Application Of Biotechnology In Environment, In Case Of Biodegradation Potential Of Bacterial Isolates From Tannery Effluent With Special Reference To Hexavalent Chromium

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ABSTRACT: Chromium is important metal due to its high corrosion resistance and hardness. The trivalent chromium (Cr (III)) is required in trace amounts for sugar and lipid metabolism in humans and its deficiency causes disease. But, due to environmental pollution caused as a result of hexavalent chromium (Cr(VI)) is a highly toxic metal pollutant that affects the environment and is abundantly available in the environment and has toxicity and harmful effect on living system and its cleanup is highly essential such as contaminants contained hexavalent chromium (Cr(VI)). To alleviate such problems by applying essential microorganisms were easily degradable by naturally occurring bacteria and the isolated one. Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries. Chromium (VI) is toxic and harmful to human health, mainly for the people who are working in industries where Cr(VI) is widely used. Therefore, this review article was initiated to study the application of isolated bacterial culture as biological tool for hexavalent chromium removal from tannery industry waste water and investigated to degrade Chromium (VI) and clean up the environments.

Keywords: biodegradation, tannery effluent, chromium, heavy metals

1. INTRODUCTION

Biotechnology, broadly defined as any technique that uses living organisms to make or modify a product, improve plants or animals, or develop micro-organisms for specific use and applicable for the processes of the protection and restoration of the quality of our environment. And used as the environmentally sound technology (EST) of choice in many applications, particularly pollution clean-up [1]. Biotechnological processes to protect the environment have been used for almost a century now, even longer than the term ‘biotechnology’ exists. Municipal sewage treatment plants and filters to purify town gas were developed around the turn of the century. They proved very effective although at the time, little was known about the biological/principles underlying their function. Since that time our knowledge base has increased enormously. However, modern biotechnology, based on the use of new tissue culture methods, and recombinant-DNA technology, or genetic engineering, is an exciting science and rich in potential and new applications are expected to include water treatment, treatment of solid wastes (including biodegradable plastics) [2]. Biotechnological techniques to treat waste before or after it has been brought into the environment are described and exemplified in the section on bioremediation. Biotechnology can also be used to develop products and processes that generate less waste and use less non-renewable resources and energy [1]. Environmental biotechnology is the used in waste treatment and pollution prevention. Additionally, environmental biotechnology can more efficiently clean up many wastes than conventional methods and greatly reduce our dependence on methods for land-based disposal. Every organism ingests nutrients to live and produces by-products as a result of different organism need different types of nutrients. Some bacteria thrive on the chemical components of waste products. Environmental engineers use bioremediation, the broadest application of environmental biotechnology, in two basic ways. They introduce nutrients to stimulate the activity of bacteria already present in the soil at a waste site, or add new bacteria to the soil. The bacteria digest the waste at the site and turn it into harmless by products. This process of bioremediation is an area of increasing interest. Through application of biotechnical methods, enzyme bioreactors are being developed that will pretreat some industrial waste and food waste components and allow their removal through the sewage system rather than through solid waste disposal mechanisms. Waste can also be converted to biofuel to run generators. Microbes can be induced to produce enzymes needed to convert plant and vegetable materials into building blocks for biodegradable plastics [3]. In some cases, the byproducts of the pollution-fighting microorganisms are themselves useful. For example, methane can be derived from a form of bacteria that degrades sulfur liquor, a waste product of paper manufacturing. This methane can then be used as a fuel or in other industrial processes. The role of microbiology in environmental biotechnology the restoration, maintenance and protection of the environment with the help of biological agents, which includes both the living organisms and their components. Environmental remediation, pollution protection, detection and monitoring are evaluated considering the achievements, as well as the perspectives in the development of biotechnology. Waste water treatment, soil waste treatment, solid waste treatment, and waste gas treatment, dealing with both microbial and process engineering aspects. The distinct role of environmental biotechnology in the future is emphasized considering the opportunities to contribute with new solutions and directions in remediation of contaminated environments, minimizing future waste release and creating pollution prevention
alternatives [3]. To take advantage of these opportunities, innovative new strategies which advance use of molecular biological methods and genetic engineering technology are examined. These methods would improve the understanding of existing biological process in order to increase their efficiency, productivity and flexibility. In case of waste water and solid industrial effluents microorganisms in sewage treatment plants remove the more common pollutants from waste water before it is discharged into rivers or the sea. Increasing industrial and agricultural pollution has led to a greater need for processes that remove specific pollutants such as nitrogen and phosphorus compounds, heavy metals and chlorinated compounds. New methods include aerobic, anaerobic and Physico-chemical processes in fixed-bed filters and in bioreactors in which the materials and microbes are held in suspension. Extensive application of heavy metals like chromium in industries particularly leather tanning industries leads to the formation of chromium-contaminated soil and ground water which pose a serious threat to living biota particularly to human health. Chromium is a potent pollutant which is mutagenic, carcinogenic and teratogenic in humans [4]- [6]. It is found to be toxic in plants also [7]. Among different forms of chromium, chromium VI is known to cause serious health hazard effects. It can cause allergic reactions, nose irritation and nose bleeds. It creates respiratory problems and weaken-immune system. It causes renal tubular necrosis and produce cytotoxic and genotoxic effects [8]. Hexavalent chromium toxicity appears to be due to its rapid permeability through biological and subsequent interaction of chromium with intracellular proteins and nucleic acids. Usually the tanning industry uses chrome liquor in the tanning process. A large number of tanning industries discharge their effluents into the environment containing chrome salts exceeding the maximum permissible limit. As a result, they provide a natural environment for enrichment of chromium-resistant bacteria and consequently these bacteria may be employed for removal of hexavalent chromium from tannery waste. Cr (VI) containing tannery effluents are commonly treated by chemical means. These methods may be a source of potential heavy metal pollution from the resultant metal-containing chemical sludge. The commonly employed methods include excavation, to pump and treat, in situ vitrification and chemical treatment with a reductant [3]. The conventional method to detoxify and remove Cr (VI) from the environment involve chemical reduction followed by precipitation, ion exchange and absorption on coal, activated carbon, alum and kaolinite [9], [10]. Several reports have indicated biological reduction of Cr (VI) by aerobic and anaerobic bacteria as biological reduction of Cr (VI) usually occurs at a neutral pH range. It generates an insignificant quantity of chemical sludge. It offers potentially cost-effective remediation strategy [11]. It does not require high energy input or toxic chemical reagents and finally it offers an economical as well as eco friendly option of metal detoxification and bioremediation. The processes by which microorganisms interact with toxic metals enabling their removal and recovery are bioaccumulation, biosorption and enzymatic reduction [12]. Recent studies have shown that certain species of bacteria are capable of transforming Cr (VI) into the much less toxic and less mobile Cr (III) [13a], [14].

2. Pollution of Water

Pollution of water by industrial effluents of process industries is a serious problem in most countries [15]. Industrial waste consists of both organic and inorganic substances. Organic wastes include pesticide residues, various hydrocarbons, solvents, cleaning fluids, dissolved residue from fruit and vegetables, and lignin from pulp and paper. Effluents can also contain inorganic wastes such as brine salts and metals. The increased industrial activities have reduced the availability of good quality water by producing a large amount of effluents to the rivers. Industrial effluents often contain various toxic metals, harmful gases, and several organic and inorganic compounds [15]. Due to discharge of toxic effluents long-term consequence of exposure can cause cancer, delayed nervous damage, malformation in urban children, mutagenic changes, neurological disorders [16]. Phenol and chromium are the major contaminant present in the effluent discharged from the various industrial processes such as wood preserving, metal finishing, petroleum refining, leather tanning and finishing, paint and ink formulation, pulp and paper industry, Textile Industry Pharmaceutical industry and manufacturing of automobile parts industries [17].

2.1. Chromium

Chromium is a chemical element discovered in 1797 by Louis Nicolas Vauquel which has the symbol Cr. Its atomic number is 24. It is a hard metal of steely gray colour and also it has a high melting point of 1907°C. It is odourless and tasteless metal. Many of its compounds are toxic and harmful effect on living system. Due to its intense corrosive resistance and hardness. The trivalent chromium (Cr(III)) is required in trace amounts for sugar and lipid metabolism in humans and its deficiency causes disease. Hexavalent chromium (Cr(VI)) is a highly toxic metal pollutant that affects the environment and is abundantly available in the environment. Due to its toxicity and harmful effect on living system its cleanup is highly essential [18].

2.1.1. Uses of chromium

Chromium metal is found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating [18], [19]. Effluents of these industries contain large quantities of chromium-laden in their wastewater [20]. Hexavalent chromium Cr(VI) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms [21]. Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries.

2.1.2. Toxicity of chromium

Considering its potential for hazardous toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries. Chromium(VI) is toxic and harmful to human health, mainly for the people who are working in industries where Cr(VI) is widely used. Chromium (VI) causes various health problems to human health hazard effects. It can cause allergic reactions, nose irritation and nose bleeds. It creates respiratory problems and weaken-immune system. It causes renal tubular necrosis and produce cytotoxic and genotoxic effects [8].
beings as reported by [22b]. It has been reported by various authors that hexavalent chromium causes lung cancer, ulcer, severe damage to the liver and kidneys, perforation of nasal septum, leukocytosis. Skin rashes in humans [20]. There is sufficient evidence for carcinogenicity of Cr(VI) in animals for the hexavalent chromium compounds like calcium chromate, chromium trioxide, lead chromate, strontium chromate and zinc chromate stated by [23]. IARC and ACGIH has also classified chromium metal and trivalent chromium compounds as not human carcinogen. According to central pollution control board the minimum permissible limits of Cr(VI) at 0.05-0.1 ppm and 2 ppm for total chromium is sited [19].

2.2. Different Physical and chemical Methods for chromium degradation and their draw backs.

Traditionally, by the use of various expensive chemical and physical processes high concentrations of both Cr(VI) and phenol are reduced from the industrial wastewater. Those methods include ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation [19], [20]. But, these processes are high energy consuming, non-economic and release effluents and waste waters which requires further treatment and thus are alarming for the environment. Also, complete removal of the pollutants cannot be possible by the use of physical and chemical processes [14].

2.2.1. Ozonization

Chemical oxidation with ozone can be used to treat organic pollutants or act as disinfectant agents. Ozone is a powerful oxidant that can oxidize a great number of organic and inorganic materials. Ozone based technologies research is also being focused on the catalytic ozonation where the presence of catalyst significantly improved the oxidation rate of organic compounds compared to non-catalytic ionization. The ozonation processes are possibly one of the most effective methods for treatment of wastewater containing organic products such as effluents from chemical and agrochemical industries, textile industry, paints, [21]. However, the characteristics of the wastewater such as pH, temperature and concentration of organic pollutant play an important role in organic degradation. Similarly [22], have studied the treatment of industrial effluent by treatment with ozone initial pH, ozone dosages and dosages of catalyst on effluent treatment. Then, organic matter remained was removed by BAF (Biological aerated filter), and the effect of hydraulic retention time (HRT) of BAF was also considered. Their result showed that under optimum pH and dose of H₂O₂ was about 30% COD could be removed by ozonization alone. The use of high oxidation potential like ozonization has recently received much attention in wastewater treatment studies. Ozonizer in which the oxygen molecules in the gas were dissociated to form ozone was used in many cases [23]. The disadvantages associated with the process are high operating cost. The cost of the equipment is very high and also it requires high voltage and electricity for its operation [24].

2.2.2. Ion exchange

Ion exchange means the removal of an ion from an aqueous solution by replacing another ionic species. There are natural and synthetic materials available which are specially designed to enable ion exchange operations at high levels. So, ion exchangers are used to perform this ion exchange for removal of organic and inorganic pollutants along with other heavy metals for purification and decontamination of industrial effluent. Synthetic and industrially produced ion exchange resins are mainly made up of polystyrene and polyacrylate are in the form of small and porous beads. Also, there are some naturally occurring minerals which have ion exchange properties. The most common one is aluminium silicate minerals which are also called zeolites [25]. There are different zeolites available made up of various ionic materials which have affinity towards some particular metals. The main features of the ionic resins include material properties such as like adsorption capacity, porosity, density [26]. The main disadvantages associated with ion exchange method are the high cost of the ion exchange resins and each resin must be selectively removes one type of contaminant only. Further, complete removal of the contaminant is not possible. Besides, it can be used for limited cycles only as by passing concentrated metal solution the matrix gets easily owned out by organics and other solids in the wastewater after several uses. Moreover, ion exchange is also highly sensitive to pH of the solution [27], [28].

2.2.3. Adsorption

Adsorption is a widely used method for the treatment of industrial wastewater containing colour, heavy metals and other inorganic and organic impurities stated by [29], [30]. Adsorbent materials are basically derived from low-cost agricultural wastes, activated carbon prepared from various raw materials such as sawdust, nut shells, coconut shells [31]. These adsorbents are basically used for the effective removal and recovery of organic and metal pollutants from wastewater streams [32]. It is a complex process affected by several factors. Mechanisms involved in the adsorption process mainly focus on the selection of the adsorbent material like their particle size surface area and porosity [33]. This method suffers from low adsorption capacity and in some cases complete removal is not possible and high cost of the adsorbent. After use the disposal of adsorbants creates problems. 2.2.4. Membrane filtration

Membrane filtration technique has received a significant attention for the wastewater treatment. It considers the application of hydraulic pressure to bring about the desired separation through the semi permeable membrane [34]. Membranes are of different pore size and it is necessary to select membranes of appropriate pore size for specific purpose so that effluent and wastewater could be purified and permeate could be recycled a number of times. Mainly three types of membrane filtration are there. They are Ultra-filtration, Nano filtration and Reverse osmosis reported by [31], [35]. Various other types of membranes such as inorganic, polymeric, and liquid membranes can be used in this process. The main problem associated with this process is incomplete removal of contaminants, high energy requirement, high cost of the
membrane and longevity of the membrane. After long term use the membrane get clogged with the contaminants present in the waste water and is damaged due to extra pressure on the membrane.

2.2.5. Chemical oxidation
In this process the waste materials from the industrial waste water are removed by the help of chemical oxidation by the use of various chemicals mainly hydrogen peroxide is widely used for this purpose as reported [36a]. There are many disadvantages associated with this process like the high cost of the chemicals, emission of various harmful by products, it creates hazardous constituent like secondary effluent problem along with the production of harmful gases.

2.3. Biodegradation
Biodegradation is the process of decaying or reduction of different organic materials and toxic metals to their non-toxic form with the help of microorganisms. In this process complete mineralization of the starting compound to simpler ones like CO₂, H₂O, NO₃ and other inorganic compounds takes place [38]. In the mixed culture of microorganism’s phenol degrading organisms utilizes phenol as sole source of carbon and produce energy, metabolites, electron donor which is used by the chromium degrading organisms to reduce chromium [39], [40]. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals are the micronutrients that also shows a significant impact on degradation behavior is reported [41].

2.3.1. Advantages of biodegradation
There are various advantages associated with biodegradation such as the process is a simple process. It is an eco-friendly and cost-effective process that requires low capital and operating cost. Being environmentally friendly process, it produces no harmful end products

2.3.2. Microorganisms involved in biodegradation of chromium
Chromium degradation can be possible with the help of variety of bacteria and fungal groups like Arthrobacter sp. and Bacillus sp. [42], E. coli ATCC 33456[43], Pseudomonas aeruginosa [44], 4, Brevebacterium casei [19], Acinetobacter sp and some unidentified species like Pseudomonas fluorescens, Pseudomonas syxanthana, Alcaligenes eutrophus. A number of bacteria Bacillus spp., Shewanella alga BrY-MT and a few unidentified strains have also been shown to reduce Cr 6+ [2].

2.3.3. Mechanism of biodegradation
The biological mechanism E. coli can directly transform the highly toxic Cr(VI) is to less toxic Cr(III) with the help of various membrane associated, NADH dependent soluble chromate reductase enzymes ([2], [43] and [45], [46] suggest the effect of different electron donors on Cr(VI) reduction using Ganooderma lucidum and Brevebacterium casei respectively. Among several electron donors and nitrogen sources screened they concluded dextrose was the optimum electron donor and peptone showed maximum Cr(VI) reduction. Similarly, maximum degradation of Cr(VI) evidenced in the presence of dextrose by [43] by using E. coli ATCC 33456. The optimum Cr(VI) reduction by E. coli ATCC 33456 within a pH range of (3-8) and the optimum pH reported was 7. Similarly, Cr(VI) reduction in E. coli ATCC 33456 was evaluated within a pH range of (3-8) by Shen and Wang, (1994) and the result showed maximum initial specific rate of Cr(VI) reduction at pH 7. Also, they have reported that effect of pH on Cr(VI) reduction were similar for both aerobic and anaerobic cultures. [19] used Brevebacterium casei for degradation of chromium. They observed the reduction of Cr(VI) using wide range of pH ranging from pH 4 to pH 9 and the maximum degradation was reported at pH 7. The above studies confirmed that pH has a significant influence on phenol as well as chromium degradation. [43] studied Cr(VI) reduction using E. coli ATCC 33456 in the temperature range of 400°C to 600°C and found optimum value at 37°C. Temperature range of 100°C to 450°C was studied by [2], and the temperature of 36°C was found to be optimum. Thus, results suggested that temperature has a great influence on both phenol and chromium degradation. The temperature is a very important factor effecting the degradation as it is an enzymatic process where the enzymes present in the microorganisms show maximum functionality at a certain temperature.

3. Sampling Method
Effluent samples were collected from a tannery industry at Kolkata, West Bengal. Samples were collected in sterilized glass bottles aseptically and transported to the laboratory in an ice bucket. Samples were analyzed within 6h of collection.

3.1. Isolation of chromium-resistant bacteria
Chromium-resistant bacteria were isolated from untreated tannery effluent on agar plates under anoxic conditions. The medium contains (per litre): casein 20g, dextrose 10g, sodium thioglycollate 2g, sodium chloride 5g, sodium formaldehyde sulphoxylate 1g, methylene blue 2mg, agar 20g, pH 7.2±0.2. These agar plates were amended with 200 μg ml⁻¹ and 400 μg ml⁻¹ Cr (VI) by standard plate method [47], [48]. The inoculated agar plates were incubated in BBL Gas Pak anaerobic Jars at 25°C for 5days. After incubation clones representing different colony types were purified on agar plates under anoxic condition. All the bacterial cultures were kept at -20°C in agar stabs.

3.2. Determination of metal tolerance activity
The anoxic bacteria were examined for their resistance to chromium by agar dilution method [49]. In this method, freshly prepared agar plates amended with Cr (VI) as dichromate at various Concentrations (50-800 μg ml⁻¹) were inoculated with overnight grown cultures under anoxic condition. All the plates were incubated at room temperature (25°C) for 48h. The minimal concentration of metal in the Petri plate showing complete inhibition of growth was considered as minimal inhibitory concentration (MIC). Tolerance to Cr (VI) was confirmed by broth-dilution method [50]. Tolerance to Zn, Cd and Pb was determined by agar dilution method. The salts used were zinc chloride, cadmium chloride and lead nitrate.
**3.4. Evaluation of Cr (VI) reduction**

The test bacteria were inoculated in diluted peptone water amended with Cr (VI) at the rate of 20μg ml⁻¹ and incubated at 25±2°C for up to 72h. The decrease in Cr (VI) concentration in the experimental flasks was measured after appropriate time intervals by 1, 5-diphenyl carbazide (50μg ml⁻¹) method [48]. The test samples were acidified (pH 1-2) and 50μg ml⁻¹ 1, 5-diphenyl carbazide was added and Cr (VI) concentration was detected by spectrophotometer (CECIL CE 7200) at 540 nm. To estimate the total chromium, samples were digested with hydrochloric acid and perchloric acid mixture (6:1). Metal concentration was determined by atomic absorption spectrophotometer.

**3.5. Characterization of bacterial isolates**

Bacterial isolates from tannery effluents were characterized according to Bergey’s Manual of Systematic Bacteriology [51].

**REFERENCES**


