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Bioremoval and Bioreduction of Chromium (VI) by the Green Microalga, *Chlorella vulgaris* Beij., Isolated from Laguna de Bay, Philippines

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A strain of the green microalga, *Chlorella vulgaris* Beij., isolated from West Bay, Laguna de Bay, Philippines was tested for its ability to biologically remove and reduce chromium (VI) at varying concentrations (0.1, 1.0, 3.0, 5.0, and 7.0 mg/L) in BG-11 medium without EDTA. The growth of the microalga was significantly inhibited at concentrations higher than 0.1 mg/L with a computed EC_{50} of 1.76 mg/L after 12 days of cultivation. The bioremoval of the Cr from the medium increased with increasing metal concentration up to 1.0 mg/L then started to decline. The cells of *C. vulgaris* bioremoved 62% (4.70 µg 100/mL) of the metal from the medium with 0.1 mg/L Cr (VI) while 0.83% (6.08 µg 100/mL) of the metal was bioremoved from the medium with 7.0 mg/L Cr (VI). A higher proportion (>80%) of Cr bioremoved was bioabsorbed inside the cells than bioadsorbed on the cell walls in all concentrations. The total Cr bioreduction also increased with increasing metal concentration but started to level off after 3.0 mg L^{-1} concentration. The microalga bioreduced a total of 57% (4.30 µg 100/mL) of the metal in the medium with 0.1 mg L Cr (VI) while a total of 5% (36.41 µg 100 mL) was bioreduced in the medium with 7.0 mg/L Cr (VI) . A higher proportion (>67%) of the bioreduced Cr was observed inside the cells for the media with Cr (VI) concentrations below 1.0 mg/L while higher proportion was observed in the culture medium for the treatments with higher concentrations. In terms of the amount of Cr (VI) bioremoved and bioreduced per mg biomass (DW), the cells that grew on the medium with 7.0 mg/L Cr (VI) exhibited the highest values.

Key Words: bioreduction, bioremoval, *Chlorella vulgaris*, hexavalent chromium, Laguna de Bay

INTRODUCTION

Hexavalent chromium and its compounds (chromates and dichromates) are considered as important pollutants in many countries because of their increasing contamination of the environment and toxicity to a variety of terrestrial and aquatic organisms (Hedgecott 1994). The main source of these pollutants are the run-offs and wastewaters of metallurgical (steel and alloys) as well as chemical (pigments, electroplating and tanning) industries. (Nriagu and Niober 1988; Kabata-Pendias & Pendia 2001).

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The usual methods employed in removing Cr (VI) ions involve chemical reduction to lesser toxic and lesser soluble Cr (III) ions followed by chemical precipitation (Kurniawan et al. 2006). However, such processes are expensive and usually produce significant amounts of unwanted secondary products. An alternative and more plausible method is the use of living cells of bacteria, fungi and microalgae for reduction of the metal ion (Hassen et al. 1998; Dursun et al. 2003; Yewalkar et al. 2007).

In our earlier work, two strains of *Chlorella vulgaris* Beij. isolated from two different areas of Laguna de Bay were tested for their resistance and ability to bioremove four

metals namely cadmium, chromium, copper, and lead (Nacorda et al. 2007). The strain from the West Bay area of Laguna de Bay, which is located near the industries, exhibited a higher resistance and removal for Cr (VI) compared with the strain from the South Bay area which is located near agricultural areas. It had a 12 day EC_{50} of 2.01 mg/L Cr (VI) and 27.96% removal at 1.0 mg/L Cr (VI) concentration in BG-11 medium without EDTA. Higher proportion of the removed Cr (VI) was detected inside the cells (19.28%) compared with those detected on the cell walls (8.68%).

However, the study was limited to the evaluation of the bioremoval capacity of the microalga for Cr (VI) using only one metal concentration. Moreover, the Cr (VI) bioreduction capacity was also not investigated. Consequently, this follow-up study was conducted to determine the bioremoval and bioreduction capacity of the microalga at varying concentrations of Cr (VI) with the aim of determining the level of metal concentration that the microalgal strain can bioremove and bioreduce.

MATERIALS AND METHODS

Chlorella vulgaris culture conditions

The freshwater microalga, *Chlorella vulgaris* Beij. from the West Bay of Laguna de Bay was cultured in 100 mL BG-11 medium without EDTA (Stanier et al. 1971) supplemented with $K_2Cr_2O_7$ to achieve final concentrations of 0.1, 1.0, 3.0, 5.0, and 7.0 mg/L of Cr (VI) designated as treatments I-V, respectively. All treatment flasks were inoculated with exponentially growing cultures to achieve an initial cell density of 1 x 10^4 cells mL. These were cultured for 12 days on open culture shelves with an ambient temperature range of 25-27°C under 12:12 dark: light cycle using cool-white fluorescent lamps that provided about 100 µmol photon ms. Cultures were agitated by regular shaking.

Growth Study

At the end of the cultivation cycle, optical cell density (OD) was determined at 625 nm using a UV-Vis Spectrophotometer (Labomed, Inc. Dual Beam 8 Autocell). The absorbance data were converted to biomass dry weight (DW) using the formula:

Biomass DW in mg/L = 0.15 ($OD_{625\ nm}$)

This formula was generated from an established standard curve for this microalgal strain using three 1.0 L cultures with different concentrations that were analyzed for optical density at 625 nm, harvested, oven-dried and weighed in a Mettler (Model H30) balance to get the dry weight.

The computed biomass (DW) for each treatment was used in the US Environmental Protection Agency (EPA) Probit Analysis Program Version 1.5 to determine the $EC_{50,}$ confidence interval and goodness of fit of the Cr (VI) ion to the strain of *C. vulgaris*.

Sample Processing and Cr (VI) Analysis

The *C. vulgaris* cells were harvested by centrifugation at 5,000 x g for 10 minutes using a table-top centrifuge (Kubota KS-5200C). Supernatants (containing the residual chromium) were collected and digested with concentrated nitric and sulfuric acids. The obtained pellets were washed three times with 20mM EDTA solution to remove metal ions that adsorbed on the cell wall and other extracellular materials. These EDTA washings (containing the bioadsorbed chromium) and the washed pellets (containing the bioabsorbed chromium) were also digested with concentrated nitric and sulfuric acids.

All three digested fractions (supernatant, EDTA washings, and washed pellets) were diluted to 100 mL and were divided into two 50 mL portions. The first portion was oxidized using potassium permanganate following the procedures of APHA (1995) in order to oxidize the reduced Cr (III) in the fractions back to Cr (VI). This portion will represent the oxidized fractions that will be used in determining the bioremoval and localization of Cr (VI) in the microalga. The other portion were directly analyzed for the Cr (VI) and will represent the unoxidized fractions. These two fractions will be used in determining the bioreduction of Cr (VI) by the microalga. The Cr (VI) in both portions was quantitatively determined by the Diphenylcarbazide method (APHA 1995).

Cr (VI) Bioremoval and Localization

The amount of Cr (VI) bioremoved per treatment was computed by deducting the oxidized supernatant fraction, which contains the remaining Cr (VI) in the media, from the Cr (VI) concentration of the control flasks. The Cr (VI) bioabsorbed and bioadsorbed by the microalgal cells are the Cr (VI) concentrations of the oxidized washed pellet and oxidized EDTA washing fractions, respectively. The percentage bioremoval, bioabsorption and bioadsorption, on the other hand, were computed using the following formula:

$$\% \text{ Bioremoval} = \frac{\text{control} - \text{oxidized supernatant}}{\text{control}} \text{ X } 100$$

$$\% \text{ Bioabsorption} = \frac{\text{oxidized washed pellet}}{\text{control}} \text{ X } 100$$

$$\% \text{ Bioadsorption} = \frac{\text{oxidized EDTA washing}}{\text{control}} \text{ X } 100$$

Cr (VI) Bioreduction

The amounts of Cr (VI) reduced to Cr (III) in the medium (supernatant), on the cell walls (EDTA washing), and in the protoplasm (washed pellet) were determined by deducting the unoxidized Cr (VI) from the oxidized Cr (VI) determined for each fraction. The total bioreduction per treatment was computed by adding the amount of Cr (VI) reduced in all fractions. The bioreduction percentages in the media, protoplasm, cell wall, and total bioreduction, on the other hand, were computed using the following formula:

$$\% \text{ Bioreduction in the Media} = \frac{\text{oxidized supernatant} - \text{unoxidized supernatant}}{\text{oxidized supernatant}} \text{ X } 100$$

$$\% \text{ Bioreduction in the Protoplasm} = \frac{\text{oxidized washed pellet} - \text{unoxidized washed pellet}}{\text{oxidized washed pellet}} \text{ X } 100$$

$$\% \text{ Bioreduction on the Cell Wall} = \frac{\text{oxidized EDTA washing} - \text{unoxidized EDTA washing}}{\text{oxidized EDTA washing}} \text{ X } 100$$

$$\% \text{ Total Bioreduction} = \frac{\text{sum of all oxidized fractions - sum of all unoxidized fractions}}{\text{sum of all oxidized fractions}} \text{ X } 100$$

Experimental design and statistical analyses

Tests were conducted using Erlenmeyer flasks that were arranged in completely randomized design (CRD). All experiments were conducted in triplicates. The data for each fraction at different Cr (VI) concentrations were analyzed using Duncan's Multiple Range Test (DMRT) and One-way Analysis of Variance (ANOVA) at 5% level of significance.

RESULTS

Growth Study

Significant growth inhibition was observed in all treatments except for the 0.1 mg/L Cr (VI) based on the result of the ANOVA test with DMRT at 5% level of significance. The inhibition was directly proportional to Cr (VI) concentration (Figures 1 and 2). Cultures with Cr

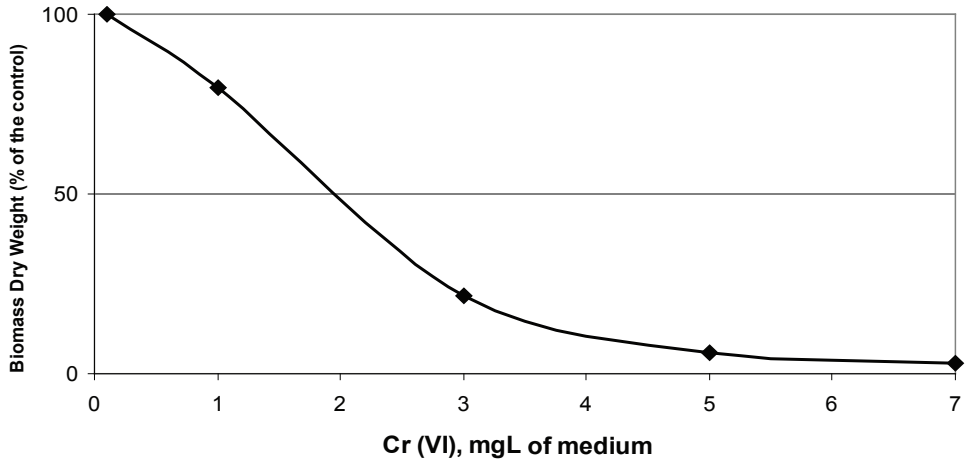

Figure 1. Effect of increasing Cr (VI) concentrations on the cell Dry Weight (% of the control) of *Chlorella vulgaris* after 12 days of incubation.

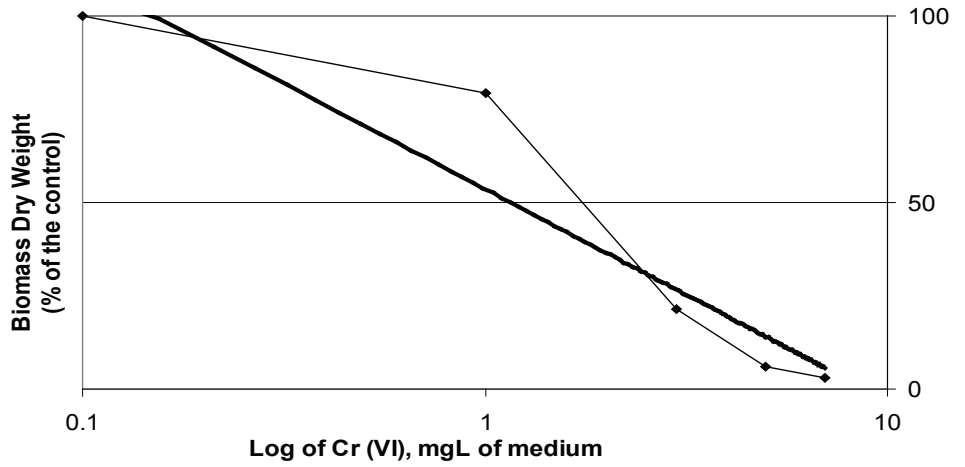

Figure 2. Logarithm of increasing Cr (VI) concentration against the biomass Dry Weight (% of the control) of *Chlorella vulgaris* after 12 days of incubation with an added trendline (thick line).

(VI) concentrations of 0.1 mg/L were not affected while those with 5.0 and 7.0 mg/L hardly grew in biomass. The effective concentration 50 value (EC_{50}) for *C. vulgaris* was determined at 1.76 mg/L of Cr (VI) with a confidence interval of 1.703 – 1.819 mg/L after 12 days of exposure. The Chi-square test for heterogeneity or goodness of fit at 0.05 level of significance had a computed value of 5.185 which is lower than the tabular value of 7.815 which indicates good distribution of data.

Cr (VI) Bioremoval

The cells of *C. vulgaris* were able to bioremove Cr (VI) ions at varying degrees at each concentration as shown in Table 1 and Fig. 3. The amount of Cr (VI) bioremoved rapidly increased with increasing metal concentration and reached its maximum between 1.0 and 3.0 mg/L and declined slowly thereafter. The highest amount of metal bioremoved was observed at 1.0 mg/L treatment with 23.06 µg Cr (VI) per 100 mL at 23 % bioremoval. The removal percentage, on the other hand, decreased with increasing Cr (VI) ion concentration in the same manner as the decrease in biomass DW in Figs. 1 and 2. The highest percentage for removal was observed in treatment I at 62% with 4.70 µg Cr (VI) removed 100 mL.

Table 1 and Fig. 3 also show the amount and percentage of Cr (VI) bioabsorption (intracellular accumulation) and bioadsorption (extracellular accumulation) by *C. vulgaris* at different concentrations. The trends for bioabsorbed amount and percentage of bioabsorption were similar with that of the bioremoval data. The highest amount of metal bioabsorbed was also observed at 1.0 mg/L treatment with 19.80 µg Cr 100 mL of medium at 20% absorption. Likewise, the highest percentage for bioabsorption was also observed in the 0.1 mg/L treatment at 52% with 3.93 µg Cr bioabsorbed 100 mL of medium. The trend for bioadsorption differed from the bioremoval and bioabsorption with a slow increase in the amount bioadsorbed that reached its maximum at the 3.0 mg/L treatment then sharply declined afterwards. The highest amount of metal bioadsorbed was observed at 3.0 mg/L treatment with 4.24 ug Cr (VI) 100 mL at 1.41% bioadsorption. The percentage of bioadsorption also differed, as it exhibited a very slow decrease as Cr (VI) concentration increased. The highest percentage bioadsorption was 10% observed in the 0.1 mg/L treatment with a value of 0.77 ug Cr (VI) 100 mL.

Based on Table 1 and Fig. 3, the higher proportion (>80%) of the Cr (VI) bioremoved from the media in all

Table 1. The amount of Cr (VI) bioremoved, bioabsorbed and bioadsorbed per 100 mL at different concentrations by *Chlorella vulgaris* after 12 days of incubation.

Cr (VI) (mg/L)	Control (µg/100 mL)	Bioremoval (µg/100 mL)	Bioabsorption (µg/100 mL)	Bioadsorption (µg/100 mL)
0.1	7.55 ± 0.18 A	4.70 ± 0.16 A	3.93 + 0.47 A	0.77 ± 0.18 A
1.0	98.67 ± 0.45 B	23.06 ± 0.84 B	19.80 + 1.89 B	3.26 ± 0.57 B
3.0	300.91 ± 0.45 C	21.38 ± 3.71 B	17.14 + 3.25 B	4.24 ± 0.38 C
5.0	513.86 ± 3.00 D	10.78 ± 3.99 A	8.79 + 1.88 A	1.99 ± 0.19 A
7.0	728.21 ± 1.34 E	6.08 ± 5.50 A	4.86 + 0.38 A	1.22 ± 0.09 A

Mean values with the same letter for each column are not significantly different based on ANOVA with DMRT at 5% level of significance
Amount = Mean + Standard Error

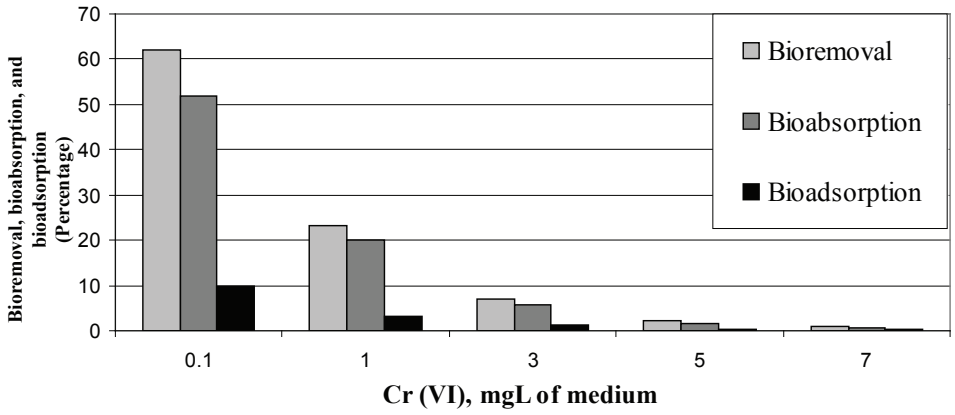

Figure 3. Percent bioremoval, bioabsorption, and bioadsorption at different Cr (VI) concentration by *Chlorella vulgaris* after 12 days of incubation.

treatments was bioabsorbed than bioadsorbed. All values of the bioabsorbed Cr (VI) were also significantly higher compared with the values of the bioadsorbed Cr (VI).

Cr (VI) Bioreduction

The cells of *C. vulgaris* were able to bioreduce Cr (VI) to Cr (III) with varying degrees at the different fractions and concentrations as shown in Table 2 and Fig. 4. The total amount of Cr (VI) bioreduced increased rapidly with increasing metal concentration and then stabilized upon reaching 3.0 mg/L (Treatment III). The total bioreduction percentages, on the other hand, decreased with increasing concentration of metal ions.

Treatment IV recorded the highest total amount of Cr (VI) bioreduced (55 µg/100 mL of the medium). However, this is equivalent to only 11% of the total Cr (VI) in the medium. Treatment I, on the other hand, had the highest percentage of the metal bioreduced (57%) but had the lowest amount bioreduced (4.30 µg/100 mL).

Of the two parts of the cell, a greater amount of the metal bioreduced was noted in the protoplasm (Table 2). The highest value noted in the protoplasm was 16.95 µg/100 mL in treatment II while the highest value observed in the cell wall was 1.11 µg/100 mL in treatment III.

The percentage reduction in the culture medium showed a bell-shaped curve response. There was a gradual increase until it reached a maximum percent bioreduction of 11% (33.32 µg/100 mL medium) in treatment III then gradually decreased thereafter (Fig. 4). The two parts of the *C. vulgaris* cell (protoplasm and cell wall), on the other hand, showed a decreasing trend in percentage bioreduction of the metal ion with increasing Cr (VI) concentration.

Based on Table 2 and Fig. 4, the higher proportion (>67%) of the Cr (VI) bioreduced from the media in treatments I and II was observed inside the protoplasm while the higher proportion was observed in the culture medium at the other treatments with higher Cr (VI) concentrations.

Cr (VI) bioremoval and bioreduction per mg DW

The efficiency of the *C. vulgaris* cells in terms of Cr (VI) bioremoval and bioreduction was assessed by determining the amount of bioremoved and bioreduced

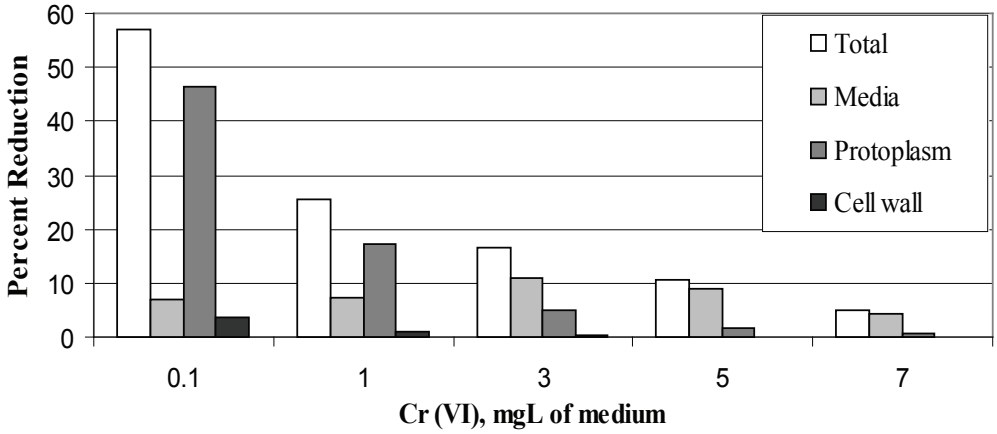

Figure 4. Percent Cr (VI) bioreduction in the media, on the cell wall and in the protoplasm at different concentration by *Chlorella vulgaris* after 12 days of incubation.

Table 2. The amount of Cr (VI) bioreduced in the culture media, on the cell wall and protoplasm at different concentrations by *Chlorella vulgaris* after 12 days of incubation.

Cr (VI) (mg L^{-1})	**Total** (µg/100 mL)	Media (µg/100 mL)	Protoplasm (µg/100 mL)	Cell Wall (µg/100 mL)
0.1	4.30 ± 0.84 **A**	0.53 ± 0.20 **A**	3.50 ± 0.45 **A**	0.27 ± 0.60 **A**
1.0	25.30 ± 3.61 **B**	7.32 ± 2.52 **A**	16.95 ± 1.86 **C**	1.03 ± 0.22 **A**
3.0	49.84 ± 2.14 **C**	33.32 ± 3.59 **B**	15.41 ± 3.30 **C**	1.11 ± 0.57 **A**
5.0	55.04 ± 12.91**C**	46.38 ± 11.73 **B**	8.56 ± 1.84 **B**	0.10 ± 0.51 **A**
7.0	36.41 ± 4.71 **C**	31.68 ± 4.85 **B**	4.67 ± 0.39 **B**	0.05 ± 0.02 **A**

Mean values with the same letter for each column are not significantly different based on ANOVA with DMRT at 5% level of significance
Amount = Mean + Standard Error

Table 3. Bioremoval and total bioreduction per mg biomass (Dry Weight) of *Chlorella vulgaris* Beij.

Cr (VI) (mg L)	Biomass (mg DW/100 mL)	Cr (VI) bioremoved (µg/mg DW)	Total Cr (VI) bioreduced (µg/mg DW)
0.1	14.63 ± 0.66 A	0.32	0.29
1.0	11.63 ± 1.03 B	1.98	2.18
3.0	3.16 ± 0.31 C	6.77	15.77
5.0	0.86 ± 0.23 D	12.53	64.60
7.0	0.45 ± 0.04 E	13.51	80.91

Mean values with the same letter are not significantly different based on ANOVA with DMRT at 5% level of significance
Amount = Mean + SE

mg DW^{-1} of the microalgal biomass. Table 3 shows an increasing trend for both bioremoval and bioreduction with increasing concentration of the metal. The highest amount bioremoved and bioreduced per mg DW were both observed at treatment V. The great difference observed between the amount bioremoved and bioreduced in treatments III – V can be accounted to the amount of Cr (VI) bioreduced in the culture medium.

DISCUSSION

The hexavalent chromium is one of the most toxic metal ions contaminating bodies of water (Hedgecott 1994). Its toxic properties arise from its ability to freely diffuse across cell membranes and also because of its strong oxidative potential that can damage enzymes and other macromolecules**.** Lately it has been shown to generate hydroxyl radicals (-OH) inside living cells from hydrogen peroxide (H_2O_2) via the Fenton mechanism (Shi and Dalal 1990; Pinto 2003**;** Shanker et al. 2005; Zsolt et al. 2006). These toxic effects were exhibited in the growth inhibitions on *C. vulgaris* at concentrations higher than 0.1 mg/L. The effective concentration value (EC_{50}) for the microalga was 1.76 mg/L after 12 days of exposure under optimum culture conditions. This value determined was lower than previously determined for the microalga (2.01 mg/L) because biomass (DW) instead of cell number was used to compute the EC_{50} (Nacorda et al. 2007). Biomass (DW) is a more accurate means of growth determination rather than counting the cell number because cells have varying sizes and weights.

To resist the toxic effects of Cr (VI) at low concentrations (1 - 10 mg/L), *C. vulgaris* is equipped with resistance mechanisms like cell wall adsorption, enzymatic reduction to Cr (III), complexation with metallothioneins, and ROS detoxification (Cervantes et al. 2001; Malik 2004). The action of these mechanisms results in the bioremoval and bioreduction of a significant amount of Cr (VI) in the medium.

The optimum concentrations for Cr (VI) bioremoval was between 0.1 - 1.0 mg/L (Treatment I- II) based on the amount of Cr (VI) bioremoved and the percentage of bioremoval. This concentration is below the EC_{50} value of the microalga. The possible reason is the high amount of biomass produced at the said concentrations. More biomass means more cells that can bioremove Cr (VI) ions in the medium. Similar results were observed by Perez-Rama et al. (2002) in the green microalga, *Tetraselmis suecica*, wherein it was noted that the highest amount of Cd (II) bioremoved was at a concentration (6.0 mg/L) lower than its EC_{50} value (7.9 mg/L).

The Cr (VI) bioremoval process of *C. vulgaris* consists of an initial rapid phase of passive extracellular bioadsorption followed by a slower active intracellular bioabsorption which is similar to the biphasic uptake observed in living cells of bacteria, fungi, and other microalgae that are exposed to different metal ions (Garham et al. 1992; Donmez & Aksu 1999; Malik 2004). The long incubation time allowed the second phase to occur to its maximum capacity which resulted in the higher amount of Cr (VI) bioabsorbed within the cell compared with the amount bioadsorbed on the cell wall. Similar result was observed by Matsunaga et al. (1999) and Perez-Rama et al. (2002) for their cultures of marine microalgae exposed to different Cd (II) concentrations for 14 and 6 days respectively. Zsolt et al. (2006), on the other hand, observed more Cr (VI) adsorbed on the cell wall compared with those absorbed inside the cells of *Chlorella pyrenoidosa* because of the shorter incubation time (three days). Another reason for the higher bioabsorption data is the high storage capacity of the protoplasm because of the several resistance mechanisms that are situated inside the microalgal cells.

Bioreduction of the toxic Cr (VI) ion to the less toxic Cr (III) ion in bacteria is facilitated directly by enzymes found in the cytoplasm and on the cell membrane (Losi et al. 1994; Kamaludeen et al. 2003; Opperman et al. 2007). These enzymes utilize different organic compounds as electron donors like NADH, low molecular weight carbohydrates, amino acids, and fatty acid**s** (Wang & Shen

1995). Information on the mechanisms of bioreduction of Cr (VI) in microalgal cells, on the other hand, is limited. Hence, the discussion will be based on bacterial mechanisms that might also be present in *C. vulgaris*.

The Cr (VI) concentrations optimum for total bioreduction was between 0.1 and 1.0 mg L^{-1} (treatments I - II) based on the amount of total Cr (VI) bioreduced and the total percent bioreduction. These concentrations are also below the EC_{50} value of the microalga. Higher proportion of the bioreduced Cr (VI) was found in the protoplasm compared with the culture media and cell wall at the said concentrations.

The bioreduction in the culture media reached its maximum at 5.0 mg/L (Treatment IV) which is three times higher than the maximum reduction in the protoplasm at 1.0 mg/L (Treatment II). This only suggests a more efficient bioreduction mechanism that is stimulated by higher Cr (VI) concentrations. Possible membrane bound Cr (VI) reductases acted on the metal ions surrounding the few remaining cells under non-growth conditions. The large amount of cellular debris observed at this concentration may also provide another reason for the higher reduction. The Cr (VI) reduced to Cr (III) inside the living cells was released back to the medium during the Cr (VI) toxicity induced cell death and degradation. Reduction of Cr (VI) in the medium was also observed by Faisal et al. (2005) who used living cells of two cyanobacteria to remove 0.03 mg/L Cr (VI) from an algal medium for 96 hours at normal culture condition. A reduction of 39.9% and 62.1% was observed for *Oscillatoria* sp. and *Synechocystis* sp. A higher bioreduction percentage (93%) was observed by Rehman & Shakoori (2001) in a strain of *Chlorella* sp. cultivated in Bold's Basal Medium with 10.0 mg/L of Cr (VI) after 14 days of cultivation. The high bioreduction percentage can be attributed to the high EDTA content (50.0 mg/L) of the medium used which reduced the availability of the toxic Cr (VI) ions to the microalga. This conditions results in the production of more cells that can bioreduce more Cr (VI) ions that are slowly released by the said metal chelator.

Bioreduction in the protoplasm, possibly through Cr (VI) reductase located in the cytoplasm, was at its maximum at 1.0 mg/L (treatment II). The value determined is 80% of the bioabsorbed Cr (VI) indicating a very active reduction mechanism for Cr (VI) detoxification inside the cells. Bioreduction on the cell wall, on the other hand, did not significantly vary and accounts for only 5 - 40% of the bioadsorbed Cr (VI). This suggests the absence of a Cr (VI) reducing enzyme in the cell wall and extracellular materials.

Both the bioremoval and bioreduction per mg DW of the microalgal cells exhibited an increasing trend that reached their maximum value at 7.0 mg/L (treatment V). This observation suggests that the cells exposed at the highest Cr (VI) concentration were still alive and were diverting all its energy and raw materials towards Cr (VI) detoxification. This non-growth condition has led to a very low biomass DW with high amounts of Cr (VI) bioremoved and bioreduced per mg DW. Similar results was observed by Perez-Rama et al. (2002) in the green microalga *Tetraselmis suecica* which had the highest Cd (II) bioremoval per cell (16.0 x 10^{-6} ug Cd cell) at highest treatment concentration of 45 mg/L. The higher values for the Cr (VI) bioreduced per mg DW compared with the Cr (VI) bioremoved per mg DW, on the other hand, were due to the higher amounts of Cr (VI) bioreduced in the media in treatments III-V. It was suggested that the reason for the higher values was due to the presence of membrane-bound Cr (VI) reductase and the release of bioreduced Cr (VI) from the protoplasm of dying cells.

Since it was observed that the cells of *C. vulgaris* can still bioremove and bioreduce significant amounts of Cr (VI) ions even in non-growing conditions, the use of higher initial densities (1 x 10^5 to 1 x 10^8 cells mL) of the microalga for the treatment with high Cr (VI) concentrations (3.0 - 7.0 mg/L) was suggested.

CONCLUSION

Living cells of the West Bay strain of *Chlorella vulgaris* at an initial density of 1 x 10^4 cells per mL could act as an effective system for the bioremoval and bioreduction of Cr (VI) ions in BG-11 medium without EDTA at concentrations below its EC_{50} (0.1 and 1.0 mg/L) after 12 days of incubation. At these concentrations, the microalga was able to produce more cells that were able to bioremove and bioreduce more Cr (VI) ions in the medium. For the treatments with higher Cr (VI) concentrations, inoculation of higher initial densities of the microalga was suggested.

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