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Editorial Open Access

Scope of *In-situ* Bioremediation for Polluted Aquifers via Bioaugmentation

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Editorial

Globally, many geographical regions have been identified specifically for polluted groundwater as shown in Figure 1. There is a need for urgent technological interventions in order to supply safe drinking water to major population dependent on groundwater. According to recent report, more than 70 countries are vulnerable to various ill effects over health due to exceeding toxic metals such as arsenic contaminants above permissible limit in groundwater for example Mongolia, Indonesia, China (As range 50-2400 µg/L), Iran, Bangladesh (As range 0.25-1600µg/L), Argentina (As range 100-2000 µg/L) Taiwan (As range 1800 µg/L). According to the reports, more than 49% area of Bangladesh has contaminated groundwater with values of 'As' >10 µg/L and also, some of the Indian states are reported with contamination of heavy metals (up to 1-1300 µg/L) especially Arsenic in West Bengal, Uttar Pradesh Bihar and some regions of Punjab (Malwa), [1].

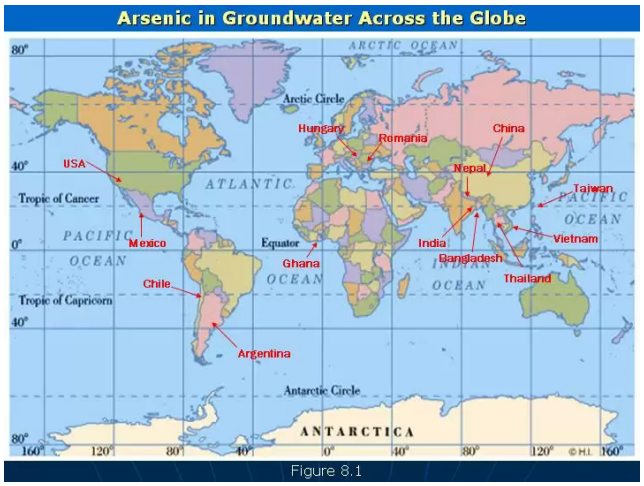

Figure 1: Global dispersion of Arsenic contamination in drinking water

Arsenic has been kept under notorious carcinogen [2] while other metals such as chromium manganese are enlisted under no carcinogenic metals while lead and nickel has been kept in possible carcinogen. The prescribed limit of non-carcinogen metal (such as chromium and manganese) may limit up to 500 µg/L in drinking water while probable carcinogen (such as lead and nickel) should not exceed beyond 20 µg/L. The most potent metals for carcinogenesis are Arsenic out of lead ,chromium and nickel which upon entry in the body (either along with drinking water or through food chain) yield following symptoms; skin lesions over foot, hands, back, hair, various other effects includes lung carcinogenesis, kidney, liver and interfering in various cells enzymes (Table 1). Recently in south west district of Punjab India, Arsenic concentration was reported more than 20-30 folds as prescribed by the WHO under safe limit [3].

Currently, purification of polluted groundwater is a great challenge because of the complex contaminants present and application of conventional technology for its remediation seems to be ineffective since they are highly expensive, lack specificity, give rise to more environmental problems. In-situ approaches or technology were extensively used for reclamation of soil in old days and in current context remediation of groundwater is possible via Bioaugmentation techniques by introducing mixed microbial consortia. In addition to its limited success, this may be highly effective because of its cost effectiveness, environmental acceptability and technically highly viable against complex pollutant in drinking water. This technique is not only very useful for polluted aquifers containing organic pollutants such as gasoline, heating oil, kerosene, jet fuel, and aviation gas, chlorinated hydrocarbons, such as 1,1,1-trichloroethene, carbon tetrachloride, chlorophenols, chlorobenzenes, tetrachloroethylene, PCBs and creosote but also is highly applicable against metal polluted aquifers since microbes are equipped with powerful detoxification metabolism via reduction/transformation catalysed by cytochrome C-reductase, specific metal reductase (actively present in anaerobic), sulphate reductase (thiobacillus which promote bioprecipitation), phosphatase (induces formation of insoluble metal phosphate coat over bacterial cell wall and helps in more trapping of toxic metals [4].

Pollutants	Major Sources	Effect on human health	Permissible Level (ppm)
Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis	0.02
Cadmium	Welding, electroplating, pesticide fertilizer CdNi batteries, nuclear fission plant	Kidney damage, bronchitis, gastrointestinal disorder, bone marrow, cancer	0.06
Lead	Paint, pesticide, smoking, automobile emission, mining, burning of coal	Liver, kidney, gastro intestinal damage, mental retardation in children	0.1
Manganese	Welding, fuel addition, ferromanganese production	Inhalation or contact causes damage to central nervous system	0.26
Mercury	Pesticides, batteries, paper industry	Damage to nervous system, protoplasm poisoning	0.01

Zinc	Refineries, brass manufacture, metal plating, plumbing	Zinc fumes have corrosive effect on skin, cause damage to nervous membrane	15

Table 1: Major sources of heavy metal pollution and their health effect [28]

Thus, introduction of microbes during Bioaugmentation in aquifer may require addition of oxygen, nutrients and other inorganic components separately (such as Nitrogen, Phosphorus, along with other elements required in micromole quantity 0.005-0.05% by weight). These conditions stimulate the growth of microbes and thus organic component degradation is possible rapidly. Initially metabolic growth of cell (lag phase) is slow and because of gene activation related to degradation of pollutants. During these processes, an on line monitoring devices must be developed so that restoration quality of aquifer may be characterized and necessary intervention can be done. In addition under anaerobic conditions addition of electron acceptor may prove beneficial since it will increase possibility of electron acceptance and thus process of biodegradation [5].

There are various techniques available for monitoring overall metabolic activity of cell during growth such as ATP, GTP, phospholipid, and muramic acid and also quality of water bioremediation can be determined by using Hydraulic conductivity (K) which should be equal to greater to 10^{-4} . Anaerobic condition is more supportive for some microbes since it helps some reaction to proceed such as Hydrogenation, Dehalogenation, and carboxylation that augments the organic molecule degradations beside metal uptake. Besides this, proper activation of Arsenate gene such as Ars R,B C, D and H M and production of Arsenic reductase (13-15 kd), a bidirectional enzyme [6] is also reported to play a key role. In addition, ArsC has very critical role in Arsenic mobilization via Dissimilatory arsenate reductase (L0MZ13), via Ars-III arsenic specific proton pump (coded by ArsB and ACR3). Arsenic reductase is a complex protein encoded by Arr system and supports conversion of As^{+3} into As^{+5} while AOX (arsenic oxidase) play opposite role as oxidation of Arsenic. *Thiobacillus ferrooxidans* have increased copy numbers of chromosomal resistance genes against Zinc and Arsenic [7] as identified by Pulsed-field gel electrophoresis (PFGE) while from *Leptospirillum ferriphilum* two arsenic resistant gene have been identified from Biooxidation tank [8] *Deinococcus indicus* has been isolated from arsenic-contaminated aquifer of west bengal [9]

Since arsenic III is toxic and presence of Ars III specific proton pump in some microbes such as *Alkaliphilus oremlandii* and *Shewanella* sp. ANA-3 [6,10,11] *Sulfurospirillum barnesii*, *Sulfurospirillum arsenophilus* [12], *Alkaliphilus oremlandii* [13], *Bacillus arseniciselenatis* [14], a *Desulfosporosinus* species and *Desulfotomaculum auripigmentum* may be a boon in increasing metal uptake strategies via In-situ approaches. The basic idea behind focussing on microbes via In-situ technology is metal-microbe interaction which is (1) capable of uptaking the toxic "heavy metals" from the environment to enhance their growth. (2) Can convert toxic metals into nontoxic form.

The major challenges of Arsenic Bioremediation is identification of key microbial community for harnessing via *In-situ* Bioremediation and fortunately various microbial diversity have been reported that can utilize As(V) as an electron acceptor to support anaerobic growth [15,16,17,12]. With advancement of new techniques, now it is possible to study structure and dynamics of microbial communities via fluorescence in situ hybridization (FISH), rRNA targeted oligonucleotide probes [18], and (ii) in situ PCR [19]. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA fragments [20] and terminal restriction fragment length polymorphism (T-RFLP) has emerged as a powerful technique for monitoring changes in bacterial diversity [21,22]

Strategy of microbes based specific oxidation and reduction of metals has resulted in successful conversion and removal of toxic selenate and selenite to the much less toxic elemental selenium [23]. Another cited example is Biomethylation which yield volatile derivatives such as dimethylselenide or trimethylarsine catalyzed by several bacteria, algae and fungi [24]. Reduction of toxic mutagenic hexavalent chromium to its trivalent form that is less toxic by use of *Bacillus* sp. *JDM-2-1*, *Staphylococcus capitis* [25,26]). Sometimes it has been observed that concentration of Arsenic increases tremendously when sample analysis is done by using certain chemicals such as acid or base [27] for instance when acetate was used to dissolve the complex soil of arsenic rich well, then arsenic concentration increases tremendously due to presence of iron (Fe). In such instance Bioprecipitation by sulfate reducing microbes may help in precipitation of metals at large, thus make separation easier at large scale.

Conclusion

Thus, for successful *In-situ* bioremediation of polluted aquifers, introduction of suitable microbial community is required along with strategic control of pH (which stimulates bioavailability). In addition to bioaugmentation approach, biostimulation is also essentially required for their growth and metabolic activity. In addition to this, specific activation of metal resistance gene, are essential part of transport phenomenon (via cotransport arsenic also removed during Uranium removal). Beside this, *In-situ* bioprecipitation (ISBP) strategies of metals before adding microbes may enhance separation efficiency of process out of aquifers (until K remain above 10^{-4}). Low cost biosensor development for rapid diagnosis of metal using specific biomarkers may improve current scenario (which may be arsenic resistance gene (Ars) unique in sequestering of metals and metalloids detoxification along with transport)

References

1. Josephson J (2002) The Slow Poisoning of Bangladesh. Env health perspect 110: 1147 -1153.
2. Cameron RE (1992) Guide to site and soil description for hazardous waste site characterization. Metals, Environmental Protection Agency (EPA)/ 600/4-91/0291).
3. Hundal HS, Singh K, Singh D (2009) Arsenic content in ground and canal waters of Punjab, North-West India. Environmental monitoring and assessment 154: 393-400.
4. Lloyd JR (2002) Bioremediation of metals; the application of micro-organisms that make and break minerals. Microbiology Today 29: 67-69.
5. Kuhn EP, Suflita JM (1989) Microbial degradation of nitrogen, oxygen and sulfur heterocyclic compounds under anaerobic conditions: studies with aquifer samples. Environmental toxicology and chemistry 8: 1149-1158.
6. Richey C, Chovanec P, Hoeft SE, Oremland RS, Basu P, et al. (2009) Respiratory arsenate reductase as a bidirectional enzyme. Biochemical and biophysical research communications 382: 298-302.
7. Kondratyeva TF, Muntyan LN, Karavaiko GI (1995) Zinc-and arsenic-resistant strains of Thiobacillus ferrooxidans have increased copy numbers of chromosomal resistance genes. Microbiology 141: 1157-1162.

8. Tuffin IM, Hector SB, Deane SM, Rawlings DE (2006) Resistance determinants of a highly arsenic-resistant strain of Leptospirillum ferriphilum isolated from a commercial biooxidation tank. Applied and environmental microbiology 72: 2247-2253.
9. Suresh K, Smith H (2004) Comparison of methods for detecting Blastocystis hominis. European Journal of Clinical Microbiology and Infectious Diseases 23: 509-511.
10. Oremland RS, Stolz JF (2003) The ecology of arsenic. Science 300: 939-944.
11. Mukhopadhyay R, Rosen BP, Pung LT, Silver S (2002) Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev 26: 311-325.
12. Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60: 107-130.
13. Fisher E, Dawson AM, Polshyna G, Lisak J, Crable B, et al. (2008) Transformation of inorganic and organic arsenic by Alkaliphilus oremlandii sp nov strain OhILAs. Am NY Acad Sci 1125: 230-241.
14. Blum JS, Bindi AB, Buzzelli J, Stolz JF, Oremland RS (1998) Bacillus arsenicoselenatis, sp nov, and Bacillus selenitireducens, sp nov: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171: 19-30.
15. Oremland RS, Dowdle PR, Hoeft S, Hoeft S, Sharp JO, et al. (2000) Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. Geochim Cosmochim Acta 64: 3073-3084.
16. Oremland RS, Stolz JF (2005) Arsenic, microbes and contaminated aquifers. Trends Microbiol 13: 45-49
17. Hollibaugh JT, Budinoff C, Hollibaugh RA, Ransom B, Bano N (2006) Sulfide oxidation coupled to arsenate reduction by a diverse microbial community in a Soda Lake. Appl Environ Microbiol 72: 2043-2049.
18. Hahn D, Amann RI, Ludwig W, Akkermans AD, Schleifer KH (1992) Detection of micro-organisms in soil after in situ hybridization with rRNA-targeted, fluorescently labelled oligonucleotides. Journal of General Microbiology 138: 879-887.
19. Hodson RE, Dustman WA, GarG RP, Moran MA (1995) In situ PCR for visualization of microscale distribution of specific genes and gene products in prokaryotic communities. Applied and environmental microbiology 61: 4074-4082.
20. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59: 695-700.
21. Liu W, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of gene encoding 16S rRNA. Appl Environ Microbiol 63: 4516-4522.
22. Garbisu C, Alkorta I (2003) Basic concepts on heavy metal soil bioremediation. European Journal of Mineral Processing & Environmental Protection 3: 58-66.
23. Garbisu C, Ishii T, Leighton T, Buchanan BB (1996) Bacterial reduction of selenite to elemental selenium. Chemical geology 132: 199-204.
24. White C, Sayer JA, Gadd GM (1997) Microbial solubilization and immobilization of toxic metals: key biogeochemical process for treatment of contamination. FEMS Microbiol Rev 20: 503-516.
25. Zahoor A, Rehman A (2009) Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. Journal of Environmental Sciences 21: 814-820.
26. Focardi S, Pepi M, Focardi SE (2013) Microbial Reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Bioremediation Applications.
27. Giloteaux L, Holmes DE, Williams KH, Wrighton KC, Wilkins MJ, et al. (2013) Characterization and transcription of arsenic respiration and resistance genes during in situ uranium bioremediation. The ISME journa 7: 370-383.
28. Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: An overview. Indian Journal of pharmacology 43: 246.