Hexavalent Chromium Reduction by Some Bacterial Strains Isolated from Some Contaminated Sites

Athmar Ahmad Abdul Hussain[1*], Aamal Ghazi Mahdi Al-Saadi[2]

Abstract

Commonly, Chromium (Cr) is a significant and unique heavy metal commonly utilized in manufacturing. Chromium is one of the greatest hazard chemicals to human health as stated by the United States environmental protection agency (USEPA). This study aimed to assess the potential of some local strains for the reduction of Cr (VI) under diverse environmental conditions. The analysis of Cr (VI) reduction was determined by assessing the absorbance of chemical complex of Cr^{+6} the purple in color linked with 1,5-diphenylcarbazide. The estimation of Cr^{+6} resistant bacteria was detected at several concentrations of Cr (VI) in various growth conditions. The results showed that *Bacillus paraflexus,Bacillus nitratireducens*and *Pseudomonas guzennei* were highly efficient to reduce 100, 500 and 1000 mg/L of Cr (VI) respectively. The growth rates of bacterial strains were decreased with increase in the Cr (VI) concentrations. These strains were able to reduce Cr^{+6} at a vast extent of temperature which is about (25 to 45°C) and with pH levels of from 6 to 8. The best conditions for Cr (VI) reduction as well as the growing isolates were at 37°C temperatures and with pH range from 6.0 to 8.0. *Bacillus paraflexus,Bacillus nitratireducens*and *Pseudomonas guzennei* could be noble applicants for the detoxification of Cr (VI) from contaminated sites. This study was the first of its kind in Iraq, both bacteria *Bacillus paraflexus,Bacillus nitratireducens and Pseudomonas guzennei* were isolated for the first time in Iraq and recorded for the first time inthe world as chromium-reducingisolates, to the best of our knowledge.

KeyWords: Dichromate Potassium Cr(VI), Resistant Bacteria, Bioremediation, Cr+6 Reduction, *Bacillus Paraflexus,Bacillus Nitratireducens, Pseudomonas Guzennei*.

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Introduction

As well known that chromium is one of the important heavy metals generally utilize in manufacturing. Cr (VI) is emitted into the environment during many manufacturing processes, such as oil refining, leather tanning, chrome plating, wood save, and textile industry (1). Chromium is one of the greatest hazard chemicals to human health among the remarkable seventeen chemicals as stated by the United States environmental protection agency (USEPA). World health organization (WHO) stated that the maximum concentration of Cr (VI) must be less than 0.05 mg/L in drinking water (2). Interestingly, the most carcinogenic and toxic type of Chromium is hexavalent Cr (VI), because of its high solubility, rapid penetrability through cell membrane, and successive interactions with nucleic acids and protiens (3). Remarkably, found numerous procedures to remove Cr^{+6}, such as precipitation and filtration (4), electrolysis method(5), solvent extraction(6) and membrane separation(7). Although, these procedures are relatively very high in cost, they have various disadvantages for example, high energy requirements, uncompleted metal removal and regenerate the same poisonous sludge and other pollutants (8). Whereas, bioremediation method that utilizes native microorganisms, is considered an important ecofriendly alternative way to the removal and detoxification of Cr (VI) (9).

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Cr^{+6} contamination that treated by bacteria has the benefits of efficiency, economy, besides no subordinate pollution. Bioaccumulation, biosorption, and enzymatic oxidation/ reduction. Are the processes carried outby the microorganisms where interact with toxic substances. The reduction of Chromium by enzymesincludes a soluble cytosolic chromate reductase or membrane-bound chromate reductase the activity of which is promoted by NADH, pentose, glutathione or FADH2as enzyme co-factors(10, 11). The aim of this study is investigation the ability of some bacterial strains to reduce hexavalent chromium as a potential source of bioremediation.

Materials and Methods

1. Identification and Characterization of the Chromium Resistant Isolates

Bacterial strains were selected and identified as mentioned in our previous study(12).

2. Cr (VI) Reduction Assay

The ability of the bacterial isolates to reduce chromium was tested by colorimetric method(13) Chromium-resistant bacterial isolates were grown in Luria Brittany liquid medium containing different concentrations of chromium (100, 500, 1000) mg/l, and incubated at 37°C, and adjust the pH at 7, then incubated for a period of (24,48,72) hours. Bacteria-free culture medium containing the same concentrations of chromium was used as a control, then 1 ml of sulfuric acid (0.2 M) was added, and 1 ml of DPC reagent. Then gently the mixture was shaked and left for 5 minutes until the purple color appears. After centrifugation, the supernatant was obtained to measure Cr (VI) concentrations. In each supernatant, the content of Chromium was detected by mensuration the purple complex absorbance of Chromium with 1,5-diphenylcarbazide (DPC) using a spectrophotometer at 540 nm (14). The DPC reagent was prepared by dissolving 0.5g of DPC dye powder in 50 ml of acetone, then the volume was completed to 100 ml with acetone to get a final concentration of 0.5 (w/v). The standard curve of $K_2Cr_2O_7$ (0, 0.4, 0.8, 1, 1.2, 1.4, 1.6, 1.8 and 2 mg/L) was as a key role to calculate the Cr (VI) concentration. In terms of % Cr (VI) reduction, the competence of chromate reduction was set by determining the variation between the Cr (VI) concentrations and control (15).

3. The Effect of Chromium on the Growth of Bacterial Isolates

Bacterial isolates were grown in Luria Brittany liquid medium containing different concentrationsof chromium (100, 500, 1000 mg/l). Isolates grown without chromate were used as controls. the cultures as well as controlswere incubated for different periods of time (24,48,72) hours and at a 37°C and pH 7. After the end of the incubation period, for each period of time, the absorbance of the optical density was measured at a wavelength of 600nm using a spectrophotometer, and the absorbance values for each concentration were compared with the control values(13).

4. Assessment ofthe Effects of pH and Temperature

The impacts of pH and temperature on the growth of nominated isolates and Chromium reduction were inspected utilizing an LB medium (20 mL) containing Cr (VI). To investigate the effect of temperature, the media were inoculated with (100, 500, 1000 mg/l) overnight. The media were incubated at various incubation temperatures at (25 and 45°C). the culture medium was sterilized and adjusted to pH of (6.0, 7.0 and 8.0) in order to invest the effect of pH, with present amounts of 0.1 ml of sterilized HCl or NaOH. After that, the tubes were incubated at 37°C. The growth of bacteria was determine through incubation time of (24, 48, 72) hours via quantifying cultures using the optical density (OD) at 600 nm. The culture was centrifuged and the supernatant was used to determine the residual Cr (VI) concentration.

Results

1. Cr (VI) Reduction Assay

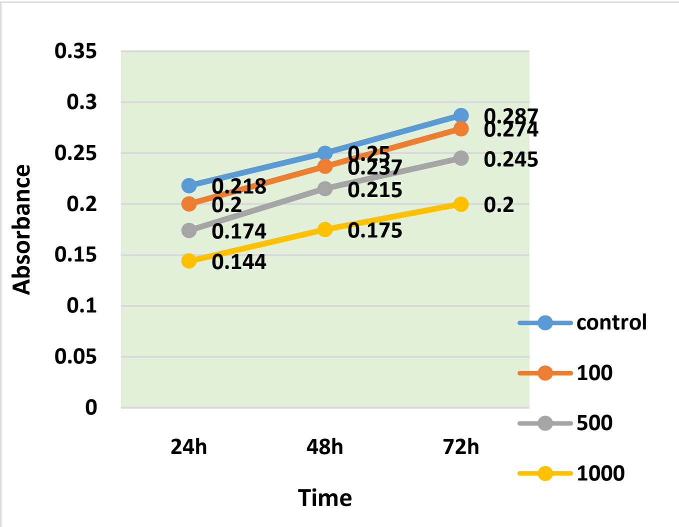
The effect of Chromium concentrations (100, 500 and 1000 mg/L) were examined in the Chromium reducing capability of *B. paraflexus,B. nitratireducens*and *P. guzennei*. The results showed that the allbacterial strains have the ability to reduce Cr (VI), and reduction rates increased with time (table 1).

Table 1. The rates of Cr(VI) reduction bythetested bacterial strains at 37C°, pH7

Conc. of Cr	**100 mg/l**			**500 mg/l**			**1000mg/l**		
Nameof bacteria	24h.	48h.	72h	24h	48h	72h	24h	48h	72h
B.Paraflexus	45%	61%	72%	60%	89%	95%	73%	84%	87%
Control	0	0	0	0	0	0	0	0	0
B.nitratireducens	74%	83%	90%	67%	75%	85%	66%	74%	80%
Control	0	0	0	0	0	0	0	0	0
P.guezennei	84%	94%	98%	80%	92%	97%	79%	88%	91%
Control	0	0	0	0	0	0	0	0	0

2. The Rate of Growth

The curves of growth of*B.Paraflexus, B.nitratireducens* and *P.guezennei* in LB broth medium with (temperature of 37°C and pH of 7.0) in the presence and absence of hexavalent chromiumat diverse concentrations (100, 500 and 1000 mg/L) are illustrated in Figures 1, 2 and 3, correspondingly. As presented in Figures the optical density (OD) of bacterial growth relied on thechromium concentration in the medium. Cr (VI) meaningfully influenced bacterial growth at the500 and 1000 mg/L concentration of Cr(VI), whereas 100 mg/L concentrations of Cr(VI) had only a small influence on their growth.



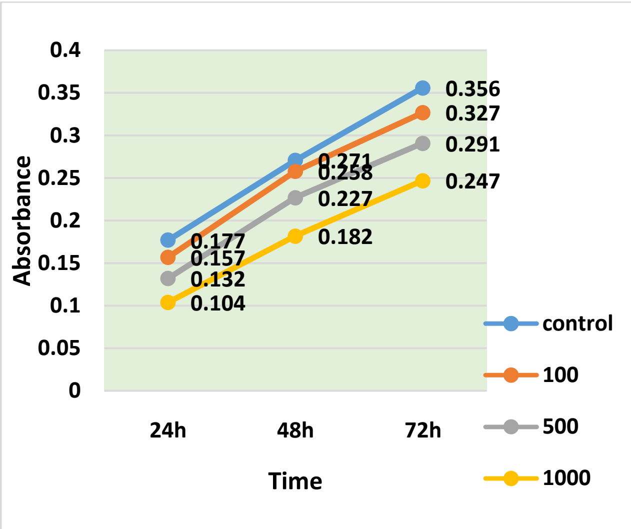


Figure 1. The growth rate ofof*B.Paraflexus***Figure 2.**The growth rate of *B.nitratireducens* ofwith and without chromium.with and without chromium.

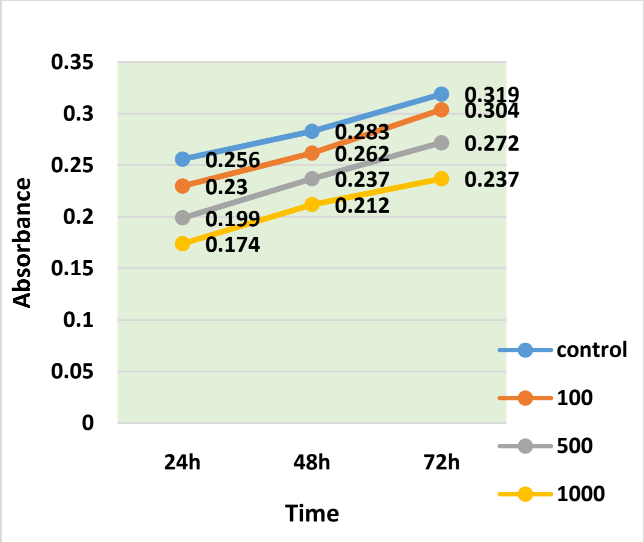


Figure 3. The growth rate of *P.guezennei* with and without chromium

3. Investigation of the impacts of Temperature and pH

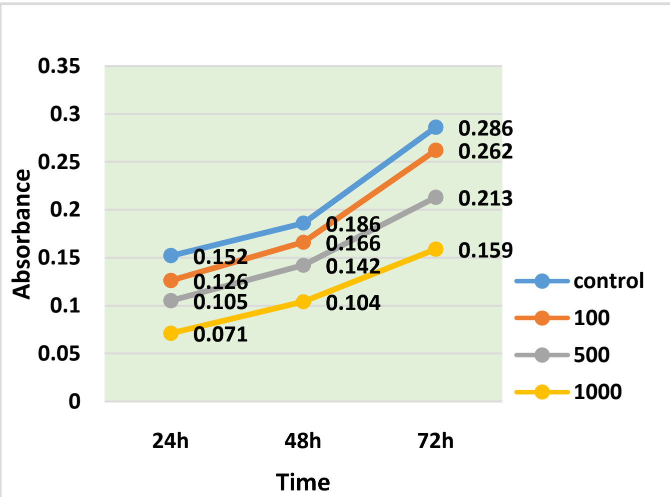
The most favorable temperatures for Cr (VI) reduction and growth were from 25°C to 45°C for bacteria, *B.Paraflexus, B.nitratireducens* and *P.guezennei*. The bacterial growth improved at37°C and then reduced at 25,45°C. The reduction of chromium(VI) by bacteria increased too at 37°C and decreased at 45°C, 25°C. On the other hand, further than 50% of Cr (VI) was reduced by isolates from 25°C to 45°C, (table 2, and table 3). Important alteration was detected in the growth of bacterial isolates in the absence or presence of Cr (VI) at altered temperatures (Figure4). Isolatesof *B.Paraflexus, B.nitratireducens* and *P.guezennei* can grow and raise to reduce about 50% of Chromium (VI) in a extensive variety of pH levels from 6.0 to 8.0, (table4, and table 5). The most favorable pH for growth of bacteria and reduction of chromium (VI) by bacterial isolates were stated to be from 6.0 to 8.0. (Figure 5).

Table2. Cr(VI) reduction at 25°C by selected isolates

Con. of Cr	**100 mg/l**			**500 mg/l**			**1000 mg/l**		
Name of bacteria	24h	48h	72h	24h	48h	72h	24h	48h	72h
B.Paraflexus	42%	55%	70%	56%	64%	73%	55%	70%	77%
Control	0	0	0	0	0	0	0	0	0
B.nitratireducens	64%	73%	79%	50%	63%	74%	44%	54%	66%
Control	0	0	0	0	0	0	0	0	0
P.guezennei	70%	77%	89%	63%	70%	80%	50%	61%	72%
Control	0	0	0	0	0	0	0	0	0

Table3. Cr(VI) reduction at 45°C by selected isolates

Con. of Cr	**100 mg/l**			**500 mg/l**			**1000 mg/l**		
Name of bacteria	24h	48h	72h	24h	48h	72h	24h	48h	72h
B.Paraflexus	38%	50%	65%	45%	66%	70%	37%	60%	69%
Control	0	0	0	0	0	0	0	0	0
B.nitratireducens	40%	50%	62%	34%	52%	55%	65%	44%	42%
Control	0	0	0	0	0	0	0	0	0
P.guezennei	60%	74%	79%	56%	60%	68%	45%	53%	60%
Control	0	0	0	0	0	0	0	0	0



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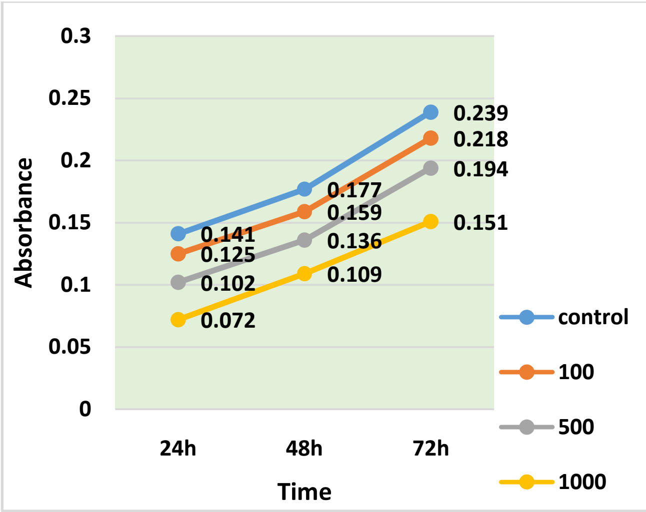

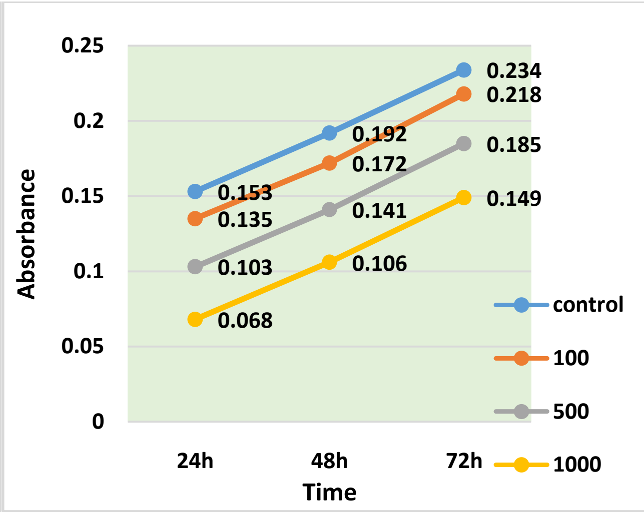

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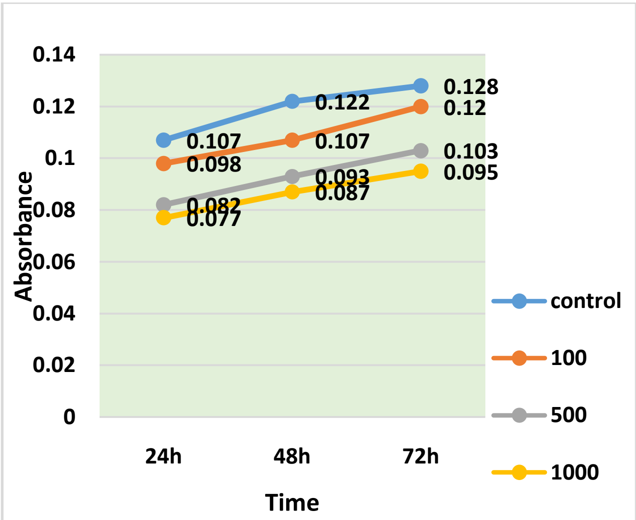
Figure 4. The growth rate of isolates at 25°C, A-*B.Paraflexus*,B- *B.nitratireducens*, C-*P.guezennei*



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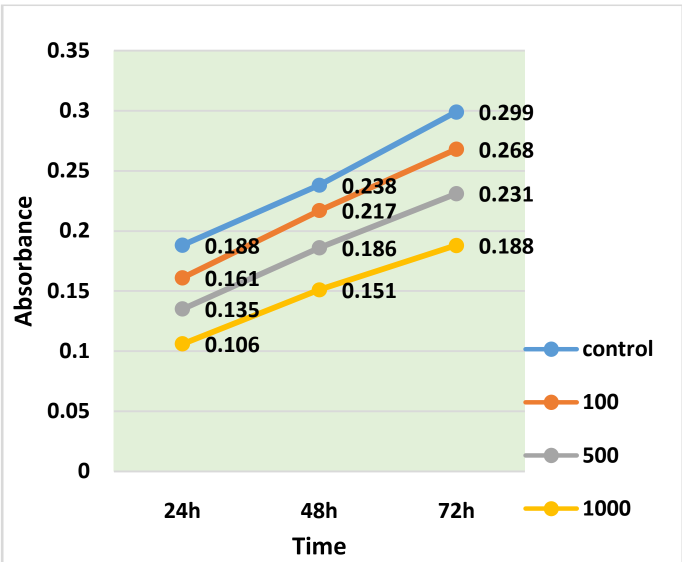

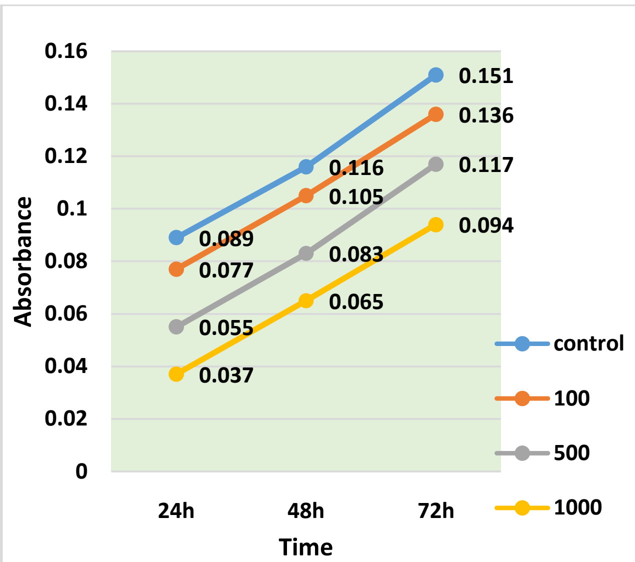

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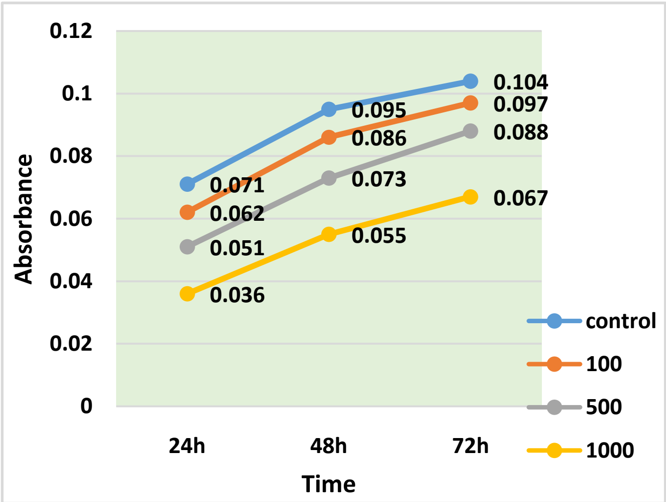
Figure 5. The growth rate of isolates at 45°C, A- *B.Paraflexus*,B- *B.nitratireducens*, C-*P.guezennei.*

Table 4. Cr(VI) reductionby selected isolates at pH 6.0.

Con, of Cr(VI)	100 mg/l			500 mg/l			1000 mg/l		
Name of bacteria	24h	48h	72h	24h	48h	72h	24h	48h	72h
B.Paraflexus	45%	61%	70%	50%	60%	74%	31%	54%	67%
control	0	0	0	0	0	0	0	0	0
B.nitratireducens	61%	78%	80%	57%	64%	70%	44%	53%	60%
Control	0	0	0	0	0	0	0	0	0
P.guezennei	76%	88%	93%	68%	76%	87%	61%	69%	74%
Control	0	0	0	0	0	0	0	0	0

Table 5. Cr(VI) reduction by selected isolates at pH 8.0.

Con. of Cr(VI)	100 mg/l			500 mg/l			1000 mg/l		
Name of bacteria	24h	48h	72h	24h	48h	72h	24h	48h	72h
B.Paraflexus	30%	58%	65%	27%	43%	60%	24%	40%	57%
control	0	0	0	0	0	0	0	0	0
B.nitratireducens	86%	93%	98%	80%	88%	94%	79%	82%	90%
control	0	0	0	0	0	0	0	0	0
P.guezennei	66%	74%	80%	63%	70%	79%	60%	70%	76%
control	0	0	0	0	0	0	0	0	0



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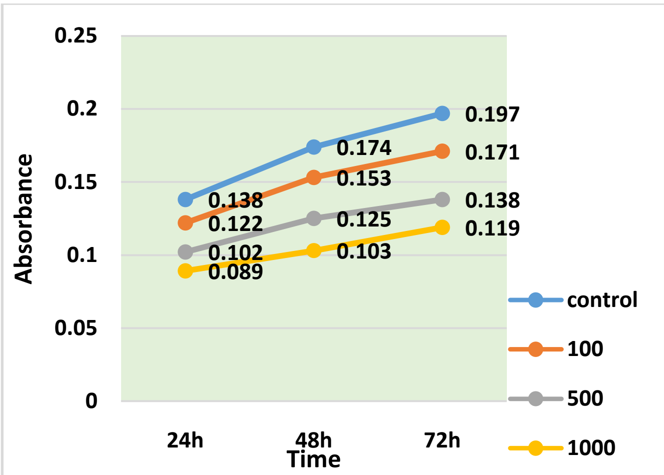

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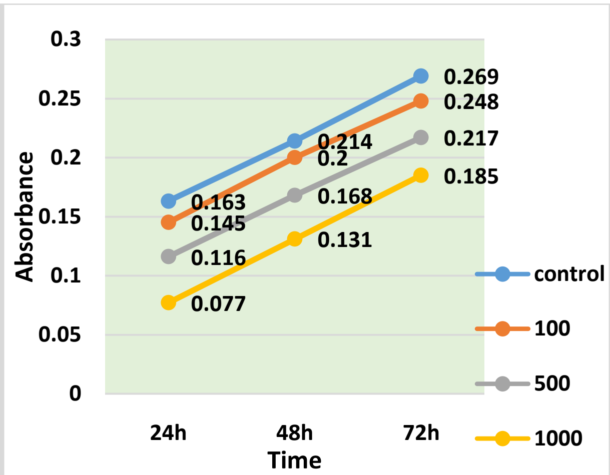

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Figure 6. The growth rate of selected isolates at pH 6.0, A- *B.Paraflexus*,B- *B.nitratireducens*, C-*P.guezennei*

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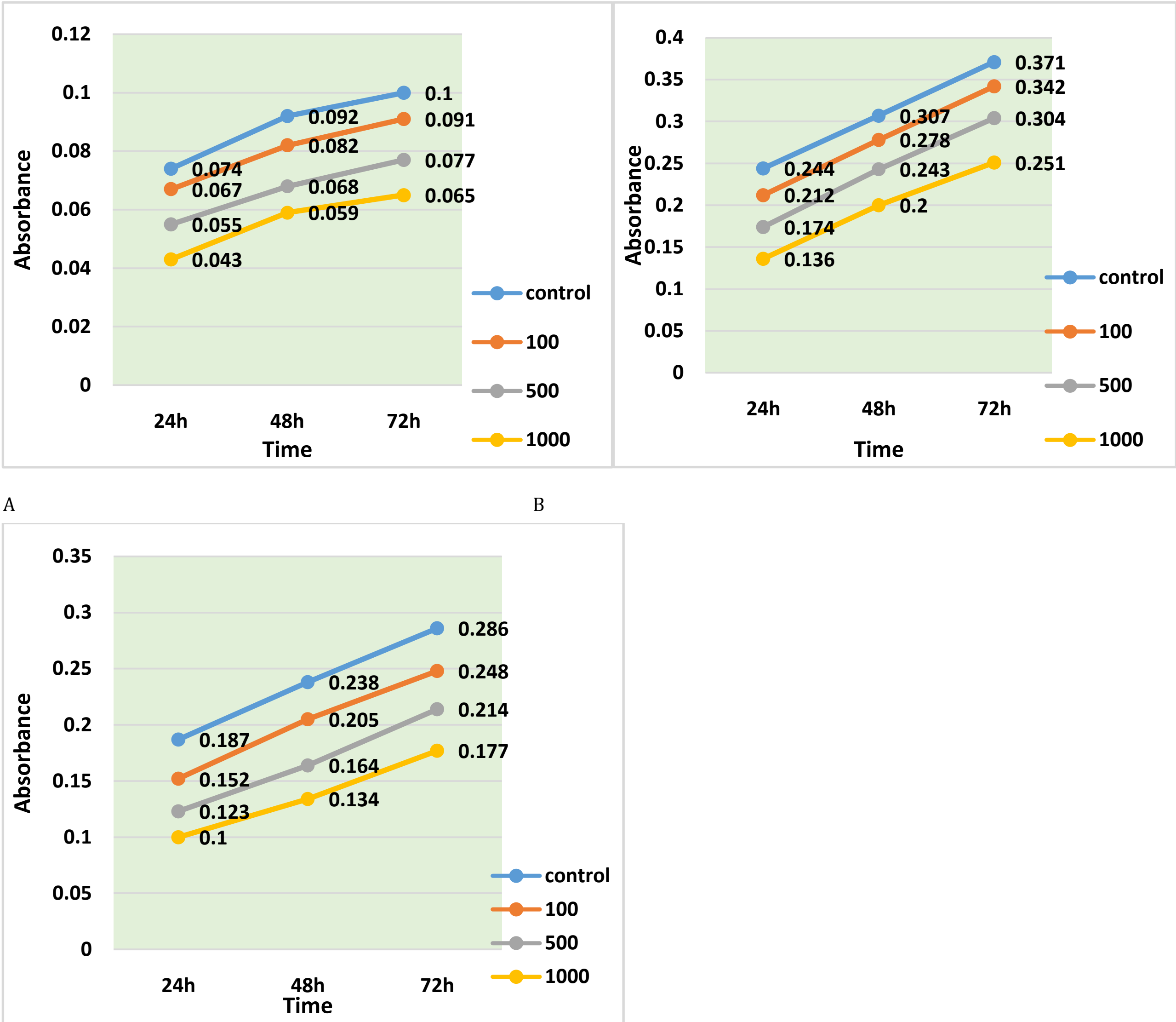

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Figure 7. The growth rate ofselected isolates at pH 8.0, A- *B.Paraflexus*,B- *B.nitratireducens*, C-*P.guezennei.*

Discussion

The bio-reduction of mutagenic hexavalent and highly toxic Cr to comparatively non-toxic trivalent chromium Cr (III) arrange by chromate-reducing bacteria. This feature helps to make an important offers as ecofriendly as well as economical choice for Cr bioremediation (16). In the present studythestrains of*B.Paraflexus, B.nitratireducens*,and *P.guezennei*that were isolated and diagnosed as mentioned in our previous study(12), showed the ability to reduce chromium (VI) at different concentrations under different conditions. Where *B.Paraflexus*displayed a good chromium (VI) reduction ratio at different concentrations, it was verified for the first time to our knowledge as a chromium – reducing and resistant bacteria. *B.nitratireducens* recorded an excellent reduction ratio for the different concentrations of chromium, and this study was the first of its kind to record *B.nitratireducens* as chromium reducing bacteria. The percentages reduction by *P.guezennei*were high, this study was the first study of *P.guezennei*as chromium – reducing bacteria.

The result indicated that hexavalent Cr is a substance that very toxic to bacteria and can easily inhibit their growth, where the growth rates of bacteria decreased in the presence of chromium related to the growth rates in the absenteeism of chromium (control). Satarupa et al (2013) have showed*Pseudomonas* sp. were able to reduce more than 50 and80% of 2 mM chromium(17). Singh (2013) reported that tolerant ofBacillus*cereus* was to 1400 µg/ml concentration of Chromium (VI), and themaximum of reduction was 72% at 1000 µg/ml Cr(VI) concentration(18). Li (2020) also report that *B. cereus* D strain reduced 87.8%of chromium (VI) through 24 h at 37 ◦C at pH 7.0 with an preliminary 2 mM concentration of chromium (VI)(19).

Thacker and Madamwar (2005) have presented that 50 mg/L of Chromium (VI) was reduced in 54 hours to 0 mg/L by bacterial isolate DM1 (20).Showed the current study the complete reduction by selected isolates unsuccessful to occur at the higher concentrations of chromium (VI),which also corroborates the findings of several(21, 22). The growth of *B.Paraflexus, B.nitratireducens*, and *P.guezennei* decreased as the Cr (VI) concentration increased.

The Cr (VI) concentrations 100 mg/L had slightly influence on the bacteria growth. Once the concentration of Cr (VI) increased to 1000 mg/L, a severe reduction of bacterial growth rate was perceived. This directs that the bacterial growth was greatly inhibited because of the Cr (VI) toxicity at the high concentrations of Cr(VI). Our results are in agreement with the study stated by Singh et al (2013) and Soni et al., (2013)(18, 23). Singh (2013) reported that the increasing of Cr (VI) concentrations contribute decreasing *Bacillus cereus* growth rate in medium. This reason belong to the inhibitory effects of greater concentrations of Cr(VI) on the organisms 's growth. Since all organism, possess a particular resistance at a specific growth conditions.

Environmental factors, like pH level and temperature were informed to affect the reduction of chromium (VI) potential of the viable cells of*B.Paraflexus, B.nitratireducens*, *P.guezennei* and control their metabolic mechanisms. The optimum temperature for bacterial growth and Chromium (VI) reduction was at 37°C for selected isolates. It is supposed that the aberration of these features from their optimum may change the chromate reductase action. This could belong to the changes in the ionization and/or conformation of the enzymes (24). In the present study, the optimum pH for bacterial growth and Cr (VI) reduction by chosen isolates was 7.0 for*B.Paraflexus* and *P.guezennei* while*B.nitratireducens* was pH 8.0. Almost greater than 50% of Chromium(VI) concentration was reduced by isolates with range of pH from 6.0 to 8.0 and temperatures range of 25-45°C. Hence, the results suggest that the most appropriates media are a slightly acidic and/or slight basal were variety to accomplish maximum reduction of Cr (VI). This because of the extremely constant nature of Cr (VI) reductase enzyme. Thus, changes in temperature and pH are unlikely to affect the structure of protein and the activity of enzyme. Interestingly, the extreme bacterial growth of Bacillus and reduction of Cr (VI) were demonstrated to be straightly associated with the optimum pH at (7.0). McLean et al.(2000) described that an best wide range of pH from 6.0 to 9.0 for Chromium (VI) reduction by *P. synxantha* (25). Our findings in the current study illustrated that the temperatures, pH levels and Cr (VI) concentrations, can be significant environmental features. These features can regulate bacterial metal tolerance as *B.Paraflexus, B.nitratireducens* and*P.guezennei*, which be able to tolerate about 1500 mg/L of Cr (VI) and have an ability of reduction about 90% at optimized conditions. By comparing the experimental data, the bacteria isolated in this study showed a higher chromium reduction efficiency, not only at the initial chromium reduction concentration but also at the time ofchromium reduction.Moreover, it can be active in the remediation approaches for ecosystems contaminated with hexavalent Cr.

Conclusion

From this study can be concluded that the original strainsfrom contaminated samples and wastes usage their essential ability to damage contaminants such as Cr(VI), which is likewise economically feasible pickwise compared to conventional approaches.*B.Paraflexus, B.nitratireducens* and *P.guezennei* have great reducing capability for Cr (VI) about 100 mg/l, 500 m/l and 1000 mg/l Cr(VI) correspondingly. Since these strains have great potential to decrease the toxic chromium(VI), this feature can be used to detoxify the chromium(VI) positions. Consequently, these results are greatly related to the tannery and mining industry from the bioremediation perspective. On the other hand, extra optimization researches are necessary to optimize the bacterial characters before they can be utilized to decrease high concentrations of chromium.

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