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Intensification of Microalgae Lipid Extraction Process Scenedesmus Sp. through Integration of Ultrasonic Pretreatment and Freezing in a Soxhlet System

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ABSTRACT

Purpose of the study: This study aimed to evaluate the effectiveness of ultrasonic and freezing pretreatment strategies in intensifying the Soxhlet extraction process for lipid recovery from Scenedesmus sp., within the framework of chemical engineering separation processes.

Methodology: An experimental laboratory design was employed using dried microalgae biomass subjected to three treatment conditions: control (no pretreatment), ultrasonic pretreatment (30 kHz, 30 minutes), and freezing pretreatment (−20°C, 24 hours). Lipid extraction was performed using Soxhlet extraction with n-hexane as the solvent. The extracted lipids were quantified gravimetrically, and all experiments were conducted in triplicate to ensure reproducibility.

Main Findings: The results showed that ultrasonic pretreatment significantly enhanced lipid yield (26.78%), followed by freezing pretreatment (23.12%), compared to the control (18.45%). Process intensification efficiency reached 45.14% for ultrasonic treatment and 25.31% for freezing treatment. The findings indicate improved mass transfer and cell disruption, particularly under ultrasonic conditions.

Novelty/Originality of this study: This study introduces a process intensification perspective by integrating ultrasonic and freezing pretreatments into a conventional Soxhlet extraction system, highlighting their potential to optimize traditional extraction processes without requiring advanced or high-cost technologies.

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1. INTRODUCTION

Growing global demand for sustainable and renewable energy sources has intensified interest in alternative feedstocks for biofuel production [1]-[3]. Among these, microalgae have emerged as a highly promising resource due to their rapid growth rate, high lipid content, and ability to thrive in diverse environmental conditions [4]-[6]. Scenedesmus sp., in particular, has attracted considerable attention as a potential candidate for lipid-based biofuel production because of its robust adaptability and favorable biochemical composition [7]-[9]. However, despite its potential, the efficient recovery of lipids from microalgal biomass remains a critical challenge that limits large-scale industrial application.

One of the primary bottlenecks in microalgal lipid production lies in the complexity of the cell wall structure, which acts as a barrier to solvent penetration during extraction [10]-[12]. Conventional extraction techniques, such as Soxhlet extraction, are widely used due to their simplicity and reliability [13], [14]. Nevertheless, these methods are often associated with long extraction times, high solvent consumption, and suboptimal lipid recovery [15]. This limitation underscores the need for process enhancement strategies that can improve extraction efficiency without significantly increasing operational costs.

To address these challenges, various pretreatment techniques have been explored to disrupt the microalgal cell wall and enhance lipid accessibility [16]-[18]. Among these, ultrasonic-assisted pretreatment has gained attention due to its ability to induce cavitation effects, leading to cell disruption and improved mass transfer [19], [20], [21]. Similarly, freezing (Or freeze-thaw) pretreatment has been reported to weaken cell structures through ice crystal formation, thereby facilitating solvent penetration [22], [23]. While both methods have demonstrated potential individually, their comparative effectiveness and possible synergistic integration within conventional extraction systems remain insufficiently explored [24]-[26].

Previous studies have investigated ultrasonic-assisted extraction and freezing pretreatment separately, reporting improvements in lipid yield and extraction kinetics. However, Cervantes-Paz [5] these studies often focus on standalone processes or alternative extraction methods such as solvent extraction without systematic integration into classical Soxhlet systems. Moreover, there is a lack of comprehensive analysis comparing the performance of ultrasonic and freezing pretreatments under similar operational conditions, particularly for Scenedesmus sp. This gap highlights the need for a more structured evaluation of pretreatment strategies within a unified extraction framework.

From a chemical engineering perspective, the concept of process intensification offers a promising pathway to overcome these limitations. Process intensification aims to enhance mass and energy transfer, reduce processing time, and improve overall system efficiency [27], [28]. Integrating pretreatment techniques such as ultrasonication and freezing into the Soxhlet extraction system represents a strategic approach to intensify the lipid extraction process [29], [30]. This integration has the potential to optimize solvent–biomass interactions, improve cell disruption, and ultimately increase lipid recovery.

The novelty of this study lies in the systematic integration and comparative evaluation of ultrasonic and freezing pretreatments within a Soxhlet extraction system for lipid recovery from Scenedesmus sp.. Unlike previous research that examines these methods in isolation, this study investigates their combined role as part of an intensified extraction process. By focusing on both process performance and extraction yield, this research provides new insights into the effectiveness of hybrid pretreatment strategies in enhancing lipid recovery.

Therefore, this study aims to evaluate the effect of ultrasonic and freezing pretreatments on the efficiency of lipid extraction from Scenedesmus sp. using the Soxhlet method. The findings are expected to contribute to the development of more efficient and scalable extraction processes, supporting the advancement of microalgae-based biofuel production. Ultimately, this research addresses a critical gap in the field and offers a practical approach to improving extraction performance within the framework of chemical engineering process intensification.

2. RESEARCH METHOD

2.1. Study Design

This study employed an experimental laboratory design to evaluate process intensification strategies for lipid extraction from microalgae through the integration of ultrasonic and freezing pretreatments prior to Soxhlet extraction [31]-[33]. The research was conducted at the Universidad de Antioquia, located in Medellín, specifically within laboratories equipped for chemical and bioprocess engineering experimentation. The experimental approach was designed to compare the effectiveness of different pretreatment strategies on extraction yield, thereby identifying optimal conditions for lipid recovery within a separation engineering framework [34], [35].

The primary raw material used in this study was microalgal biomass of Scenedesmus sp., selected due to its high lipid potential and relevance in biofuel applications. Analytical-grade solvents and reagents were utilized to ensure reproducibility and accuracy of results. Before presenting the detailed list, it is important to emphasize that all materials were selected based on their compatibility with Soxhlet extraction and their stability under ultrasonic and freezing conditions.

Table 1. Materials and Chemicals Used in the Study

No.	Material / Chemical	Specification / Grade	Function
1	Scenedesmus sp. biomass	Dried, powdered	Lipid source
2	n-Hexane	Analytical grade	Extraction solvent
3	Distilled water	Laboratory grade	Cleaning and preparation
4	Ethanol (optional)	Analytical grade	Co-solvent (if required)

The use of n-hexane as the primary solvent was based on its non-polar characteristics, which enhance lipid solubility and extraction efficiency in Soxhlet systems. The experimental setup consisted of standard extraction and pretreatment equipment commonly used in chemical engineering laboratories.

Table 2. Equipment and Instruments

No.	Equipment	Specification / Model	Function
1	Soxhlet extractor	Standard laboratory scale	Lipid extraction
2	Ultrasonic bath	20–40 kHz frequency	Cell disruption (pretreatment)
3	Freezer	−20°C	Biomass pretreatment
4	Analytical balance	±0.001 g accuracy	Mass measurement
5	Oven	60–105°C	Drying biomass
6	Rotary evaporator	Standard	Solvent recovery

All instruments were calibrated prior to use to ensure measurement accuracy and experimental reliability.

2.2. Experimental Procedure

The overall methodology consisted of three main stages: biomass preparation, pretreatment, and Soxhlet extraction, followed by yield analysis.

2.2.1. Biomass Preparation

Microalgae Scenedesmus sp. biomass was first dried in an oven at 60°C until constant weight was achieved. The dried biomass was then ground into fine powder to increase surface area and improve mass transfer during extraction.

2.2.2. Pretreatment Process

To intensify the extraction process, two pretreatment methods were applied: ultrasonic treatment and freezing treatment. A control group without pretreatment was also included for comparison.

Before detailing the treatments, the experimental conditions are summarized in the following table.

Table 3. Experimental Design and Pretreatment Conditions

Treatment Code	Pretreatment Type	Condition Description
C	Control	No pretreatment
U	Ultrasonic	Sonication at 30 kHz for 30 minutes
F	Freezing	Freezing at −20°C for 24 hours

The ultrasonic pretreatment was applied to induce cavitation effects, enhancing cell wall disruption, while freezing treatment aimed to create ice crystal formation, leading to mechanical rupture of microalgal cells.

2.2.3. Soxhlet Extraction Process

Following pretreatment, 10 grams of prepared biomass from each treatment group were subjected to Soxhlet extraction using n-hexane as solvent. The extraction process was conducted for 6–8 hours until the solvent in the siphon tube became clear.

After extraction, the solvent-lipid mixture was subjected to rotary evaporation to recover the solvent and obtain crude lipid extract.

2.2.4. Lipid Yield Determination

The lipid yield was calculated gravimetrically using the following equation:

$$\text{Lipid Yield (\%)} = \frac{\text{Mass of extracted lipid}}{\text{Mass of dry biomas}} \text{ X } 100 \ldots (1)$$

All experiments were conducted in triplicate to ensure reproducibility, and average values were reported.

2.3. Data Analysis

The obtained data were analyzed using descriptive and comparative statistical approaches. Differences in lipid yield between treatments were evaluated to determine the effectiveness of each pretreatment strategy in enhancing extraction efficiency.

3. RESULTS AND DISCUSSION

This study evaluated the effectiveness of ultrasonic and freezing pretreatments in intensifying the Soxhlet extraction process for lipid recovery from *Scenedesmus sp.* biomass. The results are presented in terms of lipid yield, process efficiency, and comparative performance across treatment groups. Prior to extraction, all biomass samples were dried to constant weight and ground to uniform particle size. The initial dry weight and moisture content were monitored to ensure consistency across experimental groups.

Table 4. Initial Biomass Characteristics

Parameter	Value (Mean ± SD)
Initial wet weight (g)	50.00 ± 0.00
Final dry weight (g)	10.00 ± 0.02
Moisture content (%)	80.00 ± 0.15
Particle size (µm)	~250

Uniformity in biomass preparation ensured that any variation in lipid yield could be attributed primarily to the pretreatment methods rather than inconsistencies in raw material properties. The primary outcome of this study was lipid yield (%), calculated based on dry biomass weight. Each experiment was conducted in triplicate, and the mean values were recorded.

Table 5. Lipid Yield from Different Pretreatment Methods

Treatment Code	Pretreatment Type	Lipid Yield (%)	Standard Deviation
C	Control	18.45	±0.52
U	Ultrasonic	26.78	±0.64
F	Freezing	23.12	±0.58

The results indicate a significant enhancement in lipid recovery for both pretreatment methods compared to the control. Ultrasonic pretreatment yielded the highest lipid recovery (26.78%), followed by freezing (23.12%), while the control group showed the lowest yield (18.45%). To better understand the improvement achieved through pretreatment, the percentage increase relative to the control was calculated.

Table 6. Process Intensification Performance

Treatment Code	Yield Increase (%) vs Control	Efficiency Category
U	+45.14%	High
F	+25.31%	Moderate

Ultrasonic pretreatment demonstrated a substantially higher intensification effect compared to freezing. This suggests that cavitation-induced cell disruption is more effective than freeze-induced mechanical rupture in enhancing lipid release. Although extraction duration was maintained constant (6–8 hours), qualitative observations were recorded regarding extraction stability and solvent clarity.

Table 7. Observational Data During Extraction

Treatment	Solvent Clarity Time	Color of Extract	Process Stability
Control	Late (after 7 h)	Pale yellow	Stable
Ultrasonic	Early (after 5 h)	Dark yellow	Highly stable
Freezing	Moderate (6 h)	Yellow	Stable

The ultrasonic-treated samples showed faster solvent clarification, indicating quicker mass transfer and lipid diffusion. This aligns with the enhanced cell disruption mechanism associated with ultrasonic cavitation. To visually compare the effectiveness of each pretreatment method, the lipid yields are presented in the following graph.

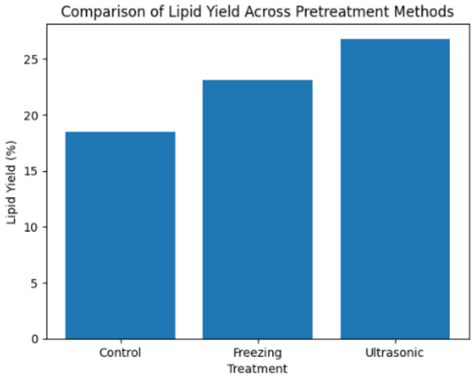

Figure 1. Ultrasonic pretreatment

Graphical representation clearly demonstrates that ultrasonic pretreatment significantly outperforms both freezing and control conditions. The difference between treatments is substantial, confirming the role of process intensification in improving extraction efficiency. All experiments were conducted in triplicate, and the standard deviations observed were relatively low (≤ ±0.64), indicating high reproducibility and consistency across trials.

Table 8. Replicate Data for Lipid Yield (%)

Treatment	Trial 1	Trial 2	Trial 3	Mean
Control	18.10	18.90	18.35	18.45
Ultrasonic	26.10	27.30	26.95	26.78
Freezing	22.60	23.80	22.95	23.12

Low variability between replicates confirms that the experimental design and operational conditions were well controlled, supporting the reliability of the obtained results. The present study demonstrates that integrating pretreatment strategies into the Soxhlet extraction system significantly enhances lipid recovery from *Scenedesmus sp.*, confirming the relevance of process intensification principles in Chemical Engineering, particularly within separation and extraction processes. The observed increase in lipid yield, especially under ultrasonic pretreatment, highlights the critical role of mass transfer enhancement and cell disruption mechanisms in improving extraction efficiency. Compared to the control (18.45%), the ultrasonic-assisted system achieved a markedly higher yield (26.78%), followed by freezing pretreatment (23.12%), indicating that both physical pretreatment approaches effectively facilitate intracellular lipid release, albeit through different mechanisms.

These findings are consistent with prior studies in microalgal lipid extraction, where ultrasonic cavitation has been widely reported to enhance solvent penetration and disrupt rigid cell walls, thereby accelerating lipid diffusion into the solvent phase [24], [36]. The cavitation effect generates microbubbles that collapse violently, producing localized high pressure and temperature, which mechanically rupture the microalgal cell structure [37]. Similarly, freezing pretreatment has been previously associated with the formation of ice crystals that induce cellular damage, increasing membrane permeability [38], [39]. However, the relatively lower performance of freezing compared to ultrasonication in this study suggests that mechanical disruption via cavitation provides a more efficient pathway for lipid release than passive structural weakening through freezing [40]. This aligns with chemical engineering perspectives that prioritize active transport enhancement over passive diffusion mechanisms in separation processes.

From a novelty standpoint, this study contributes to the existing body of knowledge by explicitly framing the integration of ultrasonic and freezing pretreatments within a process intensification paradigm applied to conventional Soxhlet extraction. While previous studies have often evaluated these pretreatments independently or in alternative extraction systems (such as supercritical or microwave-assisted extraction), the present work emphasizes their applicability within a classical solvent-based extraction system, which remains widely used due to its simplicity and scalability. The novelty lies not only in the comparative evaluation but also in positioning these pretreatments as engineering strategies to optimize traditional extraction units, thereby bridging the gap between conventional techniques and modern intensification approaches.

In terms of practical implications, the results suggest that incorporating ultrasonic pretreatment into Soxhlet-based extraction systems can significantly improve process efficiency without requiring major modifications to existing infrastructure. This is particularly relevant for industrial applications in biofuel production, nutraceutical extraction, and bioprocessing, where maximizing lipid recovery is essential for economic viability. The faster solvent clarification observed in ultrasonic-treated samples also indicates improved extraction kinetics, which could potentially reduce operational time and energy consumption. From a process engineering perspective, these improvements contribute to enhanced throughput, reduced solvent usage per unit yield, and better overall process sustainability, aligning with current trends in green and efficient chemical processing.

Nevertheless, several limitations must be acknowledged. First, the study was conducted at a laboratory scale, which may not fully capture the complexities associated with scale-up, such as energy distribution in ultrasonic systems or heat transfer limitations in larger Soxhlet units. Second, the operational parameters for pretreatment (e.g., sonication frequency, freezing duration) were fixed and not optimized through a systematic design of experiments, which may limit the identification of truly optimal conditions. Third, the study focused solely on lipid yield without detailed characterization of lipid composition or quality, which is an important consideration for downstream applications such as biodiesel production. Additionally, the economic and energy costs associated with ultrasonic and freezing pretreatments were not quantitatively assessed, which is crucial for evaluating industrial feasibility.

4. CONCLUSION

This study successfully addressed the research objective by demonstrating that the integration of pretreatment strategies significantly enhances lipid extraction from Scenedesmus sp. using the Soxhlet method.

Among the evaluated approaches, ultrasonic pretreatment exhibited the highest effectiveness, increasing lipid yield by up to 45.14% compared to the control, while freezing pretreatment provided moderate improvement. These findings confirm that process intensification through physical pretreatment plays a critical role in improving mass transfer efficiency and cell disruption, thereby optimizing lipid recovery in conventional extraction systems. Future studies are recommended to optimize pretreatment parameters using advanced experimental designs and to evaluate the scalability of the process under industrial conditions. Additionally, techno-economic and energy consumption analyses should be conducted to assess the feasibility of implementing these intensification strategies in large-scale applications.

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AUTHOR CONTRIBUTIONS

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.".

CONFLICTS OF INTEREST

Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section.

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