Lemna Minor Cultivation for Biofuel Production

T Kuznetsova¹, N Politaeva¹, Yu Smyatskaya¹, A Ivanova¹
¹Higher School of Biotechnology and Food Technology, Saint Petersburg State Polytechnical University named after Peter the Great, 194021, Novorossiyskaya Str., 48-50, St.Petersburg, Russia

E-mail: tano_lovely@mail.ru

Abstract. In this paper we consider usage of duckweed for national economy: for livestock feed, for bioethanol and biogas production, in medicine and for phytoremediation. Cultivation of duckweed Lemna minor in conditions of St.Petersburg was carried out in order to obtain biomass. Two cultivation variants were studied: in a natural reservoir and in an artificial reservoir during a period from May to July 2017. The following cultivation conditions were determined: lighting, temperature. The population growth intensity was estimated. The most favourable conditions appeared to be at natural pond in Petergof. Daylight features, lightning intensity of the Leningrad Oblast’ are less favourable than that for south Russian regions, so biomass production rate is drastically lower. The obtained L. minor biomass was dried out under the infrared lamp up to constant weight. The obtained dry biomass was used to produce lipids extracts by Sohxlet technique. Lipids output was 4% of dry biomass for duckweed grown in natural pond and 6% for duckweed grown in artificial pond. Most likely it is driven by stress factor of duckweed growth, which is limited by insufficient solar illumination. The lipid output of duckweed is not high, but we are planning to choose optimum cultivation conditions for duckweeds, which will result in increased lipids output.

1. Introduction
Duckweeds are of monocotyledon plant family. 4 genera are specified basing on morphological, biochemical and genetical characteristics: Lemna, Spirodela, Wolffia & Wolffiiella (some scientists derive the fifth one – Landoltia) and 37 types [1,2]. They are widely distributed in North and South Americas, South and Central Africa, Europe, South Asia, at the south of Australia, mostly at cultivated areas. A half of species is grown in the tropics and subtropics the rest are in the temperate zone [3].

Duckweed is quickly vegetatively divided in summer in fresh ponds with ditch-water, and covers its' surface by a continuous green cover, it duplicates its mass in 6 days [4]. L.minor is a typical species for all Central Russian areas. It may produce 200 tons of biomass from a hectare during a season at multiple gathering. At Uzbekistan 276 tons were gathered from 1 hectare during 8 months [5].

Literature analysis has shown that the major directions of L.minor cultivation investigation are defined by further usage of its biomass:
- In ecotoxicological investigations and tests[6];
- Directed cultivation for obtaining valuable components in Lemna minor tissues. Duckweeds accumulate in fronds flavonoids, diterpenoids, tannins, B1, B2 and C vitamins, steroids, unsaturated
fatty acids, including Omega-3, giving rise to its medicinal properties and wide application in medicine [7].
- Cultivation of *L.minor* with its further usage as livestock feed (duckweed has a protein content up to 45% in dry weight) [8];
- *L.minor* cultivation for biomass production as a source for bioethanol and biogas obtaining. These technological cycles are based on enzymatic hydrolysis of starch, which is contained in duckweed [9,10];
- Duckweeds are of interest as potential biofactory for fused proteins production, such as vaccine proteins, serum albumin, hemoglobin, collagen; moreover, a genetic transformation of duckweeds might be made in order to enhance its’ stability to pollutants, for example, heavy metals [11,12].

Duckweeds, together with other higher water plants, are actively used for waste water purification technologies, i.e. [13–30]. The works [5,19–21] present an ability to use duckweed *L.minor* for water purification from heavy metal ions. In order to improve efficiency of water purification, an additional magnetic field treatment was implemented.

Despite vast usage of duckweeds (for water purification, for obtaining of proteins, Omega-3, livestock feed) the authors pronounce a hypothesis on the possibility of lipid fraction obtaining from duckweed, which will be further used for biofuel obtaining.

Biofuel of the first and the second generations - ethanol and biodiesel produced from plant oils - are now broadly studied [34–44]. New direction is biofuel of the third generation: photosynthetic microalgae form the basis of biofuel obtaining. They use light energy to absorb carbon dioxide from air for production of organic compounds. Microalgae are very little, just 1.2,3,10 mm in diameter, and are able to produce a vast number of fats inside the cell, which are lipids. These lipids possess long carbon chain. They can be substracted and recycled into fuel. The conditions for high-speed synthesis of microalgae are studied in the works [45–47].

Biofuel from duckweeds, which are freely grown at many regions of our country in sufficient quantity, will possibly bring to light a new stream of biofuel generation. So, the aim of the present work is of immediate interest.

The aim of this work was to study the speed of *L.minor* cultivation in the climatic conditions of Leningrad Oblast' and obtaining of lipids from dry biomass of *L.minor*.

2. Materials and methods

As water reservoirs for cultivations we used natural pond in south part of the Leningrad Oblast' and an artificial pond, targetely created at the North of Leningrad Oblast' at the territory of Peter the Great St. Petersburg Polytechnic University (SPbPU), figure 1.

![Figure 1. An artificial pond at SPbPU territory.](image-url)

The evaluation of *L.minor* growth intensity in natural conditions was carried out in accordance with pond surface incrustation area. For this purpose, square frames were cut from expanded polyethylene:
the inner enclosure was 20×20 cm (400 cm$^2$), the external dimensions were 26×26 cm (676 cm$^2$), figure 2.

In order to achieve high cultivation efficiency, one should use pure culture. Storage of L.minor stock culture is performed at 4-10°C, using MS-environment, pH is 5.7 [10].

Duckweed is gathered using fork or shovel devices with mesh-tip.

![Figure 2. A scheme of enclosing frame.](image)

The frame is placed at the pond surface. It should not sink. 10 plants are placed in the inner part of the frame. At intense population growth the frame is photo-recorded once a day, figure 3.

![Figure 3. Photographs of L.minor population cultivated in enclosing frame: a- 15 days, b – 34 days.](image)

The photographs are contrasted using Levenhuk ToupView software. The software instruments are used for total area estimation ($S_{total}$) in pixels (px), which is enclosed by the inner frame. Also it is used for determination of total area, which is occupied by plants ($S_{p}$) in pixels (px). Pond incrustation ratio ($W$, %) is determined according to the formula (1):

$$W = \frac{S_{p}}{S_{total}} \times 100, \ [%]$$

Pond incrustation ratio is determined minimum in three frames and, using methods of statistical data processing, the average value of pond incrustation ratio and confidence range ($x_{av} \pm S_{x}$) are determined. Using the obtained data the cultivation curve is plotted: relation between pond surface incrustation area in enclosing frame (%) and cultivation time (days).
The obtained biomass was dried under the infrared lamp to get the constant weight. Dry biomass was cut in a mortar with sand for cell desintegration and more complete lipid extraction.

Lipid extraction was carried out by Soxhlet technique using extraction installation Büchi E-812 SOX. This extraction installation allows easily manipulation of time of analysis cycle. The software includes the processes of washing-out and drying, which allow one to begin the analysis directly after finishing these processes. The extraction process was as follows: 3 g of duckweed dry biomass was put into a cellulose small cup. 100 ml of solution for extraction (extracting agent) was poured into a cup for extraction collection, which was previously dried out and weighted. The solvent systems was ethanol: n-hexane (1:9).

3. Results and discussion
Duckweed cultivation was conducted in Saint Petersburg during the period from May, 29 to July, 3 at two water reservoirs:
- In a natural pond in southern Saint Petersburg (Petergof);
- In an artificial pond in northern Saint Petersburg (Saint Petersburg State Polytechnical University), figure 1.

In natural conditions of Leningrad region *L.minor* cultivation may be performed from April to November inclusive. The most efficient temperature for duckweed growth is 19-26°C. The plants withstand short-term frosts. However, at long-term water temperature decrease below 3 °C we observed slowdown of plant growth rate. Day average temperature of duckweed cultivation places are reliably equal, figure 4.

Possible pH ranges in which *L.minor* is cultivated are 3.5-8.5 [11], at this optimal pH values are 6.3-8.0.

Cultivation took place at natural light. Lightning intensity in day time is the following: - 405-1570 lx at the south of Saint Petersburg; - 1350-4000 lx at the north of Saint Petersburg. Optimum range is from 405 to 9000 lx (the average lightning intensity during our experiment was 3000 lx).

The most intensive growth of *L.minor* was observed in a natural reservoir (in pond in Petergof, figure 4) in comparison with population in artificial pond at the north of Leningradskay Oblast' (SPBPU). It can be explained by the following facts:
- Climate of the south of Leningrad Oblast' is warmer that that one in the north;
- Natural pond in Petergof is more accessible for sun;
- Natural biogeocenose contributes towards rapid duckweed growth.

Artificial pond is located between SPbPU buildings, so sun access is impeded, also it represents an artificial biogeocenose. As it is known, artificial biogeocenose is a semi-stable system and cannot self-regulate due to a small amount of species in it. Despite some discrepancies between the ponds, the time of duckweed *Lemma minor* population duplicating was 20 days both for the first and second cases, figure 4.
The obtained biomass was dried under the infrared lamp to get the constant weight. The obtained dry biomass was used to produce lipids extracts by Soxhlet technique using extraction installation Büchi E-812 SOX. Extraction process duration is about 3 hours, including:
- 15 cycles of extraction itself at 100% heating of hot plate.
- 5-minutes of washing-out at 100% heating of hot plate.
- 30 minutes of drying at 120% heating of hot plate.

Lipids output was 4% of dry biomass for duckweed grown in a natural pond of Petergof and 6% of dry biomass for duckweed grown in artificial pond of SPbPU.

Despite the fact that biomass growth in a natural pond of Petergof was significantly quicker, than that for artificial pond of SPbPU, the percentage output of lipids if higher for duckweed grown in an artificial pond. Most likely it is driven by stress factor of duckweed growth, which is limited by insufficient solar illumination. The lipid output of duckweed is not high, but we are planning to choose optimum cultivation conditions for duckweeds, which will result in increased lipids output.

The obtained lipid fractions can be used for production of technical biodiesel. This occurs during transformation of oil triglycerides into fatty acid ethyl esters [48]. The usage of such biodiesel will allow replacing of biodiesel obtained from natural mineral deposits (naphta) and save natural raw materials of our planet.

4. Conclusions
1. We have studied cultivation speed of duckweed in natural conditions of the Leningrad Oblast'. The most favorable conditions appeared to be in a natural pond of Petergof in comparison to artificial pond. This is driven by sunny location of natural pond and presence of natural biogeocenose in it.
2. Daylight features, lightning intensity of the Leningrad Region are less favourable than that for south Russian regions, so biomass production rate is drastically lower. However, the climatic characteristics (moderate climate, transient from continental to marine) allows increasing L.minor cultivation period: from May to November inclusive.
3. The lipid output from the obtained biomass is insignificant - just 4-6%. The highest lipid amount was obtained from duckweed biomass, grown in artificially created pond (SPbPU), which can be explained by stress cultivation conditions.
4. We propose to use lipid fractions from dry duckweed biomass for biodiesel production.
5. References

[1] Les D H D, Crawford D J D, Landolt E, Gabel J D J and Kimball R T R 2002 Phylogeny and Systematics of Lemnaceae, the Duckweed Family Syst. Bot. 27 221–40

[2] Les D H, Landolt E and Crawford D J 1997 Systematics of the Lemnaceae (duckweeds): inferences from micromolecular and morphological data Plant Syst. Evol. 204 161–77

[3] Tahtadjan A L 1982 Volume 6. Flowering plants Plant life [Zhizn’ rastenij] p 543

[4] Grudzinskaya I A 1982 Volume 6. Flowering plants Plant life [Zhizn’ rastenij] pp 493–500

[5] Anon Spirulina & Duckweed http://istina1888.narod.ru/20AA.HTM

[6] Ol’shanskaya L N, Sobgaida N A and Tarushkina Y A 2011 Phytoremediation technologies for protection of hydrosphere: monograph [Fitoremediacionnye tekhnologii v zashchite gidrosfery: monografiya]

[7] Khellaf N and Zerdaoui M 2010 Growth, photosynthesis and respiratory response to copper in Lemma minor: a potential use of duckweed in biomonitoring J. Environ. Heal. Sci. Eng 7 299–306

[8] Anon Duckweed – Uses, Benefits and Side Effects https://www.herbal-supplement-resource.com/duckweed-uses-benefits-side-effects.html

[9] Xu J, Zhao H, Stomp A-M and Cheng J J 2012 The production of duckweed as a source of biofuels Biofuels 3 589–601

[10] Cui W and Cheng J J 2015 Growing duckweed for biofuel production: A review Plant Biol. 17 16–23

[11] Gaidykova S E, Rakitin A L, Ravin N V, Skryabin K G and Kamionskaya A M 2008 Development of genetical transformation system of Lemma minor Ecol. Genet. IV 20–8

[12] Chhabra G, Chaudhary D, Sainger M and Jiawal P K 2011 Genetic transformation of Indian isolate of Lemma minor mediated by Agrobacterium tumefaciens and recovery of transgenic plants Physiol. Mol. Biol. Plants 17 129–36

[13] Hoseinzadeh G R, Azarpour E, Motamed M K, Ziaeidoustan H, Moraditochaee M and Bozorg H R 2011 Heavy metals phytoremediation management via organs of aquatic plants of Anzali international lagoon (Iran) World Appl. Sci. J. 14 711–5

[14] Sanghamitra K, Prasada Rao P V V and Naidu G R K 2011 Heavy metal tolerance of weed species and their accumulations by phytoextraction Indian J. Sci. Technol. 4 285–90

[15] Ikram S, Parvaiz M, Ahmed M, Ilyas U, Khalid A, Munir M, Anwar S and Afzal H 2013 Aquatic plants of Sialkot District, Pakistan World Appl. Sci. J. 28 884–96

[16] Dipu S, Kuma A A and Salom Gnana Thanga V 2011 Potential application of macrophytes used in phytoremediation World Appl. Sci. J. 13 482–6

[17] Ol’shanskaya L N, Sobgaida N A, Tarushkina Y A and Stoyanov A. 2008 Effect of a magnetic field on extraction of heavy metals from waste water with duckweed Chem. Pet. Eng. 42–5

[18] Ol’shanskaya L N, Sobgaida N A, Tarushkina Y A and Kolesnikova O N 2008 Heavy-metal uptake dynamics for higher water plants in highly concentrated solutions Chem. Pet. Eng. 44 166–9

[19] Ol’shanskaya L N, Tarushkina Y A and N.A. S 2008 Investigation of dynamics of zink, copper and cadmium accumulation from high-concentration solutions by higher water plants [Issledovanie dinamiki nakopleniya cinka, medi i kadmiya iz vysokokoncentrirovannyh rastvorov vodnymy rasteniyami] Ekol. i promyshlennost’ Ross. February 32–3

[20] Ol’shanskaya L N, Sobgaida N A and Valiev R S 2015 Heavy metal removal from contaminated runoff using adsorbents and phytosorbents [Izvlechenie tayzhelyh metallov iz zagryaznennyh stokov s ispol’zovaniem adsorbentov i fitosorbentov] Ekol. i promyshlennost’ Ross. 19 18–23

[21] Orlova T, Melnichuk A, Klimenko K, Vitvitskaya V, Popovych V, Dunaieva I, Terleev V, Nikonorov A, Togo I, Volkova Y, Mirschel W and Garmanov V 2017 Reclamation of landfills and dumps of municipal solid waste in a energy efficient waste management system: Methodology and practice IOP Conference Series: Earth and Environmental Science vol 90
[22] Il’ina K V, Gavrilova N M, Bondarenko E A, Andrianova M J and Chusov A N 2017 Express-techniques in study of polluted suburban streams Mag. Civ. Eng. 76 241–54
[23] Iakovlev A, Grishkanich A, Kascheev S, Ruzankina J, Afanasyev M and Hafizov N 2017 Inactivation pathogenic microorganisms in water by laser methods vol 10065(SPIE)
[24] Bukova M, Bondal A, Skvortsova O, Nikonova O, Kholodiakov A, Guseva I, Makarova T and Mirschel W 2016 Reclamation of the illegal dump for sustainable development the environment in Sverdlovsk of Leningrad Oblast’, Russia MATEC Web of Conferences vol 73
[25] Matveyeva A N, Pakhomov N A and Murzin D Y 2016 Recycling of Wastes from the Production of Alumina-Based Catalyst Carriers Ind. Eng. Chem. Res. 55 9101–8
[26] Bondarenko E A, Ilína K V, Andrianova M J and Chusov A N 2016 Main inorganic ions and electric conductivity of polluted urban streams Mag. Civ. Eng. 68 37–44
[27] Pavlov S, Novikov A, Pavlov A, Skvortsova O, Nikonova O, Semanina E, Zafarov R and Wenkel K-O 2016 Methods of Improving Water Treatment Systems for Individual Residential Houses MATEC Web of Conferences vol 73
[28] Umanets V, Kalitova L, Kalitov D, Chusov A and Umanets E 2015 Conditions for safe disposal of industrial wastewater into the deep aquifer International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM vol 1pp 135–44
[29] Novikov D, Molodkina L, Chusov A and Vedmetskii Y 2015 Electrokinetic and electroconductivity properties of filtering material aqualat Procedia Engineering vol 117pp 264–72
[30] Simonenko E, Gomonov A, Rolle N and Molodkina L 2015 Modeling of H2O2 and UV oxidation of organic pollutants at wastewater post-treatment vol 117(Elsevier Ltd)pp 342–9
[31] Ol’shanskaya L N, Sobgajda N A, Stoyanov A V and Valiev R S 2011 Impact of low-amplitude physical fields on electrical properties of plant cells and tissues at photoanlysis processes of wastewaters, polluted by heavy metals [Vliyanie maloamplitudnyh fizicheskix polej na ehlektricheskie svojstva tkanej i kletok rast Ekologiya - obrazovanie, nauka, promyshlennost` i zdorov’e: sbornik dokladov IV Mezhdunarodnoj nauchno-prakticheskoj konferencii. CH.1. (Belgorod: BGTU Publishing house) pp 96–9
[32] Ol’shanskaya L N, Sobgajda N A and Stoyanov A V 2011 Impact of electromagnetic radiation on the process of bioelectrochemical copper removal by eichormia [Vliyanie ehlektromagnitnych izluchenij na process bioehlektrohimicheskogo izvlecheniya medi ehjhorniej] Ekol. i promyshlennost` Ross. February 52–4
[33] Ol’shanskaya L N, Sobgajda N A and Valiev R S 2014 Impact of external physical fields energy on kinetics and mechanism of metal ion phytoremediation from effluents [Vliyanie energii vnesnih fizicheskix polej na kinetiku i mekhanizm fitoremediacji ionov metallov iz zagryaznennych stokov] Ekol. i promyshlennost` Ross. January 13–7
[34] Zubkova M, Maslikov V, Molodtsov D, Chusov A, Zhazhkov V and Stroganov A 2016 Technological Decision to Renewable Energy Usage Biogas for Off-grid Systems Consumption MATEC Web of Conferences vol 73
[35] Ryabukhin D S, Zakusilo D N, Kompanets M O, Tarakanov A A, Boyarskaya I A, Artamonova T O, Khodoroskivskiy M A, Opeida I O and Vasilyev A V 2016 Superelectrophilic activation of 5-hydroxymethylfurfural and 2,5-diformylfuran: Organic synthesis based on biomass-derived products Beilstein J. Org. Chem. 12 2125–35
[36] Vishnyakova M A, Seferova I V and Samsonova M G 2017 Genetic sources required for soybean breeding in the context of new biotechnologies: (Review) Sel’skokhozyaiistvennyaya Biol. 52 905–16
[37] Didenko N, Popkova A, Skripnuk D and Mirolyubova O 2017 Deforestation and human activity: A global perspective vol 17(International Multidisciplinary Scientific Geoconference)pp 183–90
[38] Fernandes M C, Ferro M D, Paulino A F C, Chaves H T, Evtuguin D V and Xavier A M R B 2018 Comparative study on hydrolysis and bioethanol production from cardoon and rockrose pretreated by dilute acid hydrolysis Ind. Crops Prod. 111 633–41

[39] Sirajudin N, Gusoff K, Yani S, Ifa L and Roesyadi A 2013 Biofuel production from catalytic cracking of palm oil World Appl. Sci. J. 26 67–71

[40] Mokhtana N, Daud W R W, Rahimnejad M and Najafpouy G D 2012 Bioelectricity generation in biological fuel cell with and without mediators World Appl. Sci. J. 18 559–67

[41] Mustaffa A R, Xu Hamid K H, Musa M, Salihon J and Ramli R 2016 Cultivation of microalgae using Sungai Sura water source as a medium for biodiesel production Indian J. Sci. Technol. 9

[42] Iyovo G D, Du G and Chen J 2010 Sustainable biomethane, biofertilizer and biodiesel system from poultry waste Indian J. Sci. Technol. 3 1062–9

[43] Zysin L V, Koshkin N L, Orlov E I, Sergeev V V and Steshenkov L P 2002 A study of the joint operation of a diesel engine and a gas generator processing plant biomass Therm. Eng. (English Transl. Teploenerg. 49 14–9

[44] Borovkov V M, Zysin L V and Sergeev V V 2002 The totals and technological problems of usage of vegetative biomass and organic waste in power engineering Izv. Akad. Nauk. Energ. 13–24

[45] Politaeva N, Smyatskaya Y, Slugin V, Toumi A and Bouabdelli M 2018 Effect of laser radiation on the cultivation rate of the microalg Chlorella sorokiniana as a source of biofuel IOP Conf. Ser. Earth Environ. Sci. 115

[46] Politaeva N A, Kuznetsova T A, Smyatskaya Y A, Trukhina E V and Atamanyuk I 2018 Energy Production from Chlorella Algae Biomass Under St. Petersburg Climatic Conditions Chem. Pet. Eng. 1–5

[47] Politaeva N, Kuznetsova T, Smyatskaya Y, Trukhina E and Ovchinnikov F 2017 Impact of various physical exposures on Chlorella Sorokiniana microalgae cultivation Int. J. Appl. Eng. Res. 12 11488–92

[48] Ivankin A N, Proshina O P, Oliiferenko G L, Panferov V I and Baburina M I 2014 Kinetics of biodiesel obtaining by a catalytic transformation of plant lipids into fatty acid ethyl esters [Kinetika polucheniya biodizelya kataliticheskoi transformacii rastitel’nyh lipidov v ehtilovye ehfiry zhirnyh kislot] Vestn. Mosk. Gos. Univ. lesa - Lesn. Vestn. 4 138–44

Acknowledgments
This work was performed within the implementation of Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014-2020, the project «Development and implementation of innovative biotechnologies for treatment of microalgae Chlorella Sorokiniana and duckweed Lemna minor» (Agreement № 14.587.21.0038 ), the unique project identifier is RFMEFI58717X0038.