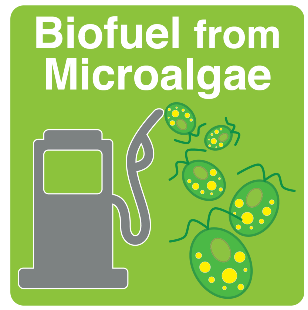
LESSON 2: BIOENGINEERING AND SUSTAINABILITY

CASE STUDY: GENE REGULATORY NETWORKS AND GREEN MICROALGAE

Part I



On a recent road trip, Jamie was excited to arrive at a gas station that was now selling biofuels. She is environmentally conscious and wants to decrease her dependence on fossil fuels. She pulled into the gas station, inserted her credit card, and started fueling up when the $$$ (cost) started to increase at an alarming rate. She thought maybe it was how the gas pump worked because she was from Oregon and had never pumped her own gas (illegal in the state). She finally stopped the pump at around $50 and realized she had only pumped in about 0.20 gallons! She thought it was an error and turned the car on. Sure enough, the gas gauge barely moved after the $50 of biofuel. She went to another gas station and filled the rest of her car up for about $30 using regular petroleum. Feeling cheated and frustrated, she went back to the other gas station with the biofuel to get her money back—because clearly the pump had malfunctioned. After talking with the employee, she learned that there was no error and, in fact, it is just much more expensive to produce biofuels.

1. Biofuel is considered a renewable resource. What makes it renewable and how does that compare with fossil fuel/natural gas extracted from the ground (think about net carbon flow)?

Biofuel is renewable because it comes from a living organism that can be grown at any time to produce more biofuel. Unlike petroleum extracted from ancient deposits, algae can be regenerated with brackish water, light, and some nutrients, all of which can be recycled. Additionally, the biofuel is being produced directly from carbon sources already in the carbon cycle, i.e., atmospheric CO_2, for photosynthesis to produce lipids and other molecules. In contrast, fossil fuels are a sequestered reservoir of carbon that has been trapped underground for millenia, and burning those fuels releases the carbon into the global cycle, adding to the overall quantity of carbon circulating.

2. The first step in the scientific process is to make an observation. Jamie observed that biofuels were far more expensive to produce than fossil fuels. She also came up with a question (the next step in the scientific process). Why is biofuel so much more expensive to produce? Come up with a list of ideas as to why biofuel might be so expensive to produce.

- Biofuel production is not efficient yet compared petroleum production. It takes a lot of algae to produce usable amounts of biofuel, so the input of resources is not yet balanced by the output of fuel and other products, making it costly
- It is not an established industry so there are a lot of startup costs involved in creating facilities for production
- Although algae requires a lot of space to grow so land must be acquired, it requires less land than corn or soil to produce the same amount of transportation fuel

Part II

Frustrated at the thought of not being able to use biofuels, purely because of cost, Jamie did some research and found that macromolecules high in carbon (like oil & carbohydrates) are ideal for harvesting biofuel. She contacted *Valenzuela-Fuels*, a local startup that researches and manufactures biofuel. She was told that they are currently using the green microalgae *Chlamydomonas reinhardtii* (nicknamed *Chlamy*) to produce oil. Microalgae grow (reproduce) quickly with light and carbon dioxide (CO_2) and only require liquid growth media with plenty of nutrients like Nitrogen, Phosphorous, and Sulfur, the similar ingredients to plant fertilizers. Figure 1 below is a recent starvation experiment from *Valenzuela-Fuels*. During the experiment the scientists grew the microalgae with all the nutrients (replete conditions) and the algae grew fast! Then they starved the cultures for either nitrate, sulfate, or phosphate, and the cells stopped growing (growth arrest), but as soon as they gave the cells their missing nutrient (Resupplementation) the cells started growing again!

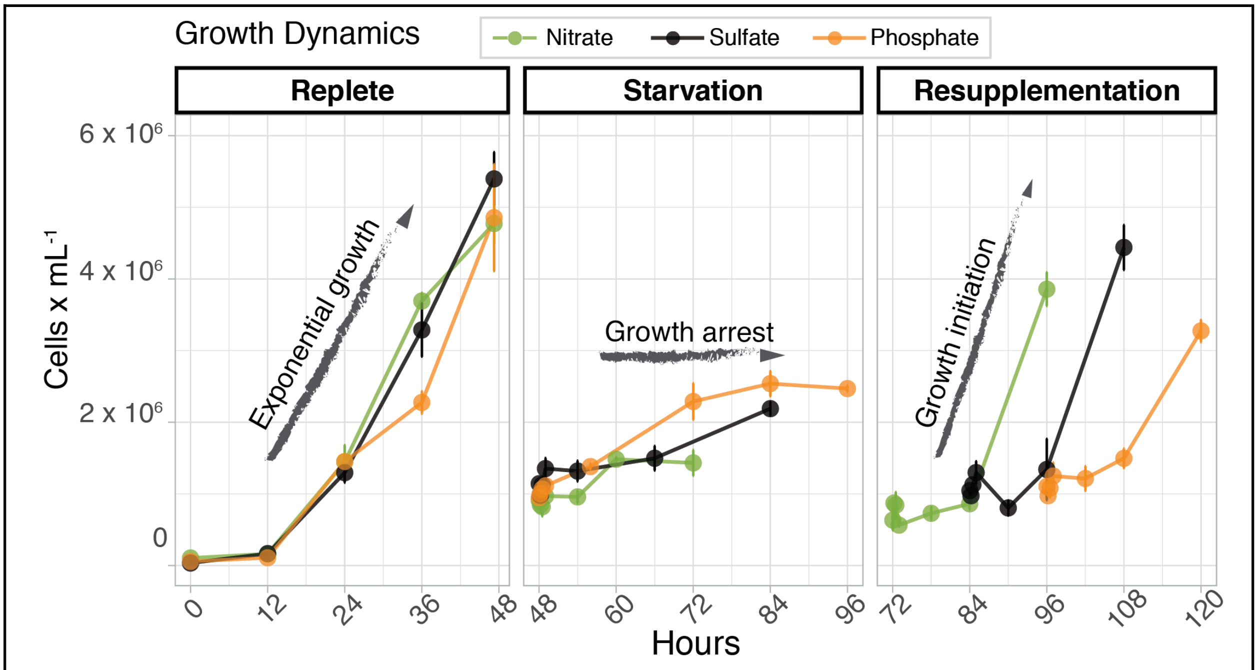


Figure 1. The growth dynamics of microalgae during a starvation experiment from *Valenzuela-Fuels*

3. Explain the growth of Chlamy using the growth curves in Figure 1. What is happening during each phase of the experiment? Explain in terms of what the algae is doing and what is present in the growth media.

- "Replete" experiment - growth media has all the nutrients needed. After a short lag phase (12-hours), the algae begin growing exponentially, rapidly reproducing and increasing the cell count. This process continues until the last end point shown, 48 hours, and may continue beyond, but researchers then washed and transferred cells into media without the indicated nutrient (i.e., starved condition).
- "Starvation" experiment - 3 different nutrient deprivation conditions shown by the 3 colors (nitrate, sulfate, and phosphate) cause the algae to grow only slightly before experiencing growth arrest, when no more cell division is occurring and cell numbers

remain mostly stagnant. The total number of cells is far less than in the replete experiment.
- "Resupplementation" experiment - The missing nutrients are added back to the media, and the algae are able to recover from their starvation-induced growth arrest to resume exponential growth and a short lag phase.

Part III
Jamie was confused. She thought all you had to do was add a bunch of nutrients and algae would grow. She thought he had a scientific breakthrough—so she called Dr. Valenzuela to tell him how he could maximize his algal yield and reduce cost.

Dr. Valenzuela (V): "Hello, this is Dr. Valenzuela."

Jaime (J): "Hi Dr. Valenzuela, my name is Jamie. I have been studying the process of making biofuel and I think I know a way to help you maximize your algal yield."

V: "That's terrific! I have been working on this for a while and anything we can do to bring the cost down would be very helpful. So tell me about your idea?"

J: "I have been studying your algal growth graph *(figure 1)* **and I was thinking all you had to do is add more nutrients and they will continue to reproduce, which should increase your yield."**

V: "That is a good thought Jamie, and in fact if I do add nutrients, that will increase the yield of algae. However, I need the cells to not only increase in total numbers, but I also need them to produce macromolecules that can be harvested for biofuel. Chlamy itself is great at reproducing if given an abundance of nutrients. However, when they are actively dividing, they are not actually producing a storage of macromolecules. We want the algae to produce a lot of lipid (fats). If we can get Chlamy to produce a lot of lipid, then we can harvest that macromolecule and create a lot of biofuel."

J: "Hmm…so how do you force a cell to produce more lipids?"

V: "Great question! We have found that if we starve Chlamy of nutrients they get stressed out and begin to produce lipids. If the nutrients are gone then Chlamy will create stores of those nutrients for later use. For example, Chlamy needs nitrogen (N), so if we grow a culture of cells in regular media with N they grow well and increase their cell concentration—however, they produce very few lipids. If we then wash those cells with -N (nitrogen starved) media, and grow them in the same -N media, they will channel their excess energy into generating stores of lipids, instead of growth."

J: "Ok, I think I am starting to understand. If the -N media is used though, then they can't divide, and so their cell concentration will stay the same and you won't get enough fuel?"

V: "Precisely. We have also done experiments in addition to the nitrogen limitation one, where we limit other nutrients such as phosphorus (P) and sulfur (S). All three of those nutrients are major nutrients needed by the cells, and starvation of those nutrients has

shown that Chlamy will begin to produce lipids. I am going to send you a graph showing the lipid accumulation from the three nutrient starvation experiments *(Figure 2)***."**

J: "Sounds great! Thank you so much."

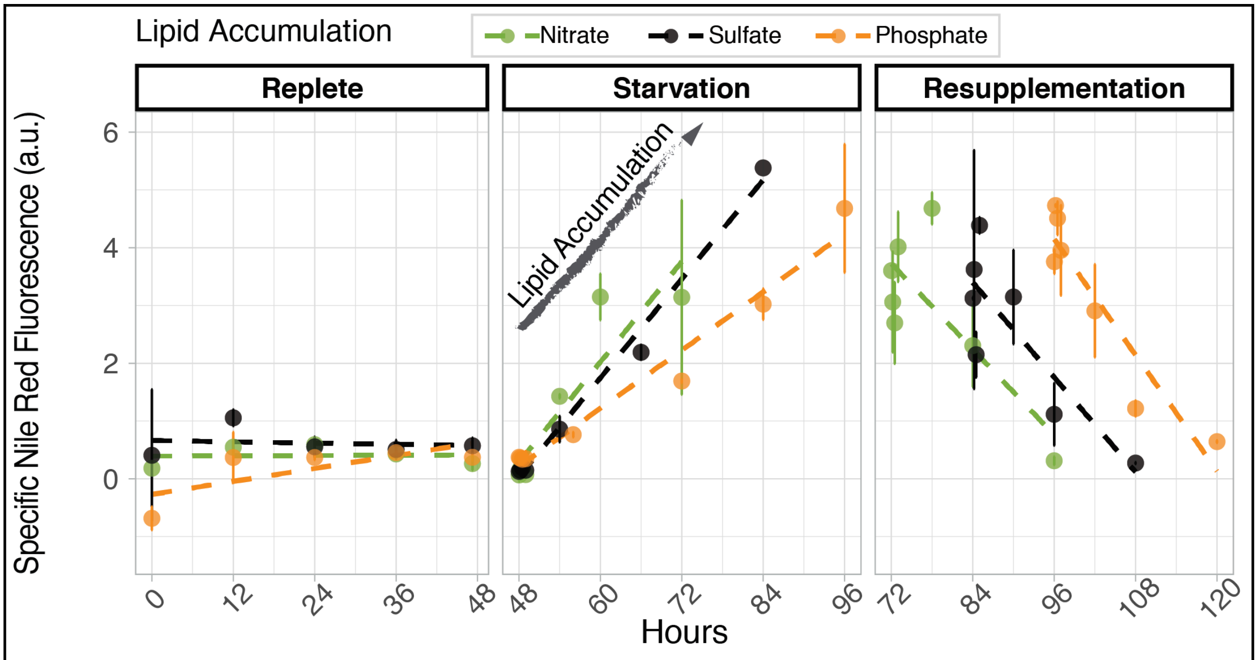


Figure 2. Lipid accumulation during the same starvation experiment in Figure 1

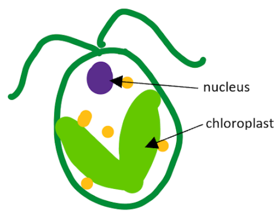
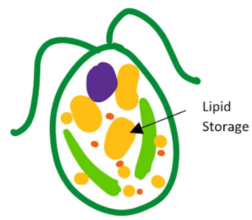
4. Describe what is happening in the three stages of Figure 2. What was the experimental condition at each step? How did the Chlamy cells respond?
   - "Replete" stage - this graph is measuring "Nile red fluorescence", which is a measurement of lipid content of the Chlamy, rather than total cell number like the previous experiment. When all nutrients are providing and cells are growing normally, they do not need to produce lipid stores, so fluorescence stays very low and constant
   - "Starvation" stage - when the Chlamy are deprived of one of the three nutrients, they switch into lipid production as a way of storing energy to survive starvation. Nile fluorescence increases steadily throughout the starvation experiment.
   - "Resupplementation" stage - when nutrients are re-introduced and the Chlamy continue growing, they stop producing more lipid and lipid reduces gradually back down to normal levels, as seen by fluorescence decreasing

5. Which nutrient deprivation experiment produced the most lipid? The least?

   - It seems like nitrate and sulfate starvation may cause slightly more lipid production than phosphate starvation.

Part IV

Jamie was beginning to realize how little she understood about the synthesis of biofuel, but was really intrigued about the science behind creating biofuel. She decided to seek clarification and called Dr. Valenzuela again to learn more about what was going on inside of these algae cells.

Chlamy cell in normal nutrient media	Chlamy cell in Nitrogen depleted media	Chlamy in phosphorous depleted media
		

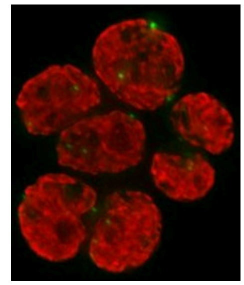


Figure 3: *Chlamydomonas reinhardtii* cells in 3 different environments. To the right--fluorescent image of Chlamy stained with Nile Red (shows lipid).

J: "Hi Dr. Valenzuela. It is Jamie again and I got a chance to evaluate your graphs and have some questions."

V: "That's great. I am glad you called me back. There is much more to this system than just growth."

J: "Yes, I am starting to understand that. I was wondering how these cells can differentiate media and adjust to the different environments."

V: "Take a look at the figure I just sent you *(Figure 3)*. **This shows different cells, all the same species (Chlamy), grown in different environments."**

J: "That is strange! If these are all the same species, how can they be so different? Don't they have the same genes? Or is the environment changing their genes?"

V: "Well they definitely have the same genetic code, but the way their genes are expressed is different. It is called a gene regulatory network and some genes get turned on and off based on their environment."

J: "How does a gene regulatory network work?"

V: "Organisms respond to their environment in many different ways. If Chlamy detects a change in nutrients within their environment, they will put more energy into different pathways in order to store energy. Usually during photosynthesis, they would use carbohydrates for energy storage. However, when deprived of nitrogen, Chlamy doesn't produce proteins for replication (like DNA polymerase), but instead turns off the genes for producing proteins and turns on the genes that are able to synthesize lipids. This allows Chlamy to continue taking advantage of the light and creates a store of lipid to ensure they survive the nutrient drought."

6. Look at Figure 3 (shown on the previous page). What is the major difference between the cells?

Normal Chlamy cells have large chloroplasts for photosynthesis and only a few small droplets of lipid. Nitrogen or phosphorous starved Chlamy have shrunken chloroplasts and multiple, large areas of lipid storage.

7. What is a gene regulatory network? What is it changing in response to? Describe it in your own words and explain how it connects to the different phenotypes of the algae in Figure 3.

Gene regulatory networks are sets of genes controlled by (in eukaryotes) transcription factors to change the phenotype of an organism. In this case, the GRNs are responding to changes in the environment, particularly nutrient starvation. Specifically, nutrient starvation causes a change in genes responsible for both chloroplast development and lipid storage - the chloroplast genes are downregulated, while lipid storage genes are upregulated, changing the cell phenotypes.

Part V
Jamie was learning a lot. She realized that genes are controlled by the environment, but she was still confused. She noticed there was a difference in the cells from Figure 3, but she didn't know exactly how the difference came about.

8. Explain to Jamie how phenotypes get expressed (think about the Central Dogma).

Genetic instructions carried in the DNA are transcribed into RNA molecules, which are then translated by ribosomes into proteins, which carry out different functions, resulting in different phenotypes.

9. Examining the different phenotypes in Figure 3, what types of genes are upregulated when Chlamy is starved of nutrients?

Genes responsible for proteins that carry out the activities necessary for producing stores of lipids are upregulated. Genes that enable growth are most likely downregulated

10. Two different organisms both do photosynthesis. They have a shared phenotype (photosynthetic activity) but different genotypes. How is this possible?

The genotype of an organism codes for all of the different traits of a cell. Therefore, if two organisms share a function, they may only share one part of their genotype, such as genes coding for proteins involved in photosynthesis. It's also possible that the two organisms carry out photosynthesis slightly differently, due to evolutionary changes, and therefore their genes for photosynthesis may be different as well.

11. Transcriptional changes occur as a consequence of changes in the environment. Why do you think that after Chlamy is subjected to -N environment, the genes coding for carbon versus nitrogen use would be upregulated?

When Chlamy is in a low nitrogen environment, it needs to adjust to using other resources to survive. Therefore any genes coding for processes that use a lot of nitrogen (like DNA or protein synthesis) would be replaced by processes that use a lot of carbon (like lipid production).

12. Let's suppose you were a bacteria living in the gut of a flashlight fish. You gain food and shelter from the flashlight fish and in return luminesce at night so the fish's shadow doesn't attract predators. However, in the morning when the fish is ready to head back to the deep it expels the bacteria (including you) into the surrounding water. How might the genes you are producing change? Think about food for survival.

The bacteria would need to switch to a genetic program for surviving in open water. This would mean upregulating pathways that allow uptake of nutrients from the surrounding water rather than from the gut of the fish.

13. Many people enjoy the summer sun and forget to apply sunscreen before going outside. TBP is a general transcription factor that binds to the TATA sequence on the promoter region of a gene. How might the regulation of a gene be changed if the harmful UV rays disrupted the TATA sequence in DNA?

If TATA is mutated, it might interfere with the binding of TBP to the promoter region. This would prevent transcription initiation of many genes, which can cause normal processes to go awry and change many phenotypes of the cell.

14. What kind of information could Dr. Valenzuela gain if he was trying to detect expression of different genes during nitrogen starvation experiments with his Chlamy?

He might learn what genes and therefore proteins play a role in increasing the lipid storage process, as well as what genes cause cell division to slow down and stop. This would help with thinking about what genes to either upregulate to increase lipid storage, or downregulate to stop the growth arrest response.

15. What about if he then could compare gene expression between starvation of phosphorus and sulfur?

Because it seems like more lipid accumulates during phosphorus starvation than sulfur, this might show which genes are responsible for the difference, helping to identify which ones allow the most efficient lipid storage, which would help efforts to make biofuel production more efficient.