Roles of microbial community on arsenic removal in drinking-water

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Abstract: The objective of the study is to evaluate the feasibility of biogenic removal of As in Bangladesh. The study had been conducted at Hazigonj, a highly As contaminated areas in the south east of Bangladesh in the period of May, 2001 to June, 2011. As-contaminated Tube-wells Water (TW) (n=16), microbial mats (MM) (n=16) mainly composed of autotrophic and heterotrophic bacteria associated with photosynthetic algae that have grown in front of As-contaminated TW, and urine (n=95) samples were collected from the locality. HG-AAS analysis of TW showed the high ranges 0.31-2.45 mg/Lₐs, (r=0.999) with an average of 1.53±0.77 mg/Lₐs. Besides this, the average of 0.60±0.70 mg/Lₐs, had been detected in urine samples that also might show the higher intake of As from the TW. Neutron activation analysis (NAA) indicated the higher concentrations of As (390 and 550 mg/kgₐs,) accumulated in two MM obtained near (0.3m) two of the tube-wells, which were several hundred times higher than those of corresponding TW (1.70 and 1.61 mg/Lₐs). Further, in a series of in situ sampling, highest As (390 mg/kg) accumulated in MM, at the upstream near the tube-well faucet (1.70 mg/L). Reduced concentrations (0.74 mg/Lₐs and 0.18 mg/Lₐs) were detected in the downstream, around 4 and 8m apart from the TW, while MM of same spots concentrated 124 mg/kg and 38 mg/kg of As respectively. This study provides the evidence of microbial As accumulation, which may help in designing an effective and economic As bio-removal procedure.

Keywords: As bio-removal; Microbial mats; Tube-well water; SEM-EDX; NAA.

INTRODUCTION

Arsenic (As), a toxic metalloid is a hazardous material for its widespread carcinogenic, mutagenic and teratogenic character, responsible for many diseases such as lung, skin, liver and bladder cancer, gangrenes of toe or some of cardiovascular and neurological effects[l-4]. Recently the evidence of arsenical chronic poisoning has been reported in many parts of the world and has become a serious public health problem[5-10].
About 95% or more of the total population of Bangladesh now use groundwater or tube-well water (TW) for drinking. Unfortunately, more than 60% of shallow and deep TW contains As above the WHO guideline value of 0.01 mg/L and more than 30% of the TW contains As above the Bangladesh standard of 0.05 mg/L. An estimated 30–75 million people in Bangladesh have been exposed to As through drinking water concentration above 0.05 mg/L. Only the high levels of As in TW cause the widespread poisoning in Bangladesh. In Hazigonj, district of Chandpur, the one of the most affected areas in the southeast of Bangladesh, almost 90–94% of wells were contaminated by As.

It is known that, microorganisms can grow and survive in the strong acidic and heavy metals-rich or As-rich conditions. Some autotrophs and heterotrophs also show the ability to use arsenic (arsenite) as their sole or auxiliary source of energy. Bacteria or microorganism in microbial mats (MM) selectively accumulate heavy metals and metalloids as having their own niche in the geo-aqua-ecosystem. In 2002, Frankenberger & Arshad suggested that bacterial or microbial methylation or some other biotransformation may generate less toxic forms of As, and thus can be used in the clean-up of As-contaminated environments. Krumbin also described that the microorganisms in MM have controlled bio-transfer process and the biogeochemical cycles during most of the history of life on the earth. Gadd (2002) proposed that the microorganisms are intimately involved in the biogeochemical cycling of metals or metalloids in the aquatic environment, and have potential applications in bioremediation. Tazaki et al. (2003) showed that microbes could play a great role in the natural bioremediation of As-polluted geo-aqua ecosystem by forming biominerals, such as lollingite (FeAs₂), at Masutomi hot-spring water in Yamanashi prefecture, Japan.

Several kinds of filters are currently available, to mitigate the drinking water problem for the treatment of As-polluted water. However, the use of such kinds of filters is still rather limited. A recent research by Islam et al. (2002) suggested a significant role of MM that grow and proliferated around the As-polluted TW in the attenuation of As-pollution. Islam et al. (2003) also reported that microorganisms in MM were enabled to produce Fe - and P-enriched biomineral, vivianite [Fe₃(PO₄)₂·8H₂O] in the As-polluted TW, which might enhance the formation process of Fe- and As-rich symplectite [Fe₃(AsO₄)₂·8H₂O].

Besides these as far as we know, biogeochemical and metabolic activities of microorganisms has not been focused for the biogenic removal of As-polluted water. It was hypothesized that microorganisms can produce As-containing minerals and/or arsenosugars concomitantly on their body surface and thus stabilize the As from its ionic form in the water.

The present study was specifically designed to evaluate the feasibility of biogenic removal of As from tube-well waters (TW), including a trial in an in situ reactor. We also describe the general situation of As pollution in the sampling area of Hazigonj, Chandpur, Bangladesh.

**MATERIALS AND METHODS**

A highly As-polluted area, Hazigonj in the district of Chandpur located (23°152’N and 90°502’E) in the southeast of Bangladesh (Figure 1). The area is alluvial deposit of old Meghna flood plain. In this study, TW(n=16), MM (n=16) of different colors (brown, green, light-green, yellowish-green, gray, and black) together with Pond Water (PW) (n=1) and River Water (RW) (n=1) were collected in cleaned polyethylene bottles and containers from 18 spouts of 4 villages (DH (1); NA (2); RY (3); BK (4)) in Hazigonj. An in situ reactor was also selected at the spout 1 of location 4, having an overflow of water from a hand-pumped tube-well. Profusely proliferated MM in front of tube-well (P₁, around 0.3m) and from other 2 points (P2 at 4m and P3 at 8m) throughout the upstream to the down were also picked up very carefully (the layer of microorganisms were about 0.5 to 1 mm in thickness on the very minute clay particles) with waters. Both of TW and MM were collected in intact or fixed (fixed 1% gluteraldehyde) and un-fixed condition.

The samples were preserved below 4°C until the assay. The water samples were tested in the field for As-concentration and its relation with Fe²⁺ or total Fe (Fe⁷) by the Pack-test kits.
Water quality

To obtain data on water conditions; pH, ORP (oxidation-reduction potential) and EC (electrical conductivity) were measured by a portable water quality inspection meter (pH; D-12, ORP; D-13, EC; ES-12, HORIBA Ltd. Kyoto, Japan). The measurement was carried out in the field on 4th June 2011. As concentration in water was tested with Pack-test kits (PT) in the field for the preliminary examination, by molybdenum blue method, which had a detection limit (DL) of 0.2-10 mg/L (Kyoritsu chemical-check Lab. Corp.). Pack-test also carried out for Fe$^{2+}$ (DL = 0.1- 5 mg/L) and total iron (Fe$^T$) (DL, 0.2-10 mg/L) concentrations in water (those samples for which readings were bellow the DL was assigned on eye estimation).

Energy dispersive x-ray fluorescence spectroscopic (ED-XRF) analyses for the chemical composition of TW and MM

Water samples were filtered through acid-cleaned nucleopore filter (0.45μm) in a closed syringe (Terumo, 20ml). After filtration, 10 ml of each water samples were pipetted into a small porcelain crucible and then allowed to dry up at a low temperature (about 50 °C) for 48 hours to obtain dried samples. The dried powdered residual samples were weighed and taken onto the miler film for analysis. Similarly, MM were dried in a decicator and then ground to obtain the fine particles. 8–10 mg of each samples was taken onto the miler film for analysis. Analysis were carried out with an energy dispersive X-ray fluorescence (ED-XRF) spectrometer (JEOL JSX 3201 using Rh Kα) operated at an accelerating voltage of 30 kV in a vacuum condition.

Microscopic observations and EDX analysis

(a) Optical microscopy

Optical microscopy was used to identify the presence and variety of microorganisms in the biomats. Pieces of microbial mats were washed with distilled water and then spread on glass slides. Both of the epicopic and DAPI (4, 6-diamidino-2- phenylindole) - stained samples were observed through epicopic fluorescence microscope (Nikon EFD3, Digital camera: COOLPIX 995). A filter UV-1A was used for epifluorescent microscopy. The DNA of bacterial cell in DAPI staining sample shows a fluorescence blue while red one indicate the presence of photosynthetic pigments under the ultraviolet ray (365 nm).

(2) Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) analyses

The scanning electron microscope (SEM) equipped with an energy dispersive X-ray (EDX) spectrometer (JEOL JSM-5200LV and PHILLIPS EDAX PV9800EX) was used to observe the micro-morphology of microorganisms and their chemical composition. Pipette-drawn prefixed (gluteraldehyde, 1%) MM were mounted onto the JEOL filter. The sample was washed again with 2.5% gluteraldehyde twice, rinsed, fixed with t-butyl alcohol, and placed into the liquid nitrogen for freezing after Suzuki et al.[29]. The sample on the filter was dehydrated in the low-vacuum chamber of SEM. After completion of freeze drying the sample was mounted on a carbon tape-posted bronze stub. Dehydrated sample was coated with carbon for analyses. Besides this the natural (un-fixed) MM also were mounted on the stub using carbon tape and air dried.

Figure 1 : Map of the study area showing sampling locations.
The natural dehydrated sample also coated with carbon. Both kinds of samples were analyzed and observed in 15 and 25kV with different magnifications.

**Determination of As in water and urine by HG-AAS**

The total As concentrations of the water and urine were determined by an atomic absorption spectrometer equipped with a flow injection hydride generator (HG-AAS; ZL-4100, Perkin Elmer, Norwalk, CT, USA). The water and urine samples were pre-reduced and analyzed according to Watanabe et al. The detection limits (DL) of the HG-AAS were 1 μg/L in the water and urine. Assay accuracy was ensured by inclusion of reference materials: NIST 1640 (trace metal in water; National Institute of Standards and Technology, Gaithersburg, MD, USA), and NIES 18 (Human urine, National Institute of Environmental studies, Tsukuba, Japan). All the measurements were within the certified ranges.

**Determination of As in MM by neutron activation analysis (NAA)**

Neutron activation analysis had been carried out to find out the As concentration in a very trace level (μg/kg). Selected MM (brown and gray) were analyzed for quantifying the As concentration. About 100 mg (dry wt.) of MM were weighted out, placed in polyethylene bags size of 10 x 10 mm, hot sealed. The analysis was kindly conducted by Dr. Yuichi Hatsuvala by NAA, in the Japan Atomic Energy Research Institute and provided us the data for our use.

**RESULTS**

**Water quality and the concentration of As, Fe^{2+} and total iron (Fe^{T})**

The water quality including the concentrations of As, Fe^{2+} and Fe^{T} in different sampling locations are given in TABLE 1.

All the TW showed slightly basic character, while the water of pond (PW) and river (RW) was slightly acidic. High concentrations of As were detected in the TW of different locations, which ranged between 2~7.5 mg/L in the field (due to the interference of PO_{4}). Further selected TW (n=8) including PW (n=1) and RW (n=1) were analyzed in the laboratory by HG-AAS. TW results showed the ranges between 0.31-2.45 mg/L_{As}, (r=0.999) with an average of 1.53±0.77 mg/L_{As}. While no trace of As was detected in the PW or RW. Most of the TW showed a circum pH value, however, the pH value (pH 7.5) of water in spout 1 of location 3, is slightly elevated that contains high As with low Fe and high EC. However, the water sample of spout 1 from location 4 also showed slightly elevated pH with high concentration of Fe and high amount of As with average EC. Besides this, in normal pH (7 or 7.1) less amount of As with high Fe and low EC was noticed. Further, the high values of ORP in all samples, confirmed the oxidative conditions of waters.

**Determination of As among the samples of TW and MM by HG-AAS and NAA**

Concentrations of As in MM and their corresponding TW are compared in TABLE 2. Selected samples of TW and MM have been analyzed by HG-AAS and NAA respectively. 550±2 and 390±8 mg/kg of As were detected in two MM (one is brown in color, from spout 4 of location 1 and the other is gray in color, from spout 1 of location 4), which were several hundred times higher than those of corresponding TW (1.61 and 1.70 mg/L of As). All of the TW showed the high concentration of As with an average of 1.53±0.77 mg/L (n=10), ranging between 0.31-2.45 mg/L. However, in the pond and river water, As was not detected. In addition, in situ experiment showed the reduction of As concentration in an overflow of a TW (one tube-well out of 16s). HG-AAS result showed that most of the As (390 mg/kg) was accumulated in MM near the TW (1.70 mg/L of As) faucet. Reduced concentrations, 0.74 mg/L and 0.18 mg/L, of As were detected in the downstream, around 4 and 8m apart from the tube-well respectively, while MM of the same spots contained 124 mg/kg and 38 mg/kg of As, respectively.

**Microscopic observations and EDX analysis**

(a) Optical microscopy of microbial mats (MM)

Sixteen MM were examined by optical microscopy, and diversities of microorganisms were found to proliferate near the As-polluted tube-well water; all data have not been incorporated in the paper. The grey and black MM were also collected from the surroundings of one of the tube-wells and from the nearest outflow points (P1~P3) at the spout 1 of location 4 (Figure 2).
TABLE 1: Water quality including the concentrations of As, Fe$^{2+}$ and Fe$^{3+}$ in different spouts of sampling locations in Hazigonj, Chandpur, Bangladesh.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sampling points</th>
<th>pH</th>
<th>ORP (mV)</th>
<th>EC (mS/cm)</th>
<th>Fe$^{2+}$ mg/L P-T</th>
<th>Fe$^{3+}$ mg/L P-T</th>
<th>As mg/L P-T</th>
<th>As mg/L HG-AAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH (1)</td>
<td>Spout 1</td>
<td>7.1</td>
<td>212</td>
<td>0.9</td>
<td>1.70</td>
<td>7.50</td>
<td>2.0</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>7.0</td>
<td>219</td>
<td>0.7</td>
<td>1.80</td>
<td>9.00</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>7.0</td>
<td>216</td>
<td>0.7</td>
<td>0.80</td>
<td>3.00</td>
<td>3.0</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>Spout 4</td>
<td>7.2</td>
<td>195</td>
<td>2.8</td>
<td>1.20</td>
<td>1.80</td>
<td>5.0</td>
<td>1.61</td>
</tr>
<tr>
<td>NA (2)</td>
<td>Spout 1</td>
<td>7.2</td>
<td>280</td>
<td>0.5</td>
<td>0.05</td>
<td>1.00</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>7.2</td>
<td>273</td>
<td>0.6</td>
<td>0.10</td>
<td>1.50</td>
<td>2.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>7.1</td>
<td>267</td>
<td>0.4</td>
<td>1.00</td>
<td>1.50</td>
<td>3.0</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Spout 4</td>
<td>7.2</td>
<td>263</td>
<td>0.4</td>
<td>1.20</td>
<td>2.50</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 5</td>
<td>7.2</td>
<td>264</td>
<td>0.4</td>
<td>0.08</td>
<td>0.06</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>RY (3)</td>
<td>Spout 1</td>
<td>7.5</td>
<td>217</td>
<td>1.3</td>
<td>0.15</td>
<td>0.06</td>
<td>7.5</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>7.3</td>
<td>208</td>
<td>1.7</td>
<td>0.06</td>
<td>0.50</td>
<td>6.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>7.4</td>
<td>222</td>
<td>1.7</td>
<td>0.05</td>
<td>0.20</td>
<td>6.2</td>
<td>2.05</td>
</tr>
<tr>
<td>BK (4)</td>
<td>Spout 1</td>
<td>7.2</td>
<td>250</td>
<td>0.5</td>
<td>1.90</td>
<td>1.50</td>
<td>3.0</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>7.2</td>
<td>251</td>
<td>0.6</td>
<td>1.50</td>
<td>1.50</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>7.1</td>
<td>238</td>
<td>0.8</td>
<td>0.20</td>
<td>0.50</td>
<td>5.0</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>Spout 4</td>
<td>7.5</td>
<td>227</td>
<td>0.7</td>
<td>1.00</td>
<td>2.50</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>PW (5)</td>
<td>Spout 1</td>
<td>6.8</td>
<td>296</td>
<td>0.3</td>
<td>0.02</td>
<td>0.55</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td>RW (6)</td>
<td>Spout 1</td>
<td>6.9</td>
<td>291</td>
<td>0.3</td>
<td>0.06</td>
<td>0.20</td>
<td>nd</td>
<td>0.00</td>
</tr>
</tbody>
</table>

pH: indexing hydrogen ion concentration; ORP: oxidation - reduction potential; EC: electric conductivity; Fe$^{3+}$: total iron; P-T: Pack-test (Kyoritsu pack-test kits); HG-AAS: hydride generation atomic absorption spectroscopy ($r=0.999$); -: no data; nd: not detected; DH: Dherra; NA: Noadda; RY: Raycho; BK: Borkul; PW: Pond water; RW: River water

TABLE 2: Arsenic concentrations in microbial mats and corresponding tube-well waters in different sampling spouts, provided with an in situ reactor, and pond and river waters in Chandpur, Bangladesh.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sampling points</th>
<th>HG-AAS TW mg/L</th>
<th>INNA MM mg/kg</th>
<th>Location</th>
<th>Sampling points</th>
<th>HG-AAS TW mg/L</th>
<th>INNA MM mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH (1)</td>
<td>Spout 1</td>
<td>0.39</td>
<td>-</td>
<td>*Spout 1</td>
<td>1.70</td>
<td>390.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>-</td>
<td>-</td>
<td>BK (4)</td>
<td>Spout 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>1.81</td>
<td>-</td>
<td>Spout 3</td>
<td>1.95</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 4</td>
<td>1.61</td>
<td>550.00</td>
<td>Spout 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NA (2)</td>
<td>Spout 1</td>
<td>-</td>
<td>-</td>
<td>Av±sd</td>
<td>1.53±0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>-</td>
<td>-</td>
<td>Range</td>
<td>0.31-2.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>0.31</td>
<td>-</td>
<td>PW (5)</td>
<td>Spout 1</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 4</td>
<td>-</td>
<td>-</td>
<td>RW (6)</td>
<td>Spout 1</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spout 5</td>
<td>-</td>
<td>-</td>
<td>*Point P1</td>
<td>1.70</td>
<td>390.00</td>
<td></td>
</tr>
<tr>
<td>RY (3)</td>
<td>Spout 1</td>
<td>2.45</td>
<td>-</td>
<td>IR</td>
<td>Point P2</td>
<td>0.74</td>
<td>124.17</td>
</tr>
<tr>
<td></td>
<td>Spout 2</td>
<td>-</td>
<td>-</td>
<td>Point P3</td>
<td>0.18</td>
<td>37.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spout 3</td>
<td>2.05</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HG-AAS: hydride generation atomic absorption spectroscopic data in mg/L; INAA: epithermal neutron activation analytical data in mg/kg; TW: tube-well water; MM: microbial mats; DH: Dherra; NA: Noadda; RY: Raycho; PW: Pond Water; RW: River Water; IR: in situ reactor; -: no data; *: in situ experiment was done in spout 1 of location 4

Optical micrograph of the grey MM (collected from point P1 of Figure 2) showed the various colonies of microorganisms (Fig not shown). DAPI-stained epifluorescence micrographs revealed the presence of bacterial cells, mainly bacilliform (about 1~2μm), cocccoid (about 2μm), rod and filamentous types of mi-
crobces, which absorbed blue and red parts, of fluorescence indicating the presence of DNA in bacterial cells, and of photosynthetic pigments in chromatophores of photo-autophytic bacteria (autotrophs) or in other algal filaments. Microbial diversities has also been observed in microbial mats from the upstream to the downstream showed a fewer bacteria without any photosynthetic pigments.

(2) Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) analyses

Two kinds of samples, fixed and un-fixed MM were observed and analyzed by SEM-EDX. Scanning electron microscopic (SEM) observation recognized the microbial colonies in the grey MM (fixed), collected from point P1 of spout 1 in location 4 (P1 in Figure 2). A common view of entire colony indicated various kinds of microorganisms, such as bacilli (b), coccoidal (c) and rods (r) associated with different types of algal (a) filaments and granular particles (A-C in Figure 3). EDX analysis (total area of A) showed the presence of chemical components such as Al, Si, P, Ca and Fe with the traces of Mg, S, K and Ti in gray MM after washed and rinsed with glutaraldehyde and with t-butylalcohol (inset of A). The colonial form of bacillus-typed bacterial cells, about 1.0µm in size, (magnified view of A) adhered onto the surroundings of filament-shaped substrate / Algal filament (B in Figure 3). Coccoidal (1.5~2µm) and filament-shaped bacteria (magnified view of A) existed in aggregation around the other filamentous microbes, might be of algae (C in Figure 3). Notably the high concentration of As was observed in gray MM by ED-XRF and INAA analysis (see Figure 4 MM1, and spout 1 of location 4 in TABLE 2). SEM photo micrograph of other fixed brown MM (spout 3 of location 3) indicated the presence of algal filaments in aggregation with various bacterial cells.

EDX analysis (total area of D) showed the presence of Mg, Al, Si, P, Cl, K, Ca and Fe with the traces of Na, S, and Mn in brown MM after washed and rinsed with glutaraldehyde and t-butylalcohol (inset of D). Further, SEM photo micrograph of an un-fixed brown MM (spout 3 of location 3) indicates the colonial forms of coccoidal (about 1~2µm), bacteria associated with a few bacilli on the filament-shaped substratum (E in Figure 3). EDX analysis (total area of E) showed the presence of chemical components such as Mg, Al, Si, P, Cl, K, Ca, Mn and Fe with the traces of Na, S and As (F in Figure 3).

Spectra of As attenuations in TW by MM

ED-XRF spectra plotted on graphs (Figure 4) showed the comparative reduction of arsenical concentration in tube-well water, which is corresponding to the predominated microbial mats produced in the different points throughout the upstream to the downstream (P1~P3 in Figure 2) in an in situ reactor. As, Fe, Si, Ca, and other elemental concentration were found higher in the microbial mats (MM1 in Figure 4) than that of water (TW1 in Figure 4) close (around 30 cm) to the tube-well flow point (tube-well; P1 in Figure 2 of location 4). Comparatively low concentration of As with other heavy metals was found in water at the point P2 (TW2 in Figure 4), and a very few As concentration (0.37 mg/L) was observed in water (TW3) at the point P3 at a distance of 8 m. Besides this microbial mats (MM2 in Figure 4) in the downstream showed a little concentration of As, while a very few traces of As was detected in MM3. However, the spectra suggesting that significant amount of dissolved As in water was uptaken by microbial mats at points P1 and P2. As a result water at the point P3 doesn’t show any significant As peak excepting the other elements.

DISCUSSION

The general situation of As pollution in Hazigonj, Chandpur, Bangladesh

In this study 16 tube-well waters collected from 4 locations (villages: (1) to (4)) of Hazigonj, Chandpur, confirmed that the sampling locality is one of the extremely As-polluted areas in the southeast Bangladesh.
Figure 3: Scanning electron microscopic images showing the presence of various kinds of microorganisms (r, rod; b, bacilli; c, coccoidal; a, algal filaments)
90-95% inhabitants of the locality use the As-polluted TW as a source of drinking and cooking purpose. As a result, a lot of people are going to be exposed to As and some of them already have been suffering from melanosis, keratosis, hyperkeratosis etc.\textsuperscript{[18]} In addition, urinary analysis of this study (n= 95) showed the ranges between 0.15 to 4.29 mg/L\textsubscript{As}, with an average of 0.60±0.70 mg/L\textsubscript{As}, which is comparable to the other studies (TABLE 3). The result of urinary As also might show some correlations between the intake of As from TW, as it is a useful indicator of exposure\textsuperscript{[30]}.

**Specific discussion on the feasibility of biogenic removal of As**

For the generation of energy some of the prokaryotes use arsenic oxyanions, either by oxidizing arsenite or by reducing arsenate. Arsenic cycling may takes place in the phylogenetically diverse and a wide ranged microorganisms in the absence of oxygen and can contribute to organic matter oxidation\textsuperscript{[31]}. Nonetheless,
our understanding on the biogenic removal of arsenic is progressing thanks to studies investigating microbial transformations of arsenic as well as their metabolism\cite{32,33}.

The main objective of the present study was to evaluate the feasibility of biogenic removal of As. In this study, 16 MM proliferating around the As-polluted TW are capable of accumulating several hundred times higher concentrations of As from the corresponding TW. For example, most of the As $\left(550 \pm 2 \text{ mg/kg}\right)$ was accumulated in MM from the TW $\left(1.70 \text{ mg/L}\right)$ faucet. It is identified that a group of coexisting microbes mainly photoautotrophic and heterotrophic bacteria and photosynthetic algae in MM are proliferating in an As-polluted TW system. They are found enable to transport As ions into their body surface with other ions. Normally, the cellular systems actively transport ion through their membrane in order to maintain osmotic stability\cite{34}. The cell wall of microbes contains proteins, polysaccharides, amines, or polyamines\cite{35-37}. Microorganisms can produce macromolecules outside of their cell wall, commonly consisting of polysaccharides together with some protein, DNA and RNA\cite{38,39}.

FTIR analysis of microbial mats showed the presence of peptide linkage functional groups on the surface of microorganisms, which can interact with metals. The importance of $\text{–COOH}$ (carboxylic) and $\text{–NH}_2$ (amino) groups has been emphasized for binding metals\cite{37,40-42}. Usually microorganisms in different kinds of MM (e.g. green, black, and reddish brown) could accumulate heavy metals and toxic elements (e.g. Fe, Mn, Cu, Zn, Cd, Pb, and As) in the geo-aquatic environments. As for example, Ledin\cite{43} reported that Scenedesmus pannonicus could accumulate As ions from the solutions. Furthermore, Labrenz et al.\cite{44} suggested that some bacteria of Disulfobacteriacea family could survive in highly toxic environment and can reduce aqueous As, Se, and Zn concentration by the geochemical and microbial process.

Tazaki et al. suggested that bacteria in reddish brown MM could produce lollingite (FeAs$_2$), an arsenic mineral on their cell and intercellular surface\cite{25}. Islam et al.\cite{27} also reported that microorganisms in MM were enabled to produce Fe - and P - enriched biomineral, vivianite $\left[\text{Fe}_3\left(\text{PO}_4\right)_2 \cdot 8\text{H}_2\text{O}\right]$ in the As-polluted geo-aquatic environment, which might enhance the formation process of Fe and As - rich symplectite $\left[\text{Fe}_3\left(\text{AsO}_4\right)_2 \cdot 8\text{H}_2\text{O}\right]$ by the isomorphic substitution between $\text{P}^{5+}$ and $\text{As}^{5+}$.

Besides this, Granchinho et al. reported that marine green algae could accumulate As and form complex arsenic-compounds of ribofuranosides derivatives, commonly termed as arsenosugar, which also showed a high As concentration\cite{45}. In addition, Lunde noted that the capacity of As accumulation in freshwater algae is comparable and similar to that of marine algae\cite{46}. Furthermore, Kaise et al. demonstrated that the freshwater algae (in culture medium) could also be accumulating As by the process of biomethylation\cite{47}. The inorganic form of As can be biomethylated by certain microbes to the gaseous arsines or to monomethyl arsenic acid (DMAA), which are believed to be a part of a detoxification mechanism in living organisms\cite{42}. However, in a clinical study Hsueh et al. reported that MA and DMA have been shown to be more genotoxic than inorganic arsenic\cite{48}.

Furthermore the notable findings (Islam ABMR., unpublished data) of phospholipids ($\text{P} - \text{O} - \text{C}$) and pyrophosphate ($\text{P} - \text{O} - \text{P}$), confirmed the existence of organic matters and living cells respectively in MM\cite{49}. Presumably, pyrophosphate maintain the ribose chain of cell membrane\cite{48,50-52}, suggesting that the possible formation of arsenosugar / arsenolipids in MM, when microorganisms accumulate As from TW.

After binding As, it cannot be released easily from the cell, as it forms an arsenic-protein complex\cite{53,54}. It might be considered for those microorganisms, which have high molecular polymeric peptide bonded protein compound in their cell surface\cite{52}. In this study SEM-
EDX analysis also indicated that As cannot be released easily from the cell surface of microorganisms without using any strong solvent/reagent in normal temperature and pressure (E-F Figure 3).

Hence, microorganisms in MM may have accumulate As with other heavy and toxic elements from As-polluted TW through adsorption, precipitation, complexation, transportation or by biomethylation\textsuperscript{[24,25,42,47,55,56]}. The similarities have been found in the present study that bacteria or algae in MM are capable of accumulating As with other elements and immobilize them by forming biominerals and arsenosugars. The data are in agreement with some previous reports for the significant role of microorganism in incorporation of As into the minerals and or to arsenosugars\textsuperscript{[22,24,25,45,57]}. In our study, suggests that MM, consisting of autotrophic and heterotrophic bacteria mainly of bacillus, coccus, rod and filamentous types, associated with photosynthetic algae, could be responsible for accumulation of As, into their cell surface from TW, by some of the complex biogeochemical processes, where contributions of polysaccharides, amines or polyamines, pyrophosphate and phospholipids are notable.

**CONCLUSION**

Microorganisms, autotrophic and heterotrophic bacteria including photosynthetic algae in microbial mats (MM) are capable of accumulating several hundred times higher concentrations of As from the corresponding tube-well water (TW), by forming biominerals and or arsenosugars. Hence the biogenic removal of As is quite feasible, where MM will play the important role as having their own niche, have the metal accumulating ability in toxic environment. The results of this study may help in designing an effective and economic As bio-removal procedure.

**ACKNOWLEDGEMENTS**

We wish to express our thanks to Dr. Yuichi Hatsukawa for providing NAA data, Prof. Alauddin Ahmed for continuous supports and helpful suggestions regarding sampling in Bangladesh, Drs. Ryutaro Ohtsuka, Chiho Watanabe, Bae Munjoo and Belkova Natalia for useful comments. This study was supported by the grants from the Japanese Ministry of Education, Science and Culture to Kazue Tazaki, Kanazawa University.

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