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BIOTECHNOLOGY

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

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Abstract. Oil shale is the main energy resource in Estonia, which generates large amounts of CO_2 and waste oil shale ash. Flue gas from oil shale combustion can also contain large amounts of SO_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_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_2. Biomass measurements of *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^{-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_2 fixation rate was 0.45 g L^{-1} d^{-1} for the control, and 0.62 and 0.83 g L^{-1} d^{-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, *Scenedesmus obliquus*, oil shale ash, flue gas, CO_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_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_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_2. In spite of the fact that CO_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

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[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 CO_2. 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 CO_2 [9]. Algae have been shown to grow under an atmosphere with a CO_2 concentration as high as 80% and 100% [10]. Flue gas contains on average 10–20% CO_2 [11,12] and flue gas from oil shale combustion approximately 12% CO_2 (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 CO_2 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, NO_x does not have a direct negative impact on microalgal growth because NO for example is transformed into NO_2 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 SO_2 (P. Rohumaa, personal communication). For this reason the effect of NO_x was not studied in the current study and the focus was placed on the possible inhibitory impact of SO_2 on algae. Although SO_2 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 SO_2 concentrations in regard to algae. In 2014, Eesti Energia emitted 24.2 kt of SO_2 [5].

For pH adjustment of acidic conditions, NaOH and $CaCO_3$ 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 CO_2 and SO_2 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^{TM}, exhibited low toxicity to ash-heap leacheates [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% CO_2 conditions with a maximum cell concentration of 10–20% CO_2. 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 $NaNO_3$ (2.5 mg L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.17 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.30 mg L^{-1}), K_2HPO_4 (0.43 mg L^{-1}), KH_2PO_4 (0.13 mg L^{-1}), NaCl (0.43 mg L^{-1}), EDTA (0.17 mg L^{-1}) + KOH (0.55 mg L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.02 mg L^{-1}) + H_2SO_4, H_3BO_3 (0.19 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.003 mg L^{-1}), $MnCl_2 \cdot 4H_2O$ (0.0007 mg L^{-1}), MoO_3 (0.0005 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.0006 mg L^{-1}), $Co(NO_3)_2 \cdot 6H_2O$ (0.0002 mg L^{-1}). The cultures were pre-grown with 2% CO_2 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% H_2SO_4). Since algal cells take up sulphur in the form of sulphate, we consider for simplicity that all of the H_2SO_4 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 H_2SO_4 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 HPLC 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 CO_2 (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 (OD_{650}) 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 OD_{650} measurements and dry biomass weight was established beforehand. The dry biomass weight was obtained by filtering 50 mL algal culture through preweighed 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:

$$M = 0.01207 + 0.0876469 \times abs, \quad (1)$$

where M is biomass (g L^{-1}) and 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):

$$P = \frac{x_1 - x_0}{t_1 - t_0}, \quad (2)$$

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_{max} and calculated from the maximum biomass yield (x_{max}) on the day.

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

$$\mu = \ln \frac{x_1 / x_0}{t_1 - t_0}. \quad (3)$$

The maximum fixed carbon dioxide (FCO_2) in a single bioreactor was calculated according to the adjusted equation from de Morais and Costa [26]:

$$FCO_2 = (x_{max} - x_0) \times M_{cbm} \times V_p \times (m_{CO_2} / m_C), \quad (4)$$

where x_{max} is the maximum biomass yield, x_0 is the initial biomass, M_{cbm} is the fraction of carbon in the biomass (g g^{-1}), derived from the typical molecular formula of microalgal biomass $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ proposed by Chisti [27], V_p is the working volume of the bioreactor, m_{CO_2} is the molar mass of CO_2, and m_C is the molar mass of carbon.

Biomass productivity was used as a proxy for calculating CO_2 fixation by applying the following equation:

$$P_{CO_2} = 1.88 \times P, \quad (5)$$

where P_{CO_2} is CO_2 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 CO_2 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 CO_2 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, Mg^{2+} 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 H_2O to $NADP^+$ [7]. Further, sulphur is crucial in

Table 1. Algae biomass productivity parameters and calculated CO_2 fixation

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 CO_2 fixation g L^{-1} d^{-1}	Mean CO_2 fixed per bioreactor, g[c]	Mean CO_2 fixed per litre, g
Control	2.97[a]	0.24	0.36	0.16	0.45	4.89	6.08
500 ppm S	3.13[a]	0.33	0.39	0.19	0.62	6.72	8.40
1000 ppm S	3.91[b]	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.

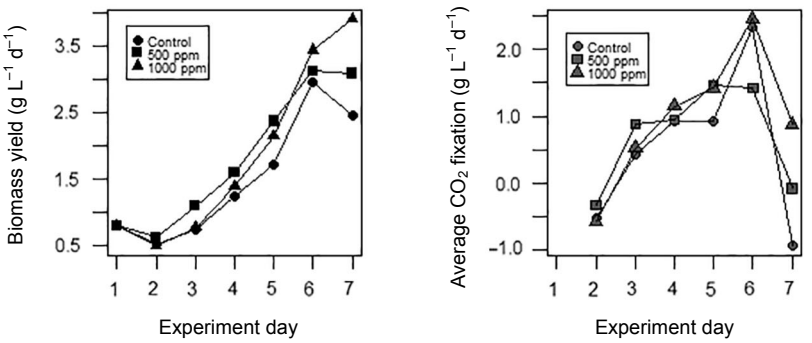

Fig. 1. Biomass yield and average daily CO_2 fixation of *Scenedesmus obliquus* during the experiment with 500 ppm S and 1000 ppm S treatments.

algal biochemical processes. It is an imperative component in the composition of the amino acids cysteine and methionine, and plays an important part in protein biosynthesis as well as in the repair cycle of photosynthetic system PSII [7,32,33]. Sulphur metabolism is also linked with RuBisCo – in sulphur-deprived cells the amount of RuBisCo declines, CO_2 exchange rate and quantum efficiency decrease and thus CO_2 fixation also declines [32,34,35]. It has been established that a moderate level of SO_x and NO_x (up to 150 ppm) is tolerated by many microalgal species [7]. However, Li et al. [36] found no inhibition on the growth of *Scenedesmus* sp. grown with 18% CO_2, 150 ppm NO_x, and 200 ppm SO_x. In fact, they suggest that NO_x and SO_x can be effectively used as nutrients for microalgae growth. When sparged through water (or algal culture medium), SO_2 dissolves according to the (simplified) equation: $SO_2 \xrightarrow{O_2} SO_3 \xrightarrow{H_2O} H_2SO_4$. The sulphuric acid further dissociates into sulphate and hydrogen ions according to the following steps $H_2SO_4 \rightarrow HSO_4^- + H^{+-}$ and $HSO_4^- \rightarrow SO_4^{2-} + H^+$. Sulphate, SO_4^{2-}, is the main bioavailable form of sulphur that is taken up by microalgae [33].

In the current study no inhibition of algal growth was noted; therefore, it is likely that sulphur is not directly toxic to *S. obliquus* once the pH has been adjusted. On the contrary, it is possible that the biomass yield and the CO_2 fixation were slightly higher in the treatments due to a higher sulphur availability, among other nutrients described earlier. The amount of sulphur used in the study can be considered slightly higher than would dissolve from flue gas, because the solubility of SO_2 is temperature dependent with more SO_2 dissolving at lower temperatures. Hence it is likely that the amount of SO_2 that would dissolve from flue gas into the growth medium would be lower, because flue gas would not be cooled to room temperature prior to sparging through the culture medium.

The maximum biomass yield achieved in the bioreactors was 2.9 g L^{-1} for the controls (on day 6), 3.1 g L^{-1} for the 500 ppm treatment (on day 6), and 3.9 g L^{-1} for the 1000 ppm treatment (on day 7). The maximum biomass productivity (P_{max}) was 0.36, 0.39, and 0.44 g L^{-1} d^{-1}, respectively. These results are comparable with similar studies. Tang et al. [37] achieved a P_{max} 0.155 g L^{-1} d^{-1} with *S. obliquus* grown with 10% CO_2 and 0.134 g L^{-1} d^{-1} when grown with 20% CO_2. Ho et al. [38] achieved a P_{max} of 0.28 g L^{-1} d^{-1} and 0.38 g L^{-1} d^{-1} with 10% CO_2 for *S. obliquus* strains isolated in Taiwan. Compared to these studies, the maximum biomass productivity of *S. obliquus* in given experiments can be considered high. However, it should be noted that because the growth conditions and the duration of experiments were different in the studies, these results should be interpreted with caution.

A high biomass yield implies good carbon fixation. It is estimated that per gram of assimilated biomass, 1.88 grams of CO_2 is fixed [7]. Carbon contained in different molecules (carbohydrates, lipids, etc.) makes up circa 50% of the dry weight of the biomass [27]. Studies of *S. obliquus* grown with 12% CO_2 at 30 °C had a CO_2 fixation rate of 0.26 g L^{-1} d^{-1} [26]. Tang et al. [37] calculated a CO_2 fixation rate of 0.288 g L^{-1} d^{-1} for 10% CO_2 and 0.246 g L^{-1} d^{-1} for 20% CO_2. 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 CO_2 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 CO_2 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 CO_2 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 CO_2 emissions are taxed, reduction in CO_2 emissions means lower costs for CO_2-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 MoO^{2-}, and Mn, which is soluble in dilute acid or as $MnCl_2$). 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 *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).

Table 2. Comparison of heavy metal concentrations in algal biomass from 1000 ppm treatment with heavy metal thresholds for animal feed and mean concentrations in biowaste compost, sludge compost, and compost from municipal solid waste

	As	Hg	Pb	Cd	Reference
Concentration in algae biomass, mg kg^{-1}	0.09	0.02	0.13	0.013	
Maximal acceptable level in animal feed[a], mg kg^{-1}	2[b]	0.1	10[c]	1	[48]
Mean concentration in biowaste compost, mg kg^{-1}	3–10[d]	0.2–2.5	40–750	0.4–5.3	[49]

[a] Maximum content relative to a feed with a moisture content of 12%.

[b] Seaweed meal and feed materials derived from seaweed have a higher threshold: 40 (2) mg kg^{-1} (ppm), while complementary and complete feed for pet animals containing fish, other aquatic animals, and products derived thereof and/or seaweed meal and feed materials derived from seaweed have a threshold of 10 (2) mg kg^{-1} (ppm).

[c] Feed materials derived from phosphates and calcareous marine algae have a threshold of 15 mg kg^{-1} (ppm).

[d] mg kg^{-1} dm.

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.

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Rohevetika *Scenedesmus obliquus* kultiveerimine kunstliku suitsugaasi tingimustes põlevkivituhaga neutraliseeritud suure väävlisisalduse juures

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Enim kasutatavaks energiatootmise kütuseks Eestis on põlevkivi. See on madala küttevääruse ja suure mineraalainesisaldusega kütus, mille põletamisel tekib suurel hulgal aluselist põlevkivituhka. Lisaks suurele süsinikdioksiidi hulgale sisaldab põlevkivienergeetikast saadav suitsugaas ka olulisel hulgal vääveldioksiidi (SO_2). Süsihappegaasi seostatakse kliimamuutustega ja seetõttu on võetud eesmärgiks CO_2 heitme vähendamine. CO_2 sidumiseks suitsugaasist ja seeläbi emissioonide vähendamist on võimalik teha mikrovetikate abil. Mitmed mikrovetikaliigid on võimelised kasvama väga suure CO_2 kontsentratsiooni juures. Antud teadustöö eesmärgiks oli kultiveerida rohevetikat *Scenedesmus obliquus* suure CO_2 ja väävli sisalduse juures ning kasutada pH neutraliseerimiseks aluselist põlevkivituhka.

S. obliquus valiti katseorganismiks seetõttu, et ta on võimeline kasvama kuni 80% CO_2 sisaldusega atmosfääris ja saavutab maksimaalse rakkude kontsentratisooni 10–20% CO_2 tingimustes. Sellesse vahemikku langeb ka põlevkivisuitsugaasi CO_2 keskmine sidaldus: ~12%. Katses kasvatati *S. obliquus*'t 14% CO_2 kontsentratsiooni tingimustes bioreaktorites mahuga 1 L. Toitelahusesse lisati väävelhapet kontsentratsioonis 500 ppm ja 1000 ppm, et jäljendada vääveldioksiidi lahustumist suitsugaasist kasvulahusesse. Lahus neutraliseeriti aluselise põlevkivituhaga. Seitsmepäevase katse käigus mõõdeti iga 24 tunni järel spektrofotomeetriliselt vetika biomassi, mida kasutati seotud CO_2 koguse arvutamiseks. Biomassi saagikus kahe töötluse puhul (maksimaalne saagikus 3,1 ja 3,9 g L^{-1} vastavalt 500 ppm ja 1000 ppm puhul) oli sarnane töötlemata kontrollrühma saagikusega (vastavalt 2,9, 3,1 ja 3,9 g L^{-1} kontrolli, 500 ppm ning 1000 ppm töötluse kohta), mis viitab sellele, et väävlil ja põlevkivituhal polnud katseorganismi kasvule pärssivat mõju. CO_2 sidumise kiirus oli vastavalt 0,45 g L^{-1} päev^{-1} kontrolli puhul, 0,62 g L^{-1} p^{-1} 500 ppm töötluse ja 0,83 g L^{-1} p^{-1} 1000 ppm töötluse puhul. Seega oleks aastas võimalik siduda 0,45 t m^{-3} kuni 0,51 t m^{-3} süsihappegaasi, tootes seejuures 123−140 kg m^{-3} biomassi. Kokkuvõtteks võib öelda, et mikrovetikat *S. obliquus* on võimalik kasutada süsihappegaasi sidumiseks suitsugaasist. Edasine uurimistöö peaks selgitama, kuidas antud protsessi optimeerida ja maksimeerida süsihappegaasi sidumist.