

Research on fluorescence detection method of *Microcystis aeruginosa*

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Abstract: The paper studied the viability determination of *Microcystis aeruginosa* by FDA and PI staining. The staining results were measured by fluorescence microscopy. The results indicated that viable and dead cells were stained as bright green and red fluorescent respectively by FDA and PI. Through PI-FDA dual color fluorescence staining, the color of green and red distinct obviously by fluorescence microscope. The staining rate has relation with the cell density. If the cell density of *M. aeruginosa* was 1.0×10^7 - 1.0×10^9 cell mL⁻¹, the staining rate would be 100.0% or 98.0% by PI and of FDA respectively.

1. Introduction

In recent years a large number of industrial wastewater and domestic sewage had been discharged into, accelerate the process of eutrophication of lakes and reservoirs in the area of diffusion, year by year, duration prolongation. Taihu, Dianchi, Chaohu, Hongze lake has a “bloom”, even the flow of the river, the largest tributary of the Yangtze River has also appeared as “algae bloom”, algae problem exists in the world of water treatment practice^[1].

Blooms of algae in water body not only causes the deterioration of water quality, the processing and safety of drinking water also have been influenced, threaten the safety of drinking water: some algae releases caused by algae toxins zoo noses or even death^[2,3], algae cells and its extracellular secretions produced three trihalomethanes, haloacetic acids, halogenated ethylene cyanide substances such as in the chloride process^[4-7]. Due to algal blooms, it poses a serious threat to the safety of drinking water for human beings, attracted great attention of researchers at home and abroad. The removal and inactivation of algae become one of the urgent problems to be solved.

The establishment of quantitative detection method to measure the algae removal effect is need for algae research, the detection method is simple and efficient will contribute to the in-depth study. Usually use the algae cell dense chemical method, algae cell density decreased the number is not large, large number of algal cells still exist in water, whether these cells with activity, culture can be determined only through again, and again cultured long time^[8,9]. There are also in water treatment, using biomass index to indicate the algicidal effect, but the index does not accurately reflect the algicidal effect, by measuring the changes of dissolved oxygen in the water containing algae, indirectly judge the survival state of algal cells^[10], the method also need to cultivate algae, consumption time is longer, the discriminant but cannot directly cell activity state^[11].

This research is based on the PI and FDA fluorescent dye single staining method, detection of *Microcystis aeruginosa* activity of PI-FDA double staining, were observed and statistical result of staining by fluorescence microscopy, in order to provide a basis and reference for



the identification of algal cell activity in water treatment.

2. Materials and methods

2.1. Culture of *M. aeruginosa*

M. aeruginosa test using was purchased from Chinese Academy of Sciences, Wuhan Institute of hydrobiology. Algae is a freshwater lakes and rivers in common algae. With BG-11 culture medium were cultured in 5 L white fine mouth bottle, a temperature of 26 ± 0.5 °C, the light intensity of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$, light dark ratio 12h:12h, shaking 1~2 times a day.

2.2 Method and equipment

2.2.1 Reagent

PI (propidium iodide, Sigma), FDA (fluorescein diacetate, Sigma).

2.2.2 Instrument and equipment

Fluorescence microscopy: OLYMPUS BX51; centrifuge HC3018 (Zhongke Zhongjia); ultra pure water system of MILLIPORE-V3.05.

2.2.3 Preparation of cell suspension

The addition of 1 ml Lugeshi solution at 10 ml of algal cells in liquid, in darkness for 48 h, the cells were fixed. Fixed cells lost all activity, but the individual cells were not broken decomposition. Centrifugal liquid fixed algal cells after 3000 r min^{-1} speed, 3~4 times of culture medium suspension with, until Lugeshi solution was clean and the solution became colorless. Reconstitution of cell death was as cells staining.

2.2.4 Preparation and dyeing dyes

FDA is soluble in acetone, match into solution concentration of 5 mg ml^{-1} . 4 °C preservation refrigerator, staining the concentration of $100 \mu\text{g ml}^{-1}$.

PI DPBS buffer solution (Dulbecco's phosphate buffered saline) with concentration of 400 g ml^{-1} solution, keep in the refrigerator 4 °C, staining the concentration of $60 \mu\text{g ml}^{-1}$.

PI single staining: take the algae cell suspension 1 mL, add $12.5 \mu\text{L}$ PI to 1mL algal cell suspension, 5 min reaction.

FDA single staining: take the algae cell suspension 1mL, $2 \mu\text{LFDA}$, evades the light

reaction of 5 min. PI/FDA double staining: algae cell suspension was 1 mL, $2 \mu\text{LFDA}$ and $12.5 \mu\text{LPI}$, evades the light reaction of 5 min.

2.2.5 Fluorescence microscopy

Take in the algal cell liquid logarithmic growth period, prepared into solution, containing different density of live cells join FDA, placed 5 min at room temperature the darkness. Take the algae cell liquid after fixation, using DPBS to prepare solution, containing different density of dead cells entering PI, but also placed 5 min at room temperature.

Take the living and dead cells in mixed cell suspension, to join the FDA, the oscillation mixing after joining PI, placed 5 min. Fluorescence microscopy of FDA detection, the maximum excitation wavelength of 493 nm, PI maximum excitation wavelength of 540 nm. In the blue light, living cells by FDA dyed bright was green. Dead cells were PI stained was red, staining with camera.

2.2.6 Cell count

Algal cell density by 0.1 mL phytoplankton counting plate count, the formula for

calculating the:

$$N = \frac{A}{B \times C} \times \frac{D}{E} \times F$$

The formula for the algal cells per liter of water in the number; for counting frame area (mm^2); as a vision of the area (mm^2); to count view number (a); 1 L water concentration (mL); volume after volume counting frame (mL) for each piece; measured by the number of algae.

On FDA and PI single stained cells, according to the number of cells under the bright field and fluorescent cells, under the field quantity, calculating the efficiency of dyeing cells.

3. Results and discussion

3.1 Algal cell morphology observation

Algae without staining, the results were shown in Figure 1, the figure shows that the algal cell is composed of a single cell or double cell individual algae mostly spherical, untreated *Microcystis* in vesicular, comparative morphology has rules, in accordance with relevant literature reports.

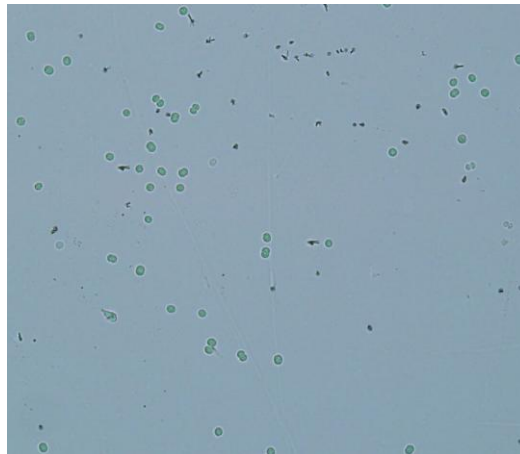


Fig.1 Micrographs of *M. aeruginosa* single-cell unstained in bright field (200×)

3.2 PI Fluorescent staining

PI staining results in Figure 2, the PI cannot enter cells, when cell apoptosis or death, can enter the cell through the cell membrane, and intracellular DNA and RNA phase function generation red fluorescent material, dead cells emit red fluorescence, as can be seen from the graph, PI at 630 nm excitation light green, dying cells were dyed red, bright red fluorescence emitted. Research shows that, the algal cell membrane integrity refused to enter the PI dying, cell membrane damaged cells to enter within the PI and combined with the nucleic acid, so the fluorescence intensity of PI was stronger that cell membrane damage is more serious in this [12-14], and research related to the previous agreement.

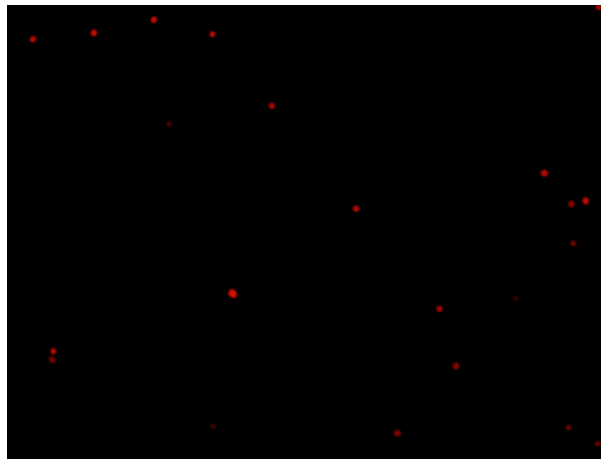


Fig.2 Fluorescence micrographs of *M. aeruginosa*' single-cell stained by PI (200×)

3.3 FDA fluorescent staining

FDA penetrates the algae cells, and esterase hydrolysis produced by transformation of fluorescein, the excitation/emission wavelength of 495nm/525 nm, the fluorescence intensity is proportional to the activity of esterase, therefore using fluorescence intensity characterization of esterase activity of fluorescein. FDA is a non fluorescent hydrophobic derivatives of fluorescein, can penetrate the cell membrane into the cell, and catalyzes the hydrolysis of two acetate groups, has a high intensity of fluorescence of fluorescein in the product, under blue light excitation (495 nm), live cells were dyed bright green FDA, cell membrane damaged, FDA can not be accumulated in the cells, cell death could not emit fluorescence. In this experiment, FDA fluorescence staining is shown in Figure 3, it is shown that the fluorescent substance full of whole cells, cells emit bright green fluorescence, according to, can not only calculate the number of cells, and can clearly distinguish the cell shape.

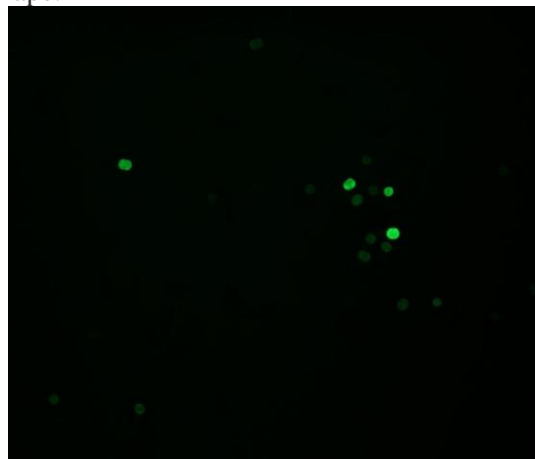


Fig.3 Fluorescence micrographs of *M. aeruginosa*' single-cell stained by FDA (200×)

3.4 FDA-PI double fluorescent staining

The $1 \times 10^7 \text{ L}^{-1}$, *M. aeruginosa* cells after fixation, respectively and live cell solution according to 1:1 into cell suspension, live cells accounted for 50%, accounted for 50% of death cells, Stained by FDA-PI double fluorescence, shown in Figure 4, it can be seen from the figure, live cells were dyed bright green, and death cells were stained red. Under the fluorescence microscope, green and red distinction is clear, can be judged on cell activity, green for living cells, and red for the death of cells.

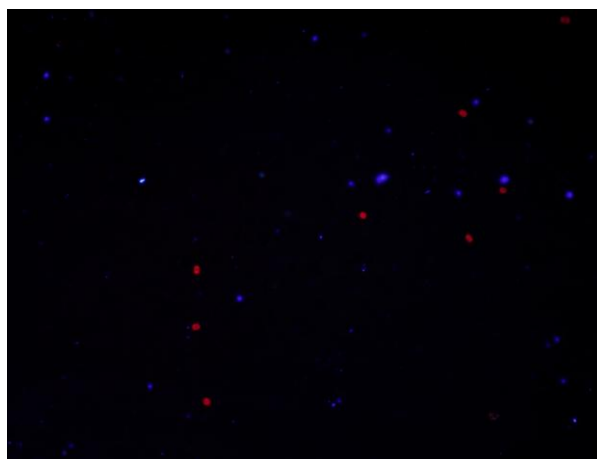


Fig.4 Fluorescence micrographs of *M. aeruginosa* single-cell stained by FDA-PI (200 \times)

3.5 Effects of *M. aeruginosa* cell density of staining efficiency on FDA and PI

FDA and PI on the growth of *M. aeruginosa* single staining efficiency results are shown in Table 1, it can be seen from the table, the cell density has little effect on the efficiency of dyeing, in $1 \times 10^7 \sim 1.0 \times 10^9$ cell mL^{-1} range, PI staining efficiency higher than FDA, PI is relatively easy to enter cells, red light emitted by the fluorescent cell death comparison, clearly visible, and the long holding time, the dying efficiency can reach above 98%.

Table 1 The staining rate of FDA and P I to *M. aeruginosa*

Cell density (cell mL^{-1})	1.0×10^7	5.0×10^7	1.0×10^8	1.0×10^9
Staining efficiency of FDA/%	92.0	94.0	97.0	98.0
Staining efficiency of PI /%	98.0	100.0	100.0	100.0

4. Conclusions

FDA hydrolysis of fluorescein accumulation in cells and live cells emitted green fluorescence under blue light, the green fluorescent cells in living cell. While the PI is just the opposite, in blue light, the death of P I cells were dyed red, red fluorescence for dead cells. Therefore, it was feasible to detect algae cell activity using PI and FDA monochromatic fluorescence.

From the experimental results, PI is relatively easy to enter cells, red fluorescent cell death sends, comparison is clearly visible, and keep for a long time, the dyeing efficiency can reach above 98%.

In the range of $1 \times 10^7 \sim 1.0 \times 10^9$ cell mL^{-1} , the cell density has little effect on the efficiency of dyeing, cell concentration is larger, the efficiency of dyeing is higher, but the difference is not very obvious.

5. References

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