Экология

UDC 579

A.E. SOLOVCHENKO, L.R. SEMENOVA, I.O. SELYAKH, P.N. SHCHERBAKOV, K.A. CHEKANOV, O.B. CHIVKUNOVA, G.A. DOLNIKOVA, and E.S. LOBAKOVA*

The Bioengineering Dept., Faculty of Biology, Lomonosov Moscow State University, *119234* Moscow Russia

e-mail: elena.lobakova@rambler.ru

Assessment of a New *Chlorella vulgaris* (Chlorophyta) IPPAS C-2015 Strain for Application in Poultry Wastewater Bioremediation

The potential of the use of a new microalga strain *Chlorella vulgaris* IPPAS C-2015 (Chlorophyta, Trebouxiophyceae) for poultry wastewater treatment has been studied. The artificial wastewater (AWW) from chicken litter that mimicked real poultry wastewater was prepared, and the efficiency of the bioremoval of the inorganic anion from it and destruction of the organic contaminant in it during the new strain of *C. vulgaris* semi-continuous cultivation was assessed. After three days of *C. vulgaris* culturing, the initial nitrate and orthophosphate levels in AWW decreased by more than 90% and more than 48%, respectively, and organic compounds were degraded by 80% on average (judging by chemical oxygen consumption). During the cultivation of the microalgae in AWW, the bacteria associated with the *C. vulgaris* pre-culture gradually replaced the bacteria characteristic of the AWW. The microalga biomass grown in AWW contained a great amount of polyunsaturated long-chain fatty acids from the C_{18} family. The capacities of the new *C. vulgaris* strain of being used in the combined poultry wastewater treatment and utilization of the resulting biomass are discussed together with the potential advantages of the microalgae-based over conventional biological wastewater treatment technologies.

Key words: bioremoval, microalgae, organic contaminants, wastewater.

Generation of large quantities of wastewater by poultry farms represents a grave environmental hazard [1]. The poultry wastewater are characterized by high concentrations of organic contaminants and biogenic elements, primarily nitrogen (N) in the form of ammonium and/or nitrate as well as phosphorus (P), and high abundance of pathogenic microorganisms. Unless treated properly, poultry wastewater dumping is illegal since it leads to eutrophication and contamination of soil and water bodies with pathogenic microorganisms [2—5].

The availability of environmentally safe techniques for utilization of litter, farm and processing wastewater is among the key constrains of poultry farming. This problem is exacerbated by the mass construction of large poultry farms in consent with the trend of intensification and industrializing of agriculture [5].

Abbreviations: AWW, artificial wastewater; BOD, biological oxygen demand; CFU, colony forming unit; Chl, chlorophyll; CL, chicken litter; COD, chemical oxygen demand; DM, dry mass; MA, microalga(e).

* Author for correspondence.

The efficient treatment of poultry wastewater is feasible with the use of contemporary treatment plants and multi-stage heterotrophic microorganism-based processes designed for municipal wastewater treatment [1, 5]. However, poultry farms now scarcely use these approaches due to their complexity and dearth preferring various combinations of sedimentation ponds and irrigation fields [5]. In addition to that, the performance of conventional treatment approaches is poor during the cold season. Moreover, the bulk of bioavailable N and P from the wastewater is irreversibly lost due to denitrification and binding to insoluble complex compounds. For more detail on the wastewater utilization, see the recent reviews [6—8].

Most of the currently known technologies for the processing of chicken litter available in Russian Federation and abroad are costly, laborious, and require appropriate equipment, which is unacceptable for the majority of marginally profitable farms [5, 6]. At the same time, single-celled photoautotrophs (further referred to as microalgae, MA) were for a long time considered as potential organisms for wastewater treatment [9]. Despite this, the investigation of deep -treatment of agricultural waste including wastewater with intensive cultures of MA only started recently. The advantages of MA-based approach include the efficient removal of biogenic elements by MA cells, the destruction of organic contaminants and suppression of pathogens by photosynthetically generated oxygen [10]. The MA-based treatment is a more environmentally friendly process due to lacking of secondary waste, e.g. spent active sludge. A further advantage comprises in the generation of microalga biomass enriched by value-added compounds (proteins, antioxidants, vitamins) with simultaneous photosynthetic assimilation of technogenic carbon dioxide [11]. The lipid-and/ or carbohydrate-enriched biomass grown in wastewater [12] can also be processed into various biofuels including biodiesel, biomethane, biohydrogen, etc. [13]. The N- and P-enriched microalga biomass can also be converted into slow-release biofertilizers [14].

Nevertheless, the development of efficient biotechnologies for poultry wastewater biotreatment demands microalga strains with a certain combination of traits which scarcely occur in nature. Thus, candidate strains for poultry wastewater bioremediation should be tolerant to high concentrations of biogenic elements [15] and other pollutants specific to a particular wastewater type. These organisms should efficiently absorb and/or decompose pollutants [10], possess a fast growth and high accumulation rates of biomass [8] enriched by value-added fatty acids. In view of the problems outlined above, we assessed the potential of a new *Chlorella vulgaris* strain IPPAS-C2015 in the bioremediation of poultry wastewater.

EXPERIMENTAL

Microalgae and cultivation conditions

The previously obtained in our laboratory *Chlorella* IPPAS-C2015 strain served as a subject in the present work. The microalga cells were batch-cultivated in 500-ml Erlenmeyer flasks with 300 ml of BG-11 [16] medium or artificial waste water (AWW, see below) at 25 °C, continuous shaking (80 rpm) and illumination by daylight fluorescent tubes (80 μmol PAR quanta m^{-1} s^{-1}) in an Innova 44R (New Brunswick, USA) shaker. To assess the potential toxic effect of ammonia, BG-11 medium was modified to the $BG\text{-}11_M$ variant by the substitution of $NaNO_3$ for NH_4NO_3 in a 2.5-fold molar excess as compared to the original medium composition [16]. In these experiments, the initial biomass content was 0.8 g l^{-1} dry mass (DM).

In the experiments with semi-batch cultivation, the cells were grown in the AWW in 1.5-l glass columns (6.6 cm internal diameter) at constant illumination with light-emitting diodes (480 μmol PAR quanta m^{-1} s^{-1}), bubbling with air (0.3 l l^{-1} min^{-1}) and 25°C. The cultures were initiated at 1.6 g l^{-1} DM.

The pre-cultures were grown in 750-ml Erlenmeyer flasks with 250 ml BG-11 medium at 25°C and 40 μmol PAR quanta m^{-1} s^{-1}. In the experiments with the AWW, the pre-culture cells were pelleted by centrifugation, re-suspended in the AWW, and cultured as described above. The culture growth was monitored by chlorophyll and DM accumulation. Dry weight was measured gravimetrically [3].

Artificial waste waster

In order to assess the potential efficiency of poultry wastewater bioremediation by the new *Chlorella* strain, AWW were prepared from the extracts of chicken litter (CL, Table 1) obtained from the Petelino Poultry Farm (Russian Federation). The AWW were prepared by incubation of CL in distilled water or BG-11 medium (10 g l^{-1}) in flasks. After the third incubation at room temperature and constant shaking (110 rpm), the homogenate was centrifuged (10 min at 3500 g); the pellet was discarded and the supernatant was used as AWW without autoclaving. The nitrogen and carbon contents in the lyophilized CL samples and resulting AWW were determined on the element analyzer CNSH Vario EL Cube (Elementar, Germany).

Table 1

Carbon and nitrogen content in chicken litter, its extraction residue, and estimation of N transfer to the AWW prepared from these substrates

Substrate	Element, % DM		Element content, g g^{-1}		C/N, mass ratio	N transferred from the litter to the AWW during the extraction	
	N	C	N	C		% of total N	Final content, mmol l^{-1} (NO_3^-)
Chicken litter	3.800 ±0.145	34.184 ±1.019	0.063 ±0.026	0.592 ±0.240	9.465 ±0.167	38.681	14.537
Residue after the litter extraction	2.330 ±0.012	38.674 ±0.716	0.023 ±0.001	0.387 ±0.007	16.596 ±0.232		

Bacterial contamination assay

To assess the bacterial contamination of the MA cultures, the culture liquid samples were applied to Petri dishes on the solidified sterile glucose-peptone-yeast medium containing, g l^{-1}: peptone, 2; yeast extract, 1; casein hydrolysate,1; glucose, 1; glycerol, 10; $CaCO_3$, 5; agar, 20 (all from Sigma, USA) made up with tap water to 1 liter. The colonies were counted to determine the colony-forming unit (CFU) number after 3 and 5 days of incubation at 37°C. The bacteria cells were investigated under a light microscope before and after Gram staining.

The bacteria cell number was counted as follows: 1-ml liquid samples were diluted 100 times with sterile tap water. The resulting samples were further diluted (stepwise, 10-fold each step) and loaded onto Petri dishes with the solid medium.

Chlorophyll and cell lipid fatty acid composition assay

Pigments were extracted from the cells with a chloroform—methanol mixture (2:1, vol); total chlorophyll (Chl) was assayed in the chloroform fraction of the extract spectrophotometrically [2, 3]; fatty acid profile of the MA cell lipids was resolved by GC/MS as described previously [2, 3]. For each cultivation conditions, at least two independent experiments were carried out, each in triplicate. Average values with their standard errors are presented unless stated otherwise.

Molecular identification of the strain

The strain under investigation was identified by comparison of the nucleotide sequences of its 5.8S rRNA gene and internal transcribed spacers ITS1 and ITS2 with the corresponding sequences of the known MA isolates from NCBI GenBank database. Isolation of DNA, PCR amplification of the selected locus, sequencing and sequence data analysis were carried out according to the earlier published protocols [17].

RESULTS AND DISCUSSION

Identification of *Chlorella* IPPAS-C2015 and assessment of its potential wastewater tolerance

Numerous reports on high tolerance of single-celled green microalgae (Chlorophyta) to eutrophic conditions of wastewater have been published [18—20]. Accordingly, a microalga isolate previously obtained in our laboratory and tentatively identified as *Chlorella* sp. was selected as an object for this study.

Multiple alignment of the sequences of rRNA gene cluster including ITS1 and ITS2 of a number of known *Chlorella* representatives was conducted using ClustalW software. As a result, conservative regions were revealed and used to design the oligonucleotide primers:

For 5′-TGGCTCATTAAATCAGTTATAG -3′,

Rev 5′-CCAAGAATTTCACCTCTGACA -3′.

Using these primers, the corresponding region of the genome DNA of the microalga isolate was amplified and sequenced; the resulting sequence was deposited in GenBank under an ID of KF006337. According to the results of the BLAST search in GenBank, the sequences of the highest homology to those obtained in this work belonged to the representatives of the genus *Chlorella* (Trebouxiophyceae). Based on the rRNA gene cluster sequence, the microalga isolate used in this work was identified as *Chlorella vulgaris* Beyerinck and deposited in the collection IPPAS (Timiryazev Institute of Plant Physiology, Russ. Acad. Sci.) under an ID of IPPAS C-2015.

Generally, the efficiency of biogenic element removal by microalga cells depends on their growth (division) rate [8] as well as tolerance to high levels of nitrogen, phosphorus, and organic pollutants. Considering the utilization of the resulting biomass, it is also important to achieve a high content of value-added compounds in cells. Screening in wastewater-mimicking model media is the optimal approach to the selection of candidate strains suitable for the farm wastewater bioremediation. Within this approach, we tested the capacity of the selected strain of growing in the model media with elevated contents of ammonium or nitrate and in the media prepared from the CL extracts (see Table 1). We monitored the biomass accumulation and pH changes during the cultivation (Table 2). Special attention was paid to the dynamics of the culture liquid pH, an important marker of the stress tolerance in MA potentially suitable for wastewater bioremediation. For instance, the uptake of dissolved inorganic carbon (bicarbonate formed from CO_2 upon its dissolving in the culture medium within the physiologically relevant pH range) [21] and nitrate by the microalga cells causes the alkalization of the culture medium [22, 23]. On the other hand, acidification of the culture medium increases the protonation of ammonium ions typically occurring in the wastewater resulting in the formation of ammonia toxic to the microalga cells [24].

The three-day cultivation of *C. vulgaris* IPPAS C-2015 in the BG-11_M medium with the excess of nitrogen resulted in a modest decline in pH (by 0.5 on average, from 7.4 to 6.9, obviously due to the uptake of NH_4^+) and biomass accumulation rate commensurate to that typical of the cultures grown in the standard BG-11 ammonium-lacking medium (data not shown). Consequently, relatively high levels of ammonium in the studied pH range did not exert a significant negative effect on the culture growth.

Further experiments were dedicated to the screening of the capaciity of *C. vulgaris* IPPAS C-2015 of growing in the AWW prepared from the CL extracts (see Table 1). The CL samples used in our work contained carbon and nitrogen in amounts of 35—40% DM and 3.8—5.3% DM, respectively, yielding the C/N mass ratio of 9—13. The C/N ratio of the CL residue after the extraction during the AWW preparation increased ca. two times due to the preferential extraction of nitrogen (mainly nitrate). Accordingly, after the three-day extraction, up to 46% of the CL total nitrogen was found in the extract (final nitrate concentration > 21 mmol l^{-1}). The extracts were later used as AWW, the model media for the estimation of the nitrate bioremoval efficiency of the MA.

In all cases studied, a similar increase in pH was observed during the growth in the model media; on the other hand, it was lower than that recorded in the control cultures grown in BG-11 medium. Gene-

Table 2

Changes in the culture medium pH and biomass accumulation in microalga cultures during the preliminary screening in AWW*

Parameter	Medium and time					
	BG-11 (control)		AWW + BG-11		AWW	
	0 h	148 h	0 h	148 h	0 h	148 h
Culture medium pH	7.4	10.3	7.4	9.7	7.4	9.7
Biomass (g DM l^{-1})	0.8	2.1	0.8	1.5	0.8	1.9

*Mean values (n = 6) are represented; in all cases SD was below 5% of the corresponding average. Initial culture density was 0.8 g DM l^{-1}.

rally, the biomass accumulation rate in the cultures grown in AWW was lower than that in the BG-11 medium. This effect might be explained by the higher nitrate content in BG-11 as compared to the AWW ([16], see also Table 1). Nevertheless, the pH values in the culture liquids as a result of cell growing in AWW were only mildly alkaline. In the presence of high ammonium amounts, these conditions can provide the formation of ammonia that is deteriorative for the microalgae growth [24]. In any case, our analysis did not reveal a high amount of ammonium in the CL samples or in AWW prepared from the CL; therefore, it is unlikely that the ammonia formation could have a lethal effect on the culture on the background of nitrate uptake.

The robustness of the *C. vulgaris* IPPAS C-2015 cultures during growth in the AWW was studied under the semi-batch cultivation mode (Fig. 1). In these experiments, the culture was diluted by 1.6 times by fresh AWW every three days. Under these conditions, the *C. vulgaris* IPPAS C-2015 strain displayed a steady biomass accumulation for at least three cultivation cycles (Fig. 1). These results suggest that *C. vulgaris* culture was robust enough under the semi-batch cultivation conditions in the AWW, which is important from the standpoint of its potential industrial cultivation in poultry wastewater.

Collectively, the results obtained in this work suggest that *C. vulgaris* IPPAS C-2015 judging from the growth rate and dynamics of the culture pH is sufficiently capable of growth in the AWW and suitable for the application to bioremediation of poultry wastewater. However, the high tolerance to wastewater components (e.g. high level of biogenic elements) is necessary but not sufficient *per se* for being a good candidate strain for wastewater remediation. The next stage of our work was dedicated to the assessment of the efficiency of the biogenic elements removal by *C. vulgaris* IPPAS C-2015 cultures.

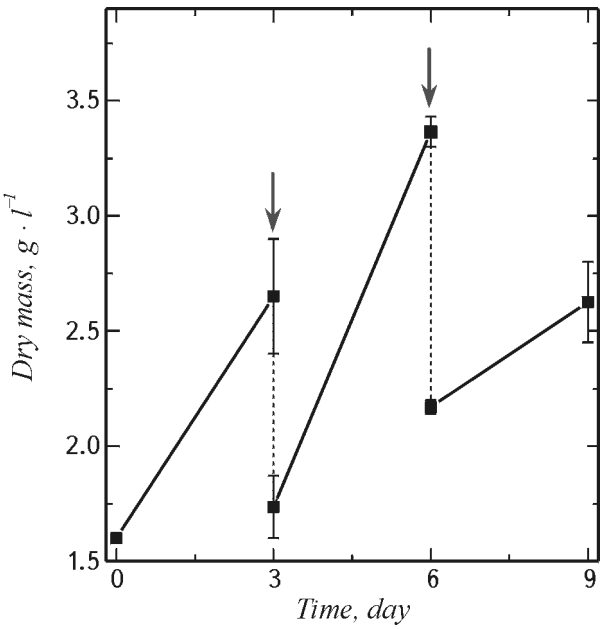

Fig. 1. Typical kinetics of dry mass accumulation by *C. vulgaris* IPPAS C-2015 culture during semi-batch cultivation in AWW. Three cycles, 72 h each, are shown. Arrows indicate the AWW replenishments

Efficiency of biogenic element removal by microalga cells

In order to estimate the biogenic element bioremoval by the microalgae, we compared the anionic composition of the AWW-mimicking poultry wastewater before and after the MA cultivation (Table 3). The cells of the studied MA strain eliminated 90% of nitrate on average from the AWW within three days of cultivation attaining the nitrate removal rate of ca. 177.2 mg l^{-1} day^{-1}. Remarkably, the highest extent of bioremoval (in terms of absolute content) was observed for nitrite; the elimination of other anions was less complete. Generally, a single 72-h cultivation cycle at the given combination of culture cell density and anion concentration (also termed as biomass load) turned to be insufficient to bring the biogenic element concentration to a value inferior to the safe-to-discharge threshold level. Obviously, to augment the bioremoval, it is necessary to decrease in the biomass load either by raising the culture density, by diluting the wastewater or by increasing the retention time of the AWW in the cultivation system. Indeed, additional tests of the nitrate bioremoval efficiency confirmed that a two-fold increase in the culture density (from 0.8 to 1.6 g DM l^{-1}) enhanced the rate and completeness of this anion elimination from the model BG-11-based medium containing nitrate in excess (data not shown).

A remarkable decrease in the chloride content (to a level inferior to the threshold allowed for discharge) was also observed most likely due to the adsorption on/absorption by the cells and/or its surface structures.

One of the most promising avenues of the utilization of MA biomass grown in the farm wastewater is its conversion to biofertilizers. It is known that ca. 3% of total nitrogen contained in dry MA biomass is available to plants immediately after the application, and after three weeks, this amount increases up to 33%; the similar dynamics was documented for the phosphorus in the MA biomass applied to soil [25]. As a result, the growth of cucumber and corn plants fertilized by the MA biomass from dairy farm wastewater was commensurate to that of plants fertilized by

Table 3

Efficiency of major anions elimination from the AWW by microalga cells after 72-h cultivation

Parameters	Concentration, mg l^{-1} (removal, % of the initial concentration in the AWW)				
	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Discharge threshold*	350	0.1	45	3.5	500
Concentration in the AWW**	455	615	589	128	106
Concentration after the cultivation	67.4±2.0 (85.9)	7.3±3.0 (98.8)	57.3±0.9 (90.3)	66.1±0.6 (48.4)	79.5±6.6 (25.0)

* For potable water open supply.
** Error < 5%.

equal (calculated by N and P content) amounts of conventional (chemically synthesized) fertilizers [3, 25]. It is important to note that regardless of the known ability of MA to over-accumulate heavy metals, no reports on the heavy metal contamination of the MA biomass grown in farm wastewater have been published [26].

The destruction of organic pollutants during cultivation of microalga cells

In modern wastewater treatment facilities, the demand of oxygen for oxidative destruction of organic pollutants estimated as chemical (COD) or biological oxygen demand (BOD) is satisfied by intense mixing or bubbling of wastewater with air [27]. When wastewater is treated by phototrophic organisms, oxygen is generated by these organisms as a result of photosynthesis thereby eliminating the need for additional aeration. This process was designated as '*photosynthetic aeration*' [10]. Apart from high inorganic ion content, poultry wastewater contains high levels of organic pollutants (2.5 g l^{-1} COD and more). In order to assess the capability of *C. vulgaris* IPPAS C-2015 to remove organic contaminants from the wastewater, we watched the dynamics of total organic compounds in the AWW (*via* COD) during the MA cultivation (Fig. 2).

As shown in Fig. 2, the cultivation of the MA in the AWW is accompanied by a sharp (by ca. 80% of the initial level) decline in the total organic compounds. This did not occur in the vessel containing AWW aerated to the same extent, but not inoculated with the MA: in this case, COD did not change significantly (data not shown). These results strongly suggest that apart from the elimination of biogenic elements, the microalga strain under investigation provides an efficient destruction of organic pollutants in the AWW.

Effect of microalgae cultivation on bacterial contamination in AWW

As noted above, decontamination from pathogenic bacteria is among the key goals of wastewater treatment [6]. In the systems using photosynthetic

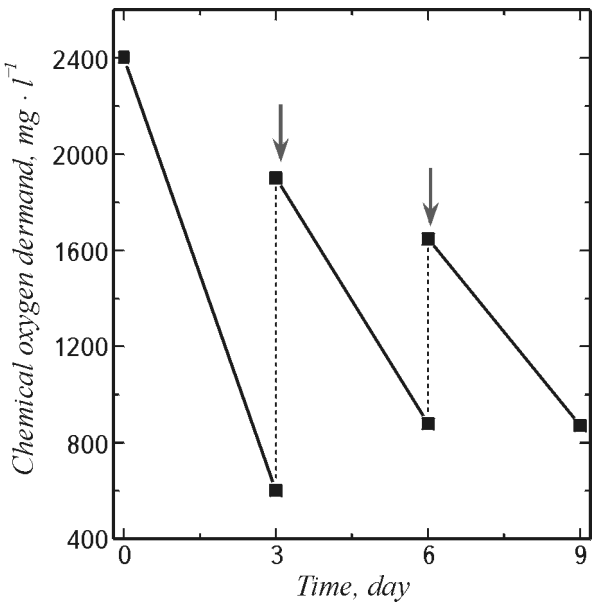

Fig. 2. Typical kinetics of decline in organic compound content in AWW during semi-batch cultivation of *C. vulgaris* IPPAS C-2015. Three cycles, 72 h each, are shown. Arrows indicate the AWW replenishments

aeration, this goal as well as organic pollutant oxidation is accomplished by photosynthetically generated oxygen and elevated pH [9, 10]. In the present work, we studied the effects of the MA cultivation on the bacteria occurring in AWW because the AWW were not sterilized to achieve a more complete reproduction of an industrial bioremediation process.

The colony forming unit (CFU) number in the freshly prepared AWW was, on average, as high as $4.2 \cdot 10^8$ ml^{-1}. Four colony types were distinguished, two of which were the dominant colony types: type 1 (olive-colored colonies with smooth edges formed by small immotile rod-shaped Gram-negative cells), and type 2 (gray smooth-edged yeast-like colonies). The tests on the standard solidified media (Czapek's medium and wort agar) confirmed the absence of fungal contamination in the AWW [28]. Judging from the colony morphotype, the AWW were characterized by a considerable presence of bacterial contaminants that only exhibited a slight diversity. Interestingly, the stock cultures of *C. vulgaris* used in this work displayed a higher bacteria diversity (up to seven visually different colony types at the mean bacterial cell number of $1.1 \cdot 10^8$ ml^{-1}).

The cultivation of *C. vulgaris* IPPAS C-2015 in the AWW brought about a two—three-fold decrease in the diversity of the associated bacteria and 300-fold decrease in CFU number. Noteworthy, after nine days of the MA cultivation, two of the bacteria types characteristic of AWW predominated in the *C. vulgaris* cultures type 1 (see above) and type 5 (gray-colored rough colonies formed by motile rod-shaped single, sometimes paired Gram-negative cells of moderate length and thickness). This evidence might indicate that the microbial consortium with *C. vulgaris* as a dominant species plays a key role in the suppression of the native microflora in AWW (as well as in the biogenic elements elimination and organic pollutant destruction).

Fatty acid composition of the microalga biomass grown in the AWW

An important feature of MA strains that makes them suitable for wastewater bioremediation is their ability to produce biomass enriched with such value-added compounds as lipids that can be converted to biodiesel [15]. Moreover, the structure lipids of thylakoid membranes of microalga cells contain valuable long-chain polyunsaturated fatty acids [29]. In view of this requirement, the fatty acid composition of the cell lipids was determined in *C. vulgaris* IPPAS C-2015 biomass grown in the AWW.

The analysis of the percentage of total fatty acids in DM of the studied strain biomass revealed no significant changes: during 72-h cultivation in the AWW, this parameter remained at the level of 8—10% DM. This trend is typical of the microalga cells grown in the absence of mineral nutrient limitation, therefore it could be expected in the biomass grown in the AWW that contain relatively high amounts of the biogenic elements (see Table 1 and Table 3). Under such conditions, mono- and polyunsaturated fatty acids from the $C1_6$ and $C1_8$ families normally predominate in the cell lipid fatty acid profile [30].

In our experiments, the cultivation in the AWW exerted a considerable effect on the fatty acid composition of cell lipids in *C. vulgaris* IPPAS C-2015 (Table 4). Specifically, there was an increase in the portion of α-linolenic acid; the percentages of other unsaturated fatty acids were also increased. In MA, α-linolenic and linoleic fatty acids are mainly associated with the structure lipids of the chloroplast thylakoid membranes [29]. It can be concluded that the observed changes in the fatty acid composition stem from the expansion of the chloroplast membrane apparatus in response to the decline in the per cell irradiance during biomass accumulation. Therefore, the biomass of the MA grown in the AWW under our experimental conditions contained a higher α-linolenic acid ($C_{18:3}$) portion in comparison with that grown in the standard BG-11mineral medium.

The microalga strains accumulating long-chain fatty acids in cell lipids are desirable in the production of feed additives with illustrious examples of the *Nannochloropsis* genus accumulating eicosapentaenoic acid [31, 32]. It is conceivable that in the absence of limitation by nitrogen, the cultures grown in wastewater would accumulate lipids enriched by value-added long-chain polyunsaturated fatty acids. The cells of *C. vulgaris* IPPAS C-2015 only contained modest amounts of fatty acids with the chain length over 18; however, the high content of linoleic and α-linolenic acids makes the *C. vulgaris* IPPAS C-2015 biomass grown in poultry wastewater a promising feedstock for the production of feed additives. It is important that heavy metals and toxic organic compounds are unlikely to occur in high amounts in poultry wastewater. As was shown by preliminary tests (see the previous section), the bacteria associated with *C. vulgaris* IPPAS C-2015 tended to displace the aboriginal bacteria in CL. However, the use of the wastewater-grown biomass for the production of feed additives would only be possible after the rigorous checking for the presence of pathogenic microorganisms and toxic components.

Thus, since the wastewater is hazardous for the environment, especially for water bodies [33], the tre-

Table 4

Fatty acid composition in cell lipids of *C. vulgaris* IPPAS C-2015 from stock culture and after 72 h of cultivation in AWW

Fatty acid, % of total fatty acids*	Stock culture	Culture after growth in AWW (3 cycles, 72 h each)		
		Cycle 1	Cycle 2	Cycle 3
14:0 (lauric)	< 1	< 1	< 1	< 1
16:0 (palmitic)	24	20	22	17
16:1 (palmitoleic)	< 1	< 1	< 1	< 1
16:2 (hexadecadienoic)	6	9	10	13
16:3 (hexadecatrienoic)	1	3	4	6
18:0 (stearic)	4	1	< 1	< 1
18:1 (oleic)	42	2	< 1	< 1
18:2 (linoleic)	22	31	27	29
18:3 (α-linolenic)	< 1	34	36	35
Unsaturation index	102	193	196	206
Sum of saturated fatty acids	29	21	22	17
Sum of monounsaturated fatty acids	42	2	< 1	< 1
Sum of dienoic fatty acids	28	40	37	42
Sum of trienoic fatty acids	1	37	41	40
Sum of polyunsaturated fatty acids	29	77	78	82

* Estimation error < 5%.

atment of agricultural wastewater including poultry farm is an acute problem. The growth of the amount of the wastewater and lack of efficient and affordable methods for their treatment are also alarming. Of special concern is the irreversible loss of biogenic elements carried by the poultry wastewater [34], mainly as a result of the denitrifying bacteria activity releasing N_2 into the atmosphere; only a small part of these nutrients could be returned to the soil so far [35, 36].

On the contrary, MA cells uptake nitrogen and phosphorus incorporating the elements into their cell components and making MA-based treatment a promising approach to bioremoval of N and P from wastewater [11, 12, 15] and their return to agroecosystems in the form of biofertilizers [3]. An additional advantage of biofertilizers from the N- and P-enriched MA biomass is the slow release of these nutrients preventing their rapid washing out of the soil and making them accessible to plants [25]. It should be emphasized that MA-based wastewater treatment with further conversion of the resulting biomass to biofertilizers would save significant amounts of '*conventional*' N and P fertilizers whose chemical synthesis is energy-intensive and hazardous to the environment [37]. The rock phosphate used for the production of the latter is a finite non-renewable resource that is expected to become scarce already in this century [30].

Despite all the obvious advantages, MA-based treatment of poultry wastewater did not yet become widespread mainly due to the limited experience in the infrastructure engineering, lack of developed processes and suitable algal strains. The present work contributes to solving these problems. In particular, the investigated strain *C. vulgaris* IPPAS C-2015 displayed a potentially high efficiency in the treatment of poultry wastewater (N and P removal as well as orga-

nic compound destruction) and was patented [38] for this application. This strain also generates valuable biomass suitable for the diversified downstream processing including conversion to biofertilizer or (after bacteriological tests) feed additives. However, additional research is required to harness the full biotechnological potential of this strain. In particular, the cultivation, harvesting, and processing of the biomass need optimization. The use of waste heat and CO_2 from natural gas-burners heating the poultry farms during the cold season is a promising avenue, although the integration of the wastewater treatment plants and heating facilities is a complex engineering task [6, 11].

Russian Science Foundation (grant no. 14-50-00029) supported the algological characterization of the strain *Chlorella vulgaris* IPPAS C-2015. The rest of the study was carried out with the financial support of the Ministry of Science and Education of Russian Federation that is gratefully acknowledged (Contract no. 14.515.11.0026).

Received 04.04.16

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