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TESTING OF *CHLORELLA/SCENEDESMUS* MICROALGAE CONSORTIA FOR REMEDIATION OF WASTEWATER, CO_2 MITIGATION AND ALGAE BIOMASS FEASIBILITY FOR LIPID PRODUCTION

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Abstract. Industry, transport and unsustainable agriculture result in the increased quantity of wastewater, release of nutrients and emission of carbon dioxide that promotes eutrophication of water bodies and global climate change. The application of microalgae for phycoremediation, their biomass use for human needs may increase sustainability and have a positive effect on the regional development. The experiments were carried out in order to establish the feasibility of treating the local municipal wastewater with microalgae consortia and their biomass potential for biofuel production. The results revealed that *Chlorella*/*Scenedesmus* consortium eliminated up to 99.7–99.9% of inorganic phosphorus and up to 88.6–96.4% of inorganic nitrogen from the wastewater within three weeks. The ammonium removal was more efficient than that of nitrate. *Chlorella* algae grew better in diluted, while *Scenedesmus* – in the concentrated wastewater. The consortium treated wastewater more efficiently than a single species. The maximum biomass (3.04 g/L) of algal consortium was estimated in concentrated wastewater. Algae accumulated 0.65–1.37 g of CO_2/L per day in their biomass. Thus, *Chlorella*/*Scenedesmus* consortium is a promising tool for nutrients elimination from the local wastewater under the climatic conditions specific to Lithuania. However, none of the two species were able to accumulate lipids under the nitrogen starvation conditions.

Keywords: phycoremediation, wastewater, microalgae, biomass, oil accumulation.

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Introduction

The demands for energy and freshwater resources are increasing with the growing human population in the world. Industry, transport, unsustainable agriculture increase the quantity of wastewater as well as the release of nutrients and emission of carbon dioxide that promotes eutrophication of the waters and global climate change (Chiu *et al.* 2009; Pittman *et al.* 2011; Olaizola *et al.* 2004; Rawat *et al.* 2011). Photosynthesizing algae are important organisms that can help to control undesirable processes in the ecosystems. Currently, the green technologies are one of the most growing sectors in the world. Algae biomass may be used for alternative application such as biofuel, natural fertilisers production, additives to animal nutrition, etc. (Munoz, Guieysse 2006; Mata *et al.* 2010; Misevičius, Baltrėnas 2011). Applications of algae for human needs are considered to have many benefits such as increasing sustainability, reduction of greenhouse gas emissions, regional development, social structure, agriculture and security of supply (Reijnders 2006).

The wastewater is a suitable resource for microalgae, because it contains large quantities of nitrogen and phosphorus that are the key elements for the algae growth (Li *et al.* 2011; Park *et al.* 2011; Pittman *et al.* 2011). Although large-scale production of algal biofuels using wastewater

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treatment was first proposed by Oswald and Golueke in 1960 (Park *et al.* 2011 and references therein), the use of microalgae in the wastewater industry is still fairly limited (Pittman *et al.* 2011; Rawat *et al.* 2011). The application of microalgae for phycoremediation and sustainable biofuels production could become economically feasible (Rawat *et al.* 2011). The algal biomass from wastewater treatment systems could be converted to biofuels: by anaerobic digestion to biogas, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol and high temperature conversion to bio-crude oil (Pittman *et al.* 2011; Craggs *et al.* 2011). Under controlled growth conditions, microalgae can accumulate up to 80% of oil in dry cell mass (Pittman *et al.* 2011). Particularly, *Chlorella* species may accumulate 14–63% and *Scenedesmus* 6–55% of oil under various conditions of cultivation (Li *et al.* 2008; Gouveia, Oliveira 2009; Mutanda *et al*. 2011 and references therein). However, lipid accumulation in wastewater grown microalgae is much lower and ranges from low (<10% DW) to moderate (25–30% DW) lipid content (Halim *et al.* 2012).

Various algae species have different ecological requirements for their development. The highly variable composition of wastewater may limit the growth of some algae, whereas the others will flourish (Bhatnagar *et al.* 2011). So, it is essential to select algal species or their consortia capable of growing in particular wastewater under changing climatic conditions that will define the success of the treatment process. Unicellular fast growing green algae are very tolerant to many wastewater conditions and efficient at accumulating nutrients from it (Ruiz-Marin *et al.* 2010). *Chlorella* (*C. ellipsoidea, C. minutissima*, *C. vulgaris*) and *Scenedesmus* (*S. bijuga*, *S. quadricauda, S. obliquus*) are the most studied algae for their application in the wastewater treatment (Lau *et al.* 1995; Gouveia, Oliveira 2009; Ruiz-Marin *et al.* 2010; Johnson, Wen 2010; Wang *et al.* 2010; Bhatnagar *et al.* 2011; Praepilas, Kaewkannetra 2011; Yang *et al.* 2011a, b). Pate *et al.* (2011) emphasized that autotrophic microalgae productivity is strongly dependent on environmental conditions most suitable for growth, especially high solar resource and temperatures, which are characteristic of particular geographical region. Thus, the actual values of daily biomass productivity and neutral lipid content that can be achieved in practice will depend on a complex combination of algal strain, cultivation system and local growing conditions.

In Lithuania with the population of about 3 mln., approximately 170 mln. m^3 of domestic-industrial wastewater are produced yearly (http://gamta.lt/cms/index). The release of wastewater around Vilnius city is about one third of the total amount in the country. Study of Gudas, Povilaitis (2013) has revealed that wastewater factor is prominent for water quality in small rivers downstream larger towns. Studies of combined use of microalgae for the treatment and biomass production are very scarce in the country. Makarevičienė *et al.* (2011) tested separately *Chlorella* and *Scenedesmus* species preference to nitrogen resource utilisation and algae biomass as the feedstock for biofuel at temperatures higher than are natural to Lithuania.

The aim of our experiments was to investigate feasibility of *Chlorella/Scenedesmus* microalgae consortia to treat local municipal wastewater and reduce greenhouse gas. We also seeked to evaluate biomass yield of the tested algae and its suitability for biofuel production under the conditions close to climate in Lithuania during summer.

1. Materials and methods

1.1. Algae cultures and growing media for the experiments

The strains of freshwater green algae *Chlorella* sp. and *Scenedesmus* sp. were isolated from Lithuanian lakes. Both cultures were grown in the BG-11 media at 20 °C and 1600 Lux illumination prior to the experiment.

Experimental studies with the mixture of *Chlorella* and *Scenedesmus* cultures were carried out in mechanically cleaned wastewater collected from the water supply and wastewater treatment company "Vilniaus vandenys". Fine soil particles, organic and mineral additives were removed from the solution in laboratory passing the water through the plankton net (pore diameter 20 μm) and additionally filtering through several layers of filter paper. Wastewater was sterilized by autoclave at 120–130 °C 1 atm. pressure for 30 min. for the elimination of bacteria. Bacteria may influence the experiment results of nutrients removal by algae due to decomposition of the organic matter in the solution. Conductivity, pH and chemical analysis of the prepared wastewater media (PO_4, NO_3, NO_2, NH_4 and BOD_5) were performed prior the beginning of the experiment.

The experiment was carried out in 250 ml sterile Erlenmeyer flasks covered with cotton-gauze plugs coated with the plastic. Four types of media with different concentrations of wastewater were used to test the optimal growth conditions for selected green algae species. Wastewater was diluted with distilled water to reach appropriate concentration: M100 – 100% of wastewater; M75 – 75% of wastewater; M50 – 50% of wastewater and M25 – 25% of wastewater in the final solution. BG-11 medium was used as a control. All experiments were carried out in triplicate.

1.2. Experiment design

The generalized experiment scheme is presented in Fig 1. Each variant of 230 ml of prepared growth media was inoculated with 5 ml mixture of *Chlorella*/*Scenedesmus* species taken from the stock algal cultures. The concentration of algae at the beginning of the experiment was 39×10^3 cells/ml (*Chlorella* sp. 31×10^3 cells/ml and

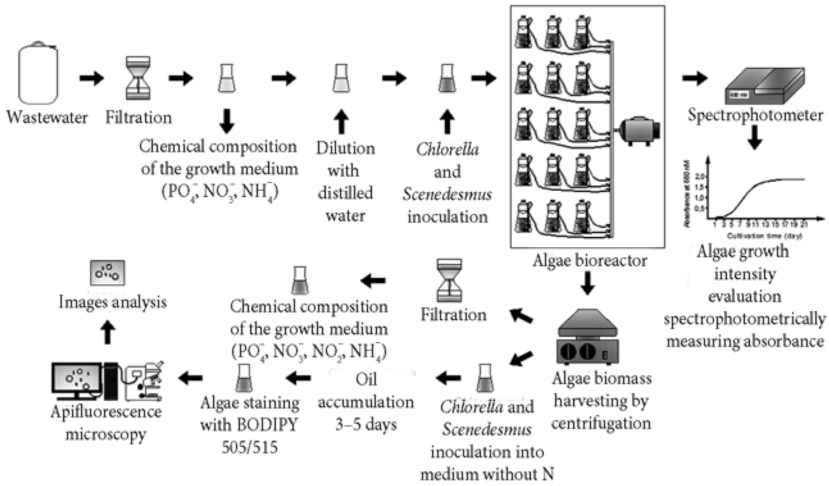

Fig. 1. The general scheme of the experiment

Scenedesmus sp. 8×10^3 cells/ml). Algae were counted in Fuchs-Rosenthal chamber using light microscope.

Climatic conditions close to the natural in Lithuania in June-August were selected to maintain algae in specialized cultivation chamber Percival *Intellus.*

The flasks were incubated at 16:8 day-night cycle with the average 2080 Lux illumination during light period. Day temperature was +20±1 °C and night temperature – +15±1 °C. The mixing of the algae cultures by air pumping (aeration intensity 2 L/min) maintained an equal gas balance and kept algae in the suspension.

Algae growth curves were developed by measuring absorption of samples at 680 nm using spectrophotometer Libra S32PC every second day on the course of 21 days experiment. Algae biomass was evaluated from cell abundance and cell volume calculated based on Olrik *et al.* (1998) recommended formula for ellipsoid with circular cross section algae cells:

$$V = \frac{\pi}{6} \cdot d^2 \cdot l, \qquad (1)$$

where: V – the algal cell volume, μm^3; d – cell diameter, μm; l – cell length, μm.

Cell volume of 10^6 μm^3 was considered as equal to 1 mg of biomass.

1.3. Nutrients and CO_2 removal

For the evaluation of nutrients removal by algae, the chemical analysis of PO_4, NO_3, NO_2, NH_4 concentrations were determined in accordance with standard methods (LST EN ISO 10304; LST EN ISO 14911) in each variant before and after the experiment. After the experiment, the solution was centrifuged 5000 rpm for 1 minute for the separation of algal biomass and media. The remaining water was filtered through 0.9 μm pore diameter filter. Nutrients residues were evaluated. CO_2 consumed and incorporated into algae biomass via photosynthesis was recalculated using formula suggested by Buehner *et al.* (2009):

$$V_{CO_2} = 1.83 \cdot V, \qquad (2)$$

where: V_{CO_2} – CO_2 consumed by algae, μm^3; V – algae cell volume, μm^3.

1.4. Oil accumulation study

Algae start to accumulate oil and other storage resources at critical for their development conditions. The analysis of oil accumulation in the algal cells was performed twice: a) at the end of the experiment when the culture reached stationary phase of growth; b) after growth in nitrogen deprived conditions. In order to create shock conditions and initiation of oil accumulation in the studied green algae cells, 5 ml of algae suspension of each variant was centrifuged (2000 rpm for 5 min.) to separate algae from the growth media. Algae biomass was resuspended into BG-11 medium without nitrogen compounds for three days under the same conditions as during the experiment.

Epifluorescence microscopy was applied to evaluate lipid accumulation in the algae. To stain lipid in the algae cells, Bodipi 505/515 (4,4-difluro-1,3,5,7-tetramethyl-4-bora-3a, 4adiaza-S-indacene; Invitrogen, USA) dyes were added to algae suspension according to Govender *et al.* (2012). Stock solution was prepared by dissolving 1 mg Bodipi 505/515 dyes into 10 ml of DMSO (0.2%). Five μl of prepared dyes was added to 6.25 ml of the sample with *Chlorella*/*Scenedesmus* culture (dye concentration 0.08 μg/ml) and incubated for 5 min. in the dark at 20 °C. Lipids stained with Bodipi 505/515 dyes fluoresce green in algae cells and were investigated with Nikon Eclipse N*i* epifluorescence microscope using a 515 nm emission wavelength filter. Images of algae were taken with Nikon DS-Ri1 camera and analyzed in the NIS-Elements BR software.

1.5. Statistical analysis

All values are expressed as mean ± standard deviation (SD). The data were checked for normality and analyzed by one-way ANOVA followed by Tukey's post-hoc test. The significant differences were considered at $p < 0.05$.

2. Results and discussion

2.1. Characteristics of the wastewater

The physico-chemical parameters of untreated municipal wastewater collected from Vilnius wastewater treatment plant are given in Table 1. The nutrient concentrations of the wastewater fell into the range from medium to strong level according to Rawat *et al.* (2011). Generally, untreated wastewater is characterized by very high concentrations of nutrients and toxic metals (Pittman *et al.* 2011). Total N (TN) and total P (TP) concentrations can be found at values of 10–100 mg/L in municipal wastewater and >1000 mg/L in agricultural effluent (Noue *et al.* 1992). The conductivity values that reach 2.68 mS/cm indicate high amount of various salts. Biochemical Oxygen Demand (BOD_5) shows high amount of easy degradable organic matter in the wastewater. In agreement, light microscopy revealed high abundance of bacteria and scarce number of heterotrophic flagellates, colourless protists.

Table 1. Physico-chemical parameters of municipal wastewater collected from Vilnius wastewater treatment plant

Contaminants	Concentration	Untreated wastewater level*
Total nitrogen, mgN/L	56.5	medium-strong
Total phosphorus, mgP/L	8.3	medium
Inorganic phosphorus, mgP/L	6.1	medium-strong
BOD_5, mgO_2/L	148	week-medium
Oxygen, mg/L	1.41	
Temperature, °C	13.6	
pH	7.74	
Conductivity, mS/cm	2.68	
H_2S, mg/l	0	
Colour	greyish	

* according to Rawat *et al.* (2011).

2.2. Growth rate of microalgae

The mixed culture of *Chlorella* and *Scenedesmus* showed similar pattern of their biomass development under various concentrations of wastewater and considerably differed from the control with BG-11 medium (Fig. 2A). Duration of the lag period was 5 days followed by the exponential growth during the next 8–12 days. Intense photosynthesis processes during the exponential phase also indicated the highest pH values in the 5–13 days (Fig. 2B). Culture slowly entered stationary phase in the 15–19 days of the experiment.

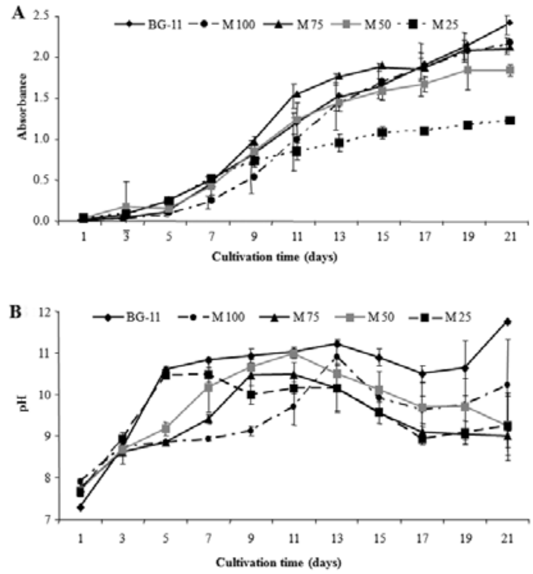

Fig. 2. The growth pattern of *Chlorella*/*Scenedesmus* algae culture under different wastewater concentrations (A) and the variation of the pH values (B) during the course of experiment

Table 2. Biomass production of *Chlorella* and *Scenedesmus* species based on references

Algae species	Biomass yield, g/L	Reference
Chlorella		
C. pyrenoidosa	2.83 in 2 days	Li *et al.* 2011
C. minutissima	0.073–0.38 in 10 days	Bhatnagar *et al.* 2010
C. vulgaris	0.21	Chinnasamy *et al.* 2009
C. vulgaris	av. 1.5, max 3.0	Gouveia, Oliveira 2009
C. ellipsoidea	0.43	Yang *et al.* 2011a
Chlorella sp.	1.9 in 17 days	Rasoul-Amini *et al.* 2011
Chlorella sp.	1.47–1.71	Wang *et al.* 2010
Scenedesmus		
S. obliquus	1.57±0.67	Abou-Shanab *et al.* 2011
S. obliquus	av. 0.9, max 2.0	Gouveia, Oliveira 2009
S. acutus	0.82	Chaichalerm *et al.* 2012

The biomass yield of mixed algae cultivated in BG-11 medium was the highest 3.7 g/L (Fig. 3). There were little differences of the algae biomass yield between concentrated (mean 2.75–3.04 g/L for M75 and M100) and diluted wastewater (mean 2.1–1.8 g/L for M25 and M50). Moreover, the growth of tested algae in concentrated wastewater was of the similar magnitude as in the chemical media, where the strains were cultured prior the experiment, indicating the wastewater as a perfect source to grow species separately or as their mixture. It seems that our results are similar to biomasses of *Chlorella* and *Scenedesmus* species recorded by the other researchers (Table 2). Nevertheless, it is difficult to compare the results from different studies due to variations of media, culturing time and regime. *Chlorella minutussima* cultured photoheterotrophically built biomass up to 0.38 g/L after 10 days growth (Pittman *et al.* 2011). Strain was defined as a good candidate for high biomass productivity in a municipal wastewater. Biomass yield of our studied *Chlorella*/*Scenedesmus* culture was 4.5 times higher that recorded by Pittman *et al.* (2011) even in the lowest biomass variant (mean 1.8 g/L) showing good perspectives of the consortia.

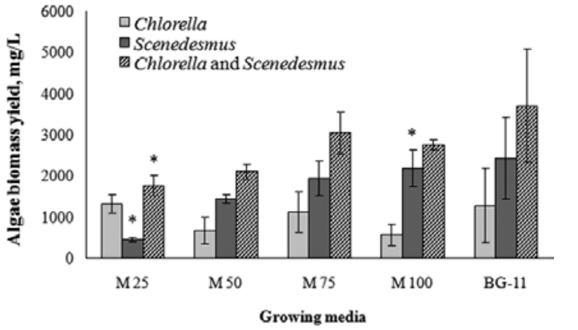

Fig. 3. Biomass yield of *Chlorella* and *Scenedesmus* species in wastewater at different concentrations

* indicate significant differences from the control (BG-11 medium) at $p < 0.05$.

2.3. *Chlorella*, *Scenedesmus* algae density and biomass variations

Control of algae species is still not achieved in wastewater treatment ponds and algal dominance, various species interactions are still poorly understood (Park *et al.* 2011). Our experiment revealed species interactions in the consortia depending on the concentration of nutrients. *Scenedesmus* showed reverse pattern of growth compared to *Chlorella* in the gradient of wastewater concentration ($p < 0.05$) (Fig. 3). Concentrated wastewater (M100) stimulated the growth of *Scenedesmus* algae giving up to 5.5 times higher biomass augmentation compared to the most diluted (M25) variant ($p < 0.05$). *Scenedesmus* cells density increased hundreds to thousands of times by the end of the experiment: up to $4.53\pm1.01\times10^6$ – $29.23\pm3.68\times10^6$ cells/mL in M25 and M100 variants, accordingly.

The small algae cells have larger ratio of surface area and volume what enabled a quicker uptake of nutrients (Hein *et al*. 1995). The average volume of *Scenedesmus* cells was nine times lower than that of *Chlorella*. It may determine the faster growth of *Scenedesmus* at higher concentrations of wastewater. Similarly, Ruiz-Marin *et al.* (2010) found that *Scenedesmus obliquus* grew better in municipal wastewater compared to *Chlorella vulgaris*.

Generally, *Chlorella* growth was suppressed by concentrate wastewater. Species growth in most diluted wastewater variants showed the highest values (Figs 3, 4A, B). Species density in M25 and M50 variants reached $2.72\pm3.68\times10^6$ cells/ml and was three times lower in the most concentrated wastewater ($0.88\pm0.28\times10^6$ cells/ml). Our results coincide with Ras *et al.* (2011) data for *C. vulgaris* which density varied from 3.0×10^6 to 5.3×10^6 cells/ml. The maximum values were stated as high density cultures.

The increase of *Chlorella* density was lower up to 28–88 times only compared to *Scenedesmus*. Possibly the weakest competition for the resources with *Scenedesmus* in the most diluted variants determined the success of *Chlorella*. Contrary to our results, Johnson, Wen (2010) showed that wastewater was feasible for biomass production of *Chlorella* sp. with pure wastewater producing the highest biomass yield. However, the species of even the same genus have different requirements and growth characteristics (Cha *et al*. 2011).

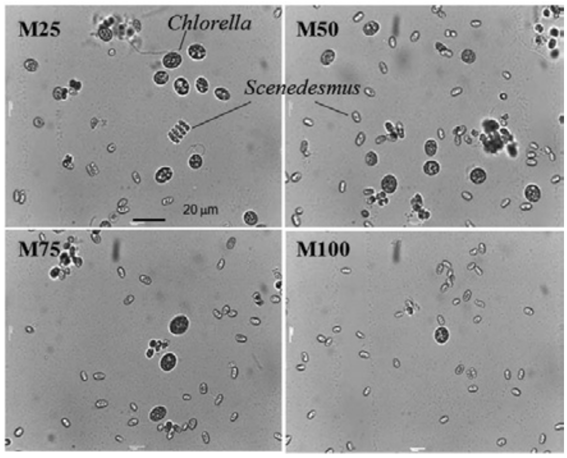

Fig. 4. The mixture of *Chlorella* and *Scenedesmus* species in the stationary growth phase at different concentrations of wastewater

Algae size is important for the biomass harvesting and desirable products extraction from the cells within the processing of the biomass (Rawat *et al.* 2011). *Chlorella* size varied less if compared to *Scenedesmus* and did not show significant differences between wastewater concentrations as well as before and after nitrogen shock application ($p > 0.05$) (Fig. 5). *Chlorella* mean cell volume increased in all variants from the beginning towards the end of the experiment and at nitrogen deprivation conditions.

Chlorella cells volume was over 30% higher in M50 wastewater variant and coincided with the lowest species culture biomass. It might be because species used a lot of energy to build up large cells slowing down the reproduction processes, what consequently lead to less culture biomass in the variant.

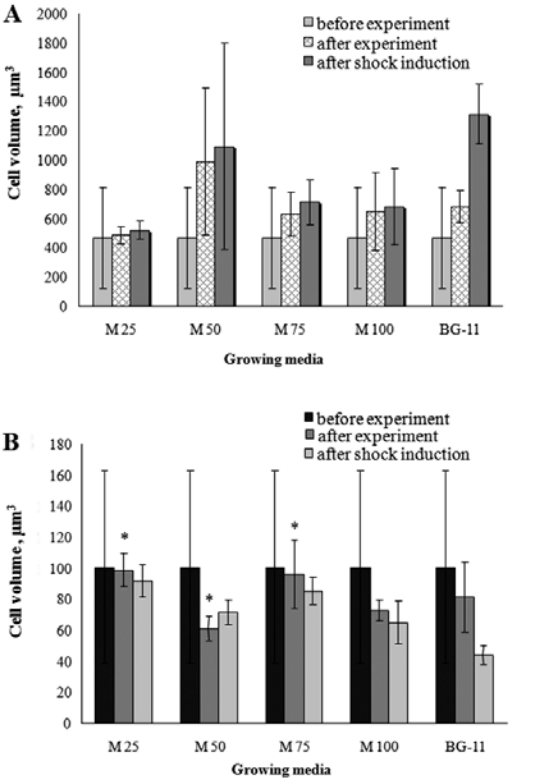

Fig. 5. Variations of *Chlorella* (A) and *Scenedesmus* (B) species cell size in wastewater at different concentrations

* indicate significant differences from the control (BG-11 medium) at $p < 0.05$.

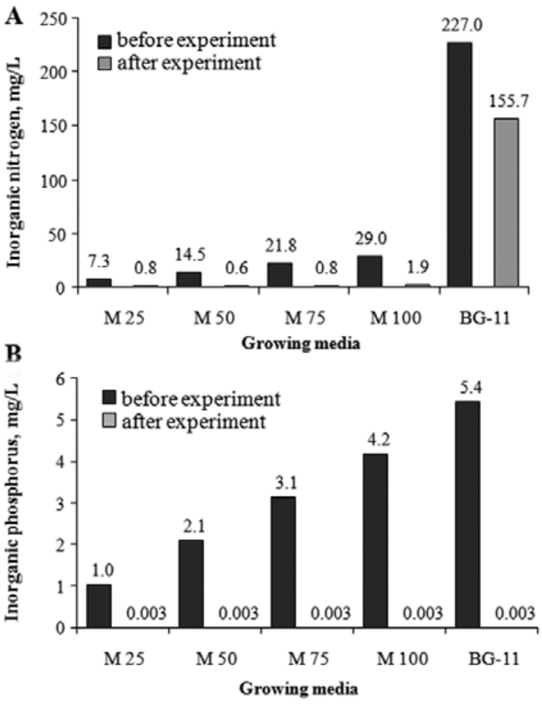

Fig. 6. The inorganic nitrogen (A) and inorganic phosphorus (B) concentrations in the media before and after the experiment

Contrary to *Chlorella*, the mean cell size of *Scenedesmus* diminished during the course of experiment and at nitrogen deprivation conditions without a clear tendency between the variants ($p > 0.05$) (Fig. 5). *Scenedesmus* built large cells and formed typical to species four-celled coenobia in diluted M25 wastewater. It is in agreement with O'Donnell *et al.* (2013) data that increase of nutrients concentration promoted more intense formation of one-celled *Scenedesmus* instead of four-cell coenobia.

2.4. Nutrients and CO_2 removal

Various species of *Chlorella* and *Scenedesmus* can remove from 80% to almost 100% of ammonia, nitrate and phosphate from treated wastewater (Pittman *et al.* 2011), however, the treatment efficiency varies depending on wastewater type and to some extent on climate peculiarities. Phosphorus is particularly difficult to remove from wastewater. Inorganic phosphorus (IP) concentration in the wastewater media variants of the experiment varied from 1.04 to 4.17 mg/L and was slightly higher in the control (BG-11 medium) (Fig. 6).

The experiment revealed that more than 99% of IP was eliminated by growing *Chlorella*/*Scenedesmus* culture within three weeks period (Fig. 7A). The elimination of inorganic nitrogen (IN) compounds from the wastewater was 88.6–96.4% indicating that the main limiting element for algae growth was IP. According to Makarevičienė *et al.* (2011), the removal efficiency of nitrogen and phosphorus of *Chlorella* sp. and *Scenedesmus* sp. reached relatively high values: TN – 91% (for both algae), TP – 94.7% and 95.6%, respectively. Similarly, Woertz *et al.* (2009) laboratory experiments showed nutrient removals of >98% for ammonium and >96% for phosphate with mixed culture microalgae grown on CO_2 supplemented primary wastewater effluent. *Chlorella ellipsoidea* eliminated 99% of nitrogen and 90% of phosphorus from the secondary effluent (Yang *et al.* 2011a). Johnson, Wen (2010) recorded slightly lower values for *Chlorella* sp. removing up to 90% of the TP and 79% of the TN contained within the wastewater.

Usually NH_4-N and PO_4-P predominate as the nutrients in wastewater, thus algae having high productivity and able to utilize these resources are ideal species for the treatment and biomass production (Park *et al.* 2011). IN concentration in the wastewater variants of the experiment media varied from 7.3 to 29.0 mg/L with dominating ammonium (Fig. 6). The concentration of IN in the control media was about ten times higher with domination of nitrate. It should be noted that ammonium from wastewater was eliminated much easier by *Chlorella*/*Scenedesmus* culture compared to nitrates showing that cultures are suitable for application in wastewater treatment. Makarevičienė *et al.* (2011) recorded the best *Chlorella* and *Scenedesmus* biomass productivity using urea as a nitrogen source or modified growing medium BG-11 with decreased concentration of $NaNO_3$. Chaichalerm *et al.* (2012) showed the lowest biomass yield of six chlorophyte species in the BG-11 medium compared to other three tested media (N-8, Kuhl, 3NBBM). They concluded that BG-11 medium has unbalanced concentrations of IN and IP, e.g. nitrogen concentration is high and phosphorous concentration is too low. Conversely, Phukan *et al.* (2011) obtained the highest biomass yield of *Chlorella* sp. (824 mg/L) in BG-11 medium compared to the other tested media (Basal, BBM, Chu-13). Li *et al.* (2008) experimentally showed that nitrate was the most favourable nitrogen source for *Neochloris oleoabundans* among the three tested nitrogen compounds, i.e. sodium nitrate, urea and ammonium bicarbonate. Even the same genus species have different preference for the nutrients. Cha *et al.* (2011) observed different growth pattern of *Chlorella vulgaris* (strain UMT-M1) and *C. sorokiniana* (strain KS-MB2) cultures at various nitrate concentrations.

The results of our experiment showed that the mixture of the culture is suitable for the effective elimination of nutrients from the wastewater. It is in agreement with Bhatnagar *et al.* (2011) that algal consortia grown in wastewater offer high production of renewable biomass for various applications, because in consortia the substantial loss of population of one algae may be compensated by the growth of the other. Chinnasamy *et al.* (2010) demonstrated that a consortium of 15 native algal isolates removed >96% of nutrients in treated wastewater.

Microalgae contain approximately 50% of carbon dry weight, thus about 1.8 kg of CO_2 is required to generate 1 kg of algal biomass (Yang *et al*. 2011b). *Chlorella*/*Scenedesmus* culture was tested for CO_2 elimination by evaluating biomass increase after three weeks of incubation. The mixture of culture growing on the wastewater incorporated from atmosphere into their biomass on average up to 1.37 g of CO_2/L per day without any significant differences between wastewater concentrations ($p > 0.05$). The values were up to two times lower if compare with the control ($p < 0.05$) (Fig. 7B). Usually room air contains 0.04% of CO_2 (Cheng *et al*. 2006). The experiments of the other researchers indicate that elevated concentrations of CO_2 may enhance the algae biomass yields by several times (Olaizola *et al.* 2004; Ota *et al.* 2009; Yoo *et al.* 2010; Jiang *et al*. 2011). Chinnasamy *et al.* (2009) found that *C. vulgaris* culture fixed 18.3 and 38.4 mg CO_2/L per day at ambient and elevated CO_2 (6%) levels.

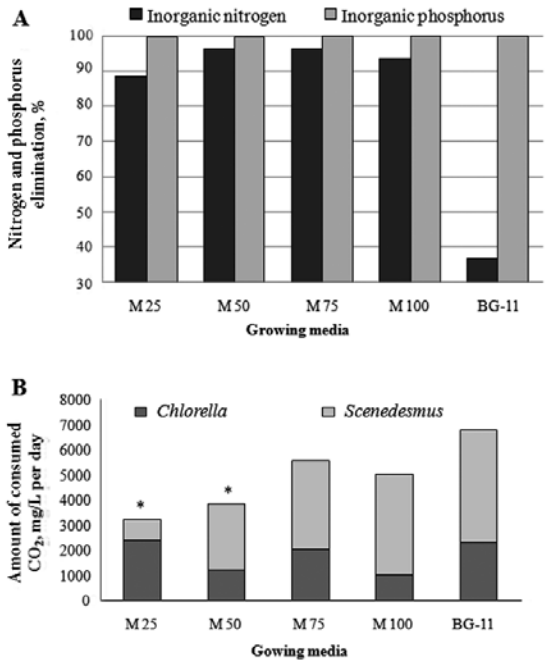

Fig. 7. Elimination of inorganic nitrogen and inorganic phosphorus (A) and the average amount of CO_2 incorporated into algae biomass via photosynthesis (B) at the different concentrations of wastewater

* indicate significant differences from the control (BG-11 medium) at $p < 0.05$.

2.5. Oil accumulation in the algae cells

Stockenreiter *et al.* (2013) found a clear correlation between light use and lipid production in functional diverse algae communities. It is considered as a powerful and cost-effective way to improve biofuel production. Nitrogen starvation was shown as one of the important factors to start oil accumulation by algae (Rodolfi *et al.* 2009; Brennan, Owende 2010; Deng *et al.* 2011; Pruvost *et al.* 2011). However, Stephenson *et al.* (2010) found that the maximal triacylglyceride productivity in *Chlorella vulgaris* was achieved by allowing the cells to deplete the nitrogen naturally instead of transferring cells to a medium without nitrogen. During our experiment period *Chlorella*/*Scenedesmus* culture reached the stationary phase in all variants of media, however, the nitrogen deprivation conditions were not achieved. The oil accumulation in *Chlorella* and *Scenedesmus* cells was additionally studied after inoculation of the algae biomass into nitrogen starvation conditions.

Under the nitrogen limitation, the growth of algae culture mixture from high concentration wastewater variants was suppressed more compared to diluted variants (Fig. 8). The experiment showed that *Chlorella* species is less susceptible to the N limitation than *Scenedesmus*. In the control variant *Chlorella* biomass increased, whereas *Scenedesmus* diminished. Nitrogen starvation might be not the best method to increase oil accumulation in the cells of particular algae, but application of high light or salt shock may give a positive result. It is also difficult to apply nitrogen starvation conditions at large operational scale to algae biomass grown in wastewater treatment plant.

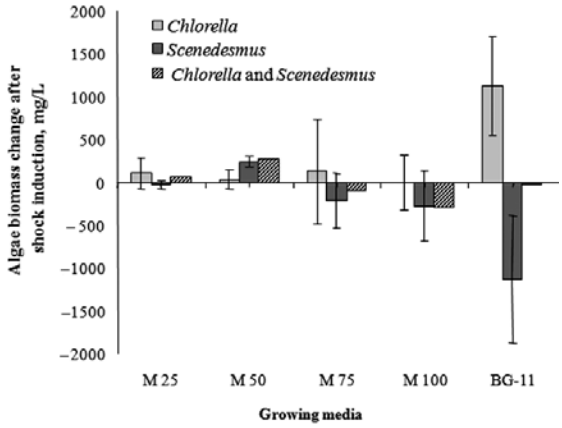

Fig. 8. Biomass yield of *Chlorella* and *Scenedesmus* species in wastewater at different concentrations after three days under nitrogen starvation conditions

Conclusions

1. *Chlorella/Scenedesmus* algae culture effectively eliminated inorganic phosphorus from the wastewater by removing up to 99.7–99.9% and inorganic nitrogen by 88.6 to 96.4% of the initial concentrations after three weeks of growth. Nitrogen effectively was eliminated in the form of ammonium, which predominated in the wastewater. Nitrate elimination was less effective.

2. The maximum biomass of algal culture consortium up to 3.04 g/L was in the concentrated wastewater. Its dilution almost twice reduced algal biomass.

3. The intensity of *Chlorella* algae growth was higher in the more diluted, while *Scenedesmus* algae – in the concentrated wastewater solutions. The consortia of *Chlorella/Scenedesmus* algae treat the wastewater more effectively than the single species, since the chemical composition of wastewater is highly variable over the time.

4. *Chlorella/Scenedesmus* algae culture accumulated on average 0.65–1.37 g of CO_2/L per day in their biomass.

5. The tested *Chlorella* and *Scenedesmus* algae have no ability to accumulate oil in the cells under nitrogen starvation conditions.

6. *Chlorella/Scenedesmus* algae consortium is a promising tool for elimination of nitrogen and phosphorus compounds from local wastewater resources as well as for diminishing CO_2 in the atmosphere under climatic conditions specific to Lithuania.

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