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Article

Studies on the mechanical properties of microalgae and their effects on growth, breeding and extraction quality

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Abstract: Microalgae is becoming a crucial research topic because of global resource shortage, pollution, and population growth make. This paper focuses on the mechanical properties of microalgae cells, including the characterization of cell stiffness, adhesion force, and deformability and their applications in growth regulation and breeding. Understanding cell behavior provides significance and serves as a basis for screening and cultivating microalgae. It analyzes the effects of mechanical factors, including light, temperature, and fluid shear stress on microalgae growth and emphasizes the importance of optimizing these conditions. It discussed high-throughput screening techniques in microalgae breeding and the correlation between mechanical properties and superior traits. With regard to microalgae processing, the paper examines the role of cellular biomechanics in harvesting, extraction, and product quality optimization, including the selection and optimization of harvesting methods, the evaluation of cell fragmentation efficiency, and the relationship between product quality and mechanical properties. The paper presents case studies on specific microalgae species like Chlamydomonas reinhardtii and highlights its potential to optimize culture conditions, promote product diversification, drive technological innovation, support environmental protection, and enhance interdisciplinary cooperation.

Keywords: microalgae; mechanical properties; microalgae cells; microalgae product; extraction; high-throughput screening; *Chlamydomonas reinhardtii*

1. Introduction

Finding sustainable and efficient biological resources has become an important topic of scientific research with the growing shortage of global resources, environmental pollution and population growth. Because of its fast growth rate, high photosynthetic efficiency, nutrient richness and ease of cultivation, microalgae has great application potential in many fields such as food, feed, medicine, bioenergy and environmental protection. Microalgae provide humans with a wealth of nutrients such as protein, fat, vitamins and minerals and are raw materials for renewable energy sources such as biodiesel and bioplastics. Therefore, microalgae are of great significance in field of sustainable agricultural development, alleviating the energy crisis and reducing environmental pollution. It makes sence of research on the cultivation and processing technology of microalgae in order to improve their yield and quality for the sustainable use of biological resources.

Microalgae contain abundant nutrients such as high-quality protein, antioxidants, vitamins, minerals and unsaturated fatty acids (including omega-3 and omega-6 fatty acids that are beneficial to human health). Microalgae are a good source of plant protein and an important ingredient for renewable energy. During growth, microalgae can accumulate a large amount of oil and absorb a large amount of carbon dioxide and this characteristic helps to address environmental challenges and support resource utilization. Cytomechanics is a cross disciplinary subject of biology and mechanics. Cytomechanics studies the structure, behavior and function of cells in a mechanical environment. It is more and more important to know microalgae's mechanical properties and how microalgae interact with their growth environment.

This research studies on the mechanical properties of microalgae and their effects on growth, breeding and extraction quality. The research is based on the basis of reviews of recent research progress in microalgae and explores the mechanical properties of microalgal cells and their application in growth regulation and breeding. The research focuses on mechanical optimization during microalgae harvesting and extraction and examines how external forces affect the cell structure and function by taking *Chlamydomonas reinhardtii* for case analysise.

2. Literature review

Microalgae are becoming research hotspot because of rich biomass components and microalgae can be applied in biorefining, wastewater treatment and production of high value-added products. On the website of Web of Science, by searching with "microalgae", the quantity of documents for microalgae over time graph is shown in **Figure 1**. This section reviews recent research progress in microalgae, including optimization and innovation of microalgae culture technology, application of emerging technologies in microalgal culture and applications of microalgae in medical and biocomposite materials.

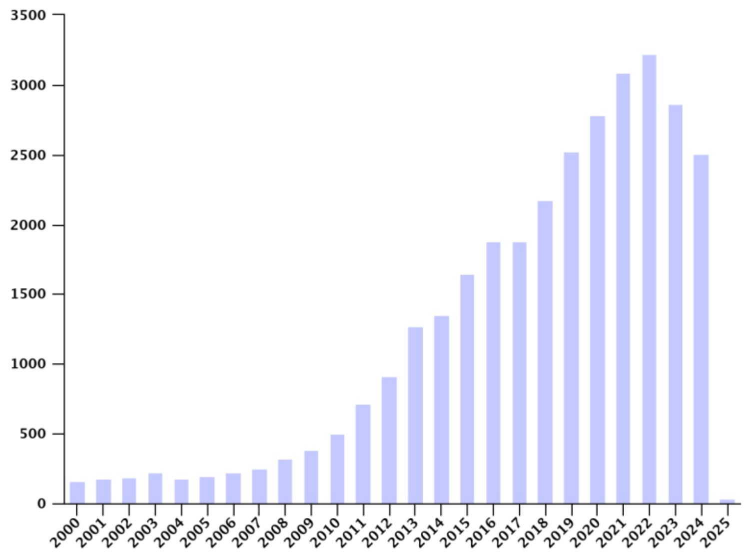

Figure 1. The quantity of documents for microalgae over time graph on the website of web of science.

2.1. Optimization and innovation of microalgae culture technology

2.1.1. Application of microalgal growth promoting bacteria (MGPB)

The application of microalgal growth-promoting bacteria (MG PBs) in microalgal cultivation has attracted increasing attention. Pathom-aree et al. [1] introduced the recent updates and progress of microalgae growth-promoting bacteria for cultivation strategies, including MG PBs for microalgae cultivation through a co-cultivation strategy, MG PBs include actinomycetes and non-actinomycetes that effectively promote the growth of microalgae by providing nutrients, regulating pH and producing growth factors, and the microalgae-MGPB co-culture system shows great potential in biomass production and wastewater treatment. Synthetic biology and metabolic network analysis provide new perspectives for optimizing these systems to further improve biomass production and biocomponent accumulation in microalgae.

2.1.2. Light quality optimization

Optimizing light quality is an effective way to promote biomass yield and biocomponent accumulation of microalgae at a low cost. Blue and red light are considered as the key spectra, and the spectral components of sunlight light are not fully utilized by microalgae. Therefore, Hong et al. [2] proposed the use of dyes, plasma scattering and carbon-based quantum dots to regulate light quality to improve the growth efficiency and quality of biological components of microalgae. Singh et al. [3] studied that LED provided continuous illumination in microalgae culture and optimized the design of photobioreactor. Although the cost was high, the cost could be reduced by means of control system and photovoltaic power supply. Mao et al. [4] investigated the use of C1 and C2 products generated by artificial photosynthesis to enhance the growth and production of pigments, starches and lipids in Chlorella pyrenoidosa, and the bioconversion of these C1 and C2 products into high-value bioproducts by the mixotrophic microalga Chlorella pyrenoidosa.

2.1.3. Waste water utilization

As nutrients and water sources, wastewater not only reduces production costs, but also promotes the recycling of resources. Pereira et al. [5] reviewed microalgae cultivation using wastewater, including production strategies and accumulation of valuable compounds and discussed emerging harvesting technologies which provides a reference for industrial production of microalgae. Alavianghavanini et al. [6] have studied that microalgae can effectively use nutrients in agricultural wastewater to grow, reduce nitrogen and phosphorus concentrations, and produce valuable biomass products at the same time. Malla et al. [7] presented information on the potential of microalgae-microbiome as a sustainable agent for wastewater and discussed microbiome engineering approaches to develop microalgae-bacteria partners for carbon sequestration and nutrient recovery. Shafiq et al. [8] studied an isolated Chlorella sp. cultivated to treat saline organic-rich fermentation effluent and concluded that mixotrophic cultivation has potential for treating high-saline organic-rich effluent as well as for producing biomass.

2.1.4. Biofilm reactor

Assis et al. [9] studied a hybrid system combining microalgae biofilm reactor (BRs) with conventional systems to optimize biomass production and overcome harvesting problems. The environmental impact was assessed using different support materials and found to be smaller for nylon and polyester. This study provides a new technical path for the cultivation of microalgae and helps to solve the harvest problems in the industrial production of microalgae.

2.1.5. Improvement of culture technology

Rozenberg et al. [10] have studied the acquisition of strains with novel properties or techniques to improve the robustness or efficiency of production. Improvements in light, nutrient delivery, and photobioreactor design have significantly increased the average productivity of microalgae. Interactions with specific bacteria may promote the growth of microalgae, but contamination needs to be effectively managed. In addition, the study of Kong et al. [11] showed that mixed microalgae and microalgae-bacteria combination had significant advantages in CO_2 fixation, wastewater treatment and production of high value-added products, which provided the possibility for diversified application of microalgae. Xu et al. [12] comprehensively evaluated the current advancements in scale-up of microalgae cultivation and its applications and evaluated the economic viability of microalgal CCU within the carbon market.

2.2. Application of emerging technologies in microalgal culture

2.2.1. Machine learning

Syed et al. [13] reviewed a variety of mechanistic and machine learning approaches for modeling and optimization of microalgal processes, covering the bioenergy, pharmaceutical and food industries. This study provides theoretical basis and technical support for the intelligent management of microalgae cultivation process.

2.2.2. Genetic engineering

Coleman et al. [14] studied genetic engineering and techniques such as random mutagenesis and genetic engineering, which significantly increased lutein production in microalgae. Through genetic engineering technology, the genetic characteristics of microalgae can be targeted to improve the content of biological components and the yield of bioactive substances.

2.2.3. Nano material

Sumathi et al. [15] studied that nanomaterials can significantly increase biomass and product yield of microalgae, and improve nutrient absorption and stress tolerance. Yuan et al. [16] studied that nanomaterials played an important role in enhancing the growth of microalgae and the production of metabolites, while assessing the effect of nanomaterials residues on the functional properties of metabolites.

2.3. Applications of microalgae in medical and biocomposite materials

2.3.1. Medical applications

The application of microalgae in the medical field has attracted more and more attention. For example, Díaz et al. [17] proposed that microalgae such as Chlorella have been incorporated into hydrogels to enhance their mechanical properties and reduce the production of reactive oxygen species (ROS), showing promise for cardiovascular tissue engineering. In addition, Caprio et al. [18] showed that defatted microalgae treatment with Alcalase shows promise for integrating the production of starch with that of nutraceuticals/biostimulants.

2.3.2. Biocomposite materials

Ren et al. [19] developed UV-responsive microcapsules to improve asphalt's self-healing. These microcapsules have a urea-formaldehyde shell with BP/DA and microalgae bio-oil as the core. They convert solar energy to thermal energy, aiding asphalt's proactive self-healing. Dimitriades-Lemaire et al. [20] developed a method to extract and purify starch from microalgae. The extracted starch was processed into TPS, which had similar properties to plant starch but smaller granules. TPS from microalgal starch was softer and more ductile than commercial TPS. Israel Kellersztein et al. [21] introduced a sustainable framework to fabricate natural biocomposites using Chlorella vulgaris microalgae as the matrix and resulted in lightweight materials with significant bending stiffness and thermal insulation capabilities. Agbo et al. [22] found that a bio-binder derived from microalgae can enhance the thermal stability and mechanical properties of epoxy resins, surpassing traditional methods and their study showed that epoxy-based composite materials made with this bio-binder have improved thermal and mechanical performance. Borah et al. [23] discussed microalgae's potential to be a sustainable and renewable resource for bio-based products.

2.3.3. Extraction of microalgae starch and preparation of bioplastics

Wiktoria et al. [24] developed processes to refine microalgal starch and create bioplastics, with chitosan enhancing mechanical properties, and demonstrated that using defatted algae or purified starch could yield films with tailored characteristics such as rigidity and water stability, depending on the biorefinery route applied.

2.3.4. Microalgae as a sustainable biofuel source

Hoang et al [25] presented advances in the production of biofuels from microalgae and solutions for industrial-scale production. Siddiki et al. [26] discussed biorefinery concept for sustainable processing of microalgae biomass, reviewed reactor technologies for cultivating microalgae and factors affecting the microalgae culture system, and presented different technologies to convert microalgae into biofuel and value-added products. Esipovich et al. [27] reviewed 10 years of research on the lipid-enriched microalga Chlorella for various applications, including transesterification to biodiesel and thermochemical conversion to bio-oil. Wang et al. [28] introduced scaling up of microalgae cultivation systems, along with cultivation modes and limiting factors and addressed microalgal lipids and related biosynthesis, harvesting and dewatering of microalgae, and extraction and transesterification of oil. Falfushynska [29] provided an overview of current cultivation and harvesting methodologies for microalgae, with a focus on their application in biofuel production, particularly highlighting sustainable aviation fuel and biohydrogen.

On the website of Web of Science, by searching with "microalgae", topic map for microalgae is shown in **Figure 2**. **Figure 2** shows the relationship between topics of microalgae, determined by documents covering the same topics and the size of the bubble indicates how many documents are on this topic while the arrows show which topics reference each other. **Figure 2** helps to see the big picture, explore more specific topics and learn terminology in the field of microalgae. On this basis, in this part, 29 references are elaborated. Among them, 12 references are studies on optimization and innovation of microalgae culture technology,4 references are on application of emerging technologies in microalgal culture and 13 references are onapplications of microalgae in medical and biocomposite materials.

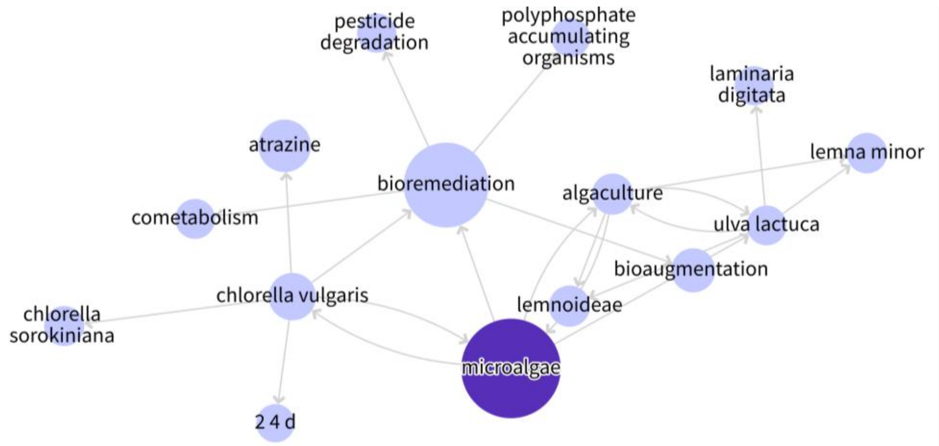

Figure 2. The relationship between topics of microalgae on the website of web of science.

3. Exploring the mechanical properties of microalgal cells and their application in growth regulation and breeding

3.1. Microalgal cell mechanical properties characterisation

In order to understand the behaviour of microalgal cells, it is key to measuring cell stiffness, adhesion and deformability while doing research on the mechanical properties of microalgal cells. The work of measuring these parameters provides the basis for microalgal screening, breeding and cultivation and helps to reveal the response mechanism of microalgal cells.

Cell stiffness is a key indicator of a cell's ability to resist deformation under external forces. Atomic force microscopy (AFM) or micropipette aspiration techniques could be used for measuring the stiffness of microalgal cells. A tiny probe or pipette is used to apply a controlled force to the cell and calculate its stiffness by observing the degree of deformation. The results show that in the stiffness of cells from different types of microalgae, there are significant differences and the differences may be related to their cell wall structure, cytoplasmic composition and growth environment. In fact, the cells of some polysaccharide-rich microalgae are relatively stiff, while those of protein-rich microalgae may be relatively soft.

Adhesion is a key parameter in the interaction between cells or between cells and the substrate. The adhesion strength of microalgal cells can be measured by using a cell adhesion tester. During cultivation, cells with stronger adhesion are easier to form stable community structures and facilitate biomass accumulation. During harvesting and processing, the differences in adhesion would affect the behaviour of microalgal cells, such as cell separation efficiency and product purity.

Deformability is the ability of cells to change shape when subjected to external forces. Flow cytometry and optical trapping can monitor microalgal cell deformation in real time, revealing significant differences between types. Subjected to external forces, showing high elasticity, some cells can quickly return to their original shape and other cells can undergo permanent shape changes or even rupture. The deformability difference may be related to the physiological state of the cell, the fluidity of the cell membrane and the structure of the cytoskeleton.

In short, measuring parameters can help people understand the mechanical properties of different microalgal cells and the parameters includes cell stiffness, adhesion and deformation capacity, etc. Changes in these parameters can reflect the diversity and functionality of microalgal cells and help to screen, cultivate and optimize microalgae.

3.2. Effect on microalgae growth of the mechanical environment

3.2.1. Lighting, temperature, fluid shear and other mechanical control factors

Light, temperature and mechanical factors such as fluid shear influence the growth and metabolism of microalgae and **Table 1** shows the regulatory effects of light, temperature, fluid shear stress and other mechanical factors. In order to optimize the growth environment and improve the productivity of microalgae, appropriate light intensity, temperature and fluid shear stress conditions should be selected according to the specific species and growth stage.

Table 1. The regulatory effects of light, temperature, fluid shear stress and other mechanical factors.

Factor of mechanics		Role of regulation
Light	Photosynthesis	Microalgae use photosynthesis to produce organic matter and oxygen. Light intensity affects photosynthesis and growth.
	Effect of light quality	The wavelength of light affects microalgae growth and metabolism. Blue and red light stimulate growth, while green light inhibits it. Light regulates synthesis of essential biological components.
	Period of light	Light needs of microalgae are regular, and too strong and too long light is unfavorable to the growth of microalgae. It is very important to control the light time reasonably.
Temperature	Enzyme activity	Microalgal enzymes are temperature-dependent. Optimal growth and activity occur at suitable temperatures, whereas high and low temperatures inhibit growth.
	Metabolism of cells	Temperature affects microalgae metabolism, nutrient utilisation and macromolecule synthesis/degradation.
	Optimum temperature range	The optimum temperature of different microalgae is different, such as 20–25 °C for Marine microalgae and different for freshwater microalgae. The appropriate temperature should be selected according to the species.
Fluid shear force	Morphology of cells	Fluid shear stress affects microalgal cells: appropriate stress helps growth and uptake, but high stress can damage or kill cells.
	Transfer of nutrients	Shear stress enhances microalgal growth by facilitating nutrient and oxygen transfer. Optimise growth by reducing light differences and preventing dark areas.
	Effect of aeration	Fluid shear stress is common in microalgae cultivation, and aeration is a common method. Optimising aeration is critical. Controlling intensity and time can improve productivity, but excessive intensity will damage cells.

Photosynthesis enables autotrophic organisms to convert inorganic carbon into carbohydrates. The literature extensively covers the molecular mechanisms that regulate carbon uptake, assimilation, and storage. Launay et al. [30] found a correlation between enzyme locations in cells and the molecular details of biochemical pathways. Perez-Garcia et al. [31] detailed four modes of microalgae cultivation—photo-autotrophic, heterotrophic, photo-heterotrophic, and mixotrophic, as presented in **Table 2**.

Table 2. Energy source, carbon source and Light availability requirements in phototrophic, heterotrophic, and mixotrophic mode of cultivation of microalgae [31].

	Energy source	Carbon source	Light availability requirements
Photo-Autotrophic	Light	Inorganic	Obligatory
Heterotrophic	Organic	Organic	No requirements
Photoheterotrophic	Light	Organic	Obligatory
Mixotrophic	Light and organic	Inorganic and organic	No obligatory

3.2.2. Relationship between mechanical factors and microalgae growth rate and biomass accumulation

Firstly, appropriate mechanical conditions, such as suitable fluid shear force and agitation speed, have a positive effect on the growth of microalgae. These conditions help the microalgae cells to remain dispersed in the culture medium and thus are able to absorb light and nutrients more efficiently. At the same time, appropriate mechanical stimulation can also promote the transfer of nutrients and oxygen in the culture medium to the cell surface of microalgae, improve their metabolic activity, and then accelerate the growth rate and biomass accumulation.

However, excessive mechanical conditions may cause damage to microalgae cells. For example, excessive shear force and stirring speed may lead to cell membrane rupture, cell structure destruction, etc., which may affect the normal growth and reproduction of microalgae. The damage would reduce growth rates of microalgae and decrease the biomass accumulation of microalgae.

Mechanical factors are one of the factors that influence microalgal growth and environmental factors such as light, temperature and nutrient concentration are also factors of the growth rate and biomass accumulation of microalgae. The growth rate and biomass accumulation of microalgae can be increased by optimizing the mechanical conditions.

3.3. Advancing microalgae breeding and trait enhancement through high-throughput screening and biomechanical optimization

3.3.1. Application of high-throughput screening techniques in microalgae breeding

The application of high-throughput screening techniques in microalgae breeding is described in **Table 3**.

In practice, high-throughput screening techniques have been successfully applied to breeding microalgae for carbon sequestration. The carbon sequestration ability of microalgae can be significantly improved by mutagenesis and modification of microalgae cells and high-throughput screening technology to domesticate the key genes of carbon fixation enzymes in microalgae cells. This technology not only helps to solve the problem of greenhouse gas emission reduction, but also provides a new direction for the development of microalgae industry.

In summary, high-throughput screening technology has broad application prospects and important value in microalgae breeding. With the continuous development and improvement of the technology, it is believed that it will play a greater role in the field of microalgae breeding.

Table 3. Application of high-throughput screening (HTS) techniques in microalgae breeding.

Improve screening efficiency	HTS technology processes many microalgae samples at once, making the screening process more efficient. This technology can quickly screen microalgae with superior characteristics.
Excellent algal strains were selected	HTS technology identifies microalgae characteristics. Mutagenesis breeding generates mutants selected using high-throughput screening technology. These strains are valuable in subsequent breeding and production cycles.
Analyze the relationship between gene and characte	HTS technology speeds up microalgae breeding and helps researchers understand microalgae, improving breeding and supporting the industry.
Promote the development of microalgae breeding research	HTS technology improves microalgae breeding research, driving innovation and progress in the microalgae industry.

3.3.2. Correlation analysis between mechanical properties and superior traits of microalgae

The correlation between the mechanical properties of microalgae and their ideal characteristics, such as high growth rates and high protein content, is a complex issue that is worth exploring.

Mechanical properties have a significant effect on the growth of microalgae. Appropriate fluid shear stress not only promotes the dispersion of microalgae cells, which is beneficial for the full absorption and utilisation of light and nutrients, but also improves the efficiency of nutrient and oxygen transfer from the medium to the surface of the microalgae, thereby increasing the growth rate and metabolic activity of the microalgae. At the same time, this shear force helps to reduce the difference in light intensity in the water body and prevent the microalgae from suffering from the self-shading effect due to long-term exposure to dark areas in the reactor. However, excessive shear stress may cause damage to the microalgae cells, including cell membrane rupture and destruction of cell structure, resulting in reduced growth rate or even death. In addition, through stirring and mixing, the microalgae cells can be evenly distributed in the culture medium, avoiding uneven growth and further improving the mass transfer efficiency, ensuring that nutrients and oxygen are quickly transported to the surface of the microalgae cells.

The correlation between the mechanical properties of microalgae and their superior characteristics is reflected in many ways. The growth rate of microalgae can be increased under appropriate mechanical conditions, such as appropriate fluid shear and agitation rates because these conditions can help microalgae cells to better absorb and utilise nutrients. While the growth rate of microalgae can be inhibited or even stopped under adverse mechanical conditions, such as excessive shear and rapid agitation because these conditions can cause damage to the microalgal cells, preventing their normal growth and reproduction. By promoting the synthesis and accumulation of intracellular proteins, mechanical stimuli, especially appropriate fluid shear stress, can affect the metabolic pathways and biosynthetic processes of microalgal cells and increase protein content. Microalgal cells may initiate repair mechanisms to restore cell structure and function when they are damaged, for example by excessive shear stress. Although this process may involve the synthesis and accumulation of a large number of proteins, thereby increasing the protein content to some extent. But this improvement may be temporary and at the expense of cell damage.

In practical applications, it is key to optimise the culture conditions which includes adjusting various parameters according to the species and growth requirements of the microalgae and echanical conditions in order to get high growth rates and high protein content. With appropriate mechanical stimulation to promote microalgae growth, it is necessary to avoid damaging the microalgae cells due to excessive shear stress and stirring speed.

4. Study of mechanical factors in microalgae harvesting, extraction and optimization of product quality

Efficient harvesting and extraction are crucial in microalgae processing, and mechanical optimisation is key. In microalgae processing, separation efficiency can be improved, energy consumption can be reduced and product recovery can be maximised by adjusting equipment parameters such as speed, pressure and frequency. The yield and quality of microalgae can be improved by choosing harvesting methods and time. Cell disruption, extraction yield and purity are key indicators of cell disruption during extraction.

4.1. Mechanical optimisation for harvesting and extracting microalgae

In harvesting and extracting microalgae, mechanical optimisation plays an important role. The separation efficiency of the microalgae cells can be improved by designing and adjusting the operating parameters, such as speed, pressure and frequency.

4.1.1. Selecting and optimising harvesting times and methods

The harvesting time of microalgae can be affected by many factors, including their growth cycle, biomass accumulation and the content of the target product. The yield and quality of the microalgae can be improved by selecting the optimal harvesting time. **Table 4** shows how to select the optimal harvesting time for microalgae.

Table 4. How to choose the best harvest timing for microalgae.

Growth cycle monitoring	The growth curve of microalgae was closely followed to understand the time point at which it reached its maximum biomass. The growth status of microalgae was determined by microscopic observation, cell counting or biomass determination.
Accumulation of target products	According to the accumulation rule of target products (such as oil, protein, etc.), the best harvest time was selected. The content of the target product can be monitored in real time by means of chemical analysis or biosensors.
Environmental Considerations	The effects of environmental factors such as light, temperature and nutrient concentration on the growth and accumulation of target products of microalgae were considered. Harvest was performed at a time point when environmental factors were suitable, microalgae were growing vigorously and the target product content was high.

The harvesting methods of microalgae mainly include centrifugation, filtration, and ultrasound. Selection of appropriate harvesting methods can improve harvesting efficiency and product quality. The principles, advantages and optimizations of the centrifugation, filtration and sonication methods are described in **Table 5**.

Table 5. Principles, advantages, and optimizations of centrifugation, filtration, and sonication.

Methods	Principle	Advantages	optimization
Method of centrifugation	Centrifugal force separated the microalgae cells from the culture medium.	Suitable for processing a large number of microalgae culture medium, can obtain relatively pure algal cells.	Modifying the centrifuge medium improves the quality of the algae cells.
Method of filtration	Separation is achieved by trapping microalgae cells on the medium using a filter medium.	Simple operation, suitable for small - scale microalgae culture.	Selecting the right filter material, pore size, filtration pressure and other parameters improves filtration speed and purity.
Ultrasonic method	Ultrasound disrupts microalgae cell walls, releasing target products.	Suitable for microalgae species with thick cell wall, can improve the release rate of target products.	Modifying the ultrasound parameters enhances cell disruption and product release.

According to the species of microalgae, growth conditions and the nature of the target product, suitable harvesting methods can also be selected for combined application. On this basis, the process parameters of various harvest methods were carefully optimized, and the best combination of process parameters was determined through experimental verification and data analysis to improve harvest efficiency and product quality. In addition, in order to further improve production efficiency and product quality, automation and intelligent technology are introduced to realize the continuous and automated microalgae harvesting process. By integrating sensors, controllers and actuators, the parameters of the harvest process are monitored and controlled in real time to ensure the efficient and stable operation of the production process.

4.1.2. Cell fragmentation during extraction: mechanical mechanism and efficiency assessment

Table 6. Physical and chemical methods for the source of external forces on cell breakage.

Methods	Description
Mechanical shear force	Strong shearing forces are applied to microalgae cells using a blender, grinder or homogeniser, rupturing the cell walls and membranes.
	Mechanical shear force has the advantages of large processing capacity and easy operation, but it may cause certain damage to the target product.
Ultrasonic wave	Microalgae cells are subjected to cavitation, a process that uses ultrasonic energy to destroy cell walls and membranes.
	Ultrasonic crushing is efficient, uniform and easy to control. However, it is expensive and requires optimised parameters.
Chemical method	Using chemicals such as surfactants, organic solvents, or enzymes, the permeability of the cell wall is altered so that the cell contents are susceptible to exudation.
	Chemical cell disruption is selective and has minimal impact on the target product, but may introduce additional chemicals and reagents.
Method of physics	Freezing and thawing destroys the hydrophobic binding structure of the cell membrane and causes intracellular ice crystals that expand and rupture the cell.
	The operation of physical method is relatively simple, but it may take a long time, and the damage degree of cell structure is not easy to control.

Table 7. Index to evaluate the efficiency of cell fragmentation when processing microalgae.

Indicators	**Description**
Breakage rate	Fragmentation rate is the percentage of fragmented cells. It can be determined by microscopy, flow cytometry or colourimetry.
	Comminution rate affects the efficiency and purity of the target product. Optimising the grinding conditions can increase the grinding rate.
Extraction rate of target product	The yield of the target product is the proportion of the total extracted from broken cells.
	The extraction rate depends on the grinding process, solvent and conditions. Choose the right solvent and conditions to optimise the rate.
Purity of product	Purity is the level of impurities in a product. Purity affects quality and value. Purification techniques increase purity.

The mechanical mechanism of cell breakage is mainly influenced by the destructive effect of external forces on the cell membrane and cell wall. The external forces include physical and chemical methods. Some methods are described in **Table 6.** During microalgal processing, the evaluation of ell fragmentation is a critical step to ensure extraction process and product quality. **Table 7** show the index to evaluate the efficiency of cell fragmentation when processing microalgae.

4.2. Quality and mechanical properties of microalgae products

Microalgae quality is affected by mechanical properties and microalgae influence product quality. High-quality microalgae contain more protein and makes the product better.

Effect of mechanical treatment on microalgae quality

First, mechanical treatment will affect the lipid quality of microalgae. Ultrasonic waves and high-pressure homogenisation can increase oil and fat production by destroying microalgae cells, and increasing solvent permeability and oil and fat release rates. Liu et al. [32] ranked the ease of cell rupture by ultrasound treatment for various microalgae species as follows: Haematococcus pluvialis < Chlorella vulgaris ≈ Chlorella vulgaris < marine algae ≈ Chlorella pyrenoidosa ≈ Rhodophyta ≈ Isochrysis galbana (no cell wall) ≈ Haematococcus pluvialis (no clear cell wall) ≈ Spirulina platensis (as shown in **Figure 3**). Cell size and cell wall composition are key factors determining the efficiency of ultrasound in rupturing cells. In addition, Liao et al. [33] used ultrasound to simultaneously extract and modify microalgal starch. In addition, mechanical treatment may also affect the oxidative stability of microalgal oil, because ultrasound treatment can produce the highest total phenol yield and increase DPPH radical scavenging activity, thereby improving the oxidative stability of the oil.

Second, mechanical treatment can affect microalgae protein quality. Mechanical crushing releases microalgal proteins by breaking cell walls and the cellulose and starch in microalgae cell walls can reduce protein extraction efficiency. And chemical extraction combined with mechanical treatment is more effective. Enzymatic hydrolysis agents and mechanical treatment dissolve microalgae cell walls so as to improve protein extraction.

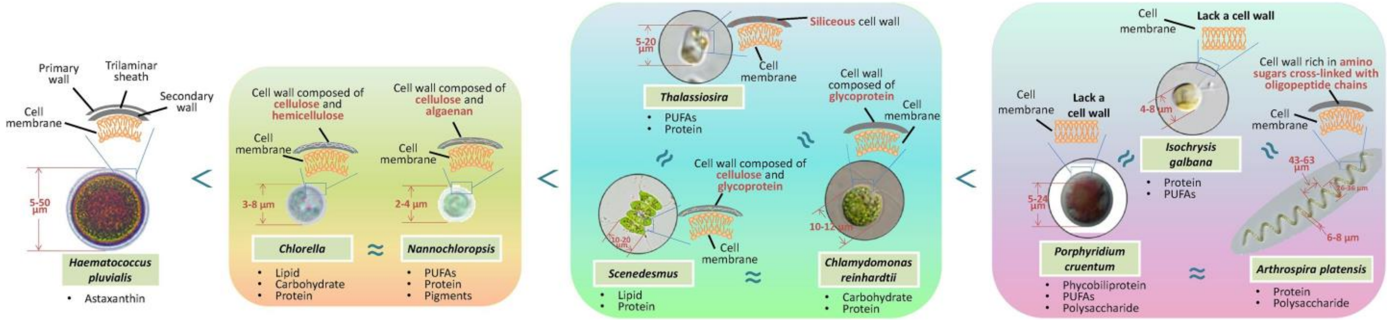

Figure 3. For the microalgal species, the ease of cell rupture by ultrasonic attack can be summarized in the following order: Haematococcus pluvialis < Chlorella ≈ Nannochloropsis < Thalassiosira ≈ Scenedesmus ≈ Chlamydomonas reinhardtii < Isochrysis galbana (lack a cell wall) ≈ Porphyridium cruentum (lack a well-defined cell wall) ≈ Arthrospira platensis [32].

Third, mechanical treatment can affect the quality of microalgal polysaccharides. As a major component of microalgal cell walls, microalgal polysaccharides have various biological activities. Microalgal polysaccharides are extracted by mechanical treatment to break down microalgal cells and release polysaccharides.

Mechanical treatment affects the purity, molecular weight and structure of microalgal polysaccharides, purified polysaccharides with low protein content and minimal polysaccharide loss could be obtained by the Sevag method combined with mechanical treatment.

In short, mechanical treatment affects the quality of microalgae products. The extraction efficiency and purity of microalgae product while enhancing quality can be optimised by controling mechanical treatment conditions and methods improves. In addition, mechanical processing may disrupt cell walls, releasing and contaminating other intracellular components and damaging microalgal products.

4.3. Safety and mechanical assessment of microalgae products

The source, production, components and risks of microalgae affect the microalgae product safety. Although mechanical treatments are not used as direct assessment indicators, they affect cell integrity and the release of compounds during processing and extraction. Inappropriate handling would release contaminants or destroy nutrients, reducing the product's safety and nutritional value. In order to ensure the treatment process is safe and controllable, mechanical treatment should be evaluated on the effect of on cell structure and composition.

4.3.1. Mechanical treatment for removing harmful substances from microalgae

Mechanical processing is important in producing microalgae products, particularly in removing harmful substances.

First, mechanical treatments destroy cell walls and membranes and would release beneficial ingredients and potentially harmful substances. Mechanical treatments include stirring, grinding and high-pressure homogenisation, ets. Mechanical treatments would exert a physical effect on the microalgae cells, destroy cell walls and cell membranes and release intracellular substances. This operation helps to extract beneficial ingredients from the microalgae and also helps to release and separate potentially harmful substances from the cells.

Second, mechanical treatment removes contaminants and debris from microalgae cells. Mechanical treatment effectively removes the contaminating organisms such as zooplankton, bacteria and viruses which adhere to the surface of microalgae cells during the cultivation of microalgae and improve the purity and safety of microalgae products.

In addition, mechanical processing can transform or degrade contaminants in microalgae products. For example, some harmful substances may undergo chemical changes and may be converted into harmless or low-toxic substances by mechanical forces. Mechanical processing can also promote the activity of antioxidant enzymes in microalgae cells and improve the cells' resistance to oxidative stress so as to reduce the harmful substances produced by oxidative stress.

However, it is worth noting that mechanical treatment, while removing harmful substances, may also have a certain impact on the beneficial ingredients in microalgae products. Therefore, in practical applications, factors such as the intensity and duration of mechanical treatment need to be considered comprehensively to ensure that while removing harmful substances, the beneficial ingredients in microalgae products are retained as much as possible.

In short, mechanical processing is effective in removing potentially harmful substances from the production process of microalgae products. Through reasonable mechanical processing parameters and process design, the safety and purity of microalgae products can be effectively improved, providing strong support for the development and application of microalgae products.

4.3.2. Application of mechanical factors in the process of disinfection and sterilization of microalgae products

Mechanical factors are useful in disinfecting and sterilising microalgae products. Treatments such as stirring, grinding and high-pressure homogenisation destroy microalgae cells, releasing contents and damaging structures. This inactivates or kills microorganisms. The specific application is as follows:

First, stirring. The shearing and impact forces generated by stirring can damage the integrity of microalgae cells, exposing harmful substances and microorganisms in the cells, which facilitates subsequent separation and removal. Stirring distributes disinfectants or biocides in microalgae products so as to improve disinfection and sterilisation.

Second, grinding. Grinding can make the microalgae cells becoming smaller and help to increase contact with disinfectants and improve efficiency. Grinding can also damage cell walls and membranes and help to remove contaminants.

Third, high-pressure homogenisation. High pressure homogenisation treats microalgae products.by using high pressure and shear forces. High pressure homogenisation can break cells and destroy the internal structure, disinfecting and sterilising so as to promote the release and distribution of nutrients and improve quality and nutritional value.

Mechanical processing has advantages and disadvantages. The advantages include that it doesn't need extra chemical disinfectants or sterilants and it is kinder to the environment and safer for humans. The disadvantages are that mechanical processing can destroy microalgae cells and make it easier to remove harmful substances and microorganisms, but also damage beneficial components.

In short, mechanical factors are broadly applied in the sterilisation of microalgae products. In practical applications, it is important to control treatment parameters and assess the impact on beneficial ingredients.

5. Case analysis

Chlamydomonas reinhardtii is easy to cultivate and a model for many biological processes, including photosynthesis, chloroplast biology, circadian rhythm systems, carbon concentration mechanisms and responses to environmental stress. This section researches into the cell biomechanics of *Chlamydomonas reinhardtii* by its cell structure and function, as well as its response mechanism to external forces.

5.1. Experimental equipment and materials

Table 8 describes the experimental equipment, materials required for this case, their functions and some equipment pictures.

Table 8. Experimental equipment, materials, functions and some equipment pictures.

Experimental equipment and materials	**Functions**	**Equipment pictures**
Flow cytometry	Used for cell sorting, enrichment and activity detection.	
Microscope: Inverted microscope or fluorescence microscope.	Used to observe cell morphology and fluorescence.	
Cell culture equipment: including shakers, centrifuges, petri dishes, etc.	Used for cell culture and pretreatment.	
Machining equipment: blenders, mills, high-pressure homogenisers, etc.	Used to apply mechanical stress to cells.	
Sorting system: WOLF G2 single cell flexible sorting system	Used for gentle sorting and enrichment of cells.	
Culture medium	TAP medium for *Chlamydomonas reinhardtii* growth.	

5.2. Methods and processes

5.2.1. Sample preparation and selection

Chlamydomonas reinhardtii was selected as the research sample and the sample was pretreated and screened by using technical means such as flow cytometry. *Chlamydomonas reinhardtii* CC-4533 (WT wild type) and CC-5416 (GFP) cells were used as samples to verify the gentle sorting capabilities of the WOLF G2 single-cell flexible sorting system.

(1) Cell culture. *Chlamydomonas reinhardtii* cells in the rapid growth phase were selected as the research sample and cultured in TAP medium until the logarithmic growth phase.

(2) Pretreatment. Impurities and dead cells in the culture medium were removed by centrifugation and washing.

(3) Screening. Cells with good activity were selected using a flow cytometer based on chlorophyll autofluorescence and other indicators.

5.2.2. Mechanical treatments

Mechanical treatments include stirring, grinding and high-pressure homogenisation used to simulate the response of cells to external forces.

(1) Stirring: The cells are stirred with a stirrer at different speeds and durations to simulate the effect of shear stress on the cells.

(2) Grinding: The cells are placed in a grinder and ground at different intensities and durations to simulate the changes in the cells when they are subjected to force.

(3) High-pressure homogenisation: The cells are subjected to high pressure using a high-pressure homogeniser to observe changes in the morphology and structure of the cells under high pressure.

After the mechanical treatments, the morphology of the green algae cells changed significantly. Wild type and GFP cells were mixed in different ratios, and the cells were sorted using the WOLF G2 system. The cells were observed and counted before and after they were sorted by flow cytometry and microscopy.

5.2.3. Observation and analysis

The cells were observed and analysed by a microscope, flow cytometer and other instruments to evaluate changes in cell morphology, structure, activity and other aspects.

(1) Morphological observation: Observe the morphological changes of the treated cells, such as cell size, shape and cell membrane integrity by a microscope.

(2) Viability test: Evaluate the chlorophyll autofluorescence and other indicators by flow cytometry so as to judge the cell viability.

(3) Fluorescence property analysis: Observe the fluorescence intensity of GFP for GFP-expressing cells by a fluorescence microscope to assess cell viability and expression levels. Mechanical treatment has a certain effect on the cell viability of green alga by detecting chlorophyll autofluorescence and other indicators. Cells can maintain a highly active state under appropriate mechanical treatment conditions. But excessive mechanical treatment can cause cell death or a decrease in viability.

5.2.4. Sorting and enrichment

Cells with specific characteristics can be isolated for subsequent research and applications by detecting indicators such as chlorophyll autofluorescence and GFP (green fluorescent protein).

(1) Flow cytometry separation: Cells with specific characteristics are isolated and enriched by flow cytometry based on indicators such as chlorophyll autofluorescence and GFP fluorescence.

(2) Single-cell sorting: The WOLF G2 single-cell flexible sorting system was used to sort single cells into 96-well plates for subsequent research.

Before sorting, the proportion of GFP expression in the green algae sample ranged from 10% to 50%. A total of five experiments were conducted to verify the enrichment effect of the WOLF G2 on samples with different target cell ratios. Chlorophyll autofluorescence (FL5; B706) and GFP (FL1; B525) to distinguish viable Chlamydomonas campestris cells [34]. The intensity of chlorophyll autofluorescence signal is a direct reflection of algal cell viability, and usually WOLF G2 single-cell flexible sorting system serves Chlamydomalga signal higher than 104 decade indicates viable cells, while low signal lower than 104 decade indicates stressed/dead cells.

5.3. Experimental parameters and reproducibility

(1) Cell concentration: Consistent cell concentration was ensured for each experiment to improve reproducibility of the experiment.

(2) Mechanical treatment conditions: Parameters such as stirring speed, grinding strength, and high pressure homogenization pressure were recorded in detail so as to be consistent across experiments.

(3) Flow cytometer Settings: The laser intensity, detection wavelength, and gating conditions of the flow cytometer were fixed to ensure data accuracy.

(4) Culture conditions: Conditions such as culture temperature, light intensity and light period were controlled to reduce the influence of the environment on the experimental results.

After three independent experiments, a large number of GFP-positive *Chlamydomonas reinhardtii* cells were successfully enriched. The enriched cells maintained high activity and successfully grew into monoclonal cells.

Researchers from TongTeng Ruijie (Shanghai) Biotechnology Co., Ltd. carried out five independent experiments within a period of three weeks to enrich a large quantity of GFP-positive Chlamydomonas lancefera. The gentle sorting capability of the sorting system was demonstrated by using Chlamydomonas reininatus as a sample. They utilized this system to separate individual Chlamydomonas reininatus into 96-well plates and successfully enriched target cells expressing GFP positivity. Five samples were prepared by blending CC-4533 (WT wild-type) and CC-5416 (GFP) cells together in different ratios. After three weeks of cultivation, these cells still maintained high growth activity and grew into monoclonal cells. This case study not only exhibited the potential application of the WOLF G2 system for cell sorting and enrichment of microalgae, but also verified the reliability and practicability of *Chlamydomonas reinhardtii* as a research model [34].

Cell biomechanics research is providing insights into the cell structure and function of *Chlamydomonas reinhardtii* and how it responds to external forces. Future research can further investigate how different mechanical treatments affect *Chlamydomonas reinhardtii* cells and explore ways to optimise the production and application of microalgae products.

This section looks at how external forces affect the cell structure and function of *Chlamydomonas reinhardtii*. It explores changes in morphology, viability, classification and enrichment techniques and their practical application. These results deepen the understanding of the biomechanical response of Rhinophylla.

6. Conclusion

This research provides an examination of the mechanical properties of microalgal cells and their multifaceted impact on growth regulation, reproduction and ultimately product quality. The study of the mechanical parameters of microalgal cells deepens the understanding of the mechanisms by which microalgal cells respond to different mechanical environments. The Changes in these parameters reflect the structural and functional diversity of microalgal cells and provide a scientific basis for the screening, breeding and optimisation of culture conditions.

Mechanical factors such as light, temperature and fluid shear play an important regulatory role in the growth and metabolism of microalgae in exploring the growth regulation of microalgae. By appropriately regulating these factors, the growth environment of microalgae can be optimised to improve its productivity and biomass accumulation. And the application of high-throughput screening technology in microalgae breeding provides a tool for targeted improvement of desirable traits in microalgae.

The research analyzes the mechanical treatment in microalgae harvesting, extraction and product quality optimisation in terms of microalgae processing and product quality optimisation. The separation efficiency can be improved, energy consumption can be reduced, and the product recovery rate can be maximised by optimising the parameters of the cutting equipment. The paper also focused on the impact of mechanical treatment on the quality of microalgae products and found that proper mechanical treatment can improve the extraction efficiency and purity of microalgae oil, protein and polysaccharides, while excessive treatment may adversely affect product quality.

Through the case study of *Chlamydomonas reinhardtii*, the study validated the significance of mechanical properties in microalgal cell structure and function. These results not only provide a novel perspective and method for the in-depth study of microalgae but also lay a solid foundation for the sustainable development of the microalgae industry.

However, this study also has certain limitations. For instance, for the characterization of the mechanical properties of microalgae cells, although the paper has described some advanced techniques, more parameters and indicators still need to be further explored to reveal the mechanical behavior of microalgae cells more comprehensively. In addition, in terms of microalgae processing and product quality optimization, it is necessary to further investigate the specific mechanisms of the influence of different mechanical treatment methods on the structure and function of microalgae cells to achieve more precise control and optimization.

Looking ahead to the future, the further research should be conducted in the following aspects: Firstly, to strengthen the multi-dimensional characterization of the mechanical properties of microalgae cells and explore more factors affecting the mechanical behavior of microalgae cells; Secondly, to further study the mechanism of mechanical treatment on the structure and function of microalgae to provide a theoretical basis for the customized production of microalgae products. Finally, to promote the integration and innovation of the microalgae industry and advanced technology to accelerate the industrial application process of microalgae research results.

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