MICRO-NANOBUBBLE TECHNOLOGY IN MICROALGAE CULTIVATION: A COMPREHENSIVE OVERVIEW

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Abstract - Microalgae cultivation is currently constrained by two major techno-economic bottlenecks: poor gas-liquid mass transfer and high harvesting costs. This review examines micro-nanobubble (MNB) technology as a strategic solution to address these barriers. Owing to characteristics such as high internal pressure, prolonged residence time, and colloidal stability, MNBs significantly enhance the volumetric mass transfer coefficient ($k_L a$) while minimizing hydrodynamic shear stress. Case studies demonstrate that MNBs not only increase biomass productivity but also facilitate efficient flotation-based harvesting with low energy consumption (below 0.5 $kWh \cdot kg^{-1}$ of dry biomass), while simultaneously improving nutrient removal in wastewater. Despite remaining challenges in scaling up, this technology offers a promising pathway for integration into circular economy models, driving the commercialization of microalgae for biofuels and high-value products.

Key words - Micro-nanobubble technology; microalgae biotechnology; gas mass transfer; nanobubble flotation; wastewater bioremediation

1. Introduction

Microalgae are versatile bio-resources with applications in carbon sequestration and wastewater treatment, biofuel production, and high-value products for food, health and biobased industries [1]-[3]. Their high lipid content, rapid growth, and ability to grow on non-arable land or wastewater make them attractive feedstocks for third-generation biofuels and integrated biorefineries [1]-[6]. Despite this potential, the transition from laboratory scale to industrial commercialization is still hindered by two fundamental techno-economic barriers: (i) limited gas–liquid mass transfer under conventional macro-bubble aeration, leading to poor CO_2 utilization; and (ii) high harvesting costs, particularly for small-sized microalgae such as *Chlorella* [3], [7], [8].

First, in dense cultures, the supply and utilization of dissolved CO_2 and O_2 are often rate limiting, especially in closed photobioreactors (PBRs) where gas liquid interfacial area is restricted [9] - [11]. Conventional coarse-bubble aeration exhibits low volumetric mass transfer coefficients ($k_L a$), short bubble residence times and high gas losses to the headspace, so a significant fraction of sparged CO_2 never reaches the cells [5], [12]. Second, harvesting dilute, small-celled microalgae (<5 μm) is technically straightforward but economically challenging: centrifugation and membrane filtration can achieve high recovery, but typically consume 1÷8 $kWh \cdot kg^{-1}$ dry biomass or incur substantial fouling-related costs, which is prohibitive for bulk commodities such as biofuels [7], [8], [13], [14].

Micro–nanobubble (MNB) technology has emerged as a powerful process-intensification tool that directly targets both bottlenecks. By generating gas bubbles in the micro- to nanometer range, MNB systems provide exceptionally high interfacial areas and prolonged residence times, thereby improving gas transfer, mixing and, when coupled to flotation, biomass separation [15]-[17], [12], [18]. This paper first summarizes the physical fundamentals of MNB generation and behavior, then discusses applications in microalgae biotechnology for cultivation enhancement, advanced harvesting and dual-purpose wastewater treatment/ bio-stimulation, and finally highlights key challenges and future directions for industrial deployment.

2. Fundamentals of Micro-Nanobubble Technology

Micro-nanobubbles (MNB) are gas cavities dispersed in liquids with characteristic diameters ranging from tens of micrometers for microbubbles down to hundreds of nanometers for nanobubbles. Owing to their small size and interfacial characteristics, MNB exhibit physicochemical properties that are markedly different from conventional macro-bubbles and can be exploited to overcome mass-transfer and separation limitations in gas liquid systems [12], [18], [19]. A basic understanding of their generation mechanisms and core properties is therefore essential for rational design and optimization of MNB assisted microalgae cultivation.

2.1. Principle of MNB Generation

The generation of MNB requires external energy input to overcome liquid surface tension and fragment the gas phase into very fine bubbles [12], [18]. In practice, two main physical mechanisms are widely used in environmental and biochemical engineering:

(1) Pressurized dissolution (decompression method): Based on Henry's law, gas is first dissolved in the liquid under elevated pressure (typically on the order of 0.3–0.6 MPa). When the supersaturated liquid is released through a fine or specially designed nozzle into ambient pressure, the sudden pressure drop induces homogeneous and/or heterogeneous nucleation, leading to the formation of a dense population of micro- and nanobubbles [12], [18], [19].

(2) Hydrodynamic cavitation: Following Bernoulli's principle, hydrodynamic cavitation is generated when the liquid accelerates through constrictions such as Venturi tubes, orifices, or high-speed rotors. The associated local pressure drop and intense shear cause pre-existing bubbles

or dissolved gas pockets to grow and subsequently collapse or break up into smaller bubbles, thereby producing micro- and nanobubbles [12], [18].

2.2. Core Physicochemical Properties of MNB

MNB display several distinctive hydrodynamic and interfacial properties that underpin their technological advantages:

(1) Long residence time and apparent stability:

In contrast to macro-bubbles, which rise and disengage rapidly due to buoyancy, nanobubbles and the smallest microbubbles have very low terminal rise velocities; diffusion and Brownian motion become important, allowing them to remain suspended for hours to weeks under quiescent conditions [18], [19]. This extended residence time greatly increases the effective gas–liquid contact time and overall gas utilization.

(2) High specific surface area and enhanced mass transfer:

Because of their very small diameters, MNB provide an exceptionally high gas–liquid interfacial area per unit volume. This increases the volumetric mass-transfer coefficient ($k_L a$) and can significantly improve the dissolution efficiency of sparged gases such as CO_2 and O_2 compared with conventional coarse aeration [18], [19].

(3) Negative zeta potential and colloidal stability:

The gas–liquid interface of MNB generally carries a negative zeta potential, with typical absolute values in the range of a few tens of millivolts depending on gas type, pH, and ionic strength. This electrostatic repulsion between bubbles suppresses coalescence and aggregation, contributing to their unusual stability in aqueous media [18], [19]. In microalgae processes, this surface charge can be exploited during harvesting, for example by using cationic coagulants or flocculants to bridge negatively charged bubbles with negatively charged algal cells and promote flotation.

(4) Self-pressurization and high internal pressure:

The internal pressure P_{in} of a bubble is higher than the ambient pressure P_{out} and increases with decreasing radius r according to the Young–Laplace equation:

$$P_{in} = P_{out} + \frac{2\sigma}{r},$$

where σ is the surface tension.

According to this equation, nanobubbles possess immense internal pressures (several tens of atmospheres) that provide a strong thermodynamic driving force for rapid gas dissolution, theoretically leading to bubble collapse within microseconds [19]. However, experimental observations confirm their stability for weeks, creating the 'Epstein-Plesset paradox' [20]. This hyper-stability is attributed to an ion shielding mechanism, where the negative zeta potential attracts counter-ions to form a dense Electrical Double Layer (EDL) acting as a diffusion barrier, alongside interfacial hydrophobic impurities that reduce the effective surface tension [21].

(5) Free-radical generation upon collapse:

The adiabatic collapse or shrinkage of microbubbles can generate highly localized temperatures and pressures, leading to the formation of reactive oxygen species (ROS), particularly hydroxyl radicals (•OH), even in the absence of strong external dynamic stimuli such as ultrasound. Takahashi *et al.* demonstrated that collapsing microbubbles (<50 μm) in water generate •OH radicals, which can act as powerful oxidants for degrading organic pollutants and inactivating microorganisms [22].

These fundamental properties, extended residence time, high interfacial area, electrostatic stabilization, elevated internal pressure, and potential for in situ ROS formation, collectively underpin the growing use of MNB to intensify gas liquid mass transfer, enhance microalgal CO_2 uptake, and enable energy efficient harvesting and water treatment processes.

3. Application of MNBs technology in Microalgae Biotechnology

The integration of Micro-nanobubble (MNB) technology spans the entire value chain of microalgae production, from upstream cultivation to downstream processing and environmental remediation.

3.1. Enhancement of Cultivation

In cultivation, micro-nanobubbles (MNB) primarily enhance gas–liquid mass transfer, improve hydrodynamics and nutrient distribution, and open opportunities for more energy-efficient and cost-effective operation in high-density microalgae systems [1]-[6], [15]-[17].

3.1.1. Optimizing Gas Mass Transfer

In dense microalgal cultures, the supply and utilization of dissolved CO_2 for photosynthesis and O_2 for respiration are frequently the primary rate-limiting steps, particularly in closed photobioreactors where gas–liquid interfacial area is restricted [9]-[11]. Conventional coarse-bubble aeration often leads to low CO_2 utilization because millimeter-scale bubbles rise rapidly, exhibit short residence times, and disengage to the headspace before the dissolved inorganic carbon can be fully assimilated by the cells [10], [12], [18].

MNB directly address this bottleneck by substantially increasing the $k_L a$. For a given gas flow rate, decreasing bubble size from the millimetre to the micro- or nanometre range dramatically increases the specific interfacial area a, while the high internal pressure and long residence time of MNB favour sustained gas dissolution [12], [15]-[18]. Experimental studies in microalgal photobioreactors have shown that microbubble-based aeration can enhance CO_2 mass transfer and growth performance. For example, a microbubble-induced airlift loop bioreactor for *Dunaliella salina* achieved approximately 20–40% higher specific growth rates than conventional fine bubble aeration, closely correlated with increased CO_2 transfer [23]. In an oscillating-flow photobioreactor, reducing aeration bubble diameter increased $k_L a$ by about 25% and significantly improved biomass productivity [24].

For *Chlorella vulgaris*, optimized bubbling strategies that combine micro- and conventional bubbles have been reported to enhance early-stage growth and subsequent

lipid valorization, demonstrating that bubble size control can be used as an operational handle to tune both biomass production and metabolite accumulation [25]. Reviews on CO_2 MNB further highlight that the combination of high surface-area-to-volume ratio, self-pressurization (Young–Laplace effect), and extended lifetime leads to higher CO_2 solubility and longer CO_2 retention in the culture medium, resulting in a more stable inorganic carbon supply for photosynthesis [15]-[17]. However, most data are still on a bench or pilot scale.

In addition to enhancing CO_2 dissolution, microbubbles facilitate the removal of excess dissolved O_2, thereby mitigating inhibitory effects associated with oxygen supersaturation in high-density cultures [23], [26]. In microbubble-driven systems, simultaneous improvement in CO_2 uptake and O_2 stripping has been observed, contributing to more favorable gas regimes for sustained microalgal growth [16], [23], [26].

Under certain operating conditions, the collapse or shrinkage of micro- and nanobubbles can generate reactive oxygen species (ROS), such as hydroxyl radicals (•OH) [22], [27]. While excessive ROS can cause oxidative stress, low and controlled ROS levels may act as physiological signals that influence growth or trigger the accumulation of storage compounds (e.g. lipids) relevant for biofuel applications. Any attempt to exploit this effect for process intensification in microalgal cultivation must therefore carefully balance ROS generation with cell tolerance [22], [27].

3.1.2. Improved Mixing and Nutrient Distribution

Beyond gas transfer, MNB modifies the hydrodynamic environment in ways that are advantageous for microalgal cultures. Owing to their small size and low terminal rise velocity, microbubbles induce gentle but pervasive micro-circulation in the liquid phase, promoting more uniform distribution of cells, dissolved gases, and nutrients without the need for intense mechanical agitation [15]-[18], [26]. This behavior helps to reduce sedimentation and prevents the formation of nutrient and light gradients that typically arise in poorly mixed photobioreactors [1], [3], [10].

Hydrodynamic studies in bubble-column and flat-panel photobioreactors show that controlling bubble size and dynamics can significantly improve mixing and biomass distribution. For example, the use of specially designed aeration devices with "bubble-cutting" elements reduced mean bubble size, decreased rise velocity, and extended bubble residence time, leading to a 33% increase in biomass productivity and a more homogeneous distribution of microalgae within the reactor column [28]. Similarly, micro-size bubble sparging in flat-panel photobioreactors has been reported to improve mixing performance and gas distribution, thereby enhancing CO_2 capture and microalgal growth compared with conventional sparger designs [16], [24], [26].

These hydrodynamic benefits are particularly important for shear-sensitive species such as dinoflagellates and filamentous cyanobacteria. Strong mechanical agitation or high gas entrance velocities in sparged reactors can cause cell deformation, membrane damage and lethal events [29], [30]. In contrast, MNB-based aeration provides more uniform gas–liquid contact and nutrient distribution at lower agitation intensities, creating a gentler environment that preserves cell integrity while maintaining adequate mass and momentum transfer [15]-[17], [26], [29], [30].

3.1.3. Energy Efficiency and Cost Reduction

A key motivation for integrating MNB technology into microalgae cultivation is the potential to increase process performance without proportionally increasing energy input. Because microbubbles and nanobubbles can deliver higher $k_L a$ at a given gas flow rate, equivalent or improved CO_2 transfer can often be achieved with lower gas throughput or reduced mechanical mixing, which directly translates into lower energy consumption for aeration and agitation [12], [18], [23], [31], [32].

Evidence from gas liquid mass-transfer applications indicates that microbubble generation, particularly when combined with energy-efficient devices such as fluidic oscillators, can significantly increase oxygen or CO_2 transfer efficiency while reducing specific energy demand [31], [32]. In microalgal systems, microbubble-driven photobioreactors have demonstrated enhanced growth and CO_2 biofixation with what has been described as relatively low energy cost compared with conventional bubbling strategies [23], [26]. Field scale demonstrations of microbubble generators for algae (e.g., "Almino" systems for raceway ponds) further suggest that substantial gains in biomass productivity and carbon utilization can be realized without expanding reactor footprint or proportionally increasing energy input [33].

When combined with optimized bubbling strategies for target strains such as *Chlorella vulgaris* including staged changes in bubble size or gas flow across different cultivation phases MNB technology offers a pathway to simultaneously maximize biomass productivity, improve CO_2 utilization efficiency, and reduce operational costs associated with gas supply and mixing [3], [11], [13], [17], [25]. This convergence of performance and efficiency is central to improving the techno-economic feasibility of large-scale microalgae production [1], [2], [4], [5], [14].

3.2. Advanced Harvesting

Harvesting constitutes a major techno-economic bottleneck in microalgae production, with dewatering and concentration steps often accounting for 20–30% of the total production costs [13], [14]. While centrifugation and membrane filtration can achieve high separation efficiencies, their energy demand and operational costs make them less suitable for large-scale, low-value applications such as biofuel production [7], [8], [13], [14]. Micro–nanobubble flotation (MNBF) has emerged as a promising alternative, leveraging the unique hydrodynamics and interfacial properties of MNB to improve separation efficiency while reducing energy consumption [7], [8], [12], [18], [34], [35].

3.2.1. Mechanism of Bubble–Cell Interaction

The efficiency of flotation harvesting is fundamentally governed by the probabilities of bubble–cell collision and

stable adhesion [7], [8], [34], [35]. Conventional Dissolved Air Flotation (DAF) systems, which generate macro-bubbles (>100 μm), often suffer from limited capture efficiency due to rapid buoyancy-driven rise velocities and insufficient contact time with microscopic algal cells [7], [8], [13], [14].

MNB possess diameters comparable to algal cells (2–20 μm). This size compatibility maximizes interception and collision probabilities consistent with Stokes-number considerations, significantly improving recovery rates compared to DAF systems [12], [18], [34]. Empirical studies by Hanotu et al. [34] demonstrated that microbubbles (10-80 μm) substantially improved algal recovery compared with conventional DAF-scale bubbles, largely due to increased bubble number concentration and more favorable bubble–cell size matching. Foam-flotation studies further highlight that appropriate cell bubble size ratios are critical for high harvesting efficiency [35].

Regarding surface modification, the natural negative zeta potential of both MNB and algal cells creates electrostatic repulsion that hinders adhesion [15]-[18], [22], [34]. To overcome this barrier, MNBF is often combined with low doses of cationic coagulants or bio-flocculants (e.g., chitosan, natural polymers), which partially neutralize surface charges and promote electrostatic bridging. Under optimized conditions, MNB act as "bridges" that link multiple algal cells and microflocs into larger, more buoyant aggregates, thereby enhancing flotation rates and improving harvesting efficiency [7], [8], [34], [35]. The critical review by Zhang and Zhang [35] emphasizes that the interplay between bubble properties (size, zeta potential, stability) and cell surface characteristics (charge, hydrophobicity, extracellular polymeric substances) is central to designing efficient foam or microflotation-based harvesting schemes.

3.2.2. Energy-Efficient and Comparative Techno-Economic Analysis

The most compelling advantage of MNBF is its drastically reduced energy footprint compared to conventional dewatering methods. While centrifugation provides robust separation, its high specific energy demand (1.4–8.0 $kWh·kg^{-1}$) renders it prohibitively expensive for bulk biofuel production [13], [14]. Similarly, membrane filtration incurs significant operational costs due to fouling and the need for chemical cleaning [7], [13], [14].

To quantify these differences, a comparative assessment is presented in Table 1. In contrast to conventional methods, MNBF achieves comparable harvesting efficiencies (>90%) with a specific energy consumption as low as ~0.005–0.075 $kWh·kg^{-1}$, which is approximately two orders of magnitude lower than centrifugation [34], [35].

Table 1. Comparative assessment of microalgae harvesting technologies based on energy consumption and efficiency

Harvesting Method	Recovery Efficiency (%)	Specific Energy (kWh·kg⁻¹ dry biomass)	Key Advantages	Key Limitations	Refs.
Centrifugation	> 95%	1.4 – 8.0	High reliability; Applicable to all strains; No chemicals required.	High energy cost; High capital/maintenance cost.	[13], [14]
Membrane Filtration	> 95%	0.2 – 1.0	Complete cell retention; High permeate quality.	Membrane fouling; Periodic chemical cleaning; Flux decline.	[7], [13]
Gravity Sedimentation	60 – 80%	< 0.1	Low energy; Simple operation.	Very slow (hours); Large land area; Biomass degradation.	[8], [13]
MNB Flotation (MNBF)	90 – 99%	0.005 – 0.075*	Low energy; Rapid separation (<20 min); High concentration factor.	Requires surfactants/coagulants; Dependent on pH and bubble size.	[34], [35]

Energy consumption for MNBF depends on the bubble generation method (e.g., fluidic oscillation vs. pressurized dissolution).

Beyond basic energy savings, advanced configurations further enhance economic viability. Hybrid frameworks coupling flocculation with low-pressure filtration have been reported to reduce energy inputs to <0.1 $kWh·kg^{-1}$ [36]. Additionally, implementing MNBF with fluidic oscillators eliminates moving parts, thereby minimizing maintenance costs compared to mechanical centrifuges [31], [32], [34]. Operationally, MNBF significantly outperforms gravity sedimentation by completing separation in minutes (<20 min) rather than hours, preserving intracellular metabolites like lipids and pigments from degradation [7], [8], [34]. These attributes position MNBF as a critical technology to resolve the harvesting bottleneck [1], [2], [4].

3.3. Dual-Purpose Applications: Wastewater Treatment and Stress Induction

Micro–nanobubbles (MNB) provide both physical mass-transfer enhancement and unique chemical reactivity, enabling simultaneous wastewater treatment and bio-resource recovery.

Chemically, the collapse of MNB generates localized energy and Reactive Oxygen Species (ROS), particularly hydroxyl radicals (·OH) [22], [27]. Takahashi et al. demonstrated ·OH production without external stimuli [22], while Liu et al. identified it as the dominant oxidative species [27]. This reactivity intensifies advanced oxidation and pollutant degradation in aqueous systems [15], [18], [37]-[39].

In wastewater-based cultivation, this underpins two key benefits. First, Ozone MNB (OMNB) achieve superior pathogen control compared to conventional ozonation by combining high mass transfer with ·OH generation [38]. Second, MNB accelerates the degradation of recalcitrant organics and reduces COD, although the specific contribution of physical versus chemical mechanisms remains under debate [37], [39]. This oxidation converts complex organics into bioavailable substrates, improving nitrogen and phosphorus removal via microbial uptake [4], [5], [7], [16].

Concurrently, MNB-induced redox perturbations act as controlled stress signals for high-value metabolite biosynthesis. In *Haematococcus* spp., astaxanthin accumulation is tightly linked to oxidative stress [40]. Zhu and Wakisaka confirmed that air nanobubbles enhance both biomass and astaxanthin content [41], supporting findings that tuned oxidative stress significantly improves yields [40].

Thus, oxygen- or ozone-based MNB serve a dual function: improving wastewater treatment and providing oxidative cues for pigment accumulation. However, practical applications require precise control of gas parameters to balance beneficial stress against the risk of irreversible oxidative damage [27], [37]-[41].

4. Discussion and Future Perspectives

The integration of Micro-nanobubble technology into microalgae biotechnology has transitioned from theoretical concepts to empirical validation. This section discusses the documented efficacy of MNB across the production chain and analyzes the critical technical hurdles that must be overcome for industrial adoption.

4.1. Empirical Evidence- What MNB Actually Deliver

Although experimental studies on MNB-assisted microalgae systems differ in strain, configuration and scale, their outcomes show a consistent pattern across cultivation, harvesting and wastewater-based applications. In most cases, micro- or nanobubble aeration leads to higher growth or biomass productivity, more efficient biomass recovery at lower specific energy consumption, and enhanced nutrient and organic pollutant removal in integrated treatment–production systems [7], [16], [23]-[26], [34]-[36], [41]-[43]. Table 2 summarizes representative case studies and highlights the magnitude of these improvements in a comparative framework

Table 2. *Representative empirical studies on MNB-assisted microalgae processes*

Case	Strain	System / Configuration	Observed gain in growth / biomass / metabolite	Energy aspect*	Key refs.
Cultivation enhancement	*Dunaliella salina*	Microbubble airlift loop bioreactor (ALB)	↑ specific growth by ~20–40%	Comparable or lower aeration power due to higher k_La	[23], [44]
Cultivation strategy	*Chlorella vulgaris*	Bubble-column PBR with optimized bubbling regime	↑ early-stage growth; ↑ lipid yield	No significant increase in aeration energy	[25]
Microflotation harvesting	Mixed microalgae	Microflotation with fluidic-oscillator microbubbles	>95–99% recovery within minutes	Much lower specific energy than centrifugation	[7], [13], [14], [31], [33], [34]
Two-stage dewatering	Mixed cultures	Flocculation + low-pressure dewatering	High concentration factor	~0.04–0.10 kWh per dewatering cycle	[36]
Wastewater + biomass	*Scenedesmus* spp., *Chlorella* spp.	Integrated wastewater-fed systems	High COD, TN, TP removal; biomass 0.3–0.4 g·L⁻¹	Aeration energy dominates; MNB proposed to reduce it	[4], [5], [42], [44]
Biostimulation (astaxanthin)	*Haematococcus lacustris*	Air nanobubble water (NBW)	↑ growth (~44%); ↑ astaxanthin content	Compatible with low-pressure NBW systems	[40], [41]

*Energy aspect: qualitative; actual values depend on system design and operating conditions.

4.2. Critical Analysis and Research Gaps

Despite the encouraging empirical data, several limitations need to be addressed before MNB can be reliably implemented on an industrial scale.

4.2.1. Scale-up limitations

Most studies operate at laboratories or pilot scale (<100 - 500 L). Scaling to large PBRs (10-50 m³) or open ponds introduces challenges in achieving uniform bubble distribution, controlling pressure drop along long piping networks, and maintaining stable bubble populations under dynamic flow conditions [15], [12], [18], [28], [37]. Novel designs such as fluidic oscillators, bubble-cutting spargers, and modular injection arrays show promise but have yet to be validated at industrial scale [28], [31], [32], [34].

4.2.2. Incomplete energy and cost accounting.

Reported gains in growth or harvesting efficiency often lack full energy balances or techno-economic analysis (TEA). Many energy values for microflotation or foam flotation are model-derived under ideal conditions [35], [36]. Comprehensive TEA/LCA (Life Cycle Assessments) for

integrated systems (cultivation + harvesting + wastewater treatment) remains scarce, making it difficult to quantify real cost advantages over conventional aeration, DAF, or membrane-based CO_2 delivery [4], [6], [13], [14], [31], [37].

4.2.3. Performance variability in real wastewater

Most MNB studies are performed in clean water or synthetic media, whereas real wastewater contains suspended solids, surfactants, and fluctuating salinity. These factors can clog fine-orifice generators, alter surface tension and zeta potential, and destabilize [5], [7], [16], [37]-[39]. Clog-resistant vortex-type nozzles and pulsed-cleaning protocols are needed for reliable long-term operation.

4.2.4. Limited understanding of biological mechanisms

While phenotypic improvements, higher growth, lipid content, or carotenoid accumulation, are well documented, the underlying regulatory networks remain poorly understood. The effects of MNB-induced redox perturbations, ROS generation, and local pressure fields on photosynthesis, stress signaling and metabolic fluxes require systematic omics-based investigation [11], [27],

[40], [41]. Without such insight, "precision cultivation" remains largely empirical.

4.2.5. Adherence to International Standards

Previously, the lack of consensus on measuring and reporting bubble properties made cross-study comparisons difficult. However, the recent establishment of the ISO/TC 281 technical committee has provided a robust framework for standardization that future research must adhere to. Specifically, ISO 20480-1:2017 [45] and ISO 20480-2:2018 [46] have unified the terminology and categorization of fine bubbles, distinguishing clearly between microbubbles and ultrafine bubbles (nanobubbles). Crucially, precise protocols for measuring number concentration and size distribution are now standardized under ISO 20480-3:2021 [47] (for microbubbles) and ISO 20480-4:2021 [48] (for ultrafine bubbles), while sampling procedures should follow ISO 20298-1:2018 [49]. To ensure reproducibility and reliable scale-up, the MNB research community must transition from ad-hoc characterization methods to strict compliance with these ISO 20480 series protocols.

4.3. Roadmap for Future Development

Based on the above gaps, several directions should guide future research and industrial deployment:

1. Engineering scale-up: Integrate CFD with empirical hydrodynamics to optimize sparger placement, bubble residence time, and uniformity in large PBRs and raceways.

2. Low-pressure, anti-clog generators: Develop vortex-type or fluidic-oscillator–based MNB generators tailored for complex wastewater matrices and renewable-energy operation.

3. Biological Mechanisms: Apply transcriptomics, metabolomics and proteomics to map how MNB conditions (bubble size, gas type, ROS levels) regulate CO_2 uptake, stress responses, lipid and carotenoid biosynthesis.

4. Standardized characterization: Establish guidelines for reporting bubble metrics and operating parameters to improve reproducibility and enable meta-analysis across studies.

5. Techno-economic and life-cycle assessment: Conduct TEA/LCA for multiple deployment scenarios (biofuels, wastewater services, nutraceuticals) to quantify the true economic and environmental impact of MNB-based systems.

With coordinated progress along these lines, MNB technology can evolve from a promising laboratory tool into a robust process-intensification platform for next-generation microalgae biorefineries in a circular bioeconomy context.

5. Conclusions

Micro–nanobubble technology offers a unifying platform to address two long-standing bottlenecks in microalgae biotechnology: inefficient gas–liquid mass transfer during cultivation and the high energy demand of harvesting. By reshaping the hydrodynamics and interfacial physics of gas–liquid contact, MNB reconcile high $k_L a$ with low hydrodynamic shear, enable energy-efficient flotation-based harvesting, and open dual-purpose opportunities in wastewater treatment and biostimulation.

From a technical perspective, MNB-enabled systems bridge the gap between the high mass transfer requirements of dense cultures and the need to maintain cell integrity. Economically, microflotation and related approaches offer a credible alternative to centrifugation and membrane processes, particularly when integrated with low-pressure, energy-efficient MNB generators. Environmentally, MNB-assisted wastewater-based cultivation improves nutrient and organic pollutant removal while reducing or eliminating the need for chemical additives.

As research progresses from phenomenological observation to mechanistic understanding and from bench-scale demonstrations to standardized industrial designs, MNB technology is well positioned to become a key enabling tool in microalgae-based biorefineries. In conjunction with rigorous techno-economic and life-cycle assessments, such systems have the potential to support commercially viable production of biofuels, wastewater services and high-value nutraceuticals within a circular bioeconomy framework.

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