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Characterization and microbial utilization of dissolved lipid organic fraction in arsenic impacted aquifers (India)

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SUMMARY

The coupled role of organic matter (OM) and microbial activity is widely acknowledged in arsenic (As) biogeochemical cycling in sedimentary environments. However, little is known about OM characteristics particularly the dissolved fraction in the Bengal Delta Plain aquifers – one of the worst As impacted regions in the world. Ongoing investigations in As-rich aquifers in Nadia district (West Bengal, India) indicate presence of arsenite {As(III)} oxidizing bacterial communities in the Grey Sand Aquifers (GSA), but absent in Brown Sand Aquifers (BSA). In this study, we investigate the key differences in dissolved organic carbon (DOC) characteristics and its relationship with differences in elemental concentrations, distribution of biomarkers, and utilization of DOC by *in situ* microbial communities in BSA and GSA. We demonstrate a new approach using ENVI™ C-18 DSK discs to pre-concentrate DOC from large volumes of water, and further extract the OM and separate it into different lipid fractions using the solid phase extraction technique. The aquifers show marked heterogeneity in terms of their DOC characteristics and elemental profiles irrespective of their grey or brown color. DOC indicates variable inputs of terrestrial derived OM sources, and OM derived from decomposition and/or microbial cellular components. DOC in the aquifers consist of predominantly *n*-alkanoic acids (~80%) followed by *n*-alkanes and *n*-alcohols. The GSAs indicate high iron (Fe) and manganese (Mn) concentrations, and presence of mature petroleum derived hydrocarbons in DOC. BSA has comparatively lower concentrations of Fe and Mn, and shows absence of mature hydrocarbons in DOC. Experiments in presence of indigenous bacteria from groundwater with DOC lipid extracts as the sole carbon source indicate higher growth in the GSA samples implying preferential use of DOC. The potential availability of DOC in these aquifers can influence the community composition of indigenous heterotrophic microbial flora, which in turn can affect elemental cycles including that of As.

1. Introduction

Dissolved Organic Carbon (DOC) is considered the largest reservoir of reactive organic carbon in the biosphere (Amon and Benner, 1996). DOC provides electron donors, which impact redox conditions and transport of nutrients and/or diffusion of microbial exo-enzymes into the surrounding environment (Thurman, 1985; Metting 1993; Marschner and Kalbitz, 2013). As a result, DOC affects sub-surface biogeochemical processes by controlling the survival of phylogenetically diverse microbial communities (Judd et al., 2006). Sedimentary organic matter (OM) in soil zone plays an important role in recharging the DOC pool in aquifers, and impacting biogeochemical cycling of different elements (Maldenov et al., 2010). Consistent with this, recent studies indicate that sedimentary OM impacts arsenic (As) contamination in the Bengal Delta Plain (BDP) aquifers of India and Bangladesh (McArthur et al., 2001; Harvey et al., 2002; Akai et al., 2004; Islam et al., 2004; Bauer and Blodau, 2006; Rowland et al., 2006). In particular, the extensive use of groundwater for agriculture, and recharge of labile OM into the sub-surface is suggested to accelerate biogeochemical interactions involving microbial release of As into shallow aquifers (Harvey et al., 2002; Akai et al., 2004). Studying groundwater DOC is however complex because its low concentration involves various pre-concentration methods involving large volumes of water (Routh et al., 2001a,b; Simjouw et al., 2005). These methods are tedious and require special sampling and/or analytical capabilities. Moreover it remains unknown if microbes can actually use the DOC fraction in groundwater for respiration in BDP aquifers.

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Previous studies in BDP aquifers suggest that DOC is transported as recharge from base of ponds, wetlands, and/or sub-surface clay/lignite deposits in aquifers (McArthur et al., 2004; Lawson et al., 2013). Hence, it is important to trace its source, chemical reactivity, and potential (bio)-availability in sustaining microbial reactions involved with As cycling in the sub-surface. The current study is part of ongoing investigations on tracing microbial communities and their role in geochemical processes associated with As cycling in aquifers in Nadia district (West Bengal). In order to understand the potential role of indigenous bacterial communities in controlling As and other elemental flux in groundwater, wells were screened and diversity of As(III) oxidizing bacteria were studied using the *aioA* gene as molecular marker in GSAs which were absent in BSA (Ghosh et al., 2014). The overall bacterial community studied in the GSA wells using 16S rRNA as a molecular marker showed predominance of As(III) oxidizing and As(V) reducing bacterial groups (Ghosh et al., 2014). In this study, we aim to: (1) assess the inorganic characteristics in groundwater, (2) identify diagnostic biomarker suites which contribute to the DOC lipid fractions, and (3) establish the capability of *in situ* microorganisms to utilize DOC for their growth. The data will be used to identify the potential role of DOC in sustaining/driving microbial processes associated with As cycling. To the best of our knowledge, this is the first study on DOC characteristics in BDP aquifers, which establishes utilization of dissolved lipids by bacteria involved in As cycling. A better understanding of factors controlling As cycling will provide knowledge toward implementing sustainable and long term cost-effective groundwater treatment methodologies.

2. Study area

The BDP aquifers dating back up to 18 ka BP have been stratigraphically divided into the Pleistocene and Holocene sedimentary deposits (Acharyya et al., 2000). The brown colored iron stained sandy aquifers referred to as the Brown Sand Aquifers (BSA) were deposited during early-mid Pleistocene (Acharyya et al., 2000; Goodbred and Kuehl, 2000). The grey colored micaceous sand bearing aquifers referred to as the Grey Sand Aquifers (GSA) were deposited later during the Holocene (Goodbred and Kuehl, 2000; McArthur et al., 2004). These shallow GSAs have been most commonly tapped into for groundwater abstraction, but unfortunately they have high As concentrations (Biswas et al., 2012; van Geen et al., 2013). High DOC in shallow GSA aquifers was reported (von Brömssen et al., 2007), however such differences in DOC concentration was absent in a detailed survey of large number of GSA and BSA tubewells (Biswas et al., 2012). BSAs have Mn (oxy)-hydroxide reducing environment and high Eh compared to GSAs due to which As mobilization is restricted. In GSAs microbial mediated reductive dissolution of As bearing Fe (oxy)-hydroxides oxidizing *in situ* organic matter leads to mobilization of As (Biswas et al., 2012). The average temperature in Nadia district is 42 °C during summer and 9 °C in winter (Statistical Handbook, 2010), and the average rainfall is 99.66 mm. The aquifers are primarily recharged by precipitation (southwest monsoons, which occur from July to September. The aquifers in this region have a shallow (ca. 2–6 m) groundwater table, which rapidly declines during summer.

Several wells in the Nadia district in West Bengal have been previously tagged as As "hot-spots" (Bhattacharya et al., 2002; Mukherjee et al., 2008; Biswas et al., 2012) because of high As concentration in groundwater, which exceeds far beyond the 10 μg/l guideline for safe drinking water as proposed by the World Health Organization (WHO, 2001). Block Karimpur II is located in the northern part of Nadia district (Fig. 1), where the GSA wells are located. The aquifer sediments vary from sand to clayey loam. The sediments of the shallow GSA wells tap fine to medium sand with large amount of mica in between the clay lenses. Haringhata block is located in the southern part of Nadia district (Fig. 1), where the BSA well is located. The sediment profile of this aquifer contains fine-to medium brown colored sand present between the clay lenses. The deeper clay lenses (after 140 m) indicate presence of un-decomposed plant matter in them.

3. Materials and methods

3.1. Groundwater sampling

The sampling locations were: (1) well 28 (N 23°55.064′, E 088°33.350′) and well 204 (N 23°56.352′, E 088°33.814′), which are GSAs located in Karimpur II Block, and (2) a BSA (well Haringhata; N 22°56.401′, E 088°32.389′) located in Haringhata Block in Nadia District, West Bengal (Fig. 1). Groundwater was collected from three pre-installed wells; a 150 m deep BSA well in Haringhata, and 50 m deep GSA wells in Karimpur II (wells 28 and 204; Fig. 1). A large volume of groundwater (roughly 3 times the well volume) was pumped out to ensure collection of fresh groundwater sample. Temperature, pH (Eco testr pH2) and dissolved oxygen (DO; Eutech DO meter 6.0) were measured at the site. Groundwater samples collected for DOC and total nitrogen was analyzed on a Shimadzu TOC-V CSH analyzer. The samples for metal analysis were immediately acidified with 1 % suprapure HNO_3 and refrigerated until further analyses. To separate As(III) and As(V) species in groundwater, 20 ml of sample was passed at a flow rate of 5 ml/min through a Disposable Cartridge® (Metal Soft Centre, Highland Park, USA, Meng et al., 2001). The filtrates were acidified with 1% HNO_3 and refrigerated until further analyses. Water quality analysis of dissolved metals was done on a Perkin Elmer NexION 300D ICP–MS. The detection limits were as follows: As 0.32 μg/l, Fe 0.2 μg/l, Mn 0.16 μg/l, K 14.6 μg/l, Ca 14.6 μg/l, Mo 3.68 μg/l, Na 3.72 μg/l and P 18.3 μg/l.

Fifty liters of groundwater was collected from each site in acid-washed low-density polyethylene containers and stored at 4 °C. Suprapure HNO_3 was added to the water samples to lower pH (ca. 5–6), and prevent iron-oxides from precipitating and coagulating the pores. The ENVI™ C-18 DSK (C-18 bonded silica; Sigma–Aldrich) discs were pre-conditioned by passing 5 ml of methanol (MeOH) and 5 ml de-ionized water before using. Groundwater was forced through the C-18 discs using a peristaltic pump and DOC was sorbed. Studies have shown that C-18 discs have good efficiency (up to 70 %) in concentrating DOC over other processes like ultrafiltration (Simjouw et al., 2005). The discs were freeze-dried, weighed and stored in the refrigerator (4 °C) until further processing.

3.2. Total lipid extraction

Lipids were extracted using a modified SPE protocol (Singhamshetty et al., 2013) with a mixture of dichloromethane and methanol ($CHCl_3$:MeOH; 9:1 v/v ratio) on a Dionex 300 automated solvent extractor programmed for a 60 min extraction cycle at 1500 psi at 100 °C, and another cycle at 140 °C. Recovery standard (50 mg/l *n*-hexatriacontane – d_{50}) was added prior to extraction. The extracts were reduced in volume on a Büchi Syncore SPE to ca. 0.5 ml, and later they were blown dry under a gentle stream of N_2. The total lipid extract (TLE) was subsequently separated into two fractions using 6 ml glass columns packed with 500 mg of Supelco Superclean LC-NH-2 (Kim and Salem, 1990). The columns were eluted with $CHCl_3$/isopropanol (15 ml of 2:1 v/v, neutral fraction) and 2% acetic acid in diethyl ether (15 ml; acid fraction). The