# Soil Redox Potential and pH Controllers

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**Abstract**
Rapid changes in redox potential (Eh) and pH occur in wetland environments. Due to frequent hydrologic fluctuations, wetland soils and sediments can have Eh values ranging from 700 mV (under drainage conditions) to −300 mV (under prolonged flooding conditions). Redox potential is probably the best available simple indicator of the oxidation–reduction status of a system, and it covers the entire range across which various inorganic redox systems function. Therefore, determining soil redox potential and pH can significantly improve our understanding in wetland biogeochemistry. One experimental approach is to determine and to closely control the redox potential and pH at which various redox couples function. A redox potential–pH controller can be designed and constructed differently to fit individual research purposes and budgets. In this chapter, we will introduce basic components of the controller, provide some modification options, and present a fully automated system for biogeochemistry research.

**Abbreviations:** ORP, oxidation–reduction potential.

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Typically, a redox potential–pH controller involves an incubation of stirred soil or sediment suspension where its redox potential and pH can be closely controlled at any point in the range of study. The soil incubation device was introduced several decades ago (Patrick, 1966; Patrick et al., 1973). Since then it has been named a *soil microcosm* because the incubated soil represents a miniature of the soil system in nature. A schematic showing its basic components and a photograph of the actual controller are presented in Fig. 7-1.

In this soil redox potential–pH controller (or soil microcosm), the soil suspension can be made by mixing soil and water in a certain ratio to form a soil slurry. For mineral soils, a soil/water ratio of 1:4 is appropriate, and for organic soils the ratio can be as low as 1:10. For example, in a 2300-mL Erlenmeyer flask, a soil suspension can be made by mixing 400 g of air-dried mineral soil with 1600 mL of deionized water (1:4 soil/water ratio). The soil suspension can be maintained homogenous by continuous stirring using a magnetic stirrer at bottom of the flask. The controller can be maintained airtight by a rubber stopper with various openings for inserting electrodes, probes, and tubing (Fig. 7-1). A gas inlet and outlet are installed to purge the incubation system when needed. Soil temperature can be monitored by a thermometer, and soil pH can be monitored by a combination pH electrode connected to a pH meter. The soil Eh can be measured by a commercially available combination oxidation–reduction potential (ORP) electrode or by a homemade Pt electrode and a calomel reference electrode that are connected to a millivolt meter. For accuracy purposes, two Pt electrodes are commonly used for each soil suspension. Construction details of the Pt electrode are available in Megonigle and Rabenhorst (2013) and Faulkner et al. (1989). The flasks should be wrapped with aluminum foil or other appropriate material to protect them against light, preventing algae growth and photooxidation reactions.

## REDOX POTENTIAL AND pH CONTROL

To study the effect of redox potential and pH on various biogeochemical processes, the soil microcosm needs to be controlled at certain Eh and pH values for a period of time during the incubation. Redox potential and pH control can be done simultaneously or separately for the same soil suspension, depending on the need of the experiment.

The redox potential of the soil microcosm system can be controlled by two approaches. One approach is to place the soil in the flask, flood it, and use the control system to prevent the redox potential from falling below a set value. The other approach is to allow the soil to be flooded for a prolong period of time in order to become fully reduced. After that, the preincubated reduced soil is placed in the microcosm flask, and the controller is used to raise the redox potential to any selected value that is higher than the redox potential of the preincubated soil.

A meter relay is needed to activate the redox potential control. In the system in Fig. 7-1, a bench pH–ORP controller (Industrial & Chemical Measurement) with an internal relay is used. The soil redox potential can be maintained within a certain range by adding either air (with $O_2$ to raise the Eh) or inert gases such as

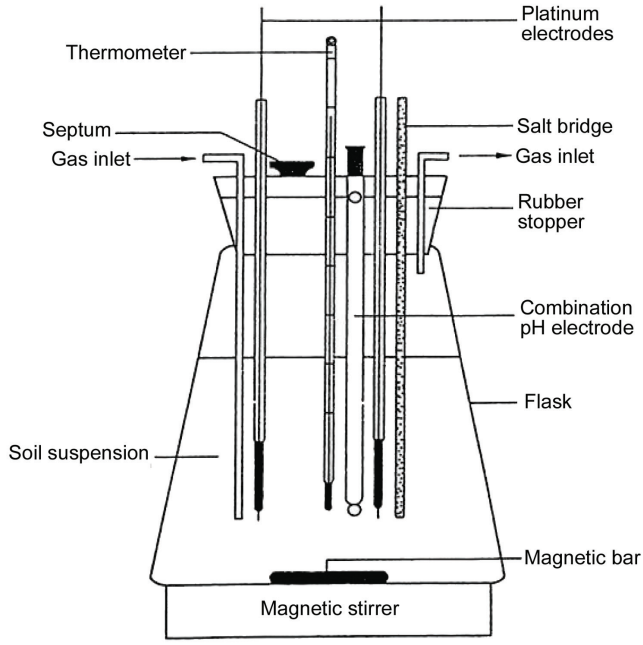


**Fig. 7-1.** Basic components of a redox potential–pH controller (top) and its photograph (bottom) (modified from Yu and Rinklebe, 2011).

$N_2$, Ar, or He (to lower the Eh) through an automatic gas pump regulation system. The control system uses $O_2$ or air to prevent the redox potential from decreasing below the set value. Normally, reduction processes in the flooded soil cause the potential to decrease, especially for wetland soils and sediments that are rich in

organic substances. When the set redox potential is reached, the solenoid value is activated by the meter relay, allowing $O_2$ to enter the soil. The flow rate of air should be controlled in such a way that a minimum amount of air is pumped into the soil suspension. To control the redox potential at lower values, a lower air flow rate should be used rather than a relatively greater air flow rate for control at higher Eh values. Air is usually adequate for controlling the redox potential. For obtaining low redox potentials, sometimes $N_2$ containing a low amount of $O_2$ (0.5–2%) will provide sufficient $O_2$ to control the redox potential without excess $O_2$. Too much $O_2$ will cause a too large and rapid increase in the redox potential. For most applications, adequate aeration to control the redox potential can be maintained by having the meter relay activate an aquarium pump to provide air to the soil suspension. At low redox potentials, the air flow should be very low, no more than approximately one bubble per second. The redox potential can be controlled within ±5 mV for long incubation periods. Two Pt electrodes are usually used so that a check can be maintained on variations in readings, or the two electrodes can be wired together to yield an average potential. Adding $O_2$ to increase Eh is a common practice because most biological and chemical processes tend to decrease the redox potential. In a rare case with a system where the redox potential tends to increase, redox potential control can be obtained using $H_2$–$N_2$ mixtures.

It is assumed that introducing $O_2$ to control the redox potential has minimal effects on the biological and chemical processes in the system. If the effect of $O_2$ is of concern, adding appropriate amounts of alternate electron acceptors can be used to reach the desired redox potentials (McLatchey and Reddy, 1998). For example, redox potential values of a soil suspension can be buffered at about 200 mV by frequent additions of $NO_3^-$ as an electron acceptor. Similarly, redox potential values can be buffered at −100 mV using $SO_4^{2-}$ as an electron acceptor. This method has limited applications to certain redox potential values because of the limitation of such alternate electron acceptors.

A similar system can be used for controlling the pH in the soil suspension. In this case, a pH electrode, instead of a Pt electrode, serves as the sensor. Acid or alkali (usually 0.5 mol $L^{-1}$ HCl or 0.5 mol $L^{-1}$ NaOH) is added manually through the serum cap (Fig. 7-1, or automatically to maintain the pH at the designated values. A very slow flow of acid or alkali from a burette or other container can be controlled using a solenoid valve. Control of the pH within ±0.05 pH unit or less can be easily maintained for long incubation periods. Situations will seldom develop where both acid and alkali will be needed during the incubation at a given pH. The system can be designed so that the amount of acid or alkali required is measured.

## MODIFICATIONS

Several modifications can be made to improve or to correct some problems in the basic setup of the redox potential–pH controller. These will increase the expense in constructing a new controller. The potential problems encountered during applications of the soil microcosm are discussed, and some remediation is suggested here.

1. In the basic setup of the controller, a rubber stopper is used to make the system airtight (Fig. 7-1). The rubber stopper also serves as a holder for electrodes, a salt bridge, and tubing for gas and liquid exchange. The size of the openings in the rubber stopper should be carefully determined. Leaking can result if they are too big, and it can be very difficult to insert the electrodes and tubing if they are too small. Even if the openings are correctly selected, it normally requires that the electrodes and tubing be moistened with water and carefully inserted to avoid breaking them or cutting the operator's hand. Replacing the Pt electrode and salt bridge tubing is occasionally needed, however. In practice, it is even harder to remove them from the rubber stopper than to insert them. Completely soaking the rubber stopper in water for hours is commonly needed to successfully remove the electrodes and tubing. One solution to avoid this problem is to use a wide-mouth bottle or a straight-sided bottle (2 or 4 L). A screw cap can be used for the bottle, instead of a rubber stopper, and the bottle can be sealed with Teflon tape. Various openings can be prepared in the cap. The electrodes and tubing can be inserted through a Nylon buck-head compression fitting union and can be sealed by tightening the screw cap of the union. This can make replacing the electrodes and tubing much easier, by simply loosening the screw cap of the union. The fitting unions can be sealed to the cap of the incubation bottle by a silicon rubber washer or an O-ring.
2. A magnetic stirrer bar is used inside the incubation flask to continuously agitate the soil suspension to make the system homogenous (Fig. 7-1). It can be difficult to keep the stirrer bar rotating properly during the study period, especially for clay-rich soils and sediments. The magnetic stirring system works perfectly with water solutions, but problems might occur frequently with soil slurries due to the higher density of the soil. Moreover, the material of which the stirring bar is made can be released into the incubation flask after long-term operation, causing potential contamination to the sample. It is suggested, when possible, to replace the magnetic stirring system with an overhead stirrer (e.g., IKA RW 16 basic). The overhead stirrer consists of a stirrer motor connected to a stainless steel stirrer shaft and blade. A solid Teflon stirrer bearing can be used to get the stirrer shaft through the cap of the wide-mouth bottle and into the soil suspension. No lubricant is needed when the stirrer is in motion because Teflon has little friction with stainless steel. The stirrer bearing can be tightened slightly to maintain a better seal, which is adequate for a soil microcosm system with a slow stirring speed. It is worth mentioning that such mechanical bearings are not perfectly airtight; however, perfect seals are normally not necessary because the controller system is slightly overpressured inside the incubation flask by purging with an inert gas, which prevents ambient air from getting into the system as well as removes the produced gases from the headspace. If the controller needs to be used in a lower than ambient atmospheric pressure (slight vacuum), magnetic coupled bearings need to be used to make a prefect seal. Various magnetic coupled bearings are commercially available but they are usually very expensive.
3. The pH–ORP controller used in Fig. 7-1 has no datalogging function. Reading of the redox potential and pH must be done manually by the operator. In this case, it is impossible to capture the dynamic changes in the redox potential and pH during the entire incubation, such as at night. Installing a datalogger for automatic monitoring of soil temperature,

pH, and redox potential will significantly improve the frequency of data acquisition to prevent any data gaps. For example, the inexpensive Sper Scientific 850059 datalogging pH meter can memorize up to 4000 records at steps between 1 s and 2 h. Resolution of at least 5 min is suggested because the datalogger may produce some errors when reading in seconds. The pH electrode is combined with a temperature probe for this datalogger. All data can be downloaded to a computer as a text file by a serial cable.

4. Both redox potential and pH measurement are temperature sensitive. The thermometer used in the soil microcosm is for calibration of the temperature compensation for the redox potential and pH measurements. The microcosm (Fig. 7-1) has no temperature control mechanism designed to study the effect of temperature on the biological and chemical processes in the soils and sediments. A heating blanket surrounding the incubation flask can be used to increase the temperature of the incubation. A heating plate placed at the bottom of flask is another option if an overhead stirrer is used. Temperature control below ambient temperature can be a challenge. A double-hull incubation vessel (KGW-Isotherm) can be used for temperature control of the incubation. Water at the desired temperature can be pumped between the two layers of the vessel to maintain temperature control of the incubation (Fig. 7-2). Various refrigerating and heating water circulators are commercially available with a temperature range between −20 and 80°C. This double-hull incubation vessel is used in the following example of a fully automated system.

## AN AUTOMATED BIOGEOCHEMICAL MICROCOSM SYSTEM

Recently, an automated biogeochemical microcosm system has been developed (Fig. 7-3). Such an automated microcosm setup has several advantages, such as a high frequency of measurements, labor and time savings, and simultaneous control of multiple microcosm systems. Redox conditions are reproducible and

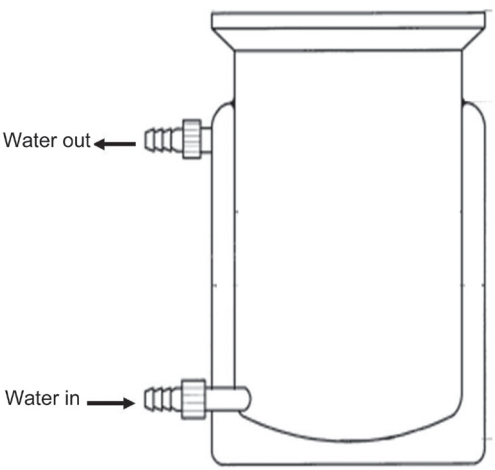


**Fig. 7-2.** Heatproof flat-bottomed glass reaction vessel with thermal jacket.

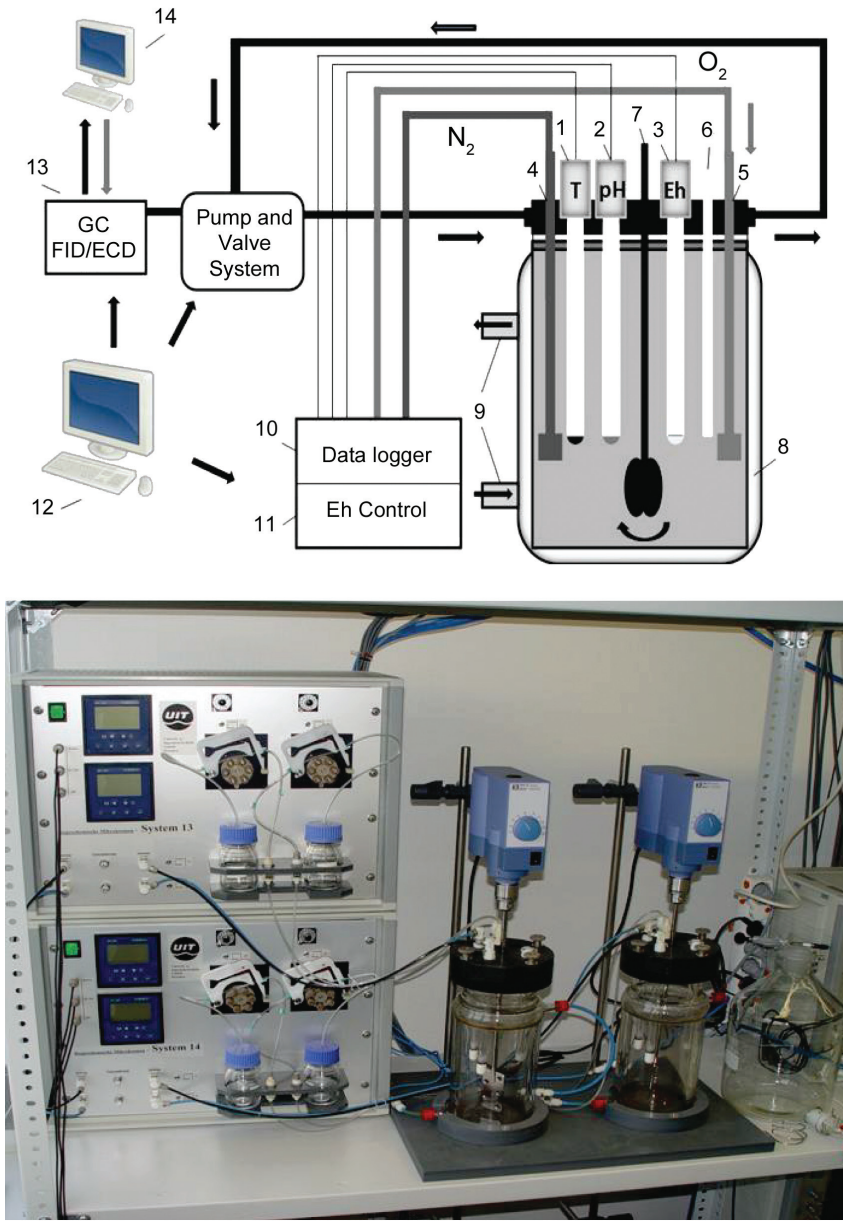


**Fig. 7-3.** Schematic of an automated redox potential–pH controller with temperature control and gas analysis system (top) and its photograph (bottom). Components of the soil microcosm system are: thermometer (1), pH electrode (2), redox potential (Eh) electrode (3), dispersion tube for $N_2$ (4), dispersion tube for $O_2$ (5), sampling tube (6), overhead stirrer (7), double-hull incubation vessel (8), temperature control by a thermostat and water circulation (9), datalogger for Eh, pH, and temperature (10), automatic redox regulation by $N_2$ and $O_2$ valves (11), control computer for datalogger, pump, and valve system (gas sampling) and gas chromatograph (start signal) (12), gas chromatograph (GC) with flame ionization detector/electron capture detector for trace gas measurements ($CO_2$, $CH_4$, and $N_2O$) (13), and computer for GC control and GC data storage (14) (modified from Yu and Rinklebe, 2011).

defined and can be changed rapidly in predefined Eh windows. Furthermore, the effect of Eh can be studied nearly independently from other parameters.

Each microcosm consists of a double-hull glass vessel (for temperature control) with a volume of 2.88 L, which can be hermetically sealed with an airtight lid. It is equipped with an overhead stirrer, a Pt electrode with a Ag/AgCl reference electrode (EMC 33), a pH electrode (EGA 153), and a temperature electrode (Pt 100) (all from Meinsberger Elektroden). These sensors allow measurements of Eh, pH, and temperature at a very high temporal resolution. Data collected by the sensors can be reported by a computer via a datalogger (for instance LogTrans 16-GPRS, UIT). The measurements can be recorded between 1 min and several hours; however, 10-min measurements are practical for many purposes.

The described system is equipped with an automatic-valve gas regulation system that allows automatic control of the Eh by adding $N_2$ (to lower the Eh) or $O_2$ (to increase the Eh). A certain number of independent biogeochemical microcosms (e.g., two, three, or four) might be used as replications to record variations, which also allows the use of statistical tests and analyses. To simulate flooding and conduct experiments, each microcosm can be filled with 200 g of air-dried soil and 1600 mL of deionized water. Depending on the question that is being studied, however, natural water can also be used. In this case, the properties (such as concentrations of the included substances) of the natural water should be carefully measured before the experiment.

For this setup, a soil/water ratio of 1:8 is selected; however, different soil/water ratios (such as 1:4 or 1:10 as described above) can also be used. The achieved slurry should be continuously stirred to reach homogeneous conditions. Redox potential, pH, and temperature in each microcosm can be automatically monitored at a very high temporal resolution. The measured redox potential values will commonly be normalized to pH 7 or alternatively to pH 5 to allow comparison among different experiments.

Experiments can be run in both directions, from reducing to oxidizing conditions or vice versa. When conducting an experiment from oxidizing to reducing conditions, it can be helpful to add an appropriate source of organic matter such as straw or glucose (for this setup, 5 g is recommend per microcosm) to provide an additional C source for microorganisms. This addition can be repeated after some days. Levels of Eh can be decreased further by continuously flushing the microcosms with $N_2$ for a certain period (e.g., 5–7 d). Redox potential can be increased stepwise by adding $O_2$. The steps can be chosen in consideration of the aim of the study. The redox potential can usually be kept within Eh windows of approximately 30 to 40 mV around the set Eh values by the automated supply of $O_2$ or $N_2$. The system also has an automated gas sampling and analysis system (Yu et al., 2007).

## APPLICATIONS

The application of a soil redox potential–pH controller has significantly contributed to our knowledge of wetland biogeochemistry. Wetland soils and sediments are

characterized by high organic matter (electron donor) and high reduction intensity (low Eh). The wide use of Eh is due to its ease of measurement and determination of the degree of anaerobicity, which can reasonably predict the stability of various compounds of biological and chemical interest. In general, the application involves the following three techniques.

### Soil Solution and Soil Suspension Samples

For soil solutions and soil suspensions under certain redox potential and pH conditions, samples can be taken using a syringe through the rubber septum without contact with the ambient air (Fig. 7-1) or through the sampling tube (Fig. 7-3). This operation is commonly used when studying the concentrations of redox-sensitive substances such as Fe, Mn, $NO_3^-$, $SO_4^{2-}$, dissolved organic C, and other metals. Soil suspension samples need to be taken while the stirrer is still in motion to maintain the soil slurry in homogeneity. This will ensure that the soil/water ratio remains the same during the experiment. Soil solution samples can be taken in two ways. The first option is to take the soil suspension as above. The sample is then immediately centrifuged for 15 min at 3000 rpm and the supernatant filtered under $N_2$ atmosphere through a 0.45-μm Millipore membrane (Whatman). The filtrate is then defined as the soluble fraction of the sample and can thereafter be divided into different subsamples for subsequent analysis. The second option is to stop the stirrer and gas influx and let the soil suspension gradually settle down without agitating. The clear soil solution at the top of the liquid phase can then be taken using a syringe. The soil solution samples still need to be filtered for future analysis.

### Gas Samples

Gas samples in the headspace of the flask can be taken using a syringe through the rubber septum (Fig. 7-1) or through the sampling tube (Fig. 7-3). The headspace is the gas phase above the soil suspension inside the incubation flask. When it is time for a gas flux measurement, the inert gas flow to purge the flask needs to be stopped to ensure a closed system. A series of gas samples can be taken at certain time intervals (e.g., 10 min). Gas samples can be analyzed to determine the headspace concentration. Minimally three samples are taken for later linear regression of the gas concentration change with time to determine the gas flux rate. If the gas concentrations are increasing with time, the gas flux rate will be positive, indicating gas emission. If the gas concentrations are decreasing with time, the gas flux rate will be negative, and gas consumption is occurring.

### Vegetation Growth

Wetland plants can be cultivated in a redox potential–pH controller to study the effects of Eh and pH on vegetation growth or the impact of plants on the soil environment (e.g., transport of $O_2$ from the atmosphere into the soil). This will need some modification of the controller as described by Reddy et al. (1976). Briefly, a desiccator (160-mm i.d.) is used as the incubation vessel and an acrylic plastic plate is used as the cap for the vessel. The plate is glued to the desiccator to

make the system airtight. In addition to the openings needed for redox potential and pH monitoring and control (Fig. 7-1), extra openings need to be made in the plate for the vegetation. The desiccator functions as a container for the soil, water, and plant roots, while the plant shoots grow above the acrylic plate. It is worth mentioning that this is technically not an airtight system because it is well known that wetland plants can serve as a conduit between the atmosphere and soil.

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