CAPÍTULO 4

Microalgal biotechnology: a new green economy?

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Resumo

The green economy emerges as a process that seeks to improve society based on natural methodologies that generate environmentally friendly technologies. Recently, it has been possible to extrapolate this concept to biological processes, one of which is microalgae cultivation. Microalgae have great potential to produce biofuels and bioactive compounds; this technology can contribute to the development of clean energy and high value-added compounds. Although microalgae biotechnology is presented as a promising technology, improvements are needed in the production processes. Thus, microalgal biotechnology seeks to maintain high yields in biomass production, which makes the microalgal industry an economically attractive company for investors.

Palavras-chave: bioproducts, cultivation, photobioreactor, microalgae, technology

1. Introduction

The green economy emerged as a process to generate improvements and sustainability in the environment. Although a relatively recent terminology, organizations, institutions, processes and technologies are included in this term, ranging from renewable energy to waste treatment, to natural resources management and water treatment, among others. Recently the green economy emphasizes the exploitation of biological systems for the benefit of mankind. This is where the microalgae biotechnological attracts the interest of many researchers and entrepreneurs in diverse areas including agricultural, food, pharmaceutical, environmental, in view of the great potentiality and attributes

presented by microalgal biomass for the production of nutraceuticals, animal and human food, biofuels, fertilizers, chemicals of high added value, among others (YAACOB et al., 2022).

There are many variations in the microalgae biochemical composition which depend on the strain type as well as the culture conditions and downstream processing of the biomass. Thus, for example, when subjecting strains of *Chlorella vulgaris* to different irradiations the growth rate varies from 0.08 d^{-1} to 1.13 d^{-1} when irradiated in a photoperiod of 16: 8 ha 37.5 and 100 Mmol photons $m^{-2}s^{-1}$ respectively (SEYFABADI et al. 2011), . On the other hand, seasonal variations may interfere with cellular performance. Open culture systems of *Nannochloropsis oculata* showed fluctuations in lipid yield. In summer and autumn were the most favorable seasons for the production of fatty acids more suitable for biodiesel production (MINHAS et al., 2023).

Microalgae present great potential to produce metabolic compounds of industrial interest. However, some aspects should be considered to make microalgal biotechnology an economically viable industry such as: (i) improvement in the strains selection, particularly those with high biotechnological potential, (ii) production of differentiated culture media for isolation, (iii) standardize biochemical modification methods, (iv) optimization of growth and biomass productivity, (v) design of photobioreactors suitable for large scale production, (vi) improvement in biorefinery processes. There are industries that develop biotechnological processes with microalgae distributed in the world, the considerations developed by them would contribute in the construction of an innovative technological platform for industries based on microalgae (YAP et al., 2021).

Microalgal culture has been used on industrial scale for decades to produce compounds that have repercussions on economic processes. In recent years, there has been an overall increase in microalgal biotechnology for commercial applications, such as the production of biofuels, bioactive compounds and bioremediation. However, few strains are used on an industrial scale, *Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis, Isochrysis galbana, Spirulina maxima* since their development and cultivation characteristics help to obtain better yields and to avoid cross contamination (ELADL et al., 2024).

Microalgae biotechnology presents advantages that catch the attention of many investors: (i) microalgal culture for biofuel production, which do not compromise the food chain, (ii) autotrophic and heterotrophic growth capacity, (iii) relatively easy obtaining of metabolic compounds, (iv) production of food compounds with nutritional and health benefits, (v) carbon biofixation, product of the microalgae photosynthetic metabolism. The production of microalgal biomass can be improved by introducing carbon dioxide (CO_2) from industrial effluents, which in addition to contributing and having high biomass productivity reduce process costs, which could help reduce greenhouse gas emissions (CHIA et al., 2021).

The process of microalgal biotechnology involves basically two steps: upstream processing and dowstream processing, each of which has several established phases ranging from nutrient composition to culture systems, types of lighting, harvesting and biorefinery (Figure 1).

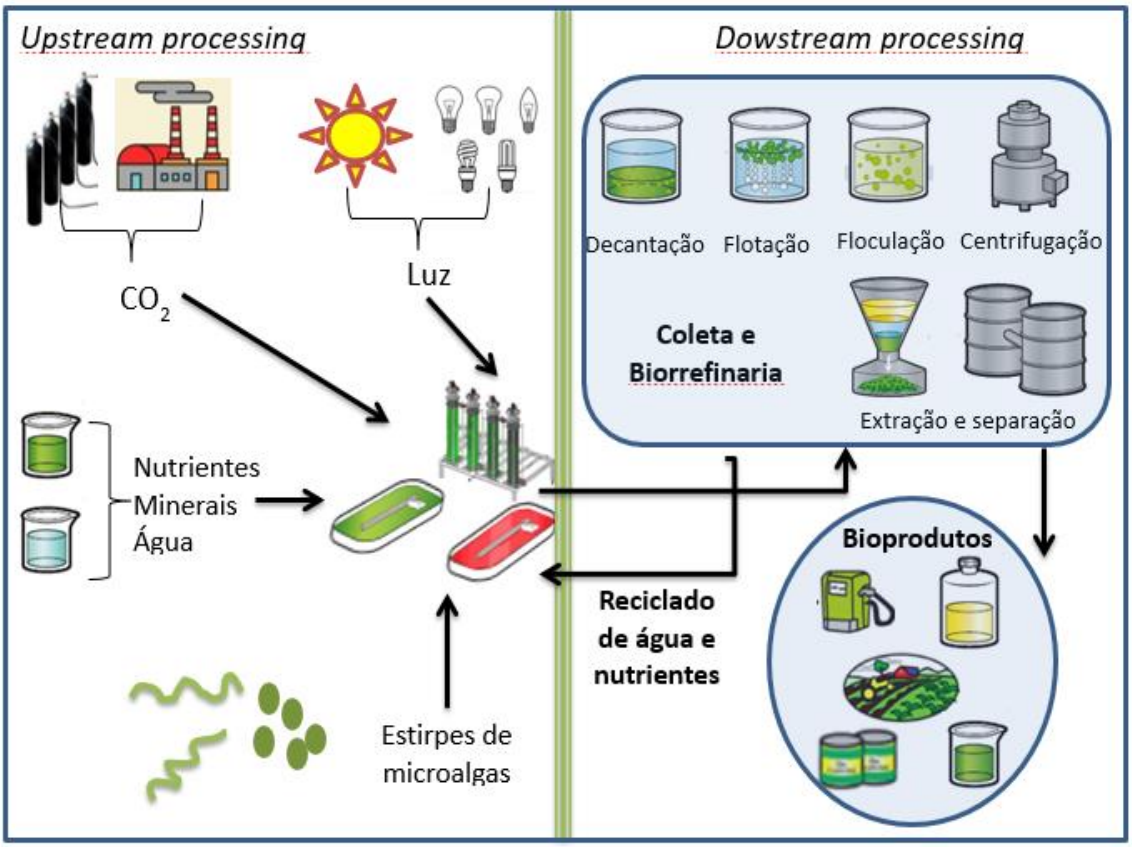

Figure 1. Scheme of the formation process of microalgae biomass and bioproducts.

In view of the fact that microalgae biotechnology offers great investment opportunities, the objective is to obtain a high production and good yield of microalgal biomass, which will serve as a source of a bioproducts diversity. The world market value of carotenoids can reach up to UD$ 1 billion per year, and bioproducts such as astaxanthin may have higher prices (SHOW, 2022). Although these types of biotech companies have great potential for profit, many challenges need to be solved. One of the major challenges for the commercial operations of microalgae is the large-scale production, since at the present time the productive processes are carried out in large measure with open photobioreactors, which limits the growth and the production to few strains types and bioproducts. The closed photobioreactors available on the market do not represent an optimal solution for the companies'needs, although the volumetric and surface productivity is higher than in the open photobioreactors, the performance does not approximate to yield obtained in laboratory scale (SARKER and KAPARAJU 2023). In this paper, we will discuss the need to optimize strains, culture systems, production costs, genetic modification, to make microalgal biotechnology economically viable.

2. Microalgae culture

Despite the great diversity of microalgae in the world, estimated at three hundred thousand, only thirty thousand have been identified and of them about fifty species are used in biotechnological research, only six to ten are used for commercial production of compounds and food additives, but not yet the number of microalgae species that can be used to produce biofuels has been defined (AHMAD et al., 2022). To initiate a microalgal culture, it is necessary to start with a mother culture which must be in adequate conditions to avoid problems of deterioration or contamination in the time. Lyophilization techniques are advantageous. Also the preservation of mother cultures in tubes or Petri plates with minimum medium is used. However, in the latter, cell degeneration can be present, which can be reduced with the addition of a new organic base and continuous peals (SARAVANA, 2023). In addition, manipulation of culture conditions in terms of lighting intensity, temperature and nutrients, produces improved cellular yields (MASOJÍDEK et al., 2021).

The culture conditions, medium type, lighting, coadjutants, temperature, directly influence the costs. For autotrophic culture there are approximately forty types of medium consisting basically of mineral salts, which depending on the culture system should be supplemented with a carbon source. Some commercial medium supplemented with vitamins, amino acids and/or phyto-hormones are used for example the F/2 medium costing UD$ 40 - 60 per liter (CHAKRABORTY et al., 2023).

The microalgal photosynthetic culture depends basically on the light energy in the cells. Inadequate illumination produces biochemical consequences, the product of photolimitation and photoinhibition that affects the kinetic performance in the reactors. The source and type of lighting in the systems is a factor to consider since, based on the characteristics of the process can use natural or artificial lighting. Other aspects that should be considered are the place of establishment of the culture, seasonal variations and variations of the photoperiod (SEYFABADI et al., 2011). Temperature is another important factor associated with microalgal cultures. In general, the optimum growth temperature is in the mesophilic region (25-35°C), although some thermophilic strains resistant to temperatures in the range of 60 °C. The great majority of culture systems assume the temperature changes as a result of the variation of the environment, although the use of heating mantles, coils and external heat exchangers can be installed in the reactors for temperature control (Satiro et al., 2016).

Regarding pH, microalgal culture is normally favored in a range that varies from neutral to alkaline (7-9), although some species show optimum growth at pH 4 (*Chlorococcum littorale*) and pH 10 (*Spirulina platensis*). There is a complex relationship between the concentration and type of the carbon and nitrogen source, since the compounds of NH_3 and NH_4^+ are strongly affected by the pH of the culture medium. Thus, pH control mechanisms must be integrated to the culture system to maintain the optimal conditions of this parameter in the process (MARKOU et al., 2016).

In photosynthetic culture systems, CO_2 is the main source of carbon used in culture; this gas has an atmospheric concentration of 0.034% which is not sufficient to maintain high biomass yields. CO_2 must be supplied through

concentrated stationary sources (compressed carbon dioxide, primary standard mixtures and industrial combustion gases) which provide inlet concentrations in levels between 3 and 15%. Ben-Amoz (2009) showed that *Dunadiella* cultures fed with flue gases increases biomass yield up to 10 times, showing to be a source of economic carbon, besides contributing to the decrease of greenhouse gases. The approximate costs of dry microalgae biomass in the 1950s were UD $ 1,000 kg^{-1}, nowadays thanks to biotechnological development can be found values up to UD $ 0.20 kg^{-1} when grown in wastewater (DE PAUW et al., 1984).

However, there is a broad expectation in the availability and use of CO_2 from industrial combustion gases to support photosynthetic microalgae cultures. However, there have been difficulties in using this technique, since these gases have high indices of impurity (CO, CH_4, NOx, SOx, H_2 and particulate matter), and the temperature of these gases is very high (JACOB-LOPES & FRANCO, 2013). In the same way, heterotrophic and mixotrophic culture have been used in environmental research and has been incorporated in studies of the industrial waste use (STREIT et al., 2015).

Microalgae culture used on large scale include two types: open and closed systems. Open systems are easier to build, more economical, and relatively simple control that closed systems. There are two types of open systems that are best known: (i) circular tanks and (ii) raceway tanks (RAMÍREZ-MÉRIDA et al., 2013). Although simple to construct, these systems have limitations in the transfer of CO_2, generate high evaporation rates producing significant variations in the composition of the culture medium, besides allowing the access of external contaminants. All this makes the use of open systems limited to extremophilic microalgae strains, which are used at industrial level basically for the production of food derived bioproducts (ROJAS-VILLALTA et al., 2024).

The closed systems provide a greater control of the physicochemical parameters during the cycles of microalgae growth, besides allowing the conduction of photosynthetic, mixotrophic and heterotrophic cultures. The main configurations of the closed systems are formed by photobioreactors type: tubular (horizontal, vertical, bubble column and air-lift), flat plate, hybrids and fermenters.

Tubular photobioreactors are the most used at laboratory, pilot and industrial level. The tubular photobioreactors (horizontal and vertical), so called based on the arrangement of their transparent parallel tubes with diameters of 0.1 - 0.2 m. The culture medium circulates through them with gravitational aid or driven with a pump. The greater the number of tubes, the greater the light energy arrangement in the medium, and therefore the surface/volume (S/V) ratio improves. Disadvantages arise when oxygen accumulates, which is toxic to cells, as well as biofilm formation in the walls of the tubes, which decreases light input and increases the cost of cleaning the photobioreactor In addition, constant recirculation of medium can cause cellular stress (SEGURA-MORALES et al. 2024).

The bubble column and airlift photobioreactors have a cylindrical shape, wide S/V ratio and a gas diffuser at the bottom of the column. They have good kinetic performance at low volume. However, their scaling is difficult because of the low reaction volume (PALADINO AND NEVIANI et al., 2021).

Flat plate photobioreactors are designed to make efficient use of sunlight; they have a high S/V ratio. This type of photobioreactors can be organized in such a way that they are oriented towards the sun, which allows better efficiency in terms of absorbed energy (PALADINO AND NEVIANI et al., 2021). The photobioreactor presents good kinetic performance and can be scaled by arranging several surface plates to gain area, but the plate elongation is not recommended for scaling, making it expensive to install on an industrial scale (Padrón and Mérida, 2025) .

The fermenters, for heterotrophic microalgae cultures, has been used in the last years, with advantages since they use substrates of industrial waste. They present high kinetic performance and are easily scalable when using configurations with 1.0-1.5 A/D ratios (RAMIREZ-MÉRIDA et al., 2015).

A comparative study of production costs per liter of microalgae oil in open and closed culture systems showed that in open systems the cost is almost three times lower than in closed autotrophic systems. On the other hand, heterotrophic crops proved to be even more economical. Production costs in autotrophic culture range from UD $ 0.1 kg^{-1} to UD $ 32 kg^{-1} compared to the heterotrophic culture UD $ 2.0 kg^{-1} to UD $ 12 kg^{-1} (ALABI, 2009). Although it is not currently possible

to compete with fossil fuels, it is expected that in the next few years, with the help of biotechnology, microalgae will produce economically viable biofuel.

3. Microalgae strains

The search for and collection of microalgae is relatively easy, although finding microalgae of biotechnological interest is more complicated. Microalgal strains must present characteristics applicable to biotechnological processes that are attractive to entrepreneurs. Thus, strains that present high growth kinetics, with high biomass yield, that are cultivated in wastewater or under natural lighting and temperature conditions, that produce metabolites of interest and that are easily separable would be desirable characteristics for the optimization of production processes. Thus, the choice of commercial microalgae strains is of utmost importance and deserves rigorous investigation. In their natural habitat, the species are well adapted to local environmental conditions and their usefulness contributes to more successful cultivation than non-native species. The cultivation of microalgae in wastewater is profitable in the production of microalgal biomass, and helps in the removal of nutrients (RAMÍREZ-MÉRIDA and RODRÍGUEZ-PADRÓN, 2023). Many research groups show attention to stremophilous strains. They have certain advantages, since they can withstand extreme conditions of temperature, toxic substances, lighting, pressure, among others, which guarantee their usefulness in biotechnological areas of interest. For example, they could directly use combustion gases from industrial furnaces, present high desiccation and photophysiological tolerance, tolerate high concentrations of heavy metals for bioremediation, present resistance to photoinhibition, produce a greater quantity of metabolites under certain conditions, allowing their use to have great potential in biotechnological processes.

4. Biochemical manipulation and high yield

The biochemical manipulation of microalgae is reflected in the composition of the chemical constituents. Several experiments have been reported, when manipulating the nutrients in *Scenedesmus obliquus* cultures, the lipid content

was five to ten times higher than the controls (MANDAL and MALLICK, 2009). On the other hand, it is interesting to note that the carotenoid content in *Dunaliella salina* cultures increased in direct proportion to the increase in salinity (Reshma et al., 2021). Takagi et al., (2006) showed that the salt content of the medium can also be a stressor in *Dunaliella*. In the early stages of the cultures, when NaCl was increased from 0.5 M (equivalent to seawater) to 1.0 M, lipids increased by 67%; when mid- or late-stage cultures were subjected to a similar stress, lipids increased by up to 70%. Thus, when selecting cells for biotechnological applications, the strain and physiological state of the microalgae play critical roles in determining production. The culture medium used influences the cellular biochemical profile as well as the rate of metabolic production. Through biochemical manipulation, lipid content can be regulated, since by placing nutrients in a limiting manner, physiological stress is generated, forcing the cell to alter its metabolism, leading to the accumulation of compounds necessary for maintenance. Some experiments show that in *Neochloris oleoabundans* cultures enriched with sodium nitrate, urea and ammonium bicarbonate as a nitrogen source, only the lowest levels of sodium nitrate showed an increase in lipids (WU et al., 2023). A limitation of inorganic phosphorus and CO2 in *Chlamydomonas acidophila* cultures resulted in high rates (BOSSA et al., 2024). In *Monodus subterraneus* cultures, as the phosphate content decreases, the concentration of lipids in the cell increases (KHOZIN-GOLDBERG AND COHEN, 2006). Nitrogen limitation in *Scenedesmus obliquus* crops produces an increase in cellular lipids from 12.7% to 43% of dry weight (MANDAL and MALLICK, 2009); similarly, an increase in CO2 concentration from 2% to 10% produces an increase in lipid content of up to 170% in seven days (MURADYAN et al., 2004; Tambat et al., 2023).

Therefore, several experiments must continue to advance in order to understand and establish optimal formulations and conditions of nutrients, temperature, and lighting that can guarantee high yields of microalgal biomass.

5. Genetic modification of microalgae

Genomic analyses of microalgae are available for only a few species. Genetic modification of microalgae is promising as a strategy to achieve higher

yields in the production of bioproducts for industrial purposes. Although several hundred microalgal strains have been cultivated, detailed investigation of cellular physiology and biochemistry is limited to less than one percent of the species, and even fewer in those whose genomes have been studied. Genetic transformation of microalgae has been limited by the presence of rigid cell walls (Shivakumar et al., 2024). However, using various techniques such as bombardment, electroporation and treatment with glass beads, several species have been genetically modified (Mosey et al., 2021).

Genetic modifications can confer properties to improve yield. For example, Li and Tsai (2008) demonstrated that the microalgae *Nannochloropsis oculata* was optimized to produce bovine lactoferricin (BLF) fused with a red fluorescent protein (DsRed), which has a bactericidal effect against *V. parahaemolyticus* infection in the digestive tract of shrimp. Microalgae engineering processes such as conversion of autotrophic systems into heterotrophic ones, improvement in photosynthetic efficiency, improvement of cultivation processes, among others, aiming at increasing cellular lipid synthesis, have generated promising results. However, the increase in lipid synthesis yield with genetic modifications has not yet been achieved at the expected levels. There are three possible strategies to improve lipid production: biochemical engineering, genetic engineering and transcription factor engineering. Biochemical engineering approaches are currently the most widely established in microalgae lipid production (BEHERA et al., 2021). Improvements in photosynthesis efficiency can be achieved by manipulating photosynthetic antennae. Since smaller antennae lead to greater photosynthetic efficiency, mutations in the genes that control their biogenesis are a possible mechanism to increase photosynthetic efficiency (WANG et al., 2022).

There is the possibility of improving the efficiency of solar energy conversion from 1-4% to 8-12%, to fully perform the metabolic process in the microalgae Chlamydomonas perigranulata, C. reinhardtii, Chlorella vulgaris, Cyclotella sp., Dunaliella salina, Scenedesmus obliquus and Synechocystis PCC 6714 (STEPHENS et al., 2010).

Microalgal biotechnological processes present an opportunity to generate methodologies and products that may be useful to society. Cell strains that have all the properties required for large-scale biotechnological processes must be

developed, and further research must be conducted in parallel with natural strains to fully understand their physiological functioning. Such modifications may confer properties to improve their yield.

6. Harvesting

There is no single best method for harvesting microalgae and reducing their water content. Cost-effective and energy-efficient harvesting methods are needed to make the biomass and bioproducts production process economical. Microalgal biomass recovery represents 20-30% of total production costs according to one source. The most common harvesting processes are flocculation, filtration, centrifugation, gravity sedimentation, and flotation. The selection of the harvesting technique will depend on the properties of the microalgae, such as density, size, and the value of the desired products (JACOB-LOPES et al., 2015).

Flocculation is a process in which the dispersion of particles from the medium is induced with the help of chemical compounds that produce aggregation in the microalgal cells. Flocculants stimulate flocculation by generating colloids and other particles in suspension in the solution to form floccules. The most commonly used for microalgae harvesting include ferric chloride, aluminum sulfate, aluminum, ferric sulfate, polyferric sulfate, as well as cationic polymers (polyelectrolytes), organic flocculants (chitosan). Bioflocculants such as Paenibacillus + aluminum sulfate, and also co-bioflocculation (Nannochloris + diatoms) are used, but require extra effort to cultivate other microalgae (MOLINA-GRIMA et al., 2003).

Pressure or vacuum filtration is satisfactory for recovering relatively large (> 70 mm) and/or filamentous microalgae, but are less effective for separating microalgae species with dimensions close to prokaryotes. Membrane microfiltration and ultrafiltration processes may be an option for recovering microalgae biomass below 30 mm (MOLINA-GRIMA et al., 2003). Cross-flow filtration allowed the recovery of 70-89% of microalgae. This procedure has the advantage of maintaining the integrity of the microalgae biomass. Filtration is an

expensive process due to membrane exchange and pumping (ZHANG et al., 1995).

The centrifugation technique is a methodology that is based on the use of centrifugal acceleration to achieve the sedimentation of microalgae in heterogeneous mixtures. The size and density of the structure determine the centrifugal separation of the element. The supernatant is a liquid located in the upper layer of the centrifuge tube, once decanted. The remaining solid represents the microalgae concentrate. The process is fast and intensive and depends on the sedimentation of the cells for the recovery of the biomass, the residence time of the cell suspension in the centrifuge and the depth of settling (MOLINA-GRIMA et al., 2003). Recovery by centrifugation is an efficient methodology for use with low volumes of fluid and high energy consumption. The disadvantages of centrifugation are high initial investment costs, noise generated during operation and electricity costs (MOLINA-GRIMA et al., 2003).

Gravity sedimentation is widely used for the separation of microalgae in aqueous solution and wastewater treatment. The sedimentation rates of microalgae are influenced by the sedimentation rate of solids and determined by the density and area of the microalgae cells (HONG et al., 2022). Gravity sedimentation preceded by flocculation is one of the most widely used techniques for harvesting microalgae biomass. The disadvantages are the time-consuming method (0.1 to 2.6 m h^{-1}) and the biomass may undergo decomposition under high ambient temperature conditions. In addition, the technique is good for use with large microalgae or with filamentous morphology (REARTE et al., 2021).

Flotation methods are based on the binding of microalgae cells using air bubbles. The resulting flocs rise to the surface of the liquid and are recovered by physical or chemical procedures. Particles as small as 500 µm can be recovered by flotation. Some strains have gas vacuoles and float on the surface of the water. The incorporation of air bubbles depends on several aspects, such as air, solids, and contact angle with the aqueous phase (FUAD et al., 2021).

6. Final considerations

In recent years, new economic and environmental opportunities have emerged thanks to genetic and process engineering that has stimulated research on microalgae. Innovative ideas emerge in research centers and are implemented on a large scale in industries.

Microalgae biotechnology is making rapid progress in the mass cultivation of microalgae and their application in various industrial processes. However, less than fifty species are used, while thousands remain unexplored. The potential roles of microalgae in genetic engineering and nanotechnology, as well as the examples of entrepreneurial researchers, have increased the prospects for the next generation of establishing biotechnological processes with microalgae to form economically viable companies. Concerted research is needed to (i) develop inexpensive media through the enrichment of wastewater; (ii) isolate and cultivate new varieties of microalgae that present high yields; (iii) improve production systems; (iv) improve biochemical and metabolic pathways through genetic engineering; and (v) improve cultivation techniques.

Furthermore, attention should be paid to high-value natural and recombinant products that can be extracted from microalgae to increase the profitability of commercial operations. Simulation and pilot-scale models will serve as a basis for optimizing processes for wastewater treatment, eutrophication, gas capture, reactor design, and biocompound extraction based on microalgae. A robust bioeconomy built on a technology platform based on innovative microalgal processes requires an interdisciplinary approach involving biologists, biotechnologists, biochemists, engineers, chemists, microbiologists, bioreactor manufacturers, aquaculturists, and modelers.

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