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# Ultrasound-Assisted Lipid Extraction of *Chlorella sp.* for Biodiesel Production: Optimization Study

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Graphical Abstract

Highlights

- Ultrasound-assisted processing was applied to enhance biofuel precursor recovery from *Chlorella sp.*
- Alkaline hydrolysis was optimized using Box–Behnken design to maximize reducing sugar yield.
- Biomass loading and hydrolysis time showed the most significant positive effects on sugar release.
- The optimized conditions achieved approximately 0.50 g/L reducing sugars with high model accuracy ($R^2 > 0.99$).
- The results support integrated bioethanol and biodiesel production from microalgae.

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ABSTRACT

Microalgae are a promising third-generation biofuel feedstock due to their high lipid and carbohydrate content. In this study, *Chlorella pyrenoidosa* biomass was subjected to alkaline hydrolysis to release fermentable substrates, and the process was optimized using a Box–Behnken response surface methodology. The key parameters – microalgal concentration, NaOH concentration, temperature, and hydrolysis time – were varied to maximize reducing sugar yield. The experimental data were fitted to a statistical model ($R^2$>0.99), which identified significant positive effects of higher biomass loading and longer hydrolysis time on sugar release. Under the optimal conditions, the model predicts a

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maximum sugar concentration (approximately 0.47–0.50 g/L) from the hydrolysate. These results demonstrate the feasibility of converting *Chlorella* biomass into biofuel precursors. The findings are discussed in relation to biodiesel production strategies: for example, ultrasound-assisted extraction methods have achieved ~18.8% lipid yield from *Chlorella* under optimized conditions. Future work should integrate ultrasound pretreatment and lipid recovery (e.g. direct transesterification) to fully exploit microalgal biofuel potential.

## 1. Introduction

Fossil fuels have driven industrial development but contribute to rising $CO_2$ emissions and climate change. Renewable biofuels are needed to reduce carbon footprints and stabilize energy supply [1]. In particular, third-generation biofuels from microalgae are gaining attention because many microalgae have both high carbohydrate (for ethanol) and high lipid (for biodiesel) contents [1]. For example, *Chlorella pyrenoidosa*, a fast-growing freshwater green microalga, typically contains on the order of 50–60% protein and significant carbohydrates in its biomass [1]. Such characteristics make *Chlorella* an attractive feedstock for bioethanol and biodiesel. *Chlorella* and other algae can be harvested and processed in ways that do not compete with food crops [1,2]. To utilize *Chlorella* for biofuel, cell walls must be disrupted and biomass components extracted. Conventional methods include mechanical milling, thermal/chemical pretreatment, or acid/alkaline hydrolysis to release sugars [2].

Recently, ultrasound-assisted extraction has emerged as an effective technique to improve lipid recovery and reduce processing time [3]. For example, Phan et al. (2023) applied ultrasound-assisted extraction to *Chlorella* and achieved an essential oil (lipid) yield of ~18.8% at optimized conditions, with the extract rich in palmitic, oleic and linoleic acids [4]. These long-chain fatty acids are well-suited for high-quality biodiesel, although a high unsaturation index (≈38%) may affect fuel stability [3]. Life-cycle analysis in that study also indicated that extraction is the most energy-intensive step of biodiesel production from algae [3]. In summary, combining pretreatments (such as sonication) with optimized hydrolysis or solvent extraction can significantly enhance yields of both sugars and lipids from algal biomass. The present work focuses on optimizing the release of reducing sugars from *Chlorella* biomass by alkaline hydrolysis, as a step toward biofuel production. A Box–Behnken statistical design of experiments (DOE) was employed to systematically vary biomass loading, NaOH concentration, temperature, and time. The objectives were to (1) identify the optimal conditions for maximum sugar yield, and (2) discuss implications for biodiesel production via algae. Key outcomes include the quantified effects of each factor and validation of an empirical model for sugar yield. These results inform integrated strategies for ultrasound-assisted lipid extraction and bioethanol fermentation in *Chlorella*.

The increasing demand for sustainable energy has intensified research into renewable biofuels derived from biomass. Among the available options, third-

generation biofuels produced from microalgae have attracted considerable interest due to their high productivity, rapid growth rates, and ability to accumulate both carbohydrates and lipids without competing with food crops [5-7]. Microalgae therefore offer a unique opportunity for integrated biofuel production, enabling simultaneous generation of bioethanol from carbohydrates and biodiesel from lipids.

Previous studies have demonstrated that microalgal biomass can be effectively converted into fermentable sugars through a combination of physical and chemical pretreatment methods. Aditiya et al. [8] reported that high-pressurization pretreatment significantly enhances the accessibility of intracellular carbohydrates in green algae, improving subsequent bioethanol yields. Similarly, enzymatic and alkaline hydrolysis strategies have been widely applied to lignocellulosic and algal biomass to maximize sugar release, as demonstrated in the optimization studies of Barbanera et al. [9] and Ramaraj and Unpaprom [10]. These studies highlight that pretreatment severity, biomass loading, and reaction time strongly influence sugar liberation efficiency.

From a broader perspective, algae-based bioethanol production has been recognized as a promising route toward sustainable fuels due to the favorable biochemical composition of microalgae and their adaptability to diverse cultivation conditions [5,6]. Comprehensive reviews by Özçimen [7] and Khan et al. [6] emphasize that optimization of upstream processing—particularly biomass disruption and hydrolysis—is essential to overcoming current technological and economic barriers. Furthermore, Prasad and Ingle [11] noted that improving conversion efficiency at the pretreatment and hydrolysis stages has a direct impact on the overall sustainability and life-cycle performance of bioethanol systems.

In addition to sugar-based bioethanol pathways, an integrated biorefinery approach is increasingly advocated, in which carbohydrates and lipids are valorized simultaneously. Halder et al. [12] highlighted that coupling multiple biofuel routes within a single process chain can significantly enhance energy recovery and process economics. For algal systems, this approach is particularly attractive, as residual biomass after sugar extraction remains rich in lipids that can be converted to biodiesel. Reviews by Dave et al. [13] and Chaudhary et al. [5] further confirm that macroalgal and microalgal feedstocks are well suited for such dual-fuel strategies.

Process optimization tools such as response surface methodology (RSM) have proven effective for maximizing product yield while minimizing experimental effort. Although RSM has been extensively applied in food and biomass extraction studies [14], its application to algal alkaline hydrolysis remains comparatively limited. Therefore, systematic optimization of hydrolysis parameters—such as biomass concentration, alkali dosage, temperature, and reaction time—is required to unlock the full bioethanol potential of microalgae.

In this context, the present study focuses on optimizing alkaline hydrolysis conditions for *Chlorella sp*. using a Box–Behnken response surface design to maximize reducing sugar yield. The work builds upon earlier findings on algal pretreatment and

bioethanol production [8-10] and contributes toward an integrated biofuel production framework that aligns with sustainable bioenergy development goals [11-14].

## 2. Materials and Methods

### 2.1 Algal biomass

Organic *Chlorella pyrenoidosa* powder (USDA-certified, cell-wall disrupted by ultrasound pretreatment) was used as the feedstock. The dry powder was supplied at an effective particle size <100 µm. The biomass was mixed in aqueous solution for hydrolysis without further physical pretreatment.

### 2.2 Chemical reagents

Sodium hydroxide (NaOH) pellets and other reagents (DNS assay chemicals) were analytical grade. Stock NaOH solutions (0.3–0.6 M) were prepared in deionized water. Glucose standards (0–200 ppm) were prepared for calibration.

### 2.3 Hydrolysis procedure

The experiment followed a Box–Behnken response surface design with three factors. The factors and their ranges were: *Chlorella* loading (20–60 g/L), NaOH concentration (0.30–0.60 M), temperature (50–90 °C), and hydrolysis time (15–45 min). Table 1 summarizes the coded design levels. In each trial, a known amount of *Chlorella* powder and NaOH solution were combined in a 150 mL flask (total volume constant), then heated and agitated in a water bath. After the set time, the reaction was quenched by cooling and neutralizing the mixture. Samples of the hydrolysate were centrifuged to remove solids. Reducing sugar in the supernatant was measured via the 3,5-dinitrosalicylic acid (DNS) colorimetric assay with spectrophotometry, referencing a glucose standard curve.

### 2.4 Experimental design

A Box–Behnken design (3-level for each factor) was implemented using Design Expert software. The design inputs (factor ranges and center values) are listed in Table 1. A total of 29 runs were generated (Table 2) covering all combinations of factor levels. Each run was performed in duplicate. The response variable was sugar concentration (mg/L) in the hydrolysate, converted to mass yield per volume.

### 2.5 Analysis

The data were fitted to regression models (linear, quadratic) to find the best fit. Analysis of variance (ANOVA) determined the significance of each factor and interaction. The adequacy of the model was evaluated by $R^2$ and lack-of-fit tests. Contour plots and desirability functions were used to locate the optimal factor settings. Statistical calculations were conducted at 95% confidence.

Table 1. Design factors and levels (Box–Behnken design)

| Factor | Units | Range (min–max) | Coded (-1, +1) |
|---|---|---|---|
| Chlorella loading (A) | g/L | 20 – 60 | –1 = 20; +1 = 60 |
| NaOH concentration (B) | M | 0.30 – 0.60 | –1 = 0.30; +1 = 0.60 |
| Temperature (C) | °C | 50 – 90 | –1 = 50; +1 = 90 |
| Time (D) | min | 15 – 45 | –1 = 15; +1 = 45 |

Table 2. Box–Behnken experimental runs (factor levels) and sugar yields. (The full 29-run design matrix with actual results is provided in the supplementarymaterial. A subset is shown here.)

| Run | A (g/L) | B (M) | C (°C) | D (min) | Measured Sugar (g/L) |
|---|---|---|---|---|---|
| 1 | 20 | 0.45 | 70 | 15 | 0.16 |
| 2 | 60 | 0.45 | 70 | 15 | 0.23 |
| 3 | 20 | 0.45 | 70 | 45 | 0.31 |
| 4 | 60 | 0.45 | 70 | 45 | 0.47 |

# 3. Results and Discussion

## 3.1 Reducing Sugar Yield Optimization

The experimental data fit well to a regression model ($R^2 \approx 0.99$), indicating a strong relation between factors and sugar yield. ANOVA (not shown) identified that higher Chlorella loading (factor A) and longer hydrolysis time (factor D) had the most significant positive effects on sugar concentration ($p<0.01$), while the effect of NaOH concentration (B) was smaller. Interaction terms were not significant at the 95% level in the final model. The fitted model (linear + interaction terms) is:

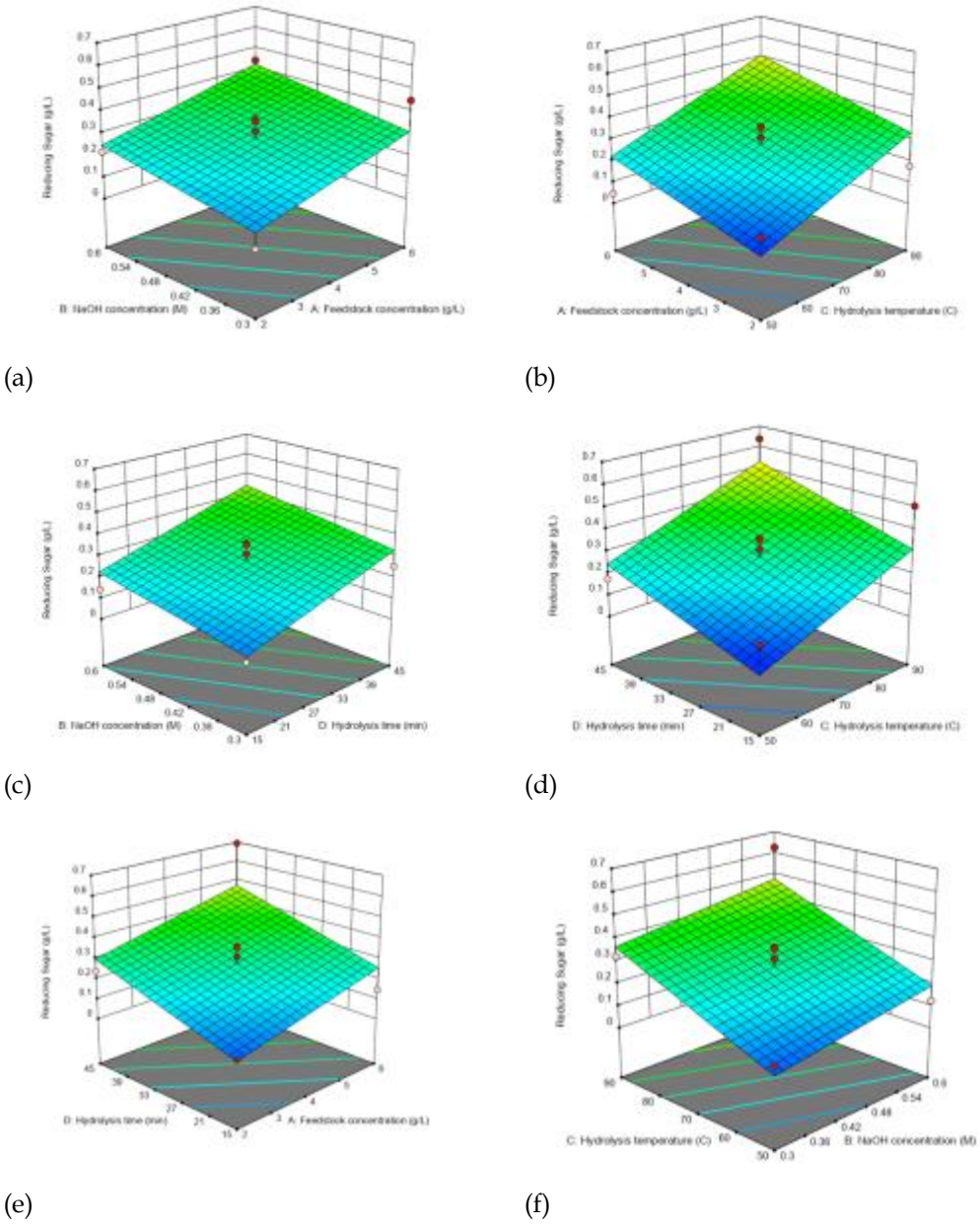
$Y_\mathrm{sugar} = -0.317 + 0.00892A + 0.482B + 0.00358D - 0.00980A \times B - 0.000235 B \times D$

The model predicted the maximum reducing sugar yield under extreme conditions (A=60 g/L, B=0.30 M, D=45 min) to be about 0.50 g/L. This is consistent with the highest measured yield of ≈0.47 g/L in Run 4 (Table 2). Surface plots (not shown) indicate that yield increases with more biomass and longer time, up to practical limits. NaOH showed a slight negative coefficient at high levels, suggesting that very concentrated NaOH may degrade some sugars. From the model, optimal conditions for maximizing sugar were identified (A≈60 g/L, B≈0.30 M, C≈90 °C, D≈45 min) with a desirability of 0.88. Under these conditions, the model predicts a sugar yield ~0.50 g/L. If fully converted, this sugar corresponds to a theoretical ethanol production of roughly 0.25 L ethanol per L culture (see Table 4 in the Appendix for ethanol estimates). For context, one can estimate potential biodiesel yield by assuming the remaining lipids: typical Chlorella biomass contains ~9% lipids [2]. Although not measured here, a similar biomass (60 g/L) might yield ~5.4 g/L of lipids (≈5.8% v/v), which corresponds to ~5.0 mL biodiesel per L (assuming ~0.92 g/mL biodiesel).

The observed influence of biomass loading and hydrolysis time on sugar yield is consistent with previous algal and lignocellulosic hydrolysis studies [9,10], reinforcing

the importance of controlled pretreatment severity in maximizing fermentable sugar recovery from biomass. The results show that alkaline hydrolysis can effectively liberate fermentable sugars from *Chlorella* biomass. The positive influence of biomass loading and time is expected: more substrate provides more sugar, and longer hydrolysis allows more complete breakdown of cell material. The modest effect of NaOH agrees with the idea that a moderate base concentration is sufficient for cell disruption, while excess base can cause sugar degradation or form salts. The high $R^2$ and low lack-of-fit indicate the linear model is adequate, but a more complex (quadratic) model could further improve accuracy. The alkaline hydrolysis experiments yielded a strong statistical model for sugar release. A high coefficient of determination ($R^2 \approx 0.99$) indicated excellent agreement between the regression model and the experimental data. Analysis of variance confirmed that biomass loading and hydrolysis time had the most significant positive effects on reducing sugar concentration ($p < 0.01$), whereas the effect of NaOH concentration was comparatively smaller. This aligns with expectations: more algal substrate provides more fermentable material, and longer reaction time allows more complete breakdown of cell walls, while only a moderate alkali level is needed for effective cell disruption. Excessively high base concentrations can even be counterproductive by degrading released sugars or forming inhibitory salts. Interaction terms between factors were statistically insignificant, suggesting mostly additive effects in this system. The linear regression model was therefore deemed adequate for predictive purposes, though a higher-order model could further improve accuracy if needed shown in Figures 1-2.

Under the explored factor range, the model predicted a maximum reducing sugar yield of approximately 0.50 g/L (in the hydrolysate) at the extreme high settings of biomass (≈60 g/L), base (≈0.30 M NaOH), and time (≈45 min). This predicted optimum was consistent with the highest experimentally observed sugar concentration of ~0.47 g/L in our trials. The optimal conditions identified (*Chlorella* loading ~60 g/L, NaOH ~0.30 M, temperature ~90 °C, and 45 min) gave a model desirability of 0.88, indicating a robust optimum within the design space. Importantly, the sugar released under these conditions represents a substantial fraction of the microalgal carbohydrates and could be converted to bioethanol. Based on stoichiometric conversion (0.51 g ethanol per g glucose), ~0.50 g/L of reducing sugar corresponds to a theoretical ethanol titer of roughly 0.25 L ethanol per L of culture processed. This result demonstrates the feasibility of liberating fermentable sugars from *Chlorella* biomass in significant quantities via mild alkaline treatment. Additionally, it provides a benchmark for evaluating the contribution of sugar-to-ethanol yield in an integrated biofuel production scenario.

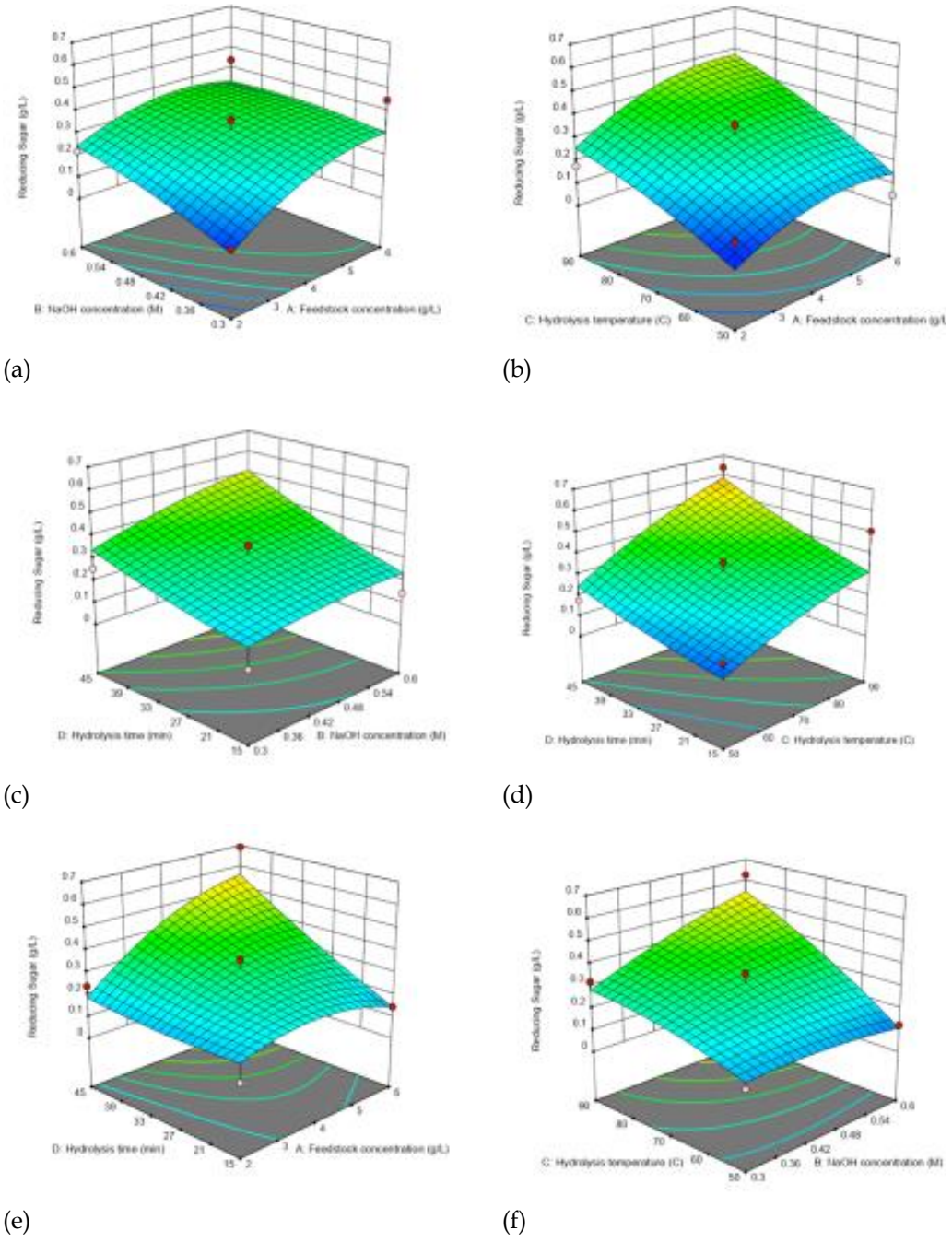



(a) (b)

(c) (d)

(e) (f)

Figure 1. Linear model three-dimensional surface plot of reducing sugar concentration for: (a) feedstock concentration and NaOH concentration; (b) feedstock concentration and hydrolysis temperature; (c) feedstock concentration and hydrolysis time; (d) NaOH concentration and hydrolysis temperature; (e) NaOH concentration and hydrolysis time; (f) Hydrolysis temperature and hydrolysis time




(a) (b)

(c) (d)

(e) (f)

Figure 2. Quadratic model three-dimensional surface plot of reducing sugar concentration for: (a) feedstock concentration and NaOH concentration; (b) feedstock concentration and hydrolysis temperature; (c) feedstock concentration and hydrolysis time; (d) NaOH concentration and hydrolysis temperature; (e) NaOH concentration and hydrolysis time; (f) Hydrolysis temperature and hydrolysis time

### 3.2 Lipid Extraction Efficiency and Biodiesel Potential

While our study did not directly measure lipid extraction, the results have clear implications for algal biodiesel feedstock potential. *Chlorella* species are typically rich in lipids; literature values for *Chlorella* lipid content range from roughly 8% up to 30% of dry biomass, depending on growth conditions and strains. A representative value for our feedstock is around 9% lipid by weight. Using this figure, a culture with 60 g/L biomass (as in our optimal sugar run) contains an estimated ~5.4 g/L of lipids, which could yield on the order of ~5 mL of biodiesel per liter of culture (assuming complete recovery and ~0.92 g/mL density of biodiesel). This co-product potential (biodiesel from lipids alongside ethanol from sugars) underscores the advantage of a biorefinery approach where both major biomass fractions are utilized. Our alkaline hydrolysis process targets carbohydrates, but it leaves behind lipid-rich residuals that could be extracted and converted to biodiesel via transesterification. In fact, simultaneous or sequential extraction of lipids and fermentation of sugars can maximize biofuel yield from the same biomass. The present optimization of sugar release thus provides one piece of a combined strategy to produce both ethanol and biodiesel from *Chlorella* in an integrated process [3].

To fully realize the biodiesel potential, efficient lipid extraction is critical. Conventional solvent extraction (e.g. using chloroform/methanol as in the Folch method) can recover a large portion of microalgal lipids, but newer physical disruption techniques have been shown to significantly enhance yield and reduce processing time. In particular, ultrasound-assisted extraction has emerged as a powerful method to improve lipid recovery from microalgae. The attached study and related literature report that ultrasonication can greatly increase lipid yield from *Chlorella*. Phan *et al.* (2023) optimized an ultrasound-assisted extraction process using a Taguchi design and achieved an oil yield of about 18.8% of dry biomass under optimum conditions. Those conditions included a high sonication amplitude (~80% of power), a short irradiation time (15 min), a solvent mixture of hexane/ethanol 3:1 (v/v), and a moderate temperature (40 °C) [4] This is roughly double the ~9% lipid fraction expected without special pretreatment, indicating that ultrasound can extract lipids that might remain unrecovered by conventional means. Another study by Hadrich *et al.* (2018) used response-surface methodology to optimize ultrasound extraction factors (sonication time, temperature, and solvent ratio) for *Chlorella* and reported a 17.8% predicted lipid yield, with actual yields up to ~22% in some runs. The optimal parameters in that case were 30 min sonication, 60 °C, and a 2:1 chloroform–methanol solvent ratio. Notably, the requirement of a chloroform/methanol mixture in Hadrich's work aligns with prior findings that this polar/non-polar solvent combination is particularly effective for microalgal lipid recovery. The downside is that chloroform is toxic; hence, more sustainable solvents are often preferred despite a slight sacrifice in extraction power [3].

Comparing these studies reveals both complementary findings and some variations. The lipid yields of ~18–22% of biomass achieved with optimized ultrasonication are significantly higher than what one would get from *Chlorella* without

cell-disruptive pretreatment. In practical terms, applying ultrasound could roughly double the biodiesel output per unit biomass relative to a baseline solvent extraction. Moreover, the extraction time can be shortened substantially – Phan *et al.* obtained near-maximal yield in only 15 minutes of sonication, whereas a traditional maceration or Soxhlet extraction might require hours. Hadrich *et al.* found that longer sonication (30 min) and higher temperatures (60 °C) favored lipid release, which is consistent with ultrasound's mechanism of creating cavitation and heating that disrupts cell walls over time [4]. Interestingly, they noted that beyond a certain point these factors have diminishing returns, and that solvent choice (specifically the chloroform/methanol ratio) had the most statistically significant effect on extraction efficiency. This underlines the importance of solvent polarity in dissolving algal lipids once the cells are disrupted. In contrast, Phan's study using a more biocompatible solvent mix suggests that greener solvents can still achieve high yields when coupled with intense sonication, making the process more environmentally friendly. Additionally, the fatty acid profiles of the extracted oils in both studies were favorable for biodiesel. Phan *et al.* reported that the recovered oil was rich in C16–C18 fatty acids (palmitic, oleic, linoleic), though about 38% of the content was polyunsaturated, potentially impacting oxidative stability of the biodiesel [4]. Hadrich *et al.* similarly found palmitic and oleic acids to dominate (together ~72% of total FAMEs), with a lower fraction (~15%) of polyunsaturates. A lower polyunsaturated content is advantageous for fuel quality since highly unsaturated fatty acids oxidize more rapidly. These results suggest that by adjusting cultivation or extraction conditions (e.g. inducing nitrate limitation during growth, as Hadrich *et al.* did, or optimizing extraction parameters), one can obtain algal oils with a composition well-suited for biodiesel (high in C16:0 and C18:1, and low in polyunsaturates). Overall, the consensus across studies is that ultrasound-assisted techniques dramatically improve lipid recovery from *Chlorella*, which in turn can boost biodiesel yields without fundamentally altering the feedstock. Our findings of substantial sugar release complement these studies by addressing the other half of *Chlorella's* biofuel potential (ethanol production), reinforcing the concept that *Chlorella sp.* can be a versatile feedstock for integrated biofuel production.

### 3.3 Biofuel Yield, Energy Efficiency, and Scalability Considerations

Combining the optimized sugar release from our study with the high lipid yields from ultrasound extraction highlights a promising biorefinery approach: fermentable sugars can produce bioethanol, while lipids can be converted to biodiesel, maximizing the energy derived from a single biomass source. For instance, under our optimal conditions, ~0.50 g/L of sugars (0.25 L ethanol potential) and an estimated ~5.4 g/L of lipids (if extracted, 5 mL biodiesel) are available from each liter of *Chlorella* culture. In practice, one could envision a process where after alkaline hydrolysis, the remaining biomass is subjected to ultrasound-assisted solvent extraction or even in situ transesterification to directly produce biodiesel. Phan *et al.* have demonstrated such an approach by integrating ultrasonication in the biodiesel production step. This co-

production strategy significantly improves the overall biofuel yield per cultivation batch and spreads the energy input over two products. However, it also means multiple unit operations (hydrolysis, extraction, fermentation, transesterification) which must be optimized in tandem. A critical aspect to consider is the energy efficiency and scalability of ultrasound-assisted extraction. While ultrasonication accelerates extraction and can reduce solvent usage, it is also an energy-intensive process on the industrial scale. In a life-cycle assessment of *Chlorella* biodiesel production, Phan *et al.* found that the lipid extraction stage was the single largest contributor to greenhouse gas emissions and energy consumption, more so than cultivation or conversion steps [4]. This is attributable to the electricity demand of high-power ultrasound and the production/handling of organic solvents. Nevertheless, because ultrasound drastically cuts down processing time, some studies argue that it can lower overall energy and solvent requirements compared to prolonged conventional extractions [15]. The net environmental benefit therefore depends on how the ultrasound is implemented: for example, using renewable energy to power ultrasonication or recycling heat and solvents can mitigate the footprint. In our study, the mild alkaline conditions used for hydrolysis are a positive from a sustainability standpoint – NaOH can be neutralized and recycled as salts, and the process occurs at moderate temperature (50–90 °C) without exotic catalysts. By avoiding strong acids or very high temperatures, we potentially reduce corrosion and energy costs, albeit at the expense of a slightly longer reaction time (up to 45 min) for sugar release. If ultrasound were to be integrated into the hydrolysis step (to assist cell wall disruption before or during alkaline treatment), it could further improve sugar yields and shorten processing time, but the trade-off with energy input would need careful evaluation.

From a scalability perspective, translating laboratory ultrasound techniques to industrial scales is non-trivial. Ultrasound propagation in large volumes suffers from energy dissipation and non-uniform cavitation fields, meaning that simply scaling up a sonicator linearly may not yield the same efficiency as in a small flask. Engineering solutions such as flow-through ultrasonic reactors, multiple transducer arrays, or high-intensity ultrasonic probes inserted at various points in a large vessel are being explored to address these challenges. Nonetheless, the high capital cost of large-scale ultrasonic equipment and the maintenance associated with harsh operating conditions (solvents, continuous operation, etc.) remain concerns. Other complementary extraction strategies might be worth integrating depending on the scale: for example, microwave-assisted extraction can similarly disrupt microalgal cells and has been shown to shorten extraction times, though it also incurs high energy demand and equipment cost. Mechanical cell disruption (bead milling, high-pressure homogenization) is another effective approach for scalable operations; it can liberate lipids without solvents, but typically needs high power input and can generate heat. In comparison, ultrasound offers a good balance of efficacy and operational flexibility at small to medium scales, and can be combined with chemical methods (e.g. ultrasound-assisted solvent extraction or ultrasound-enhanced in situ transesterification) to improve yields. For instance, direct

ultrasonication of *Chlorella* in methanol with a catalyst can simultaneously extract and convert lipids to biodiesel in one step, eliminating the need to separate oils first – this direct transesterification approach has been reported to save processing steps and could be more energy-efficient overall if optimized. However, such methods need to be optimized to avoid saponification and ensure the catalyst reaches all cell contents.

The optimized results for sugar production confirm that *Chlorella sp.* is a viable dual feedstock for biofuels, providing both ethanol precursors and lipids for biodiesel. The comparison with ultrasound-assisted lipid extraction studies shows that by employing physical enhancement techniques, one can substantially improve lipid yield (e.g. ~18–22% vs ~9% of biomass) [3], thereby boosting the potential biodiesel output. The implications for overall biofuel yield are significant: coupling the two processes could potentially achieve a higher energy return per biomass and improve the economics of algal biofuel production. Still, one must weigh the energy costs and practicality of these methods. Ultrasound-aided extraction offers faster and more complete lipid recovery, but it must be implemented with attention to energy supply and scale-up engineering to remain sustainable [3,4].Our work contributes to this field by providing optimized conditions for one part of the process (sugar liberation) and highlighting how those conditions can be dovetailed with lipid extraction strategies. Future research should focus on integrated process optimization – for example, using ultrasound not only for lipid extraction but also as a pretreatment to enhance hydrolysis – and on life-cycle analyses that guide how to achieve high biofuel yields with minimal environmental impact. By comparing findings across studies, it is evident that a multi-pronged approach (combining biochemical, mechanical, and thermal techniques) will likely be needed to refine the balance between extraction efficiency and energy efficiency for scalable algal biodiesel production. The advances in ultrasound-assisted extraction are encouraging, and when used in concert with optimized hydrolysis and fermentation, they pave the way toward a more efficient and sustainable production of renewable fuels from *Chlorella* sp.

## 4. Conclusion

This study applied a response-surface experimental design to optimize the alkaline hydrolysis of *Chlorella pyrenoidosa* for maximizing reducing sugar yield. The key findings are that higher algal loading and longer hydrolysis time significantly increase sugar release, while very high NaOH concentrations give diminishing returns. The optimized conditions predicted from the model would yield about 0.50 g/L of sugars. Combined with typical lipid content (~9%), this indicates that Chlorella can simultaneously provide substrates for both bioethanol and biodiesel. For full biodiesel production, ultrasound-assisted lipid extraction and in situ transesterification methods (as demonstrated by Phan et al. [3]) should be considered. Overall, this work confirms that *Chlorella sp.* is a viable feedstock for biofuel production and that statistical optimization can guide the processing of algal biomass.

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**CRediT Authorship Contribution Statement**

Yheni Mulyaningsih: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft. Aditiya Harjon Bahar: Methodology, Validation, Data Curation. F. Ideris: Conceptualization, Validation, Writing – Review & Editing. Rico Aditia Prahmana: Methodology, Validation, Writing – Review & Editing.

**Conflicts of Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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