Detection of potassium cyanide using *Closterium* fluorescence monitoring (CFM) system

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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 WORD: Algal biomonitoring system, CFM system, Fluorescence measurement, water pollution, potassium cyanide (KCN)

1. Introduction

C. ehrenbergii can be a desirable candidate for the evaluation of water pollution (1). This study is a water biomonitoring system using unicellular green alga, *C. ehrenbergii* , which introduced recently as a useful organism for the test of toxicity (2).

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, as well as from biological nitrogen metabolism and decomposition of complex and organocyanides in water by bacteria, fungi and algae (3,4).

Figure 1 is a simplified diagram of CFM (*Closterium* Fluorescence monitoring) system. We introduced a part of this system and evaluated the sensitivity for KCN using *C. ehrenbergii* of CFM system in this study.

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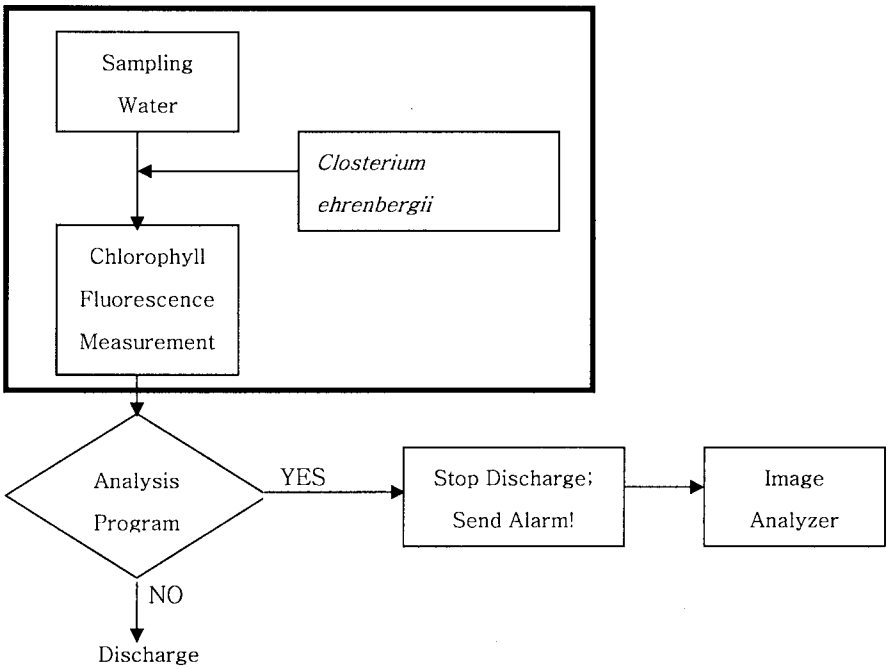

Figure 1. Schematic diagram of CFM system. Flow chart in a bold black box shows the range of this study.

2. Materials and Method

The green algae *Closterium ehrenbergii* was obtained from Toyama University (Japan) and was used as a testing organism after several asexual reproductions in the laboratory.

The ToxY-PAM Dual-Channel-Yield-Analyzer (Walz Co.) used this study is a specialized chlorophyll fluorometer for the assessment of small amounts of toxic substances in water samples using standardized photosynthetically active samples.

3. Result

The toxicity of cyanide to various aquatic organisms, particularly freshwater species, has been reported previously. However, few studies have relatively investigated the toxicity of cyanides to algae (4). It was the first time that the experiment of KCN with *Closterium ehrenbergii* was performed. We could find out the fact that fluorescence of *C. ehrenbergii* was affected by low concentration of KCN (Fig. 2). This data indicated that CFM system could detect within 0.05 mg/L KCN, for 15min, which was relatively short time.

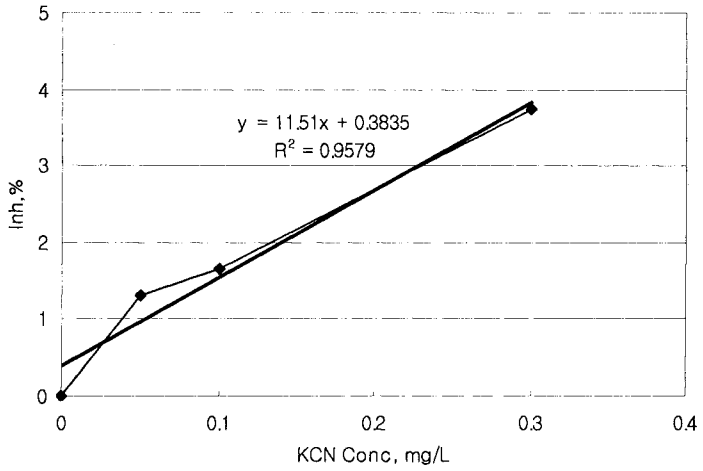

Figure2.
Influence of low concentration of KCN with *Closterium ehrenbergii* for 15 minutes.

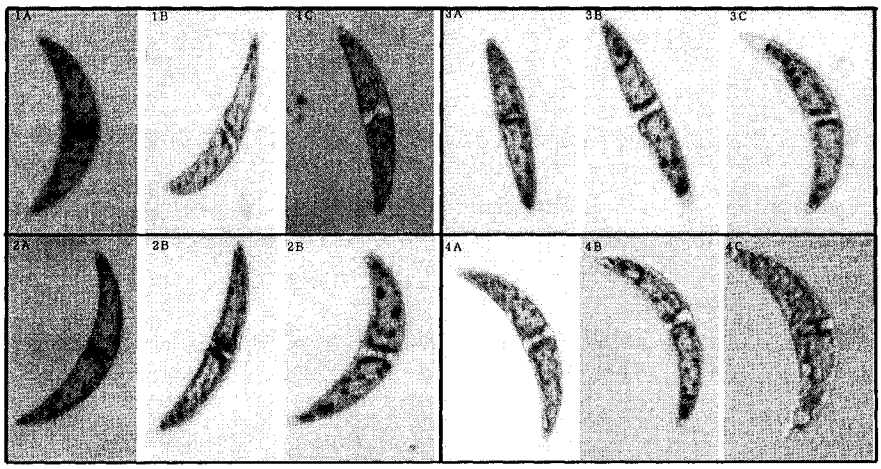

Figure 3. Morphological changes of *Chlosterium ehrenbergii* exposed to 0.1 mg/L KCN (1), 1 mg/L KCN (2), 10mg/L KCN (3), 100 mg/L KCN (4) for 30 min. (A), 60 min. (B), 120 min. (C) respectively.

On the other hand, Figure 3 shows morphological changes of *C. ehrenbergii* when it was exposed at 0.1, 1, 10, 100 mg/L of KCN. Especially it has changed in their shape of chloroplasts depend on the concentration of KCN and time series. The chloroplasts became condensed according to time series, therefore it seemed to be made a gap between two chloroplast lobes. And also it made vacuoles inside of chloroplasts looked like craters. This phenomenon is more clearly investigated in high concentration of KCN.

4. Discussion

We compared sensitivity of CFM system to similar continuous biomonitoring systems (Tab. 1), found out that *Closterium ehrenbergii* has more sensitivity for KCN than other organisms.

Also, we compared EC_{50} value of *C. ehrenbergii* with EC_{50} or LC_{50} values of other organisms from literature data (Table 2). Fish shows the most sensitivity value but it has relatively long exposure time as 48hrs, Bacteria, *Vibrio fisheri* is sensitive in short time periods but it had wide range than *C. ehrenbergii* and need high cost for the driving machines. Other alga, *Euglena gracilis* shows low precision even if short reaction time. Therefore algal biomonitoring system using *C. ehrenbergii* has high sensitivity and can expect to be used practical system.

Table 1. Comparison of the sensitivity KCN

Organism	Organism's name	Detection range (mg/L)	Reaction time	Reference
Algae	*Closterium ehrenbergii*	≥ 0.05	15min	This study
Bacteria	Ammonia oxidizing bacteria	≥ 0.05	30min	FUJI Electric Co.
Fish	*Salvelinus malma*	≥ 0.1	15min	TOSHIBA Co.
	Carp	≥ 0.05	48hr	FUJI Electric Co.

Table 2. Comparison of EC_{50}/LC_{50} values of several organisms for KCN

Species	Parameter	Endpoint	Test values (mg/L)	References
Closterium ehrenbergii	Fluorescence	15min-EC_{50}	4.31	This study
Euglena gracilis	Compactness	3min-EC_{50}	41	H. Tahedl (5)
Vibrio fishcheri	Bioluminescence inhibition	5min-EC_{50}	2.8 ~ 13.3	H. Tahedl (5)
Carp	Lethality	4h-LC_{50}	0.4 ~ 0.78	大戸時喜雄(6)

(EC_{50}: 50% Effective concentration , LC_{50}: 50% Lethality concentration)

5. References

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