

Analysis of the Prevention of Biocorrosion Caused by *Desulfovibrio alaskensis* G20

2017

Michael Boring
University of Central Florida

Find similar works at: <http://stars.library.ucf.edu/honorsthesis>

University of Central Florida Libraries <http://library.ucf.edu>

 Part of the [Microbial Physiology Commons](#)

Recommended Citation

Boring, Michael, "Analysis of the Prevention of Biocorrosion Caused by *Desulfovibrio alaskensis* G20" (2017). *Honors in the Major Theses*. 263.

<http://stars.library.ucf.edu/honorsthesis/263>

This Open Access is brought to you for free and open access by the UCF Theses and Dissertations at STARS. It has been accepted for inclusion in Honors in the Major Theses by an authorized administrator of STARS. For more information, please contact lee.dotson@ucf.edu.

ANALYSIS OF THE PREVENTION OF BIOCORROSION CAUSED BY
DESULFOVIBRIO ALASKENSIS G20

by

Michael Paul Boring

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biomedical Sciences
in the College of Medicine
and in the Burnett Honors College
at the University of Central Florida
Orlando, FL

Spring Term, 2017

Thesis Chair: William Self, Ph.D.

Abstract

Desulfovibrio alaskensis G20 and other sulfate-reducing bacteria cause significant damage to metal pipelines and other infrastructure through a metabolic pathway that releases toxic hydrogen sulfide into their surroundings. The biocorrosion that results from the release of hydrogen sulfide creates significant economic burden, and can pose health risks for those exposed to this chemical. They are commonly present in the form of biofilms, an extracellular matrix composed of bacterial cells, polysaccharides, proteins, nucleic acids, and other materials. These biofilms are difficult to remove, and they provide protection to the bacteria within from anti-bacterial treatments. *Desulfovibrio alaskensis* G20 is a strain derived from a wild-type bacterium collected from an oil well corrosion site and is a model organism for understanding biofilm formation of sulfate-reducing bacteria and how these biofilms can be prevented or inhibited by techniques such as cerium oxide nanoparticle coating. To this end, samples of *Desulfovibrio alaskensis* G20 were grown anaerobically in 24-well and 96-well plates, and the resultant biofilm growth was measured through spectrophotometry. Several different environmental parameters were tested, including temperature, electron donor molecules, basal and enriched growth media, and oxidative stress, revealing several affinities for production of biofilm growth.

Acknowledgements

I would like to express my deepest gratitude to those whose contributions made the completion of this thesis possible. My thanks to Dr. Sudipta Seal and Dr. Sean Moore for graciously donating their time to serve on my committee. My thanks also go to Dr. William Self, for your guidance and patience throughout this project, for your mentorship, for the use of your lab, and for serving as the Committee Chair for this thesis. Finally, to my friends, who have time and again gone out of their way to encourage and support me, and to my family, who have been my greatest supporters, have always been there for me and are a constant source of love, wisdom, and strength, I could not be any more grateful.

“So God has given both his promise and his oath. These two things are unchangeable because it is impossible for God to lie. Therefore, we who have fled to him for refuge can have great confidence as we hold to the hope that lies before us. This hope is a strong and trustworthy anchor for our souls.” Hebrews 6:18-19

Table of Contents

Introduction.....	1
Biofilms.....	1
Sulfate-Reducing Bacteria and Corrosion.....	1
<i>Desulfovibrio alaskensis</i> G20.....	2
Cerium Oxide	2
Materials and Methods.....	4
Growth Media and Culture Cultivation.....	4
Establishment of Biofilm	4
Measuring Biofilm Density.....	5
Results.....	7
Biofilm Experiments	7
24-Well Biofilm Experiment.....	8
96-Well Biofilm Experiment.....	15
Discussion.....	27
References.....	30

List of Tables

Table 1: Statistically Significant Data Points	27
--	----

List of Figures

Figure 1: The Effect of Varying Levels of H ₂ O ₂ in MO Media on Biofilm Density	8
Figure 2: The Effect of Varying Levels of H ₂ O ₂ in MOY Media on Biofilm Density.....	9
Figure 3: Biofilm Density with Varying Levels of H ₂ O ₂ at 25 Degrees Celsius.....	10
Figure 4: Biofilm Density with Varying Levels of H ₂ O ₂ at 37 Degrees Celsius.....	11
Figure 5: Biofilm Density with Glucose as an Electron Donor	12
Figure 6: Effect of Alternate Electron Donors on Biofilm Density at 25 Degrees Celsius	13
Figure 7: Effect of Alternate Electron Donors on Biofilm Density at 37 Degrees Celsius	14
Figure 8: Biofilm Density Using MO Media with Pyruvate.....	15
Figure 9: Biofilm Density Using MOY Media with Pyruvate.....	16
Figure 10: Biofilm Density Using MO Media with Glucose.....	17
Figure 11: Biofilm Density Using MOY Media with Glucose.....	18
Figure 12: Effect of Media on Biofilm Density with Pyruvate at 25 Degrees Celsius.....	19
Figure 13: Effect of Media on Biofilm Density with Pyruvate at 37 Degrees Celsius.....	20
Figure 14: Effect of Media on Biofilm Density with Glucose at 25 Degrees Celsius.....	21
Figure 15: Effect of Media on Biofilm Density with Glucose at 37 Degrees Celsius.....	22
Figure 16: Effect of Electron Donor on Biofilm Density in MO Media at 25 Degrees Celsius...	23
Figure 17: Effect of Electron Donor on Biofilm Density in MO Media at 37 Degrees Celsius...	24
Figure 18: Effect of Electron Donor on Biofilm Density in MOY Media at 25 Degrees Celsius	25
Figure 19: Effect of Electron Donor on Biofilm Density in MOY Media at 37 Degrees Celsius	26

Introduction

Biofilms

A biofilm is a population of cells growing on a surface and is a type of sessile cell community. Biofilms can be found on a variety of abiotic and biotic surfaces, such as metal surfaces, river stones, and human teeth. Microbial cells within biofilm structures are surrounded by extracellular polysaccharides (EPS), nucleic acids, proteins, and other materials, and are resistant to a variety of antimicrobial treatments. For this reason, it is difficult to kill microbial cells within a biofilm and remove biofilm structures. [1]

Sulfate-Reducing Bacteria and Corrosion

Approximately 10% of all corrosion to metals and non-metals are thought to be the result of microbial activities. One significant example of microbial-caused corrosion is the anaerobic corrosion of iron and steel. [2] This corrosion is largely due to sulfate-reducing bacteria, which are anaerobic microbes commonly found in nature. Sulfate-reducing bacteria generate energy in the form of ATP through electron transfer–coupled phosphorylation. To accomplish this, sulfate-reducing bacteria generally use sulfate as the terminal electron acceptor for anaerobic respiration, and they can use hydrogen, various organic acids, and sugars as electron donors. This sulfate reduction pathway results in the production of hydrogen sulfide as an end-product, which is very toxic and reactive. These bacteria are especially active in sulfate-rich environments, such as marine environments. Sulfate-reducing bacteria also have a significant economic impact due to their involvement in biocorrosion of ferrous metals in anaerobic environments. [3]

This corrosion by sulfate-reducing bacteria is a particular concern for the petroleum industry, as it can intensify the spoilage of pipelines, pumping and drilling machinery, and metal tanks, causing significant economic consequences. One study has estimated that 34% of the corrosion damage experienced by an oil company was caused by microbial biocorrosion. [4] The hydrogen sulfide product of sulfate-reducing bacteria is highly corrosive to iron, steel and other alloys, causing the observed corrosion. In addition, hydrogen sulfide causes souring of petroleum, plugging of machinery and rockpores, and is a potential health hazard. [3, 5] Sulfate-reducing bacteria are abundant in oil fields, with *Desulfovibrio* species being the primary microorganisms involved in this corrosion process. With increasing industry demands, it is necessary that the materials used in these structures be better able to deal with corrosion from microorganisms such as *Desulfovibrio*. [5, 6]

***Desulfovibrio alaskensis* G20**

Desulfovibrio alaskensis G20, like other *Desulfovibrio* species, is a sulfate-reducing bacterium that can form biofilms. It is a derivative of a wild-type strain, G100A, which was isolated from an oil well corrosion site, and it is capable of rapidly corroding mild steel. [6] These properties therefore make this strain a model organism for studying corrosion of steel from biofilms of sulfate-reducing bacteria. [5, 6]

Cerium Oxide

Cerium is a rare-earth metal that can form cerium oxide (CeO₂) nanoparticles, which have been used in a variety of biomedical purposes. These nanoparticles have high surface area-to-volume ratios and have, in some studies, been shown to have antibacterial properties, affecting multiple

structures and metabolic pathways of microorganisms. [7, 8] Not all studies, though, show inhibition of microbial growth by cerium oxide. [7] Cerium oxide coating has also shown some ability to combat corrosion in steel. [9]

We hypothesize that cerium oxide-coated steel will restrict the formation of *Desulfovibrio alaskensis* G20 biofilm and corrosion of steel compared to uncoated steel by restricting the metabolic pathway that produces hydrogen sulfide. This hypothesized restriction of corrosion of steel pipes caused by *Desulfovibrio* species would be of significant value to multiple industries, especially to the petroleum industry.

Materials and Methods

Growth Media and Culture Cultivation

Samples of wild-type *Desulfovibrio alaskensis* G20 were obtained from the library collection at the University of Missouri – Columbia. These stocks were stored in a freezer at -80 degrees Celsius.

Cultivation of *D. alaskensis* G20 was accomplished using a MO basal salt media. [10] This media contains the following components: magnesium chloride (8mM), ammonia chloride (20mM), calcium chloride (0.6mM), ferric chloride (125mM)/EDTA (250mM) (0.06mM/0.12mM final concentration), Tris-HCl pH 7.4 (30mM), Thauers Vitamins 10X, [11] a sodium phosphate-potassium phosphate buffer (2mM), and a solution of trace elements. These trace elements include the following: manganese, cobalt, zinc, molybdenum, boron, nickel, copper, selenium, and tungsten. After the addition of all reagents, the solution was autoclaved.

The cultures were grown through the addition of 250 μ L of stock *D. alaskensis* G20 into 5 mL of MO basal salt media. Incubation of the cultures occurred in an anaerobic chamber at 37 degrees Celsius for 48 hours.

Establishment of Biofilm

Six 24-well plates were inoculated using 100 μ L of *D. alaskensis* G20 culture in 2 mL of fresh growth media. A Fisher Scientific 12 mm borosilicate glass slide coverslip was also added to each well. Four different growth media were used, one of which was the same MO basal salt media described above. The second growth media used was an enriched MOY media including 1g/L yeast extract. The third growth media was a variation of the MO basal salt media, using 8.5

mM dextrose in place of pyruvate as an alternative electron donor and carbon source, [12] and the fourth media was the enriched MOY media with 8.5 mM dextrose.

Additional variables in the growth of biofilm included oxidative stress and temperature.

Hydrogen peroxide is commonly used as an antimicrobial, [13] yet it is also known that

oxidative stress from low-concentration hydrogen peroxide can enhance biofilm formation. [14]

To test the reaction of *D. alaskensis* G20 to various levels of oxidative stress, hydrogen peroxide was added to samples of the MO basal salt media and MOY media in concentrations of 0.1 mM, 0.5 mM, and 1.0 mM, in addition to controls containing no hydrogen peroxide. Half of the plates were incubated at 37 degrees Celsius, while the other half were incubated at 25 degrees Celsius.

Two 96-well plates were also inoculated with each of the four types of growth media. Each type of growth media contained hydrogen peroxide at concentrations of 0 mM, 0.1 mM, 0.5 mM, and 1.0 mM. One plate was incubated at 37 degrees Celsius, while the other plate was incubated at 25 degrees Celsius. Wells with growth media only served as negative controls for the experiment.

Measuring Biofilm Density

For the 24-well plates, the media was pipetted out of each well with micropipette set at 1 mL.

Each well was washed with 1 mL of dH₂O three times using a micropipette. 400 µL of crystal violet was added to each well to stain the biofilm growth. After ten minutes, each well was

washed with dH₂O an additional three times with a micropipette. The plates were then left to dry for 24 hours. [15]

For the 96-well plates, the media is tapped out of the wells, and each plate is wholly submerged in water three times. 125 μ L of 0.1% crystal violet was added to each well, and the plates were incubated for ten minutes. The plates were then submerged in water an additional three times to wash away excess crystal violet and were then left to dry for 24 hours.

Each coverslip from the 24-well plates was transferred to a new 24-well plate using sterile tweezers. 400 μ L of 30% acetic acid was added to each well to solubilize the contents of each well. After incubating for 15 minutes, a micropipette was used to mix the contents of each well. After mixing, 125 μ L from each well was transferred to a new, 96-well plate. The 96-well plate was then read at a wavelength of 550 nm in a plate reader spectrophotometer. 30% acetic acid was used as the comparison for data. [15]

125 μ L of 30% acetic acid was added to each well of the 96-well plates. After a 15 minute incubation time, 125 μ L was transferred from each well into a new 96-well plate. These new plates were then also read at a wavelength of 550 nm in a plate reader spectrophotometer, with 30% acetic acid as the comparison for data. [15]

Results

Biofilm Experiments

Our hypothesis is that cerium oxide-coated steel will restrict biofilm growth of *Desulfovibrio alaskensis* G20 and inhibit corrosion of steel by restricting the metabolic pathway of *D. alaskensis* G20 that produces hydrogen sulfide. Our lab was unable to obtain cerium oxide-coated glass cover slips to test this hypothesis; however, we were able to complete biofilm experiments to test the overall ability of this organism to form biofilms on glass under various conditions.

In order to establish the baseline for dye binding to the glass coverslips, 16 wells were filled with 30% acetic acid and read at 550 nm in the spectrophotometer as a control for presence of biofilm formation. The results of the acetic acid wells are as follows: average=0.0363, standard deviation 0.0028, and 95% confidence interval=0.0056. All experimental data points were above the acetic acid average, indicating some level of biofilm growth. The average, standard deviation, and 95% confidence interval for each experimental parameter were calculated.

24-Well Biofilm Experiment

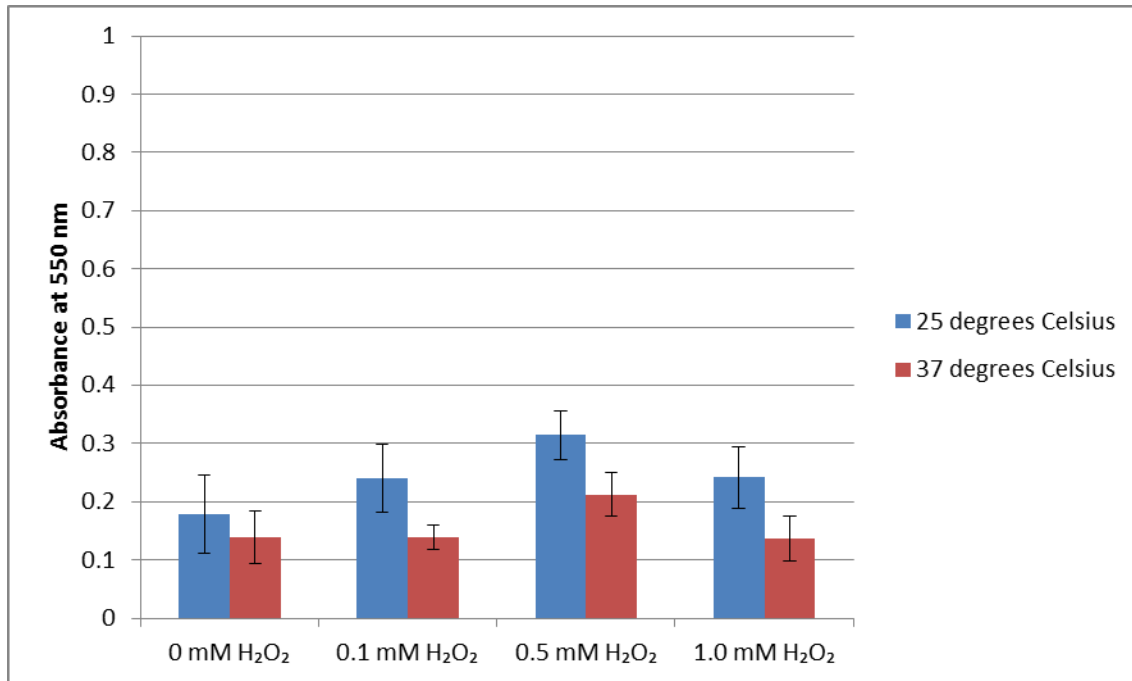


Figure 1: The Effect of Varying Levels of H₂O₂ in MO Media on Biofilm Density

Temperature is an important factor for understanding the conditions at which *D. alaskensis* G20 produces maximum biofilm growth. At all four concentrations of hydrogen peroxide, the average biofilm growth was greater when *D. alaskensis* G20 was grown at 25 degrees Celsius. The increased growth, moreover, was statistically significant with respect to a 95% confidence interval, indicated by the error bars in Figure 1 above, at concentrations of 0.1 mM, 0.5 mM, and 1.0 mM. Cultures grown with 0.5 mM hydrogen peroxide showed the greatest average biofilm density at both temperatures.

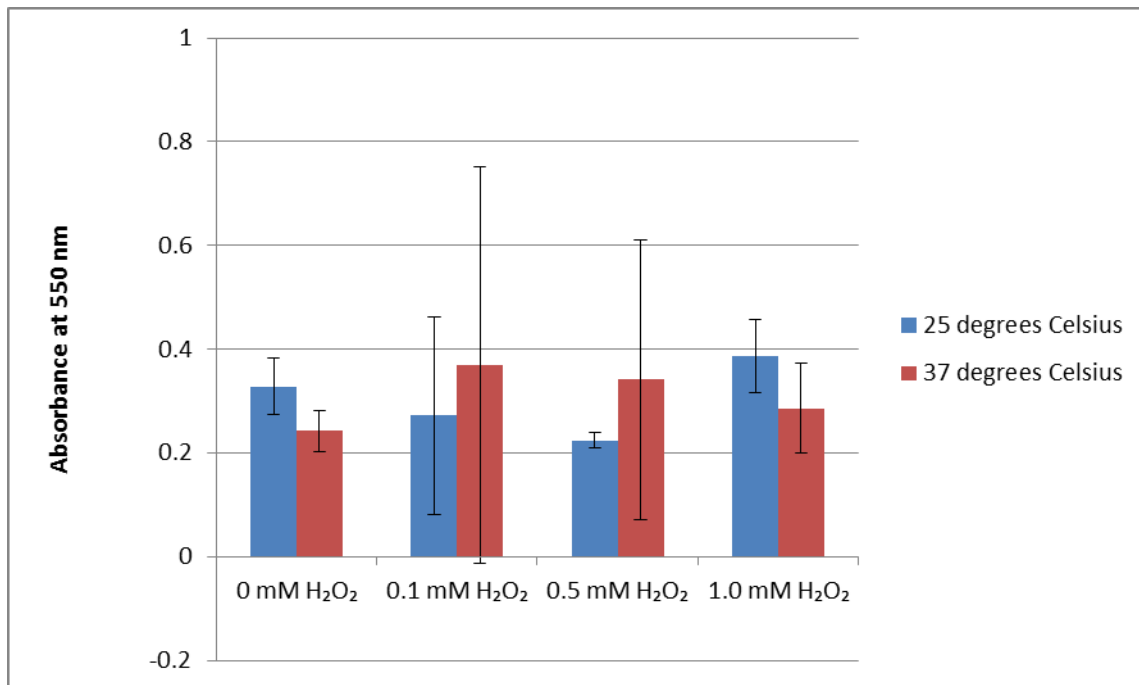


Figure 2: The Effect of Varying Levels of H₂O₂ in MOY Media on Biofilm Density

Temperature was the variable in Figure 2, as well, with data for growth in MOY media showed varied results. While greater biofilm density was present at 25 degrees Celsius in 0 mM H₂O₂ and 1.0 mM H₂O₂, greater biofilm density was present at 37 degrees Celsius in 0.1 mM H₂O₂ and 0.5 mM H₂O₂. None of these results were statistically significant, though, indicating that there is not a substantial difference between the two incubation temperatures when using enriched yeast extract MOY media.

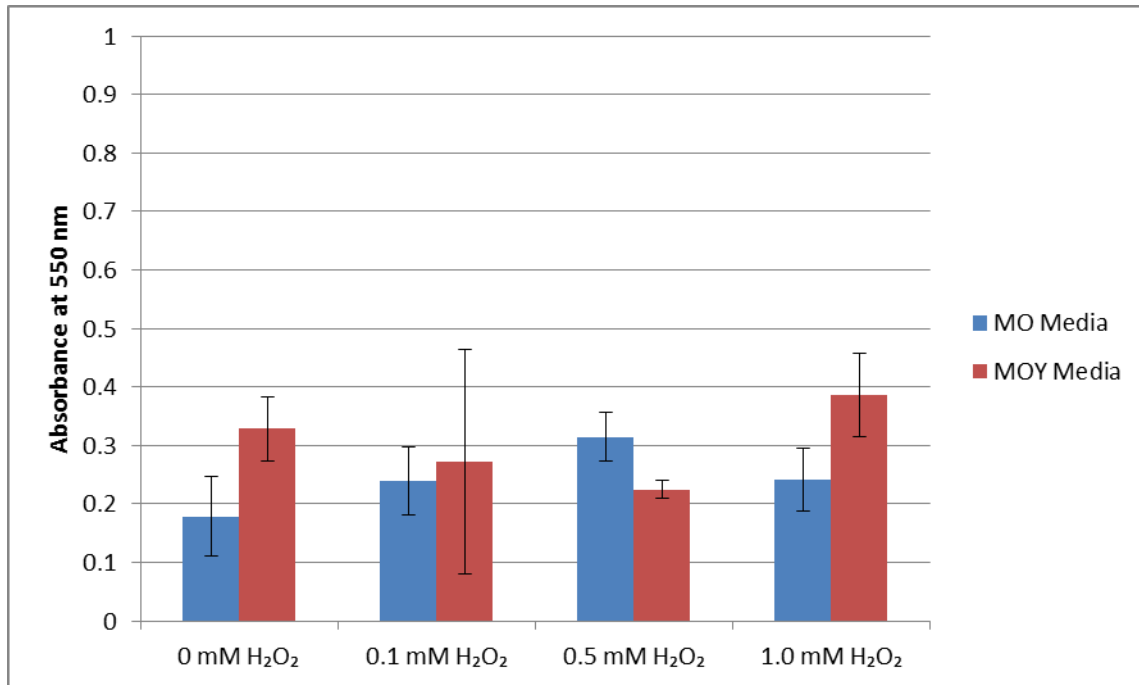


Figure 3: Biofilm Density with Varying Levels of H₂O₂ at 25 Degrees Celsius

It is also important to understand the effects that an enriched media may have on biofilm growth compared to a minimal media, whether an enriched media would promote biofilm growth, or if the stress of growing in a minimal media would better encourage biofilm formation. The samples grew with greater biofilm density in MOY media at all concentrations of hydrogen peroxide except 0.5 mM. This was statistically significant in the 0 mM and 1.0 mM concentrations; however, MO media showed a statistically significant greater density of biofilm growth than MOY at 0.5 mM. It is possible that very low concentration hydrogen peroxide may interact with *D. alaskensis* G20 in MO media to signal greater biofilm growth, while higher concentrations of hydrogen peroxide have the opposite effect.

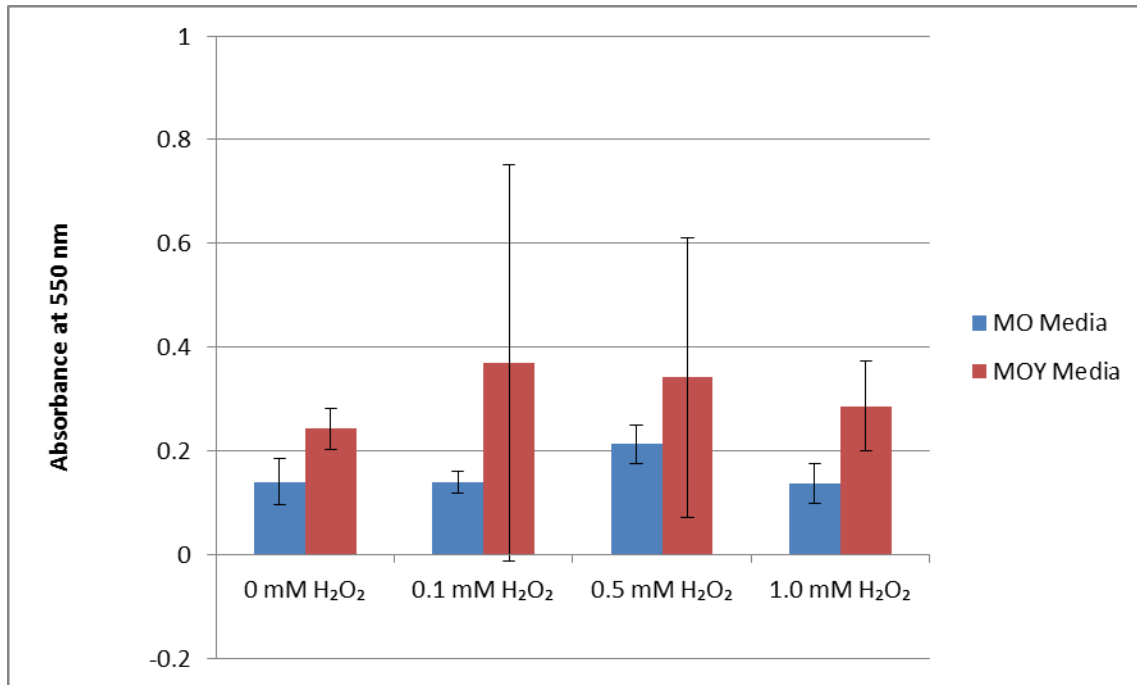


Figure 4: Biofilm Density with Varying Levels of H₂O₂ at 37 Degrees Celsius

The same variable was tested at an incubation temperature of 37 degrees Celsius. The result of this was that *D. alaskensis* G20 grew better, on average, in MOY media compared to MO media across all tested concentrations of hydrogen peroxide. At 0 mM H₂O₂ and 1.0 mM H₂O₂, the increased growth was significant on a 95% confidence interval, indicating a substantial preference for MOY media for biofilm production.

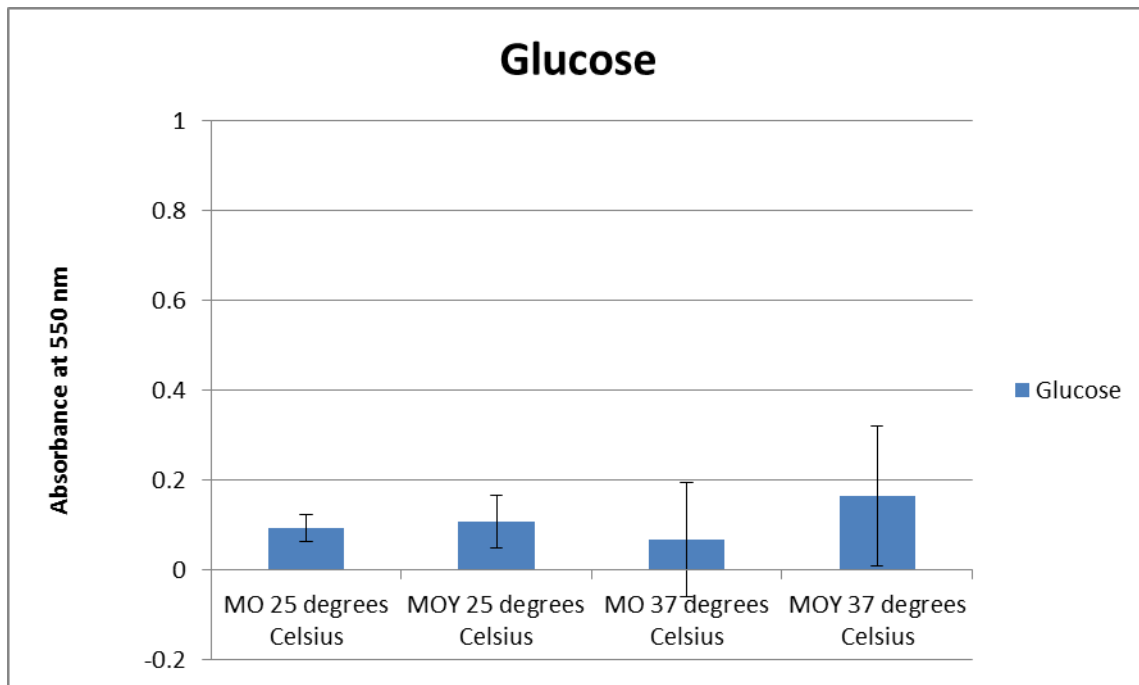


Figure 5: Biofilm Density with Glucose as an Electron Donor

Glucose was tested as an alternative electron donor in place of pyruvate to test the ability of *D. alaskensis* G20 to utilize a different substrate in its hydrogen sulfide-producing metabolic pathway. Biofilm growth using glucose as an alternative electron donor in place of pyruvate showed lower average biofilm density, as expounded upon in Figures 5 and 6, and no statistically significant features were present across media and temperature parameters for the glucose-grown samples.

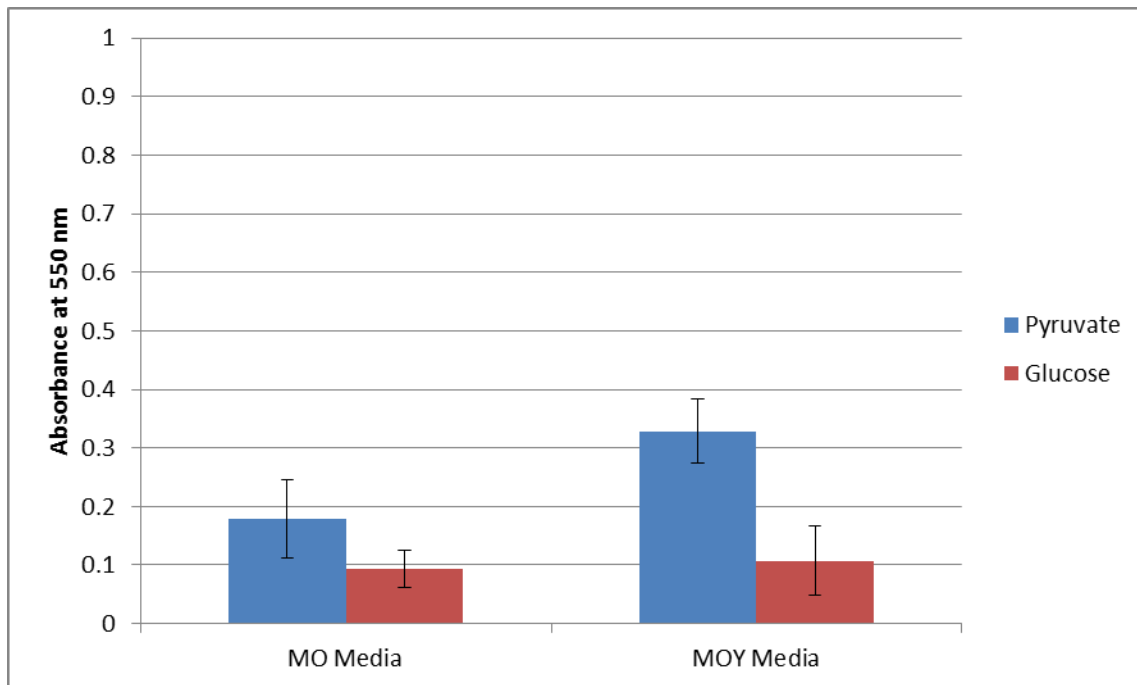


Figure 6: Effect of Alternate Electron Donors on Biofilm Density at 25 Degrees Celsius

Figure 6 shows greater average biofilm density in samples grown in media containing pyruvate as the electron donor than samples grown in media containing glucose at 25 degrees Celsius.

This difference is statistically significant in MOY media based on a 95% confidence interval.

This indicates that *D. alaskensis* G20 possibly has a reduced ability to utilize glucose as electron donor compared to pyruvate.

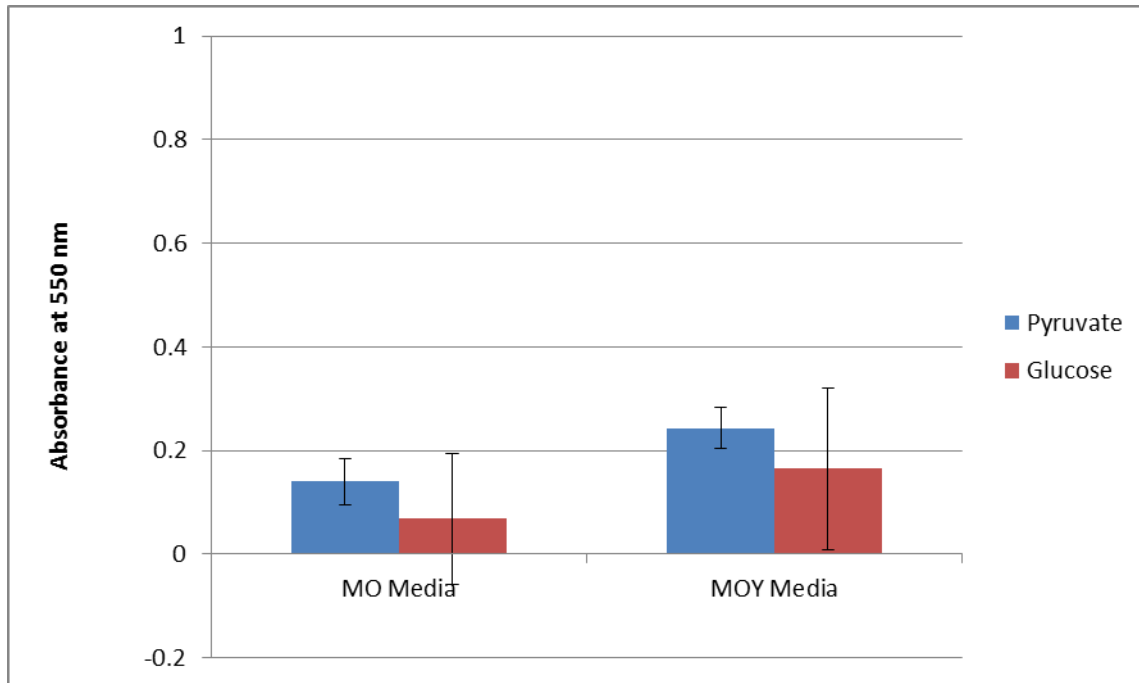


Figure 7: Effect of Alternate Electron Donors on Biofilm Density at 37 Degrees Celsius

Greater biofilm growth was also seen in samples grown at 37 degrees Celsius with pyruvate than glucose. However, these results are not statistically significant due to a large confidence interval for samples grown with glucose as the electron donor. Based on the average results, though, it would appear that pyruvate is the preferred electron donor for biofilm formation regardless of incubation temperature.

96-Well Biofilm Experiment

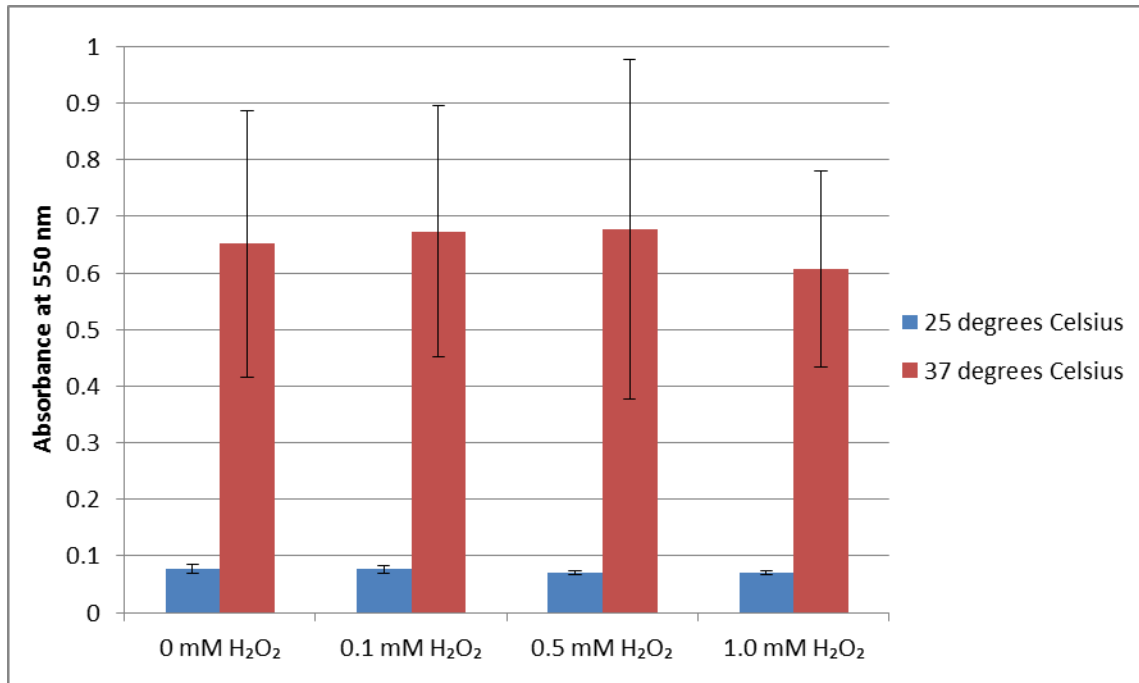


Figure 8: Biofilm Density Using MO Media with Pyruvate

As noted in the section above, temperature is a critical variable for understanding the conditions at which *D. alaskensis* G20 produces maximum biofilm growth. In contrast to the results obtained with the 24-well plates, there were statistically significant differences in biofilm density across hydrogen peroxide concentrations in MO media containing pyruvate, with the samples grown at 37 degrees Celsius having much greater biofilm density on average than samples grown at 25 degrees Celsius.

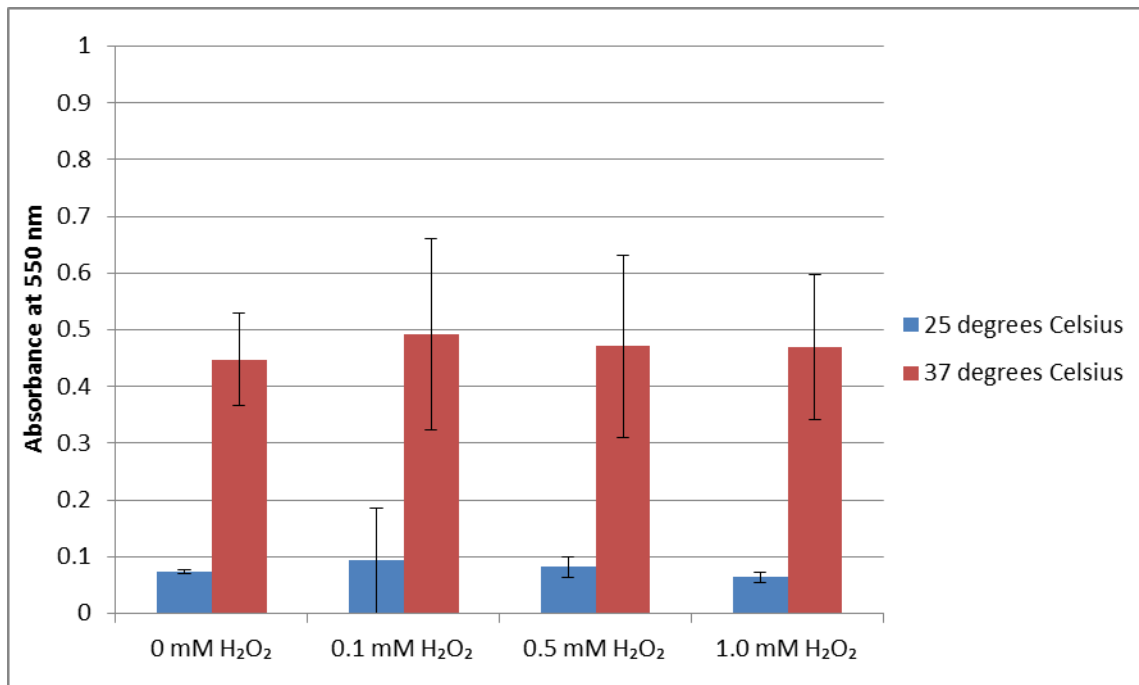


Figure 9: Biofilm Density Using MOY Media with Pyruvate

While examining samples grown in MOY media with pyruvate, there were statistically significant differences across hydrogen peroxide concentrations, with the samples grown at 37 degrees Celsius having much greater biofilm density on average than samples grown at 25 degrees Celsius. Having similar, statistically significant, results with regard to temperature between media types provides strong support for 37 degrees Celsius being a better temperature for producing biofilm growth and should be used in experiments testing inhibition of biofilm growth. This pattern continues below even when utilizing a different electron donor.

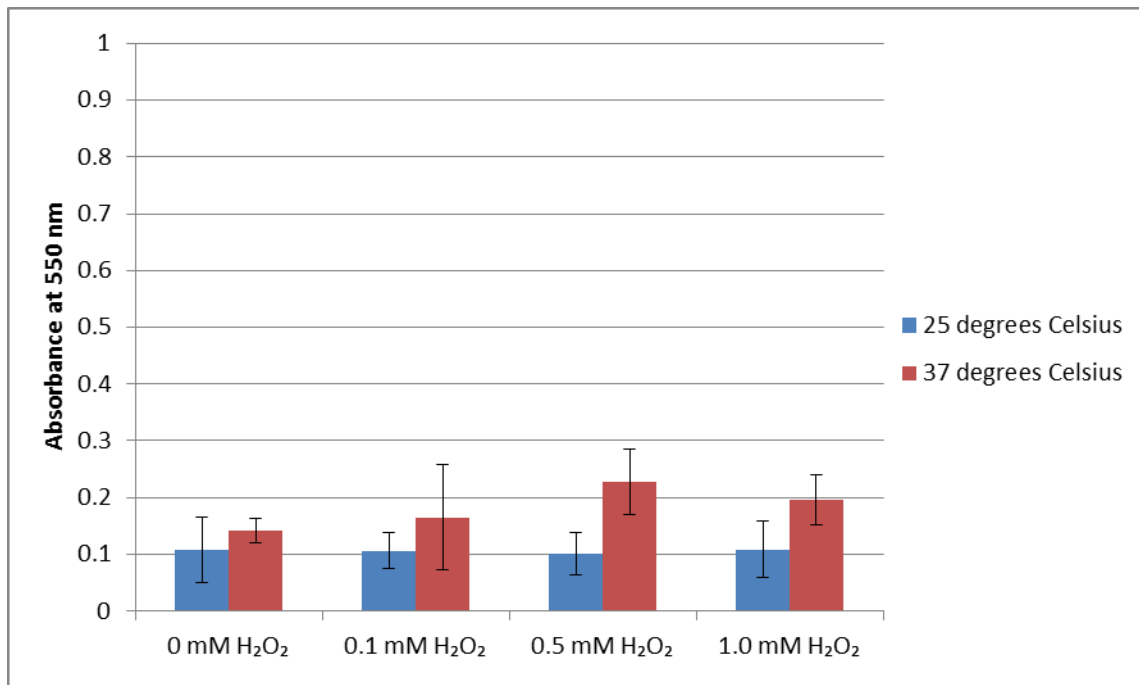


Figure 10: Biofilm Density Using MO Media with Glucose

When using glucose instead of pyruvate in MO media, the samples grown at 37 degrees had greater biofilm density on average than those grown at 25 degrees Celsius, consistent with the pattern observed above. Nevertheless, at only one concentration of hydrogen peroxide, 0.5 mM, was this increased value statistically significant.

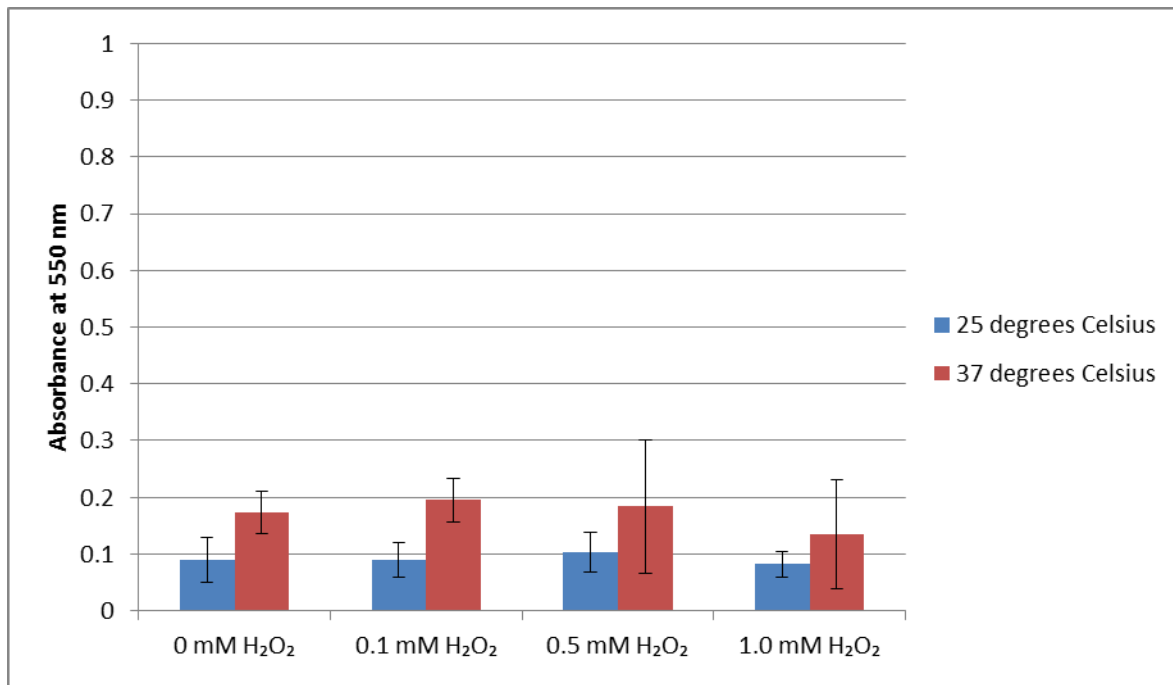


Figure 11: Biofilm Density Using MOY Media with Glucose

Likewise, when using glucose instead of pyruvate in MOY media, the samples grown at 37 degrees had greater biofilm density on average than those grown at 25 degrees Celsius. At two concentrations of hydrogen peroxide, 0 mM and 0.1 mM, was this increased value statistically significant. Combining the results from Figures 8 –11, it becomes apparent that incubation at 37 degrees Celsius produces greater biofilm density across growth media, electron donors, and hydrogen peroxide concentration. Conversely, incubation of samples at 25 degrees Celsius results in a lower degree of biofilm growth.

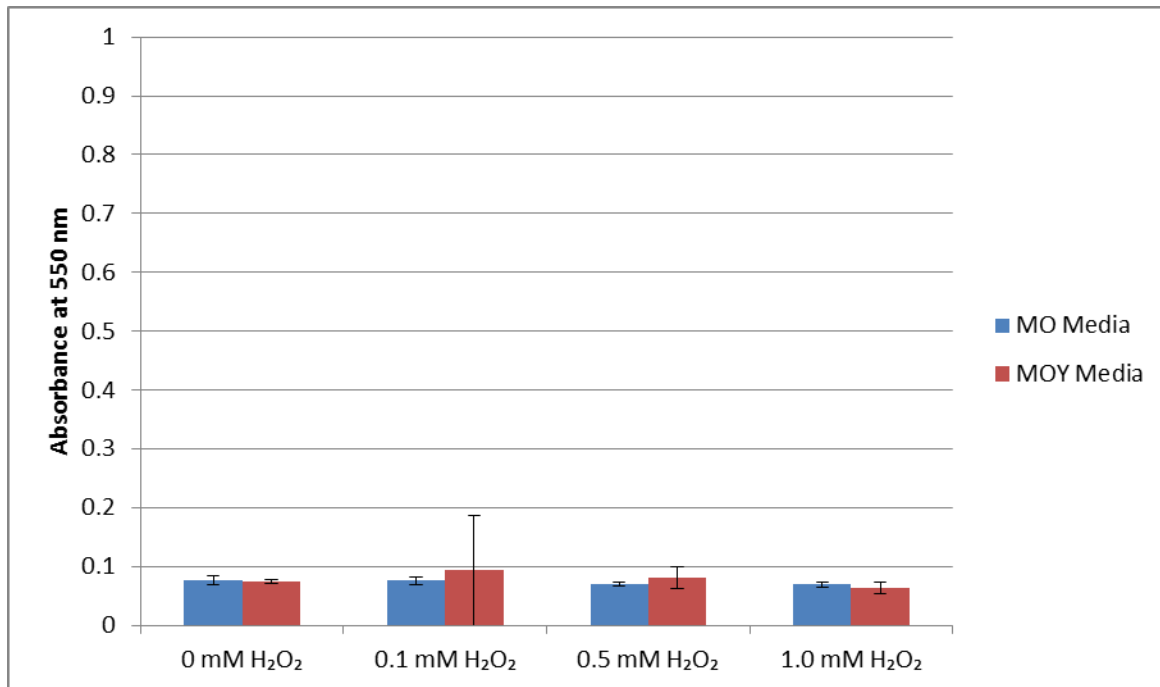


Figure 12: Effect of Media on Biofilm Density with Pyruvate at 25 Degrees Celsius

As noted in the 24-well results section, it is important to understand the effects that an enriched and minimal media may have on biofilm growth. Little difference was seen in the average biofilm density between MO and MOY media containing pyruvate at 25 degrees Celsius, with no statistically significant results. This result indicates that *D. alaskensis* G20 has little preference between the two types of media for production of biofilm, either having no effect or similar effect on biofilm inhibition or enhancement.

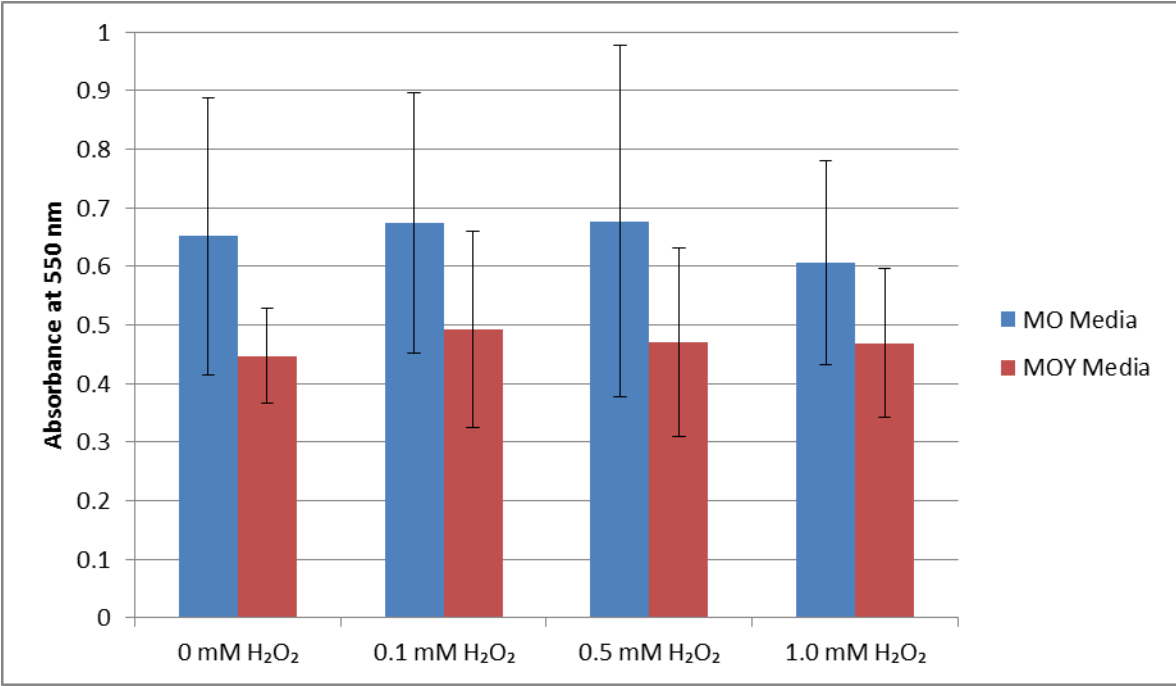


Figure 13: Effect of Media on Biofilm Density with Pyruvate at 37 Degrees Celsius

Similarly, Figure 13 shows that samples grown with pyruvate at 37 degrees Celsius are relatively uninfluenced in terms of biofilm density by choice of MO or MOY media. Samples grown in MO media have a higher average biofilm density than those grown in MOY media containing pyruvate. This difference, while consistent across hydrogen peroxide concentrations, is not statistically significant based on a 95% confidence interval.

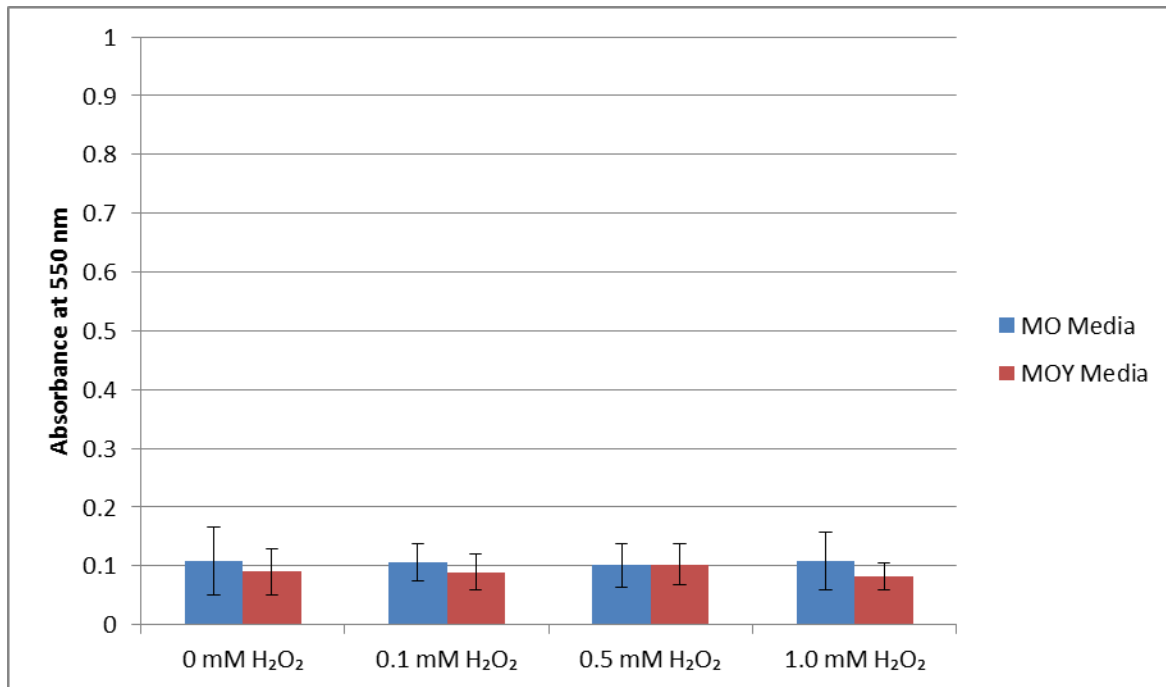


Figure 14: Effect of Media on Biofilm Density with Glucose at 25 Degrees Celsius

Using glucose instead of pyruvate, as in Figures 13 and 14, at 25 degrees Celsius resulted in a slightly higher average biofilm density for MO media-grown samples, compared to biofilm density for MOY media-grown samples. These results were not statistically significant, however, indicating that a change in electron donor does not have a substantial impact on the effect of choice of growth media.

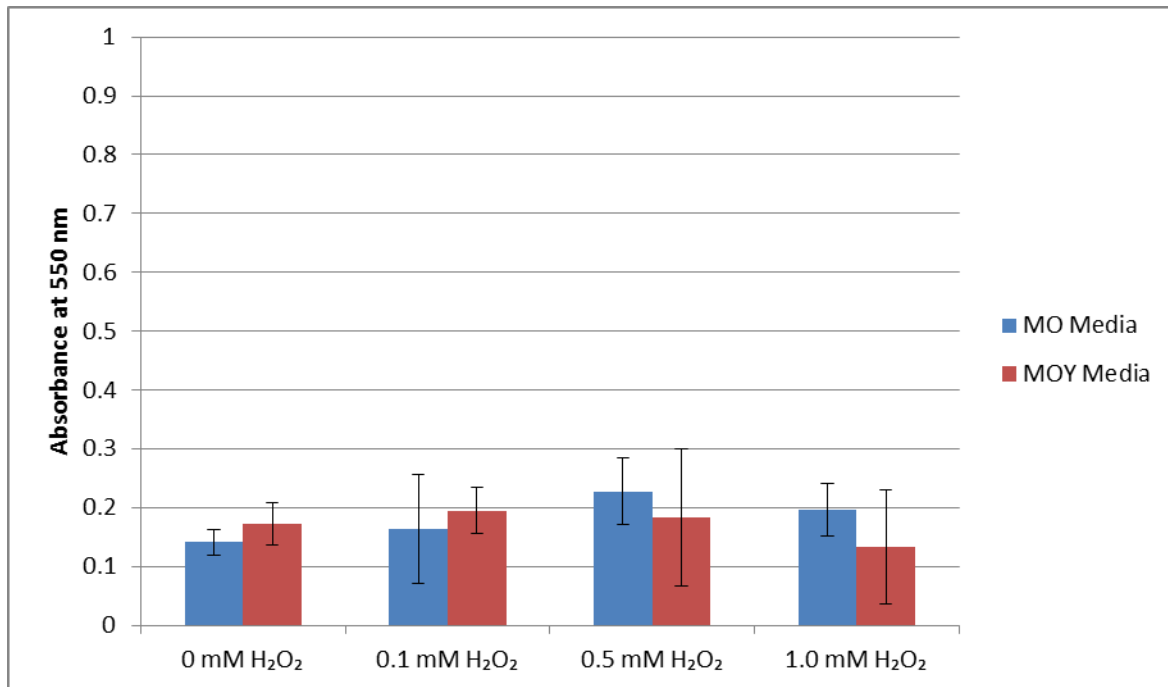


Figure 15: Effect of Media on Biofilm Density with Glucose at 37 Degrees Celsius

Using glucose instead of pyruvate at 37 degrees Celsius resulted in a slightly higher average biofilm density for MO media-grown samples in relatively high concentrations of hydrogen peroxide (0.5 mM and 1.0 mM). At lower concentrations, though, MOY media showed greater biofilm density. Neither of these outcomes was statistically significant, though. Synthesizing the data from Figures 12-15, it appears that the choice of media, whether a minimal MO media or a yeast extract-enriched MOY media, has relatively little impact on the density of biofilm growth.

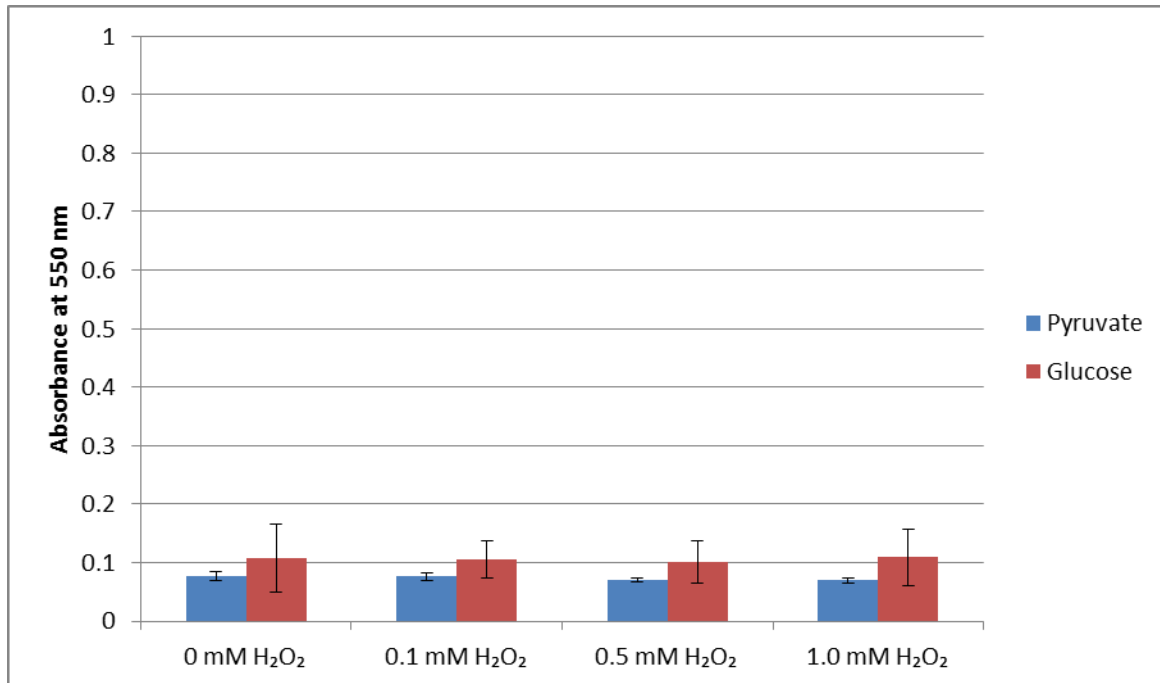


Figure 16: Effect of Electron Donor on Biofilm Density in MO Media at 25 Degrees Celsius

As indicated in the 24-well results, glucose was tested in this experiment as an alternative electron donor instead of pyruvate to test the ability of *D. alaskensis* G20 to utilize a different substrate in its hydrogen sulfide-producing metabolic pathway. In Figure 16, pyruvate and glucose are compared as alternate electron donors in MO media incubated at 25 degrees Celsius. The samples grown with glucose have higher average biofilm density than samples grown in pyruvate, but this difference in density is not statistically significant.

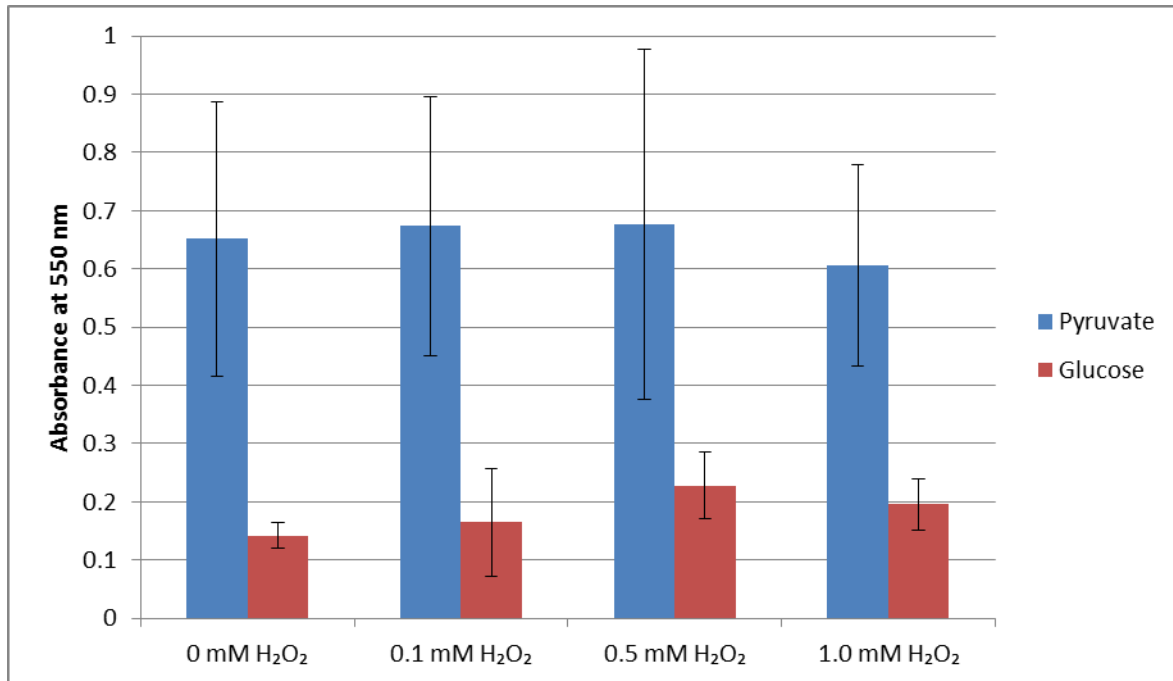


Figure 17: Effect of Electron Donor on Biofilm Density in MO Media at 37 Degrees Celsius

In contrast to samples incubated at 25 degrees Celsius in MO media, the samples that were incubated at 37 degrees Celsius in MO media with pyruvate showed much higher biofilm growth than samples grown with glucose. This difference was statistically significant across hydrogen peroxide concentrations and indicates that *D. alaskensis* G20 prefers pyruvate for biofilm growth at 37 degrees Celsius but not at 25 degrees Celsius.

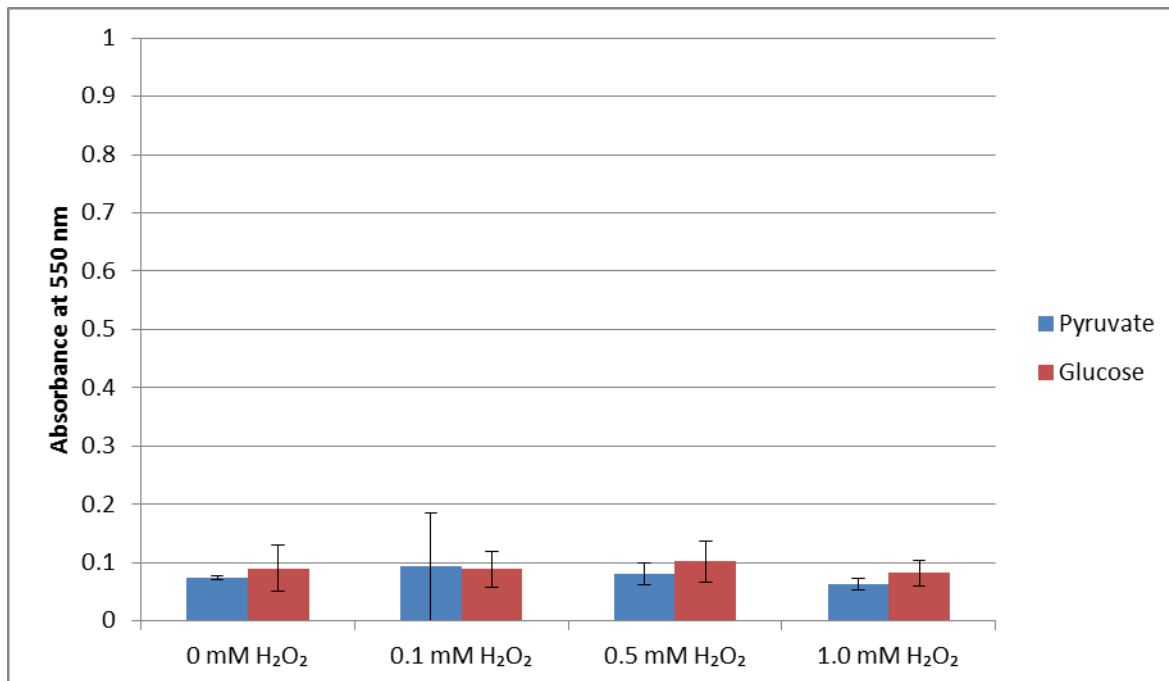


Figure 18: Effect of Electron Donor on Biofilm Density in MOY Media at 25 Degrees Celsius

As with samples grown in MO media at 25 degrees Celsius, samples grown in MOY media and incubated at 25 degrees Celsius had similar biofilm density between pyruvate and glucose.

Samples grown with glucose had slightly higher average biofilm density except at 0.1 mM hydrogen peroxide, in which pyruvate samples had a slightly higher average, but these differences in biofilm density were not statistically significant. These results are again similar to those obtained from samples grown in MO media at 25 degrees Celsius, indicating little or no preference between the electron donors at this temperature.

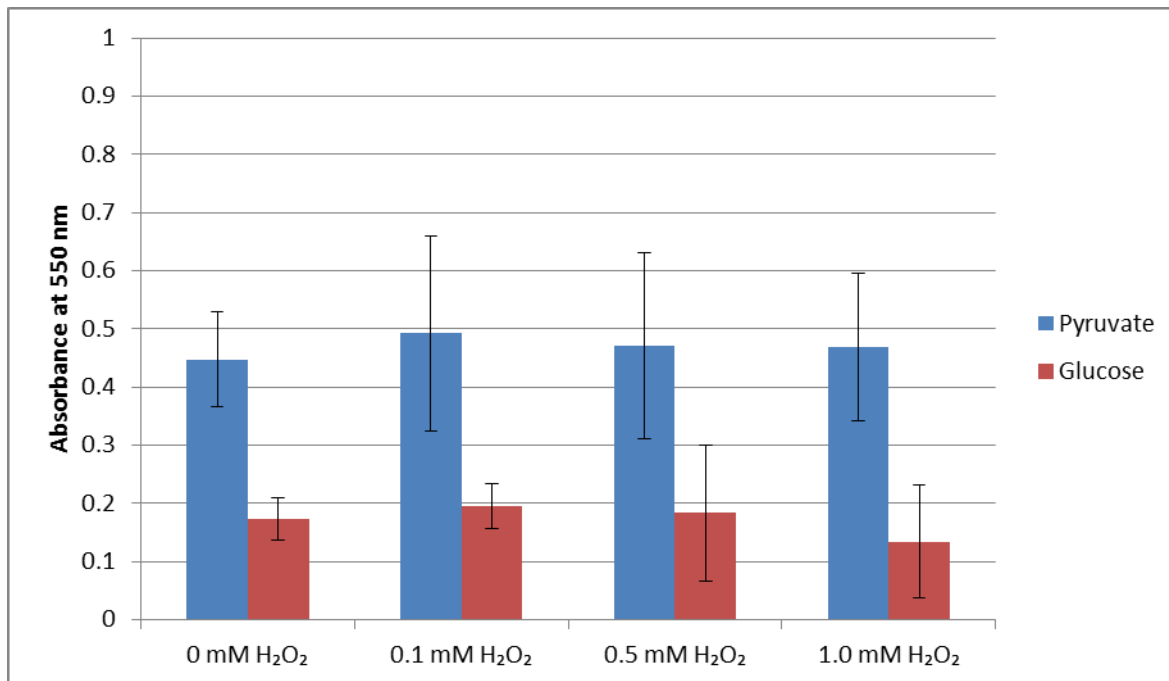


Figure 19: Effect of Electron Donor on Biofilm Density in MOY Media at 37 Degrees Celsius

However, samples grown in MOY media and incubated at 37 degrees Celsius showed a much greater difference in biofilm density between pyruvate and glucose, similar to the results obtained from samples grown in MO media and incubated at 37 degrees Celsius. Samples grown with pyruvate had higher average biofilm density across hydrogen peroxide concentrations, and this difference in biofilm density was statistically significant. Combining Figures 16–19 together, it appears that the choice of electron donor between pyruvate and glucose is not significant for *D. alaskensis* G20 when grown at 25 degrees Celsius, but the choice of electron donor is significant for the organism when grown at 37 degrees Celsius. This result is seen regardless of whether MO or MOY media is used.

Discussion

All sets of data points that were statistically significant based on a 95% confidence interval have been brought together to form the table below.

Figure #	Independent Variable	Other Growth Conditions	Concentration(s) of H ₂ O ₂
1	25 > 37 degrees Celsius	MO Media/Pyruvate	0.1 mM, 0.5 mM, and 1.0 mM
3	MOY Media > MO Media	Pyruvate/25 degrees Celsius	0 mM and 1.0 mM
3	MO Media > MOY Media	Pyruvate/25 degrees Celsius	0.5 mM
4	MOY Media > MO Media	Pyruvate/37 degrees Celsius	0 mM and 1.0 mM
6	Pyruvate > Glucose	MOY Media/25 degrees Celsius	0 mM
8	37 > 25 degrees Celsius	MO Media/Pyruvate	0 mM, 0.1 mM, 0.5 mM, and 1.0 mM
9	37 > 25 degrees Celsius	MOY Media/Pyruvate	0 mM, 0.1 mM, 0.5 mM, and 1.0 mM
10	37 > 25 degrees Celsius	MO Media/Glucose	0.5 mM
11	37 > 25 degrees Celsius	MOY Media/Glucose	0 mM and 0.1 mM
17	Pyruvate > Glucose	MO Media/37 degrees Celsius	0 mM, 0.1 mM, 0.5 mM, and 1.0 mM
19	Pyruvate > Glucose	MOY Media/37 degrees Celsius	0 mM, 0.1 mM, 0.5 mM, and 1.0 mM

Table 1: Statistically Significant Data Points

Statistically significant data points collected from the 24-well plates (found in figures 1–7) suggest that *D. alaskensis* G20 grows better at an incubation temperature of 25 degrees Celsius than 37 degrees Celsius. However, statistically significant data points collected from the 96-well plates (found in figures 8–19) overwhelming indicate that 37 degrees Celsius is the preferred incubation temperature. Data from the 96-well plates further suggest that 37 degrees Celsius is the preferred incubation temperature over a wide range of hydrogen peroxide concentrations, at least when pyruvate is present as the electron donor.

Statistically significant data from the 24-well plates indicates that different media are preferred based on other growth conditions. Basal salt MO media is preferred when hydrogen peroxide is

present at a concentration of 0.5 mM, when the media contains pyruvate and the sample is incubated at 25 degrees Celsius. However, a yeast extract-enriched MOY media is preferred at 0 mM and 1.0 mM hydrogen peroxide concentrations, regardless of incubation temperature. Also, one statistically significant data point from the 24-well plates indicated that pyruvate is preferred as an electron donor over glucose when grown in MOY media, a hydrogen peroxide concentration of 0mM, and an incubation temperature of 25 degrees Celsius. This preference for pyruvate was further implied by data from the 96-well plates, in which biofilm production was significantly greater at an incubation temperature of 37 degrees Celsius, regardless of hydrogen peroxide concentration or growth media type.

For the 24-well plates (with coverslips), the greatest biofilm density was seen in wells with MOY media containing pyruvate. An average of 0.387 absorbance at 550 nm was seen for samples grown with 1.0 mM hydrogen peroxide at 25 degrees Celsius, while an average of 0.370 absorbance at 550 nm was seen for samples grown with 0.1 mM hydrogen peroxide at 37 degrees Celsius. For the 96-well plates (without coverslips), the greatest biofilm density was seen in wells with MO media containing pyruvate. An average of 0.677 absorbance at 550 nm was seen for samples grown with 0.5 mM hydrogen peroxide at 37 degrees Celsius, while an average of 0.673 absorbance at 550 nm was seen for samples grown with 0.1 mM hydrogen peroxide at 37 degrees Celsius.

Due to circumstances beyond the control of our lab, this experiment could not be conducted at present time with cerium oxide-coated materials. However, this experiment does lay the foundation for future experimentation in cerium oxide-induced prevention of biocorrosion

caused by sulfate-reducing bacteria such as *D. alaskensis* G20 by determining ideal growth conditions and examining potential stressors of this bacterial strain.

References

1. Nakamura, K., et al. (2016). "Antimicrobial activity of hydroxyl radicals generated by hydrogen peroxide photolysis against *Streptococcus mutans* biofilm." *Int J Antimicrob Agents* 48(4): 373-380.
2. Dinh, H. T., et al. (2004). "Iron corrosion by novel anaerobic microorganisms." *Nature* 427(6977): 829-832.
3. Heidelberg, J. F., et al. (2004). "The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris Hildenborough*." *Nat Biotechnol* 22(5): 554-559.
4. Jack, R. F., Ringelberg, D. B., and White, D. C. (1992). "Differential corrosion rates of carbon steel by combinations of *Bacillus* sp., *Hafnia alvei*, and *Desulfovibrio gigas* established by phospholipid analysis of electrode biofilm." *Corr Sci* 33(12): 1843-1853.
5. Nair, R. R., et al. (2015). "Changes in metabolic pathways of *Desulfovibrio alaskensis* G20 cells induced by molybdate excess." *J Biol Inorg Chem* 20(2): 311-322.
6. Wall, J. D., et al. (2004). "*Desulfovibrio desulfuricans* G20." DOE Joint Genome Institute
7. Masadeh, M. M., et al. (2015). "Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram positive and gram negative biofilm bacteria." *Cytotechnology* 67(3): 427-435.
8. Pelletier, D. A., et al. (2010). "Effects of engineered cerium oxide nanoparticles on bacterial growth and viability." *Appl Environ Microbiol* 76(24): 7981-7989.

9. Montemor, M. F. and Ferreira, M. G. S. (2007). "Cerium salt activated nanoparticles as filler for silane films: Evaluation of the corrosion inhibition performance on galvanized steel substrates." *Electrochimica Acta* 52(24): 6976-6987.
10. Zane, G. M., Yen, H. C., and Wall, J. D. (2010). "Effect of the deletion of *qmoABC* and the promoter-distal gene encoding a hypothetical protein on sulfate reduction in *Desulfovibrio vulgaris Hildenborough*." *Appl Environ Microbiol* 76(16): 5500-5509.
11. Brandis, A., and Thauer, R. K. (1981). "Growth of *Desulfovibrio* species on hydrogen and sulphate as sole energy source." *J Gen Microbiol* 126:249-252.
12. Sim, M. S., et al. (2011). "Effect of electron donors on the fractionation of sulfur isotopes by a marine *Desulfovibrio* sp." *Geochimica et Cosmochimica Acta* 75(15): 4244-4259.
13. Yang, Y., et al. (2016). "Using *Pseudomonas aeruginosa* PAO1 to evaluate hydrogen peroxide as a biofouling control agent in membrane treatment systems." *Lett. Appl. Microbiol.* 63(6): 488-494.
14. Duan, D., et al. (2016) "Fine-tuned production of hydrogen peroxide promotes biofilm formation of *Streptococcus parasanguinis* by a pathogenic cohabitant *Aggregatibacter actinomycetemcomitans*." *Environ. Microbiol.* 18(11): 4023-4036.
15. O'Toole, G. A. (2011) "Microtiter Dish Biofilm Formation Assay." *J. Vis. Exp.* (47), e2437