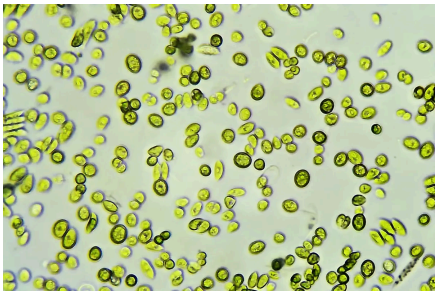
Microalgae as Carbon Sinks: Genetic Innovations for Enhanced CO_2 Fixation



Close your eyes and picture a world where we have the cleanest air to breathe, where we don't melt ourselves to death, where we cherish our environments, forests are pristine and glaciers are in perfect condition, a world where the earth is thriving, and where our planet flourishes to support future generations.

But let's not simply dream; let's turn to nature's silent heroes – algae.

With these organisms, we have the power not just to envision a better world but to make it a reality.

While mankind struggles with the implications of excessive CO2 emissions, it has become more clear that currently studied Carbon Capture and Storage (CCS) approaches and their drawbacks may be unable to manage the magnitude of the crisis on time. As a result, alternative approaches, such as biosequestration of CO2, have emerged too, but many of them also have huge downsides. Although there are exceptions, which are not as bad, a noticeable example involves microalgae. Organisms that have the particular ability to efficiently capture carbon from the atmosphere while also producing oxygen as a byproduct.

In this article, we will explore the promising world of microalgae, but especially how we can edit its genes to combat CO2 emissions in a more efficient way. We'll begin by understanding the fundamentals, including what algae are, their structure and classifications, how they do photosynthesis and how they capture carbon. Then, we will also delve into the Calvin Cycle, before talking about genetic modifications, in vivo and in vitro assembly of Crispr- Cas9 and certain methods used of algae such as electroporation, agitation transformation and biolistics aimed at enhancing algae effectiveness**.**

Time to dive in

What are algae?

Algae are photosynthetic and nucleus-bearing cells mainly found in aquatic habitats. They are known for their absence of roots, leaves, stems and the typical multicellular reproductive system found in plants. Their cells contain characteristics not seen in either plants or animals, and their photosynthetic pigments are also more diversified from those of plants.

They are considered a very important organism on Earth, due to their helpful use in the pharmaceutical industry, the great source of food that it is to marine life and most importantly for being responsible for oxygen production, they generate up to half of the O2 in the atmosphere, via photosynthesis as they are photoautotrophic organisms, meaning that they obtain all their food and nutrients from the sun energy .

(Half of the oxygen in the atm. Half Of It. Just think about it for a little, and question the importance of these organisms.)

Algae classifications

Algae can be classified into two main groups based on cellularity. On one side there are the **macroalgae** and on the other, there are the **microalgae**.

Macroalgae often called seaweed are a multicellular algal species, growing primarily in marine environments. They are large in size and can be seen by the naked eye. Some primer classifications within this group include brown algae (Phaeophyta), red algae (Rhodophyta) and green algae (Chlorophyta).

The structure of macroalgae consists of three main components:

Holdfast: Similar to the root of terrestrial plants, anchors the algae firmly. However, its purpose is not at all to absorb any nutrients.
Blades: Resemble leaves and contain chloroplasts, which are required for photosynthesis.
Stripes: They are stem-like and are responsible for supplying the seaweed with nutrients and water.

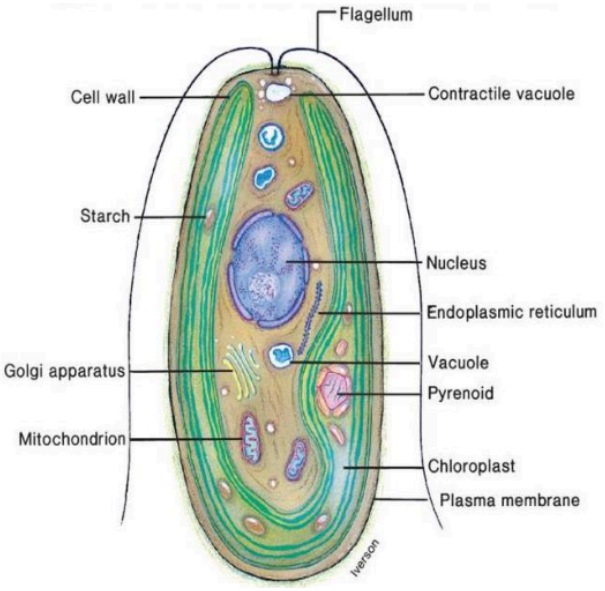
Microalgae, on the other hand, are the opposite. They are unicellular organisms, therefore they don't have a complex multicellular structure, and they are only visible through microscopic observations. They can grow both in marine and freshwater environments and some of the species include diatoms, dinoflagellates, and green microalgae.

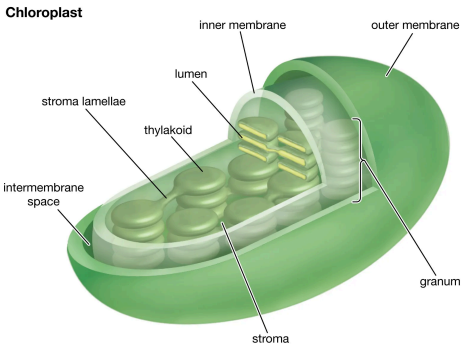
Photosynthesis and carbon capture by algae ☀️

In algal species, the pigments, responsible for absorbing as well as reflecting the diverse light wavelengths, are categorised as carotenoids which absorb green and blue light, and chlorophyll and phycobilins, which absorb red and blue light. This means numerous algae, especially microalgae, can absorb more light than terrestrial plants.

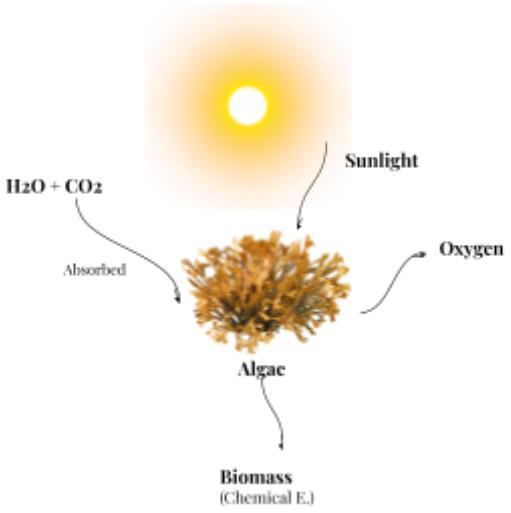
Now, let's explore how algae generate oxygen.

Algae, as previously said, produce O2 via photosynthesis, a process that takes place during the day, when the sunlight intensity is high. It takes place within the chloroplasts envelope, located in the double membrane of the chloroplasts. Here, light is absorbed and turned into chemical energy, by the pigment proteins mentioned before, which are located in the thylakoids.





During photosynthesis, water (H2O) and carbon dioxide (CO2) are crucial components. They are split apart, leading to the separation of oxygen, which is released into the atmosphere.



However, there's more, the importance of this process extends beyond oxygen production. Algae photosynthesis also plays a role in carbon sequestration, a fundamental procedure for mitigating climate change.

Through photosynthesis, algae actively collect carbon dioxide from the ocean, and since the ocean and the atmosphere are in a constant balance, once ocean CO2 is absorbed, the ocean immediately captures more carbon dioxide from the atmosphere to maintain equilibrium. The carbon collected is stored in the algal biomass. As a result, algae act as natural carbon sinks, absorbing around 45% to 50% of CO2 from the atmosphere and lowering its concentration.

Genetic modification to improve algae CO2 sequestration

Now, moving to the cool parts of this research, let's dig into genetically modified microalgae. But before, it is necessary to mention that as there are several types of algae, it's required to define which of these will be the target one. In most of the cases, regarding algae, the targeted group is green microalgae. As these are the most commonly found aquatic microalgae. They can grow rapidly, both in outdoor or indoor settings and they don't require large amounts of land to grow on. They're also pretty good at handling high CO2 levels while efficiently taking in CO2 from different sources in an eco-friendly way.

That being said, let's move to another important point before mentioning genome editing; since it's important to understand CO2 sequestration done by algae. This understanding is vital because to enhance CO2 absorption, we must first grasp the concept of **Calvin cycle** or Calvin-Benson-Bassham (CBB) cycle, (which is the key responsible for carbon fixation during photosynthesis), so we can improve the photosynthesis process effectively. By doing this, we will increase the amount of CO2 microalgae can fix. This process will also lead to increasing biomass, and with more of this, more carbon is stored in the form of organic matter rather than being released back into the atmosphere.

The Calvin Cycle

The Calvin cycle is the chemical reaction by which algae and plants convert carbon dioxide found in organic molecules from the air into lipids, proteins, nucleic acid carbohydrates and other compounds they utilise for cellular respiration such as sucrose.

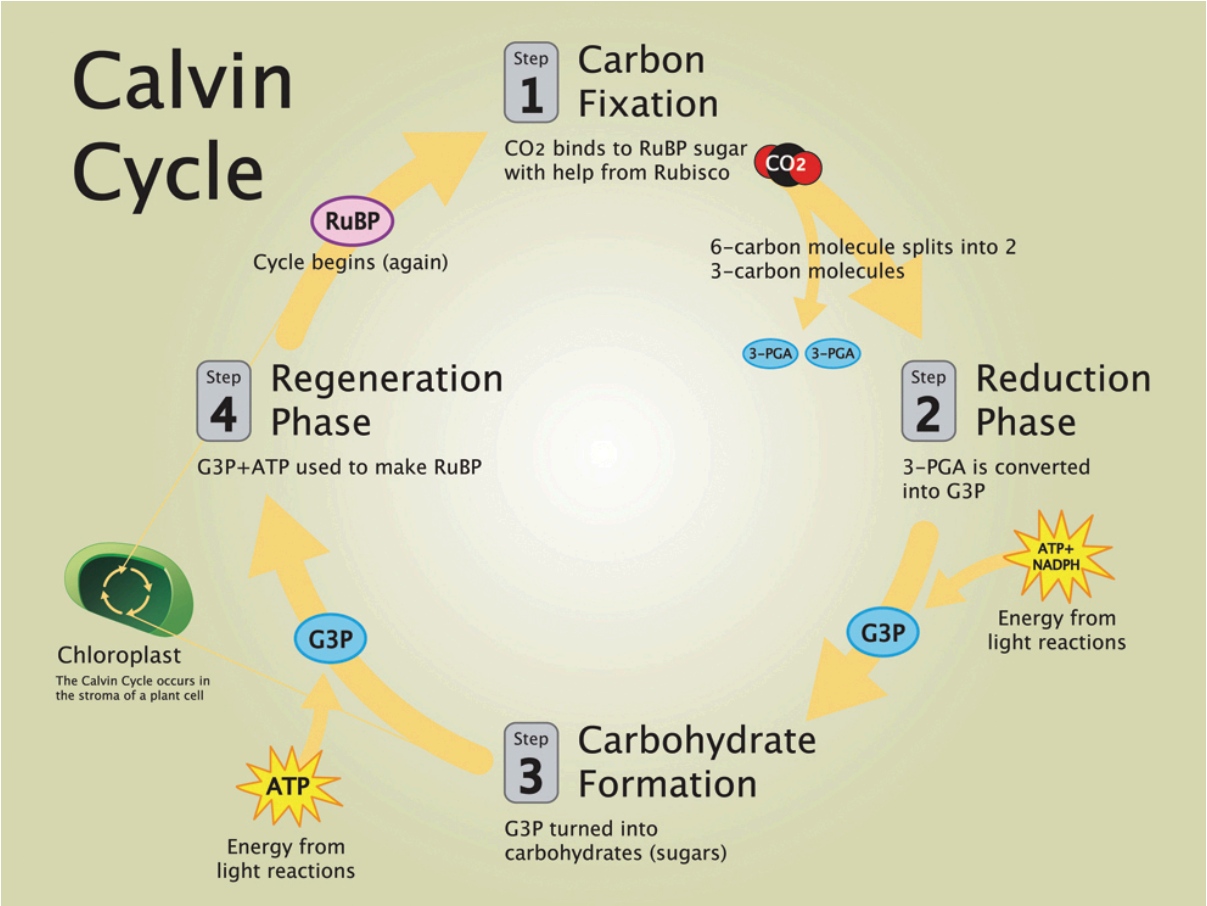
This cycle occurs in the chloroplast and involves a series of enzymatic processes conducted by 11 enzymes, and the main functions of these enzymes within the cycle are three following:

Carbon Fixation (Carboxylation): This stage is essential for further biological reactions. During it, the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme catalyses inorganic carbon (CO2) from the environment into an organic molecule called ribulose-1,5-bisphosphate (RuBP). This leads to the formation of 3-phosphoglycerate (3-PGA).

Reduction: In this phase, the organic compounds that provide energy, such as ATP (Adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate) are utilised to turn the 3-PGA molecules into a different molecule known as G3P (Glycerol-3-phosphate). An important compound which through the use of the aforementioned energy-rich molecules, carbohydrates are

synthesised. Forming the building blocks (lipids, proteins and nucleic acids) that are utilised in other cellular processes within the algae.

Regeneration: This is the final phase of this cycle, as its name says, it involves the regeneration of specific molecules, like ribulose-1,5-bisphosphate (RuBP), which have an immense role in keeping the cycle operating for they ensure that the cycle can sustain itself over multiple rounds of carbon fixation. To be more precise, G3P uses ATP to regenerate RuBP; and this, with help from the RUBisco enzyme, captures CO2.



Now that we've established the groundwork, it's time to dive into the captivating realm of gene editing, where we'll explore how scientists are modifying algae at the genetic level to enhance their CO2 sequestration abilities.

Gene editing

Gene editing is like correcting errors in a computer program's code. Just as a programmer goes over lines of code to correct problems and enhance the program's performance, gene editing involves making precise changes to the DNA sequence, which contains all the necessary information of an organism to reproduce, is similar to changing a program's instructions. Just as a programmer updates

code to build a better software version, this approach tries to improve certain features, eliminate detrimental mutations, or provide new functions, and scientists change how an organism behaves.

Gene editing is like a software program that you want to improve, but you can't directly change the source code. However, you have the option to insert new modules or lines of code that can enhance the program's functionalities. In gene editing, scientists modify the genetic makeup of algae by introducing specific genetic components that boost the production of desired molecules, enabling it to perform tasks more efficiently.

In this case, the scientists focus on a crucial part of the program, they mainly target the carbon fixation phase of the Calvin cycle in algae. They work with RuBP, a molecule known for its catalytic properties, aiming to enhance its performance for better results. Just as a programmer tries to improve the speed and efficiency of the software by altering key modules, genetic engineers attempt to optimise the catalytic properties of enzymes in algae to increase their photosynthetic capacity and CO2 sequestration. However, to the best of our knowledge, these efforts have seen only limited success thus far and scientists continue to work on refining these genetic modifications, much like how programmers persist in perfecting their software for optimal performance.

Fortunately, when it comes to how efficiently plants and some algae turn carbon dioxide into energy through photosynthesis, it's not just about that one specific enzyme called Rubisco. It's also about another process that involves a molecule called RuBP. Previous research in plants have shown that there are three enzymes, namely fructose 1,6-bisphosphate aldolase (aldolase), sedoheptulose 1,7-bisphosphatase (SBPase), and transketolase (TK), which have a big say in how fast this process happens. In fact, they have a lot more influence on this than Rubisco does. So, if we want to boost photosynthesis, these three enzymes are like the keys to the kingdom. Recent work in modifying these enzymes genetically in both plants and certain microalgae have shown that it's a promising way to make this process work even better.

In the Calvin cycle, the enzyme aldolase has a crucial role because it operates at a metabolic crossroads involving DHAP (Dihydroxyacetone phosphate), which is a key intermediate in the production of starch and sucrose. This means that aldolase plays a vital role in determining how

carbon is distributed and used within the Calvin cycle. Given its strategic position, aldolase is a highly promising target for genetic engineering aimed at enhancing photosynthetic CO2 fixation.

To perform these changes of the algae DNA there are several methods, and it's time to look at some of them, we will start with random mutagenesis .

Random mutagenesis

As strange as it sounds, one of the starting points in the gene editing algae process is causing random mutations within an alga population. This is basically, throwing some chemicals at the alga, which will cause its DNA to change. Basically what we aim with this, it's simply hope for good results.

I know, I know, it's hard to believe but when it comes to micro algae, the idea doesn't seem that bad.

When dealing with a population of microalgae in a culture, the presence of a high number of individual algae might result in differences in their traits and behaviours. Because each alga reacts differently to different conditions or stimuli, there is a good chance that a few of these algae may produce positive or desired results.
In basic terms, the variety within the microalgae population allows for the production of a few exceptional individuals with positive features, reactions, or performance , which can be useful for particular applications such as algal gene study. That's why this method is sometimes employed, yet, it can't make major changes, so if we were to target a specific part of the algae DNA, this method won't be viable.

This gives rise to several other methods, such as the targeted mutagenesis.

Targeted mutagenesis

This is a method where scientists can target and mutate a specific part of the algae's genes. One of the most efficient ways to perform this method is to harness another microbial called Clustered regularly interspaced palindromic repeats Crispr.

CRISPR-Cas9, derived from Streptococcus pyogenes, is a powerful tool that utilises the Cas9 protein to make precise cuts in DNA. Originally a defence mechanism in microorganisms, this technology has been adapted for various applications in molecular biology and genetics.
Cas9 is frequently harnessed due to its cutting ability that helps to edit the genes of all kinds of organisms. When it comes to editing algae genome, it's cost effective and easy to direct to specific target genes, it could even be used to modify multiple areas at once with the technique of multiplex editing.

But how does Cas9 cut DNA?

In the CRISPR-Cas9 system, guide RNA (gRNA) molecules play a crucial role in directing the Cas9 protein to the specific location in the genome where you want to make a genetic edit.
Here's how it works:

1 **Designing the gRNA**: Scientists design a short piece of RNA (Ribonucleic acid) called the guide RNA (gRNA). This guide RNA is engineered to be complementary to the DNA sequence of the target gene you

want to modify. In other words, it's designed to match the DNA at the specific location you want to change.

See the guide RNA molecule as a GPS system for the Cas9 protein, guiding it to the right spot in the genome for gene editing.

2. **Cas9 Binding**: The Cas9 protein acts as a pair of molecular scissors. It is guided by the gRNA to the precise location in the genome that matches the sequence of the gRNA.

3. **DNA Cutting**: Subsequently the Cas9 protein, guided by the gRNA, reaches the target DNA sequence. However, Cas9 doesn't bind to the DNA immediately, it firstly requires a proto-spacer adjustment motifs (PAM) sequence to be present adjacent to the target DNA. The presence of the PAM sequence is what ensures that Cas9 binds to the correct location in the genome. Without the PAM sequence, that consists of "NGG" meaning any nucleotide (N) which can be either adenine (A), cytosine (C), guanine (G), or thymine (T). followed by two guanine (G) bases, Cas9 won't bind to the DNA, preventing it from making unintended cuts.

4. **DNA Repair:** After the cut is done, the cell's natural DNA repair machinery comes into action to fix the cut. Depending on the desired outcome, scientists can introduce changes during this repair process or let the DNA fix itself.
Meaning, we can either cut the target part of the DNA, which is usually a gene that no longer works, that is why this process is called **gene knockout**, and then leave the algae to mutate its own genes, accomplishing the desired results, by randomly adding or removing a few bases, which stops the gene from functioning.

Or we can insert our one set of bases into the algae genome. The process is the same, Cas9 makes a cut in the target site. But here is where it changes. Instead of letting the algae mutate its own genes, we introduce synthesised DNA that hover around the target site. This DNA is then amalgamated into the genome, now the organism effectively has a new gene in its genome.

So now that we know how Cas9 works and what it does, let's talk about how it is actually inserted into the algae to produce these changes.

Cas 9 and gRNA assembly methods

We can assemble Cas9 in two different ways. With in vivo assembly or assembly or in vitro assembly.

To understand and differentiate these, let's make an analogy.

Imagine a car assembly process:
In **Vivo Assembly** is like building a car on the assembly line in the factory. All the parts are put together within the car's natural environment, with the help of machines and workers on the production line. In the case of Cas9, it's produced and functions inside a living cell, much like a car being assembled within the factory.

In **Vitro** Assembly is similar to taking car components out of the factory and assembling them in a workshop or garage. You have more control over the process and can make modifications outside of the factory setting. In the context of Cas9, it's using Cas9 that has been purified and is used outside of a living cell, allowing for more controlled and customizable experiments.

Let's look a little deeper into these two.

The first method for getting cas 9 to the algae is by using a vector. The vector acts like a delivery truck, bringing Cas9 and other necessary parts to the right place for cutting. You can think of the vector as a helpful tool that carries all the important stuff to the algae. In vivo assembly, cas9 and gRNA are brought into the same vector but on different parts of it, they will later be joined within the organism.

An important part of the vector to highlight, it's the selection market, a harmless gene that will activate when it reaches the algae DNA.

For example, some experiments will send an antibiotic along with cas9 into the algae. Then they will infect the culture with the virus associated with that antibiotic and only the organisms with the antibiotic will survive, the others will die.
…

The survivors are the same ones that cas9 successfully reached since the antibiotic was attached to the same vector. This allows scientists to select for which algae were properly edited.
With the vector fully assembled with Cas9 gRNA and selection marker, the vector is ready to be delivered into the algae.

The second method is the in vitro assembly method, also known as the RNP method, which refers to the Cas9 ribonucleoprotein.
This method is used to assemble the Cas9 protein with the gRNA to create a Cas9-gRNA complex, referred to as an RNP. This complex is then delivered into the target cells in the algae. It's a way of delivering Cas9 and gRNA without using a vector to carry the genetic information.

Ribonucleoproteins can be delivered through a process called electroporation. Which is another method of editing algae, and yeah. If you were wondering what this is, we will call about it right now. But before that, let's keep in mind that the RNP method is still an evolving field and there's still a lot of research to be done.

Electroporation

Electroporation is a laboratory technique for inserting foreign DNA or other molecules into the cells of diverse organisms such as bacteria, yeast, and mammalian cells. It involves applying an electric field to the cells, which momentarily breaks the cell membranes, resulting in the formation of tiny holes. This disruption makes it easier for chemicals like DNA to enter cells.

Here's how electroporation works:

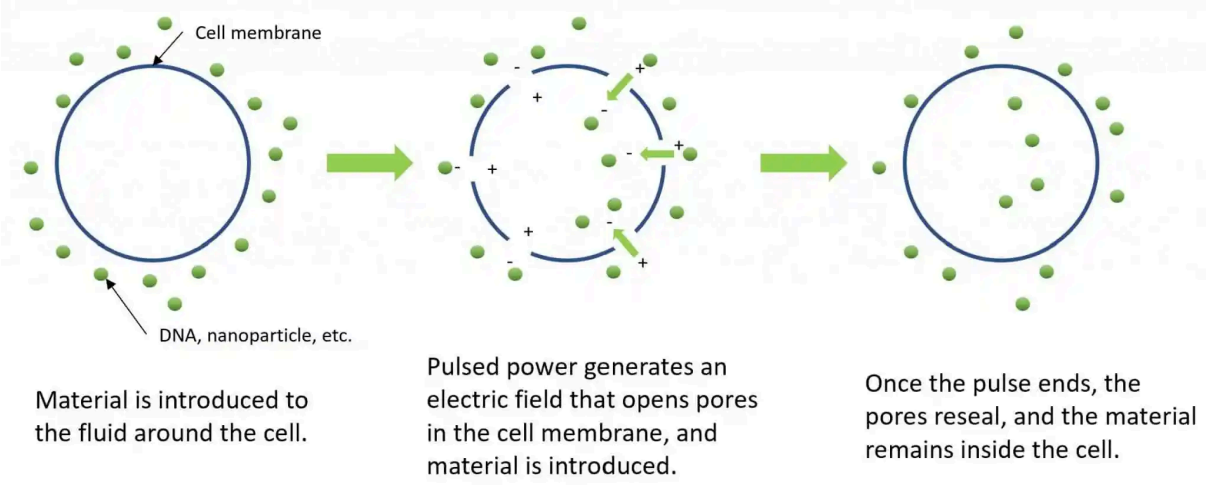
1. **Preparation of Cells**: First, the algae and the RNP, meaning the target cells are prepared for electroporation, they are placed in one specific small area.

2. **Mixture and electric pulses:** In this step electricity is used to force the algae and the RNP together.
The cas9 RNP or other molecules that need to be introduced into the cells are mixed with the cells with the help of electricity. An electrical pulse is applied to the cuvette, creating a brief and controlled electrical field across the cell membranes which force the algae to absorb the RNP. This allows Cad9 to enter the algae and begin doing its job.

4. **Membrane Pore Formation**: The electric field causes temporary pores to form in the cell membranes, allowing the molecules or in other cases also DNA to pass into the cells.

5. **Cell Recovery:** After electroporation, the cells are typically placed in a growth medium to recover. During this recovery period, the pores in the cell membranes close, and the cells return to their normal state.

Electroporation can be helpful if we want to introduce genes or genetic material into cells for improving and optimising the algae efficiency in biofuels production or to brain greater results in CO2 sequestration, for research since with electroporation we can understand how these changes affect algae growth, metabolism, and other characteristics, or for biotechnological purposes.



Here's a image to better understand how electroporation works.

Another method we can utilise to modify algae genome is the agitation transformation, so let's look into it.

Agitation transformation

Agitation transformations are a group of methods used in genetic engineering techniques used to transfer foreign DNA or genes into target cells like algae. Is a series of techniques that utilise mechanical agitation aiming to rupture the cell membranes, so that the cell can absorb genetic material.

Here's how agitation-based techniques work:

First the target algal cells are prepared for transformation, usually this means that their cell walls are severely removed or weakened to make them more receptive to genetic material.

Then, the foreign DNA or genes that scientists want to insert into the algae cell are mixed with the algae in a solution.

This is when the mechanical agitation techniques, such as vortexing, centrifugation, or using glass beads, are applied to the cell-DNA mixture. The mechanical cause disrupts the cell membranes, creating temporary openings that allow the DNA to enter the cells. Now the foreign DNA may integrate into the host genome or express its genetic information creating the desired cell behaviour.

Agitation-based approaches are particularly beneficial since they may be used for a wide range of cell types and do not require specialised equipment. These approaches can be successful for transferring

genetic material into cells with cell walls or protective barriers, making them a significant tool for genetic modification and study.

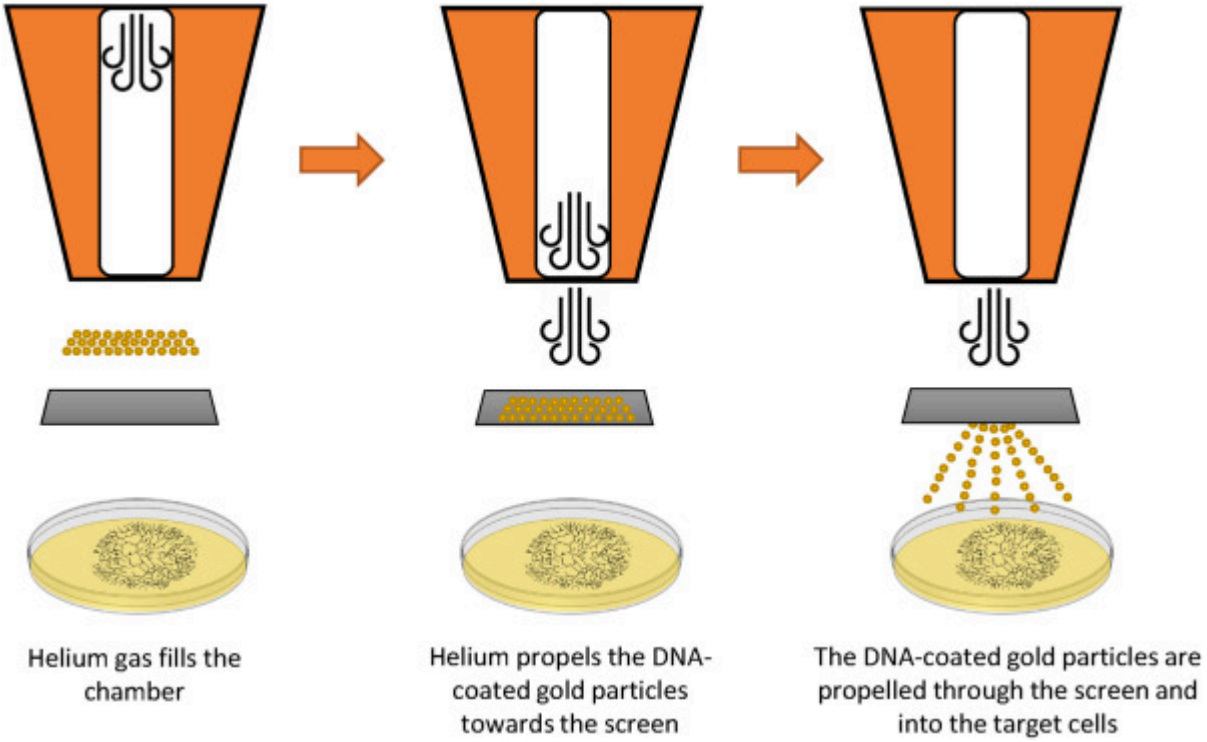
Finally, the last method we will talk about is biolistics.

Biolistics

This method is like a gene gun, is it so common but it can still be utilised for genetic modification in algae.
The process is easy and short. It begins by taking tiny metal particles, such as gold or tungsten and coating them with the DNA of interest. If you are wondering why scientists do this, it is because these coated particles serve as carriers of the foreign DNA.

Once coated, the particles are loaded into the gene gun device which aims at the target algae cells. When the gene gun is fired, the tiny particles travel at high velocity and penetrate the cell walls of the algae.

Then the algae cells take up the DNA-coated particles and release it into the cell's nucleus, where it can potentially integrate into the host genome or express its genetic information.



Example of how the biolistics method works.

The previously mentioned methods are just a way through which algae genes can be modified. As you might have perceived, it is not clearly explain how this process are done with algae, but rather how they work this is because it's important to emphasise that while many of the previous techniques have shown proof of concept, there's still considerable research and work that needs to be accomplished before CRISPR-Cas9 can be routinely utilised in algae.

Conclusion

The world of algae offers a unique chance to address the critical issues of climate change. Algae have emerged as nature's quiet fighters in our fight against global warming due to their potential for carbon absorption and oxygen generation.

Today we discussed the possibility of improving algae's natural ability to store CO2 more effectively, therefore reinforcing their position as a significant carbon sink through genetic modification. We've looked at how scientists are paving the way for a new age of sustainable solutions by diving deeply into the dynamics of the Calvin cycle and using modern gene-editing tools like CRISPR-Cas9 and we look into electroporation, agitation transformation and biolistics, as methods to insert DNA into the algae cells. Lastly, we also mention how despite the advances in gene editing technologies highlighted, it is vital to recognize that more study and development is required before these techniques can be widely used in algae.

Meanwhile, we need to persist in our efforts to unlock the full potential of algae. By doing so, we can lay the foundation for a more sustainable and promising future for generations to come.