The promotion and inhibition of chromium and kinetic analysis on the growth of Platymonas Helgolandica

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Abstract. In this work, *Platymonas helgolandica* was used as an experimental material to study the effect of chromium on it. Under the experimental ecological condition, the concentrations of Cr (VI) were set as 0, 0.05, 0.1, 1, and 10 mg/L. Every concentration designed with three parallel groups. The ecotoxicology method was used to investigate the impact of Cr (VI) on *P. helgolandica*, and (EC_{50}) was calculated by the method of linear interpolation. The results showed the growth of *P. helgolandica* had a more obvious "Hormesis" when chromium was 0.05 mg/L. However, with Cr (VI) increased, the inhibition on *P. helgolandica* was increasing, and the cell density of *P. helgolandica* decreased. The EC_{50} achieved at 24 h, 48 h, and 72 h revealed that the relative growth rate had a downward trend over time. The remodified Logistic model, the modified Gompertz model, and the Logistic model were suitable to describe cell density in an operating cycle and were beneficial to explore the growth of *P. helgolandica*.

Keywords: chromium, Platymonas helgolandica, cell density, relative growth rate, kinetic analysis.

1. Introduction

Chromium was an element existed in nature widely. It was mainly displayed in the form of tri-valent chromium Cr (III) and six-valent chromium Cr (VI) in water environment. Cr (VI) was a clear harmful substance. It had carcinogenic and teratogenic effects and was not easy to degrade [1-3]. When the wastewater containing Cr (VI) was discharged into the water body that had a large number of aquatic plants and aquatic animals, Cr (VI) could be enriched by the food chain and played a threat to biological health [4].

Platymonas helgolandica belonged to the *Chlorophyta, Chlorophyceae, Volvocales, Chlamydomonadaceae, Platymonas*, and thrived in warmer and more organic water body. Moreover, *Platymonas helgolandica* was cultivated at home and abroad widely, eaten by larvae of marine products [5].

The promotion and inhibition of chromium on the growth of the *P. helgolandica* was a prerequisite for exploring the effects of heavy metals on the marine ecosystem, and it was of great significance to investigate the effects of heavy metals on the ocean in future. Therefore, the experiment took *P. helgolandica* as a test object. The impact of Cr (VI) with different concentration gradient on the growth of *P. helgolandica* was observed. The changes of cell density and relative growth rate of *P. helgolandica* were studied under Cr (VI) stress, in order to investigate the toxic effect of chromium on it. Besides, the remodified Logistic model, the modified Gompertz model, and the Logistic model were used to describe cell density in an operating cycle and to evaluate the growth of *P. helgolandica*.

2. Materials and methods

2.1. Experimental algae species and culture conditions

Experimental algae species was *P. helgolandica*. Cr (VI) solution was composed of potassium dichromate. The natural seawater from Qingdao was filtered through degreasing cotton, and then boiled 15 min. After cooling, nutrient solution was allocated, and f/2 nutrients formula was used for culture medium. The light intensity was 3000 lx, rhythmic light was 12 h : 12 h, the temperature was 20 ± 1 °C, and pH was 8.1-8.2.

2.2. Experimental methods

On the basis of the pre-experiment, Cr (VI) solution with different concentration gradient was set up. There were four treatment groups and one control group, every processing was repeated three times and the averaged value was reported. During exponential growth cycle, *P. helgolandica* was inoculated and incubated in a constant temperature incubator. After the Lugol reagent was added, the cell density was determined, and the cell density and relative growth rate of cells were calculated by blood counting chamber. In the work, the average number of cell density of mother liquid was 16.79×10^4/mL, and the inoculation density was 4.20×10^4/mL.

The blood counting chamber principle diagram and the image under the microscope:
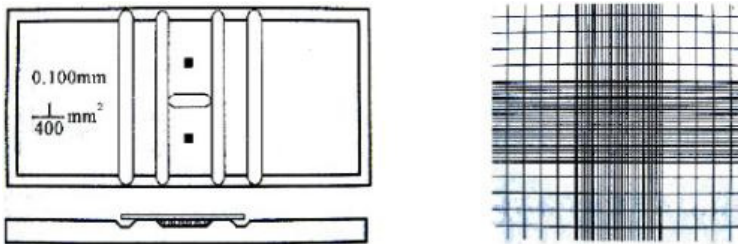
a) The blood count plate diagram and counting area schematic
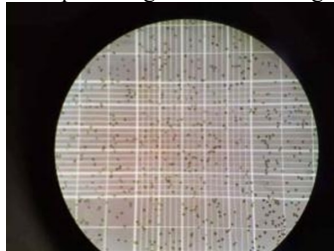
b) *P. helgolandica* image 10 x magnification under the microscope
Fig. 1. The principle diagram and the image displayed by microscope

The number of cells in every milliliter of medium:

$$A/mL = a \times 400 \times 10^4 \times b/c, \tag{1}$$

where A is cell number, a is cell number in small square, b is dilution ratio, and c is number of small squares selected.

Relative growth rate of cell [6]:

$$K = (\lg N_t - \lg N_o)/T, \tag{2}$$

where N_t is cell density at t time, mg/L, N_o is cell density at initial density, mg/L, T is culture time, h.

2.3. Kinetic analysis

The remodified Logistic model (Eq. (3)), the modified Gompertz model (Eq. (4)), and the Logistic model (Eq. (5)) were suitable to describe cell density of *P. helgolandica* in an operating cycle. The remodified Logistic model was the first to describe hydrogen production process in batch test [7]. The modified Gompertz model could be used to indicate the cell growth and product formation [8]. The Logistic model used to describe microbe growth curve.

The remodified Logistic model:

$$CD = \frac{CD_{max}}{1 + \exp\left[\frac{4R_{max(\lambda-t)}}{CD_{max}+2}\right]}. \tag{3}$$

The modified Gompertz model:

$$CD = CD_{max} \exp\left[-\exp\left(\frac{R_{max}e(\lambda-t)}{CD_{max}}+1\right)\right]. \tag{4}$$

The Logistic model:

$$CD = CD_{max} + \frac{CD_{min}-CD_{max}}{1 + \left(\frac{t}{EC_{50t}}\right)^p}, \tag{5}$$

where CD is cell density, ($\times10^4$/mL); CD_{max} is maximum cell density, ($\times10^4$/mL); R_{max} is maximum cell growth efficiency, ($\times10^4$/mL)/h; λ is lag time, h; t is experimental time, h; CD_{min} is minimum cell density, ($\times10^4$/mL); EC_{50t} is time of 50 % maximum cell density, h; p is exponent.

3. Experimental results and analysis

3.1. The effect of chromium on the growth of *P. helgolandica*

The effect of different concentrations of Cr (VI) among 72 h on cell density of *P. helgolandica* was shown in Fig. 2.
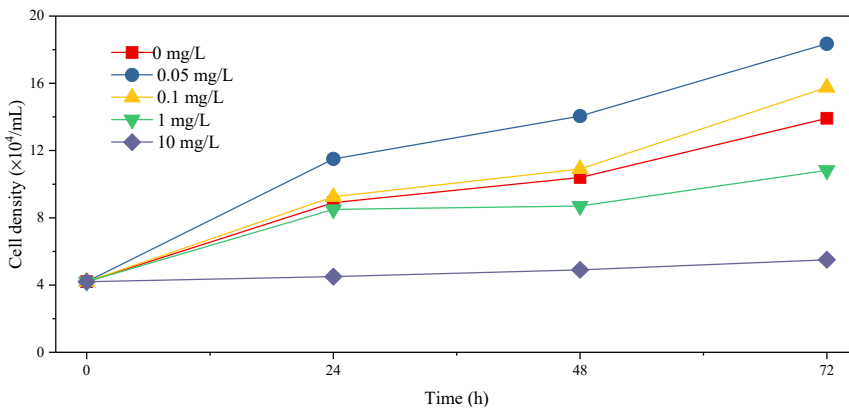

Fig. 2. The effect of Cr (VI) on the growth of *P. helgolandica*

It was known from Fig. 2 that the growth of *P. helgolandica* showed a more obvious "Hormesis" and cell density increased at low concentration of chromium. This effect was strongest

when the concentration of Cr (VI) was 0.05 mg/L. However, when the concentration of chromium exceeded 0.1 mg/L, the inhibition would present, and even threaten survival of *P. helgolandica.*

3.2. The effect of chromium on the relative growth rate of *P. helgolandica*

3.2.1. The effect of chromium on the relative growth rate of *P. helgolandica* in 24 h.

Fig.3 revealed that the relative growth of *P. helgolandica* increased first and then decreased with the increase of chromium concentration. When the chromium concentration was 0.05 mg/L, the relative growth rate of *P. helgolandica* was the highest and Cr (VI) has a significant impact on the growth of *P. helgolandica.* With the continuous increase of chromium concentration, the promotion weakened gradually and showed inhibitory effect. From the change of K value, it could be concluded that the 24 h-EC_{50} of chromium to *P. helgolandica* was 5.18 mg/L.
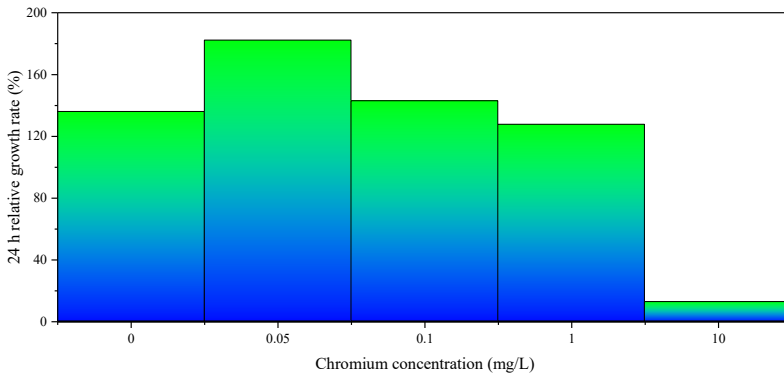

Fig. 3. The effect of chromium on the relative growth rate of *P. helgolandica* in 24 h

3.2.2. The effect of chromium on the relative growth rate of *P. helgolandica* in 48 h

It was known from Fig. 4 that the relative growth rate of *P.helgolandica* showed a tendency to increase first and then decreased. When Cr (VI) concentration was 0.05 mg/L, the relative growth rate of *P. helgolandica* was the largest, which was 109.30 %, and chromium had an obvious promoting effect on the growth of *P. helgolandica*. With the continuous increase of chromium concentration, this "excitatory effect" weakened gradually and showed inhibitory effect. When chromium concentration was 10 mg/L, the relative growth rate of *P. helgolandica* was 13.90 %. From the change of K value, it could be concluded that the 48 h-EC_{50} of chromium to *P. helgolandica* was 4.09 mg/L.
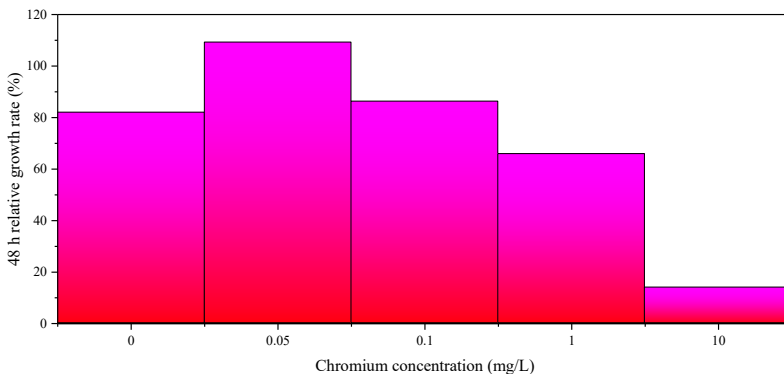

Fig. 4. The effect of chromium on the relative growth rate of *P. helgolandica* in 48 h

3.2.3. The effect of chromium on the relative growth rate of *P. helgolandica* in 72 h

Fig. 5 displayed that the relative growth of *P. helgolandica* increased first and then decreased with increasing chromium concentration. When the Cr (VI) concentration was 0.05 mg/L, the relative growth rate of *P. helgolandica* was 88.90 %, and chromium has obvious promotion on the growth of *P. helgolandica*. When chromium concentration was 0.1 mg/L, it also had "excitatory effect". With the increasing of chromium concentration, this promotion weakened gradually and showed inhibitory effect. From the change of K value, it can be concluded that the 72 h-EC_{50} of chromium to *P. helgolandica* was 3.82 mg/L.
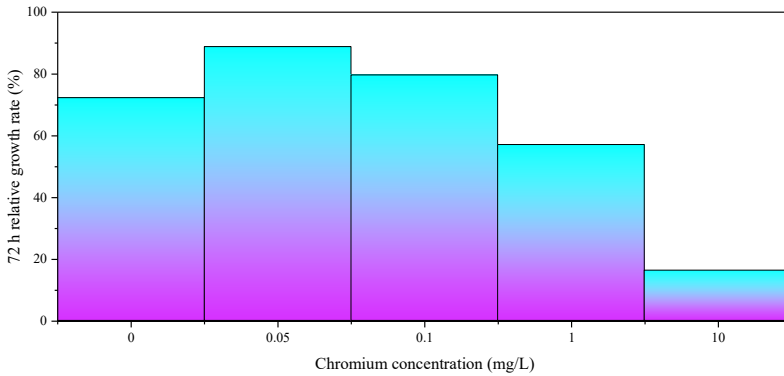

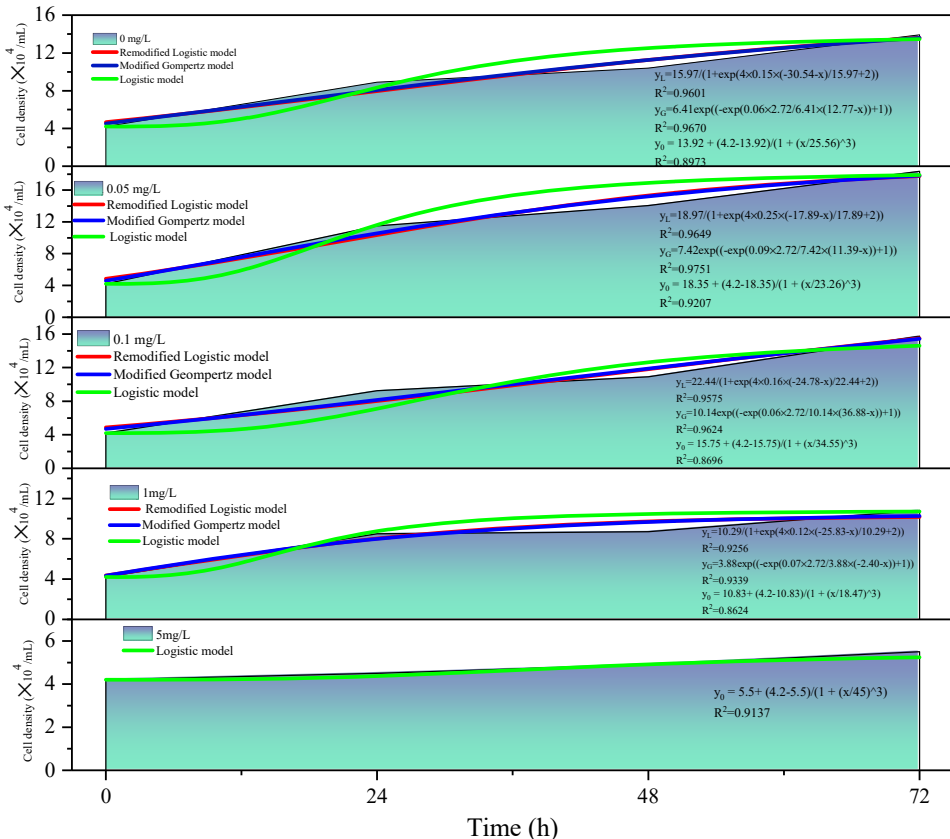**Fig. 5.** The effect of chromium on the relative growth rate of *P. helgolandica* in 72 h

Fig. 6. Kinetic analysis to cell density of *P. helgolandica* among 72 h was shown in Fig. 6

3.3. Kinetic analysis about cell density of *P. helgolandica* in an operation cycle

The effect of Cr (VI) on cell density of *P. helgolandica* among 72 h was shown in Fig. 6.

The cell density and kinetic features with Cr (VI) addition in an operating cycle were described by the remodified Logistic model (Eq. (3)), the modified Gompertz model (Eq. (4)) and the Logistic model (Eq. (5)). Kinetic parameters and fitting formulas were showed in Fig. 6.

Compared the parameters were obtained by three models, which all had relatively higher correlation coefficients (R^2). It can be obtained by multiple fitting, and the remodified Logistic model and the modified Gompertz model were suiting to describe the changes of *P. helgolandica* of cell density at 0, 0.05, 0.1, and 1 mg/L. The Logistic model could describe all Cr (VI) concentrations cell density variation.

4. Discussion

4.1. The discussion of the effect of chromium on the growth of *P. helgolandica*

From the effects on cell density of *P. helgolandic*a at different Cr (VI) concentrations, chromium showed a "low promotion and high inhibition" on the growth of *P. helgolandica*. At low concentrations of 0.05 mg/L and 0.1 mg/L, it showed a significant promotion effect compared with the control group. This phenomenon also occurred in previous studies on the toxic effect of heavy metals copper and cadmium on green algae [9]. Algae exhibited stress resistance and stimulate algal growth when disturbed by weak stresses may be a main reason. Many low concentration promotion phenomena had also been observed in some published articles [10, 11]. It indicated that the "excitatory effect" of marine microalgae caused by low concentration of heavy metals was universal.

When the *P. helgolandica* was treated with high Cr (VI) concentration showed inhibitory effect. When the chromium concentration was 1 mg/L, the inhibition began to be showed. With the increase of Cr (VI) concentration, the inhibition was more obvious. The cause of this phenomenon was that chromium destroyed the cell structure of *P. helgolandica* and destroyed its superoxide dismutase activity and the content of photosynthetic pigment, which affected photosynthesis and inhibited growth and reproduction.

4.2. The discussion of the effect of chromium on the relative growth rate of *P. helgolandica*

From Fig. 3, Fig. 4, and Fig. 5, it was found that when Cr (VI) concentration were 0, 0.05, 0.1, 1, and 10 mg/L, all were in the tolerance range of *P. helgolandica*.

EC_{50} was an important parameter to measure the biological toxicity of toxic substances in water, and EC_{50} was related to the time of exposure to toxic environment [12]. 24 h-EC_{50}, 48 h-EC_{50}, and 72 h-EC_{50} were 5.18, 4.09, and 3.82 mg/L, respectively. It was known that with the extended of contact time between *P. helgolandica* and chromium, the greater degree of damage to *P. helgolandica*.

The relative growth rate of 24 h, the relative growth rate of 48 h, and the relative growth rate of 72 h gradual declined. The cause of this phenomenon may be that the relative growth rate of *P. helgolandica* was affected by the exposure time, and the longer the exposure time of *P. helgolandica* in the toxic environment, the more bad impact. It may also be due to the growth and reproduction of *P. helgolandica* depleted part of the nutrient in the culture solution resulting in the lack of nutrients, which limited *P. helgolandica* growth and reproduction

4.3. The discussion about kinetic feature of P. helgolandica in an operation cycle

Parameters were compared obtained by three models that the remodified Logistic model (Eq. (3)), the modified Gompertz model (Eq. (4)), and the Logistic model (Eq. (5)). The modified Gompertz model owned best R^2 was 0.9596, above 0.9520 and 0.8927 provided respectively by

the remodified Logistic model and the Logistic model. However, the CD_{max} presented by the first two models were discrepant. Comparing the differences between the experimental and the predicted values, average relative error respectively are 16.37 % and 37.31 %. Average relative error of the remodified Logistic model was only 3.38 % in 0.05 mg/L, and the modified Gompertz model was little more than 0.09 % in 1 mg/L. Suitable models were good at predicting cell density at different chromium concentrations.

The Logistic model could match all the concentration of the cell density change trend, and be able to provide the time of appearance 20 %, 50 %, and 80 % maximum cell density, predict cell density at different times. In addition, other kinetic parameters (λ, CD_{min}) could also be deduced well by three models. In order to maximum enhancement for *P. helgolandica* Cr (VI) dosage had best controlled at 0.05 mg/L.

5. Conclusion

The effect of chromium on the growth of *P. helgolandica* was studied in the work. When the chromium concentrations were 0.5 mg/L and 0.1 mg/L, the growth of *P. helgolandica* showed a more obvious "excitatory effect". However, when the chromium concentration exceeded to 1 mg/L, the inhibition appeared, and the cell density of *P. helgolandica* descend. Compared with the EC$_{50}$ at 24 h, 48 h, and 72 h, it was discovered that the relative growth rate of *P. helgolandica* decreased with the extension of time. The remodified Logistic model and the modified Gompertz model were suitable for predicting CD_{max}, and the Logistic model be able to provide the time of appearance 20 %, 50 % and 80 % maximum cell density.

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