

Phytoremediation of Cd²⁺ by Marine Phytoplanktons, *Tetracelmis chuii* and *Chaetoceros calcitrans*

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Abstract

The use of marine phytoplankton, *Tetracelmis chuii* and *Chaetoceros calcitrans* as phytoremediator has already been reported. However their use as phytoremediator on the cadmium polluted marine has not yet well understood. Therefore, this study was conducted to evaluate the influence of the Cd²⁺ concentration, interacting time, and medium pH on accumulation of Cd²⁺ in the phytoplankton. Methods of analysis and data collection were carried out on 1) the growth rate, the number of phytoplankton cells, and the content of chlorophyll-A; 2) the Cd²⁺ concentration in phytoplankton at various interacting times and medium pHs; and 3) infrared spectra of phytoplankton biomasses before and after interaction with Cd²⁺. The growth of phytoplankton and the content of chlorophyll-A after the addition of Cd²⁺ into the *T. Chuii* medium decreased, while that after the addition of Cd²⁺ into the *C. Calcitrans* medium increased. The optimum accumulation occurred after 15 min at the pH of 8, i.e. 13.46 mg Cd²⁺ per g *T. Chuii* and 1055.27 mg per g *C. Calcitrans*. The functional groups of *T. chuii* involved in the bioaccumulation of Cd²⁺ are OH, -CN, S=O, N-O, S-S and M-S, while that of *C. Calcitrans* are OH, C=O, S-S, M-S and C=C.

Keywords: Phytoremediator, Cadmium, *Tetracelmis chuii*, *Chaetoceros calcitrans*

1. Introduction

Marine phytoplankton, *T. Chuii* and *C. Calcitrans*, have sizes of 7-12 and 6-8 μm, respectively (Falkowski & Raven, 2007). In order to use phytoplankton as phytoremediator of heavy metals in the marine waters, the study of toxicity, the ability to accumulate heavy metals and the potential functional groups that play an important role in the interaction in the phytoplankton from classes of dinoflagellata and diatom need to be investigated (Abe, 2001). In this research, cadmium was used as the object of research because the metal is not required in the growth process of living organism and is classified as a toxic metal. *T. chuii* and *C. Calcitrans* representing classes of dinoflagellata and diatom, respectively, were used as phytoremediators for metals.

Many researchers stated that organisms synthesize metal chelating proteins in order to give a respond to the effect of toxic heavy metals. The main small molecules in plants, algae, and fungi are considered as rich peptides called phytochelatin (Grill et al., 1985; Kawakami et al., 2006). The molecules have a structure of (Y-Glu-Cys)_n-Gly, where n can be 2-11 depending on species from which the peptides were isolated and also on the induction condition with the primary structure, as shown in Figure 1 (Hirata et al., 2001).

Phytochelatin was synthesized from a tripeptide derivative (glutathione) composed by glutamate, cysteine, and glycine. Glutathione can be found in all cells, often in a high level (Schat et al., 2002). If the environment is mediated by metal ions, glutathione will form metal chelating peptides, a phytochelatin. The phytochelatin binds metal ions to form phytochelatin-M and then enters into the vacuole (Mercado et al., 2009; Burcu, 2004).

2. Materials and Methods

2.1 Materials

Materials used in this study are as follows: *T. Chuii* and *C. Calcitrans* obtained from the pure cultures taken from Fisheries and Marine Institute, Maros, South Sulawesi, Indonesia, chemicals in analytical grade such as NaCl, MgSO₄·7H₂O, KHPO₄, CaCl₂·2H₂O, H₂BO₃, ZnSO₄·7H₂O, MnSO₄·7H₂O, CuSO₄·5H₂O, (NH₄)₆Mo₇O₂₄·5H₂O, NaFeEDTA, NaSiO₃·9H₂O, thiamine HCl, biotin, Vitamin B₁₂, CdCl₂, as well as filter paper (Whatman paper, GF/A) and aquadest.

2.2 Methods

2.2.1 Growth Pattern of Phytoplankton in the Culture Medium of Arschat

The pure culture of *T. chuii* and *C. calcitrans* was cultivated in a 500 mL erlenmeyer using the Arschat medium. Lighting with a Neon lamp of 80 watt, was given continuously under flowing CO₂ gas supplied by an air aerator at the temperatures between 20 and 22 °C and the medium pH. To determine the growth pattern of Phytoplankton, calculation of the number of cell per mm medium was performed. A sample about 0.1-0.5 mL was taken by a sterile drop pipette, dropped into Haemocytometer, and then observed under a microscope (Seafdec, 1985).

3. Results and Discussion

3.1 Growth rate and inhibition percentage of the growth of *T. chuii* and *C. calcitrans* before and after addition of Cadmium ion (Cd²⁺) in arschat medium

Results of determination of the specific growth rate (μ) for each concentration of the Cd²⁺ ion calculated using equation (1) are presented in Tables 1 and 2.

$$\mu = \frac{\ln N_t - \ln N_0}{t} \quad (1)$$

N_t = Cell density at t (cells/mL)

N_0 = Cell density at the beginning (cell/mL)

μ = specific growth rate

t = time (days) (Doshi et al., 2007)

The growth of *T. Chuii* at the culture medium added by 0.15 mg·L⁻¹ of Cd²⁺ ion tends to be similar to that of the control. The higher the concentration of Cd²⁺ ion added causes the lower the growth pattern. This phenomenon proves that the existence of Cd²⁺ ion in the culture medium of *T. Chuii* can decrease the growth of phytoplankton as can be seen in Table 1.

Table 2 shows that generally the growth rate of *C. calcitrans* increases with the increase of the exposure time until the third day except for the addition of Cd²⁺ ion at the concentration above 1 ppm showing the highest growth rate at the fourth day. It is interested to be noted that the addition of the Cd²⁺ concentration in the culture medium increases the specific growth rate until the concentration of Cd²⁺ ion equals to 1 ppm. However, further addition of the Cd²⁺ concentration causes the decrease in the specific growth rate of *C. calcitrans*.

3.2 Chlorophyll-A content in *T. chuii* and *C. calcitrans* grown in the medium added with Cd²⁺ ion at various concentration

The content of Chlorophyll-A in *T. chuii* and *C. calcitrans* grown in Arschat medium containing Cd²⁺ ion at various concentration can be seen in Table 3, Figure 2 and Figure 3.

It is clear from Figure 2 that the increase of the Cd²⁺ concentration in medium causes the decrease of the chlorophyll-A content. This result is in parallel with the results reported by Ho (2003) and Reinfelder (2000) who studied the effect of Cd²⁺ ion on the chlorophyll content of *Chlorella ellipsoidee*. Wang and Dei (2001), Baryla et al. (2001) and Inthorn (2001) reported that the addition of Cd²⁺ ion caused the decrease of photosynthesis process and the nutrient uptake.

Figure 3 shows that the addition of Cd²⁺ ion into the medium of *C. calcitrans* results in the increase of the chlorophyll-A content. This occurs because the Cd²⁺ ion can stimulate the growth of phytoplankton (*C. calcitrans*).

3.3 Effect of the medium pH on the accumulation capacity of Cd^{2+} ion on *T. chuii* and *C. calcitrans*

The ability of phytoplankton in accumulating Cd^{2+} ion as a function of the medium pH has been studied. Results of the pH effect are presented in Table 4 and Figure 4.

Based on Table 4, it is obvious that the accumulation of Cd^{2+} by both *T. chuii* and *C. calcitrans* increases gradually with the increase of the medium pH and at the pH of 8 the accumulation of Cd^{2+} achieves optimum. Unlike in *T. chuii* where the increase of the amount of Cd^{2+} accumulated as a function of the medium pH is relatively small, the increase of the amount of Cd^{2+} accumulated in *C. calcitrans* is relatively high until the optimum pH as can be seen in Figure 4.

3.4 Identification of functional groups of *T. chuii* and *C. calcitrans* Involved in the accumulation process of Cd^{2+} ion

Identification of functions groups of *T. chuii* and *C. calcitrans* was conducted by using FTIR before and after addition of the Cd^{2+} ion into both living and non-living phytoplanktons. Results showed that there were 20 and 17 absorption bands for living and non-living *T. chuii*, respectively. Functional groups which are likely to be involved in the bioaccumulation of Cd^{2+} in *T. chuii* are O-H, C=N, S=O, N-O, S-SA, M-S and M-N.

After addition of Cd^{2+} ion into the culture medium of living and non-living *C. calcitrans*, there were 16 absorption bands observed for both samples. For interaction of Cd^{2+} ion with the non-living biomass of *C. calcitrans*, there were some new bands observed and some absorption bands had different wavelengths. This indicates that the interaction of Cd^{2+} ion with living and non-living *C. calcitrans* is not the same. The functional groups of *C. calcitrans* involved in the bioaccumulation of Cd^{2+} ion are O-H, C=C, C=O, S-S and M-S.

4. Conclusion

From this study, it can be concluded that:

- 1) Addition of cadmium ion with the concentration of 0.5 ppm into the culture medium of *T. chuii* decreases the growth rate, the number of cells, the dried weight and the chlorophyll-A content of the phytoplankton. Addition of the ion with the concentration of 1 ppm into the culture medium of *C. calcitrans* increases the growth rate, the number of cells, the dried weight and the chlorophyll-A content of the phytoplankton.
- 2) Bioaccumulation of cadmium by *T. chuii* and *C. calcitrans* increases gradually with the increase of the medium pH used and achieved the optimum at the pH of 8.
- 3) Identification of functional groups on the biomass of *T. chuii* before and after addition of Cd^{2+} ion shows that functional groups of N-O, O-H, S=O, C-N, M-S and S-S plays an important role in the bioaccumulation process of cadmium. The functional groups involved in the bioaccumulation of cadmium in *C. calcitrans* are C=C, C=O, M-S, O-H and S-S.

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Table 1. Constant specific growth rate (μ) *Tetracelmis chuii* without addition of metal ions Cd²⁺ at various concentrations

[Cd ²⁺] (ppm)	μ (day ⁻¹) day to-						
	1	2	3	4	5	6	7
0.00	0.209	0.531	0.630	0.607	0.537	0.470	0.393
0.15	0.251	0.523	0.644	0.601	0.531	0.470	0.395
0.20	0.281	0.519	0.659	0.595	0.520	0.451	0.387
0.25	0.125	0.504	0.654	0.601	0.527	0.450	0.375
0.50	0.103	0.490	0.620	0.601	0.522	0.450	0.378
1.00	0.000	0.468	0.596	0.535	0.501	0.431	0.354
5.00	-0.017	0.183	0.269	0.167	0.109	0.091	0.100

Table 2. Constant specific growth rate (μ) *Tetracelmis chuii* with addition of metal ions Cd^{2+} at various concentrations

[Cd^{2+}] (ppm)	μ (day^{-1}) day to-									
	1	2	3	4	5	6	7	8	9	10
0.00	0.39	0.236	0.236	0.184	0.208	0.185	0.174	0.161	0.164	0.155
0.125	0.67	0.150	0.255	0.223	0.237	0.205	0.190	0.168	0.178	0.154
0.25	0.25	0.193	0.282	0.257	0.258	0.230	0.210	0.193	0.185	0.162
0.50	0.19	0.217	0.305	0.300	0.275	0.243	0.212	0.188	0.169	0.137
1.00	0.26	0.279	0.327	0.307	0.268	0.266	0.247	0.223	0.218	0.194
2.00	0.30	0.241	0.263	0.275	0.267	0.238	0.219	0.201	0.204	0.187
4.00	0.17	0.153	0.223	0.238	0.254	0.223	0.210	0.189	0.199	0.175
5.00	0.15	0.129	0.183	0.206	0.220	0.196	0.190	0.182	0.186	0.175

Table 3. The content of Chlorophyll-A in *T. chuii* and *C. calcitrans* as a function of the concentration of Cd^{2+} ion added in the medium

[Cd^{2+}] (ppm)	μ (day^{-1}) day to-									
	1	2	3	4	5	6	7	8	9	10
0.00	0.39	0.236	0.236	0.184	0.208	0.185	0.174	0.161	0.164	0.155
0.125	0.67	0.150	0.255	0.223	0.237	0.205	0.190	0.168	0.178	0.154
0.25	0.25	0.193	0.282	0.257	0.258	0.230	0.210	0.193	0.185	0.162
0.50	0.19	0.217	0.305	0.300	0.275	0.243	0.212	0.188	0.169	0.137
1.00	0.26	0.279	0.327	0.307	0.268	0.266	0.247	0.223	0.218	0.194
2.00	0.30	0.241	0.263	0.275	0.267	0.238	0.219	0.201	0.204	0.187
4.00	0.17	0.153	0.223	0.238	0.254	0.223	0.210	0.189	0.199	0.175
5.00	0.15	0.129	0.183	0.206	0.220	0.196	0.190	0.182	0.186	0.175

Table 4. Accumulation of Cd^{2+} ion on *T. chuii* and *C. calcitrans* as a function of the medium pH (initial concentration of Cd^{2+} ion = 0.25 ppm)

pH Medium	<i>Tetracelmis chuii</i> (mg Cd/g <i>T. Chuii</i>)	<i>Chaetoceros Calcitrans</i> (mg Cd/g <i>C. Calcitrans</i>)
4.0	0.648	1.275
5.0	0.742	2.255
6.0	0.805	3.255
7.0	0.857	4.725
8.0	0.884	5.223
9.0	0.881	5.220

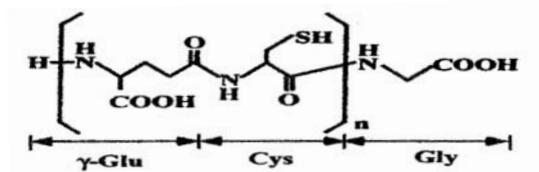


Figure 1. Fitokhelatin primary structure

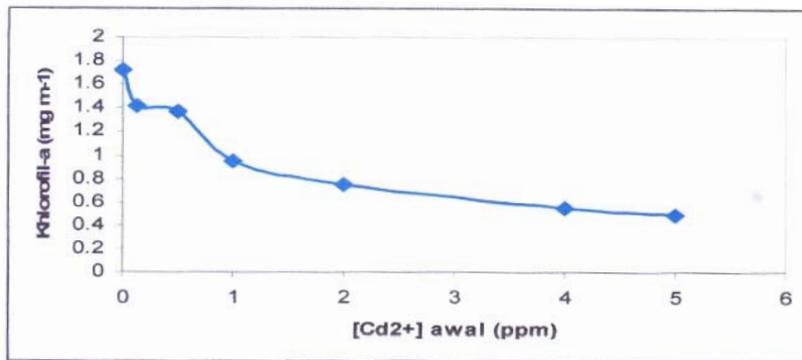


Figure 2. The content of chlorophyll-A in *T. chuii* as a function of the concentration of Cd²⁺ ion added in the growth medium

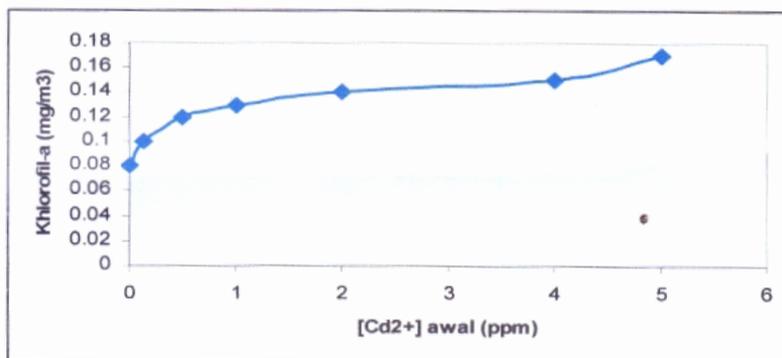


Figure 3. The content of chlorophyll-A in *C. calcitrans* as a function of the concentration of Cd²⁺ ion added in the growth medium

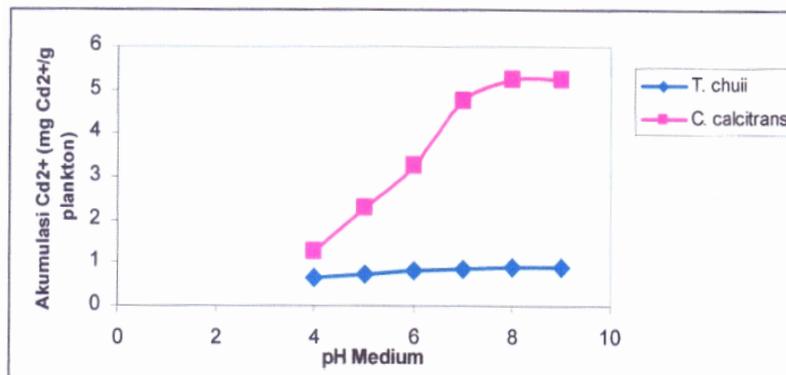


Figure 4. Effect of the medium pH on the accumulation of Cd²⁺ ion on *T. chuii* and *C. calcitrans* (initial concentration of Cd²⁺ ion = 0.25 ppm)