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Study of green microalgae as a feedstock for biodiesel production

ABSTRACT

Biodiesel as an energy source has marked an edge to a growing energy crisis issue. There are multiple ways by which biodiesel can be produced, and aggressive researchisgoing on in the field of biodiesel. In this work, we have focused on biodiesel production from microalgae. Green microalgae, a third-generation feedstock, are promising candidates for biodiesel production because of their high lipid content and rapid growth. In this study, for the cultivation of microalgae, an environment of varying temperatures that was between 27°C to 32°C was created. Also, three different concentrations (2:2, 2.5:1.5, and 3:1) of aquarium and freshwater were considered in this work for algae growth, and the lipid extraction method like mechanical cell destruction was investigated to determine its efficiency. Once the lipid extraction process was optimized, the extracted lipids were subjected to a transesterification process, converting them into biodiesel. The results of this study show that the greater concentration of aquarium water resulted in better algae production, i.e., dried weights of algae extracted from the above-considered concentrations were 2.69 grams, 2.79 grams, and 2.92 grams respectively. Biodiesel produced from the dried algae was 3.15 ml, 3.96 ml, and 4.95 ml, respectively. These results suggest that green microalgae can be considered an enticing raw material for biodiesel production. Optimizing cultivation and lipid extraction methods can improve biomass and lipid productivity, boosting overall biomass yield. This study concludes that algal biodiesel can be an alternative source to petroleum-based diesel fuel.

Keywords: *Biodiesel; microalgae; lipid extraction; biomass; temperature*

1. INTRODUCTION

The global energy consumption through energy sources like petroleum, coal, and diesel in the last thirty years has increased very drastically with an average annual increase of 3.4 %, which is almost 1.2 % points higher than the average growth of energy consumption (1). Humans fulfill their energy demand by burning fossil fuels, as they are easy to use, provide high energy density, and have a low cost compared to any other alternative energy source or non-conventional energy source. However, prolonged use of petroleum-based fuels has led to the emission of greenhouse gases like carbon dioxide at a high rate, causing acid rain and global warming—the earth's mean temperature increased by an average of 0.14 °F per decade since 1880, or about 2 °F in total (2).

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Also, because of the continuous use of fossil fuels, their reserves are depleting fast, so they may not last more than a hundred years from now. According to the Millennium Alliance for Humanity and the Biosphere (MAHB) report, the world's oil reserves will run out by 2052, natural gas by 2060, and coal by 2090(3). Therefore, several research projects and experiments are being carried out to find different alternative sources for the fulfillment of energy demand that can be renewable, economical, and environmentally friendly. It includes solar energy, wind energy, geothermal energy, and biofuels. A lot of innovative work is going on in these fields (4–7), and in the future, they will change the pattern of energy production.

The transport sector uses approximately 25% of the total energy consumption and islargely dependent on petroleum (8). Fortunately, biofuels can be used as an alternative to diesel engines. Biofuels are combustible fuels produced from renewable biomass resources, i.e., agricultural or forest products, animal waste, and biodegradable portions of industrial waste. Biofuels are classified into two categories: primary and secondary biofuels, based on whether biomass undergoes processing or remains unprocessed to be used as biofuel. Primary biofuel (solid biofuel) is an unprocessed form of biofuel generally used for cooking, heating, and electricity generation. Wood pallets, charcoal, sugarcane, corn, maze, etc. are a few examples of primary biofuels. On the other hand, secondary biofuel is produced by processing biomass through fermentation or transesterification processes(9).It includes liquid and gaseous forms of biofuel, such as biodiesel and bioethanol in the liquid phase, while biogas and biomethane in gaseous form can be used as an alternative source for petroleum-based fuels in the transport and industrial sectors. Assuming that in the future there will be high demand and extensive use of biofuel, which will require a much greater supply of feedstock on a sustainable basis. To fulfill that demand, there is considerable enthusiasm that is being taken to include many potential biomass sources other than edible crops to increase the production of biofuel at a large scale while avoiding the conflict of fuel versus food security. In this context, biofuel feedstock is classified into four generations depending on the characteristics of biomass feedstock and the technology involved in biomass processing. First-generation feedstocks like corn, hay crops, etc. undergo distillation, hydrolysis, transesterification, and fermentation technology, but they pose a threat to the food crisis. While second-generation feedstock contains all the non-edible crops such as rice husk, jatropha, agricultural residue, etc. They produce biofuels from expensive technologies like thermochemical and complex biochemical. Third- and fourth-generation biofuel feedstocks are microorganisms or microalgae and modification of microorganisms genetically, respectively.

This study focuses on third-generation biofuel feedstock microalgae. Biodiesel produced from microorganisms through the transesterification process is a better alternative to conventional diesel fuels. It is renewable and biodegradable and is considered a ‘carbon neutral fuel’ because its net emission of greenhouse gases is almost nil. Biodiesels have the property of high lubricity, which, when used in diesel engines, increases the vehicle's life span. Biodiesel can be used in its pure form as well as in blended form, where it is blended with petroleum diesel. According to the Mubarak et al.(10), the pure (unblended) biodiesel that is used is termed B100 and when it is blended with fossildiesel fuel,in different ratios, it is termedB2, B5, and B20 where the numberindicates the percentage of biodiesel blended with the fossil diesel fuel withshares2%, 5%,20% and100% in agallonofdiesel fuel.

Microalgae is a unicellular or eukaryotic photosynthetic microorganism that has a simple cell structure that uses sunlight, water, carbon dioxide, and materials like phosphorus and nitrogen as their main nutrient for its growth. They can easily be found in seawater as well as fresh water. Microalgae particularly have high oil content and grow rapidly, so they can double their mass in 24 hours(11).Microalgae have great flexibility and adaptability to grow even in diverse environments. In other words, it uses less arable land than any of the other terrestrial plants; therefore, the conflict with food production is not the issue here. The growth rate of microalgae is about 5–10 times faster than any conventional food crop. Moreover, microalgae have high lipid productivity which is the major reason for using it as analternative to biodiesel feed stock (11).According to a study, microalgae can have 15-300 times more lipid or fat productivity than any common oil crop (12).

In recent years, microalgae have attracted much more attention for producingbiodiesel.

At present, several studies are being carried out with different compositions of biodiesel from different species of microalgae. A California company, Salarymen, has already generated tens of thousands of gallons of algae-based fuels as part of their research and development agreement (13). In a study conducted by Lim et al., they successfully converted microalgal oil into bio-jet fuel using a hydroprocessing technique(14). Previous studies performed by Maria et al., Yanan et al., Sushant et al., and Jassinnee et al.have proven that different species of microalgae have a great potential to produce biodiesel(15–18). According to the literature review(19–22) marine and freshwater species of microalgae should be considered to produce biodiesel because of their high lipid content.However, in recent yearsstudies conducted by Tahir et al., Gang et al., and Alejandra et al.revealed that algae cultivated in wastewater also have the potential to produce a significant amount of biodiesel, and algae can also effectively remove the toxic elements from the wastewater, playing an important role in wastewater treatment (23–25). Their capability of wastewater treatment and being a rich source for biodiesel production makes them suitable sources to be grown on a large scale (26).

However, this study focuses on microalgae cultivation in different compositions of aquarium and freshwater and its impact on lipids. It also gives a picture of the filtration methodused for microalgae harvesting,and a brief description of thebead-beating oil extraction technique, where a household blender RPM 18000 is used for cell disruption. Furthermore, this study also discusses the production of the catalyst potassium hydroxide (KOH)through rainwater. So far, this is the first-time aquarium and freshwater composition have been discussed for algal cultivation. Though this study was performed on a small scale, it can be implemented on a large-scale project because it is economical and achievable.

2. MATERIAL AND METHOD USED

The material and methods used in this study are mentioned below:

2.1.Algae production process

Algae can be cultivated in open ponds, conventional bioreactors, and photobioreactors through phototropic, heterotropic, mixotrophic, and photo-heterotropiccultivation processes. Abrief presentation of the different stages of biodiesel production is shown in Figure 1. In this study, microalgae were cultivated for biodiesel production. The cultivation required sunlight, water, carbon dioxide, and nutrients in a 19:19:19 ratio of nitrogen, phosphorus, and potassium (19:19:19 NPK). This water-soluble fertilizer mixture is a product of the Indian Farmers Fertilizer Co-operative Limited (IFFCO).

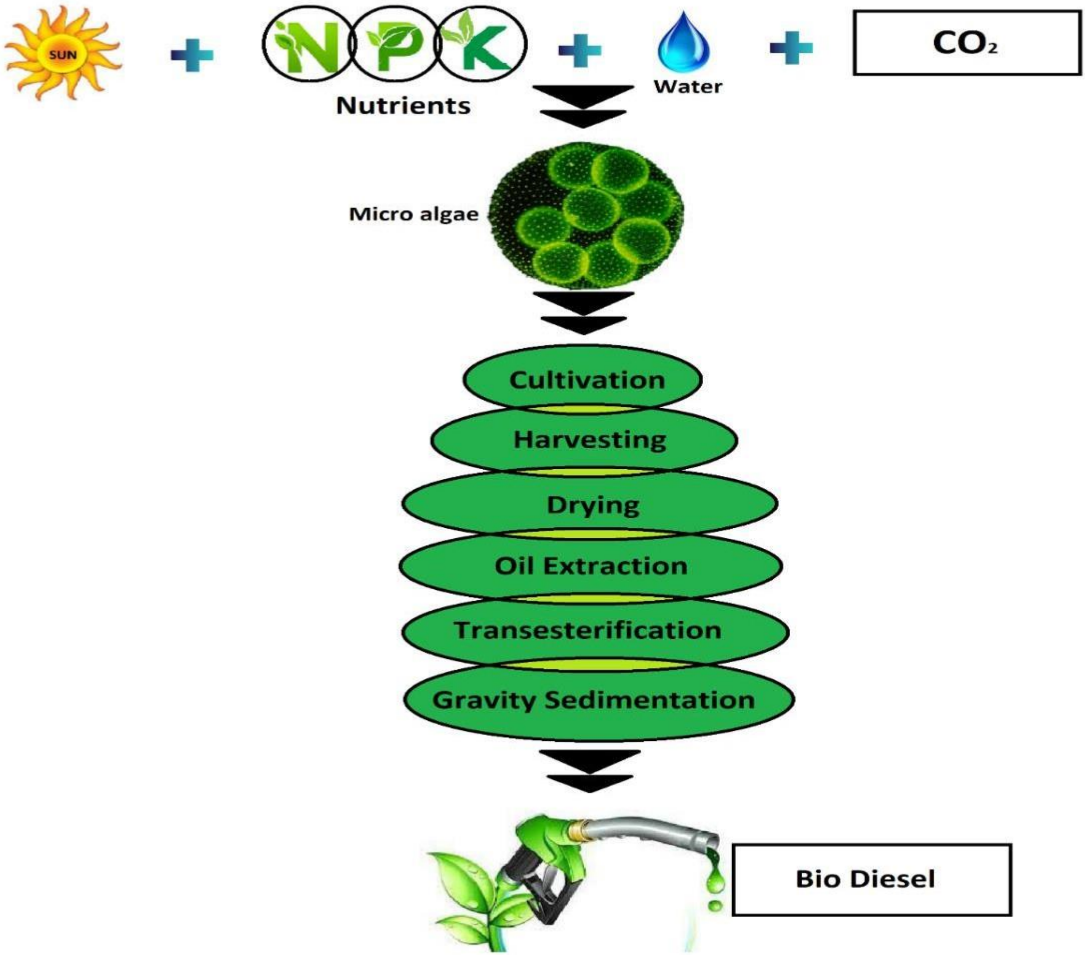


Figure 1. Different steps of biodiesel production

2.1.1.Preparation of culture media

There are two most common microalgal cultivation systems:(i) openmicroalgalcultivation systems and (ii) closed microalgal cultivation systems (27). The proper light and temperature are required for algal growth with the other specific components in both culture systems. In the closed cultivation system, different types of enclosed photo-bioreactors areused for the growth of phototrophs like microalgae under controlled conditions. In this work, for the production of microalgae, fish aquarium water that was stored for28 days was mixed with fresh water (213 TDS) in differentratiosi.e. 2:2,2.5:1.5, and 3:1 in three different glass jars (Figure2) and 5 ml of micro-algal sample were added in each concentration. Continuousshaking was administered,so it didnotproduce excessive CO_2.

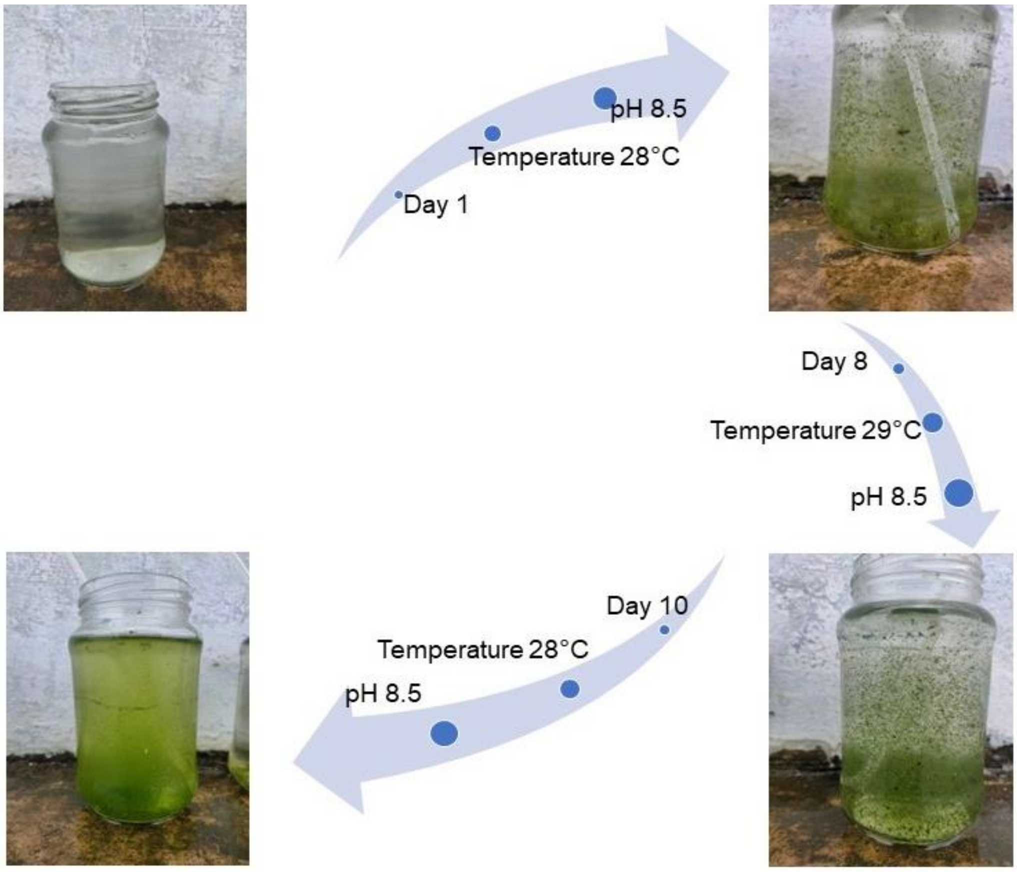


Figure 2.Stages of Microalgae Cultivation

2.1.2. Culturing Conditions

Microalgal growth depends on light, carbon dioxide, pH value, temperature,andthe presence of materials like phosphorus and nitrogen. The basic condition for algae cultivation is, the presence oflight wavelengthbetween 600nm to 700nm, a temperature range between 10°C–35°C, a pHvalue between 7-9, and a water salinity range between 12–40 g/l issuitable.During the experiment, to maintain the temperature and avoid the production of excess CO_2 in culturing media, the Orbital Shaking Incubator (tabletop VSLI-144 model) was used. In this work, microscopic green algae were used,the selection of this algae is based on itsrelatively high lipid accumulation in comparison to red algae and brownalgae. However, red algae can be used as a key for accelerating biodiesel production.

2.1.3. Monitoring culture growth

During the experiment, temperature and pH valueswere monitoredand maintained between 27°C-32°C and 8.5 respectively. This cultural growth was monitored for 16 days.

2.1.4. Microalgae Harvesting

Algal harvesting has many different harvesting techniques such as gravitysedimentation, filtration, floatation, and centrifugation (28). In this experiment, the filtration technique was used as it does not require any kind of installation, has quite high recovery efficiency, and produces much more clear material than the sedimentation process. The micro-algal culture of the jar was poured on the filter paper (Weightoffilterpaper=0.23 g) placedover the other jar and we waited till the complete draining. The green slime found on the filterpaper (Figure3)wasalgalbiomassthatwasfurther used foroil extraction. The total dry weight of algae is calculated using the given formula:

Total dry weight of algae = Weight of filter paper without filtrate - Weight of filter paper without filtrate (1)

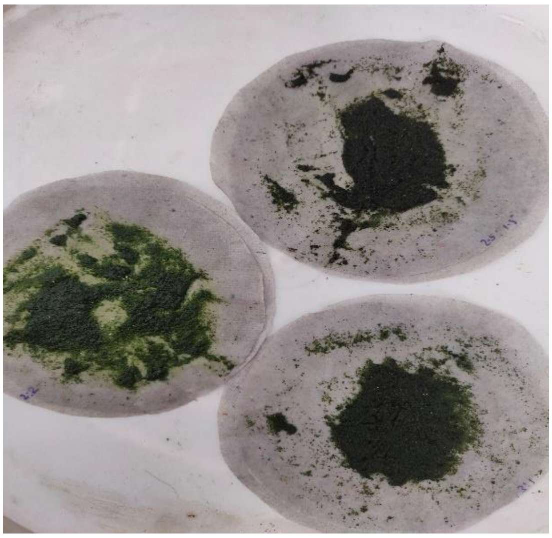


Figure 3: Algae Sample for Different Concentrations

2.1.5. Oilextraction from microalgae

There are several oil extraction methods to extractlipids from microalgae, but all those methods areclassified into two broad categories: (i) the Mechanical OilExtraction Method and (ii) the Chemical Oil ExtractionMethod.However, in recent years, some scientists classifiedthe oil extraction method for microalgae into threemore different groups that are based on Enzymes i.e. (i) Enzyme-Assisted Method, (ii) Accelerated Solvent Extraction (ASE),and (iii) Microwave-Assisted Extraction (MAE) (29)In this work, bead beating a mechanical method was used for lipid extraction during the experiment. This method refers to the disruption of microalgae cells, where damage isdirectly caused to the cell by high-speed spinning. There are two very common types of bead millmethods, the Shaking VesselMethod (SVM) and the Agitated Beads Method (ABM) (30). In shaking vessels, the cells aredamaged by continuous shaking of the entire vessel on a vibrating platform whereas a rotating agitator is fixed inside the culture ves sel which damages the cells of microalgae in ABM.

A complete ABM technique is shown in Figure 4.Duringtheexperiment,due to the unavailability of oil extraction equipment, a household blender (RPM 18000) was used for breakingcells of microalgae usinga mechanical technique and water was addedto form a solution. Then it was left to be settled. Oil was separated as itformed a layer at the top of the jar (Figure5). The volume of the algal oil was initially measured**,** and the volume yield per liter was determined using the formula given below:

$$Concentration\ of\ algae\ per\ litre = \frac{Dry\ weight\ of\ algae}{Algal\ water\ collected} \times 1000 \qquad (2)$$

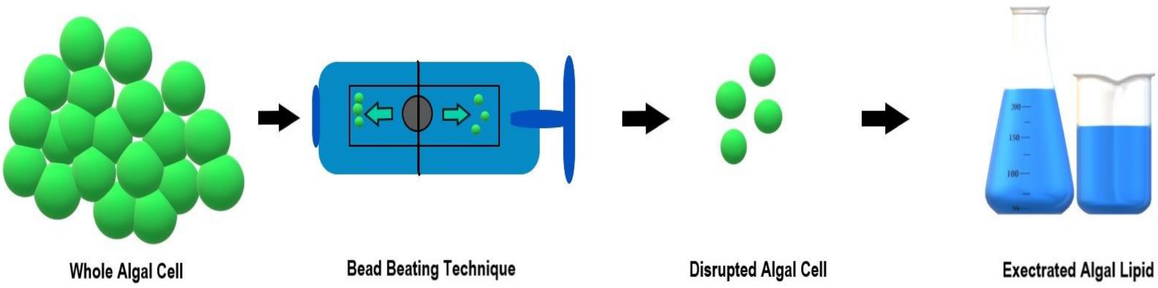


Figure 4.Bead Beating Oil Extraction Technique

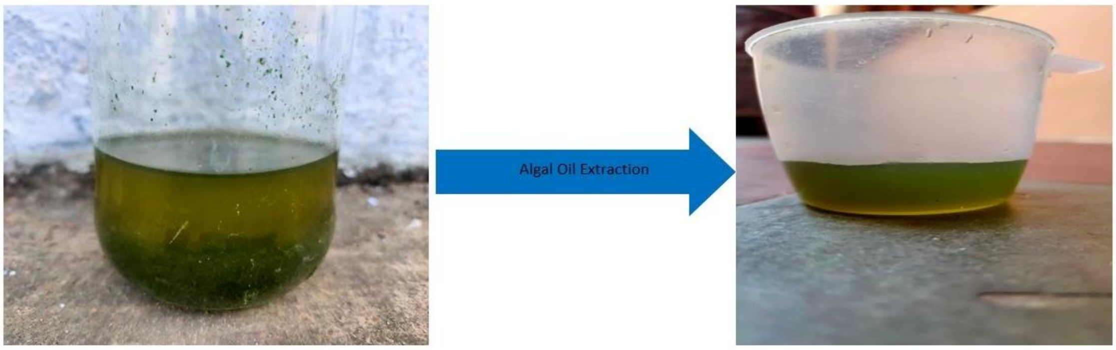


Figure 5. Oil Extraction

2.1.6. Preparation of catalyst KOH for thetransesterification process

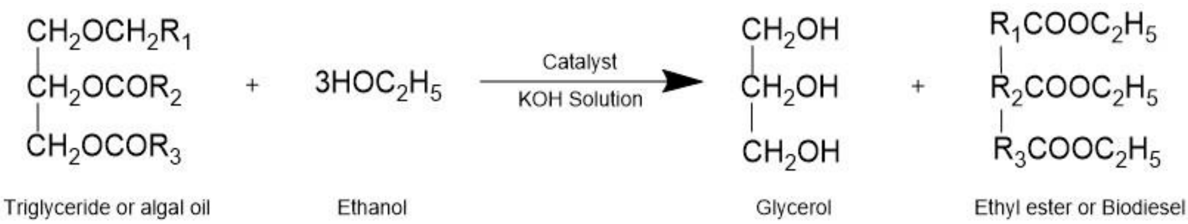
For this study potassium hydroxide solution was made from white ashes of hardwood. When hardwood trees like sugar maple, buckeye, and beech grow they extract potassium from the ground(31) In this experiment, Indian Horse Chestnut tree ashes were used as the potassium present in the wood does not burn with fire and is in the ashes.

Figure 6: Preparation of Potassium Hydroxide Solution

The second thing we need is soft water, in this work, rainwater is taken as soft water because it is ideal for preparing KOH solution as it has a lower concentration of other components and is available in large quantities. After this layers of pebbles, dried straw, hardwood ashes, and rainwater are filled in a disposable glass from bottom to top (31) as shown in Figure 6. The glass has holes at the bottom, for the drainage of water. This drained water is collected, and the pH value is testified. If the pH value is 13, it is a strong KOH solution but if its value is less, then the entire water is again poured into the barrel. In this work, 15 ml of rainwater with 10 g of hardwood ashes was taken to prepare the catalyst that produced 12.5 ml of potassium hydroxide (KOH) solution with a pH of 13.48.

2.1.7.ConversionofMicro-AlgalLipidtoBiodiesel

Following the oil extraction process, the resultant micro-algal lipidor oilcan be converted into biodiesel by any of the mechanical or chemical methods.However, in this study lipid is converted into biodiesel by theprocess of transesterification which is a chemicalprocess where triglyceride (TAG) i.e. algal oilwas reactedwith ethanolin the presence of catalyst i.e. KOH resulting in the formation of biodiesel andgly cerolasaby-product (Figure7).



R1, R2& R3 represent the chain of fatty acids in TAG and ethylester.

2.1.8. SeparationofBiodieselandGlycerinbyGravitySettlementMethod

After the transesterification process, the mixture of glycerol and ethylesteriskeptforapproximately 24 hourstoseparate biodieselandglycerin. Figure7shows the separation of biodiesel from glycerin.

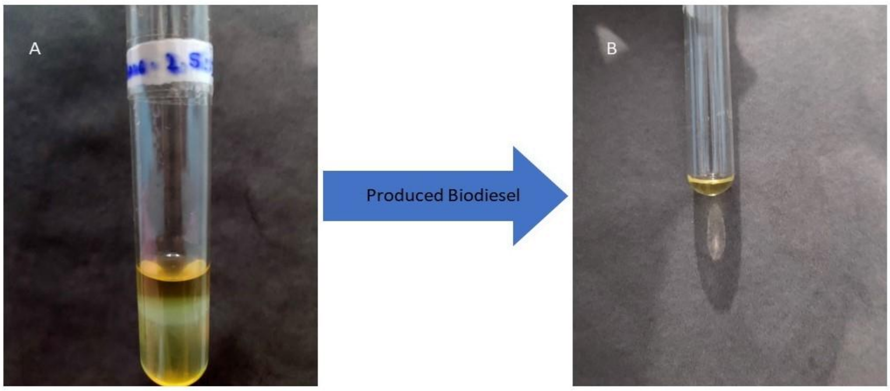


Figure 7.(a) Transesterification process, (b) Produced Biodiesel

3. RESULT AND DISCUSSION

The amount of cultivated algae, algal oil extraction, andbiodiesel produced for 2:2, 2.5:1.5, and 3:1 concentrations are shown in Figures.

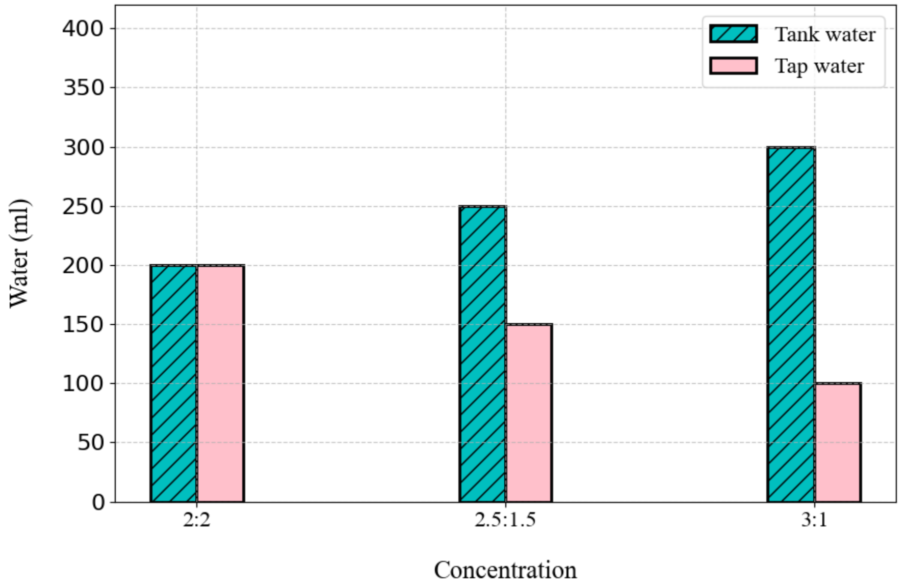


Figure 8. Proportion of aquarium and freshwater for different concentrations utilized for the experiment

The previous studies conducted by Sanchez-Bayo et al. and Osundeko et al. (19–22), they had cultivated algae in seawater, freshwater, and wastewater.However,this study cultivated algaeusingaquarium water and freshwater composition.Figure8 shows the composition of aquarium water (tank water) and freshwater (tap water) used at three different concentrations for algae production.The x-axis represents the different concentrations (2:2, 2.5:1.5, and 3:1), while the y-axis represents the volume of water in milliliters (ml).For 2:2 concentration 200 ml of aquarium and 200 ml of fresh water were taken while for 2.5:1.5 concentration, 250 ml of aquarium and 150 ml of fresh waterwere used. And for 3:1, 300 ml of aquarium water and 100 ml of fresh water wereemployed.The blue hatched bars indicate the volume of aquarium water, and the pink bars show the volume of freshwater used in each concentration.

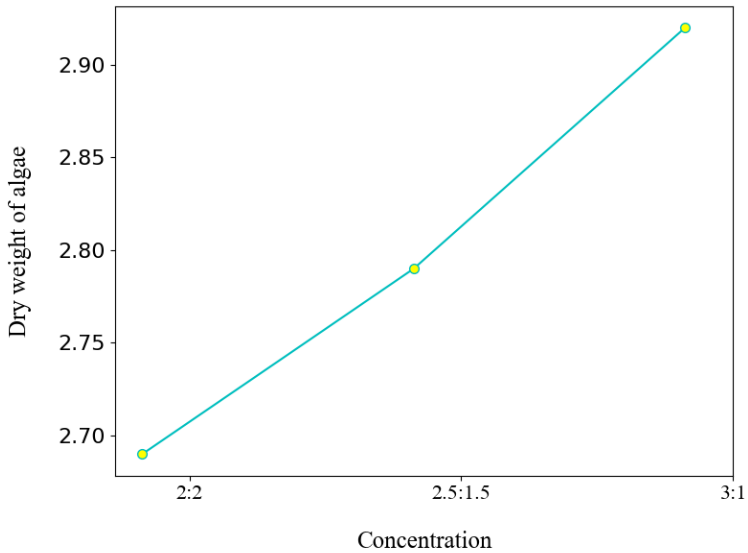


Figure 9.Dry weight of Algae Produced from different Concentrations.

The dry weight of microalgae produced from different concentrations is represented in Figure 9. The graph indicates a positive linear relationship between the concentration ratio of aquarium water to fresh water and the dry weight of algae. We can see that a higher amount of 2.92 g microalgae is produced in concentration 3:1 while in concentration 2:2 lower amount of 2.69 g microalgae was cultivated.As the proportion of aquarium water increases relative to fresh water, the dry weight of algae also increases. This suggests that higher concentrations of aquarium water led to greater algae cultivation.

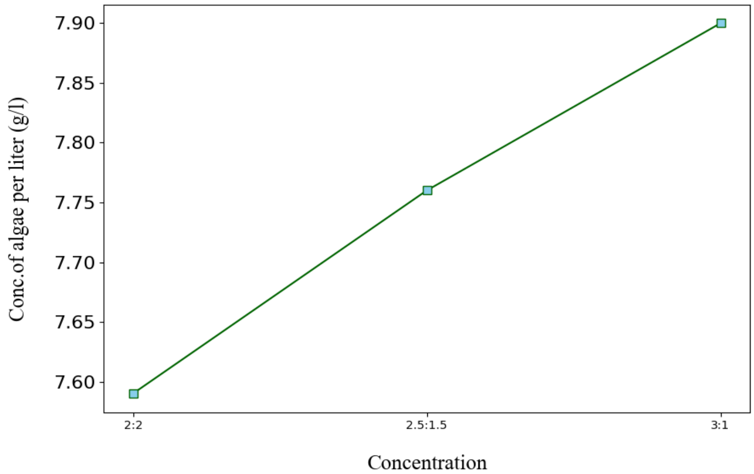


Figure 10. Concentration of Algae PerLiter (g/l) for Different Concentrations

In the experiment performed by Amit et al.(32) they took four algal strains and produced biomass yield per liter- 0.65 g/l, 0.37 g/l, 0.35 g/l, and 0.32 g/l in STS (secondary-treated sewage), PTS (primary-treated sewage), CWW (IIT Roorkee Saharanpur campus wastewater), and PWW (paper mill wastewater)respectively. In their experiment, STS had the highest algal biomass yield. Anotherexperiment performed byVíctor et al. produced 1.69 g/l and 2.25 g/l from two algae strains *Chlorella* and *Nannochloris* respectively and had the highest biomass yield in the Nannochloris algae strain(33). Whereas, in this study, algal biomass found was 7.59 g/l, 7.76 g/l, and 7.90 g/l for three different concentrations of aquarium and freshwater. Thisamount of algae per liter is calculated for the different concentrations using equation (2) and is shown in Figure 10. However, the concentration of algae per liter depends on the produced algal biomass. The concentration of algae per liter was higher for concentration 3:1 while it was lower for 2:2 concentration.

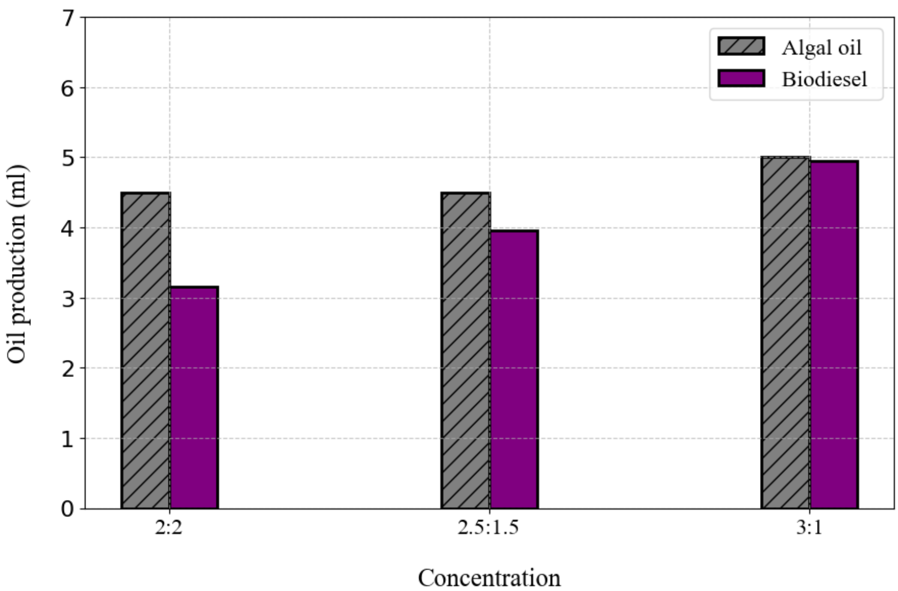


Figure 11. Algal Oil and Biodiesel Produced from Different Concentrations

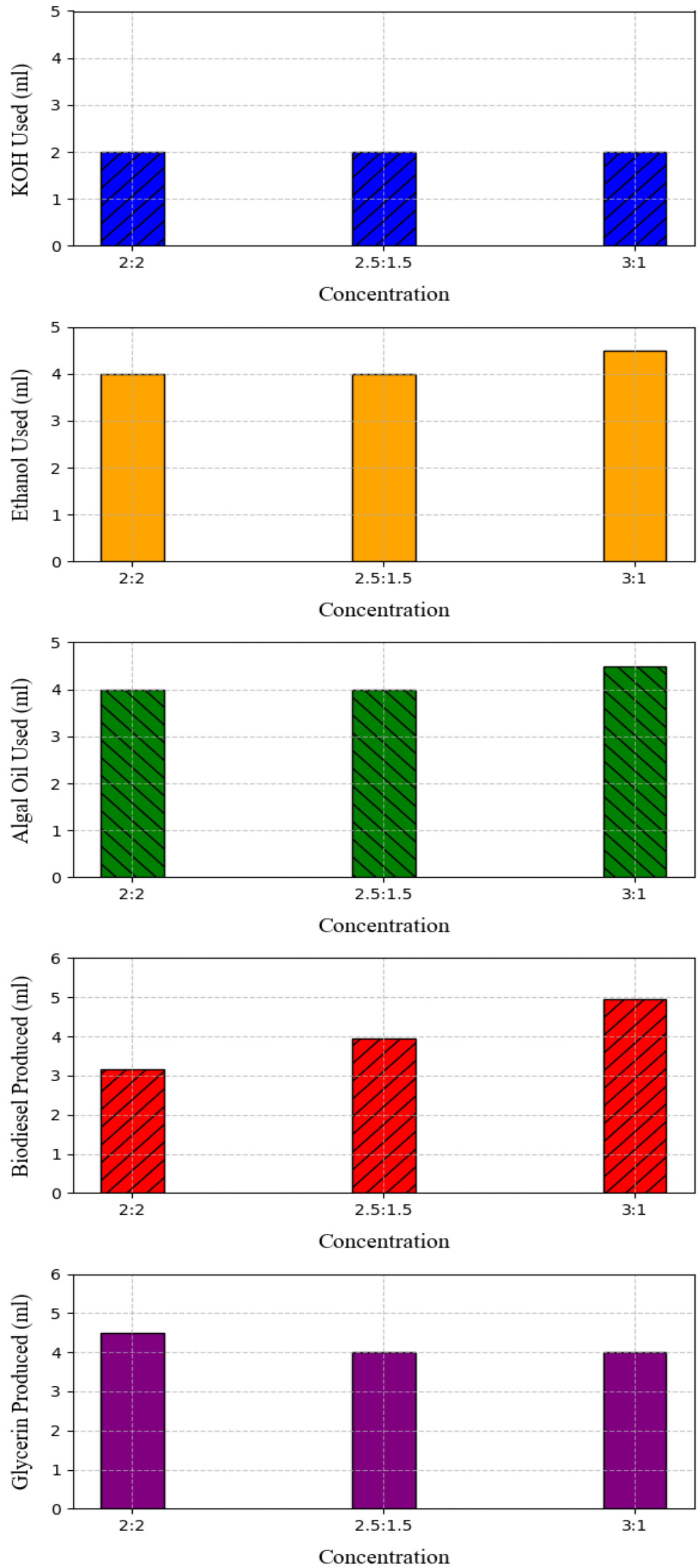


Figure 12.Chemicals Used for Biodiesel Production

The algal oil extracted from algal biomass and biodiesel produced from algal oil are shown in Figure 11. As we can see, algal oil extracted from different compositions of aquarium and freshwater is high in 3:1 concentration i.e. 5 ml, the biodiesel produced from algal oil is also high in this concentration i.e. 4.95 ml produced by bead beating oil extraction technique. Algal oil produced in 2:2 and 2.5:1.5 was in equal amounts i.e. 4.5 ml, while biodiesel produced in 2:2 was the least 3.15 ml. Also, the produced biodiesel from 2.5:1.5 concentration was 3.96 ml. On comparing our study's results with experiment results performed by Reem et al., the total algal oil content was found to be 5.8+- 0.16% through the Folch oil extraction method, and the produced biodiesel yield was 19.3% (34).

Figure 12 shows the usage of KOH, ethanol, and algal oil and the resultant production of biodiesel and glycerin across three different concentrations 2:2, 2.5:1.5, and 3:1. For each concentration 2ml of KOH was used. The algal oil and ethanol were in equal amounts for 2:2 and 2.5:1.5 concentration i.e. 4 ml each while for concentration 3:1 their amount was increased to 4.5 ml each so that we can observe the changes in the biodiesel production reaction. The production of biodiesel increased with higher concentrations, yielding 3.15 ml for 2:2, 3.96 ml for 2.5:1.5, and 4.95 ml for 3:1 concentration. The production of by-product i.e. glycerin was relatively constant for 2.5:1.5 and 3:1 concentration, both producing 4.0 ml of glycerin, whereas it was slightly higher for 2:2 concentration. We have analyzed, that as we increased the concentration of algal oil and ethanol the production of biodiesel increased while the by-product production remained constant.

4. CONCLUSION

This study concludes that microscopic green algae can be effectively cultivated in glass jars under controlled conditions. The findings suggest that algal biodiesel can be an alternative source to petroleum-baseddiesel fuel as it is environment-friendly, does not affect human health, and issustainable.

In this study, three different ratios of aquarium water to freshwater (2:2, 2.5:1.5, and 3:1) were used for microalgae cultivation. The bead-beatingoil extraction technique was used because of its high efficiency in cell disruption and shorter processing time compared to other methods. It was observed that a higher concentration of aquarium water in the considered mixture resulted in greater microalgae growth.

The results of this study showed that the highest concentration of algal oil and ethanol resulted in a higher quantity of biodiesel. However, comparing the two concentrations i.e. 2:2 and 2.5:1.5, and adding equal amounts of algal oil and ethanol to both, yields different biodiesel outcomes. In the 2:2 concentration, less biodiesel was produced, and a higher amount of by-product i.e. glycerin was generated. On the other hand, in the 2.5:1.5 concentration, more biodiesel was produced, and less glycerin was generated. The third concentration that was considered was in the ratio 3:1 and in this mixture amount of algal oil and ethanol was also increased by 0.5 ml resulting in a higher quantity of biodiesel, while the glycerin production remained the same as it was present in the 2.5:1.5 concentration.

A limitation of this study was that the processes of cultivation, harvesting, oil extraction, and transesterification process of microalgae were time-consuming. However, refiningtheseprocesses could potentiallymaximize the yield and will lead the transport sector towards a green fuel.

5. REFERENCES

[1] Z. Liu (2015) Chapter 1 - Global energy development: the reality and challenges.*Glo. Energ. Int.,* 1–64, https://doi.org/10.1016/B978-0-12-804405-6.00001-4

[2] L. Qian, J. M. McInerney, S. S. Solomon, H. Liu, A. G. Burns (2021) Climate changes in the upper atmosphere: contributions by the changing greenhouse gas concentrations and earth's magnetic field from the1960s to 2010s. *J. of Geo. Res.: Space Phy.*,126, https://doi.org/10.1029/2020JA029067.

[3] G. Kuo (2019) When Fossil Fuels Run Out, What Then?https://mahb.stanford.edu/library-item/fossil-fuels-run/

[4] S. Khatoon, S. K. Yadav, V. Chakravorty, J. Singh, R. B. Singh, M. S. Hasnain, S.M.M. Hasnain (2023) Perovskite solar cell's efficiency, stability and scalability: a review. *Mate. Sci. for Energ. Tech.*, 6, 437–59 https://doi.org/10.1016/j.mset.2023.04.007.

[5] A. Shukla, S. K. Yadav, A. Yaqub, J. Singh, R. B. Singh (2024) Performance assessment of a 1 MW grid-connected rooftop solar photovoltaic (GCRPV) plant on an institutional building in composite climate of Northern India, *Int. J. Amb. Energ*. (2024). https://doi.org/https://doi.org/10.1080/01430750.2024.2392167.

[6] S. Khatoon, V. Chakraborty, S.K. Yadav, S. Diwakar, J. Singh, R.B. Singh (2023) Simulation study of $CsPbI_xBr_{1-x}$ and $MAPbI_3$ heterojunction solar cell using SCAPS-1D. *Solar Energy*, 254, 137–57, https://doi.org/10.1016/j.solener.2023.02.059.

[7] S.K. Yadav, N. M. Kumar, U. Bajpai (2023) Quantitative impact assessment of temperature on the economics of a rooftop solar photovoltaic system. *Environ. Sci. Pollut. Res*., 30, 21900–21913,https://doi.org/10.1007/s11356-022-23592-7.

[8] P.Moriarty, & D.Honnery, (2016) Chapter 61 Global transport energy consumption. *Alt.energ. and sha. gas encyc.*, 651-656. https://doi.org/10.1002/9781119066354.ch61.

[9] O. Awogbemi, D. V. V.Kallon, E. I.Onuh, & V. S. Aigbodion, (2021) An overview of the classification, production and utilization of biofuels for internal combustion engine applications. *Energies*, *14*(18), 5687. https://doi.org/10.3390/en14185687.

[10] M. Mubarak, A. Shaija, T. V. Suchithra (2021) Experimental evaluation of salvinia molesta oil biodiesel/diesel blends fuel on combustion, performance and emission analysis of diesel engine. *Fuel.* 287, 119526, https://doi.org/10.1016/j.fuel.2020.119526.

[11] Z. Shao, D. Wu, J. Liang, W. Cong, K. Liang (2023) The effect of porous media on the thermal storage performance of salinity-gradient solar pond. *J. of Phy.: Conf. Ser.*, 2584, https://doi.org/10.1088/1742-6596/2584/1/012015.

[12] K. Venkiteshwaran, T. Xie, M. Seib, V. P. Tale, D. Zitomer (2022) (Chapter 8) Anaerobic digester biogas upgrading using microalgae. *Int. Was. Manag. and Valo. using Algal Cult.*, 183–214, https://doi.org/10.1016/B978-0-323-85859-5.00004-X

[13] A. Kose, S. S. Oncel(2016) Algae as a promising resource for biofuel industry: facts and challenges. *Int.J. of Energ. Res*., 41 (7), 924-951. https://doi.org/10.1002/er.3699.

[14] J.H.K. Lim, Y.Y. Gan, H.C. Ong, B.F. Lau, W.H. Chen, C.T. Chong, T.C. Ling, J.J. Klemeš (2021) Utilization of microalgae for bio-jet fuel production in the aviation sector: challenges and perspective. *Ren. and Sus. Energ. Rev.*, 149, p.111396, https://doi.org/10.1016/j.rser.2021.111396.

[15] M. L. N. Carneiro, F. Pradelle, S. L. Braga, M. S. P. Gomes, A. R. F. Martins, F. Turkovics, R. N. Pradelle (2017) Potential of biofuels from algae: comparison with fossil fuels, ethanol and biodiesel in Europe and Brazil through life cycle assessment (LCA). *Ren. and Sus. Energ. Rev.*, 73, 632-653, https://doi.org/10.1016/j.rser.2017.01.152.

[16] Y. Xu, P. Hellier, S. Purton, F. Baganz, N. Ladommatos (2016) Algal biomass and diesel emulsions: an alternative approach for utilizing the energy content of microalgal biomass in diesel engines. *Applied Energy*, 172, 80-95, https://doi.org/10.1016/j.apenergy.2016.03.019.

[17] S. S. Satputaley, D.B. Zodpe, N.V. Deshpande (2017) Performance, combustion and emission study on CI engine using microalgae oil and microalgae oil methyl esters. *J. of the Energ. Ins.*, 90(4), 513-521, https://doi.org/10.1016/j.joei.2016.05.011.

[18] J. Milano, H. C. Ong, H.H. Masjuki, W.T. Chong, M.K. Lam, P. K. Loh, V. Vellayan (2016) Microalgae biofuels as an alternative to fossil fuel for power generation. *Ren. and Sus. Energ. Rev.*, 58, 180-197, https://doi.org/10.1016/j.rser.2015.12.150.

[19] A.S. Bayo, D. L. Chicharro, V. Morales, J. J. Espada, D. Puyol, F. Martínez, S. Astals, G. Vicente, L. F. Bautista, R. Rodríguez (2020) Biodiesel and biogas production from isochrysis galbana using dry and wet lipid extraction: a biorefinery approach. *Renewable Energy*, 146, 188-195, https://doi.org/10.1016/j.renene.2019.06.148.

[20] R. Halim, M.K. Danquah, P.A. Webley (2012) Extraction of oil from microalgae for biodiesel production: a review. *Biotechnology Advances*, 30(3), 709-732, https://doi.org/10.1016/j.biotechadv.2012.01.001.

[21] A. Demirbas (2010) Use of algae as biofuel sources. *Energ. Con. and Man.*, 51(12), 2738–2749, https://doi.org/10.1016/j.enconman.2010.06.010.

[22] O. Osundeko, H. Davies, J. K. Pittman (2013) Oxidative stress-tolerant microalgae strains are highly efficient for biofuel feedstock production on wastewater. *Biomass and Bioenergy* 56, 284–94, https://doi.org/10.1016/j.biombioe.2013.05.027.

[23] T. Fazal, M. S. U. Rehman, F. Javed, M. Akhtar, A. Mushtaq, A. Hafeez, A. A. Din, J. Iqbal, N. Rashid, F. Rehman (2021) Integrating bioremediation of textile wastewater with biodiesel production using microalgae(chlorella vulgaris). *Chemosphere*, 281, 130758, https://doi.org/10.1016/j.chemosphere.2021.130758

[24] G. Li, J. Zhang, H. Li, R. Hu, X. Yao, Y. Liu, Y. Zhou, T. Lyu (2021) Towards high-quality biodiesel production from microalgae using original and anaerobically-digested livestock wastewater. *Chemosphere*, 273, 128578, https://doi.org/10.1016/j.chemosphere.2020.128578

[25] A.S. Bayo, D.L. Chicharro, V. Morales, J. J. Espada, D. Puyol, F. Martínez, S. Astals, G. Vicente, L. F. Bautista, R. Rodriguez (2020) Biodiesel and biogas production from isochrysis galbana using dry and wet lipid extraction: a biorefinery approach. *Renewable Energy*, 146, 188–95, https://doi.org/10.1016/j.renene.2019.06.148.

[26] S. Khan, R. Siddique, W. Sajjad, G. Nabi, K. M. Hayat, P. Duan, L. Yao (2017) Biodiesel production from algae to overcome the energy crisis. *Hay. J. of Bio*., 24(4), 163–167, https://doi.org/10.1016/j.hjb.2017.10.003.

[27] G. Zuccaro, A. Yousuf, A. Pollio, J. P. Steyer (2020) (Chapter 2) Microalgae Cultivation Systems. *Micro. Cul. for Bio. Prod.*, 11–29, https://doi.org/10.1016/B978-0-12-817536-1.00002-3

[28] G. Singh, S. K. Patidar (2018) Microalgae harvesting techniques: A review. *J. of Env. Man.*, 217, 499–508, https://doi.org/10.1016/j.jenvman.2018.04.010.

[29] Y. S. Pradana, B. R. Sadewo, S. A. Haryanto, H. Sudibyo (2021) Selection of oil extraction from chlorella species of microalgae by using multi-criteria decision analysis technique for biodiesel production. *Open Chemistry*, 19(1), 1029–42, https://doi.org/10.1515/chem-2021-0092.

[30] R. R. Kumar, P. H. Rao, M. Arumugam (2015) Lipid extraction methods from microalgae: a comprehensive review. *Front. in Energ. Res.*, 2, 125610, https://doi.org/10.3389/fenrg.2014.00061.

[31] J. Zhai, I. T. Burke, & D. I. Stewart, (2021) Beneficial management of biomass combustion ashes. *Ren. and Sus. Energ. Rev.*, *151*, 111555

[32] Amit, U. K. Ghosh (2018) An approach for phycoremediation of different wastewaters and biodiesel production using microalgae. *Env. Sci. and Pol. Res.,* 25(19), 18673–81, https://doi.org/10.1007/s11356-018-1967-5.

[33] V.M. Ortiz-Martínez, P. Andreo-Martinez, N. Garcia-Martinez, A.P. de los Ríos, F.J. Hernández-Fernández, J. Quesada-Medina (2019) Approach to biodiesel production from microalgae under supercritical conditions by the PRISMA method. *FuelPro. Tech.*, 191, 211-222, https://doi.org/10.1016/j.fuproc.2019.03.031.

[34] R. Shomal, H. Hisham, A. Mlhem, R. Hassan, S. Al-Zuhair (2019) Simultaneous extraction–reaction process for biodiesel production from microalgae. *Energy Reports*, 5, 37–40, https://doi.org/10.1016/j.egyr.2018.11.003

IZVOD

STUDIJA ZELENIH MIKROALGI KAO SIROVINE ZA PROIZVODNJU BIODIZELA

Biodizel kao izvor energije označio je ivicu rastuće energetske krize. Postoji više načina na koje se biodizel može proizvesti, a u toku su agresivna istraživanja u oblasti biodizela. U ovom radu smo se fokusirali na proizvodnju biodizela iz mikroalgi. Zelene mikroalge, sirovina treće generacije, su obećavajući kandidati za proizvodnju biodizela zbog visokog sadržaja lipida i brzog rasta. U ovoj studiji, za uzgoj mikroalgi, stvoreno je okruženje različitih temperatura koje su bile između 27°C i 32°C. Takođe, tri različite koncentracije (2:2, 2,5:1,5 i 3:1) akvarijumske i slatke vode razmatrane su u ovom radu za rast algi, a istražen je metod ekstrakcije lipida kao što je mehaničko uništavanje ćelija da bi se utvrdila njegova efikasnost. Kada je proces ekstrakcije lipida optimizovan, ekstrahovani lipidi su podvrgnuti procesu transesterifikacije, pretvarajući ih u biodizel. Rezultati ovog istraživanja pokazuju da je veća koncentracija akvarijumske vode rezultirala boljom proizvodnjom algi, odnosno, osušene mase algi ekstrahovanih iz gore navedenih koncentracija bile su 2,69 grama, 2,79 grama, odnosno 2,92 grama. Biodizel proizveden od sušenih algi bio je 3,15 ml, 3,96 ml i 4,95 ml, respektivno. Ovi rezultati sugerišu da se zelene mikroalge mogu smatrati primamljivom sirovinom za proizvodnju biodizela. Optimizacija metoda uzgoja i ekstrakcije lipida može poboljšati biomasu i produktivnost lipida, povećavajući ukupan prinos biomase. Ova studija zaključuje da biodizel iz algi može biti alternativni izvor za dizel gorivo na bazi nafte.

Ključne reči: *Biodizel; microalgae; ekstrakcija lipida; biomasa; temperatura.*

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