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厦门大学

硕 士 学 位 论 文

羟基自由基致死典型水华藻的研究

Study on the Lethal Effect of ·OH on Typical Algas in Water Bloom

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目　录

Table of Content

摘　要

我国饮用水源地富营养化加剧，高藻频发，藻类大量繁殖引起的水源污染，严重地威胁饮用水水质和城市供水。因此饮用水安全问题已成为一项重大的民生问题，而除藻技术的研发是保障饮用水安全的重要一环。目前虽已有多种较为成功的藻类控制方法，但都有各自的局限性，还需要寻找更加安全高效的除藻方法。本研究依托国家科技支撑计划项目，基于大气压强电离放电协同气液混溶技术产生羟基自由基(·OH)的方法，对·OH 对高藻水源水中的典型水华藻铜绿微囊藻、针杆藻和四尾栅藻的杀灭效果进行实验室阶段的研究。研究的主要内容和初步结论如下：

(1)分别利用荧光染色法、测定光合活性和叶绿素 a 的方法确定了 OH 对铜绿微囊藻、针杆藻和四尾栅藻的致死阈值、致死时间及 CT 值。 OH 作用于藻细胞，会使藻细胞失活，抑制光合活性及氧化降解叶绿素。

对于不同藻种和不同藻密度， OH 对其的致死阈值均不同。使藻细胞丧失光合活性的 TRO 阈值浓度等于或稍大于使细胞失活的阈值浓度，叶绿素完全氧化降解的 TRO 阈值浓度是细胞失活阈值浓度的 3~4 倍。

对于不同的藻种， OH 对其的致死时间是不同的，对铜绿微囊藻、针杆藻和四尾栅藻的致死时间分别为 2，1 和 6 s，小于其他除藻方法致死时间的 1/60，CT 值小于其他除藻剂的 1/100，OH 表现出了极强的氧化性和极高的氧化反应速率。

(2)在显微镜下观察到在较低的致死阈值下，藻细胞失活被染色，但外形基本没有改变。证明了 OH 对藻细胞的杀灭作用，同时又不会造成细胞内容物的大量溢出。根据显微镜下观察到的现象，对 OH 在生物学方面的机理进行了初步推测。

(3)建立荧光染色法检测藻细胞经过·OH 作用后的活性：使用荧光染料 SYTOX Green 对细胞进行染色，细胞荧光强烈，背景干扰很小，极易判别细胞死活，结合荧光显微镜及流式细胞仪两种手段分别对藻细胞的活性进行分析检测，效率和准确度均提高。

从本研究可知利用大气压强电离放电协同气液混溶技术产生 OH 杀灭水华藻是一种高效快速去除水华藻的方法，本研究的结果可为项目的中试实验提供方

法和数据。

关键词：强电场放电；羟基自由基；水华藻；致死阈值；致死时间

Abstract

Eutrophication of drinking water sources like lakes and reservoirs has caused frequent algae blooms. Excessive amount of algae is responsible for undesirable taste and odor, toxin generation, disinfection-by-product formation and filter-clogging in water plant, while have seriously threatened water quality and the supply of drinking water. Therefore, the safety of drinking water has become a major livelihood issues, and, the research and development of algae removal technology is an so important work to ensure the safety of drinking water. Although various methods are currently being used to control algae blooms, their successes are limited, so a safe and efficient method is still needed.

This study is supported by the National Science and Technology Support Program of China(NO. 2013BAC06B00). Based on the techniques of the atmospheric strong-field ionization electric discharge collaborative miscibility of gas and liquid method (SID method) to produce ·OH and then to kill the typical algae species *Microcystis aeruginosa*, *Synedra sp*. and *Scenedesmus quadricuauda* in water blooms. The main work and original conclusions are as follow:

(1)Determined the inavtivate concentrations, inavtivate time and the CT value of ·OH killing algae through the way of fluorescence microscope counting, flow-cytometric analysis, detecting photosynthetic capacity and chlorophyll-a.

The concentrations required for completely loss of photosynthetic capacity are equal or greater a little than that of cell viability, while that for completely degradation of chlorophyll-a are 3~4 times than that of cell viability.

The inavtivate time of ·OH on *Microcystis aeruginosa*, *Synedra sp*. and *Scenedesmus quadricuauda* at the inavtivate concentration is 2, 1 and 6 s respectively. Comparing the CT value required for complete loss of cell viability of the above algae, it is found that the CT value of *Scenedesmus quadricuauda* is relatively the highest ,and then *Microcystis aeruginosa*, last *Synedra sp*.. Comparing with other oxidants ,the Inactivate time is less than 1/60 times, and the inavtivate concentration

is less than 1/100 times, which indicate ·OH has very strong oxidability and high reaction rate.

(2)Cellular morphology is also investigated. Microscope observation demonstrates that algae cell has lose viability, while configuration of cell does not changed so much and there is no internal material goes out of the cell membrane after being treated by ·OH at the inavtivate concent. According to the phenomenon observed by microscope, there is a preliminary prediction about the biological mechanism of ·OH killing algae.

(3)Build up the way of fluorescent staining to analyze the cell viability

The SYTOX Green nucleic acid stain can easily penetrate cells with compromised plasma membranes to stain the nucleic acid and yet will not cross the membranes of live cells. It is very useful with all the algae for experiments, because of the exceptionally bright signal and almost no interfering signal from background. Fluorescence microscope and flow cytometry are used to determine the viable and nonviable cells in a sample after stained with SYTOX Green.

In conclusion, ·OH is a potentially highly effective algaecide to removing the algae in algae-ladden water. The study paves the way and provides the data for pilot scale test in the National Science and Technology Support Program of China, and prove the feasibility for mass production and application of ·OH generator.

Keywords: Strong-field discharge; hydroxyl radicals; algae in water blooms;
inactivate concentrations; inactivate time

图表目录