

Advanced Biomethane Production

from Microalgae
Harvested on the Digestate
of Biogas Plants in Ukraine

ANALYTICAL
NOTE № 3

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Acronyms

AD anaerobic digestion	MA Microalgae
AN analytical note	Mha million hectares
bcm billion cubic metres	PAR photosynthetically active radiation
C:N Carbon to Nitrogen Ratio	PBR Photobioreactor
CHP Combined Heat and Power	RED Renewable Energy Directive
COD Chemical Oxygen Demand	SAF sustainable aviation fuel
d.m., DM dry matter	SOC soil organic carbon
DAF Dissolved Air Flotation	Ths. thousand
DOM dry organic matter	TS total solids
EBA European Biogas Association	UABIO the Bioenergy Association of Ukraine
EC European Commission	UAH Ukrainian hryvna
GHG greenhouse gases	VAT value-added tax
IEA International Energy Agency	VS volatile solids

Introduction

The purpose of this Analytical Note is to analyse the prospects for microalgae cultivation with the use of biomethane production by-products (CO₂, digestate, heat) followed by subsequent production of advanced biomethane and other valuable products derived from microalgae.

The study covers an analysis of the microalgae market, technological aspects of cultivation, harvesting methods, methanization processes, and the assessment of potential economic efficiency. An estimate is provided of biomethane production potential in Ukraine, taking into account microalgae that can be harvested at biomethane plants using digestate and CO₂ produced as a result of biogas upgrading.

Currently, microalgae production in Ukraine is at an early but promising stage of development. Despite relatively small production volumes on a national bioeconomy scale, interest in this sector is growing rapidly, given the wide range of applications for microalgae - from food supplements and pharmaceuticals to alternative energy sources and wastewater treatment. At present, there are at least two active companies in Ukraine engaged in industrial-scale microalgae cultivation: LLC "Food Factory" (brand Spirulinka) with a capacity of about 12 tons/month¹, and the Aquatic farm - a local producer in the Dnipropetrovsk region, which uses open cultivation systems, with production volumes estimated at around 1 ton per month. Both companies are primarily focused on producing food-grade spirulina - one of the most researched and commercially attractive microalgae. Other companies, such as "Bionet" (a sales platform that operates in Ukraine),

offer products based on *Spirulina* and *Chlorella*, focusing on dietary supplements and functional products.

Industrial biogas production in Ukraine effectively began in 2003 with the first commercial biogas plant project using CHP (combined heat and power) with 160 kW capacity at a pig farm owned by Agro-Oven in Olenivka village, Dnipropetrovsk region. Today, 83 biogas installations are operating in Ukraine, including 33 landfill and municipal solid waste gas recovery units, and this number continues to grow². In 2024, the first biomethane production projects in Ukraine were commissioned, and dozens of new installations are expected to be built in the near future.

The biomethane production potential in Ukraine, given its status as the country with the largest arable land area in Europe and, accordingly, significant volumes of agricultural by-products and waste, is among the highest in the world. According to the Bioenergy Association of Ukraine, the potential is estimated at 21.18 billion m³ CH₄/year³, excluding the potential from microalgae (as outlined in Analytical Note 3), which amounts to about 80% of Ukraine's total natural gas consumption in the pre-war year of 2021.

As a result of the anaerobic digestion process, biogas is generated, which primarily consists of methane, CO₂, and small amounts of other gases. The predominant current use of biogas is for combined

² EBA Statistical Report 2023

³ Geletukha G.G., Zheliezna T.A., Drahnev S.V., Kucheruk P.P. Perspektyvy vyrobnytstva peredovykh biopalyv v Ukraini [Prospects for the production of advanced biofuels in Ukraine]. Energy Technologies and Resource Saving. - V. 76, № 3 (2023), P. 71-82. (Ukr.) <https://www.etars-journal.org/index.php/journal/issue/view/37/40>

¹ <https://agrotimes.ua/elevator/ukrayinskij-virobnik-spirulini-rozshiryue-potuzhnosti/>

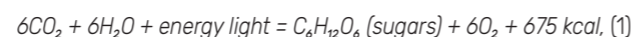
electricity and heat generation, and more recently, for biomethane production. During biogas upgrading to biomethane, a significant amount of CO₂ is separated (from 0.5 to 0.9 Nm³ CO₂ per 1 Nm³ CH₄ in biogas), which can be usefully utilized, thereby further reducing greenhouse gas emissions.

Another product of anaerobic digestion is digestate – residual raw material in the form of a concentrated mineral-organic suspension with a dry matter content of 3–8% and a high concentration of macro- and micronutrients available for microalgae growth. With the current feedstock types and volumes used for biogas production in Ukraine, the average digestate output of biogas plants is about 1.8 tons of raw mass per each MWh of biogas energy produced, or approximately 39.1 thousand tons per year per 1 MW of installed electric capacity of a CHP unit. In 2023, biogas plants in Ukraine generated a total of 1.72 million tons of raw digestate². About 85–90% of this amount consists of the liquid fraction after separation using screw separators, with a dry matter content of 1–3%. Typically, the liquid fraction is applied to the fields as a fertilizer product, but the economic viability of such application is limited to several kilometres around the biogas plant. For reference, the application rate of liquid digestate is typically around 20–30 m³ per hectare per year, which helps to illustrate how much digestate remains unused and thus could be redirected toward microalgae cultivation⁴. Enhancing the economic feasibility of utilizing the liquid fraction of digestate remains a relevant task for biogas producers. The cultivation of microalgae on digestate using CO₂ from biogas upgrading, with the return of the unseparated or settled microalgae suspension for additional biogas production, could offer one of the possible solutions.

According to EU decarbonization strategies, market preference is given to biomethane produced from sustainable feedstock listed in Directive RED III (Annex IX), creating an evident need for innovative, low-cost, and safe biomethane production using alternative high-yield biomass that can utilize waste feedstock and capture carbon. Microalgae represent such a promising biomass when cultivated on land in ponds or photobioreactors and are included in Directive RED III, Annex IX, eligible for double counting⁵.

Microalgae are photosynthetic unicellular organisms that utilize CO₂ or a combination of various inorganic and organic compounds as carbon sources. Microalgae are 10 to 50 times more efficient in CO₂ capture than terrestrial plants and can fix 1.83 tons of CO₂ per 1 ton of dry algal biomass. The photosynthetic conversion of inorganic CO₂ into metabolic energy stored as carbohydrates, proteins, and lipids is fundamental to algae productivity⁶.

Like terrestrial plants, microalgae grow and reproduce through photosynthesis. Photosynthesis is a process in which light energy is converted into chemical energy by absorbing atmospheric CO₂ according to reaction (1):



The sugars produced as a result of photosynthesis are converted into other cellular components (lipids, carbohydrates, and proteins), which form the algal biomass. Microalgae can convert sunlight into chemical energy with a high photosynthetic efficiency (6–8%), compared to terrestrial biomass (1.8–2.2%)⁷.

The concept of the technology is based on the hypothesis of the possibility of efficiently cultivating microalgae in a nutrient medium based on digestate, which contains the necessary macro- and micronutrients for microalgae growth, supplemented with CO₂ obtained from biogas upgrading to biomethane, followed by the conversion of the cultivated microalgae biomass into biogas and biomethane. As a result, through the process of photosynthesis, it becomes possible to achieve a more complete conversion of the organic carbon contained in the biogas feedstock into a gaseous energy carrier – CH₄.

It is expected that the price of biomethane from microalgae will be higher, as such biomethane is considered advanced according to EU Directive RED II (and RED III) and allows for significant greenhouse gas emission reductions. At the same time, the use of microalgae suspension for biogas production will not require mandatory thickening of the microalgae biomass (separation of microalgae from the liquid), since the moisture contained in the suspension may serve as a necessary component in the anaerobic digestion process of crop residues, whose

⁶ <https://doi.org/10.1016/B978-0-444-64337-7.00016-1>

⁷ Studies in Surface Science and Catalysis, 2020. [https://www.sciencedirect.com/topics/chemistry/photosynthetic-efficiency#:~:text=2.1%20Biological%20and%20Biochemical%20Properties,\(1.8%25%E2%80%932.2%25\)](https://www.sciencedirect.com/topics/chemistry/photosynthetic-efficiency#:~:text=2.1%20Biological%20and%20Biochemical%20Properties,(1.8%25%E2%80%932.2%25))

biogas production potential accounts for approximately 50% of the total potential. It is also known that the C:N ratio in the organic matter of microalgae is below 10, which is not optimal for anaerobic digestion but will allow balancing this ratio when combined with crop residues having a C:N ratio of 50–100. This drawback, however, can be overcome in a circular or integrated plant facility by combining microalgae with crop residues having a C:N ratio of 50–100, thereby balancing the overall ratio.

The implementation of microalgae cultivation technology using CO₂ from biomethane upgrading will also influence biomethane pricing, as CO₂

capture and utilization reduce the carbon intensity of biomethane plants.

Thus, the implementation of microalgae cultivation technology at biomethane plants, with the recycling of the liquid digestate fraction containing microalgae, will allow for reduced logistics costs of digestate handling and fresh water consumption, additional revenue from advanced biomethane produced from microalgae, and lower carbon intensity of the existing biomethane facility, while at the same time creating a circular economy approach in which no waste materials are generated, all streams are utilized, and additional value is created.

Definitions and terms

Key definitions and terms

Renewable Energy – energy that comes from non-fossil renewable sources, including wind, solar, geothermal, hydro, biomass, biogas, and biofuels. It excludes nuclear and fossil-based sources.

‘Renewable energy’ means energy from renewable non-fossil sources: wind, solar (both solar thermal and solar photovoltaic), aerothermal, geothermal, hydrothermal and ocean energy, hydropower, biomass, landfill gas, sewage treatment plant gas and biogas. – RED II, Article 2(1).

Anaerobic digestion means the process that includes a series of biological conversion processes in which microorganisms break down biodegradable material in the absence of oxygen: hydrolysis; acidogenesis; acetogenesis; and methanogenesis. The biogas produced contains methane (50–70%), carbon dioxide (30–40%), and other gases.

According to the definition of Ukrainian legislation⁵, **biomethane** is biogas that, due to its physical and chemical characteristics, meets the requirements of regulatory legal acts for natural gas for supply to the gas transportation or gas distribution system or for use as motor fuel. Biomethane is obtained by upgrading biogas, which includes the removal of

CO₂ and other impurities. Modern upgrading technologies ensure biomethane production containing 97–98% CH₄.

Microalgae – are defined as photosynthetic cells that are mostly unicellular, although some complex associations giving colonies with larger structures exist. This is a very heterogeneous group comprising prokaryotic organisms similar to bacteria (cyanobacteria, also called blue-green algae) and eukaryotic organisms, such as diatoms. They can be found in a variety of aquatic habitats, being able to thrive in freshwater, brackish, marine and hypersaline aquatic environments. They have been reported also in desert crust communities, thereby being able to endure temperature extremes and low water availability⁸. Algae are raw material from **Directive RED II, Annex 9**: «Feedstocks for the production of biogas for transport and advanced biofuels, the contribution of which towards the minimum shares referred to in the first and fourth subparagraphs of Article 25(1) may be considered to be twice their energy content: **Algae if cultivated on land in ponds or photobioreactors**».

Macroalgae, commonly known as seaweeds, are multicellular, macroscopic algae that are visible

⁸ https://knowledge4policy.ec.europa.eu/glossary-item/microalgae_en

to the naked eye. They are predominantly marine organisms but can also be found in freshwater habitats. Macroalgae are used in a wide range of industries, including food, agriculture, and bioenergy.

Advance biofuels - liquid or gaseous biofuels made from feedstocks listed in Annex IX, Part A of RED II. These are considered strategically important fuels for reducing emissions in the transport sector. RED II - Article 2, point 36: 'Advanced biofuels' means biofuels that are produced from the feedstocks listed in Part A of Annex IX.

Advanced Biomethane - biomethane produced from feedstocks listed in **Annex IX, Part A**, including microalgae, is classified as an **advanced biofuel** when used in transport.

While RED II does not explicitly define "advanced biomethane", its classification is derived from the legal treatment of advanced biofuels made from Annex IX feedstocks.

Double Counting - a mechanism allowed under RED II, whereby the energy contribution of certain advanced biofuels may be **counted twice** toward the renewable energy targets in transport. This encourages the use of non-food and waste-based feedstocks such as microalgae.

Digestate - a semi-solid or liquid by-product resulting from the anaerobic digestion of biodegradable materials. It is commonly used as a fertilizer or soil improver. Defined in Regulation (EU) 2019/1009 (Fertilising

Products Regulation): "**Digestate: a substance resulting from the anaerobic digestion of biodegradable materials**" Digestate that meets safety and quality requirements can be used as a product (not a waste) under EU law.

Sustainability Criteria - a set of requirements that biofuels and biogas must meet to be considered sustainable under RED II. These include:

Minimum greenhouse gas (GHG) emission savings (e.g., ≥65% for transport biogas).

Protection of land with high biodiversity and carbon stock.

Compliance with agricultural and environmental regulations.

Only biofuels and biogas that meet the sustainability and GHG savings criteria shall be counted toward renewable energy targets or be eligible for financial support. - RED II, Article 29

Biomass - the biodegradable fraction of biological materials - including waste and residues - originating from agriculture, forestry, fisheries, aquaculture, and industrial and municipal waste of biological origin. 'Biomass' means the biodegradable fraction of products, waste and residues from biological origin from agriculture, forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of waste, including industrial and municipal waste of biological origin. - RED II, Article 2(24).

Overview of Microalgae

Microalgae represent a broad group of photosynthetic organisms, which include cyanobacteria, diatoms, unicellular green algae, and certain other algal species. They are capable of thriving in complex agro-climatic conditions and producing a wide range of valuable products, including lipids, proteins, carbohydrates, pigments, and bioactive compounds.

SECTION 1

Classification of Microalgae

The classification of microalgae is complex and multi-level, as they are represented by both prokaryotes (such as cyanobacteria) and eukaryotes belonging to various phylogenetic lineages. Taxonomic classification of microalgae is based on morphological, cytological, biochemical, and genetic characteristics, which account for their wide variety of forms and physiological-biochemical

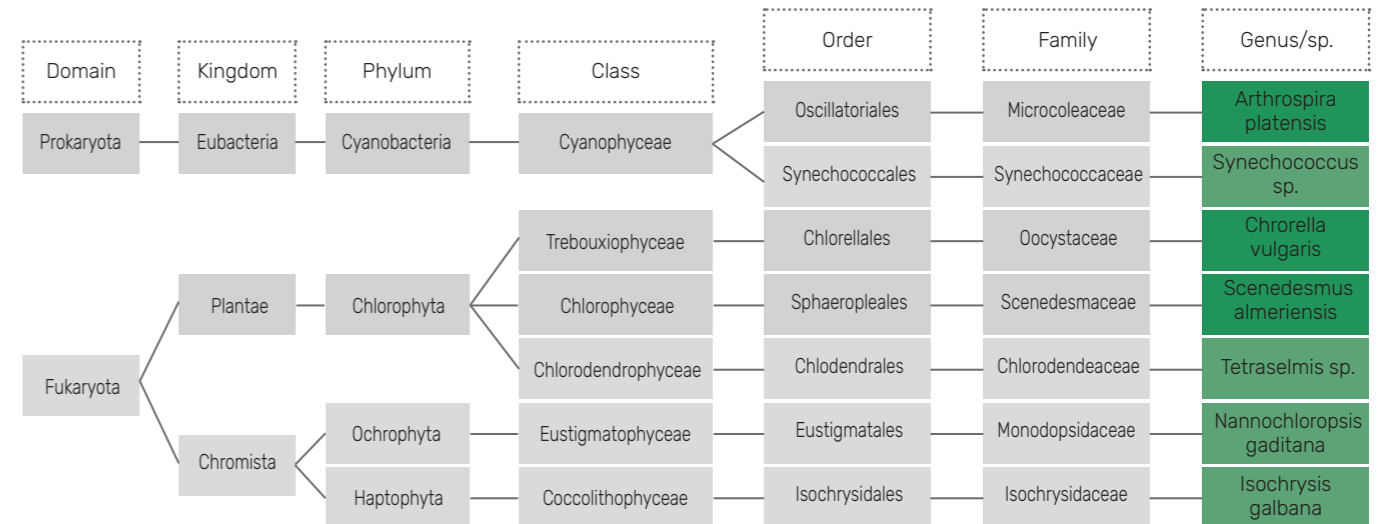


Fig. 1.1 - Taxonomic classification of widespread microalgae and cyanobacteria strains.

Species highlighted in green are associated with freshwater environments, while those highlighted in blue are related to marine habitats ?

⁹ https://www.researchgate.net/publication/359143363_Characterisation_of_the_volatile_profile_of_microalgae_and_cyanobacteria_using_solid-phase_microextraction_followed_by_gas_chromatography_coupled_to_mass_spectrometry

properties. There is no universally accepted classification system for microalgae since their taxonomic position is continuously being refined based on new molecular phylogenetic research.

Nevertheless, a generalized scheme can be presented that reflects the main groups of microalgae and their place in the system of living organisms. The taxonomic classification of widespread microalgae and cyanobacteria strains is presented in Figure 1.1.

According to modern systematics, microalgae are conventionally divided into two major groups:

Prokaryotic microalgae - cyanobacteria (*Cyanobacteria* division), which are among the oldest photosynthetic organisms on Earth. They lack a distinct nucleus and typical organelles but demonstrate high morphological complexity. Their structure includes lamellar thylakoids containing chlorophyll *a*, phycobilin pigments (phycocyanin, allophycocyanin, phycoerythrin), and in filamentous forms (e.g., *Anabaena*, *Nostoc*), the presence of heterocysts that enable atmospheric nitrogen fixation.

Eukaryotic microalgae - include numerous taxonomic groups: *Chlorophyta* (green algae), *Bacillariophyta* (diatoms), *Euglenophyta* (euglenoids), *Chrysophyta* (golden algae), *Dinophyta* (dinoflagellates), etc. The most common objects of biotechno-

logical research are green algae (*Chlorella*, *Chlamydomonas*, *Dunaliella*, *Botryococcus*) and diatoms (*Phaeodactylum*, *Nitzschia*, *Thalassiosira*).

Within green algae (*Chlorophyta* division), several classes are distinguished, including:

Chlorophyceae - includes unicellular, colonial, and filamentous forms. Representatives: *Chlamydomonas reinhardtii*, *Scenedesmus*, *Volvox*.

Trebouxiophyceae - an important group of lichen symbionts, also includes *Chlorella*.

Ulvophyceae - primarily macroalgae, with some species having microscopic stages.

Charophyceae - the group most evolutionarily related to terrestrial plants.

Typical features of green microalgae include the presence of chlorophylls *a* and *b*, starch stored inside chloroplasts as the main reserve product, cellulose cell walls, and (in some genera) the presence of flagella.

Cyanobacteria, such as *Spirulina*, *Anabaena*, *Oscillatoria*, and *Microcystis*, demonstrate high plasticity, nitrogen fixation capacity, and adaptation to extreme conditions, which makes them suitable for industrial cultivation. Their biomass is widely used in pharmaceuticals, the food industry, and as a source of proteins, antioxidants, and pigments.

It is important to note that, despite the vast taxonomic diversity, only a small portion of microalgae are currently cultivated for practical purposes. In biotechnology, priority is given to species with a high growth rate, the ability to accumulate lipids, pigments, or target metabolites. The ability to adapt to mass cultivation and optimize biomass productivity also plays a crucial role. Thus, while the classification of microalgae is complex, it remains critically important for scientific and practical applications of these organisms in bioeconomy, the food industry, and biofuel production.

Characteristics and Advantages of Microalgae

Microalgae are a polyphyletic group of photosynthetic organisms that play a key role in global biogeochemical cycles and are of considerable interest in biotechnology due to their rapid growth and accumulation of valuable metabolites. Studying the morphological structure of microalgae is fundamental to understanding their metabolism, ecological role, and potential in various fields - from pharmaceuticals to bioenergy. Microalgae may have different types of cell organization: unicellular, colonial and filamentous. Most of the unicellular cyanobacteria are nonmotile, but gliding and swimming motility may occur.

Cellular Structure and Biochemical Composition

The cell wall structure of microalgae reflects significant taxonomic diversity. In cyanobacteria, it consists of six layers, one of which is peptidoglycan, providing mechanical rigidity, while the outer layers are formed from muramic acid and a polysaccharide mucous sheath. In eukaryotic microalgae, the cell wall mainly consists of cellulose microfibrils embedded in an amorphous matrix of polysaccharides, proteins, and lipids. Certain taxa, such as *Haematococcus* or *Chlorella*, have additional multilayer structures made of algaenan - high-molecular-weight polymer resistant to chemical degradation, which complicates the extraction of target products but provides ecological stability to the cell.

Most chlorophytes (green microalgae) have complex and rigid cell walls, the cell wall contains components embedded in a matrix containing uronic acids along with other neutral sugars. 40% of the components of microalgae are readily available for methane production, the remaining 60% require pre-processing steps to make the intracellular contents accessible. The cell wall composition and

architecture of algae and cyanobacteria are highly variable, ranging from tiny membranes to multi-layered complex structures. Depending on the complexity of the surface structures, four types can be distinguished, as shown in Figure 1.2.

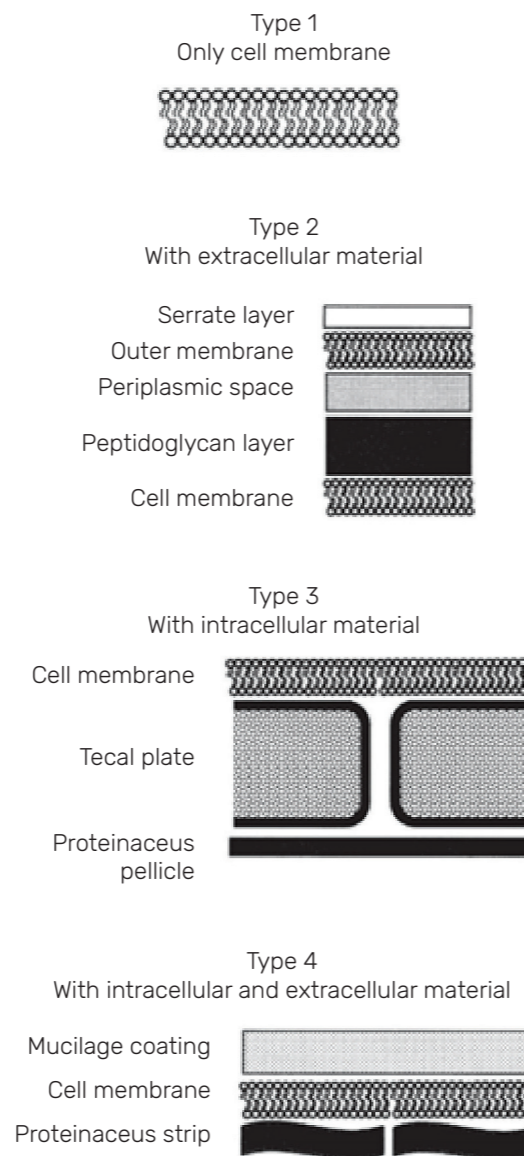


Fig. 1.2 - Schematic view of cyanobacteria and algae cell wall types. Modified from Lee, R.E., 2008. Phycology. Cambridge University Press ⁷¹

Physiological and Biochemical Properties

Microalgae are autotrophs capable of converting solar energy and inorganic substances into high-energy biomolecules. In response to stress factors, particularly nitrogen deficiency, they alter their metabolic strategy, shifting towards lipid or carbohydrate accumulation. For instance, under nitrogen-deficient conditions, the lipid content in *Chlorella pyrenoidosa* may increase from 19.7% to

over 50% of the cell's dry weight.

Microalgae can be classified based on their cultivation methods as heterotrophic, photoautotrophic, photoheterotrophic, or mixotrophic. Like most terrestrial plants, microalgae are photosynthetic and are photoautotrophs. However, some species of microalgae are also heterotrophic, using organic compounds in the growth medium as carbon and energy sources, and therefore do not require light as an energy source. Heterotrophic growth is an aerobic process in which the assimilation of organic substrates generates energy through oxidative phosphorylation, accompanied by the consumption of oxygen as the final electron acceptor. Mixotrophic cultivation is a growth method in which microalgae simultaneously utilize inorganic CO₂ and organic carbon sources in the presence of light; therefore, photoautotrophy and heterotrophy occur simultaneously. CO₂ is fixed through photosynthesis, which is influenced by light, while organic compounds are assimilated through aerobic respiration, which is influenced by the availability of organic carbon ¹⁰.

Microalgal biomass is rich in proteins, carbohydrates, lipids, pigments (such as chlorophyll, phycobilins), and secondary metabolites, serving as a source of valuable bioproducts. This versatility defines their potential for use in energy, pharmaceuticals, agriculture, and the food industry. Among the most studied and widely used species are:

Cyanobacteria (Arthrospira platensis, Aphanizomenon) - protein and antioxidant (phycocyanin) producers.

Green algae (Chlorella vulgaris, Haematococcus pluvialis, Scenedesmus obliquus) - known for high protein, antioxidant (astaxanthin), lipid, and polysaccharide content.

Diatoms (Phaeodactylum tricornutum) - a source of eicosapentaenoic acid (EPA).

Haptophytes and red algae - produce sulphated polysaccharides valuable for medicine and biomaterials.

Organelles and Intracellular Structures

Microalgal chloroplasts contain pigments specific to each group, including chlorophylls (a, b, c),

carotenoids, and phycobilins (characteristic of cyanobacteria and red algae). The presence of different pigments ensures efficient light absorption in aquatic environments at various depths. Some microalgae possess special light-sensitive organelles - stigma (or eyespot), enabling phototaxis. Cells may also contain storage inclusions: polysaccharides (e.g., starch, chrysolaminarin), lipids, and polyphosphates.

The cytoplasm contains the nucleus and various types of organelles - compartments formed by the invagination of the plasma membrane and the endoplasmic reticulum. Organelles include: chloroplast, Golgi apparatus, endoplasmic reticulum, ribosomes, mitochondria, vacuoles, contractile vacuoles, plastids, lipid globules, flagella, and microtubules. Chloroplast - contains a series of flattened vesicles, or thylakoids, containing chlorophylls, and a surrounding matrix, or stroma. Thylakoids also contain phycobiliproteins in the phycobilisomes of Rhodophyta, while in Cryptophyta the phycobiliproteins are dispersed within the thylakoids.

Thylakoids may be free or grouped in bands. Pyrenoids may occur within the chloroplast. Many motile forms have an orange-red eyespot, or stigma, made of lipid globules. A double membrane surrounds the chloroplast; in some algae, in addition to this double membrane, one or two membranes of the endoplasmic reticulum are present (Fig. 1.3).

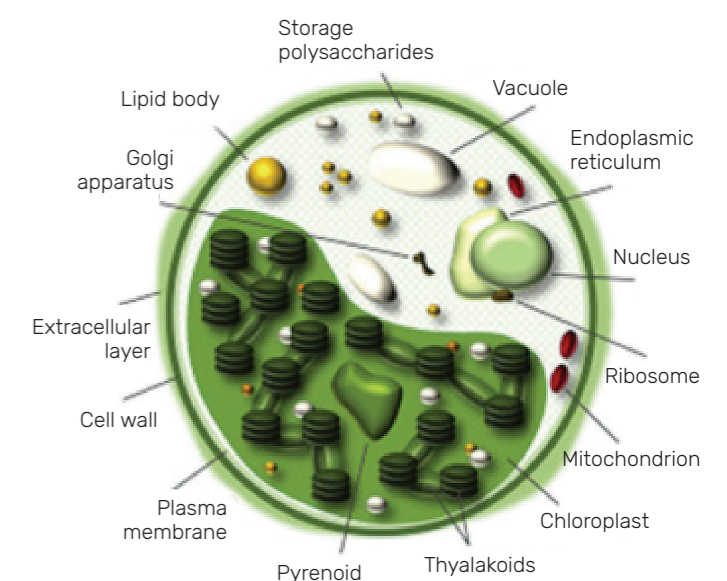


Fig.1.3 - Cell structure of eukaryotic microalgae ¹¹

¹⁰ https://www.researchgate.net/publication/283489490_Microbial_Heterotrophic_and_Mixotrophic_Culturing_for_Bio-refining_From_Metabolic_Routes_to_Techno-economics

¹¹ <https://www.sciencedirect.com/science/article/abs/pii/S0734975019301193?via%3Dihub>

Motility and External Structures

Some microalgae (e.g., *Chlamydomonas*) possess flagella, which allow active movement in the water column. Other species, such as diatoms, move through mucous excretions via a raphe slit. The mechanisms of movement are closely related to the type of cell wall and additional external structures.

Advantages of Microalgae as a Biotechnological Object

No conflict with human or animal food chains.
High in carbohydrates, proteins and oils.

Creates a long-term method of generating O₂
and uses it in photosynthetic respiration to
reduce CO₂ emissions.

Microalgae contain a higher lipid content on
a dry weight basis compared to oilseed crops
such as soybeans. In addition, the growth
and cultivation cycle of microalgae is 15 days
compared to soybeans, which have this cycle
once or twice a year.

Algae, when cultivated on land in ponds or
photobioreactors, are a double-counting
feedstock under RED II Directive, Annex 9 -
production of third generation biofuels.

Have the ability to bioremediate heavy metals.

Microalgae have higher photosynthetic
efficiency (maximum value ~ 10%) compared
to terrestrial plants (maximum value ~ 5%)

Microalgae can achieve higher biomass
productivity (50-70 t/ha per year) compared
to terrestrial plants (10-20 t/ha per year).

Microalgae can grow on non-arable lands and
in the sea.

Microalgae can grow in closed systems
and with the help of wastewater and salt
water, thus significantly reducing freshwater
consumption.

Make a potentially significant contribution to
carbon sequestration and excess nutrient
uptake quantitatively.

Thus, microalgae represent a universal biore-
source whose potential is far from exhausted. Their
environmental adaptability, biochemical plasticity,
and engineering potential open up a wide range of
applications in the context of sustainable develop-
ment.

The morphological diversity of microalgae is the
result of complex evolution and adaptation to
various ecological niches. Detailed knowledge of
cell structure allows for more accurate taxonomic
identification, prediction of physiological properties,
and effective use of microalgae in biotechnological
processes, including biofuel, biopolymer, feed, and
pharmaceutical production.

Fundamental aspects of microalgae biology

The biological features of microalgae demonstrate
their suitability for cultivation on digestate. Thus,
a major step towards understanding the potential
of microalgae is to become familiar with the key as-
pects of their life cycle, which also have influence
on future technological solutions.

The morphological, physiological and structural
characteristics of microalgae make these organ-
isms highly adaptable creatures capable of sur-
viving in a variety of environments. Microalgae
can be found in almost all environments on Earth,
including oceans, rivers, lakes, salt lakes, soil, and
as symbionts for various invertebrates¹².

From the perspective of natural microbiology,
these microorganisms play a fundamental role in
the global carbon cycle, where through photosyn-
thesis they assimilate approximately 50% of the
Earth's CO₂, while producing O₂ as a by-product,
as well as organic matter¹³. Some representatives
of microalgae, and heterocystous cyanobacteria
themselves take on an important role in the N₂
cycle in the natural environment, and diatoms take
an active part in the silica cycle¹⁴.

In aquatic ecosystems, microalgae are called phyto-
plankton and take part in nourishment for zooplank-
ton and fish, providing them with energy and organic
matter. Apart from the benefits, microalgae can pose
a threat to other organisms. Saturation of rivers, lakes
and coastal waters with nutrients from wastewater

¹² Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ. Handbook of Microalgae-Based Processes and Products. Academic Press; 2020. ISBN: 978-0-12-818536-0.

¹³ Prasad R, Gupta S, Nisha S, Oliveira CYB, Nema A, Ansari F. Role of microalgae in global CO₂ sequestration: physiological mechanism, recent development, challenges, and future prospective. Sustainability. 2021;13(23):13061. <https://doi.org/10.3390/su132313061>

¹⁴ Mishra A, et al. Role of cyanobacteria in rhizospheric nitrogen fixation. In: Cruz C, Vishwakarma K, Choudhary DK, Varma A, eds. Soil Nitrogen Ecology. Vol 62. Soil Biology. Springer; 2021:581-599. https://doi.org/10.1007/978-3-030-71206-8_25

from industrial or agricultural activities often caus-
es algae bloom (Fig. 1.4). This leads to a change in
the colour of the water, as well as a decrease in the
concentration of dissolved oxygen, which leads to
the death of other aquatic organisms. Also, some
microalgae can secrete toxins that can contaminate
drinkable water and accumulate in seafood¹⁵.



Fig. 1.4 - Algal bloom in the Dnipro River¹⁶

Microalgae include both eukaryotes and prokary-
otes, so it is quite difficult to talk about the general
structure of microalgae, but it is advisable to dwell
on morphology. Microalgae is very diverse in cell
shape and is represented by a wide range of sizes
from 0.2 to 200 microns. Among the most com-
mon cell shapes are: spherical, oval, filamentous,
spindle-shaped, stellate, spiral-shaped, disc-
shaped and many others. What is special is that
this variation is observed not only among genera
and species, but also among different phases of
cell growth (Fig. 1.5).

Microalgae are very adaptable in their nutrition
methods. Depending on the type and environ-
mental conditions where they are found, they can
implement autotrophic, heterotrophic and mixo-
trophic nutrition. Autotrophy is the most common
type of nutrition in microalgae in nature. Repre-
sentatives that implement only this type include
Porphyridium cruentum, *Chaetoceros muelleri*,
Tetraselmis suecica, *Skeletonema costatum*
and others. During such nutrition, they use solar
or artificial lighting as an energy source, and the
source of carbon is gaseous or dissolved CO₂ and
bicarbonates.

¹⁵ Thoré ESJ, Muylaert K, Bertram MG, Brodin T. Microalgae. Current Biology. 2023;33(3):R91-R95. <https://doi.org/10.1016/j.cub.2022.12.02>

¹⁶ UNIAN <https://www.unian.ua/ecology/naturalresources/10216565-zeleniy-kisil-ekologi-b-yut-na-spoloh-cherez-cvitinnya-vodi-v-dnipri-yaka-nas-vbivaye-foto.html>

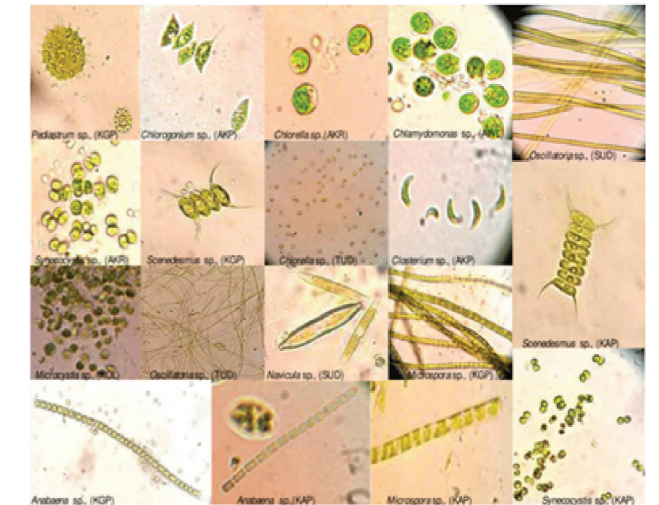


Fig. 1.5 - Microscopy of some microalgae species¹⁷

This type of nutrition is very interesting from the
point of view of utilizing CO₂, which is formed from
human activity. However, the main limitations arise
due to lighting: artificial lighting requires large
electricity costs, and with an increase in biomass
concentration, the effect of self-shading occurs,
which greatly reduces the efficiency of photosyn-
thesis and culture productivity¹⁸.

The heterotrophic type of nutrition does not require
the presence of light, and the source of carbon and
energy is organic compounds. Compared to the
autotrophic type of nutrition, heterotrophic provides
significantly higher crop productivity: if for autotro-
phic it is 0.35-0.4 g/l/day, then for heterotrophic - 1.7
- 4 g/l/day¹⁹. Typical representatives implementing
this type of nutrition are *Cryptocodinium cohnii*,
Galdieria sulphuraria, *Auxenochlorella protothe-
coides*. The biochemical composition of microalgae
implementing the heterotrophic type of nutrition is
dominated by carbohydrates and lipids, that is, this
biomass is better suited for the production of bio-
methane. The main drawback of heterotrophy is the
need to sterilize the nutrient medium, due to the risk
of contamination by foreign microorganisms that can
inhibit or completely stop the growth of microalgae.

¹⁷ Suresh A, Ayele A, Benor S. Isolation and morphological identification of some indigenous microalgae from Ethiopia for phycoprospecting. Ethiopian Journal of Science and Sustainable Development. 2019;6(2):56-60. <https://doi.org/10.20372/ejssdastu.v6.i2.2019.102>

¹⁸ Dhanasekaran D, Thajuddin N, Panneerselvam A, Rajendran R. Microalgae: A promising biological system for biofuel production and other applications. Nigerian Journal of Pharmaceutical and Pharmacological Research. 2018;8(1):1-10. <https://doi.org/10.5455/njppp.2018.8.0935625122017>

¹⁹ Yun HS, Kim YS, Yoon HS. Effect of different cultivation modes (photoautotrophic, mixotrophic, and heterotrophic) on the growth of *Chlorella* spp. and biocompositions. Frontiers in Bioengineering and Biotechnology. 2021;9:774143. <https://doi.org/10.3389/fbioe.2021.774143>

Table 1.1. Comparative characteristics of microalgae nutrition types ^{18,19,20}

Mode of nutrition	Energy source	Carbon source	Typical substrates	Advantages	Disadvantages
Autotrophy	Light	CO ₂	CO ₂ , bicarbonates	<ul style="list-style-type: none"> Utilization of CO₂ Lower risk of contamination 	<ul style="list-style-type: none"> Dependence on light Low productivity Self-shading effect
Heterotrophy	Organic compounds	Organic compounds	Glucose, fructose, acetates, sucrose, wastewater, digestate, glycerol, others	<ul style="list-style-type: none"> High productivity No need for lighting 	<ul style="list-style-type: none"> Requires sterilization
Mixotrophy	Organic compounds + light	Organic compounds + CO ₂	Glucose, fructose, acetates, sucrose, wastewater, digestate, glycerol; CO ₂ , bicarbonates	<ul style="list-style-type: none"> Reduced need for lighting Highest productivity Utilization of CO₂ 	<ul style="list-style-type: none"> Dependence on light Requires sterilization Self-shading effect

Mixotrophy combines both previous types of nutrition, allowing microalgae to simultaneously carry out photosynthesis or absorb organic compounds. The most famous representatives with mixotrophy are *Chlorella vulgaris*, *Scenedesmus obliquus*, *Euglena gracilis*. In addition, in case of a lack of necessary substances, they can be either autotrophs or heterotrophs, which gives them a strong advantage ²⁰. This type of nutrition provides the greatest biomass gains with CO₂ utilization and greater adaptation to stressful conditions. However, to use all the advantages of this type of nutrition, it is necessary to pre-treat the substrates to reduce their turbidity and increase light permeability to the cells. This method also has high risks of contamination and the effect of self-shading. Despite the disadvantages, mixotrophy allows you to get 3 - 5.18 g/L/day ¹⁹.

One of the main aspects of microalgae biology that is worth considering is photosynthesis. Photosynthesis is the main mechanism by which microalgae are able to convert light energy into chemical energy, while absorbing carbon dioxide. This process provides the cell with building material for its growth and energy. The main difference between microalgae and animals is that microalgae synthe-

size organic compounds from inorganic ones, while animals only consume ready-made substances.

In eukaryotic microalgae, photosynthesis occurs in chloroplasts, while in prokaryotic microalgae, chloroplasts are absent, and the process of photosynthesis occurs in thylakoids located directly in the cytoplasm.

This process is divided into light and dark phases. During the light phase, photons of light activate photosystem II (PS II), which leads to the release of an electron. In order to compensate for the loss of electrons, photolysis of water occurs, resulting in the formation of molecular oxygen, as well as protons H⁺, which are used by ATP synthetase to form ATP. The electron from photosystem II is sequentially transferred through plastoquinone (PQ), cytochrome complex (Cyt), plastocyanin (PC) to photosystem I (PS I), where again under the influence of light the photosystem is excited and then transfers the electron to ferredoxin, and from it to NADP with the formation of NADPH. The accumulated energy in the form of NADPH and ATP is used in the dark phase in the Calvin-Benson cycle, where CO₂ fixation occurs and carbohydrates and intermediate metabolites are formed, which are used for the biosynthesis of fatty acids, amino acids and organic acids ²¹.

²¹ Pirog, T. P. General Microbiology: Textbook, 2nd ed., revised and expanded; Kyiv: National University of Food Technologies, 2010; 632 pp.

²⁰ Zhang W, He X, Li T, et al. Recent advances in microalgae-based wastewater treatment, biofuel production, and value-added products. *Fermentation*. 2022;8(10):474. <https://doi.org/10.3390/fermentation8100474>

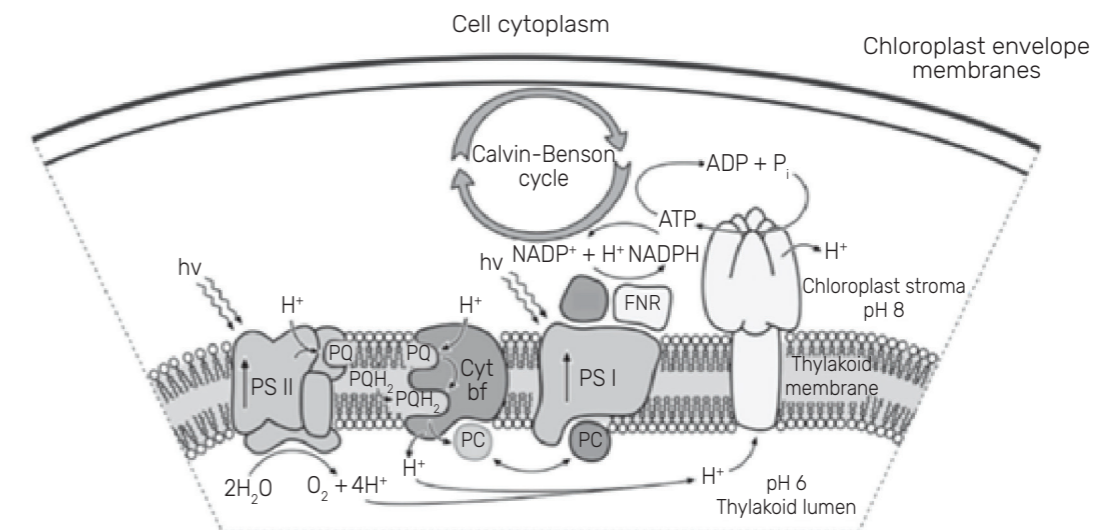
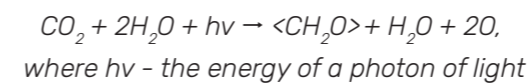


Fig. 1.6 - Scheme of the photosynthesis process in microalgae ²²

Notation: PS II - photosystem II; PS I - photosystem I; PQ - plastoquinone; Cyt - cytochrome; PC - plastocyanin; Fd - ferredoxin

²² Umen JG. Green algae and the origins of multicellularity in the plant kingdom. In: *Plant Cell Monographs*. Vol 22. Wiley; 2014:19-47. <https://doi.org/10.1002/9781118567166.ch2>

The overall reaction of photosynthesis can be described as follows:



Microalgae have a high efficiency of photosynthesis, reaching 8-10% of the theoretically possible, compared to terrestrial biomass of 1.8%-2.2% ²³. This is due to adaptations to the conditions of their existence in aquatic environments. They have a short electron transport path, a high density of photosystems, and their photosynthetic apparatus is adapted to diffuse light and short-term changes in illumination. Their photosystems can switch between linear and cyclic electron transport, which allows them to control the ratio of NADPH and ATP according to the needs of the cell ²¹.

Another adaptation is the presence of carbon concentration mechanisms.

This is possible due to the presence of pyrenoids in some microalgae and developed HCO₃⁻ and CO₂ transport systems, which ensure the concentration

²³ Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y. Microalgal Cultivation for Biofuels: Cost, Energy Balance, and Future Perspectives. In: *Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment*. Academic Press; 2016:395-416. <https://doi.org/10.1016/B978-0-12-803581-8.09259-6>

of CO₂ very close to the RuBisCO enzyme. They can effectively fix inorganic carbon even under conditions of low CO₂ concentration in the environment, and also reduce the negative effect of photorespiration at high O₂ concentrations ²².

Microalgae also have a wide range of photoprotective reactions against excessive light. They are able to convert excess chlorophyll energy into heat to prevent damage to the photosystem. In addition, they can quickly rearrange the antenna complexes of photosystems, reducing the area of light absorption. Many species of microalgae can enhance the biosynthesis of carotenoids, which protect cells from oxidative stress.

Microalgae have a side process of photorespiration. It directly competes with photosynthesis and leads to the conversion of organic substances in the cell to CO₂, serine and ammonia without any metabolic gain. This occurs due to the action of the enzyme RuBisCO, which in the absence of CO₂ or a significant excess of O₂ catalyses the oxidation reaction of ribulose biphosphate. To obtain the greatest amount of biomass, it is necessary to minimize photorespiration. This can be achieved by maintaining a higher CO₂/O₂ ratio than in air, as well as optimally illuminating the culture to avoid creating stressful conditions ²².

Cultivation and harvesting of microalgae

Large-scale production systems for microalgae and cyanobacteria are generally divided into three main types: open ponds, photobioreactors (PBRs), biofilm-based systems, or their combinations. Open ponds are relatively simple and inexpensive compared to photobioreactors; however, they require a large land area, are inefficient at capturing CO₂ emissions, are vulnerable to unfavourable weather conditions (such as rain and evaporation), and are prone to contamination. This makes them less suitable for certain applications, such as the cultivation of food and feed-grade biomass.

Photobioreactors, on the other hand, are more reliable but significantly more expensive in terms of both capital expenditures (CAPEX) and operational expenditures (OPEX). These systems are complex engineering structures designed to maintain optimal growth conditions for microalgae. They require continuous monitoring and control of numerous parameters, including aseptic conditions, temperature, pH, redox potential, dissolved oxygen levels, carbon dioxide availability, key nutrients, fluid flow rate, and mixing intensity.

PBRs are designed with a high surface-area-to-volume ratio to maximize light exposure efficiency. However, this same design principle can lead to heat loss in the absence of light. Understanding the time-dependent thermal balance in PBRs is therefore critical for accurately predicting biomass productivity²⁴.

Photobioreactors can be classified based on various design features, including orientation, culture circulation mechanism, lighting method, configuration, mode of operation (batch or continuous), and cultivation regime²⁵.

On an industrial scale, tubular systems are the most widely used; however, each type has its own

²⁴ Giovanna Salbitani, Simona Carfagna. Ammonium Utilization in Microalgae: A Sustainable Method for Wastewater Treatment. Sustainability 2021, Vol. 13(2), P.956; <https://doi.org/10.3390/su13020956>

²⁵ Anas Al-Dailami, Iwamoto Koji, Imran Ahmad1, Masafumi Goto. Potential of Photobioreactors (PBRs) in Cultivation of Microalgae. Journal of Advanced Research in Applied Sciences and Engineering Technology. 2022.Vol.27. No.1. P.32-44 <https://doi.org/10.37934/araset.271.3244>

SECTION 2

advantages and disadvantages. The choice of PBR should align with project goals, geographic location, and scale-up requirements. In the case of cultivating microalgae on digestate, the primary goal is to maximize biomass productivity. Flat plate photobioreactors are considered the most suitable for this purpose, as they can achieve the highest biomass productivity (1.5-28 g/L*day).

Open Cultivation Systems

Open cultivation systems for microalgae are the most common type of facility used for large-scale biomass production at the commercial level. Despite the availability of alternative technologies, open systems (particularly open ponds) remain relevant due to their simple construction, cost-effectiveness, ease of operation, and durability compared to closed photobioreactors (Richmond, 1999).

Classification and Types of Open Systems

There are several main types of open pond systems that differ in shape, culture mixing method, construction materials, and level of automation:

a. **Inclined systems:** The culture flows down an inclined surface due to gravity. High turbulence provides good gas exchange capacity and cell concentrations of up to 10 g/L. However, these systems are prone to CO₂ losses, evaporation, and require significant energy for pumping (Doucha & Livansky, 1999). Their commercial use is currently limited.

b. **Circular (radial) ponds:** Mostly used in Asia (Japan, Taiwan, Indonesia) for cultivating *Chlorella*, but require high investment in concrete structures and energy for rotating mixers.

c. **Raceway ponds:** These are the most widely used systems in commercial production, especially for *Arthrospira platensis (Spirulina)* and *Dunaliella salina*. They are designed as closed-loop oval channels with a plastic lining, where the culture is circulated by a paddle wheel. A schematic of a

Table 2.1. Comparison of the most common systems for microalgae cultivation^{72 71 70}

PBR	Advantages	Disadvantages	Biomass productivity g/L*day
Open ponds	<ul style="list-style-type: none"> Economy Easy to clean Low energy consumption Easily scalable Low maintenance costs 	<ul style="list-style-type: none"> Difficult to control parameters Low productivity of biomass Ability to contamination Requires a large area Inefficient mixing 	0.03-0.2
Vertical photobioreactors (Airlift and bubble column)	<ul style="list-style-type: none"> Excellent biomass productivity High efficiency of photosynthesis Limitation of photo-inhibition and photooxidation Small land required for construction Appropriate for outdoor cultivation Low contamination risk Low energy required Low cost, relatively small size and easily maintain 	<ul style="list-style-type: none"> Possibility of cell shear stress Low light exposure and low illumination area Susceptibility of biofouling on walls of reactor 	0.5-10
Horizontal tubular	<ul style="list-style-type: none"> Large lighting surface Suitable for outdoor placement Good performance Relatively inexpensive Scalable Easy control Uniform mixing Good temperature control 	<ul style="list-style-type: none"> pH gradient Losses Fouling on the walls Requires significant areas Hydrodynamic stress Possibility of low gas exchange 	0.35-1.5
Flat plate	<ul style="list-style-type: none"> Maximum exposure to sunlight High ratio of surface to volume Well-appropriate for outdoor farming High productivity of biomass Well-distributed of total light for cultivation Relatively cheap cost Easy to construct, clean and handle High photosynthesis efficacy Low concentration of dissolved oxygen 	<ul style="list-style-type: none"> Difficulty of Scalability Difficulty of cultivation temperature regulating Possibility biofouling Possibility of hydrodynamic stress in algae cells 	1.5 -28

raceway pond reactor is shown in Figure 2.1. It consists of two channels connected by bends through which water recirculates, driven by a paddle wheel. A settler is typically used for gas sparging, while the bends must be properly designed to minimize pressure losses in the system. Various settler configurations can be used—with or without baffles, and with co-current or counter-current gas-liquid contact. Simple raceway systems typically yield an average productivity of 12–13 g/m²/day, although under optimal conditions, 20–25 g/m²/day can be achieved (Borowitzka, 1999; Lee, 2001).



Fig. 2.1 - Schematic drawing and photo of a raceway ponds reactor²⁶

Natural and Semi-Natural Water Ponds

Particular interest is drawn to so-called natural or semi-modified systems. In such cases, microalgae are cultivated in natural or artificially created water bodies with minimal technological intervention.

²⁶ https://www.researchgate.net/figure/figure4-Open-ponds-for-mass-culture-of-microalgae-Race-way-and-circular-with-paddle_fig4_272510032

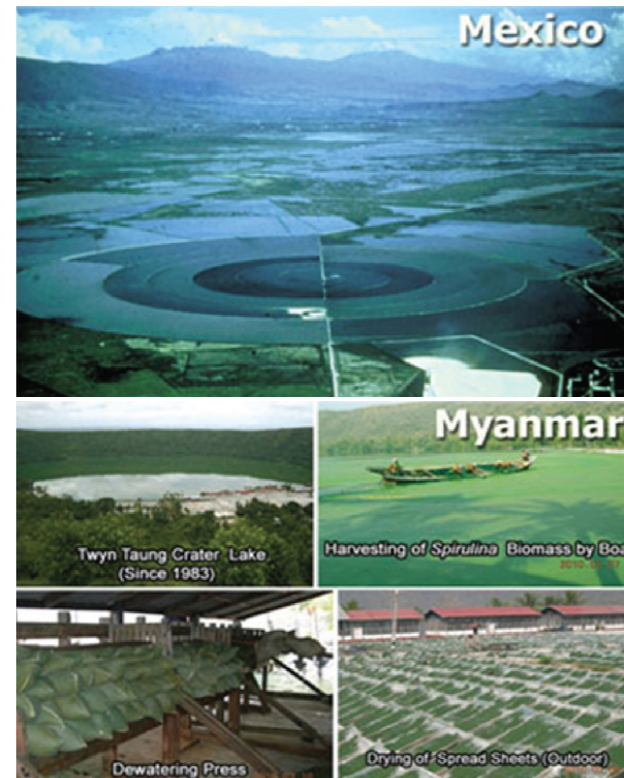


Fig. 2.2 - Spirulina Mexicana's Caracol in Lake Texcoco near Mexico City. Lake harvesting, dewatering and drying spirulina in Myanmar. Courtesy of Min Thein²⁷

Examples of Natural and Semi-Natural Water Ponds include:

Lakes in the Lake Chad region (Africa), where nearly pure cultures of *Arthrospira platensis* grow due to the high alkalinity of the water (Abdulqader et al., 2000).

Craters in Myanmar filled with alkaline water, where cyanobacteria are cultivated almost continuously: The Caracol system in Mexico - an artificial spiral-shaped lagoon over 900 hectares in size, which formerly produced up to 300 tons of spirulina annually (Fig.2.2)

Dunaliella salina lagoons in Australia - for example, the Hutt Lagoon and Whyalla facilities, with pond areas of 250–460 hectares and annual production of 6–10 tons of β-carotene.

In such systems, productivity is significantly lower (0.5–1 g/m²/day), but they remain economically attractive due to low operating costs.

Construction Materials

Materials used in open systems range from clay and sand to concrete and polymers (such as PVC

²⁷ <https://smartmicrofarms.com/spirulina/5-cultivation-worldwide/>

or polyethylene liners). The choice of material significantly impacts durability, cost, and contamination risk. Unlined ponds reduce costs but lead to leakage, sediment mixing, and contamination, making such designs less practical for most applications.

Advantages of open ponds:

- Low capital investment;
- Operational simplicity;
- Scalability potential;
- High light penetration.

Limitations:

- Limited control over environmental parameters (pH, temperature, contamination);
- Water evaporation and CO₂ loss;
- High harvesting costs due to low culture density.

Open cultivation systems remain a key technology in the microalgae production industry, especially in regions with favourable climatic conditions. The choice between raceway systems, inclined surfaces, or natural lagoons depends on the cost-benefit ratio, target product, and available resources. Further optimization is required, particularly in minimizing water loss, improving culture hygiene, and enhancing energy efficiency.

Closed Cultivation Systems (Photobioreactors)

Closed systems for microalgae cultivation, particularly photobioreactors (PBRs), are key technologies for obtaining high-quality biomass under controlled conditions. Their use ensures the isolation of cultures from the external environment, minimizing contamination risks, allowing precise control of growth parameters (light, temperature, gas exchange, pH, etc.), and increasing resource use efficiency.

General Design of Tubular Photobioreactors

Tubular PBRs are the most common type of closed systems implemented on an industrial scale (Torzillo & Zittelli, 2015). They usually consist of transparent polymer or glass tubes with a diameter of 5–10 cm, arranged horizontally, vertically, or in spiral form. Figure 2.3 shows a commercial collector-type installation with vertical photobioreactors.

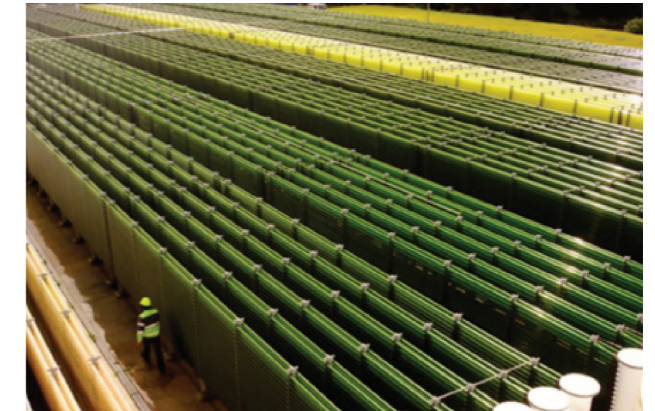


Fig. 2.3 - Tubular collector photobioreactors: commercial installation with vertical reactors at A4F-Algae for Fuel, S.A., Pataias (Portugal)⁷¹

Microalgal cultures circulate through the tubes via pumps or airlift systems. The surface-to-volume ratio (S/V) in such systems can reach 80 m⁻¹, contributing to high biomass productivity. The system typically consists of two main parts: a **photo zone**, where photosynthesis occurs, and a **mixing reservoir**, which removes excess oxygen, stabilizes temperature and pH, and facilitates CO₂ supply.

Types of Tubular Photobioreactors

Serpentine PBRs are the oldest type of tubular systems, where tubes are connected by U-shaped bends to form flat loops. A well-known example is a double-layer horizontal 4000-liter reactor with 400 m of tubing installed in Almería, Spain. Modern modifications include vertical structures cooled via heat exchangers or water baths. An innovative approach was developed by Microphyt (France), which introduced a reactor with two-phase circulation - “windy, wavy, and wiped” (www-PBR), allowing cultivation of even sensitive strains.

Manifold (comblike) PBRs use tubes connected between two collectors - inlet and outlet. For instance, Roquette Klötze (Germany) operates vertical manifold PBRs with 500 km of tubing, producing about 40 tons of Chlorella biomass annually. Key advantages include lower pressure loss, reduced oxygen concentration, and easier scalability.

Spiral PBRs consist of small-diameter tubes coiled around a vertical frame. They allow optimal space usage but present challenges related to hydrodynamic stress and cleaning. An example is the Biocoil system, tested at pilot scale with marine species and *Arthrospira*.

Table 2.2. Effect of Tube Diameter on the Productivity of *A. platensis* in Tubular PBRs³⁰

Tube Diameter (cm)	Volume per Area (L/m ²)	Surface Density (g/m ²)	S/V (m ⁻¹)	Optimal Biomass Concentration (g/L)	Volumetric Productivity (g/L/day)	Areal Yield (g/m ² /day)
14	110	50-70	9	0.4-0.6	0.20	22
13	102	60-80	10	0.6-0.8	0.23	23
7.4	58	70-80	17	1.2-1.4	0.40	23
5	39	70-90	25	1.4-2.0	0.65	25
2.5	19.6	70-120	50	3.5-6.0	1.40	27

Efficiency Evaluation of Tubular PBRs

Tubular PBRs provide high-quality biomass for pharmaceutical, food, and cosmetic industries. High productivity is achieved through careful control of tube diameter, loop length, and circulation speed. For example, a tube diameter of 5-9 cm ensures optimal light regime and cell concentration. The impact of tube diameter on the productivity of *A. platensis* cultures in tubular PBRs is shown in Table 2.2.

Optimal tube length is generally considered to be 100-150 m. In longer channels, mixing becomes problematic, as mixing time increases with tube length. This must be considered during scale-up to ensure adequate nutrient distribution (Torzillo & Zittelli, 2015). Therefore, tubular PBRs cannot be scaled indefinitely - large-scale facilities must use modular reactor units instead of overly long tubes (Eriksen, 2008). This approach becomes very costly since each module requires separate systems to control various growth factors (Kunjapur & Bruce Eldridge, 2010).

The average energy consumption ranges from 10 to 100 W/m². Major limitations include high investment costs (up to €0.51 million/ha) and the need for continuous energy supply to circulate the culture.

Large-scale cultivation in tubular systems is a proven and reliable technology for conducting production under highly controlled and automated conditions. However, due to high investment and energy demands, these systems are suitable for high-value niche products and inoculum production, but not for low-cost commodities.

Vertical Column Photobioreactors and Sleeve Systems

Vertical tubular reactors (or column photobioreactors) are among the simplest closed systems for cultivating microalgae, where mixing is achieved by air bubbling. The first such setups were developed at Stanford University (USA) in the 1940s (Cook, 1950). These glass reactors were 1.8 m high and 10 cm in diameter, with a narrowed bottom section to prevent cell sedimentation. Indoor cultivation conditions allowed a productivity of 0.48 g/L/day for *Chlorella*, whereas outdoor productivity averaged 0.28 g/L/day, with a maximum of 0.35 g/L/day. The reduction in efficiency outdoors was due to the high inclination angle relative to solar radiation, leading to significant light loss from reflection.

Researchers (Miyamoto et al., 1988) experimented with inexpensive industrial glass tubes (2.35 m high, 5 cm diameter) and achieved a productivity of 0.6 g/L/day for *Nostoc* and *Anacystis*. Using a similar approach, Hu and Richmond (1994) achieved even higher productivity - 1.6 g/L/day - during cultivation of *Isochrysis galbana*.

Modern rigid vertical reactors used in hatcheries to feed mollusc and fish larvae typically range from 2 to 2.5 meters in height and 30-50 cm in diameter. These are made of semi-transparent fiberglass and mixed with air.

Simple sleeve reactors are constructed from transparent polyethylene tubing sealed on one end. They are suspended on a frame and aerated with air. These systems can be used both indoors with artificial lighting and outdoors. Despite their short service life (due to biofouling), sleeve re-

actors are easy and inexpensive to replace. In Beer-Sheva (Israel), such reactors were used to cultivate *Porphyridium* and *Dunaliella*, yielding higher productivity compared to open ponds (Cohen & Arad (Malis), 1989).

The main disadvantages of sleeve reactors include a low surface-to-volume (S/V) ratio and intense wall fouling. An example of an improved vertical system is the concentric airlift reactor developed at the University of Almería (Spain) (2 m high, 9.6 cm in diameter), used for cultivating *Phaeodactylum tricornutum* (Garcia Camacho et al., 1999). It demonstrated efficiency comparable to horizontal systems.

Particular attention should be given to internally illuminated systems, where the cylinder is lit from within using fluorescent lamps. These achieve higher productivity due to more efficient photon absorption. For example, in Florence (Italy), a ring-shaped system was developed using two Plexiglas cylinders forming a culture chamber 3-5 cm thick and 120-150 L in volume. Gas exchange is provided by injecting an air-CO₂ mixture, and internal lighting significantly enhances photosynthesis efficiency⁷¹.

Features of Flat-Panel Photobioreactors

A typical photobioreactor (PBR) is a three-phase system that includes a liquid phase (culture medium), a solid phase (microalgae cells), and a gas phase (CO₂, O₂, N₂). Light is sometimes referred to as the fourth phase. The development of an efficient PBR requires understanding the interaction between environmental parameters and biological response²⁸.

The basic design of flat-panel PBRs consists of two parallel plates with a thin layer of algal suspension in between. Effective light transmission is usually achieved with a layer thickness of a few centimetres. PBR plates are typically made of polyvinyl chloride, polycarbonate, polymethyl methacrylate, glass, or polyethylene. The main advantage of flat-panel PBRs is the high surface-area-to-volume ratio. Figure 2.4 shows a pilot flat-panel PBR setup (1.5 m high, 10 m long, 0.10 m wide) located in Tocopilla (Chile) within the E-CL thermoelectric power plant.

Flat-panel photobioreactors demonstrate microalgae production rates of 5 to 15 g/(m²·day), although values up to 35 g/(m²·day) have been achieved using wastewater as the culture medium²⁹.



Fig. 2.4 - Pilot flat-panel photobioreactor system (1.5 m height, 10 m length, 0.10 m width), located in Tocopilla, Chile, at the E-CL power station⁷¹

These systems can achieve high photosynthetic efficiency. However, they also have some limitations. Due to their compact design, they are subject to significant temperature fluctuations and culture overheating. Flat PBRs are typically cooled by spraying water over their surface, which can be collected for reuse.

To maximize microalgae productivity, the light-saturated zone should be evenly distributed throughout the PBR volume - a challenge in large-scale operations. Mixing ensures the circulation of algal cells between zones, reducing photoinhibition at the illuminated surface and biomass loss in the dark zone³⁰.

Photobioreactors inherently have a light gradient caused by light absorption and mutual shading of cells. The light regime is defined by this gradient and the fluid circulation rate. Several zones with different light intensities can be identified simultaneously within the bioreactor volume: total darkness, light limitation, light saturation, and light inhibition. Darkness and photoinhibition zones are unfavourable for algal growth. Incident light intensity, commonly used as an indicator, poorly reflects actual light conditions in a PBR. A better metric is the average light intensity, based on the assumption that algae cells experience different light levels over short mixing intervals - effectively equivalent to constant exposure to average light intensity.

28 Clemens Posten. Design principles of photo-bioreactors for cultivation of microalgae. Engineering in Life Sciences. Vol 9. Issue 3. 2009.

29 (PDF) Microalgal Heterotrophic and Mixotrophic Culturing for Bio-refining: From Metabolic Routes to Techno-economics

30 <https://www.sciencedirect.com/science/article/pii/S2211926417306677>

Creating favourable light conditions in a PBR requires maximizing the illuminated surface-to-volume (S/V) ratio, a key parameter. The dependence of biomass productivity on the V/S ratio is shown in Fig. 1 (V - in liters, S - in m²).

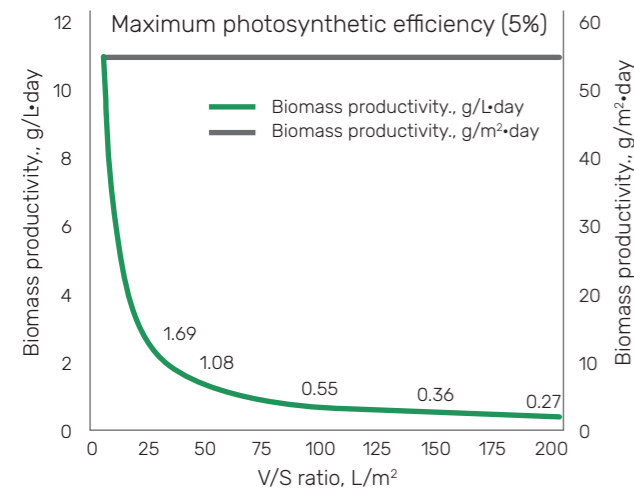


Fig. 2.5 – Biomass productivity of microalgae with varying V/S ratio in flat plate photobioreactors ³¹

Scaling up from laboratory setups to industrial systems presents major challenges. For large-scale PBRs, a microalgae concentration above 1.0 g/L is recommended. Biomass concentration significantly influences process economics and reactor size: the higher the biomass concentration, the smaller the required reactor volume. The optimal S/V ratio ranges from 43–73 m²/m³ for different types of PBRs. Higher S/V values may lead to photoinhibition and culture overheating ³³.

Tables 2.3 and 2.4 present the results of calculating the productivity of microalgae biomass cultivation for different sizes of microalgae cultivation facilities and V/S ratios, based on the data presented in Fig 2.5.

Table 2.3. Biomass productivity at boundary surface-to-volume ratios²⁸

S/V (m ² /m ³)	V/S (L/m ²)	Productivity (g/(L·day))
43	23	2
73	13	6

According to measurements by A.A. Tsygankov (2001), at a cell concentration of only 0.6 g dry

biomass/L for *Anabaena variabilis*, a culture layer 1 cm thick absorbs 90% of the incident light, with only 1% reaching a depth of 2 cm. At higher concentrations, light attenuation is even faster. This is clearly shown in measurements of photon flux density decline with depth in a *Euglena gracilis* culture. Therefore, early attempts to use containers with thick (>5 cm) culture layers for microalgae cultivation led to very low biomass yields ³².

Each culture has an optimal flat-panel reactor thickness, balancing light deficiency in deeper layers with surface light overexposure. Dual-sided lighting can double culture productivity.

Risks of biofouling and external contamination can be mitigated - or even minimized - by using disposable flat-panel reactors, significantly reducing costs. One such system is the patented Green-Wall reactor. Its design comprises a flexible transparent polyethylene bag placed between two rigid iron frames, forming a thin vertical panel. A 400 m² installation of 18 flat reactors (1.5 m³ each) was set up in Tocopilla, Chile (Fig. 2.4) and connected to a power plant to use flue gases as a carbon source for microalgae production ⁷¹.

Promising Directions for Photobioreactor Development

The further development of microalgae photobioreactor systems will follow three main directions:

- I. Improvement of existing cultivation technologies
- II. Scaling up production processes
- III. Industrialization of microalgae biomass production

A key priority is enhancing biological system efficiency by improving environmental control (pH, temperature, illumination, gas saturation) and maximizing light energy utilization. This also involves reducing capital and operating costs, for example by using inexpensive construction materials and low-power culture circulation systems.

In the context of scaling up, there is a pressing need to establish scaling criteria and adapt appropriate equipment for stable and safe operation over large areas. It is advisable to apply technological solutions from related fields such as wastewater treatment or milk processing, where similar fluid-handling technologies are well developed.

³² F. Fasaei, J.H. Bitter, P.M. Slegers, A.J.B. van Boxtel. Techno-economic evaluation of microalgae harvesting and dewatering systems. Volume 31, April 2018, Pages 347–362.

Table 2.4. Productivity of photobioreactors of various sizes

Photobioreactor	Size (m)	Volume (L)	Area (m ²)	V/S (L/m ²)	Biomass productivity (g/(L·day))
Open pond (h = 1 m)	100×100×1	10 mill	10 000	1 000	0.2
Open pond (h = 0.1 m)	100×100×0.1	1 mill	10 000	100	0.55
Open pond (h = 0.01 m)	100×100×0.01	100 000	10 000	10	6
Vertical flat-panel (dual-sided light)	100×100×0.01	100 000	20 000	5	10

Special attention should be paid to contamination control, which is critical for open systems, and to culture stability. For closed reactors, a major challenge remains the biofouling of internal surfaces, which periodically leads to culture loss.

Although global microalgae production is rapidly growing, only a synergistic advancement of these areas will enable a substantial industrial scale-up and establish microalgae as a full-fledged raw material base for the bioeconomy.

Main technological parameters of the cultivation process

Cultivation

Cultivation of microalgae is a very sensitive biotechnological process based on careful control of physicochemical parameters. The rate of photosynthesis, nutrient absorption and biomass accumulation directly depend on temperature, acidity, light level and content of dissolved gases and nutrients. In view of this, precise adherence to the optimal values of each parameter allows achieving stable and high culture growth, which is critically important for the further use of microalgae.

Since microalgae have a high adaptive capacity, but are limited by a number of biophysical laws, even minor deviations from the recommended conditions can significantly reduce the productivity of the process. Thus, controlling these parameters is one of the key factors in the effectiveness of the entire technology. This part of the analytical note systematizes the key parameters that affect the productivity of the cultivation process, indicating their optimal ranges.

Temperature

This is one of the most important parameters that has an impact on others. The solubility of CO₂ and O₂ in water depends on temperature. In addition, the activity of enzymes contained in cells and involved in the processes of photosynthesis and synthesis of cellular components depends on it. Microalgae are able to grow in the temperature range from 5 to 40°C, but each species has its own optimal growth temperature. For most microalgae, this indicator is in the range from 20 to 30°C ³³. An increase in temperature above the limiting limit causes temperature stress, which inhibits the work of enzymes and the photosynthetic apparatus, and in the case of long-term action leads to irreversible destruction of proteins, which leads to cell death. On the other hand, a decrease in temperature leads to a slowdown in metabolism and a decrease in biomass growth, when the temperature drops to 0 °C and below, the water inside the cells crystallizes and ruptures it from the inside. Considering the climatic conditions of Ukraine, temperature fluctuations during the year are significant. In the summer months, the air temperature in some regions can reach +35°C ... +38°C ³⁴, which will lead to overheating of the photobioreactors. In the cold season, especially when the temperature can drop to -18°C, there is a constant need to heat the photobioreactors.

The pH of the medium

It significantly affects the metabolism of microalgae, as well as the availability of nutrients and CO₂.

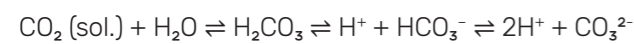
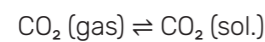
³³ Singh, S.P.; Singh, Priyanka. Effect of temperature and light on the growth of algae species: A review. Renewable and Sustainable Energy Reviews 2015, 50, 431–444. <https://doi.org/10.1016/j.rser.2015.05.024>

³⁴ <http://cgo-sreznevskiy.kviv.ua/uk/diialnist/21213/9-pidsumki-shchedrogo-na-rekordi-cherivnya-u-stolitsi>

³¹ https://www.researchgate.net/publication/323635891_Photobioreactor_Cultivation_Strategies_for_Microalgae_and_Cyanobacteria

Most microalgae have an optimum in the range from 6.0 to 9.0. During cultivation, the pH level can change: Typically, the pH increases during the light period due to the absorption of inorganic carbon and its use for photosynthesis, and decreases during the dark period due to the release of CO₂ during respiration³⁵.

This indicator also affects the form of inorganic carbon in the nutrient medium. When CO₂ is dissolved in the nutrient medium, carbon can be in 3 forms: free CO₂, HCO₃⁻ and CO₃²⁻. This can be described by the following equation:



At pH below 6, CO₂ (dissolved) predominates, from 6.0 to 8.0 CO₂ (dissolved) and HCO₃⁻ predominate, from 7.5 to 9.0 HCO₃⁻ and CO₃²⁻ begin to predominate, and from 9.0 the proportion of HCO₃⁻ decreases and CO₃²⁻ begins to predominate. Microalgae can absorb both dissolved CO₂ and HCO₃⁻, but cannot consume CO₃²⁻³⁶.

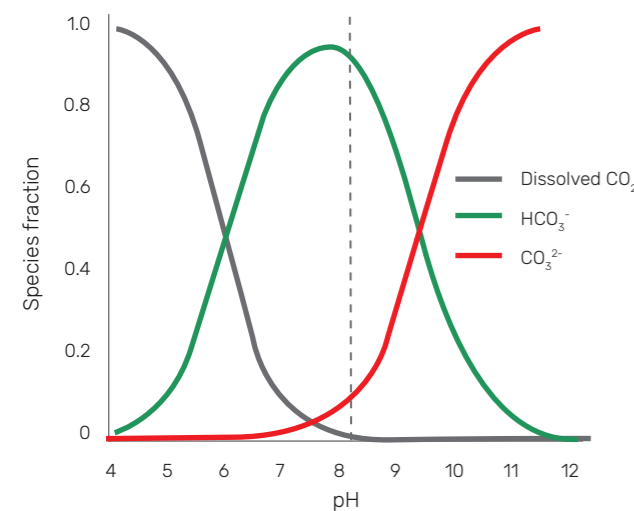


Fig. 2.6 - Dependence of the proportion of different forms of carbon on the pH level³⁷

This indicator can be regulated in several ways: in an automated mode, dose solutions of acids or alkalis or, when the pH increases, increase the supply of CO₂ to the environment, or use NaHCO₃ and other buffer substances.

³⁵ Hawrot-Paw M, Sašiadek M. Optimization of Microalgal Biomass Production in Vertical Tubular Photobioreactors. *Energies*. 2023; 16(5):2429. <https://doi.org/10.3390/en16052429>

³⁶ Sun, Z.; Bo, C.; Cao, S.; Sun, L. Enhancing CO₂ Fixation in Microalgal Systems: Mechanistic Insights and Bioreactor Strategies. *Mar. Drugs* 2025, 23, 113. <https://doi.org/10.3390/md23030113>.

³⁷ <https://andthentheresphysics.wordpress.com/2016/11/02/ocean-co2-uptake-part-2/>

Lighting

This parameter is key in photosynthesis, since light is the main source of energy for cells in autotrophic and mixotrophic cultivation. The intensity of lighting, the spectral composition of light and the photoperiod directly affect the efficiency of cultivation and the biochemical composition of microalgae.

Microalgae are able to absorb only a part of solar radiation, the so-called photosynthetically active radiation, which corresponds to the visible wavelength range from 400 to 700 nm. They do this thanks to the photosynthetic pigments contained in the cells. Chlorophyll-a absorbs most in the ranges of 380 - 470 nm and 600 - 680 nm, chlorophyll-b absorbs in the range of 410 - 480 nm, -carotene absorbs in the range of 400 - 500 nm, and phycocyanin - in the range of 600 - 640 nm, which is why LED lighting is most often used in industrial and research systems among artificial lighting. Microalgae are able to change the quantitative composition of their pigments, adapting to different lighting conditions⁶.

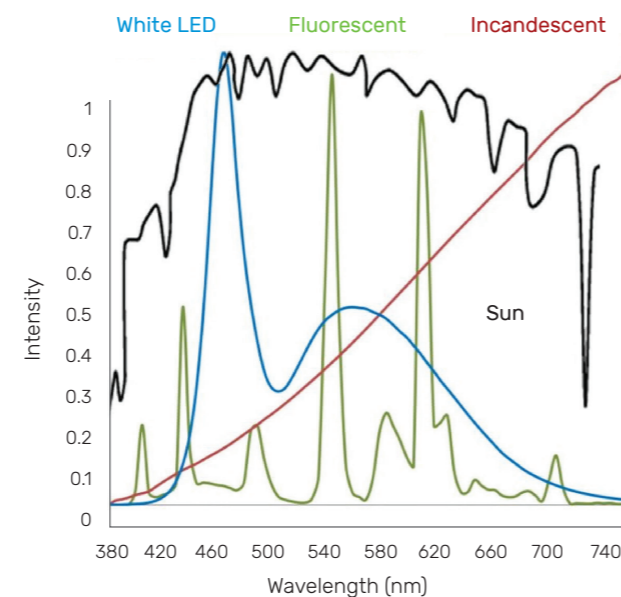


Fig. 2.7 - Comparison of radiation spectra of different lighting sources³⁸

In addition to the spectral composition of light, its intensity is also important, which directly affects the photosynthesis process. In the context of photosynthetically active radiation, this refers to the number of micromoles of photons falling per square meter per second.

³⁸ <https://blog.drwile.com/led-lights-might-pose-a-hazard-for-vision/>

The optimal light intensity for microalgae growth varies greatly for each species and is approximately in the range of 100-500 μmol photons/m²/s³⁹.

Values below the optimum lead to a limitation of photosynthesis due to a deficiency of light energy, while excessive light causes photoinhibition, caused by damage to photosystems, excessive formation of reactive oxygen species and redistribution of energy to defence mechanisms instead of cell growth.

It is also necessary to pay attention to the relationship between light intensity and culture density. In cultures with a high biomass concentration, light is absorbed mainly by cells in the outer layer, while cells in the inner layer remain shaded. Therefore, controlled lighting is often used, which is increased when the biomass concentration increases, or ensures uniform mixing.

Photoperiod. An equally important factor is the ratio of the light and dark phases. Two most often used photoperiods are: 16:8 and 12:12 for day: night⁴⁰. The presence of a dark phase is critical for the vital activity of cells. With the correct photoperiod settings, microalgae cells synchronize their circadian rhythm. During the light phase, they grow and accumulate starch through photosynthesis, and during the dark phase they use the accumulated starch for reproduction⁴¹. In addition, in the dark phase, oxidation of the photosystem occurs, which prevents photoinhibition of cells.

Violation of the day: night ratio leads to chaotic manifestations. Cells begin to reproduce during the day, which takes a certain time during which photosynthesis does not occur, so the efficiency of light and CO₂ absorption significantly decreases.

Salinity. Salinity is an important factor, especially in open culture systems, where the concentration of salts in the medium can change due to evaporation. Depending on the species, microalgae have different sensitivity to salt concentration. Based

³⁹ Palikrousis T, Manolis C, Kalamaras S, Samaras P. Effect of light intensity on the growth and nutrient uptake of the microalga *Chlorella sorokiniana* cultivated in biogas plant digestate. *Water*. 2024;16(19):2782. <https://doi.org/10.3390/w16192782>

⁴⁰ Sforza E, Simionato D, Giacometti G, Bertucco A, Morosinotto T. Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. *PLoS One*. 2012;7(6):e38975. <https://doi.org/10.1371/journal.pone.0038975>

⁴¹ de Winter L, Cabanelas ITD, Martens DE, Wijffels RH, Barbosa MJ. The influence of day/night cycles on biomass yield and composition of *Neochloris oleoabundans*. *Biotechnol Biofuels*. 2017;10:104. <https://doi.org/10.1186/s13068-017-0762-8>

on the level of tolerance to this indicator, they are divided into three main groups⁴²:

- *Oligohaline* - grow in slightly saline water with a maximum salt content of 0.5 to 5 g/kg
- *Mesohaline* - adapted to life in water with a maximum salt content of 5 to 18 g/kg
- *Polyhaline* - withstand high salinity with a maximum salt content of 18 to 30 g/kg

An increase in the salinity of the medium beyond the optimum leads to osmotic stress. In response, microalgae cells undergo biochemical changes: increased formation of lipids, which perform the function of an energy reserve, and also provide protection against reactive oxygen species, while the content of proteins and chlorophyll is significantly reduced due to inhibition of photosynthesis. In order to compensate, the synthesis of carotenoids is enhanced, and the cells also begin to accumulate soluble sugars. These changes allow microalgae to temporarily adapt to adverse conditions, but are accompanied by a significant decrease in growth rates up to its complete cessation⁴³.

In the context of using digestate as a nutrient medium, the level of salinity is of particular importance. Digestate may contain an increased amount of chlorine, sodium, sulphate, potassium, magnesium, bromine and other ions that affect the salinity of the nutrient medium and increase the osmotic pressure. To avoid growth inhibition, it is advisable to pre-dilute the digestate with water¹².

Nutrient content. For the cultivation of microalgae, it is necessary to ensure the presence of macro- and microelements in the nutrient medium in a form accessible to microalgae. General information on the necessary nutrients for their cultivation is given in Table 2.5

The lack of at least one of the nutrients will lead to a decrease in the growth rate of biomass in accordance with the Liebig law of the minimum. It is also important to consider that an excess of ammonium NH₄⁺ or nitrite NO₂⁻ can cause inhibi-

⁴² Chowdury MK, Nahar N, Deb UK. The growth factors involved in microalgae cultivation for biofuel production: A review. *Comput Water Energy Environ Eng*. 2020;9(4):185-215. <https://doi.org/10.4236/cweee.2020.94012>

⁴³ Ermis H, Altınbaş M. Effect of salinity on mixed microalgae grown in anaerobic liquid digestate. *Water Environ J*. 2020. <https://doi.org/10.1111/wej.12580>

tion or cell death ⁴⁴. Therefore, the nutrient medium must be balanced and adapted to a specific type of microalgae and cultivation conditions.

Modern studies ^{45,46} confirm that digestate from biogas plants can be used as part of a nutrient medium with a high content of macro- and microelements necessary for the cultivation of microalgae, but the concentrations of some substances can cause inhibition. Therefore, the use of digestate requires constant monitoring of its composition, as well as its pre-treatment.

44 Markou, G., Vandamme, D., & Muylaert, K. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Research* 2014, 65, 186–202. <https://doi.org/10.1016/j.watres.2014.07.025>

45 Resman L, Berden Zrimec M, Žitko V, Lazar B, Reinhardt R, Cerar A, Mihelič R. Microalgae Production on Biogas Digestate in Sub-Alpine Region of Europe—Development of Simple Management Decision Support Tool. *Sustainability*. 2023; 15(24):16948. <https://doi.org/10.3390/su152416948>

46 Nagarajan D, Lee DJ, Chang JS. Integration of anaerobic digestion and microalgal cultivation for digestate bioremediation and biogas upgrading. *Bioresour Technol*. 2019;292:121804. <http://doi.org/10.1016/j.biortech.2019.12.1804>

Dissolved CO₂ and O₂ content. Carbon is one of the key elements necessary for cell growth. Since the concentration of CO₂ in the atmosphere is insufficient for intensive culture growth, artificial carbon supply to the nutrient medium is used. It can be supplied both in the form of inorganic salts of carbonates and bicarbonates and in the form of gaseous CO₂. The second method is particularly interesting due to the capture of CO₂ by microalgae cells from the biogas upgrading plant to biomethane and the reduction of the carbon footprint of the latter ⁴⁸.

Different types of microalgae have their own optimal CO₂ concentrations at which their growth rate is maximum. Insufficient CO₂ content limits the activity of photosynthesis, respectively, reduces the overall productivity of the culture. On the other hand, excessive CO₂ concentration creates stress-

48 Geletukha, G., Hyvel, M., & Kucheruk, P. (2024). OPPORTUNITIES OF ADVANCED BIOMETHANE PRODUCTION FROM MICROALGAE GROWN ON BIOGAS PLANT DIGESTATE. Part 1. Thermophysics and Thermal Power Engineering, 46(4), 60-73. <https://doi.org/https://doi.org/10.31472/ttpe.4.2024.7>

Table 2.5. Essential nutrients for microalgae cultivation ⁴⁷

Component	Source (nutrient)	Functions	Required concentration range
Carbon	CO ₂ , HCO ₃ ⁻ , CO ₃ ²⁻ and others	Synthesis of carbohydrates, proteins, lipids	1-10 g/L
Nitrogen	NO ₃ ⁻ , urea, ammonia, N ₂ and others	Synthesis of amino acids, nucleic acids, proteins, pigments such as chlorophyll and phycocyanin	10-2000 mg/L
Phosphorus	Phosphate, hydrogen phosphate, others	Component of DNA and RNA backbone, ATP, phospholipids, nucleotides	10-500 mg/L
Sulphur	Sulphates	Synthesis of amino acids methionine and cysteine, component of coenzymes	1-200 mg/L
K, Ca, Na, Mg, etc.	Inorganic salts	Maintenance of cell structure and metabolism	0.1-100 mg/L
Fe, Zn, Mn, Pb, Cd, etc.	Inorganic salts	Co-enzyme factors	0.01-10 mg/L
Growth factors	Vitamins B, C, E, others	Part of coenzymes	0.01-1000 µg/L

47 Geletukha, H., Hyvel, M., Kucheruk, P., & Sydorenko, M. Opportunities of advanced biomethane production from microalgae grown on biogas plant digestate. Part 2. Thermophysics and Thermal Power Engineering 2025, 47(1), 65–79. <https://ihe.nas.gov.ua/index.php/journal/article/view/617>

ful conditions for cells, which can cause acidification of the environment.

Such stress causes an increase in the accumulation of lipids in cells, but significantly reduces biomass growth ⁴⁹.

The content of dissolved oxygen in the nutrient medium is equally important. During photosynthesis, microalgae actively produce oxygen, which leads to its accumulation, especially in closed-type FBI. At excess concentrations of more than 10 mg/l, unfavourable conditions arise for microalgae. An increased level of dissolved oxygen promotes the formation of reactive oxygen species and also leads to the interaction of the key enzyme RuBisCO with oxygen, rather than CO₂, which significantly reduces the efficiency of photosynthesis and growth rate⁵⁰. The optimal range of dissolved oxygen for many species of microalgae is 2 – 10 mg/l ⁵¹.

Microalgae harvesting and dehydration

Microalgae Harvesting

Biomass harvesting is no less important than cultivation. The feasibility of using microalgae also depends on the efficiency of biomass harvesting. It is estimated ⁵² that microalgae harvesting accounts for 20 to 30% of all costs.

This is due to energy consumption and the need to process large volumes of culture fluid. Some methods, although demonstrating high efficiency for biomass harvesting, require more energy than the energy value of microalgae. The quality of the obtained microalgae biomass is also extremely important.

Residues of flocculants and coagulants, excessive moisture and cell damage can affect further pro-

49 Kandasamy LC, Neves MA, Demura M, Nakajima M. The Effects of Total Dissolved Carbon Dioxide on the Growth Rate, Biochemical Composition, and Biomass Productivity of Nonaxenic Microalgal Polyculture. *Sustainability*. 2021; 13(4):2267. <https://doi.org/10.3390/su13042267>

50 Kazbar A, Cogne G, Urbain B, et al. Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Res*. 2019;101:101432. <https://doi.org/10.1016/j.algal.2019.101432>

51 Gao S, Edmundson S, Huesemann M. Oxygen stress mitigation for microalgal biomass productivity improvement in outdoor raceway ponds. *Algal Res*. 2022;68:102901. <https://doi.org/10.1016/j.algal.2022.102901>

52 Deepa P, Sowndhararajan K, Kim S. A Review of the Harvesting Techniques of Microalgae. *Water*. 2023; 15(17):3074. <https://doi.org/10.3390/w15173074>

cessing and use. Therefore, microalgae harvesting has a very strong impact on the economic feasibility and overall efficiency of the technology.

This process strongly depends on the type of microalgae and their morphological characteristics, namely: size, shape, cell wall structure, presence of a mucous layer, electrical charge determine how efficiently biomass can be collected. The same method may be unsuitable for one type of microalgae, but at the same time very effective for another.

Most microalgae have sizes in the range of 0.2 to 30 microns ⁵³, which leads to a decrease in the efficiency of natural sedimentation. According to the Stokes equation, the sedimentation rate is directly proportional to the square of the particle diameter. Therefore, such microscopic cell sizes retain them in water for a long time. In addition, microalgae cells have a density value quite close to water. Therefore, even the heaviest cells settle very slowly.

What is special is that microalgae have a negative charge on the cell surface, which arises due to the dissociation of functional groups. This creates obstacles to the adhesion of cells due to electrostatic repulsion between them without the presence of flocculants ⁵⁴.

Another problem is the presence of extracellular mucus in some species of microalgae, which consists of polymeric substances. It increases the viscosity of the suspension, reduces the filtration efficiency due to membrane clogging, and also worsens the sedimentation of cells ⁵.

Thus, the characteristics of microalgae make their collection a difficult task. That is why, first of all, the selection of methods for harvesting biomass should be based on the characteristics of the species, and only then on obtaining the target product.

Flocculation

Flotation is based on the artificial union of cells into large flocs by neutralizing the negative charge on their surface with the help of flocculants. The formed flocs eventually settle to the bottom, where they can be easily separated from the liquid. The efficiency of this method ranges from 80 to 100% ⁵²

53 Thangavel P, Sridevi G, eds. *Environmental Sustainability*. Springer India; 2015. https://doi.org/10.1007/978-81-322-2056-5_9

54 Roselet F, Vandamme D, Muylaert K, Abreu PC. Harvesting of microalgae for biomass production. *Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment*. Springer; 2019; 211–243. https://doi.org/10.1007/978-981-13-2264-8_10

and depends on the type of microalgae due to the presence of a mucous layer, the pH of the medium, the temperature, the applied dose of flocculants, and the speed of mixing. In practice, several types of flocculation are used: chemical, bio flocculation, and electro flocculation. Chemical flocculation is the easiest to use, it is based on the use of inorganic salts of aluminium or iron and synthetic or natural polymers. Although inorganic flocculants are cheap and commercially available, they need to be used in high concentrations of 120 to 1000 mg/L⁵⁵ and can leave metal ions, which negatively affects the performance of microalgae as raw material for the anaerobic digestion process. Other flocculants mentioned provide good efficiency, require much lower concentrations of substances from 5 to 50 mg/L⁴, but are much more expensive than inorganic ones. Also, natural polymers are effective for collecting only freshwater microalgae⁵⁴.



Fig. 2.8 - Chemical flocculation in laboratory conditions using Chitosan (Photo by the authors AN)

Bio flocculation is an alternative to chemical flocculation and works by releasing metabolites, which are flocculants, from other microorganisms during co-cultivation. It does not require the introduction of additional substances, but is highly dependent on the cultivation conditions. In addition, this process is more difficult to control and co-cultures can

pose a threat to the normal course of the anaerobic digestion process⁵⁶.

Electro flocculation requires electric current as a substitute for classic flocculants. It allows for careful control of the flocculation process and does not depend on the introduction of external substances, but requires significant electricity consumption and constant cleaning of the electrodes, and also has a high risk of releasing metals from the electrodes into the resulting biomass. This method works much more effectively with marine microalgae than with freshwater ones⁵².

In general, flocculation demonstrates its versatility and is often the first step in many combined methods of biomass collection due to its simplicity, efficiency and scalability.

Flotation

This method is based on the concentration of cells on the surface of the culture liquid in the form of a foam layer using air bubbles or other gas that lift the cells up. Due to the lack of intensive impact on the cells, flotation is considered promising for collecting small microalgae, such as *Chlorella vulgaris*, *Scenedesmus obliquus*, *Nannochloropsis* and others⁵².

The most common type of flotation is dissolved air flotation. In this technology, air is dissolved in the culture liquid under pressure, after which the pressure is reduced to atmospheric in the flotation tank basin, while releasing small bubbles of excess air with a diameter of no more than 100 microns. Due to this size, these bubbles have a large total surface area, which ensures their effective adhesion to the cells and their lifting upwards. This method allows to collect from 80 to 90%¹ of the total mass of microalgae.

An alternative is flotation with atomized air, which consists in simply supplying air through a diffuser. This method is much cheaper due to lower energy costs and cheaper equipment, however, the bubbles formed have a diameter of more than 100 microns, which greatly reduces the efficiency of biomass collection. In order to increase the efficiency in this technology, preliminary preparation is carried out, which consists in the use of flocculants, which allows to increase the collection efficiency to more than 90%⁵⁴.

⁵⁶ Heredia-Martínez LG, Gutiérrez-Diáñez AM, Díaz-Santos E. Bio-Flocculation: A Green Tool in Biorefineries for Recovering High Added-Value Compounds from Microalgae. *Phycology*. 2025; 5(2):19. <https://doi.org/10.3390/phycolgy5020019>

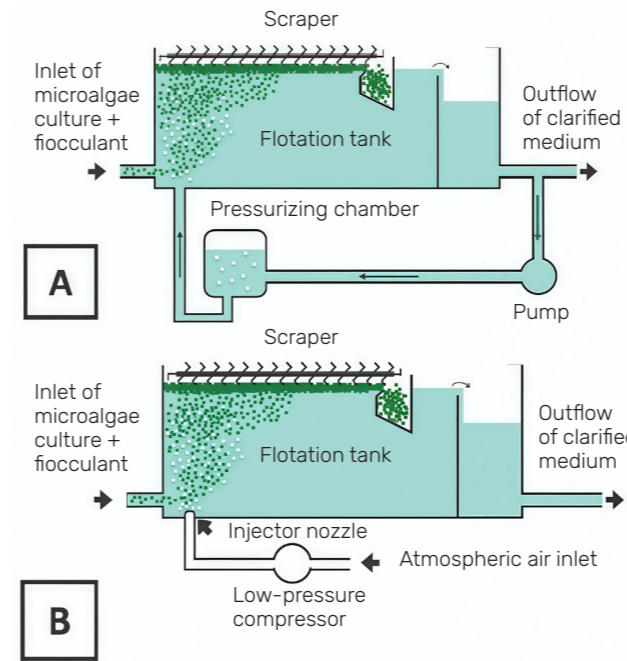


Fig. 2.9 - The principle of operation of flotation plants:⁵⁴

A - Dissolved air flotation
B - Spray air flotation

Compared to DAF, spray air flotation is cheaper and easier to operate, but is less efficient. In contrast, DAF provides efficient biomass collection at higher costs without the use of additional substances. Thus, flotation can provide high productivity and allows for the reuse of the purified medium, which is a significant advantage.

Centrifugation

Centrifugation is a mechanical method of biomass collection that is based on the action of centrifugal forces and allows for the effective separation of microalgae from the culture liquid. During centrifugation, the MV cells move to the edge of the drum under the action of centrifugal forces, forming a dense sediment. This method is widely used in both laboratory and industrial conditions.

The advantage of centrifugation is its high efficiency, which is over 95%⁵², even in a short processing time. This method also does not require the addition of various substances, it is not dependent on temperature or pH and is able to work with cultures of different densities.

Among the disadvantages, it is worth noting that this method has a high energy consumption, and centrifugation is also accompanied by heating of the medium, due to high rotation speed and friction. In addition, during intensive rotation, partial damage to the cells occurs.³ For the anaerobic

fermentation process, this is a big plus, but when storing biomass it creates great risks.

This method is not suitable for some microalgae, such as *Microcystis sp.*, *Anabaena sp.* and *Arthrospira sp.*, since these microalgae have gas vesicles that force them to be in a vertical position, as a result of which they are more difficult to collect⁵⁷.

Due to energy requirements in industry, this method is used as part of combined methods after flotation or flocculation for the final concentration of biomass. This approach allows to reduce the volume of culture liquid that needs to be processed and reduce the overall cost of collecting microalgae.

In conclusion, the use of centrifugation requires a thorough feasibility study due to its high energy consumption and technological features, but it provides one of the highest levels of microalgae harvesting.

Filtration

Filtration consists in separating microalgae cells from the culture liquid by passing the suspension through a porous membrane, with the cells retained on the surface and pores of the filter material, and the liquid passing through freely.

This method is well known and used in many types of industry, but its application to microalgae has a number of features. First of all, it allows achieving biomass harvesting efficiency of 76 to 100%¹, does not require the use of additional chemicals. In addition, due to the different pore diameters of the membranes, filtration is a highly selective method. It requires significantly less electricity than centrifugation.

However, filtration has technical limitations: during operation, the membranes become clogged, which reduces the productivity of the process and constantly requires washing or replacing the membranes. Cell morphology also has a significant impact on the process. The presence of a slime layer slows down filtration in the early stages, and the size and shape of the cells will determine the pore diameter in the membranes and the pressure gradient that needs to be created⁵⁸.

⁵⁷ Pahazri NF, Mohamed R, Al-Gheethi A, Kassim AHM. Production and harvesting of microalgae biomass from wastewater: a critical review. *Environmental Technology Reviews*. 2016;5(1):39-56. <https://doi.org/10.1080/21622515.2016.1207713>

⁵⁸ Castro-Muñoz R, García-Depraect O. Membrane-Based Harvesting Processes for Microalgae and Their Valuable-Related Molecules: A Review. *Membranes*. 2021; 11(8):585. <https://doi.org/10.3390/>

In practice, two main types of filtrations are used: dead-end and cross-flow⁵⁴. In dead-end filtration, the microalgal suspension is passed perpendicular to the membrane. This system is cheap and simple, but clogs more quickly and is more suitable for small volumes of culture fluid with a relatively low biomass concentration. In cross-flow filtration, the microalgal suspension is fed along the surface of the membrane, thereby constantly washing it from clogging and maintaining a stable filtration rate. This option is most often used on an industrial scale, although it is more difficult to implement.

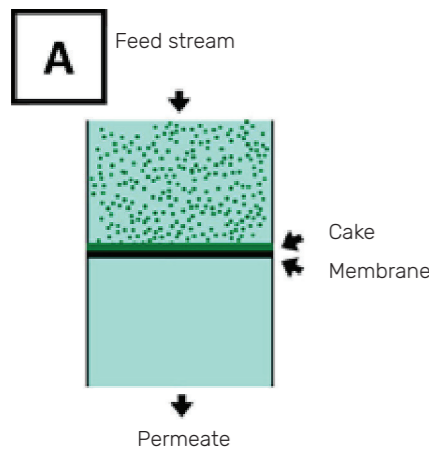
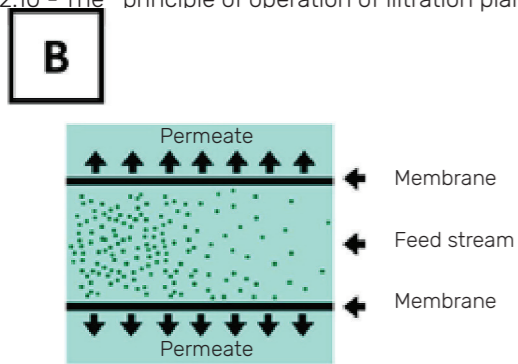


Fig. 2.10 - The principle of operation of filtration plants:⁵⁴



A - Dead-end filtration
B - Cross-flow filtration

Sedimentation

Sedimentation is the cheapest and very simple method of collecting microalgae, which is based on the natural sedimentation of cells under the influence of gravity. It does not require additional equipment or electricity consumption, but the sedimentation efficiency can reach from 66 to 97%¹ and depends on the biomass concentration, cell size and their ability to stick together. Most microalgae settle slowly or remain in a suspended state due to their adaptive features. Even heavy diatom microalgae, such as *Phaeodactylum* or

Navicula, due to their complex cell shape increase resistance and slow down sedimentation. This method is more effective for large or filamentous microalgae, such as *Arthospira* or *Spirogyra*, which are capable of aggregation⁵⁴. Thus, biomass collection by sedimentation alone requires long retention periods.

Sedimentation can be improved by pre-flocculation, which forms flocs that settle much faster. In industrial settings, sedimentation is not often used alone, but is usually a preliminary or auxiliary step before energy-dependent biomass collection methods.



Fig. 2.11 - Natural sedimentation of *Chlorella vulgaris* (Photo by the authors of the AN)

Considering the advantages and disadvantages, the most suitable methods for harvesting microalgae biomass to produce conductive biomethane are **sedimentation, cross-flow filtration** and **spray air flotation**. They provide high efficiency with minimal energy consumption, do not require the introduction of chemicals and do not leave toxic residues, which is critical for the anaerobic digestion process.

Table 2.6. Comparison of different methods for harvesting microalgae biomass^{52,54,55}

Method name	Advantages	Disadvantages	Biomass collection efficiency, %	Solids content after harvesting, %
Chemical flocculation	<ul style="list-style-type: none"> Inexpensive Fast Used on an industrial scale 	<ul style="list-style-type: none"> Requires chemical flocculants Flocculants can be toxic and need to be removed Metal contamination 	82 - 99	3 - 8
Bioflocculation	<ul style="list-style-type: none"> Inexpensive Fast Non-toxic Reusable culture medium 	<ul style="list-style-type: none"> Contamination with other microorganisms 	80 - 99	-
Electroflocculation	<ul style="list-style-type: none"> Suitable for all types Fast Without the use of flocculants 	<ul style="list-style-type: none"> Requires a lot of electricity Expensive electrodes Possibility of metal contamination 	90 - 98	10
Dissolved air flotation	<ul style="list-style-type: none"> Fast Used on an industrial scale 	<ul style="list-style-type: none"> Requires a lot of electricity Not suitable for all types 	Over 90	Up to 6
Spray air flotation	<ul style="list-style-type: none"> Fast Used on an industrial scale Affordable 	<ul style="list-style-type: none"> Not suitable for all species 	80 - 90	Up to 6
Centrifugation	<ul style="list-style-type: none"> Fast 	<ul style="list-style-type: none"> Expensive Cell damage Difficult to scale 	Over 95	22
Dead-end filtration	<ul style="list-style-type: none"> No chemicals required Simple Used on an industrial scale Reuse of culture medium 	<ul style="list-style-type: none"> Expensive membranes Membrane fouling Not suitable for all types 	76 - 100	8 - 15
Cross-flow filtration	<ul style="list-style-type: none"> No chemicals required Simple Used on an industrial scale Reuse of culture medium Self-washing 	<ul style="list-style-type: none"> Costly membranes Less membrane fouling Not suitable for all types 	76 - 100	8 - 15
Sedimentation	<ul style="list-style-type: none"> Cheap Simple Requires very little electricity 	<ul style="list-style-type: none"> Not suitable for all species Requires considerable time 	66 - 97	2 - 3

Microalgae market in the world: potential, production, technologies

SECTION 3

Microalgae market in the EU, support for the development of algae technology

According to the Algae Industry Report, there are 413 algae producers (both macro- and microalgae) across 24 EU countries, of which 153 are macroalgae producers in 13 countries, led by France, Spain, Ireland, and Norway. The majority of them rely on wild harvesting (68%). Macroalgae products are mainly used for human food (34%), followed by cosmetics (18%), nutraceuticals (15%), biostimulants (11%), and animal feed (10%). The primary markets for food and feed products together account for approximately 60% of total production⁵⁹.

⁵⁹ <https://op.europa.eu/en/publication-detail/-/publication/c759da5c-aa81-11ef-b5e9-01aa75ed71a1/language-en>

Food and feed are also the largest markets for microalgae, with cosmetics and wellbeing products representing a more significant sector than for macroalgae. The commercial utilization of algae biomass produced by companies in Europe is shown in Figure 3.1.

Most companies employ fewer than five people and carry out both production and processing of the biomass. The total production value of algae in Europe is estimated at €12.5 million. The overall turnover in the EU Member States for the period 2016-2020 was estimated at €161.4 million. The total labour costs throughout all stages of the value chain amount to approximately €12.9 million. The sector employs around 1,068 people, the majority of whom are male (62%) and under 41 years of age (55%)⁵⁹.

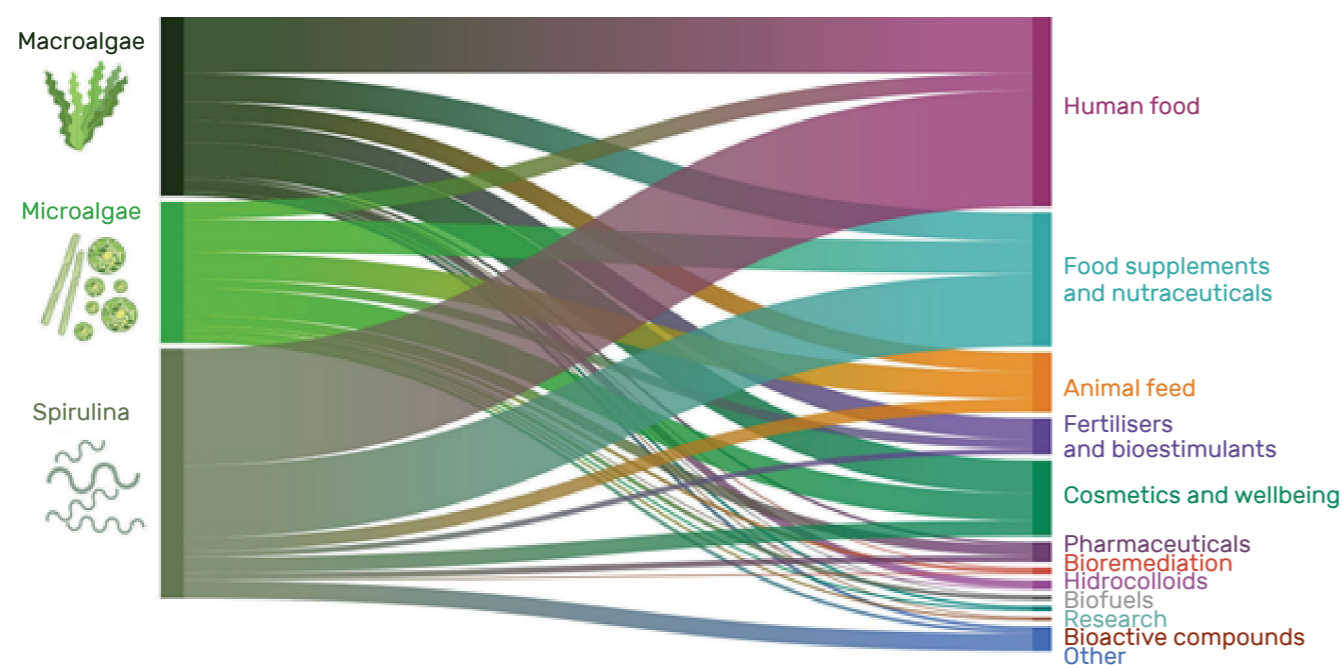


Fig. 3.1 - Algae biomass utilization based on the number of algae producing enterprises in Europe⁶⁰

⁶⁰ <https://op.europa.eu/en/publication-detail/-/publication/6af868a1-4071-11ed-92ed-01aa75ed71a1/language-en>

As of 2022, there are 87 microalgae production enterprises in 17 European countries. The main application areas for microalgae in 2022 include food products and supplements (23%), feed (19%), cosmetics (19%), pharmaceuticals (8%), fertilizers and bio stimulants (7%), together accounting for 76% of total EU production. More recently, microalgae have been identified and utilized as a feedstock to produce renewable fuels such as biodiesel, bioethanol, biogas, biohydrogen, and others⁶¹.

Currently, Germany, Spain, and Italy are the leading EU countries in terms of the number of enterprises cultivating microalgae for various purposes⁶². Official statistics on microalgae production volumes are almost entirely absent at the European level; approximate production amounts to 182 tons of dry microalgae biomass and 142 tons of spirulina. *Chlorella spp.* and *Haematococcus pluvialis* are the most produced microalgae species in terms of volume, representing over 80% of total production¹⁰.

The European microalgae market is dynamically developing due to environmental initiatives, increasing demand for natural food supplements, and support for biotechnologies. The Europe-

⁶¹ Vazquez Calderon, F., Sanchez Lopez, J., An overview of the algae industry in Europe. Producers, production systems, species, biomass uses, other steps in the value chain and socio-economic data, Guillen, J., Avraamides, M. editors, Publications Office of the European Union, Luxembourg, 2022, doi:10.2760/813113, JRC130107.

⁶² <https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2020.626389/full>

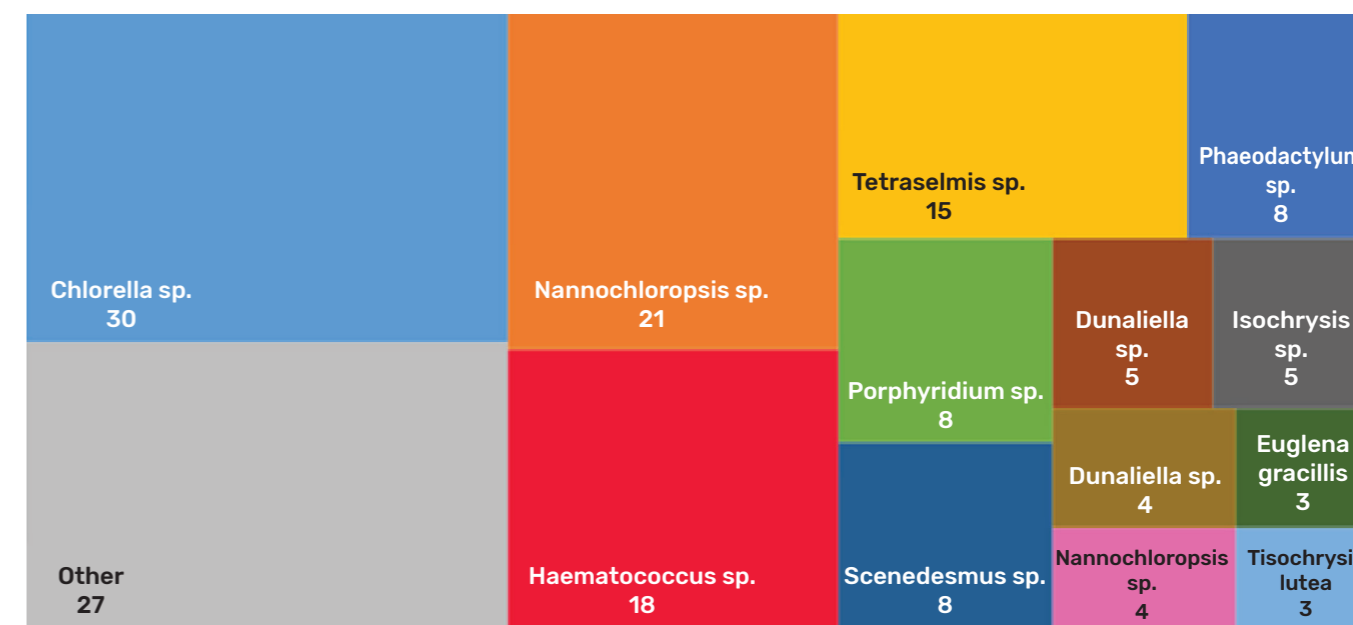


Fig. 3.2 - Distribution of microalgae species by number of companies in EU⁶³

⁶³ [https://www.europarl.europa.eu/RegData/etudes/STUD/2023/733114/IPOL_STU\(2023\)733114_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/STUD/2023/733114/IPOL_STU(2023)733114_EN.pdf)

The European Union actively supports the development of microalgae cultivation technologies through a number of funding programs and environmental initiatives for example:

EIC Programs support innovative research in the fields of biotechnology and microalgae. Table 3 provides an overview of key microalgae research support programs with brief descriptions and project examples.

The **ALGA Europe Project** develops standards for the production and application of microalgae in industry.

The **European Investment Bank (EIB)** provides support for the development of biotech startups.

Novel Food Regulation simplifies the registration procedures for microalgae in the food industry (Cruz & Vasconcelos, 2023).

Interaction platforms such as **EUA4Algae** serve as collaborative spaces for European algae stakeholders, including farmers, producers, retailers, consumers, technology developers, support organizations, investors, governmental authorities, scientists, and NGO researchers. EUA4Algae also functions as a centralized information hub for algae-related funding calls, projects, business intelligence, and best practices.

Table 3.1. Main research support programs for microalgae with short descriptions and project examples.

Program / Initiative	Area of support	Funding format	Project examples and initiatives
Horizon Europe	Research, innovation, cooperation in bioeconomy, energy, climate	Grants for consortia	ABACUS ⁶⁴ algae-based biofuel production; GENIALG - industrial exploitation of macro- and microalgae
EIC (European Innovation Council)	Deep tech, startups, commercialization	Pathfinder (research), Accelerator (deployment)	Startup AlgaeCytes - bioactive compounds from algae, supported by EIC Accelerator
EIT (Food, InnoEnergy, Climate-KIC)	Innovation, training, startups in agro, energy, climate	Grants, acceleration, training	Algae for Food & Feed - projects via EIT Food; bio-innovation training programs
Interreg Europe/ Interreg Danube	Regional innovation, sustainable development, cross-border cooperation	Partner grants for organizations from multiple countries	Project DanuBioValNet - bioeconomy value chains in the Danube region, including algae
LIFE Programme	Environment, climate, energy efficiency	Grants for pilot technologies	Project LIFE ALGAECAN - algae for wastewater treatment and biomass production
COST Action	Researcher networks, knowledge exchange	Funding for conferences, workshops, mobility	COST Action ALGAE - interdisciplinary network for algae data exchange
BIOEAST Initiative	Agro-bioeconomy in Central and Eastern Europe	Policy platform, R&I roadmaps	Includes microalgae as part of policy-level strategies

⁶⁴ <https://cordis.europa.eu/project/id/745668/reporting>.

The European microalgae market holds significant potential thanks to biotechnology support, environmental initiatives, and active investment engagement. Further development depends on simplified regulatory procedures, expanded market opportunities, and improved cultivation technologies.

Microalgae market in the world

The global microalgae market is experiencing steady growth driven by increasing demand for natural and sustainable products across food, pharmaceutical, and bioenergy sectors.

Market Size:

Estimated at **USD 782.59 million in 2024**, with a projected rise to **USD 1.38 billion by 2032**, reflecting a compound annual growth rate (CAGR) of **7.29%** ⁶⁵.

Alternative Estimate:

Another analysis values the **global microalgae based Products Market** size was valued at USD 13.25 Billion in 2023 and the total Microalgae based Products revenue is expected to grow at a CAGR of 8.2% from 2024 to 2030, reaching nearly USD 23.01 Billion by 2030⁶⁶.

North America leads with a **37.2% global market share in 2024**, driven by strong demand for natural food colorants, nutraceuticals, and significant investments in innovative technologies⁶⁶.

While there are variations in market size estimates due to differences in methodology and scope, sources (11, 15 and others) confirm robust and sustained growth. This highlights the crucial role of microalgae as a strategic resource for sustainable development and energy independence.

Key drivers of growth include demand for natural bioactive compounds for pharmaceuticals, cosmetics, and food industries, environmental trends, and investments in biotechnology. Astaxanthin (*Haematococcus pluvialis*) production for dietary supplements and cosmetics holds a key market position. The development potential of bioplastics from microalgae also stimulates market growth. Increasing investments in algae biotechnology are expected to accelerate the adoption of new solutions (Apraku et al., 2025) ⁶⁵.

⁶⁵ L. M. Casanova, A. Macrae, J. E. de Souza, A. Neves Junior, and A. B. Vermelho, "The Potential of Allelochemicals from Microalgae for Biopesticides," *Plants*, vol. 12, p. 1896, 2023. doi: 10.3390/plants12091896.

Challenges hindering the use of monomers from microalgae include the economic feasibility of producing high-quality microalgae biomass with desirable metabolites, efficient dewatering and cultivation techniques, and effective extraction and processing methods. The global market share is spread across various types of microalgae such as Spirulina, Chlorella, Nannochloropsis, Haematococcus, Isochrysis, Chlamydomonas, and others. These microalgae have diverse applications across industries, particularly in the food and feed sectors. In recent years, the global market has witnessed significant growth due to increasing R&D efforts and industrial applications, especially in the food and feed industries. Their high content of nutrients such as proteins and vitamins has attracted growing interest from food and feed manufacturers.

The rapid growth of the global population is fuelling demand for food products, while shifting consumer trends are further boosting demand for protein in both food and feed. The increasing livestock and poultry populations are also contributing to the global market growth.

North America shows a strong focus on plant-based foods, technological innovations, and sustainable development, making it one of the most prominent regions globally. Countries such as the USA, Canada, and Mexico demonstrate significant demand for functional foods and have seen notable development in poultry and livestock industries. For example, in April 2024, the U.S. Department of Energy (DOE) announced a new USD 18.8 million funding opportunity for microalgae-related R&D.

The Asia-Pacific region, including countries like India, China, and Japan, offers substantial growth potential due to its strong livestock industry and increasing demand for innovative feed products. Moreover, the rising demand for microalgae-based supplements further supports market expansion. According to the Government of India, the country accounts for about 20% of the global cattle population.

South America shows significant growth potential during the forecast period, driven by favorable environmental conditions, government support, and rapid growth of the biotechnology sector.

The Middle East and Africa offer various opportunities for market expansion, supported by government initiatives and growing awareness of microalgae-based products such as oils, powders, and more. The region holds untapped potential for research, production, and commercialization of microalgae.



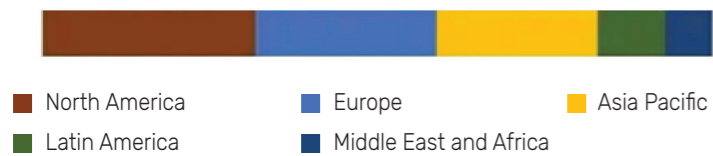
Spirulina is highly fragmented, competitive, and diverse and increasing adopting various strategies to remain competitive.



By Application, Food and Beverage segment dominated the market with a highest market share in 2023 and is expected to grow at a significant CAGR during the forecast period

Microalgae based Products Market

Microalgae based Products Market, by Region in 2023(%)



Market Size
2023: USD 13.25
2030: USD 23.01

Market Size in Billion

Average Selling Price of Carbon Dioxide Removal (CDR) Worldwide In 2023:

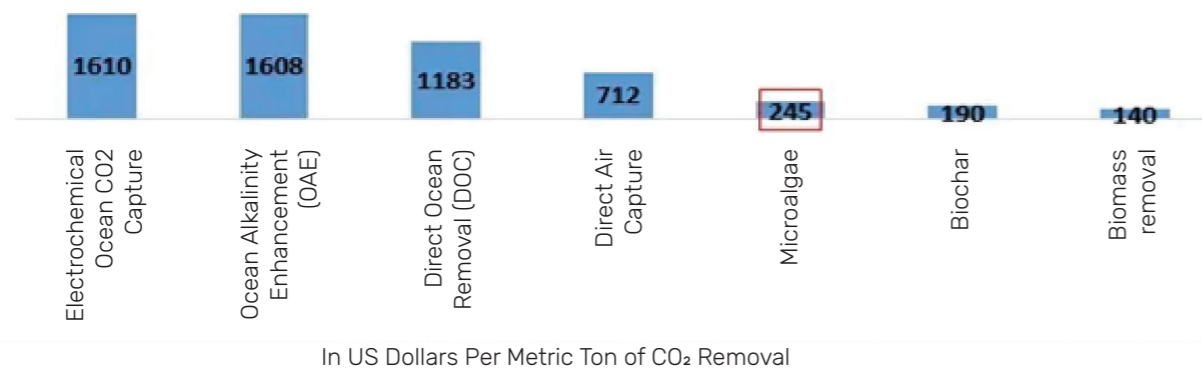


Fig. 3.3 - Microalgae market share in 2023 and forecast⁶⁶

⁶⁶ <https://www.maximizemarketresearch.com/market-report/global-microalgae-based-products-market/63970/>

The global microalgae market can be analysed from two perspectives: by species (type) and by end-use application. The following subsections outline both dimensions.

Global Microalgae Market by Type

The global microalgae market is segmented by species into *Spirulina*, *Chlorella*, *Nannochloropsis*, *Haematococcus*, *Isochrysis*, *Chlamydomonas*, and others (Fig. 3.4).

The *Spirulina* segment dominates the global market due to its high availability and widespread production. *Spirulina* is a concentrated source of fatty acids, amino acids, and vitamins, making it ideal for a variety of fish species and shrimp, which hold the largest share of aquaculture production. *Nannochloropsis*, a single-celled spherical or oval-shaped algae (2-5 microns in diameter), is rich in photosynthetic pigments, protein, and polyunsaturated fatty acids (including omega-3s). Its fast growth under typical cultivation conditions makes it pop-

ular for aquaculture feed and dietary supplements. The segment is expected to register substantial growth during the forecast period.

Global Microalgae Market by Application

The global market is segmented into food products and feed applications. The food products segment holds a significant market share and is projected to exhibit the highest CAGR during the forecast period. This segment has seen significant development with the increasing number of global publications and studies. The products have become widespread in the food sector due to their high nutritional value and the wide application of *Spirulina* and *Chlorella*. *Chlorella* is one of the most consumed microalgae species in dietary supplements, functional food ingredients, and natural food colorants. Additionally, food industry applications include products such as baked goods, noodles, plant-based foods, and others, contributing to the growth of the food segment globally (Figure 3.1).

Key Growth Drivers of the Global Microalgae Market

Rising demand for natural and sustainable ingredients. Increasing interest in natural, vegan, and organic products across food, cosmetics, and pharmaceuticals. Algae are a rapidly renewable resource with a minimal carbon footprint.

Expansion in nutraceuticals. Growing demand for superfoods (*Spirulina*, *Chlorella*), algae-derived omega-3s, and antioxidants (astaxanthin) for cardiovascular health, immunity, and vision. Growth in preventive healthcare and functional food sectors.

Use in aquaculture and agriculture. Microalgae are used as eco-friendly feed for fish, shrimp, and pets; as well as biostimulants and fertilizers, fueling interest in bio-agrotechnology.

Active use in cosmetics. Active ingredients for anti-aging, SPF protection, and detoxification. Algae-based cosmetics align with the green beauty trend. For example, demand for astaxanthin (*Haematococcus pluvialis*) for eye and skin health has surged in the USA and China (Xue et al., 2023).

Growing demand for alternative protein sources. Algae have a high protein density (up to 60%) and offer a sustainable alternative to animal proteins.

Climate resilience and circular economy. Microalgae's ability to capture CO₂ and purify wastewater

- their use is expanding in China and India for industrial wastewater treatment (Heide et al., 2024).

Government and ESG investor support. The USA and Japan are actively developing algae-based biopolymers for eco-friendly packaging (Marasca et al., 2024). North America shows strong demand for functional foods and bioplastics (Santos et al., 2024).

Technological progress and investments. New cultivation methods (photobioreactors, biorefineries), automation, and bioengineering. Increasing venture capital interest and support from international programs (EU4Algae, Horizon Europe, etc.).

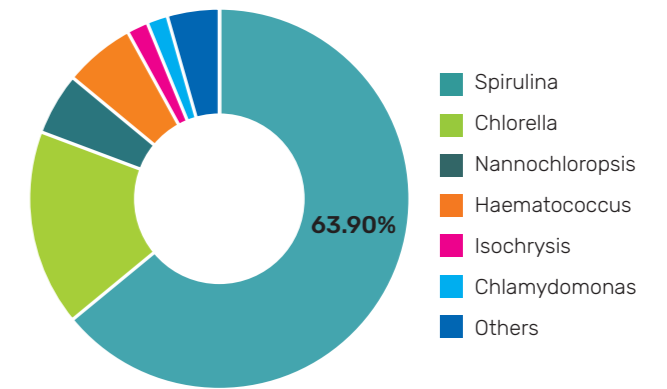


Fig 3.4. - Global microalgae market by microalgae type in 2024⁶⁷

⁶⁷ <https://www.fortunebusinessinsights.com/microalgae-market-110314>

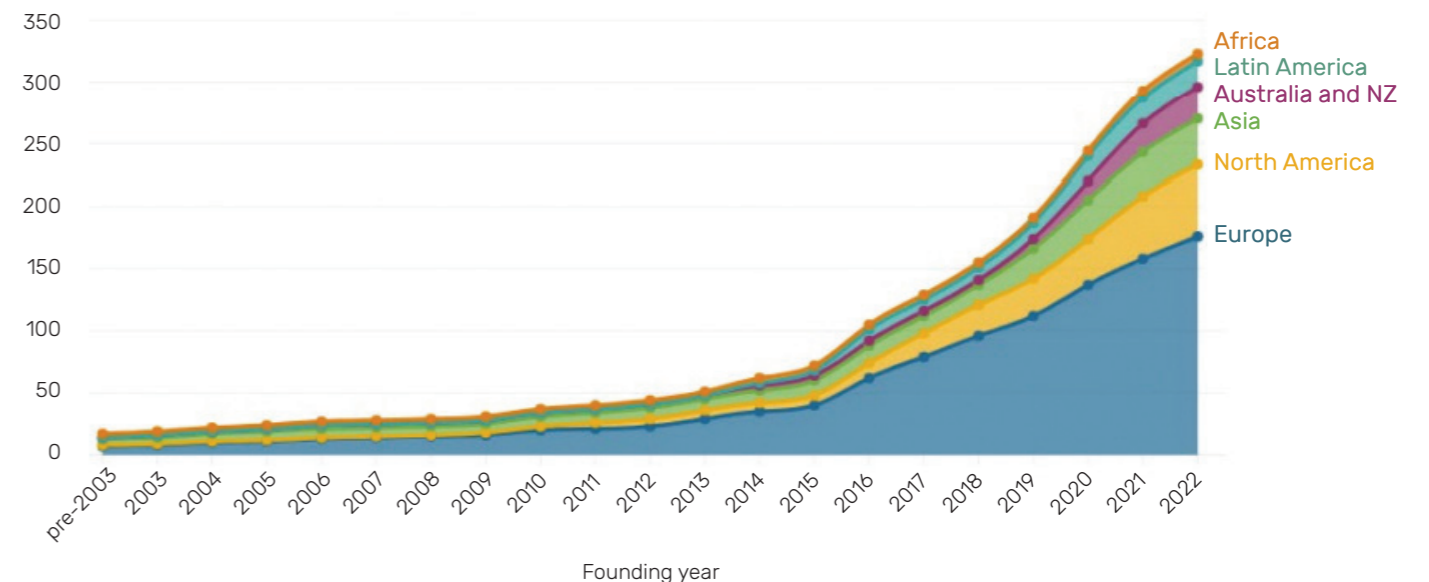


Fig 3.5 - Global development of algae start-ups by founding year, 2003-2022⁶³

Key Challenges:

High cultivation costs - energy-intensive photo-bioreactors remain a key market constraint (Thevarajah et al., 2024).

Regulation and standardization - harmonization of standards for food and pharmaceutical applications is necessary (Al-Hammadi et al., 2024).

Since 2016, there has been a rapid growth in algae farming startups, as shown in Figure 1.5, indicating high investment interest in this topic worldwide.

The global microalgae market outside the EU demonstrates high growth potential driven by biotechnology innovations, biofuel development, and the food industry. The main leaders remain Asia, North America, and Latin America. Growing demand for natural food colours, rising popularity of nutraceuticals and dietary supplements, and technological investments are expected to be the major factors driving regional growth. It should be noted that these drivers are not related to biomethane production; rather, biomethane may emerge as a parallel, additional pathway in the future but is not currently influencing market expansion.

Biomethane/biogas yield from various microalgae types

The cultivation of microalgae enables the synthesis of a wide range of valuable chemical compounds: proteins, lipids, carbohydrates, vitamins, antioxidants, enzymes, pigments, etc. The dominant compound in the chemical composition of algal biomass directly depends on the specific microalgae species and cultivation conditions. Different microalgae species demonstrate varying abilities to survive and reproduce in the presence of certain substances such as ammonia, heavy metals, etc. As a result, selecting the appropriate microalgae strain is one of the key factors when developing microalgae cultivation technologies for liquid fraction of digestate (LFD). For biogas production from microalgae biomass, it is crucial to achieve maximum biomass productivity and the ability of anaerobic bacteria to degrade biomass grown on the digestate of biogas plants. The biomass productivity and its biodegradability will determine the biomethane yield and thus the economic viability of the technology.

The optimal microalgae strain for maximum biogas production should have:⁶⁸

- a thin or absent cell wall;*
- large cell size;*
- rapid growth in non-sterile environments;*
- high specific resistance to natural contaminants;*
- a carbohydrate-based cell wall.*

Although methane yield depends on the composition of microalgae, the resistance of the cell wall is considered a limiting factor for the anaerobic digestion of microalgae. The kinetics of anaerobic digestion largely depend on the degradability of a particular microalgae strain. Low methane yields observed in some studies have been associated with poor cell degradation and a high amount of residual material that is difficult to break down during anaerobic digestion. According to these findings, easily degradable microalgae strains either lack a cell wall or have protein-based cell walls without cellulose/hemicellulose content⁶⁸.

The theoretical biomethane potential is estimated at 470-800 m³ CH₄/t VS (volatile solids). Experimental studies have shown that biomethane productivity can reach 337, 450, or 587 m³ CH₄/t VS⁶⁹. The chemical composition of various types of microalgae and the specific biogas and methane yields from them are shown in Table 3.2 and Table 3.3, respectively.

In some sources^{70,71,72}, methane yield from microalgae has been linked to the chemical composition of biomass; however, over time this assumption has proven inaccurate. Experimental data collected from the literature do not show a strong correlation between the content of lipids,

68 [Álvarez Torres, Fernando G. Feroso, Bárbara Rincón, Jan Bartacek, Rafael Borja, David Jeison. Challenges for Cost-Effective Microalgae Anaerobic Digestion, Biodegradation, Engineering and Technology. \(2013\). URL: https://www.intechopen.com/chapters/45072](https://www.intechopen.com/chapters/45072)

69 [Xiaoqiang Wang, Eva Nordlander1, Eva Thorin and Jinyue Yan. Microalgal biomethane production integrated with an existing biogas plant: a case study in Sweden. International Conference on Applied Energy, ICAE.2012.China ID: ICAE2012- A10560](https://www.intechopen.com/chapters/45072)

70 Sialve B., Bernet N. and Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances* 2009. Vol.27(4), 409-16, ISSN: 0734-9750

71 Marcia Morales, León Sánchez, Sergio Revah. The impact of environmental factors on carbon dioxide fixation by microalgae. *FEMS Microbiology Letters*, Volume 365, Issue 3, 2018 <https://academic.oup.com/femsle/article/365/3/fnx262/4705896?searchresult=1>

72 [Amos Richmond. Handbook of Microalgae Culture: Biotechnology and Applied Phycology. Blackwell Science Ltd.URL: https://algatex.org/ebook/Handbook%20of%20microalgal.pdf](https://www.blackwell-synergy.com/doi/abs/10.1080/09593330600571441)

carbohydrates, and proteins found in different algae species and the methane yield obtained by different authors. Therefore, the simple biomass composition of algae cannot be considered the primary or sole factor in selecting the best algal strain for biomethane production²¹. The decisive factors influencing methane yield are primarily the cell wall composition and biomass productivity.

Most chlorophytes (green microalgae) possess complex and rigid cell walls containing components embedded in a matrix of uronic acids along with other neutral sugars. Approximately 40% of microalgae components are readily available for biomethane production, while the remaining 60% require pretreatment to make intracellular contents accessible⁷³.

73 [Raul Muñoz & Cristina Gonzalez-Fernandez. Microalgae-Based Biofuels and Bioproducts \(Enhanced Edition\) From Feedstock Cultivation to End-Products. United Kingdom:Elsevier Science, 2017.P. 540](https://www.sciencedirect.com/science/article/pii/S0926661616300011)

Table 3.2. Chemical composition of different types of microalgae, %⁷⁴

Strain	Proteins	Carbohydrates	Fats	Nucleic acids
<i>Scenedesmus obliquus</i>	50 - 56	10 - 17	12 - 14	3 - 6
<i>Scenedesmus quadricauda</i>	47	-	1.9	-
<i>Scenedesmus dimorphus</i>	8 - 18	21 - 52	16 - 40	-
<i>Chlamydomonas reinhardtii</i>	48	17	21	-
<i>Chlorella vulgaris</i>	51 - 58	12 - 17	14 - 22	4 - 5
<i>Chlorella pyrenoidosa</i>	57	26	2	-
<i>Spirogyra sp.</i>	6 - 20	33 - 64	11 - 21	-
<i>Dunaliella bioculata</i>	49	4	8	-
<i>Dunaliella salina</i>	57	32	6	-
<i>Euglena gracilis</i>	39 - 61	14 - 18	14 - 20	-
<i>Prymnesium parvum</i>	28 - 45	25 - 33	22 - 38	1 - 2
<i>Tetraselmis maculata</i>	52	15	3	-
<i>Porphyridium cruentum</i>	28 - 39	40 - 57	9 - 14	-
<i>Spirulina platensis</i>	46 - 63	8 - 14	4 - 9	2 - 5
<i>Spirulina maxima</i>	60 - 71	13 - 16	6 - 7	3 - 4.5
<i>Synechococcus sp.</i>	63	15	11	5
<i>Anabaena cylindrica</i>	43 - 56	25 - 30	4 - 7	-

74 [Jerry D Murphy, Bernhard Drosg, Foin Allen, Jacqueline Jerney, Ao Xia, Christiane Herrmann. A perspective on algal biogas. IFA Bioenergy.2015. https://task37.ieabioenergy.com/wp-content/uploads/sites/32/2022/02/AD_of_Algae_ebook_end.pdf](https://www.sciencedirect.com/science/article/pii/S0926661616300011)

Table 3.3. Specific yield of different types of microalgae obtained as a result of batch test⁷⁴

Species	Temp. [°C]	Biogas prod. [L/kg VS]	CH ₄ prod. [L/kg VS]	CH ₄ content [%]
<i>Arthrospira platensis</i>	-	481 ± 14	293	61
<i>Chlamydomonas reinhardtii</i>	-	587 ± 9	387	66
<i>Chlorella kessleri</i>	-	335 ± 8	218	65
<i>Chlorella vulgaris</i>	28 - 31		310 - 350	68 - 75
<i>Dunaliella salina</i>		505 ± 25	323	64
<i>Dunaliella</i>	35	420		
<i>Euglena gracilis</i>		485 ± 3	325	67
<i>Nanochloropsis sp.</i>	38	388	312	80.5
<i>Scenedesmus obliquuus</i>		287 ± 10	178	62
<i>Arthrospira sp.</i>	38	556	424	76.3
<i>Arthrospira sp.</i>	35	-	310-320	-
<i>Arthrospira maxima</i>	35	190 - 340		
Mixed algae sludge (<i>Chlorella Scenedesmus</i>)	35 - 50		170 - 320	62 - 64

Also, in ⁷³ four types of cell walls are identified based on their structure and complexity. Increased resistance of microalgae cell walls to degradation is associated with the presence of sporopollenin-like biopolymers. Another non-degradable compound found in the microalgae cell wall is algaenan - a non-hydrolysable, highly resistant biopolymer consisting of long-chain n-alkyl units connected by ester bonds. The presence of algaenan in species such as *Chlorella*, *Scenedesmus*, *Haematococcus*, and *Nanochloropsis* complicates biogas

production, as pre-treatment is required to break down their cell walls, increasing operational costs²⁶.

As a result of a literature review for the analysis and selection of optimal species, six microalgae species, which are most commonly used in wastewater treatment and biofuel production, were selected: *Euglena gracilis*, *Chlorella vulgaris*, *Dunaliella sp.*, *Scenedesmus*, *Chlamydomonas reinhardtii*, and *Spirulina platensis*, as shown in Figure 3.6.

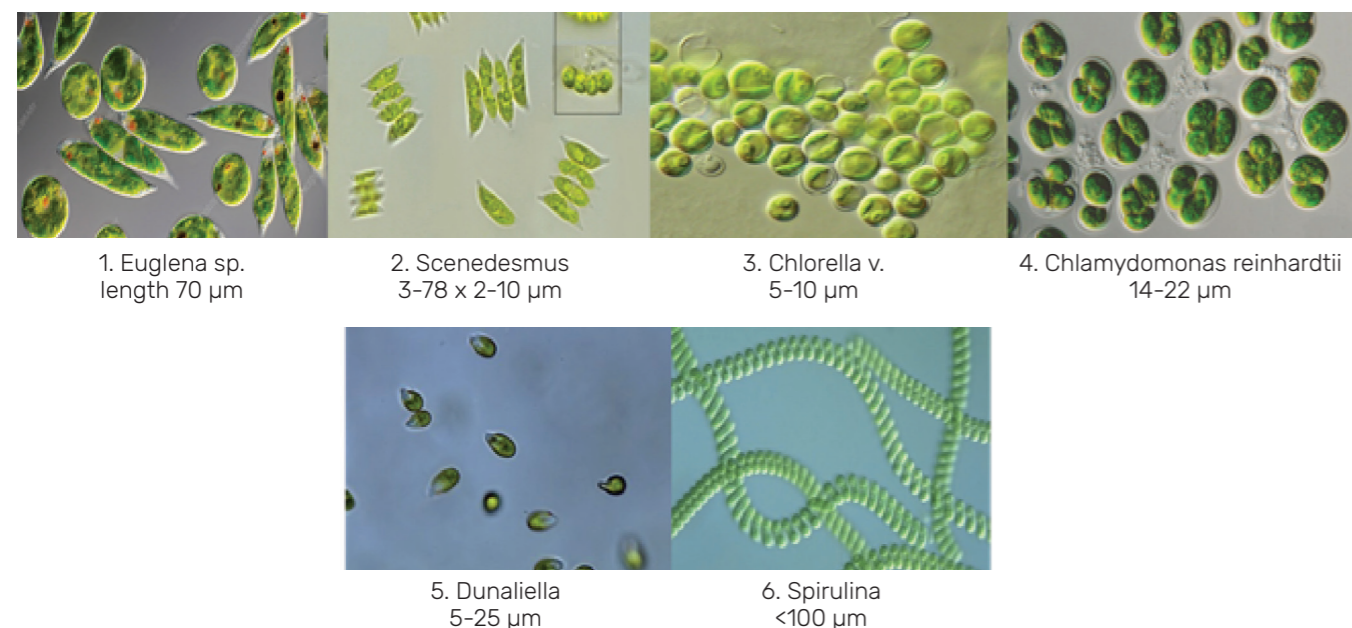


Fig. 3.6 - Microscopic appearance of selected microalgae species

Chlorella vulgaris was the first microalgae to undergo intensive industrial cultivation. However, it has proven suboptimal for biogas production due to its very strong and chemically resistant cell wall containing a sporopollenin layer. *Scenedesmus* showed better suitability, but both species require pre-treatment before anaerobic digestion due to their cell wall structure ⁷⁵.

It is worth noting that despite its complex cell wall, *Chlorella* demonstrates the highest growth rate and is the only species that can tolerate very high CO₂ concentrations in gases (40-100%) ²⁴, which may be useful for supplying concentrated CO₂ as a carbon source after biogas upgrading to biomethane. *Dunaliella* has a simple cell wall, making it optimal in terms of biodegradability for biogas production; however, it requires saline conditions for optimal growth, necessitating the addition of salts. *Chlamydomonas reinhardtii* is widely chosen for biofuel experiments due to its advantages such as rapid growth, strong adaptability, and ease of cultivation.

Euglena gracilis is characterized by high tolerance to elevated nitrogen and phosphorus concentrations. According to experimental studies, it has demonstrated the ability to effectively remove nitrogen and phosphorus compounds from domestic wastewater ⁷⁶.

Thus, from the perspective of biodegradability, *Euglena gracilis* and *Chlamydomonas reinhardtii* appear to be the most optimal species, as they possess simpler cell wall structures, lack non-degradable polymers, can tolerate high ammonium nitrogen concentrations (important when using digestate), and exhibit high specific methane yields.

Advanced biofuels from microalgae

Advanced biofuels derived from microalgae can include both liquid fuels (such as biodiesel, bioethanol, bio-jet fuel) and gaseous fuels (such as biomethane or biohydrogen). Under RED II, biomethane

⁷⁵ Kostikov I.Yu., Tsarenko P.M. Algology. manuscript of a textbook for students of the 3rd-4th year of the "Botany" specialty. - Kyiv, 2009-2013. - 377 p. <https://docplayer.net/71246758-Kostikov-i-yu-tsarenko-p-m-algologiya-rukopis-pidruchnika-dlya-studentiv-3-4-kursu-specialnosti-botanika.html>

⁷⁶ I. Nezbrzytska, S. Shamanskyi, L. Pavliukh, S. Boichenko, Z. Gorbunova, O. Horbachova, V. Repeta. Removal of Biogenic Compounds from Sewage Water in a Culture of *Euglena Gracilis* (EUGLENOPHYTA). Modern Technologies in Energy and Transport. Studies in Systems, Decision and Control, vol 510. Springer, Cham. https://doi.org/10.1007/978-3-031-44351-0_9

produced from microalgae qualifies as an advanced biofuel when used in the transport sector, while liquid microalgae-based fuels are being developed mainly at pilot and demonstration scales.

The production of microalgae-based biofuels is one of the key areas in global energy transition strategies aimed at reducing greenhouse gas emissions, mitigating competition with food production, protecting the environment, and combating climate change, partly due to their ability to utilize CO₂ from industrial or biogenic sources as a growth substrate. Many countries are conducting large-scale research in the field of microalgal bioenergy, accompanied by substantial financial and human resource investments. However, the commercialization of this sector still faces significant economic challenges, particularly due to high production costs.

Microalgae perform photosynthesis, demonstrating high growth rates and productivity. Typically, microalgae biomass contains approximately 45-50% carbon (C), 7.6% nitrogen (N), and 1.4% phosphorus (P). The production of biofuels from microalgae offers several advantages:

- No competition with human or animal food chains.
- High content of carbohydrates, proteins, and oils.

- Provides a long-term method of generating O₂ and using it in photosynthetic respiration to reduce CO₂ emissions.

- Microalgae contain higher lipid content (dry weight basis) compared to oilseed crops such as soybeans. Additionally, the growth cycle of microalgae is about 15 days, while soybeans typically have one or two harvests per year.

- When cultivated on land in ponds or photobioreactors, microalgae qualify as feedstock under RED II Directive Annex 9, eligible for double counting - production of third-generation biofuels.

- Microalgae exhibit higher photosynthetic efficiency (maximum ~10%) compared to terrestrial plants (~5% maximum).

- Microalgae can achieve higher biomass productivity (50-70 t/ha/year) compared to terrestrial plants (10-20 t/ha/year).

- Microalgae can grow on non-arable land and in marine environments.

- Microalgae can be cultivated in closed systems using wastewater or saline water, significantly reducing freshwater consumption.

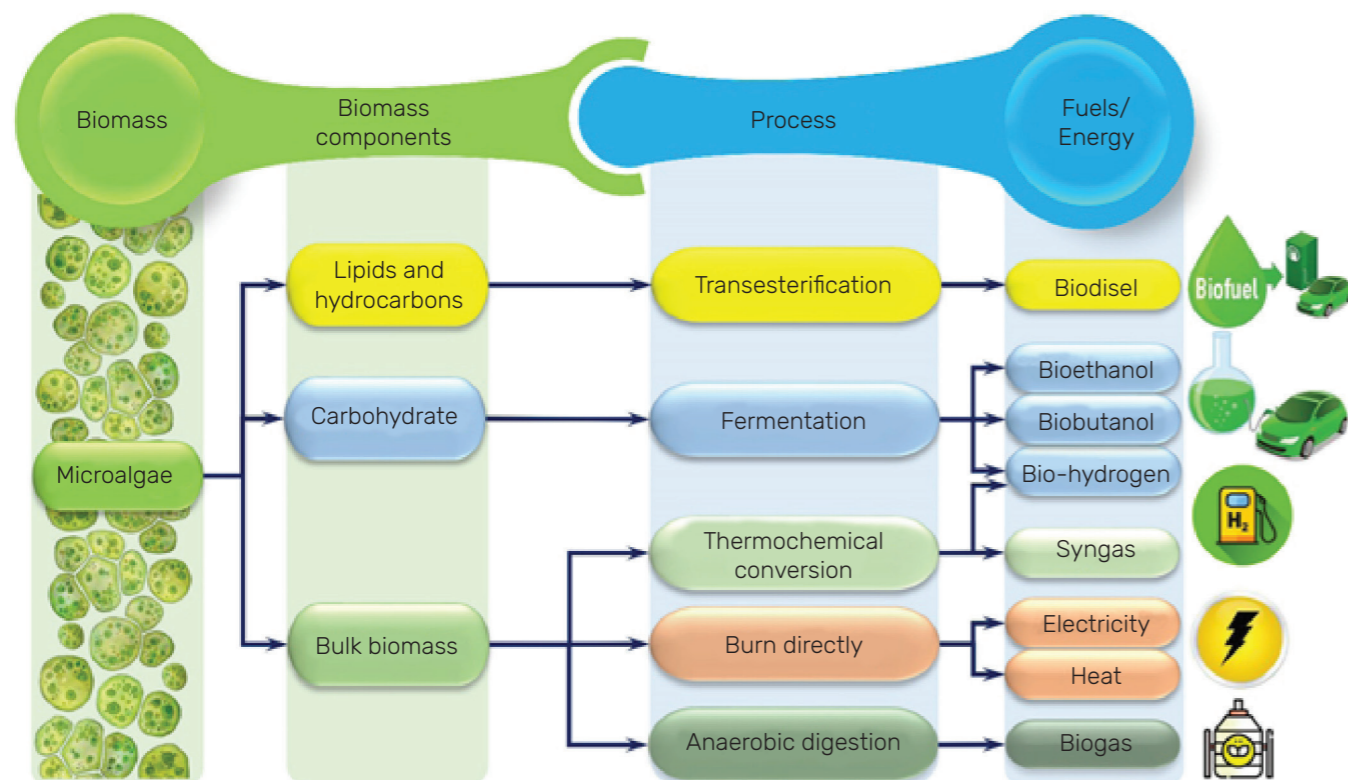


Fig. 3.7 - Overview of products and biofuels that can be obtained from microalgae biomass ⁷⁷

⁷⁷ Tiwari B., Upadhyay N. та ін. Microalgae: A potential source for sustainable development // BioEnergy Research. – 2022. – Т. 15, № 4. – С. 2035–2056. – DOI: 10.1007/s11101-022-09819-y

Figure 3.7 presents an overview of the biofuels and products that can be derived from microalgal biomass depending on processing methods and target biomass components.

Recently, the most widely studied and developed technologies include the production of biodiesel, bioethanol, and biomethane from microalgal biomass due to its high lipid and carbohydrate concentrations.

Lipids are one of the key components of microalgae, accounting for 2–60% of cell dry weight depending on species and cultivation conditions (Wijffels, 2006). They serve as membrane components, storage substances, and energy reserves. Lipids can be used directly as straight vegetable oil (SVO) or converted into biodiesel via transesterification of triglycerides and free fatty acids. Due to their higher degree of unsaturation, algal oils are less suitable for direct use in certain engine types. Energy consumption for drying accounts for approximately 85% of total energy use in the biodies-

el production process from microalgae ⁷⁸. Efficient biodiesel production requires strains with high oil content and rapid growth. Closed photobioreactors allow for stable conditions and prevent contamination. Lipids usually accumulate under stress conditions, such as nutrient deprivation. This creates a trade-off between biomass productivity and lipid content, although Rodolfi et al. (2009) demonstrated the possibility of achieving high lipid levels while maintaining culture growth.

Besides lipids, carbohydrates are also promising. Bioethanol can be obtained from algal carbohydrates after hydrolysis of starch or cell wall polysaccharides (Hamelinck et al., 2005). A key advantage of algae is the absence of lignin and their relatively homogeneous composition. Genetically **modified strains of green algae can produce ethanol directly from CO₂** and light (Deng and

⁷⁸ Lardon L., Hélias A., Sialve B., Steyer J. P. and Bernard O. Life-cycle assessment of biodiesel production from microalgae. Environmental Science and Technology. 2009. 43(17). 6475–81. 2009. ISSN: 1520-5851

Coleman, 1999), although this technology remains at an early development stage.

Botryococcus braunii produces up to 60% (and over 80% in some cases) of **hydrocarbons** in dry biomass, which can be converted into liquid fuels (Hillen et al., 1982; Wijffels, 2006). These compounds accumulate on the cell surface, facilitating extraction. However, this species grows slowly (doubling time up to 72 hours), so cost-effective cultivation is only feasible in low-cost open systems, particularly using brackish water (Qin, 2005).

Hydrogen production from algae is based on photobiological processes in the absence of oxygen (Melis and Happe, 2001). Current technologies demonstrate low productivity – up to 20 g H₂/m²/day – and require optimization of the biological pathway through genetic engineering (Kapdan and Kargi, 2006).

Anaerobic digestion is one of the most promising routes for processing marine macroalgae and microalgae, particularly after valuable compounds have been extracted (Reith et al., 2005). While hydrogen and ethanol remain at the research stage, biodiesel production is the most advanced area. Both open and closed cultivation systems are suitable for biodiesel production, and lipid transesterification to biodiesel is already a well-developed technology (Chisti, 2008a; Rodolfi et al., 2009). Biomethane production from microalgae does not require prior extraction of specific components, as the entire organic fraction of algal biomass (proteins, carbohydrates, and lipids) is converted into methane and carbon dioxide via anaerobic digestion ⁷⁹.

Biofuel production from microalgae involves several stages: cultivation, biomass harvesting, lipid extraction, and transesterification to biodiesel. Current research is focused on optimizing these processes, including:

use of hybrid photo- and heterotrophic cultivation methods to increase biomass productivity (Raja et al., 2024);

integration of harvesting and processing systems with wastewater treatment facilities;

development of enzymatic transesterification methods as environmentally friendly alternatives to chemical processing.

⁷⁹ <https://www.intechopen.com/chapters/45072>

Process integration also shows promise for improving efficiency – for example, simultaneous production of biogas or biohydrogen from residual biomass after lipid extraction.

Economic and Environmental Challenges

Despite the significant potential of microalgae, their commercial production still faces several barriers:

high costs of cultivation and harvesting equipment (photobioreactors, separators);

large volumes of water must be circulated and processed during cultivation, which increases energy demand and operational costs, even if non-freshwater sources can be used;

difficulties in scaling processes while maintaining efficiency (Raja et al., 2024).

Nevertheless, studies show that technology integration (e.g., utilization of residual streams from other industries or energy synergy with solar plants) can significantly reduce costs and improve overall production efficiency (Raja et al., 2024).

Microalgae represent a strong foundation for the development of third-generation biofuels. Their rapid growth, high lipid accumulation, and ability to grow on non-arable land without competing with food crops provide significant advantages. Although full economic feasibility has not yet been achieved, technological innovation and environmental benefits point to great prospects for this field.

Digestate as a Feedstock for Microalgae Cultivation

Considerations Regarding Digestate Characteristics

Using digestate for microalgae biomass cultivation is a critical factor in reducing the cost of advanced biomethane. Digestate, formed as a by-product of anaerobic digestion (AD), is a complex multi-component liquid that can serve as an effective source of macro- and micronutrients for microalgae cultivation. Its composition varies significantly depending on:

The type of substrate (e.g., cattle manure, poultry litter, maize silage, organic residues from the food industry);

The fermentation stage (raw or stabilized digestate);

The season - seasonal changes affect nutrient concentrations due to variations in feedstock composition.

In particular, digestate with a high content of ammonium nitrogen (NH₄-) and organic carbon is considered a promising medium for cultivating nitrogen-fixing and stress-tolerant microalgae such as *Chlorella* or *Scenedesmus*.

Biogas plants typically operate continuously throughout the year, necessitating digestate storage between spring and autumn field applications. During prolonged storage in open tanks or lagoons, methane emissions into the atmosphere increase proportionally (up to 5-10% of the methane potential in the feedstock). On most operational biogas plants, including those in Ukraine, raw digestate treatment is limited to separation into solid and liquid fractions. Further treatment requires additional costs and must therefore be justified.

Separation results in two products:

A solid fraction with 20-40% dry matter, enriched with carbon and phosphorus.

A liquid fraction with 1-8% dry matter, rich in nitrogen and potassium.

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The low dry matter content in the liquid fraction makes it economically unviable to transport over long distances. In the absence of fields for digestate application, efficient disposal solutions or large storage capacities are required. In general, the liquid digestate fraction (LDF) contains necessary nutrients for growing microalgae, but some components may inhibit growth and must be considered during digestate preparation.

Chemical Composition and Characteristics of Digestate

Digestate composition is mainly determined by the mixture of components fed into the bioreactor, including the biogas feedstock and various additives (enzymes, micronutrients, chemicals, water, etc.).

From 1 ton of maize silage, 780 kg of digestate is generated; from 1 ton of poultry litter - 890 kg; beet pulp - 910 kg; cattle manure - 920 kg; pig slurry - 990 kg. A 1 MW biogas plant can generate 40-50 thousand tons of digestate annually.

Digestate from Ukrainian biogas plants is considered a valuable secondary resource from an agrochemical perspective. However, its composition varies based on feedstock type, fermentation conditions, and post-treatment. For instance, a chemical analysis of digestate from MHP agricultural holding's biogas plants is shown in Table 4.1.

Digestate contains macro- and a range of micronutrients (magnesium, sulfur, zinc, manganese, copper, cobalt). For example, 1 ton of poultry litter digestate contains 15 kg of nitrogen, 6.6 kg of phosphorus, and 5.4 kg of potassium - the highest concentration of macronutrients among all substrate types. The nitrogen in digestate is more readily available for plants than in other fertilizers. In the liquid fraction, humic acids reach 0.21% and fulvic acids 0.07%; in the solid - 1.87% and 0.94%, respectively. The carbon-to-nitrogen ratio ranges from 20:1 to 30:1 - optimal for soil. Digestate also contains active bacteria that promote organic matter decomposition.

The solid fraction shows elevated concentrations of microelements such as Cu, Mn, and Co, which is typical of phase separation. The pH in both fractions remains within 7.7-9.3, indicating an alkaline environment - advantageous or limiting depending on the algae strain sensitivity.

Such properties confirm digestate's potential as a mineral source but also require assessment of heavy metal accumulation risks in soil from continuous application, especially without monitoring and regulation.

While digestate has strong potential as a nutrient medium for microalgae cultivation, it poses several limitations that can affect algal productivity and viability:

1. Turbidity and high suspended solids content

High turbidity reduces light penetration and impairs photosynthesis, which hinders biomass growth or halts it completely.

2. Ammonium and free ammonia toxicity

Ammonium nitrogen concentrations can reach 1000-3000 mg/L, whereas most algal strains tolerate up to ~100 mg/L. Free ammonia (NH₃) penetrates cell membranes and causes lysis. For instance, *Scenedesmus sp.* growth decreased by 77% when NH₃ concentration increased from 9 to 34 mg/L (Godos et al., 2009).

3. Phosphorus deficiency

An optimal N:P ratio ≈ 7:1 is required. Liquid digestate often fails to meet this, requiring nutrient adjustment.

4. Presence of heavy metals

Although elements like Mn, Zn, Cu, and Fe are micronutrients, their excess inhibits growth and interferes with enzymatic activity. Metal content control is essential.

5. Pathogens and foreign microflora

Using untreated digestate may introduce bacterial competition or pathogens.

Digestate from biogas plants has significant potential in closed-loop systems and waste valorization for bioeconomy applications. However, its chemical and physical characteristics require pre-treatment before direct use in microalgae cultivation.

Best Practices for Digestate Pre-Treatment

Liquid digestate from biogas plants is usually a thick, dark liquid with high turbidity and suspended solids, making it unsuitable for direct use in algae cultivation. It often contains microbial contaminants, high ammonia levels, volatile sulphur compounds, and inhibitors that suppress photosynthesis and growth.

To make digestate suitable for algae, various pre-treatment strategies are employed. The choice depends on its composition, available equipment, sterility requirements, and economic considerations.

Table 4.1. Chemical composition of MHP digestate fractions⁸⁰

Indicator	Liquid fraction	Solid fraction
pH	7.7-9.1	7.7-9.3
Total nitrogen	6.6 kg/m ³	6.8 kg/t
Phosphorus	1.9 kg/m ³	3.1 kg/t
Potassium	6.2 kg/m ³	2.7 kg/t
Sulfur	0.27%	1.56%
Manganese (Mn)	21 mg/kg	47.65 mg/kg
Zinc (Zn)	8.2 mg/kg	12.5 mg/kg
Copper (Cu)	14.1 mg/kg	34.5 mg/kg
Cobalt (Co)	7.2 mg/kg	18.1 mg/kg

⁸⁰ Добрива з біогазових установок, особливості застосування дігестатів – Пропозиція.

Based on the ALG-AD project and other studies, the most effective methods include⁵⁷:

Mechanical separation (filtration or centrifugation) reduces turbidity and removes large particles.

Dilution with water is a basic step to reduce toxic substances, especially ammonia. A 10% digestate + 90% water mix yields good N:P balance and sufficient transparency.

Thermal treatment (pasteurization at 70-90 °C) reduces microbial contamination and improves biosecurity.

pH adjustment to 6.5-7.5 is critical for species like *Chlorella vulgaris* and *Scenedesmus obliquus*.

Sedimentation is a basic but cost-effective method that has shown mixed results. For example, for raw digestate from a Langage AD plant (UK), sedimentation was ineffective even after four days due to the high dispersion of the fractions. However, when used with a different type of digestate and with an appropriate holding time, the method can be effective and enhance the efficiency of subsequent steps such as filtration or dilution. A study by Godos et al. (2009) showed that five days of settling reduced the number of suspended solids by 70%.

Flocculation using biological or chemical flocculants significantly reduces turbidity and unwanted compounds. When combined with filtration, it provides high-quality media.

Membrane technologies (ultrafiltration, microfiltration) are the most effective for removing pathogens and solids. Although costly, the ALG-AD project deemed them the most promising for scalable digestate treatment. Microfiltration (~0.1 µm pore size) improves clarity and removes bacteria.

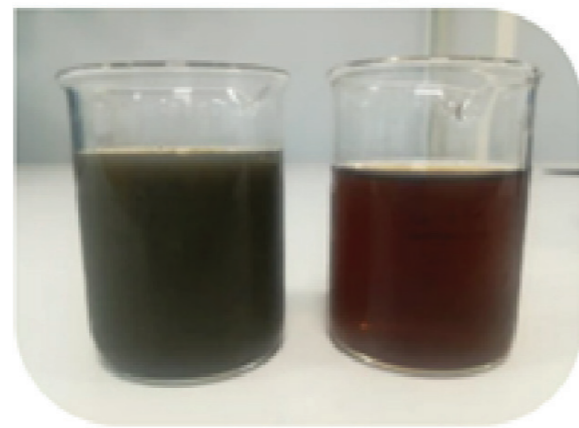


Fig. 4.1 - Digestate before (left) and after (right) microfiltration using a ceramic filter with 0.1 µm pores

The use of these practices - individually or in combination - ensures a controlled, less toxic, and nutritionally balanced substrate. This is crucial for stable microalgae cultivation and production of valuable bioproducts like pigments, proteins, fatty acids, or biofuels. From a practical standpoint, the most economically viable methods are dilution and microfiltration, as they deliver optimal media quality at low cost. These are the recommended techniques for pilot and industrial-scale systems. Reported operational costs for simple dilution are typically < 1 €/m³, whereas pressure-driven microfiltration ranges between 1-3 €/m³ depending on membrane type and energy input (Oliveira et al., 2020; Raja et al., 2024). In contrast, more advanced treatments such as ultrafiltration, nanofiltration, or reverse osmosis can exceed 5-10 €/m³, making them less attractive for pilot and industrial-scale algae cultivation systems

Biogas/biomethane production from microalgae

Selection of microalgae species and cultivation system

The efficiency of biomethane production from microalgae depends largely on the species used as feedstock. While some grow rapidly, they have poor anaerobic digestion characteristics. Others, on the

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contrary, have high bioavailability but low productivity. Species selection is always a compromise, so it is necessary to approach this issue carefully to find the most optimal solution.

The theoretical methane yield from proteins, carbohydrates and lipids is 0.50, 0.42 and 1.01 L CH₄/g

VS, respectively. Even if these values are used to estimate the potential methane yield from different microalgae species, they do not give unambiguous results. Although methane yield theoretically depends on the composition of the microalgae, the stability of the cell wall is considered to be a limiting factor for anaerobic digestion of microalgae. The kinetics of anaerobic digestion largely depends on the decomposing ability of a particular microalgae species. Therefore, when choosing a microalgae species, it is necessary to focus on other indicators.

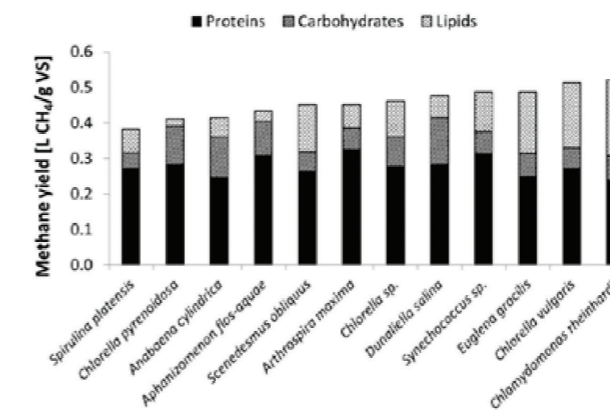


Fig. 5.1 - Potential methane yield from proteins, carbohydrates and lipids present in different microalgae species⁸¹

The ideal microalgae species for maximum biomethane production is one that has:⁸¹

1. thin or no cell wall;
2. large cells;
3. high growth rate in a non-sterile environment;
4. high resistance to natural pollutants
5. a carbohydrate-based cell wall.

Of the above factors, the quality of the cell wall is of crucial importance for anaerobic fermentation of algae. This is due to the fact that cell walls are difficult to biodegrade, and their presence prevents contact of anaerobic bacteria with the contents of microalgal cells.

⁸¹ Torres ÁL, Rincón F, Bartacek J, Borja R, Jeison D. Challenges for cost-effective microalgae anaerobic digestion. In: Chamy R, ed. Biodegradation - Engineering and Technology. IntechOpen; 2013. <https://doi.org/10.5772/55975>

The cell wall of microalgae constitutes 12-36% of the total cell mass in various microalgae. It consists mainly of carbohydrates and proteins, which constitute 30-75% and 1-37% of the cell wall, respectively⁸¹.

Most green microalgae are characterized by the presence of a complex and stable cell wall formed by a polymer matrix, which includes uronic acids and neutral sugars. This structure significantly limits the bioavailability of organic components during anaerobic fermentation. It is estimated⁸², that only about 40% of the organic mass is potentially available to the methane consortium without further processing, while the remaining 60% remains protected by the cell wall and requires additional methods of destruction. Microalgae that are more easily biodegraded usually do not have a cell wall or contain protein structures without cellulose and hemicellulose. The low bioavailability of some species is associated with the presence of algalan in the structure, which is practically not hydrolyzed and significantly complicates the destruction of the cell wall.

In addition to considering the characteristics that determine the suitability of microalgae for anaerobic digestion, it is also necessary to take into account the parameters that affect the cultivation of microalgae, namely: specific growth rate and increment, ability to different cultivation modes, resistance to ammonia. Table 5.1 shows a comparison of some microalgae species that are popular and theoretically suitable for biomethane production.

From the comparison, it can be concluded that the best for growing biomass and producing biomethane are **Euglena gracilis**, **Chlamydomonas reinhardtii**. They do not have a complex cell wall, have high biomass growth, as well as resistance to high concentrations of ammonia and aggressive media and a high specific CH₄ yield. Other species are also suitable for producing biomethane, but due to the complex structure of the cell wall and the presence of an algalan layer, *Chlorella vulgaris* and *Scenedesmus obtusus* will require pre-treatment before anaerobic fermentation. Although *Chlorella vulgaris* demonstrates the highest specific growth rate, it is also able to withstand high concentrations of CO₂, which opens up the prospect of using CO₂ after biogas upgrading to biomethane without

⁸² Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Biohydrogen production by dark fermentation using microalgal biomass as substrate. In: Pandey A, Chang JS, Hallenbeck PC, Larroche C, eds. Biohydrogen. Elsevier; 2013:105-125. <https://doi.org/10.1016/B978-0-08-101023-5.00006-6>

Table 5.1. Comparative analysis of microalgae species for biomethane production

Parameter	Type of microalgae							Ref.
	Unit	<i>Dunaliella sp.</i>	<i>Euglena gracilis</i>	<i>Chlamydomonas reinhardtii</i>	<i>Spirulina platensis</i>	<i>Scenedesmus ob.</i>	<i>Chlorella vulgaris</i>	
Complex cell wall	-	No	No	No	Yes	Yes	Yes	[1,8]
Presence of algenan layer	-	No	No	No	No	Yes	Yes	[1,8]
Presence of peptidoglycan layer	-	No	No	No	Yes	No	No	[1,8]
Presence of fibrillar layer	-	No	No	No	Yes	Yes	Yes	[1,8]
Ability to cultivation regimes,	photo	+	+	+	+	+	+	[6,7]
	mixo	+	+	+	+	+	+	[6,7]
	hetero	+	+	+	+	+	+	[6,7]
Source of org. carbon in heterotrophic cultivation	-	Acetate, lactate, glucose, glutamate	Acetate, glucose, alanine, aspartate, asparagine, glutamine, ethanol	Acetate	Glucose	Glucose	Acetate, glucose, lactate, glutamate	[7]
Concentration of proteins in biomass of MV	%	57	39-61	48	46-63	50-56	51-58	[2]
Concentration of fats in biomass of MV	%	6	14-20	21	4-9	12-14	14-22	[2]
Concentration of carbohydrates of MV	%	32	14-19	17	8-14	10-17	12-18	[2]
Pure CH ₄ yield	l CH ₄ /kg VS	420	325	387	225-293	90-178	90- 310	[2,1,5]
Resistance to high concentrations of ammonia and aggressive environment	-	+	+	+	+	+	+	[4,5]
Specific growth rate	day ⁻¹	0.25 -0.7	0.3 - 1.1	0.5 - 1.5	0.65 - 0.7	0.34 - 0.4	0.38 -4.8	[3]
Biomass growth	(g n ⁻¹ day ⁻¹)	0.12 - 0.5	0.29	0.5	0.15	0.52	0.09	[1,2,8,9]
Limiting concentration of ammonia	Mg/l	54	1 000	150	200	15	77 -324	[4,5]

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dilution with air. *Spirulina platensis* has average performance among the listed species. *Marine Dunaliella sp.* has the best bioavailability and, accordingly, a higher specific CH₄ yield, but requires higher concentrations of NaCl for its cultivation, which can lead to problems with the operation of the biogas plant.

Selection of Microalgae Cultivation System

The choice of cultivation system directly affects the biomass productivity (g/L/day), which in turn determines the potential methane yield per hectare or per unit volume of reactor. More productive systems, such as flat-plate or tubular photobio-

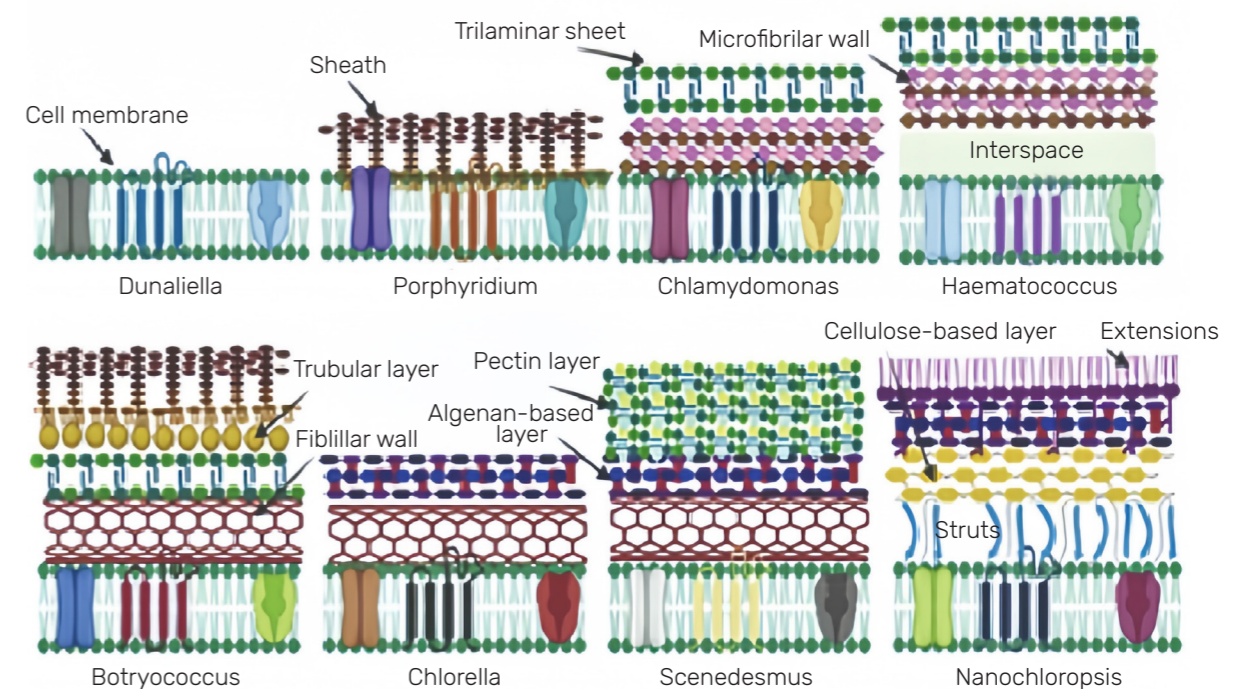


Fig. 5.2 - Comparison of the cell wall structure of some microalgae genera⁸³

83 Di Caprio F, Altamari P, Pagnanelli F. Anaerobic digestion of *Scenedesmus obliquus* biomass grown in batch photobioreactor. Heliyon. 2021;7(7):e07609. <https://doi.org/10.1016/j.heliyon.2021.e07609>

Table 5.2. Advantages and disadvantages of microalgae cultivation systems⁸⁴

Cultivation system	Advantages	Disadvantages
Open systems	<ul style="list-style-type: none"> • Lower capital costs • Easy to design and maintain • Low productivity potential 	<ul style="list-style-type: none"> • Seasonal fluctuations in productivity • High risk of contamination • Limited control over environmental factors
Photobioreactors	<ul style="list-style-type: none"> • Precise control of cultivation conditions • Low risk of contamination • High productivity potential 	<ul style="list-style-type: none"> • Higher capital and operating costs • High energy demand for artificial lighting

84 Penloglou G, Pavlou A, Kiparissides C. Recent Advancements in Photo-Bioreactors for Microalgae Cultivation: A Brief Overview. Processes. 2024; 12(6):1104. <https://doi.org/10.3390/pr12061104>

reactors, generate larger amounts of microalgal biomass with lower contamination risk, thereby ensuring a higher and more stable biomethane output after anaerobic digestion.

At the same time, the CAPEX and OPEX of cultivation systems (Tables 5.2-5.4) feed into the overall cost of biomethane production. Open ponds may appear cheaper, but their low and fluctuating productivity reduces methane yields and raises the cost per unit of biomethane. In contrast, closed photobioreactors, though more expensive to build, provide higher productivity, stable biomass supply, and better economics per unit of biomethane.

Thus, cultivation system design should be considered not only from the perspective of biomass production but also as a decisive factor in the technical and economic performance of microalgae-to-biomethane pathways.

Cultivation systems can be broadly divided into two main groups: open systems and closed systems. Open systems typically consist of large open ponds in which the microalgae are exposed to natural sunlight. Although open ponds are cost-effective, they are prone to contamination and are highly susceptible to uncontrolled environmental conditions. In contrast, closed cultivation systems involve the use of closed photobioreactors that provide a controlled environment for the cultivation of microalgae. They provide protection from contamination and allow for precise control of parameters, but can be more expensive to install and operate.

The impact of energy consumption on the microalgae biomass production process, especially biomass processing, should also be considered, as this process is the most energy-intensive. New advanced technologies are needed to reduce energy consumption, as well as to increase the productivity of microalgae biomass. Minimizing maintenance costs and increasing microalgae production are key factors to be achieved in the long term. Table 5.3 shows the cost structure of microalgae biomass production in open systems and closed photobioreactors.

Table 5.3. Cost structure for microalgae biomass production⁸⁵

System Capital Cost Structure	Open systems	PBR
Installation	15.4 %	82.7 %
Inoculation System	12.3 %	3.8 %
Earthworks	11.3 %	3.5 %
OSBL (Outside Battery Limits)	10.8 %	3.3 %
CO ₂ Supply	6.2 %	1.9 %
Hydrotreatment	12.3 %	1.4 %
Collection	21 %	6.5 %
Value Extraction	8.2 %	2.5%
Recycling	11.8 %	3.6%

85 Al-Dailami A, Iwamoto K, Ahmad I, Goto M. Potential of Photobioreactors (PBRs) in Cultivation of Microalgae. J Adv Res Appl Sci Eng Technol. 2022;27(1):32-44. <https://doi.org/10.37934/araset.271.3244>

Taking into account the above, it can be concluded that photobioreactors, despite their higher cost, are the best choice for obtaining microalgae biomass for the production of lead biomethane. Their key advantages are low risk of contamination, higher productivity and absence of seasonal fluctuations in productivity. In the context of biomethane production, photobioreactors allow to achieve the highest efficiency.

Choosing a photobioreactor type

Photobioreactors are complex systems that provide the environment and necessary condi-

tions for the cultivation of microalgae. Cultivation conditions are regulated, controlled and monitored in order to maximize the yield of microalgae biomass. FBIs can be installed both indoors under artificial light and outdoors for exposure to sunlight. Recently, dozens of new types of FBIs have been developed and manufactured. Their advantages and disadvantages directly affect the biomass yield, cost and level of scalability. Table 5.4 provides a comparison of different types of photobioreactors.

Table 5.4. Comparison of advantages and disadvantages of different types of PBR²

PBR Type	Advantages	Disadvantages	Productivity, g/L/day	Cost estimation
Fermenter	<ul style="list-style-type: none"> • Excellent mixing and aeration • Easy to scale 	<ul style="list-style-type: none"> • Mechanical wear • High energy consumption • Low surface area to volume ratio • Shear loading on cells 	1	Significantly high CAPEX High OPEX
Conical	<ul style="list-style-type: none"> • Even light distribution on an inclined surface 	<ul style="list-style-type: none"> • Difficult to install • Difficult to maintain and clean • Presence of shaded areas from supporting structures 	0.68 ⁴	Moderate or high CAPEX Moderate or high OPEX
Horizontal Tubular	<ul style="list-style-type: none"> • Efficient lighting • Easy to scale • Excellent hydrodynamics • Takes up less surface area • High surface to volume ratio 	<ul style="list-style-type: none"> • Degassing compartment required • Difficult to clean • Accumulation of dissolved oxygen • Possibility of biofouling 	1.5 ⁸⁶	Moderate CAPEX Moderate or high OPEX
Vertical Tubular	<ul style="list-style-type: none"> • Easy to scale • Excellent hydrodynamics • Efficient lighting • High surface to volume ratio 	<ul style="list-style-type: none"> • Degassing compartment required • High energy consumption • Occupies a large surface area 	0.3	Moderate CAPEX Moderate or high OPEX
Airlift	<ul style="list-style-type: none"> • Excellent mixing • Prevents sedimentation and sticking • Low risk of contamination • Low energy consumption • Relatively small size and easy maintenance 	<ul style="list-style-type: none"> • Needs periodic maintenance of mechanical parts • Scaling issues • May be unevenly illuminated 	0.8 ⁴	Moderate CAPEX Moderate or high OPEX

86 Mirazul Islam M, Alam H, Acharjee A, Mozumder-S I. Establishment of an effective photobioreactor for growing microalgae: A review. Int J Agric Res Innov Technol. 2025;14(2):153-162. <https://doi.org/10.3329/ijarit.v14i2.79511>

Column	<ul style="list-style-type: none"> • Effective aeration and mixing • Easily scalable • Low risk of contamination • Low energy consumption • Relatively small size and easy maintenance 	<ul style="list-style-type: none"> • May be unevenly lit • Requires careful control of gas supply 	1.2 ⁴	Moderate or high CAPEX Moderate OPEX
Plastic Bag	<ul style="list-style-type: none"> • Simple • Easy to scale • Easy to replace 	<ul style="list-style-type: none"> • Can be unevenly lit • Poor mixing • Not as durable • Difficult to clean 	0.23	Lowest CAPEX Low OPEX
Flat Panel	<ul style="list-style-type: none"> • Relatively easy to maintain • Easy to scale • Short light path • High surface to volume ratio 	<ul style="list-style-type: none"> • Difficult to maintain uniform temperature • Constant cleaning of panels required • Possibility of biofouling • Possibility of hydrodynamic stress 	1.38 ⁴	Moderate or high CAPEX Moderate OPEX
Membrane	<ul style="list-style-type: none"> • Highly effective due to better MV retention 	<ul style="list-style-type: none"> • Requires constant cleaning • Requires constant monitoring to prevent contamination and fouling 	0.44	Significantly high CAPEX High OPEX
Ring	<ul style="list-style-type: none"> • Optimized for better lighting and usability 	<ul style="list-style-type: none"> • Requires cleaning • Difficult to maintain 	0.22	Moderate or high CAPEX Moderate OPEX
Pyramid	<ul style="list-style-type: none"> • Design improves light capture 	<ul style="list-style-type: none"> • Difficult to maintain and clean 	0.172	Moderate CAPEX Moderate OPEX
V-Shaped	<ul style="list-style-type: none"> • Captures light better 	<ul style="list-style-type: none"> • Difficult to maintain and clean • Problems with orientation relative to natural light 	50.5 (g/m ² /day) ⁸⁷	Moderate CAPEX Moderate OPEX
Inclined	<ul style="list-style-type: none"> • Even lighting • Easily scalable 	<ul style="list-style-type: none"> • Problems with orientation relative to natural light • Requires constant cleaning • Requires careful installation 	0.276	Moderate CAPEX Moderate or high OPEX
Biofilm	<ul style="list-style-type: none"> • Easy to harvest biomass • Reduces contamination risks • Efficiently utilize dense crops 	<ul style="list-style-type: none"> • Difficult to maintain • Limited mixing • Contamination of the support matrix 	2.64 (g/m ² /day)	Low or moderate CAPEX Moderate or high OPEX

⁸⁷ Chin-On RC, Barbosa MJ, Wijffels RH, Janssen M. A novel V-shaped photobioreactor design for microalgae cultivation at low latitudes: Modelling biomass productivities of *Chlorella sorokiniana* on Bonaire. *Chem Eng J.* 2022;449:137793. <https://doi.org/10.1016/j.cej.2022.137793>

Based on the information provided, it can be concluded that the best solution would be to use **flat-plate** and **tubular photobioreactors**. Both types provide high productivity (1.38 and 1.5 g/L/day) and have moderate CAPEX and OPEX values, which makes them the most optimal solution among all the given types of FBI. The tubular has the best hydrodynamic performance, while the flat-panel reactor captures light better and has the largest surface to volume ratio. Also, a good solution is to use a **column-type reactor**, which provides high growth (1.2 g/L/day), with its simplicity and energy efficiency. In addition, it has moderate CAPEX and OPEX and is suitable for productive mixotrophic cultivation.

In turn, the least suitable types include plastic bags and conventional fermenters. Plastic bags, although the cheapest to use with the lowest CAPEX and OPEX, have poor mixing and uneven lighting, resulting in low productivity (0.23 g/l/day). A conventional fermenter demonstrates good productivity (1 g/l/day), but high energy consumption and corresponding CAPEX and OPEX.

Process parameters and modes

Energy production from microalgae in combination with biogas and biomethane production has several advantages. Microalgal biomass can be directly digested to produce biogas without energy-intensive drying, and the resulting biogas can be upgraded to biomethane for use as a transport fuel or injected into the grid. In addition, microalgae cultures can utilize the CO₂ separated during biogas upgrading, thereby both improving the quality of the upgraded gas and generating additional biomass. This creates the potential for a circular system in which microalgae growth contributes to higher overall biomethane yields⁸⁸.

The prospects for microalgae use as a raw material for biomethane production are increasing due to the its significant amount of biodegradable compounds, specifically: carbohydrates 4-57%, lipids 2-40% and proteins 8-71% of the total solids⁸⁹.

⁸⁸ Ahmad, Imran & Abdullah, Norhayati & Iwamoto, Koji & Yuzir, Ali & Mohamad, Shaza. (2020). Anaerobic digestion of micro algae: Outcomes, opportunities and obstructions.

⁸⁹ Zabed, H.M., Qi, X., Yun, J., Zhang, H. (2019). Anaerobic Digestion of Microalgae Biomass for Methane Production. In: Alam, M., Wang, Z. (eds) *Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment*. Springer, Singapore. https://doi.org/10.1007/978-981-13-2264-8_16

However, due to the significant variability in the composition of different microalgae species, the biomethane potential also varies significantly between species.

The biochemical composition of algal biomass can be altered by adapting the growth media. This change in biochemical composition happens due to stressful conditions that occur when microalgae are cultured in nutrient-limited environments. The highest calorific value of 29 kJ/g of MW biomass was found when they were cultured in a low-nitrogen environment⁹⁰. A high lipid content in biomass can be advantageous, as the theoretical biogas yield from lipids is generally higher than from proteins or carbohydrates, but excessive lipid and protein content can lead to the accumulation of ammonia and long-chain fatty acids, which are inhibitors of anaerobic microorganisms.

Measured specific biogas yields from microalgae range from 287 to 611 l/kg VS, and specific methane yields range from 100 to 450 l/kg VS⁸⁸. However, despite these advantages and potential for AD, the conversion of microalgae to biomethane is hampered by cell wall stability, low C/N ratio, and the influence of other factors, including process parameters and conditions.

The C/N ratio is one of the most important parameters in the AD process, and any significant deviation from the optimal C/N ratio reduces the efficiency of methanogenesis as well as methane yield due to the release of large amounts of total ammonia nitrogen or the accumulation of volatile fatty acids. In general, a high C/N ratio is desirable for efficient methanogenesis. However, depending on the feedstock, the effective C/N ratio varies from 20 to 30, with an optimal ratio of 25⁹¹. Microalgae biomass has a low C/N ratio due to its high protein content. In addition, protein decomposition leads to the release of large amounts of ammonium. It is presented in two forms: NH₄⁺ and NH₃, the latter is able to penetrate cell membranes and inhibit methanogenesis. The distribution of ammonium between the two forms is very dependent on temperature and pH.

⁹⁰ Murphy, Jerry & Drosig, Bernhard & Allen, Eoin & Jerney, Jacqueline & Xia, Ao & Herrmann, Christiane. (2015). A perspective on algal biogas. *IEA Bioenergy*.

⁹¹ de la Lama D, Cubero J, Rodríguez MJ, Jiménez A, Borja R. Anaerobic co-digestion of microalgae and industrial wastes: a critical and bibliometric review. In: *Progress in Microalgae Research—A Path for Shaping Sustainable Futures*. IntechOpen; 2022. <https://doi.org/10.5772/intechopen.1043378>

While using marine species of MB, it is necessary to control the salinity. A NaCl content above 10 g/l leads to a 50% drop in methanogenesis activity, and bacteria cannot adapt to this concentration even in 12 weeks. At low concentrations of 100 – 350 mg/l Na is needed, but its high content can cause inhibition or toxicity, and dehydration of bacteria is also possible due to osmotic pressure⁹². To solve this problem, the harvested biomass must be diluted or washed with fresh water.

The time of harvest and the storage conditions of the microalgae also have an impact on the AD process and methane yield. The content of biochemical components constantly fluctuates throughout the cultivation period. Harvesting the MW biomass at the appropriate time will ensure the best concentration of the main biochemical components. This time can vary greatly between microalgae species and strains, so it has to be established experimentally⁹³.

Long-term storage of microalgal biomass with high moisture content is undesirable because the processes of autolysis and lipolysis are triggered very quickly, leading to a decrease in biomass concentration and lipid content⁹⁴. It is preferable to store biomass in a dry form. Dried biomass retains its protein and lipid content unchanged for 12 months, even in a non-freezing storage facility, but a slight increase in moisture content and a decrease in vitamin content are possible⁹⁵. In the context of using microalgae as a raw material for biogas plants, it is recommended not to dry the biomass microalgae material, but simply to reduce the time interval between harvesting and loading the biomass.

Biogas formation can occur in a wide range of temperatures in psychrophilic (<20 °C), mesophilic (20–40 °C) and thermophilic (>40 °C) regimes. A

92 Torres ÁL, Feroso FG, Rincón B, Bartacek J, Borja R, Jeison D. Challenges for cost-effective microalgae anaerobic digestion. In: Chamy R, ed. Biodegradation – Engineering and Technology. Intech; 2013:139–159. <https://doi.org/10.5772/55975>

93 Feroso, F. G., Hidalgo, C., Trujillo-Reyes, A., Cubero-Cardoso, J., & Serrano, A. (2022). Effect of harvesting time in the methane production on the anaerobic digestion of microalgae. *Environmental Technology*, 45(5), 827–834. <https://doi.org/10.1080/09593330.2022.2128893>

94 Ryckebosch E, Muylaert K, Eeckhout M, Ruysen T, Foubert I. Influence of Drying and Storage on Lipid and Carotenoid Stability of the Microalga *Phaeodactylum tricornutum*. *J Agric Food Chem*. 2011;59(19):11063–11069 <https://pubs.acs.org/doi/10.1021/jf2025456>

95 Waghmare AG, Chugh N, Sagaram US, Arun S, Menon D, Venkata Subhash G, Nagle V, Dattaroy T, Dasgupta S. Characterization of storage stability of microalgal biomass for its applications as protein feed ingredients in animal and aquafeeds. *Animal Feed Science and Technology*. 2022;288:115323. <https://doi.org/10.1016/j.anifeeds.2022.115323>

decrease in temperature can cause an increase in the concentration of volatile acids, which can lower the pH value and directly affect the metabolic rate of methanogens. While at elevated temperatures, a higher metabolic rate, higher ammonium toxicity, and greater sensitivity of microorganisms to temperature fluctuations are observed. Thermophilic regime minimizes the risk of unwanted and pathogenic bacterial contamination, but requires significantly higher energy costs. There are conflicting results in the literature regarding the effectiveness of mesophilic and thermophilic regimes^{96,97}. Although the thermophilic regime potentially has a higher biogas yield, due to its high sensitivity to parameter changes, it is recommended to use the mesophilic regime, as more suitable for practical conditions.

The pH level in the bioreactor significantly affects the efficiency of biodegradation and biomethane yield through enzymatic activity and the balance of ionized and unionized forms of H₂S and NH₃. The process of methanogenesis occurs at a pH level of 6.5–8.5, but its optimum is in the range of 7.5–8.1⁹⁸. This parameter is closely related to the FOS/TAC indicator, which shows the ratio of volatile fatty acids to alkaline buffer capacity. For a stable process, the FOS/TAC indicator must be kept within 0.2 – 0.5 by increasing or decreasing the feedstock supply.

The redox potential is one of the key technological parameters for the anaerobic digestion process, as it directly affects the activity of methanogenic bacteria. The optimal range for stable methanogenesis is –350...–250 mV. At values above –250 mV, there is a risk of propionic acid accumulation, which subsequently leads to inhibition of the activity of methanogens. The pH and ORP indicators must be constantly monitored, since even minor changes will signal a violation of the stability of the process in the BGU.

Organic load and hydraulic retention time are also important parameters. The organic load determines the amount of organic matter fed into the reactor per unit volume per unit time. Exceeding this indicator beyond the optimum leads to the accumu-

96 Mora-Sánchez JF, Serna-García R, Bouzas A, Seco A, Ruano MV. Anaerobic Membrane Bioreactor for Microalgae and Primary Sludge Co-Digestion at Pilot Scale: Instrumentation, Control and Automation Implementation, and Performance Assessment. *Water*. 2023;15(18):3225. <https://doi.org/10.3390/w15183225>

97 Kinnunen HV, Koskinen PEP, Rintala J. Mesophilic and thermophilic anaerobic laboratory-scale digestion of *Nannochloropsis* microalga residues. *Bioresour Technol*. 2014 Mar;155:314–322. <https://doi.org/10.1016/j.biortech.2013.12.115>

98 Hasan MM, Mofijur M, Uddin MN, et al. Insights into anaerobic digestion of microalgal biomass for enhanced energy recovery. *Front Energy*. 2024;10:1355686. <https://doi.org/10.3389/fenrg.2024.1355686>

lation of volatile fatty acids and a decrease in pH, while too low reduces the efficiency of substrate use. The optimal organic load depends on the substrate and its characteristics. Typically, for microalgae, this indicator is in the range of 0.5 – 4 g VS/l/day⁴. The hydraulic retention time shows how long the feedstock is in the BGU. Too long a stay of the substrate in the reactor does not increase the efficiency of biomethane output, as the available nutrients are exhausted. Therefore, establishing the optimal hydraulic retention time is a necessity. This parameter largely depends on the characteristics of the feedstock, especially in co-digestion. In industrial systems, the optimal retention time ranges from 30 to 50 days⁴.

One of the easiest and most effective ways to overcome the disadvantages of mono-digestion is co-digestion. Its essence lies in mixing microalgae with substrates that have a higher C:N ratio. This strategy allows you to increase the biogas and biomethane yield, organic load and buffer capacity. Also, some substrates stimulate the synthesis of enzymes that improve the hydrolysis and decomposition processes. In addition, during co-digestion, it is possible to reduce the concentrations of toxic and inhibitory compounds due to dilution.

Microalgae and their residues undergo co-digestion with a large number of different substrates, such as sewage sludge, animal manure, food waste, agricultural waste, glycerol and others. When choosing a co-substrate, it is necessary to rely on the ease of availability and cheapness of the substrate in terms of obtaining and transporting it.

In addition, it should provide a balanced C:N ratio and should have a similar decomposition rate compared to the primary substrate⁹⁹.

99 Ganesh Saratale R, Kumar G, Banu R, Xia A, Periyasamy S, Dattatraya Saratale G. A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. *Bioresour Technol*. 2018;262:319–332. <https://doi.org/10.1016/j.biortech.2018.03.030>

Table 5.5 provides a comparison of some co-substrates that are common in Ukraine.

Analysis of the data in the table allows us to conclude that the most optimal substrate for co-digestion of microalgae is agro-waste: wheat straw, corn silage and barley straw, they improved the C/N ratio and demonstrated a moderate biomethane yield, but at the same time there is also a synergistic increase in biomethane yield compared to mono-digestion. The most effective substrate turned out to be food waste and cattle manure. Although food waste demonstrates the highest biomethane yield and a significant increase in yield, which reaches 28%, due to the unstable composition, availability and problems with transportation of raw materials, this option is not advisable to use. The use of cattle manure, although it did not demonstrate an increase in yield, has one of the highest indicators of cumulative biomethane yield of 482.54 ml CH₄/g DOM, while it was possible to increase the C/N value almost to the optimal level, and the process duration is the shortest. Chicken manure, although showing the highest yield increase of 31%, has a low C/N and cumulative biomethane yield, so its application is limited. Molasses shows a similar increase in biomethane yield as wheat straw but has the lowest biomethane yield among all substrates.

Pretreatment Methods of Microalgal Biomass

Microalgae have significant potential as a substrate for biomethane production due to their high content of organic compound carbohydrates, proteins, and lipids. However, the efficiency of anaerobic digestion of microalgae is often limited by the low bioavailability of intracellular content.

The main methods of pre-treatment of microalgal biomass are shown in Figure 5.4.

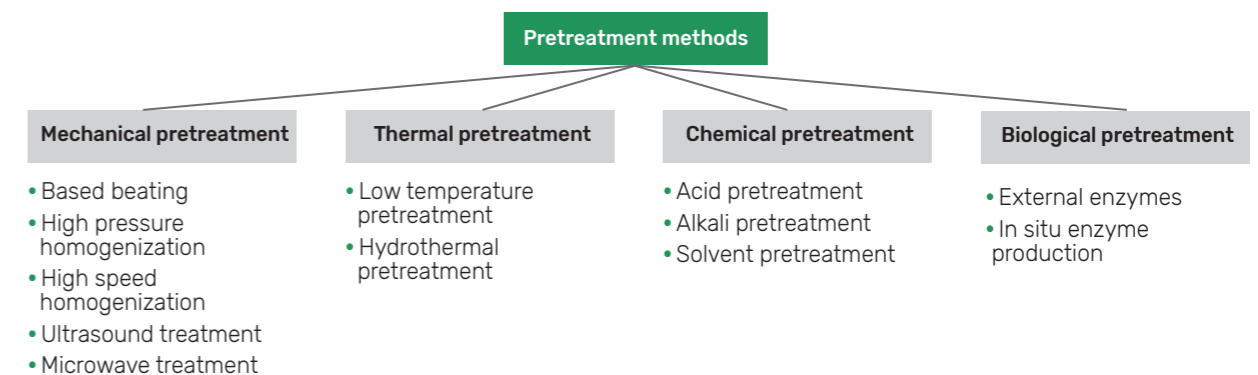


Fig. 5.4 – Pretreatment methods⁷³

Table 5.5. Comparison of substrates for co-digestion of microalgal biomass

Substrate composition	C/N	Cumulative biomethane yield, ml CH ₄ /g DOM	The increase in biomethane yield *	Process temperature, °C	Duration of the process, days	Source
Biomass of <i>Chlorella sp.</i> and <i>Monoraphidium sp.</i> , 20% and Wheat straw 80%	26.4	289	+12%	37	40	[1]
Biomass of <i>Spirulina platensis</i> 15% and Cattle manure 85%	19	482.54	0%	35	20	[2]
Biomass of <i>Scenedesmus sp.</i> 15% and Pig manure 85%	-	298	-1%	35	40	[3]
Biomass of <i>Nannochloropsis salina</i> 14% and Corn silage 86%	21.2	264	+15%	37	35	[4]
Biomass of <i>Chlorella</i> 1067 20% and Chicken manure 80%	8.94	239	+31%	35	29	[5]
Biomass of 85% and Barley straw 15%	25	347.8	+4.4%	37	30	[6]
Biomass of <i>Arthrospira platensis</i> 45% and Beet pulp 55%	25	360.9	-4.3%	37	30	
Biomass of microalgae 25% and Food waste 75%	18.2	514	+28%	35	50	[7]
Biomass of <i>Desmodesmus opoliensis</i> 50% and Molasses 50%	10	124.9	+11%	35	30	[8]

* The increase in biomethane yield refers to the difference between the cumulative biomethane yield of the co-substrate and the sum of the cumulative biomethane yields of the mono-substrates of this mixture.

- [1] Solé-Bundó M, Eskicioglu C, Garfí M, Carrère H, Ferrer I. Anaerobic co-digestion of microalgal biomass and wheat straw with and without thermo-alkaline pretreatment. *Bioresour Technol.* 2017;237:89-98. <http://dx.doi.org/10.1016/j.biortech.2017.03.151>
- [2] Álvarez X, Arévalo O, Salvador M, Mercado I, Velázquez-Martí B. Cyanobacterial Biomass Produced in the Wastewater of the Dairy Industry and Its Evaluation in Anaerobic Co-Digestion with Cattle Manure for Enhanced Methane Production. *Processes.* 2020; 8(10):1290. <https://doi.org/10.3390/pr8101290>
- [3] Astals S, Musenze RS, Bai X, Tannock S, Tait S, Pratt S, Jensen PD. Anaerobic co-digestion of pig manure and algae: impact of intracellular algal products recovery on co-digestion performance. *Bioresour Technol.* 2015;181:97-104. <http://dx.doi.org/10.1016/j.biortech.2015.01.039>
- [4] Schwede S, Kowalczyk A, Gerber M, Span R. Anaerobic co-digestion of the marine microalga *Nannochloropsis salina* with energy crops. *Bioresour Technol.* 2013;148:428-435. <http://dx.doi.org/10.1016/j.biortech.2013.08.157>
- [5] Li R, Duan N, Zhang Y, Liu Z, Li B, Zhang D, Lu H, Dong T. Co-digestion of chicken manure and microalgae *Chlorella* 1067 grown in the recycled digestate: Nutrients reuse and biogas enhancement. *Waste Manag.* 2017;70:247-254. <https://doi.org/10.1016/j.wasman.2017.09.016>
- [6] Herrmann C, Kalita N, Wall D, Xia A, Murphy JD. Optimised biogas production from microalgae through co-digestion with carbon-rich co-substrates. *Bioresour Technol.* 2016;214:328-337. <http://dx.doi.org/10.1016/j.biortech.2016.04.119>
- [7] Ferreira LO, Astals S, Passos F. Anaerobic co-digestion of food waste and microalgae in an integrated treatment plant. *J Chem Technol Biotechnol.* 2022;97(6):1545-1554. <https://doi.org/10.1002/jctb.6900>
- [8] Chen X, Mao H, Cui Y, Deng H, Zhao T, Liu J, Huang L, Shen P. An algal regulation-based molasses vinasse anaerobic digestion strategy for enhancing organic matter removal and methane production. *Renew Energy.* 2024;234:121257. <https://doi.org/10.1016/j.renene.2024.121257>

Main goals of pretreatment:

- Disruption of cell walls
- Reduction of medium viscosity
- Increased bioavailability of intracellular compounds
- Substrate optimization for stable digestion
- Reduction in digestion time

The effectiveness of anaerobic digestion is constrained by tough cell walls, high protein content, and a low C:N ratio in the biomass. Therefore, applying pretreatment methods before feeding microalgae into a bioreactor is considered essential for enhancing methane yield.

Studies show a significant increase in biomethane yield as a result of pretreatment. Table 5.7 presents the specific methane yields from various microalgae before and after treatment.

Factors affecting pretreatment method selection:

- Microalgae strain (cell wall thickness)
- Equipment availability
- Cost constraints (energy, chemicals)
- Scale (laboratory vs. industrial)
- Potential negative impact on nutrient value of digestate

Table 5.6. Effectiveness of Microalgae Pretreatment Methods Prior to Anaerobic Digestion (Methane yield increase is shown as a percentage compared to untreated *Chlorella vulgaris* biomass; Cheng et al., 2015).

No.	Pretreatment Method	Description	Methane Yield Increase (%)	Features / Notes
1	Thermal Treatment	Heating to 120 °C for 30 minutes	+41%	Disrupts cell walls; activates enzymatic processes
2	Ultrasonic Disintegration	Ultrasonication at ~20 kHz	+36%	Effective cell lysis; high energy consumption
3	Alkaline Hydrolysis	NaOH (1-2%) at room temperature	+47%	Enhances protein and polysaccharide availability; requires neutralization
4	Acid Hydrolysis	HCl or H ₂ SO ₄ (1-2%)	+20-30%	Less effective than alkaline; reduces structural carbohydrate content
5	Mechanical Disintegration	Grinding to fine suspension	+10-15%	Energy-efficient, simple; can be combined with other methods
6	Combined Thermo-Alkaline	Heating (100-120 °C) + NaOH	+70%	Highest efficiency; requires high energy and chemical input

Table 5.7. Specific Methane Yield Before and After Pretreatment ⁷⁵

No.	Microalgae species	CH ₄ Yield Without pretreatment (mL/g VS)	After pretreatment	Pretreatment method
1	<i>Chlorella vulgaris</i>	196	282 (+44%)	Ultrasound (35 MJ/kg TS)
2	<i>Arthrospira maxima</i>	210	280 (+33%)	Ultrasound (35 MJ/kg TS)
3	<i>Scenedesmus sp.</i>	150	300 (+100%)	Thermal (150 °C)
4	Mixed Cultures	170	190-220	Chemical (NaOH)

For example, on a small scale, thermal treatment is effective for *Chlorella vulgaris*, whereas industrial applications often prefer combined methods (e.g., alkaline + thermal).

Pretreatment of microalgae prior to anaerobic digestion is a **critical step** in improving the efficiency of biomass-to-biomethane conversion. Mechanical (ultrasound) and thermal methods have shown the highest effectiveness, with methane yield increases of up to 100%. However, method selection should balance energy costs, equipment availability, and algae type. Combined approaches, such as thermo-mechanical or chemo-thermal, show potential for further process optimization.

Integration of microalgae cultivation into biomethane production facilities

The symbiosis of microalgae cultivation and biogas plants opens the way to closed-loop bioeconomy systems, where the waste of one system becomes a resource for another. This approach allows to:

- reduce the environmental footprint;*
- increase nutrient use efficiency;*
- generate additional value from organic residues.*

The ability of microalgae to extract nitrogen, phosphorus, and other compounds from aqueous media is primarily determined by the physiological characteristics of the species, particularly the metabolic rate and the nutrient requirements necessary to sustain life. In natural environments, microalgae typically grow and function as a consortium of various strains and cyanobacteria, engaging in complex symbiotic exchange, assimilation, and production processes. Studies on different species of microalgae have shown that they can reduce more than 98% of COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand) in wastewater. Bioremediation's impact on wastewater purification helps reduce greenhouse gas emissions, sludge formation, and is both economically and energetically efficient¹⁰⁰.

The approach of purifying the liquid fraction of digestate through microalgae cultivation (diges-

tate bioremediation) emerged as a solution to the current problem of eutrophication in Northwestern Europe. In this region, the use of digestate as fertilizer is restricted, leading to the implementation of nitrate vulnerable zone policies under the EU Nitrates Directive 91/676/EEC, which limits the annual nitrogen load on arable land. Ammonium is the main nutrient of interest for microalgae cultivation, and increasing its availability and uptake is critical for optimal bioremediation.

In the production of biodiesel, pharmaceuticals, or cosmetic products from microalgae, the most expensive stage is the extraction of target components from the culture medium and biomass. The energy required for drying accounts for about 85% of the total energy consumption in the process of producing biodiesel from microalgae. However, biomethane production from microalgae does not require the extraction of specific components, as the biomass is suitable for anaerobic digestion (AD). In this process, all organic compounds (proteins, carbohydrates, and lipids) present in the microalgae biomass are converted into methane and carbon dioxide.

Several advantages of producing biogas from whole microalgae biomass can be highlighted:

- wet fermentation eliminates the need to dry the biomass;*
- microalgae can improve biogas quality through CO₂ bio sequestration;*
- lipid-deficient microalgae species can also serve as raw material for biogas production;*
- co-digestion with other biomass types, such as solid or liquid waste, is possible.*

The conditions required for photoautotrophic microalgae growth and their availability at biogas plants are presented in Table 5.8

Most of the essential growth conditions for microalgae are present, except for lighting, which can be addressed by installing LED lamps.

Integrating microalgae cultivation into existing biogas facilities appears to be the most effective technological solution for the utilization of the liquid fraction of digestate (LFD). In April 2024, a research project titled "*Advanced biomethane production from microalgae grown on biogas plant digestate*" was launched. It is being implemented by a consortium of companies from Ukraine and the UK: the University of Manchester (UK - admin-

istrative and technological lead), ALGAECYTES LIMITED (UK - commercial partner), MHP ECO ENERGY PJSC (Ukraine - commercial partner), and the Bioenergy Association of Ukraine (scientific partner). The project won the **InnovateUkraine** competition, funded by **UK International Development** and organized by the British Embassy in Ukraine. The main objectives of the project are:

To develop a new economically and energy-efficient process for cultivating microalgae on biogas plant digestate.

To establish a new economical and energy-efficient process for producing biomethane via anaerobic digestion (AD) of harvested microalgae.

Figure 5.5 presents the integration scheme of microalgae cultivation into a biogas/biomethane plant, as planned in the Innovate Ukraine project. After anaerobic digestion, digestate is separated into solid and liquid fractions. The liquid fraction is directed to a photobioreactor for microalgae cultivation. The culture mixture with algae biomass is then mixed with the main feedstock before being fed into the biogas unit (Fig. 5.5).

Table 5.8. Conditions required for photoautotrophic microalgae growth and their availability at biogas plants

Conditions for growth	Availability at biogas plants
Required lighting (sunlight or LED)	-/+ only in summer
Temperature (15-30°C)	+
Water	+
Carbon dioxide (CO ₂)	+
Mineral nutrients (N, P, K) Approximately 1.8 t CO ₂ , 70 kg N, 10 kg P and 8 kg K are required to produce 1 t of algae biomass.	+

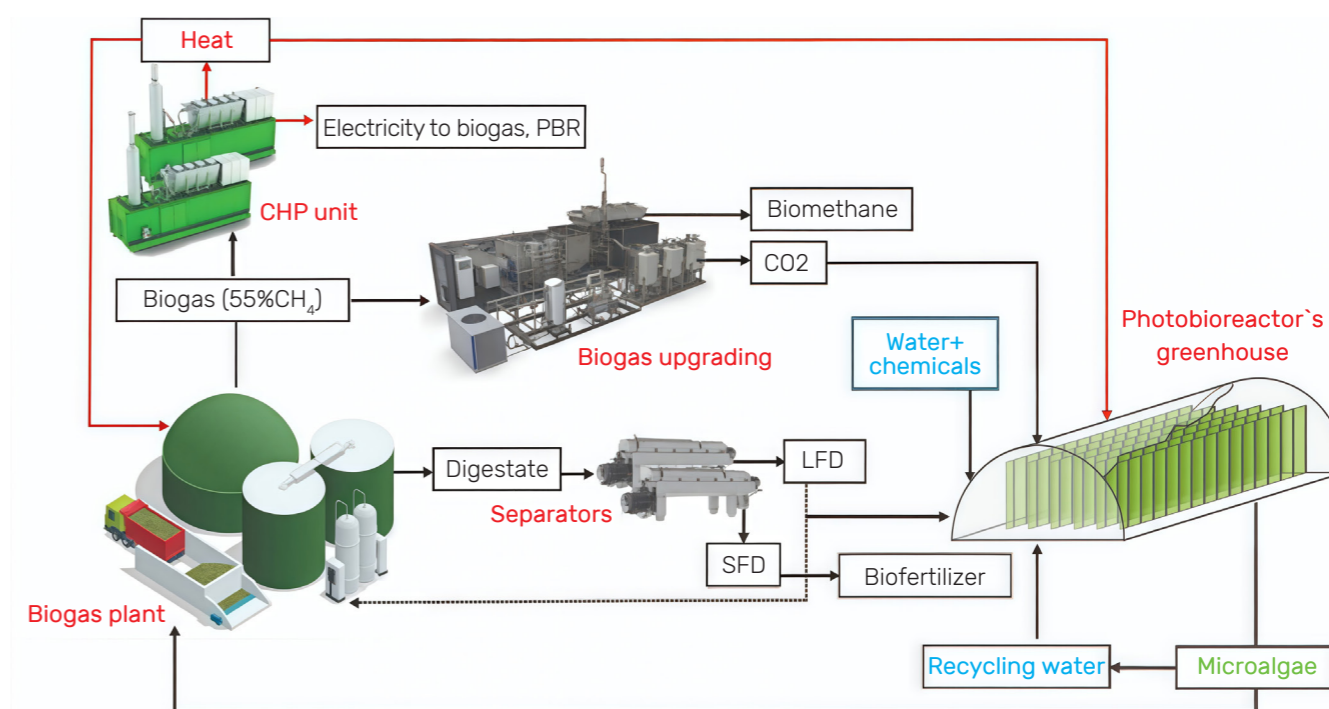


Fig. 5.5 - Scheme of integration of microalgae cultivation in a biogas/biomethane plant

¹⁰⁰ Bernhard Drosig et al. Nutrient Recovery by Biogas Digestate Processing / IEA Bioenergy, 2015

To achieve cost-effective advanced biomethane production from microalgae, the production cost must be less than € 0.2 per kg of dry weight (DW) microalgae. This goal is expected to be achieved through:

using biogas plant digestate as a free source of carbon, nutrients, and heat for cultivation;

utilizing CO₂ (a byproduct of biomethane production) for microalgae growth;

increasing cultivation productivity in photobioreactors (PBR) to 5 g / (L·day) using thin-layer flat-panel PBRs;

reducing energy consumption for biomass production to 0.02 kWh/kg due to the use of waste heat, CO₂, and minimizing dewatering needs when microalgae are used for biomethane production;

increasing the market value of biomethane by reducing its carbon intensity (to negative values) through CO₂ utilization in microalgae cultivation.

The integration of microalgae cultivation into anaerobic digestion and biomethane production technology is considered the most effective and promising approach, as most required components - process heat, CO₂, nutrients, water, and transportation - are nearly cost-free. The only significant expense is electricity for lighting, which can be minimized using solar power, energy storage, and electricity generated from biogas. As calculated for a similar project in Sweden, anaerobic digestion of microalgae biomass can potentially increase annual biomethane production at an existing facility by 9.4%¹⁰¹.

Resource needs (water, land, light, heat, CO₂ source) and nutrients (N, P, K) remain key challenges to the economic and environmental viability of microalgae cultivation. Integrating such cultivation into biomethane plants appears to be the most viable strategy.

The majority of **Ukraine's biomethane potential** - comes **agricultural feedstock**, which have a **high C/N ratio and low moisture 10-20%**, like straw, crop residues and other.

Microalgae biomass, depending on species and growth conditions, contains various proportions of

macromolecules (proteins, lipids, carbohydrates) that can be converted into additional biogas and subsequently into biomethane. Using microalgae suspension for biogas production does not necessarily require biomass concentration (separation from liquid), as the moisture content may be essential for digesting crop residues. It is known that the C:N ratio of microalgae biomass is usually below 10, which is suboptimal for anaerobic digestion, but it can help balance this ratio when co-digested with crop residues that have a C:N ratio of 50-100, resulting in a final C:N ratio within the recommended range of 15-30.

For example, mixing 1 ton of straw (DM = 80%, C = 35%, N = 0.5%), containing 200 kg water and 800 kg dry matter (4 kg N), with 8 tons of algae suspension grown on digestate (DM = 1%, C = 45%, N = 0.15%), containing 7,920 kg water and 80 kg dry matter (12 kg N), results in a 9-ton substrate mix with 90.3% moisture and a C:N ratio of 20 - within the optimal range (Tab. 5.9).

Table 5.9. Parameters of the mixture: microalgae suspension grown on digestate and straw

Indicator	Value		
	Straw	Microalgae suspension	Mixture
TS, %	80	1	9.8
C/N	70	3.0-9	19.8

Thus, co-fermentation of microalgae (C/N < 10) with crop residues (e.g., straw with C/N > 70) enables the formulation of a substrate with an optimal C/N ratio (15-30) and a suitable total solids (TS) content for anaerobic digestion (10-12%) by mixing high-TS straw (TS ≈ 80%) with low-TS microalgae biomass (TS = 1-5%).

Feasibility of biomethane production from microalgae

In the production of biomethane from MA, the key feasibility factors are economic and energy efficiency.

From the point of view of economic feasibility, the cost of produced biomethane from MA should be less than the possible sale price of such biomethane, taking into account the production cost of 1 m³ of biomethane and the minimum feasible profitability. Thus, when selling biomethane from MA at a price of 90 euros/MWh, the feasible cost of production of MA with a profitability of 20% and a production cost of biomethane of 35 euros/MWh will be 139-165 euros/tTS, with a specific methane yield of 320-380 Nm³CH₄/tVS, respectively. At a cost of MA production of 0.5 EUR/kgTS, the selling price of biomethane should be 202-233 EUR/MWh, with a specific methane yield from MA of 320-380 nm³CH₄/tVS, respectively. Given the fact that current prices for biomethane, even on the premium EU market, are significantly lower than 200 EUR/MWh, the task of finding optimal technological and conceptual solutions for MA production for biomethane production in order to minimize its cost is extremely important.

From an energy point of view, biomethane production from MA will make sense if the energy yield is greater than the total energy consumption for their cultivation, collection, supply and production of biomethane from them. Biomethane production (excluding energy demand for pre-treatment and supply of raw materials) requires approximately 0.12-0.18 MWh per 1 MWh of energy of produced biomethane. With a specific methane yield from MA of 320 Nm³CH₄/tVS (3.03 kWh/kgTS), the total energy consumption for growing, collecting and supplying MA for biogas production, taking into account the target coefficient of surplus energy yield from biomethane of 1.5, should not exceed 1.90-1.94 kWh/kgTS, with a specific yield of 380 Nm³CH₄/tVS (3.6 kWh/kgTS) - 2.28-2.32 kWh/kgTS. Minimizing energy consumption during the cultivation and harvesting of MW can be achieved only

SECTION 6

by making the most complete use of solar radiation energy as a source of light photons and thermal energy. In Ukrainian conditions, this corresponds to the warm period of the year with the highest intensity of solar radiation (May-September).

Energy production from MA (biogas, biodiesel) is a relatively new direction in the energy sector, and biomethane production from MA is generally represented by single projects in the world. Microalgae are considered as a promising raw material for the production of pharmaceuticals, cosmetics, nutraceuticals, fine chemicals, food and feed products, biofuels, as well as for low-cost wastewater treatment (Borowitzka, 2013; Spolaore et al., 2006). However, to date, industrial scales have been achieved only in high-value segments, in particular for aquaculture and human food. The main reason for this is the limited productivity of existing technologies and excessively high cost for low-value markets: world production is about 20 thousand t/year at a cost of ~20,000 USD/t (Benemann, 2013). To enter the mass energy and raw material markets, it is necessary to increase production volumes to ~10⁴ thousand tons/year and reduce the cost price below 0.50 €/kg (Chisti, 2007).

Achieving these targets is limited by both biological and engineering factors, as well as economic barriers. Key challenges include the lack of reliable data from industrial facilities (most information comes from pilot plants) and the lack of standardization of technologies (different strains, cultivation regimes, geographical conditions), which makes it difficult to compare effectiveness and draw conclusions (Richmond, 2000).

The cost is formed by investment and operating costs. For the calculation of investment and operating costs, it is essential to know the final goal and the scale of the process (i.e. production capacity). Based on this knowledge, adequate technologies can be selected for each of the required stages. Finally, it is necessary to obtain a list of the main

¹⁰¹ Xiaoliang Wang, Eva Nordlander1, Eva Thorin and Jinyue Yan. Microalgal biomethane production integrated with an existing biogas plant: a case study in Sweden

equipment required. To develop a detailed process flow diagram, it is also necessary to know the kinetic parameters of each stage to ensure correct design. In addition, the process flow diagram determines the energy and mass balances, which facilitates the assessment of the various raw material requirements required for the process.

Investment costs in MA cultivation include the cost of the main equipment and its installation, infrastructure (pipelines, electricity supply, buildings). Table 6.1 provides an overview of the available data on the cost of MA cultivation systems and photobioreactors themselves, in particular.

Microalgae (MA) production typically involves three steps: (1) biomass cultivation; (2) harvesting and dehydration; and (3) extraction, fractionation, and transformation. Cultivation is performed in open raceway ponds or in closed photobioreactors (PBRs). Due to the "dilute" nature of the cultures, starting concentrations are typically low: ~0.5–1.0 gTS/L in open systems and higher in closed systems, where MA are often harvested at 1–4 g/L (depending on design and conditions) ¹⁰².

During collection and dehydration, mechanical processes bring the dry matter content to 10–25%; for further processing, thermal drying (>85% DM) is used if necessary. In many engineering schemes, a basic thickening to ~20% TS is a project goal ¹⁰³. For biomethane production, thickening to such concentrations is not a prerequisite, and this is an obvious advantage of this direction of application of suspension with grown MA.

The largest energy consumption items are aeration/CO₂ supply, pumping/circulation of the suspension, artificial lighting (for intensive or indoor systems), and dehydration. According to generalized feasibility studies, operating costs and electricity consumption at the collection/dehydration stage are in the ranges of 0.5–2.0 €/kg and 0.2–5 kWh/kg (depending on the technology and target product quality) ¹⁰⁴.

US government models for open-pit (nth-plant) coastal farms show target minimum biomass selling prices (MBSP) of around \$450–\$700 per ton of

dry matter in the current state of technology, with an estimated reduction to 0.49/kg through productivity improvements, stream integration, and cost reductions. This highlights that fuel competitiveness requires very low fuel costs or a combination with co-products ¹⁰⁵.

Demand for MA as a feedstock is highly segment-dependent: low-cost energy markets require low biomass costs (≤\$0.5/kg of dry matter), while food/cosmetic/nutraceutical products allow for significantly higher prices per kg but with lower volumes. DOE analysis (Billion-Ton Report 2023) uses a threshold of ≤\$1000/tTS as a guideline for feasibility for widespread implementation in fuel chains ¹⁰⁶.

The IEA-Bioenergy strategic reviews consider the high cost of cultivation, which is still higher than acceptable for mass fuel markets, as the main barrier to commercialization; the "biorefinery" approach with co-products and the use of waste streams (water, nutrients, CO₂) is seen as a way to reduce costs ¹⁰⁷.

For the biomethane plant cultivation scheme (CO₂ supply from biogas upgrading, digestate heat recovery 38–42°C, nutrient input together with digestate), the key effects are:

No compression/bubbling costs (if gas pressure is already sufficient) and replacement of part of the mineral fertilizers with the digestate flow. This directly reduces OPEX compared to standard systems with separate purchase of CO₂ and salts. The generalized feasibility studies emphasize that CO₂ and nutrients often constitute a significant share of the cost ¹⁰⁸.

Reduction of heat costs by utilizing digestate heat; for moderate/cold climates, maintaining the culture temperature >25 °C has a significant impact on the energy balance. Reviews indicate that climate conditions and temperature control are sensitive cost drivers ¹⁰⁹.

¹⁰⁵ [PNNL-32695](#)

¹⁰⁶ [bioenergykdf.ornl.gov](#)The Department of Energy's Energy.gov

¹⁰⁷ https://www.ieabioenergy.com/wp-content/uploads/2017/01/IEA-Bioenergy-Algae-report-update-20170114.pdf?utm_source

¹⁰⁸ [Key Targets for Improving Algal Biofuel Production](#)

¹⁰⁹ [Microalgae cultivation: photobioreactors, CO2 utilization, and value added products of industrial importance - Shekh - 2022 - Journal of Chemical Technology & Biotechnology - Wiley Online Library](#)

Table 6.1. Examples of the cost of photobioreactors and systems based on them

Photobioreactor (PBR) type	Specific cost of photobioreactor	Cost of other components	Notes	Link
Tubular, 250, 750, 1500 and 18000 litres	Total price of systems, including PBR: from 120 thousand euros per m ³ for systems with a volume of 250 L to 8 thousand euros per m ³ for systems with a volume of 18,000 L		Based on theoretical calculations	https://doi.org/10.1016/j.aquaculture.2020.735310
"Green Wall Panel-II" (GWP@-II) - see Fig. 1. One reactor consists of twenty-four panels 48 m long. Total area of panels 800 m ² ; occupied land area 1250 m ² ; volume 39.4 m ³	79 euros/m ² of panel area	Pipelines, fittings, valves, tanks - 28% of the PBR cost Machinery and other equipment - 75% of the PBR cost Electrical equipment, instrumentation and control systems - 54% of the PBR cost	Based on implemented installation	http://dx.doi.org/10.1016/j.algal.2016.09.005
Tubular PBR, flat-panel PBR	Tubular - 86 euros/m ² , flat-panel - 101 euros/m ²		Based on theoretical calculations	https://www.acres.nl/wp-content/uploads/2018/03/3e5c6f85-d5bc-431d-ae25-85d949b327b0_WP2A7.10-report-Business-economics-microalgae-and-DSP.pdf
Tubular PBR	5000 euros/m ³	Other equipment - 44% of the cost of the PBR	Based on implemented installation	https://doi.org/10.1016/j.biotechadv.2012.02.005
Horizontal tubular reactors based on modules of type MK1-18.000. Each module consists of 24 parallel loops, with a length from the feed tray to the U-shaped bends of 120 m and a slope of 0.5%. The module has a volume of 18 m ³ and an area of 625 m ² .	6762 euros/m ³	Other equipment - 51% of the cost of the PBR	Based on implemented installation	https://rodin.uca.es/bitstream/handle/10498/34310/Techno-economic%20analysis%20of%20microalgae%20pre-print.pdf?sequence=1&isAllowed=y

Risk assessment

Although the synergy between microalgae and biogas holds significant potential for sustainable development, its implementation faces a number of technical, economic, and regulatory challenges

that require comprehensive risk management during both the planning and operational phases.

Risk assessment for the integration of microalgae cultivation and biogas plants is presented in table 6.2.

Table 6.2. Risk Assessment for the Integration of Microalgae Cultivation and Biogas Plants

Risk Name	Risk Description	Potential Consequences	Recommended Measures
1. Technical Risks			
Excessive NH ₄ -concentration	Digestate contains high ammonium levels toxic to microalgae	Growth inhibition or culture death	Dilution, aeration, pre-treatment
Variability in digestate composition	Fluctuations in feedstock input at the biogas plant	Unstable nutrient environment for microalgae	Continuous monitoring, batch standardization
Insufficient CO ₂ supply	Unstable CO ₂ generation or supply from cogeneration units	Reduced photosynthesis, slower growth	CO ₂ buffering or supplementation from cylinders
Light conditions	Inadequate or uneven natural lighting	Low photosynthetic productivity	Use of artificial lighting, LED systems
2. Environmental Risks			
Residue/waste formation	Unused microalgae biomass or metabolic by-products	Potential pollution, need for disposal	Inclusion in biogas cycle or agricultural applications
Bio-contamination risks	Culture contamination (bacteria, fungi, other algae)	Reduced quality/productivity	Sterile conditions, culture monitoring
Digestate leakage	During transport or storage	Soil and water contamination risk	Waterproofing, leak control
3. Economic Risks			
High CAPEX	Installation of photobioreactors or open ponds	Longer payback period	Government support, grants
Market volatility	Unstable demand for products (biofuels, proteins, CO ₂ credits)	Inability to market products	Contractual partnerships, product diversification
Weather dependence	In open systems - seasonal variation in light and temperature	Reduced productivity in winter	Partial transition to closed systems
4. Regulatory and Legal Risks			
Unclear product classification	Microalgae biomass may lack a defined category (feed, fertilizer, biofuel)	Certification costs, export restrictions	Select the most accessible market path
Environmental permits	Environmental impact, water use, CO ₂ emissions	Project delays, fines	Preliminary coordination with regulatory authorities

Integrated Case Studies on CO₂ Utilization, Microalgae Cultivation, and Biomethane/Biogas Production

SECTION 7

Given the need to improve the efficiency of bioenergy systems in Ukraine—especially in the context of energy decentralization and waste management—the use of digestate from biogas plants as a medium for microalgae cultivation gains strategic significance. This technology not only closes biological cycles but also offers the potential to produce high-efficiency biomethane and additional valuable products such as proteins and pigments.

Below is an analysis of the most successful European pilot projects that could be adapted to Ukrainian conditions. The integration is achieved by using digestate as a nutrient medium. After solid-liquid separation, the liquid fraction of the digestate contains significant amounts of nitrogen, phosphorus, and micronutrients essential for algae growth. Microalgae are capable of:

Absorbing CO₂ emitted during anaerobic digestion;

Accumulating lipids, carbohydrates, and proteins (up to 30-50% of dry weight) for conversion into biomethane or biodiesel;

Utilizing residual biomass after fermentation as soil fertilizer or animal feed.

Review of International Pilot Projects

Case: Slovenia - AlgaeBioGas Demonstration Centre - <https://algaebiogas.eu/>

In Slovenia, the AlgaeBioGas project established a demonstration centre that integrates algal cultivation with an existing 1 MW biogas plant primarily operating on maize silage. The goal of the initiative is to create a closed-loop system for nutrient reuse from digestate, CO₂ recycling, and the generation of additional biomass as an energy resource.

Bioreactor System consists of two open algal ponds:

A main pond with a surface area of 100 m²;

An inoculation pond for starter cultures - 10 m².

The facility is located in a greenhouse, ensuring stable microclimatic conditions and reducing seasonal productivity losses.



Fig. 7.1 - Photo of the AlgaeBioGas demonstration centre in Slovenia, implemented at an operating biogas plant.

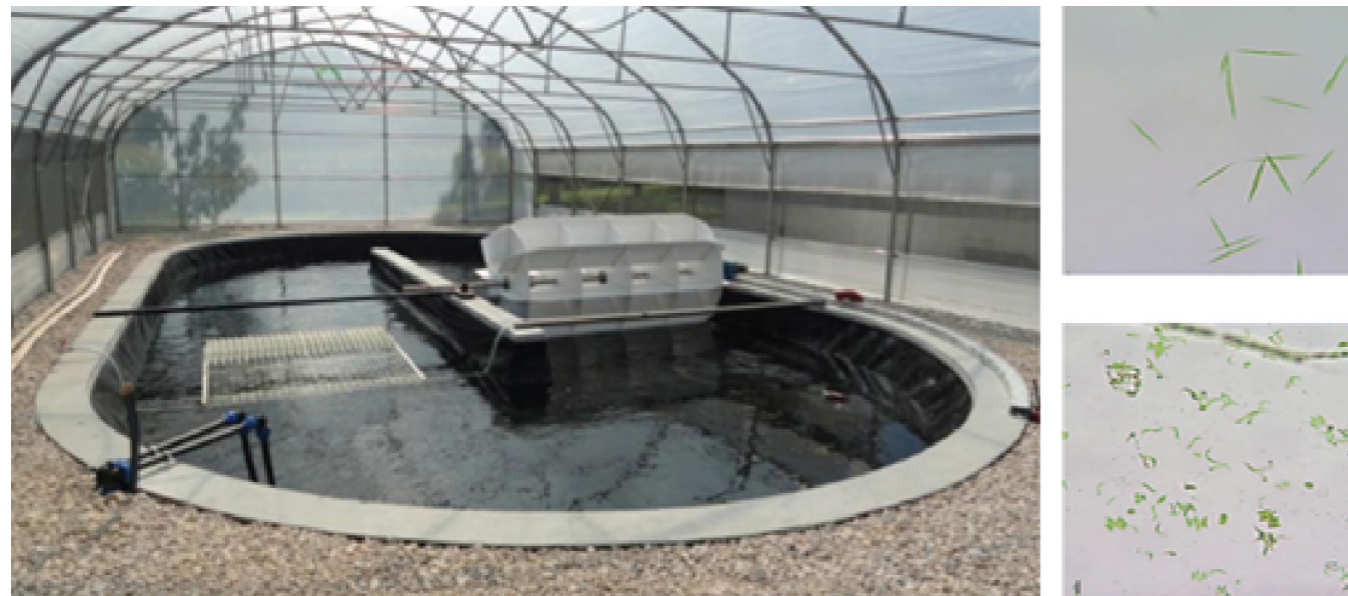


Fig. 7.2 - Photo of the AlgaeBioGas demonstration centre in Slovenia, implemented at an operating biogas plant. On the right, an image of the microalgal consortium from this installation

The process involves the following operations:

1. Liquid digestate is continuously or periodically fed into the main pond.
2. Algal-bacterial biomass is cultivated, which absorbs nitrogen, phosphorus, and CO₂.
3. Biomass is harvested daily via a sedimentation unit and pumped back to the biogas plant as a secondary substrate.
4. Residual water after dewatering can either be discharged into the environment following treatment or returned to the system.

The AlgaeBioGas project demonstrates high integration of biogas plant technology with algal-based bioremediation, achieving:

- Deep digestate purification;
- Generation of additional biomass;
- Significant CO₂ emission reduction;
- Land savings for fodder crop cultivation.

This model is fully adaptable to the conditions in Ukraine, especially in regions with high concentrations of agricultural production.

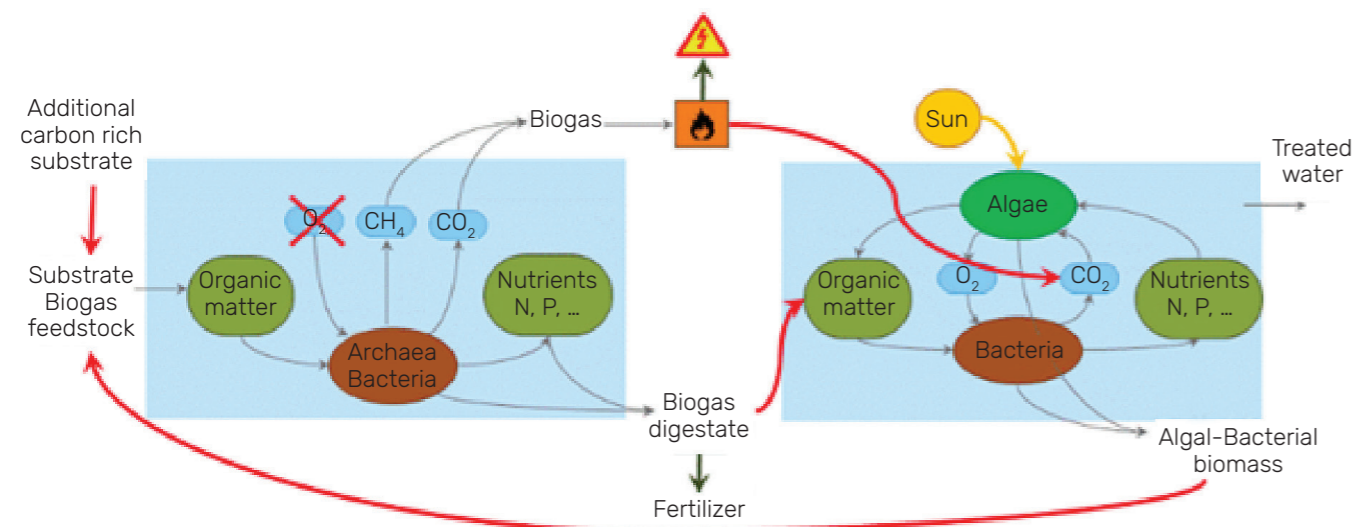


Fig. 7.3 - Process Flow Diagram of the AlgaeBioGas Project

Table 7.1. Summary of AlgaeBioGas Project Parameters

Category	Parameter	Value / Characteristic
General Information	Country of implementation	Slovenia
	Type of biogas plant	1 MW, maize silage
	Cultivation system type	Open ponds in greenhouse (100 m ² + 10 m ²)
Digestate Treatment	Volume of treated liquid digestate	30 m ³ /ha/day
	COD content in digestate	~8,000 mg O ₂ /l
	Organic matter removal efficiency	Up to 94%
	Phosphate / nitrogen removal	High, significant concentration reduction
	Odor reduction	Substantial
Biomass Productivity	Annual biomass production	30-40 tons DM/ha/year
	Biomass type	Algal-bacterial consortium
	Biomass utilization	Biogas substrate, fertilizer, or feed
Environmental Impact	O ₂ utilization from biogas plant	~240 t/year out of 13,000 t total emissions
	Equivalent land substitution (maize)	9-27 ha (replacing 335 ha of total demand)
	CO ₂ emission reduction from digestate treatment	~1,100 t CO ₂ /year
	Reduction in NO _x and N ₂ O	Yes, significant
Technological Constraints	Light dependence	High - critical factor, especially due to digestate colour
	Temperature regime	Optimized through greenhouse conditions
	Need for digestate pre-treatment	Recommended (clarification, filtration, pH correction)
Infrastructure Requirements	Area for treatment system	3-5 ha per 1 MW plant
	Water reuse	Yes, possible
	Market access	EU countries with digestate application restrictions (Germany, Italy, Sweden)

Case: ALG-AD - Integration of Anaerobic Digestion and Microalgae Cultivation (UK, France, Belgium)

<https://vb.nweurope.eu/projects/project-search/alg-ad-creating-value-from-waste-nutrients-by-integrating-algal-and-anaerobic-digestion-technology/>

The **ALG-AD project** was implemented under the Interreg North-West Europe programme to integrate anaerobic digestion (AD) systems with microalgae cultivation. The main objective was to find an effective solution for processing surplus nutrient-rich digestate, which cannot be applied to fields as fertiliser due to EU environmental restrictions. The project was carried out at three pilot sites: the UK (Swansea), France (Brittany), and Belgium (Flanders). These sites used various anaerobic digestion (AD) substrates, including food and agricultural waste, and different photobioreactor configurations to assess the performance of algae cultivation on pretreated digestate. Fig. 7.4 shows photos of the pilot sites, the photobioreactors, and the types of microalgae cultivated during the project.

Problem addressed by the project. Regulatory restrictions on digestate application, increasing accumulation volumes, need for environmentally friendly utilisation.

Solution: Microalgae cultivation based on the liquid digestate fraction, with subsequent use of biomass as feed protein concentrate, biogas substrate, or raw material for biorefining.

ALG-AD technological process:

Digestate preparation (NRD): Clarification, filtration, pH adjustment, ammonia reduction, inhibition prevention.

Algae cultivation: In photobioreactors or open systems (depending on site). CO₂ from AD plants used.

Biomass harvesting: Centrifugation or filtration, further dewatering.

Safety and quality assessment: Pathogens, heavy metals, toxin content.

Biomass utilisation: Protein feed, fertiliser, bioenergy (biomethane).

ALG-AD conclusions

Microalgae can be effectively cultivated even at low digestate concentrations, provided pre-treatment and stabilisation are applied.

Pre-treatment type critically affects cultivation results, especially lighting, pH, and ammonia levels. 3,000 L systems have proven the technical feasibility of scaling up, especially in farm settings or small biogas plants.

Chlorella sp. and *Desmodesmus sp.* are recommended strains for Europe due to their resilience and biomass productivity.

INNOLAB: Suitable for parametric optimisation; batch cultivation with precise condition control.

Langage AD (UK): Demonstrates operational stability of semi-continuous systems in farm environments.

Brittany (France): The only platform with automated flow model, closest to industrial scaling.

Table 7.2. Integrated comparison table of the three ALG-AD pilot sites (Interreg North-West Europe) This table presents key technical, biological, and infrastructural parameters for each site:

Parameter	INNOLAB (Belgium)	Langage AD (UK)	Brittany (France)
Type of AD	Food and agricultural waste	Farm biogas plant	Agro-industrial AD
Type of digestate (raw)	Liquid fraction (after DAF and filtration)	Liquid fraction	Liquid fraction
Pre-treatment	Centrifugation, DAF, 10 µm paper filter, digestate diluted to 10%	Settling, filtration, digestate diluted to 1%	Settling
Cultivation system	Vertical + horizontal tubular photobioreactor - 3000 L	Vertical bioreactor 2500 L	Horizontal bioreactors 6x500 L (3000 L)
Microalgae strains	Chlorella sp., Desmodesmus sp.	Chlorella sp., other local strains	Chlorella sp., Scenedesmus sp.
Cultivation duration	14 days	14-21 days	2 days
Cultivation mode	Photoautotrophic	Photoautotrophic and mixotrophic	Heterotrophic
Growth temperature	17-23 °C	Controlled (greenhouse)	External regulation
Lighting	LED, 100 µmol/m ² /s, 16:8 (light:dark)	LED, greenhouse, natural light	Natural + supplementary lighting
Culture density (max)	~21,800 cells/µL	Not specified, moderate	High (depending on period)
Biomass yield (estimate)	~0.3 g/L/day (lab)	0.06 g/L/day photoautotrophic; 1.6 g/L/day mixotrophic	~20-30 t DM/ha/year (estimate)
Biomass concentration	1.7 g/L	1-13 g/L	5.37 g/L
Biomass use	Protein feed, research	Feed or biogas	Biogas, fertiliser
Features	Detailed control, parametric studies	Full-scale farm biogas integration	Industrial-scale demonstration



Fig. 7.4 - Photos of ALG-AD project pilot sites

Case: ALL-GAS (Spain) - Large-scale microalgae biofuel production based on municipal wastewater

<https://www.all-gas.eu/>

The ALL-GAS project, supported by the European Commission under the 7th Framework Programme (FP7), is the world's first large-scale demonstration of microalgae-based biofuel production integrated with wastewater treatment. The project was implemented in the municipality of Chiclana de la Frontera (Spain), on the site of an operating wastewater treatment plant.

Goal: integrate the wastewater treatment cycle with biofuel production. Replace part of fossil fuel consumption with a carbon-neutral alternative -

biodiesel and biomethane from microalgae. The project parameters are presented in Table 7.6.

The ALL-GAS cycle includes:

- supplying wastewater from the treatment plant to open ponds/channels (up to 4 ha);*
- cultivation of microalgae in water rich in organics, nitrogen, and phosphorus;*
- harvesting and separation of biomass accumulating lipids and biomethane potential;*
- biomass processing: lipid fraction → transesterification → biodiesel; residues and activated sludge → anaerobic digestion → biomethane + CO₂; biogas cleaning and compression → vehicle fuel*

The **ALL-GAS** project demonstrated the feasibility of using treated wastewater and digestate as a nutrient medium for microalgae, followed by biomethane production for transport. Importantly, the project implemented a complete cycle - from biomass cultivation to its digestion. In Ukraine, such an approach could be relevant for municipalities, particularly in the context of decentralized

energy systems and integration with wastewater treatment plants. International experience generally confirms the feasibility of integrating microalgae cultivation on digestate as part of a circular bioeconomy at biogas plants. This not only reduces environmental impact but also creates new value streams in the form of biomethane, fertilizers, or bioproducts.

Table 7.3. Project parameters of ALL-GAS

Parameter	Value / Characteristic
Location	Chiclana de la Frontera, Spain
Cultivation area	Up to 4 ha
Nutrient source	Municipal wastewater
Cultivation type	Open raceway ponds, gravity circulation
Microalgae types	Scenedesmus, Chlorella, local strains
Biomass productivity	~100 t dry matter/ha/year
Oil content in biomass	~20% (for conversion to FAME - biodiesel)
Biofuel potential	Up to 40 vehicles/ha/year (20 biodiesel, 20 biomethane)
By-products	CO ₂ , treated water, bio residue
Project feature	Fully closed loop: wastewater → algae → energy → treated water
Application type	Municipal transport fuel, reduction of wastewater treatment costs

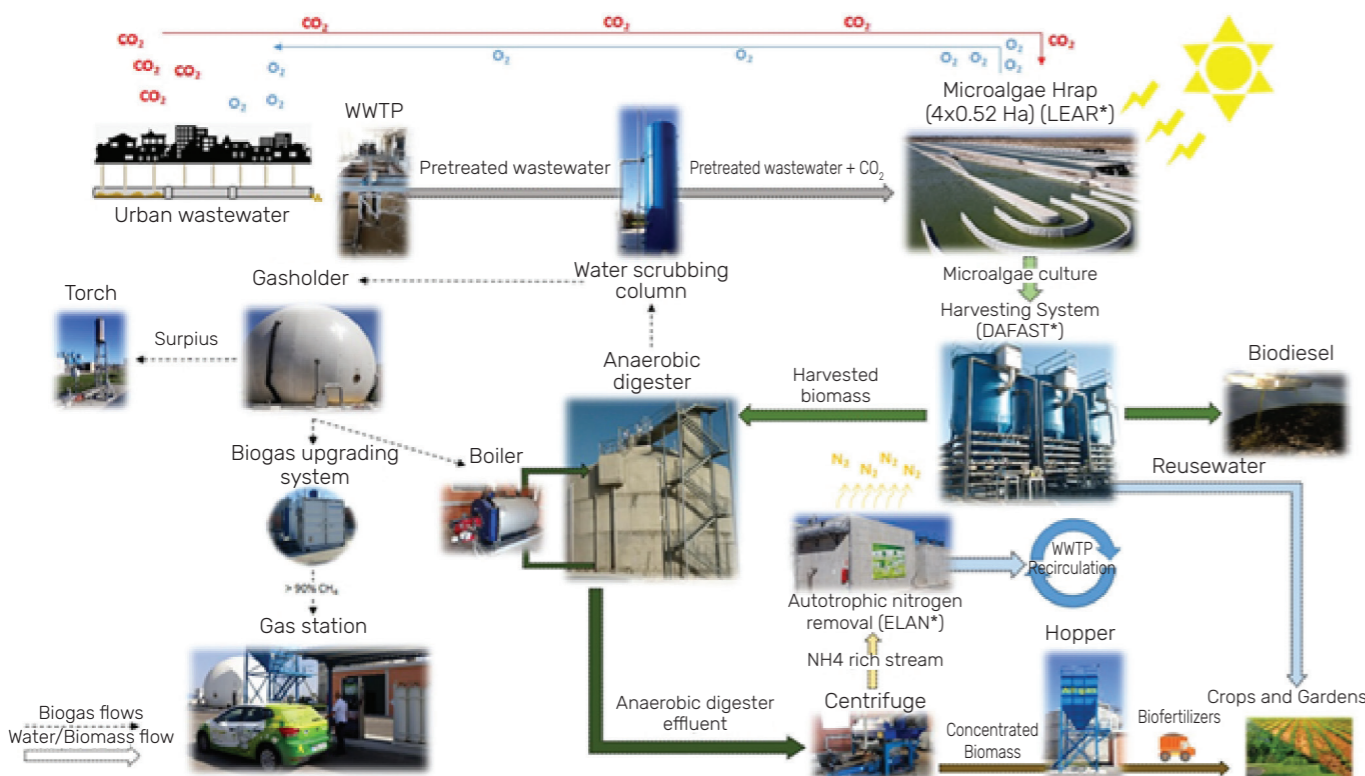


Fig. 7.5 - Process diagram of ALL-GAS project

ALGADISK - modular algae cultivation system on flue gases in biofilm reactor (Spain, FP7)
<https://cordis.europa.eu/project/id/286887/reporting/es>

The ALGADISK project (No. 286887, FP7) aimed to create an innovative biofilm-based photobioreactor providing:

- high biomass concentration at low energy and water consumption;*
- full integration with CO₂ emissions from CHP, biogas plants, or other industrial sources;*
- automated control and biomass harvesting, minimal human intervention.*

The ALGADISK system operates as a rotating biofilm photobioreactor with vertical discs partially submerged in a nutrient medium enriched with CO₂ from biogas plant flue gases or bottled reserve. Microalgae attach to the surface of these discs and, during slow rotation, alternately contact the liquid (absorbing nutrients) and the air (photosynthesizing). The system includes sensors for pH, temperature, light intensity, CO₂ content, and other

parameters, ensuring stable biomass growth. Every few days, the biofilm is mechanically scraped off the disc surface - the resulting biomass has high density (up to 100 g/l), minimizing the need for energy-intensive dewatering. The collected material can be used for biomethane production, organic fertilizers, or feed additives, creating a closed, energy-efficient, and environmentally friendly cycle.

Table 7.4 shows the main parameters of the ALGADISK project: technical characteristics, productivity, environmental and economic characteristics.

The ALGADISK system is technically feasible for implementation at biogas plants. It is suitable for the utilization of exhaust gases and mineralized digestate within closed agro-industrial cycles. Its advantages include low energy consumption, high biomass concentration, minimal water usage, and automation. However, the technology requires adaptation and practical refinement - in particular, placement in a greenhouse due to Ukraine's climatic conditions and the use of additional lighting during the winter period.

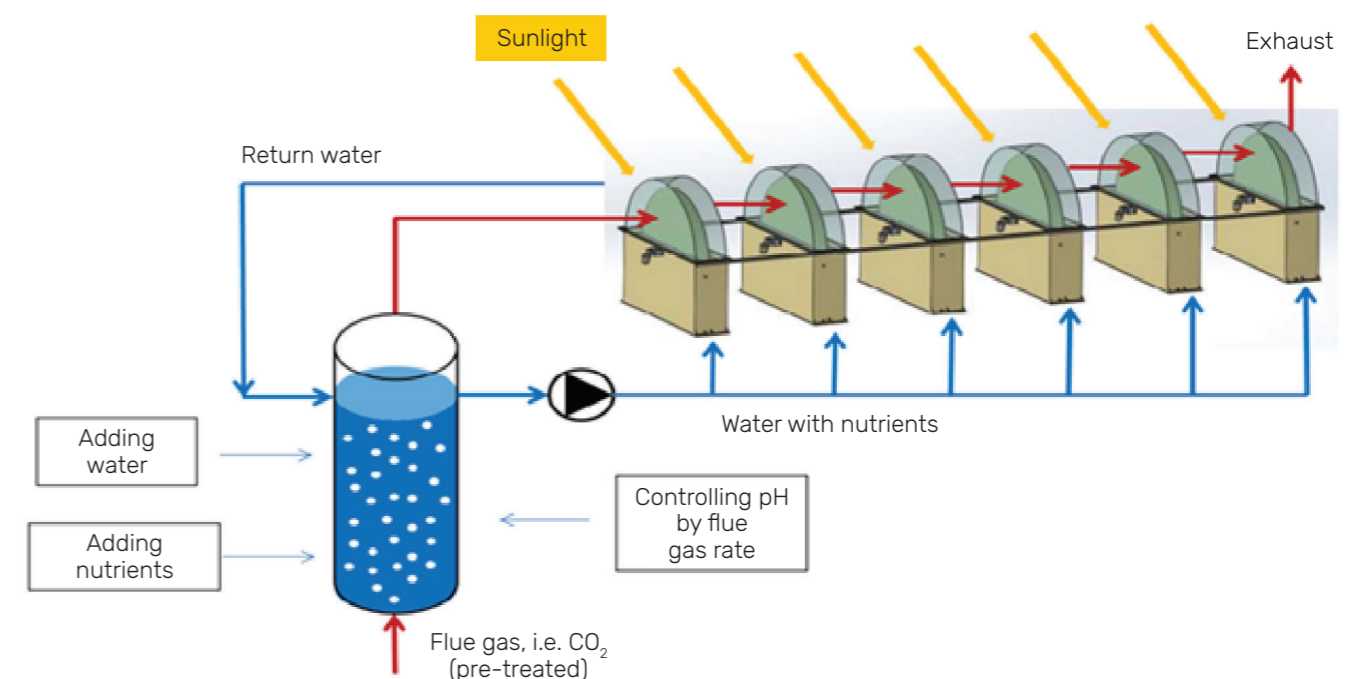


Fig. 7.6 - Schematic diagram of a microalgae cultivation plant ALGADISK ¹¹⁰

¹¹⁰ https://www.eubia.org/cms/wpcontent/uploads/2014/11/images_presentations_Presentation%20of%20Algadisk%20Technology.pdf

Table 7.4. Summary table: ALGADISK project (Spain, FP7)

Category	Parameter	Value / Characteristic
Technical concept	Reactor type	Modular biofilm disc (rotating vertical discs)
	Disc surface material	Polymer with biocompatible coating for algae adhesion
	Mechanics	Discs partially submerged, slowly rotating for light/shade cycling
	CO ₂ source	Flue gas from biogas plant (BFC, Almazán) or bottled reserve
	Control	Automated system: pH, temperature, light, CO ₂ , nutrient control
	Additional components	Antifoam system, buffer tank, heat exchanger, CO ₂ and nutrient dosing
	Emergency states	Blocked rotation → biomass drying; backup power for safety
	Modularity	1 control unit per 10 reactors; easily scalable
Performance parameters	Microalgae type	Chlorella sorokiniana
	Average biomass yield	18-20 g/m ² /day (6-7 months/year); up to 21.8 g/m ² /day (June)
	Daily reactor output	~182 g/day; or 5.4 kg/month/reactor
	Biofilm density	100-150 g/l dry matter
	Concentration after harvest	Up to 89-100 g/l without additional dewatering
	Harvest frequency	Every 2-3 days, semi-automated
	CO ₂ uptake	~0.17 Nm ³ /day (7.57 molC); efficiency 20-100% depending on load
	Nitrogen consumption	~14.8 g/day
Environmental and economic aspects	Phosphorus consumption	~2.6 g/day
	Energy consumption	2.2-3.5 kWh/day (pilot plant); potentially lower at scale
	System cost	<10,000 € / 100 kg dry matter/year (approximate)
	Product cost	~50 €/kg DM (significantly lower than commercial PBR systems)
	Market	Biofertilizers, premium feeds, supplements, bioenergy
	Water use	Low - due to biofilm immobilization
	CO ₂ reduction	Effective capture from gas and liquid phase
	Climate limitations	Not recommended for cold climates; greenhouse or seasonal use required

8. Assessment of the potential for biomethane production in Ukraine, taking into account microalgae that can be collected from the digestate of biogas plants

SECTION 8

The potential for biogas and biomethane production from microalgae is estimated based on a conceptual approach, in which the microalgae will be grown using CO₂ from biogas upgrading to biomethane, as well as, in part, nutrients in the digestate. At the same time, the availability of CO₂ from biogas upgrading is taken as the limiting factor.

Considering the estimated biomethane production potential in Ukraine at 21.85 billion Nm³CH₄/year (BAU, 2024), the theoretical potential for CO₂ formation from biogas upgrading to biomethane can be 32.46 mln tCO₂/year (Table 8.1). This estimate takes into account the average CO₂ concentration in biogas at 42.4%.

Table 8.1. Estimated potential for biomethane production from microalgae grown using CO₂ from biogas upgrading

Parameter	Unit	Value
Total potential for biomethane production in Ukraine	mln Nm ³ CH ₄ /year	21,850.0
Potential for CO ₂ formation during biogas upgrading to biomethane	mln Nm ³ CO ₂ /year	16,416.8
	mln t CO ₂ /year	32.46
Theoretical CO ₂ consumption for MA grown	T CO ₂ /tDM	1.8
CO ₂ uptake coefficient	%	80.0%
Theoretical potential for growing MA	t DM/year	14,424,882.4
VS content in MA (as for Chlorella)	%DM	94.6%
The assumed biochemical methane potential from MA	Nm ³ CH ₄ /tVS	320.0
Theoretical potential for CH ₄ production from MA	mln Nm ³ CH ₄ /year	4,366.7
Economic potential for CH ₄ production from MA	% of theoretical potential	15.4%
	mln Nm ³ CH ₄ /year	673.0

Using the potential for CO₂ formation during upgrading of the entire volume of biogas at CO₂ uptake rate of 80% will allow to cultivate 14.425 mln tDM/year of MA. At the same time, the theoretical potential for biomethane production will reach approximately 4.4 billion Nm³CH₄/year, with a specific yield of 320 Nm³CH₄/tVS MA. It should be noted that the specific methane yield assumed in the assessment can be considered to some extent a conservative value, since such a yield can reach 370–400 Nm³CH₄/tVS MA. For example, according to laboratory data of SEC Biomass (2025), the specific methane yield from *Chlorella* reaches 320–420 Nm³CH₄/tVS MA at average concentration of CH₄ in biogas at 75%.

In practice, the use of microalgae for biomethane production will make energy and economic sense under the conditions of maximum use of solar energy and minimum consumption of electrical and

thermal energy from other sources. To assess the economic potential of biomethane production from microalgae, it is assumed that in Ukraine such pre-conditions will correspond to a period of no more than 41% (150 days per year). Taking into account also the assumption that the cultivation of microalgae for biomethane production will be implemented at only 37.5% of the total biogas production capacities, the economic potential of biomethane from microalgae can be 673 mln Nm³CH₄/year. Taking into account the higher specific methane yield from microalgae at the level of 375 Nm³CH₄/tVS, the value of the economic potential can be 800 mln Nm³CH₄/year.

The regional distribution of biomethane production potential from MA grown using CO₂ from biomethane upgrading is proportional to this distribution for biomethane production potential, as shown in Fig. 8.1

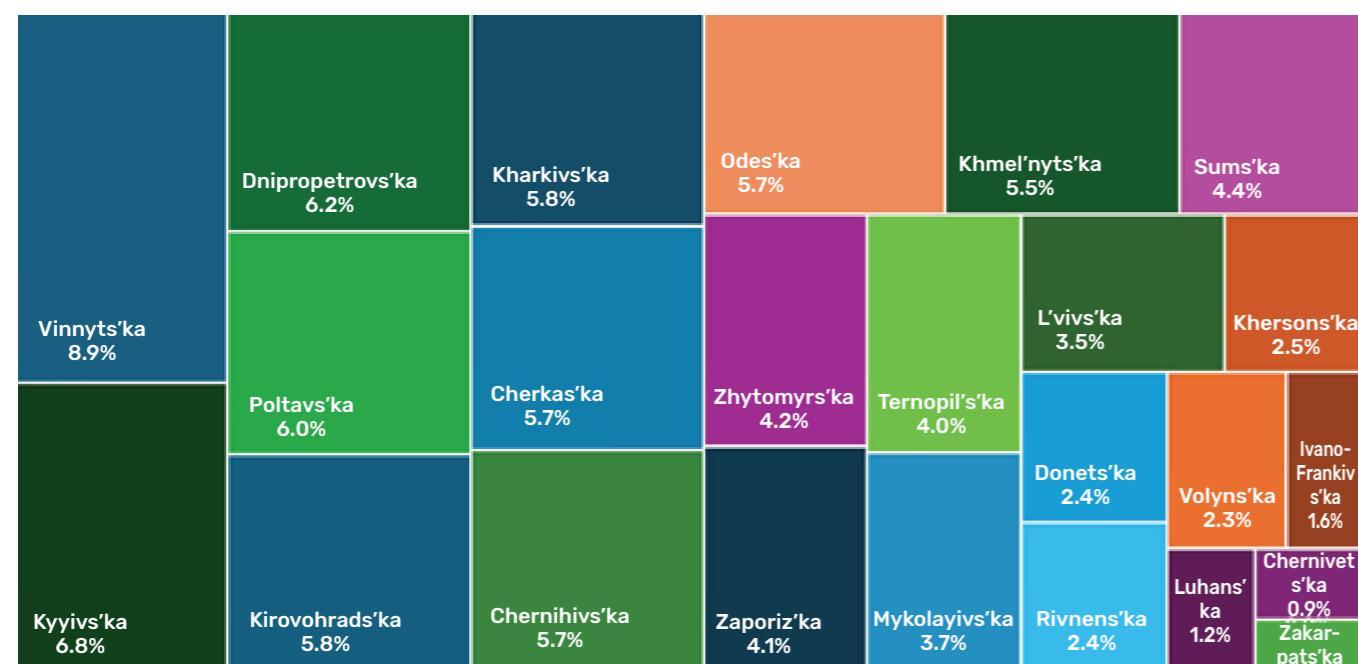


Fig. 8.1 - Regional distribution of biomethane production potential from MA grown using CO₂ from biomethane upgrading

Conclusion

Given the high reproductive activity and significantly higher conversion rate of solar radiation energy into organic matter, microalgae can become another significant source of biomass for energy production, in particular biomethane.

Microalgae are mainly produced for high-value products in other sectors, due to higher profitability and limited incentives in the energy sector. In the context of biogas plants, cultivating microalgae using digestate and CO₂ can enhance biomethane production, improve overall process economics, and reduce CO₂ emissions, even if the harvested biomass is used for energy or other non-food applications.

The main limitation for the use of microalgae for energy production is their rather high cost and significant energy costs for their cultivation and concentration. The main ways to reduce cost are to reduce energy costs (except solar), reduce CO₂ costs and nutrients for their growth. In addition, the use of high-performance photobioreactors and systems based on them will also contribute to reducing the cost of microalgae.

The integration of microalgae cultivation into the structure of biomethane projects can be considered as a feasible alternative. Several factors contribute to this, namely:

1. The presence of a significant amount of CO₂ from biogas enrichment, which in the absence of demand for it can simply be discharged into the atmosphere
2. The presence of digestate, which contains a sufficient amount of macro- and micronutrients for microalgae cultivation
3. The presence of waste heat – digestate heat and, in some cases, heat from cogeneration plants and/or biogas enrichment processes

All of the considered by-products and energy of biomethane plants usually have low demand and, accordingly, price. The use of these products and

energy for microalgae cultivation can be considered as a way to reduce their cost.

From a technological point of view, microalgae cultivation using digestate and concentrated CO₂ is possible. At the same time, the use of digestate will be limited, given the excessive content of certain compounds that may be toxic to the growth of microalgae, as well as due to the shadowing effect of microalgae growth due to the significant turbidity of the digestate.

Given the rather low nitrogen content in the composition of the most common microalgae species in the industry (e.g., *Chlorella*, ...), and therefore the high C:N ratio, the use of microalgae for biogas production is advisable together with crop residues. Given the fact that crop residues require a significant amount of moisture in the wet anaerobic digestion method, the use of microalgae suspension with a content of 0.5%–3% TS may be advisable, which also allows significantly reducing the costs of their concentration.

The cost of selling biomethane from microalgae and the cost of their cultivation are key factors determining the feasibility of this approach. Preliminary analysis shows that one of the feasible ways to reduce the cost of microalgae is to combine the use of high-performance systems with a growth rate of at least 3–5 gTS/L/day, growing microalgae only during the period with the highest solar radiation and air temperatures, which will minimize the cost of electricity and heat from other sources. With current biomethane prices at 90 euros/MWh, the cost of microalgae should not exceed 0.2–0.25 euros/t TS. From the point of view of the implementation of the microalgae cultivation project for biomethane production, such a cost value is currently a rather ambitious goal.

The economic potential, taking into account the feasibility of growing microalgae in Ukrainian conditions and only partial use of this approach at biomethane plants, will be no more than 1 billion m³/year, which, however, is also a significant amount.

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