EFFECT OF PHOTOSYNTHETIC BIOFILMS ON THE OPEN-CIRCUIT POTENTIAL OF STAINLESS STEEL

N.J.E. DOWLING

UNIREC, B.P. 50, 42702 Firminy Cedex, France

J. GUEZENNEC[1,2], J. BULLEN[1], B. LITTLE[3]

AND D.C. WHITE[1]

1. Institute for Applied Microbiology, Bldg. I, Suite 300, 10515 Research Drive, Knoxville, TN 37932 USA.
2. Institute Francais de Recherche et l'Exploitation de la Mer (IFREMER), Centre de Brest, BP 70, 29263 Plouzane, France.
3. Naval Oceanographic and Atmospheric Research Laboratory, Code 333, Stennis Space Center, MS 39529-5004 USA.

ABSTRACT

Periodic illumination of photosynthetic biofilms on AISI* 316L stainless steel resulted in evolution of oxygen (1–7 mg L^{-1}) and a corresponding increase in open circuit potential (E_{corr}) from 2 to 15 mV. The change in E_{corr} depended on the interval of illumination. When the dark cycle began, elevation in potential was followed by an immediate drop. Illumination did not affect E_{corr} in sterile systems or in systems that contained only nonphotosynthetic eubacteria. Radiated heat from illumination accounted for changes of 4 to 5 °C in temperature which, in the absence of oxygen production, should decrease dissolved oxygen by 0.75 mg L^{-1} and decrease E_{corr} by 1 mV. Positive shifts of E_{corr} induced by periodic illumination of photosynthetic biofilms is primarily the result of oxygen production.

*American Iron and Steel Institute

INTRODUCTION

Ennoblement of E_{corr} for stainless steels as a result of biofilm formation in natural seawater has been documented in the Mediterranean Sea (Scotto et al., 1985), the Norwegian Sea (Holthe et al., 1988) and the Delaware Bay (Dexter and Lin, 1988). Shifts in E_{corr} as high as +0.350 to +0.400 V have been reported in the presence of marine microflora. Susceptibility of stainless steels to localized corrosion is often predicted by the relationship of E_{corr} to pitting potential (E_{pit}). E_{pit} is defined as the potential above which pits can initiate and grow. The dependence of E_{pit} for stainless steel on the activity of the chloride ion a_{Cl^-} is as follows:

$$E_{pit} = a - b \log a_{Cl^-}, \quad (1)$$

where a and b are experimentally determined parameters (Uhlig and Revie, 1985). Ennoblement forces E_{corr} values closer to the value of E_{pit} and increases the probability that pitting will occur. Corrosion rate of stainless steel is independent of E_{corr} as long as the surface is in the passive state. Once localized corrosion is initiated, E_{corr} moves to more negative values.

Attempts to demonstrate that microbial respiration contributes to the ennoblement of E_{corr} used a selective biological respiratory inhibitor, sodium azide. After a marine biofilm formed on a stainless steel surface and E_{corr} became ennobled, the addition of sodium azide forced an immediate drop of E_{corr} to initial (abiotic) values (Scotto et al., 1985, 1986). Little et al. (1989, 1991) observed a slight positive shift of E_{corr} for stainless steels covered with microalgae and a shift in the negative direction when the metal/biofilm interface was anaerobic.

If high E_{corr} values for stainless steels are controlled by a subset of the total microbiota, such as microalgae, then light levels must influence E_{corr}. Edyvean and Terry (1983) examined oscillations of pH with respect to true day length and observed changes of 2 pH units under cultures of *Enteromorpha*. The process of photosynthesis removes carbon dioxide from water resulting in higher pH during periods of illumination. During dark periods, increasing carbon dioxide concentrations cause lower pH values. Analyses of frequency dependence for E_{corr} should reveal a diurnal oscillation during field exposures coinciding with fluctuations of natural light. In this paper a test system is described that demonstrates the effect of light on E_{corr} for a stainless steel colonized with photosynthetic organisms.

MATERIALS AND METHODS

Microbiological Cultures and Medium

A marine cyanobacterial strain, *Anabena* #33047, was obtained from the American Type Culture Collection (ATCC, Rockville, MD). *Anabena*, originally isolated from an agal mat in the Gulf of Mexico, is a photosynthetic, coccoidal, blue-green alga that forms chains, fixes nitrogen, and produces hydrogen. Maintenance conditions were those specified by ATCC. *Delaya marina* #25374, a marine, nonphotosynthetic eubacterium, was also obtained from ATCC. *Delaya marina*, formerly classified as *Arthrobacter marinus*, is a heterotrophic oxidase-positive, gram-negative organism.

The electrolyte/medium used in the exposure vessels was designed for maintenance of cyanobacteria and contained (g L^{-1}): Instant Ocean™ (Aquarium Systems, Mentor, OH) artificial seasalts, 22.5; $MgSO_4 \cdot 7H_2O$, 0.12; $CaCl_2 \cdot 2H_2$, 0.02; $K_2HPO_4 \cdot 3H_2O$, 0.02; citric acid, 0.003; ferrous ammonium citrate, 0.003; EDTA, 0.0015;

Na_2CO_3, 0.02; and trace elements, 1.0 mL. Trace elements contained (g L^{-1}) H_3B0_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO4 \cdot 2H_2O$, 0.039; $CuSO_4 \cdot 5H_2O$, 0.079; and $Co(NO_3)_2 \cdot 6H_2O$, 0.049. Final pH was adjusted to 8.5 with 2M NaOH prior to contact with the electrochemical cells.

Electrochemical and Temperature Measurements

Electrochemical measurements were made using a Schlumberger (Farnborough, England) digital voltmeter (model 7081) controlled with a personal computer. A program was written for Lotus 1-2-3 with an add-on module, Lotus-Measure (National Instruments, Austin, TX). Temperature measurements were made with resistance thermocouple probes purchased from Omega (Stamford, CT). Dissolved oxygen was measured with a mini DO probe (tip diameter approximately 3 mm) purchased from Microelectrodes Inc. (Londonderry, CT). The DO probe was calibrated, sterilized in 4% glutaraldehyde, and washed in sterile distilled water before insertion into the top of the electrochemical cell. The tip of the DO probe was positioned immediately adjacent to, and sometimes touching, the biofilm on the stainless steel electrodes.

Test System

The electrochemical cell had an operating volume of 500 mL and was fitted with a ground glass lip to which a polypropylene lid was bolted with a recessed rubber O-ring for a gas-tight seal. Holes were drilled in the lid for a medium inlet, a vent filter, a dissolved oxygen probe, two stainless steel electrode connections sealed in glass, a salt bridge fitted with a Vycor frit, and a rubber stopper from which a thermocouple was suspended. All connections were made with neoprene rubber stoppers. Electrodes were cut from AISI 316L sheet (17% Cr, 12% Ni, and 2.1% Mo).

Assembled vessels were sterilized with ethylene oxide gas. Two "Gro-lux" incandescent 20W bulbs were placed immediately on either side of the reaction vessels to illuminate the system. A mechanical timer controlled power to the lights. Light/dark cycles were established at 12-, 6-, and 0.25-h intervals. Reaction vessels were shielded with aluminum foil to limit illumination from laboratory lights. Reaction vessel tops were left uncovered by aluminum foil to allow radiation of heat generated by the lights. The autoclaved medium was aseptically pumped into the vessels and inoculated with 1 mL log phase culture of *Delaya marina* and 30 ml of a 2-week culture of *Anabena* sp.

RESULTS

E_{corr} measurements were collected from electrodes colonized with a mixed culture of *Anabena* sp. and *Delaya marina*, pure cultures of *Delaya marina* and sterile controls. Cell densities and relative proportions of each species in the mixed cultures were not determined. Attempts to grow *Anabena* without *Delaya marina* were unsuccessful. Light/dark cycles of 12-, 6-, and 0.25-h required sampling intervals of 30, 15, and 1 min, respectively, to establish sensitivity to the frequency of oscillation. During the 12-h light periods E_{corr} increased approximately 10 to 15 mV before dropping the same amount at the onset of the dark period (Figure 1). Coupons covered with *Anabena/ Delaya* biofilms achieved an E_{corr} of approximately –0.325 V/SCE* after 7 days, whereas E_{corr} values in the sterile system approached –0.210 V/SCE. The overall negative trend of E_{corr} in the presence of the mixed culture cannot be explained with the data presented in this paper. When cyanobacteria/bacterial films colonized the

*SCE is saturated calomel electrode

stainless steel surface, periodic oscillations in E_{corr} were observed as a function of illumination, while no periodic oscillation was detected in either sterile systems or coupons colonized with *Delaya marina* alone.

Temperature measurements (Fig. 2) indicated that direct illumination of unshielded reaction vessels increased the temperature of the medium by 4.5 °C. The temperature in shielded vessels increased 2 to 3 °C. Directly illuminated vessels had a median temperature of 32 °C, and shielded vessels had a median temperature of 27 °C. Shielded vessels did not provide sufficient light for the growth of *Anabena* sp.. The effect of temperature differences between directly illuminated and shielded vessels on DO was not determined. *Anabena* sp. induced a net increase in dissolved oxygen rather than the net decrease predicted for purely physical processes related to temperature increases.

The effects of sudden light/dark fluctuations at 6 h (Fig. 3) and 0.25 h (Fig. 4) on DO and E_{corr} in the presence of a photosynthetic biofilm were similar to those observed for the 12-h cycle. A short-term increase of 10 to 15 mV, measured during illumination, was followed by an equally fast drop during the dark period. Concomitant increases in DO from 1 to 7 mg L^{-1} were measured during illumination. Very sharp increases in DO were observed with little lag time after illumination. A light/dark cycle of 0.25 h combined with sampling every minute (Fig. 4) showed that DO increased from approximately 4 to 5.2 mg L^{-1}. Corresponding E_{corr} measurements oscillated between −0.242 V/SCE and −0.232 V/SCE. An increase in DO was accompanied by a slight shift of E_{corr} in the positive direction.

DISCUSSION

All wet, illuminated surfaces support the growth of a biofilm that includes an algal population (Cooksey and Cooksey, 1986). The distribution of algae in biofilms in the North Sea has been documented. Algae have been found to a maximum depth of −40 m on installations in the central and northern North Sea and to −5 m in the southern sector (Terry and Edyvean, 1986). The distribution of photosynthetic bacteria in biofilms has not been documented, although cyanobacteria have been isolated from biofilms (Callow, 1986). Most work on microbiologically influenced corrosion has focused on the impact of sulfate-reducing, acid-producing or metal-oxidizing bacteria within biofilms. The present study is the first attempt to relate photosynthesis by bacteria to an electrochemical parameter.

E_{corr} is a mixed potential resulting from oxidation-reduction reactions at the metal surface. At pH's between 8.0 and 10.0, the predominant cathodic reaction on surfaces in oxygenated media is oxygen reduction:

$$O_2 + 2H_20 + 4e^- \Rightarrow 4OH^- . \qquad (2)$$

According to the Nernst equation the reversible potential (E°) for the oxygen reduction reaction is given by

$$E = E^0(O_2/OH^-) + RT/nF \ \ln \ pO_2/(OH^-)^4 , \qquad n = 4 \qquad (3)$$

and

$$E = 1.23 - 0.059 \ pH + 0.059 \ \log \ (pO_2)^{1/4} \ \text{Volts vs. SHE* at 21 °C} . \qquad (4)$$

*SHE is standard hydrogen electrode

Ennoblement of E_{corr} is usually explained by the acceleration of the cathodic oxygen reduction reaction due to microbial activities. Either thermodynamic or kinetic effects can accelerate the rate of oxygen reduction and result in an ennoblement. In the first case, localized acidification (Zhang et al., 1989) or an increase of the partial pressure of oxygen (pO_2) increases the reversible potential of the oxygen electrode (equation 3). Catalysis by organometallic compounds has been proposed to explain ennoblement due to kinetic effects (Scotto et al. 1985; Van Den Bring et al., 1980). Acidification causes E_{pit} to become more negative with decreasing pH, making the parameter a in equation 1 more negative. Mansfeld et al., (in press) showed that E_{pit} for 304SS decreased in deaerated 3.5% NaCl from about +300 mV vs. SCE at pH 8 to about 0 mV at pH = 2. Based on these results and the fact that E_{pit} has to be more positive than E_{corr}, it is unlikely that the ennoblement of E_{corr} is the result of microbial acid production. Furthermore, cyanobacteria induce a high pH value (9.8–10.1) during photosynthesis (Terry and Edyvean, 1981).

In this paper, phototrophy was shown to influence E_{corr}. Twelve-hour light/dark cycles produced a perceptible oscillation when *Anabena* sp. was present in a biofilm on the electrode surface (Fig. 1): the presence of distinct oscillations in this case contrasted with the absence of oscillations in sterile systems. Colonization of stainless steel surfaces by *Delaya marina* did not force changes in E_{corr}, probably as the result of the limited amount of fixed carbon available to the nonphotosynthetic, heterotrophic bacteria. While the rate of response, i.e., lag, in E_{corr} produced by photosynthetic biofilms appeared to be unpredictable, the magnitude of response is determined by the corrosion mechanism and local redox potential. Using data for the bulk phase temperature and dissolved oxygen concentration (for the 6-h light/dark oscillations),

the expected magnitude of E_{corr} oscillations can be calculated, assuming that the cathodic oxygen reduction reaction was the sole contributing factor uncomplicated by anodic and/or other processes. For this calculation, all parameters other than pO_2 are assumed to be constant. If E_1 and E_2 are the reversible potentials associated with DO at 1 and 7 mg L^{-1}, respectively, then the amplitude of E_{corr} oscillation is given by:

$$E_1 - E_2 = \frac{RT}{4F} \cdot \ln\left(\frac{p(O_2)_1}{p(O_2)_2}\right) \tag{5}$$

and

$$\Delta E = \frac{0.059}{4} \cdot \log\left(\frac{7}{1}\right) = 13 \text{ mV}. \tag{6}$$

The theoretical result is in good agreement with our experimental data of an ennoblement 10 to 15 mV. E_{corr} is the result of numerous processes other than pO_2, including ionic strength, temperature, surface detritus, and degree of corrosive attack. The direct interpretation of E_{corr} as a function of the pO_2 is, therefore, questionable, given the multiple reactions that contribute to E_{corr}. For example, Dexter and Zhang (1991) evaluated the influence of light on E_{corr} and reported that E_{corr} for a given sample was usually 100 to 200 mV more negative in the daylight hours than at night. Varying the single parameter of oxygen clearly produced shifts in E_{corr} in the experiments reported in this paper.

CONCLUSIONS

We have demonstrated that reactions within biofilms, including photosynthesis, can affect E_{corr} and that environmental conditions, including light level, influence electrochemical reactions within the biofilms. An increase in interfacial pO_2 produced by photosynthetic organisms causes a sudden net increase in the E_{corr} from 2 to 15 mV. The contribution of photosynthetic organisms in biofilms on stainless steels in natural environments is in agreement with the theoretical prediction. The mechanism(s) of E_{corr} ennoblement reported by some investigators on the order of 300 mV in the marine environment is still unclear.

ACKNOWLEDGMENTS

The authors acknowledge help with the manuscript from Dr. Marc Mittelman and financial aid from the Gas Research Institute (GRI, RADIAN TMH-88-12-65), the Electric Power Research Institute (EPRI, RP8OII-O2), and the NOARL Defense Research Sciences Program (PE 0601153N). NOARL contribution number JA 333:047:91.

REFERENCES

Callow ME (1986) A world-wide survey of slime formation on anti-fouling paints. In: Evans LV and Hoagland KD (eds) *Algal Biofouling*. Elsevier, New York NY, pp. 1–20

Cooksey KE and Cooksey B (1986) Adhesion of fouling diatoms to surfaces: Some biochemistry in studies in environmental science. In: Evans LV and Hoagland KD (eds) *#28 Algal Biofouling*. Elsevier, New York NY, pp. 41–54

Dexter SC and Lin S (1988) Mechanism of corrosion potential ennoblement by marine biofilms. *Proceedings of the 7th International Congress on Marine Corrosion and Fouling*. Valencia, Spain

Dexter SC and Zhang HJ (1991) Effect of biofilms sunlight and salinity on corrosion potential and corrosion initiation. *Microbially Influenced Corrosion and Biodeterioration.* Institute for Applied Microbiology, University of Tennessee, Knoxville, TN, pp. 8-1-8-4.

Dowling NJE, Franklin M, White DC, Lee CH, Lundin C (1989) The effect of microbiologically influenced corrosion on stainless steel weldments in artificial seawater. *Corrosion '89*, National Association Corrosion Engineers, New Orleans, Paper #187

Edyvean RGJ and Terry LA (1983) The influence of microalgae on corrosion of structural steels used in the North Sea. Oxley TA and Barry S (eds) *Biodeterioration 5*. John Wiley and Sons, London

Holthe R, Bardal E, and Gartland PO (1988) *Corrosion '88*. National Association of Corrosion Engineers, Houston, TX, Paper #393

Little B, Ray R, Wagner P, Lewandowski Z, Characklis WG, Jacobus J, and Mansfeld F (1989) Electrochemical reactions on stainless steels covered with biofilms. In: National Association of Corrosion Engineers extended abstracts for the *Corrosion Research Symposium*, New Orleans, April 17–19

Little B, Ray R, Wagner P, Lewandowski Z, Characklis WG, Jacobus J, and Mansfeld F (1991) Impact of biofouling on the electrochemical behaviour of 304 stainless steel in natural seawater. *Biofouling*. In press

Mansfeld F, Tsai R, Shih H, Little B, Ray R, and Wagner P (1991) An electrochemical and surface analytical study of stainless steels and titanium exposed to natural seawater. *Corrosion Science*, in press.

Scotto V, Di Cintio R, and Marcenaro G (1985) The influence of marine aerobic microbial film on stainless steel corrosion behaviour. *Corrosion Science* 25(3):185–194

Scotto V, Alabiso G, and Marcenaro G (1986) An example of microbiologically influenced corrosion: The behavior of stainless steels in natural seawater. *Bioelectrochemistry and Bioenergetics* 16:347–355

Terry LA and Edyvean RGJ (1981) Microalgae and corrosion. *Botanica Marina* 24:177–183

Terry LA and Edyvean RGJ (1986) Recent investigations into the effects of algae on corrosion. In: Evans LV and Hoagland KD (eds) *Algal Biofouling*. Elsevier, New York NY, pp. 211–231

Uhlig HH and Revie WR (1985) *Corrosion and corrosion control: An introduction to corrosion science and engineering*, Third Edition; Wiley-Interscience, New York NY

Van den Bring F, Barendetch E, and Visscher W (1980) *Rec Trav Chim* 99:253

Zhang X, Buchanan RA, Stansbury EE, and Dowling NJE (1989) Electrochemical responses of structural materials to microbially influenced corrosion. *Corrosion '89*, National Association Corrosion Engineers, New Orleans LA, Paper #512

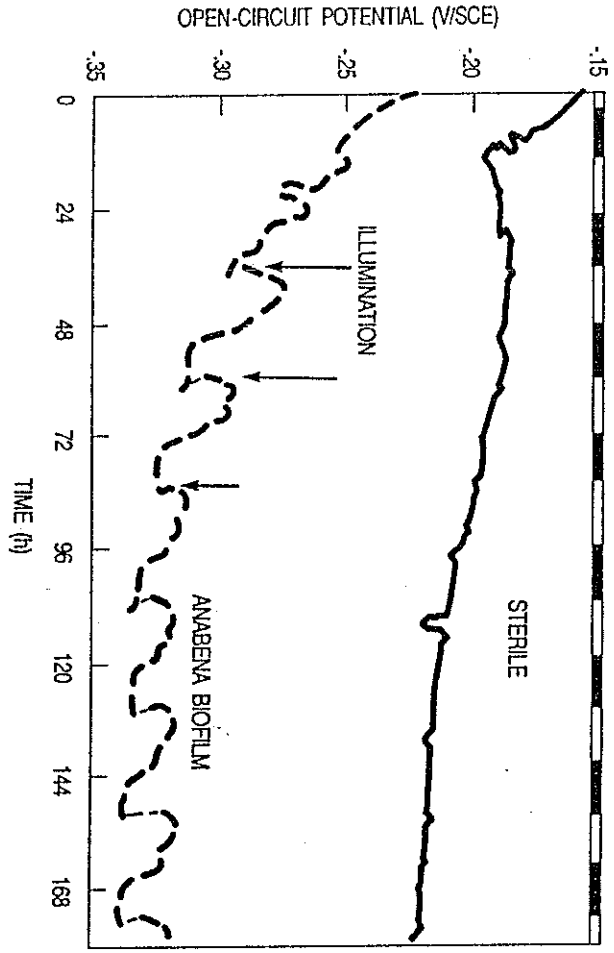
FIGURE LEGENDS

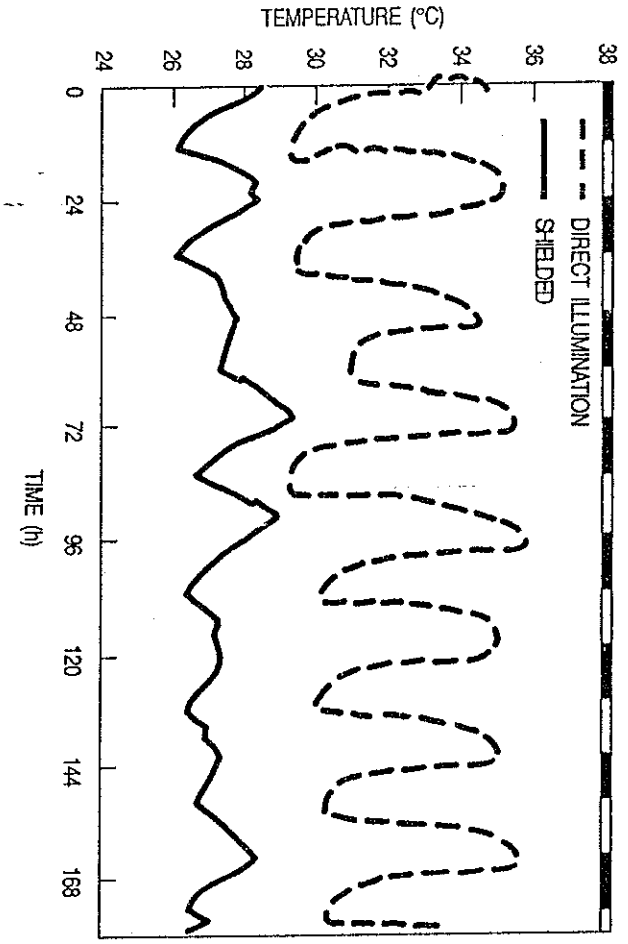
Fig. 1. Open-circuit potentials observed with and without an *Anabena* biofilm subjected to a 12-h light/dark regime. Light period denoted by white slot, dark period by black slot.

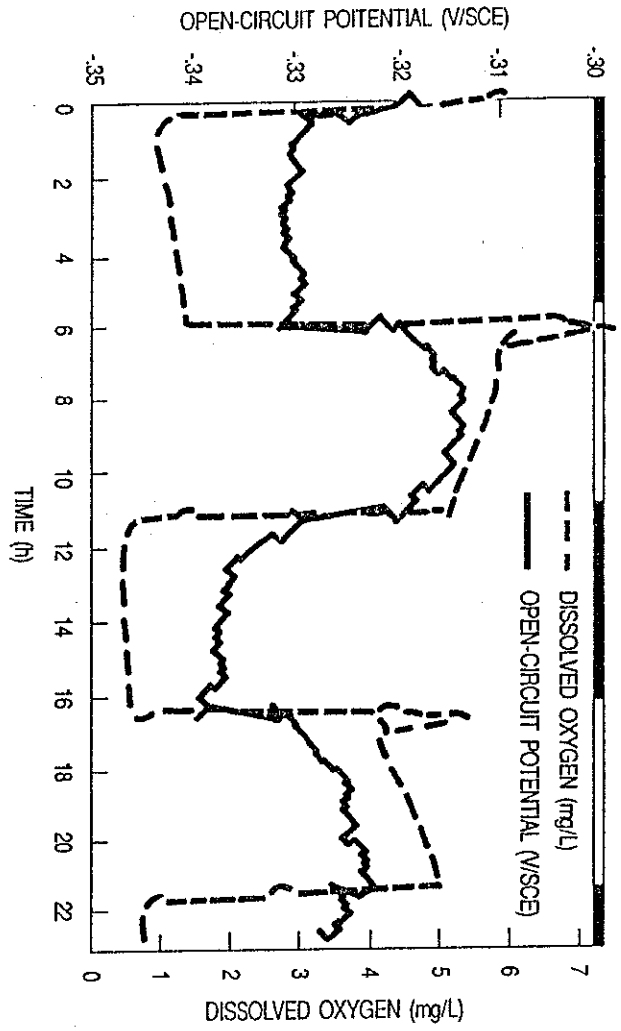
Fig. 2. Temperature oscillations of the bulk phase medium when illuminated directly or shielded by aluminum foil under 12-h light/dark regime. Light period denoted by white slot, dark period by black slot.

Fig. 3. Open-circuit potential and dissolved oxygen oscillations associated with an *Anabena* sp. biofilm under a 6-h light/dark regime. Light period denoted by white slot, dark period by black slot.

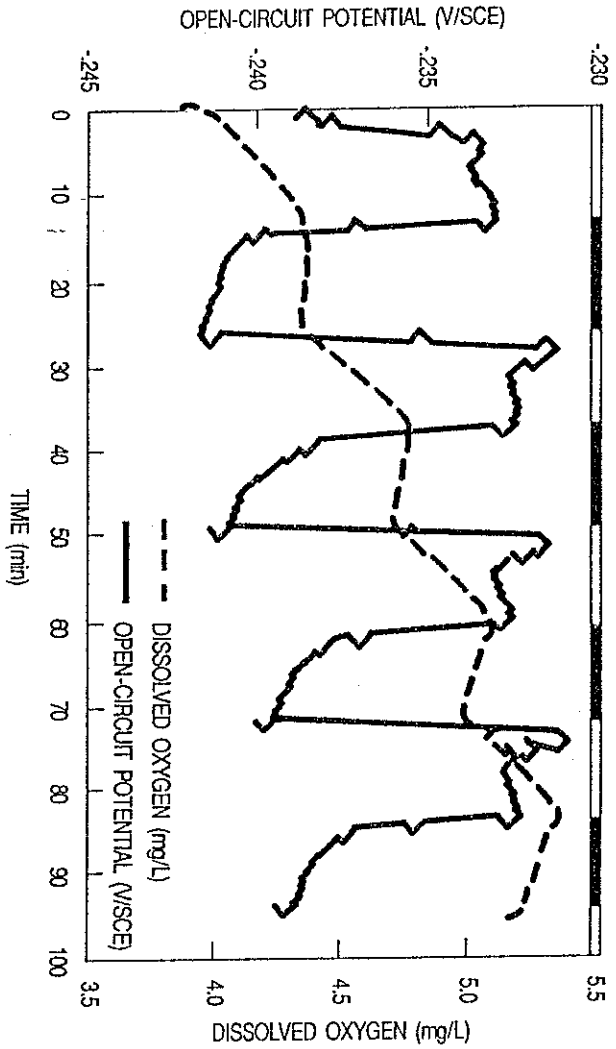
Fig. 4. Open-circuit potential and dissolved oxygen oscillations associated with an *Anabena* sp. biofilm on two separate electrodes under a 15-min light/dark regime. Light period denoted by white slot, dark period by black slot.


OPEN-CIRCUIT POTENTIAL (V/SCE)
-.35
-.30
-.25
-.20
-.15
0
24
48
72
96
120
144
168
TIME (h)
ILLUMINATION
ANABENA BIOFILM
STERILE


TEMPERATURE (°C)
24
26
28
30
32
34
36
38
0
24
48
72
96
120
144
168
TIME (h)
DIRECT ILLUMINATION
SHIELDED


OPEN-CIRCUIT POITENTIAL (V/SCE)
-35
-34
-33
-32
-31
-30
TIME (h)
0
2
4
6
8
10
12
14
16
18
20
22
DISSOLVED OXYGEN (mg/L)
DISSOLVED OXYGEN (mg/L)
OPEN-CIRCUIT POTENTIAL (V/SCE)
0
1
2
3
4
5
6
7

(3)


OPEN-CIRCUIT POTENTIAL (V/SCE)
-.245
-.240
-.235
-.230
TIME (min)
0
10
20
30
40
50
60
70
80
90
100
DISSOLVED OXYGEN (mg/L)
OPEN-CIRCUIT POTENTIAL (V/SCE)
3.5
4.0
4.5
5.0
5.5
DISSOLVED OXYGEN (mg/L)