Ускоренный новый метод SingleICIC-MS контроля эффективности лечения альгицидом водоемов при цветении токсичных водорослей

Токсичное цветение цианобактерий были зарегистрированы во всем мире в различных водных системах, в том числе в пресноводных реках, озерах, водохранилищах и в прибрежной морской среде.

1 Цианобактериальное цветение может быть вызвано сочетанием нескольких факторов, включая эвтрофикацию, солнечную радиацию, температуру, закономерности течений и другие связанные факторы.

2 Размножение токсичных водорослей в водной среде может привести к накоплению ядовитых веществ в теле водных животных и даже вызвать их смерть, и тем самым косвенно или прямо оказывает влияние на здоровье человека.

3 Цветение Microcystis aeruginosa (M. aeruginosa) является наиболее распространенным вредным цветением водорослей (ВГА) 4, поэтому его приходится учитывать в разных областях.

Медный купорос является альгицидом, часто используемым для контроля роста водорослей. Механизм действия альгицида на основе меди (Cu) для борьбы с цветением водорослей не совсем понятен. Какое количество Cu требуется для уничтожения отдельной клетки водоросли и морфологии клетки после гибели пока неизвестно. Количественное измерение этих явлений является сложной задачей из-за отсутствия общепринятой методологии.

Эта работа предлагает ускоренный и точный метод одноклеточной (СК)-ICP-MS для изучения эффективности применения сульфата меди для контроля цветения M. aeruginosa. Предложенный метод позволяет количественно определить содержание металла в отдельных ячейках, распределение металла в клеточной популяции, концентрацию металла внутри ячейки и концентрацию внеклеточного металла в ячейки одновременно, и получить эти данные в течение нескольких минут.

Предложенная методика может быть использована не только для быстрой оценки эффективности применения различных альгицидов для контроля цветения водорослей, но также имеет большой потенциал применения во многих других сферах, таких как исследование биодоступности тяжелых металлов, при разработке новых альгицидов на основе металлов, а также для быстрого мониторинга при исследованиях токсичности металлов в клетках.

ССЫЛКА НА ИСТОЧНИК:

https://www.perkinelmer.com/lab-solutions/resources/docs/app_21202-nexion-sc-icp-ms-algaecidetreatmentefficiency.pdf

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A Rapid, Novel Single Cell-ICP-MS Method to Study the Treatment Efficiency of Algaecide for Toxic Algal Bloom Control

Introduction

Toxic cyanobacterial blooms were reported worldwide in various aquatic systems, including freshwater rivers, lakes, reservoirs, and eutrophied coastal marine habitats.[1] Cyanobacterial blooms might be caused by a combination of multiple factors, including eutrophication, solar radiation, temperature, current patterns and other associated factors.[2] Their proliferation in aquatic environments can cause death or toxin accumulation in aquatic animals and then, indirectly or directly, affect human health.[3] *Microcystis aeruginosa (M. aeruginosa)* bloom is the most common harmful algal bloom (HAB)[4] and is widely studied in various fields.

Copper sulfate is an algaecide frequently used for controlling algae growth. The mechanism of copper (Cu)-based algaecide for algal bloom control is not well understood. The amount of Cu required to kill an individual algae cell and the morphology of the cell after being killed is still unknown. Quantification measurement of these phenomena is even more difficult due to the lack of appropriate methodology.

This application note describes a rapidly emerging and accurate single cell (SC)-ICP-MS method to study the efficiency of *M. aeruginosa* control by algaecide (i.e., copper sulfate). The method can quantify the metal content in individual cells, the distribution of the metal within a cell population, the metal-containing cell concentration, and the extracellular metal concentration in a cell suspension simultaneously within a few minutes. More detailed information can be found in a recent publication.[5] This technique not only can be used to rapidly evaluate the effectiveness of different algaecides in algal bloom control, but also has great application potential in many other studies, such as heavy metal bioavailability, new metal-based algaecide development and evaluation studies, as well as other toxicity studies by rapidly monitoring intrinsic metal elements in cells.

Experimental

Cell Culture and Preparation

Unicellular *M. aeruginosa* was cultured in BG-11 growth medium at 22±2 °C with 3200 lux in a 12:12 h light:dark cycle. The cell size is around 2-4 µm. It takes about two weeks for the cells to reach the logarithmic phase after being sub-cultured, and the logarithmic phase could last for one week. The late exponential phase cells were used for all experiments. Five mL of fresh cells were washed three times by 0.1 mM ethlyenediaminetetracectic acid (EDTA) aqueous solution and centrifuged at 500 g for 5 min. The cell pellets were then re-suspended in 5 mL of 0.1 mM EDTA aqueous solution to make the cell stock. The cell concentration was counted in the cell stock by a hemocytometer and then diluted for SC-ICP-MS method development and treatment studies.

SC-ICP-MS Method Development

A PerkinElmer NexION® ICP-MS equipped with a single cell sample introduction kit (Part No. B8150032 for NexION 1000/2000; Part No. N8140032 for NexION 300/350) was used, and the instrument operation and data processing were handled through the Syngistix™ for ICP-MS Single Cell

Application Software Module (Figure 1). The cell integrity after nebulization was checked with different nebulization gas and sample flow rates. Four abundant intrinsic metal elements in *M. aeruginosa* cell, i.e. Cu, Mg, Mn and Zn, were detected by SC-ICP-MS. While all of these metal levels are high enough to be monitored in a single cell and result in about the same cell concentration, transport efficiencies (TE) were determined by detecting the most abundant intrinsic metal Mg-containing cell concentration. The stability of TEs with different cell concentrations and sample flow rates was then evaluated and all method parameters were optimized. Finally, the matrix effect on Mg detection was checked by spiking the Mg standard (Mg STD) into copper- and magnesium-free BG-11 modified algae cell culture medium (MM) to make sure there were no matrix effects for extracellular metal element measurement by SC-ICP-MS when cells are broken or Mg leaches out. This test also checked that there was no particulate Mg (pulse signal) formation during the cell treatment period.

M. Aeruginosa Cell Treatment by Algaecide Copper Sulfate

Cells used for copper sulfate treatment experiments were prepared the same as for method development. Figure 2 shows the schematic of sample preparation and copper sulfate treatment. The cell concentration was determined with a hemocytometer, then cell stock was diluted to 1,000,000 cells/mL and treated by copper sulfate solution with 0, 30, 60, and 100 µg/L copper sulfate. After treatment for selected times, 1 mL cell suspension was removed and diluted three times with 0.1 mM EDTA aqueous solution, then analyzed directly by SC-ICP-MS to monitor cell status (i.e. cell lysis) and cell concentration. To ensure the cell concentration was not high enough to produce multi-cell coincidence, we also tested the correlation of cell concentration via pulse signal in the range from 3,000 to 1,000,000 cells/mL by the SC-ICP-MS method, and the result showed good linear correlation, indicating the pulse signals were from single cell events.
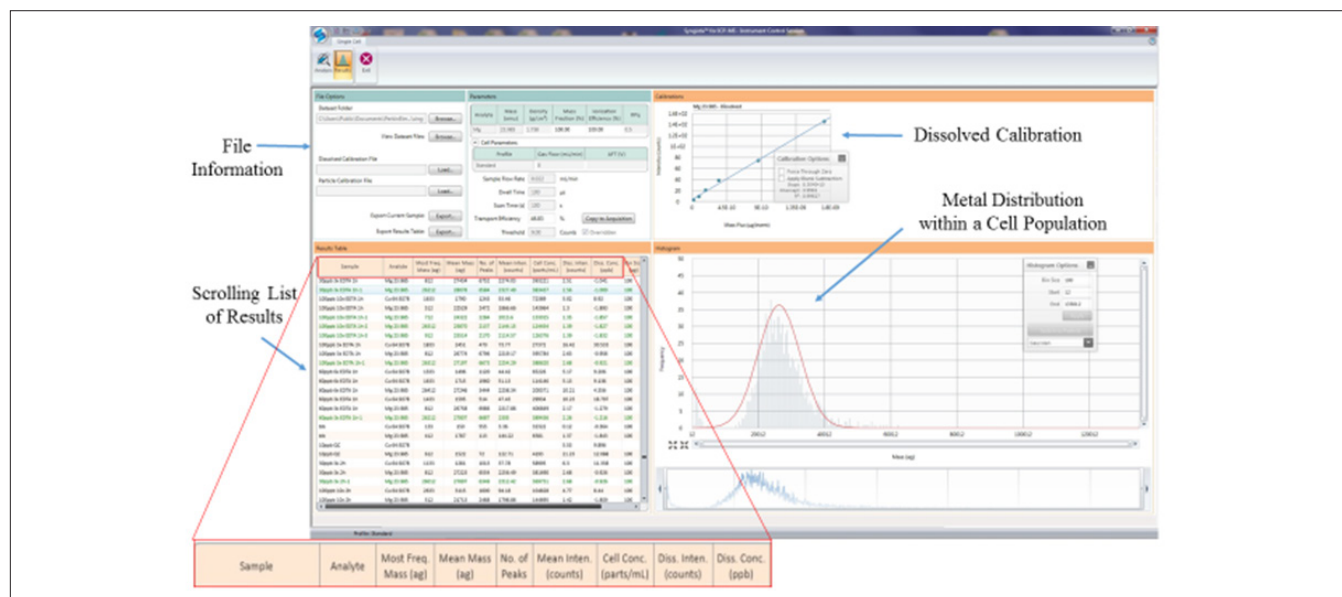

Figure 1. Example screen capture of Syngistix for ICP-MS Single Cell Application Software Module.
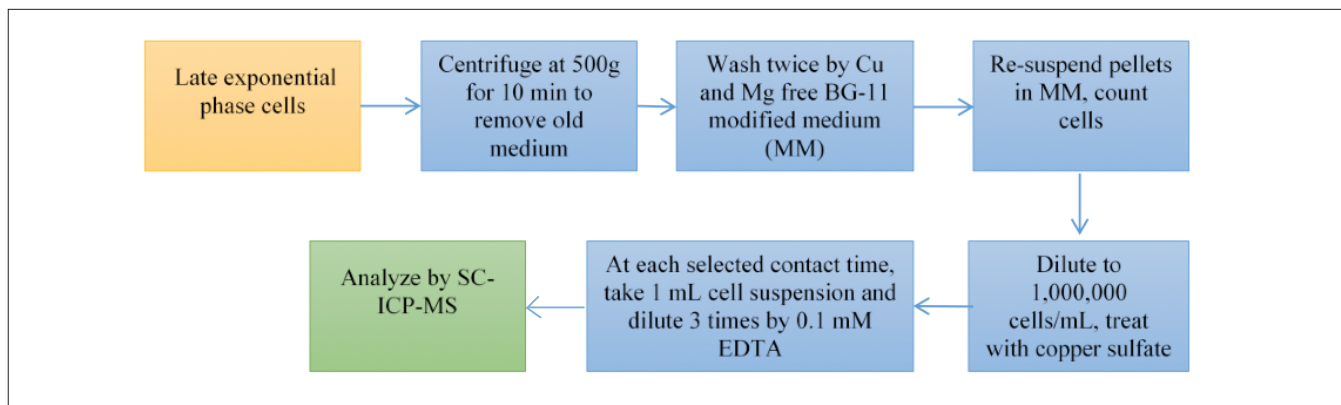
Figure 2. Schematic illustrating analysis procedure

Results

Method Development

Cells were undamaged after nebulization with sample flow rates ranging from 20 to 42 µL/min and nebulization gas flow rate at 0 to 0.7 L/min (Figure 3). *M. aeruginosa* cells contain more Mg compared to copper, zinc, and manganese, from SC-ICP-MS analysis. Thus, the transport efficiency and cell status were monitored by detecting the intrinsic metal Mg content in each cell. The optimized analysis parameters are shown in Table 1. We also detected the Mg concentration in MM by spiking standard Mg in the MM. The spike recoveries were good and indicate no matrix effect for dissolved Mg quantification measurement and no particulate Mg formation in the MM, confirming that it is suitable to analyze post-dosing samples directly. Therefore, calibration standards were prepared as suspensions in MM with 0.1 mM EDTA to matrix match the samples. To reduce the effect of the signal of extracellular Mg to the pause signals of Mg-containing cells, we made a three-fold dilution for the post-dosing samples before analysis by SC-ICP-MS.

The Efficiency of Algaecide Treatment for Toxic Algal Bloom Control

The Mg concentrations in MM, 0.1 mM EDTA solution, and copper sulfate stock solution were analyzed as controls and were found to have Mg concentrations less than the detection limit (0.2 µg/L). The Mg-containing cell concentration decreased over time in 60, 100, 200 µg/L Cu treatment groups; changes were not significant in 0 and 30 µg/L Cu treatment groups (Figure 4). These results suggest that cells are broken over time with high concentrations of Cu treatment, with the higher Cu concentrations breaking the cells faster. The extracellular Mg concentration (released to medium when the cells broke) in each treatment group increased with the decrease of intact cell concentration over time (Figure 5). In high Cu concentration treatment groups, Cu released when cells were broken, resulting in the increase of extracellular Mg concentration over time. The variation of mean mass of Mg in a single cell showed that the mean mass of Mg per cell did not change until most of the cells broke (Figure 6), which suggests that Mg release was caused by cells breaking. Mg mass distribution in cells exhibited cell heterogeneity.

Table 1. Optimized SC-ICP-MS analysis method parameters.[5]

Parameter	Value
RF Power (W)	1600
Nebulization Gas Flow Rate (L/min)[a]	~ 0.5
Makeup Gas Flow Rate (L/min)	0.7
Sample Flow Rate (µL/min)	21 - 22
Dwell Time (µs)	100
Scan Time (s)	100
Transport Efficiency (%)[b]	45.56 - 63.65
Analyte	^{65}Cu, ^{24}Mg

[a]: Parameter is re-optimized daily.
[b]: Parameter is determined daily.
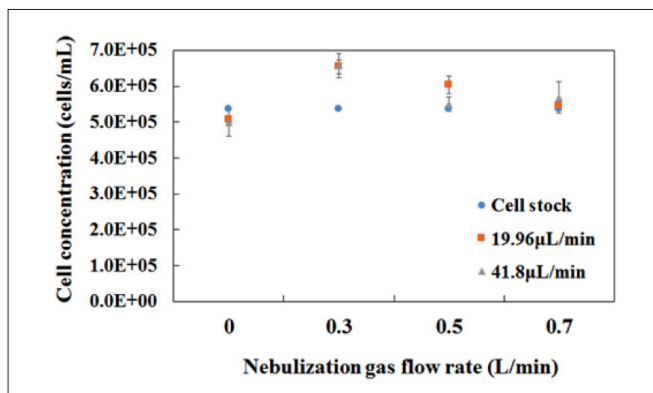
Figure 3. Cell concentration of undamaged cells before (cell stock) and after (20 µL/min and 42 µL/min) with different nebulization gas flow rates and sample flow rates.
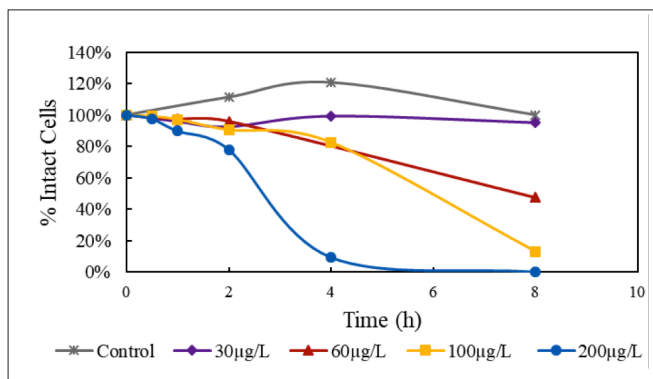

Figure 4. Variations of intact cell concentrations with different treatment times by different Cu concentrations.
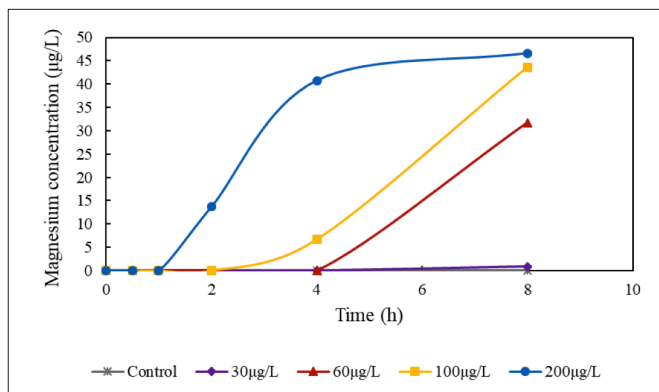
Figure 5. Variations of extracellular Mg concentrations with different contact times by treatment with different Cu concentrations.[5]
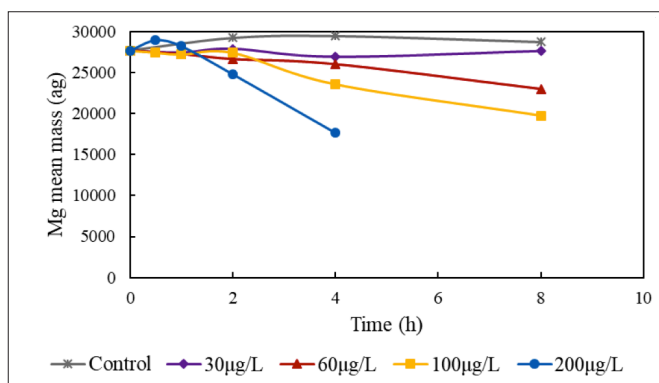

Figure 6. The variation of magnesium mean mass in a single cell with different treatment times and different copper concentrations.

Conclusions

This work has shown the ability of SC-ICP-MS to monitor cell status by rapidly detecting abundant intrinsic metal in a single cell without complex post-dosing sample preparation procedure. This method can be used to evaluate the effectiveness of various algaecides for HAB control and monitor heavy metal ion bioavailability of algal cells. The SC-ICP-MS method can be used to rapidly monitor cell status (broken/unbroken) for any types of cell treatment, such as drug treatment efficiency for drug development and evaluation, as well as nanotoxicity studies.

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Consumables Used

Description	Part Number
Sample Uptake Tubing; Orange/Red (0.19 mm id), PVC, Flared	N0773111 (NexION 300/350) N8145146 (NexION 1000/2000)
Drain Tubing; Gray/Gray (1.30 mm id), Santoprene	N0777444 (NexION 300/350) N8145173 (NexION 1000/2000)
Copper Standard, 1000 ppm	N9300183 (125 mL) N9300114 (500 mL)
Magnesium Standard, 1000 ppm	N9300179 (125 mL) N9300131 (500 mL)

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