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EVALUATION ON BACTERIA AND MICROBES CONSORTIUM FOR CHROMIUM DETOXIFICATION WITH SPECIAL REFERENCE TO SAFETY ENVIRONMENT

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ABSTRACT:
The industrial operations, including electroplating, tanning leather, mining, chemical processing, refractories, and the paint and pigment sectors, utilize the well-known hazardous heavy metal chromium. Additionally, the sludge and wastewater from these enterprises leak chromium into the environment, contaminating metals and destroying terrestrial and aquatic ecosystems. Several research also demonstrated that bacterial consortia improve beneficial traits in crops when compared to individual bacterial strains. This is the result of their growth promotion programs and thorough biological control plan. The formation of these consortiums may improve agricultural crops' salinity, pest control, drought tolerance, nitrogen uptake, and resistance to pant infections. In this article, evaluation on bacteria and microbe's consortium for chromium detoxification with special reference to safety environment has been discussed.

KEYWORDS: Bacteria, Microbes, Consortium, Chromium, Detoxification, Environment.

INTRODUCTION:

The extensive use of these forms of Cr in the chemical industry, dye manufacturing, wood preservation, leather tanning, chrome plating, and numerous other products and applications poses a significant environmental risk. [1] These usages lead to the discharge of waste or effluent containing Cr into the air, water, and land, which in turn damages the ecosystem. [2] C-compounds are well known to poison humans, animals, plants, and microorganisms. They are also mutagenic, carcinogenic, and genotoxic. [3] The health concerns associated with Cr vary according to the level of oxidation. Trivalent Cr, for instance, is not as dangerous as hexavalent Cr. [4]

With a wide range of industrial applications and concentrations from low to high, chromium significant, dangerous, and deadly metal contaminants. Condensed mineral ore is the environment's natural source of chromium. It posits that geological parent material and rock outcroppings deposit this element. The geological plant material contains an exceptionally high concentration of chromium metal. [5] In addition to natural sources, shales, sand, other sedimentary rocks, and lime stones are beneficial sources of chromium metal for the environment. The composition and structure of carbonates, sandy sediments, and parent rock determine the natural addition of chromium to the environment. [6]

Furthermore, manufacturing, rapid industrialization, and natural contamination are the main causes of anthropogenic chromium pollution in the environment. Numerous industrial processes make use of chrome. [7] The primary industries that need significant amounts of chromium compounds on a constant basis are the chemical, pharmaceutical, wood preservation, electroplating, metallurgical, refractory, and leather tanneries. [8]

Chromoxides, dichromates, and chromates are among the chemicals used in the oxidative alkaline roasting process. The synthesis of various intermediate chromium compounds employs these constituents as building blocks. [9] "Soluble compounds" are the strong oxidizing agents' chromates and dichromates because of their high-water solubility and reactivity. Sodium chromate, the oxidative forms of hexavalent chromium used in numerous industries. Dichromates, chromate pigments, chromic acid, and other kinds of chromium oxide are some examples of these forms. [10]

METHODOLOGY:

The chemicals and reagents employed in this work were of highly precise analytical and molecular quality. Before conducting experiments, all glassware was thoroughly cleaned using a 10% nitric acid solution and then rinsed with double distilled deionized water (DDW) to avoid any metal contamination or other contaminants. For achieve microbiological sterilization, autoclaved sample at 121ºC, 115 kPa for 15 minutes.

A solution of Chromium (VI) metal in water was created at conc. 1000 mg/L and added potassium dichromate salt. The current investigation included the quantification and desiccation of 2.829 grammes of salt at a temperature of 100ºC for a period of 2 hours in a hot air oven. Furthermore, the salt was dissolved in 1 liter of mili-Q water, then filtered through a 0.45 µm Whatman filter paper and subjected to sterilization at 121 ºC for 15 minutes after the filtration process. Upon appropriate dilution, the resulting solution was used for metal analysis.

The flowchart of the methodology as follows:

- Collection of tannery wastewater (TWW) sample
- Physico-chemical analysis of TWW
- Determination of pH
- Determination of Total alkalinity
- Determination of Biological oxygen demand
- Determination of Chemical oxygen demand
- Determination of Total solid
- Total dissolved solid
- Determination of Chloride ions
- Determination of heavy metals present in TWW
- Media composition for isolation and characterization of the chromium resistant bacterial strains capable for the detoxification of hexavalent chromium ion
- Isolation of chromium resistant bacterial isolates from TWW
- Screening of Chromium (VI) resistant bacterial strains
- Chromium (VI) reduction analysis
- Morphological & biochemical characterization of isolated Cr(VI) reducing bacterial isolates
- Biochemical tests
- Molecular identification and characterization of Cr (VI) resistant bacterial isolates

- Media composition of the bacterial consortium for the effective detoxification of hexavalent chromium ion
- Compatibility test of isolated bacterial strains
- Bacterial consortium development
- Reduction study of Cr(VI) by constructed bacterial consortium in LB broth medium
- Reduction of Chromium from tannery wastewater through developed bacterial consortium
- Media composition for optimization of the various environmental and nutritional parameters including carbon and nitrogen sources
- Optimization of environmental parameters (pH and temperature)
- Optimization of nutritional parameters (carbon and nitrogen sources)
- Effect of static and shaking condition
- Effect of different inoculum volume on Cr(VI) reduction studies
- Effect of varying salt concentration on Cr(VI) reduction studies
- Media composition of detoxification of hexavalent chromium ion by the developed bacterial consortium at optimized nutritional and environmental parameters conditions
- Effect of optimized environmental and nutritional conditions on the detoxification of Cr(VI) by bacterial consortium
- Preparation of Crude Cell free extracts
- Chromate reductase enzyme assay
- SDS-PAGE Analysis
- Tannery wastewater (TWW) sample preparation for toxicity evaluation
- Seed collection
- Seed germination (phytotoxicity) assay

FINDINGS, RESULTS AND DISCUSSION:

The extremely toxic wastewater from tanneries has a negative impact on the growth and metabolism of plants and animals. Upon conducting physico-chemical analysis on a tannery effluent sample, significant levels of dissolved and suspended particles, BOD, and COD were discovered. It was an extremely alkaline effluent as well. Because of the high amounts of heavy metal contamination—especially chromium, which was present in excess of what was permitted—the effluent was extremely poisonous and dangerous. When such alkaline wastewater contaminated with chromium is released into freshwater sources, it becomes dangerous for humans to consume. Animal or human consumption of this could have an impact on the marine and terrestrial biota.

Chromium (VI) is currently considered a severe environmental pollutant due to its detrimental impacts on human health and the environment. The results of the experiment showed that the isolated Cellulosimicrobium sp. combination of Microbacterium paraoxydans (SCRB 19), Kocuria flava (SCRB 17), and SCRB 10 showed improved resistance to Cr(VI). The bacteria's minimum inhibitory concentration (MIC) values, which varied depending on the heavy metal and ranged from 250 to 800 mg/L, demonstrated their antibiotic sensitivity and resistance. During the chromium reduction experiment, the isolated bacterial strains shown efficacy in lowering Cr(VI) from lower concentrations (100 mg/L) to higher values (500 mg/L). Bacterial cells exposed to Cr(VI) were found to be larger than those that were not, according to the SEM analysis. This result raises the possibility that the decreased Cr(III) precipitated or adhered to the bacterial cells. Additionally, the cells exposed to Cr(VI) had specific types of chromium peaks detected by energy dispersive X-ray (EDX) analysis. These peaks may be the consequence of reduced Cr(III) precipitating on the cells or complexing with molecules on the cell surface. The strains that had been treated with Cr(VI) underwent FT-IR spectrum analysis to assess the binding of the decreased Cr(VI) product. Cr(III) to reactive functional groups present on bacterial surfaces. The results of the experiment showed that the bacterial strain, Cellulosimicrobium sp., was resistant to several medications and metals. In order to protect the health of people, animals, and the environment, Kocuria flava (SCRB 17), Microbacterium paraoxydans (SCRB 19), and SCRB 10 have the potential to be useful bioremediation agents for the safe removal of metal-contaminated sites. They also demonstrate a stronger capacity to reduce Cr(VI).

The results of the inquiry also demonstrated that the bacterial strains SCRB 10, SCRB 17, and SCRB 19, which were screened after primary and secondary screening tests, were compatible with one another because no inhibitory zone was found in any well. These strains were therefore suitable for the growth of the bacterial consortium. The produced bacterial consortium showed a considerable increase in Cr(VI) rate in broth medium and tannery water and needed a shorter incubation period as compared to reduction using a single strain. The bacterial consortium is an extremely proactive and effective method for the repair of Cr(VI)-contaminated habitats because it functions remarkably well under environmental stress.

Chromium-contaminated environments need to be cleared by microbial reduction of Cr(VI) by bacteria resistant to chromium. It is a very practical, affordable, and trustworthy method of reducing Cr(VI). Temperature, pH, the availability of carbon and nitrogen, and other nutritional and environmental conditions can all affect how quickly chromium-reducing bacteria decompose. Several investigations have demonstrated that temperature and pH have a significant influence on bacteria's ability to carry out bioreduction. It has been demonstrated that most bacteria can lower Cr(VI) levels at neutral pH values of 7.0 or at broadly neutral pH values of 6.5–7.5. Cr(VI) forms can dissolve over a wide pH range. Since the reduction of Cr(VI) is an enzyme-catalysed reaction, variations in the optimal pH range affect the ionization rate of the enzyme, which in turn affects the structure and function of the protein.

Furthermore, the optimal temperature is very important and affects the number of viable cells that different bacterial strains can produce. The growth of germs can be hampered by both hot and low temperatures. The bacterial cell membrane becomes less fluid at low temperatures, which also change the structure of proteins, causing them to become thermally denaturized and activate the enzyme chromate reductase, which lowers Cr(VI). It is suggested that the optimal temperature range is between 30-37^{0}C. pH and temperature, sources of carbon & nitrogen are required for the proper growth and energy metabolism of bacterial strains. In order to achieve a higher rate of Cr(VI) reduction, the main objective of the research was to optimize the bacterial consortium that was formed at various nutritional & environmental parameters.

Bacterial consortiums are favoured over monocultures for reducing Cr(VI). Considering the fact that consortium cultures are more resilient to harsh environmental conditions and more proliferative. The current study's findings showed that improving environmental (pH and temperature) and nutritional (carbon and nitrogen inputs) factors is a workable way to increase consortium cultures' capacity for decrease. On the other hand, when all of the altered conditions were applied concurrently to the same bacterial consortium growing medium, there was a discernible rise in the Cr(VI) concentration. After 48 hours of incubation, it was discovered that applying all optimization parameters at once produced the greatest reduction in Cr(VI). At 100 mg/L and 200 mg/L, the microbial community completely reduced the Cr(VI) concentrations to 0%. Furthermore, at dosages of 300 and 500 mg/L, the decrease% rose significantly in comparison to typical values. In ideal conditions, a well-established bacterial consortium would offer a quicker and more effective means of detoxifying and reducing Cr(VI) while simultaneously cleaning up environments that are heavily chromium contaminated.

The experiment showed how bacteria convert Cr(VI) into Cr(III) by means of enzymes. Numerous unique bacterial species that reduce Cr(VI) include Cellulosimicrobium sp. The chromate reductase enzyme was located in the cell organisms on cellulose sp. Kocuria flava (SCRB 17), Microbacterium paraoxydans (SCRB 19), and SCRB 10 were identified to express the enzyme, according to a quantitative examination of the enzyme's activity. The soluble (cell-free extract) fraction containing Microbacterium paraoxydans showed the highest level of enzyme activity when it comes to Kocuria flava's membrane-bound chromate reductase enzyme activity. Protein with a molecular weight of 43–54 kDa was induced in the presence of Cr(VI), according to SDS–PAGE analysis.

The results of the study unambiguously demonstrate that seed germination rates were significantly lower in untreated wastewater. The detrimental effect could be brought on by the tannery effluent's higher content of suspended particles and chromium metal. Applying untreated wastewater will prevent seedlings from germinating. Compared to control and bacterially treated effluent-grown seeds, untreated sewage-grown seeds showed either very little or no root growth in the untreated seedlings. The germination of these seeds was examined, and it was found that seedlings exposed to untreated tannery effluent either never produced roots and shoots at all or did so only sporadically. On the other hand, the seedlings exposed to the tannery wastewater treated with bacteria had more fully developed roots and shoots. Untreated tannery wastewater reduced seed germination, but treated effluent did not.

Thus, using bacterial consortia to clean up tannery effluent might be a wise and ecologically friendly approach to decrease the negative effects of the waste.

CONCLUSION:

The Common effluent Treatment Plant provided an example of tannery effluent (CETP). A physico-chemical analysis revealed that the TWW sample had very little dissolved oxygen, was highly electroconductive (BOD, COD, suspended and dissolved particles), and was excessively alkaline. Along with other impurities, the wastewater from the tannery included phosphate, nitrate, chloride, and sulfate. There were also a lot of other heavy metals discovered, some of which had maximum concentrations over recommended safe bounds. The heavy metals Cr, Zn, Mn, Fe, and Ni were among them.

It was determined that the thirty bacterial isolates (SCRB 1-SCRB-30) could tolerate the metals present in wastewater collected from tanneries. Based on MIC, six bacterial strains were selected for the reduction test. Three of these six bacterial strains (SCRB 10, SCRB 17, and SCRB 19) showed the highest reduction of Cr (VI) at 100 mg/L (96.88%, 98.19%, and 93.45%) and the maximum tolerance at 800 mg/L using the agar diffusion method. The minimum inhibitory concentrations (MICs) of these three bacterial strains ranged from 250 to 800 mg/L for various heavy metals, indicating that they were both susceptible to and resistant to the antibiotics under investigation. Furthermore, these particular microbial strains. Based on data from cellular morphology, biochemical examination, and 16S rDNA gene sequencing, Microbium cellulosi sp. was assigned the numbers SCRB 10, SCRB 17, and SCRB 19. Microbacterium paraoxydans and Kocuria flava have accession numbers KY883433, KX710178, and KX710177, respectively. Additionally, FTIR and SEM-EDX analyses were performed to look into how the lowered Cr(VI) product affected the cellular structure of the bacterial cells.

It was demonstrated in the chromium reduction experiment that each of the three bacterial strains was capable of reducing Cr(VI) on its own. In an effort to promote the formation of bacterial consortiums, the compatibility of these strains of bacteria was also looked at. The three isolated strains were selected to create a bacterial consortium and were used continually throughout the experiment due to their robust mutualism. The bacterial consortia's 500 mg/L reduction capacity in real tannery effluent was considerably higher than the strain's 100 mg/L reduction capability in broth medium. The AAS analysis of the treated tannery wastewater reveals that the present bacterial population is capable of completely eliminating the chromium in TWW. Therefore, it is possible to efficiently clean up Cr(VI)-contaminated conditions and avoid the need for subsequent treatments by employing a bacterial consortium rather than a single strain.

A bacterial consortium set out to determine the effects of different environmental (pH and temperature) and nutritional (carbon and nitrogen supply) parameters in order to maximize the highest percentage of Cr(VI) that decreases at higher metal concentrations. The formed bacterial consortium showed a notable increase in Cr(VI) rate as compared to a single strain. The optimal ambient conditions for reduction experiments were determined to be 35 ºC and pH 7.0 through optimization procedures. Yeast extract (0.01%) and glucose (0.5%) were determined to be the most effective carbon and nitrogen sources to add to broth medium in order to effectively decrease Cr(VI) up to consortium culture. Additionally, research was done to find out how different salt concentrations, inoculum concentrations, and shaking and resting circumstances affected the Cr(VI) process. The results of these studies showed that the largest reduction in chromium was generated by bacterial consortium cultures cultivated under shaking conditions with increasing amounts of 2 mL inoculum.

Considering that consortium cultures are more prevalent and resilient to unfavorable environmental circumstances. The current study's findings showed that improving environmental (pH and temperature) and nutritional (carbon and nitrogen inputs) factors is a workable way to increase consortium cultures' capacity for decrease. On the other hand, when all of the altered conditions were applied concurrently to the same bacterial consortium growing medium, there was a discernible rise in the Cr(VI) concentration. Under ideal circumstances, the bacterial consortium showed a 72% and 41% reduction of 300 and 500 mg/L and a complete reduction of 100 and 200 mg/L of Cr(VI) for each parameter. Cr(VI). A well-established bacterial consortium would offer a more rapid and effective means of detoxifying and reducing Cr(VI) in all favorable conditions, as well as decontaminating environments highly contaminated with chromium.

Taking into account that Cr(VI) is converted by an enzymatic mechanism to Cr(III). Thus, in the isolated Cr(VI)-reducing bacterial strains, the activity of the enzyme chromate reductase was also investigated. The results of the present studies showed that chromate reductase enzyme activity is present in all three of the bacterial species known as Cellulosi microbium sp. Microbacterium paraoxydans and Kocuria flava. Kocuria flava, a species of microbe, showed membrane-bound chromate reductase activity; however, the most active enzyme in the soluble cell fraction was Microbacterium paraoxydans.

To assess the toxicity of the tannery effluent, phytotoxicity tests were conducted on the seeds of two common agricultural pulses, both before and after bacterial treatment. Phaseolus mungo and Cicer arietinum will serve as examples of the toxic effects of untreated tannery effluent. The phytotoxicity study's findings showed that there was significantly less germination in the untreated wastewater. The seeds grown in untreated wastewater exhibited either very little or no root growth when compared to the seeds grown in control and bacterially treated tannery effluent. This implies that untreated tannery effluent was impeding seed germination. On the other hand, the seedlings' roots and shoots that were exposed to the tannery effluent that had been treated with bacteria had expanded more fully. Therefore, the amount of Cr(VI) and phytotoxicity in tannery effluent can be decreased by employing bacterial consortia for remediation.

Thus, the entire study concludes that a more promising and successful method of reducing Cr(VI) and chromium at greater concentrations from tannery effluent was the formation of the bacterial consortium using three different bacterial strains: Cellulosi microbium sp., Kocuria flava, and Microbacterium paraoxydans. As such, it shows promise as a strong bioremediation agent in chromium-contaminated areas.

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