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Influence of live microbes on suspended sediment concentration in coastal ecosystem

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ABSTRACT

Microbes present in both fresh and saline water associate with suspended sediments by secrete extracellular polymeric substance to survive under stressful environmental conditions. Deposition of these bio-contaminated sediments affects the bed ecosystem. When re-suspended, they further affect water quality and health of both human as well as aquatic life. In this study, laboratory experiments are conducted in an annular flume to understand the interaction between suspended sediments attached with microbes (biosediment) and without microbes (non-biosediment) at different mix ratios (M_r = 0:1, 0.25:0.75, 0.50:0.50, and 1:0) and water salinity (S = 0, 1, 2, 5, 10, and 35 g/L). Results show that, rate of deposition increases with an increase in biosediment concentration for any water salinity and mix ratio. In saline water, presence of microbes on suspended sediments induces more deposition until water salinity of 5 g/L and thereafter deposition reduces. Presence of 25% and 50% of biosediment in water increases deposition up to a critical salinity of 1.72 g/L and 2.60 g/L, respectively. Suspended sediment particle size increases with an increase in biosediment concentration and decreases with an increase in water salinity. Fractal dimension results show that, when salinity increases, fragile non-biosediment and dense biosediments are retained in suspension. This study shows that presence of live microbes on suspended sediments affects their depositional behaviour.

1. Introduction

Suspended sediments in water bodies comprise of both organic substances and inorganic sediments, wherein an organic substance include plant and animal parts, animal waste and living microbes/microorganisms (McAnally, 2000). Suspended sediments present in water tend to adsorb pollutants and nutrients from the surrounding environment and aids in growth of microbes. In addition, this association of microbes with suspended sediment improves the survival rate of microbes under stressful environmental conditions such as, rise in water salinity, and temperature, etc. (Hassard et al., 2016). Lind and Dávalos-Lind (1991) reported that, number of microbes attached to suspended sediments is 4 to 7 times more than the free-floating ones. When inflow of nutrient rich sediments into coastal ecosystem increases, it causes a sudden bloom in growth of microbes and results in formation of dead zones. Around the world, about 245,000 km^2 area of dead zones have been recorded and it is increasing exponentially (Diaz and Rosenberg, 2008). Thus, it is essential to understand the interaction between microbes and suspended sediments.

Microbes attach onto surface of suspended sediment by secreting sticky extracellular polymeric substance (EPS) (Paerl, 1975). These polymeric substance exhibit different polarity such as, cationic, neutral, and anionic based on their polymeric group. Non-ionic/neutral EPS (guar gum) induces flocculation and helps to increase the floc size of suspended sediments (Tan et al., 2012). In saline water, anionic EPS enhanced flocculation of suspended sediments compared to neutral EPS (Furukawa et al., 2014). Thus, based on the EPS attachment to suspended sediments, their surface charge, settling property and flocculation ability varies in both fresh and saline water (Eisma, 1986; Droppo et al., 2001; Wingender et al., 2012).

In rivers and other fresh waterbodies, deposition of suspended sediments has direct relation with water ionic concentration (Fukuda and Lick, 1980; Cheng et al., 2006; Chandra et al., 2012). However, at low ionic concentration, deposition through EPS attachment dominates over inter-particular attractive forces (Jamieson et al., 2005). Similar observations were also noticed in saline water. At estuaries wherein fresh river water meets saline sea water, salinity increases drastically and induces deposition of suspended sediments by increasing the attractive forces between sediment particles (Krone, 1962; Mehta et al., 1989; Mikeš and Manning, 2010; Portela et al., 2013). Influence of water salinity on deposition of suspended sediment decreases when suspended sediment matrix has organic substance (Eisma, 1986; Verney

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et al., 2009). However, rise in water salinity has negative influence on attachment of microbes to suspended sediments (Bell and Albright, 1981). Deposition of these bio-contaminated suspended sediments (biosediments) increases the stability of bed (Droppo et al., 2001). This interaction between biosediment and bed has significant impact on the near bed ecosystem (Chapman, 1988). External disturbances such as tidal fluctuation, dredging and human interferences will break the polymeric bonds and re-suspension of biosediments occur (Bilotta and Brazier, 2008; Jovanovic et al., 2017). This further affects water quality and aquatic life in both fresh and saline water ecosystems (Abia et al., 2017). Once re-suspended, these biosediments interact with other suspended sediments that are present in the water column.

The significance of attachment of microbes to suspended sediments has been recognised and discussed by Bell and Albright (1981), Eisma (1986), Jamieson et al. (2005), and Verney et al. (2009). Kranck (1984) showed that suspended sediment with 50% organic matter settled much faster than pure non-biosediment. However, interaction between bio and non-biosediments has not been fully understood. Researchers have shown that, behaviour of suspended sediment alters with the presence of microbes in saline water using artificial EPS (Furukawa et al., 2009; Tan et al., 2012; Furukawa et al., 2014). Yet, the influence of live microbes on the interaction between bio and non-biosediments under different water salinity has not been addressed. Thus, the purpose of present study is to investigate the influence of live microbes on suspended sediment concentration. Laboratory experiments are conducted at different mix ratios of bio and non-biosediments under varying water salinity. Microbes for conducting the laboratory experiments are extracted from field sample and they are artificially cultured. Experiments are performed in an annular flume (Sivakumar and Chandra, 2016) using kaolin as suspended sediment (d_{50} = 6.9 μm). Results are discussed in terms of rate of deposition, equilibrium concentration (C_{eq}), average settling velocity (W_a), equilibrium mean particles size ($d_{50,eq}$) and fractal dimension (F_d). The results obtained from this study help in forecasting and predicting change in both water quality and suspended sediment flux at regions (river confluences and estuaries) wherein bio and non-biosediment interaction takes place.

2. Culturing of microbes

For performing laboratory experiments, a stable source of microbes were cultured using sediment samples collected from Adyar estuary (located in Chennai, Tamil Nadu, India). Initially, 5 g of collected sediment sample was introduced into 100 mL sterilized distilled water and it is kept in a shaker at 120 rpm (revolutions per minute) for 24 h. This process detaches microbes from sediment surface and re-suspends them in water. The supernatant liquid (10 mL) from conical flask is collected and it is reintroduced into mineral nutrient media (140 mL) for growing microbes. Mineral nutrient media for the experiments are prepared similar to Krithika and Philip (2016). Before adding microbes, for all experiments distilled water and mineral nutrient media used are sterilized by autoclaved at 121 °C for 15 min. Dextrose of 0.1 g/L is regularly added (once in every two days) as a food source for microbes. Then, the cultured mix is kept in a mechanical shaker under controlled temperature (30 °C) and it is constantly monitored. The microbial activity in the culture is traced by estimating the absorbance of UV. For this, optical density (OD) of samples (6 mL) collected before adding dextrose at different time durations (t = 0, 1, 2, 7, and 14 days) is determined using UV–Visible spectrophotometer (Techcomp, UK) at 600 nm (Fig. 1). Result shows that, OD increases exponentially with time and attains a steady state after 7 days. This increase in OD proves that microbes are growing in the sample under aerobic condition. Further, the number of microbial colony present in the sample is tested using plate count test. About 10 mL of culture sample is collected after 7 days and serially diluted using sterilized distilled water. The plate count agar medium of 20 mL is spread over petri dish and it is kept under UV light to sterilize the sample. Then, 0.1 mL of serially diluted

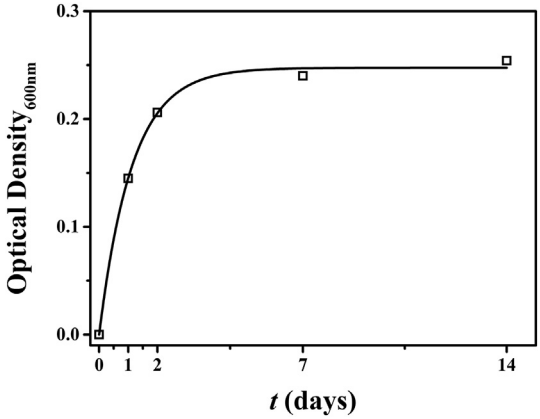

Fig. 1. Optical density (OD) at 600 nm for microbes culture at different time durations.

sample is spread on the agar medium. The dish is sealed and kept in incubation for 24 h at 25 °C. Results showed the presence of 216 × 10^3 CFU/100 mL in the test sample, which confirms presence of live microbes in the artificially grown culture from the field sample.

3. Artificial biosediments

Suspended sediments attached with the microbes are referred as biosediments. For conducting the experiments, these sediments are artificially created by mixing kaolin with nutrient media and microbial culture solution. Exactly, 20 g of artificial sediment (Kaolin) is mixed with 145 mL of sterilized nutrient media in a conical flask (250 mL). Then, 5 mL of microbes are added to the mix and it is kept in a shaker for 7 days. Once in every two days, dextrose is added to the sediment-microbes mix. The time duration and volume ratio of suspended sediment to mineral nutriment media for the experiments are fixed based on OD and volatile solids results. Based on the quantity of sediments used in the annular flume experiments and physical constrains such as difficulty in maintaining and monitoring the samples, concentration of 20 g and 40 g were used for experiments with volatile solids.

For analysing the volatile solids present in cultured sediment, 5 mL sample is collected at initial stage, time, t = 0 (plain sample contains only nutrient media and sediment), t = 5 min (after adding 5 mL of microbial solution) and after t = 7 days of culturing. The collected samples are passed through 0.45 μm filter paper and the retained particles are weighed. The filter paper is oven dried at 105 °C for 24 h to calculate total suspended solids (TSS) and further it is dried in muffle furnace at 550 °C for 1 h, to quantify the volatile suspended solids (VSS). Then, weight difference at different stages are calculated and ratio of volatile suspended solids to total suspended solids (VSS/TSS) is determined. Results showed that, for 20 g of kaolin the VSS/TSS increased by 0.007, whereas for 40 g there was no change. Thus, for all experiments, biosediments are prepared using 20 g of kaolin in 150 mL of nutrient-microbes solution. The results are further verified by repeating the experiment thrice under similar conditions and are given in Table 1. Wherein, 'S_1' is sediment + nutrient media sample at time 0 min, 'S_2' is sediment + nutrient media + microbes mix sample at time 5 min and 'S_3' is sediment + nutrient media + microbes mix sample at time 7 days. It is observed that, sediment sample without microbes has considerable amount of organic matter (~12.1%). The total suspended solids (TSS) in the sample is observed to increase by an average of 33.33% after 7 days of culturing due to the growth of microbes. To quantify the microbes growth, the VSS/TSS is analysed and it is found that on an average 0.8% microbes are present in the sample after 7 days. Although, this value is small, there is a possibility that microbes might have consumed the organic matter present in the sediment for their growth. Finding difference between organic matter contribution