A Brief Evaluation of Extraction Methods of Bioproducts from Microalgae Biomass

Elena Moore

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Abstract:

The myriad of beneficial bioproducts that can be obtained from the cultivation and harvesting of microalgae biomass prove microalgae to be a promising potential source of sustainable and useful products. Through cultivation methods of open pond systems and photobioreactors, followed by harvestation through filtration, centrifugation, or flocculation, essential bioactive compounds can be extracted from the biomass for industrial and commercial uses. The extraction of these essential products requires cellular disruption, of which there are several different methods to achieve. These methods can be categorized into mechanical, chemical, physical, and enzymatic techniques and differ in environmental impact, time, and potential bioproduct yield. Based on the utility of their applications and practicality, these methods can be evaluated for their capabilities and feasibility as effective means for the sustainable and beneficial bioproducts found in microalgae.

Introduction:

Microalgae bares a chemical composition of constituents such as essential lipids and fatty acid chains, proteins, and chlorophylls. These bioactive compounds that exist within the cell wall of these microorganisms can be applied to many different industrial and commercial uses. The proteins that can be derived from the cells can be utilized in the cosmetic industry as well as in the form of food supplements, and are even considered a potential source of livestock feed *(13)*. Certain essential lipids such as the polyunsaturated fatty acid that are found within the algae cell structure can provide health benefits to prevent certain heart diseases and autoimmune disorders upon extraction and purification *(13)*. Due to the ability of algae to perform photosynthesis, photosynthetic pigments such as chlorophylls exist within their cells, which can be utilized for their antioxidant and antimutagenic properties.

Despite the countless benefits to be gained from cultivating this resource, microalgae production and utilization has not developed in industry. This is due largely to the fact that the extraction and purification processes of the bioproducts from the microalgae biomass are extremely complex and costly *(1)*. This paper will review and discuss examples of these different extraction methods, and look to evaluate the practicality of such processes in the industry.

Such processes for extraction can be filtered into four different categories, being mechanical, chemical, physical, and enzymatic *(12)*. Mechanical techniques include using pulse electric fields for extraction as well as pressurized liquid extraction. Examples of chemical processes include supercritical fluid extraction and use of ionic liquid solvents. Physical extraction methods include microwave and ultrasound assisted extraction of bioactive compounds, and enzymatic techniques use enzymes such as trypsin to break down the cell wall for raw materials.

This paper will focus on the evaluation of these process for several factors. These include the environmental impact of the process, potential yield of bioproduct, avoidance of degradation of raw material, and time of the extraction process. The performance of each extraction method based on these criteria will provide a brief analysis of the practicality of the method in industry. This will be a suitable starting

point for the determination of the most promising method of extraction for the products that can be derived form microalgae organisms.

Chemical Methods for Extraction:

Supercritical Fluid Extraction Solvents:

Most chemical based methods for bioproduct extraction from microalgae use extractive solvents to separate the algal biomass from its bioactive compounds. A few such solvents that have been developed as of recent include supercritical fluids and ionic liquids. Supercritical fluids have both liquid-like density and solvent capabilities similar to that of a liquid, additionally because the temperature and pressure of the supercritical fluid are at the critical point, even slight changes to the temperature and pressure variables will alter its density which, in practice, allows for manipulation and control over the substrate *(14)*. Some solvents that can be used for their critical properties include ethanol, carbon dioxide, methanol, ethylene, dimethyl ether and fluorinated hydrocarbons. The specific critical properties of these substances are compared in Figure 1 below:

Solvent	Molecular weight	Critical temperature	Critical pressure	Critical density
	g/mol	K	MPa (atm)	g/cm^3
Carbon dioxide (CO_2)	44.01	304.1	7.38 (72.8)	0.469
Water (H_2O) (acc. IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH_4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C_2H_6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C_3H_8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C_2H_4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C_3H_6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH_3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C_2H_5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C_3H_6O)	58.08	508.1	4.70 (46.4)	0.278

Table 1: Overview of Properties of Supercritical Solvents

This ability for manipulation of the transfer conditions and properties makes these fluids extremely suitable as solvents for algal biomass when considering the separation of bioactive compounds from the solvent after penetration of the compound to the solvent. Supercritical CO_2 is the most effective choice as a solvent. Its relatively low critical temperature allows for the extraction to be carried out at a

low temperature which lowers risk of thermal degradation to the bioproducts and it has a high density which gives it a high solvent power compared to other supercritical fluids. It also has a low critical pressure which means the process can be carried out at a lower energy.

The advantages of using supercritcal fluid solvents for the extraction of bioactive compounds, are particularly evident when viewing their environmental impact and yield purity. This process is environmentally sustainable, as supercritcal CO_2 is a non-toxic and non-combustible substance and therefore not hazardous to workers. Additionally when used in this way, namely being extracted from the environment and sequentially used in a process and later returned to the environment, the use of carbon dioxide will not contribute to the greenhouse-gas effect *(15)*. Lastly whereas the heat input used in other methods often leads to heavy hydrolysis of the compounds being extracted and in turn oxidation which degrades the bioproduct yield *(5)*, the use of supercritical CO_2 requires little to no heat and therefore limits thermal degradation of the product. The shortened extraction time that is an effect of the high density and low viscosity of supercritcal CO_2 as well as the limited thermal impact lead to an extremely pure bioproduct yield compared to that of other discussed methods.

While supercritical fluid extraction is extremely strong in categories such as purity of yield and environmental sustainability, one area of weakness is in bioproduct yield. The strong inter-molecular forces of the supercritical CO_2 leads to agglomeration, an effect that lessens the ability of the particles to flow over large surface areas. This reduced flow leads to less penetration into the analyte, and therefore a slightly lower extraction yield.

Ionic Liquid Extraction Solvents:

Ionic liquids are essentially liquid salts with arbitrarily low melting temperatures that consist of mostly organic cations and either organic or inorganic anions. Some of their properties include melting without vaporizing or decomposing and low vapor pressure, both of these properties lead them to act as capable solvents *(4)*. Other characteristics of ionic liquids point to their potential as one of the most environmentally safe and variably manipulable solvents of algal biomass for

extraction of bioactive compounds. Ionic liquids are nonvolatile, can be made to suit various conditions by altering their composition, and due to their low vapor pressure will produce very little air pollution.

Physical Methods for Extraction:

While use of these solvents is considered a method for extraction in itself, these solvent methods are generally used alongside a different method that pre-treats the biomass by breaking down the microalgal cell *(10)*. Without first disrupting the cell wall, the effectiveness of the solvent is very limited. Once the cell wall has been degraded and the proteins that bind the bioactive compounds within the microorganism are broken down, these solvents work much more effectively to extract these bioproducts from the substrate.

Microwave-assisted Extraction:

Microwave-assisted extraction (MAE) methods of obtaining lipid bioproduct from microalgae rely on heating the biomass solvent to allow the solvent to penetrate into the cell wall. This is achieved by applying microwave energy to the polar molecules within in the biomass solution. Using microwave assisted solvent allows for significant energy transfer within the biomass material, and in turn the disruption of weak hydrogen bonds within the cell walls as shown in Figure 2 below:

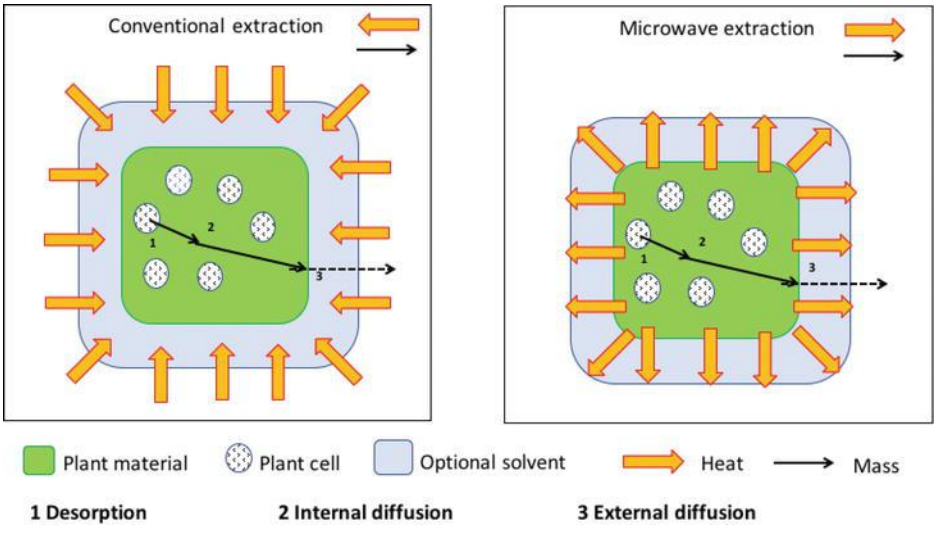

Figure 2: MAE versus Conventional Extraction Method from Plant Material *(17)*

Unfortunately the thermal addition to the substrate system risks degradation of the product. Degradation arises when the rate of reaction between the solvent and analyte is increased to the point where oxidation and other naturally degrading reactions of the compound for extraction occurs *(13)*. Nevertheless, this method is one of the most efficient based on time and bioproduct yield due to its efficient application of heat directly to the cell to acceleration of reaction rate.

Ultrasonic-assisted Extraction:

Ultrasonic-assisted extraction, (UAE), methods are effective in achieving high extraction efficiency. With good extraction time, flow rate, and algal cell concentration ultrasound is especially effective for the extraction of carbohydrates from the biomass *(7)*. This method utilizes something called ultrasonic cavitation to disrupt the cell walls for extraction of bioproducts from within. Ultrasonic cavitation occurs when high power ultrasound waves are sent through the algal biomass solution creating high and low pressure cycles of the bio-material. This is shown in Figure 3 below:

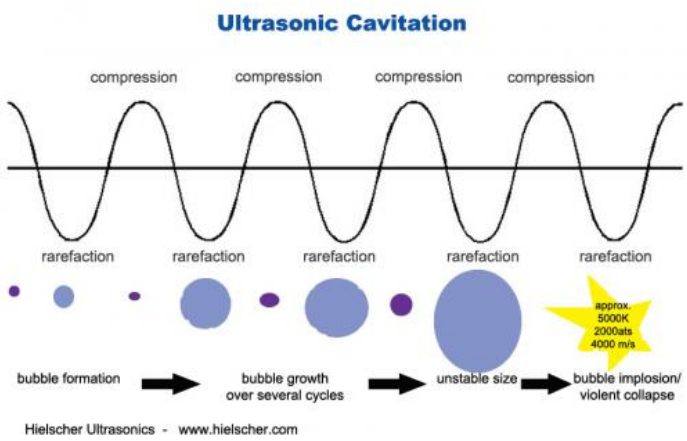

Figure 3: Ultrasonic Cavitation *(8)*

These pressure discrepancies from the ultrasonic cavitation then lead to collisions within the cell and between cells leading to rupture of the cell. The waves and pressure forces created by this process release the desired carbohydrates and lipids into the solvent. Once released, the contents can be purified from the solvent and cultivated for use.

Advantages of UAE are seen in the low environmental impact caused by the process as well as low degradation to the bioproduct yield. Specifically UAE using only ethanol as a solvent for the algal biomass which is nontoxic and biodegradable as it is formulated from a biomass itself and breaks down into harmless substances *(6)*. Because MAE requires higher temperature of the solvent for a high extraction yield, degradation of the bioproduct is likely to occur due to thermal denaturation. UAE can be preformed at lower temperatures than MAE, decreasing possible degradation of material yield from the biomass and proving it a more practical method for proper bioproduct extraction.

Mechanical Methods for Extraction:

Pressurized Liquid Extraction:

Pressurized liquid extraction (PLE) is a method for the acceleration of extraction of mainly chlorophylls and carotenoids from algal biomass *(13)*. This method utilizes high pressure and temperatures to speed up the solubility and diffusion of the biomass analyte in the extraction solvent. The high pressure helps to maintain the liquid state and temperature of the solvent, which is important for keeping consistent procedural conditions that will ensure the purity of the bioproduct yield. PLE can take one of two forms that differ in consideration of the replacement of the solvent throughout the reaction between it and the biomass analyte, one form utilizes a static flow and the other utilizes a continuous flow *(11)*, the two systems models are compared in Figure 4 below:

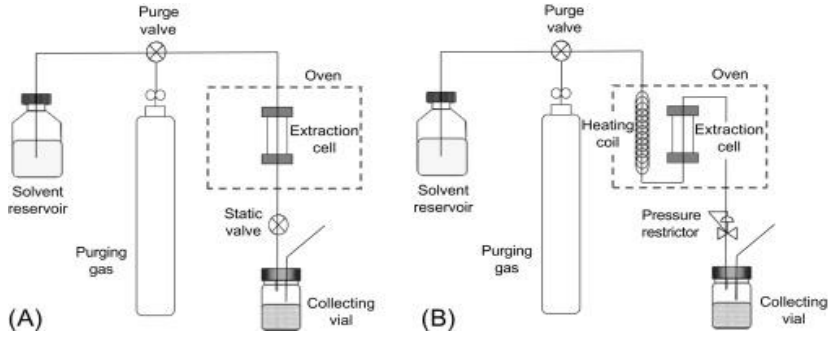

Figure 4: Configurations of (A) Static Model PLE and (B) Dynamic Model PLE *(11)*

The static flow model collects the analyte in consecutive cycles and the system is allowed to reach equilibrium, this however is not ideal as it allows for possible degradation of the bioactive compounds because at equilibrium the efficiency of the system can no longer improve. The second form of PLE, the continuous flow model, is then more favorable. Continuous flow models avoid equilibrium by continuously flowing extraction solvent through the cell, in turn allowing for the efficiency of the system to increase and degradation of the compound to be avoided. This model can be further optimized by using a high flow rate of the extraction solvent to allow the analyte to be fully penetrated by the solvent *(11)*. This optimized pressurized liquid extraction method proves promising for quickly and effectively producing high volume yield of bioactive compound from biomass that avoids degradation of material due to the high pressure and solvent flow maintenance.

Pulse Electric Field Methods for Extraction:

The break down of the cell structure of the microalgae cell is one of the most important tasks in extracting the bioactive compounds that exist within the cell walls. Pulse electric field methods for extraction acceleration are interesting in their utilization of high voltage pulses through the biomass to aid in cell disruption. The pulse of an electric field through the cell increases the permeability of the cell membrane, allowing for increased or accelerated extraction of intercellular compounds from the analyte.

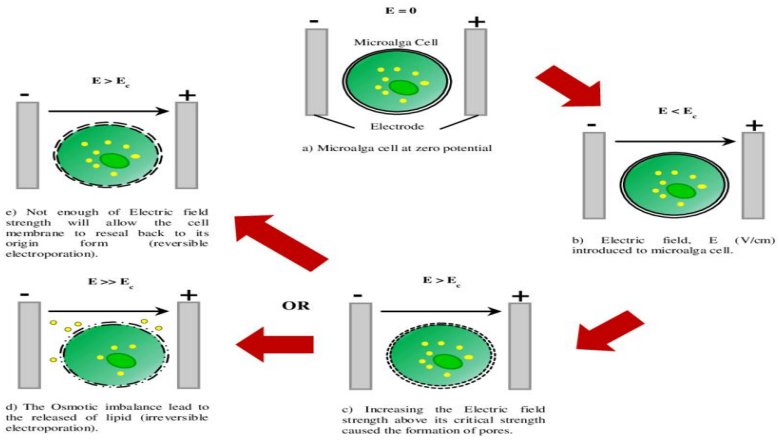

Figure 5: Flow Diagram of the Effects of an Electric Field on the extraction of lipids from the cell membrane *(18)*

Previously this method has primarily been performed for the extraction of carotenoids and proteins from the within the cell walls of the microorganism *(13)*. Since this method does not use heat, pulse electric field methods avoid degradation of the extracted compound. It also allows for large volume extraction at a short extraction time, and avoids large energy losses. These characteristics make this method an environmentally and economically efficient process for cellular disruption in the compound extraction process.

Enzymatic Methods for Extraction:

Enzyme-assisted Extraction Method:

One issue with using powerful solvents to penetrate the biomass substrate is that when attempting to recover material for human use, the toxic effects of conventionally used organic solvents must be considered. Organic solvents such as chloroform and methanol are traditionally used in extraction methods because of their immiscibility with water and ability dissolve non-polar compounds that are otherwise not readily soluble. However while these solvents are highly effective in producing high yield product quickly, they are unsafe to humans. Chloroform has been identified as a possible carcinogenic and methanol has a high toxicity *(16)*. One solution to this issue is to use a water-based solvent. When using a solvent such as this, it is particularly necessary to include a technique to increase the efficiency of the yield as water based solvents are typically less efficient*(9)*. One such technique is the addition of an enzyme to the solution to increase the rate of the reaction. Enzymes typically used in this process include cellulase, glucosidase, xylanase, and pectinase. When using enzymes the process parameters must be set-up and monitored closely. The temperature must be high enough for the enzyme to work effectively, but not too high as to degrade quickly. Maintaining the proper ratio between enzyme and solvent can be challenging. The correct ratio depends on temperature, and the amount of each changes as each is consumed in the process.

Enzymes can alternately be used to directly decompose the cell wall. One technique is to apply an enzyme called trypsin which acts by breaking down the proteins that allow the cells to associate as shown in the diagram in Figure 6:

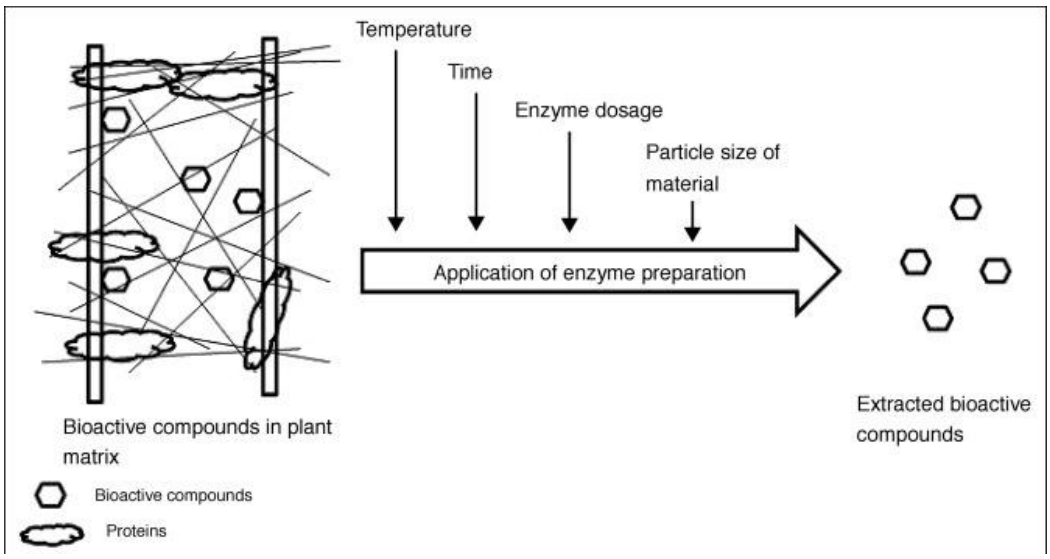

Figure 6: Enzymatic Breakdown of the Algal Cell Matrix *(9)*

Polysaccharidases such as cellulase and agarase perform a similar function of degrading the cell wall, and have been proven to effectively aid in the extraction of proteins from the microalgae cell walls *(9)*. Once an enzyme is used to break down the cell wall, solvents can react with the biomass efficiently to produce a higher yield.

Conclusive Discussion:

Typically the most efficient and practical method is a combination of the above strategies. In general the biomass will be pre-treated in preparation for extraction, this is done using one of the previously discussed mechanical, chemical, or physical methods. The goal of these pre-treatments is to break down the microalgal cell to enhance bioproduct recovery in the extraction portion of the operation. After comparing several strategies for the pre-treatment and extraction processes, ultrasound in combination with supercritical CO_2 extraction appears to be the most effective approach to the extraction of bioactive compounds from the microalgal cell. This combination achieves the best result in terms of yield volume, purity of product, and environmental impact.

Ultrasound-assisted supercritical fluid extraction optimizes high efficiency bioproduct extraction with low environmental impact in a quick and safe extraction process. Neither ultrasound-assisted extraction nor supercritical fluid extraction techniques require large thermal additives which would degrade the bioactive compound. Supercritical CO2 is considered one of the most environmentally sustainable solvents in terms of toxicity, combustibility, ease of preparation and impact on global warming.

Concerning the actual effectiveness of the combination of these procedures, the strengths of each technique compliment the weaknesses of the other. The high diffusivity of CO2 allows a very selective extraction resulting in pure bioproduct yields. Unfortunately this technique results in lower extraction yields. Pairing supercritcal CO_2 solvent with ultrasound makes up for this loss in yield. The addition of ultrasound ruptures the cell wall and increases the interaction between the solvent and the sample, thus increasing mass transfer rates and in turn the bioproduct yield. Additionally this technique allows for smaller use of the extraction solvents, making the strategy increasingly sustainable. The combination of these two techniques optimizes the respective strengths of supercritical CO_2 extraction and UAE of producing a yield that is high in volume and purity, as well as utilizing environmentally sustainable substances and procedures.

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