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Degradation of organic matter in the sediments of Hongfeng Reservoir

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Abstract **In this work, the distribution of organic carbon, DNA and lipids in the sediments of Hongfeng Reservoir were described in addition to SO_4^{2-} profile in pore water. The contents of organic carbon in the sediments range from 23.3 to 76.8 mg·g^{-1}, with the peak value appearing at the depth of 8 cm bellow the sediments water interface (SWI), and tend to decrease gradually with sedimentation depth. The concentrations of SO_4^{2-} decreased from 40.50 mg·L^{-1} to 12.00 mg·L^{-1} at SWI in top 4 cm sediment, and was kept at 12.0 mg·L^{-1} bellow that depth. Newly produced organic carbon can be conserved as long as 14 years in the sediments. The contents of DNA were relatively high in top 9 cm surface sediments, as revealed by agarose gel images, close to those of organic carbon and sulphate reduction index (SRI). This study shows that bacteria played an important role in organic matter degradation; SO_4^{2-} is the primary electron acceptor under anaerobic condition in this reservoir; DNA in the lake sediments can provide important information for the study of cycling of nutrient elements in the lake.**

Keywords: lake sediment, DNA, organic carbon, electrophoresis, sulfate.

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Lake sediments, as a valuable archive for the study of environmental change due to its continuum, high resolution and steady sedimentation, bear much global and regional environmental information, and have accommodated materials from the drainage area, air and lake itself [1]. Carbon is one of the major nutrient elements in lake sediments, so are nitrogen, phosphorus and sulphur, and all these elements are involved in lake biogeochemical cycling[2,3]. To deal with the rapid increase of carbon dioxide in the atmosphere and more serious greenhouse effect, environmental scientists have long been focusing on the study of carbon sources and sinks. Beside soils and plants, lake sediments also can play an important role in carbon cycling. So, to understand how much organic matter can be conserved in lake sediments is of special significance for global carbon balance.

Much of the organic matter produced in surface water via photosynthesis is exposed to degradation as it sinks through the water column and only a small amount of it can reach the sediment interface[4]. The diversity of microorganisms plays a key role in the diagenesis of organic matter in lake sediments[5]. The composition and molibility of these microorganisms can indicate how well an ecosystem is functioning[6]. But numerous researchers have pointed out that the majority of the bacteria in environmental samples cannot be isolated or cultured using traditional cultivation techniques only[7,8]. The extraction of microbial DNA in natural samples is a newly developed and important approach to environmental microbiological study[9], and this method can circumvent some detection biases and be complementary to the available cultivation techniques for surveying microbial systems in natural environment precisely and thoroughly. In this study, the isolation and extraction of DNA will help us to understand the process of degradation of organic matter in lake sediments by microbial activities.

1 Material and methods

1.1 Description of the study area

The Hongfeng Reservoir (106.24°E, 26.32°N) is the largest man-made reservoir in Guizhou Province. The outcrop ped strata in the drainage area of this reservoir are mainly composed of carbonate rocks, with widely distributed soils such as lime soil and yellow soil. This reservoir has a surface area of 57.2 km^2 and a maximum depth of 45 m, with the residence time of 0.325a. Its drainage area is 1596 km^2, and the average sedimentation rate is 0.17 g·cm^{-2}·a^{-1}[10].

1.2 Sampling and method

In March, 2003, five sediment cores were drilled by using the sampler developed by Wang et al.[11] at the site Houwu where the central area of Hongfeng Reservoir is located. On average, these cores are about 30cm in length, with the original soil appearing at the 25cm below SWI. The clear interface water and perfectly preserved sediments indicated that the lake sediments can reflect the processes of natural sedimentation. Sediment cores were segmented *in situ*, at intervals of 1 cm. Pore water was collected by centrifuging sediments for 30 min at 4000rpm under 4℃ within 24 h after sediments were sampled. 1mL of pore water was taken to determine NO_3^- and SO_4^{2-} by HPLC (HP1100). The sediments were dried using a Freeze Dryer (FD3-85D-MP), milled to 150 meshes, and then kept in frozen status. Part of the sediment samples were treated with hydrochloric acid to remove carbonate, followed by the determination of organic carbon on a PE2400 SERIES II Element Analyzer.

2.00 g of dry sediment samples was taken, 5ml 10 g·L^{-1} lysozyme solution was added, and culturing was conducted at 37℃ for 2 h during which the solution was vibrated 5 to 8 times. After that, DNA extractant was added in for the extraction of nucleic acids. Then the samples were centrifuged for 30min at 4000 rpm under 5℃ to collect the supernatant. The procedures were repeated three times to get three supernatants. Then the mixed supernatant was purified with equal volumes of saturated hydroxybenzene and chloroform-isoamyl alcohol. And then, the purified solution was centrifuged again to get the final supernatant, which was collected and gently mixed with ethanol so as to let DNA precipitate. After being air-dried, these DNA pellets were re-dissolved in 100 μL TE buffer. 10 μL of this solution, after mixing with 2 μL DNA dyer, was put into agarose gel containing 0.5mg·g^{-1} ethidium bromide(EB), followed by electrophoresis on agarose gel at 45V stable pressure in 0.5×TBE buffer. Finally the agarose gel was pictured in UV light on Tanon GIS-2008.

The lipid contents were determined by in 2 g of dried sediment sample, which had been wrapped with filter papers, extracted with petroleum ether for 48 h in a Soxhlet extractor, and then calculated with the mass difference.

2 Results and discussion

2.1 The profiles of organic carbon, total sulfur, NO_3^- and SO_4^{2-}

The contents of organic carbon and total sulfur in sediments of the Hongfeng Reservoir are presented in Fig. 1. The contents of organic carbon in sediments vary between 23.2 and 76.8 mg·g^{-1} and decrease slowly from the SWI to the sediment bottom. While at the 26 cm depth below SWI, with the appearance of original soil, the data show a bias from the total trend. The contents of organic carbon were found relatively constant at 15—26 cm depth, and kept at about 2 mg·g^{-1}. The contents of sulfur in sediments are within the range of 1.07—14.2 mg·g^{-1}, similar to those of organic carbon (Fig. 1).

The concentrations of SO_4^{2-} in pore water are relatively low, varying between 0.89 and 40.5 mg·L^{-1} (Fig. 2). It seems that sulfate shows somewhat increase at 0—1 cm below SWI, but a drastic decrease from 40.5 mg·L^{-1} to 12.0 mg·L^{-1} at 1—4 cm depth. Bellow that depth, the data show no obvious variation. NO_3^- concentrations, in most cases, are below 1.0 mg·L^{-1}, and kept constant at that depth.

2.2 DNA in the sediments

The contents of DNA in the sediments can reflect bacterial quantities and the degradation degree of organic matter. Agarose gel images can show the relationship between DNA contents and sedimentation depth. In Fig. 3, it is obvious that the high contents of DNA are mainly at 0—9 cm depth, and from 9 cm depth to the sediment bottom, the contents are much lower than those in top sediments, indicating that bacteria are active mostly within the range of 0—9 cm below SWI.

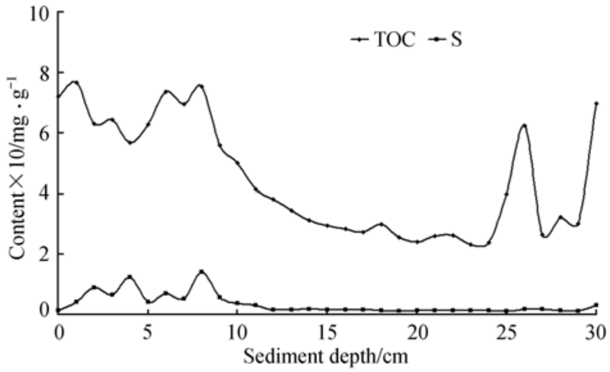

Fig. 1. The distributions of organic carbon and sulfur in the sediments of Hongfeng Reservoir.

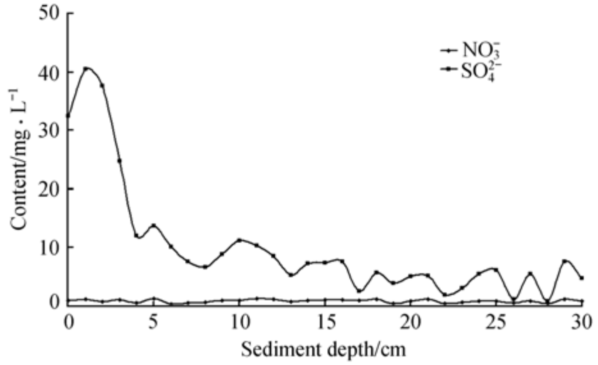

Fig. 2. The distribution of SO_4^{2-} and NO_3^- in pore water of the Hongfeng Reservoir.

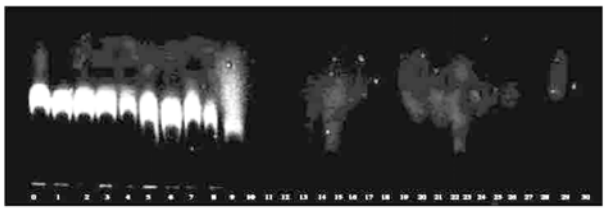

Fig. 3. The distribution of DNA in the sediments of Hongfeng Reservoir.

2.3 Lipids in the sediments

The contents of lipids in the sediments are within the range of 8—30 mg·g^{-1}, accounting for 30—50% of organic matter. The distribution of lipids shows a similar trend with that of organic carbon, i.e. decreasing gradually on the whole (Fig. 4).

2.4 Sulfate reduction and degradation of organic matter

Kristensen[12] found that under anoxic environment in natural lake sediments, the degradation rate of organic matter is slighterly higher than under oxygenic conditions. But under anoxic environment anaerobic bacteria are less

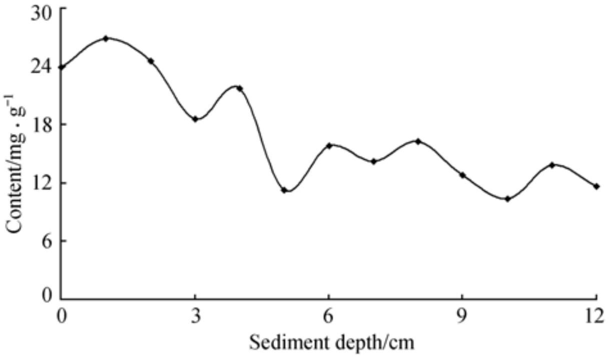

Fig. 4. The distribution of lipids in sediments of the Hongfeng Reservoir

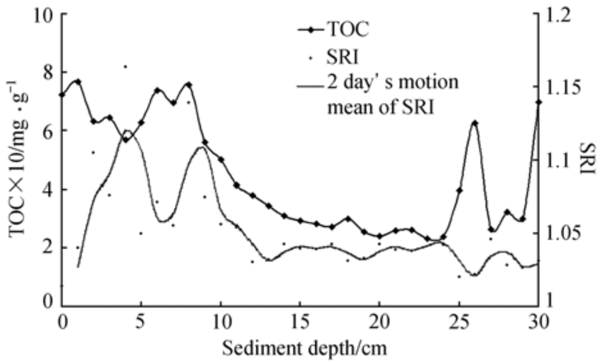

Fig. 5. The relationship between organic carbon (TOC) and sulfate reduction index (SRI).

powerful to destroy complex organic molecules, and the organic matter degradated is dominated by hard oxidizable molecules. So sulfate plays the same role as oxygen as the major electron acceptor of anaerobic microorganisms under anoxic environment conditions. Sulfur contents and the degree of sulfate reduction are important indices reflecting the degree of organic matter preservation. Sulfate reduction index (SRI) can reveal the reduction degree of sulfate[13]:

$$SRI=(TOC+C_{loss})/TOC,$$
$$C_{loss}=S(\%)\times 0.75,$$

TOC is the content of organic carbon; C_{loss} is the quantity of degraded organic matter; S(%)is the percent content of sulfur.

In March, prevails an oxygenic environment at the sediment-water interface of the Hongfeng Reservoir, around which a clear oxidation layer meaning about 1 cm in thickness is often observed. Due to the influence of aerobe, the SRI value is very low in this layer. Because of low concentrations of NO_3^- and oxygen, sulfate becomes the main electron acceptor of anaerobic micro-organisms to degrade organic matter below the oxygenic layer, and at 4 cm depth below this layer, SO_4^{2-} has been largely consumed by sulfate-reducing bacteria, causing a sharp decease in SO_4^{2-}, as indicated by SRI. Below 10 cm depth, both SO_4^{2-} and DNA are low in contents. A negative correlation is observed between the contents of organic carbon and the SRI values (Fig. 5).

2.5 Relationship between organic carbon and DNA

At the SWI, the high contents of DNA demonstrate that bacteria are so abundant as to favors the rapid degradation of organic matter. Below the 15 cm depth, electron acceptor is used up, so bacteria are less than in the upper layers. Most of the organic matter herein can be well preserved without further degradation.

Heterotrophic bacteria can degrade organic matters; on the other hand organic matter also has a significant impact on the quantity and activity of bacteria. It is found in this study that organic matter and bacterial diversity and quantity show a significant positive correlation. If organic matter is abundant, bacteria will be highly diverse, for they compete less fiercely with one another for food, so bacterial activity and quantity are high. On the contrary, bacteria will be scarce because of the less abundance of organic matter. Our findings are in agreement with those of Boetius and Damm (1998)[14].

The constant of organic matter degradation can be calculated using the following equation[15]:

$$K = Ln(C_0 / C_z)/(Z / S),$$

Z is the depth of sediment (cm); C_0 and C_z are the contents of organic carbon at the SWI and Z cm depth, respectively; K is the constant of organic matter degradation (a^{-1}), and S is the sedimentation rate ($cm \cdot a^{-1}$).

Assuming the sedimentation rate is constant, which is the case for most lake sediments, we calculated the sedimentation rate to 1 $cm \cdot a^{-1}$ in terms of the lengths of the sediment cores and of the deposition year (1961). The sedimentation rate we got is in consistency with 0.94 $cm \cdot a^{-1}$ reported by Chen et al.[16]. The constant of organic matter degradation of the Hongfeng Reservoir sediments calculated by this formula is 0.07 a^{-1}. The higher the K value, the faster the degradation rate of organic matter and the younger the age of the sediment. So only less sediment has been preserved. Younger sediments in the Hongfeng Reservoir can be preserved as long as 14 a, as evidenced by calculation.

3 Conclusions

The concentrations of sulfate in pore water of the sediment vary between 0.89 and 40.5 $mg \cdot L^{-1}$, showing a rapid decrease at the top 5 cm depth and keeping relatively constant below that depth. Under aerobic conditions, organic carbon is the main electron applier. The contents of NO_3^-, which is near the detection limit, have little difference on the whole.

The contents of organic carbon are within the range of 23.3—76.8 $mg \cdot g^{-1}$, which generally tend to decrease exponentially from the surface sediments. As viewed from

DNA data, it is indicated that bacteria and organic matter are abundant at the top 10 cm depth, leading to rapid degradation of organic carbon in this region, while below the 10 cm depth, since most of organic matter was degraded and bacterial activities were relatively weak, organic matter tended to be preserved.

The contents of sulfur are within the range of 1.07—14.12 $mg \cdot g^{-1}$ and these of lipids are, 8—30 $mg \cdot g^{-1}$, accounting for 30%—50% of organic matter. The variation trend of sulfur and lipid is in agreement with that of organic carbon mainly at the 10 cm surface. Anthropogenic activities of fish breeding have been recorded by the sediments with organic matter increasing suddenly in the sediments. It is worthy to conduct a deep-going study on the theory of bacteria in the sediments because bacteria may play an important role in the mineralization of organic carbon and the accumulation of dioxide carbon in the air, leading to greenhouse effects. This is directly related to the life and health of human beings.

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