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Chemical characterization of a marine conditioning film

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ABSTRACT

Conditioning film grown on glass panels was analyzed for organic carbon and nitrogen, total carbohydrates, aldoses, uronic acids, and proteins. These compounds showed non-linear increase over the period of immersion. Total carbohydrate-C, aldose-C, uronic acid-C, and protein-C accounted for 5.59–23.50%, 0.6–5%, 1–5%, and 4–14% of organic carbon, respectively, while aldose-C accounted for 10–72% of the total carbohydrate-C. Aldoses such as rhamnose, arabinose, xylose, mannose, and glucose were generally present in the conditioning film. A principal component analysis based on weight percentages of aldoses established three factors that contributed ~77% of the total variance. The first factor was influenced by the abundance of rhamnose and accounted for 41% of the total variance. The second factor was influenced by the concentrations of arabinose and xylose, whereas the third factor was controlled by ribose and galactose, thereby implicating the role of terrestrial and microbial input to the conditioning film. These changes in the chemistry of the conditioning film may play some role in influencing microbial adhesion to surfaces.

1. Introduction

Surfaces immersed in an aquatic environment adsorb dissolved organic matter (Baier, 1972; Loeb and Neihof, 1975; Taylor et al., 1997; Bhosle et al., 2005). This is defined as the conditioning film or the molecular film (Baier, 1972; Loeb and Neihof, 1975; Taylor et al., 1997). Conditioning film is composed of many compounds including glycoproteins (Baier, 1980), humic material (Loeb and Neihof, 1975), proteins, lipids, nucleic acids, polysaccharides, and aromatic amino acids (Taylor et al., 1997) and/or unspecified macromolecules (Zaidi et al., 1984). Of these constituents, proteins were the first compounds followed by carbohydrates to adsorb onto the stainless steel panels immersed in seawater (Compere et al., 2001; Poleunis et al., 2002, 2003). It appears that cleaning procedures and substratum surface properties may influence the chemical composition and the amount of the adsorbed material (Little and Zsolnay, 1985; Taylor et al., 1997; Compere et al., 2001). Changes in the chemical composition of the conditioning film may influence surface charge, wettability, surface free energy, and surface roughness of the substratum. These changes may play an important role in subsequent colonization by microorganisms, especially bacteria (Dexter, 1979; Baier, 1980; Fletcher and Marshall, 1982; Schneider, 1997; Bakker et al., 2004).

Carbohydrates are common and important structural and storage constituents in marine and terrestrial organisms. They account for ~10–50% of dissolved organic carbon in marine waters (Pakulski and Benner, 1994; Amon and Benner, 2003). Storage carbohydrates are rapidly utilized by the in situ organisms (Handa and Tominaga, 1969; Tanoue and Handa, 1987). This results in the accumulation of relatively fewer degradable structural carbohydrates in marine waters. Assessing the abundance and composition of neutral sugars and their composition is a useful tool in determining the sources of organic matter (Cowie and Hedges, 1984; Skoog and Benner, 1997; D'Souza et al., 2005).

Carbohydrate polymers appear to play an important role in biofilm development. They are one of the most abundant constituents of the conditioning film or molecular film (Compere et al., 2001). Carbohydrate polymers adsorbed to surfaces may also contain uronic acid, pyruvate, sulphate, and proteins (Decho, 1990). It is well known that these compounds may play an important role in the subsequent colonization by microorganisms. However, earlier studies have mostly focused their attention on the characterization of the bulk parameters such as carbon and nitrogen and/or the presence of the specific functional groups in the conditioning film using surface analytical techniques. These studies have provided useful information on the bulk chemical composition of the conditioning film. Nevertheless, little information is available on the molecular composition, nature, and short-term temporal variability of the organic matter in general, nor on the carbohydrates and non-carbohydrate constituents in particular, of the conditioning film (Bhosle et al., 2005). A proper understanding of the chemical composition and kinetics of conditioning film formation is required to better explain biofilm growth and microbial adhesion mechanisms. Furthermore, the amount and relative contribution of

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individual organic constituents associated with organic carbon such as amino acids and sugars and their temporal variations provide information on the nature and degradation state of the organic matter (Cowie et al., 1995; Bhosle et al., 2005).

The aims of the present research were: (1) to study the dynamics of carbohydrates, uronic acid, and proteins in the adsorbed organic matter; (2) to determine the changes in the composition of neutral carbohydrates over the period of immersion; and (3) to use the concentration and composition of carbohydrates to assess the nature of the adsorbed organic matter. To the best of our knowledge, this study presents the first data set on the dynamics of total carbohydrate, aldoses, and uronic acid in a conditioning film.

2. Materials and methods

2.1. Chemicals and reagents

Glass cover slips were purchased from Blue Star, India. Standard sugars and, myo-inositol were obtained from Sigma (St. Louis, MO, USA). Sodium carbonate, NaOH, HCl, and methanol were of high purity AR grades and were purchased from s.d. Fine Chemicals, Mumbai. Methanol was double distilled before use. Distilled water was purified using a UV-Milli-Q water purification system (Millipore, Bangalore, India).

2.2. Preparation and deployment of the glass cover slips

The development of the conditioning film was studied using glass cover slips (hereafter called glass panels). Before use the glass panels (22 × 60 mm) were thoroughly cleaned with chromic acid solution for 5 h, washed with UV-treated Milli-Q purified distilled water followed by methanol, and dried in an oven. Individual panels were covered with pre-ashed aluminium foil (450 °C for 5 h). The panels were then kept in a muffle furnace and ashed at 450 °C for 5 h.

2.3. Deployment of the glass panels in running seawater

Glass panels were fixed onto clean PVC racks (10.6 × 40 × 4 cm). Each rack had 28 slots to hold 56 glass panels. Before immersion, PVC racks were covered with PVC lids in order to avoid an air–water interface (Fig. 1). The entire assembly was then immersed in a glass tank (length 90.15 cm, breadth 45.75 cm, height 45.75 cm) filled with seawater. The glass tank was connected to a reservoir tank in which the surface seawater of Dona Paula Bay, on the west coast of India, was pumped. Seawater flow in the glass tank was set at 500 ml/min. Once the PVC racks containing the glass panels were properly installed in the glass tank, the PVC cover was removed. The panels were deployed during July and November 2006.

2.4. Sampling

Panels were retrieved at 0, 2, 4, 6, and 8 h following immersion. Panels that were dipped and immediately removed were defined as 0-h. During each sampling 80 glass panels were retrieved from the PVC rack using clean forceps and transferred to clean beakers kept in the tank. The beakers with the panels were removed from the tank. The panels were then rinsed thoroughly by passing through a series of four beakers (100-ml), each containing 100 ml of the UV-treated Milli-Q purified water so as to remove the loosely attached organic matter. The panels were then transferred to clean glass test tubes, covered with aluminium foil, and stored at −20 °C until analysis. A set of unused panels were used as blanks for all the parameters analyzed.

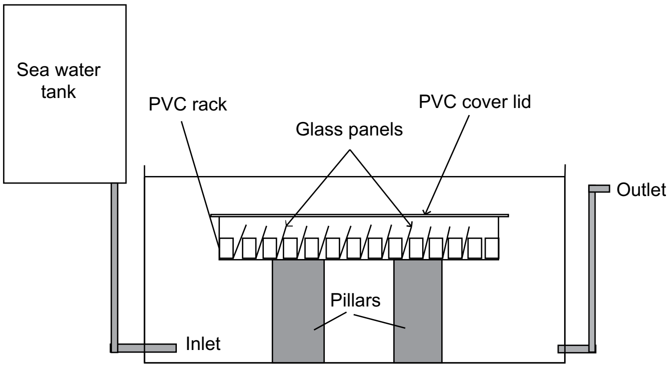

Fig. 1. Schematic diagram of the experimental setup for conditioning film development in running sea water system.

2.5. Bulk parameters analysis

Organic carbon and organic nitrogen content of the conditioning film were estimated following the methods of Parsons et al. (1984) and Smart et al. (1983), respectively. Total carbohydrate was estimated following the phenol–sulphuric acid method (Dubois et al., 1956). Uronic acids were estimated by the method of Filisetti-Cozzi and Carpita (1991). Protein content was analyzed using the method of Smith et al. (1985). The coefficients of variation of these methods based on replicate (5) analysis was better than 10%.

2.6. Aldose composition

The glass panel with the adsorbed organic matter was crushed into pieces using a clean glass rod and transferred into a glass ampoule containing 2 ml of 2 N HCl (Bhosle et al., 1995). The ampoule was flushed with nitrogen and sealed. The sample was then hydrolyzed for 3 h at 100 °C. Liberated sugars were then converted to alditol acetates and analysed using a capillary gas chromatograph (HP GC Model 6890) as described earlier (D'Souza and Bhosle, 2003; D'Souza et al., 2005). In this study the total aldoses have been defined as the sum of all the identified monosaccharides. The contribution of individual aldoses to the total was expressed as a weight percentage. Analytical variability of the GC method was greater than ±6%, whereas it was higher than ±10% for the GC plus sample preparation method.

2.7. Statistical method

A simple regression analysis was carried out to assess the relationships between various parameters (Sokal and Rohlf, 1981) using the Excel software program loaded on a personal computer. Analysis of variance (ANOVA) was carried out to understand the temporal and seasonal variations in the measured parameters. A principal component analysis (PCA) was performed on a personal computer using the Statsoft statistical software package version 5.0 (Statsoft, Inc.). The PCA was carried out using the weight percentage composition of the aldoses (D'Souza et al., 2005).

3. Results and discussion

3.1. Organic carbon and nitrogen in the conditioning film

Concentration of organic carbon and nitrogen increased for the first 4 h and then decreased for the remaining period of immersion during both the July and November 2004 sampling (Fig. 2a,b). However, the observed increase in organic carbon was not significant for time ($F = 1.87$; $P = 0.20$) or season ($F = 2.14$; $P = 0.17$), whereas nitrogen showed significant temporal variations ($F = 3.39$; $P = 0.06$). The concentration of organic carbon was generally greater than that recorded for organic nitrogen. This compares well with the earlier studies reporting the relative abundance of carbon compared to organic nitrogen. For example, Compere et al. (2001) observed a great abundance of organic carbon compared to organic nitrogen in the conditioning film developed on stainless steel. Similarly, Bhosle et al. (2005) reported relatively higher concentrations of organic carbon compared to organic nitrogen in the conditioning film developed on glass panels deployed in surface seawaters of the Dona Paula Bay, west coast of India. The C/N ratio of the conditioning film was generally high and varied from 4 to 16. A greater abundance of organic carbon relative to organic nitrogen results in a higher C/N (atomic) ratio, thereby indicating the presence of degraded organic matter and/or terrestrial organic matter (D'Souza and Bhosle, 2001; Bhosle et al., 2005). Scanning electron microscopic observations of the conditioning film did not reveal the presence of identifiable microorganisms, thereby suggesting that the organic constituents of the conditioning film were not influenced by the presence of microorganisms.

3.2. Total carbohydrates and proteins

Carbohydrates in the conditioning film showed an increasing trend over the period of immersion (Fig. 2c). Conditioning film proteins increased for the first 6 h and then showed some decrease over the remaining period of immersion. Concentrations of carbohydrates and proteins were relatively higher in July, compared to November. This was probably due to higher primary production in