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Original Paper

Possibilities of Production of Third Generation Biofuels in Slovak Conditions

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Microalgae are recognized as one of the most promising raw materials for the production of third generation biofuels because of their fast growth and high energy yields. *Chlorella sorokiniana* is a green microalgae that thrives in wastewater and is also used for its purification. The goal were to verify the effective method of cultivation and subsequent biochemical conversion of green microalgae in open bioreactors, monitoring the amount and quality of biogas produced in an experimental fermenter using the wet fermentation method at the Faculty of European Studies and Regional Development at the Slovak University of Agriculture in 2023. As a result of the experiment, we found that the specific production of biogas per unit of organic dry matter of 4.503 $m^3 \cdot kg^{-1}$ from the grown biomass of green microalgae of the monitored species several times, specifically 4.7 times exceeded the specific production of biogas from first-class corn silage. When converting the specific production of biogas per unit of dry matter, 2.728 $m^3 \cdot kg^{-1}$ represents 3.8 times more compared to the most important raw material for biogas production, corn silage. Third generation biofuels are not yet enough used in Slovakia, but there is considerable potential, as our experiment proved.

Keywords: green microalgae, *Chlorella sorokiniana*, third generation biofuels, fermentation

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1 Introduction

Energy is an important parameter for fulfilling basic human needs, from the food chain to performing various economic activities. Energy is needed at every step of production, from making fertilizers to powering tractors for planting and harvesting. High energy prices and an unpredictable energy market significantly affect input energy costs. The transition to alternative energy sources for energy management in agriculture holds great promise for improving energy efficiency and promoting sustainability in food production (Majeed et al., 2023). In pursuit of a sustainable future, the development of renewable energy technologies has become a focal point of the 21st century. Significant advances in engineering and materials science have pushed renewable resources to the forefront of energy solutions (Nazarov, Sulimin and Shvedov, 2024). An important renewable resource is biomass, which according to the authors Gaduš, Pauková and Prčík (2023) is the fourth largest source of energy after coal, oil and natural gas. Currently, it is the largest and most important renewable energy option and can be used to obtain various forms of energy, but also other bioproducts. As a result, together with other renewable energy options, it is capable of providing all the energy services required in modern society, both locally and in most parts of the world. Among many other aspects, renewables and versatility are important advantages of biomass as an energy source. Moreover, compared to other renewable resources, biomass resources are common and widespread throughout the world. Biomass is defined in the directive of the European Parliament and the Council no. 2003/30/EC as follows: biomass means biodegradable fractions of products, waste and residues from agriculture (including plant and animal substances), forestry and related industries, as well as biodegradable fractions of industrial and municipal waste (Gaduš jr. and Gaduš, 2019; Giertl and Hauptvogl, 2019). Biomass has long been considered the most important renewable energy source in Europe, the use of which contributes to increasing the energy self-sufficiency of states and economic growth (Gaduš, Pauková and Prčík, 2023). Currently, the use of biomass

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for energy purposes is being investigated, in a separate category of third-generation biofuels, which includes green microalgae, which have a wide range of uses and are not primarily needed as food for humans or livestock. Algal biofuels are more compatible with diesel engines compared to lignocellulosic and crop biofuels due to lower environmental impact (Margavelu et al., 2021). In Slovakia, the use of third-generation biofuels in motor vehicles does not exist yet (Gaduš, Pauková and Prčík, 2023).

2 Material and Methods

When processing the work, we used the method of experimental data collection realized in the form of observation of an experiment with the microalgae *Chlorella sorokiniana*, while observation is one of the techniques of data collection by systematic observation and recording of symptoms or research objects. An experiment was a deliberately induced process under controlled conditions that serves to gain or verify experience, knowledge, scientific theories and hypotheses. With the technique of experimental data collection, we gathered data through experimental methods, by manipulating one or more variables, which later affect other variables. Part of the experimental trial consisted in the first part of growing microalgae in a photobioreactor. At the workplace of the Faculty of European Studies and Regional Development of the Slovak University of Agriculture in Nitra (FESRD SUA in Nitra) experimental fermenters (Figure 1) were installed for the so-called batch experiments with basic parameters: a fermenter with a double jacket, a net volume of 100 litters, with electric heating of the heating water, with digital temperature regulation with an accuracy of ± 0.5 °C and an electric slow-moving stirrer with the possibility of setting the mixing time and pauses (in twelve cycles per day lasting 20 to 30 minutes). Subsequently, we measured the amount of biogas produced. The processing of these research data, together with their interpretation, represented the final phase of the experiment.

Figure 1 Fermenter 100 l with with Ritter gas meters at SAU in Nitra in year 2023

2.1 Characteristics of the Species Chlorella Sorokiniana

Lim et al. (2023) state that *Chlorella sorokiniana* is among the viable microalgae for large-scale production, it is also known for its ability to remove pollutants and also for its rapid growth in wastewater (Jareonsin et al., 2023). According to a study by Yadav and Prakash (2024), *Ch. sorokiniana* in combination with abiotic factors able to improve the removal of nutrients from industrial wastewater. As a result, the growth of microalgae based on abiotic factors provides an innovative strategy for successful wastewater treatment and subsequent biodiesel production.

2.2 Cultivation of Microalgae Chlorella Sorokiniana

The experiment with the microalgae *Ch. sorokiniana* took place during the monitored period from 23. 11.–6. 12. 2023 in the premises of SUA in Nitra. We started pouring water in the photobioreactor on November 21, 2023. In addition to the first day, we added liquid fertilizer (Universal liquid plant food) to the water in the photobioreactor 2 more times, on 26. 11. 2023 in the amount of 150 ml and on 3. 12. 2023 in the amount of 100 ml. In addition to fertilizer, we added glucose in the form of grape sugar in the amount of 60 g on 28. 11. 2023. We added microalgae (Figure 2) in a volume of 200 ml to a photobioreactor with a water volume of 300 liters and an area of 2 m^2. We collected the phytomass formed by *Ch. sorokiniana* from the photobioreactor (Figure 3) on 6. 12. 2023. The main advantages of the open microalgae cultivation system are: low investment costs, good gas exchange with the atmosphere (O$_2$ is released), easy operation and possible easy size expansion. On the contrary, he considers the disadvantages: high risk of pollution, high evaporation losses, the need for a large area

Figure 2 A sample of thickened microalgae material *Chlorella sorokiniana*

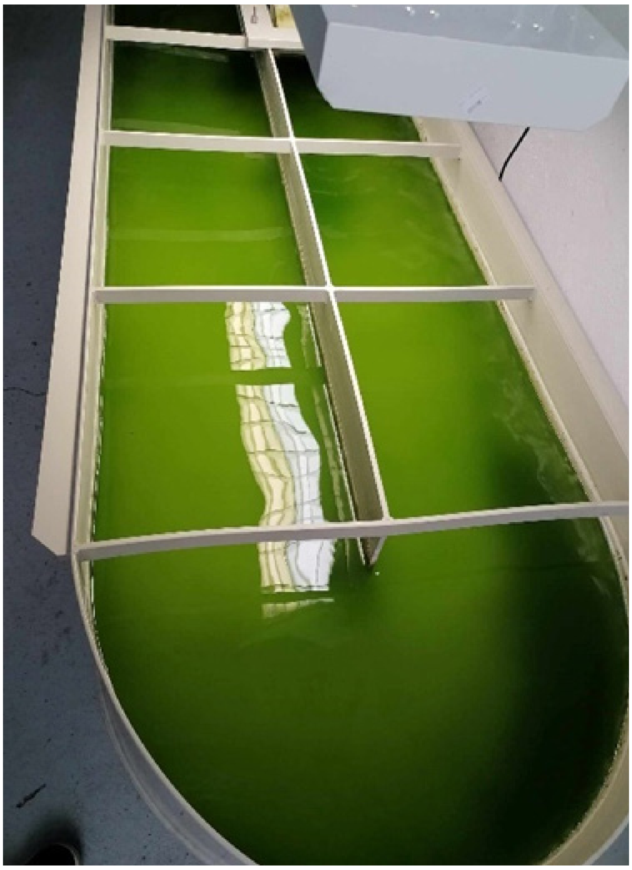

Figure 3 Photobioreactor with microalgae *Chlorella sorokiniana* after a week

and the limitation of light. Both biotic and abiotic factors can affect the yield of biomass and thus the production of biofuels. Light, temperature, pH, nutrients and carbon dioxide are among the abiotic factors, while the type of algae used for cultivation is a biotic factor that can affect yield and production (Glowacka and Gaduš, 2019; Mahmood et al., 2022). The experiment with growing the microalgae *Ch. sorokiniana* lasted 14 days. During the monitored period, we recorded the pH values, environmental temperatures, water temperatures in the photobioreactor, the duration of the additional artificial light, added nutrients, the start time of the CO_2 inflow and the possible top-up of evaporated water. Top-up of hot water took place only twice during the observed period, on 26. 11. and 3. 12. 2023, so that there is a sufficient amount of water in the photobioreactor. We switched on the pressure cylinder with CO_2 manually in the time range from 2–7 hours. We did not add CO_2 on the day of harvest.

2.3 Production of Biogas from Microalgae Chlorella Sorokiniana

We continuously measured and recorded the amount of biogas produced with a Ritter gas meter for small flows with automatic recording using the RIGAMO software. The fermenter was equipped with valves allowing the withdrawal of the substrate during experiments to carry out chemical analyses, as well as to analyze the composition of the produced biogas. Each of the experiments aimed at determining the yield of biogas of the examined substrate was carried out over a period of 30 days. Before dosing into the fermenter, we examined the following parameters in accordance with standard methodology: COD – chemical oxygen consumption ($mg \cdot l^{-1}$), DM – content of dry matter (wt. %), SO_4^2 – sulfate anions ($mg \cdot l^{-1}$) and N_{tot} – total total nitrogen ($mg \cdot l^{-1}$). After dosing the researched material into the fermenter and replenishing the inoculum (taking it from the operating fermenter of the biogas station), we performed analyzes of substrate samples from the bioreactor. We measured the following parameters: TS – content of dry matter (wt. %), ODM – organic dry matter (% TS), NH_4 – ammonia ions ($mg \cdot l^{-1}$), UFA – unsaturated fatty acids ($mg \cdot l^{-1}$), N_{tot} – total nitrogen, pH – value of acidity/alkalinity, TF – temperature in the fermenter (°C). Every day of the experiment, we analyzed the composition of the produced biogas: CH_4 – methane (vol. %), CO_2 – carbon dioxide (vol. %), H_2S – hydrogen sulfide (ppm –

expresses the number of particles per 1 million other particles), O_2 – oxygen (vol. %). In addition, we determined daily biogas production: PBP biogas production ($l \cdot d^{-1}$), CPBP cumulative biogas production (l). H_2S is already 300 ppm lethal for adults. Under certain conditions, in a confined space, hydrogen sulfide can be instantly fatal. At a concentration close to 150 ppm, the sense of smell quickly tires and the characteristic odor changes to a mild sweetness and then disappears completely. In its pure state, hydrogen sulfide can burn easily and produce a light blue flame, any leakage that occurs can cause a fire. For example, 10–500 ppm can cause various respiratory symptoms from rhinitis to acute respiratory failure (Lim et al., 2016; Portillo, 2024).

3 Results and Discussion

Microalgae are used in several sectors, from wastewater treatment, through application in the pharmaceutical industry and nutrition, to the use of biomass for biofuel purposes. An experiment with the green microalgae *Ch. sorokiniana* showed that the algae can produce 2 liters of concentrated green microalgae from the original volume of 200 ml in just 2 weeks, which is 10 times the original volume. Dry matter content 3.02% and organic dry matter content 60.53% of dry matter (Table 1). A change in pH has a variable effect on the biological responses of microalgae. Optimum pH values for microalgae range from slightly more acidic to neutral to slightly alkaline (6.0–7.0–8.0), although some species have pH optima at acidic values below 3.0. Biomass productivity decreases significantly at pH above 9.0 (Chowdury, Nahar and Deb, 2020). In our experiment, the pH value of the input substrate was 7.21. The pH level of water is a critical parameter that affects various environmental processes. The pH of water is crucial for the sustainable use and efficient production of biomass (Dewangan, 2024).

Table 1 Values of monitored parameters and chemical composition of thickened microalgae *Chlorella sorokiniana*

Parameter	Units	Input substrate – microalgae
Temperature	°C	21.00
pH	–	7.21
DM	%	3.02
ODM	% SH	60.53
COD	$mg \cdot l^{-1}$	32,000.00
N_{tot}	$mg \cdot l^{-1}$	83.00
NH_4	$mg \cdot l^{-1}$	40.00

DM – dry matter, ODM – organic dry matter, COD – chemical oxygen consumption, N_{tot} – total total nitrogen

In total, 289.6 l of biogas was produced from the substrate in the fermenter in 30 days, which represents an average daily production of 9.653 l per day, while the contribution to biogas production of 97 l of the inoculum itself was

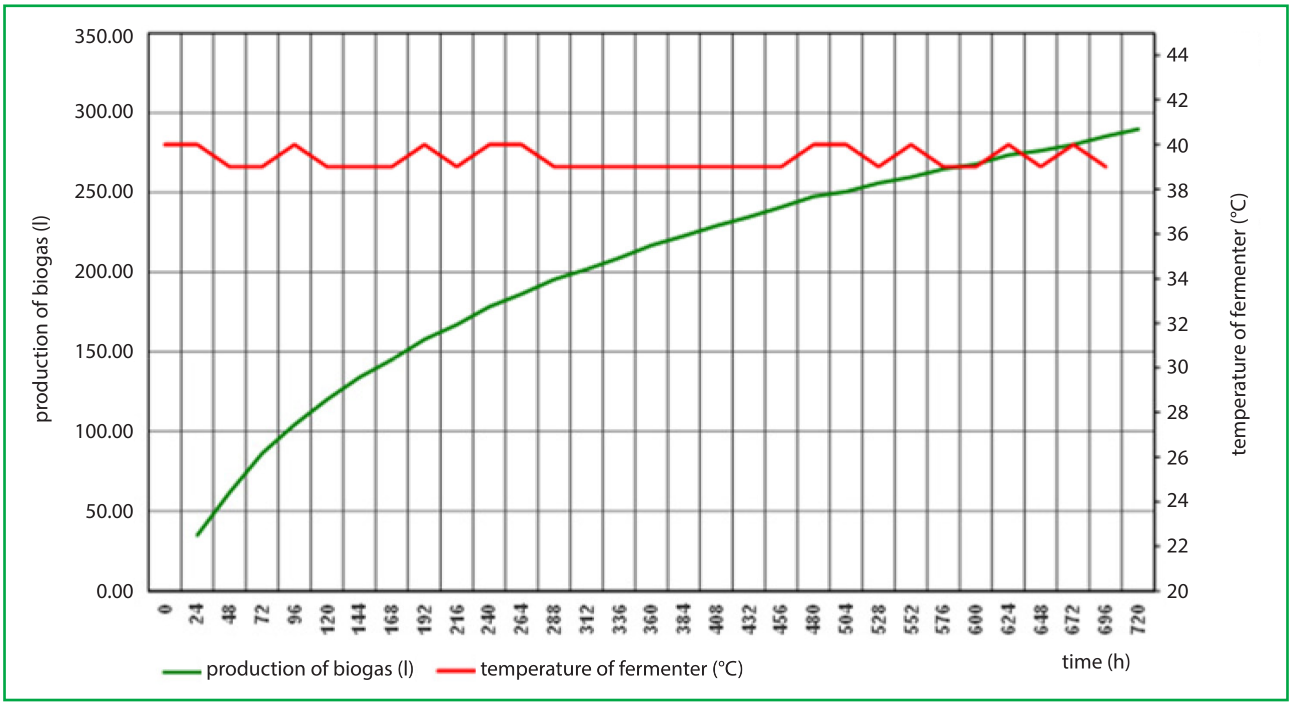

Figure 4 Cumulative production of biogas from microalgae and temperature course in the fermenter where the green line represents the biogas production and the red the temperature in the fermenter

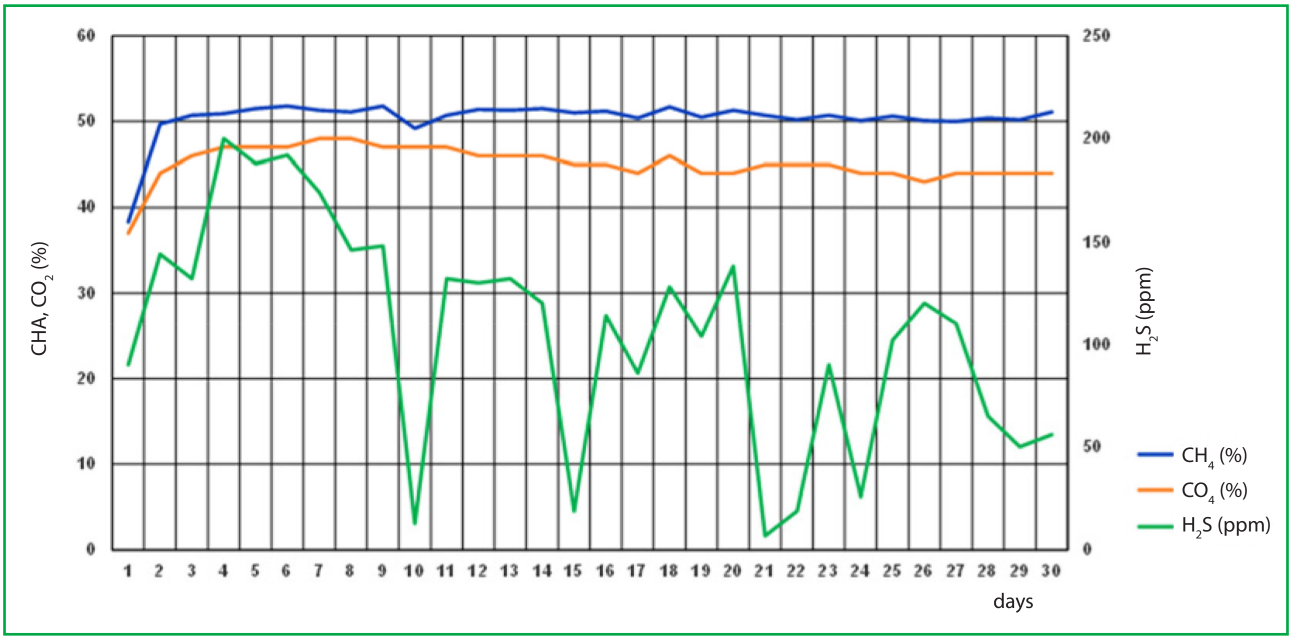

Figure 5 Course of methane, carbon dioxide and hydrogen sulphide content in biogas from microalgae

Table 2 Average recalculated values of biogas production and composition

Input substrate	Total biogas production (l)	Average dose DM substrate (kg)	Average production biogras (m^3·kg^{-1} DM)	Average methane content (vol. %)	Average carbon dioxide content (vol. %)	Average hydrogen sulfide content (ppm)
Microalgae 2 l	164.80	0.075	2.728	50.38	45.10	105.83
Manure 97 l	124.80	1.135	0.110	48.89	43.10	95.27
Corn silage 3 kg	767.09	0.988	0.714	53.98	40.40	315.17

DM – dry matter

124.8 l (Figure 4). We experimentally confirmed that thickened microalgae can be used for biogas production using the wet fermentation method. We achieved acceptable average values of methane content in biogas, namely 50.38%. However, we found the volume of hydrogen sulfide slightly higher than we expected at 105.83 ppm (Figure 5). In this case, desulfurization below 100 ppm and below will be necessary

The supply of 200 ml of microalgae for the production of biogas then represented a value of 164.8 l, which meant an average daily production of 5.493 l per day. When converting the biomass dose to the value of dry matter based on the determination of the dry matter content of thickened microalgae, which was 3.02%, the dose of dry matter and organic dry matter of microalgae of 60.53% of the dry matter (DM) to the fermenter was as follows – 0.0604 kg of dry matter of microalgae and 0.0366 kg of organic dry matter (ODM) of microalgae. The average total production of biogas per unit of dry mass of microalgae biomass was 2.728 m^3·kg^{-1} of dry mass and 4.503 m^3·kg^{-1} of organic dry mass (Table 2). The specific production of biogas per unit of organic dry matter from the grown biomass of green microalgae *Ch. sorokiniana* was 4.7 times higher than the specific production of biogas from first-class corn silage. When converting the specific production of biogas per unit of dry matter, it is 3.8 times compared to corn silage. Corn silage is produced once a year, takes up fields and is food for humans and farm animals, while the green microalgae *Ch. sorokiniana* is grown in a photobioreactor, which can be placed in any place and from a time point of view, cultivation usually has an interval of 14 days and maybe even shorter.

4 Conclusions

Biofuels of the third generation thus represent considerable progress in obtaining biofuels and at the same time in leaving important raw materials for other uses. In any case, microalgae with easy cultivation can continue to be used in various sectors of interests and it will not have such an impact on the planet Earth and its inhabitants as, for example, with corn. As a result of the experiment of biochemical conversion of green microalgae *Chlorella sorokiniana* in open bioreactors using the wet fermentation method at the Slovak

University of Agriculture, we found that the specific production of biogas per unit of organic dry matter of 4.503 $m^3 \cdot kg^{-1}$ from the grown biomass of green microalgae of the monitored species was 4.7 times higher than the specific production biogas from first-class corn silage. When converting the specific production of biogas per unit of dry matter, 2.728 $m^3 \cdot kg^{-1}$ represents 3.8 times compared to the most important raw material for biogas production, corn silage.

References

Dewangan, S. K. (2024). pH of Water and Its Relationship to Soil: A Comprehensive Analysis.

Gaduš, J. jr. & Gaduš, J. (2019). *Vplyv európskej a slovenskej legislatívy na rozvoj regionálnej bioenergetiky*. (172 p.) Verbum Praha.

Gaduš, J., Pauková, Ž., & Prčík, M. (2023). *Udržateľná výroba a využitie biomasy*. (222 p.) Verbum Praha.

Giertl, T., & Hauptvogl, M. (2019). *Verifikácia možností využívania rýchlorastúcich drevín na výrobu biopalív*. (72 p.) Slovenská poľnohospodárska univerzita v Nitre. https://doi.org/10.2478/aree-2019-0004

Glowacka, N., & Gaduš, J. (2019). Verification of the green microalgae biomass use for biogas production. *Acta regionalia et environmentalica*, 16(1), 15–19.

Chowdury, K. H., Nahar, N., & Deb, U. K. (2020). The Growth Factors Involved in Microalgae Cultivation for Biofuel Production: A Review. Computational Water, Energy and Environmental Engineering, 9, 185–215. https://doi.org/10.4236/cweee.2020.94012

Jareonsin, S. et al. (2023). Dark mode of microalga – A sustainable and economical solution for microalgal biofuel production and waste treatment. *Bioresource Technology Reports*, 23. https://doi.org/10.1016/j.biteb.2023.101574

Lim, H. R. et al. (2023). Evaluation of Real-Time Monitoring on the Growth of Spirulina Microalgae: Internet-of-Things and Microalgae Technologies. *IEEE Internet of Things Journal,* 3274–3281. https://doi.org/10.1016/j.biotechadv.2022.107931

Lim, E. et al. (2016). Effect of environmental exposure to hydrogen sulfide on central nervous system and respiratory function: a systematic review of human studies. *International Journal of Occupational and Environmental Health*, 22(1), 1–11. https://doi.org/10.1080/10773525.2016.1145881

Mahmood, T. et al. (2022). Sustainable production of biofuels from the algae-derived biomass. *Bioprocess and Biosystems Engineering*, 46, 1–21. https://doi.org/10.1007/s00449-022-02796-8

Majeed, Y. et al. (2023). Renewable energy as an alternative source for energy management in agriculture. *Energy Reports*, 10, 344–359. https://doi.org/10.1016/j.egyr.2023.06.032

Margavelu, G. et al. (2021). Transgenicism in algae: Challenges in compatibility, global scenario and future prospects for next generation biofuel production. *Renewable and Sustainable Energy Reviews*, 154. https://doi.org/10.1016/j.rser.2021.111829

Nazarov, A., Sulimin, V., & Shvedov, V. (2024). Renewable energy sources: global implementation experience. *E3S Web of Conferences* (474 p.) https://doi.org/10.1051/e3sconf/202447401030

Portillo, G. (2024). *Sírovodík*. Available: https://www.meteorologiaenred.com/sk/s%C3%ADrovod%C3%ADk.html

Smernica európskeho parlamentu a rady č. 2003/30/ES o podpore používania biopalív alebo iných obnoviteľných palív v doprave. Available: https://eur-lex.europa.eu/eli/dir/2003/30/oj

Yadav, K., & Prakash, M. (2024). Green innovation unites biodiesel production with bioremediation of waste metal cutting fluid using *Chlorella sorokiniana*. *Biomass Conversion and Biorefinery*, 1–13. https://doi.org/10.1007/s13399-024-05316-8