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Novel method to immobilize phosphate in lakes using sediment microbial fuel cells

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Title: Novel method to immobilize phosphate in lakes using sediment microbial fuel cells

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Highlights:

- Feasibility of phosphate removal in lakes by sediment microbial fuel cells.
- Efficient phosphate immobilization by steel-mesh electrodes in lab and pilot scale.
- The method was relevant in summer, during anoxia in the hypolimnion.
- Electricity production was insignificant; the focus was on environmental benefits.

Abstract

Phosphate pollution in lakes poses an intractable remediation challenge. Accumulated stocks of phosphorus in sediments cause high concentrations in the overlying water despite elimination of external sources. We propose to use sediment microbial fuel cells (SMFCs) for lake remediation by sediment phosphorus immobilization. The hypothesis is that SMFCs can increase sediment redox potential at the...
top layer, and that such changes will allow the sediment to retain phosphorus as immobile species. This study placed an emphasis on scalability, practicality, and use of low-cost materials. Stainless steel net was selected as electrode material, and modifications were tested: (i) chronoamperometric operation with anode poised at +399 mV (versus standard hydrogen potential); (ii) injection of graphite slurry; and (iii) coating with nickel-carbon matrix. Stainless steel electrodes were implemented in laboratory microcosms (1 L) and at field scale in a eutrophic freshwater lake. All tests were carried out in untreated sediment and water from Lake Søllerød, Denmark. Phosphate immobilization was shown at lab scale, with 85% decrease in overlying water using steel electrodes. At field scale maximum phosphate decrease of 94% was achieved in the water body above a 16 m² stainless steel SMFC electrode. Results are promising and warrant further study, including remediation trials at full scale. Added benefits include degradation of sediment organic matter and pollutants, inhibition of methane and sulfide emission and production of electricity.

**Keywords:** Surface water remediation, pilot-scale, in situ, low cost electrode, lake restoration, stainless steel electrode.

**Graphical Abstract**

see separate file

**Competing interests:** the authors have no competing interests to declare.

**Contributors:** all authors have reviewed and approved the manuscript prior to submission.
1 Introduction

For most lakes, phosphorus (P) limits primary production (Stumm and Morgan 1996). High emissions of P into lakes occurred with detergents and raw sewage, during the time when water toilets and sewers were installed but efficient wastewater treatment plants were still lacking. Even though those emissions have been stopped in most Western countries (EEA 2020), the legacy phosphorus now cycles between water columns and sediments of the lakes (Reitzel et al. 2003), with a slow decline in stocks over decades. High phosphate levels initiate eutrophication and algal blooms, with subsequent decreased penetration of light to benthic plants, alteration of predator-prey dynamics and oxygen deficiency. Such ecological modifications have severe consequences for the lakes as habitats, as indigenous species are replaced by a few opportunistic species adapted to such high nutrient and low oxygen levels (Singer 2016). Furthermore, eutrophic lakes lose their value for recreational activities. The problem of eutrophication occurs across all European countries and in all developed countries worldwide. The majority of Danish lakes are eutrophic, based on the indicators: chlorophyll a (70%) and phytoplankton (53%) (Danish EPA 2020).

The sediment represents the major sink of phosphate in lentic systems. With water at oxic conditions, phosphate binds to metals (Fe, Ca, Al) oxides (Nur and Bates 1979; Hansen et al. 2003; Hupfner and Lewandowski 2008) thereby being effectively retained in the surface sediment (Carlton and Wetzel 1988; Shimotori et al. 2019). In lentic waters, prolonged periods of low wind and/or high irradiance typical of summer, may cause water thermal stratification with consequent isolation of the hypolimnion leading to net oxygen consumption in the bottom water (Hupfner and Lewandowski 2008; Giles et al. 2016). Under hypoxic-anoxic conditions, reductive dissolution of iron oxides liberates phosphate which is then released to the water column (Mortimer 1942). The generated ferrous iron (Fe$^{2+}$) can be effectively scavenged by co-precipitation with $\text{H}_2\text{S}$ to form Fe-S minerals (Caraco et al. 1993; Roden and Edwards, 1997; Jørgensen, Findlay, and Pellerin 2019), resulting in the net removal of dissolved iron (Figure 1).
Figure 1. Postulated reactions. Mineralization of organic phosphorous deposited from the water to the sediment produces phosphate ($PO_4^{3-}$). In oxic sediments matter, iron-oxides are present and bind phosphate. In anoxic sediments (dark brown, bottom), Fe-oxides are reduced liberating $Fe^{2+}$, which in turn, precipitates with sulfide ($S^{2-}$) as iron-sulfur minerals ($FeS/FeS_2$). This reduces the binding capacity of the sediment towards phosphate, that may be then released to the water. By providing an alternative electron sink, the anode of a SMFC inhibits the accumulation of $S^{2-}$ and favors the reoxidation of $Fe^{2+}$ to Fe-oxides thereby maintaining an efficient binding mechanism for phosphate in the sediment (shown in red shading).

Under eutrophic conditions, stimulated primary production in the photic zone enhances the flux of highly degradable organic matter to the hypolimnion, exacerbating $O_2$ hypoxia. This will in turn increase the release of phosphate from the sediment which will further fuel primary production, leading to a positive feedback loop. Minimizing (ortho-)phosphate release from sediments is a necessary pre-requisite to restore lake water quality to good ecological status.

Aeration of sediments has been applied in lake remediation, but often with limited success (Hansen et al. 2003, Hupfer and Lewandowski 2008, Tammeorg et al. 2020), possibly because oxygen depletion at the sediment-water interface is not the sole driver of phosphate-release from the sediment but also the pH, mineralization of continuously settling organic matter and release of organic-P constitute important...
processes (Søndergaard 2007; Hupfer and Lewandowski 2008). We investigate the sediment microbial fuel cell (SMFC), as a tool to promote oxidative reactions in lake sediments with the final aim to limit phosphate release. In a SMFC, the two half reactions (oxidation and reduction) take place at separate electrodes, and the electron transfer is by an electrical current through an external circuit (Logan et al. 2006).

Organic carbon and other reduced species are oxidized by exo-electrogenic bacteria using an electrode (the anode) as electron acceptor and H\(^+\) and CO\(_2\) are produced (Kaur et al. 2014), or spontaneously oxidize on the electrode surface. This reaction is shown in Equation 1, using formaldehyde as a simple example. The electrons released by the oxidation process travel through the external circuit to the opposite electrode (cathode) where they reduce O\(_2\) completing the redox reaction (Equation 2). Thus, the anode facilitates oxidation processes under anoxic conditions using a terminal electron acceptor that can be located at cm to m distance.

Anode reaction: \(CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^-\)  
Cathode reaction: \(O_2 + 4H^+ + 4e^- \rightarrow 2H_2O\)

Studies show that the application of SMFCs favor oxidative processes such as iron and sulfide oxidation in reduced sediment (Tender et al. 2002; Matturro et al. 2017; Touch et al. 2017), with consequent inhibition of Fe-S formation (Ryckelynck et al. 2005), reduced methane emissions (Friedman et al. 2016, Liu et al. 2017) and increased sediment redox potential (Kubota et al. 2019).

The increased availability of ferric iron, induced by the SMFC (with the supply of ferric iron further reinforced by the inhibition of FeS formation), is expected to promote the binding of phosphate to the sediment and thereby limit the lake-internal cycling of phosphate, ultimately reducing the phosphate concentrations in the water column.

The objective of this study was to demonstrate the feasibility of phosphate trapping by installation of SMFCs. Electrodes were tested and optimized in laboratory reactors, and installed in a lake in northern Zealand, Denmark. Over two seasons, electrical, chemical, and biological parameters were monitored.
The results of this study may lead to novel surface water remediation techniques, both for natural and anthropogenic systems.

2 Materials and Methods

The hypothesis was tested simultaneously in eleven one-liter 2-cell laboratory microcosms (lab scale) and in two 4 x 4 m SMFC installations outdoors in a lake (lake scale, electrode area multiplied by height of water column gives 112 m$^3$).

2.1 Lake Søllerød

Lake Søllerød is a postglacial lake in the municipality of Rudersdal in Northern Zealand, Denmark (55°48'56.1"N 12°29'33.5"E). Its surface area is 13 ha with a maximum depth of 9.8 m and an average depth of 5.6 m (Jensen and Møller 2015). A thermocline regularly establishes in about 3 m depth in the period May to September. The lake receives water from four rainwater discharge pipes and through a creek (6.6 L/s) dewatering a bog area. The average hydraulic retention time is 5 to 6 years (Jensen et al. 2009).

Untreated wastewater was emitted into the lake until 1924, whereafter treated wastewater was emitted until 1975. In the 1960’s copper-sulfate was added to limit odors. Another attempt of remediation was pumping of bottom waters, which has been intermittently carried out between 1976 and 2009 (Grontmij, 2012). Between 1922 and 1975, averaged concentrations of total P during summer in the epilimnion were above 2500 μg/L. By the end of the 1970s total P steeply declined to values near 100 μg/L in surface samples, but up to 600 μg/L total P was found in the bottom water, most of it in the form of dissolved phosphate, and has since then declined very slowly (Jensen and Møller 2015). In 2018, the maximum concentration of phosphate-P in bottom water from 8 m depth was 962 μg/L (Nicolaisen 2018). The high phosphate levels lead to intense algal growth (though, during summer, nitrogen may become limiting), with Secchi depths often < 1 m and chlorophyll-a over 150 μg/L, and subsequent elevated pH-values up to 10 (Jensen and Møller 2015). Primary production has been high for several decades, resulting in high
sediment biomass (Danish Environmental Ministry 2012, Danish Environmental Ministry 2015). The top sediment layer is black muddy gel-like gyttja of about 50 cm thickness, the underlying layer is more solid silt with gas intrusions and is about 100 cm thick. The total volume of anthropogenically affected sediment has been estimated at 103 025 m³ (Hvidt 2016). In July 2017, measured concentrations of total P in sediment ranged from 1600 to 3200 mg/kg dry weight (dw). Sediment stocks of P are estimated at 9700 kg in the top 40 cm, of which 50-80% is bound to iron and the remainder mainly bound in organic matter (Jensen et al. 2009), while external P input is only 61.5 kg/year (Danish Environmental Ministry 2012). The lake and lake sediment are well studied. Its location is only 4 km north of the university campus at Kongens Lyngby. Lake Søllerød was thus chosen as field site for the lake scale test of P immobilization using SMFCs.

2.2 Laboratory microcosms (reactors)

Lab-scale SMFCs were constructed from two 650-mL chambers separated by a CMI-7000S cation exchange membrane (Membranes International Inc., USA) with aperture diameter of 70 mm. A two-chamber system with membrane separation was favored over a one-chamber system, to avoid penetration of oxygen through the open water layer to the sediment surface. Oxygen would oxidize the top sediment, allowing phosphate binding and thus mask the effect of the electrode. In field trials the membrane is not necessary, as height and stratification of the water column limit oxygen diffusion. The microcosm design is shown in Figure 2. The reactor walls were manufactured from transparent acrylic plate and tubing. O-rings were made of nitrile rubber, stoppers of natural rubber, gaskets of soft PCV, washers of brass, and rods and wingnuts of AISI304 stainless steel. Access ports to each chamber were closed with rubber stoppers and generously sealed with petroleum jelly (Vaseline) to prevent oxygen intrusion. Petroleum jelly was also applied on the anode chamber exterior at connecting joints. Electrodes were placed 15 mm from the membrane.

The lab scale experiments were conducted using sediment and water collected in one batch from Lake Søllerød. Samples were sieved to remove debris larger than 10x10 mm, and sediment (anode chamber, 60%) and water (anode chamber 40% and cathode chamber 100%) were added without further pre-
Reactors were operated at room temperature (range 19 to 26 °C) and in the dark, to avoid potential photosynthesis and oxygen formation.

Figure 2. Laboratory microcosms (reactors). Top: operating microcosm containing lake water in the cathode chamber (left), and lake sediment and water in the anode chamber (right). Cathode and anode were made of stainless steel, folded to extend throughout their respective chambers. Bottom: external components of the microcosm. Elements left to right: wing nuts, tensioning rods, disk washers, end plate, O-ring, cathode chamber and stoppers, O-ring, interface plate, gasket, membrane, gasket, interface plate, O-ring, anode chamber and stoppers, end plate, disk washers, and wingnuts.

2.3 Test of electrode materials

Anode and cathode were in all cases constructed from the same material to prevent interference from galvanic corrosion. Electrodes with specific surface area of 0.0023 m$^2$, were cut from 1 mm diameter AISI316 stainless steel wire spot-welded in 10-by-10-mm squares, and folded to distribute electrode layers evenly in each reactor chamber. Various electrode configurations were tested at laboratory scale: i) blank stainless steel electrodes (steel); ii) graphite slurry (graphite) injected as a means of increasing the
effective anode surface area and the range of a standard steel electrode. The graphite slurry consisted of 25 g pulverized graphite suspended in 50 mL lake water and was injected into the sediment in the anode chamber. iii) A coating of nickel-doped carbon (Ni-C) was tested as surface treatment, potentially leveraging carbon’s affinity for biofilm adhesion (Logan 2008) and nickel’s catalytic properties (Choi 2019, Gupta 2017). Ni-C electrodes were electrolytically coated in a carbon and nickel solution, embedding nickel particles in a carbon matrix. iv) Chronoamperometric operation was carried out using a potentiostat (Pot16, ACM instruments, UK), poising steel anodes at +200 mV vs. an Ag/AgCl electrode (4 M KCl gel, Equilibrium, France; potential against standard hydrogen electrode SHE is +399 mV) (poised). v) The control employed disconnected steel electrodes (DCtrl), to determine any effect inherent to the presence of steel. vi) A second type of controls (Ctrl) was constructed by simply omitting electrodes. Each type of reactor was run in duplicate, except DCtrl which was only one reactor.

The performance of SMFCs was determined by measuring working potential (WP, in mV) and open circuit potential (OCP, in mV). The flow of electrons (unit A·s, Ampere seconds, or Coulomb C) was calculated from WP and external resistance and subsequently used for stoichiometric calculations of substrate oxidation (1 mol e\textsuperscript{−} is equivalent to 96 485.33 A·s) (Logan et al. 2006). The external resistance (between anode and cathode) was 1000 Ω until day 24 and 200 Ω from then on, except for the Ni-C electrode, where the resistor was changed after 140 d.

In microcosm experiments, WP was measured hourly using a data logger (CR1000, Campbell Scientific). The OCP was determined only at the control reactors with disconnected electrodes (DCtrl) to avoid disturbance of the microbial community.

### 2.4 Lake installations

The first SMFC was installed in the lake in July 2019, the second in June 2020, and both are still in operation at the time of writing (November 2020). The construction and installation is shown in Figure 3. Steel wire mesh (as described previously) was suspended in 4 x 4 m frames made from heavy duty PVC tubing. Anodes were made by three layers of steel, cathodes by one layer only. Initially, anode frames
were filled with air. When in place at the lake, upon removal of rubber stoppers they filled with water and sunk to the bottom. Frames would settle a few centimeters into the sediment, placing the anode steel layers just at the surface of the sediment but avoid sinking into the sediment. Cathodes were constructed with buoyant internal chambers to achieve 5 kg negative buoyancy. Anodes and cathodes were tethered in their corners in the desired anode-cathode distance, and swimming marking buoys were connected to the four corners using ropes. Electrical wires robustly connected to each electrode were accessible from a utility box placed on the nearest bank. The first field (Field 1) was constructed with a fixed distance between anode and cathode of 3 m, the second field (Field 2) was constructed with adjustable anode-cathode distance, and the cathode was adjusted to 1.5 m depth below water surface. Electrodes were assembled on shore, and then towed in position and sunk, anode Field 1 at 4 m depth (from May 2020 7 m depth) and anode Field 2 at 7 m depth.
Figure 3. Installation of electrodes in the lake. Anode was folded (A), electrodes were installed in frames and towed in position (B). Electrodes were sunk. Cathode was suspended between surface buoys and the anode on the bottom (C), performance was monitored by measuring working potential on shore (D).

2.5 Chemical analysis

During lab scale experiments, water samples were collected from the anode chamber by inserting a syringe needle through the rubber stoppers. Due to low operating volume and the desire to limit possible disturbances, only 3 rounds of sampling were carried out. During lake scale experiments, water samples were collected from the top, middle and bottom of the water column above the anode and nearby at a control site, using Teflon tubing and a peristaltic pump from a boat. Sampling at high wind speed was avoided, to circumvent mixing of the water close to the electrode with surrounding lake water but also for safety reasons. All water samples were measured immediately for dissolved oxygen (DO), pH, electrical conductivity (EC), and temperature with hand-held electrodes (Wissenschaftlich-Technische Werkstätten Weilheim WTWW). Additional water samples were passed through 0.2-µm nylon filters and stored at –20 °C until analysis. Inorganic N and P (NH₃-N, NO₂-N, NO₃-N, and PO₄-P) were analyzed using a SKALAR San++ continuous (segmented) flow analyzer. Elemental analysis was done using Inductive Coupled Plasma with an Optical Emission Spectrometer (ICP-OES, Perkin Elmer Avio 200) or to a Mass Spectrometer (ICP-MS, Agilent 7700 Series) equipped with Syngistic™ for ICP Software v. 2.0 from Perkin Elmer. Sediment samples were centrifuged in 15-mL vials at 8800 x g for 10 minutes, freeze-dried, and digested in microwave oven (USEPA 3051A method) with 9 mL HNO₃ (65%), 3 mL HCl (30%) and 1 mL H₂O₂ (30%). Pore-water samples were taken from the supernatant of the centrifuged sediments.

2.6 Statistical analysis

The difference in the median values of measured PO₄-P concentrations above the electrode versus those at the control site was tested by the Mann-Whitney U-test (with significance level 5%) in the SPSS 22.0 software. Other differences in mean were tested using the two-sided t-test in MS Excel 2010.
3 Results

3.1 Laboratory microcosms

Figure 4 summarizes the performance of the SMFCs at lab scale. Within a few days the OCP of the disconnected control (DCtrl) reached 650 mV and remained constant above 600 mV until day 130, then slowly increased to 885 mV at the end. This OCP confirmed the well-functioning of the system, the absence of significant oxygen intrusion, and ongoing reduction processes in the untreated sediment.

Measured working potentials of the steel and the graphite electrodes showed little difference, with an initial rapid increase to values > 100 mV, and at day 24, when the resistance was lowered from 1000 Ω to 200 Ω, a decline to about 40 mV, then constant until day 170, from then on instable and circulating. We suspect that from day 170 on the reactors were no longer fully operating.

The highest WP was seen for the Ni-C electrode, which was started 64 days after the other electrodes. It quickly reached 600 mV after a few days, but from then on slowly declined over 140 days. Day 204, the resistance of the Ni-C reactor was changed from 1000 Ω to 200 Ω, and the WP fell accordingly by factor five to values lower than those measured for the other electrodes. However, the nickel electrode corroded and formed nickel(II)phosphate (see section 4.6).

Initial PO$_4$-P concentrations taken a few days after filling the reactors (immediate sampling was prohibited by particle resuspension) were between 2500 and 3500 µg P/L for all samples, except in the reactor with Ni-C electrodes, P concentrations were very low (< 1 µg/L) from the start to the end of the experiment. At day 134, half-way through the experimental period, measured PO$_4$-P had fallen in the steel and graphite reactors to about 500 µg P/L, while in the controls an increase to > 4000 µg P/L was observed. Between day 134 and 217, phosphate increased in steel, graphite, control, and DCtrl microcosms. At the end of the experiment, PO$_4$-P was 7020 and 6969 µg/L in Ctrl and DCtrl, respectively, 2663 µg/L with steel and 2198 µg/L with graphite electrodes. Poised reactors showed continuously decreasing phosphate, and Ni-C reactors exhibited consistently low phosphate concentration with maximum 41 µg/L. The variation of the measured values was small for samples taken from the steel and
graphite test initially and at \( t = 134 \text{ d} \), but towards the end, the WP got instable, and also the variation of measured P was large, hence those final values should be interpreted cautiously.

**Figure 4.** Results from lab scale experiment. Top: open circuit potential (OCP, mV, right axis) and working potentials (mV, right axis, mean of two replicates), below: dissolved phosphate in the water column \( \text{PO}_4^-\text{P} \) (μg/L, left axis, error bars denote minimum and maximum, number of replicates \( n \) is 2 to 4) at initial (day 0), half-way (day 134) and at the end (day 217). Electrode materials: stainless steel (Steel), stainless steel with addition of graphite particles (Graph), stainless steel coated with carbon embedded with nickel particles (Ni-C), stainless steel with external voltage applied (Poised), no electrode (Ctrl), and disconnected steel electrodes (DCtrl).
In Figure 5 total phosphorus (TP, mg/L) and total iron (TFe, μg/L) in pore water and water column at the end of the experiment, day 217, are shown. All SMFC-treated reactors showed lower concentrations of TP and TFe in pore water and water column than Ctrl and DCtrl. This supports the hypothesis that phosphate is eliminated by co-precipitation with iron in reactors with connected electrodes. Moreover, TFe is always below TP (note the different axes), giving iron the limiting role. An exception is the Ni-C reactor, where measured TP in the water column was low despite relatively high TFe. As reduced Fe^{2+} is soluble, but oxidized Fe^{3+} is not, this could be an indication that SMFCs affect the oxidation status of iron. TFe was only assessed when the reactors were sacrificed at the conclusion of the experiment, hence the data reflects the situation after the reactors showed instability. It is possible that the TFe concentrations were lower at day 134, when also lower PO_{4}^{3-}P concentrations were seen (compare Figure 4). TP values are similar to (Ctrl, DCtrl) or higher (steel, graphite, poised) than those for PO_{4}^{3-}P shown in Figure 4, the latter indicating the occurrence of other forms of P, probably organic P. A plausible source of organic P is the partial microbial decomposition of sediment material, which is stimulated by the electrodes.

No significant difference between the treatments could be observed for TFe and TP in sediment solid samples (not shown). The mean sediment content of TFe was 9058 mg/kg dw (standard deviation s 1495 mg/kg dw, n = 24), of Al was 4888 mg/kg dw (s 992 mg/kg dw, n = 24), while that of P was 966 mg/kg dw (s 270.2 mg/kg dw, n = 24). The analysis of heavy metals showed moderate pollution with Cu (mean 470 mg/kg dw, s 68.9 mg/kg, n = 24) and lead (mean 85.4 mg/kg dw, s 16.1 mg/kg dw, n = 24). The rank correlation between TP in water column and sediment (not shown) was significant (r = 0.50, n = 24, significant at P = 0.05) but the explained variance is low (R^{2} = 0.26), while the relation between P in sediment and in pore water was below significance (R^{2} = 0.13, n = 16).

The concentration of Ni in all sediments was low, 13.4 mg/kg dw on average (s 2.8 mg/kg, n = 21), with the strong exception of the sediment treated with the Ni-C electrode. Here, the mean was 4767 mg Ni/kg dw (s 1108 mg/kg dw, n = 3). Additionally, very high concentrations of dissolved Ni in pore water (17.5 mg/L) and water column (40 mg/L) were measured (all other water samples: 1 to 7 μg/L) in the Ni-C samples, and also Co was elevated (17 μg/L in pore water and 43 μg/L in overlying water, all other
samples < 1 μg/L). The concentration of Cr in solid samples was on average 19.8 mg/kg dw (s 3.0 mg/kg dw, n = 18), and slightly elevated in samples with poised electrodes (mean 22.5 mg/kg dw, n = 6, significantly different in a two-sided t-test, P = 0.03). However, no difference was observed for the water samples (mean of samples from poised reactors: 2.8 μg/L versus 3.1 μg/L, mean from all other reactors).

**Figure 5.** Total phosphorus (TP, mg/L, left axis) and total iron (TFe, μg/L, right axis) in sediment pore water and in the water column of microcosms with and without sediment microbial fuel cells, at the termination of the lab scale experiments on day 217. Error bars denote minimum to maximum, n is 2 to 6. Electrode materials: stainless steel (Steel), stainless steel with addition of graphite particles (Graphite), stainless steel coated with carbon embedded with nickel particles (Ni-C), stainless steel with external voltage applied (Poised), no electrode (Ctrl), disconnected steel electrodes (DCtrl).
3.2 Lake installations

Results for the lake installation are presented in Figure 6. The electrical charge (cumulative Ampere, As) shows a strong seasonal pattern, with the highest slope (and thus highest electrical current) during summer months, Field 2 also in early autumn 2020. The phosphate concentrations were significantly lower in samples taken in the proximity of the anode, compared to controls (two-sided U-test, $P = 0.00$). In late spring 2020, intense phosphate release was observed in the lake. In the controls, the increase in PO$_4^-$P continued into summer and reached values > 800 µg/L. However, concentrations as low as 41 µg/L (6% of the control at 653 µg/L) were observed above the anodes during July 2020. The rapid drop in phosphate concentration was paralleled by a sharp increase of SMFC performance, measured as cumulative ampere (Figure 6). Field 1 generated a maximum of 10 mW, causing high initial cumulative ampere, although plateauing after one month, and low or no electrical current during winter. The electrode field was towed ashore for inspection in May 2020 (day 300). A ripped cable was found, and repaired, after which the electrodes were moved to deeper waters (7 m) and re-installed.
Figure 6. Lake scale sediment microbial fuel cell performance. Cumulative ampere (A∙s) generated by the electrodes of Field 1 and Field 2, and PO₄-P concentrations (µg/L) measured at the bottom of the lake water column above the anodes and at a control site. Error bars denote standard deviation. The cable to the anode of Field 1 was broken from January to May 20th, 2020, indicated by the broken x-axis.

The generated current of both electrodes was decreased when oxygen in bottom waters was high (Figure 7, spring and autumn). In summer 2019, a thermocline with low DO at depths ≥ 3 m was observed, but was disturbed by stormy weather in mid-August. In summer 2020, a stable thermocline developed from May and lasted into late September (Figure 7, 21 July to 11 September). The current generated in the SMFCs and the phosphate measured near the lake bottom were likewise low during winter, with little difference between electrode and control, which can be contributed to the naturally aerated bottom waters.
in that period. With increasing temperature during summer, the electrode performance increased. In parallel, P concentrations increased, the increase being less at the anodes than the control.

Figure 7 Dissolved oxygen versus depth for control field, Field 1 and Field 2. Dissolved oxygen (mg/L) measured in the lake water column at varying depth. Symbols denote median, error bars are minimum and maximum of n is 5 (28-Apr to 08-Jul), 4 (21-Jul to 11-Sep) and 2 (29-Sep to 20-Oct).

4. Discussion

The underlying hypothesis of the experiments was that by increasing the redox potential of the sediment top layer with an SMFC, phosphate would be eliminated from the lake water. This hypothesis was tested at lab scale and at lake scale.
At lab scale, phosphate concentrations were significantly reduced in all SMFC reactors at t = 134 d. No effect was observed from the presence of disconnected steel electrodes, as DCtrl differed little from the controls without electrodes (Figure 4). No additional effect was observed from addition of graphite slurry: cumulative ampere and P concentrations were similar for the reactors with graphite and steel electrodes (Figure 4).

Given that the volume ratio sediment to water was 3:2 in the laboratory reactors, and that indoor temperatures higher than those at the bottom of the lake stimulate microbial processes and thus the release of phosphate from sediment, the experiment can be considered worst-case. Nonetheless, we documented partial elimination of P from the water phase. Compared to the initial concentrations measured in the water column, the decrease of PO$_4$-P at day 134 was 85%, 75%, and 31% for the steel, graphite, and poised reactors, respectively, while for Ctrl and DCtrl, the PO$_4$-P increased by 43% and 30%.

4.2 Lake Scale To quantify the effects of the SMFCs, water samples were collected near the center of the anode. This sampling method would probably fail when bottom water is not stagnant and mixing with the surrounding water occurs. Mixing can be induced by heavy winds, in particular at times with non-established thermocline from autumn to spring. In this period, no difference in phosphate concentrations between controls and test fields could be found (Figure 6). However, in this period also oxygen was present at the bottom (Figure 7), and oxidation of the top sediment layer could have masked the effects of the electrodes. With warmer weather, the lake became stratified, which is reflected in the depth profile of DO (Figure 7): during summer (21 July to 11 September), stratification was evident and DO was low below 3 m depth. Concomitantly, the PO$_4$-P concentration normally decreases during winter and early spring in nutrient rich Danish lakes (Søndergaard et al. 2005). With increasing temperatures, faster decomposition of sedimented organic matter and a higher rate of diffusion from sediment to water column led to increasing release of PO$_4$-P (Jensen and Andersen 1992; Boström et al. 1982; Gomez et al. 1998; Søndergaard 2007). The same happened in Lake Søllerød, with a steady increase of PO$_4$-P concentrations from April to July 2020, stable at the end of July, while an algal bloom occurred, and
decline from August to October. This trend occurred both for controls and test fields, but with reduced PO$_4$-P at the test fields.

The electrical charge delivered by the electrodes, but not the release of P, was inversely linked to DO at the bottom of the water column. In periods with oxygenated hypolimnion, PO$_4$-P concentrations in control samples fell to low values, similar to those near the treatments at Field 1 and 2. This was both observed in autumn and winter 2019, and at the last measurement 20th October 2020. Hence, P immobilization continues when the bottom water becomes oxygenated, but since it was measured both for control site and test fields this must be contributed to the oxygenation and not to the effect of the electrodes. The electrodes become effective during summer and until early autumn, when the bottom water is low in oxygen (also seen from the electrical current, Figure 6). As oxygen depletion increased with depth, the SMFC Field 1 was relocated in May 2020 to deeper water (from 4 m to 7 m depth).

4.3 Mechanism

A plausible underlying mechanism for the decrease of P is that iron-oxides (e.g. FeOOH), as produced by the oxidation of ferrous iron at the anode, efficiently bind phosphate, thereby preventing/minimizing its efflux from sediment to water (Roden and Edmonds 1997; Hansen et al. 2003; Lehtoranta and Pitkän 2003). Under reducing conditions, such as the one expected in our untreated lake sediment (Jørgensen, Gabriel and Boock 2000), iron and sulfur are reduced to Fe$^{2+}$ and S$^{2-}$ and co-precipitate as FeS, with phosphate being released into the water. The accumulation of FeS minerals is visible from the black color of the sediment (Jensen and Andersen 1992). A change of color from black to grey, was visible by eye in the surroundings of some electrodes (< 1 mm distance), suggestive of net depletion of FeS minerals. The ability of SMFCs to decrease the amount of hydrogen sulfide (H$_2$S) produced in the sediment has been shown (Ryckelynck et al. 2005; Touch et al. 2017; Kubota et al. 2019; Algar et al 2020). Elimination of sulfide inhibits binding of iron as FeS and FeS$_2$ (Søndergaard 2007), thus increasing the pool of dissolved iron available for anodic oxidation. Inhibition of P efflux as observed in our experiment can thus be explained in the context of previous literature showing a shift of the redox state of iron that ultimately favor P trapping in iron(III) minerals. Cable bacteria, electrically conductive filamentous Desulfobulbaceae, may
produce analogous effect (i.e., increase the sediment redox potential and favor P immobilization via promoting FeS dissolution (Marzocchi et al. 2020; Sulu-Gambari et al. 2016). However, amplicon sequencing of the variable regions V3 and V4 of the bacterial 16S rRNA gene did not identify cable bacteria in sediment samples from neither laboratory nor lake installations (data unpublished), further supporting the link between SMFC treatments and observed decrease in phosphate.

4.4 Mass balance

Measured sediment TP concentrations were close to 1000 mg/kg, while P concentrations in lake water rarely reached 1 mg/L, most of the time much less (Figure 6). Hence, the top cm of sediment contains more P than the whole water column. It is thus meaningless to place the electrode deep within the sediment, even though this may lead to a higher yield of electrical charge, because release of P from the top sediment is sufficient to eutrophicate the whole lake. Regardless, a complete oxidation of sediment (thickness up to 1.5 m, (Jensen et al. 2009)) cannot be achieved in reasonable time periods. Moreover, upon decomposition of the sediment, all nutrients and pollutants stored over decades would be released, with possibly unfavorable outcome for water quality and lake ecosystem. The goal was therefore, to establish a barrier within the top of the sediment by creating an oxidized layer that prevents the escape of P from decomposition of organic matter buried in the sediment. Consequently, the anode was designed to be placed within and on top of the sediment. As new organic matter (from dead algae and detritus) settles over the year, the anode needs a certain thickness. This was achieved by creating a stacked electrode of three layers.

4.5 Microbial Turnover to Electrons (MTE)

The total amount of electrons produced in the laboratory steel and graphite reactors over the full experimental period was similar, about 3000 As, corresponding to 31.1 mmol e\(^{-}\). The peak power production of the lake electrodes was 10.5 mW (324 mV at 10 Ohm). This is comparable to earlier power achievements with freshwater SMFCs (De Schamphelaire et al. 2008), but not an efficient electrical
power source. Thus, the benefit of the lake SMFCs is not electricity production, but improvement of the lake environment. The electrode in Field 1 produced more than 100 000 A·s over its lifetime of 15 months, or 1.04 mol e⁻. Two mol electrons correspond to the reduction of one mol elemental S to H₂S, and four mol to the reduction of one mol C to CH₄. Thus, a calculated 518 mmol (18.7 g) H₂S were not formed.

Sulfide is very toxic to higher life, such as fish, and it binds iron, thus prohibits binding of P, which is the main reason for eutrophication. The electrons produced also correspond to 260 mmol methane (4.15 g CH₄). Calculated for the whole lake area (13 ha), 539 kg CH₄ could have been avoided. In fact, lake sediments are among the most important natural methane sources on a global scale (Bastviken et al. 2004). De Schamphelaire et al. (2008) list as other potentially beneficial effects of SMFCs the avoidance of toxic mercury and arsenic species formation. Moreover, the degradation of petroleum hydrocarbons by SMFCs has previously been documented (Marzocchi et al. 2020). In the lab scale reactors, we also observed a significant reduction of dissolved lead (Pb) in pore water and overlying water (mean 4.2 μg Pb/L in Ctrl and DCtrl, n = 10 to < 0.5 μg Pb/L in reactors with MFC, n = 30, significant at P < 0.05), while no significant difference was found for Cu, Zn and As.

4.6 Corrosion resistance and long-term stability

The anodes placed in the top of the lake sediment showed no sign of corrosion, while the cathodes were overgrown by periphyton with time. This reduced the performance of the whole system, as will be described later (Haxthausen 2021, in prep.). The steel electrodes were made of Stainless Steel 316, which has superior corrosion resistance (ESPI Metals 2020). The alloy contains 12% Ni and 17% Cr (and 0.45% P). No elevated levels of these heavy metals were observed in water or sediment treated with steel electrodes. However, slightly elevated levels of Cr were observed in sediments (though, not in water) treated at lab scale with electrodes poised by a constant potential of +400 mV vs. SHE. It may be concluded that the constant flow of electrons to keep the potential high supports the corrosion of Steel 316.

A drastic increase of Ni in sediment and water samples from microcosms treated with Ni-C coated electrodes was observed. These elevated levels of Ni obviously originate from the nickel. Ni can react
with phosphate and form insoluble nickel(II)phosphate (Ni$_3$(PO$_4$)$_2$) giving off a green color that was in fact visible. The constant and very low PO$_4$-P concentration observed in the Ni-C treated reactor is hence very likely caused by Ni dissolution from the electrode. The concentration of TFe in both water column and pore water in the Ni-C reactors exceeded that of all other treatments, further indicating that the decrease in PO$_4$-P was not exclusively related to precipitation with Fe (Figure 5). The high toxicity of nickel to aquatic organisms (predicted environmental no effect concentration PNEC is at 7.1 μg/L (ECHA 2020), while the concentration of Ni in the headspace water was 40 mg/L) prevents the use of this type of Ni-C coated electrodes in open freshwater ecosystems.

In the lab reactors with steel and graphite electrodes, the measured working potential became instable after day 170 and showed cycling values (Figure 4). This instability may have been caused by fouling of the electrodes or by a malfunctioning of the membrane between anode and cathode chamber. An increase of PO$_4$-P in these reactors was observed at the final measurement, accompanied by a decrease in the NO$_3$-N concentration (not shown). This may indicate that the redox potential in the anode chamber decreased (became more negative), and nitrate became a favorable electron acceptor. Nitrate creates a redox potential high enough to sustain iron in an oxidized form and thus impacts the redox-dependent retention of phosphorus (McAuliffe et al. 1998; Duras & Hejzlar 2001, Jensen and Andersen 1992). A concomitant increase in NH$_3$ was also observed (data not shown), likely related to oxidation of organic matter. Some effect can be ascribed to the increase in temperature (day 217 corresponds to 7th of July 2020), since the concentration of PO$_4$-P and NH$_3$ also increased in the poised reactors.

4.7 Comparison to other findings

A similar effect of SMFC on phosphate and iron concentrations has been observed in lab-scale marine systems using carbon cloth as anode material (Touch et al. 2017). In that study, the decrease in pH led to release of Fe$^{3+}$ and concomitant removal of phosphate. In a laboratory study, Yang et al. (2016) observed binding of phosphate spiked to the water phase from overlying water of a SMFC containing lake sediment. Within 30 days, concentration of P in the water column fell from 0.1 mg/L to 0.01 mg/L in the SMFC-treated system, but remained almost constant in the control. Different from our study, the authors
used open 2 L glass beakers without membrane. A similar set-up with the Søllerød lake sediment in our laboratories was unsuccessful because the sediment top layer was oxidized and P was bound to sediment both in controls and SMFC-treated replicates (Nicolaisen 2018).

Previous research conducted in 15 Danish lakes showed that when the sediment TFe:TP (w/w) ratio exceeds 15, phosphate release from the sediment under oxidized conditions is low (Jensen et al. 1992; Søndergaard 2007). The measured ratio for the sediment used in the SMFC reactors was 9058 mg TFe/kg dw to 966 mg TP/kg dw, or 9.4 to 1, hence below this threshold. However, there were also 4888 mg Al/kg dw present, which also can adsorb P (Hansen et al. 2003). In earlier studies of Lake Søllerød, Jensen et al. (2009) found a less favorable ratio of TFe:TP ≤ 7. Restoration of the lake by addition of aluminum salts Al(OH)$_3$ for P co-precipitation was discussed, but dismissed due to the occasionally rather high pH levels, which can effect dissolution of aluminum salts and Al toxicity in the lake ecosystem (Jensen et al. 2009, Gensemer and Playle 1999).

4.8 Safety

The Ni-C electrodes, which released large amounts of nickel, and the poised electrodes that showed a slight increase of Cr in the surrounding sediment, were not applied at lake scale. There were no complaints from the neighborhood (contrary, persons living at the lake showed great interest and supported the project), nor from authorities. Under-water movies made periodically document that the local fish (mostly perch) were attracted to the cathode and were frequently observed swarming it in big numbers. With our current state of knowledge, we deem the electrode technique safe.

5. Conclusions and Outlook

Immobilization of phosphate in lakes is feasible using sediment microbial fuel cells. This was documented both in laboratory microcosms and field experiments at Lake Søllerød in Denmark. The SMFCs were constructed from corrosion-resistant steel, which is easy to work with, widely available, low cost and a robust material. During the lake experiment over two seasons, little maintenance was needed. Although
the electricity produced by 16 m² of electrodes is low (10 mW), the environmental effects are more significant. Apart from reduced release of P from sediments, also methane and sulfide reduction, degradation of petroleum products and other organic pollutants and precipitation of some heavy metals seem feasible. The findings presented here thus open the way to novel and promising methods for sediment and lake restoration.

The study was designed as proof of concept. To upscale to a full-scale lake remediation, the kinetics of phosphate binding to sediments by SMFCs needs to be studied, in order to calculate the electrode area required for a satisfying P reduction. Other topics not addressed in this study include methane elimination, effects on the microbial community, lake sediment redox changes, nitrogen speciation, and also optimal design and construction of SMFCs.

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Figure 1. Postulated reactions. Mineralization of organic phosphorous deposited from the water to the sediment produces phosphate (PO$_4^{3-}$). In oxic sediments matter, iron-oxides are present and bind phosphate. In anoxic sediments (dark brown, bottom), Fe-oxides are reduced liberating Fe$^{2+}$, which in turn, precipitates with sulfide (S$^{2-}$) as iron-sulfur minerals (FeS/FeS$_2$). This reduces the binding capacity of the sediment towards phosphate, that may be then released to the water. By providing an alternative electron sink, the anode of a SMFC inhibits the accumulation of S$^{2-}$ and favors the reoxidation of Fe$^{2+}$ to Fe-oxides thereby maintaining an efficient binding mechanism for phosphate in the sediment (shown in red shading).
Figure 2. Laboratory microcosms (reactors). Top: operating microcosm containing lake water in the cathode chamber (left), and lake sediment and water in the anode chamber (right). Cathode and anode were made of stainless steel, folded to extend throughout their respective chambers. Bottom: external components of the microcosm. Elements left to right: wing nuts, tensioning rods, disk washers, end plate, O-ring, cathode chamber and stoppers, O-ring, interface plate, gasket, membrane, gasket, interface plate, O-ring, anode chamber and stoppers, end plate, washers, wingnuts.
Figure 3. Installation of electrodes in the lake. Anode was folded (A), electrodes were installed in frames and towed in position (B). Electrodes were sunk. Cathode was suspended between surface buoys and the anode on the bottom (C), performance was monitored by measuring working potential on shore (D).
Figure 4. Results from lab scale experiment. Top: open circuit potential (OCP, mV, right axis), middle: working potentials (mV, right axis, mean of two replicates), below: dissolved phosphate in the water column \( \text{PO}_4^3- \) (μg/L, left axis, error bars denote minimum and maximum, number of replicates \( n \) is 2 to 4) at initial (day 0), half-way (day 134) and the end (day 217). Electrode materials: stainless steel (Steel), stainless steel with addition of graphite particles (Graphite), stainless steel coated with carbon embedded with nickel particles (Ni-C), stainless steel with external voltage applied (Poised), no electrode (Ctrl), disconnected steel electrodes (DCtrl).
Figure 5. Total phosphorus (TP, mg/L, left axis) and total iron (TFe, μg/L, right axis) in sediment pore water and in the water column of microcosms with and without sediment microbial fuel cells, at the termination of the lab scale experiments on day 217. Error bars denote minimum to maximum, n is 2 to 6.

Electrode materials: stainless steel (Steel), stainless steel with addition of graphite particles (Graphite), stainless steel coated with carbon embedded with nickel particles (Ni-C), stainless steel with external voltage applied (Poised), no electrode (Ctrl), disconnected steel electrodes (DCtrl).
Figure 6. Lake scale sediment microbial fuel cell performance. Cumulative ampere (A \cdot s) generated by the electrodes of Field 1 and Field 2, and PO$_4$-P concentrations (µg/L) measured at the bottom of the lake water column above the anodes and at a control site. Error bars denote standard deviation. The broken x-axis between winter and spring corresponds to the time the cable to the anode in Field 1 was broken.
Figure 7. Dissolved oxygen versus depth. Dissolved oxygen (mg/L) measured in the lake water column, within the SMFC fields and control area (Field 2 only from summer 2020). Green color is sampling in spring, yellow in summer, red in autumn and blue in winter.