Arsenic pollution and the probable bioremediation : An investigation on the severity and the biogeochemical activities of As-resisting microbes in biomats contributing in bioremediation by forming biominerals in the As polluted geo-aquatic environment

氏　　名	Islam ABM Rafiqul
生年月日	
本　　籍	バングラディシュ
学位の種類	博士（学術）
学位記番号	博甲第605号
学位授与の日付	平成15年9月30日
学位授与の要件	課程博士（学位規則第4条第1項）
学位授与の題目	Arsenic Pollution and the Probable Bioremediation − An investigation on the severity and the biogeochemical activities of As-resisting microbes in biomats contributing in bioremediation by forming biominerals in the As polluted geo-aquatic environment −（砒素汚染と微生物浄化の可能性－砒素汚染被害調査および生体鉱物作用により砒素汚染水の生物浄化に寄与するバイオマット中の耐砒素微生物の生物地球化学的機能に関する研究－）
論文審査委員(主査)	田崎　和江（理学部・教授）
論文審査委員(副査)	加藤　道雄（自然科学研究科・教授）神谷　隆宏（自然科学研究科・助教授） 奥野　正幸（理学部・教授）石賀　裕明（島根大学, 教授）

学位論文要旨

Abstract

Synonymous of poison 'Arsenic' is famous for its carcinogenic, mutagenic and teratogenic characters and treated as a hazards material. Most of these kinds of disease can eventually be developed in those people who drink As polluted water. The present study has focused on the severity of As pollution in Bangladesh with the special attention of probable bioremediation. Biomats found in the As contaminated areas provides clues on the biogeochemical role of specific microorganisms in bioremediation. For his study groundwater and biomats were observed and analyzed to find out the microbial ability in bioremediation of As. Samples were collected from an insitu reactor in one of the highest As polluted areas 'Hazigonj' Chandpur, Bangladesh. Most of the ground waters possess the average pH (6.5 - 7.5) and contains 0.3 - 1.7 ppm of As exceeding the World Health Organization (0.01 ppm) and Bangladesh (0.05 ppm) standards. Besides this, optical and electron microscopic observation revealed that the microorganisms are metabolically and enzymetically active in As polluted water. Epithermal Neutron Activation Analyses (NAA) showed that biomats (colony of microorganisms) can accumulate 390 - 550 ppm of As without any metabolic hazards (while water contains about 0.3 - 1.7 ppm). Further more, Energy dispersive X-ray fluorescence spectroscopy (ED-XRF) of biomats and groundwater in the in situ reactor

verifies that As attenuation occurs. Ground water sampled away (4m) from the source (hand pumped tube well) has very low as concentrations, and As was not detected farther from the source (8 – 12) meters. The As attenuation was mediated by microbes in biomats through adsorption of As on the cell surface and biomethylated to form biominerals. Vivianite, identified in the biomats may have resulted from microbe mediating biomineralization. High dissolved Fe and P concentration was further recognized to promote and enhance the As biomineralization process. The process is believed to be the parts of detoxification mechanisms mediated by the As-resistance microorganisms.

Keywords: Bangladesh; Arsenic pollution; Groundwater; Biomats; Neutron activation analysis; Bioremediation, Vivianite, Flurometric enumeration, Enzymetically active microorganism

Arsenic (As) toxicity is expressed by its widespread carcinogenicity association with diseases affecting the cardiovascular and neurological systems. These diseases develop in people who are exposed to As-contaminated drinking water. Recently, the evidence of arsenical chronic poisoning has been reported in many parts of the world and has become a serious public health problem in some of the Asian countries. Bangladesh is one of the countries that received worldwide attention in the mid 1990s when chronic As poisoning in humans was documented. Groundwater from shallow aquifers have long been used as the primary source of potable water in Bangladesh and have also been identified as the source of As. Several types of filters were used to remedy As contamination in drinking water. However, the feasibility of utilizing filters for long-term and large-scale application is impeded by its high cost and heavy maintenance. Bioremediation has attracted a growing attention in remediation efforts of As-contaminated groundwater due to its fairly low cost and high efficiency.

Present study has focused on the severity of As pollution in Bangladesh with the special attention of probable bioremediation. For this study biomats formed by bacterial colonies and developed in As-contaminated areas were collected along with groundwater, pond and river waters at different locations in Hazigonj, Chandpur, Bangladesh. The severity of As-poisoning in humans was further verified by collecting hair, nail, urine and blood samples from inhabitants (n = 95 persons) exposed to As-contaminated drinking water. These inhabitants have shown symptoms of As-poisoning as verified medically.

The high concentration of As in the collected samples confirms that Hazigonj, Chanpdur exhibits one of the most severe As pollution in southeastern Bangladesh. Drinking water contains 0.3 – 1.7 ppm As, exceeding the World Health Organization (0.01 ppm) and Bangladesh (0.05 ppm) standards. High As concentrations exceeding standard levels were also detected in hair, nail and urine in 70 out of 95 persons tested.

The biomats found in As-contaminated areas provides clues on the biogeochemical role of specific microorganisms in bioremediation. An *in situ* reactor was selected in front of a hand pumped tube-well to observe the role of microbial activity in attenuating As concentrations. Epithermal Neutron Activation Analyses (NAA) performed on the brown and gray biomats show As accumulation mediated by microbes occur nearest the source of As-contaminated groundwater (i.e. hand pumps). Microbes in biomats concentrate As ranging from 390 – 550 ppm. Optical microscopy and Scanning Electron Microscopy (SEM) reveal the presence of autotrophic and heterotrophic bacteria comprising coccus, bacillus and filamentous types closely associated with photosynthetic algae which may be responsible for As accumulation in the biomats. Energy Dispersive X-ray Fluorescence Spectroscopy (ED-XRF) of biomats and groundwater in the *in situ* reactor further verifies that As attenuation occurs. Groundwater sampled away (4 m) from the source (i.e. hand pumps) has very low As concentrations, and As was not detected farther from the source (8 – 12 meters).

The As attenuation observed was mediated by microbes in biomats through adsorption of As on the cell surface and biomethylated to form biominerals and arsenosugars. Vivianite ($Fe_3(PO_4)_2.8H_2O$) identified in the biomats may have resulted from microbe mediating biomineralization. High dissolved Fe and P concentration was further recognized to promote and enhance the As biomineralization process.

In separate laboratory experiments, sealed experiment vessels containing As minerals and sampled groundwater were stored for a year at room temperature, Results show a diversity of enzymatically active bacteria possibly enhanced by the dissolution of some As minerals. Optical microscopy with CFDA stain and SEM confirmed the diversity of the bacterial community.

Consequently, the process is believed to be the parts of detoxification mechanisms mediated by the As-resistance microorganisms. The in situ experiment proved that the bioremediation of As-contaminated areas will be assisted by the biomats containing metabolically and enzymatically active microorganisms.

学位論文審査結果の要旨

本学位論文に関し、平成15年7月31日に第1回審査会を開催、面接審査を行った後、論文の内容について検討した。さらに、8月4日に行われた口頭発表の後に第2回審査会を開き、協議の結果、以下のように判定した。

本研究はバングラデシュのヒ素汚染に焦点を当て、微生物浄化の可能性を検討した。砒素汚染被害調査および生体鉱物化作用により砒素汚染水の浄化に寄与するバイオマット中の耐砒素微生物の地球化学的機能に関する研究を行った。そして、最も砒素濃度の高い地域の4ケ所で試料採取を行った。採取した地下水のpHは6.5-7.5、砒素濃度は0.3-1.7ppmの範囲にあり、WHOの基準値0.01ppm、バングラデシュの基準値0.05ppmをはるかに超えている。また、砒素汚染水中で数種の微生物の繁殖が光学・電子顕微鏡観察により示された。さらに、地下水と数種の砒素鉱物を混合し、1年間静置してできたバイオマットにCFDA色素を加えて光学顕微鏡観察したところ、酵素活性を示す微生物が確認された。砒素濃度0.3-1.7ppmの汚染水に存在するバイオマットの砒素濃度は390-550ppmであり、砒素を汚染水から濃集していることが放射化分析で確認された。また、X線粉末回折分析によりこのバイオマットからvivianite（$Fe_3(PO_4)_2 \cdot 8H_2O$）を同定した。さらに水中の砒素をvivianiteと同じ結晶構造のsymplesite（$Fe_3(AsO_4) \cdot 8H_2O$）として鉱物化することにより、砒素を無害化し濃集していることが推測される。

以上の研究成果は砒素汚染地域のバイオマットに生息する微生物の砒素濃集機構を解明し、水質浄化におけるバイオマットの重要性を示唆した。本申請者は博士（学術）の学位を受けるに値すると判定した。