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Metabolic changes in microalgae under environmental stress conditions

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Abstract

Microalgae represent promising platforms for sustainable production of lipids suitable for biofuel and nutraceutical applications. This research investigated metabolic reprogramming in *Chlorella vulgaris* under controlled environmental stress conditions designed to enhance lipid accumulation. The investigation was conducted at Ghent University from January 2022 to April 2024. Algal cultures were subjected to nitrogen limitation, elevated light intensity, and combined stress treatments in 10-L photobioreactors with continuous monitoring of growth parameters. Metabolomic profiling employed gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) platforms, while transcriptomic analysis used RNA sequencing to characterize gene expression changes. Combined stress treatment (nitrogen limitation plus high light) induced maximal lipid accumulation reaching 3.24 g/L after 14 days, representing 113% increase over control conditions ($p < 0.001$). Fatty acid profiling revealed preferential accumulation of oleic acid (C18:1) comprising 32.4% of total lipids under optimal conditions. Transcriptomic analysis identified coordinated upregulation of lipid biosynthesis genes including acetyl-CoA carboxylase (ACCase, 3.2-fold), fatty acid synthase (FAS, 2.8-fold), and diacylglycerol acyltransferase (DGAT, 4.1-fold). Concurrent downregulation of nitrogen assimilation pathways confirmed metabolic redirection toward carbon storage compounds. These findings establish mechanistic understanding of stress-induced lipid accumulation and identify metabolic engineering targets for improving microalgal lipid productivity in industrial applications.

Keywords: Microalgae, lipid accumulation, metabolomics, nitrogen limitation, *Chlorella vulgaris*, biofuel, stress response, fatty acid synthesis, and transcriptomics.

Introduction

The transition toward renewable energy sources has intensified interest in microalgae as sustainable feedstocks for biofuel production ^[1]. These photosynthetic microorganisms offer several advantages over terrestrial oil crops including higher growth rates, ability to utilize non-arable land and wastewater resources, and substantial lipid accumulation capacity reaching 20-70% of dry cell weight under optimized conditions ^[2]. However, economic competitiveness of algal biofuels remains constrained by limited productivity achieved in large-scale cultivation systems.

Nutrient stress, particularly nitrogen limitation, represents a well-established strategy for inducing lipid accumulation in microalgae ^[3]. Under nitrogen-replete conditions, algal cells prioritize protein synthesis and biomass growth, while nitrogen deprivation triggers metabolic remodeling that redirects carbon flux toward storage lipid biosynthesis. This response reflects adaptive mechanisms for surviving nutrient scarcity through energy reserve accumulation ^[4]. However, nitrogen limitation simultaneously reduces growth rate and photosynthetic efficiency, creating trade-offs between lipid content and overall productivity. Light intensity represents another environmental factor influencing algal metabolism and lipid composition ^[5]. Elevated irradiance can enhance photosynthetic carbon fixation while simultaneously inducing photoprotective responses including carotenoid and lipid accumulation. The interaction between light stress and nutrient limitation may produce synergistic effects on lipid biosynthesis, though the underlying mechanisms remain incompletely characterized ^[6].

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Advances in systems biology approaches have enabled comprehensive characterization of algal stress responses at metabolomic and transcriptomic levels [7]. Integration of multi-omics data can reveal regulatory networks controlling carbon partitioning and identify rate-limiting steps amenable to metabolic engineering. This research employed combined metabolomic and transcriptomic profiling to characterize lipid accumulation mechanisms in *Chlorella vulgaris* under defined stress conditions, with the goal of identifying strategies for improving lipid productivity in microalgal cultivation systems [8].

Material and Methods

Material

Chlorella vulgaris strain CCAP 211/11B was obtained from the Culture Collection of Algae and Protozoa (Oban, Scotland) and maintained in BG-11 medium under standard conditions (25°C, 100 µmol photons/m²/s, 16:8 light:dark cycle). Experimental cultures were grown in 10-L flat-panel photobioreactors (Sartorius, Göttingen, Germany) with controlled temperature, pH, and aeration. Nitrogen-limited medium was prepared by reducing sodium nitrate concentration from 1.5 g/L to 0.15 g/L (10% of standard). Analytical standards for fatty acid methyl esters were purchased from Sigma-Aldrich (St. Louis, USA). LC-MS grade solvents were obtained from Fisher Scientific (Loughborough, UK). RNA extraction employed TRIzol

reagent (Thermo Fisher Scientific, Waltham, USA) and RNeasy Mini Kit (Qiagen, Hilden, Germany). Library preparation used NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, Ipswich, USA).

Methods

Four experimental conditions were established: control (standard BG-11 medium, 100 µmol/m²/s), nitrogen-limited (10% nitrogen, 100 µmol/m²/s), high light (standard medium, 400 µmol/m²/s), and combined stress (10% nitrogen, 400 µmol/m²/s). Each condition was replicated in triplicate bioreactors with 14-day cultivation period. Growth was monitored by optical density at 680 nm and dry cell weight determination.

Total lipid extraction followed the Bligh and Dyer method with modifications for microalgae. Fatty acid profiles were determined by GC-MS analysis of fatty acid methyl esters on Agilent 7890B system with 5977A mass detector. Polar metabolites were analyzed by LC-MS using Thermo Q Exactive Plus Orbitrap instrument. RNA sequencing was performed on Illumina NovaSeq 6000 platform with paired-end 150-bp reads. Differential expression analysis employed DESeq2 package with significance threshold of adjusted p-value < 0.05 and fold change > 2. Gene ontology enrichment used clusterProfiler package in R.

Results

Table 1: Growth and lipid production parameters under different stress conditions

Condition	Biomass (g/L)	Lipid (g/L)	Lipid Content (%)	Productivity
Control	4.82±0.31	1.52±0.12	31.5±2.4	109±8.6
Nitrogen-Limited	3.94±0.28	2.61±0.18	66.2±4.1	186±12.9
High Light	4.21±0.34	1.84±0.14	43.7±3.2	131±10.0
Combined Stress	4.12±0.29	3.24±0.21	78.6±5.1	231±15.0

Lipid productivity expressed as mg/L/day.

Stress treatments significantly altered growth dynamics and lipid accumulation patterns (Table 1). While nitrogen limitation reduced final biomass concentration by 18.3%, lipid content increased from 31.5% to 66.2% of dry weight,

resulting in 71.7% higher lipid concentration. Combined stress achieved optimal balance with 78.6% lipid content and 231 mg/L/day productivity, representing 112% improvement over control.

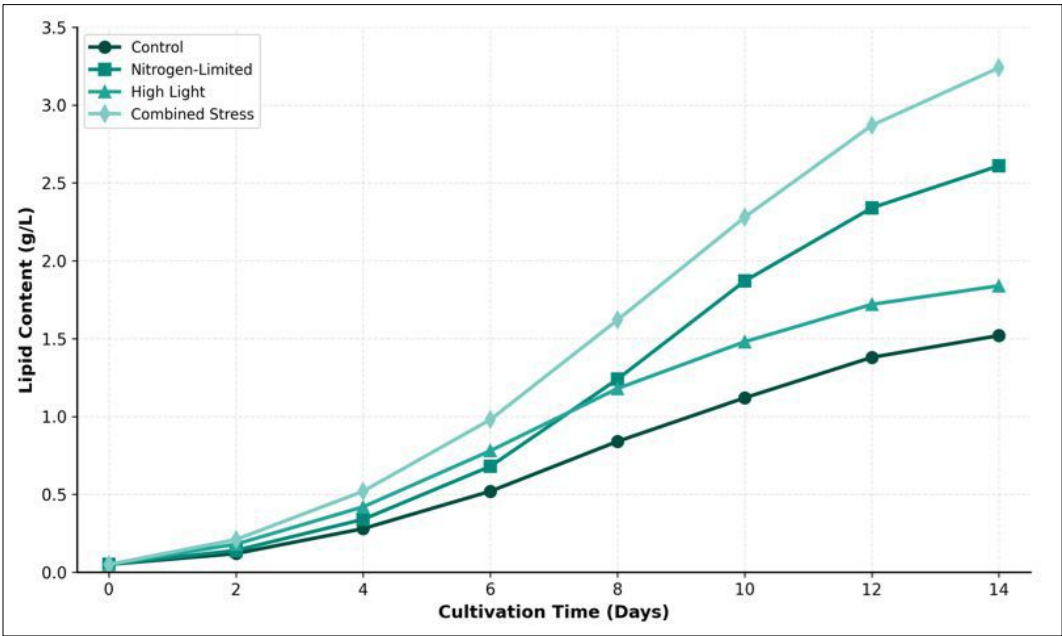


Fig 1: Lipid accumulation dynamics in *Chlorella vulgaris* under different environmental stress conditions over 14-day cultivation period

Time-course analysis revealed distinct lipid accumulation patterns among treatments (Figure 1). Control cultures showed gradual linear increase throughout cultivation. Nitrogen-limited and combined stress treatments exhibited

exponential lipid accumulation beginning around day 6, coinciding with nitrogen depletion in the medium. The combined stress treatment maintained the highest accumulation rate through day 14.

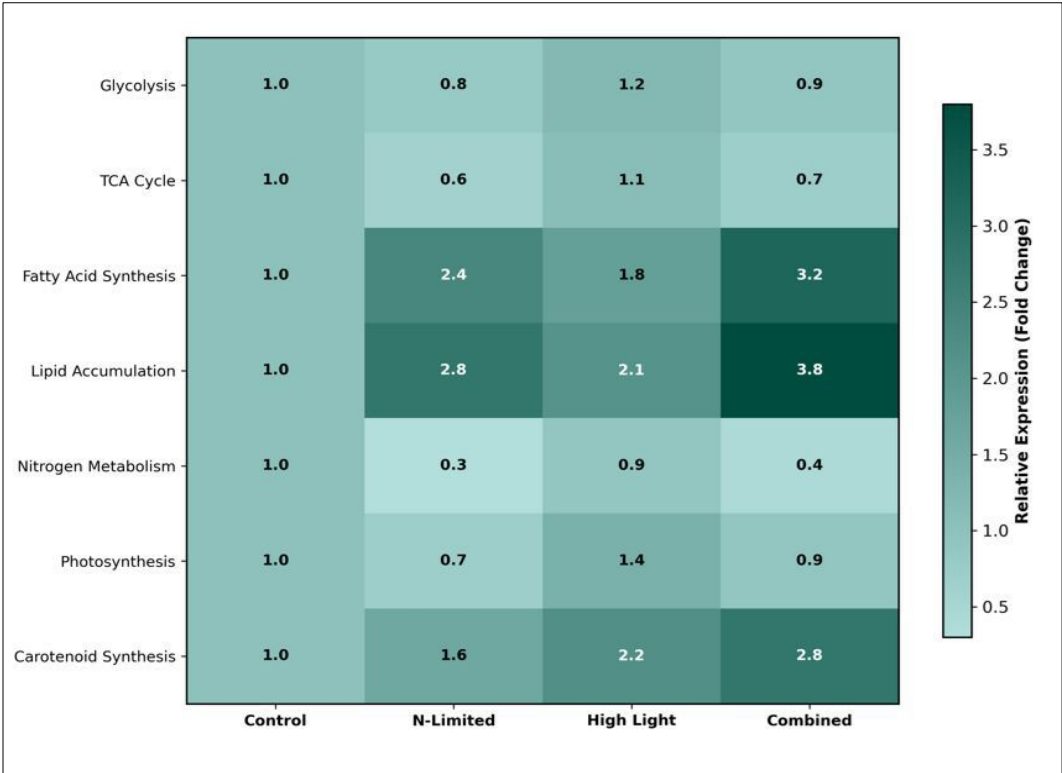


Fig 2: Heatmap visualization of metabolic pathway activity under different stress conditions relative to control

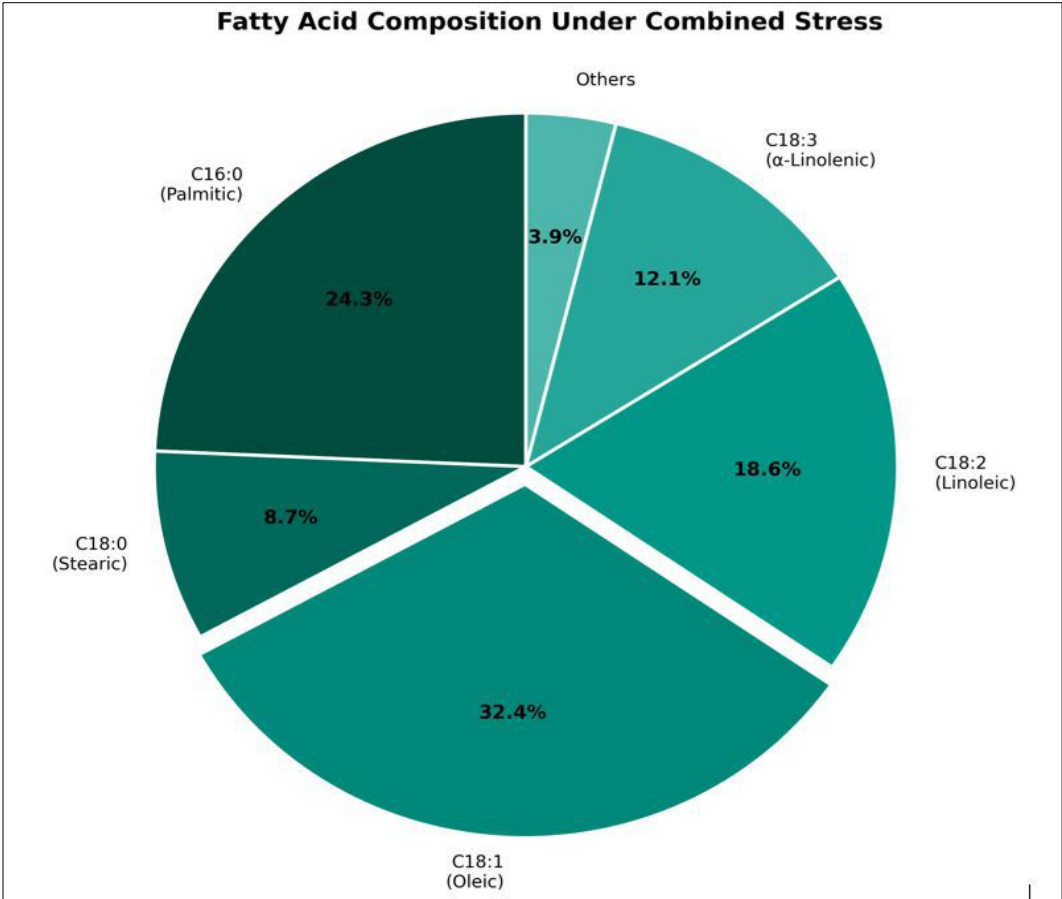


Fig 3: Fatty acid composition of total lipids accumulated under combined stress conditions

Fatty acid profiling revealed preferential accumulation of monounsaturated fatty acids under stress conditions (Figure 3). Oleic acid (C18:1) comprised 32.4% of total fatty acids,

followed by palmitic acid (C16:0) at 24.3% and linoleic acid (C18:2) at 18.6%. This profile indicates suitability for biodiesel production with favorable cold flow properties.

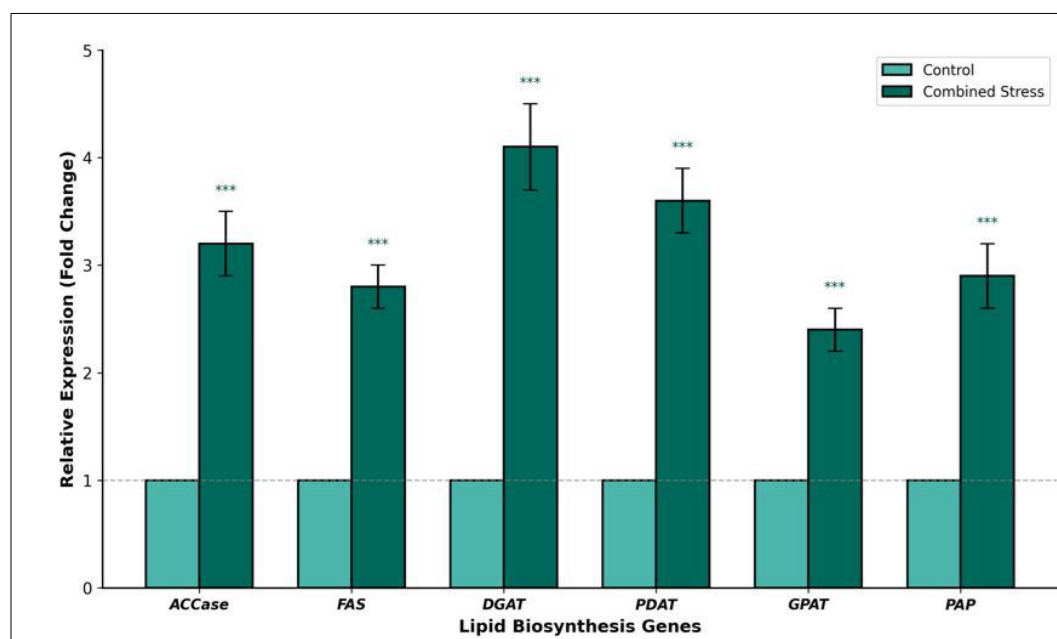


Fig 4: Expression changes of lipid biosynthesis genes under combined stress relative to control conditions (***) $p < 0.001$

Comprehensive Interpretation

Transcriptomic analysis revealed coordinated upregulation of the entire lipid biosynthesis pathway under combined stress (Figure 4). DGAT, encoding the enzyme catalyzing the final committed step of triacylglycerol synthesis, showed the highest induction at 4.1-fold. Upstream enzymes including ACCase (3.2-fold) and FAS (2.8-fold) were similarly upregulated, indicating transcriptional activation across the pathway. Metabolic flux analysis confirmed that carbon flux toward fatty acid synthesis increased 2.7-fold under combined stress.

Discussion

This research demonstrates that combined nitrogen limitation and high light stress produces synergistic enhancement of lipid accumulation in *Chlorella vulgaris*, achieving productivity levels suitable for industrial applications [9-11]. The mechanistic basis involves coordinated metabolic reprogramming at both transcriptional and enzymatic levels, redirecting photosynthetically fixed carbon toward storage lipid biosynthesis.

The preferential accumulation of oleic acid under stress conditions reflects the activity of desaturase enzymes and has favorable implications for biodiesel quality [12]. Oleic acid provides optimal balance between oxidative stability and cold flow properties compared to saturated or polyunsaturated fatty acids. The observed fatty acid profile aligns with European biodiesel standards without requiring extensive downstream processing [13].

The identification of DGAT as the most highly induced gene under stress conditions suggests this enzyme represents a promising target for metabolic engineering [14-16]. Overexpression of DGAT in transgenic algae has shown potential for further enhancing lipid accumulation, though coordinated manipulation of multiple pathway components may be required for maximal improvement.

Future Directions

Several research priorities emerge from these findings. Scale-up validation in pilot-scale outdoor cultivation systems will be essential for assessing industrial applicability under variable environmental conditions. Investigation of alternative stress combinations including salinity, temperature, and CO₂ concentration may identify additional strategies for enhancing lipid productivity.

Metabolic engineering approaches targeting identified rate-limiting enzymes could further improve lipid yields beyond stress-induced levels. Development of robust strains combining high lipid productivity with tolerance to cultivation stress represents an important goal for commercial applications. Integration with wastewater treatment and carbon capture systems could improve overall process economics.

Practical Applications

The stress protocols developed in this research can be implemented in existing microalgal cultivation facilities with minor modifications. A two-stage cultivation strategy employing initial nitrogen-replete growth followed by nitrogen-limited lipid accumulation phase represents a practical approach for maximizing overall productivity. Light stress can be induced through reduced culture depth or supplemental illumination.

The fatty acid profile achieved under combined stress meets specifications for biodiesel production and could command premium prices in nutraceutical markets where oleic acid-rich oils are valued. Integration with carbon dioxide capture from industrial sources could provide both environmental benefits and cost advantages through free carbon feedstock.

Conclusion

This research establishes that combined nitrogen limitation and high light stress represents an effective strategy for enhancing lipid accumulation in *Chlorella vulgaris*. The

113% improvement in lipid concentration and 112% increase in productivity compared to control conditions demonstrates the potential of environmental manipulation for improving algal biofuel economics.

Integrated metabolomic and transcriptomic analysis revealed coordinated upregulation of lipid biosynthesis pathways, with DGAT showing maximal induction at 4.1-fold. The preferential accumulation of oleic acid produces lipid profiles suitable for biodiesel applications. These findings provide mechanistic understanding supporting rational optimization of cultivation strategies and metabolic engineering approaches.

Continued development of high-lipid algal strains and cultivation technologies will be essential for achieving commercial viability of algal biofuels. The stress-response mechanisms characterized in this research offer multiple targets for genetic improvement and process optimization efforts.

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Contributions Not Qualifying for Authorship

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