sulphate-reducing bacteria (SRB) and MA (O'Sullivan et al., 2013). The occurrence of methanogenic archaea in estuarine sediments is influenced by the abundance of SRB and sulphate reduction is a key factor related to MA distribution (Munson et al., 1997). Further, the existence of salinity gradients in estuaries also accounts for spatial variations in methanogenesis (Verma et al., 2002). It is established that MA are prevalent upstream in the freshwater regions and decrease towards the brackish and marine ends (Munson et al., 1997; Torres-Alvarado et al., 2013). Sulphate reducing bacteria generally outcompete MA in sediments for 'competitive' substrates (acetate) when sulphate is available. However, MA can co-occur in the presence of sulphate as 'noncompetitive' substrates (methanol) are not metabolized by SRB (Lovley et al., 1982; Oremland et al., 1982; O-Sullivan et al., 2013). Acetate is considered as a competitive substrate and methanol a non-competitive substrate for MA. Hence, it is acceptable to conceive that, anaerobic organic matter decomposition, leading to methane emission in estuarine sediments will be controlled by the competitive interaction for substrates between the two groups of microorganisms *i.e.* SRB and MA (Oremland and Polcin, 1982). Spatial changes in methane emissions within the individual regime are attributed to the heterogeneity of environmental variables (Verma et al., 2002). Methanogenesis from acetate accounted for more of methane production and aceticlastic methanogens were predominant in upper sediment layers (ZeppFalz et al., 1999).

The Ashtamudi estuary is influenced by both natural and anthropogenic stresses. Most of the anthropogenic influence cannot be easily detected, as they may be derived from non-point sources of pollution, like for e.g. agricultural and urban runoff. The other visible impacts include tourism and navigation wastes and sewage disposals, discharges from coconut husk retting industries, clay factory and fish processing industries (Babu et al., 2010). Also, monsoon causes an incremental effect by river discharge and organic matter deposition (Jennerjahn, 2012) in the Ashtamudi estuary. In such conditions, it is difficult to assess the nature of anthropogenic stress (Jennerjahn and Mitchell, 2013) and also to declare an area as pristine. Nevertheless, methanogenesis is altered by change in particulate and dissolved nutrient input in Vembanad Lake, India (Verma et al., 2002). Studies on combined environmental and anthropogenic factors controlling methanogenesis have to be carried out, scaling down to the site level for adopting further mitigatory measures. Hence, the study aims to compare changes in environmental variables, as well as methanogenic abundance, and activity in selected human impacted regions of Ashtamudi estuary.

2. Materials and methods

2.1. Study area

Ashtamudi (Latitude 08° 56′ 58″; Longitude 076° 34′ 53″), the second largest brackish water system of Kerala state, India, was declared as Ramsar site in 2002. It is a good example of a wetland that plays a crucial role in hydrological, biological and ecological roles in the region (ISRO, 2013). It is the deepest among all the estuaries of Kerala, having a maximum depth of 6.4 m (near the confluence zone) and has a surface area of 32 km^2 (Babu et al., 2010). The major river discharging into the Ashtamudi is the Kallada (formed by confluence of three rivers: the Kulathupuzha, the Chendurni and the Kalthuruthy), that carries an average runoff of 76,000 million cubic metres of freshwater into the estuary every year (Anoop et al., 2008; ISRO, 2013). The lagoon is palm shaped, with eight prominent arms and hence the name Ashtamudi. It joins the sea through a 200 m wide deep gut as a permanent opening at Neendakara, which is one of the important fishing harbours in India (Babu et al., 2010). Overall, turbidity of water is low during both seasons and agriculture is the dominant land use of the catchment of this wetland (ISRO, 2013). Apart from being a major source of navigation (National Waterways 3), multiple anthropogenic pressures from chemical industries, coconut husk retting for coir industry, organic contamination from fishing and its processing industries plus urban/sewage discharges affect the estuarine environment considerably (Babu et al., 2010). The significance of sampling stations (Fig. 1) with respect to anthropogenic pressures were as follows: S1 – confluence of river Kallada and pressure owing to population, particularly, direct disposal of sewage; S2 – clay factory; S3 – part of the Ashtamudi estuary where the sediments dredged for widening of national waterways were dumped; S4 – fishing harbour, hydrocarbon discharge from mechanized fishing boats, fish processing industries; S5 – coir retting industries (retting of coconut husk to separate fibre); S6 –a solid waste plant of Kollam city in a vast area (4 acres).

2.2. Sample collection and preparation

Sediment samples were collected manually, from 6 stations of the Ashtamudi wetland, using a PVC core (10 cm length and 4 cm wide) and also a Van Veen's grab. Samples obtained by grab sampling were used for physicochemical analyses, whereas vertical microbial distribution studies (depth wise: 0–5 and 5–10 cm) were made possible with core samples. Those samples consisted of 3 replicates and were performed during summer (April, 2013) and monsoon (August, 2013). Samples of overlying water were collected in triplicate using a Dussart-flask water sampler. Sediment samples for microbial studies were collected in sterilized sample bottles, brought to the lab and stored at 4 °C. Cores obtained in each sampling station were segmented in two sections (0–5 cm and 5–10 cm) under a nitrogen atmosphere. After, each section was homogenized in sample bottles by steady shaking (Orbitary shaker - Kemi). Subsamples were immediately taken to quantify methanogenic archaea (Torres-Alvarado et al., 2013). The remaining sediment samples were maintained under 4 °C, in a refrigerator, to