

## Determining Optimal Growth Conditions for the Highest Biomass Microalgae Species in Lithuanian Part of the Curonian Lagoon for further Cultivation

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**ABSTRACT:** Anthropogenic eutrophication conditioned frequent and more pronounced blooms of toxic algae and cyanobacteria in the fresh and saline aquatic ecosystems all world around. However, blooming species could serve as a possible resource instead of a threat by exploiting them to remediate nutrients. The optimised use of algae and cyanobacteria may provide the environmental service together with economic benefit that could be obtained from their biomass. The aim of this study was to identify microalgae species from Curonian Lagoon that could be prospective candidates for cultivation in Lithuania, and statistically determine how the accumulation of their biomass correlated with the changes in environmental conditions, using five years monitoring data. Five species, frequently acquiring a much larger biomass (up to 70 mg/L) over other species, were identified: *Aphanizomenon flos-aquae*, *Planktothrix agardhii*, *Actinocyclus normanii* f. *subsalsus*, *Diatoma tenuis* and *Stephanodiscus rotula*. The optimal cultivation conditions were proposed based on results of the analysis and the potential their biomass applications are discussed.

**Key words:** Curonian Lagoon, Microalgae, Environmental condition, Hydrochemistry, Nutrients

### INTRODUCTION

Lagoon is one of the most interesting part of a coastal zone for investigation due to their valuable nature (Gönenç and Wolflin 2005). However, the increase of anthropogenic pressures in coastal lagoons makes these areas very sensitive to deterioration. Curonian Lagoon is the largest coastal lagoon in the Baltic Sea with the dominated freshwater discharges from the Nemunas river. It is a shallow, mostly freshwater, highly eutrophic semi-enclosed estuary with prolonged summer phytoplankton blooms caused by the long-term increases in nutrients. Issues related to harmful algae blooms (Pilkaitytė, 2007; Pilkaitytė and Razinkovas, 2007; Razinkovas *et al.*, 2008), odor and cyanotoxins (Paldavičienė *et al.*, 2009) as well as input of the nutrients into the Baltic Sea (Wasmund *et al.*, 2001; Stepanauskas *et al.*, 2002; Gasiūnaitė *et al.*, 2005) are still under solution. To date, no assessment has been made of the potential

economic feasibility of the Curonian Lagoon algae. The European Union requires a reduction of nitrogen and phosphorus to the Baltic Sea via Water Framework Directive and Baltic Sea Action Plan (HELCOM, 2007; Schernewski *et al.*, 2009). It was calculated that control of phosphorus would cost 0.21–0.43 and of both elements – about 3.1 billion Euros (Schindler, 2012). Odlare *et al.* (2011) showed that nutrients reduction in eutrophic inland lake may be successful applying cultivation of indigenous algae under optimized conditions. The Curonian Lagoon inorganic nutrients-rich waters can theoretically be used as a growth medium for microalgae. Gröndahl (2009) proposed modified oil booms as a device for collecting toxic cyanobacteria *Nodularia spumigena* surface blooms in the Baltic Sea. The one boom unit can harvest up to 700 kg dry weight of cyanobacteria per hour at high bloom conditions (Pechsiri *et al.*,

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2014). The collected biomass reduced nutrient concentrations in the water and has been proposed as a raw material for biogas production. Indigenous algae species thriving in the hypertrophic water ecosystems may be likely candidates in the wastewater treatment (Brennan and Owende, 2010). However, a highly competitive species are required for cultivation, otherwise they will be outgrown by other microalgae. Cultivation of dominants from the local water reservoirs could be a solution. Being native species, they also do not pose a danger in case of any leak into the natural water systems (Wilkie *et al.*, 2011). On the other hand, the costs of the ecological service should be compensated from the benefits obtained from the cultured and harvested algae. Their biomass can be used as a substrate for bioethanol, biogas and biodiesel, as a fertilizer, as a food supplement for animals or simply burned to generate heat and electricity (Pulz and Gross 2004; Ferrell and Sarisky-Reed, 2010; Amaro *et al.*, 2011; Chaichalerm *et al.*, 2011; Odlare *et al.*, 2011). However, biofuels are still not commercially viable at today's fossil fuel prices (Amaro *et al.*, 2011; Davis *et al.*, 2011). Combining nutrient removal from wastewater with biofuel production may reduce the energy, nutrient, freshwater resource cost as well as diminish the greenhouse gas emissions (Park *et al.*, 2011; Pittman *et al.*, 2011). As of 2013, most of the microalgae cultivation is located in the U.S., though smaller markets are also expected to emerge in Europe and Asia (Microalgae, 2013; SBI Reports, 2010). According to Energy policy (Reijnders, 2006), introducing a new type of industry in the local market, such as algae cultivation, will not only serve as a local energy source, but also create new jobs, which is very imperative for EU28 with a high average unemployment rate - 11.0% in September 2013 (Eurostat, 2013).

Current study focuses on the possibilities of application of native microalgae species for differently combined uses in Lithuania. The purpose of this research was to identify the dominant microalgae species of the Curonian Lagoon which would be the best possible candidates for the cultivation in the local area. The second aim was to identify environmental conditions that are the most important for an efficient algae growth and lead to the accumulation of biomass of these species.

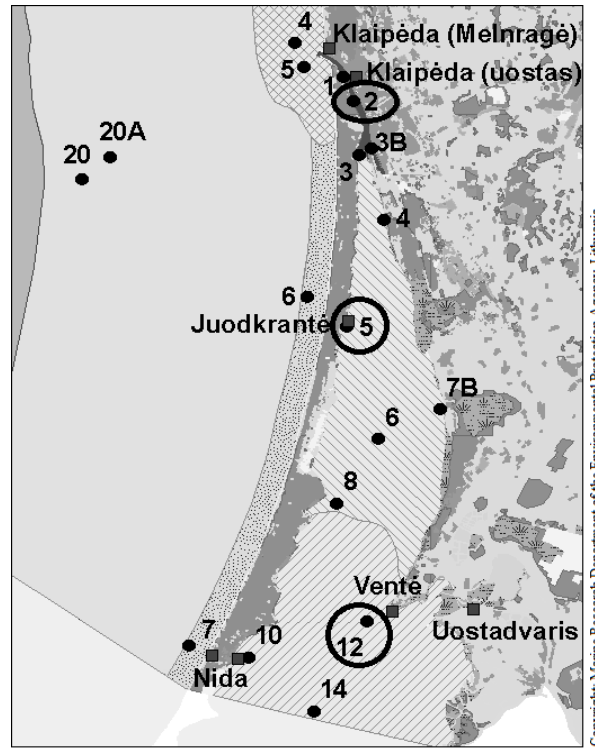
## MATERIALS & METHODS

Monitoring data on the microalgae biomass variations and the changes of water physical and chemical properties, obtained from the Marine Research Department (MRD) of the Environmental Protection Agency of the Republic of Lithuania, were used as a starting material for statistical analysis used to determine the optimal growing conditions for microalgae. The samples for the determination of microalgae species composition as well as water physical and chemical conditions, were collected during the course of six years, 2005-2010 inclusive. The water samples were taken with a Ruttner water sampler at 14 monitoring stations of the Curonian Lagoon: monthly at stations 2, 5 and 12; 3-4 times per year – at the rest stations (Fig. 1). The data (water temperature, current water level) from hydrometeorological posts located in the northern, central and southern parts of the lagoon were also analysed.

Multiple chemical, physical properties of water and other parameters were monitored:

- hydrochemical properties (pH, dissolved O<sub>2</sub> concentration, biological oxygen demand of water for 7 days (BOD<sub>7</sub>), NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub> concentrations, overall nitrogen and phosphorus concentrations, SiO<sub>2</sub> concentration, salinity);
- physical properties (water temperature, water level);
- Ecotoxicological properties (presence of oil products, detergents, Hg, Pb, Cu, Cd, Zn, Cr, Ni, and also 16 different organic pesticides and other pollutants (e.g. pentachlorophenol (PCP), benzo[k]fluoranthene, benzo[a]pyrene));
- Other factors such as wind direction, air temperature.

Phytoplankton samples were taken from the water surface layer (0-0.5 m depth) monthly at three monitoring stations 2, 5 and 12 (Fig. 1). Phytoplankton samples were treated according to the standard HELCOM methods using the light inverted microscope (magnification x100 and x400) and applying the Utermöhl technique (1958). The abundance of phytoplankton species was expressed in terms of cells, colonies, coenobiums and 100 µm filaments according to HELCOM Combine-manual (HELCOM, 2003); the best fitting geometric shape and matching equation was used for phytoplankton species biomass calculation (Olenina *et al.*, 2006). The microalgae biomass in the samples was estimated by multiplying species abundance by the mean cell volume, according to



- Monitoring station
- Hydrometeorological post

Fig. 1. Monitoring stations at the Curonian Lagoon

Table 1. Average nutrients concentrations and other hydrochemical parameters in the Curonian Lagoon across three monitoring stations in years 2005–2010. Standard deviations are shown in the brackets ( $\pm$ )

	BOD <sub>7</sub>	pH	NO <sub>3</sub>	NH <sub>4</sub>	PO <sub>4</sub>	TN	TP	Salinity
	(mg/L)		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(ppt)
<b>Station 2</b>	4.35	8.75	0.54	0.08	0.02	1.35	0.07	5.17
	( $\pm 1.74$ )	( $\pm 0.49$ )	( $\pm 0.63$ )	( $\pm 0.07$ )	( $\pm 0.02$ )	( $\pm 0.61$ )	( $\pm 0.02$ )	( $\pm 0.92$ )
<b>Station 5</b>	4.95	8.78	0.51	0.08	0.02	1.34	0.08	1.11
	( $\pm 1.90$ )	( $\pm 0.48$ )	( $\pm 0.57$ )	( $\pm 0.08$ )	( $\pm 0.02$ )	( $\pm 0.55$ )	( $\pm 0.04$ )	( $\pm 1.18$ )
<b>Station 12</b>	5.14	8.58	0.90	0.09	0.03	1.78	0.09	0.08
	( $\pm 2.09$ )	( $\pm 0.41$ )	( $\pm 0.75$ )	( $\pm 0.07$ )	( $\pm 0.02$ )	( $\pm 0.67$ )	( $\pm 0.03$ )	( $\pm 0.08$ )
<b>MAC</b>	6.00	6-9	2.30	1.00	0.40	2.50	0.10	

MAC - Maximum Allowable Concentration (Ministry of Environment of the Republic of Lithuania, 2005)

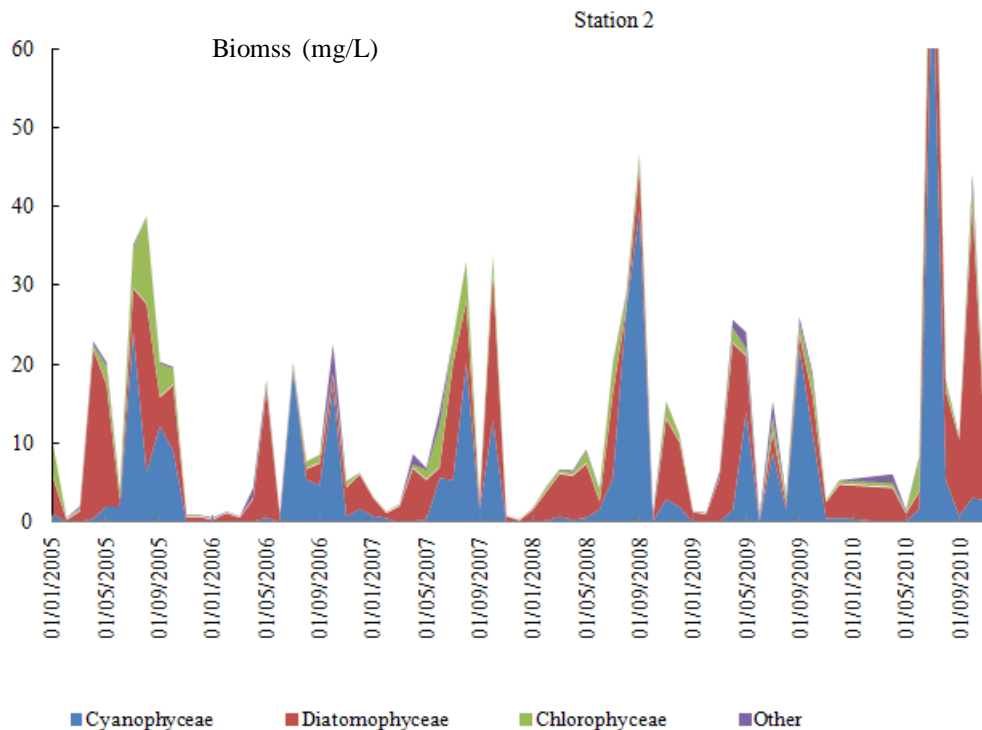
stereometrical formulas (Olrik *et al.*, 1988). The relationship between the microalgae biomass and the environmental conditions was evaluated using a statistical method known as the Canonical Correspondence Analysis to determine which environmental conditions have the strongest influence on the microalgae biomass. The data were log-transformed prior to analysis. In a case of the biomass data, “1” was added to all the values to eliminate zeroes, and then the data were log-transformed. The bi-plots show whether there is a relationship between the independent variables (environmental conditions) and dependent variables (species’ biomass): the closer the lines are to each other, the stronger is the correlation between the two variables. Opposite directions of two show a negative correlation.

## RESULTS & DISCUSSION

Curonian Lagoon is an estuary whose salinity changes from freshwater to brackish from south to north. The amounts of inorganic and total

nutrients (nitrogen and phosphorus) in water were mostly uniform across the monitoring stations, with slightly elevated levels observed at Station 12, the closest to the river Nemunas outflow (Table 1). Other parameters were also similar between the stations, only the water salinity varied from 0.03–0.47‰ (average 0.08) on Station 12 to 2.92–6.77‰ (average 5.17‰) on Station 2.

On a seasonal basis, the nutrient concentrations were the highest in spring and related with the river inflow from the catchment area as well as re-suspension from the sediments of the Curonian Lagoon. Kowalczevska-Madura *et al.* (2010) showed that a phosphorus release from bottom sediments in shallow zones may reach an average annual release up to  $8.46 \text{ mg m}^{-2} \text{ d}^{-1}$ . Nutrient concentrations in the Curonian Lagoon decreased from early summer to mid-autumn, possibly due to the nutrient uptake by the phytoplankton. The nutrients concentrations in all cases were below the Maximum Allowable Concentration (MAC).



**Fig. 2.** Annual microalgae biomass variation at three monitoring stations (January 2005 – November, 2010)

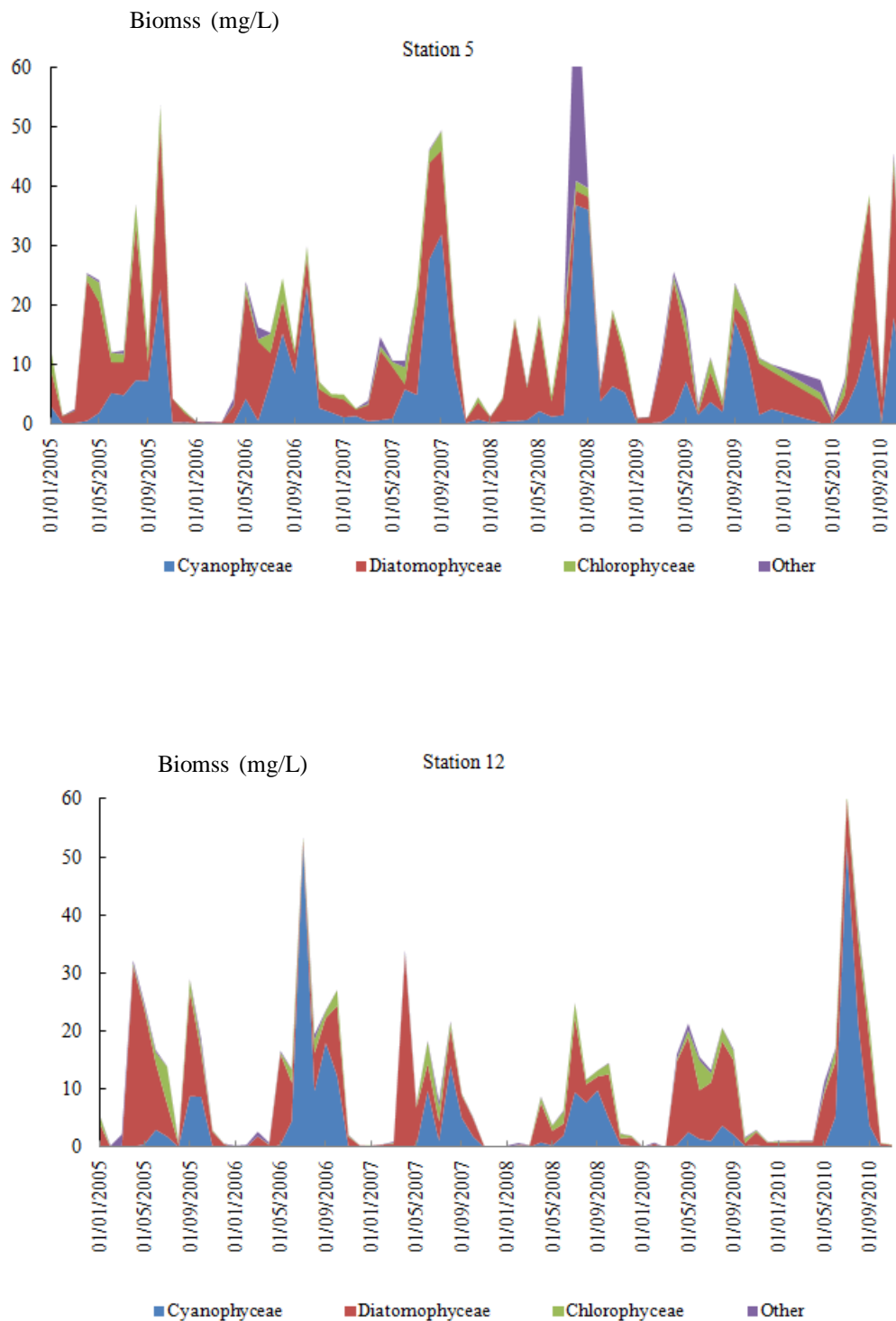
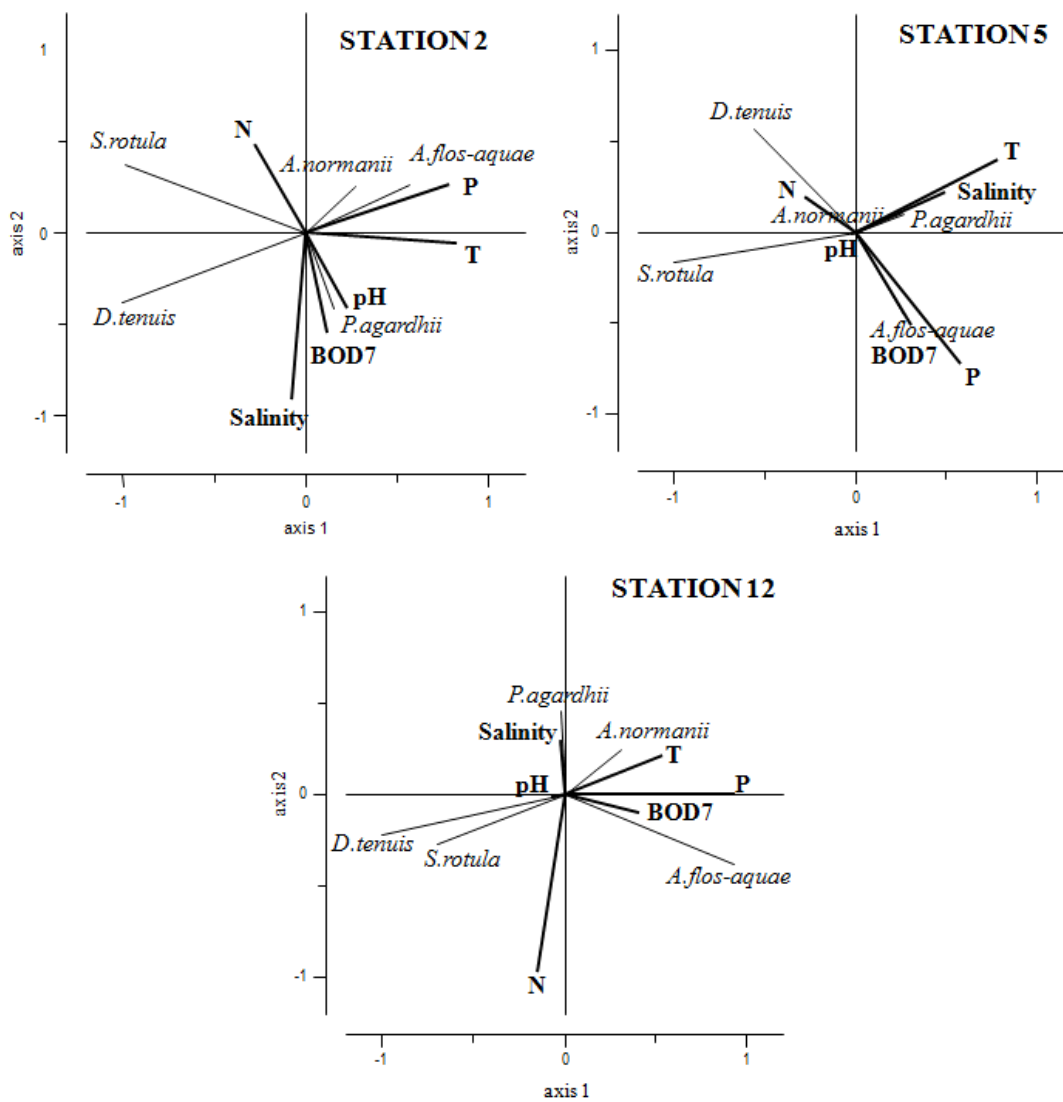


Fig. 2. Annual microalgae biomass variation at three monitoring stations (January 2005 – November, 2010)



**Fig. 3. Ordination biplots based on the microalgae biomass-environmental conditions correlation analysis. The location of bi-plots is based on the relationship of microalgae biomass and environmental conditions**

Thin lines show the species, thick lines indicate the environmental variables. BOD7 – biological oxygen demand for 7 days; N – total nitrogen concentration, P– total phosphorus concentration. T-temperature, pH – H+ concentration and Salinity-NaCl concentration.

Combined data such as total nitrogen and phosphorus concentrations, BOD<sub>7</sub>, pH, water temperature and salinity factors were selected for determining the optimal condition for most abundant microalgae species biomass accumulation. PCA analysis demonstrated that a salinity was the most important variable shaping dominating species' biomass at Station 2, whereas phosphorus and temperature – at Station five and nitrogen compounds – at Station 12 (Fig. 3).

Fig. 2 shows the regular annual variations of the microalgae biomass in three monitoring stations of the Curonian Lagoon during the time of 2005–2010. Total algae biomass increased between April and late September, reaching the maximum levels in August or September (up to 95.8 mg/L). Still, the higher maximum biomass values were recorded in the Lagoon for the previous period: from 98.74 in July–September 1999 to 152.63 mg L<sup>-1</sup> in August 2001 (Jaanus *et al.*, 2011). In winter months 2005–2010, from December to early March, microalgae biomass was much lower – from 0.13 to 13.22 mg/L.

The dominant microalgae classes within an annual cycle were *Cyanophyceae*, *Diatomophyceae* and *Chlorophyceae*. Diatoms were dominant in cold months, whereas cyanobacteria and green algae in the warmest months. During the microalgae biomass peak, usually few or even single species dominate comprising as much as 40 to 78% of the total microalgae biomass in a water sample at a given time.

From overall 526 microalgae species recorded in the Curonian Lagoon, five species were selected based on high biomass and the length period of their presence. The biomass of a single cyanobacteria species was highest if compare to the other microalgae species. Specifically *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet & Flahault (biomass up to 68.8 mg/L in July) and *Planktothrix agardhii* Gomont Anagnostidis & Komárek (up to 38.8 mg/L in August) were the most frequently high biomass reaching species in the Curonian Lagoon. According to Jaanus *et al.* (2011), *A. flos-aquae* was replaced by *P. agardhii* as the dominant cyanobacteria in the 2000s. *Actinocyclus normanii* f. *subsalsus* (Juhlin-Dannfelt) Hustedt (up to 50.5 mg/L in October), *Diatoma tenuis* C.A. Agardh (up to 10.2 mg/L in May) and *Stephanodiscus rotula* (Kützing) Hendeby (up to 24.2 mg/L in April) were dominating from the class *Diatomophyceae*. *D. tenuis* and *S. rotula* were

early spring, cold water blooming species, while all other algae and cyanobacteria species were accumulating largest biomass during the late summer and early autumn months.

The variation of the biomass of five microalgae and cyanobacteria species was compared to the data on the environmental conditions in the relevant monitoring years using the Canonical Correspondence Analysis (Fig. 3). Our study, showed a weak correlation of *Aphanizomenon flos-aquae* and *Planktothrix agardhii* with water temperature (Fig. 3), despite earlier results by Jaanus *et al.* (2011), indicated that a water temperature had the strongest impact on the summer phytoplankton community structure in combination with salinity in the Curonian Lagoon. The maximum replication rates for both cyanobacteria occurred at temperature over 20°C (Reinolds, 2006). Since *Aphanizomenon* and *Planktothrix* species prefer higher temperatures and the blooming occurred at water temperatures starting at 15°C and higher, it is better to cultivate them in shallower ponds, reservoirs or photobioreactors. Due to optimal growth temperatures, the growth period of these cyanobacteria will be short in temperate zone latitudes and restricted to summer, that diminishes their commercial applicability.

However, it seems that *Planktothrix* are less sensitive to the low temperatures than *Aphanizomenon*, as Lange (2011) listed *P. agardhii* among the dominants in the Curonian Lagoon during the winter time with the biomass of 0.01 mg/L. High biomass forming cyanobacteria showed the different response to the concentration of phosphorus: *A. flos-aquae* showed positive correlation, whereas *P. agardhii* was indifferent (Fig. 3), which proved earlier results by Pilkaityte and Razinkovas (2006), who found that phosphate concentration, temperature and wind velocity are the main factors regulating dominance of *Aphanizomenon flos-aquae* in the Curonian Lagoon. According to Dolman *et al.* (2012), *P. agardhii* attained the highest bio-volumes in lakes with the highest joint NP enrichment scores and showed a particularly strong response to phosphorus. Inorganic nutrient concentrations in hypertrophic Curonian lagoon are too high to limit total phytoplankton biomass, which is controlled mostly by ambient physical factors (Pilkaityte and Razinkovas, 2007). It may explain the weak *P.*

*agardhii* correlation with the phosphorus concentration.

Both filamentous cyanobacteria positively correlated with  $BOD_7$  levels and negatively correlated or were indifferent to variations of nitrogen concentration (Fig. 3). The lowering of nitrogen to phosphorus ratio in the environment promotes nitrogen fixing cyanobacteria growth and blooms (Vahtera *et al.*, 2007). *A. flos-aquae* reached high bio-volumes in two German lakes only when TN:TP<16:1, while the growth of *P. agardhii* seemed to be independent of seasonal variation of the TN:TP ratio (Teubner *et al.*, 1999). Donald *et al.* (2011) showed that  $N_2$ -fixing cyanobacteria exhibited little response to added nitrogen, but non-heterocystous cyanobacteria favored from increased concentrations of  $NH_4^+$  and urea. It might be that prevailing ammonium concentrations in the Curonian Lagoon favored successful *Planktothrix* development, while, under N limitation conditions, *Aphanizomenon* took the advantage. Nevertheless the both species occur and form highest biomass in low-N-high-P lakes, *P. agardhii* prefer the higher levels of enrichment with nutrients than *A. flos-aquae* (Dolman *et al.*, 2012). These facts mean that both *A. flos-aquae* and *P. agardhii* can be successfully cultivated in eutrophic waters (e.g., in semi-permeable bags), such as water from the Curonian Lagoon or water in the wastewater treatment plants. However, if *A. flos-aquae* is going to be cultivated in water taken from natural sources with the other microalgae present, nitrogen concentration has to be lowered to make them competitive over other species. Among two cyanobacteria, only *Planktothrix agardhii* showed clear positive correlation with the salinity (Fig. 3).

Fayolle *et al.* (2008) found that *P. agardhii* form high density populations in mixo-mesohaline waters and disappeared totally when the salinity exceeds, 12 g/L. They concluded that species is particularly sensitive to the high salinity. Nikulina and Gubelit (2011) observed that, with the rise in salinity, the proportion of *P. agardhii* in cyanobacteria biomass decreased, while nitrogen-fixing species increased in the Neva estuary. Ibelings (1992) recorded that *P. agardhii* dominate at exceptionally warm and stable summers with low mixing over extended periods. Contrarily, Reynolds *et al.* (2002) stated that species are quite tolerant to conditions of high turbulence. Low stability of

the water column in the Curonian Lagoon due to saline waters plumes from the Baltic Sea and freshwater discharge from Nemunas river, shallowness of the estuary exposed to wind mixing as well as low water transparency may be variables that favoured successful growth of *P. agardhii* during the last decade.

Jaanus *et al.* (2011) found that salinity mainly explains the variation of diatoms biomass in the Curonian Lagoon. However, correspondence analysis did not reveal any clear relationship between salinity and particular high biomass of diatom species (Fig. 3). Hällfors (2004) describe *Stephanodiscus rotula* as marine species that compensatory increase was seen at higher salinities. In the Curonian Lagoon, *S. rotula* formed quite dense populations in low salinity waters (Fig 3). *Actinocyclus normanii* f. *subsalsus* is a halotolerant species, however, its biomass decreases with increase of the salinity indicating that species has adapted to lower salinity inland waters (Sánchez *et al.*, 2003 Sims *et al.*, 2006). Eutrophication and turbulent conditions were defined as main factors determining species' distribution (Kiss *et al.*, 1990; Stoermer and Julius, 2003). Shallow, wind well mixed hypertrophic waters of the Curonian Lagoon are a favorable media for these diatoms to grow. Pilkaitytė and Razinkovas (2007) found that nitrogen addition favoured centric diatom species development in the Curonian Lagoon, this explains high biomasses of *Stephanodiscus* and *Actinocyclus* species in highly eutrophicated water body. According to the current analysis, *S. rotula* was positively influenced by the concentration of nitrogen and negatively – by the increase of the temperature in the case of all three stations (Fig. 3).

This means, that these diatoms are low temperature-tolerant organisms which possibly will be outgrown at higher temperatures by faster-growing species. *D. tenuis* is also cold-tolerant species; however, it is dominant at slightly higher temperatures than *S. rotula*, typically during mid to late spring. *A. normanii* f. *subsalsus*, unlike *D. tenuis* and *S. rotula*, is a warm stenothermic diatom species, also positively influenced by phosphorus concentrations (Fig. 3).

Since both cold water and warm water species are present among blooms forming microalgae species in the Curonian Lagoon, it is theoretically possible to cultivate microalgae under local conditions throughout the year, which is useful for



Lithuania with its late spring and a short summer. Although the biomass yields during the colder months will be much smaller.

*Stephanodiscus* and *Diatoma* species showed negative whereas *Actinocyclus* slight positive correlation with BOD<sub>7</sub>, thus probably only latter diatom species may be successfully introduced into wastewater treatment system. Diatoms can be used for the nitrogen compounds removal when the runoff from agricultural land dominates, but their success will be decided by the SiO<sub>2</sub> concentration in the water (a substance important for their skeleton construction).

Cyanobacteria and diatoms showed prolonged domination in the Curonian lagoon and the factors driving their growth were analysed for use these species in remediation of nutrients. On the other hand, biomass of selected species may be used for different applications that allow to cover some of the costs of ecological service. Microalgae have enormous potential for diverse biotechnological applications and are currently viewed as a prospective source of biofuel (Hempel and Maier, 2012). Odlare *et al.* (2011) successful study of indigenous algae from eutrophic lake cultivation demonstrated that algae selection and their cultivation in optimized conditions can seriously be considered as a final step to further microalgae-based biorefinery.

Cyanobacteria might be important for nutrients removal, especially phosphorus, from highly eutrophic water bodies or wastewaters. Genetic engineering of photosynthetic capability and capacity of cyanobacteria together with high species diversity and proliferation under on-going eutrophication and climate warming worldwide make them attractive candidates for use in bio-industrial applications and remediation (Deng and Coleman, 1999; Pulz and Gross, 2004; Ducat *et al.*, 2011). Vargas *et al.* (1998) found that the lipids content ranged between 8–12% DW among twelve studied N<sub>2</sub>-fixing cyanobacteria. Quintana *et al.* (2011) reviewed the cyanobacteria biomass as a feedstock for various organic (ethanol, CH<sub>4</sub> and biodiesel), inorganic (H<sub>2</sub>) and electricity biofuels. However, there are still many issues to be solved to make biofuel production from cyanobacteria economically competitive with traditional fossil fuel sources (Hu *et al.* 2008). However, hydrogen and ethanol production from a number of cyanobacteria genera under different culture conditions was

proved to be significantly more efficient than those produced from plants (Quintana *et al.*, 2011; Chadar *et al.*, 2012).

The industry for cyanobacterial applications is developing towards higher added value products (isoprene, sugars, bioactive compounds, antioxidative substances, phycocyanin, cyanobactins, compounds with antimalarial, antitumor, and multidrug reversing activities) that may be applied or were already introduced in medicine, cosmetic or food industries (Eriksen, 2008; Sivonen *et al.*, 2010; Ducat *et al.*, 2011; Chadar *et al.*, 2012).

Bloom forming *Aphanizomenon flos-aquae* are one of the most important cyanobacteria applied in biotechnology. Species are commonly used as human nutrition supplements (Pulz and Gross, 2004; Mollenhauer, 2008). Capsules with *A. flos-aquae* containing 20 antioxidants, 68 minerals and trace elements, all amino acids, B group vitamins and essential enzymes and gained popularity in a number of countries such as USA, Germany, Canada, Korea, Japan, Australia (Benedetti *et al.*, 2004; Chadar *et al.*, 2012).

However, *A. flos-aquae* have to be cultivated in closed bioreactors under strictly controlled conditions to achieve high culture purity and species toxicity should be studied first. Diatoms are among the most productive and environmentally flexible eukaryotic microalgae on the planet (Hildebrand *et al.*, 2012), probably capable of withstanding highly changeable environmental variables in the Curonian Lagoon. According to Hildebrand *et al.* (2012), 60% of studied algae strains having high growth rates and lipid yields, tolerating harsh environmental conditions were diatoms. Being environmentally adaptable and trophically flexible, diatoms out-compete other classes of algae in growth and have superior nutrient acquisition/storage and photosynthetic efficiency. Diatoms can accumulate lipid equivalently in greater amounts than other algae classes and can rapidly induce triacylglycerol under Si limitation (Hildebrand *et al.*, 2012).

After the study of *Amphora*, *Chaetoceros*, *Cyclotella*, *Nitzshia*, *Phaeodactylum*, *Thalassiosira* diatom genera, Graham *et al.* (2012) found that freshwater centric diatoms seem to be the most promising source for biofuel. They concluded that algae biomass production can outperform soybean-based biodiesel production even in a temperate climate if the algae are grown in

photobioreactor within a warmed by waste-generated heat greenhouse. Moreover, Odlare *et al.* (2011) and Krustok *et al.* (2013) research demonstrated that even small amounts of algae added to conventional biogas production plant substrates could significantly enhance the biogas production from waste products in the digester. Nehrenheim *et al.* (2010) found that a cultivation of the indigenous algae in an open system has high potential and could provide local and global protection of the environment. Pienkos and Darzins (2009) envisioned that algae biofuels development will have a regional character, and there will be a need to develop strains optimally adapted to different environments. Hence, naturally growing diatoms under bloom conditions are of particular importance. Physiological potential of diatoms to accumulate high proportions of oils that may represent a future source for fuel, long chain polyunsaturated fatty acids or eicosapentaenoic acid (Pulz and Gross, 2004; Khozin-Goldberg *et al.*, 2011). However, most of them are large size and slow growing species, thus the positive balance between oil accumulation and biomass accumulation should be studied first.

Extremely effective way of growing diatoms in controlled laboratory conditions was discovered by UK scientists who allow diatom shells to be mass-produced, harvested and mixed into paints, cosmetics and clothing to create colour-changing effects, or embedded into polymers to produce difficult-to-forge holograms (BIOPACT, 2007). Over the past several years a new interdisciplinary area – diatom bionanotechnology – emerged into research area with the applications of diatom products to biofuels, food, cosmetic and pharmaceutical industry (Gordon *et al.*, 2009).

Algae silica shells possess structural, mechanical, chemical and optical features that might be used as therapeutic agents, e.g. for the preparation of the ultra-high-purity and fraction-free silica capsules working as natural porous materials for drug delivery applications. Based on diatom morphology and mechanical and chemical properties Wee *et al.* (2005) proposed perspective applications in the future such as diatoms diatom frustules embed in a metal-film membrane, use them for pinpoint drug delivery or producing silica nanopowders. Hempel and Maier (2012) proposed using microalgae as solar-fuelled expression system for the production of recombinant proteins. They engineered the

diatom *Phaeodactylum tricornutum* to synthesize and secrete a human IgG antibody against the Hepatitis B virus surface protein. Diatoms are also widely used in aquaculture as a food for molluscs (Borowitzka, 1997).

## CONCLUSION

- Two cyanobacteria (*Aphanizomenon flos-aquae*, *Planktothrix agardhii*) and tree diatom species (*Actinocyclus normanii* f. *subsalsus*, *Diatoma tenuis* *Stephanodiscus rotula*) showed the highest biomass for the prolonged periods in the Curonian Lagoon.

- In general, high nutrient concentrations and temperature were main factors predetermining these species' prevalence over the other algae and may be successful key to using them for the remediation of nutrient rich waters or wastewaters. The other environment variables such as salinity, high turbidity and mixing activity play an accompanying role in the species' development.

- D. tenuis*, *S. rotula* will favour from high nitrogen concentrations and *A. flos-aquae*, *A. normanii* f. *subsalsus* – from high phosphorus levels. First two species are cold tolerant and the latter two warm water species, thus combining these algae and cyanobacteria into consortium it is a high probability to successful remediation of nutrients from the water.

- There are little data on accumulated biomass application of particular species for the practical use. But many research concerning other species of cyanobacteria and diatoms indicate a huge potential in the low value product market. Still a lot of investigations should be done to discover the potential of selected species to produce high value products.

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