＜特集＞

Fluorescence Measurements for Potassium Cyanide using Closterium ehrenbergii

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Abstract

This algal bioassay aims to be a water biomonitoring system using green algae, *Closterium ehrenbergii* and particularly to detecting environmental stress exerted on water by measuring fluorescence of photosynthesis processes. This method is an advantage in that the presence of the water pollution can be measured and monitored in a few minutes as short time. Moreover, this method is a further advantage in that the number of relevant equipment and manpower required for the water monitoring can be remarkably reduced. Algal biomonitoring system introduced in this study as CFM (*Closterium* Fluorescence Monitoring) system, which could be detected potassium cyanide rapidly and sensitively.

Key Words：CFM system, *Closterium ehrenbergii*, ecotoxicological biomonitoring system, fluorescence, Potassium cyanide (KCN)

1 Introduction

Chemical analyses methods required expensive equipment, large amounts of organic solvents and the purification of samples prior to ecotoxic bioassay. CFM system is a water biomonitoring system using unicellular green alga, *C. ehrenbergii*. Potassium cyanide (KCN) selected as the test material in this study, which is one of the most serious pollutants in water. It is very toxic to many life forms, which released from industrial wastes such as metal working/finishing operations, electroplating plants and chemical processes. In this study, we introduce the CFM system and evaluated the sensitivity for KCN using *C. ehrenbergii*. Ecotoxic monitoring systems have been required for detecting harmful chemicals with rapidly, stable, simple and low-cost in aquatic environment.

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This study using *Closterium ehrenbergii* and CFM system will be useful to detect toxicants in sensitivity and rapidity and to develop ecotoxicological assessment instrument.

2 Material and Method

For the algal cell culture, we used *Closterium ehrenbergii* (Chlorophyta, Chlorophyceae, Zygnematales, Desmidiaceae) EB-A1 strain. It was cultured during 3-4 days in liquid C medium on a shaking incubator at 25°C [1),2)]. We used the CFM system for measuring fluorescence parameters to KCN.

The CFM system has been developed for the purpose of detection system that uses natural aquatic photosynthetic cells as the sensing material for detection of toxic compounds in the water system.

In this system, cells and reference water and sample water were automatically injected continuously.

The used parameter is calculated according to the formula below.

Quantum Yield: (Fm−F)/Fm (F: Fluorescence, Fm: Maximum Fluorescence)

We measured quantum yield between fluorescence of cell exposed to distilled water and fluorescence of cell exposed to KCN. Exposure time of cell to respective samples is 13min. In Measure time, respective channels were measured at wavelength above 650 nm every 30 seconds.

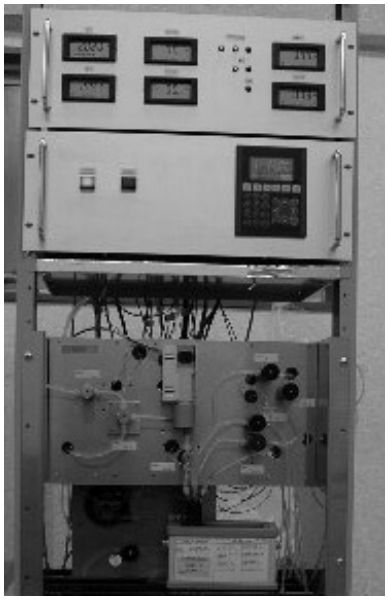

Fig.1 CFM system (EnBio Co.)

3 Results and Discussion

3.1 Quantum Yield of *C.ehrenbergii* about KCN using CFM system

The toxicity of KCN to various aquatic organisms, particularly freshwater species has been reported previously[3)−5)]. However, few studies have relatively investigated the toxicity of cyanides to algae. It was the first time that the experiment of KCN with *Closterium ehrenbergii* was performed. We could find out the fact that Yield of *C. ehrenbergii* was affected by concentration of KCN (**Fig.2**). These data were obtained by CFM system, which is developed system automatically cell and sample supplier. Compared to the control, 1 mg/L of KCN did not have toxic effect to the cell. On the contrary, the quantum yield decreased in proportion to amount of 10 and 100mg/L of KCN.

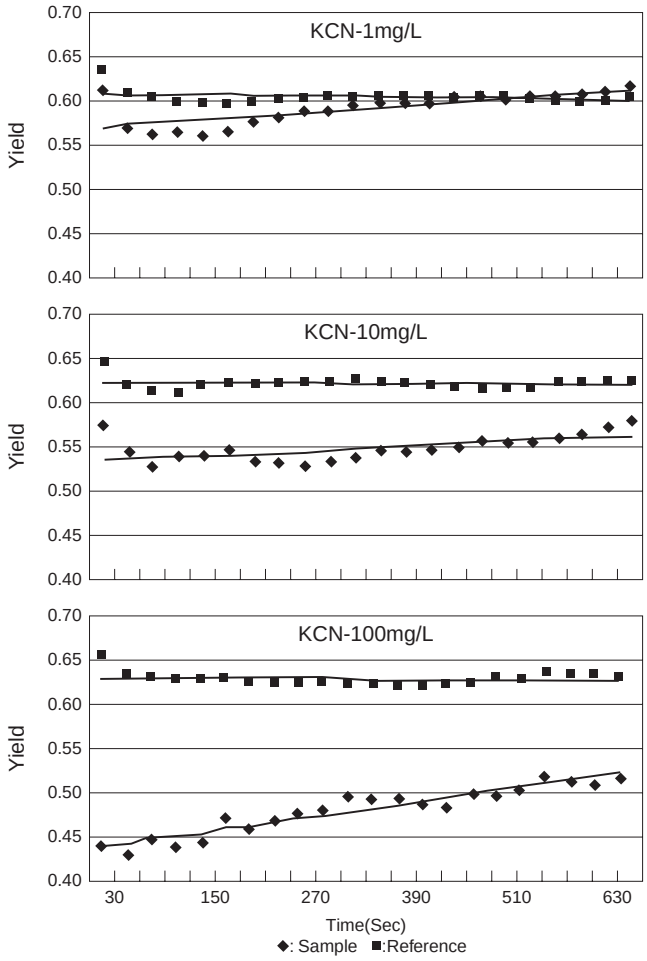

Fig.2 Yields of *C. ehrenbergii* for KCN using CFM system

3.2 Morphological changes of *C.ehrenbergii* for KCN using CFM system

Fig.3-1, 2, 3, 4. shows morphological changes of *C. ehrenbergii* when it was exposed at 10, 100 mg/L of KCN from 30min to 120min. it has changed in their shape of chloroplasts depend on the KCN concentrations and dose time series. Especially The chloroplasts were condensed according to dose time series. It seems to be made a gap between two chloroplast lobes. And also it made vacuoles inside of chloroplasts, which looked like craters. This phenomenon is more clearly investigated in high concentration of KCN.

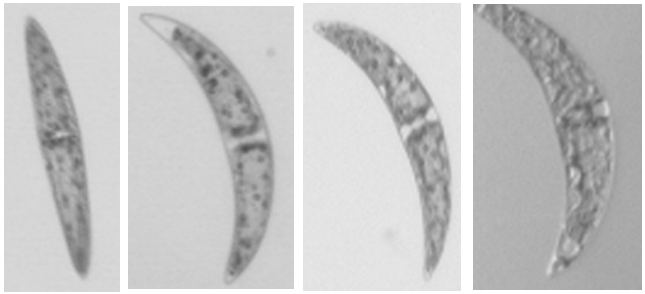

Fig.3 Morphological effect of KCN

4 Conclusion

According to the results, CFM system provides some possibilities as biomonitoring system.

1. Using *Closterium ehrenbergii* for CFM system is useful to detect potassium cyanide in sensitivity and rapidity and to develop ecotoxicological assessment instrument.
2. As KCN concentration grow higher, Quantum Yield of *C. ehrenbergii* showed decreasing tendency.

5 Acknowledgment

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