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MICROALGAE – CULTIVATION AND APPLICATION OF BIOMASS AS A SOURCE OF ENERGY: A REVIEW

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1. INTRODUCTION

1.1. General information

The resources of conventional (fossil) sources of energy that so far have been the motor of technological and civilisation development are shrinking, and some opinions expressed are such that those are the last remnants of what Man could acquire from the ground. However, in the opinion of others (Ragheb 2010, Glasby 2006), conventional fuels flow continuously from the interior of the Earth and their supplies are fundamentally inexhaustible. This view, alas, is overly optimistic since it is known that fossil fuels originate from biomass subjected to anaerobic conditions on the bottom of the oceans and to extreme temperature and pressure over millions of years. The very long waiting time for fossil fuels to be created causes that we classify those fuels as non-renewable. Nevertheless, irrespective of whether the resources of those fuels will be exhausted in the near future or not, it is a fact that their acquisition is more and more difficult and their combustion has a negative effect on the climate and the biosphere. The fundamental method of obtaining energy from organic compounds is their combustion. It causes the breaking of bonds in hydrocarbons and the release of energy and carbon dioxide. Over as short a time as several decades humanity has burned a major part of fuels formed from biomass that absorbed CO_2 and accumulated energy for millions of years. This resulted in an excessive increase of CO_2 concentration in the atmosphere. With relation to this, for decades now researchers have been searching for sources of alternative fuels. The objective of the research is the development of methods of acquisition of ecological fuel, renewable in 100%, whose production and subsequent combustion would be maximally neutral for the environment. One of the few available sources of renewable energy that meet those requirements is fresh biomass, frequently taken to mean plant biomass. Photosynthesising organisms accumulate 407 kJ of energy in each mole of bound carbon (Klass 2004). The “ecological” nature of biofuels consists in the fact that CO_2 released in combustion is absorbed by successive plantings of energy crops – and thus the biogeochemical cycle of carbon in nature is closed and does not threaten with negative climatic consequences. With the help of various chemical reactions and physical factors we can “replace” natural processes and directly utilise the energy potential of biomass. That raw material, due to its rich chemical composition, can be used for the production of various types of gaseous, liquid and solid fuels (Chisti 2007).

To acquire plant biomass, various energy crops have been introduced in cultivation. The ultimate objective of energy crops cultivation is rapid production of large amounts of biomass containing the highest possible peecentage of dry matter with high calorific value. The first ideas concerning the utilisation of biomass for energy production yielded the so-called first-generation biofuels, based on e.g. the acquisition of oil from seeds of oil-bearing plants – rapeseed, soybean, sunflower etc. Since those are edible plants, their cultivation for energy production creates a strong competition with their utilisation for food. In consequence, this has a negative bearing both on the amount of food produced and on the rise of its prices.

Second-generation biofuels largely solve those problems, as in this case the fuel is produced mainly form cellulose, e.g. straw, or from the cultivation of "energy" crops – Virginia mallow, Miscanthus, energy willow, Jerusalem artichoke etc., that are not a source of food for humans or for farm animals. However, removal of residues of those crops from fields causes impoverishment of soil in organic matter, and the "energy" crops compete for arable soil area with crops used for food production. An example here can be the production of ethanol from cellulose of energy plants, being a second-generation biofuel. That technology, however, is still a highly complicated and low-efficiency process, and under the Polish conditions not economically viable. Hence, under our conditions, the production of ethanol with that method is insufficient and cannot keep up with the growing demand for the product. Therefore, the consequences of the production of such fuels are the same as in the case of the first-generation biofuels (Abou-Shanab *et al.* 2010, Gnansounou *et al.* 2009, Oilgae Report 2009, Rosegrant 2008).

The situation is vastly different in the case of production of third-generation biofuels among which we classify fuels produced from algae. Algal cultures are characterised by extraordinary productivity, and they do not compete with agricultural production in terms of food or of area used for crop plant production. At the same time they have a beneficial effect on the natural environment as in algal production municipal sewage can be used as a source of minerals. Moreover, additional benefits result from the fact that algae absorb large amounts of carbon dioxide that is currently generated in excessive levels. The fuel produced from algae is environment-friendly as it is non-toxic and highly biodegradable (Tab.1) (Oilgae Report 2009, Tanner 2009, Schenk *et al.* 2008).

Algae are the simplest and the most widespread autotrophic organisms on the Earth. The number of algal species is still undetermined, but it is estimated at even 10 million, most of which are probably microalgae, i.e. single-celled algae

(Barsanti and Gualtieri 2006). They do not constitute an official taxonomic group, on the contrary – they are a highly diverse, polyphetic group of autotrophic species that includes eukaryotic and well as prokaryotic forms, unicellular and multicellular. Although in terms of evolution algae are precursors and predecessors of green plants, the two groups cannot be linked in any simple way. All algal species are characterised by the process of photosynthesis, and also they store similar storage substances as those stored by higher plants, but here the similarity of algae and plants ends. Algae do not produce specialised tissues like xylem or phloem, nor organs such as leaves or roots. Also the system of reproduction of algae is totally different than that of plants (Lee 2008, Barsanti and Gualtieri 2006, Burlew 1953).

Table 1. Comparison of biodiesel and diesel emissions (Khan *et al.* 2009)

Compound	B100, pure biodiesel	B20, 20% biodiesel + 80% diesel
Unburnt hydrocarbons	–93	–30
Carbon dioxide	–50	–20
Solids	–30	–22
Nox	+13	+2
Sulphates	–100	–20
Polycyclic aromatic hydrocarbons (PAH)	–80	–13
Nitrated PAH	–90	–50
Potential for ozone layer destruction	–50	–10
CO_2	–80	
SO_2	–100	

(–) lower % emission of noxious compounds compared to 100% diesel,
(+) higher % emission of noxious compounds compared to 100% diesel.

Under favourable conditions, algae utilise sunlight very efficiently and double their biomass very rapidly. Cells of certain algal species perform their cellular divisions even every 24-72 hours, and Krzemieniewski *et al.* (2009) report a division rate as fast as every 3.5 hours. Those numbers permit a rapid estimation that under favourable environmental conditions algae can be harvested many times

during a year, and their combined yield can be even 1000-fold greater than from conventional crop cultivations with yield harvested once or twice a year (Tab. 2).

Table 2. Comparison of some sources of biodiesel (Chisti 2007)

Crop	Quantity of oil ($L\ ha^{-1}\ year^{-1}$)	Area required to meet 50% of US demand for transport fuels (mln ha)
Maize	172	1540
Soybean	446	594
Coconut	2689	99
Palm oil	5950	45
Microalgae (70% of oil in biomass)	136900	2
Microalgae (30% of oil in biomass)	58700	4,5

That high productivity of algae results from their high photosynthetic effectiveness – ca. 5%, whereas plants accumulate only about 1% of solar energy (Klass 2004).

Moreover, algae do not use up the stored energy for e.g. the development of a root system, process of flowering or development of fruits. They use all their energy for the processes of cellular division (growth), which is manifested in the intensive increase of biomass. Since algae live in aquatic or wet environments, their reproductive effectiveness depends primarily on the availability of nutrients, sufficient amonts of CO_2, suitable temperature and large area of exposure to sunlight. Therefore, the latest technologies aim at ensuring such conditions for algae in so-called photobioreactors. The design of photobioreactors permits intensive breeding of algae on relatively very small areas (compared to agricultural plantations), as the adopted solutions provide for a possibility of their vertical structuring in several or even over a dozen stories. Such compact algal farm design will cause that they will require a minimum of land area compared to the area requirements of crop plants to produce a comparable yield volume. Considering the fact that certain algal species can also be applied for food or feed purposes, in these times of overpopulation of the planet this would create a virtually inexhaustible potential for the production of biomass. A distant but promising vision is also the utilisation of oceans or desert areas for the production of algae, those – from the viewpoint of human economy – being still vast non-productive areas.

Another highly positive feature of algal cultures is that they can be situated in industrial zones and algal biomass can be produced using, firstly, contaminated water, industrial and municipal wastewaters and richly fertilised runoff from arable fields, and secondly – chimney gases that emit large amounts of CO_2. Algae are characerised by very rapid growth of biomass, which involves absorption of enormous amounts of biogenic elements and CO_2, which contributes to the protection and purification of the atmospheric air, waters, soil, and the biosphere as a whole (Oilgae Report 2009, Spolaore *et al.* 2006, Klass 2004).

Under optimum culturing conditions, algae multiply very fast but they do not accumulate too much storage materials. Storage substances of algae include e.g. various carbohydrates similar to starch (e.g. laminarin, chrysolaminarin, paramylon, blue-green algal starch) (Lee 2008). They appear in the cells of micro- and macro-algae and can be successfully used for the production of **bioethanol**. Whereas, in unfavourable environmental conditions, under stress, e.g. deficit of nitrogen, phosphrus etc., algal cells accumulate lipids in the form of triacylglycerides. Often droplets of oil are situated close to the mitochondrion (Oilgae Report 2009, Hu *et al.* 2008). This ability relates basically only to microalgae. Lipids in the form of triacylglycerides are an excellent substrate for the production of **biodiesel** (Oilgae Report 2009, Hu *et al.* 2008). Other fuels that can be produced from algae include e.g. **biohydrogen, biomass – for direct combustion**, **biogas** – after fermentation, and other, e.g. **biobutanol** (Fig. 1) (Oilgae Report 2009).

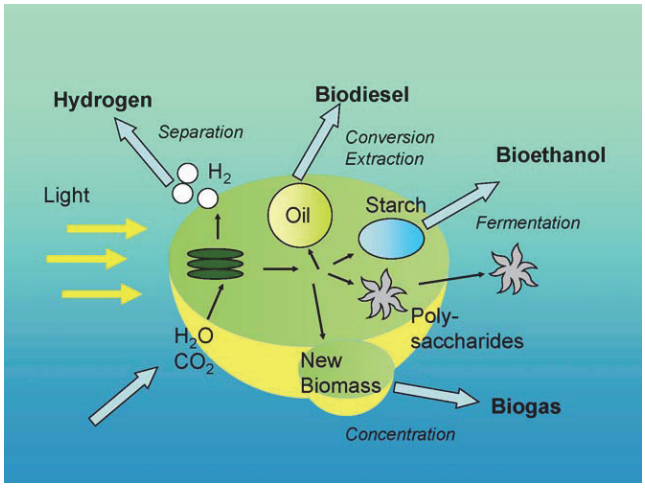


Fig. 1. Potential pathways from microalgae to fuels (Posten and Shaub 2009)

Unfortunately, today the production of fuels from algae is economically non-viable. It is, however, one of very few real possibilities of meeting the world energy requirements in the future. No other method can ensure biomass production at that level of efficiency with simultaneous positive effects on the environment. It is predicted that as soon as within the next 10 years the algal industry will develop and commercialise, giving the world the possibility of using natural, pure, truly green energy (Oilgae Report 2009, Wijffels 2009). Analysis of economic viability of algal production should be complemented, however, with the considerable requirements of algae for CO_2 and with the possibility of their utilisation for the purification of municipal sewage. Those advantages of algae can largely enhance their attractiveness and cause that interest in their production will grow both among the producers of biomass and among industrial plants facing problems with excessive emissions of greenhouse gases.

1.2. History of research on algae

The idea of using algae for human needs is not a new one (Ryan 2009). The first literature references to algae apeared ca. 2500 years ago in China (Tseng 2004). Over the years, studies on the ustilisation of algae expanded from the production of food to the production of a whole range of products: food, feed, fertilisers, cosmetics, dyes, antioxidants, as well as substances used in medical diagnostics or in biological research (Hu 2004).

Algae are also used for water treatment, and in the history there also appeared an idea of sustaining human life in space based on the use of algae (Ryan 2009). In the nearest future, however, algafarming will probably be focused mainly on the production of clean and cheap energy.

The chemical composition of algae is rich and favourable for human and animal nutrition. In numerous studies it has been demonstrated that algal protein is of comparable or even higher quality than that obtained from higher plants. Moreover, algae are a valuable source of vitamins, unsaturated fatty acids and antioxidants (Becker 2004, Spolaore *et al.* 2006, Edwards 2008). No wonder, therefore, that microalgae from the genera *Nostoc*, *Arthrospira platensis* (better known as *Spirulina*), *Aphanizomenon* have been collected for food from natural reservoirs for thousands of years (Jensen *et al.* 2001). 2000 years ago, the Chinese collected *Nostoc* algae at times of famine (Spolaore *et al.* 2006). In the 16^{th} century Spirulina was consumed by the Aztecs in Mexico and by tribes living on Lake Chad in Africa (Hu 2004, van Iersel and Flammini 2010). Macroalgae, also called

seeweed, have been harvested from natural habitats since the year 530. Since 1640 they are cultivated by man and consumed, especially in Asian countries, as sushi or an additive for soups (Pulz and Gross 2004, Edwards 2008). Microalgae, on the other hand, are added to feed for fish, snails, crustaceans and their larvae, and also more and more often to feed for farm animals (Pulz and Gross 2004).

The extensive utilisation of algae results from their great diversity, and thus also diversified chemical composition. The genus *Rhodophyta* produces agar-agar, alginic acid and carrageenins, substances necessary in microbiology and biotechnology. The first production of agar appeared in Japan in 1658. As early as in the 18th century brown algae were used for the production of sodium carbonate and iodine (Pulz and Gross 2004). The first pharmaceutical substance – chlorelline – was extracted from algae of the genus *Chlorella* in Japan during World War Two (Ryan 2009). In the nineteen fifties systematic and in-depth studies on algae were begun in search for high-value substances and proteins as well as antibiotics. Algae proved to be an excellent source of a large number of nutraceutics, pharmaceuticals, antibiotics, and also toxins (Borowitzka 1995, Spolaore *et al.* 2006).

In the nineteen fifties, Oswald *et al.* developed a method of water treatment by means of algae. The idea was well based, as algae produce oxygen, reduce eutrophicity of waters by absorbing biogenic elements, and absorb heavy metals. That method of water purification is effective and it is now extensively used (Oswald 2003).

Between the years 1959 and 1962, Professor Jack Myer and Professor Herb Ward worked on algae for the U.S. Air Force's School of Aviation Medicine (SAM) (and later for the National Aeronautics and Space Administration – NASA) in San Antonio, Texas (Ryan 2009). Their objective was to develop a system that would be capable of sustaining human life indefinitely using solar energy alone (Oswald 2003). They designed the "Algatron" – the most efficient photobioreactor ever created. It was calculated that three "Algatrons" can supply one person with 1600 kcal per day, and at the same time produce oxygen and purify water. Unfortunately, the experience gained from the project was never utilised outside of the laboratory (Ryan 2009, Oswald 2003).

The first "plantations" of algae were experimented with by Ferdinand Cohn. In 1850 he maintained for a certain time a monoculture of the species *Haematococcus* (*Chlorophyceae*) in his laboratory in Breslau (now Wrocław) and called the process a "plantation" (Preisig and Andersen 2005). In 1890, Beijerinck acquired monocultures of *Chlorella vulgaris* (Borowitzka 1999). Soon after that, there apeared ideas of creating a large and dense culture of microalgae. In1919,

Warburg (Germany) introduced into his cultures bubbles of air enriched in CO_2 and provided artificial lighting. Since the nineteen forties larger and larger structures were built and tested. Pilot semi-industrial installations were built mainly in the USA, Israel, Japan and in Germany, under the supervision of such people as Myers and Clark, Cook, Witsch, Gummert, Tamiya, Arthur D. Little. Arthur D. Little constructed several pilot photobioreactors on the roof of a building at the Massachusetts Institute of Technology (Preisig and Andersen 2005, Benemann 2008). Although ultimately the experiments of Arthur D. Little were declared to be a failure, his documentation on algae is a foundation of knowledge on the culturing of those organisms till today (Burlew 1953, Ryan 2009).

Nihon Chlorella Inc. was the first company to start producing *Chlorella* on a commercial scale, in 1961 inTokyo. The culturing was conducted in three oval-shaped ponds designed by Prof. Tamiya and occupied the area of 4000 m^2 (Hu 2004). In the nineteen seventies there appeared another algafarm, of the species Spirulina, operated by Sosa Texcoco S.A. in Lake Texcoco in Mexico (Borowitzka 1999). In 1975, Larry Switzer created Earthrise Farms in southern California, the largest Spirulina farm in the world, extending over an area of 44000 m^2 (Ryan 2009, Spolaore *et al.* 2006). A notable part of the world production of the species takes place also in China and in India (Spolaore *et al.* 2006) (Fig. 2). At present only several algal species are produced in the world on a commercial scale, for use in the production of pharmaceuticals, food supplements and animal feed – Spirulina, *Chlorella*, *Dunaliella salina* (used a source of β-carotene), and recently also *Haematococcus pluvialis* (producing a dye and a strong antioxidant, astaxanthin) (Spolaore *et al.* 2006, Lorenz i Cysewski 2000).

In recent years, the world market of microalgae has reached the level of 5000 t of dry matter per year and generates a turnover of $1.25 \cdot 10^9$ USD per year; this does not take into account products obtained from processing of microalgal biomass (Pulz and Gross 2004).

The discovery and the idea that algal biomass can be used as a raw material for the production of biofuels originated from the exeriments of Arthur D. Little from the nineteen fifties. Meier (1955) proposed the production of methane from the carbohydrate fraction of algal cells (Hu 2008). In the early nineteen sixties, William Oswald and Clarence Golueke developed the idea and presented a technical-economic analysis of decomposition of algal biomass produced in large race track-type ponds for methane production (Ryan 2009, Hu 2008). In the nineteen seventies, the cost of fuels began to rise rapidly and thus the interest in the utilisation of microalgae for the production of methane, hydrogen or liquid biofuels

increased as well (Benemann 2008, Hu 2008). That energy crisis led to the creation of the U.S. Department of Energy's Aquatic Species Program (DOE-ASP) (Ryan 2009, Sheenan *et al.* 1996). The ASP program functioned for nearly two decades (from 1978 to 1996 roku), in cooperation with the National Renewable Energy Laboratory (NREL), and during that time many aspects concerning microalgae were investigated, which resulted in the development of the fundamental knowledge on the acquisition of biofuels from algae (Sheenan *et al.* 1996). The studies on the physiology of algae were focused on the capability of manay algal species of induced biosynthesis of lipids under the effect of environmental stresses. In this respect the researchers tended to favour diatoms (*Bacillariophyceae*) and green algae (*Chlorophyceae*) (Hu 2008). The studies were conducted using algal cultivations in open ponds in Roswell, New Mexico, where it was possible to maintain long-term and stable cultures (Hu 2008). One of the conclusions formulated during the program was that it would be necessary to apply genetic modification of algae to induce increased production of triacylglycerides. The first successful genetic transformation of diatoms *Cyclotella cryptica* and *Navicula saprophila* took place in 1994 (Ryan 2009, Hu 2008). Nevertheless, even at the most opimistic assumptions, it was estimated that the cost of producing algal biofuel would be from 1.40 to 4.40 USD per gallon, which means that the proudction would be economically viable only if oil prices were higher than 60 USD per barrel (Oilgae Report 2009, Hu 2008). However, at that time oil prices were much lower, and thus, after spending 25 million dollars, the Clinton administration decided to terminate the ASP program (Oilgae Report 2009).

A Japanese project conducted by the New Energy Development Organisation and Research for Innovative Technology of the Earth (NEDO-RITE Optical Fibre Bioreactor project) revived the interest of researchers in algae. The program began in 1990 and lasted for 10 years, cooperating with 20 private companies, laboratories and research institutions. It is estimated that about 250 million dollars were spent on the program. As opposed to the earlier American program, the Japanese focused on the utilisation of closed photobioreactors and on the production of high-value products such as feed additives, antibiotics, and even paper or concrete admixtures from algae. The arguments supporting such production include e.g. much lower utilisation of land area, greater efficiency, and better control of the conditions. The project involved also the use of artificial lighting by means of fibreoptics. Unfortunately, the program did not end with a success, and NREL even declared NEDO-RITE to have been a "total failure" due to the very high production costs achieved (ca. 1000 dollars per m^2). To summarise, the

NEDO-RITE program, in spite of the huge financial outlays, did not contribute to any notable expansion of knowledge on the acquisition of biofuels from algae (Sheenan *et al.* 1995, Benemann 2008, Ryan 2009).

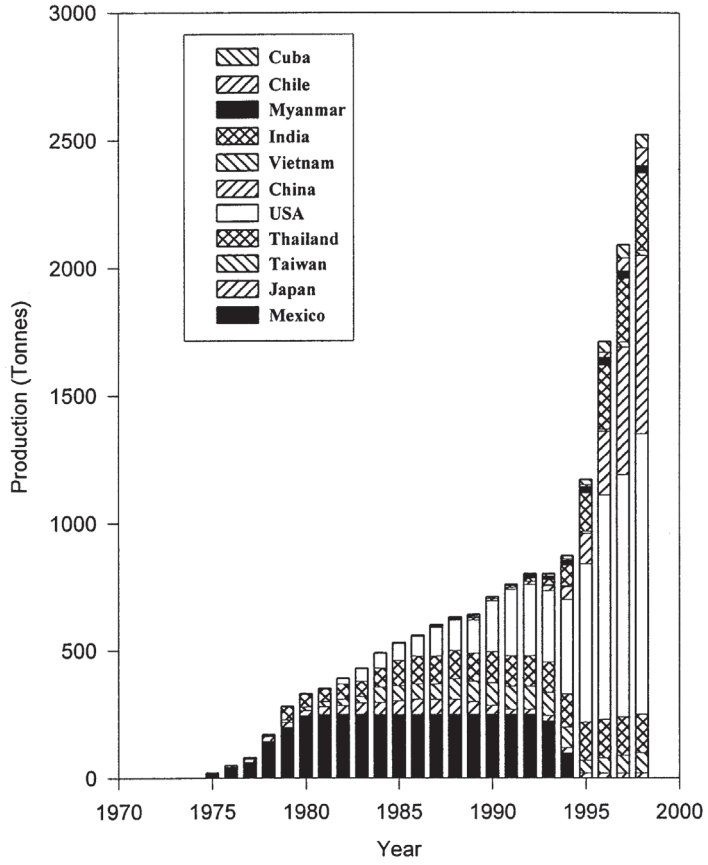


Fig. 2. Global production figures of *Spirulina* by country (Borowitzka 1999)

After the ASP program was terminated, the development in the field of algal biofuels slowed down. However, when oil prices took a significant rise in 2008 and many airlines went bankrupt due thigh fuel costs, biofuels returned to favour. On the 7th of January, 2009, Continental Airlines Inc. conducted a successful test flight of a Boeing 737 fuelled with a mixture of conventional jet fuel and biofuel obtained from algae and jatropha (Ryan 2009).

2. ALGAE

2.1. Systematics of algae

The systematics of algae is specific due to the polyphyletic nature of the group. The primary classification comprises such traits as pigmentation, storage materials, structure of cell wall, flagella and cilia, reproduction. Lee (2008) classifies algal groups with respect to the method of organisation of chloroplasts which can have from two to four membranes seperating them from the cytoplasm. At present the systematic division undergoes modifications under the effect of new discoveries in the field of cell genetics and ultrastructure. The most popular, so far, method of classification of algae is the following (Barsanti and Gualtieri 2006, Lee 2008):

Prokaryotic algae: Cyanophyta (Cyanobacteria, blue-green algae)

Eukaryotic algae: Chlorophyta (green algae) (two chloroplast membranes),
Rhodophyta (red algae) (two membranes),
Glaucophyta (two membranes),
Dinophyta (dinoflagellates) (three membranes),
Euglenophyta (three membranes),
Chlorarachniophyta (four membranes).
Heterokontophyta (four membranes) – this group includes e.g.:
Bacillariophyceae (diatoms),
Phaeophyceae (brown algae),
Chrysophyceae (golden algae),
Xanthophyceae (yellow-green algae),
Cryptophyta (four membranes),
Haptophyta (four membranes).

2.2. Forms of occurrence of algae

Algae assume diverse forms. The most common are unicellular species, the smallest of which have diameters of the order of tenths of a micrometre, and the biggest – up to several tens of micrometres. The individual cells may occur in various forms:

- in the **amoeboid form** – characterised by the lack of cell wall, its motility consisting in cytoplasm flowing to pseudopodia.
- Another motile form is the **flagellate form** – the cells have one or more flagella that ensure their motility; frequently they also have no cell wall, but they can produce other external structures (e.g. *Euglena*). Both of the above groups

include primarily mixotrophic species – during the day they stay in the upper layers of a reservoir and behave like photoautotrophic organisms, and during the night they forage on the bottom of the reservoir and absorb organic matter and biogenic elements. Thanks to those properties such species participate in the microbiological cycle and bring back decayed matter into the food chain.

- Still another form of unicellular specues is the **coccoid form** – the cells are surrounded with typically developed cell wall and have a permanent shape that is not necessarily spherical. Usually they have no capability of active motility (e.g. *Chlorella vulgaris*, photo 1.) (Wayda 2009).

Equally frequently encountered are the **colonial forms** of algae. Colonies are formed when unicellular algae undergo cell division and do not fully separate. Althouth the cells retain their independence, they are usually imbedded in mucilage or linked by means of plasmodesms. Many species form colonies with incidental numbers of lossely connected cells (*Hydrurus, Synura* (*Heterokontophyta*)), but there are also such species whose colonies are well organised and individual cells may be specialised – so-called **coenobia** (*Volvox*, *Pediastrum*). A similar form is the **filamentous colony** in which cells are linked with each other with their cell walls and assume the form of threads or beads (e.g. *Arthrospira* photo 2., *Spirogyra, Ulothrix*, *Oscillatoria, Tolypothrix, Cladophora, Stigonema*). When cells divide but no new cell walls are formed between them, there appears a unicelular organism with multiple nuclei, called a **siphonous** or a coenocytic (e.g. *Vaucheria*).

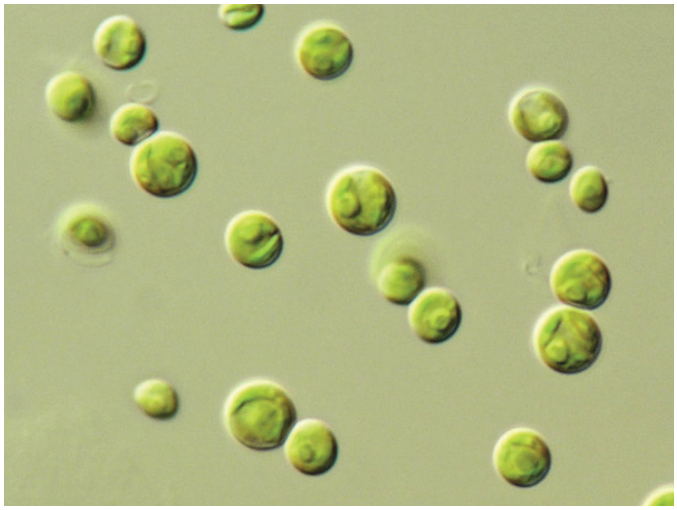


Photo 1. *Chlorella vulgaris* (Internet [1])

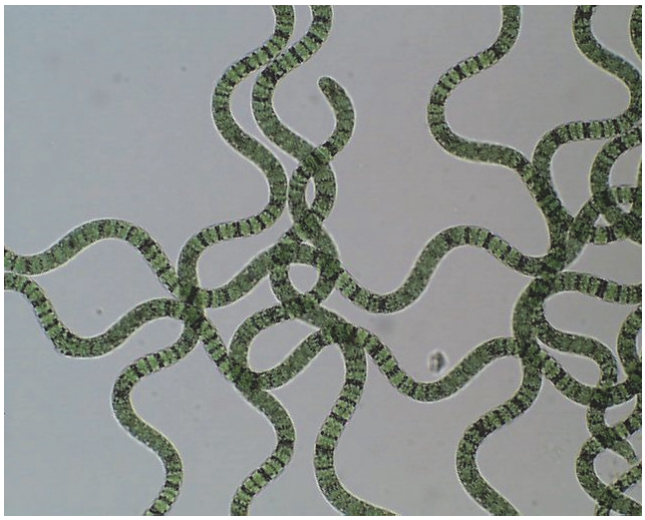


Photo 2. *Arthrospira platensis* (Internet [2])

Photo 3. *Laminaria sp.* (Internet [3])

Macroalgae, commonly called seaweed, assume the form of a simple multicellular organism whose "body" is a non-specialised or weakly specialised **thallus**. In the case of brown algae, for example, the thallus can reach lengths of tens of metres, and its morphology resembles that of the higher plants (e.g. *Fucus*, *Laminaria*, photo 3.) (Wayda 2009, Barsanti and Gualtieri 2006).

2.3. Algal cell structure

2.3.1. General information

The basic cellular structure of algae is usually similar to the structure of a typical plant cell (in eukaryotic algae) or a bacterial cell (in prokaryotic algae). The size of a single cell is usually within the range of 5-50 μm. On their surface algal cells have a negative charge (Shelef *et al.* 1984). The standard cell structures and organelles may, however, differ from the plant or bacterial ones in their shape, size, function or chemical composition. There may also appear structures that ae characteristic only for a given group of algae. Therefore, in this work we have concentrated on organelles with features characteristic for algae alone.

2.3.2. Chloroplasts

From the human point of view, the most important structures in algal cells are the photosynthesising apparatus. They are responsible for the process of photosynthesis, i.e. for the absorption and conversion of energy which is the focus of our interest. The photosythesising apparatus of prokaryotic algae are free thylakoids, embedded directly in the cytoplasm, most often concentrated peripherically in several layers parallel to the cell wall, though that is not the only possible arrangement. On the outer side of the thylakoids of cyanobacteria there are "granules", called phycobilisomes, that contain pigments – phycobilins – indirectly participating in the process of photosynthesis – phycocyanin, phycoerythrin and their variants. Phycobilisomes appear also in eukaryotic algae from the groups *Glaucophyta, Rhodophyta* and *Cryptophyta* (Tab. 3).

Eukaryotic algae have special organelles conducting photosynthesis – chloroplasts. Those structures have been formed through evolution, most probably through the absorption of a blue-green alga cell by another cell, which is referred to in the theory of endosymbiosis. Therefore, chloroplasts are semi-autonomous organelles, having their own DNA, ribosomes, and dividing indepndently from the cellular divisions. They are separated from the cytoplasm by two to four membranes (Lee 2008). The number of chloroplasts in a cell, their size and shapes are specific for a given species. As a rule, the shape of a chloroplast is oval, but e.g. algae from the genus *Spirogyra* have chloroplasts that are elongated and twisted. In almost all algal groups the chloroplast contains a pyrenoid. It is a protein structure containing the enzyme RuBisCO, responsible for carbon dioxide fixation during the process of photosynthesis. The pyrenoid may accumulate around itself storage materials, including droplets of oil. A common feature of all groups of algae is that

they contain chlorophylls – most frequently *a*, but sometimes also *b* and *c*. Apart from that, they contain also various forms of carotenoids and xantophylls (fucoxanthin, zeaxanthin, lutein, violaxanthin and others) (Tab. 3). Algae living in the surface layers of water reservoirs contain primarily chlorophyll and assume green colour. In algae living in deep water layers carotenoids and phycobilins dominate. They absorb the sparse solar radiation that penetrates into the depth of water and to which chlorophylls do not react. Those pigments transfer the absorbed energy to chlorophyll which then proceeds to perform the photosynthesis proper (Lee 2008, Barsanti and Gualtieri 2006).

Table 3. Pigments and storage materials of algae (Barsanti and Gualtieri 2006, Czerpak and Czeczuga 1978)

Phylum	Chlorophylls	Phycobilins	Carotenoids	Xantophylls	Storage materials
Cyanophyta	a, b	c-phycoerythrin c-phycocyanin allophycocyanin phycoerythrocyanin	β-carotene	myxoxanthin zeaxanthin	cyanophycin (non-protein polymer of arginine and asparagine) blue-green algae starch
Glaucophyta	a	c-phycocyanin allophycocyanin	β-carotene	zeaxanthin	blue-green algae starch
Rhodophyta	a	r, b-phycoerythrin r-phycocyanin allophycocyanin	α- and β-carotene	lutein	red algae starch
Cryptophyta	a, c	Phycoerythrin-545 r-phycocyanin	α-, β- and ε-carotene	alloxanthin	starch
Heterokontophyta	a, c	–	α-, β- and ε-carotene	fucoxanthin violaxanthin	chrysolaminarin
Haptophyta	a, c	–	α- and β-carotene	fucoxanthin	chrysolaminarin
Dinophyta	a, b, c	–	β-carotene	peridinin fucoxanthin diadinoxanthin dinoxanthin	starch
Euglenophyta	a, b	–	β- and γ-carotene	diadinoxanthin	paramylon
Chlorarachniophyta	a, b	–	–	lutein neoxanthin violaxanthin	paramylon
Chlorophyta	a, b	–	α-, β- and γ-carotene	lutein prasinoxanthin	starch

2.3.3. Stigma

The cells of motile species of algae often have the stigma, i.e. a drop of carotenoids that responds to photo stimuli. Under the effect of light a response of the cell takes place – positive or negative phototaxis (Barsanti and Gualtieri 2006).

2.3.4. External structures

In such a diverse group of organisms there occur various kinds of external cell structures. Their function is to protect the cell from any mechanical, chemical or physical damage, against pathogens, predators or from drying. By definition, therefore, those structures should be strong and reliable, and permeable only in a highly selective manner. From the viewpoint of the utility value of algae as producers of oil for the production of biodiesel or biogas, all structures that inhibit access to the protoplast are undesirable. Therefore, it is worthwhile to analyse them more colsely, so as to be able to overcome those barriers more effectively.

In principle, simple cellular membranes appear only in the gametes and spores of algae. The naked membrane encases the cells only for a short transition time, and at further stages of development various protective structures are formed rapidly. Usually the cells are covered with various cell walls, and additionally they can form mucilage layers or even hard shells. Mucilage covers most algal cells. Mucilage coats can be produced by individual cells, but also by whole colonies of cells that, thanks to the mucilage, are permanently held together. In forms producing filaments, the mucilage forms a kind of "tube" around the filaments. The mucilage is usually colourless and transparent, but it may be coloured either from external substances (e.g. from salts of iron), or from pigments produced by the cell itself. The detailed composition of mucilage has so far been determined only for a few species, e.g. in *Nostoc commune* the mucilage is formed of fibrillar glucans similar to cellulose and of simple sugars, in *Mycrocystis flos-aquae* – galacturonic acid together with pectin-like substances. The mucilage of cyanobacteria has a beneficial effect on the properties of soil – it protects the soil from erosion, enhances water retention and mobility of elements in the soil.

Among the species from the phyla *Heterokontophyta, Haptophyta* and *Chlorophyta* there are such that build various kinds of scale and plates around the cells. Those can be built of organic substances, calcium carbonate or silica. The scales can be arranged at random or in regular patterns. Another kind of such a structure is the shell called lorica – in the form of a pot from which the flaggella emerges. Diatoms (*Bacillariophyceae*) are characterised by a shell saturated with

amorphous silica, with rich ornamentation specific for each species. The unique feature of that group of organisms is that the outer structure consists of so-called bottom and cover. The shapes of the cells and their relief are usually symmetrical, and the symmetry can be radial or linear. Often on the shells there appear structures such as swellings, seams and pores that serve for contact with the external environment, for gas and nutrient exchange, and for excretion of mucilage by means of which diatoms can attach themselves to any surface and which is the means of their motility. Beneath the shell, the cell is additionally protected with coats of mucilage.

Dinoflagellates (*Dinoflagellata*) have, under the cellular membrane, a system of flattened vesicles called the *alveoli*. These are usually arranged in a single row immediately beneath the cellular membrane. The alveoles can contain cellulose plates and form an overlapping armour on the cell. The plates may differ in thickness, ornamentation, and usually they contain pores. Alveoles can also be empty, and then the cells are naked. In such cells the alveoles play a structural function. In many species, beneath the alveole layer there is a layer formed of cellulose and dinosporin, a protein similar to sporopolenin but unique to the dinoflagellates. That layer provides strengthening for the thin cellular membrane with the alveoles, and over time it becomes fossilised.

The typical cell wall appears both in prokaryotic and eukaryotic species. It is defined as a stiff structure encasing the protoplast. In blue-green algae the cell wall is of the Gram (-) type, built of peptidoglycan or of muramic acid and additionally it is covered with a coat of mucilage. The cell wall thickness is most often in the range of 1-10 nm, but in certain species from the genus *Oscillatoria* it may be even 200 nm. The cell wall of eukaryotes is always situated on the outside of the cellular membrane and is comparable with the cell wall of plant cells. Basically it is built of two components – a fibrillar component, forming the scaffolding of the wall, and an amorphous components that forms the filling between the fibres. The fibrillar component is most often cellulose, though it can be substituted with mannan, chitin or xylan (Lee 2008). The cell wall can be adcrusted with silica, calcium carbonate or acetolysis resistant biopolimer. The building of the cell wall is the responsibility of the Golgi apparatus. The cell wall of brown algae and red algae contains the largest amounts of amorphous materials filling the fibrillar scaffolding of the cell walls, and that is why those algal groups are used for the acquisiton of those components on an industrial scale. In red algae the cell wall is built in 70% of water-soluble galactans, such as agar and carrageenans, that have the ability to form gels, and thanks to that they are used in the

food and pharmaceutical industrys. In brown algae, the cell walls and the intercellular spaces contain alginic acid and fucoidan. Salts of alginic acid have emulsifying and stabilising properties.

Euglenoids are characterised by the appearance of the pellicle – a structure that is one of the strongest external structures in microorganisms. In the case of *Euglena gracilis* a pressure of nearly 138,000 hPa is required to crush that structure (Barsanti and Gualtieri 2006).

2.3.5. Structures and cells with special properties

Under unfavourable environmental conditions, especially during drought, algae form special thick-walled spore-type cells, e.g. hypnospores and hypnozygota, statospores and akinetes. Hypnospores and hypnozygota are built *de novo* from the protoplast that emerged from the mother cell wall, and protect the cell againt drought. Statospores are formed within an existing cell, and their cell walls are built of silica. Akinetes are common among blue-green algae. They are large cells encased in thick cell wall that appear under the effect of deficit of biogenic elements or light. They are extraordinarily resistant to desiccation and freezing, and can germinate even after many years of dormancy (Barsanti and Gualtieri 2006).

Heterocysts are specialised cells appearing in blue-green algae and have the ability of assimilation of atmospheric nitrogen. They have thick cell walls, thinned cytoplasm, and contain cyanophycin as storage material. The assimilation of nitrogen takes place with the help of the enzyme nitrogenase which is active only under anaerobic conditions. However, blue-green algae cope very well with the separation of nitrogenase from aerobic processes, i.e. photosynthesis, e.g. through the activation of nitrogenase only in darkness (Tomaselli 2005, Barsanti and Gualtieri 2006).

2.3.6. Genetic material and sexual processes

As concerns the genetic material there are distinct differences between the cells of prokaryotic and eukaryotic algae. Cyanobacteria do not have any typical cell nucleus. Immersed directly in their cytoplasm is a single larger molecule of DNA and numerous plasmids, i.e. smaller molecules of DNA responsible for the formation of gas vacuoles, for resistance to antibiotics and for the production of toxins. Although cyanobacteria reproduce only in the asexual manner, they have the ability of recombination of genetic material through such processes as transformation and conjugation. Transformation takes place when one cell secretes a part of its genetic

material to the environment and another call absorbs it and attaches it to its own DNA. Conjugation is a pseudosexual process in which two cells join with each other by means of pili, i.e. thin hair-like bridges of cytoplasm, and exchange plasmids.

The interphase cell nucleus of eukaryotic algae, the processes that take place in it, karyokinesis or mitosis and cytokinesis, are comparable for the cell nuclei of all eukaryotic cells.

The most frequent method of reproduction of algae is asexual reproduction through simple division of cells, longitudinal or lateral, or through the division of colonies. Numerous simultaneous divisions can take place, that lead to the appearance of a large number of small progenital cells (baeocytes). In *Volvox* or in *Pediastrum*, the successive colonies have the same number of cells as the mother colony. Other kinds of algae, e.g. cyanobacteria, can divide their colonies through special fragments – hormogonia. Another kind of asexual reproduction is the production of various kinds of spores. Zoospores are motile spores, with flagellas, while aplanospores, autospores and hypnospores and non-motile.

Certain species of algae are characterised by sexual reproductive cycles, and the diversity of the types of gametes produced and the reproductive cycles is very extensive. The gametes of two individuals of a single species may not differ from each other at all (isogamy), differ slightly in size (anisogamy) or resemble those of higher organisms – the female gamete is large and non-motile, while the male gamete is small and motile (oogamy). In various species of algae various kinds of generation succession are observed:

- isomorphic – when similarly developed diploid (spore producing) and haploid (gamete producing) organisms appear alternatively,
- heteromorphic – when there is alternation of diploid and haploid organisms, but one or the other are smaller,
- diplontic or gametic life cycle – when the diploid phase is predominant, and gametes are the only form of the haploid stage,
- haploid or zygotic life cycle – when the haploid phase is predominant, and zygote is the only form of the diploid stage,
- three-phase alternation of generations in red algae, with haploid gametophyte, diploid carposporophyte and diploid tetrasporophyte (Barsanti and Gualtieri 2006, Tomaselli 2004).

2.4. Occurrence

It is hard to imagine any water reservoir without any presence of algae. Those organisms are omnipresent – beginning with the oceans, through all fresh water reservoirs, rivers, even puddles, and ending with extreme environments such as hot springs, brines, arctic waters, or strongly polluted waters. They are also abundant in land ecosystems. Most of algal species are cosmopolitan, and – depending on the climate – the particular varieties and strains of a given species may assume various adaptations and various ranges of tolerance to environmental factors.

Algal species living in the land environment usually absorb carbon dioxide and water directly from the atmosphere. They are strongly exposed to desiccation, excessive levels of light, and to sudden changes of weather. However, they have developed certain defensive mechanisms protecting them against the harmful effect of those factors. Most frequently land-living algae develop mucilage coats that retain water and prevent desiccation of cells. Another strategy protecting against drying is thicknening of the cell wall and transition into resting spore state. There are also species that continue functioning even at low levels of water in the cytoplasm, even as low as several percent. Protection against excessive sunlight is achieved by algae by means of pigments such as astaxanthin or scytonemin. Cells of land algae species produce also large amounts of unsaturated fatty acids which, in turn, provide protection against freezing (Wayda 2009). On land, algae constitute also a component of lichens where, together with fungi, they form symbiotic relations, sometimes referred to as helotic.

However, a majority of algae are aquatic organisms. The waters of the oceans are dominated in particular by the group of diatoms (Sheenan *et al.* 1998). Thanks to their contractile vacuoles, algal cells are frequently capable of adapting, within a broad spectrum, to the level of salinity of the water. Microalgae are passively borne by the water, forming the phytoplankton. Obviously, being photosynthesising organisms, they are most abundant in the euphotic zone of water reservoirs. They stay close to the water surface thanks to various mechanisms – blue-green algae have gas vaculoes that give buoyancy, certain species of algae have flagelles that provide them with motility, other species resist sinking by means of various protrusions and cell shapes, and other still modify their weight by accumulating lipids (Shelef *et al.* 1984). In the littoral and in the shallower reservoirs there are species of algae that settle down or get fixed to the bottom forming the benthos, growing on stones (epilithes) or plants (epiphytes). On the bottoms of deeper reservoirs we encounter species with blue, brown or red colouring, less

frequently green – due to the weak penetration of blue light, absorbed by chlorolhyll, through the water (Wayda 2009). Brown thali form sometimes luxuriant underwater vegetation at notable depths. Red algae were found at the depth of 268 m (Barsanti and Gualtieri 2006). There are also various interrelationships between algae and aquatic animals. In freshwater reservoirs algal cells frequently develop mucilage coating, thanks to which they are not digested in animal organisms. They can live within the bodies of individuals belonging to zooplankton on the basis of commensalism, absorbing carbon dioxide from them. In sea waters algae are true symbionts of coelenterates. They use carbon dioxide produced by coelenterates, while coelenterates absorb oxygen and complex high-carbon organic compounds produced by algae (Wayda 2009).

2.5. Role in nature

Algae play numerous important roles in nature. As the most common autotrophic organisms on Earth they constitute the base of the trophic pyramid of oceanic, see and freshwater ecosystems. They are the food not only for zooplankton, but also for fish and other animals. At the same time, mixotrophic species of algae absorb dead organic matter, bacteria and other small organisms, which permits their inclusion in the cycle of matter – in other words they participate in the microbiological loop. Algae are pioneer organisms which are the first to colonise lands and waters poor in organic matter and create conditions favourable for the development of plants and animals with higher environmental requirements. They create ecosystems e.g. on rocks washed by the waves or situated within the tidal zone (Wayda 2009). Under favourable conditions (in fertile and warm waters) algae reproduce very fast, which in natural reservoirs or ponds is observable as algal blooms (Photo 4). That phenomenon is undesirable for two reasons. Firstly, when a large amout of algae decay simultaneously, the process of decomposition of the biomass uses up the oxygen resources in the water, which in turn results in the death of water animals and zooplankton (Jensen *et al.* 1989). Moreover, in the case of blue-green lagae blooms, the water becomes contaminated with dangerous hepato- and neurotoxins produced by blue-green algae (WHO 2003).

Algae are an important link in the biochemical cycle of various elements. Blue-green algae, as the first autotrophic organisms on Earth, formed the atmosphere of the planet over millions of years. Originally, the Earth was surrounded with a mixture of gases composed of ca. 80% of nitrogen, as much as 10% of carbon dioxide and carbon monoxide, and with no oxygen content whatsoever.

Blue-green algae saturated the atmosphere with oxygen and absorbed carbon dioxide, up to the present levels (78% of nitrogen, 21% of oxygen, 0.03% of carbon dioxide). Also now algae, as the most numerous autotrophic organisms on Earth, absorb as much carbon dioxide as the tropical forests, and produce more than 50% of atmospheric oxygen. Blue-green algae are among those few organisms that have the capability of binding atmospheric nitrogen and are highly effective in separating nitrogenase from aerobic processes.

Photo 4. Algae bloom (Internet [4])

Algal remains, under the effect of catagenesis, transformed into petroleum oil and natural gas that are now used as fuels, and the calcified shells of dead algal cells formed sedimentary rocks which are used e.g. in building. Silica shells of diatoms formed the so-called diatomaceous earth. Algae participate also in the sulphur cycle, marine algae playing in it a particular role. They produce considerable amounts of DMSP (dimethylosulphoniopropionate) from sulphuric amino acids. DMSP is a gas which, in an oxygen atmosphere, becomes degraded and oxidised to sulphur oxides that, in turn, become nuclei for condensation. Thus – indirectly – algae affect also the climate on the Earth (Wayda 2009, Barsanti and Gualtieri 2006).

3. CULTIVATION

3.1. Cultivation conditions

The factors that affect the growth, development and properties of algal cells, apart from the species-related traits, are the following:

- light, including the photoperiod,
- temperature,
- reaction
- nutrient medium, including gaseous CO_2,
- stirring.

3.1.1. Light

From the human point of view, the most important process conducted by algae is photosynthesis. Thanks to that process algae can convert the energy of photons in energy enclosed in the bonds of organic compounds. Photosyntheses is divided into the light reactions (taking place in the presence of light) and the dark reactions (taking place in darkness) (Fig. 3). For correct energy conversion, the cultivation conditions must take into account both phases of photosynthesis.

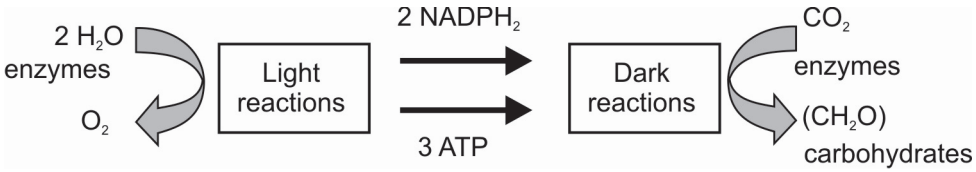


Fig. 3. Major products of the light and dark reactions of photosynthesis (Masojidek *et al.* 2004)

Light is available in varied amounts, depending on the geological region on the globe. Only about 45% of the light radiation spectrum is photosynthetically active (~ 400-700nm) and can thus be used by algae for capturing CO_2 during photosynthesis. Th theoretical efficiency of light energy converion into chemical energy in the process of photosyntheses is 11% (Gao *et al.* 2007). During the night (or under other dark conditions) algae use up the stored energy for respiration. Depending on temperature and other conditions, up to 25% of biomass produced during the day may lost again during the night (Chisti 2007).

Illumination of an algal culture does not automatically mean that photosynthesis proceeds efficiently. If the nutrient medium contains a high concentration of oxygen and low of carbon dioxide, **photorespiration** can take place. It is a process similar to the mitochondrial respiration, but it takes place in the chloroplasts, in the presence of light, and "wastes energy". The cells absorb oxygen and evolve carbon dioxide. Depending on the species, photorespiration may lower the efficiency of photosynthesis by as much as 50%. Therefore, the culture should be artificially supplemented with carbon dioxide and, at the same time, excess of oxygen produced should be taken out. Other factors inducing photorespiration include high temperature and high light intensity (Barsanti and Gualtieri 2006, Masojidek *et al.* 2004).

The relation between the rate of growth of algal cells and the intensity of light is also non-linear (Fig. 4). Up to a certain, relatively low, light intensity the growth of cells is directly proportional to increase of light intensity. Most algal species function in that manner up to light intensity of ca. 200 $\mu E\ m^{-2}\ s^{-1}$ (44 $W\ m^{-2}$). That value corresponds to a half of the optimum rate of growth of algal cultures (μ_{max}). At higher light intensity the growth is slower and slower, until it reaches its maximum value. Still higher light intensity causes **photoinhibition**, i.e. a decrease of productivity. This phenomenon is caused by excessive excitation and, consequently, destruction of the PSII system. Cultures maintained in suboptimum conditions are particularly exposed to the risk of photoinhibition. Very low or very high temperatures, nitrogen deficit, high concentration of oxygen in the nutrient medium, cause that even relatively weak light may damage the photosynthesising apparatus. Such conditions can easily lead to the occurrence of **photooxidation**, i.e. the destruction of e.g. chloroplast lipids or molecules of chlorophyll by reactive forms of oxygen (Kopcewicz *et al.* 2005). However, it is possible for algal cells to get accustomed to more intensive light, through so-called **photoacclimatisation**. Frequently, the first rapid response to high intensity of light is a change of pH. Various metabolic pathways get activated, e.g. transforming violaxanthin into zeaxanthin, which facilitates the dissipation of energy in the form of heat. Another reaction is the inactivation of PSII. Prolonged exposure to strong light results in a reduction or diminution of antenna systems and an increase of the content of carotenoids. In such a situation, carotenoids accumulate outside of the chloroplast, do not transfer energy to chlorophyll, and form a protective layer. A reverse situation takes place under weak light conditions – then the number and size of antenna systems increase. When a culture is planned in regions of the world with high insolation levels, it is advisable to

search for species with naturally small antenna systems (Masojidek *et al.* 2004, Vonshak and Torzillo 2004, Chisti 2007).

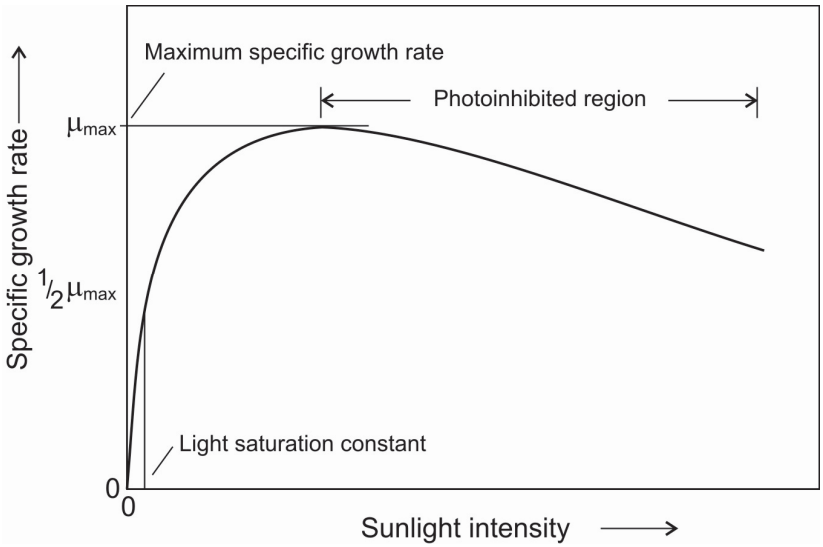


Fig. 4. Effects of light intensity on specific growth rate of microalgae (Chisti 2007)

Studies conducted by Hobson *et al.* (1979) on the species *Isochrysis galbana* demonstrated that the photoperiod directly regulates the rate of divisions. When the duration of exposure to light is short (6 h), the level of chlorophyll in the cell increases, but biomass growth is low, and there are also significant losses due to respiration taking place in the dark. With an excessively long light period (18 h) the amount of chlorophyll a in the cell is low, and biomass growth is also small due to the possibility of occurrence of the process of photorespiration mentioned above, or of inhibition of enzymes controlling the absorbtion of CO_2. On the other hand, Bouterfas *et al.* (2006) demonsrated that in three species, with illumination of 300 μmol m^{-2} s^{-1}, the rate of cell division increased with extension of the light period, up to continuous exposure to light for 24 hours a day. This was probably related to the conditions under which the cells had lived prior to the study.

Light is absorbed by algae, but with increase in their density in a culturing container that absorbtion gets weaker. For this reason all algae cultivation systems are shallow and optimised for achieving the best possible transparency. An important aspect is the ensure stable and repeatable illumination conditions for an algal

culture. That problem is best solved by the use of photobioreactors which provide much greater possibility of controlling the culturing conditions than is the case with open ponds.

There are number of light sources that can be used to supply light energy required to maintain photosynthesis:

- fluorescent tubes,
- LEDs (light-emitting diodes),
- natural sunlight.

If an algae culture is very dense, arificial light can be delivered to a photobioreactor by means of diffusers or fibreoptics (Oilgae Report 2009).

3.1.2. Temperature

For most algal species the temperature range that is suitable for effective culturing is from 20 to even 40^{o}C (Goldman and Carpenter 1974).

3.1.3. pH

The most optimum pH for the cultivation of many algal species is 7-9 (Oilgae Report 2009). An experiment conducted by Azov (1982) demonstrated that the highest levels of dry matter production were achieved from cultures maintained at pH 7.5. During the growth of a culture the reaction of the environment is changing – due to the absorption of CO_2 in the process of photosynthesis pH increases and can reach very high values, even up to 11. This should be kept in mind and pH should be maintained at a stable level, e.g. through supplementation with CO_2.

3.1.4. Nutrient medium

The nutrient medium is defined as a mixture of various nutrient substances (organic compounds and mineral components) used in the culturing of specific organisms. Such nurient media can be classified in three groups:

- Enriched – providing the best effects of mulitplication of algal biomass due to their rich composition of nutrients, e.g. Knop's medium (Tabs. 4, 5),
- Minimal – containing only such elements that are necessary to sustain the basic physiological functions (Tab. 6),
- Simple – containing a larger amount of nutrients, but guaranteeing only the growth of less demanding organisms.

Table 4. Knop's medium

KNO_3	Potassium nitrate	0.5 g l^{-1}
$Ca(N0_3)_2 \cdot 4H_2O$	Calcium nitrate 4-hydrate	0.5 g l^{-1}
KH_2PO_4	Potassium dihydrogen phosphate	0.2 g l^{-1}
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate 7-hydrate	0.15 g l^{-1}
$FeC1_3 \cdot 6H_2O$	Iron(III)chloride 6-hydrate	0.01 g l^{-1}
H_3B0_3	Boric acid	0.003 g l^{-1}
$MnCl_2 \cdot 4H_20$	Manganese(II) chloride 4-hydrate	0.002 g l^{-1}
NH_4VO_3	Ammonium metavanadate	0.0003 g l^{-1}
$ZnSO_4 \cdot 7H_2O$	Zinc sulphate 7-hydrate	0.0002 g l^{-1}
$(NH_4)_6Mo_70_{24} \cdot 4H_2O$	Ammonium molybdate 4-hydrate	0.0001 g l^{-1}

Table 5. Commercial medium

KNO_3	Potassium nitrate	10 g l^{-1}
$Ca(N0_3)_2 \cdot 4H_2O$	Calcium nitrate 4-hydrate	0.5 g l^{-1}
KH_2PO_4	Potassium dihydrogen phosphate	1 g l^{-1}
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate 7-hydrate	1 g l^{-1}
$FeSO_4 \cdot 7H_2O$	Iron(III)sulphate 7-hydrate	0.7 g l^{-1}
H_3B0_3	Boric acid	2 g l^{-1}
$MnSO_4 \cdot 4H_2O$	Manganese(II) sulphate 4-hydrate	1 g l^{-1}
$CuSO_4 \cdot 5H_2O$	Copper sulphate 5-hydrate	0.005 g l^{-1}
$ZnSO_4 \cdot 7H_2O$	Zinc sulphate 7-hydrate	1 g l^{-1}

Table 6. Baslerowa-Dvorakova medium

KNO_3	Potassium nitrate	0.1 g l^{-1}
KH_2PO_4	Potassium dihydrogen phosphate	0.01 g l^{-1}
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate 7-hydrate	0.01 g l^{-1}
$FeC1_3 \cdot 6H_2O$	Iron(III)chloride 6-hydrate	0.001 g l^{-1}

During the selection or composition of a nutrient medium for a given algal species attention should be paid to the following elements:

1. Total content of salt (salinity) and the related osmotic pressure,
2. Chemical composition of cell, taking into account the main macroelements,
3. Sources of nitrogen (nitrates, ammonia, urea),
4. Sources of carbon (organic – HCO^-_3 or inorganic – CO_2),
5. pH,
6. Micro- and ultraelements, EDTA, vitamins (Grobbelaar 2004).

In 99.9% algae biomasss is constituted by six main elements: carbon (C), oxygen (O), hydrogen (H), nitrogen (N), sulphur (S) and phosphorus (P). Calcium (Ca), potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg), iron (Fe) and silicon (Si) appear in smaller amounts. Other elements occur in trace amounts as they are only used in catalytic reactions (Barsanti and Gualtieri 2006). The nutrient requirements of algae are similar to those of higher plants. For their functioning algae use about 30 elements and compounds, but most recipes for nutrient media for algae contain only a part of those. With relation to the species, method of culturing and the purpose for which biomass in produced, different nutrient media are required. This can be best exemplified by the cultivation of *Dunaliella salina* from which at present mainly β-carotene is obtained. The first stage of the cultivation is fast multiplication of biomass, and for that purpose a nutrient medium with a high level of nitrogen and low salinity is required. In the second stage the cells are subjected to stress related with the use of another nutrient medium, with a low content of nitrogen and high salinity. That stress causes that the algae produce large amounts of the carotenoid required (Grobbelaar 2004).

Carbon

Carbon is the most important element absorbed by algae. It constitutes 46-50% of dry biomass (Schulz 2006, Grobbelaar 2004, Doucha *et al.* 2005). Depending on the manner of nutrition of particular algal species, carbon is supplied in gaseous or organic form. Photoautotrophic organisms absorb that element in the form of gaseous carbon dioxide and build it into hydrocarbons on the pathway of photosynthesis. Mixotrophic or heterotrophic algae absorb – instead of CO_2 – ready organic substances, most often simple sugars, e.g. glucose, organic acids (e,g, acetic acid, lactic acid), alcohols or amino acids (Lee 2004). It has been demonstrated that in the case of *Chlorella* sp. mixotrophic nutrition is a more effective method of cultivation than hetero- or autotrophic nutrition alone (Lalucat *et al.* 1984).

The cultivation of microalgae for energy generation should be based on photoautotrophic nutrition, even if only for ecological reasons. Those organisms are capable of absorbing 1.65-1.83 g of carbon dioxide per 1 g of biomass produced (Doucha *et al.* 2005), contributing to a considerable reduction of the content of that greenhouse gas in the atmosphere. In spite of the good water solubility of CO_2, the low content of the gas in atmospheric air (0.03%) does not ensure optimum growth of algal biomass (Grobbelaar 2004). Therefore, in algal cultures pumping of carbon dioxide is applied, at concentrations from several to even several dozen percent (Ono and Cuello 2003). During the culturing, carbon dioxide is quickly dissolved in the nutrient medium and assumes the forms of CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}. Carbon is then absorbed by algae in the gaseous form, in the following reactions:

$$2HCO_3^- \Leftrightarrow CO_3^{2-} + H_2O + CO_2$$
$$HCO_3^- \Leftrightarrow CO_2 + OH^-$$
$$CO_3^{2-} + H_2O \Leftrightarrow CO_2 + OH^-$$

As we can see, an excessive level of OH^- groups appears in the solution, which causes an increase of the nutrient medium pH, frequently to the value of pH 11. The best method of correction and maintenance of stable reaction is to provide a continuous supply of CO_2 to the culture, which will rapidly acidify the environment. A similar effect can be achieved through the addition of acetic acid (Grobbelaar 2004). The pumping of gas causes not only its better dissolution in the nutrient medium, but it also prevents the sedimentation of algal cells through evoking a stirring effect in the culturing reservoir.

Nitrogen

This element can constitute 10% or more of dry matter of albal biomass, depending on the species, culturing conditions and its availability for the cells. Nitrogen deficit is usually manifested by a change in the culture coloration to yellow-orange (due to a drop in the level of chlorophyll and an increase in the content of secondary carotenoids) and by the accumulation of organic compounds – e.g. carbohydrates or lipids (Grobbelaar 2004).

Nitrogen can be supplied to algae in the form of:

- nitrates (NO_3^-), nitrites (NO_2^-) – when nitrogen is absorbed from such a form the pH of the nutrient medium increases,

- ammonium (NH_4^+) – duing nitrogen absorption from such a form the pH of the nutrient medium decreases due to the evolution of H^+ ions, wich appears to be an ideal solution taking into account the general tendency towards an increase of pH during the growth of a culture; however, there is a risk that ammonium ions will transform into gaseous ammonia which easily escape from the nutrient medium,
- urea – Berman and Chava (1999) demonstrated that various species of algae grow notably better in the presence of urea nitrogen than nitrate or ammonium nitrogen. The reason for that, however, is not fully known. One of the hypotheses is that algae ustilise not only nitrogen but also carbon contained in molecules of urea,
- other organic forms, e.g. amino acids (Flynn and Butler 1986).

All forms of nitrogen are intracellularily decomposed (organic forms) and/or reduced (nitrates, nitrites), and transformed into the ammonium form, and only in that form nitrogen is used for the production of cells' own amino acids and proteins. Therefore, from the energy point of view, the most favourable is direct use of the ammonium form of nitrogen for algae nutrition (Flynn and Butler 1986, Reynolds 2006).

Cyanobacteria have the ability of binding atmospheric nitrogen (N_2) into chemical compounds. In specialised cells – heterocysts – they conduct the following reaction:

$$N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2$$

The reaction is catalysed by the enzyme nitrogenase. For its proper activity, nitrogenase requires anaerobic conditions, therefore the processes of photosynthesis and nitrogen binding must be separated from each other.

Phosphorus

Biomass of microalgae contains up to ca. 1-1.2% of phosphorus in its dry matter (Reynolds 2006). It is primarily a component of nucleic acids, ATP and cell membranes in the form of phospholipids. Algae absorb phosphorus supplied as orthophosphoric ions $H_2PO_4^-$ and HPO_4^{2-} (Oilgae Report 2009). The characteristic fact is that algae absorb that element in the so-called luxury manner, i.e. in largely excessive amounts, store it in their cells and use up when the level of phosphorus in the environment becomes insufficient. The effects of phosphorus deficit are similar to those of nitrogen deficit. However, additionally there appears an inhibition of synthesis of phycobilisomes (Hu 2004a).

Elements such as nitrogen (N) and phosphorus (P) can be supplied to cultures in the form of an agricultural fertiliser, which is simple, easily available, but may notably increase the costs of algae cultivation (Braun and Reith 1993, Chisti 2008). There are several options of cheaper sources of those elements. The literature mentions the use of sewage, e.g. from fisheris, for that purpose (Aresta *et al.* 2005). It also provides the description of a system in which nitrogen and phosphorus are eliminated from sewage from a pigsty (Olguín *et al.* 2003). Another option of obtaining nutrients is their recovery from residues after various technological processes, e.g. anaerobic fermentation (Braun and Reith, 1993) or gasification (Minowa and Sawayama 1999).

An interesting example of nutrient media are the so-called conditioned media, CM. They are formed through the secretion, by a suspension of algae, of organic substances to the substrate on which the coltivation is conducted – that phenomenon is known as conditioning. Nutrient media obtained in this manner have a high content of minerals and organic matter secreted by algal cells, and not as it was thought before produced through the decay or damage to cells. Conditioned media are most frequently used to augment the proliferation of cells in low density cultures, as the initial density of cells has a considerable influence on the rate of growth of plant cells (Schröder and Knoop 1995).

There are numerous reports confirming that conditioned media (CM), obtained after the cultivation of various cells of prokaryotic and eukaryotic microorganisms, display biological activity towards a large number of organisms (Bassler 1999). Microalgae produce bioactive compounds with varied biological activity as well as chemical structure (Borowitzka 1995; Pulz and Gross 2004; Ördög *et al.* 2004; Stirk *et al.* 2002).

Algae, both in cultivation and in natural conditions, produce and secrete diverse organic substances with a low molecular weight that display activity during the reduction of CO_2 in the process of photosynthesis (Fogg 1983). Microalgae produce also large amounts of secondary metabolites, especially under stress conditions, such as limited availability of nitrogen, high salinity, high intensity of light or low temperature (Wijffels 2007). Algae produce, and sometimes also secrete numerous substances that display antioxidant properties, e.g. polyunsaturated fatty acids and proteins (Pulz and Gross 2004).

An addition of CM to an algal cultivation may cause a very strong increase in the size of the cell population. That stimulation can most probably be attributed to regulator substances of universal effect, present in CM media, displaying strong autostimulating properties.

Such a medium, like other culturing media, loses its properties with the passage of time. But the interesting thing is that an addition of a culture of algae that are active and secrete organic substances may cause the preservation of all original parameters of the CM.

A culture augmented in this manner is called a “nurse culture”, and the supporting cells are referred to as “feeder cells”. An additional advantage of nutrient media of this kind is the possibility of their use in relation to an organisms that did not produce the medium, and that efect is called “cross-conditioning”.

In suspension cultures of algae the growth of cell populations takes place through the assimilation of compounds absorbed from the culturing medium. With the passage of time the growth slows down, until at a certain point it ceases due to the exhaustion of nutrients.

3.1.5. Stirring

Stirring time can be defined as the time required to achieve a homogeneous mixture. Stirring is a very important parameter relating to photobioreactors. Its primary function is to maintain algal cells in suspension. It is also responsible for the distribution of nutrients and of heat generated within the reactor, it improves the trasfer of CO_2 to the reactor and mass flow between cells, removes oxygen formed during photosynthesis, facilitates the flow of cells to the best illuminated parts of the photobioreactor, and additionally reduces the risk of occurrence of the process of photoinhibtion (Janvanmardian and Palsson 1991). Studies conducted so far demonstrated differences in biomass growth for stirred and non-stirred cultures maintained under identical conditions. The rate of growth of cells subjected to stirring is in this case greater (Bosca *et al.* 1991). Currently various stirring methods are applied, depending on the species of algae in cultivation and on the cultivation system used. In open ponds paddle-wheels are used, causing the flow of algal suspension (Boussiba *et al.* 1988, Hase *et al.* 2000). In photobioreactors, on the other hand, rotors are employed (Ogbonna *et al.* 1999, Mazzuca, Sobczuk *et al.* 2006), stirring through direct or indirect flow of air through the suspension (Ogbonna and Tanaka 2001), or static stirrers installed inside a system of tubes (Ugwu *et al.* 2002). The growth rate of most photosynthesising cells is very low, therefore for their correct development only a slight amount of stirring is necessary to achieve the required production of biomass. This kind of stirring of algal suspension is ensured by exposing the algae to the effect of sunlight for only 1/10 of the duration of the cultivation. That method additionally causes a faster growth of algae.

3.1.6. Cultivation in sterile conditions

The risk of algal culture contamination by heterotrophic microorganisms is reduced when the nutrient medium does not contain a source of organic carbon. However, in many other objects where photoautotrophic cells are cultivated infection with other photoautotrophs may prove to be a major problem. Therefore, before starting a new culture, the photobioreactor should be sterilised, and all types of photobioreactors should be resistant to the procedures of steam, physical or chemical sterilisation (Oilgae Report 2009).

During cultivation there may appear incteractions between algae and certain bacteria. Bacteria can stimulate the growth of algae through the secretion of vitamins, organic substances that can form chelates, available forms of nitrogen, and may affect the pH and the redox potential of the nutrient medium through the evolution of CO_2. On the other hand, bacteria can also inhibit the growth of algae through competition against algae for elements, secretion of algicides, degradation of polysaccharidde and decomposition of algal cells (Banerjee *et al.* 2002).

3.2. Methods of cultivation

3.2.1. Batch culture

This is the simplest and the most frequently used method of cultivation. The cultivation is conducted in batches. It consists in the inoculation of a portion of algae in a fresh environment, incubation at suitable conditions, and after the algae have grown the cultivation is ended with the harvest of all biomass grown from the medium. The next cultivation is prepared totally from the beginning. That method has numerous advantages, e.g. simplicity of maintenance, possibility of rapid change of species cultivated or of adopted parameters in the new cultivation. At the same time, however, it is also a low-efficiency method. In addition, with that method of cultivation there is a high risk of infection of the culture in the initial stage of growth, when there is a small number of cells in a highly fertile environment which can then be easily dominated e.g. by undesirable other algal species, bacteria, fungi etc. (Lee and Shen 2004, Oilgae Report 2009)

Algal cells introduced into a fresh and fertile environment display a typical kinetics of growth which is composed of six phases (Fig. 5):

1. **Lag phase** – in this phase cells do not multiply; it is a preparatory period during which algae adapt physiologically to cell divisions – e.g. the level of the necessary enzymes increases; the phase can be shorter or longer, depending

on how drastic a change of environment takes place, and on the growth stage of the inoculated cells,

2. **Acceleration phase** – this is a short phase during which cell divisions begin and accelerate to the highest rate of division (shortest cell cycle),
3. **Exponential growth phase** – the rate of divisions is maintained at a constant maximum level, and the number of living cells increases logarithmically; this phase is relatively short due to e.g. the fact that the cells soon begin to overshadow each other and then the rate of growth drops,
4. **Retardation phase** – the rate of divisions decreases, mainly due to the decreasing amount of light penetrating the suspension,
5. **Stationary phase** – the rate of division and the number of cells is maintained at a constant level; the quality of the cells decreases with time, toxic secondary metabolites may appear, and the chemical composition may become poorer,
6. **Decline phase** – the number of cells begins to drop due to e.g. depletion of biogenic elements and other nutrients, excessive changes of pH or infection; cells are virile until they use up their storage materials; sometimes the process of decay of cells is accompanied by the production of various kinds of spores that will germinate as soon as the external conditions improve.

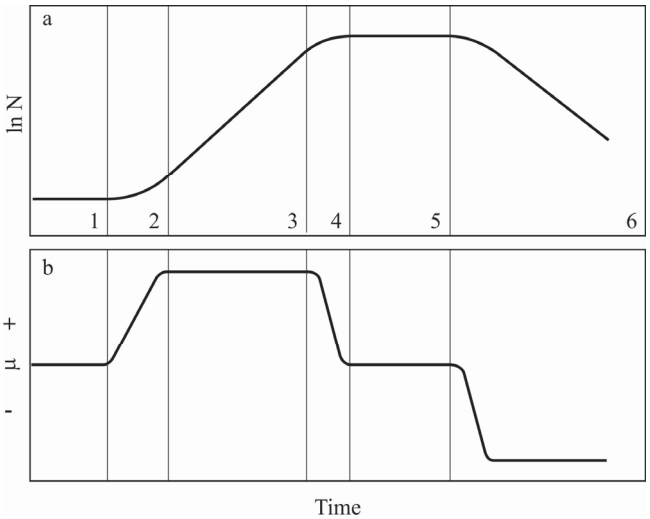


Fig. 5. Growth curve of an algal population under batch culture conditions (a) and corresponding variations of the growth rate (b) (Barsanti and Gualtieri 2006)

The key that will allow algae to be used for continuous production is the permanent maintenance of the culture in the phase of exponential growth (Barsanti and Gualtieri 2006, Lee and Shen 2004).

3.2.2. Continuous culture

This method of cultivation consists in continuous harvesting of a part of grown cells and addition of small amounts of fresh nutrient medium. The chemcial environment, the day and night cycles and the culture density are maintained at a constant level. In this manner we achieve a culture that is permanently maintained in the phase of exponential growth and that can be supported for much longer than a non-continuous culture. However, the cultivation cannot be continued infinitely. Over time there may appear bacterial or fungal infestation, there may also appear zooplankton feeding on algal cells. Moreover, in the course of the cultivation secondary metabolites and growth inhibitors produced by the algae themselves accumulate in the medium. That is the defense of algae against excessive culture density, and in intensive large-scale cultivations it may constitute an important factor limiting the growth (Richmond 2000, Lee and Shen 2004, Oilgae Report 2009).

4. CHOICE OF SPECIES FOR CULTIVATION

The group of green algae is related the closest with the higher plants and species from that group are the most frequently studied and recommended for cultivation. At the sime time, however, e.g. NREL (National Renewable Energy Laboratory in USA) favours diatoms (Sheenan *et al.* 1998), and for years now *Spriulina*, a species of blue-green algae, has been in cultivation (Oswald 2003).

Most of the species of algae are common all over the globe, but always there is certain local occurrence of characteristic varieties and strains of a given species. They are adapted to the conditions of their region, and therefore may display unique traits, e.g. in their metabolism (US DOE 2010). Therefore, it is advisable to collect, isolate and select strains from e.g. extreme environments, as they may be naturally adapted to the cultivation that we plan to operate (Mata *et al.* 2010). The more so if we plan to conduct algae cultivation in open ponds it is important that the species to be cultivated is adapted to the local climatic and atmospheric conditions etc.

The choice of species for cultivation is the key moment, as the traits of a given organism will have an effect on every stage of cultivation and on further processes involved in the production of biofuel. Even before we select a specific species for cultivation, it is necessary to decide what kind of fuel we want to ob-

tain from the biomass produced. Depending on the type of fuel we should search for algal species with specific chemical composition – e.g. if our target is biodiesel we should search for a species that produces large amounts of lipids. The succession of criteria used in the choice of a suitable algal species is, therefore, as follows:

1. Making the decision on what type of fuel we want to produce,
2. Analysis and choice of the most suitable chemical composition of biomass and content of dry matter,
3. Determination of the method of cultivation that will be favourable under the local climatic conditions,
4. Confrontation of nutrient media which can be used with the nutrient requirements of a given species of algae,
5. Rate of growth of the species measured as the amount of biomass produced in time,
6. Range of ecological tolerance of the species (to temperature, pH, salinity, elevated saturation with oxygen),
7. Behaviour in culture with high population density,
8. Structure and size of cell,
9. Possibility of acquiring other valuable substances from a given species (Mata *et al.* 2010).

At present the cultivation of algae solely for energy purposes is not economically viable. Therefore, for a long time still one should search for solutions that would permit the utilisation of a single culture for multiple purposes – e.g. for the production of valuable pharma- and nutraceuticals etc. (item 9.) (Wijffels 2009).

A compilation of desirable traits of algae for mass culture is presented in Table 7 below.

Chemical composition is the key element in choosing the species, and also determines the method of cultivation and the type of fuel to be produced. Species with a high content of lipids are the most suitable for the production of liquid esters and hydrocarbons, and moreover the high calorific value of their biomass permits the acquisition of the largest amounts of methane (Fig. 6). The only drawback of the production of methane from lipids is their long time of degradation compared to other substrates (Tab. 8). A high content of carbohydrates in biomass permits the production of bioethanol (Fig. 7). Cells with a high content of proteins are characterised by the lowest amounts of produced biogas due to greater amounts of emitted NH_3 which can inhibit the process of fermentation (Fig. 8) (Sialve *et al.* 2009, Feinberg 1984).

Table 7. Desirable characteristics of algae for mass culture (US DOE 2010, Griffiths and Harrison 2009)

Feature	Benefit
Fast cell division	Gaining domination in the environment, suppression of competitive species, reduction of culture area
High content of the product of interest	Increased profits, but usually production of valuable substances slows down the growth
Adaptation to extreme conditions	Reduced risk of infection and appearance of predators, but such conditions may be hard to maintain
Resistance to pathogens	Reduced risk of infection
Large cells, forming colonies and filaments	Reduced costs of harvesting and further production processes
Ability of spontaneous flocculation without addition of chemical agents	Facilitates harvesting
Wide range of tolerance to environmental factors, including high intensity of light	Permits less intensive control of conditions and cutivation in various seasons of the year
High tolerance to high levels of CO_2 and ability to absorb large amounts of CO_2	Permits cultivation in the presence of high concentration of CO_2 and its efective sequestration
Resistance to mechanical stress	Permits the use of pumps and various methods of stirring without danger of damage to the cells
Resistance to contaminants	Possibility of cultivation in polluted wastewaters and with the use of exhaust gases containing NO_x and SO_x
Non-secretion of autoinhibiting substances and ability of living in high-density cultures	Greater density of the culture

Table 8. Methane yields for three types of organic compounds and time of hydrolysis (Sialve *et al.* 2009)

Substrate	Methane yield (L CH_4 g s.m.o. $^{-1}$)	Time of hydrolysis (days)
Proteins	0,851	0,43
Lipids	1,014	3,2
Crbohydrates	0,415	0,18

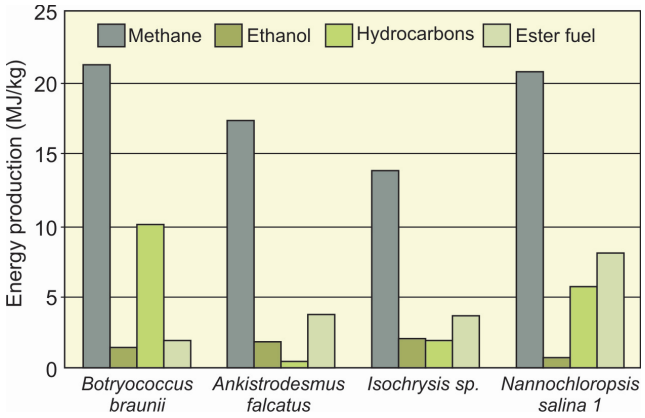


Fig. 6. Fuel production from representative high-lipid producers (Feinberg 1984)

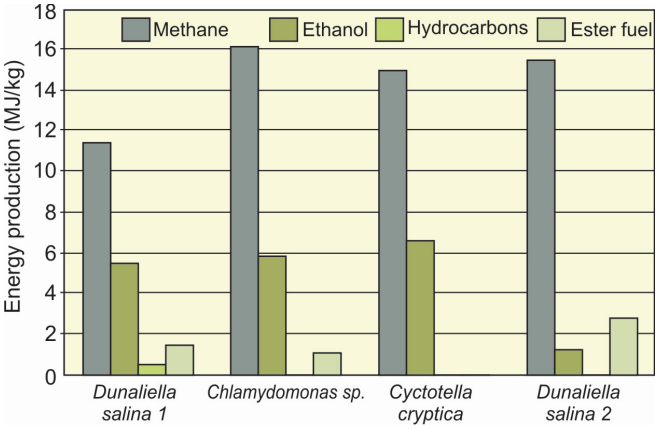


Fig. 7. Fuel production from representative high-carbohydrate producers (Feinberg 1984)

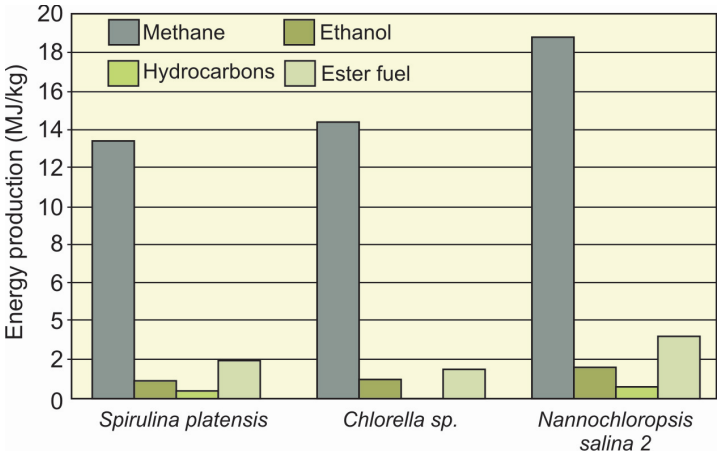


Fig. 8. Fuel production from representative high-protein producers (Feinberg 1984)

The growth rate of a culture is defined as biomass increase in time. Biomass increase is understood as increase in the mass of cells through their cell divisions and denoted as μ. The maximum growth rate is achieved by algae in the phase of the so-called logarithmic growth and it may amount to even 5.65 divisions per day (Goldman and Carpenter 1974). Another way of defining the growth rate is the increase of dry matter yield. Doucha and Livansky (2006) achieved dry mater yield of 4 g m^{-2} per day (*Chlorella sp.*) in their open-pond cultures.

An extremely important parameter that should be taken into account is the culture density that can be achieved. It is expressed in dry matter per litre or per unit of photobioreactor area. Many algal species have the ability of self-control of excessive growth by producing autoinhibiting substances. Whereas, there are also species for which the primary limitation of growth is the availability of light. Doucha and Livansky (2006) observed very high density of culture of the species *Chlorella sp*. in open photobioreactors with depth of 6 and 8 mm – ca. 40 g of dry mater l^{-1}.

Under optimum culture conditions microalgae produce only small amounts of lipids (accounting for 5 – 20% of dry cell matter). Usually those lipids are incorporated in the cellular membranes, chloroplast and plasmatic. They contain esters of glycerol with medium, long and very long molecules of monounsaturated fatty acids. They can also be composed of glycosylglycerides or phosphoglycerides (Hu *et al.* 2004).

Under stress conditions algae cells produce notably greater amounts of lipids (20-50% of dry matter, and even more) (Tab. 9). Stress reactions can be caused by such factors as nitrogen and phosphorus deficit in the nutrient medium, and in

some species – elevated salinity (Mutlu *et al.* 2011, Pick 2002). In such cases an alternative metabolic pathway of lipid production is activated, and as a result lipids are synthesised in the form of storage triacylglycerides. They are stored as droplets of lipids ("lipid bodies") in the cytoplasm or in the chloroplast. A negative effect of cultivation under unfavourable conditions is simultaneous slowing down of the growth rate (Sheenan *et al.* 1998). The species *Botryococcus braunii* is one of few species in which high levels of long-chain hydrocarbons were discovered, amounting to as much as 80% of dry matter. The molecules were similar to those found in petroleum oil. It is probable that it was cells of that algal species that formed a considerable part of todays' oil resources in the world (Hu *et al.* 2004).

4.1. Examples of algal species of interest in terms of cultivation for energy

Botryococcus braunii (Trebouxiophyceae)

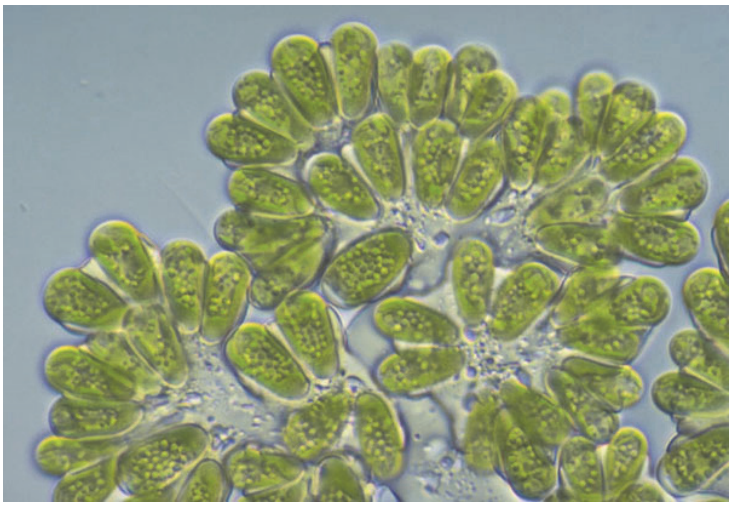


Photo 5. *Botryococcus braunii* with lipid droplets (Internet [12])

Species belonging to the group of green algae (*Chlorophyta*); its name appears in all discussions and studies concerned with biodiesel. It produces hydrocarbons in amounts reaching even 86% of its dry matter. *Botryococcus* most probably accounts for the greater part of all fossil fuel resources. There are three varieties of *B. braunii*, distinguished on the basis of the hydrocarbons they produce – A, B and L.

The cell wall of *B. braunii* is composed of a biopolymer which is resistant to non-oxidative degradation, especially to acetolysis, which may reduce effectiveness in the process of methane fermentation. It is also characterised by fluorescence under the effect of UV. Beneath the cell wall *B. braunii* has an intracellular layer of mucoid polysaccharides, mainly galactose-related, that migrate to the substrate increasing its viscosity. Cells are immersed in that extracellular substance, forming a kind of colonies, which has its advantages as well as disadvantages. Larger cell aggregates mean easier harvesting, but the viscous substance may clogg filters and other equipment elements (Lund University and Statoilhydro 2008, Banerjee *et al.* 2002).

The species is characterised by relatively slow growth (Tab. 9). When *B. braunii* absorbs CO_2 from clean air, the generation time is 6 days, and when a gas mixture is supplied that contains 0.3% CO_2, the growth period is 40 h. Towards the end of the phase of logarithmic growth, the cells contain numerous chloroplasts and their cytoplasm is filled with droplets of lipids (Banerjee *et al.* 2002), even when no stress conditions are applied (Lund University and Statoilhydro 2008). During the cultivation there takes place a change of the colour of the culture due to the accumulation of secondary carotenoids formed under the effect of stress conditions – e.g. nitrogen deficit in the environment or excessive level of illumination. Nitrogen deficit causes degradation of photosynthesising pigments and thus inhibits the process of photosynthesis. Therefore the cell accumulates those substabces as storage material. Production of polysaccharides and lipids is the highest under stress conditions, e.g. lack of nitrogen in the substrate. The species has a unique ability of spontaneous secretion of lipids outside of the cell (Photo 5). When the source of nitrogen is ammonia, pH of the culture drops drastically, causing a reduction of photosynthetic activity and of the production of hydrocarbons, but at the same time there is an increase in the production of amino acids. Utilisation of those latter causes a sudden drop of pH, decrease in the viscosity of the substrate and transition of the culture into a stationary state. Phosphorus often disappears from the substrate already in the initial phase of cultivation and is accumulated by the cells. Even though the level of phosphorus in the substrate is very low, the growth rate of the culture remains high. In the final phase of cultivation phosphorus can be released during the lysis of the cells. The N:P ratio has an effect on the content of lipids in algal cells. High intensity of light causes an increase of the level of carotenoids and affects the colour of the culture. Cells adapted to high intensity of light produce greater amounts of biomass (ca. 7 kg m^{-3}) and a larger amount of hydrocarbons. Such cells are not sensi-

tive to reaction changes within a broad range. During the cultivation an increase of pH is observed, due to the utilisation of CO_2. The optimum temperature is about 25°C. Sodium fluoride enhances the growth of the culture when its concentration is 0.1 g m^{-3} in the medium. *B. braunii* is frequently cultivated in the modified medium Chu –13. Using the modified Prat medium the achieved biomass productivity was 3.9 kg per m^3 during 3-4 days. A mature culture of the species appears on the surface of the reservoir as a bloom that is easy to harvest (Banerjee *et al.* 2002, Oilgae Report 2009, Lund University and Statoilhydro 2002).

Chaetoceros muelleri (Bacillariophyceae)

Species belonging to diatoms. Like all diatoms, it requires silica in the nutrient medium (it is supplied in the form of sodium silicate). Studies conducted by McGinnis *et al.* (1997) and within the ASP program (Sheenan *et al.* 1998) show that the optimum conditions for *C. muelleri* are temperature of 30-35°C and conductivity of 25 mS cm^{-1}. In the phase of logarithmic growth, under such conditions, the growth rate may be even 3-4 divisions per day. This species has a high content of lipids in the cells – under optimum conditions from 66.7 to 73.3 mg l^{-1}, and under stress conditions from 400.1 to 497.8 mg l^{-1} (in terms of percentage values, during a stress period the content of lipids in a cell increases from 19% to 38-39% – ASP subjected the cells to the stress of N and Si deficit).

Chlorella protothecoides (Trebouxiophyceae)

It is a Chlorophyta species which attains high productivity of biomass and lipids under heterotrophic conditions – when it uses glucose or acetates as a source of carbon, and when there is a deficit of nitrogen, the content of lipids in the cells reaches 55.2%, and the production of lipids amounts to 654 mg l^{-1} day^{-1}. Purely autotrophic growth causes a high content of protein in the cells (ca. 54%) and a low level of lipids (ca.14.5%) (Xu *et al.* 2006).

Dunaliella tertiolecta (Chlorophyceae)

Species belonging to green algae, characterised by two flagella, with cells of ca. 10 μm in diameter. It is characterised by a broad range of tolerance to salinity and, therefore, it is possible to safely cultivate the species in open cultures (Tsukahara and Sawayama 2005). It achieves the greatest increase of biomass at a maximum salt concentration of up to 10 g l^{-1} and at light intensity of 300 μmol m^{-2} s^{-1} (Fig. 9).

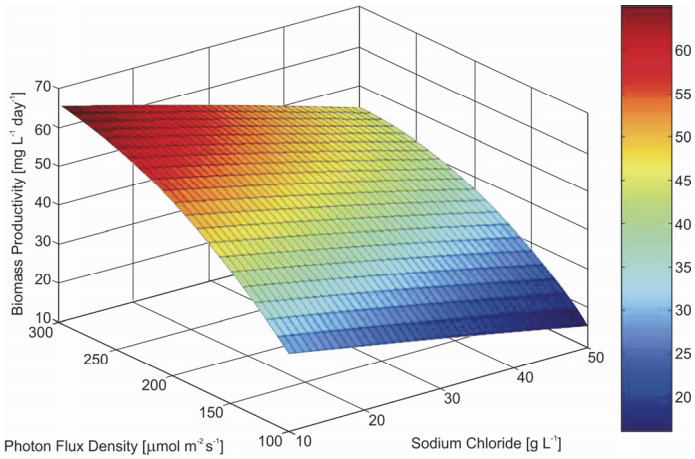


Fig. 9. Evolution of biomass productivity in function of NaCl concentration and illumination (Massart and Hantson 2010)

However, at such a configuration of culture conditions the production of oil is not high. It increases under less favourable conditions – at salinity of about 50 g l^{-1} and less intensive illumination (ca. 200 µmol m^{-2} s^{-1}) (Fig. 10). This is attributed to the production of glycerol as a substance that equalises osmotic pressure within the cell (Massart and Hantson 2010). Detailed carbohydrate composition in the cell of *Dunaliella* indicates that the production of fuel requires catalytic conversion, which is a limitation on the utility value of the species (Sheenan *et al.* 1998).

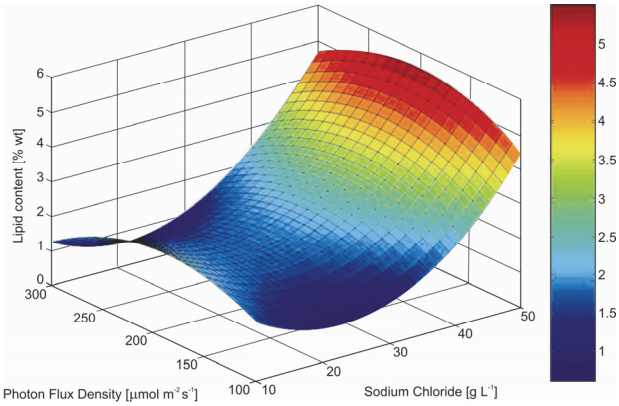


Fig. 10. Evolution of lipid content in function of NaCl concentration and illumination (Massart and Hantson 2010)

Euglena gracilis (Euglenoidea)

Like all euglenoids, this is a mixotrophic species. It lives in both fresh and salt water, as well as in the soil. Cell diameter is ca. 24-35 μm (Chae *et al.* 2006). When the environment becomes too dry, it assumes the resting spore form (develops a thick cell wall) and remains dormant until the conditions improve. The storage materials it produces include lipids (mainly in the form of wax ester, an ester of myristic acid) and carbohydrates (in the form of paramylon – beta-1.3-glucan) (Sheenan *et al.* 1998, Oilgae Report 2009). The optimum culture temperature is ca. 27^{o}C (Oilgae Report 2009, Chae *et al.* 2006). *Euglena gracilis* is adapted to living at high carbon dioxide concentrations (up to 45%) (Ono and Cuello 2003). It has a lipid content of 14-20% in dry matter (van Iersel 2010). Studies conducted by ASP on, among others, *Euglena gracilis* (Sheenan *et al.* 1998) revealed the mechanism of accumulation of lipids in cells of microalgae. In *Euglena gracilis* nitrogen deficit causes chlorophyll losses and thus inhibition of cell divisions. It does not, however, affect the accumulation of lipids. Therefore, the synthesis of lipids continues all the time at a constant level, and only the number of cells steadily decreases. As a result the percentage content of lipids in a single cell increases. The maximum rate of cell division of this species under autotrophic conditions is 0.06 per hour (1.44 division per day) (Chae *et al.* 2006). A higher rate of cell division is observed under mixotrophic conditions and in mixed cultures (Friday *et al.* 2010).

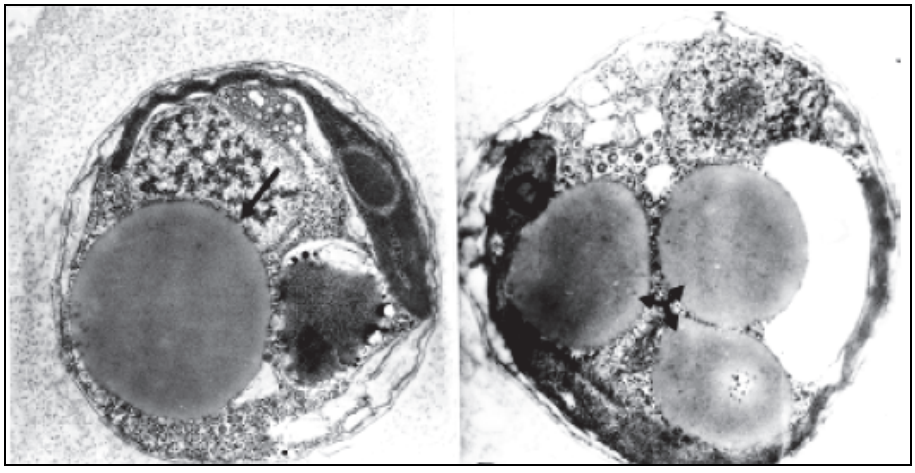


Photo 6. Lipid droplets in *Isochrysis galbana* cells (marked with arrows) (Liu and Lin 2001)

Isochrysis galbana (Prymnesiophyceae)

Golden algae species, with two flagella. It has no typical cell wall, but it is not particularly sensitive to hydrodynamic stress. Cell diameter is ca. 5-6 μm (Falinski 2009, Liu and Lin 2001). Optimum temperature for the species is 27^{o}C. *I. galbana* has a broad range of tolerance with relation to substrate salinity, but it is highly sensitive to iron deficit. In a closed culture, under conditions of nitrogen deficit the content of lipids increases from 24% to 47% of dry matter (Kaplan *et al.* 1986). Lipid droplets are formed within the chloroplast, then migrate to the cytosol and reach the size of up to 3 μm in diameter (Photo 6) (Liu and Lin 2001). The growth rate in the phase of logarithmic growth is ca. 0.0288 h^{-1} (0.69 division per day). The cells are rich in EPA, PUFA and DHA acids and they are used to supplement the diet of zooplankton (diatoms, Artemia); they are also used as feed in hatcheries of small crustaceans, molluscs, shrimp (Falinski 2009).

Nannochloropsis salina (Eustigmatophyceae)

A green algae species with cell diamater of ca. 3.3 μm, with particularly high content of eicosapentaenoic acid (EPA) and violaxanthin. It is used in the cultivation of zooplankton, and is a source of nutrition for corals and other filtrators. It is a species resistant to unstable conditions of temperature, illumination and pH and to high salinity (Sukenik *et al.* 2009, Lund University and Statoilhydro 2008). Under optimum conditions its content of lipids is ca. 28%. Under stress conditions it increases even up to 54%, in which 34.4% is accounted for by fatty acids. Thanks to its favourable chemical composition, it permits the production of greater amounts of energy than in the case of *B. braunii*. Under optimum conditions the species displays a high content of proteins (55.8%) (Feinberg 1984). The similar species *Nannochloropsis oculata* is characterised by the highest growth of biomass when cultivated with the use of an air mixture with 2% content of CO_2; higher levels of carbon dioxide cause inhibition of further growth (Chiu *et al.* 2009).

Neochloris oleoabundans (Chlorophyceae)

Green algae species with a high content of lipids in the cells (ca. 23%, and with nitrogen deficit that value increases to 37-40%) (Pruvost *et al.* 2009, Li *et al.* 2008). Li *et al.* (2008) demonstrate, moreover, that nitrates (e.g. sodium nitrate) are the best sources of nitrogen. The species achieves the highest effects of biomass production at sodium nitrate concentration of 10 mM (0.63 g l^{-1} day^{-1}), and the highest production of lipids at 5 mM (0.133 g l^{-1} day^{-1}).

Phaeodactylum tricornutum (Bacillariophyceae)

A diatom whose genome has been sequenced (Yu *et al.* 2009). The lipid content is relatively low, at 15-20%, maximum up to 30%, but the species displays very rapid growth of biomass, especially under mixotrophic conditions, when glycerol is the source of carbon and urea of nitrogen (up to 1.87 g l^{-1} day^{-1}). Under photoautotrophic conditions productivity at the level of 1.5 g l^{-1} day^{-1} has been achieved, with up to 0.08 divisions per hour, and high photosynthetic effectiveness reaching up to 14% (Fernandez *et al.* 2003). Optimum conditions are pH 7.7, temperature of 21.5-23^{o}C (temperature above 35^{o}C is lethal), oxygen below 400% of saturation (Lund University and Statoilhydro 2008). It is a fresh-water species, and although there is no drastic drop in biomass production during an increase in the level of NaCl, there is a drop in the production of fatty acids. The growth of biomass and the content of fatty acids are positively affected by an admixture of vitamin B_{12}, magnesium sulphate, deficit of silicon, supplementation with CO_2 at concentrations of up to ca. 1% (Yongmanitchai and Ward 1991). Lipids are accumulated only in the stationary phase of the culture (Yu *et al.* 2009)

Pleurochrysis carterae (Prymnesiophyceae)

A unicellular flagellate motile alga that has the ability of forming calcium plates, scales covering the cell. It belongs to the class *Haptophyta*. Its rate of growth at 28^{o}C is 0.55 division per day, and at elevated salinity even 0.92 division per day. The species is characterised by productivity at the level of 0.54-0.63 g l^{-1} day^{-1}, and lipid content in dry matter reaches 33%. Biomass production can be additionally enhanced by an addition of vitamin B_{12}. When the growth rate is high, the volume of the cell decreases (Moheimani 2005) (Fig. 11).

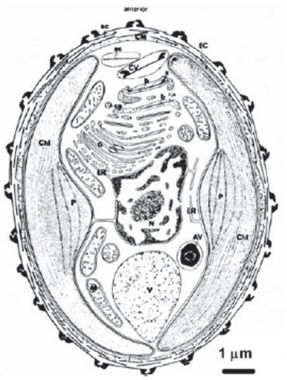


Fig. 11. *Pleurochrysis carterae* cell (Moheimani 2005)

Prymnesium parvum (Prymnesiophyceae)

A unicellular Chrysophyte (Golden alga). It has two flagella that allow its mobility. It produces toxins dangerous to fish, but without any greater effect on humans and cattle. The production of those toxins takes place under physiological stress, e.g. at N or P deficit, and also when there appear variations of tempera-

ture, salinity and insolation. The species grows within salinity range of 0.1-10%, but the most optimum level is between 0.3 and 6%. The species produces dimethylsulphoniopropionate and other unknown polyols, most probably for purposes of osmoregulation. It lives in a broad range of temperatures, 2-30^{o}C, and also of pH – growth has been observed even at pH 5.8, though higher pH values are preferred. It is capable of heterotrophic growth in darkness in the presence of glycerol, it feeds on bacteria (especially with phosphorus deficit it satisfies its demand for that element feeding on bacteria) and on other algae. It can absorb nitrogen from a variety of sources: ammonia, nitrates, amino acids, kreatin, but not from urea. The content of lipids is 22-38%, and the rate fof growth at 27^{o}C amounts to even 0.94 divisions per day (Oilgae Report 2009, Baker *et al.* 2007).

Scenedesmus dimorphus (Chlorophyceae)

A unicellular Chlorophyta species. It is one of the preferred species for the production of biodiesel (lipid content from 16 to as much as 40%). The species has heavy cells that tend to sediment when not in constant motion. Optimumu temperature is 30-35^{o}C. A widespread species (Oilgae Report 2009).

Tetraselmis suecica (Chlorophyceae)

A unicellular marine species with productivity at the level of 0.2 g l^{-1} day^{-1}, which includes ca. 27-32 mg of lipids l^{-1} day^{-1}. The rate growth is ca. 0.39 division per day. What is important, lipids appear in the call already in the phase of logarithmic growth (Montero *et al.* 2010). Under conditions of nitrogen deficit there is an increase in the level of carbohydrates (from 10.7% to 47.1%), and a drop in the content of proteins and lipids (from 67.6% to 28.3% and from 23.1% to 14.6%, respectively), while under combined conditions of N and P deficit the level of lipids increases (from 20% to 40%). The photosynthetic efficiency is 9.1% (Sheenan *et al.* 1998).

Table 9. Lipid content and productivities of different microalgae species (Mata *et al.* 2010, Pruvost *et al*. 2009)

Species	Oil content in d.m. (%)	Biomass production (g/L/day)	Species	Oil content in d.m. (%)	Biomass production (g/L/day)
Botryococcus braunii	25.0-75.0	0.02	*Monallanthus salina*	20.0-22.0	0.08
Chaetoceros muelleri	33.6	0.07	*Nannochloris sp.*	20.0-56.0	0.17-0.51
Chaetoceros calcitrans	14.6-16.4/39.8	0.04	*Nannochloropsis oculata.*	22.7-29.7	0.37-0.48
Chlorella emersonii	25.0-63.0	0.036-0.041	*Nannochloropsis sp.*	12.0-53.0	0.17-1.43
Chlorella protothecoides	14.6-57.8	2.00-7.70	*Neochloris oleoabundans*	29.0-65.0	0,55 - 0,63
Chlorella sorokiniana	19.0-22.0	0.23-1.47	*Nitzschia sp.*	16.0-47.0	–
Chlorella vulgaris	5.0-58.0	0.02-0.20	*Pavlova salina*	30.9	0.14
Chlorella pyrenoidosa	2.0	2.90-3.64	*Pavlova lutheri*	35.5	0.003-1.9
Crypthecodinium cohnii	20.0-51.1	10	*Phaeodactylum tricornutum*	18.0-57.0	0.36-1.50
Dunaliella salina	6.0-25.0	0.22-0.34	*Porphyridium cruentum*	9.0-18.8/60.7	0.004-0.74
Dunaliella primolecta	23.1	0.09	*Scenedesmus obliquus*	11.0-55.0	0.19
Dunaliella tertiolecta	16.7-71.0	0.12	*Scenedesmus quadricauda*	1.9-18.4	0.03-0.26
Euglena gracilis	14.0-20.0	7.70	*Skeletonema costatum*	13.5-51.3	0.06-4.3
Haematococcus pluvialis	25.0	0.05-0.06	*Spirulina platensis*	4.0-16.6	0.21-0.25
Isochrysis galbana	7.0-40.0	0.32-1.60	*Thalassiosira pseudonana*	20.6	0.12-0.32
Monodus subterraneus	16.0	0.19	*Tetraselmis suecica*	8.5-23.0	0.30

5. TYPES OF CULTIVATION SYSTEMS FOR ALGAL BIOMASS

5.1. Introduction

The cultivation of energy crops and the technologies of processing their biomass are a subject that is commonly known and extensively described in literature (Kościk 2003). The production of various types of fuels from algal biomass, however, is based in part on other systems and processes that have not been fully researched and developed. While the commonly used processes related with the processing of plant biomass can be successfully applied in the processing of algal biomass (e.g. transestrification in the production of biodiesel), the earlier stages of the production are a totally different issue. The stages of production of algal biomass and energy comprise the following:

a) Choice and selection of suitable species,
b) Cultivation in special culturing systems, taking into account the specific physiology of algae,
c) Separation from liquid nutrient medium (harvesting),
d) Further processing depending on the purpose of cultivation – dehydration, extraction, conversion.

Due to the specific size of those organisms and their living conditions, their cultivation requires the use of special systems.

5.2. Systems for cultivation of algae

The systems used for the cultivation of algae can be natural or artificial farming ponds or photobioreactors which provide the possibility of controlling all parameters necessary for rapid growth of algal biomass (Fig. 12) (Tab. 10).

The cultivation of algae with the use of natural and artificial open ponds is simple in terms of technology, but not always cheap when we compare the costs of production with the productivity achieved.

5.2.1. Open ponds

The cultivation of algae in open farming ponds is not a new idea. That kind of algae farming has been already well researched, and then developed and used over the recent years (Hase *et al.* 2000, Bousiba *et al.* 1988, Tredici and Materassi 1992). The first attempts at open production of algae were undertaken in Germany during World War II, when they were used as a suplement for the daily diet.

Table 10. Comparison of different culture devices

Parameter/problem	Cultivation pond	Photobioreactor
Required area	Large	Small
Loss of water	Very high, may cause the precipitation of salts	Low
Loss of CO_2	High, depending on depth of the pond	Low
Oxygen concentration	Usually sufficiently low due to continuous spontaneous degassing	Accumulation of O_2 in the closed system requires gas exchange in dedicated devices
Temperature	Highly variable, in certain ponds control possibile through regulation of depth	Cooling frequently required
Stress on cells	Usually low (gentle stirring)	Usually high (fast flow rates required for good stirring of the culture and for gas exchange)
Cleaning	Very simple, non-complicated	Required (contaminations and algal growth on walls limit the acess of light; however, cleaning leads to abrasion of photobioreactor walls)
Risk of contamination	High (limitation of number of species that can be cultivated)	Low
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g l^{-1}	High, usually from 0.5 to 8 g l^{-1}
Flexibility of production	Possibility of cltivation of only a few species, problems in change of species cultivated (contamination of new colony)	High, possibility of frequent change of algal species ultivated
Process control and reproducibility	Limited, regulation of flow rates, mixing and temperature possible only through change in the depth of the pond	Possible under suitable conditions
Dependence on weather conditions	High (intensity of light, temperature, precipitations)	Medium (intensity of light, required cooling)

Start-up cycle	6-8 weeks	2-4 weeks
Investment costs	High, ca. 100 000$ per hectare	Very high, ca. 250 000$-1 000 000$ per hectare
Operation costs	Low	Higher (addition of CO_2, removal of oxygen, cooling, cleaning)
Costs of cultivation	High, depending on species cultivated	Lower due to high concentration of biomass and better control of species cultivated

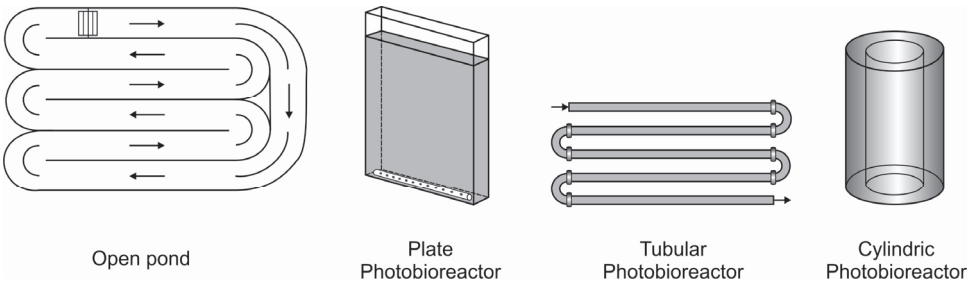


Fig. 12. Various types of algae growing systems (Schenk *et al.* 2008, Sanchez *et al.* 1999)

However, the ponds were used not only to produce food, but also e.g. for the purification of wastewaters. The first country to apply that method of wastewater treatment was the USA. Biomass acquired in that manner was used for the production of methane which was applied as a source of energy (Burlew 1953, Oswald and Golueke 1960). Two types of algae farming ponds are distinguished: natural, among which we include lakes, lagoons and ponds, and artificial ponds or other man-made water reservoirs. As a rule, algae farming is conducted in shallow ponds, artificial reservoirs or in special ponds in the form of a recirculation loop.

An algae farming pond has the form of a recirculation loop (Photo 7). The depth of the channel forming the loop is ca. 0.3 m. Suspension of algae is set in motion by means of a turbine, which eliminates the phenomenon of sedimentation of algae in the reservoir. Such production ponds are lined with white plastic. Their primary advantage is simple design and operation. However, with the use of such systems one should take into account such phenomena as diffusion of CO_2 to the atmosphere, or water evaporation from the pond. Moreover, they require notable area for the cultivation, do not provide the possibility of controlling the culturing

conditions such as temperature or lighting. Algal vegetation depnds largely on the location and, apart from tropical zones, is limited to the warmer months, due to which the production of biomass is not overly effective (Ugwu *et al.* 2007). This type of algae cultivation system is additionally exposed to contamination with other microalgae, algae or bacterial species.

Photo 7. Open ponds (Internet [5])

5.2.2. Closed ponds

This system is, in a way, a combination of open ponds and closed photobioreactors. In this system the open culture of algae is enclosed within a greenhouse structure. Such systems are equipped with filtration devices in which water is purified and then used again. Structures of this kind use biological filters, supplemented with chemical and mechanical methods of purification. There is also a possibility of cultivation of more species than in the open systems, with simultaneous control of their domination in the culturing medium. The system creates fewer problems than the open ponds, it is easier to maintain and operate, and provides greater guarantee of obtaining pure cultures of algae. Another advantage is the possibility of increasing the amounts of carbon dioxide supplied to the cultures, which leads to faster and greater increase of biomass. The costs of building

a closed pond are higher than those of an open pond, but lower than the costs of building a photobioreactor.

5.2.3. Photobioreactors

The necessity of achieving greater productivity and maintaining monoculture of algae have led to the development of closed cultivation systems - photobioreactors. Every process and every living cell requires a different kind of bioreactor (Photos 8, 9, 10). Before designing a new photobioreactor is it required to identify the purpose for which it will be used. Various types of designs present various problems, but at the same time they provide beneficial solutions. At present there are many types and variants of photobioreactors that differ in terms of design, method of illumination or the way of stirring of the culture. Generally, however, we can identify elements common for all kinds of photobioreactors, such as:

- system of illumination,
- optical transfer system,
- ventilation and gas exchange systems,
- stirring system,
- nutrient supply system,
- measurement systems (pH, illumination intensity, temperature),
- electrical system.

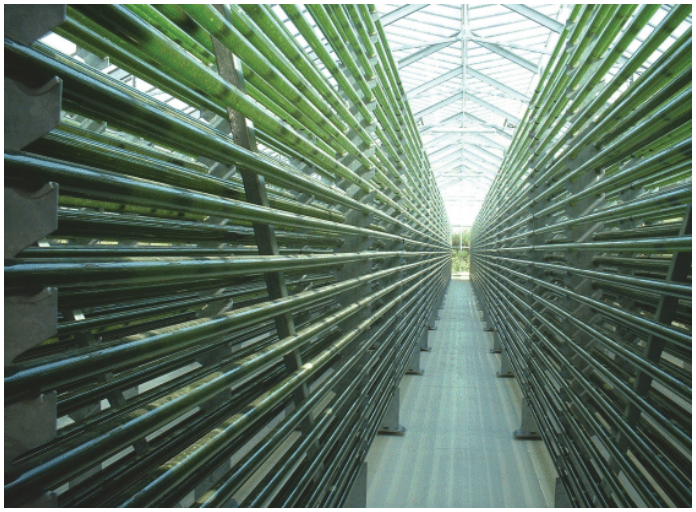


Photo 8. Tubular photobioreactor (Internet [6])

Photo 9. Column photobioreactor (Internet [7])

Photo 10. Flat-panel photobioreactor (Internet [8])

a) Flat panel photobioreactors

As early as 1953 a work was presented that described flat-panel containers as a good system for cultivation of algae (Milner 1953) (Fig. 13). Following that path, in 1985 a photobioreactor equipped with a fluorescent lamp was designed (Samson and Leduy 1985), and a year later an outdoor-use reactor was designed, built of thick-gauge transparent PVC (Ramos de Ortega and Roux 1986). With the passage of time, more and more extensive research on reactors of this type was conducted, and results describing the conditions of culturing of various types of algae were presented in numerous publications (Tredici and Materassi 1992, Hu *et al.* 1996, Zhang *et al.* 2002, Hoekema *et al.* 2002). The primary advantage of flat-panel photobioreactors is the large area of illumination of the culture. Flat panel photobioreactors are usually made of transparent plastics permitting the maximum utilisation of solar energy. Oxygen accumulation in reactors of that type is lower compared to cylindrical photobioreactors. It has also been demonstrated that in flat panel photobioreactors it is possible to achieve highly efficient photosynthesis (Hu *et al.* 1996, Richmond 2000), thanks to which they are highly useful for the production of biomass. Nevertheless, with all of their advantages they also have certain shortcomings that limit their usefulness (Tab. 11)

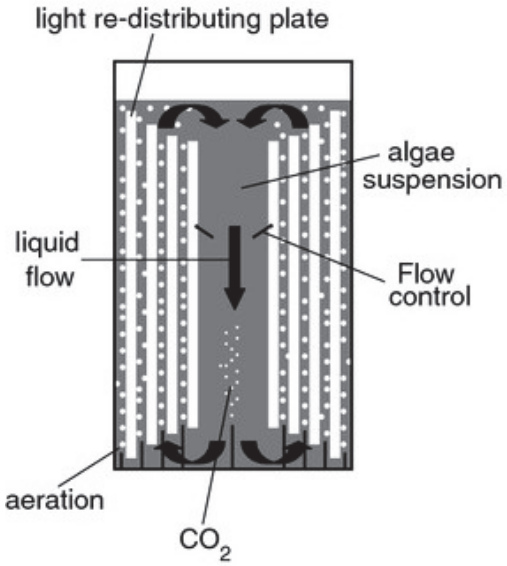


Fig. 13. Schematic of flat-panel photobioreactor (Internet [9])

b) Tubular photobioreactors

This is one of the most suitable types of photobioreactor for outdoor cultivation of algae. Such reactors are usually built of glass or plastic. Biomass contained in the tubes circulates within the system under the effect of air pumps and an air system. The reactors are built in the form of horizontal tubes or serpentines (Chaumont 1988, Molina Grima 2001), vertical tubes (Pirt *et al.* 1983), as well as sloping tubes (Lee and Low 1991, Ugwu 2002). The aeration and stirring of cultures is effected usually by means of air pumps (Fig. 14). One of the fundamental shortcomings of tubular photobioreactors is their low efficiency of biomass production that decreases with increasing scale of the system. Another limitation of biomass production in such system is the phenomenon of photoinhibition (Vonshak and Torzillo 2004) and problms with controlling the temperature of the culture. It is possible to use thermostats to set the required temperature of the biomass produced, but this is a solution that is dificult to apply and costly to operate.

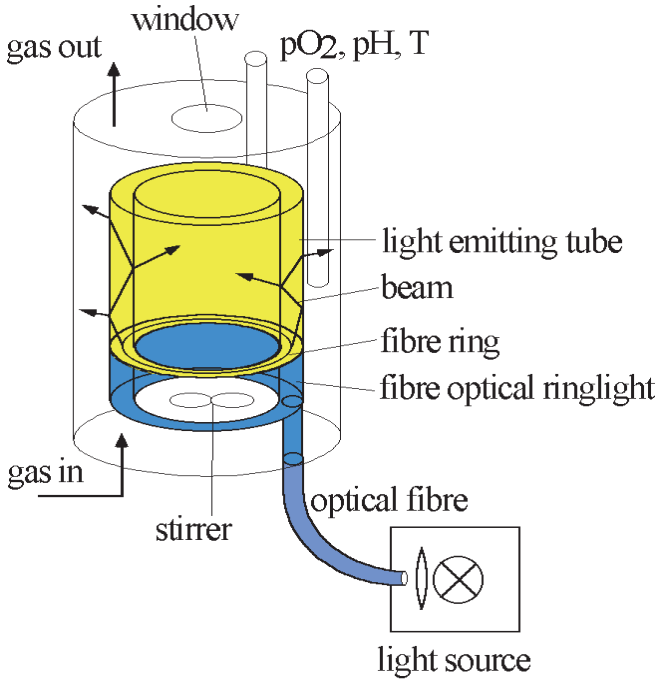


Fig. 14. Schematic of tubular photobioreactor (Internet [10])

c) Cylindrical photobioreactors

Up till now photobioreactors of this type have been designed in various kinds and sizes (Choi *et al.* 2003, Vega-Estrada *et al.* 2005, Garcıa-Malea Lopez *et al.* 2006, Kaewpintong *et al.* 2007). For the purpose of developing outdoor cultivation of algae, extensive research has been conducted on the illumination systems in reactors of this type (Evers 1991, Eriksen *et al.* 1996). Cylindrical photobioreactors occupy a small area, are fairly inexpensive in operation, and relatively easy to maintain (Sanchez Miron *et al.* 2003). They have a simple structure, as they are composed of a glass tube that gives the possibility of illuminating the culture with either natural or artificial light (Fig. 15). The simple internal structure is well adapted to the cultivation of photosynthesising organisms (Katsuda *et al.* 2000), therefore reactors of this type appear to be a good tool for the production of algae at an industrial scale. The limitations involved in the application of cylindrical photobioreactors are presented in Table 11.

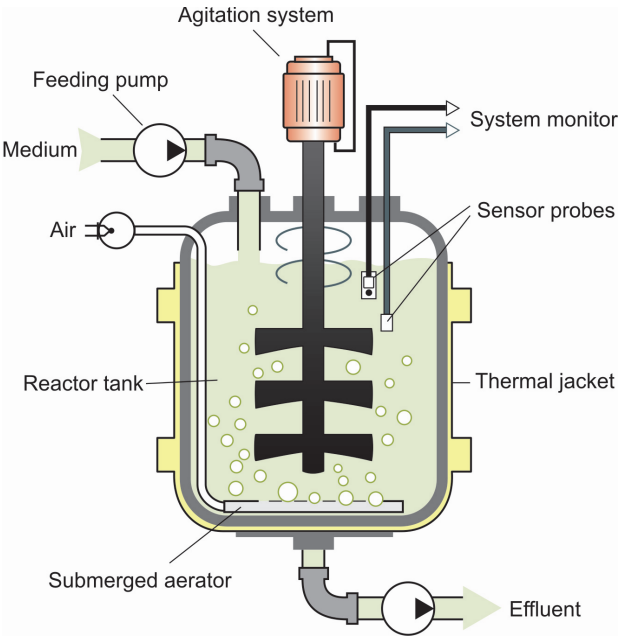


Fig. 15. Schematic of cylindrical photobioreactor (Internet [11])

a) Photobioreactor Sartorius Stedim Biotech BIOSTAT® PBR 2S

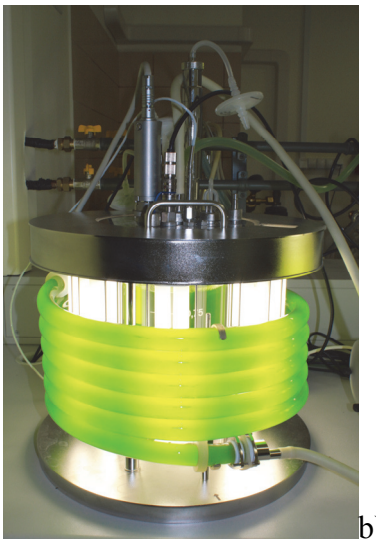
a)  b) 

Photo 11. Laboratory photobioreactor Sartorius Stedim Biotech BIOSTAT® PBR 2S a) cultivation unit, b) control unit

Photobioreactors Sartorius Stedim Biotech BIOSTAT® PBR 2S are the basic equipment of the laboratory dealing with algae at the Institute of Agrophysics, PAS, Lublin. The laboratory photobioreactor with capacity of 3 l permits the cultivation of microalgae under sterile conditions. It is composed of a cultivation module (Photo 11.a) and a control unit (Photo 11.b). The cultivation module consists of an inner container with capacity of 1 l, in which the electrodes and the gauges measuring the culturing parameters are placed, and of a system of glass tubes that permit effective utilisation of light by algal cells. The light is provided by fluorescent tubes and its intensity is fully controllable. The algal culture cultivated in the photobioreactor is in constant motion thanks to the application of a peristaltic pump. Temperature is maintained thanks to a heating water jacket surrounding the inner container. The control unit permits comprehensive control of parameters during the production cycle and maintaining specific values of pH, temperature or oxygen level. The advantage of those devices is the possibility of setting various parameters in so-called cascades, making them dependent on one another, e.g. automatic regulation of the intensity of light in relation to the optical density of the culture or regulation of the flow rate in relation to oxygen content.

The values of the parameters are recorded in time and the data can be exported to other computer programs.

Table 11. Comparison of different algal biomass production systems (Ugwu *et al.* 2007)

System	Advantages	Disadvantages
Open pond	Relatively economical, easy to clean after a cultivation cycle, good for cultivation of algal biomass.	Low level of control of cultivation conditions, hard to maintain over long period of time, poor production of biomass, limited number of cultivated species, large area of cultivation, high risk of contamination of algae.
Flat-panel photobioreactor	Large area and high degree of illumination, suitable for outdoor cultivation, good for immobilisation of algae, high productivity, relatively cheap, easy to clean and conduct cultivation, low increase in oxygen level.	Larger scale production requires many panel modules and support-structure materials, difficult temperature control, walls covered with growth to a certain degree, possibility of appearance of hydrodynamic stress in certain species.
Tubular photobioreactor	Large area of illumination of culture, relatively cheap, suitable for outdoor cultivation, relatively good increase of biomass.	Appearance of pH gradient, oxygen and CO_2 dissolve in the reactor, sludge and algal growth on the walls, requires large area.
Cylindrical photobioreactor	High efficiency of biomass production, good circulation of culture, low strain during stirring, low energy consumption, easy to sterilise, easy to operate, good for immobilisation of algae, reduces the effect of photoinhibition and photooxidation.	Relatively small area of illumination that further decreases with increase of the scale of production, design requires the use of special materials, possibility of appearance of strains within the photobioreactor.

6. METHODS OF HARVESTING OF ALGAL BIOMASS

6.1. Introduction

The next stage of algal biomass production, after the cultivation of a suitable amount of cells, is the harvesting. As opposed to the harvesting of conventional crops, the harvesting of algae is – so far – a much more troublesome and a more expensive process. It is estimated that the cost of this stage of algal biomass production constitutes 20–30% of the total costs (Gudin and Therpenier 1986). The main aspects that differentiate this stage of algal biomass production from crop plants include:

- cultivation in liquid nutrient medium,
- microscopic size of the cells; often the fastest growing species of microalgae are very small and motile, which precludes e.g. simple filtering (Oilgae Report 2009),
- considerable dilution of the culture (from 0.02 % in open ponds 0.5% in tubular bioreactors, which means 1 ton of dry matter of algae per 200–5000 m^3 of nutrient medium) (van Iersel 2009),
- fast growth causes that harvesting can be made almost every day, and not once a year.

The aim of harvesting is the separation of cells from the liquid medium in which the cultivation is conducted (Fig. 16). That process should lead to the obtainment of concentrated suspension with 5-25% content of dry matter (Shelef *et al.* 1984). An excessive content of water in the separated biomass has a negative bearing on the feasibility of further processing, if it turns out that subsequent thermal dehydration is necessary (Molina Grima *et al.* 2003). Taking into account the initial density of the culture, this is an important problem.

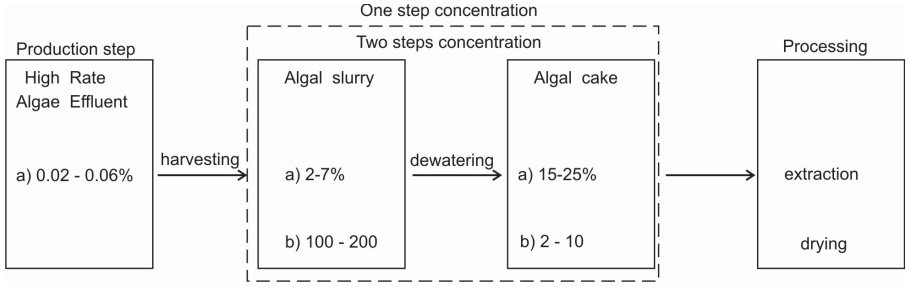


Fig. 16. Steps of algae harvesting; a) algal concentration (% TSS), b) concentration factor (Shelef *et al.* 1984)

The most frequently applied methods of harvesting are the following:

- Centrifuging,
- Filtration,
- Sedimentation,
- Flocculation,
- Flotation.

6.2. Centrifuging

Centrifuging is one of tghe fastest and most efficient methods of biomass separation. However, it requires very high investment in terms of energy and finance. Due to its energy requirements, it can be used only in the production of microalgae for the acquisition of valuable substances, e.g. pharmaceutical, or for feed for aquacultures (Molina Grima *et al.* 2003). Over 95% of biomass can be centrifuged at acceleration of 13000 g, and with decrease in acceleration the rate of recovery of biomass decreases as well (Haesman *et al.* 2000). Speeds that high usually damage the cells, and therefore should not be used in laboratory practice for the maintenance of collections of cultures (Knuckey *et al.* 2006).

6.3. Filtration

Filtration is a method of biomass separation consisting in the application of filters or screens with suitable mesh size, on which the cell are retained while the liquid passes freely. The process be conducted by gravity, under pressure or under vacuum. There are numerous filter designs – disc filters, drum filters, belt presses, microstrainers and other (Shelef *et al.* 1984). The advantage of the method is primarily its low cost and the lack of a need of using chemical reagents (Rossignol *et al.* 1999). However, due to the small sizes of microalgae cells (Chlorella, Dunaliella, Scenedesmus – average of 5–10 μm), it is necessaru to use filters of very fine mesh that are prone to rapid clogging (Shelef *et al.* 1984). Therefore, filtration is the most suitable method for the harvesting of colony-forming algae or of species with exceptionally large cells, e.g. *Arthrospira platensis* or *Coelastrum proboscideum* (Mohn 1980). In a paper by Molina Grima *et al.* (2003) it was proposed to use diatomaceous earth as an auxiliary filtration layer on the filter. Such a solution permits the recovery of even small microalgae cells (e.g. *Dunaliella*). The shortcoming of the method, however, that the recovered material will have a content of the filtration layer.

Filtration is an economical method of microalgae harvesting at a relatively small scale (up to 2000 l per day), whereas on the industrial scale centrifuging is more efficient and more economically viable (Molina Grima *et al.* 2003).

6.4. Sedimentation

Sedimentation permits free deposition of cells on the bottom of the sedimentatikon tank. This is not easy as algal cells are naturally adapted to stay in a suspension close to the surface of the culture reservoir due to the competition for light. We should mention here the electric charge on the cell surface that causes mutual repulsion, and also the fact that algal cells maintain density similar to that of the nutrient medium. Additional mechanisms that passively keep the organisms close to the surface include the formation of gas vacuoles by blue-green algae, active movement, generation of various shapes and sizes of cells. The rate of settlement of cells with spherical shape (e.g. *Chlorella*) is defined by the Stokes' Law (Shelef *et al.* 1984). The method can be applicable for the harvesting of cells with high density, e.g. diatoms which have a silica armour (Oilgae Report 2010). However, sedimentation as a method is neither efficient nor fast. The average concentration that has been achieved in tests amouted to 1.5% total suspended solids (TSS) (Shelef *et al.* 1984). One of the possible ways of making the process of sedimentation more effective is the addition of flocculants.

6.5. Flotation

Flotation is a method of separation which is based on the difference in the affinity of the surfaces of the bodies to be separated to gas or air bubbles. The process causes adhesion of cells to gas bubbles and the formation of froth on the surface of the liquid culture that can be easily harvested (Shelef *et al.* 1984). Flotation can be applied with relation to particles with diameters below 500 μm (Matis *et al.* 1992). To facilitate the separation of biomass with the method, it can also be combined with the addition of flocculants (Shelef *et al.* 1984). Flotation can proceed in various manners, and we distinguish the following, among others:

- Dissolved Air Flotation – consisting in the saturation of the suspension with air under conditions of high pressure, and then subjecting the suspension to the effect of atmospheric pressure; the air bubbles produced have average diameters of 40 μm; in conjunction with flocculation, this method can provide separation of 80-90% of biomass with dry matter content of 6% (Shelef *et al.* 1984, Uduman *et al.* 2010)

- Dispersed Air Flotation – in this method air bubbles have diameters of 700–1500 μm (Uduman *et al.* 2010); Chen *et al.* (1998) demonstrated that in combination with certain surfactants this is a highly efficient method of separation of microalgae; the process is related with the pH of the culture – pH 4 is the value at which the properties of the cell surface change
- Electroflotation – bubbles are generated through electrolysis – chlorine on the anode, and hydrogen on the cathode; in this method constant conductivity should be maintained in the culture at all times; it is possible to obtain biomass with 5% dry matter content (Shelef *et al.* 1984).

In their report, Koopman and Lincoln (1983) also mention autoflotation, i.e. a method consisting in the utilisation of oxygen produced by the algae in the culture as the flotation gas. However, that method can only be applied in situations where the level of saturation with oxygen is not less than 16 mg l^{-1}.

6.6. Flocculation

Flocculation is a method of separation consisting in inducing particles or cells to form larger aggregates (Molina Grima *et al.* 2003). The process facilitates further harvesting through other processes – sedimentation, centrifuging, flotation or filtration (Shelef *et al.* 1984). The essence of the process of flocculation of cells of microalgae is the neutralisation or reduction of the negative charge on the surface of cells that causes their mutual repulsion and maintenance in the state of permanent dispersion in the suspension (Molina Grima *et al.* 2003). There are a number of factors that initiate the process, and they can be divided into the following:

a) inorganic compounds containing polyvalent cations, e.g. Fe^{+3}, Al^{+3} – $FeCl_3$, $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$; for those to have the desired effect, the reaction of the environment must be suitable (usually acid) (Uduman *et al.* 2010, Papazi *et al.* 2010); another kind of an inorganic flocculant is calcium hydroxide ($Ca(OH)_2$) which causes increase of pH to 11, inducing the formation of $Mg(OH)_2$ which has a flocculation effect; the method has its limitations – the concentration of magnesium ions must by higher than 10 mg l^{-1}, and the aggregates formed must have a high content of calcium (Shelef *et al.* 1984). Lee *et al.* (1998) demonstrated that an excellent method of separation of the species *Botryococcus braunii* is just the regulation of pH to the value of 11 (e.g. by adding NaOH), without any adition of specialised chemical flocculants. The advantage of this procedure

is the absence of reagents in the recovered biomass, and moreover the reaction can be safely reversed by adding HCl.

b) organic cationic polymers; apart from the neutralisation of the cell surface charge, polymers physically attach cells to their large molecules through the process of bridging; that process, however, takes place only in environments with salinity not higher than 5 kg m^{-3}; at a high ionic force the polymers assume compact arrangement of molecules which then cannot attach algal cells; the general rule is such that the greater the molecular weight of the polymer the more efficient the flocculation (Molina Grima *et al.* 2003, Uduman *et al.* 2010); an example of an organic flocculant is chitosan – a non-toxic and edible compound.

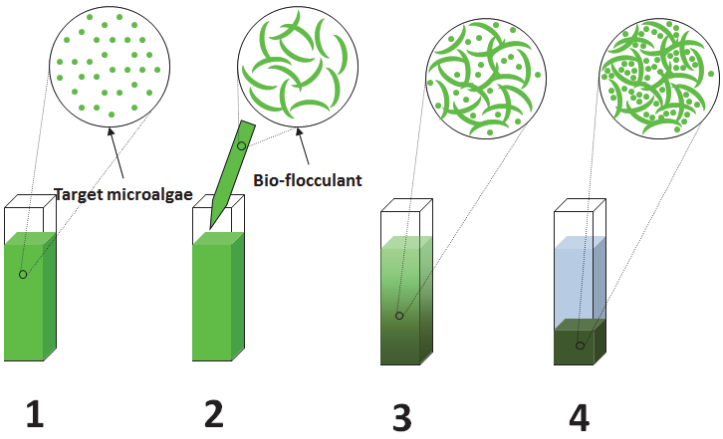


Fig. 17. Bioflocculation (Internet [13])

Apart from flocculation caused by physical and chemical factors, other kinds of flocculation include also autoflocculation and bioflocculation. Autoflocculation is the phenmenon of spotaneous joining of cells under the effect of raised pH which, in turn, is caused by the consumption of CO_2 from the medium by the cells themselves. The necessary factor for that process to take place in an adequate level of orthophosphates and calcium in the nutrient medium (Sukenik and Szelef 1984). Bioflocculation is a process consisting in the joining of algal species (or other organisms, e.g. bacteria) that have the ability of autoflocculation with species that do not have that ability (Fig. 17). The result of that operation is effective flocculation of both species through the retention of cells of the species without the ability of autoflocculation in the aggregates of the other species (Salim *et al.* 2010).

7. ENERGY PRODUCTS

7.1. Conversion of microalgae biomass

Microalgae biomass is one of the potential sources of biofuels. The basic way of utilisation of microalgal biomass is its conversion for energy purposes. The conversion can take place through thermochemical, biochemical and chemical processes, and through direct combustion. The technologies of algal biomass conversion are presented in Figure 18.

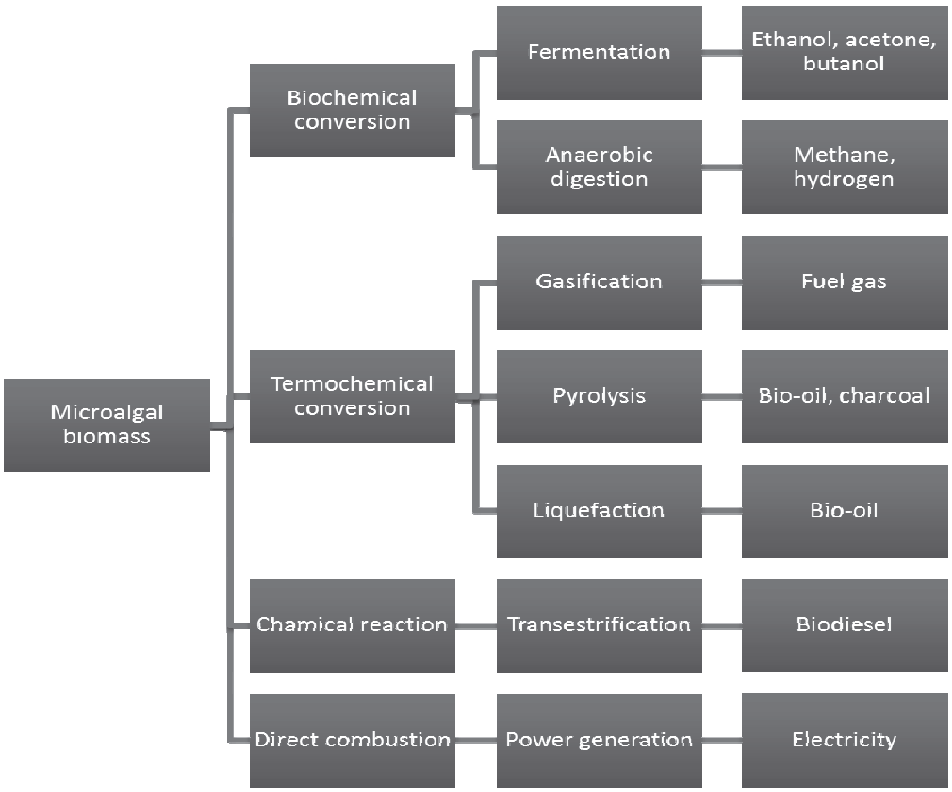


Fig. 18. Microalgal biomass conversion processes (Wang *et al.* 2008)

The processes of thermochemical conversion of microalgal biomass include its gasification, pyrolysis and liquefaction. The products of the process of biochemical conversion can be ethanol, biogas, and of the chemical conversion - biodiesel (Lewandowski 2007).

Pyrolysis is thermal conversion of biomass under conditions of high temperature and absence of oxygen. Pyrolysis can be an autonomous process or a stage of the gasification of biomass. The end products of pyrolysis are pyrolytic oils (bio-oils) and small amounts of pyrolytic gases and solid products. The process of pyrolysis was used for the first time to obtain liquid fuels from microalgae in 1986. The technology is used primarily in the conversion of lignincellulose biomass for the production of bio-oils. However, literature reports indicate that the process of pyrolysis can be more suitable for microalgal biomass due to the fact that pyrolysis of microalgae proceeds at a lower temperature than that of lignincellulose biomass, and the bio-oils obtained are of higher quality. Microalgae are characterised by a high content of lipids, soluble polysaccharides and proteins, which undergo pyrolysis more easily than lignincelulose biomass. Moreover, the costs of pyrolysis of lignincellulose are relatively higher than those of pyrolysis of algae (Huang *et al.* 2010).

With relation to the rate of the process of decomposition of organic components, we distinguish so-called fast and slow pyrolysis. Compared to the slow (conventional) pyrolysis, fast pyrolysis is a technology where the production of biofuels proceeds directly to the liquid form, at relatively low temperatures (450°C-550°C), high heating rates (103°C s^{-1}-104°C s^{-1}) and a short time in the reactor. The oil produced as a result of fast pyrolysis of microalgal biomass amounts to ca. 70% of the weight of the fuel. Experimental results indicate that fast pyrolysis is an effective method of production of biofuels and gases from microalgae (Tab. 12) (Huang *et al.* 2010). They also indicate that the best for the pyrolysis of micoalgal biomass is the reactor with a fluidal deposit (Peng and Wu 2000).

Gasification is a process of biomass conversion into gaseous products through its partial oxidation. It is a high-temperature process usually taking place in the temperature range of 800°C-900°C, leading to the obtianment of synthesis gas. Synthesis gas contains hydrogen, carbon oxide, and small amounts of methane, carbon dioxide, water vapour and nitrogen. The amount and composition of the gas produced depend on the gasification factor, temperature, pressure, and the technology of gasification (Wang *et al.* 2008).

The synthesis gas produced is converted, e.g. through the Fischer-Tropsch synthesis, into biofuels. The process of gasification is conducted in ordinary reactors or in reactors with fluidal deposit (Lewandowski 2007).

Microalgae biomass is characterised by high moisture, and the conventional thermochemical methods require dry raw material. To skip the dehydration stage, which is a process with high energy requirements, it is proposed to apply the

technology of catalytic gasification of high-moisture biomass at low temperature. In that process microalgal biomass undergoes direct gasification to methane. As during gasification nitrogen contained in algal biomass is converted into ammonia, it can be recovered and re-used in algal cultures (Minowa and Sawayama 1999). A schematic diagram of catalytic gasification of microalgal biomass at low temperature is presented in Figure 19 (Amin 2009).

Table 12. The application of fast pyrolysis for some microalgal species (Huang *et al.* 2010)

Samples	Type of pyrolysis	Optimal pyrolysis temperature (K)	Heating rate	Oil yield (% biomass dry weight)	References
*C.prototheco*ides	Fast pyrolysis	773	–	52	Peng and Wu 2000
*C.prototheco*ides	Fast pyrolysis	773	600°C s^{-1}	18	Miao and Wu 2004
Microcystis aeruginosa	Fast pyrolysis	773	600°C s^{-1}	24	Miao and iWu 2004
Heterotroficzna hodowla *C. prototheco*ides Heterotrophic *C. prototheco*ides	Fast pyrolysis	723	600°C s^{-1}	57,9	Miao and Wu (a) 2004
*C.prototheco*ides	Fast pyrolysis	773	10 K s^{-1}	53,3	Demirbas 2006

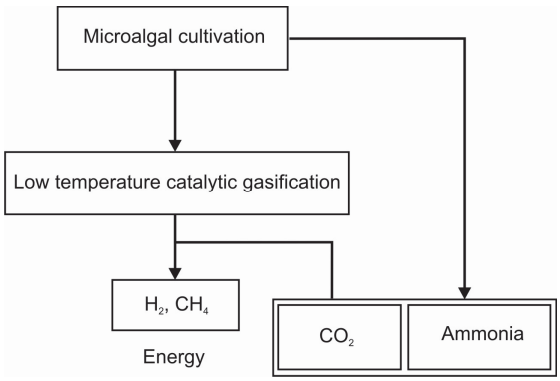


Fig. 19. Diagram of a microalgal system for fuel production by gasification at low temperature (Amin 2009)

7.2. Biodiesel

The energy crisis enforces the search for new biological sources of biomass rich in lipids. Particular interest is focused on microalgae that are natural producers of triacylglycerols (TAG) which are the material used in the production of biodiesel.

Triacylglycerols are esters of higher fatty acids and glycerol, and they are present in all vegetable oils. As opposed to the higher plants, algae are characterised by greater diversification of fatty acids. Under optimum growth conditions, algae synthesise fatty acids with medium-long carbon chains (C_{10}-C_{14}), long carbon chains (C_{16}-C_{18}) and very long chains ($\geq C_{20}$), as well as derivatives of fatty acids which are included in the composition of the cell membranes. The fatty acid profile can vary with relation to the species or strain and to the conditions in the growth environment. Under unfavourable conditions or due to the aging of cells the fatty acid metabolism of microalgae undergoes a change towards the biosynthesis and accumulation of neutral lipids, mainly in the form of triacylglycerols. Triacylglycerols do not play any structural function, but they constitute an energy reserve. As a result of the change in the lipid metabolism, triacylglycerols may account for as much as 80% of the total lipid content in the cell and can be converted into biodiesel (Miao and Wu 2006). Assaying of the fatty acid profile is of key importance for the determination of the applicability of a given species for biodiesel production. Studies conducted so far indicate that one of the most important factors affecting the lipid metabolism in microalgal cells is the limitation of nitrogen availability (Hu *et al.* 2008, Tang *et al.* 2011). As opposed to the higher plants, in which particular lipids can be synthesised and accumulated in specific cells, tissues or organs, the synthesis and accumulation of various kinds of lipids by microalgae take place in a single cell.

Among algae, the highest potential for biodiesel production is displayed by microalgae, primarily green algae, due to their ability of accumulating considerable amounts of lipids in their cells. Lipid content in the cells of the most common algae (*Chlorella, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Nitzschia, Phaeodactylum* and *Porphyridium* sp.) varies from 20% to 50%, but under certain conditions it may even reach 80% (Amaro *et al.* 2011; Mata *et al.* 2010). Literature data indicate applicability for biodiesel production of, among others, the fresh-water microalgae *Scenedesmus* sp., *Chlorella* sp., *Spirogyra* sp., *Spirulina* sp. and marine species from the genera *Synechccocus* sp., *Dunaliella* sp. (Scott *et al.* 2010, Singh *et al.* 2011). The lipid content and the achieved productivity of *Chlorella* indicate that it can be the optimum species for biodiesel production (Amaro *et al.* 2011, Lv *et al.* 2010).

7.2.1. Factors affecting accumulation of lipids

7.2.1.1 Method of cultivation

The types of nutrition most frequently used in microalgae cultivation for energy purposes are photoautotrophic and heterotrophic. Whereas, mixotrophic and photoheterotrophic growth is less frequently used in the production of microlagae.

A. Phototrophic cultures

The most frequently applied methods of microalgae cultivation, utilising solar radiation or artificial illumination, are those based on phototrophic growth of microalgae. The fundamental requirements for the phototrophic nutrition of microalgae are the availability of carbon dioxide, micro and macroelements, and light. Phototrophic cultivation of microalgae can be conducted in systems of open cultures (e.g. ponds) and closed cultures (photobioreactors). The closed system of the photobioreactor is favourable for those species of microalgae which can easily succumb to infection by other microorganisms. The exception are those species that can survive under extreme conditions, such as e.g. high pH (e.g. *Spirulina*), salinity (e.g. *Dunaliella*), or those characetrised by rapid growth rates (e.g. *Chlorella*) in open culture systems. In the production of long-chain fatty acids (DHA, EPA) it is suggested to use photobioreactors due to the possibility of better control of environmental conditions (Huang *et al.* 2010). The high costs of operation and capital investment and the small production scale compared to the open systems of cultivation indicate that the use of photobioreactors for large-scale biodiesel production may not be feasible or profitable. Open ponds are characterised by relatively low costs of operation compared to the closed photobioreactors, and in this respect are more favourable for use in biodiesel production.

Cells of microalgae from phototrophic cultures, depending on the species, are characterised by varied content of lipids within the range from 5% to 68% (Amaro *et al.* 2011). Increase in lipid content in the cell is an effect of depletion of the source of nitrogen or exhaustion of nutrients (Mata *et al.* 2010). Limitation of the availability of e.g. nitrogen increases the content of lipids but reduces biomass productivity. Therefore, lipid content cannot be the sole factor determining the possibility of utilisation of microalgae for oil production. Instead, we should consider lipid productivity which represents jointly the content of lipids and biomass production. Lipid productivity is a more suitable index indicating the possibility of utilisation of microalgae for oil production. The highest lipid productivity achieved in a phototrophic culture of *Chlorella* sp. was 179 $mg^{-1}L^{-1}d^{-1}$ with 2% CO_2 (Chen *et al.* 2011).

The slow growth of microalgae in photobioreactors is mentioned as a shortcoming of phototrophic cultivation. This is related with the phenomenon of photoinhibition or reduced accessibility of light in cultures with high cell density. Whereas, the utilisation of CO_2 as a source of carbon for the production of micoalgal biomass is given as the primary advantage of the use of phototrophic cultures (Chen *et al.* 2011).

B. Heterotrophic cultures

Certain species of microalgae display the ability of not solely phototrophic nutrition but, like bacteria, can also grow in darkness, using organic compounds as a source of energy.

The primary advantages of utilising heterotrophic cultures in the cultivation of microalgae include the elimination of the necessity of light, good control of the cultivation processes, and low costs of harvesting resulting from the higher density of cells produced through heterotrophic growth as compared to phototrophic growth. In heterotrophic cultures oriented at the production of lipids the most important component of the nutrient pool is the source of carbon. Microalgae can utilise organic sources of carbon such as glucose, fructose, saccharose, lactose, galactose, mannose, acetic acid, acetates, glycerol (Liang *et al.* 2009).

Cultures with heterotrophic growth are characterised by considerably higher lipid productivity than cultures with phototrophic growth (Tab. 13). Lipid content increase of 40 % was obtained in a culture of *Chlorella protothecoides* after the change from phototrophic conditions to heterotrophic cultivation conditions (Liang *et al.* 2009). However, the heterotrophic system of microalgae cultivation, utilising sugars as the source of carbon, is much more susceptible to infection with heterotrophic species of bacteria and fungi than cultures with phototrophic growth (Chen *et al.* 2011). Multiple experiments demonstrate, however, that properly managed cultures of microalgae are fairly resistant, and infections are often an indicator of poor cultivation conditions (De Pauw *et al.* 1984).

C. Mixotrophic cultures

Mixotrophic microalgae can conduct the process of photosynthesis and also utilise for their growth both inorganic carbon (CO_2) and organic compounds as a source of carbon. This means that they have the ability of growth in phototrophic or heterotrophic conditions, using either or both types of growth. Compared to the phototrophic and hetetrophic cultivation conditions, cultures with mixotrophic growth are less frequently used in biodiesel production (Chen *et al.* 2011).

In the mixotrophic cultivation, microalgae require the presence of light during the utilisation of organic compounds as a source of carbon. The requirement of availabiliy of light as a source of energy and of organic compounds at the same time is the main difference between mixotrophic and photoheterotrophic cultures (Chojnacka and Marquez-Rocha 2004). The utilisation of photoheterotrophic growth, as in the case of mixotrophic growth, for the needs of biodiesel production is very rare (Chen *et al.* 2011).

D. Comparison of various cultivation conditions

Although the ability to accumulate lipids in the cells of microalgae, and thus the possibility of obtaining oil, is a species-related feature, literature reports indicate that cultures with heterotrophic growth have considerably greater efficiency of oil production than algae cultivated in other conditions (Chen *et al.* 2011). Heterotrophic cultures can, however, be very easily infected, especially in open culture systems, which is a notable problem in the case of large-scale production. Comparison of various cultivation conditions is presented in Table 13.

Table 13. Comparison of the characteristics of different cultivation conditions (Chen *et al.* 2011)

Cultivation conditions	Source of energy	Source of carbon	Cell density	System of cultivation, type of reactor	Costs	Remarks
Phototrophic	Light	Inorganic	Low	Open ponds or photo-bioreactors	Low	Low cell density
Heterotrophic	Organic	Organic	High	Fermentor	Average	Contamina-tions, high costs of substrates
Mixotrophic	Light and organic	Organic and inorganic	Me-dium	Closed photobio-reactors	High	Contamina-tions, high costs of substrates
Photohetero-trophic	Light	Organic	Me-dium	Closed Photobio-reactors	High	Contamina-tions, high costs of substrates, high costs of equipment

In the cultivation of microalgae for biodiesel production the phototrophic cultures are the most frequently used. They are the easiest in industrial-scale application, and at the same time they are the most prospective due to the possibility of utilisation of CO_2 from combustion gases. Cultures of microalgae based on the phototrophic growth are, however, characterised by considerably lower oil productivity than hereotrophic cultures, mainly due to the slow growth of cells and low biomass productivity (Tab. 14). On the other hand, low costs of intensification of phototrophic cultivation cause that the method is still highly attractive. There is only sparse information in the literature on the utilisation of mixotrophic and photoheterotrophic cultures for oil production. The primary limitation to the utilisation of those two methods of microalgae cultivation is the high risk of infections. The cultivation conditions of such cultures may also require the use of photobioreactors, which significantly increases the costs (Chen *et al.* 2011).

Table 14. The lipid content and productivities of different microalgae species under different cultivation conditions (Chen *et al.* 2011)
a-CO_2, b- glucose, c-acetate, d-glycerol

Species	Cultivation conditions	Mass ($g\ L^{-1}\ d^{-1}$)	Lipid content (% d.m.)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	Source
Chlorella protothecoides	Phototrophic [a]	0,002-0,02 [a]	11,0-23,0	0,2-5,4	Illman *et al.* 2000
	Heterotrophic[b]	2,2-7,4	50,3-57,8	1209,6-3701,1	Xiong *et al.* 2008
	Heterotrophic[b]	1,7-2,0	43,0-48,7	732,7-932,0	Li *et al.* 2007
Chlorella vulgaris	Phototrophic[a]	0,01	33,0-38,0	4,0	Liang *et al.* 2009
	Heterotrophic[b,c]	0,08-0,15	23,0-36,0	27,0-35,0	Liang *et al.* 2009
	Mixotrophic[b,d]	0,09-0,025	21,0-34,0	22,0-54,0	Liang *et al.* 2009

7.2.1.2. Temperature

Temperature is one of the main factors affecting the fatty acid profile of microalgae. For many algae and blue-green algae a general trend has been observed consisting in an increase in the content of unsaturated fatty acids with decreasing temperature and an increase in the level of saturated fatty acids with increase of temperature (Lynch and Thompson 1982; Raison 1986; Sato and Murata, 1980). Temperature also has an effect on the total content of lipids in the cell. It was observed that increase of temperature was accompanied by an increase of the content of lipids in cultures of *Ochromonas danica* (Aaronson 1973) and *Nannochloropsis salina* (Boussiba *et al.* 1987). Whereas, in the cultivation of *Chlorella sorokiniana* at various temperatures no significant changes in lipid content were observed (Patterson 1970). The literature provides only a limited amount of information on the effect of temperature on the metabolism of lipids, which makes it impossible to identify a general trend (Hu *et al.* 2008).

7.2.1.3. Light intensity

Algae growing under various light conditions display differences in their chemical composition, content of pigments and photosynthetic activity. Low light intensity induces the production of polar lipids, mainly membrane lipids related with chloroplasts. High intensity of light causes a reduction in the total content of polar lipids, and a simultaneous increase in the level of neutral lipids, primarily triacylglycerols. Light intensity has also an effect on the degree of saturation of fatty acids. It is assumed that the conditions of poor light intensity are conducive to the production of PUFA which are incorporated in the structures of cell membranes. Whereas, high intensity of light stimulates the synthesis of fatty acids, causing an increase of production of saturated and monounsaturated fatty acids (Brown *et al.* 1996; Khotimchenko and Yakovleva 2005; Napolitano 1994; Spoehr and Milner 1949; Sukenik *et al.* 1989).

7.2.1.4. Growth phases and physiological status

The content of lipids and aftty acids is also subject to change during the particular growth phases of microalgae. As indicated by research, the biochemical composition of microalgae in the exponential phase differs from that in the stationary phase. In studies on the fatty acid profile of mciroalgae *Thalassiosira* sp., *Tetraselmis* sp., *Dictyosphaerium pulchellum, Stichococcus* sp*., Chlorella* sp*.,*

Scenedesmus falcatus, Anacystis sp., *Synechococcus* sp. and *Synechocystis* sp. higher content of fatty acids was found in the stationary phase than in the exponential phase. In the stationary phase an increase in the content of triacylglycerols was observed for many species of microalgae. In cells of *Parietochloris incise* the content of triacylglycerols increased from 43% in the exponential phase of growth to 77% in the stationary phase, and in the case of a culture of *Gymnodinium* sp. the level of triacylglycerols increased from 8% in the exponential phase to 30% in the stationary phase. The effect of transition from the phase of exponential growth to the stationary phase was a decrease in the share of PUFA in the total lipid content (Bigogno *et al.* 2002, Mansour *et al.* 2003).

The composition and content of lipids and fatty acids are also affected by the ageing of the culture. With increasing age of the culture an increase was observed in the total content of cellular lipids, e.g. in cultures of *Chlorococcum macrostigma* and *Nitzschia palea* (Hu *et al.* 2008). Whereas, in the case of a culture of *P. tricornutum* no effect of culture ageing on the total cotnent of fatty acids was observed, although the accumulation of triacylglycerols was notable and the content of polar lipids was reduced (Alonso *et al.* 2000). A distinct increase of the level of saturated and monounsaturated fatty acids and simultaneous drop of the level of PUFA with the ageing of the culture was demonstrated on the basis of analysis of fatty acid composition of cultures of *P. tricornutum* and *Chaetoceros muelleri*. A majority of studies on the lipid metabolism of microalgae were conducted in batch cultures. For this reason it is difficult to separate the true effects of culture ageing from the effects of nutrient deficiency on the metabolism of lipids (Hu *et al.* 2008; Liang *et al.* 2009).

The synthesis of triacylglycerols and the process of their accumulation in the cytosol can be, with few exceptions, the response of algae to stress conditions. Under the effect of stress, excess of electrons accumulated in the photosynthetic chain of electron transport may cause excessive production of reactive forms of oxygen, which may lead to inhibition of the process of photosynthesis and to damage to lipids of the cell memberanes, to proteins and other macromolecules. The triacylglycerol synthesis pathway is coordinated with the secondary synthesis of carotenoids. Carotenoids are located in the granular and lamellar structures of photosynthesising chromatophores. Lipid-rich carotenoids play a protective role against the processes of photooxidation, controlling or reducing the amount of light falling on the chloroplast. The synthesis of TAG can also make use of PC (phosphatidylcholine), PE (phosphatidylethanolamine), galactolipids or toxic fatty acids excluded from the system of membranes as donors of the acyl group, and

thus play the role of a mechanism of detoxification of lipids of membranes (Hu *et al.* 2008, Rabbani *et al.* 1998).

7.2.1.5. Nutrients

Limited availability of nutrients is one of the stress conditions that lead to the accumulation of lipids coupled with reduced rate of growth (Roessler 1990). Among the nutrients, nitrogen deficit is one of the most important factors affecting the metabolism of lipids in algae. A general tendency of the accumulation of lipids, especially triacylglycerols, in response to nitrogen deficit has been observed for numerous species or strains of various taxa of algae. Illman (2000) found in his study an increase in the content of lipids, in response to reduced levels of nitrogen in the substrate, in all five strains of *Chlorella* included in the study, among which the increase of lipids was 63%, 56% and 40%, respectively, in the dry matter of *C.emersonii, C. minutissima* and *C. vulgaris.* The availability of nitrogen in the nutrient medium should be balanced so as to optimise the production of lipids and biomass.

Other nutrients whose deficit stimulates the accumulation of lipids include phosphates and sulphates. Limitation of phosphorus availability caused an increase in the level of lipids, mainly triacylglycerols, in cultures of *Monodus subterraneus* (Eustigmatophyceae) (Khozin-Goldberg and Cohen, 2006), *P. tricornutum* and *Chaetoceros* sp. (Bacillariophyceae), *I. galbana* and *Pavlova lutheri* (Prymnesiophyceae), and a decrease of the content of lipids in cells of *Nannochloris atomus* (Chlorophyceae) and *Tetraselmis* sp. (Prasinophyceae) (Hu *et al.* 2008, Reitan *et al.* 1994).

Research shows that limitation of the availability of sulphur leads to an increase in the total lipid content in green algae *Chlorella* sp. and *C. reinhardtii* (Sato *et al.* 2000).

Iron availability in the substrate is another factor which affects the metabolism of lipids in microalgae cells. It has been demonstrated that high concentrations of iron induced lipid accumulation in *Chlorella vulgaris* (Liu *et al.* 2002).

7.2.1.6. Carbon dioxide

Sources of CO_2 for microalgae can include atmospheric carbon dioxide, exhaust gases, industrial gases, or soluble carbonates. Assimilation of CO_2 by microalgae is pH-dependent. For optimum growth, microalgae require at least 1% of CO_2 in the atmosphere. Literature reports indicate that certain species of microal-

gae are characterised by good tolerance to high concentrations of CO_2 (and high temperature) and low levels of SO_x and NO_x (up to 150 ppm) (Matsumoto *et al.* 2003). Research shows that *Chlorella kessleri* and *Scenedesmus obliquus* display tolerance to high contents of CO_2 (up to 18%) (de Morais and Costa 2007). As follows from literature reports, the fatty acid profiles and the content of fatty acids in cells of microalgae may differ notably with relation to various concentrations of CO_2. Tsuzuki noted that increase in the concentration of CO_2 from 0.036% to 2% might cause an increase in the content of saturated fatty acids in *C. vulgaris* (Tsuzuki 1990). In a study on the effect of high concentrations of CO_2, within the range from 0.03% (air) to 50% CO_2, on the production of lipids and the ability CO_2 binding by *S. obliquus* and *C. pyrenoidosa* it was found that high level of carbon dioxide (30%-50%) was conducive to the accumulation of polyunsaturated fatty acids. Whereas, low concentrations (0.03%) of CO_2 were favourable for the accumulation of saturated fatty acids such as palmitic acid and short-chain fatty acids (Tang *et al.* 2011). Increase in the content of lipids in microalgae cultures was also observed at CO_2 levels from 2% to5% (Yue and Chen 2005).

Studies on the efficiency of photosynthesis of various microalgae, taking into account CO_2 binding and lipid productivity, conducted on 27 algal strains, revealed the highest growth rates for *Chlorella* sp.*, Chlamydomonas* sp. and *Synnecococcus* sp. At CO_2 concentration of 3% the effectiveness of lipid production of the microalgae under study amounted to 0.322 g d^{-1}, and at 0.03% CO_2 their lipid productivity was 0.190 g d^{-1} (Fulke *et al.* 2010). The content of CO_2 in the atmosphere is not sufficient for the achievement of the fast growth rates and high productivity of microalgae, required for the production of biofuels. Gases from the processes of combustion contain usually up to 15% CO_2. Therefore, processes of combustion are capable of providing sufficient amounts of CO_2 for large-scale production of microalgae (Kumar *et al.* 2010). An important problem is the content of pollutants in exhaust gases, SO_x, NO_x, that have to separated from CO_2 due to their toxic effect on the growth of algae. However, the process of purification increases the costs of biomass production, where the source of carbon accounts for ca. 60% of the total costs of nutrients used in the cultivation.

7.2.2 Stages of biodiesel production

Biodiesel production from wet biomass of microalgae takes place in stages and comprises the dehydration of algae, extraction of oil, and oil coversion to biodiesel (Chisti 2007). The stages of biodiesel production are presented in Figure 20.

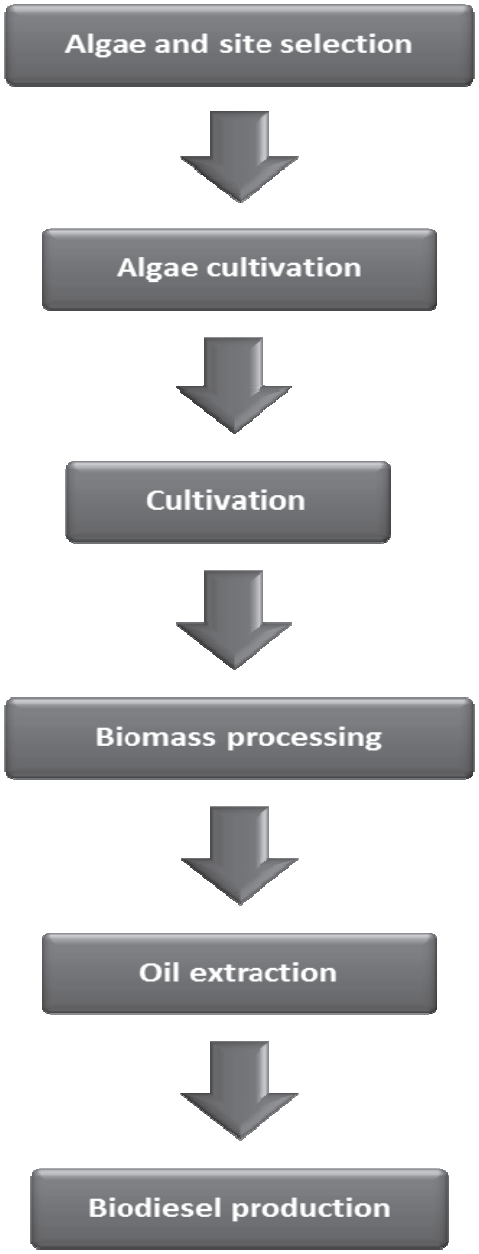


Fig. 20. The stages of biodiesel production from microalgae (Mata *et al.* 2010)

7.2.2.1. Disintegration of cells

The process of biodiesel production requires prior liberation of lipids from the intracellular structures. The process of lipid liberation should be conducted at high efficiency, with cost- and energy-saving methods, thus permitting limitation of the use of organic solvents and maximising the yield of liquid fuels. The proces of liberation of lipids to the surrounding liquid is conducted with the use of various methods of disintegration. The disintegration of cells of microalgae is performed with the use of e.g. microvawes, the bead-beating method (mechanical disintegration), ultrasonic methods and the addition of 10% NaCl (Amaro *et al.* 2011). However, none of these methods is sufficiently effective. Lee studied the application of various methods of disintegration and the yield of lipids from *Botryococcus* sp., *C. vulgaris* and *Scenedesmus* sp. (Lee *et al.* 2010). The most efective for *Botryococcus* sp. was the application of the method of bead-beating and microvawes, the yield of lipids being 28.1 % and 28.6%, respectively. The lowest yield of 8.8% was obtained in the case of sonification. For *C.vulgaris* and *Scenedesmus* sp. the most effective method was the application of microwaves. The effectiveness of the methods of cell disintegration in the extraction of lipids varies with relation to the various species of microalgae, but the application of microwaves appears to be the simplest and the most efficient method (Amaro *et al.* 2011).

7.2.2.2. Extraction of lipids

The ideal method of extraction of lipids from microalgae should not only be specific to lipids, in order to minimise co-extraction of non-lipid compounds, but also selective towards specific fractions of lipids.

The literature provides descriptions of numerous methods of lipid extraction from microalgae, among which the best known include oil expression, fluid-fluid extraction, supercritical fluid extraction (SFE), and the application of ultrasounds (Singh and Gu 2010). Table 15 presents the advantages and limitations of the most popular methods of extraction.

Solvents most frequently used for lipid extraction include chloroform-methanol, hexane, ethanol (96%), and hexane-ethanol mixture. Also a mixture of hexane with isopropanol is used. Hexane is as good a solvent for the extraction of lipids as the classic chloroform-methanol mixtures. In addition, it is characterised by lower extraction efficiency than chloroform, but it is also less toxic. It has marginal affinity to non-lipid contaminants (it extracts considerably less non-lipid

material, including undesirable pigments) and displays greater selectivity towards neutral lipid fractions. Extraction with ethanol may lead to the isolation of undesirable compnents, e.g. sugars, amino acids, hydrophobic proteins or pigments (Amaro *et al.* 2011, Mata *et al.* 2010).

Table 15. Advantages and limitations of various extraction methods for algae oil (Harun *et al.* 2010)

Method of extraction	Advantages	Limitations
Oil press	Easy in application, does not require the use of a solvent	Large number of samples required, slow process
Extraction with a solvent	Relatively low costs of solvents, replicable process	Most organic solvents are combustible and /ot toxic; Recovery of solvents is cost and energy consuming; Large amunt of solvents required
SFE	Non-toxic (no organic solvents), high selectivity of the process	Insufficient interaction between supercritical CO_2 and sample, fails in quantitative extraction of polar analytes
Ultrasounds	Reduced time of extraction, reduced use of solvent; improved solvent penetration into cell materials, improved liberation of cell contents to the substrate	High energy consumption

A promising and pro-ecological technology, that may replace conventional solvents in lipid extraction, is the extraction with carbon dioxide in supercritical state. The main advantage of that technology is the low toxicity of the solvent. Extraction in the supercritical state is much more effective that the traditional methods of separation with the use of solvents. Supercritical fluids are selective, thus ensuring purity and high concentration of the product. In the extraction with CO_2 in supercritical state, carbon dioxide – after exceeding the critical parameters

(through heating and pressure changes) – is introduced into the extractor where is acts as a solvent in oil extraction. The primary limitation of this method of extraction is the very high cost of the process (Amaro *et al.* 2011).

7.2.2.3. Process of transesterification

Due to the similar physical and chemical properties of vegetable oils and microalgae oil, the technologies used for biodiesel production from crop plants can also be applied for the production of biodiesel from microalgae. Table 16 presents a comparison of properties of microalgae oil, diesel fuel, and the requirements of the ASTM (American Society for Testing and Materials) standard for biodiesel.

Table 16. Comparison of properties of microalgae biodiesel, diesel fuel and ASTM standard (Amin 2009)

Properties	Biodiesel microalgae oil	Diesel fuel	ASTM biodiesel standard
Density (kg l^{-1})	0.864	0.838	0.86-0.90
Viscosity (mm^2 s^{-1}, at 40°C)	5.2	1.9-4.1	3.5-5.0
Flash point (°C)	115	75	Min.100
Solidifying point (°C)	–12	–50 do 10	–
Cold filter plugging point (°C)	–11	–3.0 (max. –6.7)	Summer max 0 Winter max<–15
Acid value (mg KOH g^{-1})	0.374	Max. 0.5	Max. 0.5
Heating value (MJ kg^{-1})	41	40-45	–
H/C ratio	1.81	1.81	–

Among the available methods of biodiesel production from microalgae, transesterification with methanol is the most frequently applied. That process leads to

the obtainment of methyl esters of higher fatty acids, commonly known as biodiesel. The main problem involved with the use of vegetable oils and microalgae oils as fuels for diesel engines is their greater viscosity compared to diesel fuels produced from petroleum oil (Tab. 16). The process of transesterification causes a reduction of the initial vicsosity and improves fluidity. Biodiesel production from microalgae requires large amouts of biomass. The reaction of the process of transesterification is presented in Figure 21.

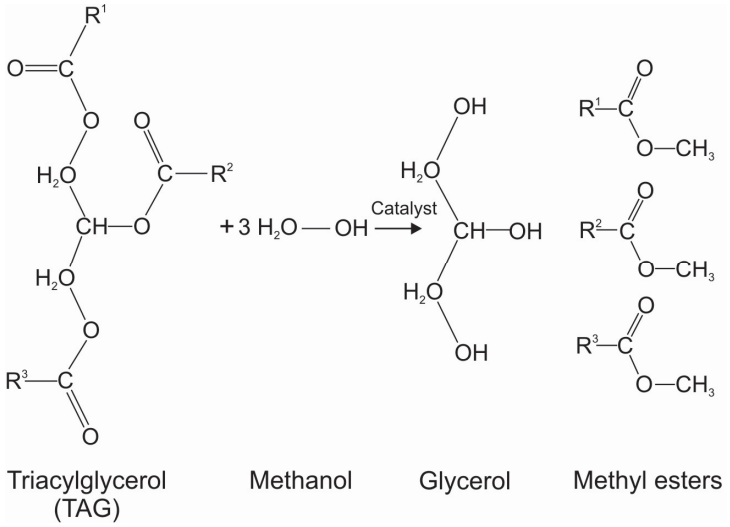


Fig. 21. Transesterification of triacylglycerides with methanol. R^1-R^3 – bicarbonate groups (Mata *et al.* 2010)

As a result of the reaction of tranesterification, the glycerol contained in the molecule of lipid (triacylglycerol) is replaced with aliphatic alcohols. The products of the reaction are methyl esters of fatty acids and glycerol as a by-product. Alcohols commonly used for transesterification include methanol, ethanol, propanol, butanol and amyl alcohol, but due to its low cost methanol has the most extensive application. The reaction of transesterification proceeds in stages, with the production of intermediate products - diacylglycerols and monoacylglycerols. The effectiveness of the process of transesterification depends on such factors as the catalyst used, the molar ratio of alcohol to oil, temperature, and purity of reagents. The catalysts in the reaction of transesterification can be acids, bases and enzymes (lipases). Akaline catalysts of transesterification provide higher reaction and con-

version rates than acid catalysts. Transesterification conducted with the use of alkaline catalysts is approximately 4000 times faster than a reaction with acid catalyst (Chisti 2007, Mata *et al.* 2010).

The conventional methods of dehydration and extraction of oil from biomass of microalgae are costly and require large inputs of energy. An alternative for the traditional methods of extraction and transesterification is the method of conversion of wet biomass of microalgae into biodiesel with supercritical methanol. Transesterification of oils with supercritical alcohols significantly reduces the time of transesterifiiation and is characterised by lower energy requirements compared to the conventional methods thanks to the elimination of the processes of dehydration and the processes of extraction. The cost of biodiesel production in the supercritical conditions is lower by ca. 50% as compared to the conventional methods. Supercritical methanol ensures maximum conversion of triacylglycerides into suitable esters of fatty acids in a single-stage process. The technology ensures energy-efficient biodiesel production from microalgae biomass without the need of using a catalyst (Anitescu *et al.* 2008, Patil *et al.* 2011, Warbi *et al.* 2004).

7.3. Production of biogas from microalgal biomass

The production of biogas is one of the simplest direct methods of the utilisation of wet biomass of microalgae for energy purposes with the exclusion of the energy-costly process of dehydration and subsequent chemical extraction. The low content of cellulose, lack of lignin and presence of polysaccharides (alginate, mannitol, laminarin) make the biomass of microalgae an easy material for the process of methane fermentation. Under the conditions of anaerobic fermentation, those features guarantee good stability of the process and high efficiency of processing (Sialve *et al.* 2009).

The utilisation of the carbohydrate fraction of cells of microalgae for the production of methane was first proposed by Meier (Dembiras 2010). The idea was developed by Golueke *et al.* who performed a technical and economic feasibility analysis of the fermentation of biomass of microalgae (primarily *Chlorella* and specfies of *Scenedesmus*) cultivated in large ponds (Gouleke *et al.* 1957). Those authors demonstrated that the rate of biodegradation of biomass of microalgae was slower by 60%-70% with relation to the rate of degradation of active sludge. They also indicated possible limitations of the process: high pH, toxic effect of ammonia or resistance of algal cells. Golueke and Oswald were the first to present the process of methane fermentation in conjunction with the processes of produc-

tion of microalgae (Golueke and Oswald 1959). The energy crisis of the nineteen seventies caused the reappearance of interest in algae as a source of fuels. Detailed technical analyses conducted by Benemann demonstrated that microalgae cultivation systems could produce biogas at costs competitive to the projected prices of fossil fuels (Dembiras 2010, Posten and Schaub 2009).

Table 17. Proteins and carbohydrates content of various species of microalgae (Demirbas 2010)

Algae strain	Protein (% dwt)	Carbohydrate (% dwt)
Scenedesmus obliquus	50-56	10-17
Scenedesmus quadricauda	47	–
Scenedesmus dimorphus	8-18	21-52
Chlamydomonas rheinhardtii	48	17
Chlorella vulgaris	51-58	12-17
Chlorella pyrenoidosa	57	26
Spirogyra sp.	6-20	33-64
Dunaliella bioculata	49	4
Dunaliella salina	57	32
Euglena gracilis	39-61	14-18
Prymnesium parvum	28-45	25-33
Tetraselmis maculate	52	15
Porphyridium cruentum	28-45	40-57
Spirulina platensis	52	8-14
Spirulina maxima	28-39	13-16
Synechoccus sp.	46-63	15
Anabaena cylindrica	43-56	25-30

One of the methods of estimating the potential of microalgae in the process of methane fermentation is the determination of their cell composition. Changes in the cell composition may have an effect on the efficiency of the process of fermentation. The content of proteins, lipids and carbohydrates is a species-related trait, but it also depends on environmental factors (Sialve *et al.* 2009). In the process of biomethane production, as in the case of biodiesel production, lipids play an important role. The possibility of their conversion into biomethane is greater (1390 L kg^{-1} t.d.m.: 72% CH_4, 28% CO_2) than in the case of proteins (800 L kg^{-1} t.d.m.: 60% CH_4, 40% CO_2) and carbohydrates (746 L kg^{-1} t.d.m.: 50% CH_4, 50% CO_2) (Schenk *et al.* 2008). Table 17 presents the content of proteins and carbohydrates in various species of microalgae.

The mineral composition of microalgae cells meets the nutrient requirements of anaerobic bacteria. Moreover, the main compoments of algal cells: carbon, nitrogen and phosphorus, and such elements as iron, cobalt and zinc have a stimulating effect on the process of methanogenesis. Of particular importance in the process of methane fermentation is the C/N ratio determining the availability of carbon and nitrogen contained in the raw material. Compared to the land plants, many species of microalgae are characterised by a high content of proteins, denoted by a low value of C/N ratio. For the land plants that ratio is 36, while for fresh-water algae its value is 10.2 (Elser *et al.* 2000; Sialve *et al.* 2009).

The process of methane fermentation taking place under anaerobic conditions leads to the production of biogas whose main components are methane and carbon dioxide.

Anaerobic fermentation of algae is conducted by three groups of bacteria:

1. hydrolysing bacteria, that transform polymerised organic compounds into monosacharides, carboxyl acids and alcohols,
2. acidogenic bacteria, that transform the products of hydrolysis into acetates, carbon dioxide and hydrogen,
3. methane bacteria, that convert the products of acidogenesis to methane and carbon dioxide.

The duration of the process of fermentation is from 10 days to several weeks (Vergara-Fernadez *et al.* 2008).

Table 18 presents the yield of methane from various species of algae.

Methane obtained from the process of fermentation can be used as gas fuel and also can be converted into electric energy. A schematic diagram of the production of biogas from microalgae biomass in presented in Figure 22. Biomass

residues from anaerobic fermentation can be further processed and utilised e.g. as a fertiliser.

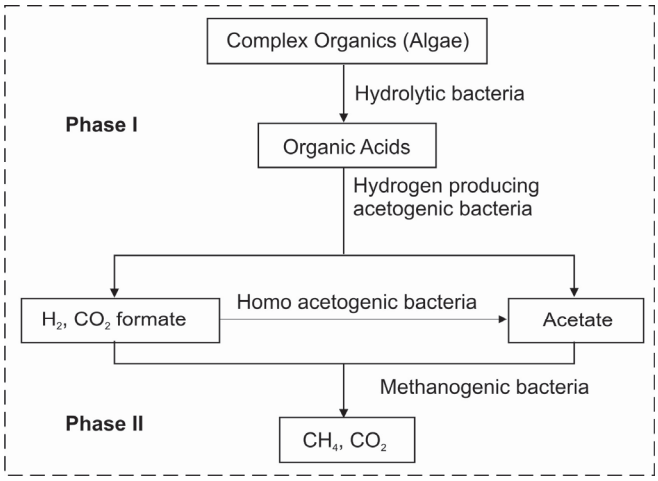


Fig. 22. Biogas production process (Vergara-Fernadez *et al.* 2008)

Table 18. Methane yield from differnent algae strains (Harun *et al.* 2010)

Biomass	Methane yield (m^3 kg^{-1})	Reference
Laminaria sp.	0.26-0.28	(Chynoweth 2005)
Gracilaria sp.	0.28-0.4	(Bird *et al.* 1990)
Macrocystis	0.39-0.41	(Chynoweth 2005)
L.Digitata	0.5	(Morand and Briand 1999)

Factors affecting the efficiency of methane production include the following:

- content of organic substances,
- pH,
- temperature,
- hydraulic retention time (HRT).

Literature data indicate that increase in temperature of the fermentation process from 35°C to 50°C can increase the rate of biodegradation of algal biomass by 5% to 10%. The results of studies by Chen (Sialve et al, 2009), who achieved the highest methane yield at temperature of 40°C, indicate that the temperature range of mesophilic fermentation is opimal for the fermentation of microalgae biomass.

At the same time they indicate slower degradation of organic compounds under the conditions of mesophilic fermentation (Sing and Gu 2010). The key questions for the process of fermentation of microalgae biomass, emphasised in scientific piublications, are the following:

- the presence of sodium (salts) in the biomass of marine algae may inhibit the process of anaerobic fermentation
- the efficiency of methane production from microalgae biomass can be increased through breaking of the cell walls and liberation of organic compounds contained in the cells
- nitrogen content in the cells of certain algae can be high, which increases the level of ammonia in the process of fermentation and may have an inhibiting effect on the process (Demirbas 2010).

The content of methane in the biogas obtained in the process of methane fermentation is similar in most studies and falls within the range of 69-75%. This indicates that biomass of microalgae is a good substrate for methane fermentation (Sialve *et al.* 2009).

The costs of methane production from microalgae biomass are higher compared to other sources of biomass, e.g. grasses, wood. The optimum solution providing a reduction of the costs of production and increasing profitability of methane production is the application of integrated processes of cultivation of microalgae and sewage treatment. In spite of the fact that microalgae biomass has a high potential for biogas production, no industrial-scale production systems have been implemented so far (Sing and Gu 2010).

8. ECONOMICS OF PRODUCTION OF MICROALGAE

The existing cultivations of algae most frequently provide biomass for the production of valuable chemical substances (e.g. β-carotene from *Dunaliella salina*, astaxanthin from *Haematococcus pluvialis*). Algae as a source of energy are not yet produced at a large scale, but that potentiality will start to develop.

Wijffels *et al.* (2010) calculated the cost of production of algae under European conditions (Holland). The factors taken into account included the following:

- natural illumination – insolation conditions,
- moderate climate,
- replacement of plastic elements every year,
- productivity of algae at levels realistically achieved at present,
- purchase of biogenic elements and CO_2.

The costs of cultivation of microalgae vary primarily with relation to the scale of production. The costs of production on an area of 1 ha in flat-panel photobioreactors amount to ca. 9 EUR per kilogram of biomass. The main cost items are human labour (24%) and energy (18%). Considerable reduction of costs appears at the moment of increasing the scale of production – on the area of 100 ha the unit cost drops to 4 EUR per 1 kg of biomass, notable cost savings being achieved in the costs of employment of personnel, while the costs of energy increase to 35% of the total costs. That cost item includes, among other things, the stirring of the cultures and the pumping of gases. Under such conditions, the amount of energy consumed in the cultivation is greater than the energy accumulated in the bonds of the biomass, which of course causes that the whole process is unsuitable for the production of energy.

Assuming that CO_2 and fertilisers were delivered at no cost (as e.g. residual material from incineration plants and sewage treatment plants), the costs of energy were reduced by 10%, the photosynthetic efficiency raised to 7%, and the whole cultivatoin transferred to a location with greater insolation (e.g. the Caribbean) – unit cost of 0.4 EUR kg^{-1} could be realistically achieved. Chisti (2007) calculated that the cost of production reaching 10,000 tons a year would attain unit costs of $ 0.47 and $ 0.60 per kilogram of biomass, respectively, for production in photobioreactors and ponds, and one litre of oil produced from micoalgae biomass would cost $2.80, which is more or less in conformance with the calculations presented in the Oilgae Report (2009). Taking into account that the price of diesel fuel in the USA, without taxes etc., is $0.49 per litre (in 2006)(Chisti 2007), and in Europe the price of biodiesel is 0.5 EUR per litre (Wijffels 2009), it turns out that the production of microalgae focused on biodiesel production is unrealistic. If the biomass contained 40% of lipids, the costs of production would need to be within 0.2 EUR/kg, and that would have to include the costs of extraction of oil conversion into biodiesel.

Chisti (2007) provides a formula that can be used to calculate the threshold of profitability of microlage oil production (per litre), in comparison to the price of crude oil (per barrel):

$$C_{o.a.} = 6.9 \cdot 10^{-3} C_{r.}$$

$C_{o.a.}$ – cost of production of microalgae oil (per litre),

$C_{r.}$ – crude oil price (per barrel).

The average crude oil price over the recent period (March 2011) is $116 per barrel, which gives a threshold of profitability at the level of $0.80 for the production of 1 litre of microalgae oil.

Cultivation of microalgae will certainly not be based on the production of biodiesel as the sole product, as that would not be profitable. For microalgae cultivation and energy production from microalgae to be profitable it is necessary to undertake activity and research in many fields. First of all, the necessary objective of such activity is to ensure that all the components of algal cells are utilised (proteins, lipids, carbohydrates and others).

- Lipids – biodiesel, chemical industry, fatty acids omega –3
- Carbohydrates, proteins – for food, feed, chemical reagents
- Pigments, vitamins and other valuable components – e.g. for supplements
- Oxygen – should also be recovered

Wijffels *et al.* (2010) analysed the following example: algae with lipid content of 40%, 50% proteins, 10% cabohydrates, being utilised as follows:

- 25% of lipids for feed and for food components (e.g. omega-3 acids) (2 EUR per kg) and 75% for diesel (0.5 EUR per kg)
- 20% of water-soluble proteins for food (5 EUR per kg), 80% of insoluble proteins for feed (0.75 EUR per kg)
- Carbohydrates can be utilised in various ways, having a low content of cellulose, and more of fructans, glucans, glycerols – ca. 1 EUR per kg.
- Apart from that, algae reduce the levels of nitrogen and phosphorus. The cost of conventional purification is ca. 2 EUR per 1 kg of nitrogen. Algae have a content of 70 kg of nitrogen per 1000 kg of biomass, thus we can save 140 UER per 1 ton of produced biomass. Likewise for phosphorus.
- One ton of algae produces 1600 kg of gas rich in oxygen; the value of that gas is ca. 0.16 EUR per kg.

The calculated total value of all products from algae would be ca. 1.65 EUR per 1 kg of biomass, with projected unit cost of production of ca. 0.4 EUR per 1 kg of biomass. The example is illustrated by a pie chart (Fig. 23). Moreover, Chisti (2007) proposes fermentation of the remaining material, which would yield an additional profit.

Further steps that should be undertaken to improve the profitability of microalgae production relate to genetic and metabolic engineering. Thanks to those modern techniques it is potentially possible to:

- increase the photosynthetic efficiency to 7-9%,
- increase the rate of biomass production,

- increase the content of lipids in the cell,
- broaden the tolerance of algal species to e.g. high temperatures,
- increase the capacity of light absorption, so that production can increase with increase in light intensity,
- eliminate photoinhibition,
- eliminate susceptibility to photooxidation.

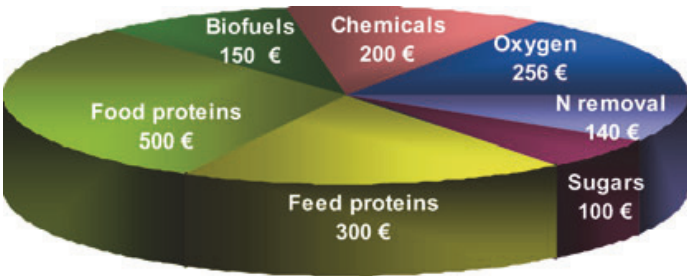


Fig. 23. Value of algal biomass per 1000 kg after biorefining (Wijffels 2010)

Moreover, a lot remains to be done in the area of the design of photobioreactors. Particularly important problems that need to be solved include the following:

- adaptation of stirring intensity so that every cell spends adequate periods of time exposed to light and in the dark part of the photobioreactor,
- equally important, adaptation of stirring to such intensity that no damage is caused to the cells,
- another problem is the deposition of cells on the walls of photobioreactors, which inhibits light penetration to the deeper areas of the structure (Chisti 2007).

Oilgae Report (2009) gives the following costs of the production of microalgae:

Open ponds:
Investment costs - $125,000-$150,000 per hectare
Operating costs - $15,000-20,000 per hectare per year ($2.27 per one gallon of biodiesel)

Closed ponds:
Investment costs - $200,000 per hectare per year
Operating costs - $20,000-25,000 per hectare per year

Photobioreactors:
Investment costs - $0.4-1 mln per hectare
Operating costs - $ 25-30000 per year at production of 1 T of biomass per day ($9.83 per gallon of biodiesel)

Cost of CO_2 – from $2 to $25 per 1 ton of algal biomass
Cost of nutrient medium – ca. $40–50 per 1 ton of algal biomass

Separation of biomass (operating costs):
Filtration: ca. $100 per 1 million gallons
Centrifuging: ca. $400 per 1 million gallons
Flocculation: ca. $1000 per 1 million gallons
Flotation: from ca. $50 to $200 per 1 million gallons

Acquisition of oil (operating costs):
Mechanical oil press: $ 35 / T (ca. 12 c per gallon)
Extraction with a solvent: $ 55 / T (ca. 20 c per gallon)

Conversion to biofuel (operating costs):
Biodiesel production – transesterification $0.25 per gallon
Production of ethanol from starch (through fermentation) $0.7 per gallon
Production of ethanol from cellulose (through fermentation) $1.3 per gallon
Anaerobic fermentation 2-3 c per 1 kWh of generated energy
Gasification/pyrolysis/Fischer-Tropsch, gasification + FT: ca. $0.8 per gallon
Direct combustion: 6c per 1 kWh

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10. SUMMARY

World's energy demand has been constantly increasing for decades. Yet, fossil fuels stock, which we use the most extensively, will probably be depleted soon. Moreover, combustion of these conventional fuels leads to excessive carbon dioxide emission. This process causes a multitude of unfavorable consequences for Earth's climate and biosphere. That is why the scientists have been searching for alternative fuel sources for years. These fuels have to meet some requirements, and general goal is that they must be neutral to the natural environment, truly clean and "green". One of the possibilities of obtaining friendly energy is biomass processing. Nowadays, a few species of energy plants (e.g. rapeseed, soybean, corn) are used for this purpose. However, traditional cultivation productivity is poor and insufficient and also it occupies the land that would preferably be destined for food and feed production.

Algae are potentially a much more efficient source of biomass than all energy plants. They are simple plant-like, photoautotrophic organisms, predominantly single-celled. These so-called microalgae have the ability to grow and divide very fast. In optimal conditions, their cell cycle could last only a few hours. This means that biomass increase is extremely rapid. Cultivation can be supplied with exhaust gases and wastewater, because algae absorbs great amounts of carbon dioxide and nutrients.

Some species of microalgae accumulate large amounts of lipids, when treated by nitrogen-starving conditions. Composition of fatty acids is often appropriate for biodiesel production. Biofuels that we can obtain from algal biomass include: bioethanol, methane, hydrogen and more.

This review presents fundamentals about morphology, anatomy, physiology and biochemistry of microalgae as well as describes basic information about the cultivation. It characterises some species which could be suitable for energy production, the cultivation devices, methods of cultivation and harvesting and also products that can be obtained from the biomass of microalgae. In the end, there is the balance sheet for the production of microalgae for energy purposes.

11. STRESZCZENIE

MIKROGLONY – HODOWLA I WYKORZYSTANIE BIOMASY NA CELE ENERGETYCZNE

Obecna trudna sytuacja energetyczna na świecie zmusza naukowców do poszukiwania alternatywnych, odnawialnych źródeł energii. Biomasa roślinna jest substratem, który można wykorzystać w sposób wszechstronny, z którego można wyprodukować wiele różnych rodzajów paliw. Począwszy od oleju rzepakowego używanego jako komponent biodiesel'a, skrobię kukurydzianą do produkcji etanolu, skończywszy na roślinach typowo celulozowych np. wierzba energetyczna,

które mogą służyć do spalenia bezpośredniego. Uprawa roślin energetycznych nie jest jednak na tyle wydajna, aby zaspokoić światowe potrzeby energetyczne, a ponadto stanowi konkurencję dla upraw żywieniowych i paszowych. Składa się na to m.in. mała efektywność fotosyntetyczna, umiejscowienie wartościowych substancji tylko w niektórych organach rośliny oraz powolny wzrost i plonowanie jedynie raz, dwa razy do roku.

Mikroalgi są to autotroficzne organizmy jednokomórkowe, które swą fizjologią przypominają rośliny. Posiadają one jednak wiele pozytywnych cech, które kwalifikują je jako potencjalne niezwykle wydajne źródło biomasy. Mikroalgi mają bardzo wysoką efektywność fotosyntetyczną, co przekłada się na szybką produkcję biomasy. W sprzyjających warunkach podziały komórkowe przeprowadzane są nawet co kilka godzin, przy czym wymagania środowiskowe tych organizmów są stosunkowo niewielkie. Do hodowli można z powodzeniem wykorzystywać ścieki komunalne oraz gazy spalinowe, co dodatkowo korzystnie wpływa na środowisko.

Niektóre gatunki mikroalg, w odpowiednich warunkach, gromadzą duże ilości tłuszczu w komórkach, który ma odpowiedni profil kwasów tłuszczowych do produkcji biodiesel'a. Biomasę alg można także poddać przetworzeniu na inne paliwa, np. bioetanol czy biogaz.

W opracowaniu zawarto podstawowe informacje na temat budowy i fizjologii mikroalg, a także opisano zagadnienia związane z hodowlą na cele energetyczne. Wyróżniono gatunki mikroalg, które przejawiają korzystne cechy w hodowli, przedstawiono rodzaje urządzeń hodowlanych, metody hodowli oraz czynniki wpływające na efektywność hodowli. Ponadto omówiono podstawowe metody separacji biomasy z płynnego środowiska, produkty, które można uzyskać po przetworzeniu biomasy oraz przedstawiono przykładowe zestawienie ekonomiczne produkcji alg na cele energetyczne.

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