Microalgae cultivation for wastewater treatment and biogas production at Moscow wastewater treatment plant

N. Shchegolkova, K. Shurshin, S. Pogosyan, E. Voronova, D. Matorin and D. Karyakin

ABSTRACT

The process of cultivation of microalgae on purified and clarified wastewater of Kuryanovo wastewater treatment plants (KWWTP) was studied. The studies were conducted on monoculture (*Scenedesmus quadricauda* and *Chlorella sorokiniana*) and on polyculture, the composition of which was formed from microalgae present in the wastewater. The authors created and investigated the columnar photobioreactor (PBR), which acted as a pilot project on the purified and clarified water of KWWTP and allowed the removal of total nitrogen and phosphorus phosphates with an efficiency of up to 90%. The formation of a stable biocenosis from 22 species of algae (with 3–4 dominant species) and 31 species of zooplankton organisms belonging to six systematic subdivisions was recorded. The optimal retention time of the microalgae polyculture for the most effective wastewater treatment has been determined. The conducted studies have shown that the depth of decomposition of ashless matter and the ultimate biogas potential of untreated microalgae biomass is 15% lower than the corresponding values obtained with digestion of activated sludge, which necessitates studies in the field of pretreatment of algal biomass. The paper shows: connections between chlorophyll-a content, algal biomass and fluorescence index F_0 and between biomass increment and Fv/Fm value.

Key words | biogas, biomass, fluorescence, methane, photobioreactor, wastewater treatment

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INTRODUCTION

Numerous studies have shown that the cultivation of microalgae is at the center of solving many environmental problems: the production of renewable biofuels, the production of biomass for livestock feed or fish farms, the assimilation of greenhouse gases, and the wastewater treatment (Benemann 2003; Chen *et al.* 2003; Yen & Brune 2007; Park *et al.* 2013; Marchello *et al.* 2015; Mehrabadi *et al.* 2016). The most important of these problems is the production of biofuels from the biomass of microalgae with simultaneous assimilation of carbon dioxide. Underestimation of the limiting role of nitrogen and phosphorus for obtaining biofuels reduces to 'no' all the grand plans for replacing fossil fuels with biofuels. For the production of microalgae biomass, these elements can be supplied with mineral fertilizers and from various kinds of waste and sewage. Estimated calculation (Shchegolkova 2012) has shown that the energy potential of biomass, which can be obtained by using all nitrogen fertilizers produced in Russia, is about 8% of all energy produced in Russia. This means that the assimilation of CO_2 in the cultivation of microalgae on artificial nutrient media is unlikely to be profitable in the long run. However, nitrogen and phosphorus-containing wastes (primarily domestic wastewater) available in each megapolis are a 'free' source of these elements and, of course, can and should be used to produce biofuel from microalgae. Earlier calculations (Shchegolkova 2012) have allowed estimation of the amount of biogas, which can be obtained from domestic wastewater of such a megacity as Moscow. The total possible production of biogas energy is 1.6–2.2 billion kWh. This makes up 3.2–4.3% of the consumed energy in Moscow. An important point here is that the use of microalgae in the field of wastewater treatment allows the achievement of not only high quality purification, but also an energetic payback of the enterprise, in some

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cases due to the generation of biogas during anaerobic digestion of the excess algal biomass that is formed. In addition, lately houses with closed cycles of nutrient elements are increasingly spreading. Photobioreactors in these houses are part of the architectural ensemble, a wastewater treatment plant and a biomass source at the same time.

Photobioreactors and biofuel production from algae are being actively studied all over the world. Monoculture of algae can be used in the secondary treatment process and get productivity up to 93 dry weight (DW) g/L/day with 100% reduction of nitrogen and phosphorus (Abdelaziz *et al.* 2014). Strains of algae monoculture can produce biogas – up to 671 mL/L/day (raw biogas) and up to 86 mL/L/day (methane) (Mahdy *et al.* 2016). Several studies have reported the polyculture biomass productivity on pretreated wastewater up to 141 DW g/L/day (Prandini *et al.* 2016) and 12 g/m^2/day (Park *et al.* 2013). Algae strains were carried out removing 68% of nitrites, 54% of nitrates, 45% of ammonium and 94% of phosphates (Marchello *et al.* 2015). Biogas production described as 130 mL/g (Passos *et al.* 2016). Also, studies reported the polyculture productivity on initial (raw) wastewater up to 11 g/m^2/d (Mehrabadi *et al.* 2016). Efficiency of removal by algae achieved 87% for TOC, 85% for nitrogen, 92% for phosphorus and 49% for zinc (Hodges *et al.* 2017). Biogas production achieved from 317 to 1,170 mL/L/day (Yen & Brune 2007) and up to 0.9 g/m^2/day (Mehrabadi *et al.* 2016).

These data were mainly obtained on laboratory devices in short-term experiments. Data on the long-term monitoring of the functioning of the PBR on actual operating water treatment plants is extremely limited.

According to economic analysis conducted by the University of California at Berkeley (Lundquist *et al.* 2010), anaerobic digestion and the generation of biogas are the most beneficial option for utilizing the excess biomass of microalgae. Thus, the efficiency of the entire process and the very possibility of using microalgae directly depend on the completeness of digestion and output of biogas. The depth of the process of anaerobic digestion can be regulated by changing its external conditions (the residence time of the substrate in the reactor) and the properties of the substrate (species composition and pretreatment). The properties of the inoculum (a culture adapted to algomass digestion) are important for initiating the digestion process and the initial stage of the process (Lundquist *et al.* 2010).

Therefore, the study of the processes of microalgae cultivation in domestic wastewater and the processes of digestion of this biomass is of great importance. The main objective of our work was to determine the main technological parameters for the cultivation of microalgae on domestic effluents of a large water treatment plant and to assess the biogas generation potential of the grown biomass of algae. For this purpose, the main characteristics of biomass growth were determined, as well as the biogas composition and the depth of decomposition of the microalgae biomass during digestion. In addition, the biopotential of algomass and the mass of activated sludge as substrates for anaerobic thermophilic digestion were compared.

MATERIALS AND METHODS OF RESEARCH

The research was carried out at KWWTP, which receive sewage from half of the city of Moscow. For the cultivation of microalgae, purified and clarified water with the composition showed in Table 1 was used.

To assess the growth rate of monoculture in the first day, *Chlorella sorokiniana* and *Scenedesmus quadricauda* were used. Cultivation was carried out under artificial illumination in conical flasks with continuous mixing (with a shaker) at different ratios of the initial biomass of algae and wastewater. To an equal volume of biologically purified wastewater (BPW), a suspension of algae *Chlorella sorokiniana* or *Scenedesmus quadricauda* was added in ratios of 7, 13 and 27% of the total volume of liquid in the reactor.

During the 24-h period, the absorption spectra measurements were made. Measurements of absorption spectra of algal suspensions were carried out in the range from 350 nm to 850 nm on a single-beam spectrophotometer with an integrating sphere based on spectrometer USB2000 (Ocean Optics, USA). The absorption spectrum of algal suspension was calculated from the two spectra of light attenuation obtained at the installation of the sample cuvette, placed at different distances from the integrating sphere, that allowed evaluation of light scattering in the sample (Merzlyak & Naqvi 2000). The initial values of the optical density of the algae suspension corresponded to

Table 1 | Chemical composition of biologically purified and clarified wastewater

Indicators	Biologically purified wastewater (BPW)	Clarified wastewater (CW)
N-NH_4, mg/Ll	2.50 ± 0.61 mg/L	24.20 ± 1.31
N-NO_2, mg/L	0.36 ± 0.23 mg/L	0.00 ± 0.00
N-NO_3, mg/L	13.63 ± 1.45 mg/L	0.04 ± 0.01
P-PO_4, mg/L	1.66 ± 0.27 mg/L	5.65 ± 0.56
Chlorophyll *a*, mg/L	0.001 ± 0.001 mg/L	0.001 ± 0.001

Chlorella –2.18, and for *Scenedesmus* – 2.46. The values of the optical density at a wavelength of 678 nm show the relative content of chlorophyll in the suspension of algae, and the optical density at 550 nm demonstrates the relative number of cells in the culture. The values of the optical density normalized at the initial instant of time determine the relative increase in the number and content of chlorophyll 'a' in the suspension.

Laboratory photobioreactor

The pilot plant, which is a photobioreactor with microalgae, was created and put into operation in the Engineering and Technological Center of the MGUP 'Mosvodokanal' on the Kuryanovo Wastewater Treatment Plants. The plant (Figure 1) consists of columns with algal culture; illumination system (3000 lux, determined with the help of a luxometer); an air supply and distribution system (to supply algae with CO_2 and mix biomass within columns); a system for supplying purified wastewater once daily to replace water discharged from the reactor; a system for biomass dehydration by filtering with the use of a vacuum pump (a filter 3 μm pore size). Air is supplied 24 h a day and columns are illuminated 12 h a day; illumination is regulated by a controller. Water temperature in the reactors was 22–25 °C. Columns are completely mixed batch reactors with once daily partial exchange of fluid. In the columns, different regimes were set, differing in the *volumetric exchange ratio* (VER – the volume of the reactor liquid, exchanged for wastewater, in percent from the total volume of the column) – 17, 25, 33, 40 and 50%.

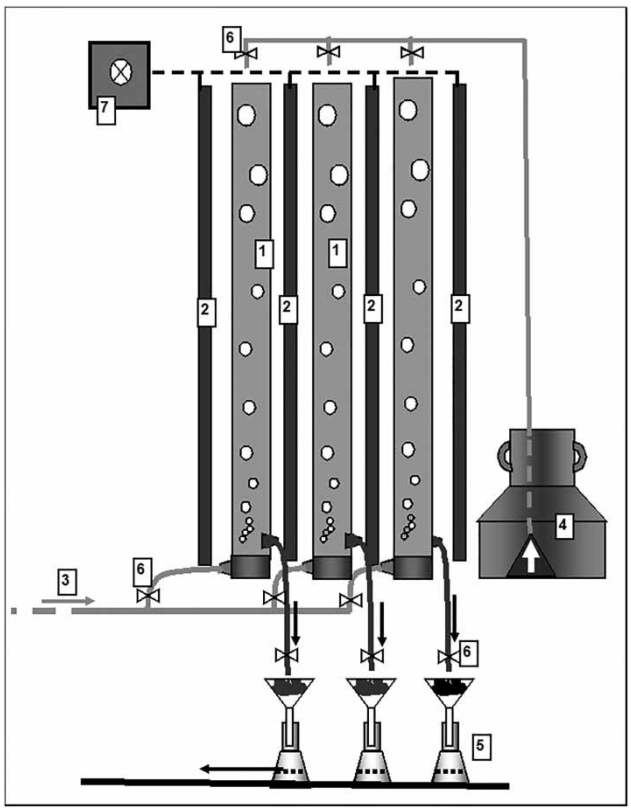

Figure 1 | Diagram of photobioreactor: 1 – reactor columns with algal culture; 2 – illumination system; 3 – the system of air input and distribution for supply of algae with CO_2 and for culture mixing; 4 – the system of treated waste water supply, once daily (reservoir with a pump); 5 – the system for biomass dehydration and culture sampling from cultivators; 6 – valves; 7 – controller.

Determined parameters: the composition of water entering the PBR and outgoing from the PBR (daily: pH, NH_4, NO_3, NO_2, PO_4, suspended matter, chlorophyll; weekly: species composition and the number of microalgae, species composition and the number of zooplankton). The content of chlorophyll 'a' was determined after pigment extraction with organic solvent with subsequent spectrometric measurement. The filtered biomass was weighed.

The amount of biomass was also determined through fluorescence measurements.

Biomass samples were collected into non-sterile, 1-L plastic bottles and analyzed for pigment concentration and fluorescence. Fluorescence was measured within 2 h after sampling. Parameters of chlorophyll fluorescence in algae were measured with a certified pulse fluorometer 'Mega-25' for measurements in highly diluted suspensions of microalgae. The fluorometer has been constructed at the Department of Biophysics, Faculty of Biology, Moscow State University (Pogosyan *et al.* 2006).

Fluorescence was measured using an electronic photomultiplier equipped with light filter CS 18 transmitting light at wavelengths longer than 680 nm. Fluorescence excitation was provided using LEDs having the emission peak at the wavelength of 455 nm. Fluorescence signals from opened (F_0) and closed (F_m) PS II reaction centers were measured at the excitation light intensities with photon densities of 0.8 and 6000 $\mu mol \cdot m^{-2} s^{-1}$, respectively. The potential efficiency of primary photosynthetic processes was calculated using the formula:

$$\frac{F_m - F_0}{F_m} = \frac{F_v}{F_m},$$

where $F_v = F_m - F_0$ is variable fluorescence.

The efficiency of the primary processes of photosynthesis (Fv/Fm) is a dimensionless characteristic of energy efficiency of photosynthesis, which represents output–input

ratio and does not depend on algae species (Rubin 2005). The level of constant fluorescence, Fo, highly correlates with the total content of light-harvesting pigments in the photosynthetic apparatus of phytoplankton, and, correspondingly, it also correlates with the abundance of algal cells (Matorin *et al.* 2004). Hence, it can be used to estimate culture growth. The use of fluorometric method for estimation of chlorophyll content in phytoplanktonic algae does not require any pretreatment of water for measurement. Fluorescence was also measured with a WaterPAM (Walz, Germany) fluorometer.

To study fluorescence, three modes of PBR reactors were chosen: the first reactor was supplied with BPW; culture age was 3 days (one third of reactor volume was replaced daily with biologically purified water), the second and third reactors were supplied with clarified water; culture age in the second reactor – 3 days, in the third reactor – 2 days (VER: half of the reactor volume per day).

Digestion substrate and inoculums for gas generation

A microalgae biomass and activated sludge of KWWTP were used as a digestion substrate. The separation of biomass was carried out by sedimentation. The digested sludge, that was used as a inoculum, was additionally digested in serum bottles under anaerobic (53 °C) conditions for 240 h. Additional digestion was carried out for minimization of an impact of available organic substance in inoculum in general gas generation.

Gas generation experiment conditions

Ultimate biogas potential of algae biomass and activated sludge was determined in 500 cm^3 glass serum bottles. They were filled with inoculum (KWWTP digested sludge) and substrate (suspended algae biomass or KWWTP activated sludge) in a range of ratio (I:S) from 5:1 to 1:1 by ashless substance (AS), and added with liquid nutrient medium for mixed anaerobic cultures (Speece & McCarty 1964; Owen *et al.* 1979). Volume of substrate, inoculum and nutrient solution were calculated to make gas headspace in the bottle of 200 cm^3. 1 g of $NaHCO_3$ was added in each bottle in order to provide medium buffer capacity. The bottles were flushed with nitrogen for 5 min. After that, all bottles were sealed up with rubber plugs and applied with aluminium caps. The bottles were kept in a thermostat at constant temperature 53 °C during 76 days. Biogas volume was calculated by pressure measurement results in the bottles' headspace. Biogas composition was determined in the maximum biogas generation period by gas chromatography (chromatograph Crystal 2000M (Russia), equipped with a conductometer, a column filled with Porapak Q sorbent, carrier gas: He). The measurements were carried out in five replicates. The data were statistically analyzed by Fisher's test.

RESULTS AND DISCUSSION

Investigation of the growth of monoculture on purified wastewater in the first day

Incubation in BPW KWWTP with suspensions of *Chlorella sorokiniana* and *Scenedesmus quadricauda* was carried out at different ratios of algal biomass and wastewater (Figure 2).

During the entire cultivation period, all the reactors retained high fluorescence variable values Fv/Fm (0.62–0.69 for *Chlorella* and 0.53–0.62 for *Scenedesmus*), indicating a good physiological state of the algal culture in the wastewater. During the day, there was a significant decrease in the content of ammonium and nitrate nitrogen in the BPW KWWTP. The greatest increase in the content of chlorophyll 'a' (D678) and the number of cells (D550) in the suspension during the day was observed with a greater dilution of the inoculum in both *Chlorella* and *Scenedesmus* (Figure 2). However, in *Chlorella*, this dependence was expressed much more sharply. If in *Scenedesmus* the difference between the final concentrations in the three variants of the experiments was 0.2–0.3 normalized units of optical density (D678), then for *Chlorella* this difference between the variants at the end of the experiment was 1.0–1.5 normalized units. That is, the optical density at the wavelength of 678 (the content of chlorophyll 'a') doubled.

Within 24 h, the pH values of algal cultures increased significantly in both *Chlorella* and *Scenedesmus* at different amounts of inoculum (Figure 2).

Table 2 presents data on the reduction of ammonium and nitrate nitrogen in the reactors during the first day of cultivation. During 24 h of cultivation, there is a significant decrease in ammonium nitrogen, and the amount of nitrates also decreases. The greatest decrease in the content of ammonium nitrogen was observed for *Chlorella*, which confirms the observed sharp increase in the chlorophyll 'a' content and scattering for this culture. The difference in the rate of growth for these two species of algae is probably due to the reaction of *Scenedesmus* to specific (specifically for this species) growth inhibitors contained in the wastewater.

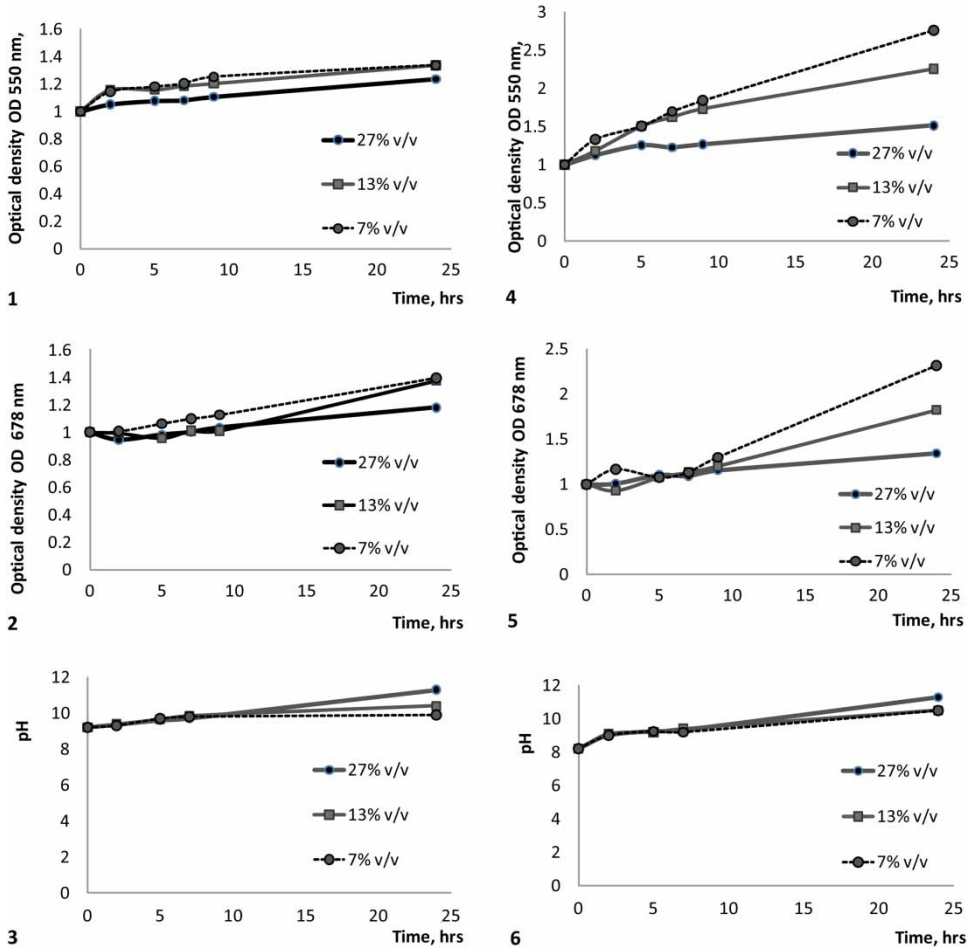

Figure 2 | Changes in the optical density at the wavelength of 678 and 550 nm and pH level of *Scenedesmus* (1)–(3) and *Chlorella* (4)–(6) during 24-hr cultivation (average of five variations).

The incomplete reduction in the amount of nitrates with the growth of culture on BPW KWWTP may depend on the short cultivation time (24 hours) and the small age of the initial culture chosen as an inoculum.

Investigation of the growth of polyculture on purified wastewater in a long-term experiment

Species composition of algae in photobioreactor

The formation of species composition in the reactor was the major problem since the beginning of the work. It is known that reactors with single species of alga are most productive. However, maintaining sterility in a reactor for post-treatment of domestic wastewaters is impossible. Algae, which grow in all wastewater treatment constructions (open channels of supplied water and secondary settling) are constantly coming into reactor.

The experiment lasted about 3 years. Culture of green alga *Scenedesmus* was added to photobioreactor at the very beginning of the experiment. The reactors then functioned as batch complete reactors with one daily wastewater discharge. Routine examination of the species composition of incoming and post-treated water began after the stabilization of the species composition of the PBR, approximately 1 month after the launch of the PBR. This examination has shown that species diversity in the incoming water was significantly higher, than in the reactor. Whereas 89 species were found in incoming water for the total observation time, only 22 species were found in the reactor. In both cases, green and diatomic algae are dominating (Figure 3). Species composition in photobioreactor is rather stable during the whole time of observation.

Dominating species of algae in water, coming into reactors, are, among diatomea: *Nitzschia palea (Kutz.) W. Sm.*, *Stephanodiscus sp*, among green algae: *Scenedesmus quadricauda Chod.*, *Scenedesmus opoliensis P. Richt.*, among

Table 2 | Parameters of ammonium nitrogen proportion and nitrates during 24 h of cultivation – average of five variations (*Scenedesmus* and *Chlorella*)

***Scenedesmus* cultivation time**	**2**	**5**	**7**	**9**	**24**
27 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	102	82	73	60
$N\text{-}NO_3$, mg/L	100	102	99	99	52
13 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	96	89	74	39
$N\text{-}NO_3$, mg/L	100	94	88	94	48
7 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	94	88	84	42
$N\text{-}NO_3$, mg/L	100	104	94	93	50
***Chlorella* cultivation time**	**2**	**5**	**7**	**9**	**24**
27 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	90	86	63	36
$N\text{-}NO_3$, mg/L	100	101	96	99	73
13 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	101	82	83	24
$N\text{-}NO_3$, mg/L	100	107	96	94	72
7 v/v of inoculum					
$N\text{-}NH_4$, mg/L	100	96	89	85	14
$N\text{-}NO_3$, mg/L	100	101	96	99	74

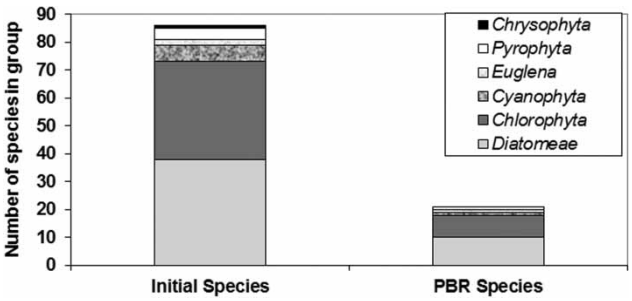

Figure 3 | Algae species characteristic of BPW and CW.

blue-green algae (cyanobacteria): *Oscillatoria amphibia f. tenuis (Anissim.) Elenk., Oscillatoria tenuis Ag. ex Gom, Chroococcus minutus*. Dominating species of algae in the reactors are, among diatomea: *Dicthyosphaerium ehrenbergianum Nag., Navicula viridula Kutz,* among green algae: *Scenedesmus obliquus (Turp.) Kutz., Scenedesmus parvus*, among blue-green algae: *Oscillatoria amphibia f. tenuis (Anissim.) Elenk*., among pyrrophyta: *Didymocystis lineata Korsch.,* among euglenoids: *Astasia* sp.

As seen from analysis of species composition, structure of algal community in the photobioreactor is altered. Most biomass is composed by the following algae: *Oscillatoria, Scenedesmus* and *Astasia.* Total biomass of these species in discharged water was as high as 500–1,000 g/m^3.

Efficiency of purification from nutrients

Daily measurements were made of water quality and algal biomass growth in each column. Table 3 shows the statistically processed water quality data at the outlet of the reactor for different reactor loading options (with different retention times of the algal culture, which was set by a different fraction of the daily purified water).

The water, which has been purified in the photobioreactor, reaches Russian standards for nitrogen ammonium, nitrates and phosphates. The efficiency of their removal in the experiments was 34–48% for ammonium nitrogen, 33–87% for nitrogen of nitrates, 89–91% for phosphates, and 40–94% for total nitrogen.

The nitrogen content of the nitrite after post-treatment was at the level of 2–11 MPC, increasing with the VERs in the reactors (Table 3). It should be noted that the presence of a relatively high content of nitrites is due to the fact that the nitrification process in the reactor proceeds simultaneously with the assimilation of biogenic elements with microalgae. It was noted that the content of nitrite in the drain water from the reactor reached the normative values when in the storage tank for 1 h. This is due to the presence of a high oxygen content in the drain water (due to photosynthesis), which was spent on the ongoing process of nitrification (second stage).

Table 3 | Chemical composition treated wastewater by photobioreactor after algae biomass separation

Dilution, %	**17**	**25**	**33**	**40**	**50**	**MPC (maximum permissible concentrations, Russia)**
$N\text{-}NH_4$, mg/L	0.20 ± 0.07	0.24 ± 0.05	0.15 ± 0.02	0.04 ± 0.03	0.05 ± 0.04	0.4
$N\text{-}NO_2$, mg/L	0.07 ± 0.06	0.13 ± 0.08	0.15 ± 0.05	0.29 ± 0.08	0.28 ± 0.08	0.024
$N\text{-}NO_3$, mg/L	1.10 ± 0.99	1.55 ± 0.60	4.23 ± 1.35	6.73 ± 2.29	10.63 ± 1.29	9.1
Ntotal, mg/L	1.37 ± 1.01	1.91 ± 0.59	4.54 ± 1.41	7.06 ± 2.29	10.95 ± 1.26	–
$P\text{-}PO_4$, mg/L	0.09 ± 0.02	0.12 ± 0.02	0.13 ± 0.06	0.13 ± 0.04	0.14 ± 0.04	0.20

Removal of nitrogen forms by microalgae is based on the assimilation of this element, whereas purification of water from phosphates occurs both through assimilation and through another process – mediated chemical removal of phosphates. With intensive consumption of CO_2 by microalgae, the pH of the purified water increases (up to 8.0–9.5), which leads to the formation of sparingly soluble phosphate salts. The pH level in the PBR stably kept at values of 9.0–10.5. Thus, both biological and mediated chemical–biological removal of phosphates occurred simultaneously. That is why the efficiency of removal of phosphates in the variants of the experiment (with different biomass increments) did not differ and was 89–91%. The second process 'leveled out' the action of the first. The efficiency of the reactors was estimated by the following technological indicators: the efficiency of total nitrogen removal, the achievement of normative values for nutrient elements, the daily biomass increment per unit volume of the reactor (Table 3, Figure 4).

At VER of 17–33% the highest values of the purification efficiency from total mineral nitrogen (75–95%) are observed. The reduction in the efficiency of nitrogen removal occurred when VER had exceeded a third of the volume of the reactor. If with VER of a third of the volume per day, 75% of nitrogen is removed from the water entering the reactor, then in the case of an exchange rate of a half the volume, only 50% is removed.

VER of a third of the volume of the reactor was characterized by the greatest increase in biomass per unit volume of the reactor: an average of 250 mg/L/day by wet weight of biomass or 50 mg/L/day by dry weight. The maximum increase in raw biomass was 960 g/(m^3 day) or 190 mg/L/day on a dry basis.

As experiments in the pilot photobioreactor have shown, VER of 33% (of the total volume per day) is characterized by the maximum biomass increment with high water purification efficiency.

Disinfection of water and species composition of zooplankton

For three variants of experiments (VER: 17, 25 and 33%), studies were conducted on the efficiency of water disinfection in reactors. The content of total coliform and thermotolerant bacteria was determined in the entering the PBR and purified water (Table 4).

The purified water in three experiments after passing through the reactor has bacterial contamination values corresponding to the standards for wastewater. The disinfecting 'agent' is the organisms of zooplankton inhabiting the reactor. During the observation period, 31 species of zooplankton organisms belonging to six systematic subdivisions have been identified in the reactors: rotifers (16 species), cladoceras (eight species), copepods (one species), crustaceans (one species), nematodes (one species), chironomidae (one view). The dominant species with the maximum biomass (up to 700 mg/m^3) and the number (up to 800,000/m^3) are: crustaceans *Ostracoda*, rotifers *Philodina acuticornis Murray, Lecane (Monostyla) bulla Gosse, Colurella* sp., *Lepadella ovalis Mull., Lepadella* sp., *Cephalodella gibba Ehrenberg, Asplanchna priodonta* Gosse and cladoceras *Oxyurella tenuicaudis Sars., Alona rectangula Sars*. It is

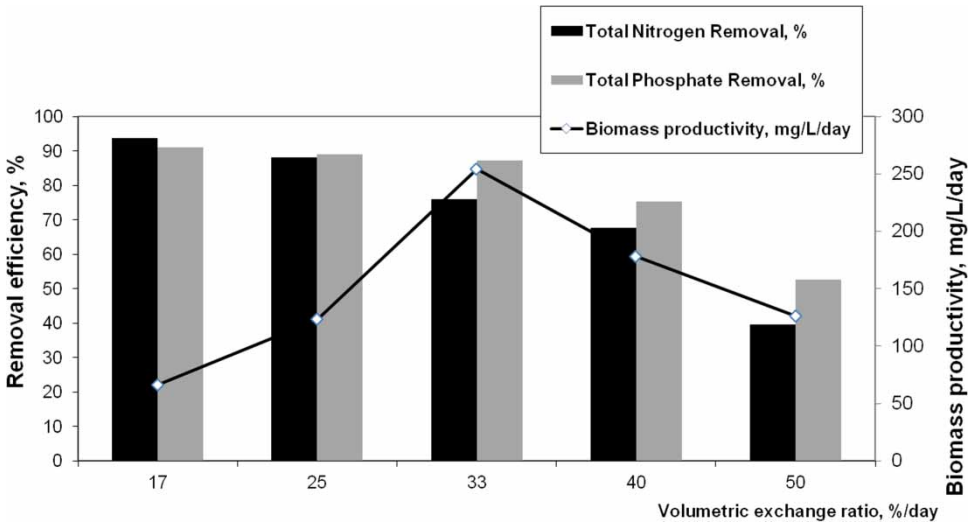

Figure 4 | The main indicators of the photobioreactor for different VER: (1) total nitrogen and phosphate removing efficiency, %; (2) biomass productivity, mg/L/day.

Table 4 | Bacterial characterization of the purified wastewater after photobioreactor

VER. %	Efficiency of disinfection	Total coliforms index, CFU/100 mL	Thermotolerant coliforms index, CFU/100 mL
33	Input value	68,000	44,000
	Output value	**420**	**18**
	Reduction, %	99	100
25	Input value	18,000	13,000
	Output value	**110**	**3**
	Reduction, %	99	100
17	Input value	7,600	5,900
	Output value	**220**	**2**
	Reduction, %	97	100
Russian standard (SanPiN 2.1.5.980-00)		***500***	***100***

these species that primarily disinfect water, consuming bacterial biomass. Their role is also important in the production of algae, since zooplankton transforms organic matter (bacteria, detritus) into nitrogen and phosphorus mineral compounds accessible to algae.

Evaluation of the application of the fluorimetric method for technological control of a photobioreactor

Technological indicators of productivity and fluorescence characteristics

The principal technological parameter of photobioreactor is biomass growth per unit reactor volume per unit time. Productivity depends on several factors: nutrient content, culture age, and the toxicity of incoming water. For 1 month, experiments were conducted in the PBR, where the fluorescence indices were compared for the cultivation of algae on purified and on clarified wastewater. The first and second reactors differed in the quality of incoming water and had the same culture age (3 days). The second and third reactors were supplied with clarified water and had culture age 3 and 2 days, respectively. Purified water (first column) had a lower nutrient content and lack of toxicity. The latter is confirmed by biotesting experiments; toxicity index with infusoria was 0.26 (Kozlov *et al.* 2006). Clarified water contains much nutrients, mostly in the form of ammonia salts, which are first consumed by algae. However, the toxicity level of this water is higher than that of biologically treated water. Toxicity index varied from 0.35 to 0.89 in biotests with infusoria (Kozlov *et al.* 2006). The most toxic water (in accordance with the high content of clarified water) was the water of the third column. All these factors resulted in different average values of productivity of the three reactors: 149, 206, 135 mg/L/day by wet weight with variability of the values not more than 25% of absolute values (Table 1).

Densities of the algal cultures (determined daily in discharge fluid) were also different. Wet weight of algae in the three reactors was 287, 125, and 165 mg/L, respectively.

Fluorescence parameters (Fo) and (Fv/Fm) were measured in samples from reactors, differing in the rate of culture growth and in the extent of growth suppression by toxic substances. It was found that coefficient of correlation between average values of Fo in the three reactors and chlorophyll 'a' concentration was 0.98, and coefficient of correlation between Fo and biomass content was 0.99.

The activity of PS2 reaction centers, related to oxygen evolution in photosynthesis, can be estimated from fluorescence parameter Fv/Fm. This parameter, which reflects the maximum quantum of PS2, can be used to estimate photosynthetic activity of the culture of microalgae in photobioreactor. In natural water bodies with a high nutrient content, Fv/Fm values may be as high as 0.6–0.7. Under the effect of stress conditions and pollutants, this parameter decreases. In dead cells, it is equal to 0.

Fv/Fm values in the three reactors varied within a range of 0.46–0.58. There were no statistically significant differences between the three reactors (Table 1), however, a tendency to direct correlation was found between Fv/Fm and reactor production (Table 5, Figure 5).

Thus, fluorometric method can be used not only for quantitative estimation of algal biomass in photobioreactor (by parameter F_o), but to also assess reactor productivity. An increase in production is associated with a higher Fv/Fm ration. Correspondingly, a decrease in the latter

Table 5 | Chlorophyll concentration, biomass content, fluorescence parameters F_o and photosynthetic activity (Fv/Fm) in PBR

	Column 1	Column 2	Column 3
Kind of wastewater	Biologically purified	Clarified	Clarified
Daily dilution, %	33	33	50
Toxicity of water	no	low-toxic	moderately toxic
Chlorophyll 'a' mg/L	1.48 ± 0.24	0.72 ± 0.39	0.90 ± 0.28
Total biomass, mg/L	287 ± 41	125 ± 28	165 ± 62
Productivity, mg/L/day	149 ± 36	206 ± 48	135 ± 34
F_0	4,475 ± 484	2,029 ± 901	2,918 ± 942
F_v/F_m	0.51 ± 0.07	0.55 ± 0.05	0.50 ± 0.02

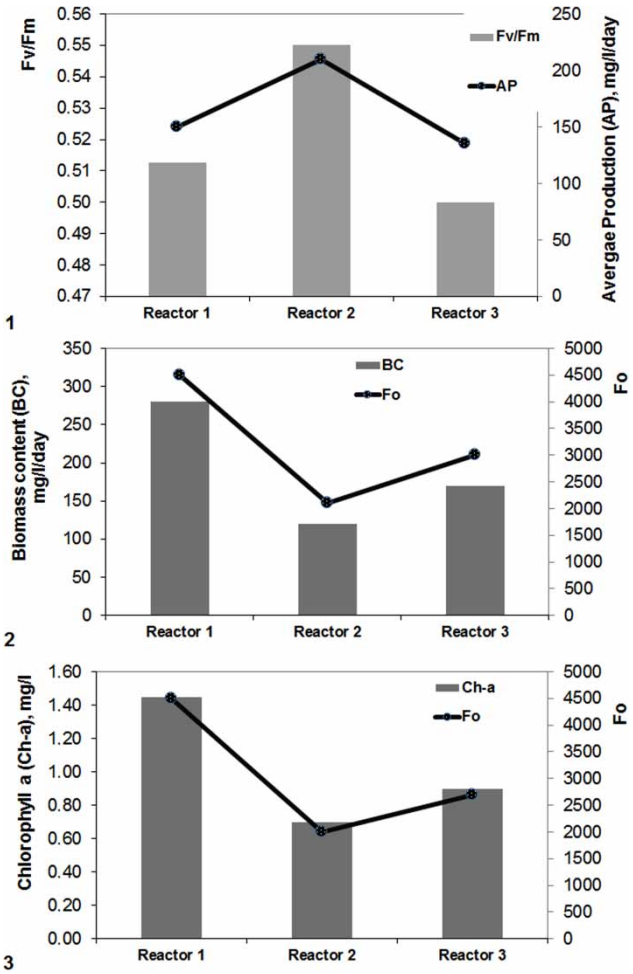

Figure 5 | Value of average production (1), biomass content (2), chlorophyll concentration (3) and fluorescence parameters (Fo and photosynthetic activity Fv/Fm) in photobioreactors.

parameter can be used as an indicator of water toxicity, nutrient deficiency, or other changes in technological mode, which leads to a lower efficiency of photobioreactor operation. Continuous instrumental control of the two parameters will help to timely apply technological measures for stabilization of photobioreactor production.

Evaluation of the energy potential of microalgae biomass

Impact of inoculums: substrate ratio on a value of specific methane yield in anaerobic digestion

The value of specific methane yield (dm^3 per kg of initial AS) depended on I:S ratio and inoculum type. When KWWTP digested sludge was used as inoculum, the increase of I:S from 1:1 to 3:1 resulted in the increase of specific gas yield from 110 to 134 dm^3 CH_4/kg VS, I:S ratio expansion from 3:1 to 5:1 did not result in significant increase of biogas yield (Table 6).

Absolute values of specific methane yield with use of digested sludge varied from 110 to 136 dm^3/kg AS, which corresponds to data (84–217 dm^3/kg AS) of other researchers studying anaerobic digestion of algae (Mussgnug *et al.* 2010; Ras *et al.* 2011; Gonzalez-Fernandez *et al.* 2012; Mehrabadi *et al.* 2016). Comparison of the ultimate biogas yields of algae and active sludge showed that the algae biogas production was 15% lower (at I:S ratio of 3:1).

Thus, methanogenic community of digested sludge is capable of relatively deep digestion of algae biomass. The specific methane yield of algae was found to be slightly less than that of excess activated sludge. An increase in the specific biogas yield and algae biomass degradability can be achieved after biomass pretreatment, such as thermohydrolysis (Gonzalez-Fernandez *et al.* 2012).

Biogas composition. Biogas obtained in the algae digestion process was composed by 57.0–59.7% of methane and by 40.3–43.0% of carbon dioxide. The biogas volume fraction

Table 6 | Parameters of anaerobic digestion of microalgae biomass

			Biogas composition, %	
I:S ratio	**Specific methane yield, dm^3/kg AS**	**AS biodegradability, %**	**CH_4**	**CO_2**
Algae with digested sludge as inoculum				
1:1	110	14	59.1	40.9
2:1	121	15	58.8	41.2
3:1	134	13	59.7	40.3
5:1	136	22	57.0	43.0
Active sludge with digested sludge as inoculum				
3:1	157	30	67.5	32.5

of methane for algae digestion corresponded to the literature data (Gonzalez-Fernandez *et al.* 2012), but was lower than that in the digestion of activated sludge. Variations in biogas composition may be caused by different chemical composition of digested substrates (microalgae and activated sludge), and also, possibly, by different availability for the methanogenic community of the internal contents of algal cells and activated sludge.

Biodegradability. High cost of waste utilization makes algae biomass decomposition degree one of the most important indicators of the process. Substrate biodegradability (based on AS) varied from 13% to 22% with expansion of I:S ratio. A tendency of increase of microalgae biomass degradability was observed with growth of inoculum ratio in the initial mixture. Active sludge AS degradability was higher, reaching 30%.

Possibility of inhibition with ammonium ion. Destruction of substrate cell walls and further fermentation of cell content in some cases may cause an increase of ammonium concentration to the levels inhibiting methanogenesis. Ammonium nitrogen concentration in centrifuge effluent of digestate (105.0–652.5 mg/dm^3) did not exceed toxic threshold for methanogens (Bruno *et al.* 2009). Thus, possibility of inhibition of anaerobic microalgae digestion process was excluded.

Solving technical problems in the development of an industrial photobioreactor

The technical and economic feasibility of the industrial implementation of the creation of an industrial PBR was assessed according to the following positions:

1. Total energy consumption for the operation of column reactors.
2. Possible volume of capital expenditures of the photobioreactor and the area occupied by the reactor: they are inversely related to the daily water exchange ratio in the columns.
3. Reducing the wastewater discharge fee: this indicator depends on the efficiency of removal of nitrogen and phosphorus.
4. Compensation of a part of the energy costs for water treatment by obtaining fuel from the biomass of algae.

The main energy costs in the pilot project are to illuminate the columns with fluorescent lights. In the pilot project, the specific energy consumption (for lighting and aeration) is about 70 kWh for additional cleaning of 1 m^3 of wastewater. For comparison, the energy costs for the complete treatment of 1,000 m^3 of wastewater in the Moscow treatment plant are 160–180 kWh. Therefore, the industrialization of a large photobioreactor with artificial lighting is unrealizable due to the astronomical high cost of purification. However, there are already ways to replace artificial lighting with natural – solar. In the world, modern technologies such as the 'light well' are actively developing. This technology concentrates the solar stream and distributes it through the system of fiber-optic transmitters to the right spot for lighting. The technology allows the maintenance of sufficient illumination in the room even on cloudy days. Using this technology for lighting, it is possible to reduce the specific energy consumption of a photobioreactor to 1,000 kWh/1,000 m^3 of wastewater with water (VER of 33% of the volume of the reactor and up to 700 kWh/1,000 m^3 of wastewater with VER of 50% of the reactor volume.

Calculate the area occupied by buildings with a system of 2-m column-reactors at the expense of treated waters of about 2 million m^3 per day (similar to a water treatment facility for a city with 10 million inhabitants). Estimated calculations when taking into account the annual dynamics of solar radiation show that at the latitude of Moscow with VER of 33%, the area of buildings will be 3 km^2, with VER of 50% – 2 km^2. For more southern cities of Russia (for example, for the city of Sochi), in order to provide additional treatment of effluents from the city with 0.5 million inhabitants, only 15–20 buildings with an area of about 10–12 thousand m^2 are needed. In warm solar regions, the treatment system does not require the construction of buildings and can be carried out 'in the open.'

Estimated economic calculations show that when using a photobioreactor, wastewater discharge fee on nitrogen and phosphorus compounds are reduced by 100 times according to modern standards. Also, biogas from the biomass of algae produced in the reactor will allow to compensate up to a third of the total energy expended for wastewater treatment.

CONCLUSIONS

The treatment plant in the classical sense can be transformed into an ecological complex for water purification and biogas production from microalgae. The most promising for the development of this technology are warm regions with high solar radiation. The application of this technology in all regions is very promising.

The principal difference between a photobioreactor and other treatment systems is as follows:

- Simultaneously, the water is treated and disinfected to the normative values.
- Purification by this method (using clarified water in PBR) occurs without waste, which must be taken out for deposition, as in the case of activated sludge. The resulting biomass is the raw material for a number of valuable products: biofuel and/or organic fertilizer and/or animal feed.
- The treatment plant has income items in the form of sales (or use for internal needs) of energy from biomass.

The data obtained in the present work demonstrated that the fluorescent method of photosynthesis assessment is applicable for the evaluation of technological processes in growing microalgae biomass. The method allows to significantly reduce the effort required to monitor and implement process control with continuous surveillance.

It was found that the ultimate biogas potential of microalgae is 15% less than that of activated sludge. The composition of the biogas obtained during digestion did not depend on the ratio of I:S and the type of inoculum, while the volume fraction of methane in biogas during digestion of the activated sludge was slightly higher than when digesting the algomass.

According to the obtained data, the optimal ratio of I:S should be recognized as 3:1. Inhibition of methanogenesis by ammonium ions was not observed.

Microalgae have a low potential for digestion, so further studies of increasing the efficiency of digestion should be aimed at finding various methods of preliminary treatment of algomass to increase the availability of organic matter.

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