



Beyond enhancement of macrophyte litter decomposition in sediments from a terrestrialized shallow lake through bioanode employment



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HIGHLIGHTS

- SMFC enhanced the decomposition of macrophyte litter in sediments.
- Enhanced degradation of recalcitrant carbons by SMFC improved humification.
- SMFC enriched aromatic compounds-degrading bacteria.
- Methanogen growth was inhibited by microbial anode during experiments.
- SMFC might be exploited against lake terrestrialization process.

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ABSTRACT

The accumulation of excess decayed macrophyte litter in sediments of shallow lakes can lead to ecological deterioration. This work investigated the impact of sediment microbial fuel cell (SMFC) employment in macrophyte litter degradation in sediments and is based on 700 days of lab experiments. The results showed removal efficiencies of total organic carbon (TOC) at three depths (0–8 cm, 8–18 cm, and 18–28 cm) in SMFC-amended sediments, which increased by 18.9%, 20.9%, and 20.8%, respectively. Moreover, SMFC enhanced the degradation of recalcitrant carbons and stimulated humification of organic matter in sediments, which might benefit carbon sequestration in the long term. Pyrosequencing analysis showed that a relatively high proportion of aromatic compounds-degrading bacteria were detected in SMFC-employed sediments, although SMFC did not noticeably change the bacterial and archaeal community structures in sediments. Also, the methanogen growth in sediments over the whole experimental period was inhibited with SMFC employment. Considering both enhanced litter biodegradation and methanogen depression, SMFC could be exploited as a new and promising way to delay lake terrestrialization.

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1. Introduction

Lake eutrophication is one of the most prevalent global problems [1–3]. As eutrophication can create a favorable condition for aquatic vascular plant growth in shallow water areas [4,5], macrophytes usually spread rapidly in these ecosystems. Once decayed, a

substantial part of dead plant tissues eventually settle into sediments as litter.

The major component of macrophytes is refractory lignocelluloses with low nutrients; hence, biodegradation of macrophyte detritus is relatively low, and only a small fraction of these materials is consumed by grazers. Then, the settlement of macrophyte litter increases carbon storage, and also is responsible for increasing the sediment layer and accelerating lake terrestrialization [6,7]. Over the past decades, the terrestrialization of aquatic environments due to the deposition of macrophyte-related organic matters has attracted considerable attention [8,9]. Furthermore, accumulated organic matter also causes the depletion of oxygen and accelerates the production of greenhouse gases such as methane.

Abbreviations: DNA, deoxyribonucleic acid; ER, electron recovery; FA, fulvic acid; HA, humic acid; LPI, labile pool I; LPII, labile pool II; OTUs, operational taxonomic units; PCoA, principal coordinates analysis; PCR, polymerase chain reaction; RNA, ribonucleic acid; RP, recalcitrant pool; SMFC, sediment microbial fuel cell; SPSS, statistical package for social sciences; TOC, total organic carbon.

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In organic-rich sedimentary environments, degradation of organic matter is generally limited due to a lack of suitable electron acceptors. To address this problem, biostimulation has been used to introduce oxygen and/or other chemical electron acceptors to affect the redox state and improve the native microbiological activity within sediments thereby leading to organic compound degradation [10,11]. A redox state also independently affects the degradation of more refractory organic matters via stimulated oxidative enzyme activities and energy benefits that elevated redox conditions provide to heterotrophic microbial communities [11,12]. While continuous aeration or adding chemicals to replenish electron acceptors can improve organic degradation, these methods are expensive and can lead to further pollution of the aquatic ecosystems [13,14]. A more environmentally friendly way to provide long-term electron acceptors and thereby induce microbial respiration in sediments is the sediment microbial fuel cell (SMFC).

SMFC has an anode electrode embedded in anaerobic sediments and a cathode electrode suspended in the aerobic water column above the sediment [15]. Electrons released during substrate oxidation are transferred via anode-respiring bacteria to the anode, which serves as a terminal electron acceptor. As a result, an electric current is generated [16,17]. SMFC can be used as power sources for instruments deployed for aquatic environment monitoring. Furthermore the employment of only SMFC [15,18,19], has led to both the mitigation of methane emission [20] and enhanced removal of aromatic and petroleum hydrocarbons in contaminated sediments [21,22]. This remedial activity of SMFC is achieved possibly by creating new anaerobic metabolic pathways through altering the redox state of the sediments. However, the removal of macrophyte detritus settled in the sediments and subsequent organic matter alteration after SMFC employment were unknown.

In this study, SMFC employment in sediment remediation led to the use of its anode as a supplementary electron acceptor. This technology allowed an investigation of the decomposition and transformation of submerged macrophyte litter. The results show the litter effect on the sediment and ecological status of an area in the East Taihu Bay. The microbial community composition in sediments was analyzed with 16S rRNA pyrosequencing, and methanogen growth was also quantified. This study concludes with a discussion of the potential of microbial anode in lake terrestrialization prevention and sediment carbon sequestration.

2. Materials and methods

2.1. Lake characteristics and sampling

Taihu Lake (31°10'N, 120°24'E) is the third largest freshwater lake and is a typical large shallow lake with an area of 2340 km². The increasing nutrient inputs associated with population and economic growth has led to eutrophication of the lake. In this study, samples were collected from Eastern Taihu Bay (31°10'N, 120°25'E) with a depth of less than 1.5 m and with a dominance of submerged aquatic vegetation [23,24]. Terrestrialization has been become serious in Eastern Taihu Bay mainly because of the excessive growth of macrophytes followed by the accumulation of macrophyte detritus.

Submerged macrophyte *Potamogeton malaianus*, which is one of the dominant submerged plants in Taihu Lake, was taken manually. Surface sediments were collected from the top 20 mm below the sediment–water interface using a gravity coring device onboard of a river boat. Samples of surface water (20 cm depth) were also collected. Samples were transported to the laboratory within several hours. Then, the sedimentary samples were sieved through a standard 2-mm mesh screen to obtain a uniform size

for experiments. Water samples were pre-filtered through a 200- μ m-mesh to remove zooplankton and large detritus.

The physical and chemical properties of the sediments were measured as follows: moisture, 46.7%; total nitrogen, 1250.3 mg kg⁻¹ dry sediment; total phosphate, 763.6 mg kg⁻¹ dry sediment; cellulose, 269.3 mg kg⁻¹ dry sediment; and total organic carbon, 15.7 mg kg⁻¹ dry sediment. Meanwhile, the properties of the water were measured as follows: pH 8.0, and conductivity 0.50 mS cm⁻¹.

2.2. Experimental setup

Four plexiglass columns, each with a capacity of approximately 4 L (12 cm \times 35 cm, diameter \times height), were used to perform the biodegradation experiment in a dark environment at 25 °C, which contained two non-SMFC controls and two SMFC treatments. In this experiment, *P. malaianus* biomass was crushed after air-drying and sifted through a 0.45 mm sieve. 3000 g of wet sediment and 48 g of crushed plant litter were mechanically mixed with a litter/sediment ratio of 3% (dry weight/dry weight). The mixture was then added into each column, followed by filling with water taken from Taihu Lake. The overlying water was continuously circulated above the sediment layer at 10 L per day⁻¹ through peristaltic pumps.

For SMFC employment, carbon felt electrodes (Sanye Carbon, China) were used as both the anode and cathode for each column experiment. Prior to experiments, carbon felts were soaked in 1 M HCl for 24 h, and then washed with deionized water several times until pH reached neutral. The anode (22 cm \times 20 cm, long \times wide) was affixed to plastic stents and buried about 8 cm from the sediment–water interface and parallel to it. The cathode (9.5 cm, diameter) was suspended above the overlying water and its upper surface was exposed to air. A copper wire was used to connect the external circuit containing a resistance of 100 Ω . All exposed metal surfaces were sealed with silver and nonconductive epoxy resin. The voltage signal was measured using a multimeter (Model 2700, Keithley Instruments, Cleveland, OH, USA).

The sediment samples were collected about every three months during experimental operation and sliced into three layers (0–8 cm, 8–18 cm, and 18–28 cm), and parts of them were stored in –80 °C refrigerator for chemical and microbial analysis.

2.3. Chemical analysis

The total organic carbon (TOC) in sediment samples was measured using Walkley and Black's rapid titration method [25]. Humic acid (HA) and fulvic acid (FA) were isolated from the sediments based on differences in solubility in alkaline and acid solutions according to the procedure recommended by the International Humic Substances Society [24,26]. The labile and recalcitrant carbon pools in sediment organic matter were measured using the three-pool approach [27] and included three parts: (1) labile pool I (LPI), which consisted mainly of noncellulosic polysaccharides, (2) labile pool II (LPII), which consisted mainly of cellulose, and (3) recalcitrant pool (RP), which consisted of mainly lignin and humic substances.

2.4. DNA extraction for 454 pyrosequencing and real-time quantitative PCR

At the end of the experiment, bulk sediments and electrodes samples were taken for 454 pyrosequencing. Electrodes were rinsed with sterile water to remove visible sediments on the surface. Then a sterile razor blade was used to acquire electrode-associated microbes for subsequent DNA extraction. Real-time quantitative PCR was used to analyze the contents of

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