THE EFFECTS OF USING DIFFERENT CONCENTRATIONS OF ALCOHOL ON MICROALGAE CELLS' STRUCTURAL INTEGRITY AND HOW CAN THESE EFFECTS IMPACT FETAL ALCOHOL SYNDROME

By

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ABSTRACT

The effects of alcohol on microalgae cells using different alcohol concentrations and how those impact the cell's structural integrity will be studied. This study is significant because I will be connecting the results to fetal alcohol syndrome in humans. When alcohol is consumed by a pregnant woman, potential damage could occur to the fetus. In my research, I will be determining at which point in time and amount of consumption greatly effects the organism. The reason I am studying microalgae is due to accessibility and ethicality. My expectations for this project are that with any amount of alcohol present, the cells will be impacted and that their membrane will deteriorate as the mass flow rate and concentration increase. With the help of Dr. Ke Du, an Assistant Professor in the Chemical and Environmental Engineering Department, and with $8,000 in funding from the National Science Foundation, I will be executing my experimental research.

The way I am going to conduct this research is by using nano-sieve equipment which has a canal where the microalgae cells will rest. The gaps will be filled with alcohol and the mass flow rate of alcohol and concentration of alcohol will vary as different trials are completed. I will potentially be working with two of Dr. Ke Du's PhD students where one will help interpret my results using photolithography and the other will assist me with the cell culturing process.

ACKNOWLEDGEMENTS

I would like to thank many individuals for helping me develop my ideas and execute my capstone project. I would first like to thank Dr. Richard Cardullo for his guidance for the past four years in University Honors and supporting this project as well as always being accessible. I would also like to thank Dr. David Cocker for helping me start this capstone project and offering suggestions early on to better execute my project. In addition, Dr. Ke Du and his PhD student Dr. Can Wang helped me greatly throughout this whole project including developing the ideas of my project to spending countless hours helping me interpret my data and execute the experimental portion of my project. I will forever be grateful for their extensive support. Finally I would like to thank my family for supporting my educational endeavors and constantly pushing me to do my best work as well as have a strong work ethic.

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INTRODUCTION

CONTEXT AND IMPORTANCE

Alcohol, especially ethanol in the context of consumption, effects many biological systems by disrupting cellular processes and overall health [1]. These effects are particularly concerning during pregnancy, leading to prenatal deficiencies such as fetal alcohol syndrome (FAS). FAS is a severe condition causing physical, behavioral, and cognitive problems in children exposed to alcohol before birth [2]. Despite extensive research, the exact ways alcohol causes these issues at the cellular and molecular levels are still unclear and further research and funding is necessary to understand the direct effects [3].

To understand these mechanisms better, researchers use simple organisms, such as microalgae in controlled experiments [4]. Microalgae, which are single-celled photosynthetic organisms, are ideal for this research [5]. Microalgae have well-understood cellular processes and are easy to grow, making them suitable for studying how ethanol impacts cells [6]. In addition, the use of microalgae is ethical as this type of research would be unethical to pursue in pregnant individuals. By examining how ethanol effects microalgae, researchers can gain insights into the broader effects of alcohol, potentially uncovering mechanisms relevant to human health and FAS [7]. Specifically, comparing the cell membrane to the human amniotic sac will gather insight on the impacts of consuming alcohol during pregnancy.

This research, done for the University of California, Riverside's University Honors Program, will look at how different concentrations of ethanol effect microalgae health by measuring changes in chlorophyll and carotenoid levels to assess the viability of the organism

after being exposed to ethanol. Understanding these effects in microalgae can help us learn more about how alcohol disrupts cells in other organisms, including humans.

RESEARCH PROBLEM AND OBJECTIVES

The common drinking of alcohol in general, especially during the prenatal state, can lead to serious problems for the fetus, and eventually the born baby, such as FAS. However, the exact interaction by which alcohol causes these issues at the cellular level is not fully understood [8], [9]. Looking into this, scientists often use simple organisms like microalgae because they are easy to grow, study, and are easily accessible [10], [11].

The purpose of conducting research in regards to this topic is to develop a better understanding of how different concentrations of ethanol effect microalgae cells. More specifically, the focus is on measuring changes in chlorophyll a, chlorophyll b, and carotenoids. The health of these pigments are essential for the health and function of the cells [12]. By examining changes in the health of the microalgae, the purpose of conducting this research hopefully provides insights into how alcohol impacts cells. The results of the research will then be compared to the effects of alcohol on a fetus [13].

Main Objectives:

1. To measure changes in chlorophyll a, chlorophyll b, and carotenoid levels in microalgae cells that are exposed to different ethanol concentrations.
2. To examine cell damage or stress response in microalgae due to ethanol exposure.
3. To use these research findings to relate effects to potential impacts on human fetal development and FAS.

Overall, the main goal is to provide valuable insights into how alcohol exposure harms cells in general.

HYPOTHESIS

Before conducting an experiment, it is important to have a general idea going in of predictions, and expected outcomes. These predictions don't necessarily have to be correct as a hypothesis can change throughout the researching processes. The hypothesis for this experiment is that higher concentrations of ethanol will harm microalgae cells than lower concentrations. This could be shown as lower levels of chlorophyll a and b become present, which are important for photosynthesis, and changes in carotenoid levels, which protect cells [17], [18]. Previous studies suggest that ethanol disrupts cellular processes and damages the photosynthetic machinery in plants and algae [19].

By looking at these effects in microalgae, the study aims to understand how ethanol effect cells and at what point does ethanol effect the cells. Is any ethanol okay to consume whether it's one drink or multiple? Gaining insight into this could help in understanding similar impacts in human cells, especially those related to FAS. The results from this experiment may help explain how alcohol exposure during pregnancy can harm fetal development and provide education to women by showing them the effects that drinking exposes their body to.

BACKGROUND

Introduction to the Research Topic

Alcohol, commonly consumed as ethanol, can disrupt biological systems and negatively impact an individual's health. During pregnancy, exposure to ethanol can lead to fetal alcohol syndrome (FAS), which can cause significant physical, behavioral, and cognitive challenges in children especially if consumed in the prenatal period [20]. Although there has been extensive research on this concern, the exact relationship in which ethanol causes these cellular and molecular issues is still unknown.

Current Knowledge and Previous Research

Ethanol can enter through a cell's membrane. This makes them more fluid and disrupts proteins such as receptors, enzymes, and ion channels [21]. This disruption can interfere with the cell's communication and metabolism, whether it's immediate or long-term, leading to cell damage and ultimately death. Ethanol's impact on membrane permeability can also weaken the cell's structure and ultimately make it vulnerable to other environmental factors, causing further damage.

One major way ethanol harms a cell is by producing reactive oxygen species (ROS) creating a radical oxygen state. When the human body processes ethanol, it generates ROS. This can potentially cause damage to lipids, proteins, and DNA in cells [22]. This oxidative stress state causes the ethanol to become toxic and could lead to inflammation, cell death, and other various diseases, including liver cirrhosis and cardiovascular diseases. In addition, ROS can also cause cell mutations and genomic instability, which further disrupts cellular function.

During prenatal fetal development, ethanol can disrupt pathways that control cell growth and survival. For example, ethanol can block the MAPK/ERK pathway, which is essential for cellular growth. This could lead to developmental issues and increased cell death [23]. These disruptions contribute to the developmental problems seen in FAS, such as growth deficiencies, facial anomalies, and neurodevelopmental disorders. Ethanol exposure during critical prenatal periods of development can also lead to long-term cognitive and behavioral deficits. This highlights the importance of understanding these mechanisms and potential threats if specific pathways are altered.

Microalgae as a Model Organism

Scientists use simple organisms to study how factors like ethanol effect cells due to their accessibility, economic purposes, and ethicality. Microalgae, single-celled organisms that use photosynthesis, are ideal prospects for this area of study because they are easy to grow and their cellular processes are well understood [24]. Microalgae could also be useful for studying ethanol's effects on cell structure and viability because they are sensitive to their environment and can provide quick and clear results which can be later applied to other areas of study.

Microalgae have pigments including chlorophyll a, chlorophyll b, and carotenoids. These elements are crucial in their photosynthetic process and allow for the protection of the cells from environmental damage. Chlorophyll pigments capture light and convert it to energy, while carotenoids protect cells from ROS through a process of neutralizing the cell [25]. By studying how ethanol effect these pigments, insight can be gained into its broader impact on cell health in relation to healthy cells. Changes in these pigment levels can indicate disruptions in photosynthesis and cellular health, making them valuable biomarkers for assessing ethanol toxicity.

Microalgae also offers practical advantages for laboratory research. They have short generation times and can be cultured in large quantities under controlled conditions. This makes them cost-effective and efficient for high-throughput screening of various environmental stressors, including ethanol. Furthermore, the genetic and physiological simplicity of microalgae allows for detailed mechanistic studies that might be challenging in more complex organisms.

Gaps in Existing Knowledge

While there is valuable research on how ethanol effect cells, there is limited information available on how different ethanol concentrations impact microalgae specifically. Most research has been focused on complex and larger organisms. This leaves a gap in understanding the effects of simpler organisms like microalgae. Due to this gap, it may be difficult to relate more simple and small organisms such as microalgae to organisms that are more complex such as humans.

Current studies on ethanol's impact on microalgae focus on high concentrations of ethanol and often overlook the smaller effects of lower doses that might be more relevant to environmental and physiological conditions. Additionally, the variation in experimental conditions, such as differences in species and culture methods, makes it difficult to compare results across studies and draw overall conclusions.

Significance of the Study

The research this capstone entails aims to fill the missing gaps by providing insight into how different ethanol concentrations effect microalgae cells. By examining changes in chlorophyll a, chlorophyll b, and the carotenoid levels, this experiment gives insight into the

extent of cell damage and/or stress response caused by the cell being exposed to ethanol. Understanding the effects alcohol has on microalgae can help scientists make connections to more complex organisms, potentially revealing mechanisms relevant to human health and fetal development.

Additionally, using microalgae as a model system gives ethical and practical advantages. Microalgae are easy to alter in a laboratory setting and do not raise an ethical concern than if larger, more complex organisms were used. Conclusion gathered from this experience can provide different strategies to weaken the effects of ethanol exposure in more complex systems, including potential treatments for FAS.

THEORY

Understanding Ethanol's Impact on Cells

Ethanol effects various biological systems by potentially altering pathways that impact cells' function. It's important to understand how ethanol impacts a cell's structural integrity in order to help interpret the experiment's results. Ethanol's ability to disrupt cell structure and function has long term effects, especially for fetal development and alcohol-related diseases.

Membrane Fluidity and Ethanol

Ethanol can enter through cell membranes because it can interact with both the hydrophobic and hydrophilic components of the cell's lipid bilayer. This makes the membrane more fluid, which disrupts the function of proteins in the membrane and ultimately effect different pathway functions of the cell. When these proteins don't work properly, cell signaling and metabolism can be faulty, leading to cell damage and death [26]. Changes in membrane structure can also make cells more susceptible to other harmful environmental factors.

Reactive Oxygen Species (ROS) Accumulation

When cells metabolize ethanol, they produce a species called reactive oxygen species (ROS). These ROS are highly reactive molecules that can damage cell parts such as lipids, proteins, and even DNA. The buildup of ROS in a cell can exhaust the cell's defense system ultimately leading to oxidative damage and cell death. This oxidative stress is a major reason why ethanol is toxic and contributes to conditions such as liver damage and possibly neurodegeneration [27]. Long-term exposure to ethanol can lead to constant oxidative stress and long-term cell damage.

Disruption of Signaling Pathways of the Cell

Ethanol can heavily damage signaling pathways that control important cell processes such as cell growth, development, and survival. For example, ethanol can block an important pathway such as the MAPK/ERK pathway. This pathway is essential for cell growth and survival. Disruption of this pathway can severely impair cell function and increase the changes of cell death [28]. Ethanol can also interact with other signaling pathways such as the PI3K/Akt pathway. This particular pathway is involved in cell survival and metabolism. By damaging this specific signaling pathway, ethanol can significantly impact cell health and development. This could also potentially lead to diseases such as cancer and neurodegenerative disorders.

Photosynthetic Pigments

Photosynthetic organisms, specifically microalgae, are dependent on pigments such as chlorophyll a, chlorophyll b, and carotenoids to take in the light energy and protect the cell from the oxidative damage present. Ethanol's impact on these pigments provide great insight into its wider effects on a cell's health.

Chlorophyll a and b: Chlorophyll a and b are pigments that are crucial in the photosynthetic process. They absorb light energy and convert it into chemical energy. If the cell is exposed to ethanol, or any alcohol in general it can degrade its chlorophyll pigments. This may result in the reduction of photosynthetic activity and ultimately impair the cell's ability to produce energy. The degradation of a cell is often linked with an increased level of ROS production and therefore oxidative stress. This will ultimately cause damage to chlorophyll molecules [29]. The decrease in chlorophyll pigments in the microalgae can negatively effect growth and survival of the organism.

Carotenoids: Carotenoids protect cells from oxidative damage through the neutralization of ROS and stabilizing chlorophyll pigments in the molecules. Radical oxygen molecules are very unstable and it is essential for them to be stabilized to ensure it isn't damaging to the organism. Ethanol-induced oxidative stress can lower carotenoid levels. This causes a reduction in the cell's ability to defend itself against ROS and leads to further damage. A decrease in carotenoids can compromise the cell's protective mechanisms, making it more at risk to light-inflicted damage [30]. Reduced carotenoid levels can also effect other cell processes in other aspects than photoprotection.

Comparative Analysis: Microalgae and Human Cells

Studying ethanol's effects on microalgae can provide valuable insight into similar processes present in human cells. Both human and microalgae cells share basic mechanisms such as their membrane structure and ROS generation. By understanding how ethanol negatively impacts important processes in microalgae cells, researchers can connect these findings to potential impacts in human cells and overall conditions such as fetal alcohol syndrome (FAS).

Mitochondria, similar to chloroplasts in plant cells, are involved in an energy production process and have the potential to be exposed to oxidative stress. When ethanol damages the mitochondria structure, in human cells, it can lead to a decrease in energy levels resulting in an increase in cell death. This disruption can contribute to developmental problems associated with FAS [31]. By understanding these similarities, researchers can develop ways to alter the negative effects of ethanol on human health.

Concluding Thoughts

Understanding how ethanol impacts cells is essential for interpreting the experimental data and drawing further conclusions and applications. Through examining the effects of ethanol on microalgae, this experiment strives to reveal the cellular mechanisms behind ethanol toxicity in a cell. This insight can help in connecting basic cell research and the complex effects of alcohol on human health. This could also assist in helping to better understand fetal alcohol syndrome in humans. This capstone is essential in developing ways to prevent and treat FAS and other alcohol-related conditions.

METHODS

To measure the effects ethanol has on microalgae, a series of experiments took place in Dr. Ke Du's lab with the assistance of his PhD student, Dr. Can Wang. The experimental procedures consisted of two parts. Part one includes measuring the cell growth while part two consists of measuring the pigments extracted including chlorophyll a, chlorophyll b, and the carotenoids. The data gathered from this experiment will help interpret the effects the ethanol has on wildtype microalgae cells, specifically the strain CC-125. This experiment took place over the course of approximately three days. If more time was allowed, the experiment would have continued for longer.

Part 1: Measuring Cell Growth

One day one of the experiments, there is a set up part of the experiment required. It is necessary to have six test tubes for this part. The first step is to label each of these tubes. Each tube will have the same amount of microalgae cells and a varying amount of ethanol. The six test tubes are labeled 2 µL, 20 µL, 200 µL, 2000 µL, control, and negative. The negative is for the ethanol because it is the negative control of this portion of the experiment. The control is the pure microalgae. The amount in these tubes isn't important, but it is important that there is enough in the tubes to carry out the experiment. For the other four tubes put 2 µL, 20 µL, 200 µL, 2000 µL into its corresponding tube. In each of these testing tubes 10 mL of pure microalgae is added into each tube and mixed with the ethanol previously added.

A 96 well plate was utilized during this portion of the experiment. Each sample was run a total of three times to get a total of three trials to ensure accuracy. Six rows are utilized and labeled on the well plate for the negative control, control, 2 µL, 20 µL, 200 µL, and 2000 µL

sample. Start by taking each sample and micropipetting 200 µL in each well of each sample. Row A corresponds to the negative control, row b corresponds to the control, row c corresponds to 2 µL, etc. Each row will have three total samples. At the end of this part of the experiment there should be a total of 18 rows filled with the samples. After filling the wells, insert the wells into a spectrophotometer to measure the optical density. The wavelength used to measure this specific strain of microalgae is 680 nm. This is done for three days.

Part 2: Extracting the Pigments

The second part of this experiment consists of extracting the pigments. First, add 2 mL of each tube except the negative control to five centrifuge tubes. Then centrifuge the tube for 5 minutes at 6000 revolutions per minute. After the tubes are done in the centrifuge, carefully pipette the extra fluid on top. After the fluid is removed from each tube, add 2 mL of 80% acetone in each tube and use a vortex to ensure it is well mixed into the microalgae. This is done to extract the pigments. After the cells and acetone are well mixed, they must be placed into a water bath at 55°C for 30 minutes.

After the 30 minutes, centrifuge the samples at 12000 RPM for 5 minutes. The procedures that follow are similar to filling the well plates in part 1. Fill the 96 well plate as before but instead of using ethanol as the negative control, acetone will be used instead. When filling the wells with the centrifuged tubes, ensure that the supernatant is the part that is used. Insert into the spectrophotometer like before to measure the OD once again. For this section, OD will be measured at 663 nm, 645 nm, and 470 nm. This will later be useful when calculating the pigments extracted. This experiment will also occur over the course of three days.

RESULTS

Results From Part 1 - Measuring Cell Growth

Day 1

	1	2	3	Average	Day 1	Comparison Day 1
Negative	0.0040	-0.0020	-0.0020	0.0000		
Control	1.5860	1.6580	1.6260	1.6233		
2	0.0801	0.1128	0.0566	0.0831	0.000144293418	0.0001442934189
20	0.1456	0.1725	0.1574	0.1585	0.0000806375010	0.00008063750193
200	0.1961	0.1954	0.1986	0.1967	0.000216404714	0.0002164047147
2000	0.4111	0.4397	0.4114	0.4207	0.000133396823	0.0001333968231

Table 1: Percent Inhibition Calculated For Day 1 for the Samples. To the right, a T-Test was performed and if the T-Test was significant, it is highlighted in yellow.

Day 2

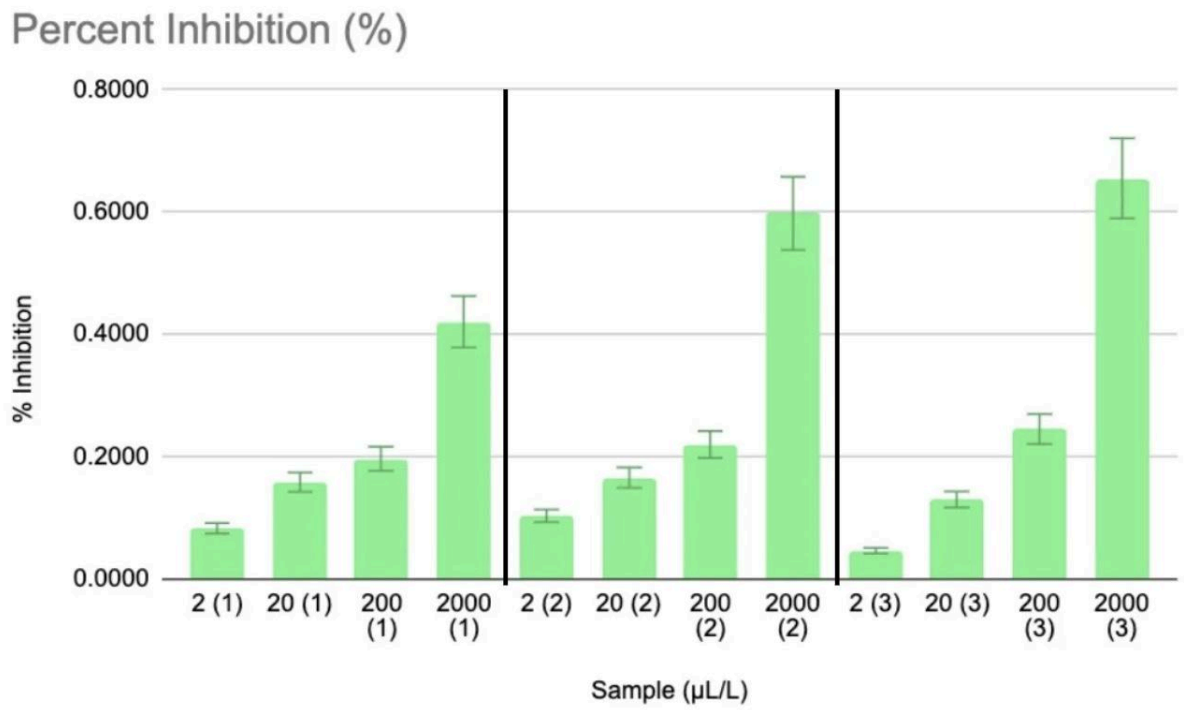
	1	2	3	Average	Day 2	
Negative	0.0030	-0.0020	-0.0020	-0.0003		
Control	1.7970	1.7930	1.8660	1.8187		0.02473832355
2	0.0996	0.0864	0.1238	0.1033	0.000063411868	0.000296208533
20	0.1619	0.1467	0.1892	0.1659	0.000056833112	0.0003480941466
200	0.2398	0.1539	0.2663	0.2200	0.000218743701	0.00129419282
2000	0.6071	0.5800	0.6066	0.5979	0.00028010046	0.0007860341842

Table 2: Percent Inhibition Calculated For Day 2

Day 3

	1	2	3	Average	Day 3	
Negative	0.0000	-0.0010	0.0010	0.0000		
Control	1.6420	1.6750	1.6190	1.6453		0.3535001907
2	0.0238	0.0639	0.0513	0.0463	0.000097421320	0.0000346285961
20	0.1255	0.1445	0.1204	0.1301	0.000037118841	0.0001222047579
200	0.2253	0.2579	0.2526	0.2453	0.000144533688	0.00007097873474
2000	0.6693	0.6543	0.6418	0.6551	0.000238824102	0.0007403139262

Table 3: Percent Inhibition Calculated For Day 3



Graph 1: The Change in Percent Inhibition Over Three Days with Different Concentrations of Alcohol Used

Results From Part 2 - Extracting the Pigments

Day 1

	Trial 1	Trial 2	Trial 3			
	Ca	Ca	Ca	Average	T-Test Same-Day	T-Test Vs Day1
N	0.0122	0.0188	-0.0310	0.0000		
C	3.3184	3.4547	3.4331	3.4021		
2	2.9229	3.0619	3.0459	3.0102	0.00003891073375	0.00003891073
20	2.8816	2.7801	2.9370	2.8662	0.01732651198	0.01732651198
200	2.5198	2.6175	2.6279	2.5884	0.0002145426816	0.00021454268
2000	3.3542	3.4341	3.4162	3.4015	0.9780069154	0.9780069154

Table 4: Amount of Chlorophyll A Present in Samples on Day 1

Day 2

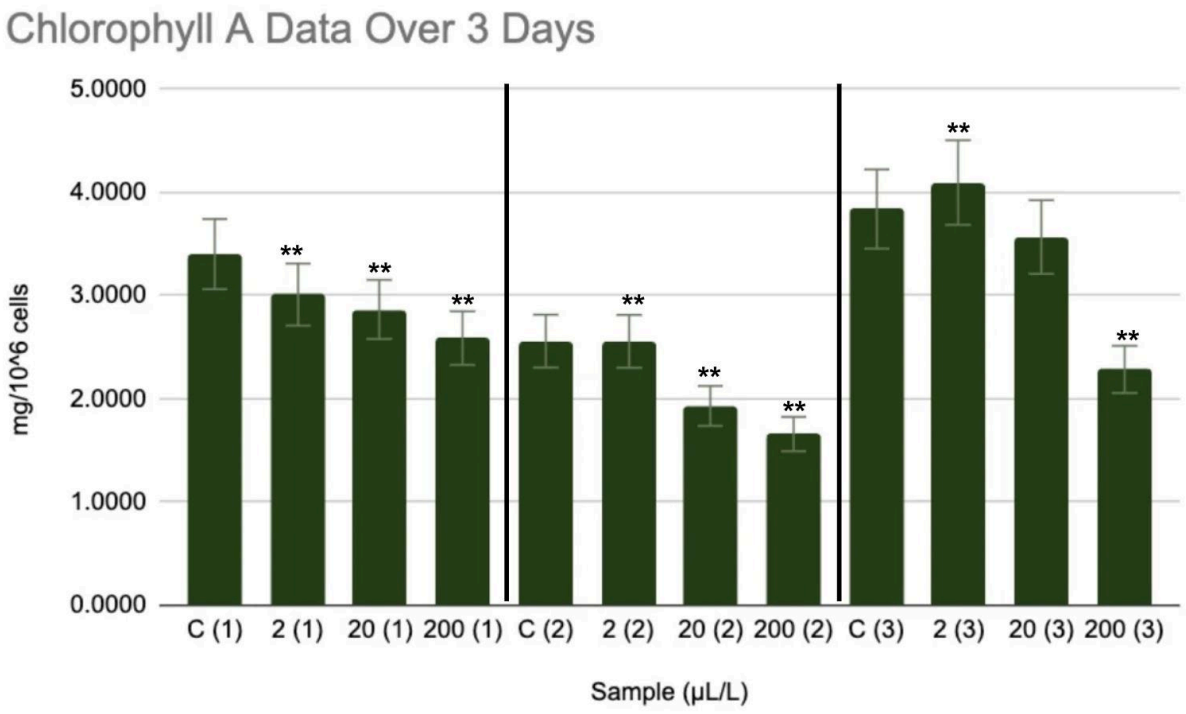
	Ca	Ca	Ca	Average	T-Test	
N	0.0366	0.0132	-0.0498	0.0000		
C	2.0416	2.3686	3.2683	2.5595		0.1335650526
2	2.5321	2.4757	2.6645	2.5574	0.9954415527	0.006303330016
20	1.8377	1.9242	2.0322	1.9314	0.1815484197	0.000658021850
200	1.5756	1.6930	1.7081	1.6589	0.1151595605	0.000036946986
2000	2.4702	2.5717	2.5473	2.5297	0.9402587466	0.000192144894

Table 5: Amount of Chlorophyll A Present in Samples on Day 2

Day 3

	Trial 1	Trial 2	Trial 3			
	Ca	Ca	Ca	Average	T-Test	
N	-0.0028	-0.0066	0.0094	0.0000		
C	3.8004	3.7515	3.9648	3.8389		0.0257400587
2	3.6829	4.2682	4.3368	4.0960	0.3122798603	0.05318064017
20	3.2686	3.7093	3.7243	3.5674	0.1963941663	0.2657256057
200	2.2211	2.3141	2.3207	2.2853	0.001539946214	0.000129073735
2000	1.4301	1.4292	1.4574	1.4389	0.0005335466463	0.000416945809

Table 6: Amount of Chlorophyll A Present in Samples on Day 3



Graph 2: Amount of Chlorophyll A Present in Samples Over Three Days with Different Concentrations of Alcohol Used

Day 1

	Trial 1	Trial 2	Trial 3			
	Cb	Cb	Cb	Average	T-Test Same Day	T-Test Vs Day1
N	0.0000	0.0004	0.0006	0.0003		
C	4.3094	4.7504	4.6886	4.5828		
2	3.2442	3.6006	3.4904	3.4451	0.001164865906	0.001164865906
20	3.3809	3.1883	3.4993	3.3562	0.02173589718	0.02173589718
200	2.5027	2.5894	2.7330	2.6084	0.002695153668	0.002695153668
2000	4.6618	4.8314	4.8799	4.7911	0.1183264402	0.1183264402

Table 7: Amount of Chlorophyll B Present in Samples on Day 1

Day 2

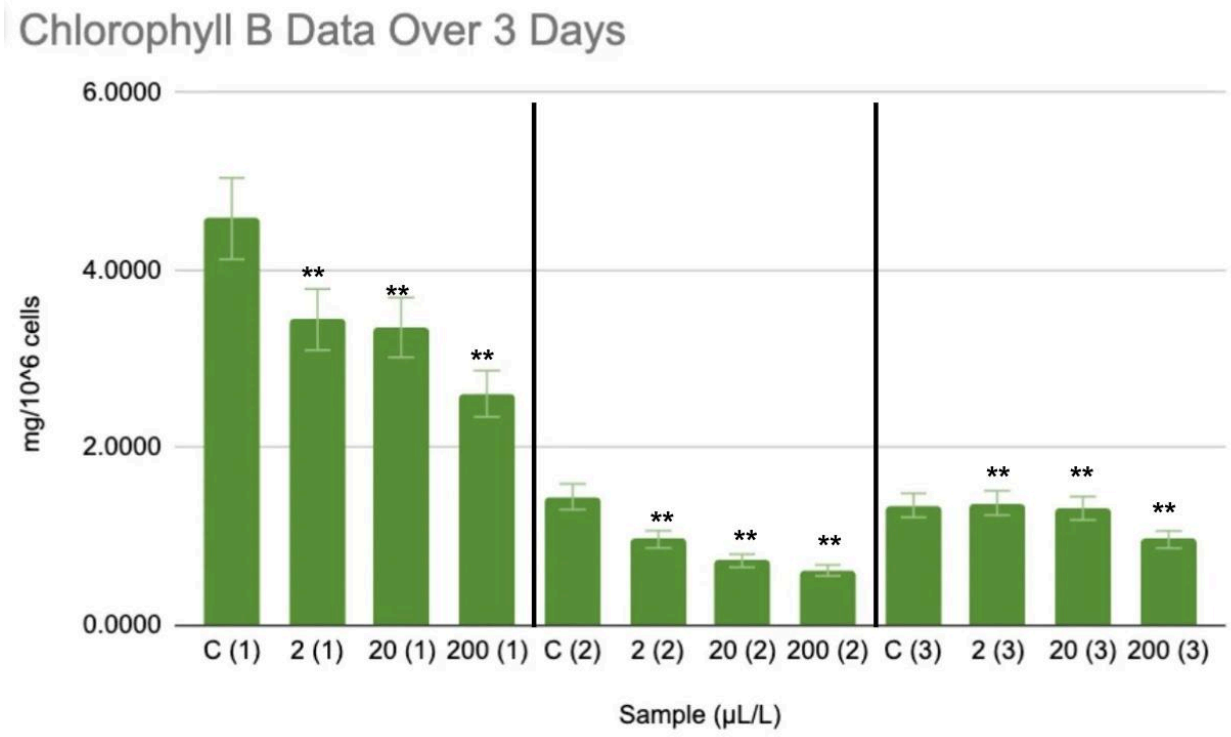
	Trial 1	Trial 2	Trial 3			
	Cb	Cb	Cb	Average		
N	-0.0151	0.0705	-0.0554	0.0000		
C	0.7906	0.9215	2.6378	1.4500		0.02929928159
2	0.9870	0.8964	1.0272	0.9702	0.4879105114	0.001844966937
20	0.6848	0.7251	0.7755	0.7285	0.3337250333	0.000957396281
200	0.5841	0.6496	0.6244	0.6194	0.2955239587	0.000908346420
2000	1.4298	1.4449	1.4550	1.4432	0.9919165202	0.001753183401

Table 8: Amount of Chlorophyll B Present in Samples on Day 2

Day 3

	Trial 1	Trial 2	Trial 3			
	Cb	Cb	Cb	Average		
N	0.0201	-0.0352	0.0151	0.0000		
C	1.2993	1.3194	1.4402	1.3529		0.001420424446
2	1.2338	1.4101	1.4956	1.3798	0.6272578794	0.000571074628
20	1.2337	1.3747	1.3496	1.3193	0.533137617	0.000837083085
200	0.9064	0.9819	1.0171	0.9684	0.004222644403	0.000913479784
2000	0.9666	0.8911	0.9364	0.9314	0.01350952573	0.001852602897

Table 9: Amount of Chlorophyll B Present in Samples on Day 3



Graph 3: Amount of Chlorophyll B Present in Samples Over Three Days with Different Concentrations of Alcohol Used

Day 1

	Trial 1	Trial 2	Trial 3			
	Cc	Cc	Cc	Average	T-Test Same Day	T-Test Vs Day1
N	-0.0045	0.0039	-0.0042	-0.0016		
C	1.4366	1.3784	1.3501	1.3884		
2	1.5286	1.5132	1.5329	1.5249	0.03497262632	0.03497262632
20	1.4758	1.4730	1.4518	1.4669	0.05789500597	0.05789500597
200	1.5567	1.6338	1.5684	1.5863	0.03915880247	0.03915880247
2000	0.6646	0.6563	0.6127	0.6446	0.000393500655	0.000393500655

Table 10: Amount of Carotenoids Present in Samples on Day 1

Day 2

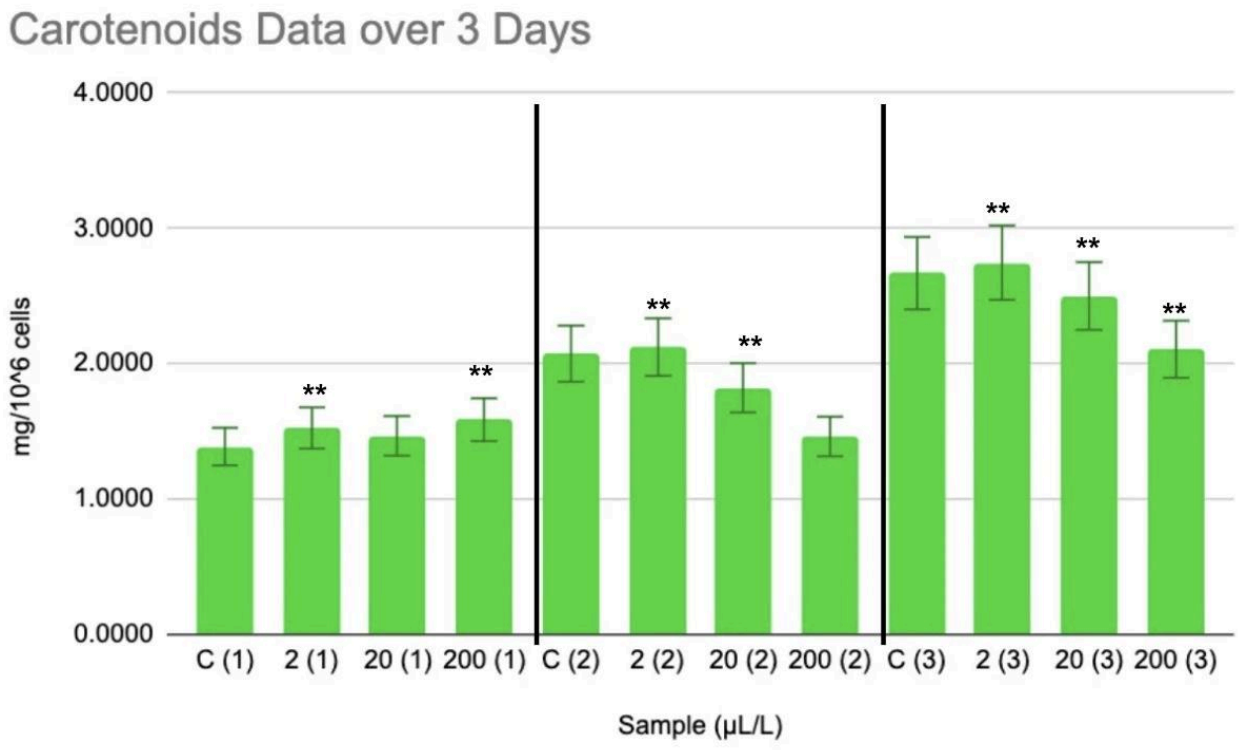
	Trial 1	Trial 2	Trial 3			
	Cc	Cc	Cc	Average		
N	0.0063	-0.0235	0.0171	0.0000		
C	1.9655	2.2944	1.9606	2.0735		0.02831861362
2	2.0527	2.1296	2.1854	2.1226	0.7090100686	0.007477309238
20	1.7282	1.8310	1.9070	1.8221	0.1679132843	0.03022938365
200	1.3934	1.4756	1.5174	1.4621	0.03098283325	0.3555252723
2000	0.5599	0.5953	0.5823	0.5792	0.004688849055	0.001764654857

Table 11: Amount of Carotenoids Present in Samples on Day 2

Day 3

	Trial 1	Trial 2	Trial 3			
	Cc	Cc	Cc	Average		
N	-0.0091	0.0161	-0.0114	-0.0015		
C	2.6395	2.6311	2.7347	2.6684		0.001788506061
2	2.4832	2.8707	2.8833	2.7457	0.5846722501	0.01292500834
20	2.2708	2.6022	2.6265	2.4998	0.2421537804	0.01538190537
200	2.0501	2.1237	2.1469	2.1069	0.002315976498	0.005720587389
2000	0.1389	0.1688	0.1653	0.1576	0.0001556926424	0.000773883449

Table 12: Amount of Carotenoids Present in Samples on Day 3



Graph 4: Amount of Carotenoids Present in Samples Over Three Days with Different Concentrations of Alcohol Used

DISCUSSION

Part 1: Measuring Cell Growth

In part 1, the main calculation that indicates the heath of the cell is percent inhibition. Percent inhibition in a cell gives an accurate representation of the biological activity over time as the cell is being exposed to ethanol. A higher percent inhibition indicates the cell's function is impaired at a higher extent than a lower percent inhibition. Table 1, Table 2, and Table 3 all indicate different data collected over a three day period for ethanol, pure microalgae, 2 µL, 20 µL, 200 µL, and 2000 µL. Given the OD calculated during the experiment, Percent inhibition can be calculated.

$$\%\, I \;=\; (\, Cc \;-\; Ct)/C\mathrm{c}$$

Where % I is Percent Inhibition

Ct is Cell Density of the Sample

Cc is Cell Density of the Control

Percent inhibition was calculated for all three days and all three trials. Given this data all three trials for that particular sample were averaged out. Looking at raw data isn't an indicator if the experiment had significance or not. To indicate where or not the experiment was successful/has any significance, it is important to run a T-Test. No T-test was run on the negative control as the purpose of this is to calibrate the spectrophotometer. On Day 1, the samples were all compared to the control and a T-Test value was given. On Days 2 and 3 the samples were compared to that day's control. In addition to this, these samples were controlled to the control sample from Day 1. All of the data is significant that was obtained according to the T-Test

indicating there was a significant change in the data over the course of 3 days. As shown in Graph 1, the higher the concentration of alcohol present in the microalgae, the higher the percent inhibition is. A 2000 µL sample has a higher percent inhibition than a 2 µL sample. In addition a sample of 2000 µL had a higher percent inhibition on day 2 than a sample of 2000 µL on day 1. This indicates that the higher the concentration is consumed and as more time goes on, the cell's function is impaired. Any amount of alcohol present in the cell has the ability to impair a cell shown by the 2 µL sample on day one. This is important in pregnant individuals because a 2 µL sample is equivalent to one drink in an individual that is 70 kg. If a 70 kg individual consumes even one alcoholic beverage during pregnancy, it has the ability to cause damage not only to their cells but to their unborn fetus.

Part 2: Extracting the Pigments

The pigments extracted in this experiment are chlorophyll a, chlorophyll b, and the carotenoids. Chlorophyll a is the primary photosynthetic pigment in plants and algae, absorbing light most effectively in the blue-violet and red parts of the spectrum. Chlorophyll b is an accessory pigment that broadens the range of light wavelengths that can be used for photosynthesis, reflecting yellow-green light. Carotenoids is a chlorophyll variant found in certain algae, with absorption properties that suit their specific environmental needs.

Table 4, Table 5, and Table 6 all give the reading corresponding to chlorophyll a. Chlorophyll a is calculated by an equation.

$$Ca = 12.21\,A663 - 2.81\,A646$$

A663 is the absorbance at 663 nm

A646 is the absorbance at 646 nm

Table 7, Table 8, and Table 9 all give the reading corresponding to chlorophyll b. Chlorophyll b is calculated by an equation.

$$Cb = 20.13\,A646 - 5.03\,A663$$

A644 is the absorbance at 646 nm

A663 is the absorbance at 663 nm

Table 10, Table 11, and Table 12 all give the reading corresponding to carotenoids. Carotenoids are calculated by an equation.

$$Cc = (1000A470 - 3.27Ca - 104Cb)/229$$

A470 is the absorbance at 470 nm

Ca is Chlorophyll a

Cb is Chlorophyll b

After conducting this experiment, it is concluded that the data gathered from Day 3 may not be accurate. The ethanol damage on the final day may have been so great that it resulted in inaccurate reading in the results. Information about what could be done differently the next time this experiment is conducted is in the future research section. For the purposes of this report, only Day 1 and 2 are being compared to determine the effects of ethanol on the pigments. Graph 2 and 3 show the chlorophyll a and chlorophyll b pigment data respectively. As shown from Graph 2 and 3, as the concentration of alcohol increases, the less successful chlorophyll a and chlorophyll b becomes. This is because as alcohol is exposed to the microalgae, the less the cell is able to function like normal. For higher concentrations of alcohol, the pigment is even lower indicating that the higher the alcohol concentration, the larger it has an effect on the cells. Any

concentration of alcohol has an effect on the microalgae as well. This can be seen when comparing the data for 2 µL between Day 1 and Day 2.

According to Graph 4, the Carotenoids increase as alcohol is being exposed to them. Since carotenoids act as a defense system it increases as more alcohol is added to try to "fight" the effects of the alcohol and maintain some level of homeostasis. These results are present in this experiment. The higher the concentration of alcohol, the higher the carotenoids concentration present in the cell to try to maintain the health of the cell. Overall this part of the experiment's results were expected because the chlorophyll pigments concentration decreases as being exposed to alcohol which indicates that the cell's ability to function is impaired. Because of this the carotenoids concentration increases as it serves as a defense mechanism for the cell against the alcohol.

CONCLUSION

In conclusion,this capstone report indicated how different concentrations of ethanol effect microalgae cells by measuring changes in chlorophyll a, chlorophyll b, and carotenoid levels. The results showed that a higher level of ethanol present in the cells disrupts these important pigments, which are crucial for the microalgae's growth and survival. An increase in carotenoids was found during the experiment, indicating an increase in the cells' defense mechanism against stress caused by ethanol. Through the use of microalgae, a better understanding of how ethanol disrupts cellular functions was discovered, which can be related to similar effects in human cells. Insight into fetal alcohol syndrome (FAS) was gained through scientific research which highlights the need to avoid alcohol during pregnancy to protect the developing fetus and the mother. The results of this experiment can help guide future research and develop ideas to reduce the negative impact of ethanol on human health.

FUTURE RESEARCH

This experiment wasn't carried out as expected before due to accessibility of the items and the time strain. Originally the microalgae strain was supposed to be a mutation of the wildtype, CC-400. This is because this type of microalgae doesn't have a cell wall but rather has a cell membrane to help further mimic the effects of a fetus in the amniotic sac. The membrane is more comparable to an amniotic sac than a rigid cell wall. This strain was difficult to source and won't arrive until much later so the wildtype microalgae was utilized. In addition, due to instrumental issues, the use of an auto fluorescent lamp wasn't utilized. An auto fluorescent lamp allows researchers to measure viability in a cell. Although this was measured via pigments in the new experiment, it would have been useful to incorporate it into the results. Further research will be done during the summer under the guidance of Dr. Ke Du with alterations to this experiment to better enhance the results and ensure the data being published is very accurate as more data is necessary to ensure the data collected is very accurate. After making these changes to the experiment during the summer, there are hopes of publishing a paper by the end of the summer with Dr. Ke Du and his PhD student Dr. Can Wang.

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