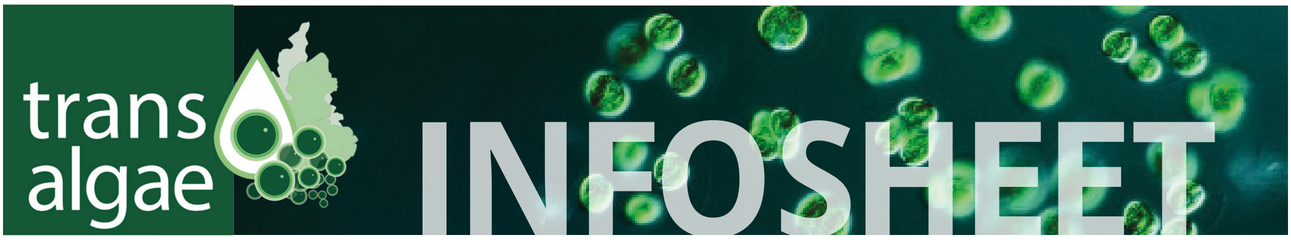

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Lipid and fatty acid production by microalgae

In a laboratory experiment, microalgae were cultivated in wastewater from three different points of the municipal wastewater treatment plant in Vaasa. Subsequently, we investigated the potential to produce biodiesel from the algal biomass. The preliminary results showed that water from all three points of the wastewater treatment process was equally suitable for algae cultivation with the purpose of biodiesel production.

ABOUT THE MUNICIPAL WASTEWATER

The municipal wastewater treatment plant Pått in the city of Vaasa employs an activated sludge biological treatment process. Wastewater samples were taken from three different points of the treatment process: after the pre-sedimentation, after the sedimentation and the effluent, i.e. the cleaned water. Water from after the pre-sedimentation contains nitrogen foremost in the form of ammonium-nitrogen, while water from after the sedimentation contains nitrogen mainly in the form of nitrate. The concentration of total nitrogen in the waters was between 24 mg L-1 and 41 mg L-1 in the beginning of the experiment. (Martonen, 2017)

CULTIVATING THE MICROALGAE

In the experiment, the green algae *Scenedesmus dimorphus* was employed. Samples of wastewater from the three different points of the treatment process were taken to the laboratory, filtered and sterilized in an autoclave. Wastewater was filled into measuring cylinders and algae were added to the water. The cultures were maintained in the laboratory at ca. 24 °C, aerated with ambient air, and continuously illuminated with cool white fluorescent lamps at an intensity of 147 µmol m-2 s-1. The color of the algae suspension changed from green in the beginning to ochre in the end over the course of 18 days (Fig. 1). After the experiment, the algae were let to settle, the sediments were centrifuged and the pellets dried for 22 hours in an oven at 88 °C and stored in a freezer at -20 °C.

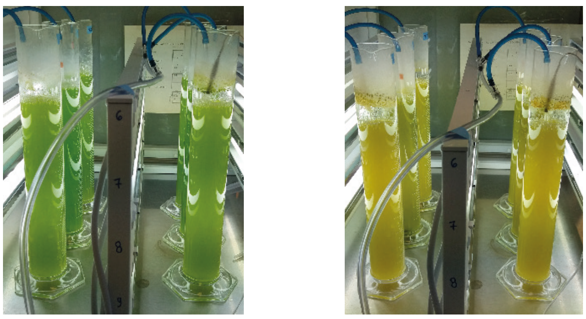

Figure 1. Algae cultivation on day 5 (left) and on day 15 (right).

PRODUCING BIODIESEL

The biodiesel potential is determined by measuring the lipid content and the amount of fatty acid methyl esters that can be produced from the lipids. The total lipids of the dried algae biomass were extracted with a single-step method based on the Folch et al. (1957) method, previously developed at the SLU laboratory (Axelsson and Gentili, 2014). The total lipid content per dry weight was determined gravimetrically. In the next step, the crude lipids extracts were purified with solid-phase-extraction (SPE) in order to isolate neutral and polar lipids. These two lipid fractions were then trans-methylated into fatty acid methyl esters (FAMEs) and subsequently characterized and quantified with a TRACE™ 1310 GC system equipped with a flame ionization detector (Lage and Gentili, 2018).

PLENTY OF LIPIDS AND AVERAGE LEVELS OF BIODIESEL

After two and a half weeks of cultivation, the algae dry biomass had increased from 0.9 g L-1 to 2.6 g L-1 (Martonen, 2017). In the end, between 53 % and 70 % of the dried biomass were lipids (Fig. 2) and about 23 % of the harvested algae dry biomass could be refined into FAMEs (biodiesel) from neutral lipids and free fatty acids (Fig. 3). Similar numbers have been reported elsewhere in research literature.

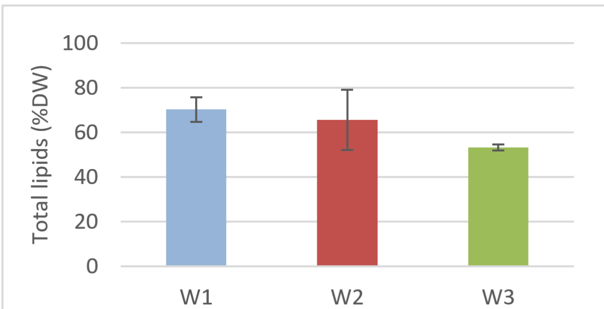

Figure 2. Total lipids as percentage of algae biomass dry weight (mean ± standard deviation, technical replicates n = 3). W1: water after the pre-sedimentation, W2: water after the sedimentation, W3: cleaned water.

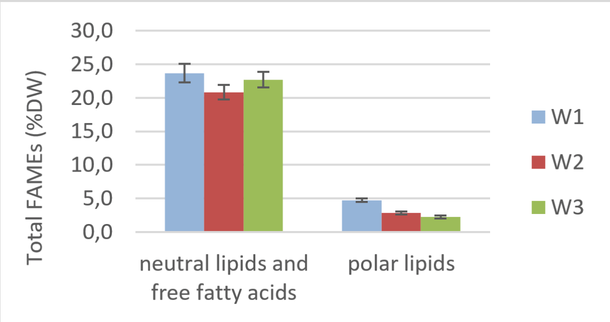

Figure 3. Total amount of FAMEs refined from neutral lipids and free fatty acids, and polar lipids as percentage of the dried algae biomass (mean ± standard deviation, technical replicates n = 3). W1: water after the pre-sedimentation, W2: water after the sedimentation, W3: cleaned water.

COMPOSITION OF THE BIODIESEL

Biodiesel quality is affected by the degree of saturation (or unsaturation) of the fatty acids involved. For instance, unsaturated and especially polyunsaturated fatty acids are prone to oxidation and this leads to a deterioration of oxidation stability and biodiesel storage time. The profile of the FAMEs from the present study is shown in figure 4. The majority of FAMEs had chains of 16 and 18 carbon atoms with no or only one double bond. This complies with rather satisfying composition of biodiesel. However, fuel property is determined by numerous more parameters.

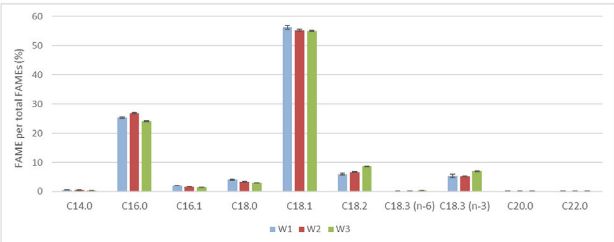

Figure 4. Profile of FAMEs produced from neutral lipids and free fatty acids as percentage of total FAMEs. W1: water after the pre-sedimentation, W2: water after the sedimentation, W3: cleaned water.

There are only minor variations in FAMEs composition from algae cultivated in water from the three different process points. Thus, it does not seem to matter which one of the three tested qualities of wastewater is being used to cultivate the microalgae *Scenedesmus dimorphus*. All of them resulted in similar amounts of biodiesel. More research will be done to confirm the results.

NITROGEN DEFICIENCY IS KNOWN TO ENHANCE LIPID PRODUCTION IN MICROALGAE

In this experiment, the nitrogen and phosphorous concentration in the wastewater was low from the beginning and already after the first 24 hours, the algae seemed to have taken up most of the nitrogen and phosphorous from the wastewater. Since no nutrients were added to the waters, most likely, the algae became nutrient deficient.

When growth conditions become unfavorable for microalgae or even stressful and nitrogen availability is limited, carbon is used in the synthesis of carbohydrates and lipids instead of proteins.

Moreover, in natural conditions light intensity varies during the course of the day. In this experiment, cultivations had been illuminated at medium intensity but non-stop for 24 hours per day. This might have caused additional stress.

ACKNOWLEDGEMENTS

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