Growth of Scenedesmus obliquus under artificial flue gas with a high sulphur concentration neutralized with oil shale ash

Lara Podkuiko\textsuperscript{a,b*}, Jüri Olt\textsuperscript{a}, and Timo Kikas\textsuperscript{a}

\textsuperscript{a} Institute of Technology, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 56/1, 51014 Tartu, Estonia
\textsuperscript{b} Department of Marine Systems, School of Science, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

Received 26 October 2016, revised 26 January 2017, accepted 27 January 2017, available online 6 April 2017

© 2017 Authors. This is an Open Access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/).

Abstract. Oil shale is the main energy resource in Estonia, which generates large amounts of CO\textsubscript{2} and waste oil shale ash. Flue gas from oil shale combustion can also contain large amounts of SO\textsubscript{2}. Microalgae can be used for biological sequestration of carbon from flue gas. In this research, green algae Scenedesmus obliquus were grown with 14% CO\textsubscript{2} in 1 L bioreactors. Sulphuric acid was added with a concentration of 500 ppm and 1000 ppm in order to imitate the dissolution of sulphur dioxide from flue gas into the growth medium. Oil shale ash was used to neutralize SO\textsubscript{2}. Biomass measurements of \textit{S. obliquus}, carried out every 24 hours for 7 days, were used as a proxy for carbon fixation. The biomass yields of the untreated control and of the treatments were similar (maximum yield 2.9, 3.1, and 3.9 g L\textsuperscript{-1} for the control, 500 ppm, and 1000 ppm treatment, respectively), suggesting that neither the sulphur nor the ash had an inhibitory effect on algal growth. In fact, the biomass yield was slightly higher in the treatments, which implies that minerals contained in waste ash could be utilized by algae. The calculated CO\textsubscript{2} fixation rate was 0.45 g L\textsuperscript{-1} d\textsuperscript{-1} for the control, and 0.62 and 0.83 g L\textsuperscript{-1} d\textsuperscript{-1} for 500 ppm and 1000 ppm treatment, respectively. Therefore, microalgae can be used for carbon sequestration from flue gas. Further research should be done in order to optimize the growth conditions and maximize carbon fixation.

Key words: microalgae, \textit{Scenedesmus obliquus}, oil shale ash, flue gas, CO\textsubscript{2} sequestration.

1. INTRODUCTION

Oil shale is the main energy resource used in Estonia with annual use approximately 15 million tonnes [1]. Oil shale is a low-grade fossil fuel with a mineral content of 40–50%; therefore approximately half of the oil shale used for generating electricity ends up as waste oil shale ash [2,3]. According to the report of Eesti Arengufond (Estonian Development Fund), 6.9 million tonnes of oil shale ash was generated in 2012 [4]. As with any fossil fuel, the combustion of oil shale generates high amounts of carbon dioxide: in 2014, 12.8 million tonnes of CO\textsubscript{2} was emitted by Eesti Energia (the largest energy provider in Estonia) [5]. Due to climate change concerns, measures are taken to mitigate the emissions of CO\textsubscript{2}. This can be achieved by decreasing energy consumption, increasing the proportion of renewable energy, and sequestering the carbon from flue gas. Sequestering methods vary depending on the storage time and quantity of the fixed CO\textsubscript{2}. In spite of the fact that CO\textsubscript{2} mitigation by biological systems is marginal in terms of storage time and global emission rates [6], it offers many advantages.

Although methods based on chemical (using metal oxides or aqueous amine solutions) and physical (e.g. geological sequestration) reactions often require special equipment, are energy-consuming and costly if scaled up for commercial use, and involve disposal problems...
[7,8], the end product of biological mitigation – biomass – can be refined into various products or converted into energy, thereby making it cost-efficient.

Agricultural and forest ecosystems are able to mitigate atmospheric CO2. However, autotrophic microalgae can be used to sequester carbon straight from flue gas. Autotrophic microalgae are microscopic, mostly unicellular photosynthetic organisms. Many microalgae species exhibit a higher photosynthesis rate than terrestrial plants and thus can fixate larger quantities of CO2 [9].

Algae have been shown to grow under an atmosphere with a CO2 concentration as high as 80% and 100% [10]. Flue gas contains on average 10–20% CO2 [11,12]. Flue gas from oil shale combustion approximately 12% CO2 (P. Rohumaa, personal communication). Additionally, bio-mitigation with microalgae can be combined with urban wastewater treatment to establish a cost-effective and sustainable biomass production [7,13].

However, there are some constraints in using microalgae to mitigate CO2 straight from flue gas. Firstly, the temperature of flue gas is usually 100–120 °C, which is unsuitable for algal growth. Therefore, the flue gas has to be cooled to an adequate temperature. This may be achieved with different heat-exchange systems. Secondly, flue gas contains additional gases such as sulphur and nitrogen oxides, which together with carbon dioxide decrease the pH of the growth medium to pH 2–3, and thereby inhibit algal growth.

It has been argued, however, that besides lowering the pH, NOx does not have a direct negative impact on microalgal growth because NO for example is transformed into NO2 in the culture medium and serves as a nitrogen source for microalgae [14,15]. On the other hand, high amounts of sulphur have been found to be toxic to many algal species [16]. Flue gas from oil shale combustion contains on average 550 ppm and maximally up to 1500 ppm of SO2 (P. Rohumaa, personal communication). For this reason the effect of NOx was not studied in the current study and the focus was placed on the possible inhibitory impact of SO2 on algae. Although SO2 emissions need to be reduced according to the set limits (for Estonia, 100 kt by 2010 and 10 kt by 2020) [17] and desulphurization units are being integrated with power plants, flue gas may still have quite high SO2 concentrations in regard to algae. In 2014, Eesti Energia emitted 24.2 kt of SO2 [5].

For pH adjustment of acidic conditions, NaOH and CaCO3 are widely used with microalgae. In this research, oil shale ash was used for pH adjustment. Oil shale ash, as a waste product of oil shale based energy generation, is readily available in Estonia. It is water-soluble and its pH is 12–13. Therefore, it can be used for the neutralization of acidic conditions caused by high CO2 and SO2 concentrations. Additionally, oil shale ash contains traces of Ca, Fe, K, Mg, etc. [18,19], which are important elements in algal nutrition. On the other hand, shale ash contains variable quantities of such metals and polyaromatic hydrocarbons that may, depending on their concentration, inhibit algal growth [20–22]. Oil shale fly ash eluate has been shown to be toxic to green algae 
Pseudokirchneriella subcapitata, while green algae Selenastrum capricornutum, used in Algaltoxkit F®, exhibited low toxicity to ash-heap leachates [23,24]. Although oil shale ash has been used for liming substrates of agricultural crops, to the best of our knowledge no research papers cover the use of oil shale ash for the cultivation of microalgae.

The aim of our study was to investigate the growth of the microalga Scenedesmus obliquus in conditions of high carbon dioxide supply and sulphur concentration. This species was chosen as a test organism because it has been reported to grow under 80% CO2 conditions with a maximum cell concentration of 10–20% CO2. Oil shale ash was used as a neutralizing agent and the content of four heavy metals in the biomass was analysed.

2. MATERIALS AND METHODS

2.1. Microalgae cultures and medium composition

Cultures of S. obliquus were obtained from the University of Göttingen (Georg-August-Universität Göttingen Algae Collection). The cultures were pre-grown in Bold Basal Medium (BBM) [25] containing NaNO3 (2.5 mg L–1), CaCl2·2H2O (0.17 mg L–1), MgSO4·7H2O (0.30 mg L–1), KH2PO4 (0.43 mg L–1), KH2PO4 (0.13 mg L–1), NaCl (0.43 mg L–1), EDTA (0.17 mg L–1) + KOH (0.55 mg L–1), FeSO4·7H2O (0.02 mg L–1) + H2SO4, H3BO3 (0.19 mg L–1), ZnSO4·7H2O (0.003 mg L–1), MnCl2·4H2O (0.0007 mg L–1), MoO3 (0.0005 mg L–1), CuSO4·5H2O (0.0006 mg L–1), Co(NO3)2·6H2O (0.0002 mg L–1). The cultures were pre-grown with 2% CO2 under cool fluorescent lamps with an irradiation of 2500 lux (measured with TES 1335). The ambient temperature was 24–25 °C.

2.2. Preparation of treatment solutions

Treatment solutions were prepared with sulphuric acid (96% H2SO4). Since algal cells take up sulphur in the form of sulphate, we consider for simplicity that all of the H2SO4 dissociates into sulphate and is thus bioavailable to microalgae. For dilute solutions, 1 ppm is considered equal to 1 mg L–1. In order to achieve a 500 ppm concentration, 0.9615 mL/L of H2SO4 is required. Because the working volume of the reactor was 0.8 L, ~0.77 mL was used for the preparation of the 500 ppm treatment solution and twice the amount for the 1000 ppm treatment solution. Sulphuric acid was
dissolved in 20 mL of BBM. Treatment solutions were prepared in triplicate. Oil shale ash obtained from the Põlevkivi Kompetentsikeskus (Estonian Oil Shale Competence Centre) was added for the neutralization of the pH. Around 3.1 g and 6 g of oil shale ash was used for the neutralization of the 500 ppm and 1000 ppm treatment solutions, respectively (the exact amount varied between the replicates and was adjusted depending on the pH, which was measured constantly). The paste was transferred quantitatively to a vacuum pump and filtered through 2 μm glass microfibre filters (Whatman, GF/F) into 100 mL of BBM. The pH of the solution was measured with a pH meter (SevenCompact™ pH/Ion S220, Mettler Toledo) and the pH was adjusted with 1 M HCl or 1 M NaOH, as necessary. The final pH was 6.5–6.7. As the control, BBM with a similar pH (6.2–6.5) was used.

2.3. Operation of the photobioreactor

The experiments were operated in batch mode. As bioreactors 1 L Schott Duran HP LC high pressure glass bottles with ports were used. The culture of S. obliquus with an initial concentration of ~0.8 g L⁻¹ was added to the neutralized solutions and BBM was added to the final volume of 800 mL. A mixture of air and CO₂ (14% of total), serving as the carbon source, was sparged through 2 μm filters (Whatman, FP30/0.2 CA-S) into the culture medium with a flow rate of 1 L min⁻¹ (set using gas flow meters); at the same time the culture was mixed. The bioreactors were placed under cool fluorescent lamps with an irradiation of 2500 lux under a continuous light cycle.

2.4. Algal biomass measurements

The experiment lasted for 7 days. Measurements were carried out every 24 hours. Optical density (OD) measurements were made with a spectrophotometer (Spectronic 200, Termo Scientific) at 650 nm (OD₆₅₀) with proper dilutions to give an absorbance in the range 0.1–1.0 in case of OD greater than 1.0, and were used as a proxy for determining algal biomass. A calibration scale using OD₆₅₀ measurements and dry biomass weight was established beforehand. The dry biomass weight was obtained by filtering 50 mL algal culture through pre-weighed and predried (100 °C, 24 h) filters with a 2 μm pore size (Whatman, GF/F), and dried at 100 °C for 24 h or until the weight was invariable. Then the filters loaded with biomass were weighed on an analytical scale (ME204, Mettler Toledo) and the weight of the blank dried filters was subtracted to obtain the weight of the dried biomass. The biomass was calculated from the following equation:

\[ M = 0.01207 + 0.0876469 \times \text{abs}, \]  

where \( M \) is biomass (g L⁻¹) and \( \text{abs} \) is the optical density measured at 650 nm.

2.5. Determination of kinetic parameters of growth

Biomass productivity was calculated from the change in biomass concentration (g L⁻¹) within a cultivation time according to Eq. (2):

\[ P = \frac{x_1 - x_0}{t_1 - t_0}, \]  

where \( P \) is biomass productivity (g L⁻¹ d⁻¹), \( x_1 \) is the final biomass determined in grams per litre, \( x_0 \) the initial biomass, \( t_0 \) is the starting time of the experiment, and \( t_1 \) is the end time in days. The maximum biomass concentration achieved in the bioreactors is denoted \( P_{\text{max}} \) and calculated from the maximum biomass yield (\( x_{\text{max}} \)) on the day.

Specific growth rate of the microalgae was calculated using the following equation:

\[ \mu = \ln \frac{x_1}{x_0} \frac{t_1 - t_0}{t_1 - t_0}. \]  

The maximum fixed carbon dioxide (FCO₂) in a single bioreactor was calculated according to the adjusted equation from de Morais and Costa [26]:

\[ \text{FCO}_2 = (x_{\text{max}} - x_0) \times M_{\text{cbm}} \times V_p \times \left( \frac{m_{\text{CO}}}{m_c} \right), \]  

where \( x_{\text{max}} \) is the maximum biomass yield, \( x_0 \) is the initial biomass, \( M_{\text{cbm}} \) is the fraction of carbon in the biomass (g g⁻¹), derived from the typical molecular formula of microalgal biomass CO₉H₁₈O₅N₀.₁₁P₀.₀₁ proposed by Chisti [27], \( V_p \) is the working volume of the bioreactor, \( m_{\text{CO}} \) is the molar mass of CO₂, and \( m_c \) is the molar mass of carbon.

Biomass productivity was used as a proxy for calculating CO₂ fixation by applying the following equation:

\[ P_{\text{CO}_2} = 1.88 \times P, \]  

where \( P_{\text{CO}_2} \) is CO₂ fixation (g L⁻¹ d⁻¹) and \( P \) is biomass productivity.

At the end of the experiments the biomass from the 1000 ppm treatments was submitted to the Estonian Veterinary and Food Laboratory for heavy metal analyses. The results were compared to the standard threshold of heavy metals in animal feed and mean heavy metal concentrations in organic composts used as agricultural fertilizers. The results were analysed using R [28].
3. RESULTS AND DISCUSSION

3.1. Biomass productivity and CO₂ fixation

During the experiments, algal biomass was monitored for 7 days. The results are summarized in Table 1. Repeated measures ANOVA was used to analyse the data. The results show that in general the biomass yields for the control and treatments were similar ($p > 0.05$). This suggests that neither the added sulphur nor the oil shale ash had an inhibiting effect on the algae. A linear model was fitted to confirm if there was any difference in biomass yield between the treatments and control in regard to the experiment day. The results showed that although the biomass yield was time-dependent, the group-means were not significantly different (adjusted $R^2 = 0.84$ and $p < 0.05$). The biomass yields were similar up until the last day of the experiment, when the biomass yields became notably different (Fig. 1).

The initial decrease in biomass as shown in Fig. 1 could be explained by the sudden change in environmental conditions: the change in CO₂ concentration from 2% in the pre-growth phase to 14% in the experimental conditions. After acclimatization, the algae entered an exponential growth stage. The control and the 500 ppm treatment reached their maximum on day 6, the 1000 ppm treatment on day 7. Although the biomass yields did not differ significantly, the biomass yield was slightly higher in the treatments (Fig. 1). It is likely that the minerals contained in oil shale ash (Ca, Fe, K, Mg) were utilized in algal nutrition. While Ca and Fe are micronutrients, K and Mg are macronutrients and are essential in algal nutrition. For example, Mg²⁺ controls the distribution of excitation energy between photosystems and regulates the activity of the enzyme RuBisCo, which regulates carbon fixation [29]. It is also required for nitrogenase activity [7]. Potassium is important in growth and photosynthesis processes; potassium-deficient cells show higher rates of respiration [30]. Iron is involved in the chlorophyll manufacturing process and is contained in different proteins such as ferrodoxin, which is used for electron transfer in a range of metabolic reactions [31]. It is also involved in the electron flow from H₂O to NADP⁺ [7]. Further, sulphur is crucial in

| Treatment      | Maximum biomass yield, g L⁻¹ | Biomass productivity, g L⁻¹ d⁻¹ | Maximum biomass productivity, g L⁻¹ d⁻¹ | Specific growth rate, d⁻¹ | Average daily CO₂ fixation, g L⁻¹ d⁻¹ | Mean CO₂ fixed per reactor, g | Mean CO₂ fixed per litre, g |
|----------------|-------------------------------|---------------------------------|----------------------------------------|---------------------------|--------------------------------------|-----------------------------|-----------------------------|
| Control        | 2.97ᵃ                       | 0.24                            | 0.36                                   | 0.16                      | 0.45                                 | 4.89                        | 6.08                        |
| 500 ppm S      | 3.13ᵃ                       | 0.33                            | 0.39                                   | 0.19                      | 0.62                                 | 6.72                        | 8.40                        |
| 1000 ppm S     | 3.91ᵇ                      | 0.44                            | 0.44                                   | 0.23                      | 0.83                                 | 9.11                        | 11.39                       |

ᵃ On day 6 of the experiment.
ᵇ On day 7 of the experiment.
ᶜ Calculated per bioreactor with a working volume of 0.8 L.

Fig. 1. Biomass yield and average daily CO₂ fixation of *Scenedesmus obliquus* during the experiment with 500 ppm S and 1000 ppm S treatments.
Scenedesmus

Li et al. [36] found no inhibition on the growth of tolerated by many microalgal species [7]. However, yield and the CO₂ fixation were slightly higher in the was noted; therefore, it is likely that sulphur is not adjusted. On the contrary, it is possible that the biomass of 0.288 g L⁻¹ d⁻¹ [26]. Tang et al. [37] calculated a CO₂ fixation rate of 0.288 g L⁻¹ d⁻¹ for 10% CO₂ and 0.246 g L⁻¹ d⁻¹ for 20% CO₂. The calculated fixation rates of carbon dioxide in this study were 0.45 g L⁻¹ d⁻¹, 0.62 g L⁻¹ d⁻¹, and 0.83 g L⁻¹ d⁻¹ for controls, 500 ppm and 1000 ppm treatment, respectively. The mean CO₂ fixed per litre was 6.08, 8.40, and 11.39 grams for the control, 500 ppm and 1000 ppm treatments, respectively.

If we consider the mean CO₂ fixation value per litre from the experiments (the overall mean from control and treatments was 8.62 g L⁻¹ per week and the mean from treatments 9.90 g L⁻¹ per week), then we can say that roughly 0.45–0.51 tonnes m⁻³ of CO₂ could be fixed annually. According to the results of biomass productivity, 123–140 kg m⁻³ of biomass could be produced and harvested annually. Because CO₂ emissions are taxed, reduction in CO₂ emissions means lower costs for CO₂-emitting companies.

If waste ash could be used as an additional nutrient source for algal cultivation, it could lead to savings as a result of minimizing the use of chemicals in cultivation. Fly ash from municipal solid waste and bituminous coal combustion has been used in agriculture for the enrichment of soils with phosphorus, potassium, and several micronutrients (Ni, Co, Mo, Se, etc.), and additionally as a liming agent for acidity reduction in acidic soils [39,40]. It is also common practice to use oil shale ash for agricultural liming in Estonia, and attempts have been made to use oil shale ash for the treatment of damaged pine forests growing on acid sandy soil in order to reduce the acidity of the soil and improve the health of pine stands [41,42]. According to Häsanen et al. [19], oil shale ash sampled from different parts of different oil shale power plants contains (per 20–100 mg of ash) 3.7–7.7 ppm Co, 8.1–17.9 ppm Cu, 335–700 ppm Mn, 2.8–18.6 ppm Mo, 18.9–44.5 ppm Ni, and 27.7–380 ppm Zn. All these are essential micronutrients for algae. Most of these elements are soluble in water (except Mo, which is soluble if bound with oxygen as MoO₄²⁻, and Mn, which is soluble in dilute acid or as MnCl₂). Additionally oil shale ash contains up to 2800 ppm phosphorus [19], which could be utilized by algae. Therefore oil shale ash could be used as a substitute for commonly used reagents or as fertilizer in culture medium preparation, thereby reducing cultivation costs.
For further cost reductions, wastewater could be used as a source of nitrogen and phosphorus, which are the main macronutrients required by algae. Urban wastewaters may contain up to 100 mg L\(^{-1}\) N and P, while the concentration in agricultural wastewater may be as high as 1000 mg L\(^{-1}\) [43]. The cultivation of microalgae in wastewater has been studied extensively [13,44–47].

However, because oil shale ash needs to be filtered out as it increases turbidity and thus the permeation of light through the culture medium, ash could be replaced by oil shale ash leachate, which is a clear liquid with high pH values originating in oil shale ash deposits. Further research should establish the elemental composition of the leachate and its usability for algae nutrition.

3.2. Heavy metal content and potential use of biomass

The biomass from the 1000 ppm treatment was analysed in order to determine the concentration of arsenic, mercury, lead, and cadmium to evaluate its usability as animal feed or aquafeed or as organic agricultural fertilizer. The results are summarized in Table 2.

Microalgal biomass is typically rich in nitrogen and phosphorus, which makes it suitable for use as organic fertilizer. The biomass is also rich in minerals and typically has a sufficient protein content to be used as an inexpensive addition to traditional animal feed and aquafeed. However, the protein content in microalgae is species specific. For example, the protein content in the dry biomass of \textit{Scenedesmus almeriensis} can reach 55\% [50]. Heavy metals and other pollutants can limit the use of algal biomass as agricultural fertilizer or feed. The concentrations of As, Hg, Pb, and Cd in the experimental biomass were much lower than the European accepted thresholds. This enables the use of biomass as fertilizer or feed; however, a full analysis of nutrient content should be carried out beforehand.

Another way to utilize algal biomass from the CO\(_2\) sequestration process is to turn it into energy – ferment into bioethanol or turn into methane through anaerobic digestion [51]. Therefore the biomass can be sold to biorefineries. Profits from biomass sales as well as cost reductions associated with lower CO\(_2\) emissions should, in the long-term, offset the initial investment costs of establishing an algae farm. This implies, however, the investment and operating costs of an algae farm to be minimal, which requires process optimization and operating cost reductions (e.g. using wastewater as discussed previously).

4. CONCLUSIONS

We found that a high sulphur concentration did not have an inhibitory effect on algal growth when the pH drop caused by sulphuric acid was neutralized by oil shale ash. Additionally, the oil shale ash did not inhibit algal growth either. On the contrary, the biomass yield from the treatments was slightly higher, suggesting that a higher sulphur availability and minerals in the ash could serve as nutrients for algae. Considering the results, it can be estimated that 450–510 kg m\(^{-3}\) of sulphur could be fixed and 123–140 kg m\(^{-3}\) of sulphur biomass could be produced annually. Using flue gas as a carbon source and oil shale ash as an additional nutrient source for microalgae cultivation can provide inexpensive biomass, which can be used as fish feed or as an agricultural fertilizer. Further research needs to be carried out in order to optimize the growth conditions and CO\(_2\) fixation, to study the possibility of using oil shale leachate as an additional nutrients source.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge the financial support of the Environmental Investment Centre of Estonia, project No. 4910. We would also like to thank the Estonian Oil Shale Competence Centre for providing oil shale ash for the experiments. The publication costs of this article were covered by the Estonian Academy of Sciences.
REFERENCES

1. Keskkonnaministeerium. Põlevkivi kasutamise riiklik arengukava 2016–2030 [Estonian national oil shale development plan for 2016–2030]. Tallinn, 2015. https://www.riigiteataja.ee/aktiilisa/3180/3201(6002/RKo_16032016Lisa.pdf (accessed 2017-02-02).

2. Tamm, K., Kuusk, R., Uibu, M., and Kallas, J. Transformations of sulfides during aqueous carbonation of oil shale ash. *Energy Procedia*, 2013, 37, 5905–5912.

3. Laja, M. Põlevkivituhk, omadused ja käitumine vesikeskkonnas [Oil shale ash, characteristics and behaviour in an aqueous environment]. University of Tartu, 2005 (in Estonian).

4. Eesti Energia: Environmental report, 2014. Eesti Energia, 2015 http://www.digar.ee/id/nlbi-digar:239879 (accessed 2017-02-02).

5. Lackner, K. S. A guide to CO2 sequestration. *Int. J. Chem. Res.*, 2011, 5, 110–117.

6. Sudhakar, K., Suresh, S., and Premalatha, M. An overview of CO2 mitigation using algae cultivation technology. *Int. J. Chem. Res.*, 2013, 3, 110–117.

7. Ono, E. and Cuello, J. L. Selection of optimal microalgae species for CO2 sequestration. *Nat. Conf. Carbon Sequestration*, 2003, 1–7.

8. Indian Council for Agricultural Research (ICAR). *Indian Soil, Weather and Water Resources*, New Delhi, 2005.

9. Kumar, K., Banerjee, D., and Das, D. Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. *Bioresource Technol.*, 2014, 152, 225–233.

10. Zhao, B. and Su, Y. Process effect of microalgal-carbon dioxide fixation and biomass production: a review. *Renew. Sust. Energy Rev.*, 2014, 31, 121–132.

11. Christenson, L. and Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv.*, 2011, 29, 686–702.

12. Bhola, V., Swala, F., Ranjith Kumar, R., Singh, M., and Bus, F. Overview of the potential of microalgae for CO2 sequestration. *Int. J. Environ. Sci. Technol.*, 2014, 11, 2103–2118.

13. Cheah, W. Y., Show, P. L., Chang, J.-S., Ling, T. C., and Juan, J. C. Biosequestration of atmospheric CO2 and CO2-containing CO2 by microalgae. *Bioresource Technol.*, 2014, 184, 190–201.

14. Ronda, S. R., Kethineni, C., Parupudi, L. C. P., Thumuguntla, V. B. S. C., Venmula, S., Settali, V. S., et al. A growth inhibitory model with SOx influenced effective growth rate for estimation of algal biomass concentration under flue gas atmosphere. *Bioresource Technol.*, 2014, 152, 283–291.

15. Directive 2001/81/EC of the European Parliament and of the Council on national emission ceilings for certain atmospheric pollutants. *OJ*, 2001, L309, 27.11.

16. Uibu, M. and Kuusk, R. Carbon capture and fixation using lime-containing wastes: the influence of aqueous phase composition on Ca dissolution from oil shale ash. *Energy Procedia*, 2013, 37, 5913–5920.

17. Directive 2001/81/EC of the European Parliament and of the Council on national emission ceilings for certain atmospheric pollutants. *OJ*, 2001, L309, 27.11.

18.Directive 2001/81/EC of the European Parliament and of the Council on national emission ceilings for certain atmospheric pollutants. *OJ*, 2001, L309, 27.11.

19. Hässänen, E., Aurela-Tapola, L., Kinnunen, V., Larjava, K., Mehtonen, A., Salmikangas, T., et al. Emission factors and annual emissions of bulk and trace elements from oil shale fueled power plants. *Sci. Total Environ.*, 1997, 198, 1–12.

20. Alkar, E., Ababna, H., and Fathi, A. A. Toxicological response of the green alga *Chlorella vulgaris* to some heavy metals. *Am. J. Environ. Sci.*, 2010, 6, 230–237.

21. Pérez, P., Fernández, E., and Beiras, R. Fuel toxicity on *Isochrysis galbana* and a coastal phytoplankton assemblage: growth rate vs. variable fluorescence. *Ecotox. Environ. Safe.*, 2010, 73, 254–261.

22. Djomo, J. E., Dauta, A., Ferrier, V., Narbonne, J. F., Monkedje, A., Njine, T., et al. Toxic effects of some major polyaromatic hydrocarbons found in crude oil and aquatic sediments on *Scenedesmus subsipicatus*. *Water Res.*, 2004, 38, 1817–1821.

23. Blinova, L., Bityukova, L., Kasemets, K., Ivaska, A., Kääkäinen, A., Kurvet, I., et al. Environmental hazard of oil shale combustion fly ash. *J. Hazard. Mater.*, 2012, 229–230, 192–200.

24. Manusadzianas, L., Balkołęty, L., Sadauskas, K., Blinova, I., Pöllumaa, L., and Kahru, A. Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices. *Aquat. Toxicol.*, 2003, 63, 27–41.

25. Andersen, R. A. *Algal Culturing Techniques*. Elsevier Academic Press, 2005.

26. Morais, M. G. and Costa, J. A. V. Bio fixation of carbon dioxide by * Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *J. Biotechnol.*, 2007, 129, 439–445.

27. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.*, 2007, 25, 294–306.

28. R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2008. http://www.R-project.org. (accessed 2016-10-20).

29. Andersson, I. Catalysis and regulation in Rubisco. *J. Exp. Bot.*, 2008, 59, 1555–1568.

30. Myers, J. Physiology of the algae. *Am. Rev. Microbiol.*, 1951, 5, 157–180.

31. Cammack, R., Rao, K. K., Bargeron, C. P., Hutson, K. G., Andrew, P. W., and Rogers, L. J. Midpoint redox potentials of plant and algal ferredoxins. *Planta*, 2002, 214, 190–201.

32. Zhang, L., Happe, T., and Melis, A. Biochemical and morphological characterization of sulfur-deprived and H2-producing *Chlamydomonas reinhardtii* (green alga). *Planta*, 2002, 214, 552–561.

33. Hell, R., Dahl, C., Knaff, D. B., and Leustek, T. (eds). *Sulfur Metabolism in Phototrophic Organisms*. Advances in Photosynthesis and Respiration, Vol. 27. Dordrecht, Springer, 2008.

34. Giordano, M., Pezzoni, V., and Hell, R. Strategies for the allocation of resources under sulfur limitation in the green alga *Dunaliella salina*. *Plant Physiol.*, 2000, 124, 857–864.

35. Sexton, P. J., Batchelor, W. D., and Shibles, R. Sulfur availability, Rubisco content, and photosynthetic rate of soybean. *Crop Sci.*, 1997, 37, 1801–1806.
Enim kasutatavaks energiatootmise mõjuks Eestis on põlevkivi. See on madala kütteväärtuse ja suure mineraalaine-koguse arvutamiseks. Biomassi saagikus kahe töötluse puhul (maksimaalne saagikus 3,1 ja 3,9 g L−1) on võimalik optimiseerida ja maksimeerida süsihappegaasi sidumist.

S. obliquus valiti katseorganismiks seetõttu, et ta on võimeline kasvama kuni 80% CO2 sisaldusega atmosfääris ja saabutatavaks energiatootmise mõjus. See on madala kütteväärtuse ja suure mineraalaine-koguse arvutamiseks. Biomassi saagikus kahe töötluse puhul (maksimaalne saagikus 3,1 ja 3,9 g L−1) on võimalik optimiseerida ja maksimeerida süsihappegaasi sidumist.

S. obliquus valiti katseorganismiks seetõttu, et ta on võimeline kasvama kuni 80% CO2 sisaldusega atmosfääris ja saabutatavaks energiatootmise mõjus. See on madala kütteväärtuse ja suure mineraalaine-koguse arvutamiseks. Biomassi saagikus kahe töötluse puhul (maksimaalne saagikus 3,1 ja 3,9 g L−1) on võimalik optimiseerida ja maksimeerida süsihappegaasi sidumist.