The Impact of Microplastics on Algae (Respiration)

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Executive Summary

Microplastics are extremely harmful to everything. Microplastics can introduce toxic substances in humans, animals, or plants,as well as absorb undesirable chemicals that when released out of the microplastics can have harmful effects. Photosynthesizing algae is very important in the global ecosystem as it is responsible for 70% of earth's oxygen. It is also very important in the marine environment as it is the food base for most marine organisms. We wanted to research if or how microplastics affect algae and learn about various solutions to the microplastic problem.

Our experiment tried to model a real world interaction between microplastics and algae, and we did that by having 8 jars with the same amount of our algae and adding different amounts of microplastics. We tested the dissolved oxygen in each jar to test the productivity of the algae.

We created an object oriented model to represent algae, microplastics, and their interaction. We also created a program which takes inputs for dissolved oxygen levels and determines the total carbon productivity from and imputed data table

Our results were fascinating, the algae that had more interactions with the microplastics was producing more oxygen. Until the production plateaued for all the algae.

Introduction

a. Project goal

We investigated how microplastic affected the respiration of algae. If microsplastics have harmful effects on algae, they may harm everything in the food chain that depends on them.

b. Microplastics

Plastic pollution is a worldwide issue on account of microplastics having been found everywhere, even in the most remote regions. Microplastics are defined as being smaller than 5mm, while nano plastics are defined as being smaller than 100nm. Microplastics have been found to have negative consequences on organisms including humans.[1].

Microplastics occur during the process of breaking down larger pieces of plastic such as water bottles or plastic packaging and particles break off from the larger pieces. It is estimated that there are now trillions of microplastic particles in the oceanic environment. Microplastics can take hundreds or thousands of years to break down, and scientists are saying that there is no one square mile of the ocean's surface that is free of plastic pollution.

Microplastics carry harmful toxins from when they were manufactured and release them when physically damaged. According to the article *Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies,* microplastics also have the ability to absorb and transport harmful substances as well as act as a gathering point for certain “microbial assemblages or even pathogenic bacteria” [31]. Microplastics can absorb organic contaminants, mainly Persistent Organic Pollutants (POPs), which can lead to the introduction of other materials in the food web. Studies have found the combination of microbes and microplastics to be even more toxic than they would be on their own. This combination of microbes and microplastics has even been found to be common in an urban river.

c. Importance of Algae

Photosynthesizing algae is a major oxygen source in the world as it contributes to 70% of the oxygen on the Earth. Algae is also very important in the oceanic ecosystem as it serves as the food base and provides micronutrients for almost all aquatic organisms. Other than providing lots of oxygen for humans to live, algae can also be used for supplements, for example omega-3 fatty acids as found in algae oil. They are also used in dyes, fertilizers, food, protein-rich animal/aquaculture feed, pharmaceuticals, and algal fuel.

d. Existing Studies

There have been many studies showcasing the different interactions between microplastics and algae. One important factor is the size of the microplastics. Larger microplastics have the ability to block the sun, preventing photosynthesis, while smaller microplastics are more prone to leaching chemicals which are toxic to living organisms.

In the article *Effect of microplastics exposure on the photosynthesis system of freshwater algae* [1], Wu *et al.* investigated the effects of on the photosynthetic system of freshwater algae. They tested the plastics polyvinyl chloride (PVC) and polypropylene's (PP) effect on the algae *Chlorella pyrenoidos*a and *Microcystis flos-aquae*. This study found that microplastics harm the algae, consequently negatively influencing and reducing photosynthesis. However, the algae ended up photosynthesising more than algae without microplastics. The study found that, "algae may resist microplastics stress through self-regulation, leading to an increase in photosynthetic activity in low-concentration groups (5–10 mg/L) and even higher than that in the control group in some cases" [1].

Model and Implementation

a. Algae Processes Modeled in Data

To help us calculate the growth of algae to determine how it varies under stress we collected dissolved oxygen from living samples, which allows us to calculate the total carbon productivity of the algae. This equation helps us figure out how much the algae has grown. Our model inputs the current amount of dissolved oxygen and subtracts the initial amount of dissolved oxygen to determine the change in oxygen levels. That value is then divided by the amount of time elapsed to determine the net primary productivity. Using the net primary productivity we can multiply it by .375, the ratio of the mass carbon absorbed to the mass of oxygen released during photosynthesis [11], to calculate the total carbon productivity. We can take the total carbon productivity and multiply it times the initial mass to give us approximately the current mass of the algae.

```
def net_primary_productivity (l_bottle, elapsed_time):
 npp = (l_bottle - initial_dissolved_oxegen) / elapsed_time
 # npp = gpp-rr
 print(npp)

initial_dissolved_oxygen):
 npp = (l_bottle - initial_dissolved_oxygen) / elapsed_time
 tcp = npp * 0.375
 return(tcp)

original_oxygen = 6.1
glass_num = [1, 2, 3, 4, 5, 6, 7, 8]
dissolved_ox = [8,7,9.6,8.6,7.8,7.8,7.2,6.9]
subtract_water = 64
time_elapsed_days= [3,3,3,3,3,3,3,3]
alldata = pd.DataFrame(list(zip(glass_num, disolved_ox,
time_elapsed_days)))
alldata.columns =['glass_num', 'disolved_oxegen', 'time_elapsed_days']

for i, element in enumerate(glass_num):
 my_ttc = total_carbon_productivity(dissolved_ox[i], time_elapsed_days[i],
original_oxygen)
 print(element, my_ttc)
Last_ttc.append(my_ttc)
print(Last_ttc)
1 0.23750000000000004
2 0.11250000000000004
3 0.4375
4 0.3125
5 0.21250000000000002
6 0.21250000000000002
7 0.13750000000000007
```

```
8 0.10000000000000009
[0.23750000000000004, 0.11250000000000004, 0.4375, 0.3125,
0.21250000000000002, 0.21250000000000002, 0.13750000000000007,
0.10000000000000009]

Classroom = pd.read_csv('/content/Algae - Sheet1 (1).csv')

rows = [list(row) for row in Classroom.values]
print(rows)
[['1', 6.1, 9.6, 9.8, 7.0, 5.6, 10.0, 6.8, 4.8, 9.4, 9.6], ['2', 6.1, 8.0,
9.0, 7.5, 6.2, 7.2, 6.6, 5.6, 10.0, 8.8], ['3', 6.1, 7.0, 8.0, 7.0, 6.2,
7.6, 7.2, 6.2, 10.0, 9.0], ['4', 6.1, 8.6, 10.4, 5.6, 6.0, 6.4, 6.6, 5.8,
9.0, 9.2], ['5', 6.1, 7.8, 8.8, 6.8, 6.2, 6.8, 7.2, 5.8, 8.8, 9.0], ['6',
6.1, 7.8, 8.8, 6.8, 6.2, 6.8, 6.6, 5.0, 8.2, 9.0], ['7', 6.1, 7.2, 6.8,
6.4, 6.2, 6.2, 6.4, 5.8, 8.4, 8.2], ['8', 6.1, 6.9, 8.4, 6.1, 6.0, 6.2,
7.6, 5.6, 8.4, 7.0], ['Days Since Last Test', 0.0, 2.0, 4.0, 3.0, 7.0,
7.0, 5.0, 2.0, 14.0, 14.0]]

Classroom
      Jar#  2/6   2/8   2/12  2/15  2/22  2/29  3/5   3/7   3/21  4/4
0     1     6.1   9.6   9.8   7.0   5.6   10.0  6.8   4.8   9.4   9.6
1     2     6.1   8.0   9.0   7.5   6.2   7.2   6.6   5.6   10.0  8.8
2     3     6.1   7.0   8.0   7.0   6.2   7.6   7.2   6.2   10.0  9.0
3     4     6.1   8.6   10.4  5.6   6.0   6.4   6.6   5.8   9.0   9.2
4     5     6.1   7.8   8.8   6.8   6.2   6.8   7.2   5.8   8.8   9.0
5     6     6.1   7.8   8.8   6.8   6.2   6.8   6.6   5.0   8.2   9.0
6     7     6.1   7.2   6.8   6.4   6.2   6.2   6.4   5.8   8.4   8.2
7     8     6.1   6.9   8.4   6.1   6.0   6.2   7.6   5.6   8.4   7.0
8     Days Since Last Test   0.0   2.0   4.0   3.0   7.0   7.0   5.0   2.0
14.0  14.0

clasi1 = rows[0]
clasi2 = rows[1]
clasi3 = rows[2]
clasi4 = rows[3]
clasi5 = rows[4]
```

```
clasi6 = rows[5]
clasi7 = rows[6]
clasi8 = rows[7]
Days_Past = rows[-1]

print(Days_Past)
print(clasi2)

print(Days_Past)
print(clasi2)
['Days Since Last Test', 0.0, 2.0, 4.0, 3.0, 7.0, 7.0, 5.0, 2.0, 14.0,
14.0]
['2', 6.1, 8.0, 9.0, 7.5, 6.2, 7.2, 6.6, 5.6, 10.0, 8.8]

print(clasi1)
print(Days_Past)
['1', 6.1, 9.6, 9.8, 7.0, 5.6, 10.0, 6.8, 4.8, 9.4, 9.6]
['Days Since Last Test', 0.0, 2.0, 4.0, 3.0, 7.0, 7.0, 5.0, 2.0, 14.0,
14.0]

alldata = pd.DataFrame(list(zip(glass_num, clasi1[1:10],
Days_Past[1:10])))
alldata.columns =['glass_num', 'disolved_oxegen', 'time_elapsed_days']
print(alldata)
   glass_num  disolved_oxegen  time_elapsed_days
0          1              6.1                0.0
1          2              9.6                2.0
2          3              9.8                4.0
3          4              7.0                3.0
4          5              5.6                7.0
5          6             10.0                7.0
6          7              6.8                5.0
7          8              4.8                2.0

Prev_ttc = Last_ttc
def initial_dissolved_oxygen(Prev_ttc):
```

```
  enumerate(Prev_ttc)

def total_carbon_productivity(l_bottle, elapsed_time,
initial_dissolved_oxygen):
  npp = (l_bottle - initial_dissolved_oxygen) / elapsed_time
  tcp = npp * 0.375
  return(tcp)

Last_ttc = []
for i, element in enumerate(glass_num):
  my_ttc = total_carbon_productivity(dissolved_ox[i],
time_elapsed_days[i], original_oxygen)
  print(element, my_ttc)
  Last_ttc.append(my_ttc)
print(Last_ttc)
```

b. Algae interaction

For our object oriented code of our model the we have three classes: one representing algae, one representing mico plastics, and a class describing the model. Our algae duplicates every time it is called or every tick making one tick equivalent to the amount of time it takes algae to duplicate. Our microplastics are created with a random size below 5 mm. It is important to have this variation as different sizes of microplastics can have different effects on algae.

```
!pip install --quiet mesa
# The exclamation points tell jupyter to do the command via the command
line
from mesa.visualization.modules import CanvasGrid
from mesa.visualization.ModularVisualization import ModularServer
import mesa
import random
from mesa.time import RandomActivation

# Data visualization tools.
import seaborn as sns
from mesa.space import MultiGrid
```

```
# Has multi-dimensional arrays and matrices. Has a large collection of
# mathematical functions to operate on these arrays.
import numpy as np


class AlgaeAgent(mesa.Agent):
   """A singular ball of algae."""

   def __init__(self, unique_id, model):
       # Pass the parameters to the parent class.
       super().__init__(unique_id, model)
       self.poision= False

       # Create the agent's attribute and set the initial values.
       self.size = 1

   def move(self):
     x, y = self.pos
     x_offset = self.random.randint(-1, 1)
     y_offset = self.random.randint(-1, 1)
     new_position = (x + x_offset, y + y_offset)
     self.model.grid.move_agent(self, new_position)
     # The agent's step

     if self.size > 0:
         self.size *= 2



   def step(self):
       self.move()
       print(f"agent {self.unique_id}; pos {self.pos}; poision
{self.poision}")
       print(f"Hi, I am an agent, you can call me {str(self.unique_id)} I
have {self.size} cells.")
class MicroplasticAgent(mesa.Agent):
```

```
    """A singular pice of microplasic."""

    def random_microplastic_size():
        x = random.randint(0,20)
        return x

    def __init__(self, unique_id, model):
        # Pass the parameters to the parent class.
        super().__init__(unique_id, model)
        self.poision = True

        # Create the agent's attribute and set the initial values.
        self.size = random_microplastic_size()

    def move(self):
      x, y = self.pos
      x_offset = self.random.randint(-1, 1)
      y_offset = self.random.randint(-1, 1)
      new_position = (x + x_offset, y + y_offset)
      self.model.grid.move_agent(self, new_position)
        # The agent's step will go here.
        # For demonstration purposes we will print the agent's unique_id

    def step(self):
      self.move()
      print(f"agent {self.unique_id}; pos {self.pos};poision
{self.poision}")
      print(f"Hi, I am an plastic, you can call me {str(self.unique_id)} I
am {self.size}mm big.")

class AlgaeModel(mesa.Model):
    """A model with some number of microplastics."""

    def __init__(self, N, p, width, height):
        super().__init__()
        self.num_agents = N
```

```
        self.num_plastic = p
        # Create scheduler and assign it to the self
        self.schedule = RandomActivation(self)
        self.grid = MultiGrid(width, height, torus=True)
        self.running = True


        for i in range(self.num_agents):
            a = AlgaeAgent(i, self)
            x = self.random.randrange(self.grid.width)
            y = self.random.randrange(self.grid.height)
            self.grid.place_agent(a, (x, y))
            self.schedule.add(a)
        for i in range(self.num_plastic):
            a = MicroplasticAgent(i, self)
            x = self.random.randrange(self.grid.width)
            y = self.random.randrange(self.grid.height)
            self.grid.place_agent(a, (x, y))
            self.schedule.add(a)


    def step(self):
        self.schedule.step()
    #def step(self):
     #   """Advance the model by one step."""

        # The model's step will go here for now this will call the step
method of each agent and print the agent's unique_id
      #  self.schedule.step()
```

Live Experiment

We started our experiment by reviewing relevant literature. We needed to understand what kind of algae to use, what to contain the algae in, how to feed the algae, how to test their respiration, and so many other things. We would like to say at the beginning of this section that we were unable to avoid plastic as certain materials we used were plastic or shipped in plastic. Plastic and microplastics are so integrated in our society that there is no way to live plastic free.

In our search for the best algae to grow in a lab at school there weren't a lot of options available. The one we ended up choosing, *Anabaena,* was the best option that fit our situation. They have many habitats and can survive under many conditions. For example, *Anabaena* is under the Blue-Green algae family, who are known for living everywhere. *Anabaena* specifically are, "benthic; mat-forming in littoral zone, or on sediments or aquatic plants; in freshwater pools, ponds, or saline lakes." This means that they live on the bottom of a body of water. There are about 110 different species of *Anabaena* that have been found world-wide in different pools, ponds, or salt lakes.

In nature *Anabaena* photosynthesize so we kept them under grow lights. *Anabaena* don't need a lot to survive in the wild, and end up taking about 4 hours to duplicate, but in our experiment we needed to add a solution of Alga-Gro Freshwater, from Carolina Biological Supply so the *Anabaena* could get the micronutrients they need to survive.

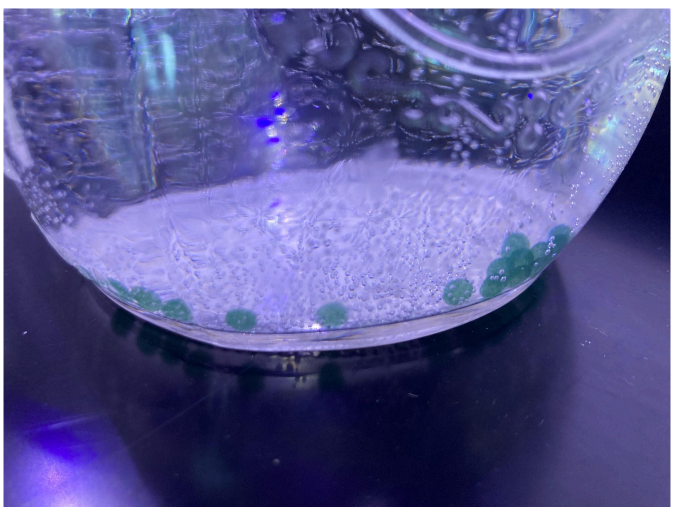


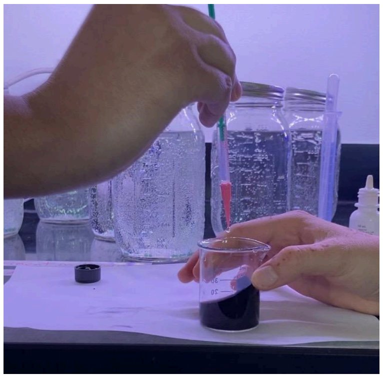
Figure 1: *Anabaena* beads from Carolina Biological Supply, in an 800 ml mason jar.

After choosing *Anabaena* we were looking into storage containers for growing and testing the algae and mason jars were the best option. We had 8 jars that could hold 800 ml each. We then sterilized the jars using boiling water and set up our station. After sterilizing we added 750 ml of filtered water and 50 ml of our Alga-Gro solution, took our first base test of how much dissolved oxygen was in the jars, and added our algae (Figure 1). We tested the parts per million (ppm) of dissolved oxygen twice a week.

For testing dissolved oxygen we used the Dissolved Oxygen Water Quality Test Kit from LaMotte. The steps go as follows;

1. Fill Water Sampling Bottle with 32 ml of test water
2. Add 4 drops of Manganous Sulfate Solution
3. Add 4 drops of Alkaline Potassium Iodide Azide
4. Cap and mix
5. Allow precipitate to settle
6. Add 4 drops of Sulfuric Acid
7. Cap and mix until reagent precipitate dissolve
8. Fill test tube too the 20 ml line
9. Fill Titrator with Sodium Thiosulfate
10. Titrate until sample color is pale yellow
11. Add 8 drops of Starch Indicator (Figure 2)
12. Continue titration until blue color just disappears and solution is colorless (Figure 3-4)
13. Read result in ppm Dissolved Oxygen

Figure 2: Adding the Starch Indicator to the testing solution



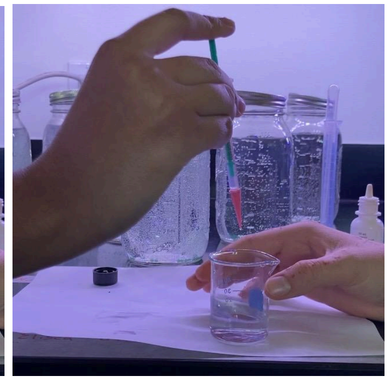


Figure 3: (left) Adding the Sodium Thiosulfate to the testing solution

Figure 4: (middle) Adding the Sodium Thiosulfate to the testing solution and watching the solution turn lighter

Figure 5: (right) Adding the Sodium Thiosulfate to the testing solution until the solution turned colorless

On March 6th we added the microplastics. (Figure 6). We added a different amount to each jar. We added 15ml of microplastic plastic to Jars #1 and# 2, 10ml to #3 and #4, 5ml to # 5 and #6, an no plastics to #7 and #8.

Figure 6: Sanded down Polyvinyl chloride, aka microplastics

We gathered our data on a spreadsheet and made several graphs based on the data. (Figure 7)

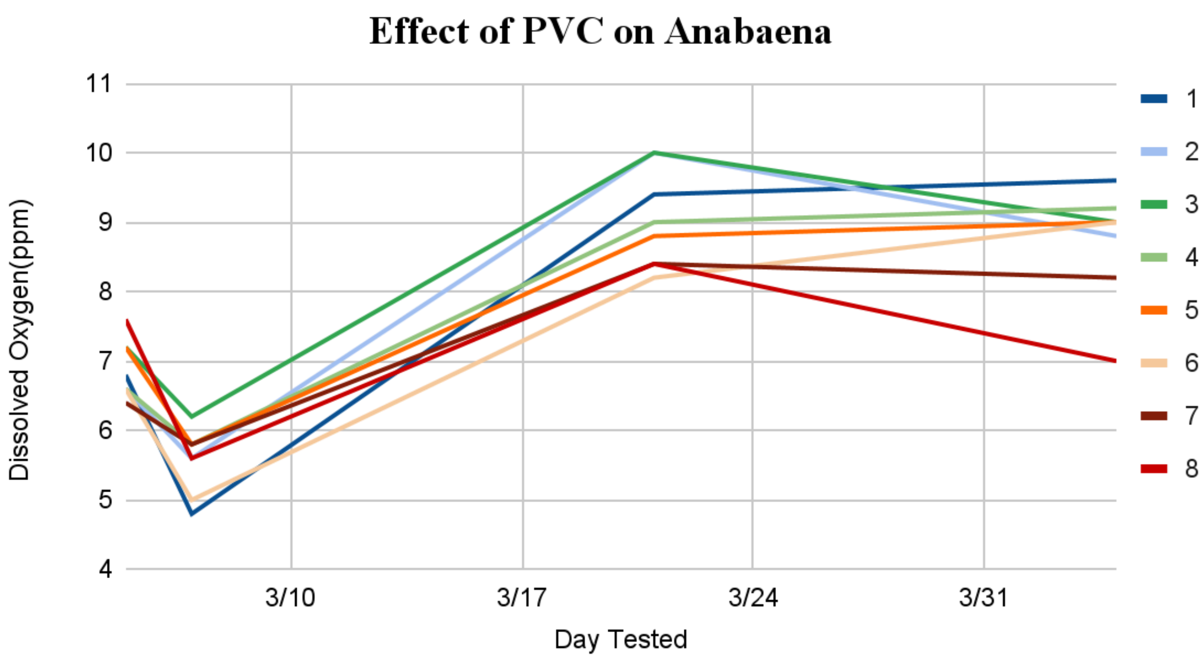


Figure 7: Graph on the Effect of PVC on *Anabaena*

Results

Contrary to what one might believe, the *Anabaena* that interacted with the microplastics were more efficient in producing oxygen than the other *Anabaena* that didn't interact with the microplastics. There was an initial dip in the oxygen production as seen in Figure 7 but after there was a steady increase until the production levels out. We hypothesized that the increase in production was due to a stress factor in the algae and after the algae had coexisted with the microplastics for about 5 days their production plateaued. An observation we made was a couple of days after we added the microplastics we noticed that the algae was growing on the microplastics. (Figure 8) We also noticed in Jar's 3 and 4 white fuzz that looked like mold on March 7th, but it went away as quick as it came. By recent date the microplastics have integrated themselves with the algae and the algae is growing rapidly. (Figure 9)

Figure 8: *Anabaena* growing on the microplastics

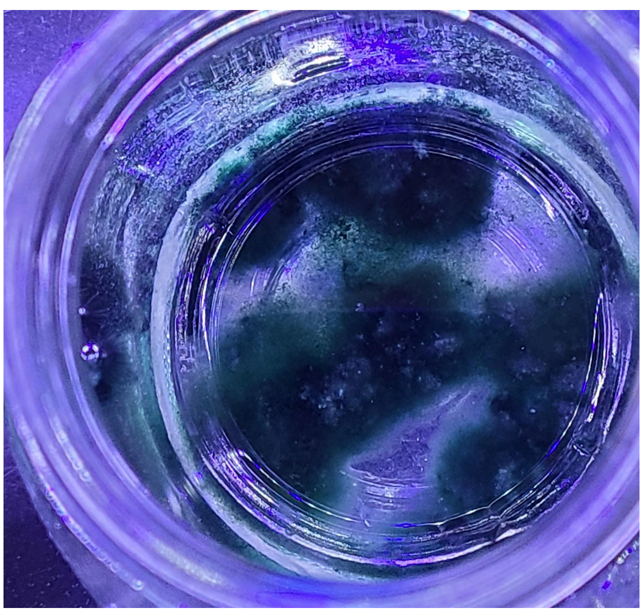


Figure 9: most recent picture of the microplastics and the *Anabaena* living together harmoniously

Conclusion

a. Limitations and Errors

Experimental practices were limited due to budget and time. We ran the risk of miscalculation as we were experimenting due to human error. While doing our best to avoid contamination, experimental contamination was possible in a classroom environment. There was a chance for exposure by accident to other organisms which may have influenced the oxygen results in our tests. Additionally, measurements relied on precise measuring, mixing, and documentation which were subject to error.

Creating an environment for the experiment with no exposure to plastic was impossible. This means that in the beginning when we set up the experiment with no plastic and the control group for the experiment likely were exposed to plastic. The containers transporting the *Anabaena* contained plastic. Our water used in the experiment was stored in plastic bottles. Additionally, while transporting the algae and collecting water samples, we used plastic pipettes. Across the jars exposure the exposure to plastic other than the intentionally added PVC was consistent amongst all the jars.

While the algae did grow and shrink at certain points in time the amount of oxygen in the water generally didn't exceed above 10 ppm. At the surface of the water there is an exchange in materials that can't be completely avoided. Having a very sedentary set up where the top of our container was mostly covered helped prevent this exchange of materials but didn't completely stop it from occurring. We hypothesize that this exchange was responsible for a gradual loss of oxygen in the water over time, meaning that the algae may have grown more than we were able to record.

b. Future Plans

i. Furthering the Code

Currently our code only allows for us to input numbers and receive results in the form of numbers. We would like to in the future have our model operate in a three dimensional plane.

We would like to further our model to make it more representative of our in class experiment and demonstrate the production of oxygen in the water. Currently the exchange of energy and materials in the water is lacking the factor of producing oxygen and taking in carbon dioxide.

In the future we would like to further the complexity of our code by including an equation to represent the stress caused by microplastics. This stress caused by microplastics lead to *Anabaena* increasing photosynthesis, likely in an attempt to resist the impact of the micro plastics. We would like to implement a mathematical model to represent the increase in photosynthesis with the amount of stress.

Currently the rate at which the algae duplicates is completely dependent on the amount of ticks in the model. This creates a constant ratio between the algae growth and the distance traveled by the algae or turbidity of the water. While in our experiment the water is still so there is no concern of including calculations of turbidity we would like to give the model independent variable for thies factors which could possibly influence the algaes growth and oxygen levels in the water.

Complex systems that occur in algae were not able to be modeled.

ii. Solutions to Microplastics

When starting our project we had a few solutions in mind: filters, microplastic eating algae, and upon further research we found that boiling water and then filtering it “helped remove up to nearly 90 percent of the tiny plastic particles”. With the results of our experiment we would like to, in the future, figure out what effect *Anabaena* had on the microplastics.

Some things that anyone can do in their own life to help reduce the amount of microplastics in our society is to reduce, reuse, recycle. Reduce: avoid single-use plastics, buy plastic-free items, use public transportation every once in a while. Reuse: refill and return product containers, use more durable products, share, lend, rent. Recycle: people can recycle plastic and anything that their local dump takes.

Acknowledgements

We would like to acknowledge our teacher, Mrs. Rowe, for providing our team with tremendous support including providing materials to conduct experiments, a place to work, and guidance with the scientific research involved in our project. Also we would like to thank Ms. McKinnley for providing help with the coding aspect of our project. We would like to express our appreciation for the SuperComputing Challenge and all the resources and support they have provided.

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