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Producing transportation fuels from algae: In search of synergy

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ABSTRACT

The study found that promising algae biofuels R&D breakthroughs (hydrothermal liquefaction technology, high-frequency magnetic impulse cavitation reactors, etc.) and industry milestones (technologies of hydrorefining and catalytic selective oxidation among others), in order to move forward, require for implementation of new synergies and further innovations needed to improve economical production of advanced biofuels that are not applicable today. It seems that already viable state-of-the-art findings must be re-examined extensively in all of the different aspects in order to hasten the commercialisation of algal biofuels production in sustainable biorefineries. The same could be said about the feedstock selection for algal biomass production and its cultivation. It is the first step to successful large-scale algae cultivation in new regions of the world. Based on the above mentioned we identified fourteen promising algae species that can successfully grow in various regions of Russia under local climatic conditions. Samples collected during expedition were analysed at Lomonosov Moscow State University. Providing predetermined alternate periods of light and darkness and for temperature control of the different mediums to improve photosynthetic responses we investigated two different microalgal production systems: open ponds of the volume $V=500$ l and closed bioreactors of the volume $V=1.0$ l. Later on, a review on interdisciplinary synergies between biology and technology to open up new avenues of R&D in the field of algae-for-transport was carried out by leading universities of Lithuania, Russia, and Ukraine. In summary, we found that it is already possible to reduce the price of the 3rd and 4th generation biodiesel fuel from algae by applying the synergistic approaches to sustainable energy production highlighted in this paper, and probably some other ones as well.

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1. Introduction

Nowadays, an interest in alternative fuels has become an important cornerstone to deal with, and it is likely to be driven by further research and development in upcoming decades [1,2]. The impact of using bio-fuels in road transportation is an important topic to debate on [3–5]. Biofuels derived from sources such as non-food biomass and crops, termed "second generation" (2G) biofuels, do not compete directly with arable land and so are thought to be sustainable [6,7]. However, the low conversion rates of plant-matter to fuel means that 2G biofuels have a limited ability to contribute to fulfilling energy demand, unless substantial areas are devoted to the cultivation of such crops [8]. Partly due to some of the issues mentioned, growing interest is now focused on the development of "third generation" biofuels obtained from microalgae organisms [8–11]. Recently, microalgae based biodiesel is a newly emerging field, because high potential biodiesel could be derived by microalgal biotechnology [12–15].

A review of literature reveals that the technical aspects of development and deployment of algal biofuels in the world have gained much scholarly attention [9,11,16–18]. One of the actual world's directions and works in bioenergy is related to development of engineering–geographical technologies for effective accumulation of solar energy into biomass of microalgae and its further transformation to biofuel [17,19–21]. Bioenergy potential of algae attracts much attention of biofuel producers investing substantial funds in research projects. The interest to algae is defined by the fact that its biomass has a number of attractive characteristics such as natural bio-productiveness and high energy content, that may be at least 10 times the size of energy accumulated in the oilseed crops (in the form of accumulated fatty acids). Fuel produced from algae is attributed to biofuels of the third (algae grown especially for energetic purposes) and fourth (genetically modified algae that convert CO_2 directly to fuel) generations [17,19–21].

First of all, a term microalgae reads into the context of lipid extraction, and finally chemical transesterification for the formation of biodiesel. With the introduction of new technologies, the same type of biomass may serve as a source for the different types of fuel. There is a range of new technologies that are being applied to production of the third generation biofuels from algae (see Fig. 1a) [19]: (1) biosynthesis of ethanol and hydrogen by fermentation using algae; (2) biosynthesis of (a) carbohydrates (production of bioethanol or biobutanol via acetone–butanol–ethanol fermentation), (b) hydrocarbons (with further processing to diesel fuel, kerosene, gasoline motor fuels, etc.), (c) triacylglycerides (transesterification of algae oil to biodiesel/derivation of the fuel used to power aircraft from the hydroprocessing of fats and oils), etc. Meanwhile, waste algal biomass may serve for sustainable production of second-generation biofuels, such as methane, hydrogen, bio-oil, and liquid biofuels.

Many types of green alga can grow well photoautotrophically as well as heterotrophically [22] utilising sugars as carbon and energy sources (see Fig. 1b). Many of the current intensive algae production systems (natural ponds, man-made ponds, closed photo-bioreactors, etc.) were adapted to photoautotrophy (capable of growing photolithoautotrophically and control inorganic nitrogen accumulation) [19]. More recently, zero-exchange management systems have been developed, which encourage heterotrophic growth and have been promoted for production of algae in closed fermenters (see Fig. 1b) [19]. Liu et al. [22] came to the conclusion that the heterotrophic algal cells demonstrate much higher yields of total lipids, neutral lipids and oleic acid than the photoautotrophic cells. The collection of these insights taken as a synergetic whole suggests that the oil from the heterotrophic cells are more feasible for biodiesel production.

2. Literature review on algal biology and growing systems

In favourable conditions, algae grow very quickly and accumulate up to 50 per cent of oil of their total weight. There are thousands of algae species in the nature – from microscopic ones to the ones of 60 m length [23]. Algae are widespread all around the world, yet in the Arctic snow it is possible to find microalgae even thought, usually, algae live in water. Hence, nature sufficiently endowed algae with special vital forces. The majority of them may breed twice if they live in favourable for that conditions. The world Algae Base includes about 55,000 species of marine, brackish, fresh water and terrestrial algal species [23,24]; these lists are continuously supplemented with new species. In the opinion of some scientists who are well-versed in gathering, processing, analysing and evaluating information on the physiology and biochemistry of algae [25], it is possible to collect 100 tons of algae biomass from one hectare in one year. Accordingly, the essence lies in the process of photosynthesis and the function of the photosynthetic apparatus (the purpose of which is to absorb light energy and transform it to chemical energy with the help of chlorophyll molecules and pigments) of the different types of algae [26]. Composition of the pigments and their quantitative ratio predetermine the colour of cells. Cytokinesis – process by which a parent cell divides into two or more daughter cells – is an important cell cycle of monocellular or multicellular organisms (algae). According to compatibility of cytokinesis and mitosis (nuclear division), the following reproductive strategies of algae are recognised (see Fig. 2) [26]: (i) successive division (simultaneously after the division of the nucleus (karyokinesis), partition wall formation in cells occurs), (ii) simultaneous division (partition walls are formed only after the process of the division of the nucleus in plant cells is over), and (iii) gradual-segregative division. Algae grow by repeated cell division within a chain of cells. After the repeated divisions of nucleus follows the formation of the partition walls that divide the whole plait into multinucleate segments. Thereafter, the newly formed segments are able to divide further on.

One of the prerequisites for the economical use and an efficient conversion of algae is high level of biomass content. Phytoplankton chlorophyll *a* concentration is the most widely used indicator of algal biomass, determination of which is very important in limnological research for gathering information so that obtained data could be used for identification of algae biomass on a weight or volume basis, in the form of photosynthesis performance index (see Fig. 3) [27].

Ref. [27] proposes a simple equation for describing the dependence of algae biomass on chlorophyll *a* concentration [27]:

$$y = 2.68 + 1.17x \quad (1)$$

where y is the chlorophyll *a* concentration, mg/m^3 and x is biomass, g/m^3.

The correlation coefficient of 0.929 (n=58) indicates a strong positive correlation between variables [27].

A major bottleneck to algae-based fuels production lies in the fact that the algae culture has to grow fast, to accumulate substantial quantity of lipids including hydrocarbons and triglycerides, and to be easily adapted to meet the requirements of advanced technologies. The basic idea of finding new native species of algae is preconditioned by the objective to design and construct biofuel production plants that remove more CO_2 than they generate and develop future solutions through synergies between biology and technology, as well as to highlight the most outstanding discoveries across this field. Besides that, efficiency of most bioenergy technologies is site-specific [1,2]. Thus, detailed information on available resources and their relative economics is very important for their successful development [3,16,28]. Presently, first industrial and pre-industrial projects of algae production are under development [28]. Two systems are commonly used

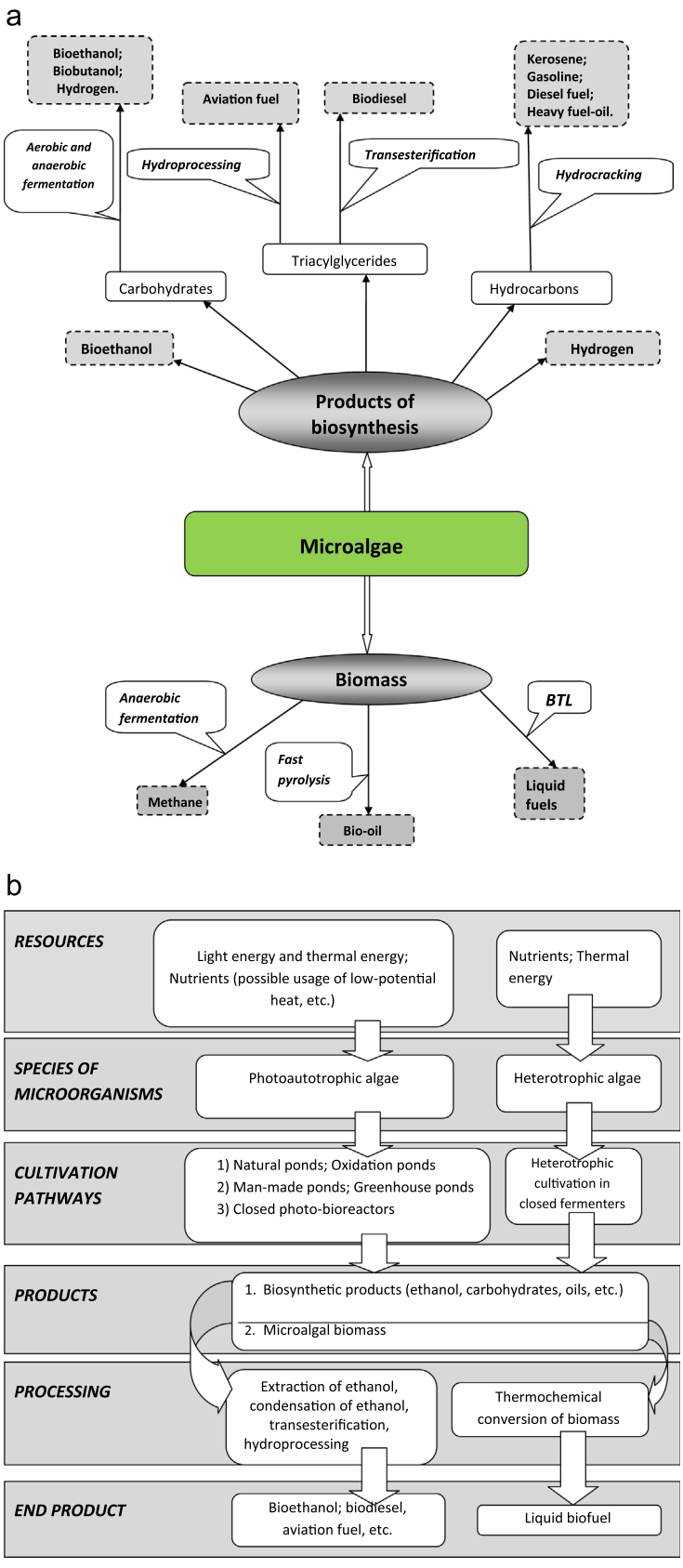

Fig. 1. Third generation biofuels from microalgae: a – modern trends in energy-from-microalgae, b – production of fuels from a microalgal feedstock.

to cultivate algae: the open system (e.g., open raceway ponds) and the closed system (photobioreactors) [8,11,18,29,30]. Their advantages and disadvantages are presented in Fig. 4.

Many agree that while industrial symbiosis (the use of wastewater or industrial flue gas) and various other synergies have the potential to offset algae's high cultivation and harvesting costs,

with each additional interdependent synergistic technology comes a level of complication that may challenge the performance, reliability, resilience, and viability of the system [29,31].

There is a clear rationale for the search of synergies in various techniques of resources cultivation and biofuel production that makes the process more efficient, although it should not be assumed that they will be the most effective means to improve conditions in all cases. Meanwhile, co-authors will focus mainly on transport biofuel (biodiesel) from algae, as this is the area where the most intensive research and development activity is being carried out and that is likely to generate also the great technological fallout on the entire transport sector and bioenergy sector as integral parts of today's economy.

3. Methodology and collected data analysis

3.1. Research on algal biotechnology

During 2008–2012, numerous expeditions having been made by LMSU researchers to various regions of Russia (Karelia (63°N 32°E), The Kamchatka Peninsula (57°N 160°E), Lake Baikal (53°30′N 108°0′E), Valdaysky District (57°58′N 33°15′E), etc.) to collect new native samples of lipid-containing algae species and investigate them by using the cell wall cytochemistry methods covering the use of Sudan dyes (Black B: $C_{29}H_{24}N_6$). Data received using this qualitative method agree with quantitative metrics for lipids in microalgae earlier described by co-authors in pre-feasibility study, patent document [32] and Refs. [19,20].

Acclimated to low temperature, two *Arthrospira platensis* [32] strains from the collection of the Laboratory of Renewable energy sources at LMSU and twelve new strains of algae were analysed for the following tests: (1) identification at the genus level and the species level; (2) selection of technological parameters in order to obtain growth kinetics and biomass yield of these strains at standard conditions of cultivation (1st stage of cultivation); (3) selection of technological parameters in order to switch from standard conditions of cultivation to stressful conditions (2nd stage of cultivation); (4) biochemical analysis of algal biomass obtained during the 1st and 2nd stages of cultivation; (5) development of laboratory-technical regulations for the microalgal biomass production of the certain biochemical composition.

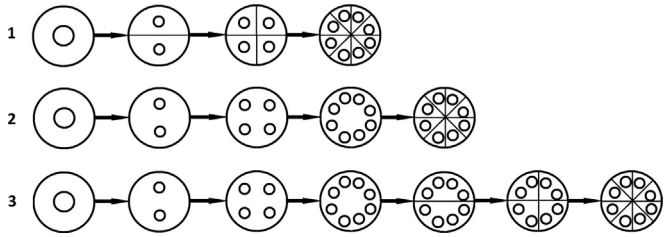

Fig. 2. Reproductive strategies of algae [26].

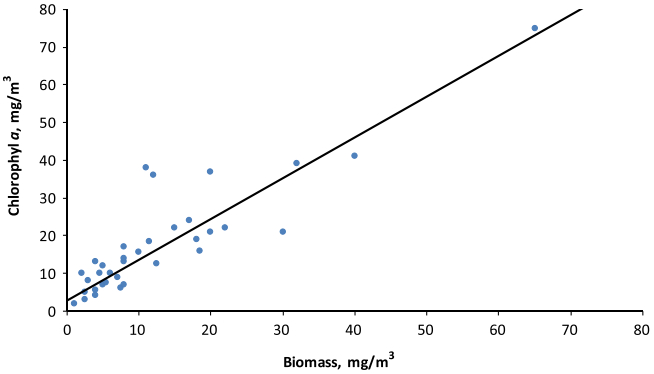

Fig. 3. Correlation between chlorophyll *a* concentration and biomass of phytoplankton [27].

Gas–liquid chromotography and thin layer chromotography have been used to separate, identify and in some cases quantify lipid extracts [17]. Several forms of spectroscopy have also been applied to analyse algal lipids [17]: the content of lipids in the obtained biomass was recorded on a RF-5301PC spectrofluorimeter (Shimadzu) with a 150 W Xenon lamp as the excitation source using the stock solution Nile Red of dark purplish-red powder (Sigma N-3013) and fluorescent microscope MICMED-2 LOMO equipped with a digital camera and micro-analysis software (LLC TeleMedTechnika/ТелеМедТехника, OOO). For the laboratory tests, four synthetic mediums formulated for cultivation of the selected species of algae were used: (i) Blue-Green Medium BG-11 ($NaNO_3$ – 1.5 g/l, K_2HPO_4 – 0.04 g/l, $MgSO_4 \cdot 7H_2O$ – 0.075 g/l, $CaCl_2 \cdot 2H_2O$ – 0.036 g/l, Citric acid – 0.006 g/l, Ammonium ferric citrate – 0.006 g/l, $EDTANa_2$–0.001 g/l, Na_2CO_3–0.02 g/l, Trace metal mix A5 solution – 1.0 ml/l per 1.0 l of distilled water) [33], (ii) Zarrouk's medium ($NaHCO_3$–16.8 g/l, KNO_3 – 3.0 g/l, $K_2HPO \cdot 3H_2O$ – 0.66 g/l, K_2SO_4 – 1.0 g/l, $MgSO_4 \cdot 7H_2O$ – 0.2 g/l, NaCl – 1.0 g/l, $CaCl_2$. $2H_2O$ – 0.04 g/l, $FeSO_4 \cdot 7H_2O$ – 0.018 g/l, EDTA – 0.08 g/l, Zarrouk's trace metal solution – 1.0 ml/l, per 1.0 l of distilled water) [34], (iii) Optimal Haematococcus medium (OHM): KNO_3 – 0.41 g/l, Na_2HPO – 0.03 g/l, $MgSO_4 \cdot 7H_2O$ – 0.246 g/l, $CaCl_2 \cdot 2H_2O$ – 0.11 g/l, Fe(III) (citrate)$\cdot nH_2O$ – 2.62 mg/l, $MnCl_2 \cdot 4H_20$ – 0.98 mg/l, $ZnSO_4 \cdot 7H_2O$ – 0.22 mg/l, $CuSO_4 \cdot 5H_2O$ – 0.012 mg/l, $Na_2MoO_4 \cdot 2H_2O$ – 0.12 mg/l, Cr_2O_5–0.075 mg/l, NH_4VO_3 – 0.023 mg/l, $CoCl_2 \cdot 6H_2O$ – 0.011 mg/l, SeO_2 – 0.005 mg/l, biotin - 25 mcg/l, thiamine - 17.5 mcg/l, vitamin B12 - 15 mcg/l per 1.0 l of deionized distilled water, and (iv) Tamiya medium: KNO_3 – 5.0 g/l, KH_2PO – 1.25 g/l, $MgSO_4 \cdot 7H_2O$ – 2.5 g/l, $FeSO_4 \cdot 7H_2O$ – 0.009 g/l, EDTA– 0.037 g/l, H_3BO_3 – 2.86 mg/l, $MnCl_2 \cdot 4H_20$–1.81 mg/l, $ZnSO_4 \cdot 7H_2O$ – 0.22 mg/l, MnO_3–0.018 mg/l, NH_4VO_3–0.023 mg/l per 1.0 l of distilled water.

3.2. Algal biodiesel technology roadmap and production synergies

We generally assess the current technical status of algae biofuel technologies in relation to the production of the most common fuel for transport, i.e. biodiesel, and describe the appropriateness of promoting the growth of an algae biofuel industry if the synergy among different technological levels would be achieved. Research activity data were collected from both primary (presentation of scientific exploratory developments at LMSU (Russia) and state-of-the-art technologies developed in Ukraine) and secondary sources. An extensive review of R&D projects, international academic and patent publication records, relevant books, journals, reports, and case studies from elsewhere in the developing world provided the theoretical basis for analysis of the data. Obtained data constituted an input for further calculations of algal biomass potential for transport needs. We also examine the potential role of modern biotechnology in improving commercial viability of algae biodiesel.

4. Discussion

4.1. Patent research and analysis

One proxy measure which demonstrates the relatively low level of R&D funding in many EU countries, and globally, is offered by the marginal increase in the number of algal biofuel technology patents. The low number of patents describing algal biofuel production technologies is an advantage for researchers and potential investors as it leaves opportunities for further developments. A significant

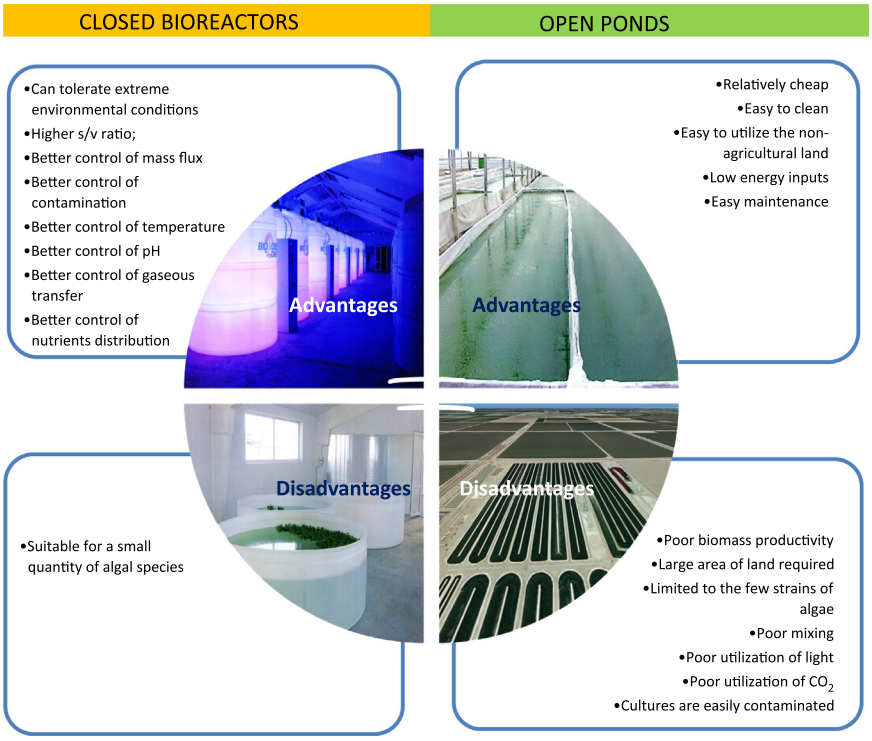

Fig. 4. Closed bioreactors vs. Open ponds [11].

part of the research activities remain modest in the international context. It is however difficult to understand the high levels of commercial activity and investment into the algal biofuel production technologies at present as a biofuel resource, in light of the research advances still required [35]. Notwithstanding this fact, several key challenges suited to the worldwide application are already solved this is likely to remain the case.

The preliminary design in the patent RU 2 487 920 C1 [36] aimed to determine the advanced operating condition by using "Method of producing biodiesel fuel from reservoir mud and/or sewage treatment plant sludge" [36]. Presently, the main method of utilisation of reservoir mud and sewage treatment plant sludge lies in mechanical dewatering and storage of dehydrated precipitates in silt(-detention) basins where during a long period of time their biodegradation takes place. This method of utilisation for mud and waste water precipitations leads to long-term and, most commonly, irrevocably alienated land plots as well as does not meet the requirements of the ecological standards.

Substance [36]: material cells are mechanically disintegrated; lipids are extracted using a Folch method, involving extraction of lipids with a mixture of chloroform and methanol (2:1 by volume), followed by washing the extract with KCl solution (0.88%) and, after demixing and removing the top phase, with a mixture of 0.88% KCl solution and methanol (1:1 by volume); the KCl solution and the mixture are added in an amount of 1/4 part of the obtained and remaining volume of extract, respectively; the ready lipid extract is dried by passing through a layer of anhydrous Na_2SO_4; the dry extract is then re-esterified with a mixture of methanol and acid catalyst, where the methanol is mixed with the catalyst in ratio of 50:1 by volume; biodiesel is extracted from the reaction mixture with hexane; the biodiesel extract is dried by passing through a layer of anhydrous Na_2SO_4 [36].

Effect [36]: obtaining biodiesel using a cheap and simple method by processing mud or sludge from treatment facilities.

All the key distinguishing features of this innovative technical solutions enabled obtaining biodiesel according to EN 14214 and EN 14213 requirements; proposed method complies with the patentability criterion of inventive step and non-obviousness. The main advantages of the claimed methods of biodiesel fuel production from reservoir mud and/or sewage treatment plant sludge are as follows [36]:

- Raw material in production will cost nothing in comparison with other expensive raw material currently used to create the same products.
- Two-component mixture enables a more complete extraction of lipids.
- Application of acid catalyst while carrying out interesterification reaction causes no soap in the process.

Result [36]: reduction in biodiesel production cost.

4.2. *Ways to increase effectiveness of growing microalgae biomass as a commodity for production of biofuels*

During cultivation of microalgae, it is impossible to achieve high accumulation rate of lipids and productivity of biomass at the same time [17,19,20]. Induction of biosynthesis and accumulation of lipids may be achieved under the physiological stress conditions: nitrogen-starvation, phosphorus-starvation, osmotic shock (stress), intensity and spectral composition of light, pH-changing (acidity or basicity of an aqueous solution), temperature regimes, impact of heavy metals and other chemical matters [19–21]. One of the possible ways to accumulate microalgal biomass having an optimal content of lipids is cultivation in two stages [19,37,38]. The first stage includes biomass accumulation under optimum growth conditions. On the second stage of microalgae cultivation, the high cell biomass yield is affected to stress conditions; an increased insolation level coupled with nitrogen and phosphorus deficiency in a nutrient medium directly influences synthesis induction and intracellular accumulation of lipids. For implementation of the two-stage technology for microalgae cultivation a prototype photobioreactor module equipped with light-emitting-diode lighting (LED) [17] systems and carbon dioxide (CO_2) supply system for

Table 1
Content of lipids in biomass of microalgae grown in various conditions of cultivation (LMSU data).

No	Specie/strain	Cultivation conditions	Content of lipids, %
1	*Arthrospira platensis* rsemsu 1/02-P	1/2 Zarrouk's medium; semi-continuous cultivation in open ponds of the volume $V=500$ l equipped for near-surface mixing process; lighting $I=(55\pm5)$, μE/(m^{2}s); steady-lighting conditions; $T=21\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$.	54.9
2	*Arthrospira platensis* rsemsu 1/02-T	Zarrouk's medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=21\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 10 days.	36.1
		Zarrouk's medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(400\pm25)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions; $T=21\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	47.1
3	*Nannochloropsis sp.* rsemsu N-1/11-B	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/ (m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	25.0
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	23.5
4	*Chlorococcum sp.* rsemsu Ccc-7/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	25.2
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	35.5
5	*Chlorococcum sp.* rsemsu Ccc-24/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	37.8
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	20.3
6	*Chlorococcum schwarzii (Ettl et Gartner)* rsemsu Chcc-14/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	43.2
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	34.0
7	*Stigeoclonium sp.* rsemsu St-k-13/11	BG-11 Medium having an increased by 10 times phosphorus content; cultivation in shake flasks of the volume 750 ml filled with 300 ml of medium (frequencies of orbital revolution of the stirrer's platform 120–130 min^{-1}); lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 21 days.	17.6
8	*Stigeoclonium sp.* rsemsu St-m-13/11	BG-11 Medium having an increased by 10 times phosphorus content; cultivation in shake flasks of the volume 750 ml filled with 300 ml of medium (frequencies of orbital revolution of the stirrer's platform 120–130 min^{-1}); lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 21 days.	22.0
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	25.7
9	*Chlorella vulgaris (Beier.)* rsemsu Chv-20/11	Tamiya Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	21.0
		Tamiya Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	33.0
10	*Chlorella sp.* rsemsu Chl-1/11-B	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	31.1
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	22.5
11	*Chlamydomonas sp.* rsemsu Chlam-10/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	26.2
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	31.9
12	*Chlamydomonas sp.* rsemsu Chlam-15/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	58.1
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 2 days.	68.1
13	*Haematococcus pluvialis (Flotow em. Wille)* rsemsu Hp-1/11	Optimal Haematococcus Medium (OHM); cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 14 days.	4.0
		Optimal Haematococcus Medium (OHM) that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(450\pm50)$, μE/(m^{2}s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$; growing and photosynthesis (duration) – 1 day.	16.8

Table 1 (*continued*)

No	Specie/strain	Cultivation conditions	Content of lipids, %
14	*Coenocystis sp.* Coen-11/11	BG-11 Medium; cultivation in closed bioreactors of the volume $V=1.0$ l including the air barbotage (containing 2% CO_2) tank in the reactor; lighting $I=(55\pm5)$, μE/(m^2s); lighting requirements: light cycles (night/day)=16:8; $T=25\ ^\circ C\pm1\ ^\circ C$; growing and photosynthesis (duration) – 14 days.	16.6
		BG-11 Medium that does not contain nitrogen and phosphorus; cultivation in open ponds of the volume $V=1.0$ l having a magnetic stirrer (speed 800 min^{-1}); lighting $I=(350\pm50)$, μE/(m^2s); lighting requirements: steady-lighting conditions, $T=25\ ^\circ C\pm1\ ^\circ C$; growing and photosynthesis (duration) – 2 days.	18.9

Table 2
Synergistic solutions of commercialised prototype HFMICR [45].

Complex solutions	Proof of concept
Low-temperature reaction	• In HFMICR, the reaction takes place on molecular level at room temperature and there is no need to heat the algal oil. • All components are exposed to magnetic-directed cavitation pulses. Fatty acid molecules are split using micro-explosions. This results in a decrease of viscosity, increase of the Cetane number, an improvement in fuel characteristics as well as in speed and completeness of the etherification reaction.
Minimal quantities of catalyst and alcohol	• Avoidance of using alcohol recovery process. One of the main problems with biodiesel processors based on outdated tank technologies is that, the methanol in the water phase must be recovered and recycled back to the front-end of the plant. This requires additional equipment (still, distillation column, condenser, etc.) which increases total power consumption. • Avoidance of using re-etherification reaction which requires additional expensive equipment and protective systems intended for use in explosive mediums during methanol recovery process. • No alcohol losses during methanol recovery process. • Improvement of the environmental and economic characteristics.
One pass through the process	The time taken to obtain algal biodiesel is reduced by a factor of 8–10.
Reduce in total power consumption	Usually, biodiesel production is based on heating of oil up to 67 °C–70 °C that can lead to higher power consumption caused by the vacuum drying, re-etherification, methanol recovery, etc. The use of HFMICR processing eliminates all the stages mentioned above, which means that 5–7 times less power to be consumed.
Improvement in technical architecture	• Avoidance of the initial water washing step/no additional expensive and energy consuming washing equipment. • Avoidance of the expensive and energy consuming vacuum drying equipment. • Avoidance of using purifying sorbates.

the medium has been developed at LMSU. After the first stage of cultivation (of the best candidate strains with potential for lipid production) microalgae biomass production has taken place in an open cultivators of the prototype photobioreactor module containing nitrogen- and phosphorus-free nutrient medium and illumination option range from 350 to 450 μE/(m^2s). Shortage of nitrogen and phosphorus and relatively high lighting caused a physiological stress for the investigated cultures. These factors affected further growth of algae yet stimulated lipid synthesis and accumulation in cells [21,38]. Biomass productivity (yield) values during the first stage of cultivation and obtained lipid content during the final stage were the main indicators for the investigated algal species. During the growth cycle of microalgae, biomass production and total lipid content of the aforementioned fourteen strains cultivated under different conditions showed that significant increases in lipid contents have been observed in microalgae (see Table 1); nine strains reacted positively to stress; five strains also reduced the lipid levels to some extent [21,38].

The stress response (a stressed alga) is species- and strain-specific; hence stress parameters require thorough experimental selection for every culture. The performed research on cultivation of various species of microalgae in various environments also opened a possibility for practical realisation of technology intended to the efficient utilisation of anthropogenic CO_2 [21]. In addition, optimal conditions for growth, maintenance, and storage of culture collection of algae have been worked out (see Table 1).

4.3. Algae-based fuel technologies as market innovations

A large range of suitable compounds can, in principle, be produced from the different matter of biological origin. Nevertheless, market expansion strategy and further economic growth requires commercialisation of the advanced technologies. Since conventional biofuel production processes, though already commercially available, continue to improve in efficiency and economics, the advanced conversion routes currently are at early and late stages of the research and development. Examples of advanced technologies include, but are not limited to the following: hybrid technologies of syngas production; synthetic fuels for diesel engines (Fischer–Tropsch biodiesel, bio-DME (bio-Di-Methyl-Ether), biomethanol); Koch biodiesel production from organic waste; biodiesel from algae; hydrogen production processes from biomass; conversion of cellulose containing biomass into alcohols, etc. Hastening the manufacture of bio(diesel)fuels from algal biomass is seen as development of sustainable biorefineries, including the establishment of the necessary infrastructure. However, it is obvious that only a portion of the technical potential can be realistically met. Moreover, the estimates of technical potential are only indicative and are likely to change over time. The technical potential will likely grow with the development of available technologies. The economic potential of algal biofuels in the medium and long term will very much depend on their cost compared to prices for fossil fuels. The latter are difficult to predict.

Use of bio-liquids to fuel transport vehicles does go some way toward solving the oil depletion problem, but will only reduce Greenhouse gas emissions if their kg CO_2-e/MJ over the full fuel LCA (life cycle) is less than that for petroleum-based transportation fuels. A considerable amount of scholarly literature [11,39–43] has been published on the different pathways for alternative diesel fuels production from lipids. The most advanced are (see Fig. 5):

1. Transesterification with methanol.
2. Hydrorefining (including hydrocracking and hydrogenation).
3. Catalytic selective oxidation.

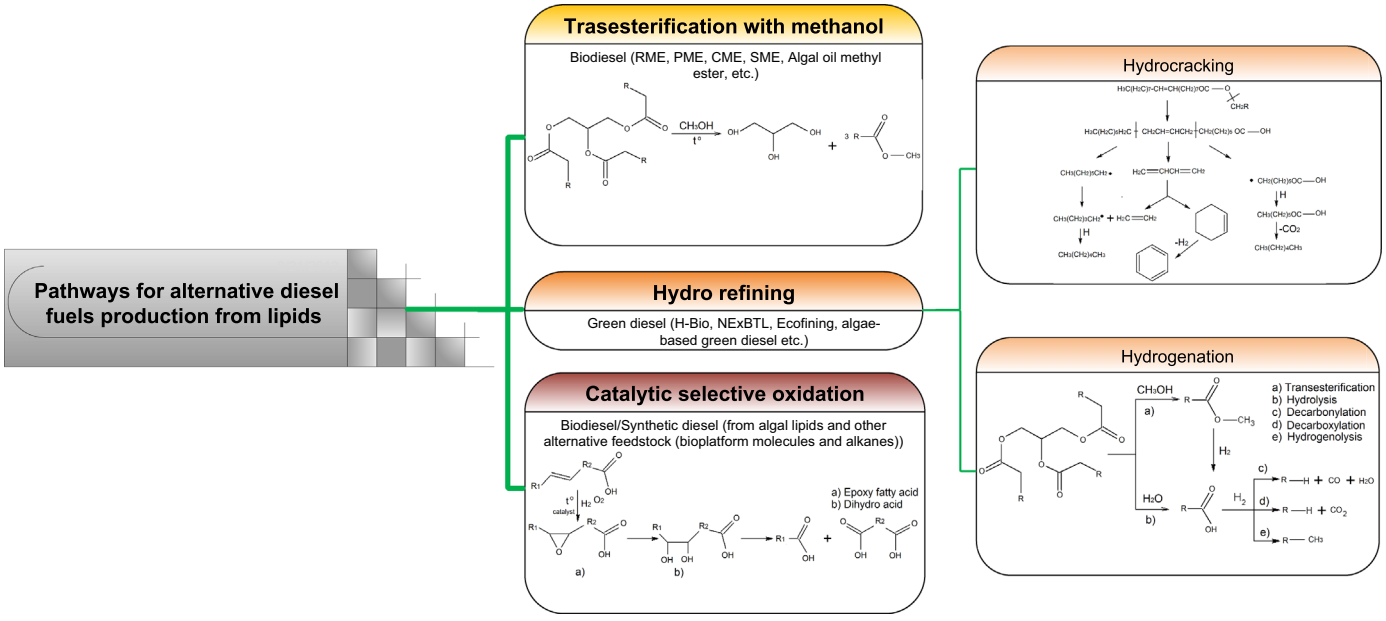

Fig. 5. Possible pathways for alternative diesel fuels production from lipids [11].

Oil and starch received from algae may be used in industry. Both these materials are transformed into liquid fuels, bio-ethanol and biodiesel quite easily. By-products of algae oil and starch may also be used: transformed into biogas, forage additives or agricultural fertilizers. On this understanding, the algae-based fuel technology can be called a market innovation. Conversion of triglyceride fatty oil of algae to fuels has generally been performed by transesterification using an alkali catalyst to produce biodiesel. Selective catalytic oxidative (see Fig. 5) cleavage of the olefin bond of monounsaturated fatty acids has been offered as a step of the complex processing microalgae [39]. High content of monounsaturated fatty acids in microalgae is favourable to the catalytic selective oxidative cleavage of olefin bond to proceed with environment friendly oxidants in mild conditions [39].

Algal oil refining (see Fig. 5) is a process to transform algal oil into fuel by hydrocracking or hydrogenation. Hydrocracking breaks big molecules into smaller ones using hydrogen while hydrogenation adds hydrogen to molecules. The refining of algal oil into motor-fuels (usually–green diesel, naphtha, light fuel gas) usually requires a two-step process for conversion to produce diesel fuel with the required properties. The first step involves removal of O_2, along with N_2 and sulphur. During this step, straight-chain alkanes from the fatty acid of the algal oil are produced. The cracking and isomerization of the (n-) alkanes into a mixture of (iso-) alkanes (highly branched) is the second step of conversion. Some of the larger molecular weight paraffins can also be cracked into smaller molecular weight paraffins [44]. This flexibility allows the yield of jet and diesel product to be tuned towards either fuel, depending upon the current market value of jet fuel versus diesel fuel. The treated product was then fractionated by distillation into naphtha, synthetic paraffinic kerosene jet fuel and diesel fractions [44]. Along with naphtha, some light fuel gas is also produced. Both of these products have value as fuels, and the naphtha fraction can be a feedstock for a reforming unit for gasoline or polymer-grade olefins [44].

Among the innovative synergistic solutions for algal biodiesel production, the state-of-art technology – Ukrainian-made high-frequency magnetic impulse cavitation reactors (HFMICR) [11,45] which include several innovative synergistic solutions for the process design must be discussed to give readers full picture of what it is (see Table 2). The commercialised prototype HFMICR proves out some technical assertion that is the key to the feasibility of the preferred alternative [11,45]:

HFMICR can operate both as the part of fully automated system and separately. It is possible to arrange both mobile production of biodiesel plant for own needs and stationary complexes designed for mass-production capable of producing 500 l of biodiesel per hour and processing efficiency 0.002 kW hour/liter [45].

Hydrothermal liquefaction of algal biomass provides another direct and synergetic pathway for liquid biocrude production. Until today, the processing option usually was applicable to dry biomass feedstock, such as algae. Most current processes require the algae to be dried (as is required in other thermochemical conversion processes) – a process that takes a lot of energy and is expensive [46]. The most important cost-saving step in the hydrothermal liquefaction technology is that the process works with wet algae: the new process, described by Elliot et al. [46] works with the algae slurry that contains as much as 80–90% water and eliminates the need to expend energy to dry the feed before processing. The proposed system also abele to eliminate another step required in today's most common algae-processing method: the need for complex processing with solvents like hexane to extract the energy-rich oils from the rest of the algae [46,47]. Instead, researchers work with the whole algae, subjecting it to very hot water under high pressure to tear apart the substance, converting most of the biomass into liquid and gas fuels [46,47].

5. Conclusions

The synergies between the different production steps in biofuel industry are evident. These synergies have the potential to offer an approach to bioenergy development that is both sustainable and beneficial. Microalgal strains with lipid content are of great interest in the search for a sustainable feedstock for the 3rd and 4th generation biodiesel production. Usually, they contain proportionally high levels of lipids (over 30%). Algae cultivation can be done in a variety of environments. During biochemical studies co-authors identified the most dramatic increases in the lipid content of the following cultures at specific cultivation conditions: (1) *Chlamydomonas sp.* rsemsu Chlam-15/11 (68.1%) – BG-11 medium, steady-

lighting conditions $I=(450\pm50)$, μE/(m^{2}s), $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$, growing and photosynthesis (duration) – 2 days, (2) *Chlamydomonas sp.* rsemsu Chlam-15/11 (58.1%) – BG-11 medium, lighting $I=(55\pm5)$, μE/(m^{2}s); lighting requirements: light cycles (night/day)=16:8, $T=25\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$, growing and photosynthesis (duration) – 14 days, (3) *Arthrospira platensis* rsemsu 1/02-P (54.9%) – 1/2 Zarrouk's medium, steady-lighting conditions $I=(55\pm5)$, μE/(m^{2}s), $T=21\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$, and (4) *Arthrospira platensis* rsemsu 1/02-T (47.1%) – Zarrouk's medium, steady-lighting conditions $I=(400\pm25)$, μE/(m^{2}s), $T=21\ ^\circ\mathrm{C}\pm1\ ^\circ\mathrm{C}$, growing and photosynthesis (duration) – 2 days. The examples reported in this paper are aimed at demonstrating that algae biofuel production is one of the branches in the liquid biofuel sector that is undergoing profound structural changes, which are aimed not only at achieving better economic returns, but also better sustainability. There is not so long way until algae will entail a profitable contribution as source of transportation biofuels in general and biodiesel in particular. Major breakthroughs are indeed necessary towards design and development of advanced technologies able to increase product yields and at the same time to decrease processing costs.

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