SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17133; Implementing nature based solutions for creating a resourceful circular city
STSM title: Cultivation Of Microalgae For Functional Food Ingredients
STSM start and end date: 22/01/2020 to 31/01/2020
Grantee name: Dr.-Ing. Mehmet Ali Küçüker

PURPOSE OF THE STSM:

Microalgae feedstocks have been utilized for biorefinery concept due to their fast growth potential, coupled with high lutein, lipid, carbohydrate, nutrient and protein contents in a third-generation biorefinery. Advantages of the cultivation of microalgae compared to terrestrial plants can be listed, most notably: a high biomass productivity (a rapid process), eco-friendly technology (a 10 to 50 times higher CO_2 fixation rate) and reduced required land. Institute of Environmental Technology and Energy Economics (IUE) at Hamburg University of Technology (TUHH), provides research and education activities on microalgal products and technologies for food, pharmaceutical, energy and environment sectors. IUE has extensive expertise on algal research under the supervision of Prof. Dr.-Ing. Kerstin Kuchta. The goal of this STSM is for Dr.-Ing. Mehmet Ali Küçüker, is to gain first-hand experience on the cultivation of microalgae that contain large amounts of structural biopolymers, including lutein, proteins and carbohydrates and its specific process for the obtaining functional food ingredients. The specific tasks of this STSM are listed as follows: *Training for cultivation of the microalgae with high rate of cultivation efficiency in the photobioreactors (PBRs);* Extraction of functional food ingredients from cultivated microalgae.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The algae species *C. sorokiniana* (211-8k) from the Collection of Algae Cultures (SAG) was used for the investigations carried out in this work. The cultivation of *C. sorokiniana*, embedded in a biorefinery, has a high potential for becoming a resource-saving alternative for the production of valuable products such as lutein and protein. Cultivation of *C. sorokiniana* was conducted using lab-scale and flat panel photobioreactors (PBRs). The stock culture was kept in a test tube at a temperature of 21 ° C and a light intensity of 25 µmol m^{-2} s^{-1}. For the preparation of the culture, 5% (w / v) agar was added to the culture medium before autoclaving. The precultures were grown by transferring the stock culture into 500 mL Erlenmeyer flasks and then swirling (150 rpm) at a light intensity of 300 µE m^{-2} s^{-1}. The pre-cultures were grown up to a volume of 10 L. The culture was also supplied with filtered (Sartofluor MidiCaps, Sartorius, Göttingen) and CO_2 (4 vol%, v / v) enriched air. The species were filled in 10 L column airlift photobioreactor, incubated for 15 days under continuous illumination at an average intensity of 300 µE m^{-2} s^{-1} at room temperature. The DS-medium was used (after supplementation with phosphate and nitrate) to grow the microalgae. The concentrations of the additional salts were 5 g/L KNO_3 and 0.75 g/L K_2HPO_4. The initial pH was between 6.8 and 7.2. The temperature and pH were measured online by WTW instruments. The harvested microalgae were concentrated by means of a concentrative separator (Westphalia GEH). Total crude protein was determined according to AOAC International Method 2001.11, where the TKN was multiplied by a conversion factor of 6.25. Organic chromophores were detected for lutein. The chromophores were first extracted, separated chromatographically and finally quantified. For chromatographic detection, the sample was also initially centrifuged for 4 minutes. at 10,000 rpm. The pellet was then resuspended in EtOH and the cells were disrupted using a ball mill. For this purpose, glass

beads were added to the suspension (2 x 1.7-2 mm; 4 x 0.25-0-3 mm). The digestion was carried out 5 times for 55 s at 6000 rpm. The suspension was then centrifuged again (4 minutes at 10,000 rpm) and the supernatant filtered (2 µm) and transferred to the HPLC. In addition, enzymatic hydrolysis of algal cells were tested for algae protein hydrolysate.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Cultivation of *C. sorokiniana* with standard culture medium was conducted using PBRs. A typical growth curve of this cultivation is presented in concentration increased from 0.92 to 4.00 $g\,L^{-1}$. After 9 days the biomass concentration of *C. sorokiniana* reached 5.32 $g\,L^{-1}$ with average productivity of 0.32 $g\,L^{-1}\,d^{-1}$. The growth curve flattens with progressing time. While the productivity within the first four days was 0.47 $g\,L^{-1}\,d^{-1}$, it declined to productivity of 0.23 $g\,L^{-1}\,d^{-1}$. This reveals a limitation of light availability for the algae cells with increasing cell density. The recycling of medium could be beneficial in terms of increasing the efficiency of nutrient effort and decreasing the nutrient and water requirements. The harvested microalgal biomass (*C. sorokiniana*) was contained 41.6%, 15.4% and 13.4% of proteins, lipids and carbohydrates on a dry weight basis, respectively. Lutein concentration was detected by 0.3 %. An increase in lutein concentration as a result of an increase in temperature or light exposure. In general, it should be noted that the amino acid concentration in the cultures examined was in the range between 350 and 431 g kg^{-1}. This order of magnitude correlates with the detection of the protein concentration. There is no significant changes in the spectrum of amino acids due to external factors could be determined.

The studies carried out on the contents and concentrations of the microalgae *C. sorokiniana* allow the following conclusions:

• The examination of the primary cell components has shown that these are not significantly influenced by external factors or by the type of culture medium. A concentration of about 410 g kg^{-1} for proteins, 305 g kg^{-1} for saccharides, 130 g kg^{-1} for lipids and 52 g kg^{-1} for pigments was found as the mean value.

• The compounds lutein, neoxanthine, zeaxanthin, violaxanthin, carotene and carotene were detected in the microalgae *C. sorokiniana* with the chromophore analysis. The average concentration of lutein was about 2.8 g kg^{-1}.

Main outcomes of the STSM: Manuscript under review carried out during and after the STSM
Nils Wieczorek, Mehmet Ali Kucuker, Niclas Büscher, Kerstin Kuchta, Outdoor cultivation of *Chlorella sorokiniana* in third generation biorefinary: Resource savings through medium recycling. It was submitted to Bioresource Technology Journal on 23rd of February, 2020.

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FUTURE COLLABORATIONS

The main possible implementation is linked to the future and possible co-operation on the same subject (for example using other financial channels to support this research) or on other related items linked in the environmental and biotechnological aspect for microalgae cultivation. It is well know that Turkey is a very interesting region for the raw and secondary material production and future collaboration in these ambits will be possible also considering private and public companies present in Turkey and Europe. Other implementations will be possible in the ambit of the scientific co-operation and in the future further exchange of researchers and/or students.