Enhancement of Survival and Electricity Production in an Engineered Bacterium by Light-Driven Proton Pumping††

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Microorganisms can use complex photosystems or light-dependent proton pumps to generate membrane potential and/or reduce electron carriers to support growth. The discovery that proteorhodopsin is a light-dependent proton pump that can be expressed readily in recombinant bacteria enables development of new strategies to probe microbial physiology and to engineer microbes with new light-driven properties. Here, we describe functional expression of proteorhodopsin and light-induced changes in membrane potential in the bacterium Shewanella oneidensis strain MR-1. We report that there were significant increases in electrical current generation during illumination of electrochemical chambers containing S. oneidensis expressing proteorhodopsin. We present evidence that an engineered strain is able to consume lactate at an increased rate when it is illuminated, which is consistent with the hypothesis that proteorhodopsin activity enhances lactate uptake by increasing the proton motive force. Our results demonstrate that there is coupling of a light-driven process to electricity generation in a nonphototrophic, synthetic engineered bacterium. Expression of proteorhodopsin also preserved the viability of the bacterium under nutrient-limited conditions, providing evidence that fulfillment of basic energy needs of organisms may explain the widespread distribution of proteorhodopsin in marine environments.

Classic experiments in microbial bioenergetics used light-driven reactions from halobacterial bacteriorhodopsin or the photosynthetic reaction center to provide a temporary driving force for understanding transport and chemiosmotic coupling (6, 7, 19, 35). However, light-driven reactions have not been used in metabolic engineering to alter microbial physiology and production of chemicals. The recent discovery of proteorhodopsin (PR) in ocean microorganisms and the ease with which this membrane protein can be functionally expressed by recombinant bacteria have made possible many engineering strategies previously not available (1, 16). In this paper, we describe progress toward the goal of integrating light-driven reactions with biocatalysis.

In contrast to the situation for established industrial microorganisms, such as Escherichia coli, our current understanding of less-studied algal and phototrophic bacteria may limit metabolic engineering strategies which require genetic manipulation. Metabolic engineering strategies using photosynthetic bacteria have focused largely on methods to increase hydrogen production, and improvements rely mainly on engineering of nitrogenase and hydrogenase to produce H2. Algae appear to be suited to large-scale cultivation for lipid production, but so far little has been done to engineer these organisms (36). In principle, platform microbial hosts capable of producing a diverse range of products could be boosted by addition of light-driven processes from phototrophic metabolism.

To demonstrate the feasibility of transferring a light-driven process into a nonphototrophic bacterium, we chose to study proteorhodopsin (PR) first because it is one of the simplest mechanisms for harnessing the energy from light. The proteorhodopsins are a group of transmembrane proteins that use the light-induced isomerization of retinal, the oxidative cleavage product of the carotenoid β-carotene, either to initiate signaling pathways or to catalyze the transfer of ions across cell membranes (8). PR was discovered by metagenomic analysis of marine samples (1) and is related to the well-studied bacteriorhodopsin of archaea (33) and rhodopsin (34), a eukaryotic light-sensing protein. The membrane potential generated by light-driven proton pumping by PR has been confirmed to drive ATP synthesis in a heterologous system (25). However, bacteria expressing heterologous PR were shown not to benefit from this pumping activity, as no significant increases in growth rates were observed (9). This led to the suggestion that PR may benefit the organism only under starvation conditions. In agreement with this hypothesis, Gomez-Consarnau et al. (10) have reported that the light-dependent growth rates of a marine flavobacterium that has a native PR are increased only when the organism is cultured under energy-limited conditions.

Studies of both native and recombinant systems in which rhodopsins are expressed have generated light-dependent membrane potentials. In membrane vesicles isolated from a native host, the light-dependent membrane potential gener-
ated by bacteriorhodopsin provides the driving force for ATP synthesis (35) and uptake of leucine and glutamate (20, 22). More recently, studies of recombinant systems have coupled the membrane potential to other transport processes. In one example, the membrane potential-dependent export of specific toxic molecules increased when E. coli cells expressing both an archael rhodopsin and a specific efflux pump were exposed to light (17). In another experiment, starved E. coli cells expressing PR increased the swimming motion of their flagella when they were illuminated (44). Based upon measurements of flagellar motion as a function of light intensity and azide concentration, the proton motive force generated by PR was estimated to be ~0.2 V, a value similar to the value for aerobic respiration in E. coli (42).

As a nonphotosynthetic host for recombinant PR expression, we chose the dissimilatory metal-reducing bacterium Shewanella oneidensis strain MR-1, which is genetically tractable for engineering and is able to use a variety of terminal electron acceptors, including insoluble metal oxides (11, 30). Key to the ability of this bacterium to reduce metal oxides is a multicomponent extracellular respiratory pathway that transports electrons from menaquinol to cytochromes in the outer membrane. This pathway is composed of a cytoplasmic membrane tetraheme protein (CymA), a periplasmic decaheme protein (MtrA), an integral outer membrane protein (MtrB), and a decaheme lipoprotein (MtrC) that is associated with MtrB (14, 37, 40). The ability of S. oneidensis to reduce extracellular metal oxides has made it possible to harvest electrons from this organism by coupling it to an electrode which serves as the electron acceptor (21). The electron flow to the outer surface allows respiration rates to be measured directly by electrochemistry.

In the current work, we introduced PR into an electricity-generating bacterium, S. oneidensis strain MR-1, and demonstrated that there was integration of a light-driven process into the metabolism of a previously nonphotosynthetic organism that resulted in a useful output. We show here that PR allows cells to survive for extended periods in stationary phase and that the presence of light results in an increase in electricity generation. A possible physiological model to explain these effects is discussed.

MATERIALS AND METHODS
Microbiological methods. E. coli JM109 was used for all molecular biology procedures, and plasmids were transferred to S. oneidensis strain MR-1 by conjugation with the mating strain E. coli WM3064. S. oneidensis was cultivated at 30°C either in Luria-Bertani (LB) medium or in minimal medium containing 20 mM lactate (15, 23). Anaerobic cultures of S. oneidensis contained 40 mM fumarate as an electron acceptor when they were not grown in electrochemical chambers with an electrode as the electron acceptor. When required, 50 µg ml⁻¹ kanamycin and 10 µM retinal were included for plasmid selection and PR reconstitution, respectively. Optical densities at 600 nm (OD₆₀₀) of cell cultures were determined with a Bausch and Lomb Spectronic 20. Estimates of the number of cells ml⁻¹ in a culture were obtained by counting the number of colonies after a dilution series of the culture was plated on LB agar.

Gene cloning. The PR gene of a marine bacterium in the SAR86 phylogenetic group (original contig GenBank accession no. AF279106; proteorhodopsin gene GenBank accession no. AA910475) was amplified by PCR from a plasmid containing the PR gene (1) using gene-specific oligonucleotides (5'-GGCTTAG AAGGACTGATCTACATATAAGATTTCTGTTACGTTG-3' and 5'- ACTAGGGCGCGGCTTAAAGCATTAGAAGATCTTAC-3') with added XbaI and NotI restriction sites and a Shine-Dalgarno sequence. The PCR product was cloned into the constitutive expression vector pUCMod (the lac repressor binding sequence was deleted) (39) and sequenced to ensure that the PR gene was not changed. The PR gene and the upstream constitutive lac promoter then were subcloned into the BamHI site of the broad-host-range vector pBBR1MCS-2 (18) for expression in S. oneidensis (3). Subsequent digestion of the pBBR1MCS-2 plasmid containing the fragment cloned into the BamHI restriction site revealed that the modified promoter and PR gene were oriented opposite the endogenous pBBR1MCS-2 promoter sequence.

Cell adhesion. Cultures of S. oneidensis harboring pBBR1MCS-2 containing the PR gene or an empty control plasmid were incubated anerobically in minimal medium containing 10 µM retinal at 30°C for approximately 16 h. The cells were centrifuged and resuspended in 50 mM Tris-HCl (pH 8.0), 0.1 M NaCl. It was difficult to measure the difference spectrum because of the background caused by scattering and absorption by native cytochromes expressed by S. oneidensis. To reduce the scattering of a sample, resuspended cells were sonicated using 10-s pulses at 30% power for a total of 90 s (Branson Sonifier). Absorption spectra were measured (Varian CARY 50 spectrophotometer), and the effects of scattering were removed by subtracting a cubic polynomial fit to the data at 310 nm and 700 to 750 nm. To remove absorption by native cytochromes, the difference spectrum for cultures of S. oneidensis containing pBBR1MCS-2 with the PR gene and cultures of S. oneidensis containing an empty control plasmid was calculated. To account for minor differences in cytochrome absorbance at 400 nm and the numbers of cells in each culture, the two baseline-corrected spectra were normalized at the cytochrome absorption peak (λ₅₇₆ of 410 nm) by scaling the spectrum for the culture of S. oneidensis containing the empty plasmid. To estimate the number of PR molecules cell⁻¹, the concentration of PR in the sample was calculated by assuming that the molar extinction coefficient at 520 nm was 50,000 M⁻¹ cm⁻¹ (2), and the number of viable cells was determined by plating serial dilutions on agar plates. The estimate was based on three replicate experiments.

Illumination methods. Light for both the growth rate measurement experiments and the electrochemical experiments was generated by circuit boards containing arrays of green (λmax 530 nm) high-power light-emitting diodes (LEDS) (Luxeon III Emitter; LXHL-PM09; Future Electronics) powered by a direct current power supply (Mastech model HY3003E). In the growth experiments, the samples were illuminated by LEDs spaced approximately 3 cm apart. In the electrochemical experiments, both faces of the electrode were illuminated by separate arrays of LEDs (see Fig. 4A). To provide sufficient light intensity, the circuit boards were coated with thin layers of clear epoxy (EPO-TEK 302-3 M; Epoxy Technology) and were submerged in the temperature-controlled water bath <1 cm from the electrochemical chamber. Light intensities were measured with a light meter (AEMIC Instruments model CA813), and photometric units (lux) were converted to radiometric units (mW cm⁻²) using the standard photosensitive dye and power spectrum of the LEDS.

Membrane potential measurement. The membrane potential for S. oneidensis was measured using a membrane potential-sensitive dye and flow cytometry (31). Cells grown aerobically in minimal medium with 20 mM lactate were centrifuged and then washed and resuspended in an equal volume of minimal medium without lactate as the electron donor. After incubation at 30°C for 30 to 40 h in the dark, the cells were diluted to obtain a concentration of ~106 cells ml⁻¹ and then washed and resuspended in a medium containing the empty pBBR1MCS-2 plasmid containing the fragment cloned into the BamHI restriction site. Optical densities at 600 nm (OD₆₀₀) for the cultures were determined. A 2-ml sample of each culture was diluted 1:3 with growth medium containing either 0 or 10 mM lactate. Following incubation for 1 h at 30°C either in the dark or in the presence of approximately 3 mW cm⁻² of light, the cells were exposed to the cyanine dye DioC₂(3) at a concentration of 10 µM for 5 min in the dark. Fluorescence intensities at wavelengths greater than 640 nm were measured for 10,000 cells using a FACScalibur flow cytometer (Becton Dickinson). An EDTA treatment that is often required to disrupt the cell membrane and allow the dye to enter the cells of Gram-negative bacteria was not required for S. oneidensis. For depolarization of the membrane potential, the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was added at a concentration of 5 µM.

Electrochemical studies. Electrochemical measurements for S. oneidensis were determined using 20-ml electrochemical chambers as described previously (23, 24). Ten milliliters of an anaerobic culture of S. oneidensis grown to an OD₆₀₀ of 0.4 to 0.5 at 30°C in minimal medium containing 30 mM lactate, 40 mM fumarate, 50 µg ml⁻¹ kanamycin, and 10 µM retinal was transferred to a sterile, oxygen-sparged, stirred electrochemical chamber. During medium exchanges, the medium surrounding the electrode was replaced with fresh medium containing 30 mM lactate, 50 µg ml⁻¹ kanamycin, 10 µM retinal, and 0.5 µM riboflavin (23). During periods of light, both faces of a graphite working electrode (AXF-5Q; Poco Graphite, Inc.) were illuminated. For experiments in which period illumination was used, the electrochemical chambers (with 15 ml of medium) contained electrodes that were 2 cm by 2 cm by 0.1 cm and the chambers were flushed with nitrogen gas. Heat from the LED circuit was dissipated using a temperature-controlled circulating
water bath (NESLAB, RTE-5B), and the measured temperature did not vary by more than 0.3°C. Radiant heating due to light was negligible because the LED light sources do not emit infrared light. The potential at the working electrode was maintained at 0.245 V versus standard hydrogen electrode, and the current production at 30°C was measured at 30-s intervals.

To measure metabolite concentrations in the medium from electrochemical chambers, samples were removed from the chambers, centrifuged to remove cells, and loaded (2 μl) onto an Agilent Zorbax SB-Aq C18 column (5 μm; 4.6 by 250 mm), and chromatography was performed using an Agilent 1100 high-performance liquid chromatography (HPLC) system equipped with a photodiode array detector. Metabolites were separated with an isotropic mobile phase containing 20 mM sodium phosphate (pH 2) and 0.5% acetonitrile at a rate of 1 ml min⁻¹, and the absorbance at 210 nm was monitored. Standard curves were generated using known concentrations of each chemical, and peak areas were integrated using the Agilent ChemStation software.

**RESULTS**

**Functional expression of PR by *S. oneidensis***. We first established that expression and reconstitution of PR bound to retinal in *S. oneidensis* lead to accumulation of red pigment in the cell membrane (Fig. 1A). While wild-type cultures are naturally pink due to expression of c-type cytochromes under O₂-limiting conditions, quantitative changes in absorption due to functional reconstitution of PR were measured by calculating the difference spectrum for retinal-expressing PR cultures and cultures containing an empty control plasmid (Fig. 1B). A comparison of the difference spectrum obtained to previous PR spectra suggested reconstitution of recombinant PR in *S. oneidensis* (1). Using an extinction coefficient for rhodopsins absorbing in the visible region and the number of cells ml⁻¹ culture, we estimated that there were 40,000 molecules of PR per *S. oneidensis* cell grown anaerobically in minimal medium containing 10 μM retinal.

Expression in bacteria belonging to the SAR86 phylogenetic group and in *Pelagibacter* cells, which contain 24,000 and 10,000 molecules of PR per cell, respectively (2, 9).

Having established that there was expression in *S. oneidensis*, we next examined generation of light-dependent membrane potentials by monitoring the fluorescence of cells labeled with the lipophilic cyanine dye DioC₂(3), which aggregates at high concentrations, causing its fluorescence to shift to longer wavelengths (31). Because the interior of energized cells has a negative potential relative to the exterior, the positively charged dye accumulates in the cytoplasm and the fluorescence intensity increases at longer wavelengths. Membrane potentials of cells deenergized through starvation or addition of the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP) become depolarized and no longer drive accumulation of the dye in the cytoplasm.

Cultures of *S. oneidensis* expressing PR were incubated in the dark without an electron donor to starve the cells and to deenergize the membrane potential. Cells then were incubated in the light, and fluorescence intensities of in-
individual cells were measured by flow cytometry (Fig. 2). While cultures incubated in the dark without lactate contained both energized cells (high fluorescence intensity) and deenergized cells (low fluorescence intensity), cultures incubated either in the dark with lactate or in the light without lactate contained only energized cells. Addition of the protonophore CCCP depolarized the membrane potential, demonstrating that PR pumps protons out of the cytoplasm to increase the membrane potential. Incubation of starved cells expressing the empty control plasmid in the light did not generate a population of energized cells (see Fig. S1 in the supplemental material).

**PR enhances survival during stationary phase.** Although the results described above showed that recombinant PR operates as a light-driven proton pump, it is not clear how PR influences the physiology of *S. oneidensis*. In bacteria that naturally contain PR, light-driven proton pumping is hypothesized to provide a selective advantage during stationary phase (9, 10, 25). To explore whether this benefit can be observed in an engineered bacterium that does not express PR naturally, we measured the anaerobic growth of cultures of *S. oneidensis* expressing PR in minimal medium supplemented with retinal both in the light and in the dark (Fig. 3). While the optical densities at 600 nm of both cultures grown in the light and cultures grown in the dark peaked at 25 h, only cultures grown in the light without lactate contained both the working electrode and the counter electrode. So that details of the interior of the chamber could be seen, it was necessary to reduce the light intensity compared to the intensity used during the experiments and to remove reflections of the LEDs on the glass surfaces. (B) Oxidation current of an electrochemical chamber inoculated with *S. oneidensis* expressing PR (red trace) or containing a control plasmid (black trace). The arrows indicate the beginning of each 1-h illumination period. The light intensity was 10 mW cm$^{-2}$, and the traces are representative of three independent experiments.

**PR enhances electricity generation.** With the demonstration that recombinant PR is functional and integrated into the physiology of *S. oneidensis*, we investigated whether light-driven proton pumping by PR can influence extracellular respiration and generation of electricity. In a series of reactions in *S. oneidensis* that have not been fully elucidated, oxidation of lactate to acetate leads to reduction of menaquinone and formation of biosynthetic precursors. In the presence of an electrode at an appropriate potential, the redox loop is completed by oxidation of menaquinol and subsequent transfer of electrons via several cytochromes to the outside of the cell, where small-molecule mediators assist in transfer to the electrode (21, 23). Electron transfer to the electrode can be observed directly by measuring the oxidation current (24). After inoculation, cells attach to the electrode, which is a requirement for respiration and growth because mediators do not efficiently carry electrons from planktonic cells to an electrode (23).

Illumination of recombinant *S. oneidensis* cultures expressing PR growing anaerobically on electrodes (Fig. 4A) resulted in light-dependent increases in the oxidation current (Fig. 4B). Light-dependent changes were not observed in cultures containing an empty control plasmid. The oxidation current measured in electrochemical chambers inoculated with *S. oneidensis* either expressing PR or containing the empty control plasmid increased for approximately 70 h as bacteria colonized the electrode. After reaching asymptotic values that did not depend on expression of PR and were sensitive to conditions during inoculation, the current remained steady until lactate was depleted from the medium.

The effect of light-dependent proton pumping by PR on the respiration rate of anaerobically growing cells was measured by illuminating the cultures periodically, and the sawtooth re-
response to changes in light intensity shown in Fig. 4B was representative of all experiments. Typically, after 10 min of illumination the current increased to a value that was 0.04 ± 0.01 A m\(^{-2}\) greater than the value before illumination; when the light was removed, the current decreased within 1 h to a value expected for an identical experiment in which there was no illumination. The magnitude of the light-dependent increase in current represented a 100% increase during early stages of growth. As the absolute light-dependent current was relatively constant as more cells attached to the electrode, illumination at later stages resulted in a 30% increase in the respiration current, indicating that there may have been changes in light penetration for thicker biofilms.

The light-dependent increase in current was proportional to the intensity of the light used to illuminate fully colonized electrodes (Fig. 5). Saturation of the light-dependent increase did not occur with intensities of 10 mW cm\(^{-2}\). Previous studies have shown that saturation of the light-dependent increases for planktonic E. coli expressing PR (44) and halobacteria expressing bacteriorhodopsin (13) occurs at approximately 20 mW cm\(^{-2}\). Addition of exogenous riboflavin, which was previously identified as an extracellular electron mediator produced by S. oneidensis (23, 43), did not alter the magnitude of the light-dependent change in current.

The effects of continuous illumination on the current and metabolite concentrations for an electrochemical chamber containing S. oneidensis expressing PR were measured (Fig. 6 and Table 1). After inoculation and attachment of cells to the electrode, planktonic cells and the medium surrounding the electrode were removed and replaced with fresh medium (zero time). In the dark, the current increased as cell growth continued on the electrode. After a second medium exchange and illumination of the chamber, the average rate of current production increased, and lactate was consumed more rapidly.

For both light and dark periods, decreases in lactate concentration were matched by increases in acetate and pyruvate concentrations. To obtain precise carbon recovery values and to prevent complete oxidation of lactate and acetate to CO\(_2\) by aerobic respiration or consumption of H\(_2\) produced at the counter electrode, it was necessary to sparge the chambers with N\(_2\). Under these conditions and regardless of illumination, the number of electrons recovered at the electrode was less than the expected value. As observed previously (4), it is likely that high N\(_2\) flushing rates decrease the H\(_2\) partial pressure and increase the rate of hydrogenase-catalyzed oxidation of menaquinol and reduction of protons to form H\(_2\) (26). Regardless of the mode of electron disposal and variations in cell growth and attachment to the electrode, illumination of PR-containing chambers was always correlated with an increased rate of oxidation of lactate to acetate.

**DISCUSSION**

As evidenced by the occurrence of proteorhodopsins in environments and clades of bacteria, a PR is expected to provide a selective advantage. Given the role of this molecule as a light-dependent proton pump, it was hypothesized that PR could provide an advantage under nutrient-limited conditions (9, 10). Despite attempts to measure this advantage, most growth rates of bacteria expressing PR are not higher in the light (10). We provide evidence here that PR allows cells to remain viable for extended periods. Our results not only suggest that PR provides a selective advantage by maintaining a

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**TABLE 1. Carbon balance for electrochemical chamber in dark and light conditions**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time (h)</th>
<th>Lactate (mM)</th>
<th>Acetate (mM)</th>
<th>Pyruvate (mM)</th>
<th>Lactate consumption rate (μmol h(^{-1}))</th>
<th>Avg current (A m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>0</td>
<td>32.0</td>
<td>0.0</td>
<td>0.4</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>13.7</td>
<td>15.3</td>
<td>2.5</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>Light</td>
<td>68</td>
<td>32.4</td>
<td>4.6</td>
<td>0.2</td>
<td>2.5</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>6.7</td>
<td>25.2</td>
<td>0.4</td>
<td>2.5</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*For experimental details, see Materials and Methods and the legend to Fig. 6.*
membrane potential during stationary phase but also show that this benefit can be transferred easily into a bacterium that does not contain a PR naturally, which may explain the widespread occurrence of this protein in many different groups of marine bacteria that live under long-term nutrient limitation conditions in the open ocean (38).

The results of our electrochemical studies demonstrate that light energy can be used by cultures of \textit{S. oneidensis} expressing a light-dependent proton pump to enhance generation of electric current. Compared to the time required for \textit{S. oneidensis} to divide during anaerobic growth or to increase its biomass, light-induced changes in current were rapid, suggesting that rather than altering protein expression, illumination increased the rates of extracellular respiration by individual cells. Because all electrons deposited on an electrode must originate from lactate, any increase in current is proportional to an increase in lactate consumption. Our measurements demonstrate that electrochemical chambers containing \textit{S. oneidensis} expressing PR generate more current and consume more lactate in the light than in the dark (Table 1).

Well-established observations have demonstrated that small changes in membrane potential lead to large increases in transport rates and accumulation ratios for small molecules (6, 7, 19). Small increases in the intracellular pH also result in increased accumulation of weak acids, such as lactate. For example, for symport of a molecule such as lactate compensated by one charge (32), the Nernst equation (12) predicts that a 7- to 18-mV increase should cause a 30 to 100% increase in the intracellular concentration. Similarly, 0.1- to 0.3-unit ΔpH changes can result in a similar accumulation ratio. Such modest changes in the membrane potential or ΔpH are consistent with the proton-pumping capacity of PR (27, 29), suggesting that illumination leads to increased lactate uptake, increased reduction of electron carriers such as NAD+/NADH and menaquinone/ menaquinol, and a need for faster regeneration of oxidized electron carriers (Fig. 7). A similar mechanism may be involved in the light-induced increases in current in a photosynthetic \textit{Rhodopseudomonas} species isolated using amorphous iron(III) as an electron acceptor (45).

While the increase in lactate uptake due to PR could be predicted, the increase in the \textit{S. oneidensis} respiration rate was unexpected. Previous bioenergetic experiments with respiring \textit{E. coli} and mitochondria have shown that the membrane potential and the rate of respiration are inversely related (5, 28). In a regulatory loop known as respiratory control, increases in the membrane potential slow processes that transport protons out of the cell by increasing the energy required to pump protons across the membrane. As electron transport is linked to pumping of many protons (10 H⁺ pumped for 2 e⁻ transferred from NADH to oxygen) (41), a high membrane potential feeds back to limit respiration. However, if the net respiratory reaction is coupled to less proton pumping, respiratory control would not be expected to have such a powerful influence. For anaerobic growth of \textit{S. oneidensis}, the 4-electron oxidation of lactate should not result in translocation of more than 2 net protons (2 H⁺ pumped for 2 e⁻ transferred from formate to menaquinone) (41), and the translocation could be less if protons are also used in import and export of solutes. The observation that \textit{S. oneidensis} does not exhibit respiratory control makes this bacterium an attractive platform for strategies combining electron transport and proton motive force generation and suggests further experiments to examine the role of the membrane potential of \textit{S. oneidensis} during anaerobic growth.

While the amount of electrical energy generated is smaller than the amount of light energy used to illuminate the electrode under laboratory conditions, our work shows that it is possible to integrate light-driven reactions into a nonphotosynthetic bacterium to alter survival and metabolism, with effects such as acceleration of electricity generation. In addition to increasing the rates of oxidation of carbon sources, light-induced proton gradients incorporated into engineered bacteria may be used to drive energy-demanding synthetic reactions for production of fine chemicals. More sophisticated light-converting systems, such as photosynthetic reaction centers, may further improve the efficiency and yield of light energy capture and conversion.

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