Application Status of Chlorophyll-a Determination Method in Seawater

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Abstract: As an important indicator of water eutrophication and water ecological health, the monitoring methods of Chlorophyll-a are being increasingly valued by environmental supervision departments and relevant scientific research institutions. This paper reviews several commonly used monitoring methods, and systematically analyzes the advantages and disadvantages of each method. It is found that each monitoring method has certain restrictions. Therefore, when the environmental protection department conducts routine monitoring or scientific research institutions conduct long-term tracking monitoring, they should choose the appropriate method according to the different monitoring objects, or even combining different monitoring methods to determine chlorophyll-a, so as to provide reliable data support for water environmental management and scientific research work.

1. Introduction
Chlorophyll a is an indispensable investigation item in water ecological survey, and is the most direct and effective indicator reflecting the biomass of phytoplankton and even the degree of eutrophication of water body [1]. Chlorophyll a is an essential measurement parameter in field observation [2]. At present, the monitoring method of phytoplankton chlorophyll-a mainly include spectrophotometry, chromatography, fluorescence method, due to the different research and accuracy for determination of chlorophyll a, time, adopts the research methods are also different, furthermore, due to the current research method is numerous, often bring confusion for researchers in terms of methods, therefore, this article will focus on the phytoplankton chlorophyll-a monitoring methods were summarized that reference and reference for the related research work in the future.

2. Spectrophotometry
The maximum absorption peak of chlorophyll a is located at 663nm. According to Lambert Beer law, within a certain concentration range, the absorbance value of chlorophyll a is directly proportional to its concentration. After the water sample is filtered, concentrated, extracted and constant volume, its absorbance value is determined at the maximum absorption wavelength, interference value is removed, and the concentration of chlorophyll a can be calculated according to the standard curve of chlorophyll a. Currently, the determination of chlorophyll generally adopts spectrophotometry for accurate quantification. Spectrophotometry is an analytical method based on the selective absorption of light by substances and molecules, which belongs to spectrophotometry [1]. The most commonly used method for determination of phytoplankton chlorophyll in domestic water bodies is the spectrophotometric method recommended by water and wastewater monitoring and analysis methods (the fourth edition) [2]. Spectrophotometry is the most widely used method because of its low price, simple operation and stable results.
However, this method has the disadvantages of long time consumption, unstable operation and so on, which leads to a large error in the result. Moreover, contact with acetone, an organic reagent, is easy to cause harm to human body. Xiao-yan zheng [3], etc., on the basis of in the method, put forward the chlorophyll a and the determination of magnesium content of chlorophyll a, and the method of acetone extraction, grinding method of cell disruption was improved, using extraction instead of grinding, not only avoid the acetone in the process of grinding damage to people, reducing the chlorophyll-a in light exposure, and to reduce the repeated transfer in the process of the loss of chlorophyll a, greatly improve the efficiency of the experiment; Li Huiling et al. simplified and improved the determination method of chlorophyll a in water and waste water monitoring and analysis method (the fourth edition) [2], and extracted chlorophyll a by grinding free method on the basis of the original method. The experimental results showed that the extraction rate and stability of chlorophyll a were improved, and the accuracy of the experimental results was ensured [4].

The current spectrophotometric method is mainly divided into acetone method and ethanol method according to different extraction agents, among which, acetone method is less used at present due to the larger toxicity of acetone, and the use of ethanol method is gradually increasing. According to the different methods, the method can be divided into monochrome method and tricolor method. As a conventional method for the determination of phytoplankton chlorophyll a, spectrophotometry is mostly applied to the routine monitoring of scientific research and environmental protection surface water, and suitable for the determination of indoor water environment samples in large quantities. In order to improve the accuracy of the method, the spectrophotometry can be improved from the following aspects: changing the way of cell fragmentation, such as ultrasound, repeated thawing; Select better extraction reagent, such as mixed reagent; Optimize extraction methods, such as delayed or heated extraction; Select better filter membrane, such as glass fiber filter membrane, freeze and store in dark, etc. [5].

3. Chromatography

Chromatography is the principle of the separation of material in the mobile phase and stationary phase adsorption force, when the samples through the chromatographic column, separated material takes place continuously in the current phase and fixed phase adsorption, desorption, of the different time to components through chromatographic column, different material will successively outflow of chromatographic column, enter into the detector is analyzed, so as to achieve the purpose of separation [6]. Many scholars, including Mantoura [7], Suzuki and Wright [8], have successively established an HPLC method for the analysis of photosynthetic pigments in Marine phytoplankton. High performance liquid chromatography (HPLC) has been widely used in the determination of phytoplankton pigment characteristics, the method is reproducible, the instrument is relatively cheap, and can meet most of the field sample detection. However, there are still three deficiencies in HPLC method, which affect the accuracy of pigment qualitative and quantitative analysis: pigments with the same or similar spectral characteristics are difficult to be distinguished by HPLC [9], and false negative or false positive are likely to occur. In quantitative analysis, chromatographic peaks with low concentration are easily disturbed by their isomers, pigment degradants and adjacent high concentration chromatographic peaks [10]. It is impossible to distinguish different pigments in the co-efflux components, and the co-efflux unknown pigment components are often mistaken for known pigments [11]. The detector was not sensitive enough, and some important pigments with low content, such as Allo indicating micro phytoplankton and zeaxanthin indicating micro phytoplankton (Zea), tended to be near the detection limit of HPLC method with low quantitative accuracy.

4. Remote Sensing Imaging

Based on geographic information system of remote sensing technology as a new type of regional water environmental investigation and monitoring method, has a strong advantage in space and time continuity, commonly used method is through the study of remote sensing image spectral characteristics and the relationship between the chlorophyll a concentration, building mathematical model inversion chlorophyll a concentration [12].

Chen Chuqun et al. [13] selected several combinations of bands from TM1 to TM4 as the
dependent variable, analyzed the correlation degree between combinations of bands and chlorophyll mass concentration, modeled the combinations of bands with the highest correlation degree, and obtained five inversion models for estimating chlorophyll a mass concentration in surface seawater. Andreo et al. [14] used MODIS chlorophyll product data and GIS to discuss and analyze chlorophyll a mass concentration in the gulf of Patagonia, Argentina, and obtained the maximum and minimum variation range of phytoplankton distribution, which was used to monitor the changes of water environment. Watanabe et al. [15] used Landsat8 OLI images to combine band values to form a single factor, establish a regression equation, and invert chlorophyll a mass concentration. Dalu et al. [16] used tm1-tm3 band data and the verified 3-band empirical formula to calculate the mass concentration of chlorophyll a and suspended solids, and discussed the correlation between the mass concentration of chlorophyll a in urban reservoirs and the distribution of the mass concentration of suspended solids and the change of reservoir water level.

5. The Fluorescence Method

Chlorophyll molecules will absorb the energy of photons when exposed to excited light, and transition from the most stable and lowest energy ground state to an unstable and high-energy excited state. Since the excited state is unstable, the molecule loses energy by emitting fluorescence and quickly returns from the excited state to the ground state. Fluorescence determination of chlorophyll a content is the use of natural chlorophyll fluorescence characteristics, with a particular wavelength excitation light chlorophyll samples, chlorophyll by stimulating emit red fluorescence, according to the law of lambert - than the ear, in the range of certain concentration, the fluorescent light intensity and the concentration of chlorophyll is proportional relationship, using fluorimeter to issue fluorescence intensity on determination of the sample, can be quantitatively chlorophyll concentration [1].

Fluorescence method has the advantages of fast measurement speed, high sensitivity, good real-time performance and free from interference of other commonly used pigments. It is mostly used in field measurement or online monitoring, especially for eutrophication measurement of water bodies, so as to timely monitor the growth distribution and change status of phytoplankton in water bodies. At present, the determination of chlorophyll a in seawater by fluorimeter is relatively mature. In 2012, Ma Longshan et al. [17] established a derivative synchronous fluorescence detection method for chlorophyll a. This method is simple, fast and does not require complex pretreatment. Thus, the linear range of chlorophyll a is 0.02-125 microns /L, the detection limit is 0.25 microns /L, and the recovery rate is 97.0% ~ 103.8%. In the same year, Dong Dasheng et al. [18] studied the technology and design of chlorophyll a and turbidity sensor. The sensor technology is designed based on the principle of fluorescence induced chlorophyll a detection and the principle of scattering turbidity detection technology, which can measure the relevant parameters of seawater in real time and realize the function of weak optical signal detection required by the design. Now the common seawater chlorophyll a fluorescence sensor on the market mainly includes YSI type sensor developed by the institute of oceanography and instrumentation of Shandong academy of sciences, Alec chlorophyll a sensor from Japan, and YSI sensor from the United States, with resolution up to 0.05 microns /L and accuracy up to 6.72%.

6. Conclusion

With the rapid development of society, the problem of cyanobacteria has become more and more prominent, which has become a key factor threatening the ecological environment of river, sea, lake and reservoir. Chlorophyll a monitoring is an important indicator to evaluate eutrophication level of river, sea, lake and reservoir as well as water ecological health. Its monitoring method has been paid more and more attention by environmental supervision departments and relevant scientific research institutions. Although many monitoring methods have been applied in practice, each monitoring method has some limitations. Therefore, when environmental protection departments carry out routine monitoring or scientific research institutions carry out long-term tracking monitoring, they should choose appropriate methods according to different monitoring objects, or even choose several monitoring methods combined to measure chlorophyll a, so as to provide reliable data support for water environment management and scientific research.
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