

Research Article
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Isochrysis Crisis

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ABSTRACT

Isochrysis galbana, a brown algae, was trialed to grow at the CFK aquaculture lab but failed until we tried comparing different tank salts to create a well-balanced base. Our objective was to demonstrate and compare the different saltwater mixes that can be used in cultivating brown algae.

Isochrysis galbana and other green algae that are grown at the CFK lab play a very important part in the aquaculture system. Algae is used as a good source of food and nutrients for many primary producers. Algae are one of the most important animals on earth. They create most of the oxygen that we breathe, and they filter every part of the water around the world. Brown algae is known around Florida because of certain species, like sargassum.

We are currently having an issue with sargassum, so learning about different brown algae species can benefit everyone around the world. If *Isochrysis sp.* is kept at a stable salinity of 35ppt then it will easily and successfully grow. The samples were grown according to the standard measures but with different salt bases one was raw salt water, live aquaria and REEF salt. The raw seawater sample grew over a 1–2-day period with salinity that usually stayed around 40. The samples mixed into each artificial reef salt took about a week or more to grow color and cultivate the algae.

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Received: March 27, 2024; **Accepted:** April 01, 2024; **Published:** April 10, 2024

Introduction

Isochrysis sp. is a single-celled brown algae and a primary producer in oceanic ecosystems. “Brown algae and brown seaweeds are the most dominant organism in many of the world’s coastal regions” [1]. As aquaculture becomes more common knowledge, the complexity of recreating an oceanic environment is also known. Some sort of food chain is needed for successful cultures, and primary producers or their energy is used to help with this process. Algae, being a primary producer, is a perfect option for this necessity. Larval staged organisms are most commonly fed algae, which provides a nutritious food source. Algae help with the survival and growth of these marine organisms, which is vital for the aquaculture industry. At my school’s aquaculture lab, green algae are grown at a constant level to keep up with fish production. We miss out on key nutrients and energy that can be harnessed by not adding brown algae to their feed, as it is so abundant in the ocean. Brown algae samples were attempted to be cultured but failed. In this project, I will be developing a brand-new culture of *Isochrysis sp.* at the CFK Aquaculture Lab. The starters were ordered and began culturing once they were delivered. Once the algae began reproducing, I observed their salinity and patterns. We have two different salts used to create a dilution to observe which created the better brown algae cultures. “This species could tolerate a wide range of salinity in phototrophy, but 35‰ salinity was optimal for algal growth in mixotrophy.” [2]. The salinity remained at 35 ppt, but different salt mixers were used.

Methods

This study examines the success of culturing *Isochrysis sp.* using two different salt mix brands. *Isochrysis sp.* samples were purchased on February 10th. *Isochrysis sp.* cultures were grown

in 10000 ml (about 2.64 gal) glass beakers using standard algal culture techniques, using algal density microscopy. This process includes using a microscope to measure the *Isochrysis sp.* blood cells and using a formula to calculate the volume. This is then calculated to the flask using cell density = $e^{\{[\ln(\text{absorbance}_{684}) + 16.439]/1.0219\}}$ (n=130; r²=0.9998). *Isochrysis sp.* cultures are subject to two different salt mixes, Instant Ocean®. The results from this study will benefit the aquaculture lab and future research on *Isochrysis sp.* Other materials included a large light, a pump, tubes, pipettes, 1800 ml (about 60.87 oz) flasks, and a Carolina sample. A hemocytometer and a glass slide were used to place the samples onto a microscope. A counter was then used to help keep track of counting.

Results

Isochrysis sp. samples were cultured using the standard methods used by the CFK Aquaculture Laboratory using three different saltwater mixes. Raw seawater, water sourced from the ocean containing all-natural nutrients but no living organisms, was used for our first sample in our first beaker. This batch was created on March 3 and cultured by March 7. Measurement was done on March 7 using a hemacytometer to average cells, which found an average of 12.8 cells per square unit. This equates to 3,200,000 cells/ml for the first measurement which can be found in Table 1. On March 15, a measurement of cells was completed with an average of 17.4 cells per square unit and 4,350,000 cells/ml. On March 15 two samples were also used to create 2 different cultures, one using instant ocean and one using reef salts. On March 17, there was no change in the beaker’s color, so two more samples were used to make our fourth and fifth cultures. They followed the same order of two different salt mixtures. By March 23 two

samples had succeeded in culturing, one from the March 15 batch and the other from the March 14 batch. On March 27, the second sample from the batch created 3-15 had successfully cultured, proving both salt mixtures worked. On April 1, measurements were taken from all sample beakers. The first sample, created on March 7th, had 29.4 cells per square and 7,350,000 cells/ml. The second sample created on March 15 revealed 12.2 cells per square and 3,100,000 cells/ml. The other sample created on March 15 had 18.4 cells per square and 4,600,000 cells/ml. The successful third sample created on March 17 showed 28 cells per square and 7,000,000 cells/ml. The final sample that was created on March 17 never completed its culture; it stayed white as the photo shows. This culture was still measured and showed 0.6 cells per square unit and 150,000 cells/ml. Table 1, shows the data, including the amount of cells/ml in each measurement.

of the two cultures was observed to be successfully growing by March 23. The other culture never got pigmentation and stayed a white hue, with less than 4 cells identified within the entire hemacytometer. This culture was discarded on April 1st. There are many variables concerning the growth rate between cultures. Due to lab overcrowding, all the cultures were grouped closely together, causing half of the cultures to be directly in the light and the others to be in front of those cultures. When removing samples from the beakers, they may have been exposed to lab ventilation and contaminants. A pipette was placed in the beaker, suctioned by a human finger, and then placed on a microscope slide. The samples were also cultivated in the main laboratory with other lab projects where different solutions could have dripped into the dilution buckets. The CFK laboratory assistant Jessie app helped me tremendously to cultivate these samples safely and effectively as they would hopefully be used in the lab. Algae is used all around the lab as feed for many different species, including copepods and clownfish. The brown algae will help the lab test new nutrients and improve mixed feed solutions for these organisms. The brown algae will create new opportunities for the lab to be more of a controlled oceanic ecosystem.



Figure 1

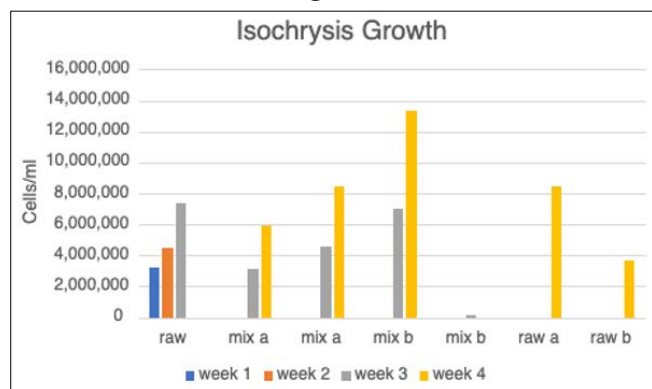


Table 1: Represents the Data Including the Amount of Cells/ml in Each Measurement.

Discussion/Conclusion

On March 3rd, the first set of *Isochrysis sp.* cultures were grown with raw seawater. It was split on March 27. The split was successful and resulted in two new cultures identified as Raw A and Raw B. Raw A had more algae cells when compared to Raw B. This may be due to many issues with splitting algae. Dilutions used to properly separate the algae into two separate samples can be incorrect due to human error and other variables. On March 15th, the second set of *Isochrysis sp.* cultures were created and had a steady rate of growth over time. One of the cultures had a higher number of cells when compared to the other. On March 17th, the third set of *Isochrysis sp.* cultures were created. One

References

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2. Yousef A, Jian G Q (2013) Cultivation of *Isochrysis Galbanain* Phototrophic, Heterotrophic, and Mixotrophic Conditions. BioMed Research International. 2013: 1-9.

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