Engineering Perspectives in Filtration and Separation, Vol. 2, No. 4, December 2024
ISSN:3107-5290

Techniques for Creating, Extracting, Separating, and Purifying Food and Feed Using Microalgae

Dr. Jonathan Whitmore[1] and Dr. Isabella Fontaine[2]*

[1]Senior Researcher, Department of Environmental Engineering, Massachusetts Institute of Technology (MIT), USA.*

[2]Professor, Department of Environmental Engineering, Massachusetts Institute of Technology (MIT), USA.

Abstract--- Numerous Microalgae (MA) species are utilized for their significant biotechnological possibilities in generating various biomolecules applicable across multiple industrial sectors. The primary issue of biotechnology processes is achieving economic viability by producing highly valuable chemicals. Many of these chemicals are sequestered within the cells, necessitating efficient gathering, wellness, and purifying methods. New developments in physicochemical therapies, such as supercritical liquid extraction, ultrasound-assisted gathering, pulsed electrical fields, and solvent-free processes, aim to develop sustainable and adaptable methods for efficient and pure extraction of the desired goods. This article examines the existing methodologies, emphasizing select instances of recently granted patents about gathering, rehabilitating, and purifying microalgal elements at small and large levels, in alignment with the global shift towards bio-based outcomes.

Keywords--- Microalgae**,** Separation Process**,** Purification**,** Food.

Received: 19 - 09 - 2024; Revised: 25 - 10 - 2024; Accepted: 21 - 11 - 2024; Published: 27 - 12 - 2024

I. Introduction

Algae cultivation is a potential approach that addresses population expansion and rising anthropogenic emissions (Tahir et al., 2024). Microalgae (MA) are a heterogeneous group of autotrophic microorganisms capable of utilizing anthropogenic emissions as nutritional sources and converting tiny atoms, such as carbon dioxide or ammonia, into valuable biomolecules like proteins (Ray et al., 2022). MA has elevated photosynthetic efficiency and rapid growth rates, leading to substantial productivity of sugars, proteins, or fatty compounds. Algae cultivation can occur in areas with unsuitable soil, water, or climatic conditions for traditional agriculture. MA does not directly compete with agricultural produce, facilitates the remediation of unfit water for farming, and aids in revenue distribution in arid areas.

The cornerstone for establishing MA as a feedstock for various uses is the creation of economically viable biorefineries (Tong et al., 2023). Algae cultivation is a nascent field that remains in the developmental stage relative to traditional agriculture. The manufacturing of MA significantly diverges from conventional crops, severely restricting the transfer of innovation and machinery from existing agricultural practices. Algae cultivation is expensive due to the necessity of specialized and intricate systems that maintain microalgal cells at elevated productivity levels of biomass and compounds (ElFar et al., 2021). These expenses are also linked to the substantial energy consumption of specific later steps, such as biomass drying and extracting lipids.

The extraction and purifying of biomolecules hinder the construction of biotechnological procedures, rendering them less profitable than chemical alternatives (Anwar et al., 2023). The advancement of innovative, more efficient, and sustainable methods and techniques is pivotal in facilitating the transition to goods made from biomass. The subsequent sections examine the primary technologies for destroying cells, selective gathering, recovery, and purifying of MA components. This research examines the prevalent techniques for obtaining and purifying various components from microalgal feedstock. It provides instances of different kinds of MA and the methods employed to extract the needed ingredients from each.

II. Commercial Cultivation of MA

The food marketplace has revealed the potential for profit of MA, which continues to stimulate its cultivation in different nations. The production of MA has been consistently rising at remarkable rates during the past several decades (Figure 1). Between 1990 and 2024, the MA market underwent a sevenfold increase, culminating in an annual production of 8000 tons of MA feedstock. Recent projections indicate that the yearly manufacturing of MA fluctuated between 18000 and 20200 tons from 2017 to 2020. Rapid growth is anticipated for 2023, with a predicted manufacturing rate of 28500 tons per year and an estimated market value of USD 1.2 billion.

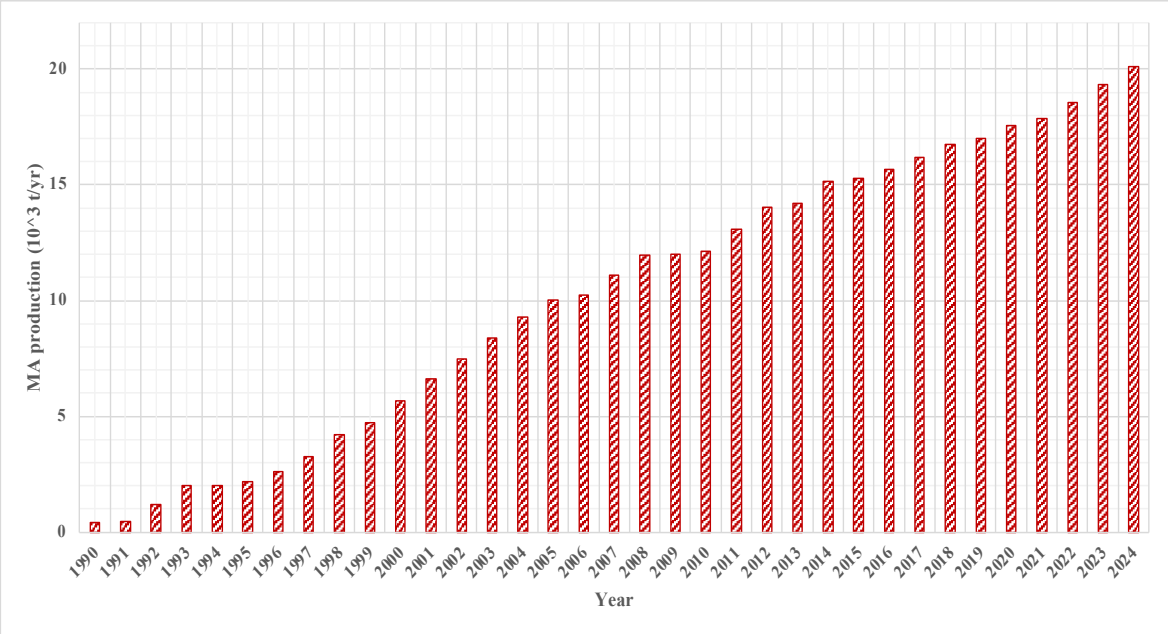


Figure 1: MA Production Analysis Over the Years

Numerous MA strains have notable phenotypic characteristics for enhanced biofuel generation, attracting the interest of various academics and companies throughout the 2000s. Creating a viable manufacturing chain and integrating biofuels into a nation's energy matrix is arduous. Notable instances include the manufacture of bioethanol in the United States and Brazil, which commenced in the 1990s and continues to substitute fossil fuels partially. Research and viewpoints on MA for enhanced biofuel manufacturing have shifted towards long-term outcomes, mainly receiving funding from authorities and significant energy corporations aimed at reducing greenhouse gas emissions (Usmani et al., 2023).

MA is unlikely to be an outlier among other biomasses evaluated for enhanced biofuel generation, necessitating further research to create viable procedures. An up-and-coming option is the progressive fractionation of complete MA biomass into several classes of proteins utilizing the biorefinery idea, facilitating the expansion and diversification of algal farm goods for the marketplace (Ahmad & Ashraf, 2023). It is crucial to emphasize that while numerous compounds can be recovered from MA matter, the advancement of MA breeds or biorefinery techniques in algae farms must consider the supply chain and fluctuations in demand for these compounds. Table 1 shows the nutrients of different samples.

Table 1: Nutrients Analysis

Items	Protein (%)	Carbohydrate (%)	Lipd (%)	Energetic value
Soybean	36	32	21	19.3
Corn	12	78	5	17.3
Wheat	16	67	2	15.4
Vulgaris	61	14	7	21.4
Platenis	58	17	15	16.8
Reisseri	27	14	17	17.4
Bacillaris	18	16	32	18.3

The protein needed for 7.6 billion individuals is roughly 200 million tons, with around 850 million tons of cereals utilized for livestock feed. MA can be integrated into these marketplaces as protein gathers, feed products, or dehydrated MA, which refers to the leftover biomass acquired post-lipid collection. An exemplary instance of effective co-production of biofuels and protein transpired in Brazil throughout the 2010s.

Nutritive Quality of MA Proteins

A critical criterion for assessing protein quality is the analysis of the amino acid composition. Additional criteria, including protein absorption and digestion, are significant, with multiple variables potentially affecting protein digestion, such as shape, antinutritional substances, and the extraction procedure employed. Indicators have been suggested to aid in assessing protein quality, grounded in the concentration of vital amino acids and their bioavailability (Nosworthy et al., 2023).

The World Health Organization (WHO) endorsed the Protein Digestion Adjusted Amino Acid Rating. It is the proportion of the restricting vital amino acid, defined as the amino acid with the most significant disparity relative to the guidance, to the quantity of that amino acid in the WHO standard. This proportion is increased by the protein's digestion (Equations 1 and 2).

$$P(\%) = \frac{mg\ of\ bounding\ amino\ acids\ in\ 1g\ of\ testing\ sample}{mg\ of\ amino\ acids\ in\ 1g\ of\ standard\ sample} * (TD) \quad (1)$$

$$TD = \frac{X*(R_N - R_{EN})}{X} * 100 \quad (2)$$

If TD represents fecal actual digestion, X denotes total nitrate consumption, and RR_N and R_{EN} signify total fecal nitrate and exogenous fecal nitrate, correspondingly.

The Digestive indispensable Amino Acid Rating was introduced as a more accurate quality of protein metric than the previous one. It evaluates the digestion of each vital amino acid individually (Equation 3), whereas the last measure (P) assesses protein digestion based on nitrate content. It offers a superior depiction of the accessibility of individual amino acids throughout digestion.

$$D(\%) = \frac{mg\ of\ digesting\ amino\ acids\ in\ 1g\ of\ samples}{mg\ of\ the\ amino\ acids\ in\ 1g\ of\ the\ standard\ sample} * 100 \quad (3)$$

Besides actual digestion, another metric that effectively assesses protein bioavailability is the Biological Vitality (BV), which considers nitrogen removal in urination in place of fecal nitrate (Equation 4).

$$BV(\%) = \frac{X-(R_N - R_{EN})-(V_N - V_{EN})}{X-(R_N - R_{EN})} * 100 \quad (4)$$

$R_N$represents total fecal nitrate, R_{EN} Denotes endogenous fecal nitrogen, V_N indicates total nitrate excreted in urine and V_{EN} signifies endogenous nitrate excreted in urine.

The assessment of the impact of eaten protein on livestock weight increase can be readily determined using the Protein Effectiveness Ratio (PER). PER denotes the correlation between weight increase and protein intake (Equation 5).

$$PER = \frac{mass\ gain\ in\ g}{Sample\ intake\ in\ g} \quad (5)$$

The Elementary Amino Acid Indicator is a widely utilized metric for assessing the quality of proteins, relating each vital amino acid's concentration to that in an egg protein (Equation 6). A protein grading approaching 100 indicates superior quality. It does not permit inferences regarding the heterogeneity that arise among amino acids or the digestibility of proteins.

$$E(\%) = \frac{mg\ of\ amino\ acids\ in\ 1\ g\ of\ samples}{mg\ of\ amino\ acids\ in\ 1g\ of\ egg\ sample} * 100 \quad (6)$$

III. Methods of Extraction

Microalgal cells can include numerous beneficial chemicals that require separation from less desirable elements. Various extraction procedures can be employed with cellular disruption or applied directly to the entire cell. These encompass solvent extraction processes employing chemical solvents, ionized liquids, deep eutectic chemicals, supercritical fluids, etc.

Extraction Using Organic Solvents

Solvents made from organic matter are a recognized method for extracting biomolecules from MA. This type of extraction is sometimes preceded by a cell-disruption phase to improve solvent access to intracellular molecules, increasing the extraction yield. Numerous patents filed in the Library have incorporated natural solvents to extract MA biocomponents. The research outlined a solvent-free extracting lipids method utilizing an alkaline solution for cell damage with sodium hydroxide, succeeded by successive aqueous phase division, attaining process efficiency and bioproduct purity akin to conventional solvent-based extraction using hexane.

Extraction Using Alternative Solvents

In contrast to traditional solvents, other solvents are expected to have reduced safety, ecology, and health implications. Alternative fluids include bio-based fluids like terpenes, ionic fluids, deep eutectic chemicals, and liquid plastics. Polyethylene Glycol (PEG) is one of the most extensively researched liquid polymers, recognized for its biodegradability and prevalent application in aqueous two-phase processes.

Ionized Liquids (ILs) are organic matter salts with melting values at or below 100 °C. Not all ionic liquids possess "green" attributes and should be circumvented. Studies provide a comprehensive overview of the possible environmental risks associated with ILs to enhance comprehension. These chemicals typically consist of an organic charge and an anion, which is organic or inorganic. This property considerably expands the use of ILs and has garnered substantial interest from the contemporary scientific community, particularly regarding the removal and separation of compounds.

Supercritical Fluid Separation

Supercritical Fluid Extraction (SFE) has become an environmentally friendly and contamination-free extracting technique, particularly in the food and pharmaceutical sectors. SFE integrates extracting and segregation by meticulously regulating process variables, including pressure, temperatures, fluid flow, and the processing duration. This selective capability facilitates the acquisition of goods with enhanced purity and quantities. Table 2 shows the different minerals available in the sample.

Table 2: Minerals Analysis Over Different Samples

Items	Soybean	Corn	Wheat	Vulgaris	Platenis	Reisseri	Bacillaris
Ca	5285	8333	4003	5676	7868	4177	1830
Mg	143	517	885	383	794	608	1011
P	3086	2401	5262	5489	3143	84	3484
K	3366	2221	7132	7896	1570	4190	6774
Cu	33.03	25.49	26.22	41.8	15.68	55.52	41.91
Fe	668.92	849.17	870.17	0.61	382.45	651.63	883.59
Mn	20.18	9.22	17.27	33.88	13.01	2.91	2.26
Se	0.003	0.07	0.001	0.01	0.013	0.034	0.02
Zn	12.56	15.66	2.45	29.58	21.16	5.15	7.61

Substances with temperatures and pressures exceeding their critical point are called Supercritical Fluids (SCFs), which effectively amalgamate the characteristics of gaseous and liquid forms. In this condition, SCFs exhibit intriguing properties, including reduced viscosity compared to liquids, enhanced solvation capabilities relative to gases, and increased diffusivity. SCFs can facilitate challenging or unfeasible extractions employing natural solvents. Research conducted a comprehensive evaluation of SCFs as solvents for the foreseeable future, showing that, from a sustainability point of view, SCFs represent an attractive alternative to traditional chemical solvents, offering goods with outstanding purity and less consumption of energy, particularly as advancements in high-pressure methods continue.

IV. Methods for Separation and Purification

Post-extraction, biomolecules are typically blended with a solvent or integrated into one stage, necessitating the separation of essential compounds from those of lesser significance or removing contaminants that diminish their value or introduce toxicity. Separation techniques are necessary to purify the chemicals that are obtained. This encompasses electrophoresis, membrane segregation, and ultracentrifugation, among others, whose attributes, benefits, and drawbacks will be delineated.

Electrophoresis

Electrophoresis utilizes the molecular charge to facilitate their migration via a magnetic field. This approach is frequently employed for the separation of peptides and other biomolecules. When all particles are compelled to traverse the column, particles navigate via the column pore spaces in electrophoresis, whilst bigger particles stay primarily stationary. High-resolution divisions can be accomplished by two-dimensional electrophoresis (2DE), which has been documented to resolve over 1000 molecules from the Escherichia coli bacteria in just one study. This method's advantage lies in separating molecules by their charges, followed by their molecular masses. Research indicates that phoresis and 2DE have been employed to resolve water-soluble and insoluble peptides from MA. The study delineated a technique utilizing ultrasonography and acoustophoresis for the gathering, lipid removal, and separating Dunaliella salina. The procedure consists of three consecutive compartments; in each room, a designated ultrasonic transducer generates acoustic waves parallel to the flow to facilitate gathering, lipid removal, and lipid segregation accordingly.

Membrane Separation Techniques

Membrane procedures facilitate separating various chemicals according to their size or energy (e.g., electromembrane). In traditional membrane filtering, compounds exceeding the membrane pore dimensions are trapped, while smaller molecules navigate the pores indiscriminately, without selection.

- ***Ultrafiltration***

Ultrafiltration (UF) is extensively employed for the concentration of macromolecules and the exchange of buffers. Utilizing membranes with reduced pore sizes (i.e., 1-100 nm) under mild temperatures can produce excellent retention of peptides and activities. In contrast to chromatographic procedures, UF lacks selectivity and is prone to fouling and absorbing target chemicals during filtration. It is a cost-effective method that is easily scalable for commercial uses.

The research assessed the purification of crude isolated from Spirulina sp. by a two-step process. Initially, conduct microfiltration using filters with pore sizes of 5 and 0.9/0.1 μm at flow rates of 140 and 120 mL min^{-1}, accordingly, followed by ultrafiltration with a membrane having a 50 kDa Molecular Mass Cut-Off (MWCO) at 67 kPa and a flow speed of 05 mL min^{-1}. Under these circumstances, the purity ratio improved from 0.52 ± 0.21 (before UF) to 1.15 ± 0.02, qualifying it as food-safe.

The pigment purifying study examined ultrafiltration as an adjunct to alcohol-salt aquatic two-phase processes for the purifying of fucoxanthin, resulting in a roughly 15% decrease in contaminants. They observed that elevating the ethanol content in the biphasic system resulted in a more significant entrapment of pollutants within the membrane. In this instance, fouling is deemed a favorable occurrence.

- ***Electromembrane Filtration (EF)***

EF serves as an alternative to traditional membrane filtering. The primary benefit of EF is its ability to facilitate separating based on charge and molecular mass without the need for pressure, exhibiting more excellent selectivity than traditional membranes. Research has demonstrated that EF is an effective method for collecting Chlorella. When power is utilized, KR-1 enhances the Concentration Factors (CF) fourfold (6.52). The highest CF attained was 1.45 without power and 1.61 using a commercial polyvinylidene membrane.

Ultracentrifugation

The distinct sedimentation of atoms, based on their concentrations and influenced by gravitational force, is the fundamental basis of particle separation through spinning. At the laboratory size, the force of ultracentrifugation can attain levels up to 200,000 x g, enabling the removal of contaminants (e.g., chlorophyll, cellular debris) from C-phycocyanin extracts and the estimation of molecular weights of amino acids and enzymes derived from phytoplankton. Ultracentrifugation is now obsolete for this function.

Ternary Partitioning Plan (TPP)

TPP is an efficient technique for purifying proteins. T-butanol and sulfuric acid are introduced to an aquatic protein mixture. Ammonium sulfate facilitates protein precipitation and subsequent gentle spinning results in protein concentration within a transition zone that includes two indistinguishable phases: t-butanol above and water below. T-butanol is typically miscible in freshwater; however, specific quantities of salt, such as ammonium sulfates, render it immiscible. In TPP, -butanol enhances protein buoyancy by functioning as a co-precipitating reagent and facilitating triphasic production. Recent studies employed an amalgamation of TPP and LBF, utilizing Liquid Triphasic Floatation (LTF), to extract proteins from the bacterium Ch feedstock. LTF,

the three phases consist of a combination of microalgal material, saline solution, and t-butanol. The proteins are most significant in the transitional zone and TPP, and the air pockets utilized in LBF expedite extraction, hence lowering processing time. In this study, they attained over 92% recoveries and over 65% efficiency when separated.

V. Conclusion

Microalgal biomass is a prolific supply of biomolecules applicable across several industrial sectors, including medicine, food, and aesthetics. Akin to any biotechnological procedure, it is imperative to devise measures to ensure its economic viability. Recent advancements using hypercritical, ionic, and deep eutectic liquids have demonstrated prospective alternatives to conventional extractive procedures reliant on organic solvents. Physicochemical therapies, including supercritical liquid gathering, ultrasound-assisted gathering, and pulsed fields of electricity, are promising techniques for large-scale use, demonstrating excellent efficiency and yielding extracts of elevated purity. The therapies can be applied in upstream procedures as growth stimulants and downstream procedures to improve extracting yield. In biomolecule purity and separation, chromatographic techniques are extensively utilized at the laboratory level; however, advancements in non-chromatographic approaches utilizing multiphase processes have broadened their applicability and rendered them scalable. The requirement for substantial quantities of polymer or organic solvents remains the primary disadvantage of these approaches. Environmentally friendly innovations integrating high effectiveness, selection, and minimal energy consumption, such as extracting supercritical fluid, are the preferred methods in large-scale MA operations. Recent advancements in microalgal feedstock have illustrated its significant potential as a resource for innovative biorefineries and the feasibility of a new, ecologically friendly bio-based society. Although the emergence of competitive biological products in the market remains challenging and necessitates significant commitment from researchers, the shift from chemical to organic goods is an undeniable global trend.

References

[1] Tahir, F., Ashfaq, H., Khan, A. Z., Amin, M., Akbar, I., Malik, H. A., ... & Malik, S. (2024). Emerging trends in algae farming on non-arable lands for resource reclamation, recycling, and mitigation of climate change-driven food security challenges. *Reviews in Environmental Science and Bio/Technology*, *23*(3), 869-896. https://doi.org/10.1007/s11157-024-09697-0

[2] Ray, A., Nayak, M., & Ghosh, A. (2022). A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production. *Science of the Total Environment*, *802*, 149765. https://doi.org/10.1016/j.scitotenv.2021.149765

[3] Tong, K. T. X., Tan, I. S., Foo, H. C. Y., Show, P. L., Lam, M. K., & Wong, M. K. (2023). Sustainable circular biorefinery approach for novel building blocks and bioenergy production from algae using microbial fuel cell. *Bioengineered*, *14*(1), 246-289. https://doi.org/10.1080/21655979.2023.2236842

[4] ElFar, O. A., Chang, C. K., Leong, H. Y., Peter, A. P., Chew, K. W., & Show, P. L. (2021). Prospects of Industry 5.0 in algae: Customization of production and new advance technology for clean bioenergy generation. *Energy Conversion and Management: X*, *10*, 100048. https://doi.org/10.1016/j.ecmx.2020.100048

[5] Anwar, A., Imran, M., & Iqbal, H. M. (2023). Smart chemistry and applied perceptions of enzyme-coupled nano-engineered assemblies to meet future biocatalytic challenges. *Coordination Chemistry Reviews*, *493*, 215329. https://doi.org/10.1016/j.ccr.2023.215329

[6] Usmani, R. A., Mohammad, A. S., & Ansari, S. S. (2023). Comprehensive biofuel policy analysis framework: A novel approach evaluating the policy influences. *Renewable and Sustainable Energy Reviews*, *183*, 113403. https://doi.org/10.1016/j.rser.2023.113403

[7] Ahmad, A., & Ashraf, S. S. (2023). Sustainable food and feed sources from microalgae: Food security and the circular bioeconomy. *Algal Research*, 103185. https://doi.org/10.1016/j.algal.2023.103185

[8] Nosworthy, M. G., Medina, G., Lu, Z. H., & House, J. D. (2023). Plant Proteins: Methods of Quality Assessment and the Human Health Benefits of Pulses. *Foods*, *12*(15), 2816. https://doi.org/10.3390/foods12152816