CROATICA CHEMICA ACTA CCACAA **70** (1) 141–150 (1997)

ISSN–0011-1643
CCA–2411

Original Scientific Paper

Polarography of Marine Bacteria: A Preliminary Study*

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Received April 4, 1996; accepted July 17, 1996

We report the first investigations of marine bacteria interactions with the mercury electrode/seawater interface. The effect of marine bacteria on the oxygen electro-reduction process in seawater was studied. The presence of bacteria ($N > 10^9$/mL) is manifested in a high surface activity and rapid oxygen consumption. In more diluted suspensions of bacteria ($N < 10^9$/mL), attachment of aggregated cells produces specific current transients that can be distinguished from the background response caused by the collective effect of adhesion of single cells and/or adsorption of their release products. The general pattern of current-time curve sequences has not been described before; it seems to simultaneously reflect a heterogeneous volume distribution of cells and their release products, as well as their interfacial properties.

INTRODUCTION

Bacteria contribute substantially to the ocean carbon pool and their role as organic particles should be taken into account in studies designed with the aim of understanding the biogeochemical cycles of elements.[1]

The role of bacteria in the formation, transport and degradation of particulate organic matter is a complex and unsolved problem in oceanography:

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* Dedicated to Marko Branica on the occasion of his 65th birthday.

they prolifically create fine new particles, bacteria, by assimilating dissolved organic matter. Bacteria may solubilize and fragment larger flocculant organic aggregates, »marine snow«[2] with their hydrolytic enzymes,[3] while bacterial mucilaginous adhesives can cement particles together,[4] producing marine snow.[5,6]

In order to understand how bacteria influence the spatial-temporal distribution of organic matter, it is important to devise direct experimental methods of probing organic matter in the aqueous environment without perturbing its original heterogeneous distribution. Microelectrodes[7,8] seem to be particularly suited for such a purpose.[9] Alldredge and Cohen, used pH and oxygen microelectrodes to study chemical gradients around marine snow.[10] They presented experimental evidence that microscale chemical gradients persist in the ocean against processes of advection and diffusion on a scale significant to microorganisms.

We have been using the dropping mercury electrode (for a recent review see Novotny and Heyrovský, this issue) to probe the heterogeneity of organic matter distribution created by bacteria. Here, we report a preliminary study of marine bacteria grown as laboratory monocultures. The electrochemical approach has been described in more detail in the preceding communication (Ivošević and Žutić, this issue).

EXPERIMENTAL

Bacterial Cultures

Strains of marine bacteria isolated from seawater, collected off Scripps Pier, and from a station ~ 1 km offshore,[11,12] were obtained by courtesy of David Smith, Scripps Institution of Oceanography. The bacteria were grown as batch monocultures at 18 °C. The growth medium was commercial Marine Broth, Difco or ZoBell medium[13] (5 g peptone, 1 g yiest extract in 1 L of raw seawater autoclaved at 120 °C for 30 minutes). The seawater was collected in Northern Adriatic about 1 km off the Center for Marine Research, Ruđer Bošković Institute at Rovinj, Croatia. Bacteria were counted by the standard epifluorescence microscopy after DAPI (4,6-diamidino-2-phenylindole) staining.[14]

The cells were harvested after 3 days and the maximum cell density in culture was 4×10^9 /mL. To separate cells from the growth medium, cells were pelleted (4000 g, 3 minutes), the supernatant was decanted and the pellet washed with seawater. This procedure was repeated 3 times to remove all traces of the growth medium. The cleanup was controlled polarographycally by measuring the supernatant surface activity until the surface activity of supernatant decreased to that of seawater.

The pellets were then resuspended in 4 mL of seawater or organic-free electrolyte (0.6 M NaCl + 5 mM $NaHCO_3$, pH 8.4). Cell density in the suspension was in the range $5 \times 10^9 - 5 \times 10^{10}$/mL. Aliquots of the suspension were added directly to 20 mL of seawater or organic-free electrolyte in the electrochemical vessel. Each series of electrochemical experiments was completed within 30 minutes.

Model Cell Cultures

Naked nanoflagellate *Dunaliella tertiolecta* (Plymouth Culture Collection) was grown in seawater sterilized and enriched with F/2 nutrients[15] in batch cultures. Cells were separated after 6 days of growth (stationary phase) by mild centrifugation and aliquots of the resulting dense suspensions were added to organic-free electrolyte. Viability of cells was controlled in all stages of the experiment by microscopic observation of cell motility.

Electrochemical Set-up

The dropping mercury electrode (DME) had a flow rate of 6.1 mg/s, drop time of 2.08 s (at –0.4 V) and maximum surface area of 4.7 mm^2. All potentials are referred to an Ag/AgCl (0.1 N NaCl) reference electrode which was separated from the measured solution, dispersion by a ceramic frit. Its potential was + 2 mV *vs.* calomel electrode (1 N KCl).

The electrochemical measurements were performed with a PAR 174A Polarographic Analyser. The polarograms were recorded at 5 mV/s scan rate, using a 70045 Hewlett--Packard *x-y* recorder. The current-time curves (*I-t*) at constant potential were recorded with 50–200 μs per point time resolution and stored using a Nicolet 3091 digital oscilloscope connected to a PC computer.

All measurements were performed in a standard Metrohm vessel with 20 mL of cell suspensions in seawater or organic-free electrolyte saturated with air and termostated at 25 °C. The vessel was open to air throughout the measurements.

RESULTS AND DISCUSSION

The dropping mercury electrode is particularly suited as a probe for colloidal systems and heterodispersions of fluid particles[16–25] because of its continuously renewable surface and efficient transport of solutes and particles by convective streaming (streaming polarographic maxima of the first and second kind[26–29]).

At DME, reduction of molecular oxygen dissolved in seawater takes place in a broad potential range (for a review see Grasshoff [30]), featuring polarographic maxima of the first and second kind, which are particularly sensitive to surface active constituents of seawater.[31] Adsorption of organic molecules at DME is manifested as a regular suppression of the current of polarographic maxima, while adhesion of fluid particles (≥ 1 μm) yields pronounced current spikes[32,33] that appear as irregular oscillations on polarograms. At the ionic strength of seawater (Figure 1, curve A), the current amplitude of the polarographic maximum and hence its supression is smaller than in a more diluted electrolyte.[28,31–33] However, the unique advantage of this method is the possibility to measure unperturbed distributions of adsorbable solutes and particles directly in seawater.

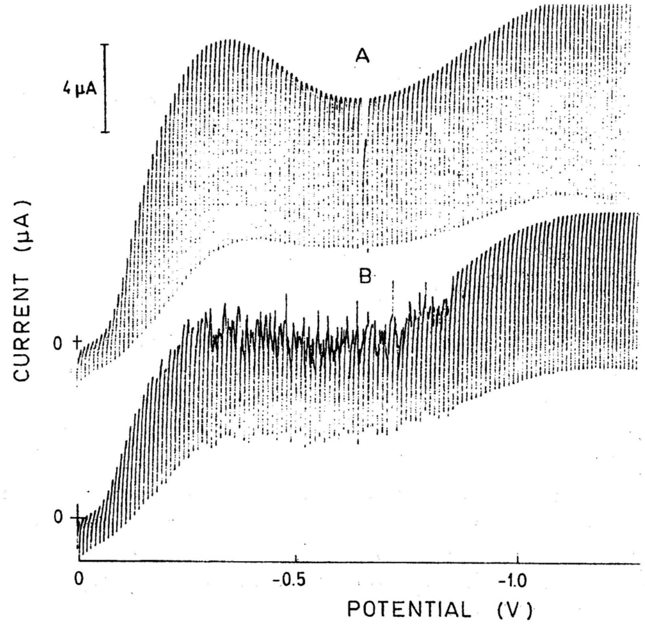

Figure 1. Polarogram of oxygen reduction recorded in air-saturated, organic-free electrolyte 0.6 M NaCl + 5 mM $NaHCO_3$, (curve A); and after addition of *Dunaliella tertiolecta* particles, 2.7×10^5 cells/mL, (curve B).

The effect of particles on the shape of the oxygen reduction polarogram by dispersion of living phytoplankton cells *Dunaliella tertiolecta* (d = 5–10 µm) is shown in Figure 1. Curve B is the polarogram of oxygen reduction in a dispersion of 2.7×10^5 cells/mL in organic-free electrolyte. Irregular current suppression appears in the potential range from –220 to –880 mV, where the attachment and subsequent spreading of the cells at the electrode surface takes place. We can also identify a broader range (–120 to –1550 mV) of regular current suppression, which can be interpreted as adsorption of dissolved surfactants released by *Dunaliella tertiolecta* cells.[17]

Marine bacteria are known for their adhesion to both hydrophobic and hydrophilic surfaces[34] and could be regarded as surface active constituents of seawater. We have selected two different strains of marine bacteria that are simple to grow in batch cultures and easy to separate from organic rich growth medium. The strain BF3 is a typical representative of marine free-living bacteria. It was isolated from the bulk water of the microcosmus after a phytoplankton bloom.[11] At the time of electrochemical experiments, cell dimensions were 1.0–3.2 µm in length and 0.7–1.0 µm in width. Yellow pigmented bacterium S-3 is related to *Cytophaga / Flavobacteria* (according to a high 16S rRNA sequence similarity values[12]) which are known to attach to aggregates and have surface dependent gliding motility. Cell dimensions were 1.4–4.0 μm in length and 0.4–0.6 μm in width.

Polarograms were first recorded in a dense BF3 suspension (3×10^9 cells/ /mL) in seawater. Curve a in Figure 2 was recorded in air-saturated seawater before additon of bacteria. Recording of curve b started 5 minutes after addition of bacteria. The main effect of bacteria is manifested as a dramatic decrease of the oxygen reduction current (although the surface of suspension was exposed to air throughout the measurements). The subsequently recorded polarogram (Figure 2, curve c) shows that the dissolved oxygen is completely depleted from the surrounding medium by bacterial respiration. Culture of bacterial strain S-3 showed a similar time dependence for the oxygen reduction current. The oxygen-reduction current, at constant potential at –650 mV, plotted against time, is shown in Figure 3. The result indicates intensive bacterial metabolic activity and viability.

The electrochemical characterization was further performed in a more diluted suspension (5×10^8 cells/mL) where no measurable decrease of oxygen concentration due to cell metabolism takes place during the electro-

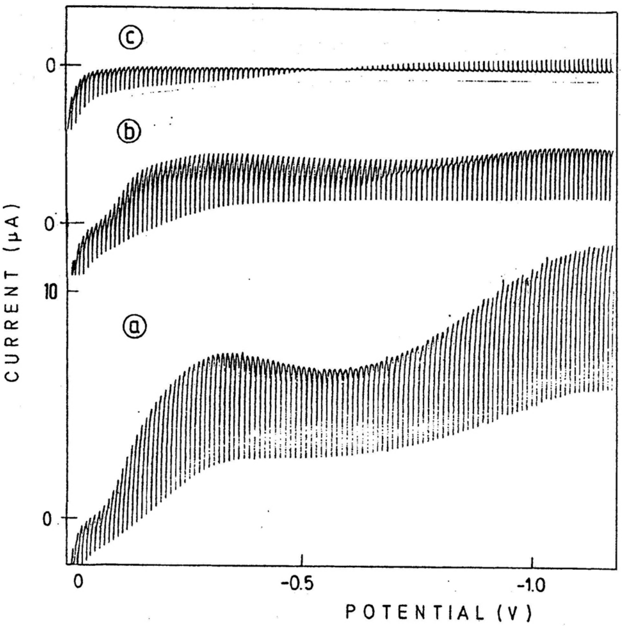

Figure 2. Polarogram of oxygen reduction in
Curve a: air-saturated seawater
Curve b: air-saturated seawater, 5 minutes after addition of 3×10^9 cells/mL of marine bacteria BF3.
Curve c: same as (b), 5 minutes later.

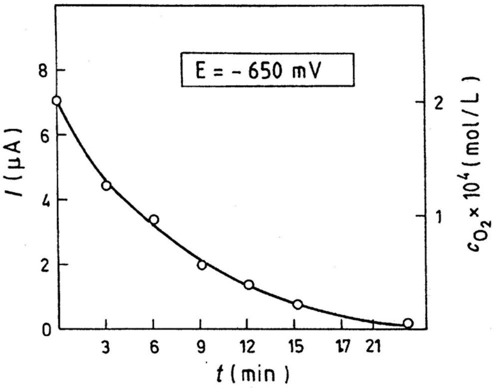

Figure 3. Bacterial consumption of oxygen expressed as the decrease of oxygen-reduction current at constant potential (–650 mV) in the dispersion of bacteria S-3, 5.4 × 10^8 cells/mL in seawater.

chemical measurement (Figure 4). Comparison with the polarogram of organic-free electrolyte shows clearly the potential range where the current is suppressed by adsorption of organic constituents (–100 to –1350 mV). The suppression is irregular in the potential range from –300 to –1100 mV, indicating particle attachment. The effect of particles is much less pronounced

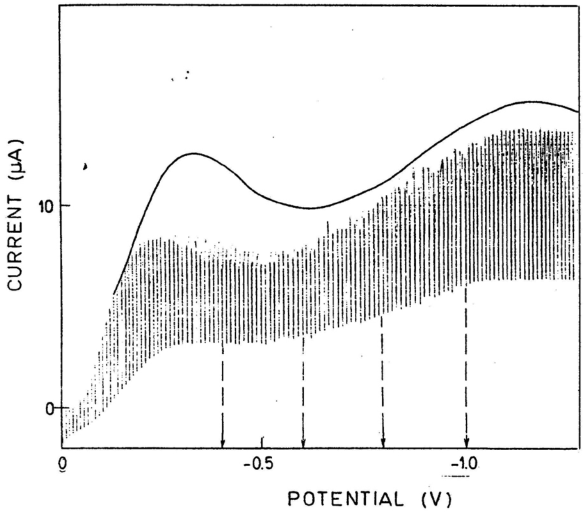

Figure 4. Polarogram of oxygen reduction in the dispersion of bacteria S-3, 5.4 × × 10^8 cells/mL in 0.6 M NaCl + 5 mM $NaHCO_3$. The thin line is the envelope of a polarogram in organic-free electrolyte.

than in the case of *Dunaliella tertiolecta* where the cell density was only 7×10^5/mL.

For a more detailed analysis, we recorded the current-time transients at selected constant potentials and at a higher time resolution. *I-t* curves were recorded at –400 mV where the electrode is positively charged and at –600, –800 and –1000 mV with increasingly negatively charged electrode.[35]

I-t curves recorded on subsequent mercury drops are known to be perfectly smooth and reproducible in organic-free electrolyte as well as in the presence of adsorbable organic molecules. Such a regularity and reproducibility of *I-t* curves indicates a homogenous distribution of surface active constituents at the interface.

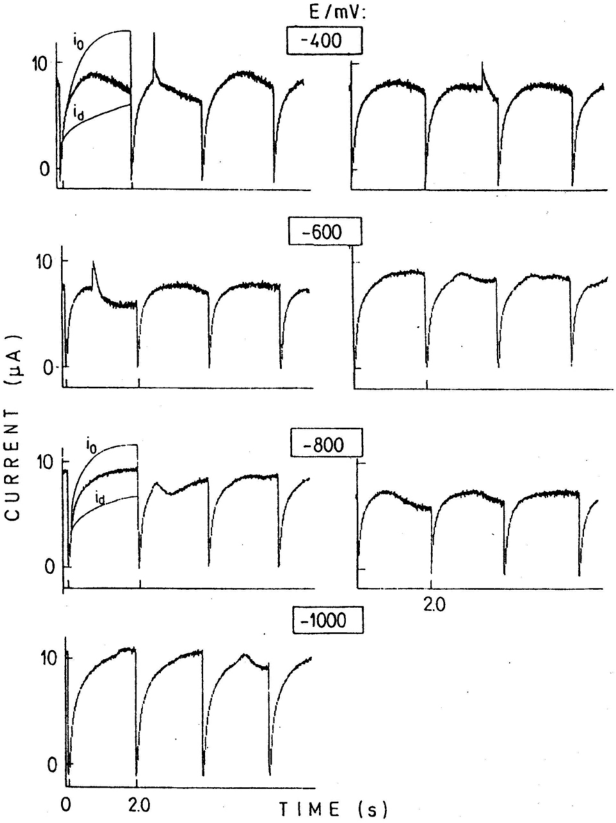

Figure 5. Current-time curves of oxygen reduction in the dispersion of bacteria S-3 (same as in Figure 4) at different constant potentials. Each set of *I-t* curves at three consecutive mercury drops was selected at random. Current in organic-free electrolyte (i_0) and the current for surface coverage with organic molecules approaching unity (i_d) is indicated by thin lines for potentials: –400 and –800 mV.

Let us consider *I-t* curves in Figure 5. Curves i_0, indicated for potentials –400 and –800 mV, were recorded in organic-free electrolyte and they correspond to the surface coverage $\theta = 0$, while curves i_d correspond to total suppression of polarographic maximum when surface coverage appoaches unity, $\theta \approx 1$.

At a positively charged electrode surface (–400 mV), each *I-t* curve is different and some show very pronounced spikes which end up in a significant decrease of the base-line current. At a negatively charged electrode surface (–600, –800 and –1000 mV), spikes degerate into humps. At all these potentials, the *I-t* curves exhibit two types of variability: smooth base-line current varying from drop to drop and a random appearance of discontinuities. The spikes at the positively charged electrode surface and humps at the negatively charged electrode suraface, could be assigned to the attachment of aggregated cells. The decrease of current (ΔI) at the end of the attachment signals is caused by an increase in surface coverage $0.1 \leq \Delta\theta \leq 0.5$.

The general pattern just described has not been observed before, although numerous heterogeneous systems have been studied so far. It seems to be specific to bacteria[36] reflecting volume distribution and interfacial reactions of single cells and aggregates.

CONCLUSION

We have demonstrated that the dropping mercury electrode can be used as a probe in studying interfacial interactions of marine bacteria. High surfactant activity of bacterial suspensions is revealed by the extent of suppression of the oxygen-reduction current in a range of positive and negative charges of the electrode surface.

The electrochemical response of bacterial suspension differs from the pronounced attachment signals of single algal cells but also from simple adsorption curves of dissolved surfactants. The general pattern of the current-time curve sequences recorded for bacterial suspensions in seawater (5×10^7 – – 5×10^8/mL) has not been described before; it seems to simultaneously reflect a heterogeneous volume distribution of cells, their release products and larger cell aggregates, as well as their interfacial properties.

Acknowledgments. – This research was sponsored by the Ministry of Science and Technology of the Republic of Croatia and the UNEP/IOC project, Link between eutrophication and interfacial processes. We thank Dr. David C. Smith, Scripps Institution of Oceanography, for the isolates of marine bacteria, and Dr. John Green, Plymouth Marine Laboratory, for monocultures of marine phytoplankton. We also thank Richard Long for proof reading.

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SAŽETAK

Polarografija morskih bakterija: Preliminarno istraživanje

Vesna Svetličić, Nadica Ivošević, Vera Žutić i Dragica Fuks

Opisana su prva istraživanja interakcije morskih bakterija s međupovršinom živina elektroda/morska voda praćenjem utjecaja bakterija na elektrodnu reakciju otopljenog kisika u morskoj vodi. Prisutnost bakterija ($N > 10^9$/mL) očituje se u visokoj površinskoj aktivnosti i brzoj potrošnji kisika. U razrjeđenoj suspenziji bakterija ($N < 10^9$/mL) pojedinačni događaji prianjanja nakupina stanica bakterija uzrokuju specifične odzive na krivuljama struja-vrijeme, koji se mogu razlučiti od postupnog sniženja struje redukcije uzrokovanoga skupnim učinkom adhezije pojedinačnih stanica i/ili njihovih produkata lučenja.

Opći oblik uzastopnih krivulja struja-vrijeme na živinoj kapajućoj elektrodi, kakav nije do sada opisan u literaturi, istovremeno odražava heterogenost raspodjele stanica bakterija i neposrednih produkata lučenja kao i njihovih međupovršinskih svojstava.