

Intracellular distribution of chromium and toxicity on growth in *Chlorella pyrenoidosa*

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ABSTRACT The effect of chromium on the growth response on green alga *Chlorella pyrenoidosa* were examined. An increase in chromium concentration caused a significant reduction in the growth of *Chlorella* cells, and the the EC₅₀ value was 1.6 mg/L, while lethal concentration of chromium appears to be approximately 20 mg/L. We have investigated the intracellular distribution of chromium, and the elemental composition of the treated cells. Bulk of the chromium is localized in the cell wall, while the membrane and soluble fraction contains the smaller part of the total chromium.

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KEY WORDS

Chlorella
chromium
growth response
intracellular distribution
elemental composition

Chlorella pyrenoidosa is an unicellular green alga, which is occur both in the fresh and marine waters. Its physiology, biochemistry and photosynthetic apparatus is similar to higher plants but its growth is very quick. For these reasons *Chlorella* often was used as a medium of various metabolic and stress investigations (Lusstigman and Lee 1995).

Within the normal pH range in natural waters the CrO₄²⁻, HCrO₄⁻ and Cr₂O₇²⁻ ions are the forms expected. They constitute a lot of Cr(VI) compounds which are quite soluble and thus mobile in the environment (Nieboer and Jusys, 1988). Many laboratory studies dealt with toxic effects of chromium in higher plants and algae (Bishnoy et al. 1993; Rachlin and Grosso 1993).

Materials and Methods

The alga used in this study was *Chlorella pyrenoidosa* IAM-C128 obtained from the collection of the Institute of Applied Microbiology, University of Tokyo.

When cultures reached approximately 0.1g/l dry matter weight (OD_{750nm} 0.1) in the nutrient medium, the suspension was divided into series of 500 ml glass tubes. The algae were treated with 1-50 ppm of chromium (VI). Sodium dichromate was used as hexavalent chromium. Algae were autotrophically propagated for 96 hours after chromium treatments.

Algal cell fractionation was achieved following methods of Okamura (Okamura and Aoyama, 1994) The elemental composition was measured by a Spectroflame-type inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (Spectro GmbH Kleve, Germany).

Results and Discussion

The effects of chromium (VI) to *Chlorella pyrenoidosa* was investigated using increasing concentrations of chromium (0-50 ppm). An increase in chromium concentration caused a significant decrease in the cell density and cell number of *Chlorella pyrenoidosa* (Fig. 1). The influence of chromium on cell density and cell number followed very similar trends,

indicating that the growth responses might be correlated.

The cells from 20-50 ppm cultures showed chlorotic symptoms, and the cells did not grow when we transferred them to fresh medium without chromium. The lethal concentration of chromium appears to be approximately 20 ppm for chromium.

We have investigated the concentration of chromium, calcium, magnesium and iron. The cells were fractionated into 3 fractions. Cell wall, membrane and soluble fraction was measured after the cells were disrupted. The amount of metals in whole cells and in each fraction was determined (Table 1.)

Intracellular distribution of chromium is not between fractions. Generally 70% chromium is localized in the cell wall region, while the amount of accumulated chromium was almost the same in the membrane and in the soluble fraction. Although chromium accumulation increased with increasing concentrations, however the response was not linear. High concentration chromium in the algal cells causes a higher

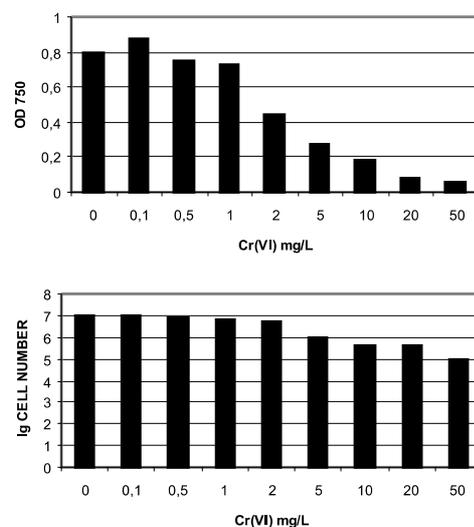


Figure 1.

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	Control				1mg/L				5mg/L			
	Cr	Ca	Mg	Fe	Cr	Ca	Mg	Fe	Cr	Ca	Mg	Fe
Whole alga cell	0,2	48	1423	148	17	64	1116	114	28	74	931	78
Cell wall	0,2	45	1512	114	14	60	972	100	23	77	902	82
Memb. Fract.	Nd	4,8	111	32	2,5	1,4	98	26	4,1	6,4	44	11
Soluble fract	Nd	3,4	102	41	2,1	1,7	68	10,5	3,4	1,7	49	2,5

concentration calcium and a lower concentration of magnesium and iron, the reason of this phenomena can be an ion exchange. This effect is more considerable in the case of magnesium.

In summary, chromium is toxic to *Chlorella*, although according to some report low, chromium concentration can stimulate the growth of plants and algae. *Chlorella pyrenoidosa* can bind and accumulate chromium. The bulk of accumulated chromium is in the cell wall while the membrane and soluble fraction contains a smaller part of chromium.

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