

Case Study on Innovative Aquatic-Based Bioeconomy for *Chlorella sorokiniana*

Iryna Atamaniuk, Hannah Boysen, Nils Wiczorek, Natalia Politaeva, Iuliia Bazarnova, Kerstin Kuchta

Abstract—Over the last decade due to climate change and a strategy of natural resources preservation, the interest for the aquatic biomass has dramatically increased. Along with mitigation of the environmental pressure and connection of waste streams (including CO₂ and heat emissions), microalgae bioeconomy can supply food, feed, as well as the pharmaceutical and power industry with number of value-added products. Furthermore, in comparison to conventional biomass, microalgae can be cultivated in wide range of conditions without compromising food and feed production, thus addressing issues associated with negative social and the environmental impacts. This paper presents the state-of-the art technology for microalgae bioeconomy from cultivation process to production of valuable components and by-streams. Microalgae *Chlorella sorokiniana* were cultivated in the pilot-scale innovation concept in Hamburg (Germany) using different systems such as race way pond (5000 L) and flat panel reactors (8 x 180 L). In order to achieve the optimum growth conditions along with suitable cellular composition for the further extraction of the value-added components, process parameters such as light intensity, temperature and pH are continuously being monitored. On the other hand, metabolic needs in nutrients were provided by addition of micro- and macro-nutrients into a medium to ensure autotrophic growth conditions of microalgae. The cultivation was further followed by downstream process and extraction of lipids, proteins and saccharides. Lipids extraction is conducted in repeated-batch semi-automatic mode using hot extraction method according to Randall. As solvents hexane and ethanol are used at different ratio of 9:1 and 1:9, respectively. Depending on cell disruption method along with solvents ratio, the total lipids content showed significant variations between 8.1% and 13.9%. The highest percentage of extracted biomass was reached with a sample pretreated with microwave digestion using 90% of hexane and 10% of ethanol as solvents. Proteins content in microalgae was determined by two different methods, namely: Total Kjeldahl Nitrogen (TKN), which further was converted to protein content, as well as Bradford method using Brilliant Blue G-250 dye. Obtained results, showed a good correlation between both methods with protein content being in the range of 39.8–47.1%. Characterization of neutral and acid saccharides from microalgae was conducted by phenol-sulfuric acid method at two wavelengths of 480 nm and 490 nm. The average concentration of neutral and acid saccharides under the optimal cultivation conditions was 19.5% and 26.1%, respectively. Subsequently, biomass residues are used as substrate for anaerobic digestion on the laboratory-scale. The methane concentration, which was measured on the daily bases,

showed some variations for different samples after extraction steps but was in the range between 48% and 55%. CO₂ which is formed during the fermentation process and after the combustion in the Combined Heat and Power unit can potentially be used within the cultivation process as a carbon source for the photoautotrophic synthesis of biomass.

Keywords—Bioeconomy, lipids, microalgae, proteins, saccharides.

I. INTRODUCTION

MICROALGAE are unicellular algae with a size range from several micrometers (µm) to a few hundreds of micrometers, which can usually be found in natural water bodies, individually or in group-formed communities. Similar to most of the flora species, microalgae are also autotrophic organisms that utilize carbon dioxide by photosynthesis. Due to unique cell composition and a rapid growth rate microalgae have recently recognized as a valuable feedstock for the pharmaceutical, food and feed industry.

Depending on the biomass type as well as cultivation conditions, high quality chemicals such as proteins, saccharides, vitamins, lipids and pigments can be obtained from different microalgae species. A summary of potential products and their application is presented in Fig. 1.

As a natural source of protein, microalgae *Chlorella sorokiniana* have been applied as a food and feed supplements over the last 2000 years [11]. Due to high concentration of proteins (35–45%) they can potentially be used as an alternative animal protein replacement [9], [10]. However, even though numbers of methods for protein concentration and isolation have developed, namely precipitation, ultrafiltration, centrifugation and dialysis, there is still a lack of process integration with extraction of other fine chemicals, thus, enhancement of economic feasibility of the whole process.

From the commercial point of view, sustainable energy and biofuels production to replace fossil fuels is another promising approach of aquatic-based bioeconomy. Microalgae are reported to be cultivated in wide range of climate and weather conditions, and therefore do not compromise food and feed production [11], [12]. Under certain cultivation conditions such as a lack of nutrients or/ and high light intensity, aquatic biomass synthesizes large amount of lipids which can further be converted into biodiesel. Besides lipids, microalgae have high carbohydrates content and can potentially be used for bioethanol production. In addition, under anaerobic conditions organic biomass can be utilized for biohydrogen and biomethane generation. It also needs to be mention that combination of different processes, for instance extraction of

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lipids for biodiesel production and further fermentation of residues has certainly caught much attention over the last years.

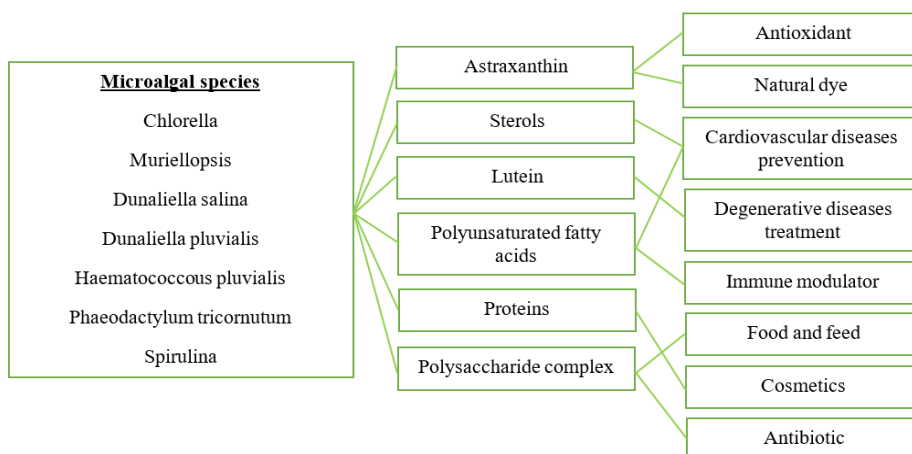


Fig. 1 Microalgae potential products and their application [1]-[10]

Apart from the possibility to produce different energy carriers and high quality fine chemicals, microalgae are capable to reduce and fix anthropogenic emissions. As a fact, green microalgae such as *Chlorella sorokiniana* are able to fix CO₂ 10-15 folds more CO₂ compared to terrestrial plants without exploitation of arable land [13]. At the same time, representatives of the genus *Chlorella* are characterized by high productivities and frugal nutritional requirements. Additionally, these species are featured by an increased thermophile tolerance until 46°C and a high resistance against contaminants [14].

Despite, number of advantages of microalgae bioeconomy, its commercial exploitation is generally limited only to processes related to proteins and biodiesel production, whereas pharmaceutical and food applications are only at the early stage of the development. Therefore, the main purpose of this study is to demonstrate a possibility of technological application of microalgae *Chlorella sorokiniana* for the production of value-added components and CO₂-neutral energy.

II. MATERIALS AND METHODS

A. Microalgae Cultivation and Downstream Processing

Microalgae *Chlorella sorokiniana* (strain SAG 211-8k) were cultivated in the pilot-scale system in Hamburg (Germany) in open pond (5000 L) and flat panel reactors (8 x 180 L) with contentious monitoring of process parameters such as light intensity, temperature and pH. Micro- and macronutrients were added into a medium according to recommendation provided by Culture Collection of Algae at Göttingen University [15]. After repeated-batch cultivation processes to biomass dry matter content of 4 g·L⁻¹ to 5 g·L⁻¹ efficacious harvesting by centrifugation and dehydrating using freeze-drying method. Obtained samples were further used for extraction of lipids, proteins and saccharides as described below.

B. Lipids Extraction and Characterization

For the lipids extraction from microalgae *Chlorella sorokiniana* hot extraction according to Randall with hexane and ethanol solvents mixture was used. Solvents that are used in the study were of GC/ HPLC grade of purity (puriss. p.a., reag. Ph. Eur., Sigma-Aldrich) with purity of ≥ 99 % and ≥ 99.8 % for hexane and ethanol, respectively.

As a blink sample, 1 g of freeze-dried microalgae powder was directly used for the lipids extraction.

In order to ensure sufficient cell disruption, 1 g of freeze-dried microalgae powder was mixed with 30 mL of mixed solvent using the high speed homogenizer (IKA® Werke, T25 Basic) with 21500 rpm for 15 minutes. The obtained suspension was then mixed with 45 mL solvent mixture of hexane ethanol to achieve the proportional ratio of 9:1 or 1:9 w/w, respectively. Alternatively, microwave assisted cell disruption using CEM One Touch™ Technology Mars 6 was applied. The process parameters were set as followings: power - 200 W, pressure - 1 psi, 10 minutes of ramp time and 10 minutes of hold time at 100°C.

Lipids extraction was carried out using Behr Extraction Apparatus E 4 unit at the temperature of 150 °C. The total time of decocting, rinsing and evaporation was adjusted from 60 min to 180 min depending on the solvent type. All experiments were conducted in triplicate.

The total lipid content was determined gravimetrically. Moreover, the fatty acids that were present in extracts were converted into the corresponding fatty acid methyl esters (FAME) by suitable derivatization method. Subsequently, the gas chromatographic (GC) separation of FAME and their detection by flame ionization detector (FID) was carried out. Sample quantification was achieved by means of an internal standard Supelco 37 Component FAME Mix, 10 mg/mL in dichlormethane and Supelco PUFA No.1 (Marine Source) (Sigma-Aldrich).

C. Proteins Determination by Total TKN Method

Determination of total proteins in microalgae was carried out by TKN method. In this method, organically bound nitrogen is catalytically digested and converted into the ammonium form and distilled with already preexistent ammonium nitrogen by addition of sodium hydroxide and ammonia. Kejadahl Nitrogen-protein conversion factor was 5.95 [16]. All experiments were conducted in duplicate for each sample.

D. Isolation and Characterization of Soluble Protein Fraction

Proteins isolation from microalgae biomass was carried out using alkaline lysis with 0.5 M NaOH assisted by high speed homogenization (21500 rpm for 15 minutes). The ration of biomass to sodium hydroxide was kept at 1:100. Furthermore, re-suspended biomass was incubated for 90 minutes at 55°C, and then centrifuged (14500 rpm at 4°C for 20 minutes). A supernatant that contains crude soluble protein underwent de-colorization by pH adjustment with 0.5 M HCl. Obtained acidified proteins fraction was first kept at 4°C for 60 minutes, and then followed a few centrifugation (14500 rpm at 4°C for 10 minutes) and washing stages. A pellet was dissolved in deionized water and pH value was adjusted to 7.4–7.6. Soluble protein isolates were kept at -20°C for 24 h and then freeze-dried.

Soluble fraction of protein in *Chlorella sorokiniana* was analysed according to Bradford method using Coomassie Brilliant Blue G-250 agent (0.01% Coomassie Brilliant Blue G-250, 4.7% C₂H₅OH, 8.5% H₃PO₄) [17]. Calibration curve for quantitative characterization of the obtained optical density was done using standard stock solution of albumin (fraction V) with the concentration of 0.05 g/L, 0.1 g/L, 0.25 g/L, 0.5 g/L and 1.00 g/L.

All experiments were conducted in duplicate for each sample.

E. Saccharides Determination

The procedure for saccharides determination was conducted according to phenol-sulfuric acid method [18]. Thus, 50 mg of freeze-died microalgae was re-suspended in 10 ml deionized water by high speed homogenizer (21500 rpm for 15 minutes). Obtained suspension was filtrated using syringe filter with the pore size of 0.45 µm. Further, the sample aliquot of 1 mL was mixed with 1 mL 5% w/v phenol solution and 5 mL concentrated H₂SO₄. Obtained solution was mixed immediately and incubated at 30°C for 30 minutes. After the level of obtained solution was adjusted to 50 mL by distillate water, photometric determination at 480 nm and 490 nm for acid and neutral saccharides took place.

Quantitative characterization of acid and neutral saccharides was done using the calibration curve for alginate and dextran stock solutions, respectively.

All experiments were conducted in triplicate.

F. Biogas Formation Potential (GB21) Test

The biogas formation potential test (or GB21 test) for microalgae sample after the extraction of valuable components was done in three parallels using a batch test under mesophilic

conditions described in German standard procedure VDI 4630 [19]. The biogas quantity was controlled on the daily bases by GC with Thermal Conductivity Detector (TCD).

All experiments were carried out for at least 21 days until less than 1% of the total biogas volume that was produced up to that time was formed.

III. RESULTS AND DISCUSSION

A. Results of Lipids Characterization in *Chlorella sorokiniana*

As has already been mentioned above, the total lipids determination was carried out using hot extraction method with or without microalgal cell disruption by high speed homogenization or microwave pre-treatment method. Obtained results for 10 samples using 9:1 and 1:9 w/w hexane-ethanol solvents are presented in Fig. 2.

As can be seen in Fig. 2, under the same extraction conditions (solvent type and cell disruption method), the average concentration of lipids in microalgae *Chlorella sorokiniana* has shown variations within different samples, which might be explained by influence of physical factors such as light intensity and average day/ night temperature over the cultivation season.

A comparison between different solvents for the hot extraction has shown that 9:1 w/w hexane – ethanol solvent tends to have a positive impact on the total lipids yield, along with the time reduction, which is needed for the solvent evaporation (see Section II B). In this case, using the same cell disruption method the total lipids yield can be increased by 0.5% – 1.84%. At the same time, microwave assisted cell disruption improves the total lipids yield compared to not pre-treated samples and high speed homogenization by up to 1.86 % for the same solvent type. Nevertheless, high temperature within the extraction might generally affect fatty acids pattern in obtained lipids extracts, no significant difference was determined by chromatographic FAME analyses of samples that underwent different cell disruption techniques followed by extraction using 9:1 and 1:9 w/w hexane-ethanol solvents. The average fatty acid composition in microalgal lipids extracts is shown in Fig. 3.

From Fig. 3 it can be seen that predominant fatty acids in microalgae are palmitic (C16:w0), Oleic acid (C18:w1), Linoleic acid (C18:w2) and α -Linolenic acid (C18:w3). Therefore, they can be used as a valuable primary component in various food and feed industries.

B. Results of Proteins Determination

In this study, the protein determination in microalgae *Chlorella sorokiniana* was conducted by two different methods, namely TKN and Bradford methods. Obtained results and the comparison of those methods is presented in Fig. 4.

Based on results presented in Fig. 4, the protein concentration in microalgae *Chlorella sorokiniana* was determined to be 39.8% - 47.1%. Similar to lipids, such variations might be explained by weather conditions during the outdoor cultivation process. In also needs to be mentioned, that a relatively good correlation between TKN and Bradford

method can be summarized for all samples, which verify a usage of Kejadahl Nitrogen-protein conversion factor of 5.95 [16].

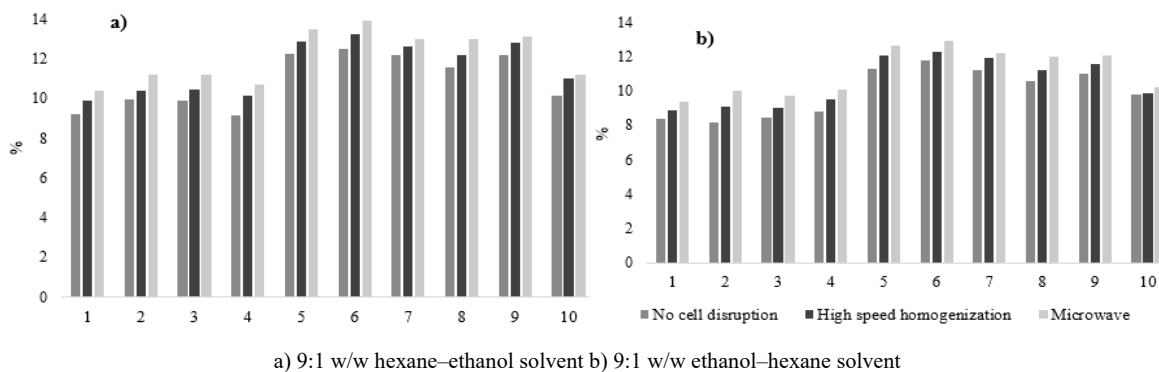


Fig. 2 Total lipids concentration in microalgae *Chlorella sorokiniana* depending on cell disruption method and solvents type. The total lipids concentration presented in Fig. 2 is an average value for triplicate experiments ($p < 0.05$)

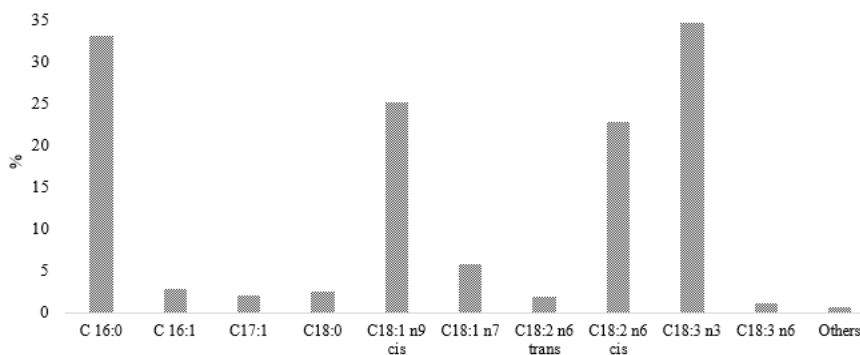


Fig. 3 Fatty acids profile in lipids extracts from microalgae *Chlorella sorokiniana*

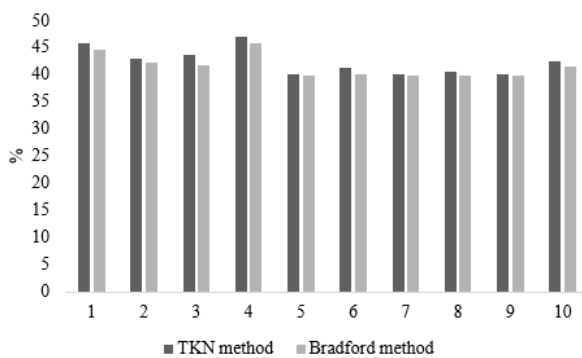


Fig. 4 Protein concentration in microalgae *Chlorella sorokiniana* according to TKN and Bradford methods. The protein concentration presented in Fig. 4 is an average value for duplicate experiments ($p < 0.05$).

C. Results of Saccharides Determination

As has been mentioned in previous chapter (see Chapter II–E Saccharides determination) acid and neutral saccharides were determined by phenol-sulfuric acid method [18]. The saccharides distribution and total saccharides content is shown in Fig. 5.

From Fig. 5, it can be seen that the average concentration of neutral and acid saccharides under the described above

cultivation conditions was 19.5% and 26.1%, respectively. The total concentration was showing slight variations between 42.24% and 46.87%.

D. Biogas Formation Potential of Microalgal Residual Biomass

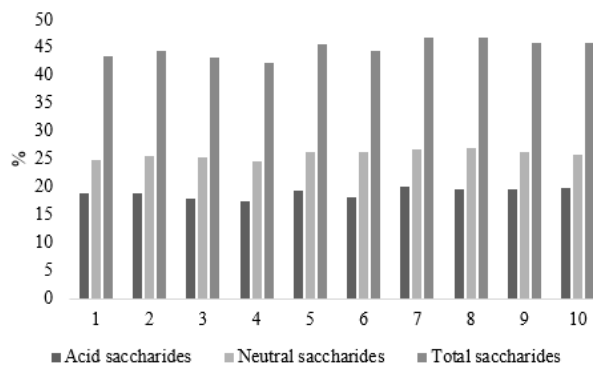


Fig. 5 Saccharides concentration in microalgae *Chlorella sorokiniana*. The saccharides concentration presented in Fig. 5 is an average value for triplicate experiments ($p < 0.05$)

After the extraction of lipids, proteins and saccharides, the residual biomass was consequently used for energy generation by means of anaerobic digestion. Results of the GB21 test for

samples after a certain extraction step is presented in Fig. 6.

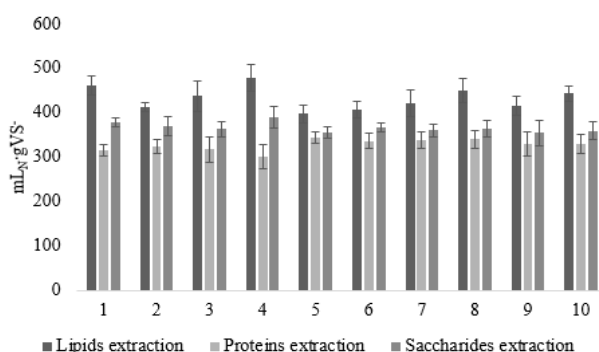


Fig. 6 Biogas formation potential of microalgae *Chlorella sorokiniana* after lipids, proteins and saccharides extraction. Quantification of variation between triplicate experiments is presented as standard deviation (SD)

From Fig. 6, it can be seen that the higher biogas formation potential was observed when residual biomass after lipids extraction was used. In the case of proteins or saccharides extraction, the biogas production drops significantly due to their high concentration in the microalgal cell as well as easier uptake mechanisms by microorganisms.

The biogas composition was mainly represented by methane CH_4 and carbon dioxide CO_2 with the concentration of 48-55% and 45-52%, respectively.

E. Interpretation of Obtained Results Potential and Development of Bioeconomy Concept

Under favorable cultivation conditions with sufficient supply of micro and macro nutrient, microalgae *Chlorella sorokiniana* have shown similar biochemical composition for 10 analyzed samples. For some samples, major environmental effects, which are mainly related to the light intensity and temperature, have caused increase of lipids fraction as a protection mechanism along with decline of protein concentration (see Figs. 2 and 5). As a part of the bioeconomy concept, it might be possible to vary cultivation conditions in order to allow preferable modifications in algal cellular composition. In this study, the cultivation conditions were chosen with an aim to allow step-wise extraction and isolation of valuable components, thus to balance main components such as lipids proteins and saccharides.

Based on obtained results, as a first step of microalgae-based bioeconomy lipids extraction with hexane and ethanol as a co-solvent shall be carried out. Nevertheless, microwave assisted cell disruption is reported to be more efficient method (see Fig. 2), associated costs and its complicity has to be considered for economical assessment of the overall process. Therefore, high-speed homogenization can potentially be used for pre-treatment of microalgae cell before the extraction. Residuals, after lipids extraction might further be used for the proteins isolation. Finally, based on the desirable product residuals after the second bioeconomy step could further be processed to obtain pure saccharides fraction or be used for anaerobic fermentation with an aim to produce CO_2 -neutral

energy. The last option will also allow closing the process loop, since carbon dioxide can be used as a carbon source for the photoautotrophic synthesis of biomass.

IV. CONCLUSIONS

In this research a potential for the development of bioeconomy concept for microalgae was studied. Obtained results have shown that under favorable cultivation conditions, microalgae cell is well balanced and consists of three components that can be extracted in a step-wise approach, namely proteins (40-45%), saccharides (43-47%) and lipids (9-14%). Moreover, with an aim to produce carbon dioxide neutral energy, biomass residues after extraction step might be used for biogas production. In this case, CO_2 can be recirculated as a carbon source for microalgae cultivation, thus closing the loop of the overall technological process.

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