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REVIEW

Label-free processing of microalgal biomass for high-throughput bioprocess development using external fields and microfluidics

[version 1; peer review: 2 approved with reservations]

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Abstract

Microalgal biotechnology has the potential to provide carbon-neutral and even carbon-negative solutions for a plethora of applications, including human nutrition, animal feed bioactive ingredients, and CO_2 capture. However, current technologies are unable to compete with classic approaches because of their high investment and operational costs as well as technical challenges, especially when dealing with multiproduct biorefinery models. Developing and optimizing these novel bioprocess technologies is extremely resource-intensive because of expensive infrastructure, lack of automation, and reduced data acquisition and processing. Microfluidic platforms can deal with individual cells and components, allowing their precise manipulation, transformation, and separation, particularly in external fields such as electric, magnetic, electromagnetic (e.g., light), and acoustic fields. External fields, in conjunction with microfluidics, could provide an all-in-one chip solution for the production, extraction, and purification of high-value microalgal components for enhanced process development, including monitoring and control. Recent breakthroughs in the fabrication technologies of these microfluidic chips, as well as their modular nature, allow for scale-outs to suit anything from vast industrial applications to smaller-scale research and development purposes, bringing us to the brink of achieving the full potential of microalgal biotechnology foreseen decades ago.

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microalgae, microfluidics, lab-on-a-chip, external fields, acoustics, multiproduct biorefinery, high-throughput process development

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Introduction

Microalgae are an interesting source of functional ingredients because of their small footprint and ability to efficiently utilize CO_2 to produce various valuable compounds. Microalgal cells can have a high-protein content (~50% DW) as well as lipids, sugars, pigments and other high-value components (Barkia *et al.*, 2019; Koller *et al.*, 2014; Tibbetts *et al.*, 2015). Globally, hundreds of algal species are used as food, feed, and fertilizers, with recent applications in pharmaceuticals and cosmetics. However, in Europe, the adoption of microalgae as a novel source of functional ingredients is currently limited to only a handful of species (Caporgno & Mathys, 2018; Mendes *et al.*, 2022). The global production value of dried whole-cell biomass of species such as *Arthrospira platensis* cyanobacteria and *Chlorella vulgaris* microalgae, species with novel food status in Europe, is worth hundreds of millions of euros (Fortune Business Insights, 2025). In contrast, high-value microalgal molecules, such as omega-3 fatty acids and β-carotene, could open up a significantly larger market (Selvam *et al.*, 2024). However, their extraction and purification require highly complex processes involving cell lysis followed by intensive post-treatment (Figure 1). The costs of these downstream processes vary considerably, depending on the employed microalgal strain and growth strategy (autotrophic, mixotrophic, or heterotrophic). For instance, the cost of extracting and purifying soluble proteins ranges from 4 to 14 €/kg_{DW} (dried weight), pigments from 5 to 70 €/kg_{DW}, and oil from 4 to 15 €/kg_{DW} (Ruiz *et al.*, 2016). Their market value is often in the same order of magnitude as the downstream processing costs while the biomass production costs are at between 3 and 7 €/kg_{DW} under large-scale cultivation using vertical flat panels (Lam *et al.*, 2018). This makes it challenging to generate profitable chains under current circumstances (Chen *et al.*, 2024; Show, 2022). Heterotrophic microalgal cultivation can lower biomass costs by allowing denser cultures while reducing contaminants. However, these require a constant stream of carbon source (often sugar), which could offset these advantages unless carbon-rich side-streams are used as growth media (Udayan *et al.*, 2022). Thus, developing a precise and rapid process development, monitoring, and control platform to explore novel downstream processing approaches is critical to enable microalgal biotechnologies to reach their full potential.

One way to achieve this is to devise automated high-throughput process screening, development, optimization, and even production platforms. The current process development automation efforts involve the use of expensive liquid-handling robots that can automate repetitive tasks, boost productivity, ensure consistency, and manage large amounts of data. However, the initial setup costs for automated systems can be high, especially for small-scale operations and complex functionality (Torres-Acosta *et al.*, 2022). Moreover, liquid-handling robots can lack flexibility and the ability to adapt to a new task, workflow, or substrate. These systems might require significant modifications, making them less adaptable than manual or semi-automated systems in specific research environments. Moreover, some liquid handling systems may not be compatible with every type of sample or material, such as microalgae cultures, which can have varying viscosities and properties. Thus, exploring less expensive microfluidic systems and external

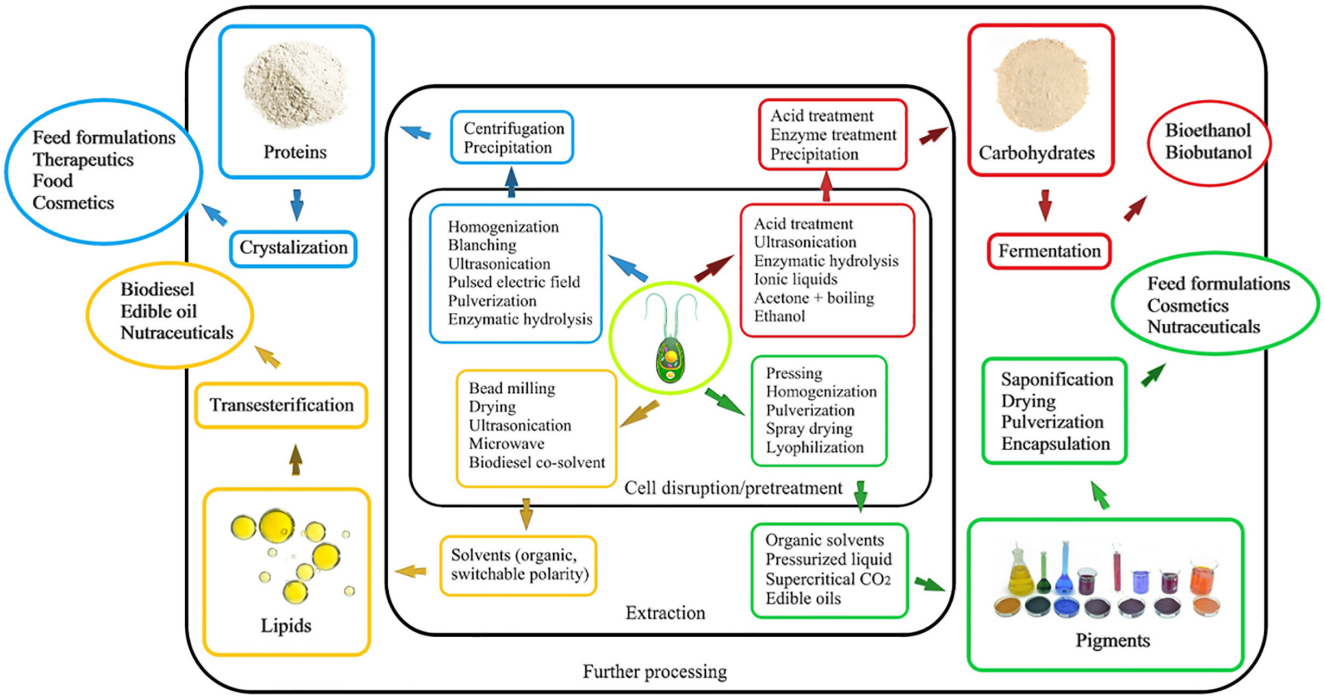


Figure 1. Downstream processing steps for the recovery of microalgal components such as proteins, carbohydrates pigments and lipids, commonly employed by single product biorefinery models.

fields could lead to affordable process development and large-scale data acquisition. Moreover, these high-precision platforms can elucidate specific phenomena that are otherwise impossible using conventional systems. This will allow the integration of multiple production and processing steps at single-cell resolution for increased precision in cell transformation, cultivation, and manipulation, as well as the extraction of specific target components within one chip (Mehendale *et al.*, 2020). Current high-precision 3D printing, laser cutting, and polydimethylsiloxane (PDMS)-based microfluidic production methods have reached technological maturity and are thus ready to mass-produce these devices, ensuring that the spike in demand is met (Saggiomo & Velders, 2015; Sanchez Noriega *et al.*, 2021).

High-throughput process development using microfluidic technologies

Manipulating particles and fluids in the submillimeter region is generally encompassed by the umbrella term of microfluidics and lab-on-a-chip (LoC) concepts. Although the microfluidic field exploded in the late 90ies thanks to the PDMS replica molding invented by Whitesides (Xia *et al.*, 1997), it has started fulfilling its promise as an analytical tool (Fonzo *et al.*, 2025; Gurkan *et al.*, 2024). These systems provide a wide range of advantages over traditional approaches, such as reduced costs, footprint, waste, precise control, and possible monitoring at single-cell resolution (Ongaro *et al.*, 2022). Moreover, they can be scaled out through stacking multiple chips together and using them in parallel to increase their throughput in case larger quantities of product are required (Dubay *et al.*, 2019; Wu *et al.*, 2021). Although using microfluidics for high-throughput screening and even production systems seems counterintuitive considering the small amount of liquids involved in the chip, this approach has been used in commercial applications by companies such as Capsum (Capsum, 2025). Their proprietary technology uses parallel microfluidics for the large-scale fabrication of microdroplets used in beauty products, such as creams. These can be found in high-end products, such as 'Hydra Beauty Micro Crème' of CHANEL (V.G., 2017). This example demonstrates that the high-throughput production of ingredients is possible using microfluidics. Moreover, as stated before, the simplicity and lack of movable components could translate in relatively low production and maintenance costs (Battat *et al.*, 2022; Nge *et al.*, 2013).

These platforms allow the integration of multiple unit operations on a single chip, thus enabling less expensive and automated complete process development platforms. These approaches have become increasingly popular in recent decades and have been successfully employed in various applications such as cell culture (Iuliano *et al.*, 2020), molecular tools for genome manipulation, sample processing and detection, biomass harvesting, compound extraction, and purification (Huh *et al.*, 2010). Notable breakthroughs have also been reported in organ-on-a-chip and human-on-a-chip biomedical applications. A number of reviews are thoroughly addressing most of these applications and innovations (Huang *et al.*, 2024; Ingber, 2022; Kumar *et al.*, 2024; Shoji *et al.*, 2024). The present manuscript focuses on novel concepts for microbes in general, and microalgae more specifically, high-throughput process development, and even production platforms incorporating LoC concepts and various external fields. This could provide an innovative alternative to traditional process screening and the high-precision production of functional ingredients.

Microalgal cultivation

Cultivating microalgal biomass represents a central part of microalgal biotechnology because of its impact on expressed metabolic pathways, and thus the growth and production of target metabolites. However, classic high-volume cultivation approaches reveal only the impact of these variables on the overall population and not at the cellular level (Westerwalbesloh *et al.*, 2019). In addition, these systems are relatively complex and expensive, even at the bench scale. Thus, more targeted and accessible growth and analysis strategies are required to circumvent these limitations and to adequately understand these processes. Recent decades have brought a number of breakthroughs in the field through the development of microfluidic bioreactors that allow the cultivation and targeted analysis of a small number of algal cells and even single-cell approaches (Anggraini *et al.*, 2022; Enders *et al.*, 2024; Ozdalgic *et al.*, 2021). As in the case of traditional cultivation, microfluidic strategies can be characterized as continuous-flow systems that use mechanical traps and batch techniques, represented by droplet microfluidics and microchambers (Figure 2).

Continuous-flow cultivation strategies most often use a series of microstructures to trap single cells or small cellular clusters, whereas media containing the required nutrients are continuously circulated. These so-called mechanical traps come in a variety of forms, each with their advantages and disadvantages. Small poles for instance arranged in semicircular shapes, with gaps smaller than the cell size, were used to trap microalgal cells flowing through (Kim *et al.*, 2014). Moreover, microfluidic devices with integrated pneumatic valves have been developed to selectively release single cells from specific traps (Chai *et al.*, 2021; Gong *et al.*, 2023). In contrast, picoliter scale droplets and microchambers have been employed to develop microfluidic batch systems, allowing for high-throughput screening assays. Droplet-based microfluidics, including immiscible phases and hydrodynamic traps, were initially used for this purpose (Kim *et al.*, 2017; Luan *et al.*, 2020). More recent developments have focused on incorporating digital elements, such as electrowetting, allowing for the addition of fresh media to the droplets, as well as different reagents, such as fluorescent dyes (Karcz *et al.*, 2022; Liu *et al.*, 2023). Although these approaches have been successfully used to investigate the growth profile and metabolite accumulation of small groups of cells, even at single-cell levels, clogging issues and strict requirements in fabrication precision remain important hurdles.

Upstream process development and control

Advancements in microfluidic algal biotechnology are beyond the cultivation scope. Most of the advantages come from more precise manipulation, transformation, monitoring, selection,

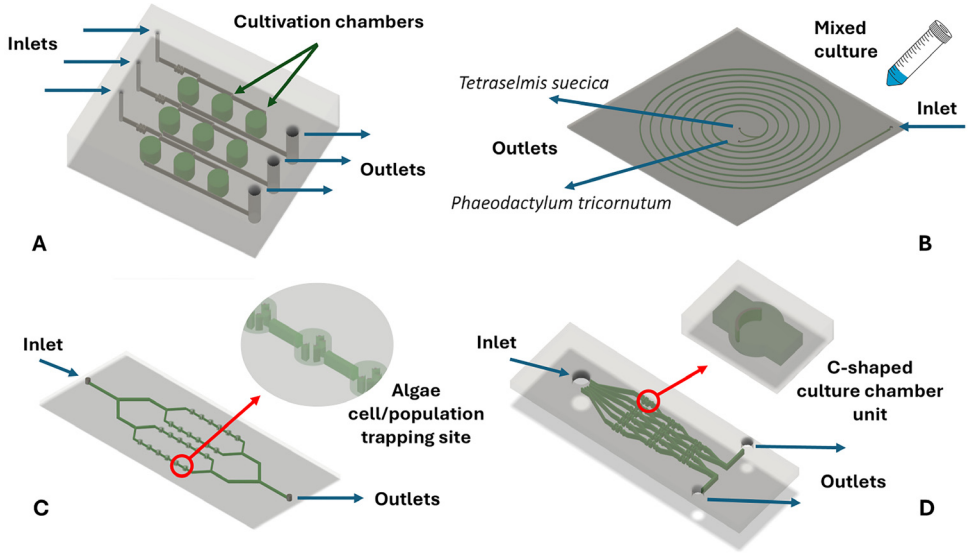


Figure 2. LoC systems develop to cultivate microalgal cells. Batch and fed-batch approaches have been studied using micro-reactors (**A**) and spiral channels (**B**). Moreover, continuous systems have been developed using mechanical traps such as micropillar arrays (**C**) or small semispherical obstacles (**D**). These retain small populations of cells or even single cells while fresh medium is continuously provided.

and control of individual cells, allowing a deeper understanding of their biology and responses. This allows for the faster and more efficient development of novel microalgal-based processes and products. The microalgal industry, as well as the microbial biotechnological sector, could benefit directly from these advancements. This could be achieved by enabling research and development activities with minimum operating costs and even full automation, similar to practices commonly found in the biomedical industry, but at a fraction of the costs.

Cell transformation | There is a significant push towards biosourcing most of the building blocks presently used by various industries, including manufacturing, clothing, construction, food, pharmaceutical, and cosmetics, to reduce the environmental strains of our society (Beigbeder *et al.*, 2019). Thus, there is a need to develop microbial and microalgal cell factories capable of converting readily available and waste substrates into these platforms and specialty molecules by inserting target gene constructs into these organisms. Presently, there are a range of delivery pretreatment strategies that are relatively invasive and can alter the exogenous DNA to be inserted or subsequent cell viability. Thus, LoC variations of established methods and completely new approaches have been developed in an attempt to circumvent these limitations. For instance, higher transgene expression levels have been achieved by employing a digital microfluidic electroporation system with an integrated on-chip cell culture (Im *et al.*, 2015). Moreover, higher transformation efficiencies were achieved when using a ZnO nanowire-incorporated microdevice and pneumatic PDMS microvalve for high-throughput delivery of the hygromycin B resistance gene cassette (Hyg3) into *Chlamydomonas reinhardtii* cells (Bae *et al.*, 2015). Furthermore, a fully-automated digital microfluidic method was developed for arrayed gene-editing in mammalian cells (Sinha *et al.*, 2018).

Deep monitoring and machine learning | LoC devices allow for a precise evaluation of the changes taking place during the cultivation of these microorganisms (Cabanelas *et al.*, 2015; Gao *et al.*, 2020). A series of traditional as well as novel approaches have been specifically developed for microfluidic applications for smooth integration and analysis (Gao *et al.*, 2021; Sá *et al.*, 2020). Some of the first such attempts consisted of integrating microfluidic flow cytometry using an external photodetector and illuminating glass chips with a laser. This allowed for rapid and reliable identification of phytoplankton species (Benazzi *et al.*, 2007; Schaap *et al.*, 2012). Moreover, droplet-based LoC culturing, staining, and analysis set-ups were used to select lipid overproducers from a microalgal mutant library through an integrated optical detection system in a flow-through manner (Kim *et al.*, 2017). More recent efforts have led to the development and integration of optical sensors into microfluidic platforms consisting of organic light-emitting diodes and organic photodetectors, which can efficiently determine the optical, electrical, and geometric characteristics of microalgal cells (Lefèvre *et al.*, 2015). Cellular capacitance and impedance changes were used to further distinguish between morphologically similar species and even among living and dead microalgal cells (Song *et al.*, 2014; Wang *et al.*, 2013). The big data generated by these sensing elements can be used, for instance, to train genetic algorithms for machine learning to better understand these processes.

Screening and isolating overproducers | There is a largely untapped potential for microalgal biotechnology considering the sheer biodiversity of species, as well as specifically engineered strains. Thus, there is an evident need for either bioprospecting local candidates or separating engineered viable overproducers from untransformed cells. For instance, it was recently demonstrated that by employing a spiral microchannel, lift

and Dean flow drag forces, microalgal species close in size were separated (Schaap *et al.*, 2016). Recently, a dielectrophoresis-based (DEP) microfluidic sorting platform has been developed to separate and quantify *C. reinhardtii* microalgal cells into 6 different outlets based on their intracellular lipid content (Han *et al.*, 2019). Direct current dielectrophoresis was additionally developed and successfully employed to separate viable from non-viable microalgal cells using microchannels containing insulated microstructures generating spatially defined nonuniform electric fields (Gallo-Villanueva *et al.*, 2011; Song *et al.*, 2012). These sorting strategies can also be used as process control strategies to remove bacterial contaminants from the media. For instance, some authors reported a purification efficiency of 99.8% when passing a mixed sample containing *Coenochloris signiensis* and bacterial cells through a 3-inlet microchannel by exploiting the different inertial forces experienced by these cells (Zhang *et al.*, 2016).

Optimization of growth conditions and production of target metabolites | These single-cell manipulation and monitoring strategies also hold great potential for precise process characterization and improvement (Figure 3). The combination of high throughput and small sample size makes these microfluidic strategies faster and less expensive tools to determine the optimal regions of interest. Several studies have used LoC approaches to investigate the impact of process variables such as light regimes, temperature, pH, and various nutrients and CO_2 concentrations on specific desired outputs. For instance, great emphasis was made on the impact of light on cell growth and production of various metabolites due to the fact that the small volumes involved translate into reduced self-shading effects (Paik *et al.*, 2017). Recently, a droplet-based LoC platform was successfully used to determine the effects of nutrient availability and temperature on the growth of *C. vulgaris* (Saad *et al.*, 2019). Similarly, an array of microchambers was used to maximize the growth of *Scenedesmus obliquus* under non-limiting CO_2 conditions (Castaldello *et al.*, 2019). This game-changing study proved that CO_2 can be effectively delivered under precise conditions to microalgal cells cultivated in microchambers. Moreover, the large amount of potential data continuously generated by such platforms could be used to train neural network algorithms, allowing for a better understanding of these processes and predictions regarding outputs, such as maximizing growth or production of certain targeted compounds.

Downstream process development

Microalgal biomass contains a cocktail of valuable functional molecules that must be gently extracted and separated prior to their formulation and use. However, these steps are very energy-intensive and technologically challenging, amounting to most of the overall process costs (Boboescu *et al.*, 2018). For instance, various harvesting techniques have been conventionally employed, including flocculation, filtration and centrifugation (Fasaei *et al.*, 2018). Flocculation has the lowest energy requirements, but requires chemicals, and the results vary based on the targeted species. Combinations of flocculation, membrane filtration, and centrifugation have been explored to further increase dewatering efficiency while reducing energy requirements (Kumar *et al.*, 2023; Zhao *et al.*, 2022). Cell

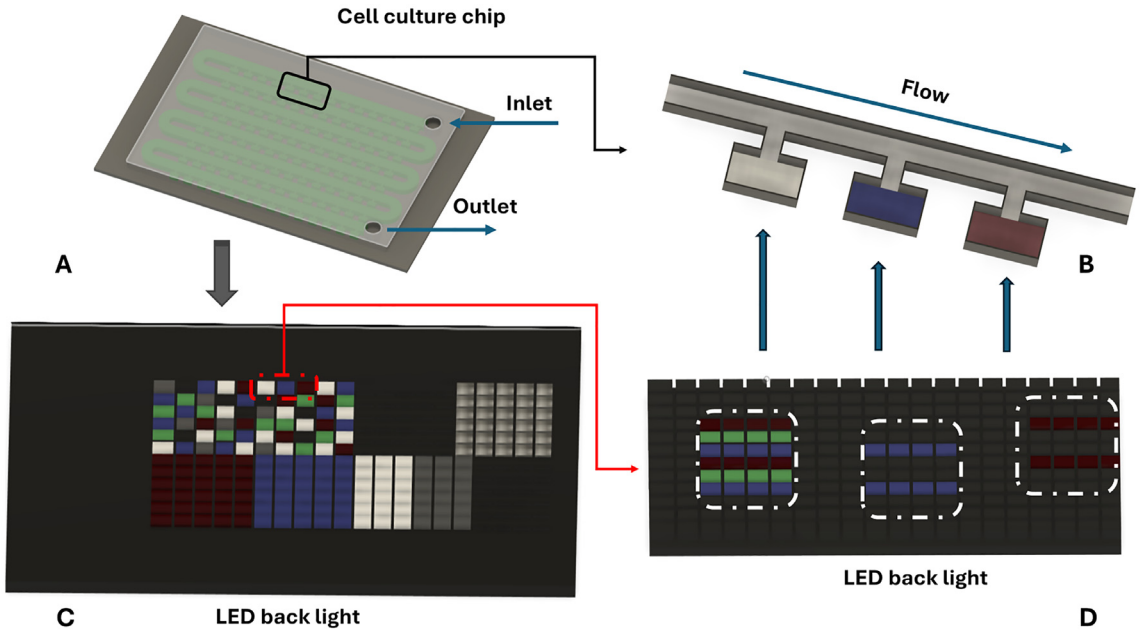


Figure 3. High-throughput LoC screening system to optimize growth parameters in microalgal cultivation. A series of small cultivation chambers (**A**) are arranged along microfluidic channels providing fresh growth media (**B**). Below these chambers there is a platform with small LED lights (**C**) providing different combinations of wavelengths to each of the individual cultivation chambers (**D**). This allows the screening of numerous light conditions and regimes within one single LoC system (Bodénès *et al.*, 2019).

lysis and product recovery, on the other hand, usually require harsh conditions, such as high temperature and pressure, as well as corrosive chemicals and hazardous solvents, often leading to the denaturation of more sensitive metabolites, making multi-product recovery efforts very difficult (Boboescu *et al.*, 2016; Slegers *et al.*, 2020). Thus, there is a strong incentive to develop alternative approaches to circumvent these limitations. Among these, several LoC approaches have yielded promising results, suggesting that alternative solutions are available.

Cell harvesting has proven to be a challenging task prior to biomass processing because of its small size and similar density to that of water. This represents a critical step toward reducing the cost of subsequent treatment operations. For instance, a microfluidic chip has been developed to continuously concentrate microalgal cells by integrating microstructures retaining the cells while allowing the media to flow unhindered (Hønsvall *et al.*, 2016). This allowed the successful concentration of *Rhodomonas baltica*, *Chaetoceros sp*., and *Thalassiosira weissflogii* microalgae, and could be an alternative for species that are difficult to harvest due to their properties or reduced size. Inertial forces are an efficient way to separate particles based on their different properties using hydrodynamic forces. The same mechanism was also used to concentrate various microalgal cells by driving them towards known equilibrium positions, while the excess media was removed through separate outlets, at a concentration factor of 3.28 (Wang & Dandy, 2017). The approach was demonstrated at large scale as well using *Chlorella sp.* and *Spirulina sp.* suspensions, reaching concentration factors of up to 3 (Carbonell Chacón, 2019; Liu *et al.*, 2018; Salazar-González *et al.*, 2024).

Once harvested, the microalgal biomass must be treated to extract and subsequently fractionate the targeted components. Some of the earlier attempts simply miniaturized existing techniques such as high-pressure homogenizers (Halim *et al.*, 2012). However, the energy requirements for these approaches are still relatively high and very limited for LoC applications, as the subsequent use of solvents and acids would require compatible building materials such as glass and ceramics, further increasing production costs. Thus, alternative attempts have used microfluidic electroporation to weaken and permeabilize the cellular outer compartments by exposing cells to an electric field (Elersek *et al.*, 2020; Kashyap *et al.*, 2024; Li *et al.*, 2024; Zhang *et al.*, 2021). Exposing *C. vulgaris* cells to a continuous pulsed electric field successfully allowed the recovery of lipids at 51% efficiency in commercial solvents such as hexane. Thus, the focus shifted towards combining these electrical treatments with other approaches, such as mechanical stresses such as cyclic pressure, resulting in enhanced lipid extraction efficiencies (Bensalem *et al.*, 2018; Carullo *et al.*, 2022; Khoo *et al.*, 2023).

Thus, these breakthrough innovations emphasize the potential of microfluidic approaches in providing high-throughput single-cell transformation, monitoring, and sorting, as well as product extraction and purification. Moreover, the nature of these devices allows the development of microfluidic chips that integrate several of these unit operations and work seamlessly together under continuous automated regimes for overall process optimization. Reliable integrated in-line process monitoring approaches were developed and used to, for instance, select different microalgal overproducers and optimize their growth conditions to maximize the production of target metabolites (Barata *et al.*, 2016; Kerk *et al.*, 2021; Zhou *et al.*, 2021). The large volume of data generated by these platforms can be used within multifactorial or neural network models to design and optimize complete biotechnological processes with minimal investment and time. Moreover, if the production of larger quantities of biomass or product is required, the stacking and simultaneous operation of multiple microfluidic platforms can proportionally increase their throughput (Li *et al.*, 2022; Riordon *et al.*, 2019). Novel technologies, such as 3D printing, can be used to centrally or locally manufacture these platforms at a fraction of the costs required by automated liquid-handling robots. However, because these microfluidic platforms are usually made of resins, conventional processing agents such as organic solvents cannot be employed; thus, alternative strategies, such as external fields, are required.

External fields in LoC platforms

Using external fields throughout the different upstream and downstream steps to replace or enhance the effect of specific geometries and microstructures holds great potential for LoC process development. These represent the physical forces that can interact with specific particles based on their properties, intensity, and microfluidic geometries (Boboescu *et al.*, 2018; Kieffer *et al.*, 2025). The effects of these fields decrease with increasing distance between the source and target particles. Thus, operating microfluidic systems keeps these forces relevant and targeted. Under these conditions, the forces generated by these fields can sort, deflect, or trap particles, thus overcoming their specific gravity, Stokes drag force, and random Brownian motion. Consequently, they are perfect candidates for alternative downstream harvesting, extraction, and separation strategies (Table 1). Several previous studies have dealt with these forces in detail, characterizing them into various classifications such as active and passive techniques for particle separation (Ebrahimi *et al.*, 2024; Mansor *et al.*, 2025; Zhang *et al.*, 2020). Here, however, we focus on the use of various external fields in conjunction with microfluidic channels and geometries to develop novel high-throughput process development platforms for microalgal biotechnology applications.

Laminar and asymmetric flow

Using passive separation principles, such as drag forces, considerably simplifies the process because it does not require the generation of other external fields. In these cases, the driving force for separating the microparticles was generated by the architecture of the microfluidic device. Inertial microfluidics, for instance, represents a commonly used passive separation technique that employs straight, spiral, or serpentine microchannels (Tang *et al.*, 2020). Shear inertial lift and wall lift are mostly present in devices with straight microchannels, whereas centrifugal forces and secondary flows dominate curved-channel platforms. These principles have been used to separate

Table 1. External forces used for the continuous separation of particles. These can be used to harvest and even sort microbial and microalgal cells of desired traits, as well as separate different compounds from a mixture once these cells have been lysed. Moreover, some of these fields such as acoustics and electric can be used for the cell lysis step as well (Liu *et al.*, 2022).

Fields	Force size (r) dependence	Separation principles	Responsible property of particle	Advantages	Disadvantages
Gravity	Centrifugal force, gravitational force	Sedimentation field-flow fractionation (FFF), centrifugal Chromatography	Density	High separation efficiency	Require specific sample, solvent or membrane
Electric field (uniform)	Electrostatic force	Electrophoresis (EP)	Charge, ζ potential	Very high separation and efficiency	Flow rates change with chemistry
Electric field (nonuniform)	Dielectric force	Dielectrophoresis (DEP)	Complex dielectric constant (conductivity, permittivity)	High throughput and separation efficiency	Requires high voltage. Depends on medium conductivity
Laser	Scattering force	Photophoresis (PP)	Complex refractive index (extinction coefficient, refractive index)	High separation efficiency	Heating and photodamage to particles, low throughput
Magnetic field (nonuniform)	Magnetic force	Magnetophoresis (MP)	Magnetic susceptibility	Very high throughput, requires relatively low cost	Requires long time for magnetic bead antibody labelling
Magnetic field and electric current	Lorentz force	Electromagnetophoresis (EMP)	Conductivity	Very high efficiency	High cost
Ultrasonic wave	Acoustic radiation pressure	Acoustophoresis (AP)	Density, compressibility	High separation efficiency, controlled cut off separation	Complex fabrication, limited device material to transmit acoustic power efficiently

polystyrene beads of sizes ranging from 590 nm to 7.32 μm in a spiral microfluidic channel (Bhagat *et al.*, 2008). Similarly, the insertion in a laminar flow of a structure of pillar arrays tilted at an angle generates unique flow streamlines, often exploited as deterministic lateral displacement of particles based on their size (Bhagat *et al.*, 2008). Thus, larger particles displace laterally, while smaller particles tend to flow in a zigzag pattern through the pillars without experiencing lateral displacement, effectively achieving particle separation. This was demonstrated for instance using a microfluidic device to separate label-free viable from non-viable mammalian cells at 100% efficiency (Tottori *et al.*, 2016). Moreover, a clog-free tunable microfluidic filter was developed to concentrate microalgal cells by exploiting hydrodynamic interactions around trilobite-shaped structures (Tottori *et al.*, 2016). Although these passive approaches are promising, there are several significant limitations, such as particle-particle interactions, which can disrupt the equilibrium between the fluid and wall lift forces, severely impacting their efficiency.

Gravity and centrifugal forces

Gravity is one of the most commonly used external field methods for harvesting microalgal biomass. This exploits the gravitational and centrifugal forces acting on microalgal cells to concentrate them prior to their dewatering and subsequent treatment steps. Cell shape, size, and density determine whether using gravity represents a viable harvest method, as most of these species measure only a few microns and have a negligible specific gravity difference from that of their culture media (Roy & Mohanty, 2019). Most reported studies using gravitational forces for these purposes used conventional technologies, such as settling tanks. For instance, it had been demonstrated that both *Scenedesmus dimorphus* and *C. vulgaris* microalgae can be harvested through gravity sedimentation using a downward-flow settling tank with a maximum separation efficiency of 80% separation efficiency (Roy & Mohanty, 2019). However, this approach is limited by the morphology of the microalgal cells, their ability to aggregate, and long settling times. In addition to their incompatibility with continuous processes, LoC applications using passive sedimentation are not feasible.

One way to circumvent these limitations is to use centrifugal forces. These strategies can significantly accelerate the process and can be developed to work under continuous modes. However, one cannot simply scale down a centrifuge because the sedimentation phenomena are weak in microfluidic devices owing to the small Stokes values of the microalgal cells. Among the first microfluidic centrifugation attempts were the so-called "lab-on-a-disk" (LoD) systems, in which a disk, acting as the device foundation is rotated by a motor, generating a primary flow in a linear, radially outward direction. These platforms were specifically successful in processing blood samples to separate their components in a gradient

medium (Kinahan *et al.*, 2014). However, only recently have truly LoC centrifugation devices been successfully demonstrated. For instance, vortical flows have been generated by passively exploiting the geometry and topology of microfluidic devices without requiring motor-activated centrifugal forces. When a side chamber or repeating topological features, such as post arrays, are incorporated in microfluidic devices, hydrodynamic rotation and flow recirculation generate inertial effects that can be exploited to separate particles based on their size and density (Equation 1 and Equation 2) (Ahmed *et al.*, 2020). For instance, a "vortex gear" was developed and successfully demonstrated for on-chip applications, by specifically generating two adjacent vortices opposite to each other, similar to a set of gears (Tsai *et al.*, 2018). Moreover, microfluidic vortex shedding has been used to deliver mRNA into primary human T lymphocytes through cell membrane poration by integrating post arrays into microfluidic chips (Jarrell *et al.*, 2019). Thus, these approaches hold great potential for LoC algal applications, not only for downstream separation strategies but also for the upstream genetic transformation of overproducing cell lines.

$$u = \frac{\rho_p - \rho_f}{18\mu} D_p^2 r_d \omega^2 \tag{1}$$

$$v = \frac{1}{2}\rho_f \omega^2 (r_2^2 - r_1^2)\frac{1}{R_{tot}} A \tag{2}$$

Buoyancy force is also present in addition to centrifugal and hydrodynamic drag forces. These forces create a steady-state lateral velocity (u) that depends on the particle diameter (Dp), particle, fluid density (ρp, ρf), and distance from the center of rotation (rd). The downstream velocity (v) depends on the hydrodynamic resistance of the channel (Rtot), input and output channel distances, and area of the channel (A).

Optical forces

A focused beam of light can be used to manipulate the particle towards which is directed by exploiting the mismatch in the refractive index between the cells and the medium. This is triggered by two forces: optical scattering, which tends to push the cells and particles away, and gradient forces, which attract particles with the highest refractive index towards the beam maxima (Equation 3 and Equation 4) (Yan *et al.*, 2017). Thus, an optical sorter, which exploits the interaction of cells and other particles with an optical lattice, has been successfully employed, yielding sorting efficiencies of close to 100%, exceeding thus values reported for fluorescence-activated cell sorting (MacDonald *et al.*, 2003). Moreover, efficient identification and sorting of individual leukemia cell lines have been demonstrated by combining multichannel microfluidics with laser tweezers Raman spectroscopy (Lau *et al.*, 2008). The design was adjusted by identifying the optimal hydrodynamic focusing and pinch-flow fractionation based on device architecture, flow velocity, and laser intensity. Recent attempts have combined photoconductive substrates with transient electrodes to manipulate microparticles (Wu, 2011). These so called "optoelectronic tweezers" have been thus reported to deliver high-specificity single-particle manipulation under 100 000 times less optical intensity. However, integrating these devices into existing LoC platforms has proven challenging owing to the lack of fluidic components (Yan *et al.*, 2017). Although there have been no studies using these separation principles in the context of LoC microalgal process development, the untapped potential is noteworthy because of its relative simplicity, low energy requirements, and easy integration on microfluidic platforms.

$$F_{grad}(r) = 2\pi n_2^2 \varepsilon a^3 \left(\frac{m^2-1}{m^2+1}\right) \nabla |E^2(r)| \tag{3}$$

$$F_{scat}(r) = \frac{n_2}{c}\frac{128\pi^5 a^6}{3\lambda^4}\left(\frac{m^2-1}{m^2+1}\right)^2 I(r) \tag{4}$$

where c is the speed of light, m is the ratio between the refractive indices of the particle and the medium, and r is the vector of the position.

Electric field

The use of electric fields for various LoC biotechnological applications is very popular, owing to their relative simplicity and high tunability. The electric field manifests three forces on the cells and other microparticles: electrophoresis and dielectrophoresis. Electrophoresis refers to the migration of charged particles in the presence of a uniform electric field. This migration is primarily determined by the intensity of the applied electric field, electrophoretic mobility of the particle (determined by its surface charge), and electroosmotic flow (Equation 5). Among these approaches, capillary electrophoresis and miniaturized free-flow electrophoresis are the most commonly used methods. These proved efficient in separating nanometer-sized particles such as fluorescent molecules, proteins, enzymes, and even gold nanoparticles (Jeon *et al.*, 2016; Jozanović *et al.*, 2023; Salafi *et al.*, 2017).

$$F_{EP} = 6\pi\zeta_p \varepsilon a E \tag{5}$$

where a is the radius and E is the electric field.

Dielectrophoresis refers to the migration of a polarizable particle under the influence of an inhomogeneous electric field based on the difference in polarizability between microparticles and microfluidic media (Equation 6). Given these phenomena, a wide range of particles, such as cells and biomolecules, can be manipulated using dielectrophoresis. Dielectrophoresis phenomena have been successfully developed and used in different microalgal biotechnology applications, such as strain selection, analysis, separation, and concentration (Abt *et al.*, 2020). Thus, these approaches allow for a wide range of highly tunable cell manipulation techniques such as focusing, trapping, concentration, filtering, and separation, which are often critical steps in microalgal biotechnology processes. However, certain limitations must be overcome, such as the large electric field requirements for smaller targeted particles to achieve forces of sufficient magnitude.

$$F = 2\pi r^3 \epsilon_m \left\{ \frac{(\epsilon_p - \epsilon_m)\omega^2\tau^2}{(\epsilon_p + 2\epsilon_m)(1+\omega^2\tau^2)} + \frac{(\sigma_p - \sigma_m)}{(\sigma_p + 2\sigma_m)(1+\omega^2\tau^2)} \right\} \nabla |E_{rms}|^2 \tag{6}$$

where r is the radius of the spherical particle; ε_m is the permittivity of the medium; ∇ is the del operator; and $|E_{rms}|^2$ is the squared electric field intensity (root mean square), which is

equal to $E_p^2/2$. The terms in the braces are the real parts of the Clausius–Mossotti factor, which is usually denoted by Re[*Ke*]. Here, ε_p, σ_p, and σ_m are the permittivity of the particle, conductivity of the particle, and conductivity of the medium, respectively; ω is the angular frequency defined by $2\pi f$, where f is the frequency of the field, and $\tau = (\varepsilon_p + 2\varepsilon_m) / (\sigma_p + 2\sigma_m)$. The dielectrophoretic force depends on the frequency; when $\omega < 1/\tau$, the force is controlled by the conductivity, and when $\omega > 1/\tau$, the force is governed by the permittivity.

Magnetic field

The magnetic force exerted on a particle is mostly governed by the difference between the magnetic susceptibility of the particle in question and the medium (Munaz *et al.*, 2018). Additional factors such as particle volume and various properties of the magnetic field are also significant for the extent to which the magnetic force is felt by the particles. These can move towards a higher magnetic field (positive magnetophoresis) or away from the magnetic source (negative magnetophoresis) owing to the mismatch between the magnetic susceptibility of the particle and the media (Equation 7). Since most biological particles, such as cells and molecules, are diamagnetic, strategies such as magnetic labels and tweaking of the magnetic susceptibility of the media have been developed to achieve a sufficient particle-migration force (Sung *et al.*, 2017). Numerous studies have reported efficient separation of microalgal cells using these techniques. For instance, the harvesting efficiency of *S. dimorphus* microalgal cells has been increased from 60% to 85% by optimizing the magnetophoretic separation process and the characteristics of the magnetic nanoparticle labels (Ge *et al.*, 2015). Moreover, recent efforts have demonstrated label-free separation of biological compounds by negative magnetophoresis. Microfluidic devices have been developed to continuously separate paramagnetic cells such as deoxyhemoglobin red blood cells and diamagnetic cells such as white blood cells from blood samples based on their native magnetic properties, with efficiencies of 93.5% and 97.4%, respectively (Han & Frazier, 2006).

$$F_M = \frac{\Delta_x V_p}{\mu_0}(B\nabla)B \tag{7}$$

Thus, the force on a particle in a magnetic field depends on the difference in the magnetic susceptibility of the particle and the fluid ($\Delta\chi = \chi p - \chi f$), the magnetic flux density (B), and its gradient (∇B). μ_0 represents vacuum permeability.

Electromagnetophoresis exploits the Lorentz force that manifests when a magnetic field is combined with an electric current (Equation 8). Even though the phenomena are known for quite some time, more recent publications have revisited these strategies to exploit their potential to manipulate micro- and nanoparticles. For instance, the electromagnetic migration of polystyrene particles with various diameters ranging from 3 to 22 μm has been demonstrated (Namba *et al.*, 2000). More recently, the electromagnetophoretic migration of nonconductive particles such as yeast cells has been observed in a capillary flow system and used to measure the dynamic binding force of the interaction between mannose chains outside the yeast cell and Concanavalin A (Iiguni & Watarai, 2010) thus, even if not yet proven directly in microalgal biotechnology processes, these approaches hold great potential for a wide range of upstream and downstream applications.

$$F = \mu HjV\left\{\frac{2(\sigma_1 - \sigma_2)}{2\sigma_2 + \sigma_1}\right\} \tag{8}$$

where μ is the magnetic permeability of the liquid medium, H is the magnetic field strength, j is the current density, V is the volume of the particle, and σ1 and σ2 are the conductivities of the spherical particle and medium, respectively.

Ultrasonic waves

Ultrasound is a mechanical acoustic wave with a frequency range of approximately 10 kHz–20 MHz. Water is an excellent medium for the propagation of acoustic waves, as indicated by its low attenuation coefficient compared with electromagnetic or light waves. At low frequencies (20–100 kHz), sound waves convey high energy to algal cells by cavitation and shear effects (Chia *et al.*, 2019). On the other hand, high-frequency ultrasound waves of 1–10 MHz, could interact with the resulting microalgal particles through acoustophoresis (Figure 4). Two superimposed high-frequency sound waves can constructively interfere, generating a standing wave characterized by regions of maximal pressure oscillation amplitude, known as antinodes, and regions of zero displacement, known as nodes. The susceptibility of a suspended particle to a resonant ultrasonic field depends on the acoustic contrast factor (determined by its density and compressibility), relative to the suspending fluid (Urbansky *et al.*, 2019).

The acoustic contrast factor (ϕ) can be calculated from the density (ρ) and compressibility (β) of the particles (p) and medium (m), which are suspended in (Equation 9) (Yosioka & Kawasima, 1955).

$$\phi = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m} \tag{9}$$

Particles with $\phi < 0$ move towards the antinodal plane, whereas particles with $\phi > 0$ move towards the nodal plane. In case the acoustic contrast factor is 0, the particles will not move in any direction (Leong *et al.*, 2013). The underlying forces causing this movement and the accumulation of particles at the nodal or antinodal planes are called primary and secondary radiation forces, respectively. The former is a second-order nonlinear force that can be calculated from the particle radius (R), wavenumber (k), specific energy density (E_{ac}), distance from the nodal point of the standing wave (x), and acoustic contrast factor (Equation 10).

$$F_{ac} = \frac{4\pi}{3}R^3 k E_{ac}\phi \sin(2kx) \tag{10}$$

Secondary radiation force plays a key role in harvesting microalgae through acoustic forces. This force moves particles within a plane together, which can result in the formation of larger aggregates and, therefore, easier harvesting through enhanced sedimentation forces (Leong *et al.*, 2013). When an aqueous emulsion is subjected to a standing ultrasonic wave field, the

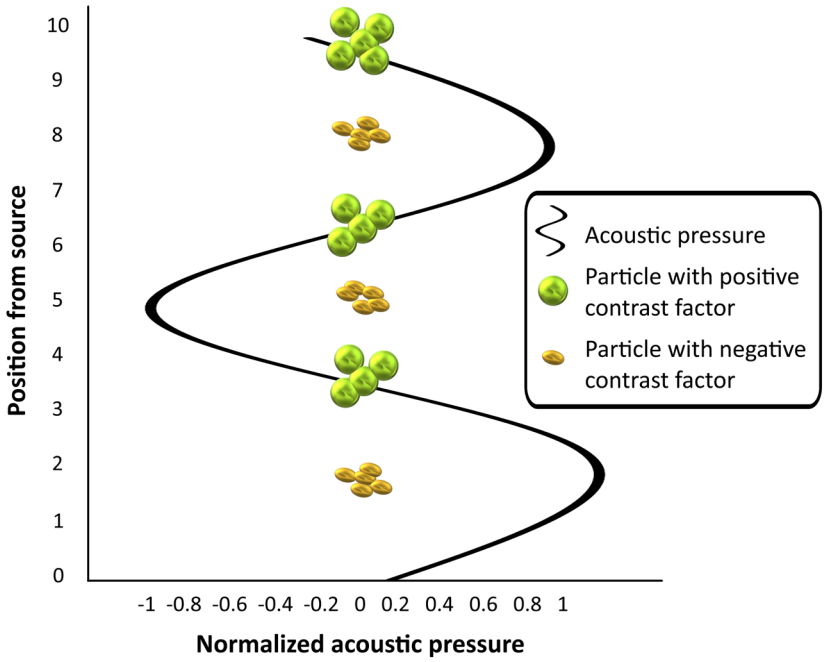


Figure 4. Particle response to a standing acoustic wave based on their acoustic contrast factor. A positive contrast factor triggers particle migration towards the acoustic pressure nodes, while a negative contrast factor determines the particles to migrate towards the acoustic pressure antinodes.

primary acoustic radiation force (particle – acoustic field interactions) is expected to drive some of these particles towards the pressure antinodes of the standing wave field, while others migrate to the nodes, effectively separating them based on their different properties. Additionally, some of these components, such as oil droplets, coalesce owing to secondary acoustic forces (particle-particle interactions) and hydrophobic interactions between the triglyceride molecules, further aiding their separation from the rest of the mixture. These principles have been successfully applied to separate microalgal cells from growth media in small batch volumes and low-throughput systems (Hincapié Gómez & Marchese, 2015; Kumar *et al.*, 2023). The cells were observed to migrate towards the nodes or antinodes of the standing wave and form agglomerates, which ultimately precipitate (Aligata *et al.*, 2019). This could ultimately reduce the cost of algae harvesting by approximately two orders of magnitude.

Novel LoC high-throughput process development platforms

Combining these microfluidic systems with one or more external fields could provide a comprehensive high-throughput automated process development platform or even complete ingredient extraction, fractionation, and stabilization technology. Recently, we proposed a combination of acoustic fields and microfluidic channels for sorting microalgal cells of the desired biomass composition, as well as mildly disrupting and separating most of their main components (Kieffer *et al.*, 2025). When coupled with a cell cultivation stage, this could lead to the development of a complete LoC solution to develop new microalgal biotechnological processes or even achieve high-precision production of functional ingredients (Figure 5). For instance, multiple small cultivation chambers or microreactors can be used for high-throughput screening of different process parameters, media compositions, and even mechanical stress to evaluate their impact on small populations of microalgal cells or even single cells. We recently demonstrated that by subjecting *Arthrospira platensis* cells to mild acoustically induced mechanical stimuli, biomass composition can be precisely manipulated with minimal negative impact on their growth (Marsousi *et al.*, 2025). In- or online monitoring solutions can detect if the presence of desired characteristics, such as overexpression of a certain compound, is present among these cells and release them to the downstream stage of the LoC platform (Havlik *et al.*, 2013; Porras Reyes *et al.*, 2024). High-frequency acoustic standing waves have been envisioned as a label-free approach to sort the desired cell or group of cells and send them to the subsequent cell lysis stage, whereas the rest of the cells can be deviated back to the cultivation chambers or discarded (Kieffer *et al.*, 2025). High-frequency acoustic pulses can be used to lyse microalgal cells, releasing their lipid droplets, chloroplasts containing photosynthetic pigments, protein-rich pyrenoids, nuclei, and starch granules. We recently demonstrated the increased efficiency of this cell disruption method compared to established benchmark technologies (Boboescu *et al.*, 2022). Subsequently, the shear forces aid in breaking the different non-covalent bonds, keeping the cell components together, while preserving at the same time their structural integrity. This represents a critical condition for acoustic fractionation of purified compounds. Subsequently, by increasing and fine-tuning the acoustic frequencies, these particles can be separated into different spatial locations (nodes and antinodes), allowing the recovery of each of these fractions in distinct outlet streams (Figure 6).

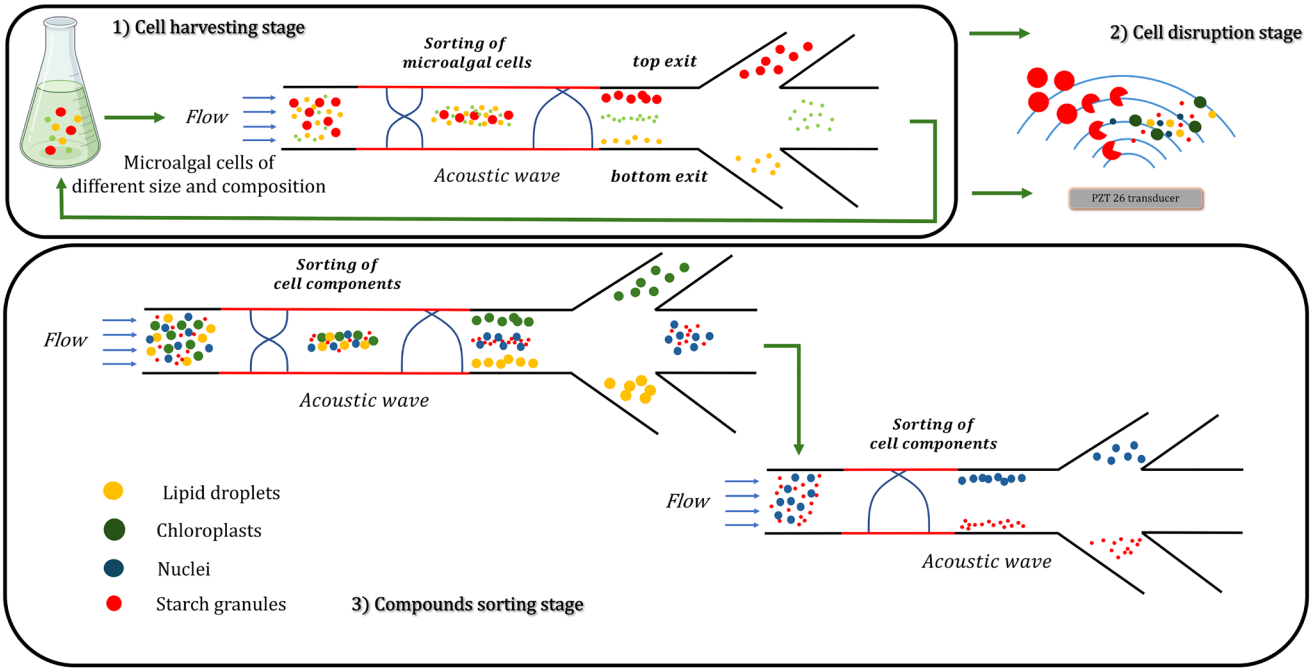


Figure 5. Acoustofluidic process to concentrate microalgal cells and extract their main components. A heterogenous population of microalgal cells of the same species but with different sizes and biomass composition pass through a cell sorting stage where they are being separated based on their differences in acoustic properties (specific density and compressibility). The cells having the desired traits (e.g. high lipid content) are being diverted to the cell lysis stage via high-frequency acoustic standing waves. Here, lower frequency pulses mildly disrupt the microalgal cells and release their main components which are being further separated via successive stages based on their size and acoustic properties using higher frequency acoustic fields. Adapted from (Kieffer *et al.*, 2025).

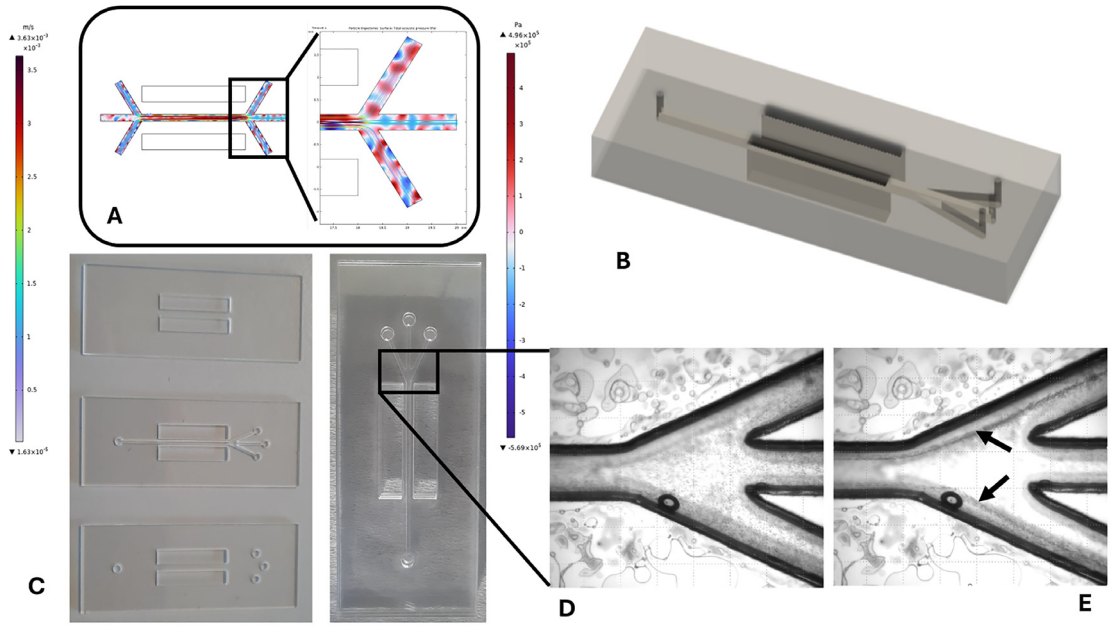


Figure 6. The process of designing, fabricating and testing acoustofluidic sorting systems. The devices are first designed and modeled (**A**) to understand the nature of the generated acoustic radiation (right acoustic pressure bar) and how will this interact with the microalgal cells flowing through the microchannel (left particle velocity bar). This stage allows for further design optimization to increase the separation efficiency of the target cells and/or components. Next, the data is used to design (**B**) and fabricate (**C**) the microfluidic chip. This is used to perform subsequent tests to elucidate these interactions. Particles are observed flowing through the microchannel in an equally dispersed manner when the acoustic field is switched off (**D**), as well as being concentrated towards the two lateral outlets (highlighted by the two arrows) when the acoustic field is turned on (**E**).

We recently demonstrated that various fine structures can be 3D printed using alternative bio-based materials such as algal oils, which could be suitable for mass manufacturing of microfluidic structures, including acoustic LoCs (Fonzo *et al.*, 2025). Microfluidic systems have been used to perform various processes under a higher output. For instance, high-throughput blood cell focusing and plasma isolation have been reported using spiral inertial microfluidic devices, increasing throughput from ml/hour levels to ml/min (Xiang & Ni, 2015). Moreover, stacking together multiple microfluidic platforms allows a significant throughput increase by scaling-out the process, for example, the company Capsum (Wu *et al.*, 2021). Simple microfluidic devices have been successfully stacked to achieve the high-throughput electrokinetic manipulation of particles and cells (Xuan, 2019; Zhu *et al.*, 2016). Researchers from the University of Maine and Harvard University Wyss Institute for Biologically Inspired Engineering have developed a low-cost microfluidic water purification system intended for developing countries. These devices are designed to operate continuously in parallel for prolonged periods of time at dozens of milliliters of treated water per minute per chip.

Two main drawbacks to the process economy are foreseen: manufacturing costs and productivity. The chip cost can decrease from >100 euros/device to <1 Euro per device if the production volume of chips increases from <100 up to millions of units (Jenkins *et al.*, 2015; Su *et al.*, 2016) with standard manufacturing technology, or even lower when using novel manufacturing approaches such as 3D printing (Felton *et al.*, 2021). Furthermore, in contrast to standard equipment that can depreciate over the years, chips can be considered consumables; therefore, they are expected to be replaced after a certain time. Productivity is determined by the flow conditions per chip, which are intrinsically limited by the channel size. The flow rate typically ranges from a few mL min^{-1} to hundreds of mL min^{-1} however, it has been proven that a value of several mL min^{-1} can be achieved with novel technologies (Kojic *et al.*, 2019). However, even if these advancements in chip manufacturing and parallelization could make this approach suitable for the production of specialty ingredients and process development applications, actual industrial-scale processing would require scaled-up technological solutions for the bulk products. Thus, the question remains whether the electrified, non-thermal, and solvent-free processes developed at the microfluidic scale can be translated into an industrial scale and if they are feasible.

Scalability and feasibility of novel electrified and non-thermal processes

Even if the promise of a microbial-based economy has often been alluded to, it always seems to be just out of reach, even if pilot facilities have been successfully operated for quite some time now (Barceló-Villalobos *et al.*, 2019; Fuentes-Grünewald *et al.*, 2021). One of the main reasons for this is that extracting each of the targeted cell components requires a different set of unit operations, each with specific parameters and solvents. Moreover, most often, once a certain compound is extracted, the rest of the biomass, representing more than 90% of the initial amount, cannot be further valorized, becoming thus a waste (Banu *et al.*, 2020). Therefore, the only way microalgal-based specialty components can become a reality is to develop multiproduct biorefineries, with most of the different unit operations condensed into a single processing step (Lam *et al.*, 2018; Slegers *et al.*, 2020). The solution can be used to exploit specific forces triggered by external fields such as electrical fields, lasers, microwaves, and ultrasound to process microbial cells and their components (Pipliya *et al.*, 2025).

At this stage, it is very difficult to provide accurate predictions of how these novel biorefinery approaches are compared to traditional technologies. Our recent observations in high-frequency acoustic harvesting, disruption, and separation of *T-iso* microalgae cells and their components revealed that lower frequencies of 20 kHz required a minimum of 4500 J to disrupt 90% of the microalgal cells, while only 1000 J was required at the highest investigated frequency of 1146 kHz (Boboescu *et al.*, 2022). This translates to a reduction in energy requirements by up to six times when compared with current industry standards, such as bead milling. More than 90% of the energy introduced into the system for cell disruption is dissipated as heat in classical mechanical operations (Lee *et al.*, 2017; Postma *et al.*, 2015; Postma *et al.*, 2017). Thus, electrical or acoustic fields can reduce the input of energy with respect to mechanical techniques, while preserving product functionality (Günerken *et al.*, 2015; Lee *et al.*, 2017). Similar energy requirements are extrapolated for the acoustic harvesting of microalgal cells as well, the harvesting efficiency can reach up to 70 ± 5% with an energy consumption of 3.6 ± 0.9 $kWh \cdot m^{-3}$ using an ultrasonically enhanced settler (Hincapié Gómez & Marchese, 2015). While these values are higher than passive approaches such as settling and flocculation, they could become significantly lower than active ones such as centrifugation (8 kWh·m-3) and even membrane filtration (9.7 kWh·m-3) (Castro-Muñoz & García-Depraect, 2021; Najjar & Abu-Shamleh, 2020). Moreover, filtration technologies are plagued by fouling issues, and flocculation is slow and not suitable for all microalgal species, whereas industrial centrifuges are expensive and require high maintenance. Acoustic harvesters are easily adaptable to continuous processes, have no moving parts, and are suitable for most microalgal species by tuning the frequency and intensity of acoustic standing waves.

Novel approaches include the extraction and separation of various microalgal components. If microalgal multiproduct biorefineries, for instance, could be developed using current benchmark extraction and purification technologies for carbohydrates, proteins, pigments, and lipids, these would represent up to 80% of the total process costs (Lam *et al.*, 2018). Moreover, the harsh conditions used during these approaches could cause the denaturation of many of these compounds, and thus, their techno-functional and bioactive properties. Replacing these with milder electrified approaches such as acoustics, compound extraction, and purification would not only reduce microalgal biorefinery costs but also potentially enhance the functionality of the recovered ingredients

(Corrêa *et al.*, 2021; Pereira *et al.*, 2023). Some non-thermal electrified processes, such as acoustic, pulse electric field, microwave, pressure, and cold plasma, have been demonstrated on a large scale under continuous or semi-continuous modes, suggesting their potential in industrial applications (Gusbeth, 2025; Rao *et al.*, 2023; Yadav & Gogate, 2023). Even if the impact of these emerging technologies on the process economy has never been quantitatively addressed in the literature, it can be speculated that the sharp condensation of the classical cascade of unit operations also reduces labor and investment costs. In addition, the energy costs, especially for cell disruption and solvent evaporation, are expected to be significantly reduced.

Conclusions

Significant breakthroughs are required to fully enable biotechnological applications to generate some of the most used functional ingredients and platform molecules. This often translates into large, expensive, and time-consuming process development platforms that are out of reach for most parties involved. Microfluidic devices present several advantages over these classic approaches by providing inexpensive systems with a small footprint for high-throughput screening and optimization campaigns with single-cell resolution for analysis and control. Moreover, these platforms can be used in conjunction with external fields to retain, stress, transform, and sort cells and even target components in unique ways. This makes them perfect candidates for cost-effective microalgal biotechnology development platforms and production of specialty ingredients. This could lead to the development of novel large-scale electrified microalgal processing technologies to circumvent the need to use multiple complex unit operations and hazardous chemicals. An acoustofluidic device was proposed and briefly described as a potential novel automated process development and production platform. In addition, the scaling-up feasibility of such technologies is discussed, including how they can be compared from a financial perspective with benchmark approaches. We expect that these novel precision cultivation, control, and extraction techniques will enable new biotechnological solutions to replace much of the conventional carbon-intensive processes.

Ethics and consent

Ethical approval and consent were not required.

Data availability

No data are associated with this article.

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General comments:

The review article discusses the potential of using microfluidics for the study of microalgae and in the context of developing high throughput bioprocess techniques. This subject is highly interesting and significant for the community of microalgae bioprocessing and particularly in the context of developing novels techniques for the improvement of current performances at the large scale. The paper discusses promising aspects of microfluidic technology such as the possibility to integrate external fields (electric fields, magnetic fields and ultrasonic waves) to further characterize or treat microalgae cells for developing efficient large scale production purposes.

The review is structured into six distinct sections: Introduction, High-throughput process development using microfluidic technologies, External fields in LoC platforms, Novel LoC high-throughput process development platforms, Scalability and feasibility of novel electrified and non-thermal processes and Conclusions.

It seems important to highlight the originality of this review, which relies on discussing the application of microfluidics and its coupled techniques on particularly microalgae cells and its potential as a high throughput technique for bioprocess productions.

The proposed review should clearly emphasize on its originality compared to the current existing reviews on microfluidic technology and its potential to integrate different external fields techniques in general and on “microfluidics for microalgae applications”. This review should include the optimizations required when choosing to use microfluidic technology for the study and culturing of microalgae cells.

The structure of the article could be redefined to highlight the potential on microalgae. Indeed, the parallelism between microfluidics and external electric field isn’t evident or doesn’t seem justified in the context of this review. Microfluidics is a technology in itself and it can integrate

such physical techniques; it is coupled to external fields for microalgae studies but it's not independent in this case.

As a second major comment, some important points could be mentioned and discussed in this review:

- Fabrication techniques, how do we obtain such devices? What are the parameters to be taken into consideration… a short description could be an additional input in this review.
- The link between the discussed techniques and the microalgae application. What are the limits when trying to use them on microalgae? What are the particularities of microalgae that can impact the efficiency of treatments such as electric and/or mechanical solicitations?
- Are there any results of extraction yields using acoustic fields?
- Automatization is required when moving towards high throughput processing: could the authors discuss this further?

There are some articles and book chapters dealing with this subject that can be helpful, for example:

- Wolf et al. (2023). *High-throughput screening to accelerate microalgae-based phycochemical production.* Value-added products from algae: phycochemical production and applications. Cham, Switzerland: Springer Cham.273-319. https://doi.org/10.1007/978-3-031-42026-9_10 ;
- **Lab Chip**, 2024,**24**, 244-253, https://doi.org/10.1039/D3LC00851G;
- Syed et al., 2024, « A review on machine learning approaches for microalgae cultivation systems », 10.1016/j.compbiomed.2024.108248).

What would be the requirements for using machine learning with microalgae microfluidics and how would it be conducted?

Reviewing comments on the article :

- *« One way to achieve this is to devise automated high- throughput process screening, development, optimization, and even production platforms ».* Could the authors please add references to this comment ? Such as **:** Wolf et al. (2023). *High-throughput screening to accelerate microalgae-based phycochemical production.* Value-added products from algae: phycochemical production and applications. Cham, Switzerland: Springer Cham.273-319. https://doi.org/10.1007/978-3-031-42026-9_10
- *« Moreover, liquid-handling robots can lack flexibility and the ability to adapt to a new task, work- flow, or substrate. These systems might require significant modifications, making them less adaptable than manual or semi-automated systems in specific research environments. Moreover, some liquid handling systems may not be compat- ible with every type of sample or material, such as microalgae cultures, which can have varying viscosities and properties ».* Could the authors please add references involving such studies?
- **Figure 1 :** the representation of downstream processing steps for the recovery of microalgal components isn't obvious through this box-in-box scheme. Could this be revised to show clearly the sequential evolution of the chain of production with the different processing steps ? Maybe by adding arrows to indicate the sequential steps, or numbers ?
- *« Presently, there are a range of delivery pretreatment strategies that are rela- tively invasive and can alter the exogenous DNA to be inserted or subsequent cell viability ».* Could the authors please add a reference here ?
- « *The big data generated by these sensing elements can be used, for instance, to train genetic*

algorithms for machine learning to better understand these processes ». The section including this comment is named : ***Deep monitoring and machine learning.*** However this aspect doesn't seem developed enough in the corresponding text. Please add more detail, see for examples the references above-mentionned on machine learning for microalgae applications.

- *« Reliable integrated in-line process monitor- ing approaches were developed and used to, for instance, select different microalgal overproducers and optimize their growth conditions to maximize the production of target metabolites (Barata et al., 2016; Kerk et al., 2021; Zhou et al., 2021). »*

The references here are review articles. Would it be possible for the authors to add case studies ?

- *« Novel technologies, such as 3D printing, can be used to centrally or locally manufacture these platforms at a fraction of the costs required by automated liquid-handling robots ».*

What are the limits of 3D printing for microfluidics ? Dimensions ? Resolution, fabrication challenges…

- *« However, because these microfluidic platforms are usually made of resins, conventional processing agents such as organic solvents cannot be employed; thus, alternative strategies, such as external fields, are required ».*

The link expressed here between external fields and limiting aspect of resins in mirofluidics is not evident here. How about the other materials that are investigated ? The external fields are mentionned here as a solution for extraction purposes. However, later in the text, they are also related to the purpose of concentrating microalgae?

- *« Although these passive approaches are promising, there are several significant limitations, such as particle-particle interactions, which can disrupt the equilibrium between the fluid and wall lift forces, severely impacting their efficiency ».*

Could the auhtors also mention the particularities related to microalgae structure ? Cell wall composition for example ?

- *« For instance, a "vortex gear" was developed and successfully demonstrated for on-chip applications, by specifically generat- ing two adjacent vortices opposite to each other, similar to a set of gears (Tsai et al., 2018) ».*

Please note here that as the authors Tsai et al., have expressed in their article, this technique has potential for drug delivery or medium refreshment for cell cultures, it seems less adequate for the separation of cells, or at least much more challenging.

- *« Thus, these approaches hold great potential for LoC algal applications, not only for downstream separation strategies . »*

It seems however difficult for large scale productions.

- *« Moreover, efficient identification and sorting of individual leukemia cell lines have been demonstrated by combining multichannel microfluidics with laser tweezers Raman spectroscopy (Lau et al., 2008) ».*
- In the section « Optical forces », please also consider in the discussion the observations published by the reference Lau et al., 2008 that is referred to in the text by the authors.

Please see this section from the article of Lau et al., 2008 : « Although both our proposed RACS device and commercial FACS systems share similar principles (i.e. microfluidic delivery of cells, laser analysis of single cells, cell sorting), the RACS system in its current form is unlikely to compete with the FACS system in terms of throughput or efficiency. This is due to the inherently weak scattering signal of Raman spectroscopy, a fundamental limitation that requires many seconds to minutes to interrogate a single cell. However, the RACS technique may be a more suitable technique to complement FACS systems…».

- In section « Electric field», could the authors also mention : What would be the

considerations to be taken for microalgae? There are some articles published on this application for microalgae. Dielectrophoresis can also be applied for the evaluation of microalgae content using an electrorotating field for example and other articles show other techniques.

- « Dielectrophoresis refers to the migration of a polarizable particle under the influence of an inhomogeneous electric field based on the difference in polarizability between microparticles and microfluidic media ... » it would be preferrable to replace « microfluidic media » by external media for example.
- In the following text : « More recently, the electromagnetophoretic migration of nonconductive particles such as yeast cells has been observed in a capillary flow system and used to measure the dynamic binding force of the interaction between mannose chains outside the yeast cell and Concanavalin A (Iiguni & Watarai, 2010) thus, even if not yet proven directly in microalgal biotechnology processes, these approaches hold great potential for a wide range of upstream and downstream applications. ». Could the authors please add more content on the potential of this technique for microalgae. Particularly, by describing the possible interaction that we could find between the cell wall and another target. By adressing for example the composition of the microalgae cell wall. It would be a very interesting addition to this section.
- These principles have been successfully applied to separate microalgal cells from growth media in small batch volumes and low-throughput systems (Hincapié Gómez & Marchese, 2015; Kumar *et al.*, 2023).

Could the authors also discuss further this aspect ? What is the applicability of this technique on the particular case of microalgae cells ? The chip from the article Kumar et al., 2023 (referred to in the text), for example was able to concentrate rigid cells and to sort them according to size. However, the authors conclude that optimization with respect to cell properties, such as size and flexibility, needs further work. Although no clogging of the chip was seen for R. baltica and Chaetoceros sp., production of exopolymers by T. weissflogii made harvesting challenging for this species. The TrilobiteTM microfluidic chip appears to be a promising tool for pre-concentrating microalgae that are difficult to harvest due to their repelling properties or small size.

- Aspects that could be interesting to mention from the reference used in the text: Cell diameter influence: « Bosma and coworkers demonstrated that the diameter of the microalgal cells influences the aggregation of microalgae. Microalgae Monodus subterraneous, which has a cell diameter of 3.8 mm, was harvested efficiently up to 93% using ultrasonication [68]. Since ultrasound of low amplitude and high frequency (1–2MHZ) causes the aggregation of cells while ultrasound of higher amplitude and low frequency (<1MHz) causes the disintegration of microalgal cells through acoustic streaming and cavitation [70]. »
- What about the upscaling of such technique ? : « Despite these advantages, ultrasound-mediated harvesting is not viable for application at the commercial scale as this method is energy extensive and requires further evaluation for improvement in the separation efficiency [24]. »
- For the following sentence : « Acoustic harvesters are easily adaptable to continuous processes, have no moving parts, and are suitable for most microalgal species by tuning the frequency and intensity of acoustic standing waves », could the authors please provide references to this. Can you please give examples of species and studies ?
- As mentionned by the authors, microfluidics can bring the advantage of conducting high throughput processes. This must be accompanied by Deep learning and machine learning techniques to make it feasible at the large scale. Could the authors comment on this ? Some

examples can be given for machine learning on high throughput studies using microfluidics.

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microalgae bioprocessing, Microfluidics, Electrical engineering

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 22 August 2025

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Referee Report
This review presents an in-depth examination of lab-on-a-chip (LoC) systems and external field-based technologies (acoustic, electric, magnetic, optical, etc.) for the high-throughput, label-free processing of microalgal biomass. The authors cover upstream (cultivation, transformation, monitoring), midstream (screening, sorting), and downstream (harvesting, lysis, separation) stages of microalgal bioprocessing.
The review emphasizes how microfluidic and external field techniques could enable low-cost, small-footprint, and high-resolution processing platforms suitable for multiproduct microalgal biorefineries. The authors also discuss recent advances in fabrication, integration, and scalability, presenting the concept of acoustofluidic LoC systems as a potentially disruptive alternative to conventional approaches.

Overall, this manuscript is clearly a comprehensive review article and highly relevant to researchers working in microalgal biotechnology, process intensification and automation, microfluidics, and lab-on-a-chip development.

Revisions required:
This manuscript demonstrates a board understanding of current technologies but would benefit from a more balanced treatment and critical reflection on the limitations of the discussed technologies. 1) **Clarify novelty**: Explicitly position this review against other existing reviews. What unique angle is being taken? A short comparative paragraph or summary table would help. 2) **Improve critical depth**: Some claims about LoC scalability, replacement of industrial unit operations, or low-cost chip production are currently speculative. These should be clearly qualified or supported with stronger citations. 3) **Assess technological maturity**: Statements suggesting these systems are ready for large-scale deployment may overstate the readiness level. Add a section or table indicating the Technology Readiness Level for each approach (e.g., acoustic sorting). 4) **Machine learning & automation**: The review touches on integrating LoC platforms with ML/big data but lacks specific examples or citations. Including one or two case studies would strengthen this section.

Factual Accuracy and Citations
The majority of factual statements are well supported by citations. However, some quantitative claims—such as cost reductions in chip fabrication and energy savings (e.g., “6× improvement”)—require clearer referencing or methodological justification. Similarly, statements such as “chips can be used as consumables” or “eliminate the need for solvents” are plausible but need to be framed with boundary conditions and proper citations.

The **conclusions** are generally aligned with the literature but should be tempered to avoid overstatement. 1) Reframe speculative future directions more cautiously. For example, rather than saying these technologies "could replace entire microalgal biorefineries," consider phrasing such as “have the potential to complement or transform specific stages of microalgal bioprocessing workflows.” 2) Add a brief **Limitations and Outlook / Future Perspectives** section summarizing unresolved challenges such as chemical compatibility of materials, cost and scalability barriers, standardization and reproducibility, limited throughput in bulk processing.

Literature Coverage
While the review presents a broad and technically rich overview, it omits some recent and highly relevant publications—especially in the areas of label-free cell analysis, machine learning, and high-throughput screening.

References
1. Guo B, Lei C, Kobayashi H, Ito T, et al.: High-throughput, label-free, single-cell, microalgal lipid screening by machine-learning-equipped optofluidic time-stretch quantitative phase microscopy. *Cytometry Part A*. 2017; **91** (5): 494-502 Publisher Full Text
2. Cao J, Russo D, Xie T, Groß G, et al.: A droplet-based microfluidic platform enables high-throughput combinatorial optimization of cyanobacterial cultivation. *Scientific Reports*. 2022; **12** (1). Publisher Full Text
3. Kim H, Devarenne T, Han A: Microfluidic systems for microalgal biotechnology: A review. *Algal Research*. 2018; **30**: 149-161 Publisher Full Text

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microalgal Biotechnology, Bioprocess Engineering, Lab-on-a-Chip / Microfluidics, Microflow Through optical Sensing, Environmental Biotechnology, Process Intensification and Automation, High-Throughput Screening Technologies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.