ALGAL BIOTOXICITY ASSAY USING µFLOW CYTOMETER FOR ENVIRONMENTAL MONITORING

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ABSTRACT

Algal biotoxicity assays using laboratory flow cytometers are useful for water pollution monitoring. They can provide quantitative valuation of harmful aquatic toxicants such as heavy metals. However, these assays are often performed only in a laboratory because of the bulky sizes of most flow cytometers. Here we report the first algal biotoxicity assay run on a briefcase-sized portable microflow cytometer developed in our lab. Inside our portable cytometer, laser-induced fluorescence is used for the analysis of the esterase-activity and an algal assay on the biotoxicity of copper, a common pollutant in water, is demonstrated.

KEYWORDS: Algae, Biotoxicity assay, Microflow cytometer

INTRODUCTION

The contaminants in natural waters such as heavy metals tend to interact with organic matters to form the metal-organic complexes that are difficult to analyze by traditional analytical methods. Instead, indirect methods using algal biotoxicity assays are often employed to monitor the environmental impact of contaminants in aquatic systems. For an example, [1] reported an algal biotoxicity assay using a flow cytometer for quantitative measurement of the bioavailability of copper. The flow cytometer is an important part of the algal assay. The flow cytometer can perform multi-parameter analysis on a wide range of cellular properties and monitor the response distribution of individual cells within a population. By using biochemically specific fluorescent dyes, flow cytometry can also provide information about the physiological statuses of cells and the action mechanisms of aquatic toxicants. However, traditional laboratory flow cytometers are often too bulky so portable flow cytometers are needed for real-time in-field algal assays. For example, the discrimination of algae cells in a microfluidic channel has been reported by measuring fluorescence emission and electrical impedance [2]. Here, we report the first complete algal biotoxicity assay run on a briefcase-sized, portable micro-flow (µflow) cytometer. The µflow cytometer is designed around a disposable microfluidic chip and the complete algae-based copper biotoxicity assay is demonstrated.

THEORY

The esterase activity has been proven to be an accurate indicator for algal biotoxicity assays [3]. It relates closely to the algal metabolic activity, which can be reduced or even inhibited entirely after exposure to the toxicants in water. The level of the esterase activity can be quantitatively evaluated by using a fluorogenic staining, i.e., Flourescein Diacetate (FDA). The non-fluorescent FDA, after entering the cell membrane, is hydrolyzed by nonspecific esterase in the cytoplasm and transformed into fluorescein [3], which then can be precisely measured by a fluorescence-based µflow cytometer.

Accordingly, a two-color laser-induced fluorescence detection scheme is developed for this algal biotoxicity analysis. The green fluorescence of the FDA staining is measured as an indicator of the esterase activity level. Meanwhile, the intrinsic fluorescence (Red) is measured to identify algae cells among other particles in the water sample. The intrinsic red fluorescence originates from a pigment, Chlorophyll α, which is ubiquitous and essential for the photosynthetic function of algae cells.

The µflow cytometer also uses a disposable PDMS microfluidic chip previously reported in [4]. However, the flow cell used here is optimized in terms of the sample volume needed for each test.

Figure 1: System overview and the microfluidic chip.

Figure 2: Schematic view of the optical configuration.

EXPERIMENTAL

Fig.1 shows our microflow cytometer system and the microfluidic chip. The disposable chip is made by the standard PDMS soft lithography process. The detection zone of the microfluidic channel is 28µm high and 21µm wide. These dimensions can be optimized for different algal species. A solid-state 488nm laser module is used as the excitation
source. The green fluorescence (510-560nm) and the red fluorescence (>590nm) emissions are measured simultaneously by two photomultiplier tubes, as shown in Fig.2. The test sample is drawn through the channel by a mini peristaltic pump. The whole system is housed within a briefcase-sized aluminum case (12”x9”x5”) and the data can be read out through a standard USB port [4].

In our algal assays, the microalgae, *Dunaliella* (UTEX, USA), was chosen for its ubiquitous existence in marine systems. The algae samples are cultured in the standard medium f/2-Si before testing. For the demonstration of biotoxicity test, the algae cells are harvested and suspended in 5X diluted f/2-Si medium with additional Cu^{2+} toxicant (0ppm, 13ppm and 32ppm, induced by CuSO4 stock solution 4% w/v). The algae are cultured in a constant temperature of 20°C with a white fluorescent bulb lighting (dark/light cycle of 12hour/12hour) to support their photosynthetic function. The esterase activity of the algae samples is evaluated by FDA staining, which uses this recipe: 30µl sample stained by 6µl FDA solution (1µg/ml) for 5minutes, then loaded for test.

**RESULTS AND DISCUSSION**

Fig.3 shows a portion of the typical signals measured from the FDA stained algae samples from the cytometer system. Each peak of the intrinsic chlorophyll fluorescence (Red) represents an algae cell being counted. Correspondingly, the green intensity of the FDA staining measures the esterase activity level of each cell. The heights of the peaks are then recorded for analysis.

![Figure 3: Measured fluorescent signals: Chlorophyll fluorescence (Left) and FDA staining (Right). Each red peak is a counted algae event. Each green peak measures the esterase activity level in the algae cell.](image)

The exposure of the algae samples to toxicant Cu^{2+} leads to a lowered esterase activity, as shown in Fig.4. Without toxicant exposure (i.e., in the control sample with 0ppm Cu^{2+}) the algae cells stained by FDA show strong green fluorescence. However, after an 8hour exposure to the medium with 32ppm Cu^{2+}, the fluorescence from the cells becomes barely visible. The inhibition can be quantitatively evaluated from the cytometer system, as shown in Fig.5.

![Figure 4: Fluorescence microscopy pictures of algae cells with normal (Left) and copper-inhibited (Right) esterase activity levels. For easier visualization of the cells with inhibited esterase activity, the picture is contrast enhanced and the cells are marked with white circles.](image)

![Figure 5: Scatter plots of the measured signals from two samples: 32ppm Cu^{2+} (Left) and 13ppm Cu^{2+} (Right). Each dot is a counted algae event. Red dots are cells from the experimental sample. Blue dots are cells from the control sample (0ppm Cu^{2+}). Esterase activity levels (i.e., normal, lowered and inhibited) are determined by the fluorescence from FDA staining.](image)
Fig. 5 shows the scatter plots (red fluorescence vs. green fluorescence) of the measured results from two different samples (i.e., 32ppm or 13ppm Cu²⁺). Each dot represents a counted algae event. In each plot, the blue dots represent data from the control sample (i.e., 0ppm Cu²⁺), whereas the red dots represent data from the experimental sample. In the 32ppm sample, most cells show very weak FDA fluorescence (lower than 5% of the averaged value from the control sample) after 8hours, which indicates strongly inhibited esterase activity. On the contrary, algae cells in the 13ppm sample did not exhibit significant changes in terms of FDA staining after 8hours (which also can be seen in Fig.6). After 56hours, however, the esterase activity of the algae shows a broader distribution with a lower average level, and strongly inhibited esterase activity levels are observed from a significant amount of cells (as shown in Fig.5). In brief, the differences in the algae populations’ esterase activity levels under various copper concentrations are clearly visible in those two samples.

![Figure 5: Scatter plots of measured results from two different samples.](image)

CONCLUSION

This work reports the first algal copper-biotoxicity assay performed by a portable µflow cytometer using two-color laser-induced fluorescence detection. The FDA staining is used to quantitatively evaluate the esterase activity levels of algal cells affected by copper contamination. The measurement of the biotoxicity of copper to algae cultured under different copper concentrations is demonstrated.

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REFERENCES


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