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Bacterial Mediated Bio-degradation of Chromium Cr(VI) and Relative Effects of Varying Temperature and pH on Degradation Potential

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ABSTRACT: *During present study, three bacterial strains viz; Brevibacterium, Ochrobacterium intermedium and Bacillus cereus were isolated from tanneries and electroplating industrial effluents from Kasur, Pakistan and were screened and utilized for In-vitro bio-degradation of toxic chromium. Different temperature and pH ranges were investigated for maximum chromium reduction. Ochrobacterium intermedium was found to be more efficient in reduction of Cr (VI) to Cr (III) as compared to B. cereus and Brevibacterium. The growth responses of bacterial strains to environmental stresses were also observed.*

Keywords: *Environmental stresses, Kasur, Ochrobacterium intermedium, Tanneries*

INTRODUCTION

The major sources of chromium pollution are chrome ores, stainless steel industries, ferro-chrome production, ore refining, chemical and refractory processing, manufactures of alloys, wood preservatives, photographic sensitizers, mordents and pigments for rubber and ceramics (Sexana et al., 1990; Khalil et al., 1991; Panov et al., 2003). Tanneries are one of major source of chromium contamination in Pakistan.

Kasur is 55 kilometers southeast of Lahore and has become well known for the pollution problems mainly caused by large volume of untreated effluents discharge by tanneries. Tanning of leather results in 9000 cubic meters of heavily polluted waste water. An estimated amount of 160 tons/ year of poisonous chromium is discharged only from Kasur tanneries (Khalil et al., 1991). These industries are responsible for reduction of agricultural crops productivity (EPA, 2017). Major vegetable species grown in Kasur are potato, onion, chillies, tomato, melons and other cucurbits. Among these, potatoes are reported to be cultivated at large scale in Kasur and surrounding areas irrigated with chromium contaminated water (Ahmad et al., 2004). Faisal and Hasnain (2001) reported the adverse

effects of hexavalent chromium and lead to the accumulation in *Triticum aestivum*. They further reported the similar adverse effects of chromium on germination and growth of *Helianthus annuus*.

Microorganisms can accumulate chromium and reduce it from Cr(VI) to Cr(III), although high level of Chromium are toxic to these organisms (Ozdemir et al., 2004). Accumulation and sorption of Chromium has been reported in yeast, bacteria (*Helice crassa*) and in seaweed fungus (Coleman, 1988), which may contribute to the presence of chromium in food chain. Genetically engineered bacteria have been used as biosorbent for the removal of heavy metals from contaminated water (Paziradeh et al., 1998). Specific metabolic pathways are involved in bioprecipitation or biotransformation of toxic heavy metals. Accumulation and reactivity of cell wall of microorganism are important for bioremediation of heavy metals, Therefore, introduction of metals binding peptides to the cell wall of these organisms enhances their ability to remediate toxic heavy metals.

The present investigation is based on the use of indigenous bacterial strains to reduce in-vitro chromium toxicity reduction.

MATERIALS AND METHODS

Three bacterial strains; *Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium* isolated from tanneries effluents from Kasur and identified by Faisal and Husnain (2001) were used.

Growth Response:

Growth response of selected three strains of bacteria (*Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium*) was monitored at three different pH levels (5, 7 and 9) with three different temperature variables (28, 37 and 45 ^{o}C). Overnight incubated bacterial cultures adjusted to a uniform O.D (optical density) at 600 nm, were used to inoculate the L-Broth medium supplemented with K_2CrO_4 kept fot 48 hours at 150 rpm. O.D of bacterial cultures at different intervals (0, 8, 24 and 48 hours) was monitored at 600nm.

Temperature Range of Bacteria

Sterilized L-Broth (with and without K_2CrO_4) was inoculated with fresh cultures of bacterial strains after adjusting uniform O.D at 600 nm. The media containing flasks were incubated at three different temperatures 28, 37 and 45 ^{o}C for 24-48 hours. Effect of temperature on bacterial growth was monitored spectrophotometerically (by recording O.D at 600 nm).

pH Range of Bacteria:

pH of the L-Broth (amended with and without K_2CrO_4) was adjusted to different pH levels ranging from 5-11. Sterilized flasks were inoculated with adjusted mass of bacterial cells and incubated at 28 ^{o}C, 37 ^{o}C and 45 ^{o}C on orbital shaker (150 rpm) for 24-48 hours. Optical densities were monitored spectrophotometerically at 600 nm on the Beckman D-2 spectrophotometer.

Cr Metal Resistance Range of Bacteria:

For the determining Cr metal resistance of the selective strains, Nutrient Agar (Peptone 3 gl^{-1}, Beef extract 5 gl^{-1}, NaCl 5 gl^{-1} and Agar 12 gl^{-1}) medium was supplemented with different concentrations (0.5 – 45 mg ml^{-1}) of K_2CrO_4 and poured into plates. Streaked plates were incubated at 37 ^oC and results were recorded after 24 to 48 hours.

Chromium Reduction:

Chlorimetric method by (DeLeo and Ehrlich 1994) was used to analyze the chromium reduction intervals 24 and 48 hours. Reduction medium (Tryptone 10 gl^{-1}, Yeast Extract 5 gl^{-1}, NaCl 5 gl^{-1}, Citric Acid 1 $gl^{-1}$and Na_2HPO_4 6.9 gl^{-1}) inoculated with adjusted bacterial concentration in the test tubes with pH adjusted at 5, 7 and 9 and supplemented with known concentration (500 μg ml^{-1}) of K_2CrO_4 were incubated at 28, 37 and 45 ^oC on orbital shaker (120 rpm). Harvesting of cultures was performed at the intervals of 24 & 48 hours. Chromium reduction was monitored by centrifuging the cultures and analyzing them chlorimeterically.

RESULTS

At different ranges of temperature (28, 37 and 45 ^oC) and pH (5, 7 and 9), the bacterial strains (*Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium*) have shown variations in growth response.

Growth response of *Bacillus cereus*:

Bacillus cereus is a rod like Gram + ve bacterium. These bacteria showed the decrease in the growth response at different physical conditions (temperature and pH) under chromium stress as compared to control. Beside K_2CrO_4, temperature and pH also have significant role in the growth response. *Bacillus cereus* exhibited reduction in growth with the application of the chromium. At acidic pH growth of this strain was very poor at all temperature ranges (28, 37 and 45 ^oC) relative to other pH. Best growth response under chromium free medium was at neutral pH at all temperature ranges whereas under chromium stress alkaline pH was preferred at all temperatures (Table 1, Fig. 1). Nevertheless the growth was significantly better at alkaline/neutral pHs when compare with that of pH 5. Maximum growth was recorded at 37^{o}C which declined at other temperatures.

Growth response of *Brevibacterium*:

Brevibacterium is also rod like Gram +ve with lustrous yellow colonies. It showed a uniform growth response at 37 ^oC at all pH ranges (Table 2, Fig. 2). Acidic pH reduced the growth at all temperatures except 37 ^oC. The reduction in growth response was also observed at the initial stages of the growth at 37 ^oC but in later stages the growth became uniform at all pH levels. At neutral pH, growth was encouraging relative to the other pHs (5 and 9) at all temperatures. The alkaline pH also reduced growth at low temperatures (28 ^oC) and addition of chromium further reduced the growth except at elevated temperature 45 ^oC where the growth response increased as compared to the other pHs (Table 2, Fig. 2).

Under all levels of pH, maximum growth for *Brevibacterium* was observed at 37^{o}C. While the minimum growth was

observed at 28 °C and pH 5 under K_2CrO_4 stress and under stress free medium the minimum growth was observed at 45 °C and pH 9. At all temperature ranges, the reduced growth of *Brevibacterium* was observed under K_2CrO_4 stress conditions except at pH 9 and temperature 45 °C, where the growth increased as compared to stress free conditions.

Growth response of *Ochrobactrum intermedium*:

Ochrobactrum intermedium is a rod shaped gram +ve bacterium, having transparent appearance of colonies. This strain appeared more tolerant as compared to *Brevibacterium* and *Bacillus cereus. Ochrobactrum intermedium* has great ability to grow at all temperature and pH levels at 500 µg ml^{-1} K_2CrO_4 stress and stress free conditions. The growth was found almost uniform at acidic pH under all temperatures (28, 37 and 45 °C) under Cr free and Cr stress media. Under neutral to alkaline pH, the growth of *Ochrobactrum intermedium* was uniform in the later stages of the growth recovering from initial stages, where the significant reduction was observed with the addition of K_2CrO_4 at all temperatures and pHs except at pH 9 temperature 37 °C. Acidic pH reduced the growth under all temperatures 28, 37 and 45 °C. pH 5 seemed to be more sensitive for *Ochrobactrum intermedium* specially when K_2CrO_4 supplemented media was used (Table 3, Fig. 3).

Conclusively, among *Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium,* maximum growth response was exhibited by *Ochrobactrum intermedium* at all temperature and pH ranges. *Bacillus cereus* seemed to be comparatively more sensitive towards K_2CrO_4 at all temperature and pH ranges than *Ochrobactrum intermedium.*

CHROMIUM REDUCTION

Chromium resistant bacterial strains viz; *Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium* showed the tendency of reducing hexavalent form of chromium (initial Conc. 500 µg ml^{-1}) to trivalent chromium at all temperature and pH ranges. Chromium reduction was determined after 24 hours and 48 hours for each strain.

Chromium Reduction Potential of *Bacillus cereus*:

Bacillus cereus reduced 67% of Cr(VI) at 28 °C and 52 % at pH 7 and 9 respectively, after 24 hours of incubation. Only 5 % of the chromium reduction was recorded at pH 5. After 48 hours relatively more chromium reduction at all pH (about 79, 82 and 79 % respectively) was recorded.

At 37 °C, relatively more chromium reduction (about 67 %) was recorded at pH 7 as compared to pH 5 and 9 (~ 53 and 49 % respectively) after 24 hours. *Bacillus cereus* reduced 100 % chromium after 48 hours at pH 7 and 9. Only 71% reduction was recorded at pH 5.

At 45 °C, nearly the same reduction (48, 51 and 47 %) pattern was observed at all pH ranges after 24 hours. But after 48 hours, maximum chromium reduction potential was recorded at pH 9 (81 %) as compared to pH 5 and 7 (71 and 55 % respectively). The most favorable temperature for the chromium reduction for *Bacillus cereus* was 37 °C and the favorable pHs were 7 and 9. (Table 4, Fig. 4)

Chromium Reduction Potential of *Brevibacterium*:

Brevibacterium reduced 89% of initial concentration of chromium at pH 7 and temperature 37°C. Reducing ability/ attribute was observed to be delineated at all other ranges of temperature and pH. Minimum reduction (50.7%) was recorded at same temperature and alkaline pH (9) (Table 4, Fig. 5). After 48 hours of incubation, 100% reduction was recorded at 37°C at pH 5 and 7. This response decreased at other ranges of temperature and pH. Lowering of temperature (28°C) at pH 7 decreased the chromium reduction potential.

Chromium Reduction Potential of *Ochrobactrum intermedium*:

O. intermedium presented to be the most efficient strain to reduce chromium, applied initially. Up to 100% chromium reduction was recorded after 24 hours at 37°C and pH 7 while minimum (58.5%) reduction was recorded at 28°C and pH 5. Increasing incubation time to 48 hours, increased the chromium reduction potential. The strain reduced 100% of applied chromium at 37°C at all pH ranges. Alkaline pH favoured the reduction response of (100%) at elevated temperature (45°C) and low pH decreased the reduction potential compared with other ranges of pH and temperature (Table 4, Fig. 6).

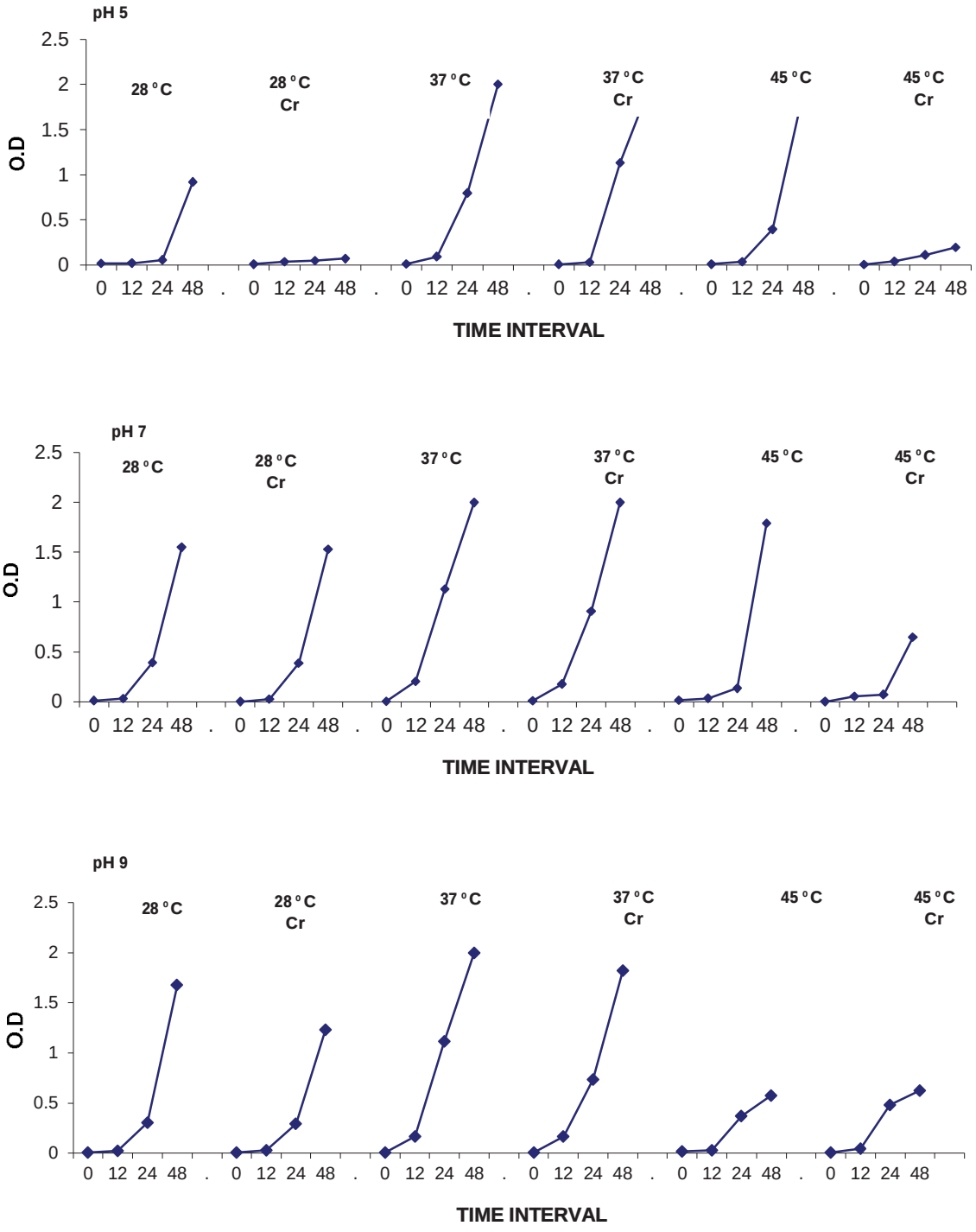

Fig. 1: Growth response of *Bacillus cereus* at 600 nm.

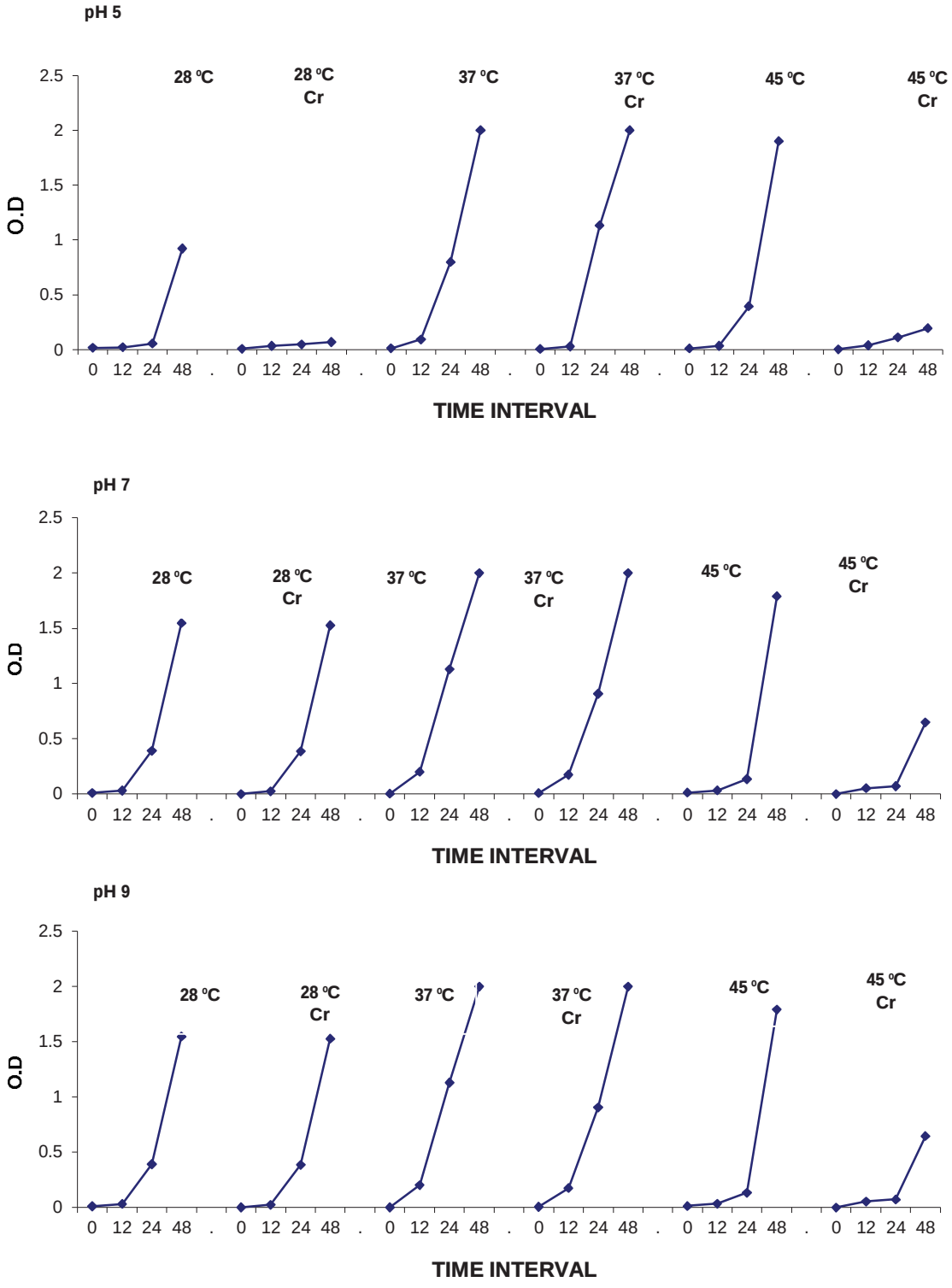

Fig. 2: Growth response of *Brevibacterium* at 600 nm.

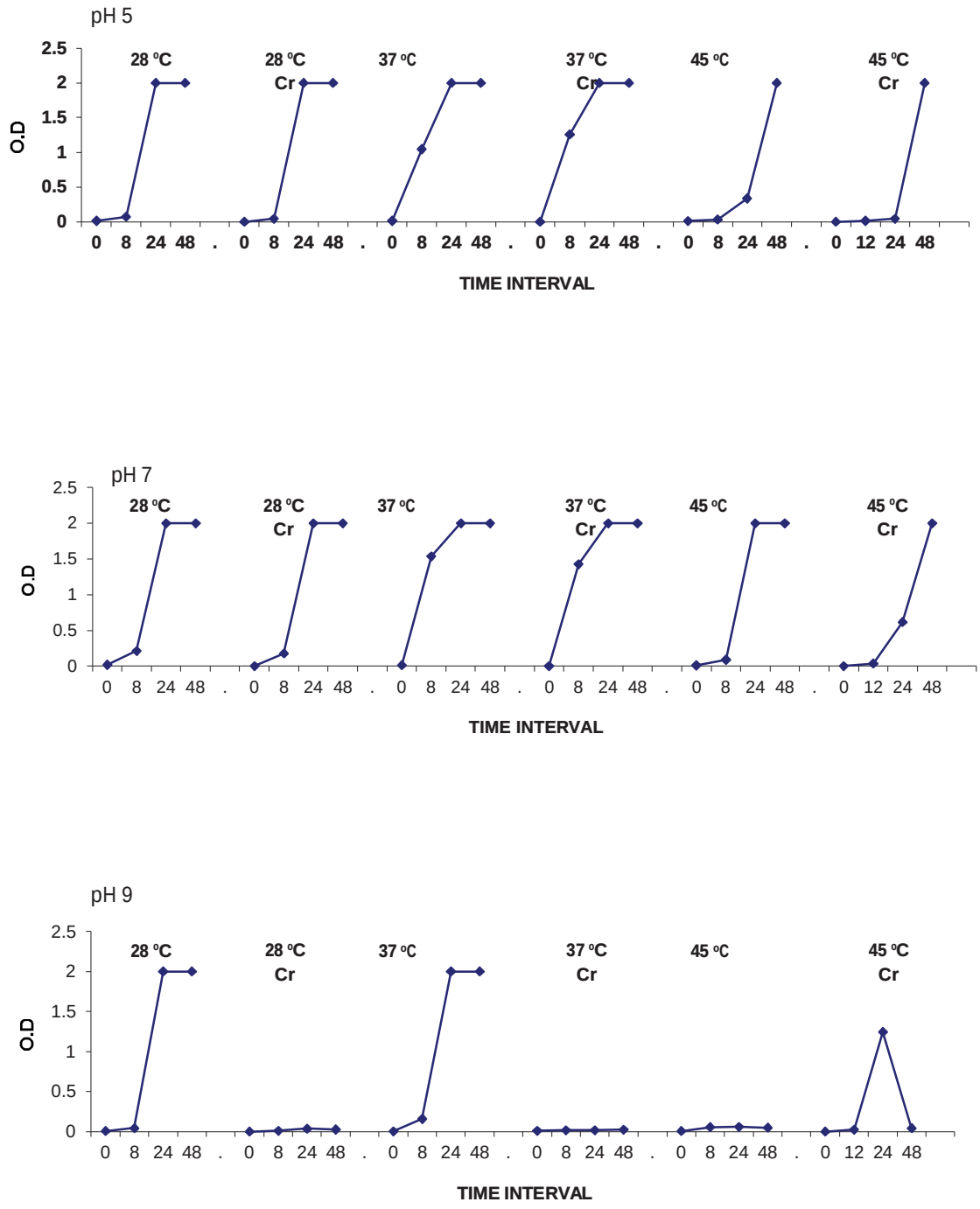

Fig. 3: Growth response of *Ochrobactrum intermedium* at 600 nm.

Table 1: Chromium reduction in % after 24 and 48 hours in bacterial strains Bacillus cereus, *Brevibacterium* and *Ochrobactrum intermedium.*

TIME	Strains	Temp	pH		
			5	7	9
24 HOURS	*Bacillus cereus*	28 °C	5.0 ± 0.5	67.0 ± 1	52.2 ± 1.5
		37 °C	52.8 ± 0.4	67.5 ± 2	49.4 ± 0
		45 °C	47.6 ± 1	51.2 ± 3	46.6 ± 1
	Brevibacterium	28 °C	62.9 ± 0.6	64.8 ± 0	59.6 ± 0.6
		37 °C	67.0 ± 0.4	89.0 ± 0	50.7 ± 0
		45 °C	77.0 ± 3	64.5 ± 2.6	66.6 ± 2
	Ochrobactrum intermedium	28 °C	58.5 ± 0.4	85.9 ± 1.2	65.0 ± 3
		37 °C	83.0 ± 1	100.0 ± 0	86.0 ± 0.75
		45 °C	55.5 ± 0	59.0 ± 0	59.4 ± 2
48 HOURS	*Bacillus cereus*	28 °C	79.5 ± 2	82.5 ± 2	79.1 ± 0.8
		37 °C	70.9 ± 0.6	100 ± 0	100 ± 0
		45 °C	54.5 ± 0.8	70.8 ± 1.2	80.9 ± 1.8
	Brevibacterium	28 °C	71.5 ± 1.4	69.8 ± 2	83.3 ± 0.2
		37 °C	100 ± 0	100 ± 0	85.8 ± 1
		45 °C	84.2 ± 1	80.1 ± 1.2	76.3 ± 2
	Ochrobactrum intermedium	28 °C	83.5 ± 1.6	100 ± 0	100 ± 0
		37 °C	100 ± 0	100 ± 0	100 ± 0
		45 °C	79.4 ± 1.8	94.4 ± 2	100 ± 0

Table 2: Growth response reading of *Bacillus cereus* at 600 nm.

Sr.no	Temp	pH	Culture Condition	Optical Density (OD) at 600 nm			
				0 hour	8 hours	24 hours	48 hours
1	28 °C	5	Without Cr	0.014	0.013	0.035	0.065
2			With Cr	0.008	0.020	0.041	0.036
3		7	Without Cr	0.000	0.100	0.673	0.735
4			With Cr	0.016	0.077	0.226	0.269
5		9	Without Cr	0.051	0.100	0.687	0.919
6			With Cr	0.003	0.123	0.607	0.677
7	37 °C	5	Without Cr	0.011	0.026	0.336	0.932
8			With Cr	0.011	0.341	0.652	0.623
9		7	Without Cr	0.000	0.704	0.865	1.592
10			With Cr	0.016	0.632	0.651	0.975
11		9	Without Cr	0.007	0.045	0.255	0.671
12			With Cr	0.008	0.404	0.874	1.112
13	45 °C	5	Without Cr	0.007	0.017	0.046	0.126
14			With Cr	0.010	0.035	0.042	0.068
15		7	Without Cr	0.001	0.695	0.862	1.175
16			With Cr	0.016	0.184	0.198	0.275
17		9	Without Cr	0.001	0.367	0.687	0.999
18			With Cr	0.010	0.395	0.570	0.785

Table 3: Growth response reading of *Brevibacterium* at 600 nm.

Sr.no	Temp	pH	Culture Condition	Optical Density (OD) at 600 nm			
				0 hour	8 hours	24 hours	48 hours
1	28 °C	5	Without Cr	0.015	0.019	0.053	0.919
2			With Cr	0.006	0.031	0.046	0.067
3		7	Without Cr	0.011	0.031	0.392	1.548
4			With Cr	0.001	0.025	0.386	1.528
5		9	Without Cr	0.007	0.024	0.302	1.682
6			With Cr	0006	0.028	0.295	1.233
7	37 °C	5	Without Cr	0.009	0.091	0.797	2.000
8			With Cr	0.004	0.027	1.130	2.000
9		7	Without Cr	0.002	0.201	1.130	2.000
10			With Cr	0.007	0.176	0.907	2.000
11		9	Without Cr	0.007	0.165	1.114	2.000
12			With Cr	0.004	0.167	0.734	1.824
13	45 °C	5	Without Cr	0.008	0.031	0.393	1.900
14			With Cr	0.001	0.036	0.110	0.192
15		7	Without Cr	0.013	0.034	0.134	1.791
16			With Cr	0.000	0.053	0.071	0.648
17		9	Without Cr	0.019	0.025	0.371	0.576
18			With Cr	0.005	0.045	0.480	0.622

Table 4: Growth response reading of *Ochrobacrum intermedium* at 600 nm.

Sr.no	Temp	pH	Culture Condition	Optical Density (OD) at 600 nm			
				Zero hour	8 hours	24 hours	48 hours
1	28 °C	5	Without Cr	0.007	0.042	2.000	2.000
2			With Cr	0.000	0.010	0.035	0.026
3		7	Without Cr	0.018	0.212	2.000	2.000
4			With Cr	0.000	0.177	2.000	2.000
5		9	Without Cr	0.014	0.071	2.000	2.000
6			With Cr	0.000	0.043	2.000	2.000
7	37 °C	5	Without Cr	0.004	0.160	2.000	2.000
8			With Cr	0.008	0.015	0.016	0.023
9		7	Without Cr	0.013	1.537	2.000	2.000
10			With Cr	0.000	1.426	2.000	2.000
11		9	Without Cr	0.013	1.044	2.000	2.000
12			With Cr	0.000	1.255	2.000	2.000
13	45 °C	5	Without Cr	0.006	0.055	0.059	0.049
14			With Cr	0.000	0.024	1.246	0.044
15		7	Without Cr	0.011	0.088	2.000	2.000
16			With Cr	0.000	0.038	0.617	2.000
17		9	Without Cr	0.009	0.034	0.334	2.000
18			With Cr	0.000	0.013	0.043	2.000

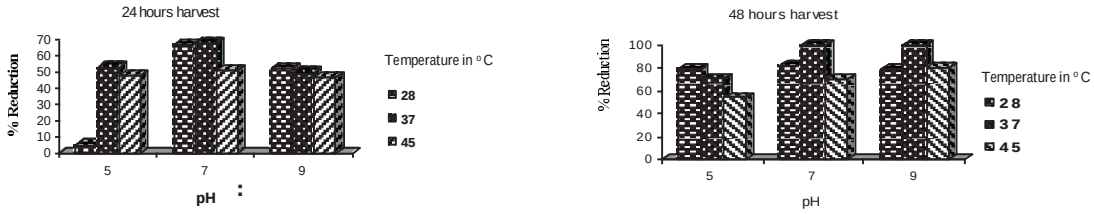

Fig. 4 % Cr(VI) reduction by *Bacillus cereus* at 24 hrs and 48 hours

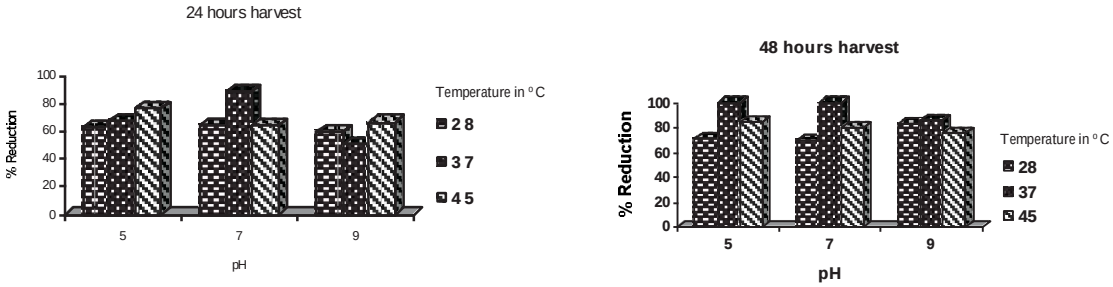

Fig. 5: % Cr(VI) reduction in *Brevibacterium* at 24 hrs and 48 hours.

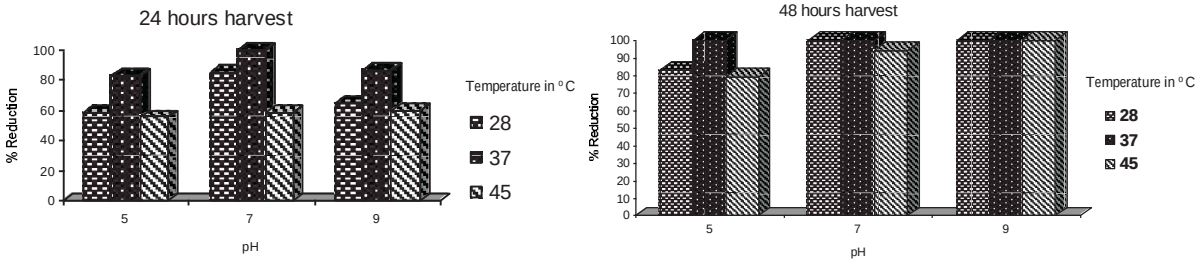

Fig. 6: % Cr(VI) reduction by *Ochrobactrum intermedium* at 24 hrs and 48 hours.

DISCUSSION

Present work deals with the effect of varying temperature and pH on growth and chromium reduction of three bacterial strains *Brevibacterium, Ochrobactrum intermedium* and *Bacillus cereus*. These strains isolated by Faisal and Hasnain (2001) from the tanneries and electroplating industries in Kasur were the subject of this study. All the parameters mentioned were studied under completely sterile conditions. Chromium appeared to reduce the growth of bacterial strains. (Figs. 1-3). Though heavy metals act as limiting factor for the growth of microbes but certain microbes can tolerate the heavy metals by various metabolic pathways. Protein engineering of bacterial chromate reductases can generate improved enzymes that reduce chromate more efficiently, thereby minimizing chromate toxicity to the remediating bacteria and can function in the presence of other pollutants (Matin, 1994; Matin et al., 1995).

To survive under metal-stressed conditions, bacteria have evolved the several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of metal ions inside the cell and reduction of heavy metals ions into less toxic state (Cooksey, 1994; Nies, 1999). Bacterial strains *Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium* {showed different behaviour at extreme pH and temperature ranges}were able to grow under chromium stress with varying conditions of temperature and pH. (Fig. 1-3). In general growth is affected by extreme temperature and pH (Bakerman and Nealson, 2004; Nascimento and Lemos, 2004; Richard and Foster, 2004; Yuk and Marshall, 2004). In alkalophiles bacteria the heat treatment at low pH destroy heat resistant mechanism of spore and induce low survival rate, and optical density decrease and DPA release (Kudo and Horikoshi, 1983). In the presence of chromate, the growth of bacteria reduced at pH 5 (Faisal and Hasnain, 2001). The addition of $K_2 CrO_4$ at higher temperature 45 ^oC reduced the growth of *Bacillus cereus* at pH5. Low temperature 28 ^oC also reduced the growth of *Bacillus cereus* by the addition of $K_2 CrO_4$ in the L-Broth. Both high temperature and low pH are responsible for the reduction of growth of *Bacillus cereus*. (Fig. A). Iqbal (2000) has reported that density of bacteria can be affected by pH. All the strains were able to grow from acidic to neutral range (7-6). The cell surface play a key role in keeping the intercellular pH value ion range between 7 and 8.5, allowing alkalophiles to thrive in alkaline environment (Horikoshi, 1999). In case of *Bacillus cereus*, low pH (5) is the limiting pH which reduce the growth in presence of chromate at low temperature (28 ^oC). The growth of bacteria was better in chromate free nutrient broth as compared to chromate supplemented nutrient broth (Faisal and Hasnain, 2001). Ithoh et al. (1994) reported that the viability of *E.coli* cells showed the decreased growth in the presence of chromate. All the strains showed the reduction in the growth in the presence of chromate at all pHs (except pH9). At higher pH in the presence of chromate, the growth response increased significantly (Figs. 1-3). Although heavy metals are important and essential trace elements, at high concentrations, they can be toxic to microbes. Microbes adopt to tolerate the presence of metals or can even use them to grow (Anne, 2003). The most favourable temperature for

the chromium resistant bacterial strains *Bacillus cereus, Brevibacterium* and *Ochrobactrum intermedium* is 37 ^{o}C. (Figs. 1-3). *Ochrobactrum intermedium* is the most tolerant bacterial strain at all temperatures and pH at 500 $\mu g\ ml^{-1}$ of K_2CrO_4 and *Bacillus cereus* appeared most sensitive strain (Fig. 1) towards K_2CrO_4.

Faisal and Hasnain (2001) reported that *Ochrobactrum intermedium* and *Brevibacterium* at initial K_2CrO_4 concentration of 750 $\mu g\ ml^{-1}$ with inoculum concentration 2.4 x 10^7 and 9.6 x 10^7 respectively are able to reduce 73 and 62 % from the medium after 96 hours. The chromium reduction potential of bacterial strains were examined at different temperatures and pHs. (Fig. 4-6). At pH 7.5 and 37 ^{o}C, a consortium of sulfate-reducing bacteria (SRB) showed a first-order Cr(VI) removal constant of 0.15/h for an initial Cr(VI) concentration of 1000 mg/L due to H_2S production and subsequent precipitation of metal sulfide (Fude et al., 1994). Few studies provide quantitative information about Cr(VI) reduction by indigenous microbes in soil. Organic amended soil in pots reduced Cr(VI) in ground water from 1 mg/L to 50 g/L (Losie et al., 1994). Presence of other heavy metals did not affect the Cr(VI) reduction potential of *O. intermedium* and *Brevibacterium* (Faisal and Hasnain, 2001). At low concentrations different heavy metals (Ni, Mn, Zn, Co and Ag) had no significant effect on the reduction potential of the bacteria strains (Faisal and Hasnain, 2002). Present work is conducted to optimize the maximum reduction potential under various physical conditions. The reduction potential of bacteria was examined. The time factor played major role in the biotransformation of hexavalent chromium to trivalent chromium. The reduction potential was determined after 24 and 48 hours. Julia and Angela (2002) reported that biotransformation occur in the presence or absence of wide range of electron acceptors including oxygen, nitrate, sulfate and ferric iron. The optical conditions are anaerobic at lower chromium (VI) concentrations and aerobic at higher chromium (VI) concentrations. Chromate tolerance conferred by the ChrA protein was associated with reduced accumulation of CrO_4^{-2} in both *Pseudomonas aeruginosa* and *Alcaligenes eutrophus* and it was hypothesized that ChrA was involved in the extrusion of chromate ions (Cervantes et al.,1990) (Cervantes et al., 1988). 100 % chromate reduction was observed at 37 and 45 ^{o}C with pH 7 and 9 after 48 hours for *Bacillus cereus*. For *Brevibacterium*, 100 % reduction of chromium was observed 37 ^{o}C and pH 5 and 7. *Ochrobactrum intermedium* could reduce Cr(VI) to Cr(III) under all conditions of temperature and pH of three strains. All the bacterial strains favoured the maximum chromium reduction at 37 ^{o}C with neutral pH. (Fig. E, F, G). *Ochrobactrum intermedium* was found to have maximum chromium reduction potential.

CONCLUSION

Present study suggests the effective use of indigenous bacterial strains for the bioremediation of toxic effluents released from different textile and tanning industries. As evident from current investigation, three bacterial strains *viz*; *Brevibacterium, Ochrobacterium intermedium* and *Bacillus cereus* were isolated, screened and utilized for *In-vitro* bio-degradation of toxic chromium. These strains not only removed chromium but

also had different growth responses towards different concentrations of pollution load. The ability of these strains could also be used to treat other heavy metals added in our environments as pollutants.

REFERENCES

1. Ahmad B, Bakhsh K and Hassan S (2004) Economics of growing carrot, Faculty of Agricultural Economics and R.S., University of Agriculture, Faisalabad. A report submitted to Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan.

2. Anne S (2003). Implications of Microbial Heavy Metal Tolerance in the Environment. Reviews in Undergraduate Research, 2: 1-6.

3. Bakerman C, Nealson KH (2004). Relationship of critical temperature to macromolecular synthesis and growth yield in *Psychrobacter crypegella.* J. Bacteriol., 186: 2340-2345.

4. Cervantes C, Ohtake H, Chu L, Misra TK and Silver S (1990). Cloning, nucleotide sequence, and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. J. Bacteriol. 172: 287–291.

5. Coleman RN (1988). Chromium toxicity: effects on microorganisms with special reference to the soil matrix. In: *Chromium in the Natural and Human Environments,* Nriagu, J. O. and Nierboor, E., Eds., John Wiley and Sons, New York: 335–368.

6. Cooksey DA (1994). Molecular mechanisms for copper resistance and accumulation in bacteria. FEMS Microbiol. Rev., 14: 381-386.

7. DeLeo PC, Ehrlich HL (1994). Reduction of hexavalent chromium by *Pseudomons flurescence* LB300 in batch and continuous cultures. Appl. Microbiol. Biotechnol., 40: 756-759.

8. Environmental Protection Agency of Pakistan (2017). Chromium in Drinking Water. (http://www.environment.gov.pk)

9. Faisal M, Hasnain S (2001). Reduction of toxic hexavalent chromium by bacterial strains isolated from the effluents of tanneries. Pak. J. Bot., 33: 65-671.

10. Faisal M, Hasnain S (2002). Bacterial mediated aerobic reduction of toxic Cr(VI) in industrial effluents. Pak. J.Microbiol., 2: 27-32.

11. Faisal M, Hasnain S (2004). Bacterial reduction of toxic Cr(VI) into Cr(III). Chin. J. Biotech., 20: 774-778.

12. Fude L, Harris B, Urrutia MM, Beveridge TJ (1994). Reduction of Cr (VI) by a consortium of sulfate-reducing bacteria (SRBIII). Applied and Environ. Microbiol. 60: 1525 1531.

13. Horikoshi K (1999). Some applications of their products for biotechnology. Microbiol. Mol. Bio. Reviews., 63: 735-750.

14. Iqbal A (2000). Variation in morphology. Physiology and biochemical attributes of bacterial strains from polluted water, soil and rhizoplane of plants. M.Sc. Thesis, University of the Punjab, Lahore, Pakistan.

15. Ithoh M, Nakamura M, Suzuki T, Kawai, K, Horitsu H and Takamizawa, K (1994). Mechanism of chromium (VI) toxicity in *E. coli*. In: *hydrogen peroxide essential in Cr (VI) toxicity.* J. Biochem., 117: 780-786.

16. Julia KT, Angela RB (2002). Low-Temperature Chromium(VI) Biotransformation in Soil with Varying Electron Acceptors. J. Environ. Qual. 31:1831–1841.

17. Khalil M, Adeeb F, Hassan S and Iqbal J (1991). Annual Progress Report of EPA Research Laboratory. Environmental Protection Agency, Hons. Phy. Environ. Plan. Dept. Government of Pakistan.

18. Kudo T, Horikoshi K (1983). Effect of pH on heat resistance of spores of alkalophilie *Bacillus* No 2b-z. Agric. Biol.Chem., 47: 403-404.

19. Losie E, Amrein C and Frankenberger WT (1994). Environmental biochemistry of chromium. In: *Reviews of Environmental Contamination and Toxicology* (Ware, G.W., ed.), 136: 91-121.

20. Matin A (1994). Starvation promoters of *Escherichia coli.* Their functional regulation and use in bioprocessing and bioremediation. Ann. N.Y. Acad. Sci., 721: 277-291.

21. Matin A, Fraley CD, Little CD and Keyhan M (1995). Use of starvation promoters to limit the growth and selectivity enrich expression of trichloroethylene and phenol transforming activity in recombinant *Escherichia coli.* Ann. Appl. Environ. Microbiol., 61: 3323-3328.

22. Nascimento MM, Lemos JAC and Abranches J (2004). Goncalves RB and Burne, RA, Adaptative acid tolerance response of *Streptococcus sobrinus.* J. Bacteriol., 186: 6383-6390.

23. Nies DH (1999). Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol., 51: 730–750.

24. Ozdemir G, Ceyhan N, Ozturk T, Akirmak F and Cosar T (2004). Biosorption of chromium (VI), cadmium (II) and copper (II) by *Pantoea* sp. TEM18. Chem. Eng. J., 102: 249-253.

25. Panov VP, Gyul Khandan, Yan EE and Pakshver AS (2003). Regeneration of Exhausted Chrome Tanning Solutions from Leather Production as a Method Preventing Environmental Pollution with chromium. *Russian Jour.* Appl. Chem., 76: 1476-1478.

26. Paziradeh M, Wells MB and Ryan LR (1998). Development of bacterium based heavy metal biosorbants: Enhanced uptake of cadmium and mercury by *Escherichia coli* expressing a metal binding motif. Appl. Environ.

Microbiol., 64: 4068-4072.

27. Richard H, Foster JW (2004). *Escherichia coli* Glutamate and Arginine dependent Acid resistance system increase internal pH and reserve transmembrane potential. J. Bacteriol., 186: 6032-6041.

28. Sexana DK, Jain VK and Chandra SV (1990). Fetoplacental maternal uptake of hexavalent chromium administrated orally in rates and mice. Bull. Environ. Contam. Toxicol., 45: 430-435.

29. Yuk HG, Marshall DL (2004). Adaptation of *Escherichia coli* O157 : H7 to pH alters membrane lipid composition, verotoxin secretion and resistance to simulated gastric fluid acid. Appl. Envron. Microbiol., 70: 3500-3505.