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Biodegradation of Hexavalent Chromium from Paint Industry Effluent by Indigenous Bacteria

Tofa Begum
Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh
Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Md. Imranul Hoq
Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

Md. Zahidul Haque Bulbul
Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

Jumana Mahmud
Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh
Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

Md. K. Pramanik
Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Md. Abu Hena Mostofa Kamal
Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Md. Naimul Islam
Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Ruhul A. Khan*
Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Abstract

Hexavalent chromium (Cr-VI) is toxic, mutagenic and carcinogenic chemical, whereas its reduced trivalent form (Cr-III) is much less toxic. Cr-VI is widely used in paint industry, tannery industry, and so on. In the present study an attempt was made to isolate naturally occurring bacteria from paint industry effluent possessing high potentiality to reduce Cr-VI. Seven efficient chromium reducing bacterial strains were isolated as Bacillus korlensis, Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Micrococcus varians, Enterobacter intermedius and Tatumella terrea. These bacteria reduced chromium in culture media at maximum 5 mM concentration within a period of 24–72 h as determined by 1, 5-diphenylcarbazide (DPC) colorimetric method. However, significant Cr-VI reduction or biodegradation was observed at 1.25 mM substrate concentration within 24 h at 37°C. The research was very promising for development of a microbiological process to be used in the removal of toxic hexavalent chromium from the environment.

Keywords: Chromium; Biodegradation; Degradation /reduction rate.

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1. Introduction

The paint industries around the world have become the focus of attention due to the increasing demand of paint. Beginning in early 50s, the paint industries in Bangladesh have grown in different zones and are still growing with time because of the continual increase of demand of paints. Environmental legislators as well as researchers because of the enduring contamination of soil, water and air by the discharge of hazardous and non-hazardous wastes, which include liquid effluent, solid waste and volatile organic compounds. Paints, lacquers and varnishes are among the products that have distinct effects on environment and health [1]. Similar other industrial sector of Bangladesh, most of the paint industries have no effluent treatment plant or solid waste management plant, and the untreated effluents are either directly or eventually disposed into drains, canals or rivers, and the solid wastes are dumped into surrounding land or water bodies. Hence, the natural water bodies are being continuously contaminated with toxic organic and inorganic substances, which result in deterioration of the natural water parameters, reduce the dissolved oxygen level, change the composition of organic constituents, and increase the load of heavy metals. A number of heavy metals are used in paints, and paint industrial discharges are reported to contain lead (Pb), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) [2], and concerned due to their toxicity at high levels of exposure. Chromium (Cr) is widely used in paint industry in hexavalent form (Cr-VI) as pigments in paints and inks or as anticorrosive agents to paints, primers and other surface coatings.

*Corresponding Author

Cr-VI used in paint industry includes sodium dichromate ($Na_2Cr_2O_7$), chromium trioxide (CrO_3) and various salts of chromate and dichromate, for example, as lead chromate ($PbCr_2O_4$), potassium dichromate ($K_2Cr_2O_7$), etc. More than 170,000 tone of Cr waste are discharged into the environment annually as a consequence of industrial and manufacturing activities [3].

It is, therefore, crucial to treat heavy metal-contaminated waste water or solid waste prior to its discharge to the environment. A number of physicochemical techniques, such as including precipitation, flotation, ion exchange, solvent extraction, adsorption, cementation onto iron, membrane processing, and electrolytic methods, etc. have been established for eliminating heavy metals from the industrial effluents [4]. These processes have significant disadvantages, which are, for instance, incomplete removal, high-energy or labor requirement, and production of toxic sludge. Due to such constraints, alternative treating techniques, such as those using microbial processes are gaining more considerations, while bacteria or fungi break down or reduce pollutants during their metabolic activities by a process known as bioremediation. Also, several other bacteria including species of *Bacillus* [5], *Pseudomonas* [6], *Streptomyces* [7], *Arthobacter* [8], *Ochrobacterium* [9], *Providencia* [10] and *Leucobacter* [11] use enzymes to convert highly toxic Cr-VI to less toxic trivalent chromium (Cr-III) compounds.

This study thus aims to explore an astounding potential of natural inhabitant bacteria in reducing highly toxic heavy metals from paint industrial discharge using them as energy source and developing highly prospective bioremediation approaches in near future.

2. Materials and Methods

2.1. Sampling

Samples were collected from 10 different points of Elite Paint Industry, 92, Baizid Bostami Road, Chittagong, Bangladesh. It was transported to laboratory and analyzed within 5 hours.

2.2. Isolation of the Bacteria

Paint industry effluent samples were serially diluted up to 10^{-6}. Samples (1 ml) from 10^{-3} to 10^{-6} were spread on the nutrient agar (NA) medium plate. The plates were incubated at 37°C for 24h at inverted position. After incubation, identical single colonies were selected based on their colony characteristics such as color, shape, elevation, margin and surface of bacterial colony, etc., and picked up and transferred into nutrient agar slant.

2.3. Screening of Chromium (VI) Resistant Bacteria by Diphenylcarbazide Colorimetric Method (APHA, 1998)

The ability of isolates in reducing Cr-VI was evaluated by measurement of hexavalent Cr (Cr-VI) reduction in a chromium salt solution. Each strain was inoculated in minimal salt medium (MSM) broth, pH 7, and incubated for time required to obtain mid logarithmic cells of density 10^6/ml as the appearance of optical density (OD) of 0.002 and 0.008 for Gram-positive and Gram-negative strains, respectively, at 625 nm in a UV-visible spectrophotometer (Shimadzu UV-VIS 1800, Japan) [12], which was used as inoculum in the biodegradation assay.

The method is based on the principle that a colorimetric indicator 1, 5- diphenylcarbazide (DPC) reacts with Cr-VI in strongly acidic solution and results in the formation of highly colored carbazone inner complex salt of a chromous ion (Cr-II). This magenta colored complex can be measured spectrophotometrically at 540 nm with extremely high detection power.

In the course of the preparation of calibration curve, the standard solution was diluted ranging the concentrations of 0.2 – 1mM by an interval of 0.2mM, and the absorbance was measured at 540 nm in the spectrophotometer (Shimadzu UV-VIS 1800, Japan).

The efficiency of bacterial isolates in reducing the concentration of Cr-VI was determined by measuring the absorbance at 540 nm of the culture supernatant following centrifugation at 4000 rpm for 5 min at 4°C using DPC reagent as described by APHA. [13].

2.4. Identification of Bacterial Isolates

The bacterial isolates were identified up to species on the basis of colony morphology, microscopic characteristics, and biochemical tests using standard microbiological methods “Bergey’s Manual of Determinative Bacteriology”, 8th edition [14].

2.5. Effect of salt Concentrations and Time

The experiments were conducted by inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37 °C for 24, 48 and 72 h, and determination of Cr-VI reduction by 1,5-diphenylcarbazide colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

3. Result and Discussion

3.1. Screening of Chromium Reducing Bacteria

10 samples were collected in sterile glass bottle from 10 different selected points from a paint industry situated in Chittagong, Bangladesh. Sample vials were transported in laboratory for further investigation.

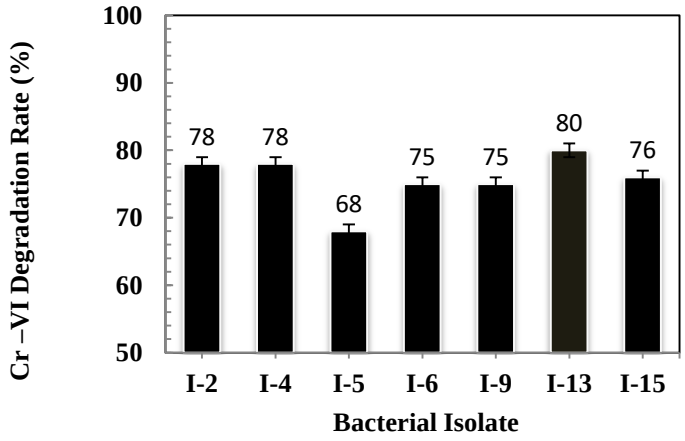
Nutrient agar (NA) media were used to isolate the bacteria from samples by streak plate method. Total 17 isolates were selected on the basis of colony color, shape, elevation, margin and surface bacterial colony, and termed

as I-1 to I-17. The pure single colony of isolates were transferred to nutrient agar slant for investigation and kept at 4°C.

Seven potential strains were found after screening depicted in Figure 1. Here, Cr-VI detoxification rates (%) are plotted against the seven bacterial isolates. Isolate no. 13 (I-13) exhibits highest degradation rate are 80%, and I-5 exhibits lowest degradation rate are 68%. The bacterial isolates I-2, I-4 exhibit 78%, I-15 exhibits 76%, and I-6, I-9 exhibit 75% Cr-VI degradation.

The results obtained from the inoculation of minimal salt medium supplemented with 1 mM $K_2Cr_2O_7$, incubation at 37° C, for 24h and determination of Cr-VI detoxification by 1,5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-1. Bacterial Isolates from Paint Industry Effluent Exhibiting Significant Degradation of Chromium



3.2. Identification of Chromium-VI Reducing Bacteria

The results obtained from morphological, cultural and biochemical tests are shown in **Table 1**. Total 7 bacterial strains were identified including *Bacillus korlensis*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Micrococcus varians*, *Enterobacter intermedius*, and *Tatumella terrea*.

Table-1. Results of Cultural, Morphological and Biochemical Characteristics of Cr (VI) Reducing Bacteria

Tests	**Bacteria**						
	Bacillus korlensis	***Bacillus cereus***	***Bacillus subtilis***	***Micrococcus luteus***	***Micrococcus varians***	***Enterobacter intermedius***	***Tatumella terrea***
Gram stain	+	+	+	+	+	-	-
Spore stain	+	+	+	-	+	-	-
Arrangement	single or in chain	in pair or in chain	single	in pair or cluster	in pair or tetrads	single or in chain	single or in chain
Colony color	brown	opaque	cream	yellow	yellow	yellow	pale orange
Motility	+	+	+	-	-	-	-
Indole	-	-	-	-	-	-	-
Voges-proskauer	+	+	+	-	-	-	+
Methyl-red	+	-	+	+	+	+	+
Simmons' citrate	-	-	+	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	-	+
Oxidase	+	+	-	-	+	-	-
Triple sugar iron	A/A	K/A	K/A	A/A	K/A	A/A	A/A
H_2S Production	-	-	-	-	-	-	-
oxidative-fermentative	F	F	F	F	F	F	F
Glucose fermentation	+	+	+	+	+	+	+
Sucrose fermentation	+	+	+	+	-	+	-
Lactose fermentation	+	-	-	-	+	+	-
Urease	-	+	-	+	+	+	-

Legend: +, positive; -, negative; A/A, Yellow color/acidic slant; K/A, alkaline red slant/ acidic yellow butt; F, fermentative

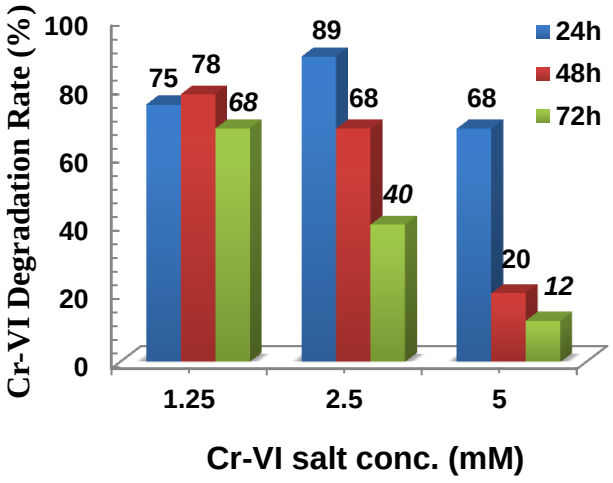
3.2. Degradation/ Reduction Rate of Cr-VI with Time by *Bacillus korlensis*

The reduction rate of hexavalent chromium by ***Bacillus korlensis*** *is shown in* **Figure 2**. Here, Cr-VI detoxification rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. *B. korlensis* exhibited the highest degradation rate (%) is 89% at 2.5 mM salt concentration at 24 h, and the lowest degradation rate is 12% at 5 mM salt concentration at 72 h.

At 1.25 mM salt concentration, the degradation or reduction rate increased 75% to 78% with time at 24 h to 48 h, and at 72 h the degradation rate decreased at 68%. With time at high co ncentration of salt 2.5 mM and 5 mM, the degradation rates were decreased. At 2.5 mM conc., the degradation rate was 68% and 40% at 48 h and 72 h, respectively. At 5 mM salt conc., the degradation rate was 68% and 20% at 24 h and 48 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer. It has been reported that the optimal temperature of Cr-VI reduction could be in the range of 25– 37 °C [15].

Figure-2. Cr-VI Degradation Rate Against Different Cr-VI Salt Conc. by ***Bacillus korlensis***



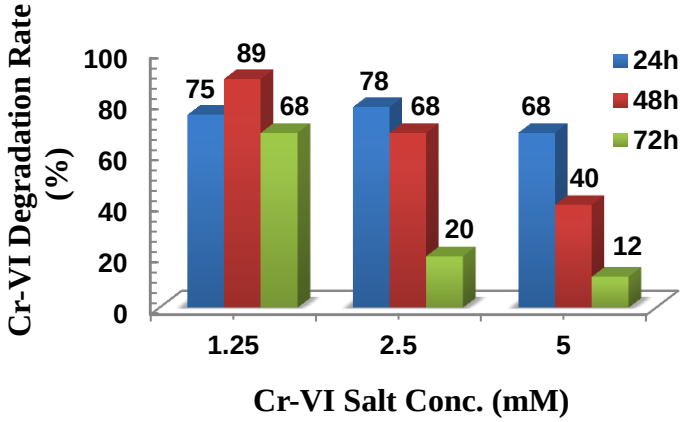
3.3. Degradation/Reduction Rate of Cr-VI With Time by *Bacillus Cereus*

Figure 3 depicted as the degradation rate of hexavalent chromium by ***Bacillus cereus***. Here, Cr-VI degradation rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. *B. cereus e*xhibited the highest degradation rate (%) was 89% at 1.25 mM salt concentration at 48 h, and the lowest degradation rate was 12% at 5 mM salt concentration at 72 h.

At 1.25 mM salt concentration, the degradation or reduction rate increased 75% to 89% with time at 24 h to 48 h, and at 72 h the degradation rate decreased at 68%. With time at high concentration of salt 2.5 mM and 5 mM, the degradation rates were decreased. At 2.5 mM conc., the degradation rate was 78%, 68% and 20% at 24 h, 48 h and 72 h, respectively. At 5 mM salt conc., the degradation rate was 68%, 40% and 12% h at 24 h, 48 h and 72 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-3. Cr-VI Degradation Rate Against Different Cr-VI Salt Conc. by ***Bacillus cereus***



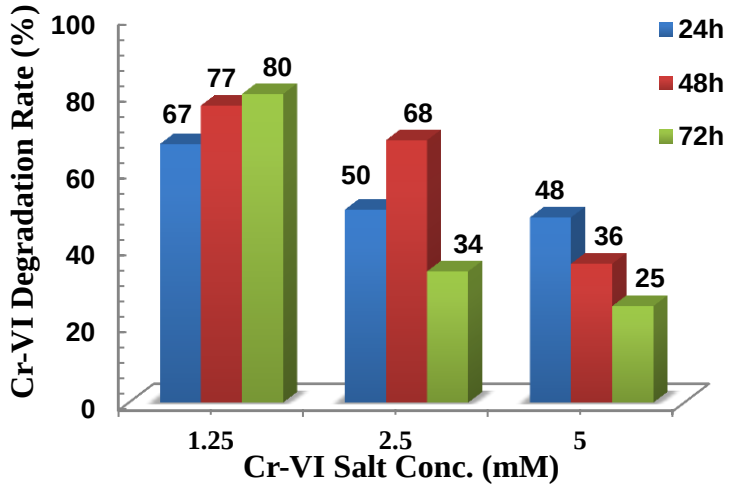
3.4. Degradation/ Reduction Cr-VI With Time by *Bacillus Subtilis*

Figure 4 depicted as the degradation rate of hexavalent chromium by ***Bacillus subtilis***. Here, Cr-VI detoxification rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. *B. subtilis e*xhibited the highest degradation rate (%) was 80% at 1.25 mM salt concentration at 72 h, and the lowest degradation rate was 25% at 5 mM salt concentration at 72 h.

At 1.25 mM salt conc., the reduction rate was 67%, 77% and 80% after 24h, 48h and 72h, respectively. At 2.5 mM of Cr concentration, the degradation rate was Cr (VI) at 50%, 68% and 34% after 24h, 48h and 72h, respectively. At 5 mM of salt concentration, the bacteria reduced 48%, 36% and 25% after 24h, 48h and 72h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-4. Cr-VI Degradation Rate Against Different Cr-VI Salt Conc. by ***Bacillus subtilis***



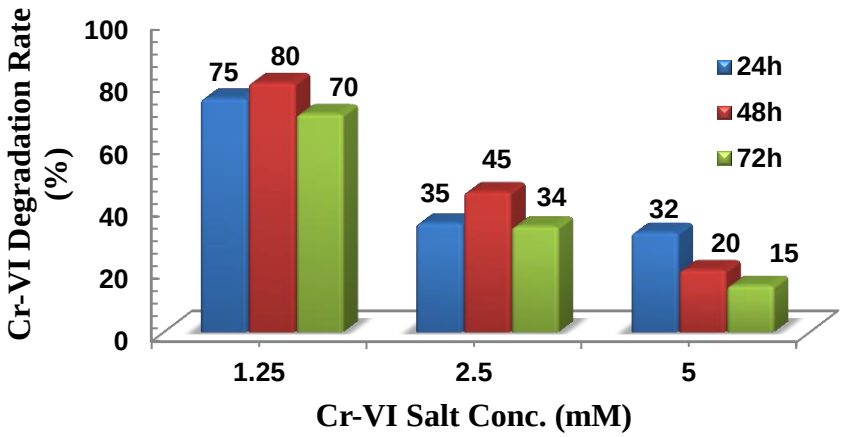
3.5. Degradation or Reduction Rate of Cr-VI with time by *Micrococcus varians*

The degradation or reduction rate (%) of Cr-VI at different Cr salt concentration by ***Micrococcus varians*** is shown in **Figure 5**. Here, Cr-VI degradation rates were plotted against different conc. of Cr salt (1.25 mM, 2.5Mm, 5 mM). The highest degradation rate was 80% at 1.25 Mm salt conc. at 48 h and the lowest rate was 15% at 5 mM salt conc. at 72 h exhibited by *M. varians*.

The reduction rates were increased with time with little fluctuation at 72 h at 1.25 mM and 2.5 Mm salt conc. But degradation rate significantly decreased from 1.25 mM to 2.5 mM and 5 Mm. At 1.25 mM salt conc., the reduction rates were 75%, 80% and 70% at 24 h, 48 h and 72 h, respectively. At 2.5 mM salt conc., the reduction rates were 35%, 45% and 34% at 24 h, 48 h, 72 h, respectively. At 5 mM salt conc., reduction rates were 32%, 20% and 15% at 24 h, 48 h and 72 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-5. The Degradation Rate of Cr-VI over Different Salt concentration by ***Micrococcus varians***



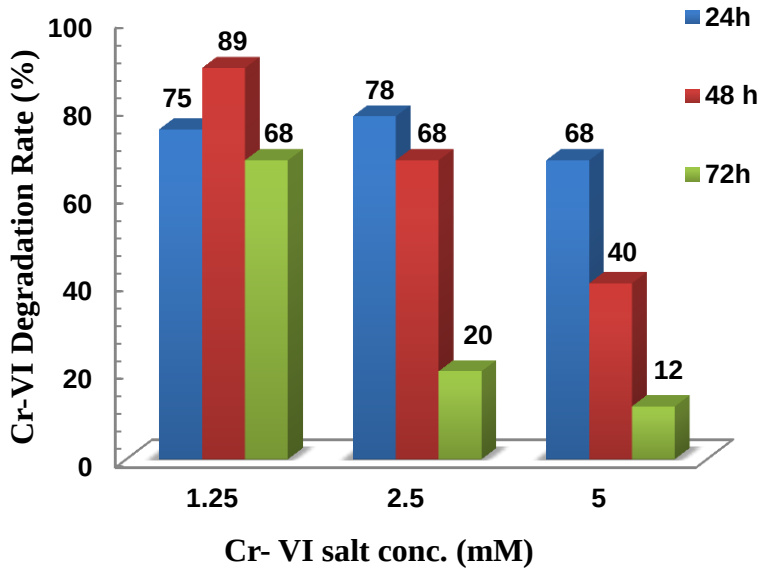
3.6. Degradation or Reduction Rate of Cr-VI With Time by *Micrococcus Luteus*

The degradation rate of hexavalent chromium by *Micrococcus luteus is shown in* **Figure 6**. Here, Cr-VI degradation rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. *M. luteus* exhibited the highest degradation rate was 89% at 1.25 mM salt concentration at 48 h, and the lowest degradation rate are 12% at 5 mM salt concentration at 72 h.

At 1.25 mM salt concentration, the degradation or reduction rate increased 75% to 89% with time at 24 h to 48 h, and at 72 h the degradation rate decreased at 68%. With time at high concentration of salt 2.5 mM and 5 mM, the degradation rates were decreased. At 2.5 mM conc., the degradation rate was 78% , 68% and 20% at 24 h, 48 h and 72 h, respectively. At 5 mM salt conc., the degradation rate was 68% and 40% and 12 % at 24 h, 48 h and 72 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-6. The degradation rate of Cr-VI over different salt concentration by ***Micrococcus luteus***



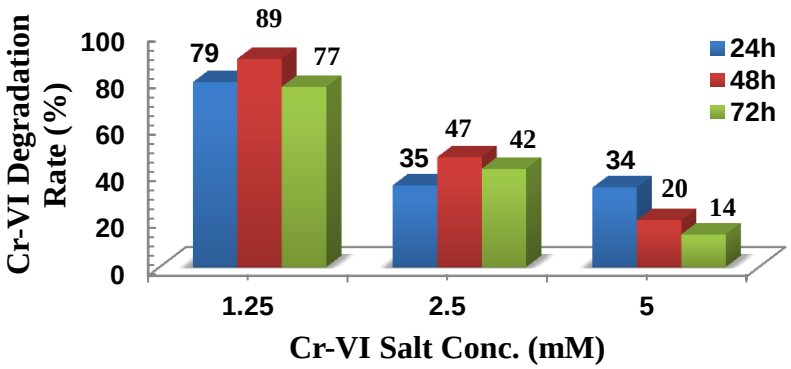
3.7. Degradation or Reduction rate of Cr-VI With Time by *Enterobacter Intermedius*

The degradation rate of hexavalent chromium by ***Enterobacter intermedius*** *is shown in* **Figure 7**. Here, Cr-VI degradation rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. *E. intermedius* exhibited the highest degradation rate was 89% at 1.25 mM salt concentration at 48 h, and the lowest degradation rate are 14% at 5 mM salt concentration at 72 h.

With high salt conc., the degradation rates were decreased. At 1.25 mM salt concentration, the degradation or reduction rates were 79%, 89% and 77% at 24 h, 48 h and 72 h, respectively. At 2.5 mM conc., the degradation rate was 35%, 47% and 42% at 24 h, 48 h and 72 h, respectively. At 5 mM salt conc., the degradation rate was 34% and 20% and 14 % at 24 h, 48 h and 72 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI detoxification by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-7. The Degradation Rate of Cr-VI over Different Salt Concentration by ***Enterobacter intermedius***



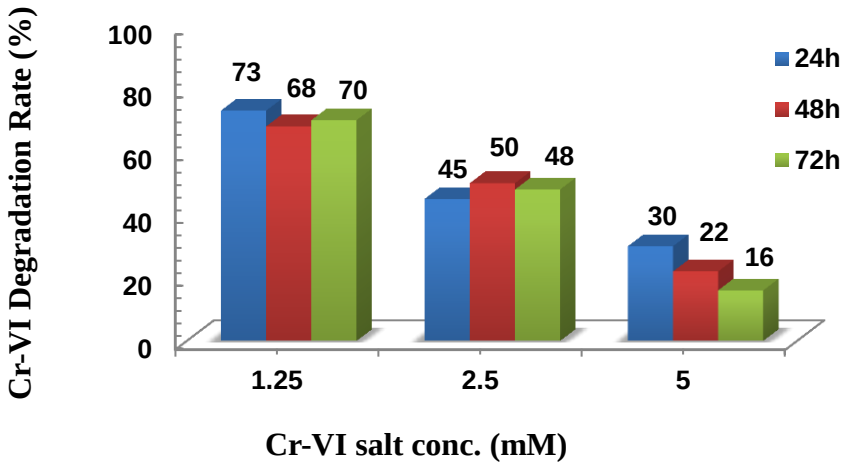
3.8. Degradation or Reduction rate of Cr-VI With Time by *Tatumella Terrea*

The degradation rate of Cr-VI by ***Tatumella terrea*** *is shown in* **Figure 8**. Here, Cr-VI degradation or reduction rates (%) are plotted against $K_2Cr_2O_7$ salt concentration as 1.25 Mm, 2.5 Mm and 5 Mm at multiple time period 24 h, 48 h and 72 h. ***T. terrea*** exhibited the highest degradation rate (%) was 73% at 1.25 mM salt concentration at 24 h, and the lowest degradation rate was 16% at 5 mM salt concentration at 72 h.

The degradation rates were decreased when the salt conc. increased. At 1.25 mM salt concentration, the degradation or reduction rate was 73%, 68% and 70% with time at 24 h to 48 h, and at 72 h. With time at high concentration of salt 2.5 mM and 5 mM, the degradation rates were decreased. At 2.5 mM conc., the degradation rate was 45%, 50% and 48% at 24 h, 48 h and 72 h, respectively. At 5 mM salt conc., the degradation rate was 30%, 22% and 16% at 24 h, 48 h and 72 h, respectively.

The results obtained from the inoculation of minimal salt medium supplemented with 1.25 mM, 2.5 mM and 5 mM of $K_2Cr_2O_7$, incubation at 37° C for 24, 48 and 72 h, and determination of Cr-VI degradation by 1, 5-diphenylcarbazide (DPC) colorimetric method measuring absorbance at 540 nm in a UV-visible spectrophotometer.

Figure-8. The Degradation Rate of Cr-VI over Different Salt Concentration by *Tatumella terrea*



The study revealed that with the increasing of Cr-concentration, and incubation period, the rate (%) of Cr-detoxification or reduction was decreased with little fluctuations at 48hours. It is clear from our results that increase in chromate reduction was growth dependent; higher reduction were noticed during the first 24hours corresponding to log phase of the microbial growth. Such growth dependent chromate reduction has also been earlier reported by [16]. Decrease in reduction ability at high substrate concentration might be due to the toxicity of the Cr-VI.

It is thus obvious the maximum reduction and detoxification by the selected six isolates was achieved at 1.25Mm substrate conc. and 24 hours time interval at 37^0C, and one isolate showed the maximum reduction and detoxification at 2.5mM and 48hours at 37^0C.

4. Conclusion

The present study revealed the capacity of the bacterial isolates to reduce or detoxify of hexavalent chromium and convert them to as non-toxic trivalent chromium. The potential bacterial strains are able to tolerate high concentration of 5mM/1474.25mg/l of hexavalent chromium. Thus the study can be effective in the removal of chromium where high concentrated chromium is present.

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