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MICROALGAE – CULTIVATION METHODS*

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A b s t r a c t

Investigations into the use of algae for energy production have been carried out for many years. A key issue is the selection of technology for culture and acquisition of algae biomass for energetic purposes. The proliferation and culture of algae may be conducted with a variety of methods, beginning from strictly monitored methods in closed laboratory systems, to less predictable methods in open systems. Though many systems have been developed so far, unfortunately none of them may be found cost-effective. Photobioreactors are expensive and require high exploitation inputs (lighting, supply of carbon dioxide), they additionally pose some difficulties in exploitation e.g. due to overgrowing and restricted light penetration. In contrast, some technological systems are applied in the technical scale that merge certain elements of open and closed systems. There is also a possibility of algae biomass proliferation and culture in dark systems.

MIKROALGI – METODY HODOWLI

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S ł o w a k l u c z o w e: mikroglony, hodowla, stawy otwarte, fotobioreaktory, systemy ciemne.

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Abstrakt

Badania nad możliwością wykorzystania glonów do celów energetycznych prowadzone są od wielu lat. Jedną z najważniejszych kwestii, warunkujących opłacalność produkcji glonów, jest wybór odpowiedniego systemu hodowlanego. Stosowane są zarówno reaktory zamknięte, jak również technologie oparte na systemach otwartych. Testowane w warunkach laboratoryjnych oraz w skali technicznej rozwiązania technologiczne są zwykle mało opłacalne. Fotobioreaktory są drogie i stwarzają trudności eksploatacyjne, np. ze względu na zarastanie oraz ograniczenie dostępu światła. Systemy otwarte charakteryzują się niską efektywnością i produktywnością. W skali technicznej stosowane są również rozwiązania, które łączą pewne elementy systemów otwartych i zamkniętych. Istnieje również możliwość namnażania biomasy glonów w systemach ciemnych.

Introduction

Investigations into the use of algae for energy production have been carried out for many years. The first attempts to culture and exploit algae for fuel production purposes have been undertaken during the II World War by German researchers. The possibility of using algae biomass in processes of methane fermentation was discovered in the fifties of the XXth century by, among others (*Algae culture*. 1953). In the sixties, algae biomass production has been started in a technical scale in open systems. In the eighties the USA Department of Energy has initiated a research on the use of algae in energy production (Aquatic Species Program). In the successive years technologies have been developed for biodiesel production and algae culture in photobioreactors, and commercial bio-refineries have been established in, among others, Turkey and the United States. Nowadays, a number of research and implementation programs have been underway worldwide, including the EU Member States, that are aimed at boosting the effectiveness of algae biomass production and conversion into biofuels (LO et al. 2010, MUSSATTO et al. 2010, VIJAYARAGHAVAN 2009). A few thousands of patents linked with technologies of algae biomass production, separation and conversion into biofuels are registered annually, which indicates a great global interest of scientists in this respect (GALLAGHER 2011, STEPHENS et al. 2010).

A key issue is the selection of technology for culture and acquisition of algae biomass for energetic purposes. The proliferation and culture of algae may be conducted with a variety of methods, beginning from strictly monitored methods in closed laboratory systems, to less predictable methods in open systems (MOLINA-GRIMA 1999).

Owing to the site the process is being run at, systems are divided into:

– open systems (outdoor systems), making use mainly of open aquifers, ponds, though under favorable environmental conditions (light exposure and high temperature) they may also refer to photobioreactors (PBRs). Applied

mainly for economic concern, they provide no possibility for monitoring conditions of the culture process, and are sensitive to environmental pollution, predators and competitive species. The open systems include (BOROWITZKA 1999):
  – traditional ponds, ground or concrete with large (up to 250 ha) area and depth of up to 0.5 m;
  – circular ponds with mechanical or convective mixing;
  – race track-type ponds with a paddle wheel;
  – cascade ponds.
– closed systems, making use mainly of various types of photobioreactors or small tanks. Such cultures assure, most of all, the possibility of constant monitoring over lighting and temperature, protection against predators, parasites and competitive species of algae. The closed systems include:
  – the sack system of "large bags", operating in a sequential or semi-continuous mode,
  – tubular photobioreactors with horizontal or vertical orientation, or inclined at any angle. A technological solution of this type may be arranged in parallel or spirally as the Biocoil type reactor,
  – plate photobioreactors.

Owing to the duration and method of running a culture, systems may be divided into:
– batch systems, where the culture is inoculated in a single dose into the culture medium and cultivated until the moment when the population of cultured organisms reaches its maximum or when cell density in the culture approximates the maximum. Then harvest occurs, and the cultured biomass is separated from the culture medium;
– continuous systems with continuous inflow and outflow of culture medium and continuous reception of biomass produced;
– semi-continuous systems with partial reception of biomass and continuous addition of culture media so as to maintain algae growth rate close to the maximum.

Open systems

A very important issue is the choice of technology for culture and production of algae biomass for energy purposes. Owing to economic concerns, the systems used currently in the industrial scale are open ponds. In most cases, their design is very simple. These are usually ground tanks with a large surface area and depth of up to 0.5 m, stirred mechanically by means of a paddle agitator. They are built in the form of a round pond or a racetrack. The culture

media applied usually include chemical substances or sewages containing appropriate quantities of biogenic compounds, if necessary supplemented with microelements. Carbon dioxide is acquired directly from atmospheric air through simple diffusion. An advantage of this technological solution is simple and inexpensive construction, its drawbacks however include high water losses as a result of evaporation, low yield of biomass production, limited possibilities of culture of specific algae species susceptible to various infections, diseases and parasites. Systems of this type prove successful in regions with high insolation and unlimited access to water, hence at the seaside areas. These technologies are commonly applied in the Asian countries, Mexico, the USA (e.g. in Arizona) as well as in Europe, including e.g. Italy, Spain and even the Netherlands, mainly for cultures of Spirulina and Chlorella genera algae (DEMIRBAS et al. 2011).

Due to the very good results are obtained as breeding ponds in a race track that we decided to just describe the type of open systems. This type of technology not only high productivity but also is probably the most reasonable choice in economic terms.

The system consists of shallow open ponds racetrack type with a width of 2.0–3.0 m and depth 0.1–0.3 m, made of PVC, clay or paved area from 1000 to 5000 m^2 (MOLINA-GRIMA 2003). Pond type of race track design is based on

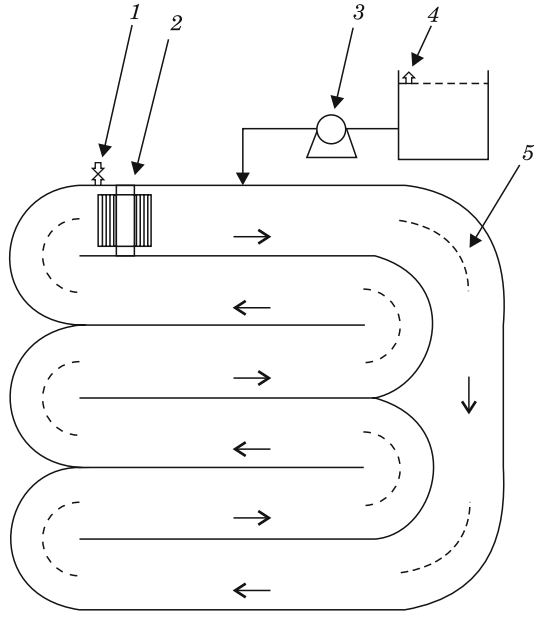

Fig. 1. Rays ways reactor: *1* – discharge of biomass; *2* – puddlewheel; *3* – nutrient dosing pomp; *4* – medium tank; *5* – baffle

a series made in a closed loop recirculation duct. Excitation processes of mixing and flow in such a device there, using the paddle wheel. Channels of the pond can be constructed of concrete or compressed earth and are lined with white plastic. The medium can be fed into the system on a continuous basis during the day when the culture is efficiently radiated. Nutrients are administered before the paddle wheel, where the stream begins breeding. Grown biomass is received from the device before the paddle wheel at the end of the loop. To prevent falling of algae stirrer is maintained constantly in motion. Rays ways reactor is shown in Figure 1.

If the wastewater system are introduced, it can remove up to 35 g BZT/m^2 d (175 g BZT/m^3 d pond with a depth of 0.2 m) compared to 5–10 g BZT/m^2 d (5–10 g BOD/m^3 d pond at a depth of 1 m) achieved in conventional stabilization ponds (RACAULT and BOUTIN 2005). This project also requires a much shorter hydraulic retention time (HRT) in the system, amounting to 2–6 d (MARA and PEARSON 1998) compared to 10–40 d in traditional ponds (CRITES, TCHOBANOGLOUS 1998). Despite a much better cleaning efficiency, few such systems are currently used for wastewater treatment in the world.

Photobioreactors

A completely different approach to the problem of proliferation and culture of alga biomass is the application of closed systems, the so-called photobioreactors. A variety of these systems have been developed so far, including: horizontal tubular photobioreactors, horizontal tubular photobioreactors or sloping under any angle, biocoil type reactors, continuous or semi-continuous big bag systems, or flat-plate photobioreactors (BOROWITZKA 1999, AMIN 2009) – Figure 2.

Photobioreactors are much more universal devices that may be applied under various climatic conditions. The closed character of bioreactors restricts evaporation, eliminates the problem of parasites and predators, whilst artificial lighting assures optimal conditions for photosynthesis. Such conditions afford the possibility for running cultures of specific algae species, e.g. these with a high concentration of oil in biomass (CUARESMA et al. 2011, DEMIRBAS 2011).

The first closed photobioreactor in use was a big gags photobioreactor (BAYNES et al. 1979, WATSON 1979). It consisted of large sterile plastic bags ca. 0.5 m in diameter with an adjusted aeration system. Most of those systems have been designed to operate in the batch mode, however semi-continuous systems do happen as well. This variant of the system was developed by COHEN and ARAD (1989) and its modification consisted in using bags with a smaller

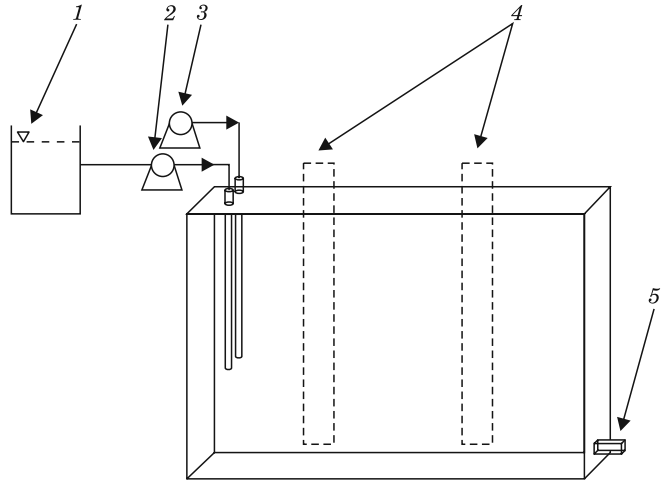

Fig. 2. Plate photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – air pomp; *4* – light source; *5* – discharge of biomass

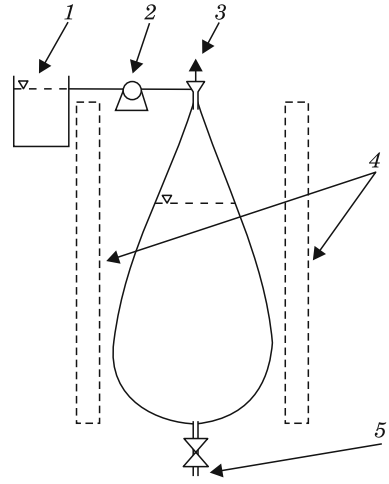

Fig. 3. Big bags photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – gas outlet; *4* – light source; *5* – discharge of biomass

diameter. So far, however, a few companies have been exploiting this system (Figure 3).

The key problem linked with its exploitation is the necessity of running the process indoor, for there is no possibility of controlling temperature. In addition, a relatively large diameter of the bags poses problems with culture lighting, which in turn constitutes a factor diminishing system's productivity.

A reactor of this type requires high workload and does not assure thorough mixing, which may cause the collapse and reduction of process effectiveness.

Most of tubular reactors are made of glass or polycarbonate (PC), and the flow of medium and supply of gases proceed through pumps or, preferably, by means of the airlift system. They may be constructed in the horizontal (MOLINA et al. 2001), vertical or inclined (TREDICI and ZITTELLI 1998, UGWU et al. 2002), and conical orientation (WATANABE and SAIKI 1997). The aeration and mixing of culture in a tubular photobioreactor is usually conducted with the use of air pumps or the airlift system. This reactor may be exploited also outdoor, as it possesses a vast illumination surface area. In contrast, one of its main drawbacks is a low mass transfer. This phenomenon occurs as a result of an increasing level of oxygen along with increasing sizes of this type of reactors. Investigations have shown that a very high level of dissolved oxygen (DO) may easily be reached in the tubular reactors (MOLINA et al. 2001). Furthermore, of key significance is the process of photoinhibition ongoing in the tubular reactor under external (outdoor) conditions (VONSHAK and TORZILLO 2004). Once the system is scaled up by increasing tubes' diameter, the ratio of illumination surface area to system volume is decreasing. In this case, the cells at the lower part of the tube will not receive enough light for cell growth (due to light shading effect) unless there is a good mixing system. Then, the effectiveness of providing light to cells may be achieved through the improvement of the mixing system (UGWU et al. 2003, UGWU et al. 2005).

An additional difficulty in a tubular photobioreactor is temperature control. Though it is feasible to apply a thermostat, it is an expensive and difficult to implement solution. Worthy of notice is also the possibility of adherence of algae cells to the walls of the reactor. In addition, a long tubular reactor is characterized by gradients of oxygen and CO_2 transported alongside the tubes (CAMACHO RUBIO et al. 1999, UGWU et al. 2003). An increase in pH value would lead to the necessity of frequent re-carbonization, which in turn would increase algae production expenditures.

A Biocoil type photobioreactor is a tubular photobioreactor composed of a transparent, plastic tube with a small diameter (2.4–5.0 cm), which is screw-wrapped around a vertical tube with a large diameter (BOROWITZKA 1999). A few parallel systems of tubes are coupled through collectors with a pumping system that may be realized through the airlift system or a variety of pumps. The type of the pump applied depends on algae type. The reactor may be equipped in a gas exchange system. Temperature may be controlled manually or automatically. Reactor's design assures uniform mixing and minimizes the adherence of algae cells to the internal walls of the tubes. Reactor's operation may be fully automated, which enables reducing costs of the production process. The system may also be designed so as to assure the axenic conditions (Figure 4).

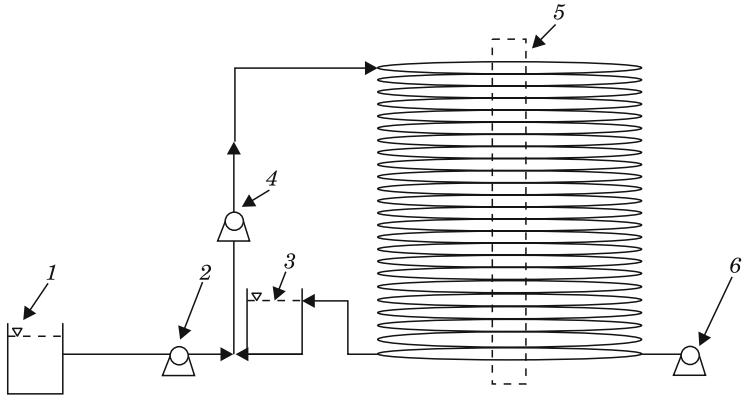

Fig. 4. Biocoil photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – discharge of biomass; *4* – circulation pump; *5* – light source; *6* – air pomp

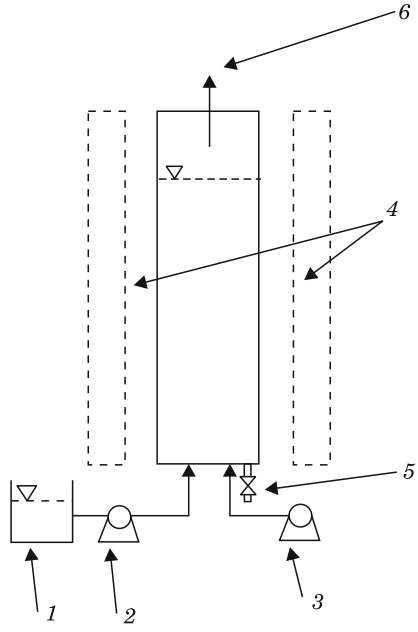

Fig. 5. Column photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – air pomp; *4* – light source; *5* – discharge of biomass; *6* – gas outlet

Various configurations of vertical-column photobioreactors have been extensively studied in view of their applicability for cultivation of algae (CHOI et al. 2003, VEGA-ESTRADA et al. 2005, GARCIA MALEA LOPEZ et al. 2006, KAEWPINTONG et al. 2007). They are compact, low-cost, and easy to operate monoseptically (SANCHEZ MIRON et al. 2002). In addition, they are very promising for

large-scale cultivation of algae. Studies have shown that bubble-column and airlift photobioreactors (up to 0.19 m in diameter) may attain the final biomass concentration and specific growth rate that are comparable to values typically reported for narrow tubular photobioreactors (SANCHEZ MIRON et al. 2002). Some bubble column photobioreactors are either equipped with two draft tubes or constructed as split cylinders. In the first case, the mixing occurs between the riser and the downcomer zones of the photobioreactor through the walls of the draft tubes (Figure 5).

Some photobioreactors may be equipped in external lighting with fluorescence lamps or light-emitting diodes (LED), which has become the focus of interest in recent studies (WANG et al. 2007). Those reactors are also equipped in stirrers to assure the transfer of algae cells in the whole volume of the system. Air and CO_2 are supplied to the reactor from the bottom, so as to assure the maximally long time of algae cells contact with gases. This type of photobioreactor may also be equipped in automated sensors measuring the intensity of lighting, to enable the exploitation of solar energy of both natural and artificial origin (OGBONNA et al. 1999). In this case, the artificial source of solar energy is switched on only at diminished intensity of the natural source (in cloudy whether or at night). There is also a possibility of applying light pipes for distribution of radiation in cylindrical photobioreactors (MATSUNAGA t al. 1991). One of the main advantages of this type of bioreactors is the feasibility of hot- or pressure-sterilization of their contents. Furthermore, solar energy may be supplied to the reactors in a continuous mode (both in daytime and at night) by coupled application of artificial and natural sources of light.

Though many systems have been developed so far, unfortunately none of them may be found cost-effective. Photobioreactors are expensive and require high exploitation inputs (lighting, supply of carbon dioxide), they additionally pose some difficulties in exploitation e.g. due to overgrowing and restricted light penetration. In contrast, some technological systems are applied in the technical scale that merge certain elements of open and closed systems. For instance, in Turkey the racetrack-type ponds were located in greenhouses, which has a positive impact on reduced evaporation and restricted access of predators, enables achieving temperature stability and applying additional lighting if necessary. It also affords the possibility of introducing an additional source of CO_2 in the form of e.g. combustion gases, to the greenhouse's interior. It seems that these types of solutions may prove successful also in Poland (UGWU et al. 2008). The table 1 shows advantages and disadvantages of commonly used systems for the cultivation of algae.

Table 1

Advantages and disadvantages of commonly used systems for the cultivation of algae

Type of system		Advantages	Disadvantages
Open systems	round ponds, racetrack-type ponds	relatively economical, easy to use and clean after completion of culture, good for the cultivation of algae on a large scale	low ability to control culture conditions, difficulty with cultivation algae in the long term, low productivity, large size, limited number of culture species, cultivation susceptible to external factors (predators, disease, pollution)
Closed systems	photobioreactors column (vertical)	the high mass transfer, good mixing and low stress, low energy consumption, high potential for scalability, easy to ensure sterility, good for the immobilization of algae, reduce photoinhibition and photooxidation	a small area of exposure, their construction requires the use of sophisticated materials, the possibility of hydrodynamic stress, decrease in surface exposure with increasing diameter of the column
	photobioreactors plate	the large surface area exposure, suitable for outdoor culture, good for the immobilization of algae, good availability of light, good productivity, biomass, relatively cheap, easy to clean, low concentration of oxygen	increasing the size of the reactor requires the use of multiple chambers and supporting structures, the problems of controlling the culture temperature, the risk of fouling the walls, the possibility of hydrodynamic stress in some species of algae
	horizontal tubular photobioreactors	the large surface area exposure, suitable for outdoor culture, good productivity, biomass, relatively cheap	fluctuations in pH, dissolved oxygen and CO_2 in the pipe length, the risk of fouling the walls, requires a large surface

Technological parameters of autotrophic cultivation

Irrespective of the fact whether algae biomass is produced in open systems or in closed photobioreactors, appropriate technological parameters ought to be assured that determine fast development and growth of the algae biomass (COHEN 1991). For optimal growth algae need appropriate lighting at the level of 200–400 fmol photons m^{-2} s^{-1}, which corresponds to ca. 1/10 of the lighting provided directly from the Sun. Under conditions of intensive culture, the quantity of CO_2 that has to be supplied to the system reaches ca. 1.83 kg per 1 kg of produced biomass. In this case, wastewaters, fresh or salty water

supplemented with nitrate and phosphate fertilizers may serve as the culture medium. Significant microelements in such a culture include nitrogen, phosphorus, iron and in the case of selected species also silicon. Unlike that of nitrogen and phosphorus, the availability of carbon rarely suppresses the growth of algae. Production performance in fresh waters is often diminished by the availability of phosphates (CHISTI 2007). In turn, the availability of silicon is a factor reducing the growth of diatoms. A number of algae species require an external source of vitamins, often thiamine, biotin, B_{12} and riboflavin, purins, pyrimidine and other growth factors. The temperature of the culture should oscillate in the range of 20–30°C, whereas pH value in the range of 6 to 8. Water saturation with oxygen should not exceed 400% owing to the arrestment of photosynthesis and ongoing processes of photoinhibition. Worthy of notice is also that the life cycle of algae used for biofuels production reaches 7 days (TAMBURIC et al. 2011).

Dark systems

There is also a possibility of algae biomass proliferation and culture in dark systems (BOUARABA et al. 2004) (Figure 6). These are the so-called heterotrophic cultures, in which appropriate carbonic compounds are fed to bioreactors as a culture medium for the algae. Such cultures are run with acetate or glucose as a source of carbon. This system has for the first time been used in *Chlorella* culture, which was described by KAWAGUCHI and SOONG in 1980. As reported by LEE (1997), in the year 1996, approximately 550 t of this alga were produced in Japan. The Martek Inc. company (USA) runs a heterotrophic culture of *Crypthecodinium cohnii* to be used for the production of long-chain unsaturated fatty acids (KYLE et al. 1998). Typical conditions of the heterotrophic culture include:

- temperature: 26–28°C;
- no light;
- agitation with the rate of 200–480 rpm;
- pH from 6.1 to 6.5;
- culture medium containing ca. 20 g of glucose or acetate/L of culture;
- necessary supply of nitrogen and phosphorus compounds in a quantitative C:N:P ratio of 9:1.25:1.25.

A culture in the heterotrophic system has several advantages. Systems of fermentation are well recognized, much is known about their design and operation. Another advantage is a high concentration of biomass that may range from 20 to 100 g d.m./L (RADMER and PARKER 1994, RUNNING et al.

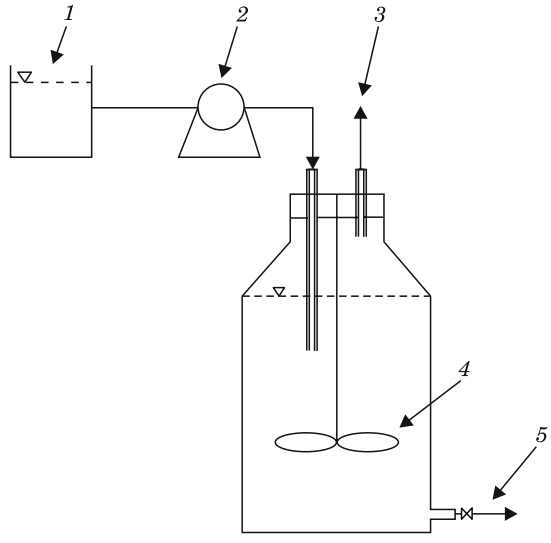

Fig. 6. Fermentation tank: *1* – medium tank; *2* – nutrient dosing pomp; *3* – gas outlet; *4* – stirrer; *5* – discharge of biomass

1994). The major drawbacks of this system are that it cannot be applied to all species of algae and that the process itself poses some technological complications (BOUARAB et al. 2004, OGBONNA 1996, PEREZ-GARCIA 2011).

Conclusion

Nowadays algae are perceived as one of the types of biomass with a very high energetic potential. In the technical scale, they are cultured mainly for feedstuff or foodstuff purposes, however there are also some biorefineries operating in the technical scale. The exploitation of this type of substrate in the systems of methane fermentation seems also promising, which has been proved by results of worldwide investigations.

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