Supporting Information

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SI Text

Calibration Curves for H_2O_2 in CDM. Calibration curves for H_2O_2 were collected in the presence of a *Sg* biofilm. The conditions are described in detail in *Materials and Methods*. In order to be consistent with our real-time measurements, we strictly followed the same procedure and carried out the calibration curve measurements in the same way. The *Sg* biofilm was incubated in 1.5 mL CDM for at least 1 h before different aliquots of H_2O_2 solution (0.03%, v/v) were added into CDM solution to make H_2O_2 concentrations ranging from 0.06 mM to 1.6 mM. The H_2O_2 oxidation currents were measured by pulsing the Au ultramicroelectrode (UME) from 0.55 V to 0.80 V, 5 min after H_2O_2 was added each time. No glucose was added to the CDM so that *Sg* cells in the biofilm would not produce any extra H_2O_2 during calibration measurements.

Fig. S1 shows the results obtained in the presence of a *Sg* biofilm. A linear relationship ($I = -0.299C - 0.0069$, $R^2 = 0.99533$) between the current and H_2O_2 concentration was observed at H_2O_2 concentrations smaller than 1.0 mM (Fig. S2). As concentration goes above 1.0 mM, the curve levels off and deviates from the linear portion. Most of the H_2O_2 concentrations obtained in our real time in vitro measurements were within 1 mM, so we used the linear portion of the calibration curve to calculate H_2O_2 concentration (Fig. S2).

In order to test if our calibration curve was working properly, we intentionally added 0.5 mM H_2O_2 to a 1.5 mL *Sg* biofilm suspended in CDM in a Petri dish, and the current response was measured to be 0.15 nA, which corresponds to 0.5 mM based on the calibration curve in Fig. S2. This indicated that our calibration curve was indeed valid for our experiments.

Stability Tests. Stability is a very important issue since we normally need to run the experiments for a long duration. Because CDM is a defined culture medium with many components, and the temperature needs to be maintained at approximately 37 °C during the experimental process, in such conditions, the SECM tip (Au UME) is prone to contamination, and H_2O_2 tends to decompose as well. As a result, a current decay is usually observed as time proceeds. To make sure that our measurements were valid during this long duration, we carried out a series of stability tests.

Fig. S3 shows the results of stability tests performed in the absence of *Sg*. The current response for 0.2 mM H_2O_2 in 1.5 mL CDM with 10 mM glucose was measured as a function of time. As shown in Fig. S3, the H_2O_2 oxidation current decreases slowly with time. After 30 min, the current decreased only approximately 20%, which means that the tip has relatively good stability in low H_2O_2 concentration.

Another stability test was carried out in the presence of a *Sg* biofilm. A relatively high H_2O_2 concentration (0.6 mM) was tested this time. H_2O_2 current was measured every 5 min up to 35 min, and the result is shown in Fig. S4. The current of H_2O_2 decreased gradually with time. The rate for the current decay is faster (43% current drop in first 20 min) in the presence of *Sg* cells.

Effect of the Number of *Sg* Bacteria on H_2O_2 Current Response. We also tested to see if the amount of *Sg* cells would affect H_2O_2 measurements. In this experiment, 0.5 mM H_2O_2 was added to 1.5 mL CDM solution. The current response of 0.5 mM H_2O_2 was measured 10 min later each time when one aliquot of 0.5 mL of *Sg* suspension (2.5×10^8 *Sg* cells) was added into the above solution. To make the concentration of H_2O_2 constant during the measurements, extra H_2O_2 was added (28 μl 0.03% H_2O_2) each time to compensate the volume change due to the addition of 0.5 mL *Sg* suspension. A total of 4 aliquots of *Sg* suspension were added, and it was found that the current responses did not show any significant changes (Fig. S5). This indicated that the number of *Sg* did not significantly affect H_2O_2 detection.

Simulation. Because the electroactive species hydrogen peroxide or *P* moved toward and away from the electrode surface only because of a concentration gradient, Fick's second law of diffusion was used in the simulation. The concentration of species P was given as $C(r,z,t)$ and the diffusion equation in cylindrical coordinates was described as

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r} + \frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial z^2}\right)$$

where r and z are the coordinates as shown in Fig. S6; t represents time; C and D represent the concentration and diffusion coefficient of species P.

The simulation model described above was solved by a finite element method in 2D axial symmetry dimension where the mesh was increased in exponential grid fashion to generate a two-dimensional grid. A finer mesh distribution was used at the regions where sharp changes in the concentration gradients were noticed.

Initially the hydrogen peroxide concentration in the solution was zero and the flux in the bulk boundary was set as zero. The diffusion coefficient of hydrogen peroxide in the solution was taken as 1.5×10^{-9} m^2/s. The hydrogen peroxide generated from the biofilm surface was assumed to be a constant flux problem. Henceforth, the flux ($mol/m^2/sec$) value was adjusted at the biofilm surface to fit the experimental hydrogen peroxide response curve or hydrogen peroxide concentration vs. time (s) measured by SECM tip. The 25 μm tip (RG = 10) was located 100 μm away from the substrate to record hydrogen peroxide concentration and was held at diffusion controlled potential to avoid kinetic complications. The current at the electrode was calculated as

$$I_{\text{tip}} = \int_{r=0}^{r=a} 2\pi nFDr\frac{\partial c}{\partial z}dr$$

where $n = 1$; $F = 96485$ C/mol; and $D = 1.5 \times 10^{-9}$ m^2/s; a = tip radius, m. The current obtained by the simulation was converted to hydrogen peroxide concentration using the same calibration curve used in experimental hydrogen peroxide measurement.

Confocal Laser Scanning Microscopy. Cells were grown and prepared as described above, except for the replacement of *Aa* Y4 with a constitutive GFP-producing *Aa* Y4 strain. Membranes were stained in 3 mL of PBS containing 60 μM propidium iodide and incubated for 10 min prior to rinsing in PBS 3x at 3 mL each. Membranes were then resuspended in 3 mL PBS and observed through a 40x water immersion lens with an Olympus FV1000 confocal microscope. Excitation was performed using a 488 nm Ar laser and green and red channels were separated using standard filter settings. Micrographs were acquired at 400x magnification in 1 μm z-axis step sizes. Image processing was performed using Imaris software.

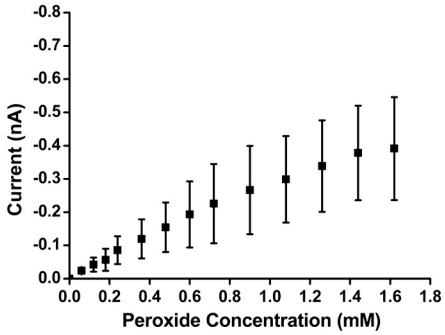

Fig. S1. Calibration curve for H_2O_2 with concentrations ranging from 0.06 ~ 1.6 mM in CDM with a *Sg* biofilm at 37 °C. Error bars were calculated based on three independent measurements.

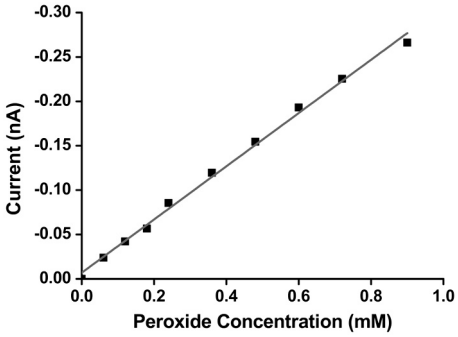

Fig. S2. Calibration curve for H_2O_2 over the concentration range from 0.06 ~ 1.0 mM in CDM with a *Sg* biofilm at 37 °C.

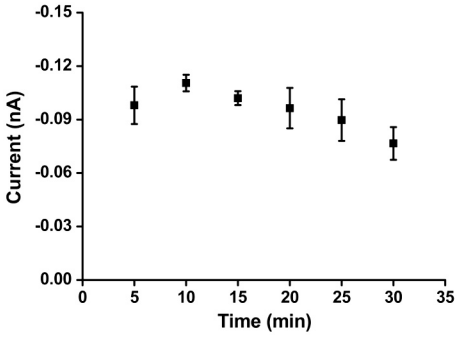

Fig. S3. Amperometric current of Au UME as a function of time in 0.2 mM H_2O_2 CDM solution at 37 °C. Error bars were calculated based on at least three independent measurements.

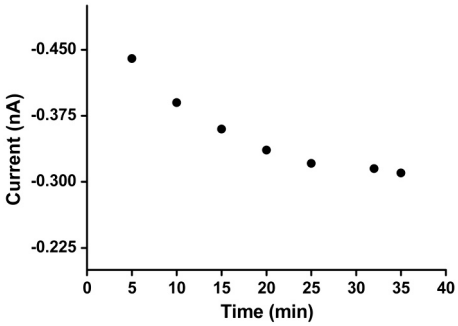

Fig. S4. Current response of 0.6 mM H_2O_2 as a function of time in the presence of a *Sg* biofilm.

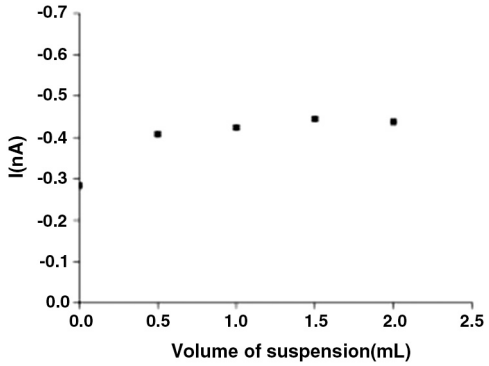

Fig. S5. Effect of *Sg* numbers on H_2O_2 detection. 1.0 mL of *Sg* suspension contain 5×10^8 *Sg* cells.

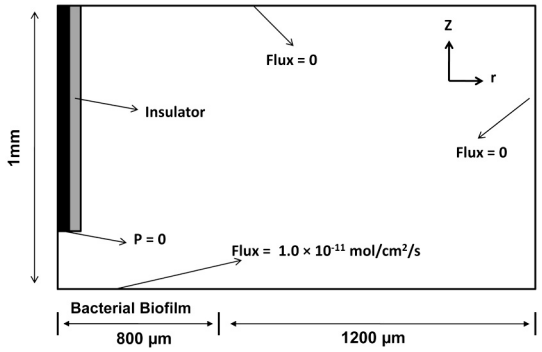

Fig. S6. The schematic diagram of Comsol Multiphysics model used for simulation.

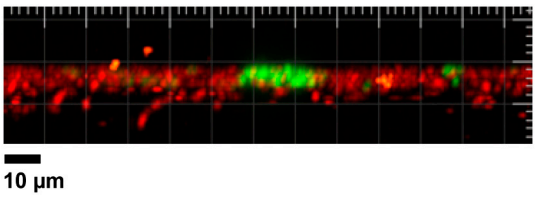

Fig. S7. Confocal laser scanning micrograph of a mixed species biofilm. *S. gordonii* (red) and *Aa* (green) were cocultured in a colony biofilm. Green and red channel fluorescence are due to *Aa* GFP expression and propidium iodide staining respectively. Images were taken at 400x magnification with a z-step interval of 1 μm.