Review Paper: Algae Biofuels — A Sustainable Alternative to Fossil Fuels

For a long time now, we have relied on the remains of dead plants and animals that lived millions of years ago to power our world. We have used it in every form we've found – coal, oil, natural gas – to produce electricity or use it for heating and transportation. Little by little we began to use them even more frequently, to power nearly every aspect of our modern life, thinking it was The Big Thing, the step toward evolution.

Little did we know about the environmental impact that this unrenewable source was causing to the world. Because not much longer after starting its insane production and use, pollution marred our skies with greenhouse gases. The consequences then rippled through ecosystems. The effects started triggering disruptions in weather patterns, endangering wildlife, and leaving a permanent wound on the delicate balance of our planet.
The very resources we utilised to accelerate progress became our enemy, and after realising this, we were forced to evaluate the cost of our unrestrained reliance on fossil fuels.

Innovation and technology commenced to play a huge role in this fight, in which many scientists were seeking alternatives. Turning to microalgae and algae as a sustainable option, laboratories buzzed with exploration. While gene editing emerged as a key tool to boost biofuel production, offering hope for a cleaner and more sustainable future.

Yet, as ambitions soared, financial constraints posed challenges and gene editing seemed to be not as easy as expected. The slow advancements, and little production incapable of achieving the desired demand, caused several labs and investors to hesitate, questioning whether algae biofuels were actually a good alternative to fossil fuels. While others haven't lost hope and await discovery in the algae gene modification and biofuels production industry to be able to change the world.

So what's going to happen now? Are algae biofuels really a good alternative? Does the money invested compensate for the results we are obtaining? What are the long-term sustainability prospects of algae biofuels?

There are lots of questions to be asked and a lot of answers missing, but let's learn a little about it and conclude by ourselves.

This paper covers the production of genetically modified algae for biofuel generation, including the cultivation process and examples of experiments conducted to enhance microalgae's ability to secrete and produce lipids, as well as the challenges associated with both production methods and genetic modifications of microalgae for biofuel production.

Outline of Paper

Introduction

In 2023, worldwide CO_2 emissions from burning fossil fuels reached nearly 37 billion tons, reaching a record. Studies reveal that around 45% are caused by coal, 35% by oil, and 20% by gas.

The issue here is, that when fossil fuels are burned, they emit significant amounts of greenhouse gases, such as methane (CH_4), carbon dioxide (CO_2), sulphur oxides (SO_x) and nitrogen oxides (NO_x), into the air. These **anthropogenic** gases trap heat in the atmosphere and lead to global warming, ocean acidification and environmental pollution. Already, the average global temperature has risen by 1°C! If it reaches 1.5°C to 2°C, it could give rise to greater environmental issues such as biodiversity loss, species extinction, and food scarcity, which endangers global health and may worsen poverty for millions of people worldwide, and one of them could be us.

And yes, we are setting goals to solve these issues, we are taking matters about it. We aim to reach net zero but to do that, the use of fossil fuels needs to decrease drastically. That's why several kinds of sources such as plants, algae, organic wastes and biofuels, with their chemical characteristics—liquid, solid, or gaseous— which produce biofuels, are being deeply reached. Among these, the fourth generation of biofuel, produced mainly from algae, has given rise to lots of controversial studies, regarding its effectiveness compared to cost production.

Before delving into specific experiments and challenges, it's important to have a solid grasp of the background and relevance of algal biofuels and genetic changes. I don't want anyone to get brain rot, so the essential concepts and terms are defined below for clarity.

I'll also provide more definitions at the start of some sections to make sure everything is as clear as water.

Definitions

Algae:

Algae are photosynthetic organisms, which can be single-celled bacteria or multicellular seaweeds. They are found in various aquatic locations, including freshwater and marine habitats, as well as wet terrestrial settings.
Algae have critical functions in ecosystems, acting as primary producers and supplying oxygen via photosynthesis. They are also useful in a variety of applications, including as dietary additives, biofuels, and environmental remediation.

Lipids

They are a significant component of algal cells, serving as a storage form of energy, they carry out a variety of roles, including providing energy to the cell, keeping its structure and membrane fluidity intact, and protecting it from stress by stabilising the membrane bilayer and serving as cellular recognition markers.

Biofuels

Short and simple, they are liquid fuels derived from renewable biological sources. Algae biofuel is the same thing but made out of algae, a type of aquatic plant that has the ability to convert sunlight, carbon dioxide, and water into oil, or biofuel.

Gene editing

By default, gene editing is the *alteration of the genetic material of a living organism by inserting, replacing, or deleting a DNA sequence, typically with the aim of improving some characteristic of a crop or farm animal or correcting a genetic disorder.*

It sounds simple, but trust me, it is much more complex than it seems.

DNA sequence

To genetically modify an organism, we have to look into its **DNA sequence**. A specific arrangement of nucleotides that make up the genetic code of every living organism and varies greatly in length.

DNA sequences are studies to identify specific genes responsible for certain traits or functions and target precise genes to achieve desired outcomes.

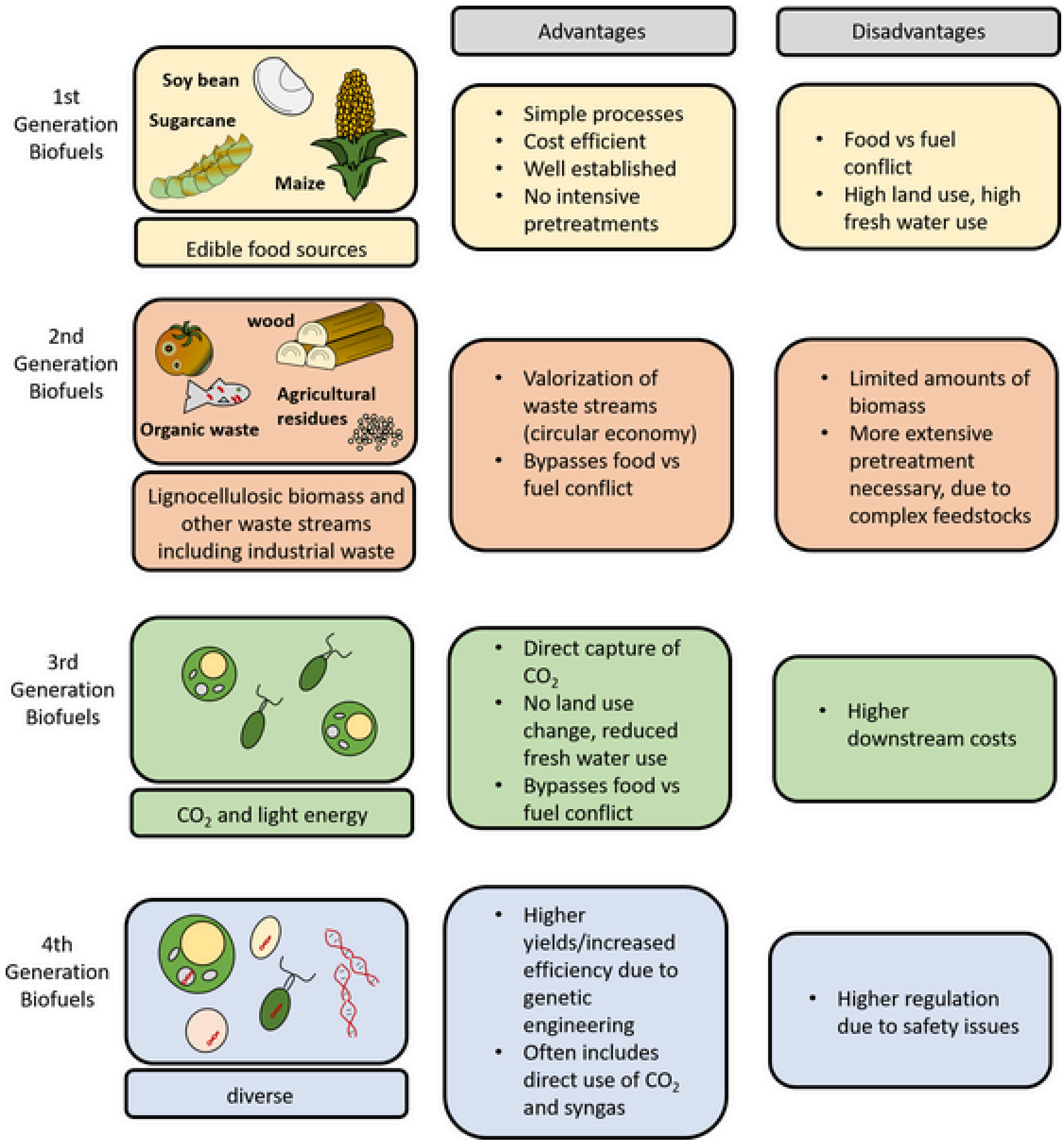
At this point, you must be wondering what's the correlation between algae biofuels and genetic modifications. Well allow me to explain to you

Over the past years, biofuels have gained popularity as a viable alternative to fossil fuels due to their lower emissions of greenhouse gases, essentially CO2. Derived from renewable biological sources, they provide a promising solution for sustainable energy.

Biofuel generations

Based on the particular type of feedstock utilised in their production, biofuels are divided into four generations.

The **first-generation** biofuels are produced with edible feedstocks such as corn and sugarcane. Thus, it has arisen concerns regarding food insecurity as it threatens food supplies and biodiversity. The **second-generation** biofuels are made with inexpensive, plentiful non-food biomass sources like food waste and agricultural residues. **Third-generation** biofuels are produced using feedstocks like algae. Lastly, **fourth-generation** biofuels are made from genetically modified microorganisms and feedstock, done to enhance **biofuels' feedstock effectiveness.**



Among the various generations of biofuels, algae has gained significant attention, due to its **rapid growth rates, high photosynthetic efficiency, and capacity to produce lipids** and carbohydrates needed for the production of a wide range of high-value bioproducts (i.e., biodiesel, bioethanol, biomethane, biohydrogen, bioenergy, biofertilizer, algae-feed and supplements) from the algal biomass.

Notably, algae cultivation has no special requirements. All it needs is water, simple salts, minerals, and sunlight. Not just that, but biofuel production from algae cultivated in wastewater offers the added advantage of wastewater treatment and CO2 sequestration from the environment.

Briefing everything up, we modify the algae genes to increase their lipids production and boost the manufacture of biofuels. Since they grow rapidly and their cultivation is severely simple.

That being said and understood, it's time to move on to algae biofuel production

Algae biofuel production

There are different ways of cultivating algae. Some algae are capable of growing in the dark. We referred to them as heterotrophic algae because they use organic carbon such as acetate or glucose as energy and carbon sources. For this kind of algae, the cultivation methods have high capital and operating expenses, so it's hard to justify biodiesel production.
Then, we also have photosynthetic algae. They are a little more normal because, like most living organisms, they rely on light and carbon dioxide as energy and carbon sources and because they're easier to maintain, algal biofuel production often depends on photoautotrophic growth.

Now, there are a variety of systems available for photoautotrophic microalgal culture. For now, let's focus on the main ones: open ponds and bioreactors. ***Allow me to explain how they work.***

Open ponds

In open ponds, baffles are necessary in the channel to guide the flow around curves, which are made to conserve space. The system operates continuously, employing a paddlewheel to circulate and blend the algal cells and nutrients, preventing the biomass from settling.

As the methods necessitate additional nutrients, a feed comprising nitrogen, phosphorus, and inorganic salts is introduced before the paddlewheel.

After completing the loop, the algal broth is gathered behind the paddlewheel. Depending on the specific nutrient requirements of the algal species, a variety of wastewater sources can be utilised for algal cultivation, including dairy lagoon effluent and municipal wastewater.

While open ponds are not as expensive to create or maintain as enclosed photobioreactors, this culture method has drawbacks. To start with, as they are open-air systems, they
continually evaporation and cause a significant amount of water loss. So, microalgae grown in an open pond are unable to properly absorb CO_2. This leads to a reduction in algal biomass production.

Then, biomass is also affected by contamination with undesirable algae species and other organisms from feed. Meaning, proper growing conditions are difficult to maintain in open ponds, and recovering biomass from such a diluted culture is costly.

Enclosed photobioreactors

The most common type of photobioreactor typically takes on a tubular shape, often positioned to maximise exposure to sunlight. However, when photobioreactors are placed indoors artificial lighting becomes necessary, leading to elevated production expenses.

To ensure continuous motion of the algae biomass a turbulent flow within the reactor is employed, supported by a circulation mechanism involving pumps that move the medium broth through the tubes. Within these tubes, the biomass undergoes photosynthesis under light exposure.

However, photosynthesis generates oxygen, which can accumulate and poison the algae. To address this the culture is periodically directed to a degassing zone, where excess oxygen is removed from the algal broth through bubbling with air. Additionally, to enable successful large-scale cultivation of microalgae, CO2 is also introduced into the system to prevent carbon starvation and an increment in the pH.

	Advangades	**Disadvantages**
Open ponds	• Low cost construction • Easily scaled • Low cost maintenance • Relatively low energy input • Easy maintenance	•Easily contaminated • Overrun by alien algae • Grazing by zooplankton •Lower productivity •Evaporative water loss • Large areal requirement • Poor mixing, CO, and light utilisation
Enclosed photobioreactors	• Higher level of process control possible • More resistant to contamination • Little evaporative water loss • Higher yield of biomass • Outdoor and indoor capabili (winter)	• Expensive •Scale up difficult • Wall growth • Cooling may be required • Energy intensive •Sophisticated construction • pH, dissolved CO, and CO, gradient within the tubes, depending on the model

Graph of advantages and disadvantages of both

Lipids extraction

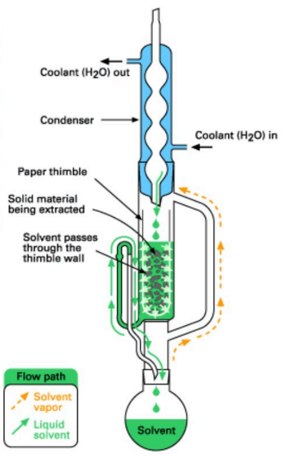
Definition

Reflux

Is a technique involving the condensation of vapours and the return of this condensate to the system from which it originated.

There are various methods for extracting lipids, and one commonly used for large-scale algae biofuel production is the **Soxhlet extraction** method.

To start this method, the algae needs to be dried, which can be done using **air drying** or **spray drying** techniques. In air drying, the algae are spread out in thin layers to naturally evaporate moisture. It's simple, energy-efficient, and cost-effective but takes up space and time. Meanwhile, spray drying breaks the algae into small particles and quickly evaporates moisture in a heated chamber, producing a fine powder. Although more efficient, it requires more energy.



For Soxhlet extraction, we use a specific apparatus divided into three main parts. At the bottom, we add the solvent, often **hexane**, chloroform, or others. Hexane is favoured for its low boiling point and high extraction rates **(≈10.78 %).**

This process involves continuously cycling the heated solvent through the dried algae sample to dissolve the lipids present in the algae, and after numerous cycles, the solvent containing the extracted lipids is collected and concentrated, and the lipid residue is recovered for further analysis or processing.

Cell disruption

This process also involves cell disruption or lysis since to dissolve and extract lipids from the algal biomass, the solvent penetrates the cell walls and membrane of the algae to extract lipids trapped within the cells.

The lipids we are left with are TAGs, which by a process called **transesterification,** we can obtain biodiesel.

As seen, traditional Soxhlet extraction is noted for it**s low cost and simple operation**, but it has limitations such as long extraction periods and significant chemical use. Although, in the past few years, innovative variations of Soxhlet extraction have arisen to overcome these concerns and examples include high-pressure Soxhlet extraction, automated Soxhlet extraction, and microwave-integrated aided Soxhlet extraction technology, among others.

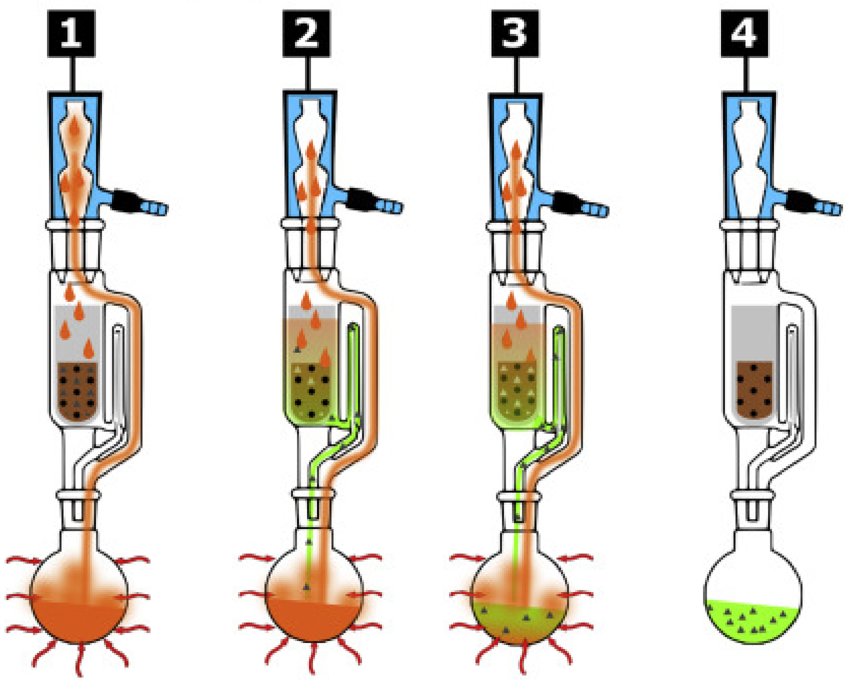


Image depicting the continuous extraction process and its progression.

But what's the matter with lipids?

Allow me to give you a better and clearer understanding, starting with the very beginning.

Lipids importance

As mentioned before, algae do photosynthesis. But it's a bit more complex than absorbing sunlight. There are actually tons of processes and pathways involved, which occur during the daylight. To keep it simple, you can think about microalgae as tiny solar-powered factories. They utilise absorbed light to **split water into oxygen**, protons, and electrons. Basically, what they're doing is using sunlight as their energy source to break down water molecules into essential components.

During nighttime, in photosynthesis, protons and electrons are used to degrade CO2 into starch through the **Calvin cycle.**

*If you're unfamiliar with the Calvin Cycle, you should check it out **here**!*

At this point, you might still be confused about what the Calvin Cycle and starch have to do with lipids. And the thing is that, well, this **starch can be broken down into lipids to form fatty acids and Triacylglycerols (TAGs).**

Process briefly explained

Starch stores energy, which is used to perform metabolic processes without extra energy sources. Lipids, however, are **secondary sources**.

An example of a lipid is the previously mentioned TAGs, which throughout every growth stage of the microalgae accumulate energy. Although, their highest accumulation rate happens during the stationary phase.

Now, a point to highlight is that because lipids are secondary sources, they hav**e less energy output and accumulate more energy.** That's why, blocking metabolic pathways that produce starch and other energetic compounds is a strategy to increase TAG content.

Yes, I know, I was previously mentioning TAGs quite a lot already, but that is because they play a relevant role in the genetic modification of microalgae.

TAG Importance

Triacylglycerols are a chemical compound that is a key factor for biodiesel production since they have a high-density capacity of 9 kcal/g. Meaning they have the **ability to store large amounts of energy per unit mass**. Being an efficient source of fuel once turned into biodiesel, bioethanol, and biogas.

The main aim of modifying the genes of algae is to boost their production of TAGs. When algae produce more TAG, it means more biodiesel can be made from them.

The reason why TAG plays a big role in algae biofuels is because as mentioned before, lipids are a source of energy to the algae, so just as is needed to produce biofuels, it is also needed by the algae.

This issue can be seen as a matter of supply and demand where TAGs are the supply, and it's demanded both by the algae and by the biofuel producers, so to balance this up, different methods to increase TAG production are being studied.

Some methods used are exposing the algae to stress, as it has been shown that algae synthesise between 20-50% more TAG when exposed to stress or nutrient deprivation, in particular, when they are under nitrogen deficiency.

However, when the wild microalgae strains are nutrient-starved, the biomass yield is decreased, so we turn to genetic modification as a strategy for producing strains that can accumulate lipids without affecting biomass productivity or impairing growth.

*During the last few years, we mainly conducted research on a few microalgae species. But today we won't complicate it. We will be examining four experiments conducted on Nannochloropsis oceanica, aiming to **improve lipid accumulation** and **increase TAG** and later conclude which of them we consider to be the most efficient.*

Case study conducted on Nannochloropsis oceanica

Oleaginous microalgae are a promising source for biofuel production, although not many share this view. The commercial use of microalgal biofuels faces challenges because there are not enough strains that grow quickly and produce a lot of lipids.

Fortunately, significant genetic advances have been made in the field. Despite the large number of algal species and only a few being characterised, Nannochloropsis stands out among the few examined species due to its **high adaptability** and thriving in various marine and freshwater environments. They're considered a **model microalga for studying TAG metabolism** due to their **rapid growth, high TAG content, capacity to accumulate storage TAGs** to half of their dry weight under nitrogen-starved conditions, and the availability of genetic tools, holding great promise for mass cultivation and biofuel production. As mentioned, if we want to commercialise large-scale algae biofuels, improving cost and efficiency is a must and developing strains with enhanced lipid productivity without compromising growth and biomass yield or causing any other physiological effect is essential.

Now, the following are a few genetic modification experiments conducted on Nannochloropsis oceanica.

Experiment 1

Definitions:

Transcription factors:
Transcription factors are proteins that help turn specific genes "on" or "off" by binding to nearby DNA.

This binding activates or represses the transcription, which is the process of copying DNA into RNA, of the associated gene. Casing alterations in the amount of messenger RNA (mRNA) and the amount of protein produced from that gene.

Overexpression
Is the process of creating higher than normal levels of a specific protein or gene within a cell or organism. This can be achieved through genetic manipulation techniques such as gene amplification, introducing extra copies of the gene into the cell, or using strong promoters to drive the expression of the gene.

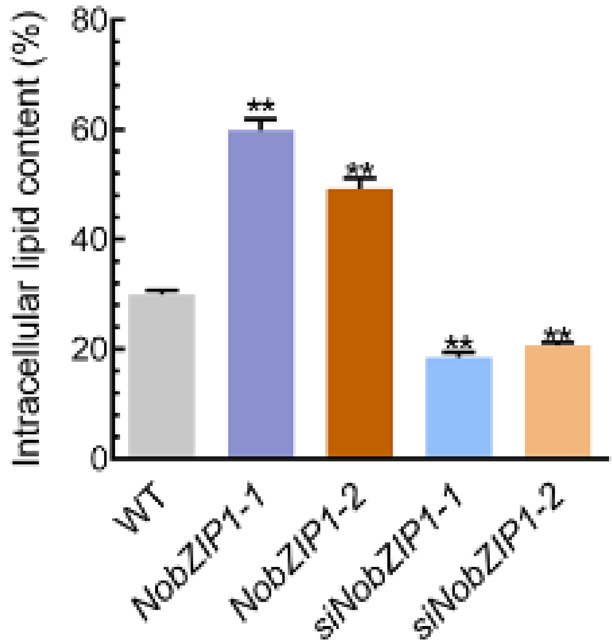
Overexpression of TF NobZIP1

The purpose of this experiment was to enhance the accumulation of lipids in the microalgae without causing any other effect during cellular functions, and this was obtained effectively.

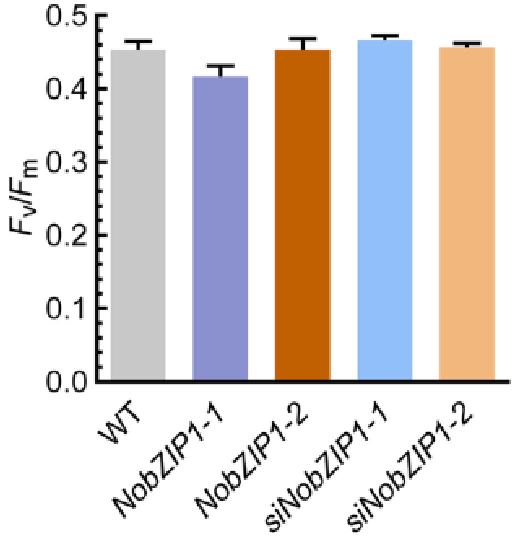
Through transcriptomic analysis done on the microalgae **Nannochloropsis oceanica**, a **TF** called **NobZIP1** was identified. To investigate its function, NobZIP1 gene expression was manipulated by using overexpression and **RNA interference (RNAi)** techniques. Consequently, NobZIP1 was silenced and it was found that NobZIP1 regulates key genes involved in **lipogenesis** (the production of lipids) and cell wall polymer synthesis.

The overexpression showed an increase of **50%** in the production and secretion of lipids. In addition, UDP-glucose dehydrogenase, one of the genes regulated by NobZIP1, showed a change in the cell wall composition, further contributing to the increased lipid secretion.

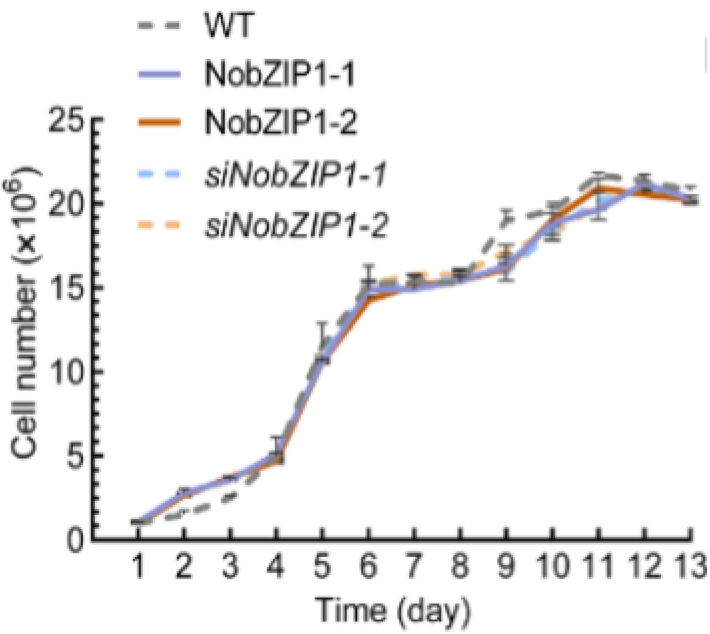
Not just that, the results indicated that the overexpressing strains did not lead to any significant alterations in cell biology parameters, such as cell growth and photosynthetic efficiency, compared to wild-type strains.
The results obtained emphasise **NobZIP1's** promise as a tool for increasing lipid synthesis while maintaining overall cellular health.



Gray: WT
NobZIP1-1 and NobZIP1-2 **NobZIP1 overexpress**
siNobZIP1-1 and siNobZIP1-2 **NobZIP1** silence with RNAi
As seen in the second and third column, examples of the overexpression of **NobZIP1** in **Nannochloropsis oceanica** there's an increment of nearly **50% increase of lipids content in the algae,** meanwhile the algae **NobZIP1** gene is silence there is decrement in lipids production inside the algae cell.



The graph shows the efficiency of photosynthesis, and there is any difference among the modified algae compare to WT



We can see in this graph that the cell growth analysis, which maintains almost the same as wild algae (wild type, **WT**) in the for algae that were modified.

A similar experiment was done but this time targeting a different gene. Let's have a look at it

Experiment 2

Overexpression of NoDGAT1A to increase TAG production

The goal of the experiment was to enhance TAG synthesis and alter fatty acid composition without **compromising cell growth.**

The experimental procedure consisted of knocking out the **NoDGAT1A** gene in N. oceanica, and the findings showed that there was a decrease in TAG production by 25%. However, when NoDGAT1A was overexpressed, it led to significant increases of **39%** in **TAG content per cell,** particularly under nitrogen deficiency conditions. What's even better is that the microalgae didn't experience any adverse effect on cell growth, meaning that cells continue to grow normally despite the increased TAG accumulation.

NoDGAT1A
Found in the chloroplast endoplasmic reticulum (cER), is an enzym**e involved in the production of TAG.** Its main purpose is to catalyse the conversion of a fat molecule, diacylglycerol (DAG), into TAG.

Experiment 3

Using a native gene from the LC-PUFA to enhance the production of lipids

Definitions:

Long-Chain Polyunsaturated Fatty Acids (LC-PUFA)

There are fatty acids with long carbon chains and many double bonds. There are primarily omega-3 fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid).

The experiment aimed to enhance the production of long-chain polyunsaturated fatty acids (**LC-PUFA**) in the marine microalga **Nannochloropsis oceanica**, particularly targeting eicosapentaenoic acid (**EPA**), which is essential for human nutrition and biofuel production.

This was done by overexpressing a gene in the microalgae called **NoD12**, responsible for a key step in **LC-PUFA** biosynthesis and under nitrogen starvation conditions, that lead to significant changes in fatty acid composition.

The procedure involved cloning and overexpressing the **NoD12** gene in N. oceanica, resulting in an **increase of TAG content** by **39%** under nitrogen scarcity and by **47%** when nitrogen was plentiful, compared to the wild strain.

Experiment 4

Overexpressing the putative Malonyl CoA-acyl carrier protein transacylase

The aim of this study was to look into the involvement of **Malonyl CoA-acyl** carrier protein transacylase (**MCAT**), found in the marine microalga **Nannochloropsis oceanica (NoMCAT**), in fatty acid production.

The technique entailed identifying the putative MCAT in N. oceanica and then developing microalga lines that overexpressed NoMCAT. These engineered lines were then compared to wild-type strains to determine any variations in growth rate, photosynthetic efficiency, lipid content, and fatty acid composition, and the results revealed significant increases in growth rate and photosynthetic efficiency. But besides that, engineered lines also caused an **increase in neutral lipid** content, up to **31%** more than the wild type, indicating increased lipid accumulation.

Besides these four experiments on Nannochloropsis oceanica, there have been many others where the algae were genetically altered. Scientists now employ various methods, including molecular and biochemical studies, to identify genes for manipulation. They also investigate how cells respond to stress by analysing their genes, proteins, and other molecules through **genomic, transcriptomic, and proteomic analyses.** One focus is on finding transcription factors and regulators that control lipid production. These are key because changes in them can impact many other genes involved in metabolism, leading to increased lipid production.

But if there have been numerous experiments conducted on algae and the results haven't been that bad, why aren't GM algae biofuels more commonly used? Here is why

The limited adoption of GM algae for biofuels production

Although several experiments have been conducted in certain algae spices, this isn't an easy process, and there are still plenty of improvements that need to be done in order to address high production costs, efficient lipid extraction methods, and the challenges associated with large-scale cultivation and nutrient supply, not to mention the ethical and ecological concerns of GMOs. And yes, we have made improvements over the last decades thanks to the use of genetic engineering tools such as microRNA (miRNA), silence RNA (siRNA), and CRISPR-Cas. However, it's still not sufficient to enable large-scale cultivation, because all those previous barriers prevent the widespread commercialization of biofuels from gene-edited algae.

Let's examine these issues thoroughly to understand them better.

Genetic Limitations

When we talk about GM algae for biofuel production, we don't look at increasing the number of TAGs they produce, but we have to overcome **rate-limiting steps, over-express synthesis, and decrease catabolic reactions**. But that's not all, because while the number of genome-sequenced species is on the rise, the genetic makeup of several other species is still lacking. So who knows, it's possible that more highly effective algae for biofuel production exist, yet remain undiscovered. This highlights the importance of **further DNA sequencing** developments. The cost of these analyses, along with expenses for algae cultivation and lipid extraction, significantly hampers the competitiveness of algae biofuels. In fact, it falls far short of even competing with the current national average gasoline price of **$3.53 per gallon.**

Even if we were to solve the immediate challenge at hand, another crucial issue arises:

Ethical and ecological issues

Conducted analysis conducted through **screening in silico** to verify the implications for **predator–prey** interactions if they escaped into the natural environment into surrounding water bodies, were accidental release or leakage, they could form harmful algal blooms (**HABs)** on a scale greater than naturally occurring species do.

HABs are a conserve because they can produce toxins that sicken and cause harm to animals and human beings.

Moreover**,** because algae **proliferate** rapidly, they could lead to competition with native species, causing an **ecological disruption** by changing nutrient dynamics, disrupting food webs, and affecting biodiversity.

Though, there are more worries, such as the scarce **regulatory frameworks** regarding their use, the long-term effects of releasing GM algae into the environment and the public perception and acceptance of GMOs in the environment.

And we keep adding to the list…

Water use

Another important consideration is water usage. In open ponds, water evaporation is a common issue, but we can address this by utilising wastewater for algae cultivation. This approach not only mitigates water wastage but also provides additional benefits. Beyond biodiesel production, algae cultivation aids in effectively cleaning water through wastewater treatment operations.

Land utilisation

The utilisation of land has long been a contentious issue, and algae cultivation may not represent the most viable method for biofuel production, particularly in terms of land usage. Since if we were to fulfil only US oil consumption, we would need to dedicate an estimated 30 million acres to production facilities. Besides, the production procedure, like developing the infrastructure to grow and process the algae and providing the power to run the operation, for algae-based biofuels has been discovered to need **more energy** than the finished product can produce, resulting in a **higher carbon footprint.**

*But is this really an issue? Don't they currently utilise about **24 million** acres of onshore and offshore subsurface minerals for fossil fuel production?*

Point of view

DNA sequencing
DNA sequencing is the process of identifying the exact sequence of nucleotides within a DNA molecule. This method allows for the finding of genetic variants, the identification of gene functions, and the understanding of genetic disorders.
From my perspective, every step toward a more sustainable future, including innovations, often comes with negative consequences. However, for once in all it's time to shift our mindset and start thinking more positively. Yes, the production of biofuels might still emit CO2 and require considerable land use, but what about the extraction, transportation, and burning of these fossil fuels which produce around **1.4 billion metric tons of carbon dioxide,** accounting for **over a quarter of yearly US greenhouse gas emissions**? Meanwhile, algae have the ability to **capture** and re-use up to **1.8 kg of CO2 per kilogram of algal biomass.** Not to mention that algae carbon sequestration is being explored further through gene editing to increase its efficiency.

Algae biofuels are relatively new and may initially be costly, but with more people working in this field, researching deeper **DNA sequencing**, more sustainable and effective methods of cultivating the algae, and extracting its lipids from it, algae biofuels could yield substantial returns on investment.

One approach scientists could explore is combining different strategies to enhance lipid production. For instance, doing the first and third experiments concurrently could lead to great improvements. By overexpressing the **TF NobZiIP1** and the **NoD12c** gene simultaneously, we could potentially **increase lipid production and secretion by 50% and TAG production by 43% without affecting cellular function.** Furthermore, it is necessary to highlight the need for ongoing research and collaboration within the scientific community to progress these results. Collaborative efforts with other laboratories could provide valuable insights and resources for accelerating algal biofuel production. Likewise, successful outcomes from the first experiments may guide future research, perhaps leading to the overexpression of genes NoDGAT1A and NoMCAT found in experiments 2 and 4 for greater efficiency.

However, before implementing such strategies, we could utilise **screening in silico** to predict the outcomes and minimise risks and costs. This proactive approach ensures that any alterations made do not compromise cellular function and allows for informed decision-making in optimising algae biofuel production. This will ensure that any changes made do not jeopardise cellular function and enable informed decision-making in algal biofuel production.

Companies working in the field

There are several businesses in the field of biotechnology that are exploring new solutions to battle climate change and solve global concerns by harnessing the potential of microalgae for a variety of purposes, including sustainable biofuel generation.

Below, we will look at a few examples.

Viridos

Viridos is a biotechnology company dedicated to combating climate change by developing renewable algae biofuels. Their goal is to implement a breakthrough solution on a global scale to shift heavy transportation towards sustainability, aiming t**o reduce greenhouse gas emissions** by nearly **70%**. Utilising an advanced algal strain and agronomic platform**, they have achieved seven times the oil productivity of wild algae**, laying the foundation for scalable and sustainable algae oil production.

AlgaEnergy

AlgaEnergy is committed to addressing challenges such as food security, environmental sustainability, and energy efficiency, aligning with Sustainable Development Goals (SDGs). Through the scale-up of microalgae cultivation processes, innovative product development derived from microalgae, and a focus on CO2 fixation, they are actively engaged in genome engineering and gene editing technologies such as CRISPR/Cas9 systems to optimise the properties and capabilities of microalgae, aiming to enhance productivity, resilience, and sustainability across various industries.

Aurora Algae

Aurora Algae, a producer of algae-based products for a variety of applications including medicines, nutrition, aquaculture, and fuels, is committed to sustainable product development. Their major objective is to improve the productivity and characteristics of microalgae for biofuel generation and other applications through gene editing methods. They have adapted a CRISPR/Cas9 system for gene editing in marine algae, allowing precise modifications in the DNA of microalgae to optimise their productivity and properties specifically for biofuel production, consequently improving the efficiency and sustainability of algae farms and progressing their algae-based product development processes towards greater sustainability and efficacy.

Conclusion

The potential of algae biofuels is quite exciting, and it could be enormous only if research evolves further.

These biofuels are not currently delivering benefits at the expected rate, but with help of DNA sequencing and other advancement in the field of gene editing, soon this biofuel could be viable to produce at large scale.
As for the current knowledge about all algae species and their genes, it is challenging to determine their potential as a replacement for fossil fuels. Although we've seen that certain experiments conducted in the one of the most efficient algae for biofuel production, Nannochloropsis oceanica, have shown hardly any issues regarding the dilemma of causing impactful changes in cellular physiology, which marks a significant advancement.

Now, we cannot overlook the cost requirement for further research in biotechnology and the need for greater efficiency in algae harvesting and lipid extraction. But despite this, algae possess many capabilities. They grow rapidly, absorb more CO_2 than any terrestrial plant, and their biofuel pollutes less than fossil fuels since they do not produce as much greenhouse gas emission. Thus, algae biofuels present a promising alternative, especially when used alongside other alternatives to replace fossil fuels.