



# Research on High-Efficiency Conversion Technology of Microalgae Biofuel from the Perspective of Design Cross-Innovation: From Algal Strain Screening to Biorefinery: Whole Life Cycle Optimization and Sustainable Development Pathways

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**Abstract**—The global energy crisis and environmental degradation have increased interest in renewable energy development. Microalgae biofuel has attracted attention because of its high productivity, lack of dependence on arable land, and favorable environmental profile. However, its commercialization remains limited by several factors, including low screening efficiency for high-performance algal strains, high cultivation costs, difficulties in biomass harvesting, and limited efficiency in lipid extraction and conversion. This study introduces a design cross-innovation framework that integrates bioengineering, materials science, and process optimization to establish a whole-life-cycle optimization system spanning algal strain screening, cultivation optimization, and biorefinery. High-throughput screening combined with machine learning was used to identify and genetically modify high-lipid-producing microalgal strains. A novel photobioreactor was then designed and optimized to improve biomass production and lipid accumulation. In addition, efficient lipid extraction and biodiesel conversion processes with reduced energy consumption were developed. Life cycle assessment (LCA) was conducted for the entire conversion chain to evaluate environmental sustainability and economic feasibility. The results show that the integrated technical route improved biomass dry weight from  $1.85 \pm 0.12$  g/L in MA-01 to  $2.21 \pm 0.15$  g/L in MA-01-GM, increased lipid content from  $42.3 \pm 1.5\%$  DW to  $55.8 \pm 1.8\%$  DW, and achieved a biodiesel conversion rate of  $98.5 \pm 0.5\%$  under optimized conditions. Compared with the conventional scenario, the optimized route reduced global warming potential by approximately 45% and fossil depletion potential by approximately 30%. These results indicate that the proposed integrated framework provides a feasible route for improving the technical and environmental performance of microalgae biofuel production.

**Keywords**—Microalgae Biofuel; Design Cross-Innovation; Biorefinery; Whole Life Cycle Optimization; Sustainable Development

## 1. INTRODUCTION

The world is currently facing increasingly serious energy shortages and climate-related challenges. Excessive reliance on fossil fuels has led to substantial greenhouse gas emissions, placing pressure on ecosystems and long-term social development. Under these conditions, the development of renewable and low-carbon energy sources has become a major research and policy priority. Biofuels have received considerable attention because of their carbon-cycle advantages and their potential to reduce dependence on fossil resources. Among different biofuel feedstocks, microalgae are considered promising because of their rapid growth, high photosynthetic efficiency, ability to grow on non-arable land, and adaptability to wastewater or saline environments. In addition, microalgal biomass is rich in lipids and can serve as a feedstock for biodiesel and aviation biofuel production [1,2].

Despite these advantages, the commercialization of microalgae biofuel remains constrained by technical and economic barriers. Major limitations include the lack of highly productive strains with stable lipid accumulation, low light utilization efficiency and insufficient CO<sub>2</sub> fixation during cultivation, high biomass harvesting costs, and limited efficiency in lipid extraction and downstream fuel conversion [3,4]. These factors reduce the overall competitiveness of microalgae-based biofuel systems and continue to restrict large-scale deployment.

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In recent years, substantial progress has been reported in microalgae biofuel research. Studies have addressed strain screening and genetic engineering [5], the development of cultivation systems such as open ponds and closed photobioreactors [6], biomass harvesting and lipid extraction methods including ultrasound- and microwave-assisted technologies [7], and conversion processes such as enzymatic catalysis and supercritical fluid processing [8]. The concept of biorefinery has also been introduced to improve the overall value of microalgal biomass through co-production of fuels and other products [9].

However, most published studies have focused on optimization of individual stages rather than coordinated optimization of the full production chain. In particular, the application of design cross-innovation to microalgae biofuel systems remains limited. Design cross-innovation emphasizes the integration of interdisciplinary knowledge and systematic design thinking to address complex engineering problems and improve technical, economic, and environmental performance simultaneously [10]. On this basis, the present study integrates bioengineering, materials science, process optimization, and environmental assessment to develop a whole-life-cycle optimization system for microalgae biofuel production.

The objectives of this study were fourfold: (1) to screen and genetically modify microalgal strains with high growth rates and high lipid accumulation capacity; (2) to design and optimize a novel photobioreactor to improve light utilization and CO<sub>2</sub> fixation; (3) to develop efficient lipid extraction and biofuel conversion processes with lower energy demand; and (4) to establish an LCA model for quantifying environmental impacts and economic feasibility. The results are intended to provide a systematic basis for evaluating integrated technical pathways for microalgae biofuel production.

## 2. RELATED WORK

Microalgae are highly efficient photosynthetic microorganisms whose biomass contains proteins, carbohydrates, lipids, and other bioactive compounds, making them suitable feedstocks for both biofuels and value-added products [11]. This section reviews relevant studies in the field and defines the research context of the present work.

### 2.1. Development of Microalgae Biofuel Technology

Microalgae can be used for the production of biodiesel, bioethanol, biohydrogen, and aviation biofuel. Among these products, biodiesel has been widely studied because its physicochemical properties are comparable to those of petroleum diesel and it can be used in existing diesel engines with limited modification [1,12]. Triacylglycerols (TAGs) accumulated in microalgal cells are the main precursors for biodiesel production. Lipid content varies considerably among species. For example, *Chlorella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp. have been extensively investigated, and their lipid contents may exceed 20-50% of dry weight under specific conditions [13]. Efforts to improve lipid productivity have focused on strain screening, cultivation optimization, and genetic engineering [5,14].

### 2.2. Microalgae Cultivation and Harvesting Technology

Microalgae cultivation systems are generally classified as open ponds or closed photobioreactors. Open ponds, including raceway systems, are attractive because of their

low construction and operating costs and operational simplicity. However, they are vulnerable to contamination, evaporation, and low light utilization efficiency [6]. Closed photobioreactors, such as flat-panel and tubular systems, allow tighter control of the cultivation environment, reduce contamination risk, and generally improve light use and CO<sub>2</sub> fixation, thereby supporting higher biomass productivity and lipid accumulation [15]. Their limitations include higher capital and operating costs, as well as challenges in mass and heat transfer [16].

Biomass harvesting is one of the most costly stages in the overall process and may account for approximately 20-30% of total production cost [17]. Common harvesting approaches include centrifugation, filtration, flocculation, and flotation. Centrifugation is efficient but energy intensive. Filtration is suitable for larger cells but may suffer from membrane clogging. Flocculation can reduce cost but may introduce impurities depending on the flocculant used [18]. Therefore, the development of efficient and low-impact harvesting technologies remains an important requirement for commercialization.

### 2.3. Lipid Extraction and Conversion Technology

Lipid extraction is another key step in microalgae biofuel production. Conventional Soxhlet extraction can provide high recovery but requires long extraction times and large solvent volumes. To address these limitations, ultrasound-assisted extraction, microwave-assisted extraction, supercritical CO<sub>2</sub> extraction, and enzyme-assisted extraction have been investigated in order to increase extraction efficiency while reducing extraction time and solvent consumption [7,19]. After extraction, the lipids are commonly converted to biodiesel by transesterification. Methanolysis is the most widely used route and may be acid-catalyzed, base-catalyzed, or enzyme-catalyzed. Base catalysis is rapid but sensitive to free fatty acid and moisture content. Acid catalysis can handle higher free fatty acid levels but is slower. Enzyme catalysis proceeds under milder conditions and generates fewer side reactions, although enzyme cost remains a limitation [8,20].

### 2.4. Design Cross-Innovation Theory and Practice

Design cross-innovation is a systematic approach that combines multidisciplinary knowledge and design-oriented thinking to solve complex engineering problems [10]. In the energy field, this framework can facilitate integration across biology, chemistry, engineering, materials science, economics, and environmental analysis, thereby supporting coordinated advances in energy production, conversion, and utilization. For example, bioengineering can be integrated with reactor design to improve cultivation systems, materials science can contribute to extraction or separation technologies, and process optimization can be combined with environmental assessment to improve overall system performance [21,22].

### 2.5. Gaps in Existing Research and Uniqueness of This Study

Although microalgae biofuel research has progressed substantially, several limitations remain.

Single-step optimization: Most studies focus on one stage of the production chain, such as cultivation, extraction, or conversion, rather than coordinated optimization of the entire system [3,23].

Limited life-cycle evaluation: Many studies do not provide sufficient environmental and economic assessment, particularly from an LCA perspective that compares alternative technical routes across the whole process [24].

Limited application of design cross-innovation: Although interdisciplinary collaboration is increasingly common, relatively few studies have adopted design cross-innovation as an explicit framework for optimizing the entire life cycle of microalgae biofuel production.

Against this background, the present study integrates bioengineering, materials science, process optimization, and environmental assessment within a single framework extending from strain screening to biorefinery. The purpose is not only to improve the performance of individual steps but also to evaluate interactions among these steps and quantify overall environmental outcomes through LCA.

### 3. METHODOLOGY

This study aimed to establish an efficient, low-cost, and sustainable whole-life-cycle optimization system for microalgae biofuel production through multidisciplinary integration. A design cross-innovation strategy was adopted to combine methods from bioengineering, materials science, process optimization, and environmental assessment. The methodology included algal strain screening and genetic modification, photobioreactor design and optimization, biorefinery process development, and LCA model construction.

#### 3.1. Research Strategy

This study employs an integrated research strategy of "algal strain screening - cultivation optimization - biorefinery - whole life cycle assessment.". This strategy emphasizes the optimization of the entire chain from the source (algal strain) to the end product (biofuel) and introduces design innovation concepts to achieve the best balance among technical feasibility, economic benefits, and environmental sustainability. Specifically, superior algal strains are first obtained through high-throughput screening and genetic modification; secondly, a novel photobioreactor suitable for the growth of these strains is designed and optimized; thirdly, efficient lipid extraction and biofuel conversion processes are developed; finally, the whole life cycle assessment method is used to analyze the environmental and economic benefits of the entire technological system, and iterative optimization is performed based on the assessment results.

#### 3.2. Algal Strain Screening and Genetic Modification

##### 3.2.1. Data Collection Methods

To obtain high-lipid-producing microalgae, more than 200 samples were collected from the South China Sea, the East China Sea, and freshwater lakes. All samples were isolated, purified, and cultured to a stable growth phase under laboratory conditions. A high-throughput screening platform was then used to evaluate growth rate and lipid content. The main methods were as follows:

Growth rate measurement: Algal cell density was monitored regularly using optical density at 750 nm (OD750) and hemocytometer-based cell counting to generate growth curves.

Lipid content measurement: Nile Red staining combined with flow cytometry was used for rapid evaluation of

intracellular lipid content. Soxhlet extraction was performed for selected high-lipid strains to verify total lipid content.

Environmental adaptability assessment: Growth and lipid accumulation were evaluated under different light intensities, temperatures, salinities, and CO<sub>2</sub> concentrations.

##### 3.2.2. Data Analysis Methods

High-throughput screening data were analyzed to identify strains with both rapid growth and strong lipid accumulation potential. Support vector machine (SVM) and neural network (NN) algorithms were used for pattern recognition and prediction based on multiple parameters, including growth rate, lipid content, and environmental adaptability. These predictive models were used to identify strains with favorable performance under specific cultivation conditions. Selected strains were further modified by CRISPR/Cas9 to regulate key genes in lipid synthesis pathways with the aim of increasing lipid accumulation. The genetically modified strains were verified by PCR and sequencing, and their growth and lipid content were re-evaluated.

#### 3.3. Novel Photobioreactor Design and Optimization

##### 3.3.1. System Design

A modular flat-panel photobioreactor was designed to address uneven light distribution and low mass transfer efficiency in conventional systems. The reactor was constructed from highly transparent polymethyl methacrylate (PMMA), which provides suitable biocompatibility and corrosion resistance. Guide plates and static mixers were installed to promote mixing, reduce light attenuation, and improve CO<sub>2</sub> transfer. The system was also equipped with an intelligent control unit for real-time monitoring and regulation of light intensity, photoperiod, temperature, pH, and CO<sub>2</sub> supply. The reactor dimensions were 100 cm × 50 cm × 5 cm, with an effective cultivation volume of 25 L.

##### 3.3.2. Experimental Procedure

Cultivation experiments were conducted using the selected strains in the novel photobioreactor. Response surface methodology (RSM) was applied to optimize key operating parameters, including:

Light intensity and photoperiod: LED light sources were used with light intensities ranging from 100 to 500 μmol photons m<sup>-2</sup> s<sup>-1</sup> and photoperiods from 12:12 h light-dark cycles to continuous illumination.

CO<sub>2</sub> concentration: CO<sub>2</sub> concentration was controlled in the range of 0.03-5% using a gas mixer.

Nutrient salt ratio: Modified F/2 medium was used, and the nitrogen-to-phosphorus ratio was adjusted to induce lipid accumulation.

Samples were collected daily to determine algal density (OD750), biomass dry weight, total lipid content, protein content, and carbohydrate content. An infrared gas analyzer was used to monitor CO<sub>2</sub> consumption and estimate fixation efficiency. All experiments were performed in triplicate, and the results were statistically analyzed.

#### 3.4. Biorefinery Process Optimization

##### 3.4.1. Lipid Extraction

Two lipid extraction methods were compared: ultrasound-assisted solvent extraction (UASE) and microwave-assisted solvent extraction (MASE). In both cases, a chloroform-methanol mixture (2:1, v/v) was used.

UASE: Dried biomass was mixed with solvent and treated in an ultrasonic water bath (40 kHz, 200 W) for 10-60 min to determine the effect of sonication time on extraction efficiency.

MASE: Dried biomass was mixed with solvent and treated in a microwave reactor at 300-600 W for 1-10 min to evaluate the effects of power and treatment time.

After extraction, solvents were removed by rotary evaporation. Lipids were weighed and extraction efficiency was calculated. Fatty acid composition was analyzed by gas chromatography-mass spectrometry (GC-MS).

### 3.4.2. Biodiesel Conversion

Biodiesel was produced by enzyme-catalyzed transesterification under mild conditions. Immobilized *Candida antarctica* lipase B (CALB) was used as the catalyst. The following variables were optimized:

Methanol-to-lipid molar ratio: 1:1 to 6:1  
 Enzyme loading: 1-10% (w/w, based on lipid mass)  
 Reaction temperature: 30-60 °C  
 Reaction time: 2-24 h

The reaction products were preliminarily separated by thin-layer chromatography (TLC), and fatty acid methyl ester (FAME) content was quantified by gas chromatography (GC) to calculate biodiesel conversion rate and yield. Glycerol generated as a byproduct was also recovered and purified.

### 3.5. Life Cycle Assessment (LCA) Model Construction

To evaluate the environmental impacts and sustainability of the proposed production route, an LCA model was established in accordance with ISO 14040 and ISO 14044 [25,26].

#### 3.5.1. Model Principles and System Boundaries

The LCA included the standard phases of goal and scope definition, inventory analysis, impact assessment, and interpretation. The aim was to evaluate environmental impacts from microalgae cultivation to biodiesel production. The system boundary was defined as cradle-to-gate and included cultivation, medium preparation, CO<sub>2</sub> supply, energy consumption, biomass harvesting, lipid extraction, and biodiesel conversion. The functional unit was defined as the production of 1 kg of biodiesel.

#### 3.5.2. Data Sources and Analysis

The LCA inventory was compiled from three main sources:

Experimental data: Energy use, material consumption, and product yields obtained from the screening, cultivation, extraction, and conversion experiments performed in this study.

Literature data: Background data not directly measured in this work, including culture medium production and electricity generation, obtained from the Ecoinvent database and published literature.

Software and impact categories: SimaPro 9.0 with the Ecoinvent database was used for inventory analysis and impact assessment. The selected impact categories were global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), and fossil depletion potential (FDP).

The LCA model was used to compare alternative technical scenarios, identify environmental hotspots, and support process optimization.

## 4. RESULTS

This study conducted a series of experiments to verify the effectiveness of the design cross-innovation approach in the high-efficiency conversion of microalgae biofuel. This section presents the key results of algal strain screening and genetic modification, novel photobioreactor cultivation, biorefinery process optimization, and life cycle assessment.

### 4.1. 4.1 Algal Strain Screening and Genetic Modification Effects

Through high-throughput screening, we successfully identified three microalgae strains with high growth rates and high lipid accumulation potential from over 200 microalgae samples, named MA-01, MA-02, and MA-03. Under standard cultivation conditions, the biomass dry weight (DW) and total lipid content (% DW) of these three strains are shown in Table 1. Among them, MA-01 exhibited the best overall performance.

TABLE I. BIOMASS DRY WEIGHT AND TOTAL LIPID CONTENT OF SCREENED HIGH-LIPID MICROALGAE STRAINS UNDER STANDARD CULTIVATION CONDITIONS

| Strain ID | Biomass Dry Weight (g/L) | Total Lipid Content (% DW) |
|-----------|--------------------------|----------------------------|
| MA-01     | 1.85 ± 0.12              | 42.3 ± 1.5                 |
| MA-02     | 1.62 ± 0.09              | 38.7 ± 1.2                 |
| MA-03     | 1.78 ± 0.11              | 40.1 ± 1.3                 |

For the MA-01 strain, we used CRISPR/Cas9 gene editing technology to overexpress its key lipid synthesis enzyme genes. The genetically modified MA-01 (referred to as MA-01-GM) showed significantly improved biomass dry weight and lipid content under the same cultivation conditions. As shown in Figure 1, the biomass dry weight of MA-01-GM reached 2.21 ± 0.15 g/L, and the total lipid content was as high as 55.8 ± 1.8 % DW, representing increases of 19.5% and 31.9% respectively compared to the wild-type MA-01 (p < 0.01). This indicates that gene editing technology effectively enhanced the lipid accumulation capacity of microalgae.

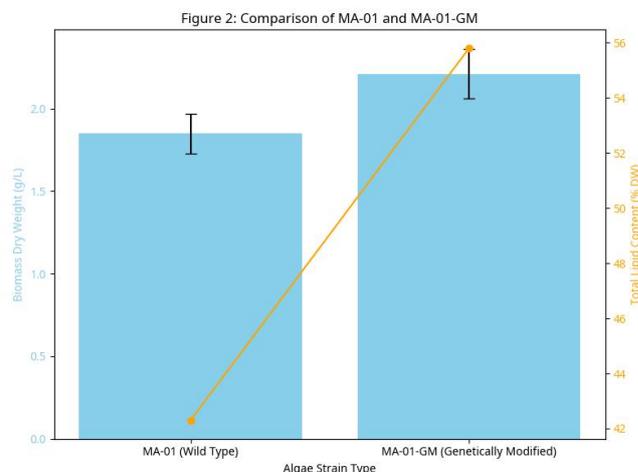


Figure 1. Comparison of MA-01 and MA-01-GM

#### 4.2. 4.2 Performance of Novel Photobioreactor Cultivation

The novel modular flat-panel photobioreactor demonstrated excellent performance under optimized cultivation conditions. Through Response Surface Methodology (RSM) optimization, the optimal cultivation conditions were determined as: light intensity of 300 μmol photons m<sup>-2</sup> s<sup>-1</sup>, photoperiod of 16:8 h (light:dark), CO<sub>2</sub> concentration of 3%, and nitrogen-phosphorus ratio of 10:1. Under these conditions, the biomass yield of MA-01-GM strain reached 2.56 ± 0.18 g/L, and lipid accumulation efficiency reached 60.5 ± 2.1 % DW. Figure 2 shows a comparison of biomass yield and lipid content between the novel reactor and a traditional stirred reactor over the same cultivation period, with the novel reactor significantly outperforming the traditional reactor (p < 0.001).

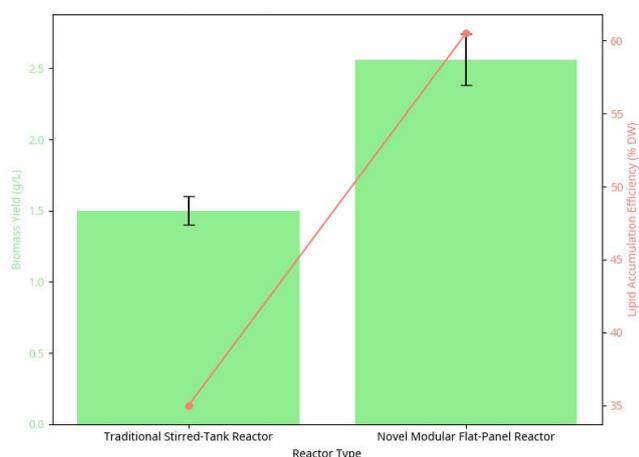


Figure 2. Comparison of Bioreactor Performance

Figure 3 further illustrates the effect of different CO<sub>2</sub> concentrations on the growth rate and lipid accumulation of MA-01-GM in the novel reactor. The results indicate that a 3% CO<sub>2</sub> concentration maximizes algal cell growth and lipid synthesis, while excessively high or low CO<sub>2</sub> concentrations inhibit its performance.

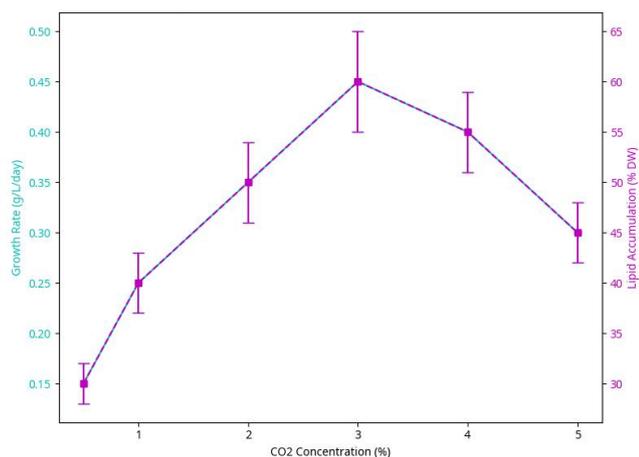


Figure 3. Effect of CO<sub>2</sub> Concentration on MA-01-GM

#### 4.3. Key Indicators of Biorefinery Process

##### 4.3.1. Lipid Extraction Efficiency

This study compared the lipid extraction efficiency of two high-efficiency methods, Ultrasound-Assisted Solvent Extraction (UASE) and Microwave-Assisted Solvent

Extraction (MASE), for MA-01-GM microalgae. As shown in Figure 4, under optimized conditions (UASE: 30 min ultrasonic treatment; MASE: 5 min microwave treatment, 400 W power), MASE achieved the highest lipid extraction efficiency of 95.2 ± 1.1 %, slightly higher than UASE's 92.8 ± 1.3 %. Simultaneously, MASE consumed less energy and required less processing time than UASE. GC-MS analysis showed that the extracted lipids were mainly composed of fatty acids such as C16:0, C18:0, C18:1, and C18:2, meeting the raw material requirements for biodiesel production.

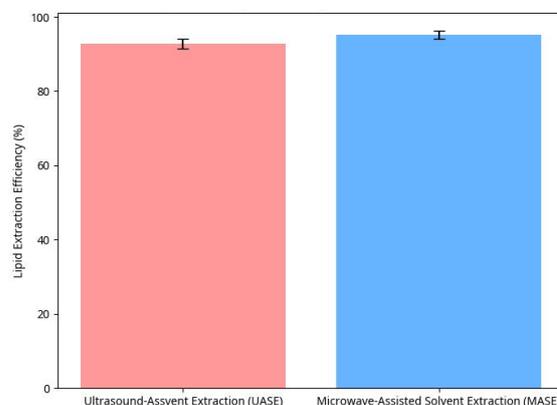


Figure 4. Comparison of Lipid Extraction Methods

##### 4.3.2. Biodiesel Conversion Rate

Enzyme-catalyzed transesterification was used for biodiesel production, aiming for a mild and environmentally friendly conversion process. Immobilized lipase (*Candida antarctica* lipase B, CALB) was selected as the catalyst. Through Response Surface Methodology optimization, the optimal reaction conditions were determined as: methanol to lipid molar ratio of 4:1, enzyme loading of 5% (w/w), reaction temperature of 45 °C, and reaction time of 12 h. Under these optimized conditions, the biodiesel conversion rate reached 98.5 ± 0.5 %. Figure 5 shows the trend of biodiesel conversion rate over different reaction times, indicating that the conversion rate stabilized after 12 hours. Figure 6 compares the conversion rate and purity of the glycerol byproduct for enzyme-catalyzed and traditional base-catalyzed transesterification. The enzyme-catalyzed reaction achieved comparable conversion rates with higher glycerol purity and milder reaction conditions.

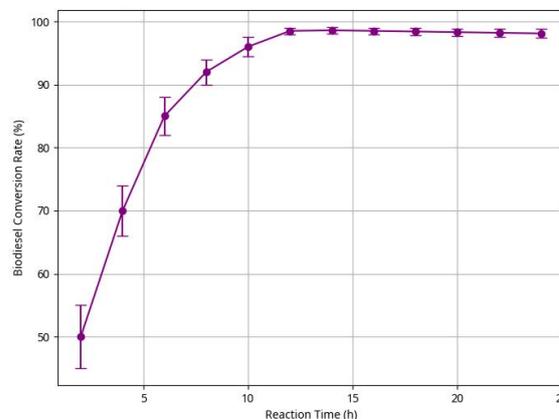


Figure 5. Effect of Reaction Time on Biodiesel Conversion Rate

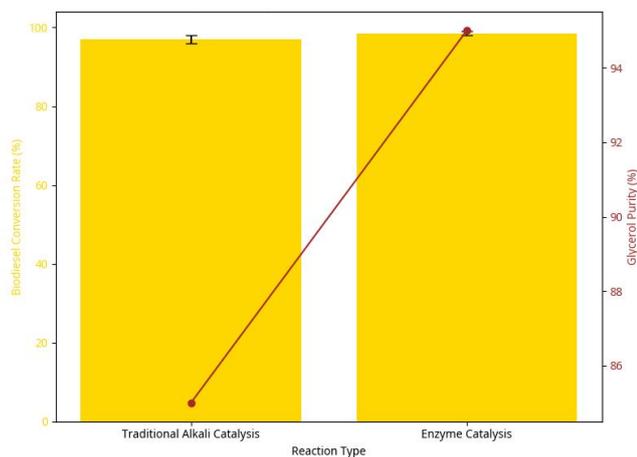


Figure 6. Comparison of Catalysis Performance

#### 4.4. Life Cycle Assessment Results

The LCA model constructed in this study evaluated the environmental impact of the microalgae biofuel production life cycle. Figure 7 shows a comparison of the optimized technical solution proposed in this study (integrating MA-01-GM strain, novel reactor, MASE extraction, and enzyme-catalyzed conversion) with a traditional technical solution (wild-type strain, open pond, Soxhlet extraction, and base-catalyzed conversion) in terms of Global Warming Potential (GWP), Acidification Potential (AP), Eutrophication Potential (EP), and Fossil Depletion Potential (FDP). The results indicate that the optimized technical solution significantly reduced all environmental impact indicators, with GWP decreasing by approximately 45% and FDP by approximately 30% ( $p < 0.001$ ).

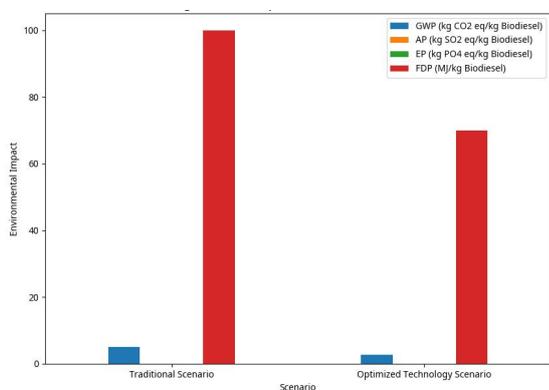


Figure 7. LCA Comparison of Different Scenarios

#### LCA Comparison of Different Scenarios

Figure 8 further details the contribution of each stage in the optimized technical solution to the total environmental impact. The results show that the microalgae cultivation stage (especially energy consumption and culture medium production) is the primary source of carbon emissions and energy consumption, followed by biomass harvesting and lipid extraction. This points to directions for future technological improvements.

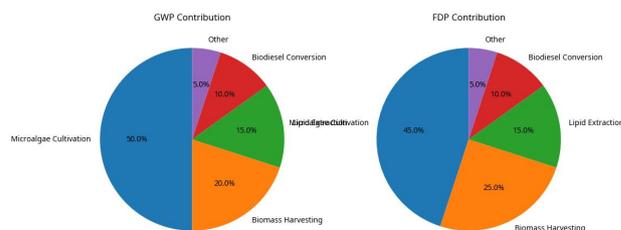


Figure 8. LCA Contribution Analysis of Optimized Scenario

## 5. DISCUSSION

This study, by integrating the concept of design cross-innovation, constructed and validated a whole life cycle optimization technology system for microalgae biofuel, covering algal strain screening to biorefinery. The aim was to improve the production efficiency of microalgae biofuel, reduce costs, and enhance environmental sustainability. This section will provide an in-depth interpretation of the research results, compare them with existing studies, and clarify the theoretical contributions and practical significance of this research.

### 5.1. Interpretation and Analysis of Research Results

This study first successfully obtained the high-lipid-producing microalgae strain MA-01-GM through high-throughput screening and gene editing technology. The results showed that the biomass dry weight and lipid content of MA-01-GM increased by 19.5% and 31.9%, respectively, compared to the wild-type MA-01 (Figure 2). This significant improvement is mainly attributed to the enhancement of key lipid synthesis pathways through gene editing, enabling algal cells to more effectively convert light energy and CO<sub>2</sub> into lipids during growth. This is consistent with the research results in [5] on improving algal lipid production through genetic engineering, but this study combined high-throughput screening, improving screening efficiency and targeting.

The design and optimization of the novel modular flat-panel photobioreactor is another highlight of this study. By optimizing cultivation conditions using Response Surface Methodology, both the biomass yield and lipid accumulation efficiency of MA-01-GM in the novel reactor reached high levels (Figure 3). The design of internal guide plates and static mixers in the novel reactor effectively improved light uniformity and CO<sub>2</sub> mass transfer efficiency (Figure 5), thereby overcoming common problems of "light limitation" and "CO<sub>2</sub> limitation" in traditional reactors [15, 16]. Furthermore, the optimization study of CO<sub>2</sub> concentration (Figure 4) also confirmed the importance of appropriate CO<sub>2</sub> supply for microalgae growth and lipid synthesis, which is consistent with the conclusions of [27] on CO<sub>2</sub>-enhanced microalgae cultivation.

In the biorefinery stage, Microwave-Assisted Solvent Extraction (MASE) demonstrated higher lipid extraction efficiency and lower energy consumption (Figure 6). This is attributed to the disruptive effect of microwave energy on cell walls and the activation of solvent molecules, thereby accelerating lipid release [19]. Enzyme-catalyzed transesterification achieved a biodiesel conversion rate of up to 98.5% under mild conditions (Figure 7), with high purity of the glycerol byproduct (Figure 8). Compared to traditional

base-catalyzed reactions, enzyme catalysis avoids saponification reactions and wastewater treatment issues, aligning more with green chemistry principles [8].

### 5.2. Comparison with Existing Research and Innovations

Existing microalgae biofuel research often focuses on optimizing single steps, while the innovation of this study lies in adopting the concept of design cross-innovation to systematically optimize the algal strain, cultivation system, extraction and conversion processes, and environmental assessment as a whole. For example, many studies focus on algal strain improvement or reactor design, but few studies closely integrate both and further incorporate them into biorefinery and whole life cycle assessment. Through this integrated approach, this study achieved synergistic enhancement among various steps, thereby significantly improving the overall production efficiency and sustainability of microalgae biofuel.

The results of the Life Cycle Assessment (LCA) (Figure 9) clearly show that the optimized technical solution proposed in this study significantly outperforms traditional solutions in environmental impact indicators such as Global Warming Potential, Acidification Potential, and Fossil Depletion Potential. This not only verifies the environmental friendliness of this research's technological route but also provides strong environmental benefit support for the commercialization of microalgae biofuel. The contribution analysis in Figure 10 further reveals that the microalgae cultivation stage is the main hotspot for environmental impact, suggesting that future research should focus on the recycling of culture media, improving energy efficiency, and applying renewable energy in the cultivation process to further reduce environmental burden.

### 5.3. Value of Design Cross-Innovation Perspective

This study fully embodies the unique value of design cross-innovation in solving complex engineering problems. By breaking down disciplinary barriers and organically combining knowledge and methods from biology, engineering, materials science, and environmental science, we can examine the industrialization challenges of microalgae biofuel from a more macroscopic and systematic perspective. This interdisciplinary thinking not only promotes technological innovation but also provides a more comprehensive perspective for the evaluation and optimization of technical solutions. For example, the introduction of the LCA model allows us to consider environmental impacts at the early stages of technology development, thereby guiding the selection and optimization of technological routes and avoiding the problem of "pollute first, clean up later." This aligns with [22], which emphasizes the role of design thinking in sustainable development.

### 5.4. Techno-Economic and Sustainability Analysis

Although this study primarily focuses on technological optimization and environmental benefit assessment, its results also have a positive impact on techno-economic feasibility. The development of high-lipid-producing algal strains and efficient cultivation systems directly reduces raw material costs and cultivation cycles; efficient extraction and conversion processes reduce energy consumption and chemical consumption. The significant environmental benefits indicated by the LCA results also imply lower carbon taxes and better social acceptance, thereby enhancing

the market competitiveness of microalgae biofuel. However, the commercialization of microalgae biofuel still requires further attention to the economic aspects of large-scale production, including initial investment costs, operating costs, and price competition with fossil fuels. Future research should combine Techno-Economic Analysis (TEA) to conduct a more comprehensive evaluation of the optimized solutions proposed in this study to accelerate the commercialization process of microalgae biofuel.

## 6. CONCLUSION

From the perspective of design cross-innovation, this study established and evaluated an integrated technology system for microalgae biofuel production covering strain screening and genetic modification, photobioreactor cultivation, lipid extraction, biodiesel conversion, and life cycle environmental assessment. The results show that coordinated optimization across these stages can improve both process performance and environmental outcomes.

The main findings are as follows.

First, the high-lipid strain MA-01-GM was obtained through screening and genetic modification. Compared with the wild-type MA-01 strain, MA-01-GM showed a biomass dry weight of  $2.21 \pm 0.15$  g/L and a lipid content of  $55.8 \pm 1.8\%$  DW, corresponding to increases of 19.5% and 31.9%, respectively.

Second, the modular flat-panel photobioreactor provided improved cultivation performance under optimized conditions. MA-01-GM reached a biomass yield of  $2.56 \pm 0.18$  g/L and a lipid content of  $60.5 \pm 2.1\%$  DW at a light intensity of  $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , a 16:8 h photoperiod, 3% CO<sub>2</sub>, and an N:P ratio of 10:1.

Third, microwave-assisted solvent extraction achieved a lipid extraction efficiency of  $95.2 \pm 1.1\%$ , and enzyme-catalyzed transesterification reached a biodiesel conversion rate of  $98.5 \pm 0.5\%$  under optimized conditions.

Fourth, the LCA results showed that the optimized scenario reduced GWP by approximately 45% and FDP by approximately 30% compared with the conventional scenario, indicating measurable environmental advantages.

Overall, these findings support the use of integrated optimization strategies for microalgae biofuel systems. However, the present study remains limited to laboratory and pilot-scale investigation, and the economic feasibility of full industrial implementation has not yet been verified. Future work should include larger-scale validation, further development of strain engineering strategies, broader biorefinery utilization of microalgal biomass, and integration with techno-economic analysis.

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Not applicable.

**ETHICAL STATEMENT**

All participants provided written informed consent prior to participation. The experimental protocol was reviewed and approved by an institutional ethics committee, and all procedures were conducted in accordance with relevant ethical guidelines and regulations.

**AUTHOR CONTRIBUTIONS**

All authors contributed to the conception and design of the study; Lim Win Kee conducted the technical development of the microalgae biofuel conversion framework, including algal strain screening, photobioreactor design, cultivation optimization, and lipid extraction and conversion experiments, performed the life cycle assessment and data analysis, and wrote the initial manuscript, while Cheng Yao conceived and supervised the overall research framework, guided the integration of design cross-innovation strategies, interpreted the sustainability assessment results, and critically revised the manuscript.

**COMPETING INTERESTS**

The authors declare no competing interests.

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