

**К ВОПРОСУ БЕЗОТХОДНОЙ ТЕХНОЛОГИИ ИСПОЛЬЗОВАНИЯ БИОМАССЫ
МИКРОВОДОРОСЛЕЙ *CHLORELLA SOROKINIANA* ДЛЯ ПОЛУЧЕНИЯ
ЛИПИДОВ И СОРБЕНТОВ**

Н.А. Политаева, И.В. Атаманюк, Ю.А. Смятская, Т.А. Кузнецова, Туми Амира, П.Б. Разговоров

Наталья Анатольевна Политаева *, Юлия Александровна Смятская, Татьяна Алексеевна Кузнецова, Туми Амира

Научно-исследовательская лаборатория «Экологическая биотехнология», Санкт-Петербургский государственный политехнический университет Петра Великого, ул. Политехническая, 29, Санкт-Петербург, Российская Федерация, 195251

E-mail: politaevana1971@gmail.com, makarovayulia169@mail.ru, tano_lovely@mail.ru, toumi.amira@hotmail.com

Ирина Викторовна Атаманюк

Гамбургский университет технологий, Шварценбергштрассе, 95, Гамбург, 21073 Гамбурга, Германия

E-mail: iryna.atamaniuk@tuhh.de

Павел Борисович Разговоров

Кафедра технологии пищевых продуктов и биотехнологии, Ивановский государственный химико-технологический университет, ул. Жиделёва, 1, Иваново, Российская Федерация, 153002

E-mail: razgovorov@isuct.ru

*Обозначены области применения биомассы микроводорослей *Chlorella sorokiniana* в народном хозяйстве (кормовые и биологически активные добавки и др.). Указано, что термическая и химическая модификация отходов растительного сырья может обеспечивать получение высокоэффективных материалов для водоочистки. Схематически представлены основные этапы безотходной технологии переработки указанных микроводорослей с выделением из них ценных липидов и получением сорбционных материалов. Определен жирнокислотный состав липидной фракции (77 мг/г), полученной методом Сокслета из лиофилизированной биомассы микроводорослей. Выявлено, что она включает 83,7% непредельных (в основном C_{18:1}, C_{18:2}, C_{18:3}) жирных кислот, а предельные жирные кислоты преимущественно представлены рядом C₁₆ – C₂₀. В качестве наиболее ценных биологически активных соединений в составе лиофилизированной биомассы, необходимых для полноценной жизнедеятельности человека, обнаружена α-линоленовая кислота (Омега-3, 28,3%). Проведенный микроструктурный анализ остаточной биомассы выявил ее пористую поверхность и возможность концентрирования на ней молекул различных адсорбатов из растворов. При изучении сорбционных свойств остаточной биомассы с использованием показателей начальной и конечной концентрации оценили эффективность очистки модельных вод от ионов Fe³⁺ (47,5%), что указывает на необходимость модифицирования сорбционных свойств биомассы. Установлено, что степень очистки сточных вод, включающих соединения железа (III), может быть значительно (в 1,8 раз) увеличена путем формирования из остаточной биомассы гранулированных материалов с дополнительным введением в состав композиции хитозана, выполняющего функции связующей и модифицирующей добавки при соотношении основных компонентов в смеси 4:1.*

Ключевые слова: микроводоросли *Chlorella sorokiniana*, липиды, жирно-кислотный состав, Омега-3, сорбенты, очистка сточных вод

WASTE-FREE TECHNOLOGY OF *CHLORELLA SOROKINIANA* MICROALGAE BIOMASS USAGE FOR LIPIDS AND SORBENTS PRODUCTION

N.A. Politaeva, I.V. Atamanyuk, Yu.A. Smyatskaya, T.A. Kuznetsova, Toumi Amira, P.B. Razgovorov

Nataliya A. Politaeva *, Yuliya A. Smyatskaya, Tatiana A. Kuznetsova, Toumi Amira
Scientific Research Laboratory «Environmental biotechnology», St. Petersburg State Polytecnic University,
Polytekhnicheskaya st., 29, St. Petersburg, 195251, Russia
E-mail: politaevana1971@gmail.com*, makarovayulia169@mail.ru, tano_lovely@mail.ru, toumi.amira@hotmail.com

Irina V. Atamanyuk

Hamburg University of Technology, Schwarzenbergstrasse, 95, Hamburg, 21073 of Hamburg, Germany
E-mail: iryna.atamaniuk@tuhh.de

Pavel B. Razgovorov

Department of Food Technology and Biotechnology, Ivanovo State University of Chemistry and Technology,
Zhidelyova st., 1, Ivanovo, 153002, Russia
E-mail: razgovorov@isuct.ru

In the present article we introduce application areas of Chlorella sorokiniana microalgae biomass for national economy (feed and biologically active additives, etc). Here it is shown that thermal and chemical modification of plant material waste might result in obtaining of highly-efficient materials for water purification. Major stages of waste-free technology of microalga processing with further extraction of valuable lipids and obtaining of sorption materials are schematically shown. We have determined fatty-acid content of lipid fraction (77 mg/g), obtained by Soxhlet method from lyophilized microalga biomass. It was revealed, that it includes 83.7% of unsaturated fatty acids (generally, C_{18:1}, C_{18:2}, C_{18:3}), whereas saturated fatty acids are mostly presented by C₁₆ – C₂₀ family. In lyophilized biomass we discovered α -linolenic acid (Omega-3, 28.3%), which is one of the most valuable biologically active compounds, required for full-value human life and activities. Microstructure analysis of residual biomass of C. sorokiniana microalgae has shown pore surface, formed by destroyed cells. In the course of studying the sorption properties of residual biomass with the use of initial and final concentration parameters, the efficiency of purification of model water from Fe³⁺ ions (47.5%) was evaluated, which indicates the need to modify the sorption properties of biomass. It has been established that the degree of wastewater purification containing iron (III) compounds can be significantly increased (by 1.8 times) by forming from the residual biomass granular materials with additional introduction of chitosan in the composition, which using as a binder and a modifying additive (mass ratio 4:1).

Key words: microalgae *Chlorella sorokiniana*, lipids, fatty-acid content, Omega-3, sorbents, wastewater purification

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INTRODUCTION

Wastewater purification using sorption materials is a highly-effective way for removal of various chemical and biological contaminants from water, at the same time leading to improvement its smell and

taste. Also sorption materials might be used for purification of vegetable oils from undesirable ingredients [1, 2]. The following substances are widely used as sorption materials: activated carbons, silicagels, numerous compounds of aluminium and silicon oxides, etc. Besides this, usage of modified waste of plant

material for solving of the above-mentioned tasks is environmentally sound issue and makes economic sense. In particular, now there is widespread experience [3-10] of sunflower, wheat and millet threshing husk inclusion into wastewater after its' thermal and chemical modification. Besides modification of initial material, the aforesaid issues can be solved by efficient usage of bonding additives at creation of composite sorption materials [11]. For example, papers [12-18] present the studies on the possibility of sorption-active materials production from agriculture wastes with addition of chitosan. There was revealed that chitosan inclusion improves sorption capacity for heavy metal ions for 15-20% [12-18].

In that context technological aspects of microalga *Chlorella (C.) sorokiniana* processing for implementation as a feed or biologically-active material are of great interest [18-20]. In 2017 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 N 14.587.21.0038 of 17 July 2017, the unique project identifier is FMEFI58717X0038) was supported. Implementation of the project provides the possibility to join the technologies of obtaining the valuable components from plant material and sorption materials for wastewater purification.

At this the general resolution concerning development of innovative biotechnology of microalgae *C. Sorokiniana* processing, requires consequent solutions of a number of technological tasks, namely the following:

- Selection of biomass cultivation conditions [19, 20];
- Selection of appropriate methods for extraction of valuable phytocomponents (lipids, pigments, carotenoids, pectine substances);
- Usage of residual biomass for obtaining sorption materials for efficient water purification;
- Usage of spent materials as a substrate for fermentation of organic waste, separation of the obtained biogas into methane and carbon dioxide;
- Usage of methane for household needs, and carbon dioxide for intensification of microalgae cultivation process.

The aim of this work was to obtain lipids from dry biomass of *Chlorella sorokiniana* microalgae, as well as investigation of sorption properties of this biomass towards Fe (III) compounds, which are presented in effluents.

EXPERIMENTAL PART

Microalgae *C. sorokiniana* was grown in natural conditions of Saint Petersburg in summer. Further we have obtained lipid fraction (77.3 mg/g), by Soxhlet method from lyophilized microalga biomass. Chromatographic analysis of this fraction was carried out using gas chromatograph Agilent 7820A.

After extraction of lipid fraction we obtain spent material, which is known as residual biomass. Microstructure of the obtained biomass was studied using Scanning Electron Microscope Zeiss Leo 1530. In order to investigate the sorption properties of the obtained biomass, it was added in the amount of 20 g/l to the model solutions containing iron (III) ions of 50 mg/l concentration. The mixture was held in static conditions for 20 min. Further, the model solutions were filtered and the residual content of iron ions was analyzed using spectrophotometric technique PND F 14.1:2.4.50-96.

RESULTS AND DISCUSSION

The conducted chromatographic analysis allowed us to estimate fatty-acid content in the lipid fraction of the biomass (Fig.1), obtained from *C. Sorokiniana* microalgae.

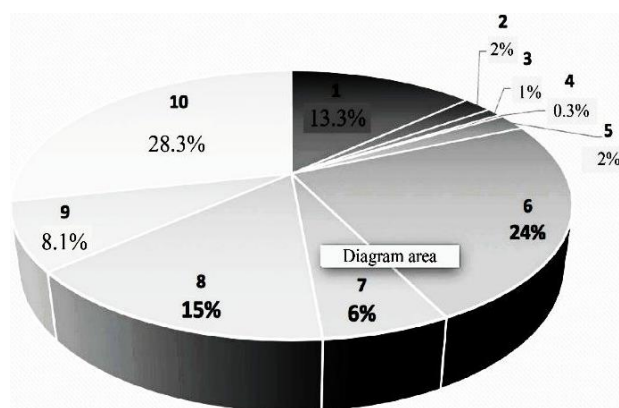
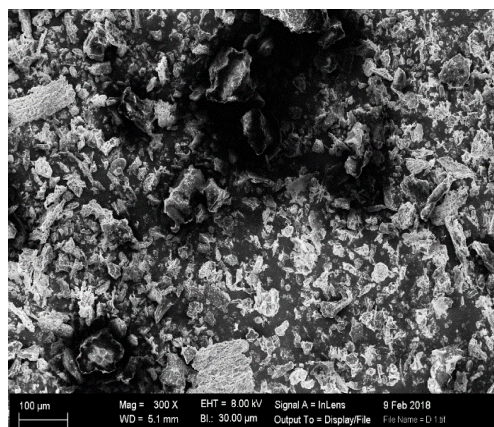


Рис. 1. Состав липидной фракции биомассы *C. sorokiniana*: 1 – C_{16:0} (пальмитиновая); 2 – C_{18:0} (стеариновая); 3 – C_{20:0} (арахиновая); 4 – C_{16:1} (пальмитолеиновая); 5 – C_{16:2} (пальмитолинолевая); 6 – C_{18:1} (олеиновая); 7 – C_{18:1} (элаидиновая); 8 – C_{18:2} (линолевая); 9 – C_{18:3} (γ-линоленовая); 10 – C_{18:3} (α-линоленовая)

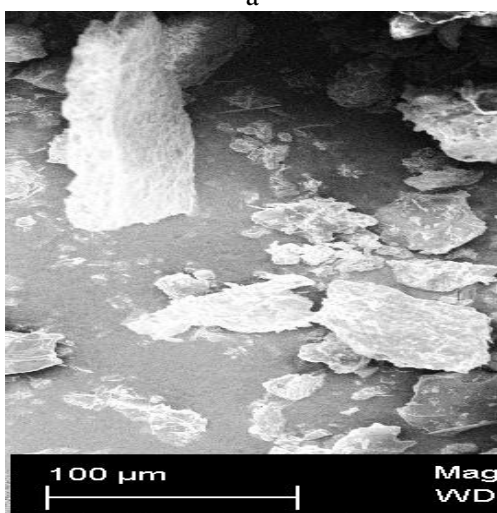
Fig. 1. Lipid fraction content of *C. sorokiniana* biomass: 1 – C_{16:0} (palmitic); 2 – C_{18:0} (stearic); 3 – C_{20:0} (arachidic); 4 – C_{16:1} (palmitoleic); 5 – C_{16:2} (palmitolinoleic); 6 – C_{18:1} (oleic); 7 – C_{18:1} (elaidic); 8 – C_{18:2} (linoleic); 9 – C_{18:3} (γ-linolenic); 10 – C_{18:3} (α-linolenic)

It was found that lipid fraction is predominantly made up of unsaturated fatty acids (C_{18:1}, C_{18:2}, C_{18:3}), whereas the amount of saturated acids having 16-20 carbon atoms in the chain is 16.3%.

Among unsaturated fatty acids we discovered α -linolenic acid in the amount of 28.3% (Fig. 2) of lipid fraction mass, which is related to polyunsaturated family (Omega-3). Omega-3 acid is one of 8 basic elements for human health, youth and beauty. It regulates fat metabolism and life cycle of the positive bacteria, which inhabit the human guts. Also it improves human vision, reduces inflammatory processes in joints, improves brain functions, serves for immune stimulation, helps at skin disease, eczema, allergy and Alzheimer disease treatments. It should be noted, that Omega-3 acid isn't produced in human body and enters it solely together with food of special composition (oily fish, vegetable oils). In this context the issue concerning technology of Omega-3 obtaining from *C. sorokiniana* microalgae is reasonable both from economic and comprehensive points of view.



a



b

Рис. 2. Микроструктура поверхности остаточной биомассы *C. sorokiniana* после выделения липидов: а – увеличение 300; б – увеличение 500

Fig. 2. Surface microstructure of residual biomass of *C. sorokiniana* after lipid extraction: a – magnification is 300; б – magnification is 500

After lipid extraction from biomass we obtain spent material (so-called "residual biomass"). According to the literature data [18], the initial microalga biomass, along with lipids and pigments, contains cellulose (23.5%) and hemicellulose, as well as starch, chitin- and pectin-like substances, which govern its sorption properties.

Microstructure analysis of residual biomass of *C. sorokiniana* microalgae (Fig. 2 a,b) has shown pore surface, formed by destroyed cells (Fig. 2a).

Magnification $\times 500$ allowed us to fragmentarily clearly see pore surface of the cell membrane (Fig. 2b). Investigation of sorption properties of residual biomass using initial (C_0) and final (C_{fin}) biomass concentrations, showed that the efficiency of model wastewater purification from Fe^{3+} ions was 47.5% (Table).

Table

Efficiency of wastewater purification from Fe^{3+} compounds using obtained granulated sorbents

Таблица. Эффективность очистки сточных вод от соединений Fe^{3+} с использованием полученных гранулированных сорбентов

Sorbent composition	Initial Fe^{3+} cation concentration, C_0 , mg/l	Concentration after purification, C_{fin} , mg/l	E, %
Residual biomass	50	26.25	47.5
Residual biomass + chitosan (1:4)	50	6.57	86.9



a b c d

Рис. 3. Материалы, получаемые на основе микроводорослей *C. sorokiniana*: а – лиофилизованная биомасса; б – экстракт липидов; в – остаточная биомасса; г – гранулированные сорбенты из остаточной биомассы и хитозана

Fig. 3. Materials obtained from microalgae *C. sorokiniana*: а – lyophilized biomass; б – lipid extract; в – residual biomass; г – granulated sorbents made from residual biomass and chitosan

Such relatively low value denotes the necessity of biomass sorption properties modification. For this purpose we prepared granules from residual biomass and chitosan (mass ratio 1:4) according to the technique described in [12, 13]. Granules external view is presented in Fig. 3.

Drastic increase of effluent purification efficiency from Fe (III) ions using composite granulated sorbent can be described taking into account chemo-

sorption properties of chitosan, namely the presence of active NH₂- and OH-groups in its molecular structure [12, 13]. Fig. 3 presents external view of materials obtained from *C. sorokiniana* microalgae biomass. A schematic diagram for usage of such biomass, presented in Fig. 4 provided the basis for energy- and resource-saving technology of material processing and obtaining of valuable components (lipids and sorbents).

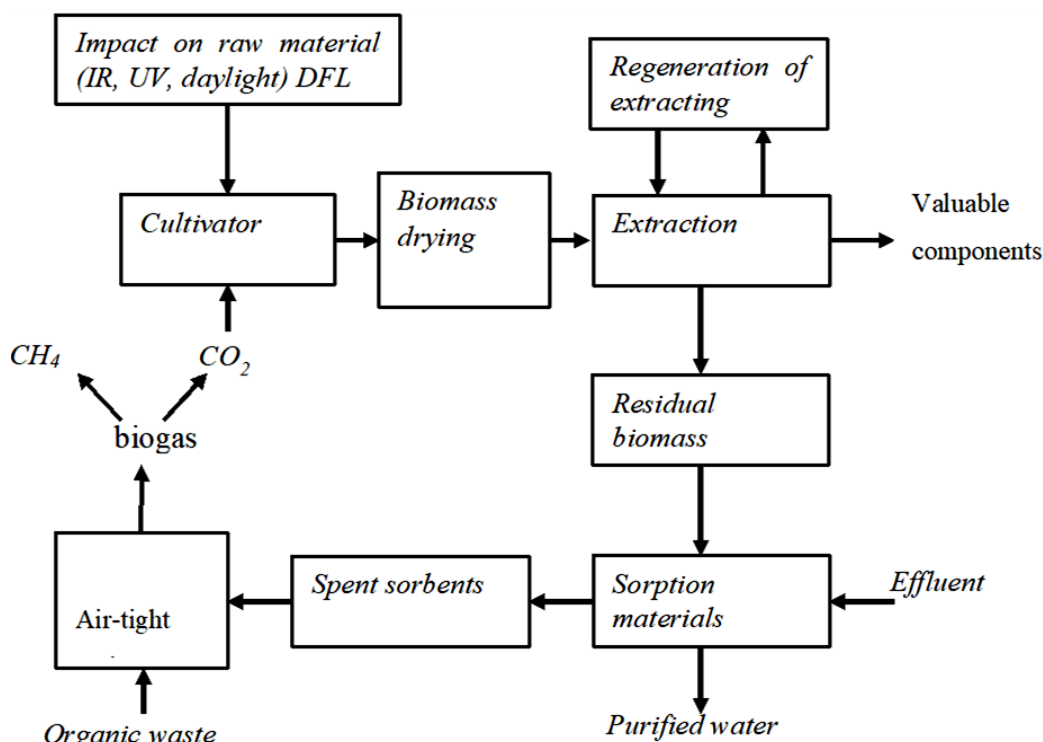


Рис. 4. Принципиальная схема технологической переработки микроводорослей *C. sorokiniana* и использования сухой биомассы
Fig. 4. Basic scheme of technological processing of microalgae *C. sorokiniana* and dry biomass usage

The uniqueness of the proposed waste-free technology is of biomass circuiting [19, 20]. This technology is waste-free, as the residual biomass acts as secondary material for sorbent obtaining. At this, even shallow analysis of the scheme from Fig. 4, reveals its specific features: In order to obtain favorable conditions for microalga *Chlorella sorokiniana* cultivation, the raw material is treated by infrared or ultraviolet radiation, and the process is performed at daylight. It should be noted that investigation of various physical impacts on *Chlorella* microalgae is the subject of a separate investigation and isn't considered in details here. However, we expect that the impact of such treatment with a high probability will be driven by initial response of cells to heat (or other factors). At this, as was mentioned above, the obtained biomass possesses moderate sorption properties (E = 47.5%). How-

ever, the additional usage of chitosan in the scheme for granulated sorbent formation at mass ratio of the major components (4:1), allows one to solve the problem. Also it provides increase of efficiency of effluent purification from Fe³⁺ for 1.8 times (E = 86.9%).

CONCLUSIONS

Consequently, gas chromatography technique was used to estimate fatty-acid content (16.3% of saturated acids, 83.7% of unsaturated acids) of lipid fraction, obtained from lyophilized biomass of *C. sorokiniana* microalgae. In lyophilized biomass we discovered biologically-valuable α -linolenic acid in the amount of 28.3% of total lipid fraction mass, which belongs to family of polyunsaturated fatty acids (Omega-3).

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