

# Preservation of *Geobacter sulfurreducens* at Room Temperature

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## Introduction

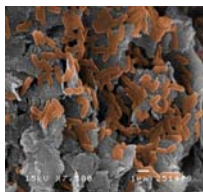


Figure 1. *G. sulfurreducens* colonizing on an electrode surface. (Bond)

### What is *G. sulfurreducens*?

*G. sulfurreducens* is an electricity producing bacterium, used in applications varying from bacterial fuel cells and microbial biosensors to catalytic surfaces for bioremediation. Successful development of such applications requires stabilization and preservation of *G. sulfurreducens* as thin films on surfaces.

### How does *G. sulfurreducens* produce electricity?

In the production of ATP, *G. sulfurreducens* oxidizes acetate or hydrogen compounds, producing CO<sub>2</sub> and electrons as byproducts. The electrons are transported through the cell membrane by protein electron carriers and dispersed by pili "nanowires" onto an electron acceptor (Fig 1). In a fuel cell, electrons are donated to an anode where they flow through a resistor to a cathode. The cathode is submerged in water with dissolved oxygen. The electrons are stripped from the cathode by an oxygen-cation reaction, which produces water. Currently, 0.5 W/m<sup>2</sup> of power can be produced.

### Why are we interested?

We are interested in preserving the fuel cells at room temperature. Therefore, in this project we aim at quantifying the desiccation response of *G. sulfurreducens*. We hypothesize that membrane phase transition is the most prominent stress affecting the viability of *G. sulfurreducens* during desiccation and re-hydration. We test this hypothesis using a fluorescence-based viability assay and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Cell membranes can transition between the liquid crystalline and gel phases when exposed to a change in temperature or level of hydration (Fig 2). The membrane phase transition temperature ( $T_m$ ) is the temperature at which the phase change rate is the highest. Cell membranes going through a phase transition can experience packing defects, which could cause the membrane to become leaky [1]. It is hypothesized that osmolytes suppress the change in  $T_m$  by replacing the water molecules between the lipid head groups [2], and therefore eliminate membrane phase transition, and the accompanying leakage upon re-hydration [1].

Previous studies showed that many types of bacteria have increased post-desiccation survival if they are dried in the presence of osmolytes such as trehalose, sucrose, or glycerol [3]. For example; *Bacillus thuringiensis* experience a significant reduction in  $T_m$  when dried in the presence of sucrose [4]. *Saccharomyces cerevisiae* also experiences a reduction in  $T_m$  when dried in the presence of trehalose [4]. However, addition of disaccharides to the media the bacteria is desiccated in also increases the extracellular osmotic stress. Studies with *E. coli* show that increasing osmotic stress in the presence of glycerol can increase  $T_m$  [5].

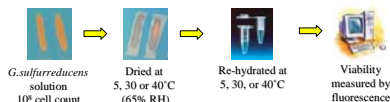


Figure 2. Molecular dynamics simulations of two different membrane phases: gel and liquid crystalline. (www.physik.uni-bielefeld)

## Methods

### Viability Assay Measurement

*G. sulfurreducens* were dried and re-hydrated in an anaerobic glove bag at 5, 30, or 40°C and 65% relative humidity (RH). In the experiments, 10 µl of *G. sulfurreducens* solution was deposited on precut micro slides and dried. Post re-hydration viability was determined using a fluorescence based membrane permeability (Live/Dead) assay.



### FTIR Analysis of the Membrane Phase Change

IR spectra of *G. sulfurreducens* was collected using a Thermo-Nicolet 6700 FTIR spectrometer while the temperature was incremented. The fluidity of the membrane was monitored by observing the change in peak position (Fig 3) of the symmetric CH<sub>2</sub> stretch of the membrane lipids with respect to temperature.

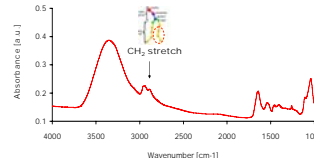


Figure 3. FTIR spectra for *G. sulfurreducens*

In order to apply the 65% RH and 5°C condition an environmental chamber was constructed and placed inside the glove bag. A peltier (thermo electric cooling device) was used to cool the desiccating bacteria solution to 5°C. Psychrometric analysis was conducted to adjust the conditions in the environmental chamber.

## Results

### Osmotic Stress Decreases Viability

In order to explore the effects of acute osmotic stress on *G. sulfurreducens* viability, bacteria were suspended in 4 different sucrose solutions (0, 6.75%, 13.5%, 27% w/w). The viability was measured without drying (Table 1) the bacteria. It was found that media and medium containing 6.75% sucrose had approximately the same viability. However, with increasing extracellular sucrose concentration, the viability decreased significantly.

Table 1. *G. sulfurreducens* viability (hydrated) at different sucrose concentrations

Sucrose [%w/w]	Osmolarity mOsm	Viability [%]
0 (n=4)	300	100 ± 0
6.75 (n=4)	500	95 ± 8.9
13.5 (n=2)	700	64 ± 1.6
27 (n=3)	1100	40 ± 6.5

### Membrane Phase Change Affects Viability

*G. sulfurreducens* dried for 45 minutes in media, or in a medium containing 6.75% w/w sucrose were re-hydrated either at 5, 30, or 40°C (to ensure or eliminate membrane phase transition during de-hydration) (Fig 4). During drying, the  $T_m$  increased from 21°C to 35°C in media or medium containing 6.75% sucrose dried for 45 mins (Fig 4).

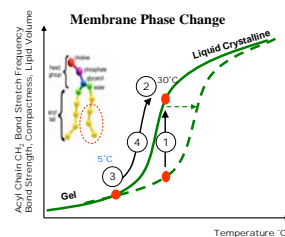


Figure 4. The CH<sub>2</sub> stretch versus temperature, and corresponding  $T_m$  for different conditions. Red dots indicate the state of the membrane during desiccation; solid arrows indicate re-hydration; dashed arrow indicates shift in membrane phase behavior when dried.

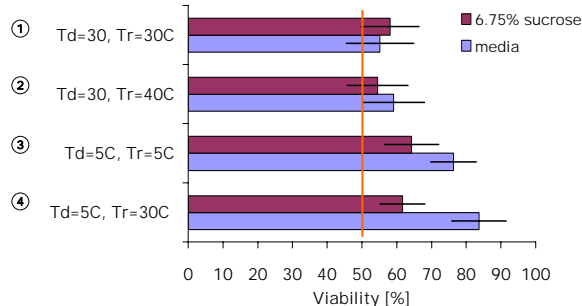


Figure 5. Post re-hydration viability of *G. sulfurreducens* dried for 45 min at 65% RH. ( $T_d$ : drying temperature,  $T_r$ : re-hydration temperature)

## Discussion

### *G. sulfurreducens* is Sensitive to Extracellular Osmotic Stress

Results showed that sucrose did not have any interaction with the cell membrane. In fact, the viability of the bacteria decreased with the addition of sucrose, these results can be seen in Table 1. This decrease in viability is attributed to the extracellular osmotic stress on the cell membrane due to the addition of sucrose. The decrease in viability is thought to be due to the morphological changes [5] as a result of dehydration. Since the osmotic stress is minimum in the case of media, viability is always higher for *G. sulfurreducens* dried in media compared to sucrose.

### Sucrose Did Not Suppress Membrane Phase Transition Temperature

FTIR analysis of the cell membrane showed that *G. sulfurreducens* dried at 30°C increased  $T_m$  from 21°C to 35°C. Hence, during drying at 30°C the membrane transitioned from the liquid crystalline to the gel phase, and then back to a liquid crystalline phase during re-hydration. *G. sulfurreducens* dried at 5°C (which corresponds to the gel phase) only experienced membrane phase transition during re-hydration. Minimizing the membrane phase transitions increased viability, probably due to limiting the membrane defects [5]. Re-hydration temperatures of the desiccated bacteria were also investigated, but the results showed that they had little effect on viability. This points to the importance of desiccation conditions, as opposed to re-hydration conditions on viability.

## Conclusions

1. *G. sulfurreducens* is susceptible to increasing concentrations of extracellular sucrose prior to desiccation. Addition of sucrose over 6.75% w/w results in a drastic decrease in viability. During desiccation, presence of extracellular sucrose does not increase the post re-hydration viability of *G. sulfurreducens*. It decreases it further possibly due to the additional osmotic stress.

2. *G. sulfurreducens* dried at 5°C had significantly higher viability compared to 30 or 40°C drying and post re-hydration temperatures. This shows that the membrane phase transition during drying (as opposed to re-hydration) has a more pronounced effect on the viability of the bacteria.

## References

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