The Effect of Increased Oxygen Concentration on the Luminosity of Bioluminescent *E. coli*

Purpose

The purpose of the study is to alleviate the pressures of light pollution and reduce energy consumption caused the standard usage of lights by developing bioluminescent bulbs that can be used as an economically and environmentally as a sustainable substitute to low intensities of light that cause light pollution.

Introduction

Light pollution is a significant problem that goes unnoticed by many. It disrupts natural cycles, can cause unforeseen health issues, and affect the environment. Light also requires a large amount of energy to maintain, which leads to more airborne pollutants and greenhouse gases in the atmosphere. This experiment will mainly focus on alleviating the symptoms of over-illumination by using bioluminescence.

Light pollution has negative impacts on plant and animal physiology. Many flowering light-sensitive plants are affected because the lights prohibit them from flowering and reproducing. This affects the pollinators that rely on these flowering plants, stripping them of a food source. Excessive lighting further affects animals by altering migratory patterns, competitive interactions, predator-prey relations, and can cause physiological harm. Often, birds and insects navigate by moonlight and starlight. Over illumination causes them to wander off course and towards cities. It also causes declining insect populations, leading to devastating and lasting effects in some food webs.

Light pollution also causes adverse health effects in humans. Exposure of light during the night can lead to sleeplessness or low melatonin production. Low levels of melatonin can lead to sleep disorders, migraines, fatigue, medical stress, depression, anxiety, and obesity. Over-illumination has been linked to an increase in high blood pressure and a greater risk of having certain carcinomas. Health effects are also caused by improper spectral composition. Artificial light tends to emit more blue light than natural sources. Blue light also inhibits melatonin. Blue light exposure at night has been linked to bipolar disorder, post-traumatic stress disorder, generalized anxiety, and other mood disorders.

A way to curb light pollution and its effects is the usages of bioluminescence. Bioluminescence is the natural production of light. The reaction of bioluminescence requires a tremendous amount of energy. When reactions in nature are required to be faster, an enzyme is used. An enzyme is a natural catalyst that lowers the activation energy of a reaction. The catalyst in bioluminescence is luciferase. Reactions can also be sped up due to different variables such as amount of reactant. This study will focus on the effect of varying amounts of oxygen, which is the reactant of bioluminescence. *E. coli* encoded with pVIB will be used to make the culture bioluminescent. pVIB is a plasmid, which is a genetic structure that is a small circular piece of DNA.

Bioluminescence has tremendous benefits over standard lighting. Bioluminescence produces a soft light, which does not have an improper spectrum. This eliminates many of the dangerous effects observed on wildlife affected by artificial lights. Art exhibits in France and New York have found, through qualitative assessments, that the natural lighting feels calming and reduces stress and anxiety. Another benefit is that natural lighting is also that it is cost-effective. This is because bacteria cultures can be easily cultivated and grown. As society modernizes, more light is used, and natural lighting can curb many of the harmful effects of artificial lighting, leading to a brighter future.

Hypothesis

The highest concentrations of oxygen will yield the highest lux (a). The highest concentration of oxygen will also be able to sustain a higher lux over a longer period of time (b). This is due to oxygen being the reactant in the bioluminescence reaction.

Data/Results

Lux of 0% Concentration over Time				
Time (Days)	Trial 1	Trial 2	Trial 3	Average
0	0.1	0	0	0.03
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0

Table 1: Graph of Averages of 0% Oxygen Concentration (control)

Lux of 25% O Concentration over Time				
Time (Days)	Trial 1	Trial 2	Trial 3	Average
0	12.2	12.3	12.2	12.23
1	11.4	11.2	11.2	11.27
2	10.7	10.9	10.8	10.8
3	10.2	10.1	10.1	10.13
4	9.8	9.9	10	9.9
5	9.6	9.6	9.8	9.67
6	9.2	9.2	9.1	9.17

Table 2: Graph of Averages of 25% Oxygen Concentration

Lux of 50% O Concentration over Time				
Time (Days)	Trial 1	Trial 2	Trial 3	Average
0	17.4	16.9	17	17.1
1	16.4	16.1	16.4	16.3
2	15.8	15.6	15.6	15.67
3	15.4	15.4	15.3	15.37
4	15.3	15.1	15.2	15.2
5	15.2	15	15.1	15.1
6	14.8	14.7	14.9	14.8

Table 3: Graph of Averages of 50% Oxygen Concentration

Lux of 75% O Concentration over Time				
Time (Days)	Trial 1	Trial 2	Trial 3	Average
0	17.5	17.4	17.3	17.43
1	17.2	17.2	17.1	17.17
2	16.7	16.7	16.7	16.73
3	16.6	16.6	16.4	16.57
4	16.4	16.4	16.3	16.4
5	16.3	16.3	16.1	16.23
6	16.1	16.1	16	16.1

Table 4: Graph of Averages of 75% Oxygen Concentration

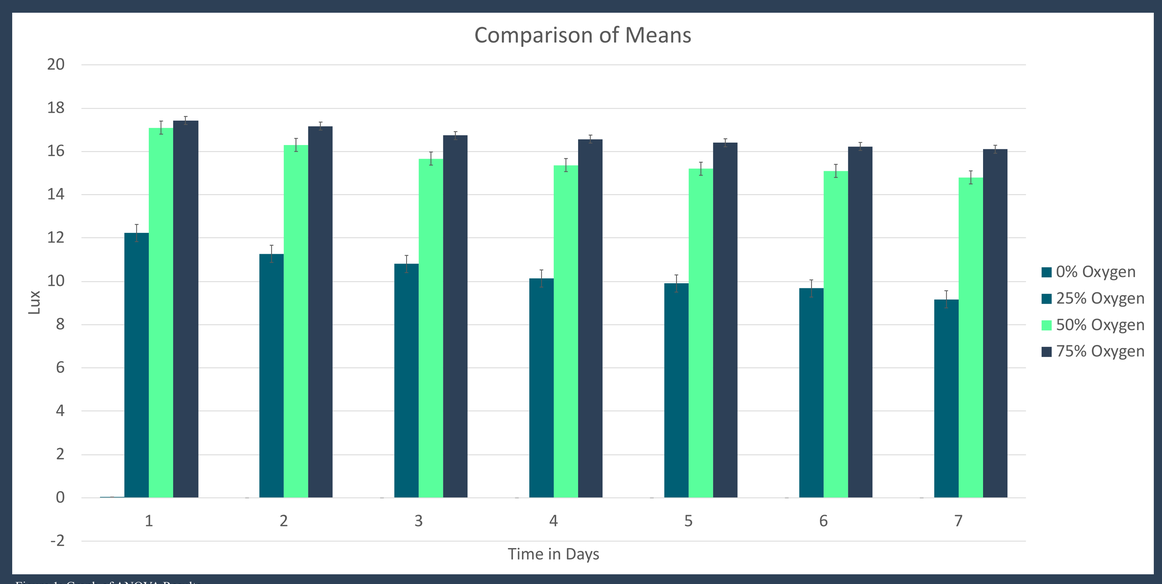

Figure 1: Graph of ANOVA Results

Scheffé Test			
Treatment Pair	Scheffé T-Statistic	Scheffé p-value	Scheffé Inference
0% vs 25%	39.3934	1.1102e-16	** p<0.01
0% vs 50%	58.8327	1.1102e-16	** p<0.01
0% vs 75%	62.6417	1.1102e-16	** p<0.01
25% vs 50%	19.5393	1.4372e-11	** p<0.01
25% vs 75%	23.3483	3.0576e-13	** p<0.01
50% vs 75%	3.8090	0.0910393	Not significant

Table 4: Scheffé post hoc test to Determine Specific Comparisons Between Groups

Bonferroni and Holm results: only pairs relative to 25% simultaneously compared					
Treatment Pair	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni Inference	Holm p-value	Holm inference
0% vs 25%	27.7847	0.0000e+00	** p<0.01	0.000e+00	** p<0.01
0% vs 50%	41.6010	0.0000e+00	** p<0.01	0.000e+00	** p<0.01
0% vs 75%	44.2944	0.0000e+00	** p<0.01	0.000e+00	** p<0.01

Table 5: Bonferroni and Holm post hoc Specific Values Against Control Significance

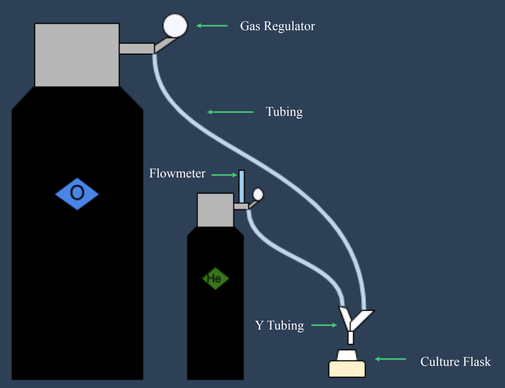

Figure 2: Diagram of Apparatus Used to Fill Culture Flasks

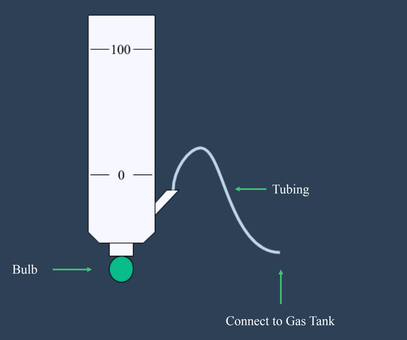

Figure 3: Diagram of Soap Film Flow Meter Used to Calculate Flow Rates

Methodology

1. Introducing and growing pVIB culture- Grow *E.* coli at 37°C for two days. Once two days have elapsed remove the three Petri dishes and look for colonies of *E. coli* 2 mm to 3 mm in diameter. Remove these colonies using an inoculation loop, and place in a sterilized tube containing 10 µl calcium chloride, which will make the cells porous. Suspend the colony in a tube using a sterilized pipette till the mixture is homogenous. Using a different sterile inoculation obtain pVIB. A small bubble should form in between the circle. Mix in the pVIB in the tube and suspend using a pipette. Do this for a second tube as well. Once finished let rest in an ice bath while heating up a water bath to 42°C. After the water bath has been heated place the tubes for 90 seconds in the bath. Heat shocking will allow the pores to open up and take in the plasmid. After heat shocking place the tubes in an ice bath and let them rest for at least a minute. Cold shocking will make sure the pores close up and no plasmid leaks out. Now add 250 µl of Luria broth to the mixture. Place five sterilized glass balls into both of the prefilled ampicillin petri dishes. Take 100 µl of the mixture a tube and place into a dish. Do this for the second tube as well. Now shake the dishes on a flat surface to roll the beads for 5 minutes then remove beads, and place the dishes upside down in an incubator at 30°C for two days. After two days pour the Luria broth into a sterilized 1000 mL container, and grow the pVIB culture in the broth for three days.

2. Measuring flow rates and calculating percentages- Use a clamped soap film meter to calculate flow rates. Record a video as bubbles pass the zero line and reach the 100 mL line. For best results, form several bubbles, five to six seconds apart, and time the last bubble. Make sure excess froth does not form from pressing the bulb continuously. After taking the video use the equation flow in $\frac{mL}{min} = \frac{60 \bullet (v\ travlled)}{s}$. The oxygen flow rate was found to 6000 $\frac{mL}{min}$. The helium tank is attached with a flow meter. The ratio between flow meter reading and $\frac{mL}{min}$ is found to be 1 unit to 20 $\frac{mL}{min}$. Keep the oxygen flow rate at a constant 6000 $\frac{mL}{min}$. Multiply the oxygen constant by .25, .50, and .75 to find helium to oxygen percentages of 25% He to 75% O, 50% He to 50% O, and 75% He to 25% O. Have 100% He to 0% O be a control. The products of the oxygen flow rate and decimals yields the helium flow rates that correspond to the above oxygen to helium percentages. The calculate helium flow rates will be 1500 $\frac{mL}{min}$ for 25% He, 3000 $\frac{mL}{min}$ for 50%, and 4500 $\frac{mL}{min}$ for 75%. The 100% He flow rate can be any rate.

3. Preparing the apparatus- Gather 12 culture flasks and separate into four groups of three. Label the groups with masking tape and for every three write 0% O, 25% O, 50% O, and 75% O. Pour 75 mL of Luria broth into each culture flask. Now attach sterilized tubing to both gas tanks. Connect both of the tubes with a sterilized Y tubing. After connecting all the tubing set the flow rates of both the tanks. Fill each culture with a gas mixture using the corresponding flow rates found in step 4: 100% He to 0% O, 25% He to 75% O, 50% He to 50% O, and 25% He to 75% O.

4. Gathering results- Use a Vernier LabQuest 2 with a light sensor attachment. Move the culture flasks to a dark area with no light intrusion. Swirl the flask for five seconds to cause bioluminescence. Point the light sensor at a distance of 10 cm and take note of the lux displayed on the screen of the lab quest. Do this for seven days.

Conclusion

Hypothesis (a) was supported. Data pre-analysis showed that the highest oxygen concentrations (75% and 50%) were the brightest. Post-analysis also showed that there was no significant difference between these two groups, demonstrating that each performed equally. Hypothesis (b) was supported. It was seen that the highest concentration of oxygen (75%) was able to remain brighter over time than any other time. Post-analysis showed there was a significant difference between the data sets over time demonstrating that the highest concentration did remain the brightest over time.

All figures and tables were made by Malhar Amin