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Metabolic engineering of Cyanobacteria & micro algae for enhanced production of bio fuels & high value products

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Abstract
Cyanobacteria and microalgae has been the subject of extensive study with the aim of producing variety of biotechnological products. Native strains, on the other hand, have a few flaws, such as limits in growing, harvesting, and product extraction, which hinder them from achieving optimal output value at the lowest possible cost. To overcome these restrictions, genetic engineering must be used to create strains with improved traits. Improved photosynthetic efficiency by increased RuBisCO activity & shortening of light-harvesting antenna has resulted in promising advances in the growth of Cyanobacteria & microalgae. Using genetic engineering to induce autolysis & product secretory systems has also helped with ultimate product extraction, allowing for direct product improvement without the need for costly taking out processes. We outline the various enzymes & pathways that has been addressed for optimising culture aspects harvesting & product extraction in Cyanobacteria & micro algae thus far in this review. Genetically modified strains can be created using synthetic biology technologies to tackle difficult process issues and attain economic viability. Researchers in the field will benefit from this detailed overview of gene alterations, which they can utilise on their strain in order to increase yields & make the manufacturing process more economically viable.

Keywords: microalgae; cyanobacteria; bio fuel; biological contamination; controlling strategy.

Introduction:

There is a potential to employ breakthroughs in many sectors of science and engineering in the development of upcoming fuels and high-value products ensure that they are maintainable in mutually production & consumption. The exploit of bioengineering of photosynthetic microbes using synthetic biology can manufacture novel compounds directly from CO2 is one such advancement. Cyanobacteria and Microalgae as "versatile photosynthetic organisms with many valuable physiological features that make them suitable platforms for the development of diverse biotechnological products." Some of these species can withstand salt, high temperature, water shortage & UV radiation in harsh conditions. Apart from their ability to adapt to various environmental conditions, cyanorbacteria & micro algae can develop photo autotrophically, hetero trophically & mixo trophically depending on carbon supply available. Cyanobacteria & microalgae are useful in biotechnology because of their ability to adjust to changing

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environmental condition and their self-sufficiency. Cyanobacteria and microalgae have been widely used for biotechnological purposes as a result of these features.

The growing environmental pollution challenges linked by the usage fossil fuels, as well as the projected shortage of these resources in the not-too-distant future have prompted researchers to look into novel alternative fuels such as biofuels [1–4]. Biofuels, which are made up of, Biodiesel, bioethanol, and jet fuel, for example, are now being recognised as drop-in fuels sourced from manufactured by microbial cell factories from biological resources and their use has soared, particularly in industries where electricity is not accessible [5,6].

Four generations of bio fuels have been produced based on feedstock sources and bioprocess technology. Grain crops and oleaginous plants are used to make first-generation biofuel [7]. The use of non-edible lingo cellulosic materials in second-generation bio fuel is intended [8]. However, “competing with humans for food crops” or “competing with agricultural plants for fertile land” are big disadvantages for these two generations. Bio fuels are produced by the third and fourth generations using photosynthetic microbes such as eukaryotic microalgae & prokaryotic cyanobacteria, which cannot compete for food & arable land [9]. The Third Generation is bio fuel focuses on increasing biomass output to raise lipid accumulation, even as the fourth generation intends to improve the direct synthesis of algae bio fuels using systems biology and genetic engineering methods and methodologies [10–12].

Microalgae and cyanobacteria could have a lot in common, like a faster growth rate & higher photosynthetic good organization than plants, the capability to live in locations where agriculture isn't possible, and a high level of environmental tolerance [13,14]. Microalgae, on the other hand, offer a fully bio degradable & Sulphur free liqid for bio fuels [15]. Increased knowledge of algae physiology and advancements in bioprocess engineering have cleared the road for their utilisation in bio fuel applications [16]. Cyanobacteria are gaining popularity as a high-capability photosynthetic raised area with simple backdrop & straightforward inherent modifications. The photosynthetic generation is wide spectrum of small molecule bio fuels such as ethanol, butanol & isoprene have been enabled by modifying the native Cyanobacteria's metabolism might benefit from the addition of heterologous metabolic pathways [5,19–24].

Microalgae & cyanobacteria bio manufacturing have both proven on the way to be potential solutions for long-term bio fuel generation, resulting in large number of research & development activities in the field [25,26]. Commercialized uses of microalgaland cyanobacterial-based photosynthetic bio fuels, on the other hand, are still a long way off [27]. Aside from Another urgent problem is to develop biogenetic techniques cultivation process improvements & improved photo bioreactor designs for scale-up production [32–34] impeding microalgae or cyanobacteria scaling-up processes is the inevitability of biological contamination [35–37].

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In large-scale mass cultivations, different types of biological contaminants usually inhibit or destroy cellular biomass accumulation of microalgae or cyanobacteria depending on the microalgae/development cyanobacteria's stage and decreasing biomass results in a related in liqid production. In the case of direct “algae-to-bio fuels” is conversion The target products might influence and modify environmental conditions and contribute to certain contamination patterns via photosynthetic chassis cells. That's why a deeper knowledge of biological contaminants and their contamination patterns in large-scale microalgae and cyanobacteria cultivations for the production of biofuels is becoming increasingly apparent. More importantly, the need for quick solutions to biological contamination control has emerged as a critical issue that must be addressed immediately.

The goal of this analysis is to provide an update on microalgae & cyanobacteria for biofuels production: biological contamination and controls. We emphasise the significance of avoiding and regulating contamination related to biomass as well as contamination related to synthetic products in the future production of direct algae-to-bio fuels (Figure 1)

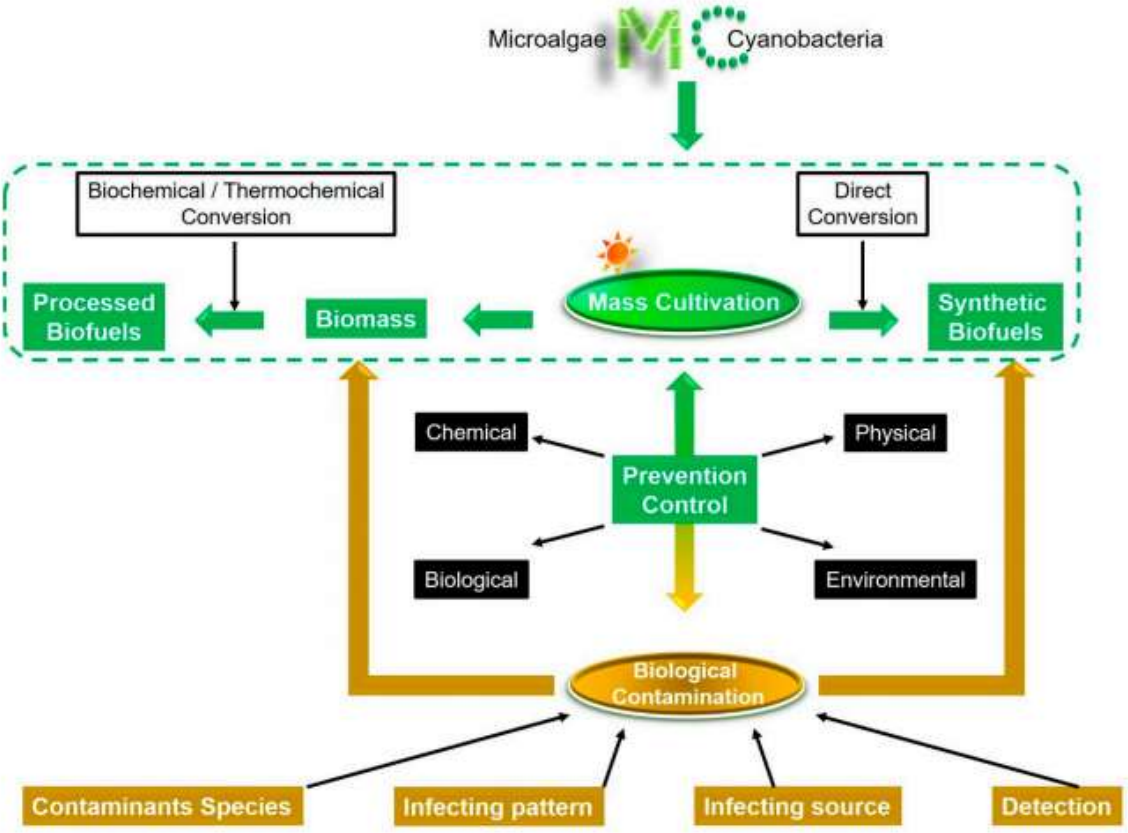

Figure 1. Rising the economic viability of microalgae & cyanobacteria based bio fuel production requires preventing & controlling biological contamination as a major concern.

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As considerate of synthetic biology have progressed cyanobacteria & microalgae have be modified to generate powder, medicines & other high value goods as well as bio fuels. However, there are still fundamental impediments that must be overcome in arrange to efficiently utilise those organisms in a way which is both cheaply possible and suited for industrial processes. As a result, substantial research has been undertaken using native cyanobacterial & microalgal strains to achieve ideal growing conditions. To reduce the cost of mass production of Chosen strains of cyanobacteria and microalgae are commonly cultivated under naturally changing sunlight.

Variable beam intensity, shading effects & photo damage due to heat dissipation are all factors to consider could all affect production under these conditions. Another source of worry is the time-consuming and energy-intensive process of harvesting. During large-scale manufacturing, substantial cyanobacterial and algal biomass is produced. The Harvesting cost of cultivate bio mass must be reduced the employing most cost-effective procedures. Harvesting is expected to account for 20–30% of total expenditures. To minimise spoilage, the gathered biomass must be dried in a few hours in hot areas, which may necessitate specialist equipmentIt was shown that downstream operations such as harvesting and product extraction of cyanobacterial or microalgal biomass accounted for 70–80 percent of the total cost and energy consumption.

Selective genetic engineering of strains can help reduce costs and increase yields in a given process, allowing Cyanobacteria and microalgae to attain their full potential as a multi-component producer (Fig. 1).

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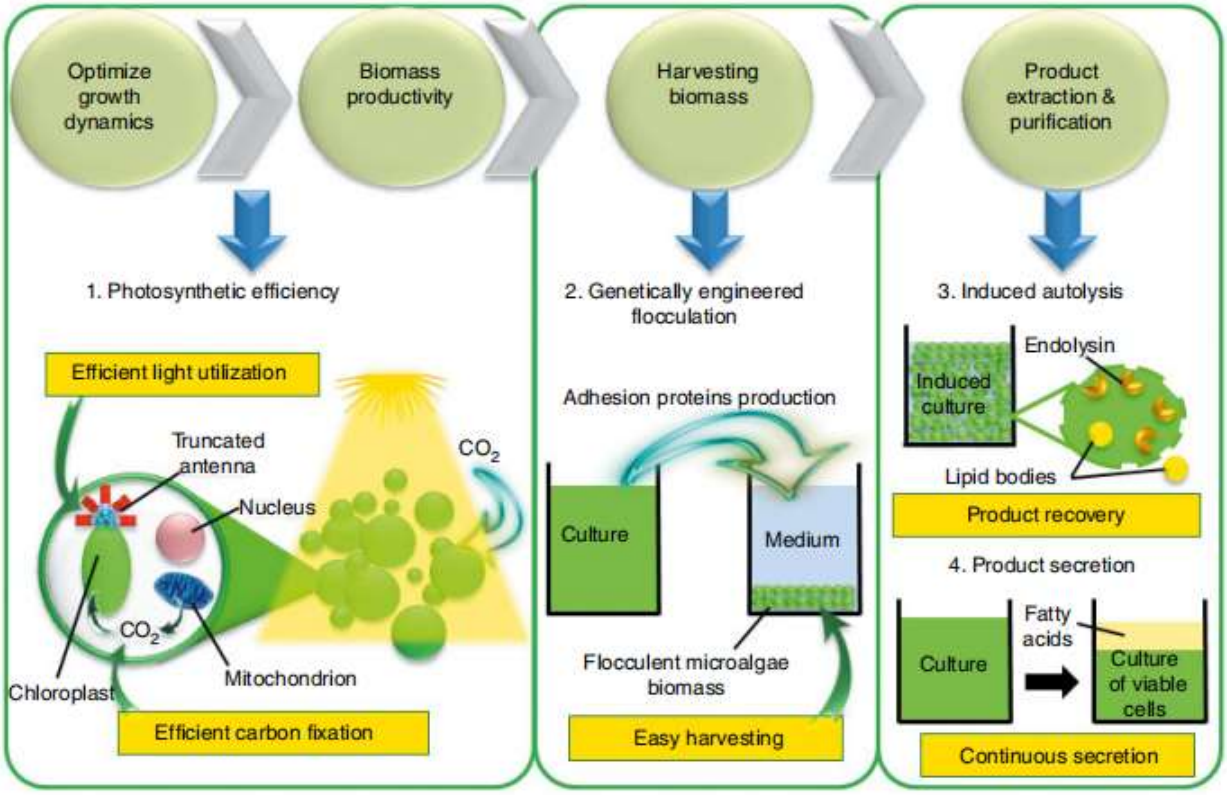

Figure 2. A diagram depicting the various phases involved in Cyanobacteria and microalgae producing biotechnology goods. Increased carbon fixation and light consumption efficiency might increase growth and cultivation, while inducing flocculation, autolysis, and continuous product secretion could improve harvesting and extraction of end products.

To create genetically modified strains with better traits, new molecular techniques were used.
To genetically alter phototrophs, researchers have used established techniques such as homologous recombination & small interfering RNAs, as well as newer ones such as the adaptative immune system based on clustered regularly interspaced short palindromic repeats (CRISPR) and random mutagenesis adaptative immune system based on clustered regularly interspaced short palindromic repeats & random mutagenesis (Kindle 1990; Bartel 2009; Kilian 2011; Cong 2013; Iwai 2013; Iwai 2013; Iwai 2013; Iwai 2013; IW Certain steps & enzymes) (Table 1) has emerged as potential target for improving various aspect of phototrophic organism production processes with the introduction of these methods and the rise the number of cyanobacterial and microalgal genomes that have been completely sequenced (see Lu€ et al. 2011 for a list of sequenced genomes). This study highlights the many therapeutically targeted enzymatic , biochemical pathways & enzymes cyanobacterial & microalgal biomass production, harvesting, and downstream processing. Unlike earlier research that focused on specific chemicals & strains the genetic engineering methodologies & target genes reported that may be used to any photosynthetic organism-based biotechnological compound production process. Rather of focusing on a single strain or product, this evaluation attempts to improve any production process.

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It can be used by field researchers to modify cyanobacterial & microalgal strains to improve yields & make harvesting & product extraction easier. Those benefits will help the industrial process's economic feasibility & overall viability.

2. The cyanobacteria/microalgae-to-biofuels opportunity

Figure 3 depicts a graphic diagram of cyanobacteria microalgae-to-bio fuel potential. Growing primary biomass and processing biomass are both part of the entire system for producing bio fuels. According to studies performed over the last six decades cyanobacteria & algae create a wide spectrum of chemical intermediates & hydrocarbons that are precursor to bio fuels. As a result, cyanobacteria-to-fuel offers assure as a possible alternative for fossil-fuel-derived goods.
Biomass from cyanobacteria can be utilised as a food source or a variety of feed stocks. Antioxidants, colouring agents, medicines, and bioactive chemicals are among the major biomolecules that can be obtained. Anaerobic digestion can convert biomass to biomethane (biogas). The photosynthetic system of cyanobacteria can diverge electrons from two main processes, resulting in the creation of H2. Carbohydrates, proteins, lipids, and fatty acids are all produced by the Calvin cycle. Fermentation may transform carbohydrates into bio-ethanol. Biodiesel can be made from lipids. Fermentation of fatty acids produces acetate, butyrate, and propionate, which are stabilised to generate CH4, H2, and e.

3. Improving production by metabolic engineering:

Photosynthetic organisms production is determine by their carbon complex rate & light usage ability whose range since one Cyanobacteria or microalgae sprain to the next (Rosgaard 2012). The photosynthetic process is not a entirely optimised to function properly in bright light (Pirt 1980). As a result, various attempts have been made to change genes involved in CO2 fixation and light use using genetic engineering methods. The following is a list of the genes that have been manipulated, the technique of manipulation, the target organisms & the results of this gene handling thus far.

4. Improving carbon fixation

Cyanobacteria and microalgae use thetic processes to determine the saturation limit of light intensity, which affects the pace of the whole process (Sukenik 1987). Carbon fixation's process have carefully studied & the enzymes & genes involved has been discovered (Smith 1983). Several targets have been changed in Cyanobacteria & microalgae to increase carbon fixation, increasing photosynthesis efficiency by increasing the amount of carbon fixed by the cells as a whole.

The Calvin cycle of enzyme ribulose-1, 5-bisphosphate carboxylase oxygen is involved in photosynthesis and hence has a major influence on the rate of photosynthesis (Furbank 1996).

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RuBisCO's inefficiency has been blamed on its poor specificity and sluggish catalytic rate. To compensate for RuBisCO's inefficiencies, Carbon concentrating mechanisms (CCMs) are active processes that use energy in oxygenic phototrophs. RuBisCO formI which is made up of eight large & small sub units is the most frequent in Cyanobacteria and microalgae. Genes coding for the big and small subunits, respectively, are rbcL & rbcS. In Cyanobacteria & genes both are share the same promoter, however in algae, the chloroplast genome expresses rbcL whereas the nuclear genome produces rbcS. The rbcL & rbcS genes are from Synechococcus elongates sp. PCC6301 were effectively over expressed in Synechococcus elongatus PCC7942, increasing RuBisCO activity. RuBisCO activity increased 14-fold as a result of the overexpression, resulting in a 2-fold rise in isobutyraldehyde production. A comparable improvement in RuBisCO performance was achieved by using two distinct promoters to generate type I from the purple-sulphur bacterium Allchromatium vinosum into Synechococcus sp. PCC 7942. It increased RuBisCO activity by fourfold and O2 evolution by half even though heterologous RuBisCO was located elsewhere in the cytoplasm than carboxysomes.

Another Calvin cycle rate-limiting enzyme with promise for carbon fixation optimization are sedoheptulose-1, 7-bisphosphatase. The photosynthetic carbon decline sequence which is regenerates RuBisCO's precursor, includes this enzyme (Fig. 4). The SB Pase of Chlamydomonas reinhardtii was transformed into a glycerol-producing halotolerant Dunaliella bardawil transformant (CrSBP). As a result, the activity of SBPase in CrSBP was around 80% higher than in wild-type. CrSBP growth rates were shown to be slower than wild type growth rates at various salinities, but with reduced volatility and salinity sensitivity. It was found that the transgenic Dunaliella had 50–100% higher rates of photosynthetic efficiency (nmol O2 per cell per lmol photons per m2) and CrSBP rates than the wild type at 2 mol l 1 NaCl. This was in comparison to the natural type. Overexpression of SBPase increased total organic carbon content by 37% and glycerol synthesis by a comparable amount in environments with high salinity, according to the

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research.

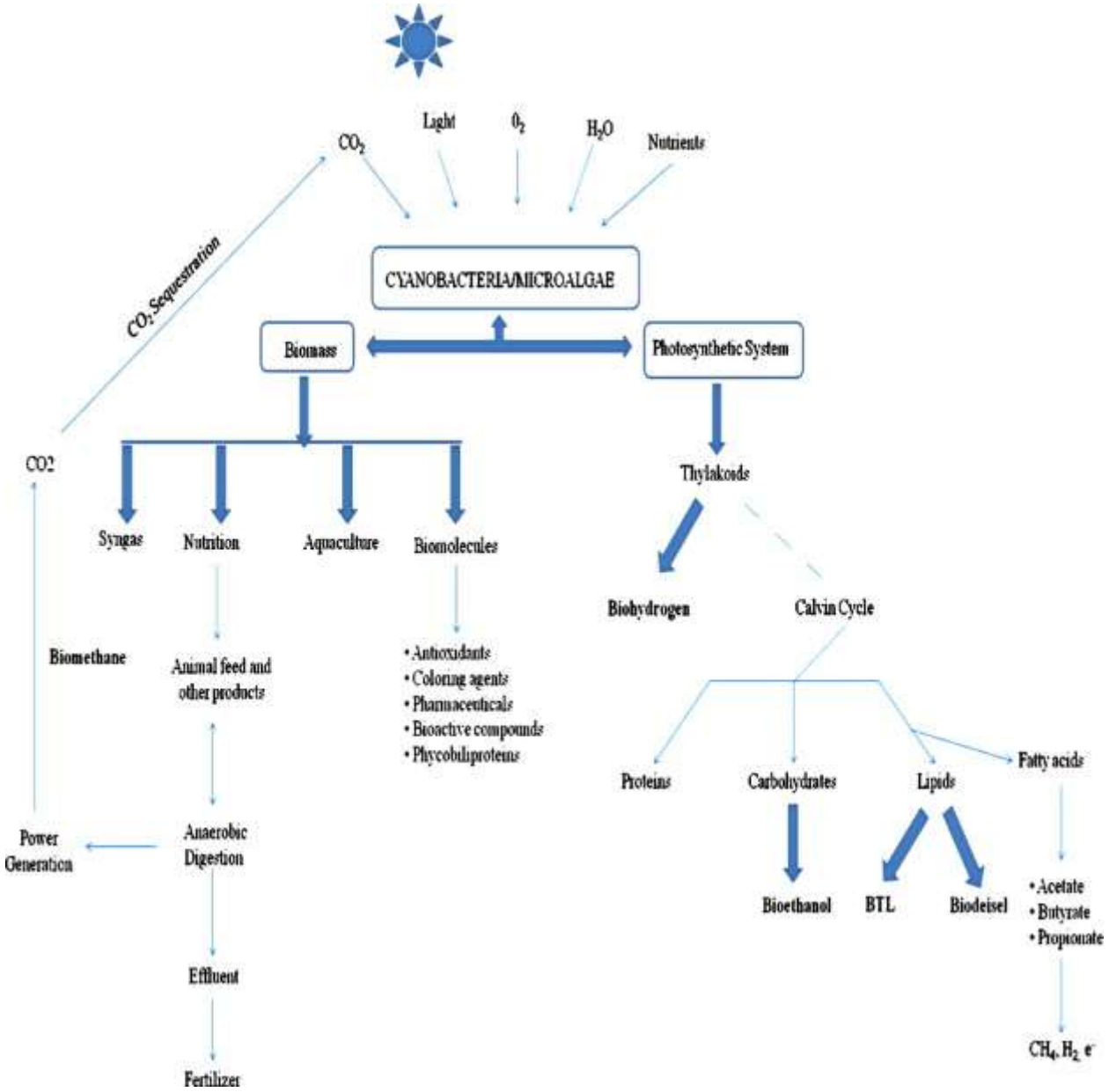

Fig. 3. Cyanobacteria & microalgae have the potential to produce a wide range of biofuels and by-products.

Apart from a Calvin cycle, microalgae may fix CO2 by generating Oxaloacetate for the tricarboxylic acid cycle through the phosphoenolpyruvate carboxylase (PEP Case) enzyme. PEPCase converts phosphoenolpyruvate (PEP) to oxaloacetate which then enters the TCA cycle (Oleary 1982). Despite the fact that many bacteria's activity is dependent on Acetyl-CoA, glutamine activates and glutamate inhibits PEP Case from green algae. PEP Case activity in Cyanobacteria is considered to be related to malic enzyme via massive CO2 fixation, which accounts for 20–25% of total ingested CO2.

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According to Yan et al., a recent work on Synechocystis PCC6803 led to the development of a regulated expression system for the pepc gene under the copper-induced Pete promoter (2014). There was no evidence of pepc gene overexpression in this study, however it was discovered that at 0 nmol l 1 copper levels, carbohydrate content rose and TCA cycle carbon flow through PEPC reduced.

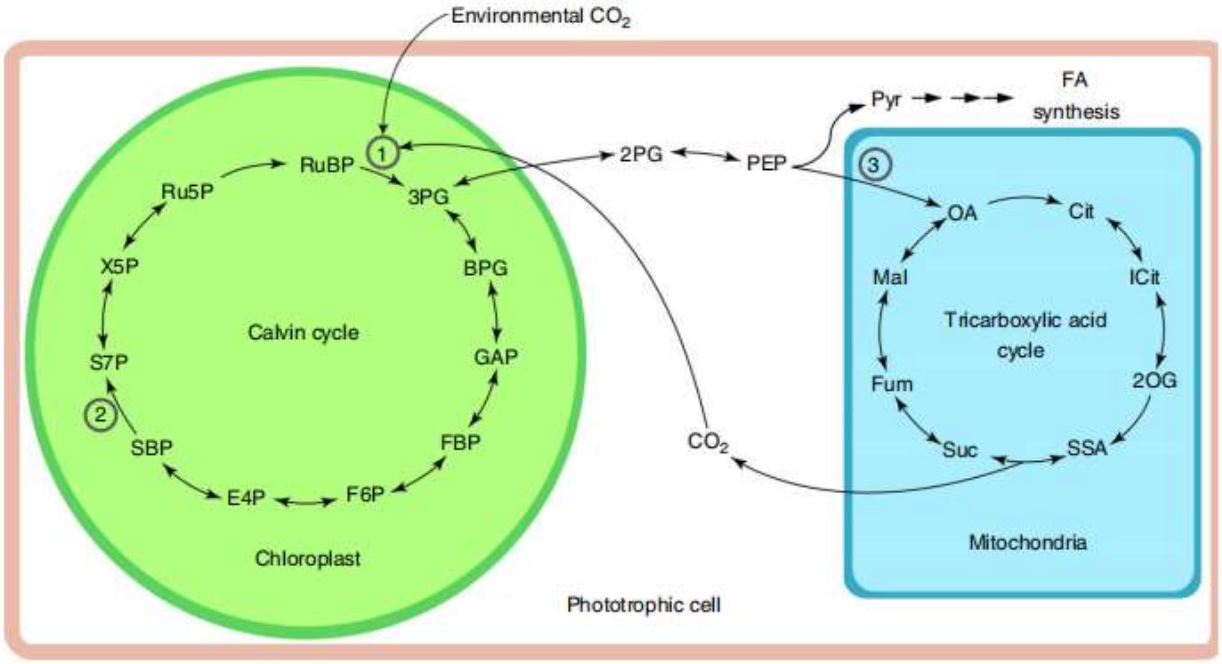

Figure 4 Phototrophs' carbon fixation is aided by the Calvin and tricarboxylic acid (TCA) cycles. To boost the activity of these enzymes, scientists used genetic engineering techniques (e.g., RuBisCO, sedoheptulose-1,7-bisphosphatease, phosphoenolpyruvate carboxylase). The numbers reflect the increased activity of these enzymes. 2,2-Oxoglutarate is the chemical name; 2,2-phosphoglycerate is the chemical name; 3,3-phosphoglycerate is the chemical name; 1,3-bisphosphoglycerate is the chemical name. the chemical compound citrate is known by its abbreviation citrate. GAP is for glyceraldehyde-3-phosphate, and X5P stands for xylulose-5-phosphate. F6P stands for fructose-6-phosphate, and FBP for fructose-1,6-bisphosphate. Isocitrate stands for isocitrate, and Mal is malate. Pyruvate is pyruvate. FA is a fatty acid.

The PEPC1 gene, which codes for a PEP Case component, has been linked to fat buildup and growth rates. The use of interfering RNA to down regulate CrPEPC1 result in a transgenic alga with a 20 percent rise in TAG content, a 39–49 percent decrease in PEP Case activity & overall decreased growth rates. CrPEPC1 over expression, nevertheless, result in increased growth rate over the first five days such as a 157–184 percent increase in PEP Case activity and a 37% drop in TAG content. Mutants with a down-regulated pepc gene is the cyano bacterium. Anabaen PCC7120 had no effect on total photosynthetic activity, but dark respiration was reduced in the mutants. Continued photosynthetic activity is considered to be due to a reduction in dark respiration. With Anabaena sp. PCC7120, however, over expression of pepc led in enhanced photosynthetic activity and dark respiration.

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Phototrophic organisms now have a new approach to increase carbon complexes thanks to the development of a synthetic carbon fixation pathway. Recent research by Bareven & colleagues has smooth the door for the use of synthetically designed pathway to boost photosynthetic production in biotechnologically important organisms. The most effective carbon complex routes were devised by screening and analysing around 5000 known enzymes.

Four criteria were used to evaluate these pathways:
Specific route activity, energy cost, thermodynamic favorability, and topological compatibility are all factors to consider. The pathways Malonyl-CoA-oxalo acetate-glyoxylate (MOG) and Malonyl-CoA-oxaloacetate-glyoxylate (MOG) were named after the routes with the greatest specific activity. The MOG pathways have a similar metabolic core structure and utilise the highly efficient carboxylating enzyme PEPC. Lactoyl-CoA dehydratase has to be oxygen-sensitive among the enzymes tested; however, current variations of the enzyme are less sensitive to aquarobics settings. MOG routes have a 2–3 times greater route specific activity than the Calvin cycle. Other synthetic routes for carbon complex in photo trophs have been attempted; one example is Shih and his coworkers' recent work, in which they bypassed the normal photo respiratory mechanism by introducing six genes from the 3-hydroxypropionate pathway into Synechococcus sp. PCC7942. The results showed that all six genes were expressed and active, indicating via the enzyme acetyl-CoA carboxylase, a net increase in carbon complex (AC Case). However, a few obstacles must be solved before the introduced channel may reach its full potential. The expression enzymes were first acquired from a thermophile, which operates at higher temperatures than mesophilic cyanobacterial enzymes. Second the transformant strain must perform a substantial amount of assembly work due to the high size of the changed enzymes. Third, native ACCase activity may be inadequate, necessitating the introduction of exogenous ACCase with enhanced activity to optimise process efficiency.

5. Major biological contaminations and the infection patterns:

Because contaminants will always penetrate the culture through water or air, biological contamination is a common problem when growing microalgae and cyanobacteria in large quantities.
Microalgae and cyanobacterial strains produce and secrete a wide range of products, some of which can be significantly inhibited or consumed by biological contaminants. The other group of contaminants be able to significantly inhibit or consume the cell growth of microalgae and cyanobacterial strains, depending on the objectives.

5.1. Cell Growth-Affecting Contaminations:

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Zooplankton, bacteria, fungus, and other eukaryotes as well as even viruses have been found in large-scale cultivations of microalgae and cyanobacteria, all of which impact cell development [39–41].

Wang summarized various contamination mechanisms used by biological contaminants, stating that zooplankton used a feeding system that is either positive or negative, Direct or indirect assault was employed by phytoplankton-lytic bacteria, direct cell contact, nutrient competition, or allelopathy was used by toxic algae, and viruses used a host-specific infection strategy. According to Molina, biological contamination processes are connected to natural relationships including mutualism, commensalism, and parasitism [41]. These massive pollutants in the cultures are detrimental to bio mass buildup & liqid formation and usually result in the complete loss of bio fuel production (Table 1). In fact, a greater increase rate is more significant than a higher lipid content of the microalgal or cyanobacterial cells when it comes to increasing liqid output [38]. Contaminations induced by zooplankton grazing are still the most problematic examples, with rotifers such as Brachionus calyciflorus and Brachionus plicatilis being the most abundant yet harmful grazers [42]. According to Fischer, rotifer B. Per rotifer, calyciflorus may devour about 500 Chlamydomonas reinhardtii cells per hour [43]. According to Lubzens' research, rotifer B. plicatilis may consume up to 4800 Nano chloropsis sp. cells each hour, causing pond collapse in a couple of hours [44–46].

5.2. Products Accumulation-Affecting Contaminations:

Biosolar cell factories, such as designed microalgae and cyanobacteria have received notice as "biosolar cell factories" for the direct conversion of carbon dioxide into bio fuel products thanks to the advent of systematic metabolic engineering and synthetic biology [27]. Many biofuel compounds' economic viability has been assessed through experimental pilots. Instead of hindering direct conversion, biological pollutants, such as cyanobacteria or microalgae designed to produce biofuel, might cause cell growth to be contaminated during photosynthetic synthesis. Due to concerns about biological contamination, we turned our attention to increasing the production of synthetic ethanol rather than expanding our lab's stockpile of cyanobacterium Synechocystis cells [24,47]. There was at least one invading contaminant bacterium identified by 16S RNA as Pannonibacter phragmitetus in the unsterilized outdoor culture system that was completely eaten and eliminated eliminated ethanol accumulation in the non-sterilized environment. Ethanol was used as the only carbon source for P. phragmitetus' growth in the lab, and it was shown to be 68% more productive for that organism than the currently established species of the cyanobacteria [37]. It is impossible to overestimate the gravity of synthetic product contamination despite the fact that just a few cases have been reported so far. Biofuel mass production may make extensive use of "biosolar cell factories," however while scaling-up operations, special attention should be paid to synthetic product-related contaminations.

As a potential alternative to or complement to conventional fossil fuels, both the third and fourth generations of biofuel are now being explored. After that, there will be biomass-based biofuels created from microalgal and cyanobacterial biomass, and finally, The fourth generation of

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biofuels will be made up of directly converted biofuels derived from microalgal and cyanobacterial cell transformation platforms. Contaminants that influence cell development and accumulation of products (Table 1), such as those listed above have negative impact on the productivity of microalgae and cyanobacteria-based biofuel outputs, particularly in large-scale open systems. In order to achieve commercial-scale biorefinery of these biofuels, it is critical to ensure that the microalgal or cyanobacterial cells develop properly from the start. As a result, it's critical to learn about biological contaminants that limit cell development and might endanger mass-culture accumulation of microalgae or cyanobacteria biomass. In recent years, advances in metabolic engineering & systematic biology have made some eukaryotic micro algae and prokaryotic cyanobacteria attractive as biosolar cell factories for the generation of biofuels in future. This has led to the emergence of new and distinct biological contaminations during the technology's scaling-up phase.

As a result, it's important to improve our understanding of biocontaminants that impact product accumulation but don't impair microalgae or cyanobacteria cell development yet It's capable of destroying the entire algae-to-biofuels manufacturing process.

Improving light utilization

It's critical to improve light usage in Cyanobacteria and microalgae in order to boost their development and output. Chlorophylls and carotenoids, for example, operate as light harvesting antennas absorbing light may be employed in photochemical reaction centre. In Cyanobacteria the existence of a phycobili some allows for light absorption and unidirectional energy transmission to photo system II (PSII) reaction centre, similar to the light collecting antenna found in green algae. Photosynthetic apparatus regulation capability was identified in the 1970s, although the technique or genes involved were unknown at the time.. It was recently revealed that a few genes involved in the regulation of photosynthetic machinery may be utilised to improve the use of light by microalgae.

Tetali & Colleagues found a novel gene that controls antenna size, affecting photosynthetic organisms' light harvesting complex (LHC). A tla1 mutant of the green alga C. reinhardtii with a shortened light-collecting antennae was produced using DNA insertional mutagenesis. The deletion of tla1 in C. reinhardtii resulted in a 50% reduction in PSII antenna size and a 67% reduction in PSI.
The tla1 mutant of C. reinhardtii has lower levels of total chlorophyll, LHC polypeptide abundance, and chlorophyll b. This led to a 130 percent increase in cell density and a 45 percent increase in mass culture productivity depending on the amount of chlorophyll present. C. reinhardtii alterations As the antenna size regulating mechanism is shared by all photosynthetic species, it might be extended to other creatures. They found that Scenedesmus obliquus and Chlorella vulgaris could be treated successfully with this method, as well as the bacterium Botryococcus braunii and Botryococcus sudeticus. Cyanobacteria and microalgae growth might

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benefit from a strategy that transitions between different light intensities, Unless artificial lighting is used to provide a steady level of light for growth. This would allow them to alter the size of their antenna in response to changes in light intensity by switching between the two different chlorophylls (von Wettstein 1995; Tanaka 1998; Masuda 2003). Chlorophyll an is converted in C. reinhardtii via the chlorophyllide an oxygenase (CAO) gene, which codes for the enzyme. CAO-RNAi transformants were created after this gene was silenced using RNAi-mediated silencing (CR). It was shown that these CR transformants had a 30–48 percent lower concentration of chlorophyll b than wild type, which led to reduced cell sizes while retaining the photoprotective and state transition capacities of the light collecting antennaes. As a result of the reduced shadowing influence, the photosynthetic rates of these transformants were 2–2 6-fold higher, and the culture densities were 15–35 percent higher than those of the wild type. For effective light utilisation at high light intensities, the size of light-collecting antennas must be decreased, but not completely eliminated, according to this study. Another gene that might be used to modify microalgae's LHC is the CAO gene. The prospect of inhibiting CAO in other species to improve light use may be illuminated with more research.

In Phycobilisomes & Phycocyanin rods are heterodimers of CPC-a & CPC-b proteins linked by linker polypeptides. They're all encoded by the CPC-operon, a collection of genes contained in a single person. The CPC- person of Synechocystis PCC6803 was knocked out by homologous recombination using a DNA construct containing a codon-optimized kanamycin resistance gene. The quantity of light required to attain half-saturation photosynthetic intensity was roughly doubled for the cpc transformants. Kirst et al. (2014) discovered that the production of the cpc transformants was 57 percent greater than the wild type under high light intensity. The cpc transformants have an advantage more than the wild type when employing open systems in a naturally sunny geographical area.

6. Improving harvesting:

A more effective manufacturing method requires the use of specialised equipment for dewatering and perhaps recycling huge micro algal cultures. Several micro algal biomass extraction methods have been studied. Although popular, techniques like centrifugation and ultra filtration are expensive; others, including sedimentation and flotation, generate low yields and are strain specific. Auto flocculation is a potential technology that may be used on its own or in conjunction with other methods.

The term "auto flocculation" was coined by Sukenik and Shelef (1984) to explain the spontaneous flocculation of microalgae caused by variations in the culture's pH and/or ionic strength. Auto flocculation isn't an option with some strains. To allow cells to adhere and flocculate, Volvox carteri, an alga, produces fibrous hydro xyproline-rich glycoproteins (HRGPs). To begin, Algal-CAM is an HRGPs member with two homophilic cell adhesion domains at the C terminus, one of which attaches to the surface of cells in a manner similar to extensin (Huber and Sumper 1994). In

this work, glycoproteins present in the extracellular matrix (ECM) of C. reinhardtii were synthesised exogenously, allowing the previously non flocculating alga to self-flocculate (Fig. 2). When C. reinhardtii was mutated into the wallless CC-400 strain, algal-CAM was produced and released into the extracellular matrix (ECM). When adhesion proteins are synthesised exogenously, previously nonflocculating organisms might become flocculating. To illustrate, making adhesion protein expression inducible would allow for timely flocculation to maximise biomass or product outputs by increasing the amount of time available for research in this area. Because of the increased shadowing impact and access to nutrients, autoflocculation may impede biomass accumulation. It is preferable to induce autoflocculation during the height of biomass accumulation.

7. Improving final product excretion:

When it comes to the cost of the procedure, extraction accounts for roughly 60% of the total cost of the product, whether it's intracellular lipids, free fatty acids, or a heterologous one. Genetically modifying Cyanobacteria and microalgae to enable direct product recovery from growing medium has been attempted as a result, avoiding costly and energy-intensive manufacturing procedures.

Because of the poor procedures used, cell disruption was previously considered uneconomical for large-scale production of microalgal biomass. A new genetic engineering discovery has made it possible to extract desired compounds from microalgal or cyanobacterial cells without harming the cells themselves, as long as an inducible lytic mechanism is used (Fig. 2). The cyanobacterium Synechocystis sp. was studied in a single cell model. A nickel detection and response signal system, PCC6803, was developed. The lytic proteins holin and endolysin from bacteriophages P22 and k were generated under the control of the nrsB promoter via a method devised by Young et al. (2005), resulting in the mutant SD127. To allow endolysin to reach the protein-peptidoglycan layers, holin forms tiny membrane lesions, disrupting covalent bonds and impairing the integrity of the cell wall. Death rates of 7, 20, and 50 lmol l 1 nickel concentrations were all about 60% per hour when nickel was added to the culture. Cell permeability was assessed using the SYTOX Green nucleic acid dye, which can only enter cells with damaged cell walls.

On an industrial scale, using significant quantities of nickel to trigger cell lysis raises the expense of production process. An option to exploiting The photo-inducible lytic mechanism for nickel signalling was just recently developed & discovered in Synechocystis PCC6803. The photo-inducible cpcG2 promoter is used for Lytic genes that express holin and endolysin, while the J23116 promoter is used for antiholin in this system. To avoid premature lysis due by uninduced cpcG2 expression Antiholin was added with weak promoter. CcaS & CcaR are homologous two-component light responsive units that control cpcG2 expression, which is up-regulated by green light (550–600 nm) & down-regulated by red light (672 nm). Because no chemicals are administered and must be removed later, this approach holds more potential in terms of water recycling and economic viability. Light-induced lysis can protect the ecosystem by ensuring that

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any unintentionally released mutant strain cannot survive under sunlight. This method is viable because of the use of a green light filter over the culture pond or photo bioreactor.

It is also possible to enhance product release by genetically altering the fatty acid machinery, which would allow the fatty acids to be released directly into the medium (Fig. 2). Acetyl-CoA, a precursor to the Calvin cycle, is converted to free fatty acids in the plastids of cyanobacterial and microalgal cells (FFA). A mutant of Synechocystis sp. PCC6803 is that can emit FFA into the culture medium was created using a combination of gene regulation, deletion, and addition. SD277 is the result of six generations of mutants with gene deletions that redirect energy away from FFA synthesis, shift necessary substrates to other pathways, or impede excretion. ACC subunit overexpression and the inclusion of a codon-optimized tesA gene that produces thioesterase I (TE) enhance FFA production in this mutant. The periplasmic enzyme TE promotes the synthesis of fatty acids when the signal sequence peptide is absent, allowing FFA to be excreted into the medium.

The mutant expelled almost 80 times more FFA than the wild type is allowing for direct improvement of significantly more excreted FFA from the medium. The development rate of SD277 was reduced, and the ultimate cell density of the culture was one-third that of wild type. Nonetheless, like with previous modified Synechocystis sp. PCC6803 & S. elongatus sp. PCC7942 strains studied, the changed strain's health was substantially damaged.

Ethanol and b-phellandrene are two more transgenic microalgae compounds that have passively spread into the media. The ars1 sequence, which is an endogenous secretion signal, has also been described as a method for emission of remerging proteins produced in transgenic C. reinhardtii.

Conclusions

Techniques for growing cyanobacteria and microalgae need to be improved significantly, which might be aided through genetic engineering. The ability to modify various features of phototrophic organisms to improve their qualities has become simpler because to advances in genetic engineering technology. Denser cultures and reduced photoinhibition were the outcome of improved carbon fixation rates and more efficient light utilisation, which led to increased production. Generating flocculating properties in a chosen strain by genetic engineering can make harvesting easier and more effective without the need of pesticides or pH adjustments. The inducible auto-lysis system and direct product secretion are two examples of low-cost product extraction techniques that use genetically altered strains. It may be easier to use high-throughput methods like genomics, proteomics, and metabolomics now that newly sequenced genomes are readily available to identify new genes and pathways that might be used as genetic alteration targets in the future Newly identified targets can be altered to improve production performance and lower total costs. The production process usually includes growth, harvesting, and down-term processing.

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Using genetically modified strains with improved traits like growth or production raises environmental concerns because of the potential for escape into the surrounding ecosystem. Inducible microalgal strains with improved control and greater growth capacity may be a better alternative for mass production than traditional microalgae. To obtain better knowledge of environmental consequences of those genetically engineered strains more study is needed. The main barriers were to using genetically engineered photosynthetic organisms in the industrial setting are: I a lack of knowledge about stability, growth, & metabolism; (ii) expression of foreign genes frequently disrupts the native metabolism network, potentially causing competition for essential precursors and affecting the cells' overall health; and (iii) functional issues. As a result, research should concentrate on extending the database of transgenic photosynthetic organism designs. Future study should aim to combine two or more of effective uses described earlier into a single microalgae "super-strain" to maximise manufacturing procedure profitability.

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