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Impact Of Millions Of Tones Of Effluent Of Textile Industries: Analysis Of Textile Industries Effluents In Bhilwara And An Approach With Bioremediation

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Abstract : It is well known that cotton mills consume large volume of water for various processes such as sizing, desizing, scouring, bleaching, mercerization, dyeing, printing, finishing and ultimately washing. Contaminated air, soil, and water by effluents from the industries are associated with heavy disease burden (WHO, 2002) and this could be part of the reasons for the current shorter life expectancy in the country (WHO, 2003) when compared to the developed nations. Some heavy metals contained in these effluents (either in free form in the effluents or adsorbed in the suspended solids) from the industries have been found to be carcinogenic (Tamburlini et al., 2002) while other chemicals equally present are poisonous depending on the dose and exposure duration (Kupchella and Hyland, 1989). These chemicals are not only poisonous to humans but also found toxic to aquatic life (WHO, 2002) and they may result in food contamination (Novick, 1999). There are sulphide and metal pollutant like fluoride, Arsenic, Molybdenum etc cause several harm effect to the life directly on indirectly.

Bhilwara has 4000 Textile manufacturing units which production exports to 70 countries specially in Europe, South Africa and North American. It has highest number of register private motor vehicle (4 wheeler) in Asia. This is second largest producer of polyester fiber in India, third largest producer of Salt i.e 1/10th production of country and only center in country for producing insulation bricks. This project is characterised of textile industries' effluents in Bhilwara and its Fluoride pollutant separation by Microbes.
Key Words: Effluents, Pollutant, fluoride, Arsenic, Bioremediation.

Introduction To Bhilwara

Presently known as textile Industry came to be known as Bhilwara because it was mostly inhabited by Bhils in old days. Over the years it has emerged out as the TEXTILE CITY of Rajasthan. It is 260 km away from Jaipur known as **"Pink City".**

The town of Bhilwara has extreme climatic conditions and summer and winter season are the main seasons, while monsoon is short lived, and sometimes does not even occur in this place. The place gets limited rainfall throughout the year and draught conditions are common in the area. The summer season is from the months of March to May and temperatures range from 28°C to45°C

The formation of Rajasthan, a uniform pattern was evolved for the administration of the entire Rajasthan State and it is divided into six divisions. Bhilwara district is included in Ajmer division. The district is now divided in to twelve sub-divisions; Bhilwara is having 1783 villages as per census of 2002.

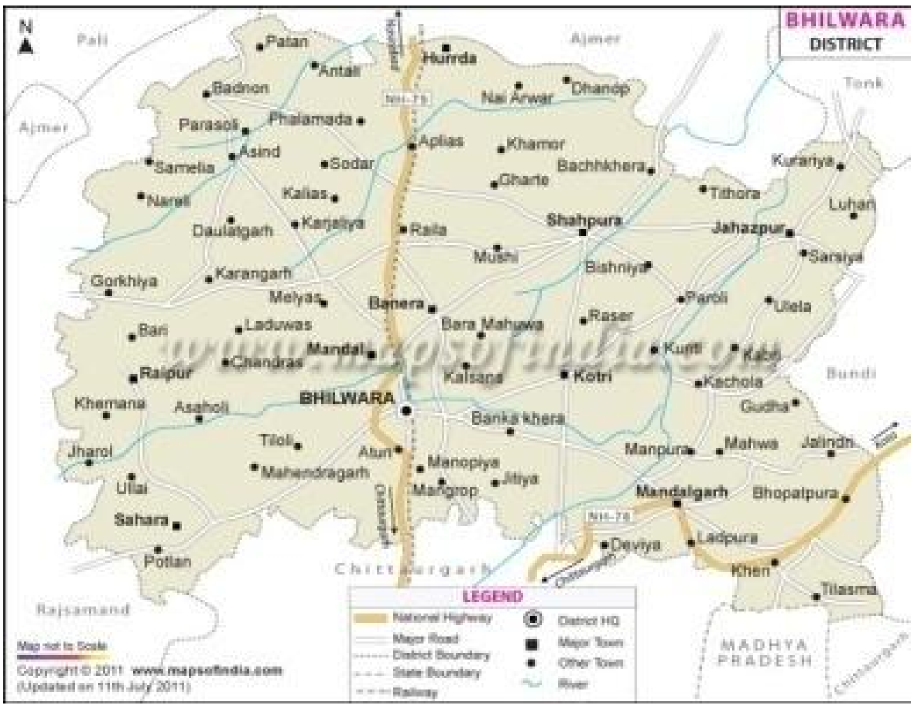
Bhilwara district lies on the south eastern part of Rajasthan. The geographical area of the district is 1047451 hectares. There is only one main seasonal river in the district – **Banas.** Bhilwara district has made an important place for itself in the industrial map of the state.

During the last decade the district has developed in to a leading place in the textile industry the in the country. Bhilwara is the hub of textile industries in the state and known as **"Textile City"**. In 1978 when the District Industrial Centre (DIC) was established, only 1059 small scale industries were registered. However by March 2002 this figure has risen to 12739. Bhilwara accounts for about 47% of the net valued added of textiles in Rajasthan. There are presently 22 processing houses and 8 dye houses in Bhilwara. Besides textiles, insulation bricks, A.C. Conductors, tractors & compressors, china clay, 'Hozari' Products, fertilizers and 'Niwar' Industries are other main industries in Bhilwara. This industry requires **18 MLD** of water and produces about **7.5 Million Litres** of **effluent** per Day**.**

Small Scale and Cottage Industries: There are total of 15734 units were registered with the DIC, Bhilwara till on 31st march 2006 out of which 5747 were sido units. An investment of Rs. 212.09 crores had been made in these units. The number of persons employed was 59383**.**

Agro Based Industries: Oil Mills, Flour Mills, Ice Candy manufacturing units, Dal Mills and Units producing Biscuits Confectionary items, Khandsari, Masala and Cattle Feed. As on 31st March 2006 there were 1361 units producing such items. They provide employment to 4972 persons and had an investment of 831.52 lacs.

Present Situation In Bhilwara



A 2004 study by the state public health engineering department found that most open wells in villages near the Kothari river that flowed beside the industrial belt had chromium, lead, iron, zinc and sodium above the norms set by the Bureau of Indian Standards.

In December 2005, the Rajasthan Pollution Control Board ordered the textile mills to operate as zero discharge units. Since then, units have been growing eucalyptus trees in 2-5 hectares of agriculture land in its premises, using wastewater for irrigation.

Agricultural activity in 8-10 other villages located downstream of the btm processing unit on Gangapur road has reached a standstill. Even the air smells foul. Villagers again protested in July 2006. Even the units, which earlier drew water from tube wells, no longer use the areas' groundwater because it is polluted. They buy water from private tankers. About 1,000 private tankers supply water to the units. The Ground Water Table (GWT) position within a few years has dropped from 100 ft. to 600 ft. Pollution continues even cattle could not drink the water. Villagers complain of health problems like stomach disorders, gastroenteritis and skin diseases. The effluent discharge have high quantity of Cl, Pb, Fe , Fl and other heavy metal which are harmful to human life.

Result And Discussion

Constituents Of Textile Effluent

Physical and chemical parameters of Textile Waste Water

S. No.	Parameters	Textile waste water	Standards (ISI 2490-1981)
1.	Colour	Brownish-Black	
2.	Odour	unpleasant	
3	Ph	8.3	5.5 to 9.0
4	BOD mg/L	350	100
5	COD mg/L	770	
6	TDS mg/L	2352	2100
7	TSS mg/L	270	200
8	Oil & grease	60	10
9	Iron mg/L	0.37	
10	Manganese mg/ L	0.070	
11	Sodium mg/L	520	5(ISI 2490)

12	Potassium mg/L	24	60(ISI 2296)
13	Calcium as Ca mg/L	62.4	75
14	Magnesium as Mg mg/L	61.5	50
15	Total Hardness as CaCO3 mg/L	408	
16	Chloride mg/L	378	600
17	Sulphate mg/L	348	1000

The quantities and characteristics of discharged effluent vary from industry to industry depending on the water consumption and average daily product. Untreated or incompletely treated textile effluent is notoriously known to contain i) large amount of total suspended solids which increases the turbidity in water and prevents the light from reaching aquatic plants and animals, ii) large amount of total dissolved solids limiting the industrial and agricultural use of water, iii) high levels of chemical oxygen demand (indicating high degree of pollution) and biological oxygen demand (leads to lowering of the level of dissolved oxygen thereby inhibiting aquatic habitats) and iv) elevated temperatures which lower the rate of dissolution of atmospheric oxygen in the water and affects the sustainability of the aquatic habitats due to reduction in the level of the dissolved oxygen.1 It also causes problems of foaming and color persistence, having a highly fluctuating pH affecting the solubility and chemical forms of most substances in water. Most of the heavy metals are essential for growth of organisms but are only required in low concentrations.2 the increasing concentration of heavy metals leads to Bioaccumulation of metals in fauna and flora if the rate of uptake of heavy metals by the organisms is

More than the excretion phase. Heavy metals are not biodegradable so they accumulate in primary organs in the body and over time begin to fester, leading to various symptoms of diseases.3 Thus, untreated or incompletely treated textile effluent can be harmful to both aquatic and terrestrial life by adversely affecting on the natural ecosystem and long term health effects.

Impact Of Metal On Life

About fluoride:

Nearly 25 countries in the world are suffering from excess of fluoride content in the groundwater and India is one of them. Approximately 20 states of India are facing the problem of excessive fluoride in the ground water. 62 million people including 6 million children suffer from fluorosis(UNICEF, 1999). Dental Fluorosis(in children), Skeletal Fluorosis(17-22 age group) and neurological Complication(more than 50 years old).

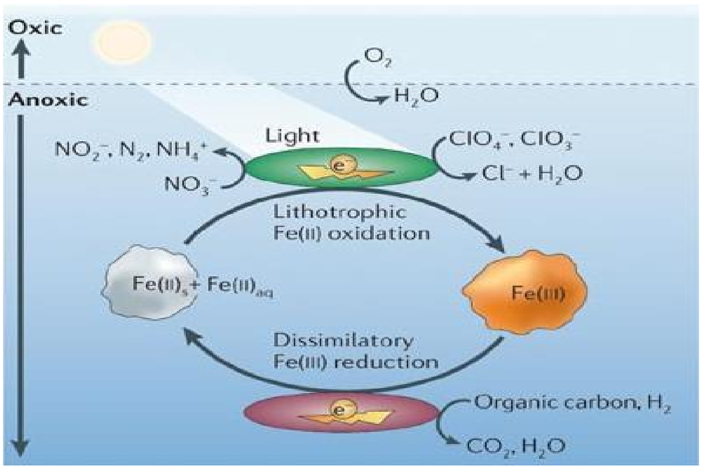
About sulphur:

There are following impact on life- Neurological effects and behavioral changes, Disturbance of blood circulation, Heart damage, Effects on eyes and eyesight, Reproductive failure, Damage to immune systems, Stomach and gastrointestinal disorder, Damage to liver and kidney functions, Hearing defects, Disturbance of the hormonal metabolism, Dermatological effects, Suffocation and lung embolism

About other metals:

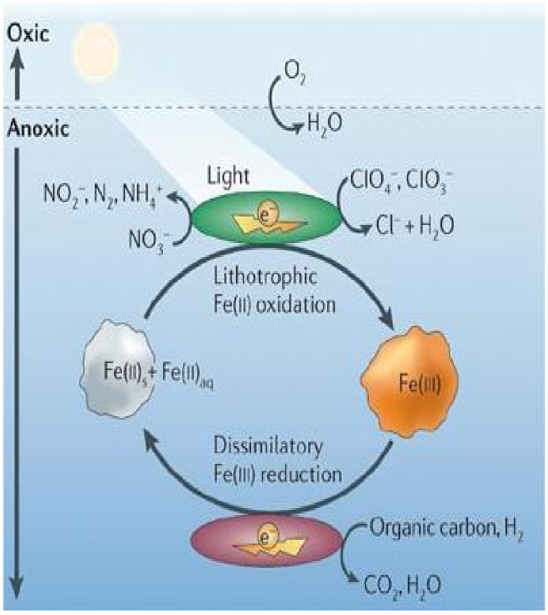
In 2004, a study by the state public health engineering department found near the Kothari River had chromium, lead, iron, zinc and sodium. Lead is especially injurious to the brain and nervous system of developing children. The Ground Water Table (GWT) position within a few years has dropped from 100 ft. to 600 ft. The water also had high levels of chlorides making it hard. Arsenic is well-known as a poison as well as a carcinogen and can cause cancer of the skin, lungs & liver and bladder. Cadmium damage to the lungs through breathing and also irritates the stomach and leading to vomiting and diarrhea. Laboratory studies show that repeat exposure to chlorine can affect the immune system, the blood, the heart, and the respiratory system of animals.

An Approach With Bioremediation



Description

Geobacter metallireducens was first isolated by Derek Lovley in 1987 in sand sediment from the Potomac River in Washington D.C. Geobacter have been found in anaerobic conditions in soils and aquatic sediment[1]. **Geobacter metallireducens** is the first known organism that can oxidize organic compounds to carbon dioxide with iron oxides as an electron acceptor Its a rod shaped Gram-negative, anaerobic bacteria and may have flagella and pili. The first G. metallireducens (initially known as strain GS-15) was first isolated from freshwater sediment, and was able to gain energy through dissimilatory reduction of iron, manganese, uranium and other metals[1]. G. metallireducens can also oxidize short chain fatty acids, alcohols, and monoaromatic compounds such as toluene and phenol using iron as its electron acceptor[2]. G. metallireducens also plays a role in carbon and nutrient cycling and bioremediation, enabling the metabolism of soluble harmful (sometimes radioactive) contaminants into insoluble harmless forms. These characteristics, in conjunction with its predominance in many sediment environments, make G. metallireducens a possible agent for bioremediation can be use the metabolism to remove pollutants It gains energy for survival and growth through the oxidation of organic compounds and the reduction of iron oxides. Geobacter metallireducens specifically expresses flagella and pili, only when grown on insoluble Fe(III) or Mn(IV) oxide, and is chemotactic towards Fe(II) and Mn(II) under these conditions (Childers et al 2002) [5] . Insoluble materials like iron, magnesium, and uranium oxides, that can't be broken down into soluble subunits can be metabolized by Geobacter.



Geobacter species have proven to be an excellent model for the development of genome-scale analysis of natural environments, bioremediation, and bioenergy applications.

Genome Structure

The genome of G. metallireducens is 4.01 Mbp long with a (circular) chromosome length of 3,997,420 bp encoding 3,621 genes with a GC content of 59.51%. It also has a plasmid of 13,762 bp encoding 13 genes with a GC content of 52.48% [3]. The plasmid contains a gene for an addiction module toxin, RelE/StbE, which produces toxin and gives resistance to the bacteria. The plasmid also contains a gene that encodes for a plasmid stabilization system protein, RelE/ParE, that allows the bacteria to adapt to new environmental conditions (ie: a change in nutrients)[4]. The chromosome encodes for various housekeeping pathways including metabolism, organism cell structure, sensor proteins (chemotaxis), as well as genes that encode for flagella and pili synthesis[3.]

Cell structure and metabolism

G. metallireducens contains genes for flagella synthesis. G. metallireducens was originally thought to be immotile because they were grown in labs under ideal conditions where the bacteria had plenty of soluble metals. Flagella synthesis does not initiate unless nutrient conditions are poor. Under conditions where soluble metals were replaced with less favorable iron oxide, G. metallireducens was seen to grow flagella and swim[5.]

G. metallireducens also contains genes that allow the bacteria the ability of chemotaxis which allows it to sense compounds & to move towards metallic compounds or favorable environments where nutrient supply is favorable and away from less favorable environments where nutrient supply is poor[5.] G. metallireducens is chemotactic to solublelise electron acceptors Fe (II) and Mn(II) and expresses flagella and pili only when grown on insoluble Fe(III) or Mn(IV) oxide[5.] These results suggest that G. metallireducens senses when soluble electron acceptors are depleted and will promote synthesis of flagella and pili allowing it to search for, and establish contact with, insoluble Fe(III) or Mn(IV) oxide [5.]

Further experimentation showed the importance of flagella and pili as of the deletion of gene fliC & pilA, which encodes the flagellin protein & structural protein of the type IV pili, resulted in a strain that did not produce flagella, was non-motile, and was defective for the reduction of insoluble Fe (III). As well as inhibited the production of lateral pili as well as Fe(III) oxide reduction and electron transfer to an electrode. These results demonstrate the importance of flagella and pili in the reduction of insoluble Fe (III) by G. metallireducens and provide methods for additional genetic-based approaches for the study of G. metallireducens.[10]

Geobacter species are examples of such Fe(III) reducers that lack c-cytochromes and must directly contact Fe(III) oxides to reduce them[6.] They produce pili that were proposed to aid in establishing contact with the Fe (III) oxides[6.]

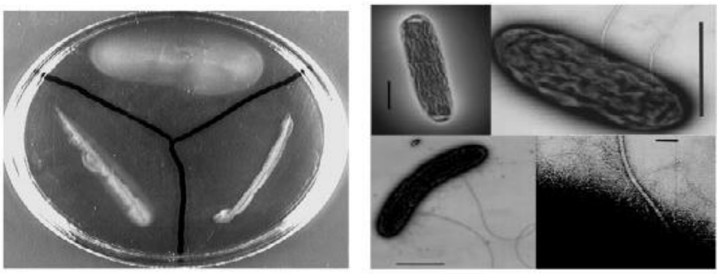
Ecology

G. metallireducens has been known to take part in bioremediation of organic and metal contaminants in groundwater and participates in the carbon and nutrient cycles of aquatic sediments. Aside from using Fe (III) oxides, the G. metallireducens uses metals such as plutonium and uranium to metabolize food [5.] G. metallireducens consumes these radioactive elements and breaks down the contaminants. When G. metallireducens metabolizes uranium, it changes the metal from a soluble to an insoluble form. The insoluble uranium drops out of the groundwater--decontaminating streams and drinking water. The insoluble uranium remains in the soil and could then be extracted[5.] The use of an insoluble electron acceptor may explain why Geobacter species predominate over other dissimilatory iron-reducing bacterial species in a wide variety of sedimentary environments.

As we are concentrating in the problems of Bhilwara but similar problems will be there in industrial areas so this approach will solve many such problems.

Culture methods

G. metallireducens has been grown anaerobically in fresh water acetate (FWA) medium as described [12]. Motility plates were composed of FWA medium containing 50mM Fe (III)- citrate and 0.35% agar. All plate manipulations were done in a 30 °C heated anaerobic glove box (Coy) containing a N2:CO2:H2 (in per cent, 83:10:7) atmosphere.



a, Motility agar plate of swimming motility in response to growth with the electron acceptors., The appearance of a light-grey haze extending outward from the initial streak indicated swimming. **b,** Electron micrographs showing the absence of flagella on cells grown with Fe(III)-citrate (top left), in contrast to cells grown with Fe(III) (top right) or Mn(IV) (bottom left) oxides as the terminal electron acceptor.

JEOL 100S Microscope - Expression Analyses

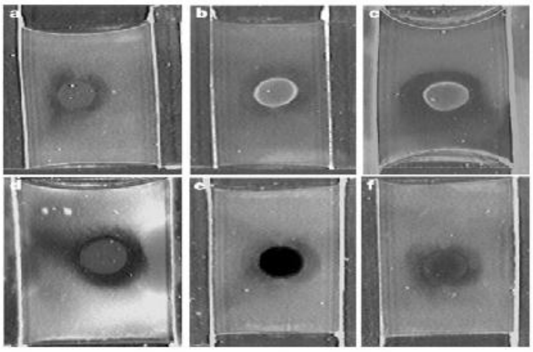
Surface proteins were prepared as described previously 20. Cells were collected by centrifugation, resuspended in 1–2 ml 10mM Tris, pH 7.4, and vortexed for 2min. Cells were removed by centrifugation at 16,000g for 1 min. The supernatant was transferred to a new tube and centrifugation was repeated for 5min. The resulting cell-free supernatant was transferred to a new tube and MgCl2 was added to 100 mM. Tubes were kept at 4 8C overnight and precipitated proteins were collected by centrifugation at 16,000g for 15min at 4 8C. We used primers for pilA (forward primer, 5 0-GGATCCATGCTGAAACGTTTT-3 0; reverse primer, 5 0 GGGATCCTCACCAGTCCTTGTACGGAG-3 0) to amplify the gene from genomic DNA of both G. sulfurreducens and G. metallireducens. Cells were collected from cultures of G. metallireducens grown on various electron acceptors, and mRNA was

extracted using an RNeasy kit (Qiagen). In the case of Fe(III) oxide, cultures were first treated with oxalate to solubilize the insoluble Fe(III) oxide particles21. Isolated mRNAwas reverse transcribed with OmniScript

(Qiagen), and the resulting complementaryDNAwas subjected to PCR amplification using primers internal to pilA (forward primer, 5 0-GGC AATTGCCGTCCCCAACT-3 0; reverse primer, 5 0 TTACCCTTGAT CTCCT CGGT-3 0).

Chemotaxis assays

Chemotaxis assays were performed using a modified agarose plug method[13.] The sides of a chemotaxis chamber consisted of two plastic strips 15-mmapart on a glass slide. A drop of molten low-melting-point agarose containing the substrate to be tested was placed on a coverslip that was inverted onto the plastic strips to form a chamber Cells for Chemotaxis. This culture processs was described.[14]



Bright ring of cells around agarose plugs in b and c is indicative of chemotaxis.

Current Research

Wiatrowski (et al) determined that some dissimilatory reducing bacteria, such as Shewanella oneidensis, Geobacter sulfurreducens, and Geobacter metallireducens, can also reduce ionic mercury (Hg[II]) to elemental mercury (Hg[0]) without having to use a mercury reductase[7.] The reduction of mercury was determined to be metabolically similar to how Fe (III) is reduced in the tested dissimilatory reducing bacteria where reduction required the presence of electron donors and acceptors. It was concluded that the discovery of mercury reduction indicated possibilities of mobilizing mercury and producing methylmercury in anoxic environments[7.]

Tang (et al) analyzed the central metabolic pathway in G. metallireducens, specifically the carbon fluxes using isotopic carbon (13C). Acetate was used as the primary carbon source and Ferric nitrilotriacetate (Fe-NTA) was the electron acceptor. G. metallireducens was found to have complete biosynthesis pathways for essential metabolism and an additional (and unusual) isoleucine pathway, which used acetyl-CoA and pyruvate as precursors[8.] The isotopomer modeling indicated that acetate was oxidized to carbon dioxide via TCA cycle while also reducing iron. The main biosynthesis pathways employed were the pentose phosphate pathway and gluconeogenesis but only accounted for less than 3% of carbon consumption[8.] The model also indicated high reversibility in the reaction between oxoglutarate and succinate, which was not expected. This metabolic step where oxoglutarate is converted to succinate was found to be the rate limiting reaction/step[8.] All in all, it was concluded that this rate limiting step determines G. metallireducens' carbon metabolism and explains why it is low.

Jahn (et al) discovered that even very stable iron complexes could be reduced by dissimilatory iron-reducing bacteria (G. metallireducens). Cyanide-metal complexes are frequent contaminants found in the soil or aquifers of industrial sites that may be released and spread by outgassing or transport with the groundwater. Cyanide forms very stable complexes with iron called Prussian Blue (Fe(4)[Fe(CN)(6)](3)). Jahn (et al) indicated that Prussian Blue could be used as the electron acceptor in dissimilatory iron-reducing bacteria such as G. metallireducens and Shewanella alga strain BrY[9.] Prussian Blue is reduced to Prussian White (Fe(2)[Fe(CN)(6)]). However, Prussian White could reoxided by exposure to air and become Prussian Blue again [9]. Both G. metallireducens and Shewanella alga strain BrY were shown to be able to grow with Prussian Blue, using it as its primary electron acceptor.

The abundance of Geobacter species in contaminated aquifers in which benzene is anaerobically degraded has led to the suggestion that some Geobacter species might be capable of anaerobic benzene degradation, but this has never been documented. A strain of Geobacter, designated strain Ben, was isolated from sediments from the Fe (III)-reducing zone of a petroleum-contaminated aquifer in which there was significant capacity for anaerobic benzene oxidation. Strain Ben grew in a medium with benzene as the sole electron donor and Fe (III) oxide as the sole electron acceptor. With benzene as the electron donor, and Fe(III) oxide (strain Ben) or Fe(III) citrate (G. metallireducens) as the electron acceptor, cell yields of strain Ben and G. metallireducens were 3.2 × 109 and 8.4 × 109 cells/mmol of Fe(III) reduced, respectively. Strain Ben also oxidized benzene with anthraquinone-2,6-disulfonate (AQDS) as the sole electron acceptor with cell yields of 5.9 × 109 cells/mmol of AQDS reduced. Strain Ben serves as model organism for the study of anaerobic benzene metabolism in petroleum-contaminated aquifers and G. metallireducens is the first anaerobic benzene-degrading organism that can be genetically manipulated.[11]

Potential and actual applications on Bhilwara

Research on the potential of Geobacter is underway and on-going. Geobacter's ability to consume oil-based pollutants and radioactive material with carbon dioxide as waste byproduct has already been used in environmental clean-up for underground petroleum spills and for the precipitation of uranium out of groundwater. Geobacter metabolize the material by creating pili between itself and the food material.

It has been shown that species of Geobacter are able to cooperate in metabolizing a mixture of chemicals that neither could process alone. Provided with ethanol and sodium fumarate, G. metallireducens broke down the ethanol generating an excess of electrons which were passed to G. sulfurreducens via "nanowires" grown between the species, enabling G. sulfurreducens to break down the fumarate ions[.2]

Microbial biodegradation of recalcitrant organic pollutants is of great environmental significance and involves intriguing novel biochemical reactions.In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of hitherto unknown anaerobic hydrocarbon-degrading and reductively dehalogenating bacteria during the last decades provided ultimate proof for these processes in nature. Many novel biochemical reactions were discovered enabling the respective metabolic pathways, but progress in the molecular understanding of these bacteria was rather slow, since genetic systems are not readily applicable for most of them. However, several complete genome sequences are now available for bacteria capable of anaerobic organic pollutant degradation. The genome of the hydrocarbon degrading and iron-reducing species Geobacter metallireducens (accession nr. NC_007517) was determined recently. The genome revealed the presence of genes for reductive dehalogenases, suggesting a wide dehalogenating spectrum of the organisms. Moreover, genome sequences provided unprecedented insights into the evolution of reductive dehalogenation and differing strategies for niche adaptation.[4]

Geobacter species are often the predominant organisms when extracellular electron transfer is an important bioremediation process in subsurface environments. Therefore, a systems biology approach to understanding and optimizing bioremediation with Geobacter species has been initiated with the ultimate goal of developing in silico models that can predict the growth and metabolism of Geobacter species under a diversity of subsurface conditions. To date, these studies have included sequencing the genomes of multiple Geobacter species and detailed functional genomic/physiological studies on one species, Geobacter sulfurreducens . Genome-based models of several Geobacter species that are able to predict physiological responses under different environmental conditions are now available. Quantitative analysis of gene transcript levels during in situ uranium bioremediation has demonstrated that it is possible to track in situ rates of metabolism and the in situ metabolic state of Geobacter in the subsurface. Initial attempts to link in silico Geobacter models with existing subsurface hydrological and geochemical models are underway. It is expected that this systems approach to bioremediation with Geobacter will provide the opportunity to evaluate multiple Geobacter -catalyzed bioremediation strategies in silico prior to field implementation, thus providing substantial savings when initiating large-scale in situ bioremediation projects for groundwater polluted with uranium and/or organic contaminants[. 5]

Geobacter species to grow in subsurface environments with insoluble Fe(III) oxides as the electron acceptor, and effectively remediate groundwater contaminated with hydrocarbon fuels or uranium and similar contaminants associated textile industries which are polluting the ground water of Bhilwara and surrounding as well as contaminating the water bodies flowing through the region The important mechanism for microorganisms to exchange electrons in syntrophic associations, such as those responsible for the conversion of organic wastes to methane in anaerobic digesters, a proven bio-energy technology and clean energy for the area.

Conclusion

Geobacter metallireducens is an important microorganism to optimize in anaerobic bioremediation. These studies summarized here demonstrate that microbial metal reduction has the potential to be a useful technique for the bio-restoration of environments contaminated with organics and metals. Geobacter can reduce metals such as Iron, lead, chromium, uranium, fluoride, mercury etc. They are able to metabolize mixture of chemicals that neither could process alone. Removal of organic and metal contaminants in groundwater can be achieved. Geobacter species have proven to be an excellent model for the development of genome-scale analysis of natural environments, bioremediation, and bio-energy applications. Its potential or production of electricity during this process has also led scientists to theorize that Geobacter could act as a natural battery which itself increases the need of more extensive research on topic. As we are concentrating in the problems of Bhilwara but similar problems will be there in industrial areas so this approach will solve many such problems.

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