Progress of On-line Biological Monitoring Technology in Different Water Environment

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Abstract. In recent years, there have been frequent occurrences of vicious water pollution incidents, and the technical requirements for water environment monitoring have greatly increased. The use of biological monitoring technology to assess the water environment has developed rapidly. This article analyzes the basic principles of different aquatic biological monitoring technologies, introduces the methods and characteristics of different aquatic biological monitoring, and summarizes the advantages and disadvantages of various biological monitoring technologies, and finally prospects the future development of biological monitoring technologies.

1. introduction

Due to the serious increase in water pollution in my country, traditional physical and chemical online detection methods are difficult to comprehensively assess the quality of water bodies, especially ineffective monitoring of comprehensive toxicity, hygiene indicators, and water ecological health[1]. Aquatic organisms are an important part of the aquatic ecosystem and are closely related to the aquatic environment. Biological monitoring judges the pollution status of water bodies through changes in the structure of biological communities. The close relationship between organisms and the aquatic environment can be used for comprehensive monitoring[2]. Through nearly a hundred years of scientific and technological development, online biological monitoring technology for the aquatic environment has been rapidly developed[3].

Inspired by biology, biological monitoring uses the reaction of different types of aquatic organisms to water to monitor and analyze water pollution from a biological perspective[4]. Online biological monitoring technology uses biological or ecological changes to monitor and evaluate water pollution[5].

The main purpose of the biological early warning system is to initiate a comprehensive analysis program to identify toxic substances and detect peaks of toxic substances in the environment. Online biological monitoring technology can conduct research based on biological physiological changes, biological behavior changes and comprehensive biological indicators. For example, changes in the number and species of individuals in the biological community can monitor the quality of water bodies; large fleas and small fishes collect their swimming speed, swimming distance, and height, using a 3D tracking infrared reflection system; The mussel is to observe the opening and closing of the shell, using high-frequency electromagnetic induction sensors or similar sensors for continuous monitoring,
luminescent bacteria acquire weak light, using photomultiplier technology; green algae use continuous analysis of the influence of water on its metabolism, using chlorophyll Fluorescence monitoring technology.

2. Online biological monitoring technology biological selection
The organisms selected for online biological monitoring should meet the following conditions: 1. The organisms have a large amount of research background data; 2. The organisms can live in a large amount in a certain area of the water environment; 3. The organisms should be in the middle of the food chain; 4. The organisms It is an important representative in the monitored water. Combining the principle of biological screening, bivalve mollusks mainly choose organisms with bivalve opening and closing movement, Vibrio qinghai and luminescent bacillus are commonly used domestic luminous bacteria; algae mainly choose green algae that rely on chlorophyll for photosynthesis; zooplankton Some small fish and large fleas are used.

3. Microbial community monitoring method
Microorganisms have a simple biological structure and a short survival time, and can react quickly to water pollutants. The changes in the community can effectively reflect the changes in the current water environment. Therefore, it plays an irreplaceable role as the main monitoring method to monitor the instantaneous and long-term continuous changes of water quality. The microbial community monitoring method can continuously sample and monitor most protozoan species, and its advantage is that the monitoring results are reliable and highly repeatable.

With the development of methods for monitoring microbial communities, mathematical analysis occupies an important position in the monitoring methods of microbial communities. The development of computers is more conducive to finding the changing laws of biological communities, making the monitoring methods of microbial communities more and more accurate. There are many specific methods to use the biological community monitoring method. For example, Cheng Ding et al used the microbial community monitoring method to monitor papermaking wastewater, and judge whether the wastewater can be used for farmland irrigation through the Shannon-Wiener diversity index. But PFU detection method (foam plastic block detection method) is the most common biological detection method.

The community species diversity index is calculated according to the Margalef formula:

$$D = \frac{(S-1)}{\ln S / N}$$

and the pollution intensity is calculated according to the Shannon-Wiener formula:

$$H = -\sum_{i=1}^{S} \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

where S is the total number of biological species in the sample, N is the total number of protozoan individuals, \(n_i\) is the total number of individuals in a certain organism in the sample. D and H can be used as evaluation indicators of microbial community structure and water pollution degree in static toxicity test. The larger the value of D, the smaller the degree of pollution of the water body. The value of H: 0-1 is a heavy pollution zone, 1-2 is a \(\alpha\)-medium pollution zone; 2-3 is a \(\beta\)-medium pollution zone; > 3 is a low pollution zone.

4. Camera Tracer Monitoring System
The camera tracking monitoring system uses a camera to monitor and record the behavior changes of aquatic organisms, and combines the behavioral response of aquatic organisms to realize online water quality monitoring. The advantage of fish as a biological test is that fish exist in all aquatic ecosystems, and most fish are easy to identify, which is very suitable for ecotoxicological assessment. In addition, fish are a useful indicator of chronic toxicity because they have a long life cycle. Like many aquatic animals, zebrafish swim in 3D space. Therefore, it is best to use a 3D recording system to study its behavior. The automatic video tracking system proposed by Maaswinkel H et al. achieves this by using a mirror system and a correction program. Before this system can be used, the system must be calibrated so that it can record angle and flocks of adult zebrafish at the same time. Chunlei Xia et al. proposed a fish head and tail image recognition method based on gray-scale features. Tracking experiments were performed on 2-5 individual zebrafish, the simplified posture of the fish was
calculated from the head and the center of mass of the fish, and a multi-fish behavior monitoring program based on the measurement of the fish position was made. Martin Føre et al. [18] developed and tested two new types of sonic transmitter devices to reflect the feeding behavior of farmed fish, and ultimately maximize economic benefits and reduce environmental pollution. During the modeling process, Kai Lin et al. [19] used two cameras (top view and side view) to identify the three-dimensional coordinates (xyz) of each fish. The main challenge is the distortion caused by the perspective effect and the boundary of the water body: refraction. Then, based on Lambert absorption law and statistical data analysis, an L-Z correlation model was established, and the model was estimated by monitoring the diurnal and night three-dimensional trajectories of four fishes.

At present, the new infrared reflection (IREF) system for fish tracking has been widely used. The IREF system consists of a camera fixed with an external illuminator and a personal computer with data processing and visualization software. An infrared reflection system combined with a near-infrared range camera and an external illuminator is used to obtain the back image of the fish. The camera and illuminator module of the system are fixed above the center of the fish school container, and the optical axis of the camera and illuminator should be perpendicular to the water surface to simplify the calculation of fish coordinates [20]. The geometric features of several fish backs were extracted and used for model development, and the model was optimized and verified by the ten-fold cross-validation method. The IREF system is based on the amount of near-infrared light reflected by underwater objects. In other words, the sensor can use the brightness of the fish body to estimate the depth of the fish, thereby estimating the true dimension of the fish. The IREF system can be used as a reliable, inexpensive, pressure-free and accurate sensor. In addition, the IREF system can detect and separate overlapping fish through the difference in pixel intensity. By identifying the different brightness of adjacent pixels, the separation of overlapping targets can be achieved [21]. The three-dimensional camera monitoring technology is shown in Fig.1.

The use of three-dimensional camera monitoring technology can clearly reflect the movement of the fish, whether the water quality is normal or not, there will be significant differences in the condition of the fish. In the case of a sudden deterioration of water quality, the movement speed of the fish body will be significantly accelerated, and the acceleration changes frequently. The movement speed and acceleration changes of the fish body are studied to detect the pollution of the water quality [22].

5. Bivalve software monitoring system
The monitoring system for bivalve mollusks is based on bivalve organisms to avoid the influence of external environmental pressure and maintain the stability of internal environment by eliminating external environmental stimuli through the closure of bivalves by organisms themselves [23]. Schyring B J et al. [24] firstly used electromagnetic induction technology to analyze the water pollution situation according to the change of the distance between the mussel bivalves. The Musselmonitor instrument from the Netherlands was made according to the principle that the tension frequency of the biological bivalves changed with the change of the water pollution situation. Electromagnetic conversion system is used to detect the tension of bivalves, carry out online
monitoring and early warning of toxicity of aquatic organisms, and this instrument has been successfully applied to offshore pollution monitoring [25-26]. Ana Carolina Ruiz-Fernandez et al [27] measured the concentrations of Cr, Fe, Mn and other elements in mussels to monitor the pollution of seawater. Adria A. Elskus et al [28] concluded that the mussel pollutant data is likely to be sufficient to provide information on the distribution of chemical pollutants in coastal waters under different environmental conditions and pollutant levels.

The bivalve software monitoring system is an on-line biological monitoring system which uses high frequency electromagnetic induction sensor to continuously monitor the condition of bivalve opening and closing. Dreissena-Monitor is a water quality early warning system based on mussel valve movement (Fig. 2). The system consists of two channels, and the opening and closing of bivalve mollusks is connected to the computer by sensors and acquisition cards fixed to the two shells. Then, the measured results are compared with the natural opening and closing behavior of shells. If there is a sudden increase in the number of opening and closing of shells or a decrease in the ratio of opening and closing of double shells occurs simultaneously in two channels, the system will trigger an alarm [29]. During toxicity testing, one channel is used as a blank control and the other is treated with a toxic substance. Both channels run in parallel, and one would expect the same response from both channels if there were a real toxic effect. Dreissena-Monitor is the only biological early warning system that can measure temperature to compensate for the temperature-dependent activity level of the measured organisms. It also meets the key functional requirements of the early warning system: 1. Reliable, with no personnel on duty for at least a week; 2. Easy to operate; 3. Less than 3 hours of maintenance per week on average; 4. Automatic detection of alarm within 30 minutes [30].

The double shells of bivalve organisms are in the open state 80% of the time under normal conditions, and occasionally close for a short time; the frequency of double shell opening and closing changes under the pollution state of the bottom concentration; the double shell will remain in the state for a long time under the worsening condition. Closed state, only occasionally opened; serious pollution will cause the death of bivalves, the bivalves will always be in the open state, and the scale of the opening is large. Therefore, the water pollution situation can be judged by monitoring the bivalve expansion and closing conditions of bivalve organisms.

6. Luminescent bacteria online monitoring system

The online monitoring system uses luminescent bacteria as indicator organisms, and judges the water quality from the changes in the luminous intensity of the bacteria. Luminescent bacteria emit light in clean water substances, and toxic pollutants in the water will destroy its metabolic process and inhibit luminescence. The degree of inhibition is related to the total amount of toxic pollutants and can be used to evaluate the toxicity of wastewater [31].

Luminescent bacteria use changes in luminescent intensity as an indicator of biotoxicity in liquids. The light source of bacterial bioluminescence is a flavin mononucleotide derivative.
The visible light emitted by luminescent bacteria has a wavelength of 450～490nm. When luminescent bacteria come into contact with toxic substances, the luminescent intensity of bacteria will decrease with the increase of toxic substances concentration. Mojtaba Mohseni et al. isolated a glowing, curved rod-shaped gram-negative bacterium from the Caspian Sea and proposed it as a good choice for commercial kits for monitoring toxic substances.

Luminescent bacteria method has many advantages, such as speed, sensitivity, low cost, simple operation and intuitive measurement results. In recent years, it has received extensive attention from related scientific researchers. Ma Yong et al. improved the experimental conditions and steps of the existing luminescent bacteria, and added the original luminescence intensity and luminescent natural variation factors during data processing, thus reducing the experimental error. Chen Jihong et al. conducted an experimental study on the optimal experimental conditions for rapid recovery of bacterial liquid from freeze-dried bacterial powder, and found that the bacterial liquid could be effectively tested within seven days at 2～5℃, and the optimal experimental time and temperature for monitoring Vibrio fiscali were 15min and 15℃ respectively. Lili Zhang et al. used Vibrio qinghai to conduct luminescence inhibition analysis in the test tube to evaluate the surface water pollution of Huangpu River.

### 7. Algae fluorescence analysis system

Chlorophyll fluorescence technology is based on the principle of photosynthesis, which reflects the photosynthetic metabolic state of cells through the change of fluorescence. Algae are important to the environment and are often used in toxicity testing because they are sensitive to many substances, particularly herbicides and heavy metals. Rapid and accurate determination of chlorophyll content of planktonic algae in natural water can provide basic data for water quality monitoring and early warning as well as protection and management of aquatic ecology. When algae toxins which meets in the external environment, the photosynthesis is affected, the total energy released in chlorophyll in energy reduction for photochemical reaction, but offers chlorophyll fluorescence energy increase, expressed as rising trend on fluorescence kinetics curve, the peak, fluorescence intensity will decrease with the increase of time and slow.

Algae can adapt to different environments, have high fertility and are very sensitive to poisons. Light is one of the most important environmental factors that affect the growth and physiological activities of algae. Toxicity tests used microalgae cells as biosensitive elements, and the change of chlorophyll fluorescence as a response index. In order to fully grasp water quality, planktonic algae must be monitored, and environmental quality can be clarified by the response of individual, population or community of algae to environmental changes.

Alexandre Camuel et al. studied the use of oxygen productivity measurements to assess the short-term response of algae to herbicides. Zheng Kai et al. proposed the application of a new chlorophyll fluorescence imaging system to investigate the toxicity test of herbicide to Chlorella proteinosa, and the detection time was shortened from 96 h to 2 h, but for the overall experimental process, the time of algal culture before detection was still long. The online algae monitor AOA, developed by BBE Company, uses four light sources to measure chlorophyll fluorescence, and has achieved intuitive results in monitoring chlorophyll, changes in the number of algae and population change. Algae Toximeter® is based on online fluorescence spectrum analysis of chlorophyll content of Algae in water to analyze the concentration of chlorophyll in Algae. Herbicides interfere with algae metabolism and reduce chlorophyll content, causing the system to sound an alarm. The system is sensitive to herbicide identification and can achieve high temporal resolution. Cunha Isabel et al. developed a biosensor of Aptamer for the detection of aquatic algal toxins, which can be used for real-time on-site detection of aquatic toxins produced by Marine and freshwater microorganisms (cyanobacteria, dinoflagellates and diatoms). Lee Seunguk et al. developed a 3D printing smartphone platform integrated with optoelectronic wetting (OEW) operated by optical drive microfluidic chip. The OEW-powered microfluidic chip not only transmits droplets, merges, mixes, and holds multiple paths in the detection area, but also fluorescent detection and counting of target algae cells.
using a smartphone. A 3D-printed smartphone platform integrated with OEW was used to test various water samples and conduct field analysis of the test data. Because algal cells can be quickly detected, it is possible to detect and screen for microbial contaminants early, preventing the spread of harmful microorganisms in the aquatic environment.

8. Conclusions

Various online biological monitoring technologies have their advantages and disadvantages. Microbial communities require a long sampling time. Static water sampling usually takes four weeks, and flowing water sampling takes about two weeks. The advantage of the new 3D tracking infrared reflection system is low hardware cost and intuitive reflection of fish movement, but the disadvantage is low accuracy; double The opening and closing of the test organisms of the shell mollusc monitoring system will be affected by the noise of the external environment, which will affect the accuracy and stability of the monitoring, the luminous bacteria online monitoring system has a large difference in the luminous intensity of bacteria. During the detection period, the luminous variation range is wide and the error is relatively large. Large, the algae fluorescence analysis system is sensitive to herbicides and heavy metals in polluted water, and does not react strongly to other pollutants. Therefore, it is necessary to continuously develop relevant online biological monitoring technologies to support the monitoring and analysis of water pollution emergencies and realize water pollution emergency management.

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