Samyu Vakkalanka

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mCURE Lab Report Final

Background

Marine Hypoxia

As reliance on man-made innovations in industries such as agriculture and energy production increases across the world, the adverse side effects of these methods and their impact on nature become more prominent and fatal. A notable example of this is marine hypoxia. This byproduct of eutrophication ultimately results in the damage of ocean habitats, leading to increased “dead zones,” or the inability for species to survive in large plots of water, across the globe.

Marine hypoxia can be linked to the combination of many different harmful practices and their resulting processes. At its core, it is an insufficiency in the dissolved oxygen in areas in the ocean, causing these areas to be uninhabitable to much of marine biodiversity. Oxygen is important in cellular respiration, a vital method for energy production in organisms that feeds into many other biological processes. When these individuals don’t have access to dissolved oxygen in the water, they will not be able to survive, causing mass migration from these areas with low oxygen concentration, resulting in what are known as dead zones (Vaquer-Sunyer *et al., 2008*).

The specific causes, however, can become much more complicated. One important factor to note is that oxygen concentration fluctuates naturally and has both seasonal cycles and changes over long periods of time as a result of the respiration and photosynthesis of organisms living in the water. In addition, a process known as stratification, or the inability of freshwater and seawater to mix within a single column of water, is known to cause hypoxia due to the oxygen from the top waters not being able to mix with what’s underneath (Lee *et al.,* 2018). However, the major causes of hypoxia are anthropogenic. One of the largest dead zones in the

world, the Gulf of Mexico (among other major dead zones around the world), is caused primarily by nutrient pollution as a result of eutrophication. An influx of specifically nitrogen and phosphorus are deposited in the ocean by the Mississippi river as a result of runoff from agricultural materials such as fertilizer farther north (Rabalais 1999). Increased nutrient availability leads to an overgrowth of phytoplankton. These large blooms ultimately die and sink to lower levels of the ocean before being eaten by other organisms, especially heterotrophic bacteria. This results in an increased respiratory need of the organisms and not enough dissolved oxygen to support them (Chan *et al.* 2019). In combination with stratification, this increased oxygen consumption at the surface leads to dead zones underneath.

The Gulf of Mexico is particularly important to consider due to its economic importance in the seafood industry. The dead zone causes many of these harvested organisms to die out or migrate (Petrolia *et al*. 2006). By studying microbes in this hypoxic zone, information about the future of microbial ecosystems can be predicted. Organisms from different layers with different dissolved oxygen levels can be observed to determine how they exist as anthropogenic interference increases (Stewart, 2016).

Cellular respiration

Bacteria (specifically the organisms studied in this experiment) need oxygen to do cellular respiration. Cells exist to carry out predetermined functions that are necessary for the survival of the organism, ranging from reproduction to the assembly of organic materials used in cell growth. In order to accomplish these, cells need energy in the form of ATP. This chemical energy is produced through cellular respiration. The process occurs in the cytoplasmic membrane of bacterial cells (Breeuwer, Abee, (2004)).

The basis of cellular respiration is the breaking down of glucose to use as an oxidizing agent. Glycolysis is the process that uses glucose to make two three-carbon pyruvate molecules. These molecules are then transferred to the citrus cycle. Here, the molecule undergoes a series of oxidation reactions which results in the reduction of a number of electron

carrier molecules (Buchanan *et al.* 1990). All of these are then transferred to the electron transport chain, the biggest ATP producing process in respiration.

The ETC is powered by NADH and $FADH_2$ oxidizing to form NAD^+, FAD, electrons, and H^+ ions, which are essentially just protons. As the electrons move across various proteins embedded in the cytoplasmic membrane, they release some energy to allow the protons to be transported into the intermembrane space where they begin building a steeper concentration gradient. Once the electrons reach the end of the chain, they are transferred to oxygen (O_2), the final electron acceptor. This splits the oxygen and allows it to bond with hydrogen ions, resulting in water. In the end, the built up protons are allowed back through the membrane through the ATP synthase molecule. This flow of ions essentially spins a rotor, resulting in ATP production (Liu *et al.* 2002).

The molecules of glucose and oxygen are the most effective in producing energy in bacteria due to their high difference in reduction potential. However, the redox tower shows different possibilities with many other molecules that can be used in this process in the absence of oxygen molecules. For example, Fe^{3+} or NO_3^- can be used as replacements for oxygen in hypoxic zones because there is still a significantly large difference in potential between these molecules and glucose. However, if this difference is not enough, the ETC (and consequently the Krebs cycle) can't occur (Chen *et al.* 2017).

Introduction

This experiment studied the effects of varying temperatures (4°C, 12°C, 16°C, 22°C, 30°C, 35°C, 40°C) on the growth rate (and ultimately oxygen uptake rate) of LSUCC0684, LSUCC0719, and LSUCC0744, three different strains of SAR 116. These bacteria were found and collected from the hypoxic zone in the Gulf of Mexico. However, they were specifically chosen because they can be found all over the world. Microbes (bacteria and protists) make up the majority of the biomass in the oceans. As a result of this, they are the most affected by changes in the ocean biome. In addition, they are contributors to marine hypoxia as described

above (Bar-on et *al*.) In addition, the studied bacteria need oxygen to do cellular respiration. Living in a hypoxic zone makes this a significant challenge, yet they still survive. Studying them can provide insight into how these changes are affecting the microorganisms living in these environments.

In addition to figuring out how these strains' oxygen uptake rate vary between strains and growth rates, the specific growth rate at varying temperatures was determined and an optimal temperature for each strain's growth could be calculated. Observing this type of data lays the groundwork for future experiments with this bacteria. Knowing the optimal temperature for growth could allow future researchers to conduct experiments at a much faster pace. However, this variable also provides insight into how rising ocean temperatures affect the growth of the strains of the bacteria. If the effect of temperature on the organisms is understood better, researchers can gain a better understanding of what might happen if the oceans continue on their current trajectory.

Method

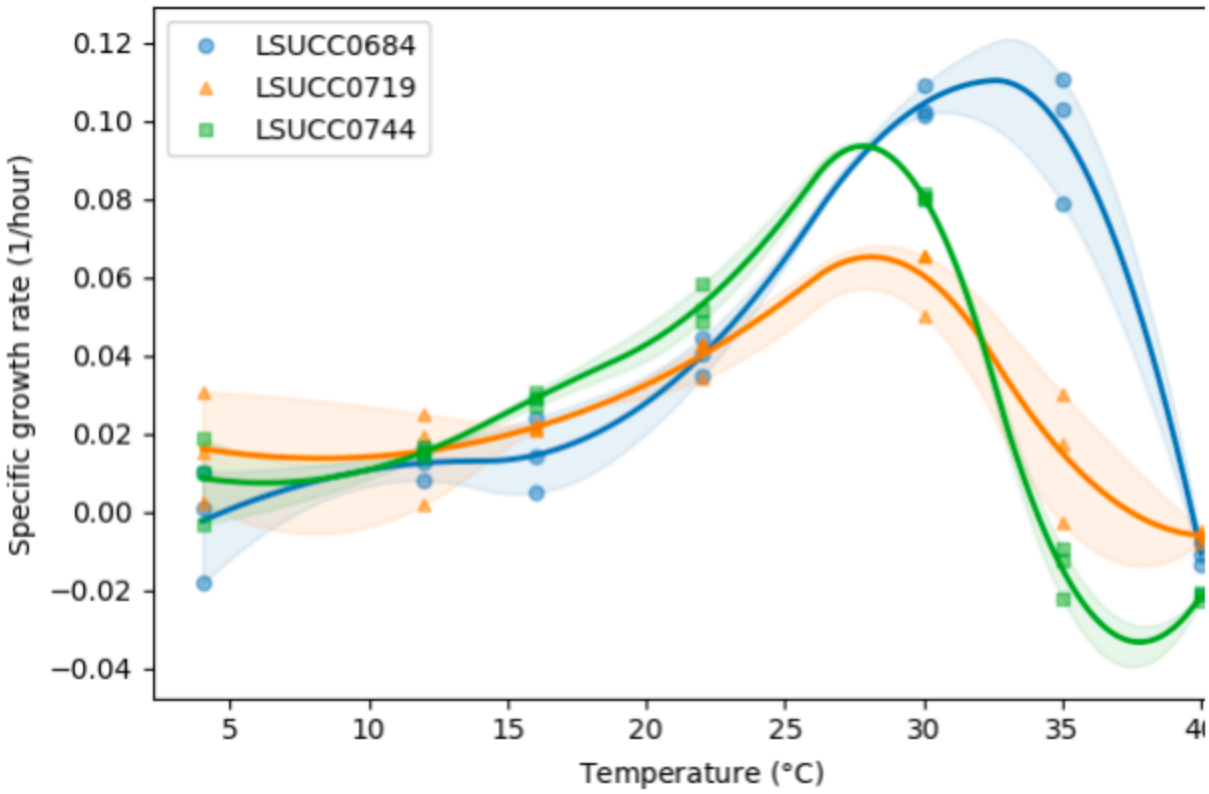
To obtain the various observations set out for the purposes of this experiment (determine the effect of temperature on the specific growth rate as well as predict the oxygen uptake rate of the three strains at different temperatures), a growth rate needed to be found. This was accomplished through the use of bacterial isolation. First, the bacteria samples from the hypoxic zone were diluted to near extinction. This was done by repeatedly adding artificial seawater to a large number of samples with the hopes of diluting the sample to just have one organism. Artificial seawater with set amounts of vitamins, salts, and trace metals (among other ingredients) were used in order to help standardize the experiment and ensure repeatability. These were then stored at the various temperatures and the cell density was collected at various time intervals using flow cytometry. This was graphed with the time to make a growth curve (cell density (cells/mL) vs. time (hr))

In order to calculate the specific growth rate (1/hr), the natural log transform of the cell density was graphed over time. The exponential phase of the curve was isolated and a linear regression model was created based on this data. From here, the slope of the line was the specific growth rate. The specific growth rate of each trial of each strain was graphed with the temperature they were at, allowing an optimal temperature to be determined.

From here, since no data regarding oxygen consumption was collected at this time, a prediction was made based on known data regarding cell specific respiration data in the Louisiana shelf. This allowed the comparison of a particular strain at a certain temperature and it's possible change in oxygen concentration at three different predictions for oxygen uptake rates.

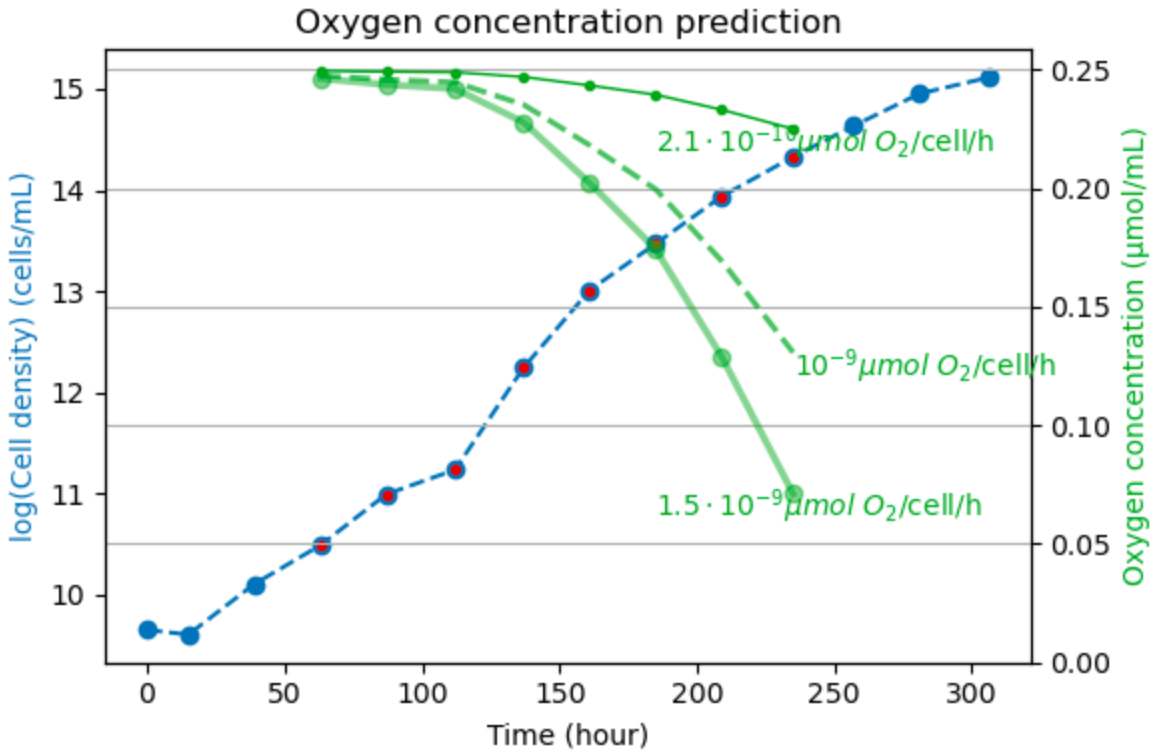
Results

Temperature

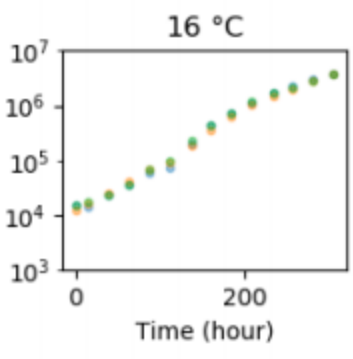


This is the resulting graph from the experiment. The specific growth rate of each of the three trials of the three strains at all of the temperatures was plotted. From here, it can be determined that the optimal temperature for LSUCC0684 is between 30°C and 35°C (around 34°C). LSUCC0719 and LSUCC0744 both had peaks between 25°C and 30°C (around 27°C). Other important notes on this graph were that the specific growth rate dropped significantly after the optimal temperature as opposed to the steady climb that occurred in temperatures leading up to their optimal temperature. This indicates that higher temperatures will result in the almost complete inability of these strains of bacteria to grow. In addition, LSUCC0719 had a maximum specific growth rate that was lower than both LSUCC0744 and LSUCC0684. This is interesting note because, even though they all have an optimal temperature for growth, they still had different growth rates in these conditions which means that there are other (possibly external) factors affecting their growth that need further investigation or research).

Oxygen Uptake



This graph indicates the prediction for change in oxygen concentration over time at three different oxygen uptake rates retrieved from a different study. This data used the specific growth rate of the first replicate of LSUCC0719 at 16°C (the growth curve of which is shown below).



This prediction is found using data collected in the field, meaning that it is probably inaccurate due to discrepancies between the lab and the actual location in the field that might affect repeatability of this growth curve. The main reason why this prediction is inaccurate is because of the introduction of oxygen to the flasks in which the bacteria were grown in the lab. A true experiment with the collection of oxygen concentration data would have required these flasks to be completely sealed off after the bacteria were added. However, in order to collect samples to put through the flow cytometer, the flasks had to be opened and exposed to an external oxygen source. This would mean that oxygen is reintroduced into the system and a plot of the decrease in oxygen concentration based on this lab setup would not be accurate or resemble the predictions.

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