The Use of *Vibrio fischeri* as an Indicator of Water Quality

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Introduction

Water quality problems have been widely studied; various tests have been created, and efforts have been made world-wide to improve the condition of the world's water supply. *Vibrio fischeri* are a potential indicator of water quality, but exactly what aspect of the water quality this organism is capable of indicating has not been determined. This experiment aims to find factors that are able to limit the bioluminescence of *V. fischeri.* The results of this experiment (the single pollutant that causes the *V. fischeri* to decrease in bioluminescence), may replace the chemical tests and indicate which factor of water quality is limiting for the *V. fischeri*.

Purpose

The purposes of this experiment are to:

- determine which component of the water the *V. fischeri* are most reactive to and
- determine the baseline toxicity of that component that will cause the *V. fischeri* to have a decrease in the level of bioluminescence.

Research Question: How will varying concentrations of naturally-found compounds impact *Vibrio fischeri*?

Experimental Parameters

Research Hypothesis: If *V. fischeri* vary the production of bioluminescence because of the environment, and the researcher alters the environment, then the bioluminescence of the *V. fischeri* will change.

Null Hypothesis: If *V. fischeri* are exposed to non-ideal levels of compounds present in water, then the bioluminescence produced by the *V. fischeri* will not change between the different environmental condition groups.

Alternate Hypothesis: If *V. fischeri* are exposed to non-ideal levels of toxins present in water, then the bioluminescence produced by the *V. fischeri* will change between the environmental condition groups.

Independent Variables: KNO_3, pH, and NH_4OH

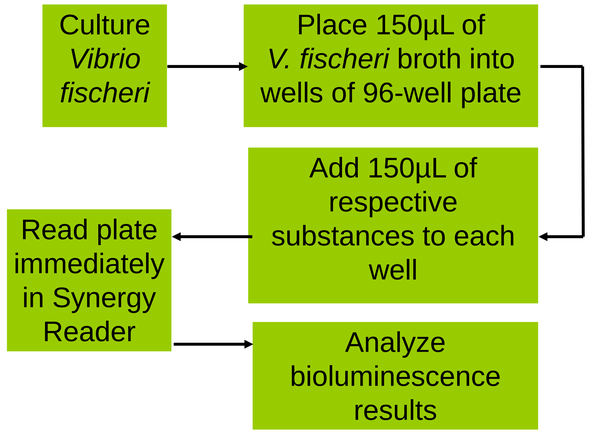
Dependent Variable: level of bioluminescence

Constants: temperature, agar, broth, volume of added substance, volume of combination, time

Materials

- Broth culture of *Vibrio fischeri*
- Agar culture of *Vibrio fischeri*
- Inoculating loops
- Bleach
- Ethanol
- Incubator
- Latex gloves
- Photobacterium broth
- 1L Beaker
- Culture bottles
- dH_2O
- Balance, 0.1g precision
- Nine 250mL beakers
- LoggerPro pH Probe

Procedure



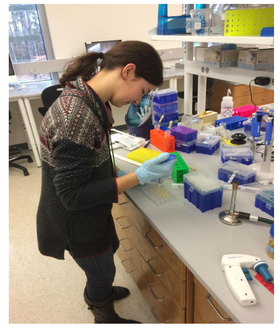
Results

Experimental Matrix

	1	2	3	4	5	6	7	8	9	10	11	12
B	pH 6.0, LA	pH 6.0, LA	pH 6.0, AvA	pH 6.0, AvA	pH 6.0, HA	pH 6.0, HA	pH 6.0, LN	pH 6.0, LN	pH 6.0, AvN	pH 6.0, AvN	pH 6.0, HN	pH 6.0, HN
C	pH 6.5, LA	pH 6.5, LA	pH 6.5, AvA	pH 6.5, AvA	pH 6.5, HA	pH 6.5, HA	pH 6.5, LN	pH 6.5, LN	pH 6.5, AvN	pH 6.5, AvN	pH 6.5, HN	pH 6.5, HN
D	pH 7.0, LA	pH 7.0, LA	pH 7.0, AvA	pH 7.0, AvA	pH 7.0, HA	pH 7.0, HA	pH 7.0, LN	pH 7.0, LN	pH 7.0, AvN	pH 7.0, AvN	pH 7.0, HN	pH 7.0, HN
E	pH 7.5, LA	pH 7.5, LA	pH 7.5, AvA	pH 7.5, AvA	pH 7.5, HA	pH 7.5, HA	pH 7.5, LN	pH 7.5, LN	pH 7.5, AvN	pH 7.5, AvN	pH 7.5, HN	pH 7.5, HN
F	pH 8.0, LA	pH 8.0, LA	pH 8.0, AvA	pH 8.0, AvA	pH 8.0, HA	pH 8.0, HA	pH 8.0, LN	pH 8.0, LN	pH 8.0, AvN	pH 8.0, AvN	pH 8.0, HN	pH 8.0, HN

Symbol	Meaning (Concentration)
LN	Low Nitrate
AvN	Average Nitrate
HN	High Nitrate
LA	Low Ammonia
AvA	Average Ammonia
HA	High Ammonia
Units	Relative Light Units (RLU)

All charts and tables created by researcher.



Above: Researcher places bacteria in well plate. Photo credits to Dr. Scott Nowak.

Statistics

Table 1: T-Test of Bioluminescence between Group A (pH 7.0, avg. NO_3) and Group B (pH 6.0, high NO_3)
Null Hypothesis 1: If *V. fischeri* are exposed to non-ideal levels of nitrate present in water, then there will be no difference in mean bioluminescence produced by the *V. fischeri*.

Treatments	T-Value	P-Value	Conclusion
[pH 7.0, avg. NO_3], [pH 6.0, high NO_3]	-2.193	0.03992	Statistically Significant

Results indicate that the mean bioluminescence of the two groups were not the same. Therefore, the null hypothesis can be rejected.

Table 2: T-Test of Bioluminescence between Group A (pH 7.0, avg. NO_3) and Group C (pH 8.0, high NO_3)
Null Hypothesis 1: If *V. fischeri* are exposed to non-ideal levels of nitrate present in water, then there will be no difference in mean bioluminescence produced by the *V. fischeri*.

Treatments	T-Value	P-Value	Conclusion
[pH 7.0, avg. NO_3], [pH 8.0, high NO_3]	-2.071	0.04656	Statistically Significant

Results indicate that the mean bioluminescence of the two groups were not the same. Therefore, the null hypothesis can be rejected.

Table 3: T-Test of Bioluminescence between Group D (pH 7.0, avg. NH_3) and Group E (pH 6.0, high NH_3)
Null Hypothesis 1: If *V. fischeri* are exposed to non-ideal levels of ammonia present in water, then there will be no difference in mean bioluminescence produced by the *V. fischeri*.

Treatments	T-Value	P-Value	Conclusion
[pH 7.0, avg. NH_3], [pH 6.0, high NH_3]	1.563	0.08942	Not Statistically Significant

Results indicate that the mean bioluminescence of the two groups were not different enough to be statistically significant. Therefore, there is a failure to reject the null hypothesis.

Table 4: T-Test of Bioluminescence between Group D (pH 7.0, avg. NH_3) and Group F (pH 8.0, high NH_3)
Null Hypothesis 1: If *V. fischeri* are exposed to non-ideal levels of ammonia present in water, then there will be no difference in mean bioluminescence produced by the *V. fischeri*.

Treatments	T-Value	P-Value	Conclusion
[pH 7.0, avg. NH_3], [pH 8.0, high NH_3]	1.964	0.05337	Not Statistically Significant

Results indicate that the mean bioluminescence of the two groups were not different enough to be statistically significant. Therefore, there is a failure to reject the null hypothesis.

Conclusion

The data conclusively show that the non-ideal nitrate conditions had a significantly negative impact on the bioluminescence of the *Vibrio fischeri*. The data also show that the non-ideal ammonia conditions did not have a significant impact on the bioluminescence of the *Vibrio fischeri*. The toxic levels of nitrate did affect the bioluminescence of the *V. fischeri*, though the toxic levels of ammonia did not. Possible sources of error could have come from human-error when inserting the substances into the well plate. Error could also stem from the inability to control the exact number of bacteria in each culture sample.

Future Work

Vibrio fischeri could be used as a water quality test for the factor to which it reacts. Further trials and research will be done to determine a baseline toxicity for the nitrate compound for the *Vibrio fischeri.* The difference in bioluminescence of the *Vibrio fischeri* in optimal conditions and sample conditions could be indicative of water quality. Bioluminescence results of the *V. fischeri* will be compared with the Georgia Adopt-A-Stream measures of stream health. These two methods of analysis of stream health will be compared for correlation.