Research Article

Characterization of biodiesel produced from microalgae grown on fish farm wastewater

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Abstract

This project investigated the influence of fish wastewater nutrients on properties of fatty acid methyl esters (FAME) of three microalgae species (*Tetradesmus obliquus*, *Heterochlorella luteoviridis* and *Chlamydomonas reinhardtii*) cultivated in unmodified and modified fish farm wastewater (FFW). The fuel properties obtained from the FAME of microalgae cultivated in FFW were then compared to the fuel properties obtained from FAME of the same microalgae species cultivated in a standard growth media of modified tris-acetate-phosphate. FFW nutrients supported the accumulation of desirable biodiesel fatty acids. C16–C18 were the main fatty acid component in all samples making up to 95%. All samples had saturated fatty acids above 22% and over 60% unsaturated fatty acids. Fuel properties of biodiesel produced from microalgae species cultivated in FFW were similar to fuel properties of biodiesel from the same species cultivated in the standard growth media. When compared to ASTM D6751-02 and EN 14214 biodiesel standards, biodiesel from FFW had desirable fuel properties (cetane number, kinematic viscosity, density, higher heating value, cold filter plugging point and iodine value). Overall, *Chlamydomonas reinhardtii* (in both FFW media) had the most suitable biodiesel property, especially when looking at the cold filter plugging point.

Graphic abstract

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Abbreviations

CN	Cetane number
CFPP	Cold filter plugging point
DU	Degree of unsaturation
FA	Fatty acids
FAME	Fatty acid methyl esters
IV	Iodine value
HHV	Higher heating value
LCSF	Long-chain saturated factor
MSFA	Monosaturated fatty acid
PSFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TAG	Triacylglycerol
ν	Kinematic viscosity (mm^2/s)
ρ	Density (g/cm^3)

1 Introduction

Biodiesel is the best replacement for fossil diesel and is also more environmental friendly than the petrodiesel [1]. Biodiesel has other advantages over petrodiesel as renewable, biodegradable, has low exhaust emissions, not toxic and essentially free from sulphur and aromatics [2–5] and can be used in any engine without modification because it has similar properties with diesel fuel [6, 7]. Biodiesel most often is used in different ratio blends with petrodiesel 2% (B2), 5% (B5) or 20% (B20). Also, it can be stored and transported using diesel tanks and equipment. Furthermore, it has better lubricating properties than diesel fuel because biodiesel is oxygenated, is more completely combusted and increases the life of engines. In addition, it is safer to use, store and handle due to high flash point of biodiesel. In sensitive environments like heavily polluted cities, biodiesel is an ideal fuel to use due to relatively low emissions [5].

The quality of biodiesel obtained is directly influenced by the composition of the FAs produced by the algal species [8]; this makes lipid composition an important parameter in species selection for commercial microalgae biodiesel production [9]. The structural properties of the fatty acids, such as level of saturation, chain length and branching, affects the chemical and physical properties of biodiesel [10, 11]. These fatty acid properties vary with species and nutrient conditions of the algal culture [12] and in turn, determine the main parameters of the biodiesel including cetane number, oxidative stability, kinematic viscosity, cold flow and lubricity [11]. FAs are the building blocks of triacylglycerol (TAG) and every other cellular lipid. Under environmental stress conditions, some microalgae can accumulate TAGs up to 20–50% dry cell

weight. TAGs serve as energy and carbon storage and do not perform structural roles like other lipids [13]. There is more diverse chain length distribution in different microalgae species than in higher plants. This makes it possible for selection of certain species for selected fuel properties, like selection of species with less than C_{16} chain length for jet fuels and selection of species with very long chain length C_{20} for lubricants [12].

Transesterification of animal fats and vegetable oil is the most commonly used method for biodiesel production [3]. Transesterification is achieved by reacting biologically derived oils (animal fats and vegetable oils) that are made of fatty acids (FA), with monohydric alcohols in the presence of a catalyst. During the transesterification reaction, the FAs bond with the methyl group to produce fatty acid methyl ester (FAME) and glycerol [14].

Equation 1 is adapted from Chisti et al. [15].

In recent years, aquaculture has been a rapidly growing sector with an increasing demand for fish [16]. With this, aquaculture releases nutrient-rich effluent, associated with increased eutrophication [17], which can lead to shortage of freshwater [18]. A growing world population will lead to even higher volumes of effluent discharged from aquaculture as the dependence on farmed fish increases. Farmed fish supplies about 50% of fishes consumed by humans [19]. Aquaculture wastewater is rich in organic nutrients especially nitrogen and phosphorus. This nutrient-rich aquaculture wastewater can be used to replace the enormous amounts of synthetic fertilizers required for cultivation of microalgae biofuel feedstock [20]. Some benefits of using FFW nutrients for microalgae cultivation include but not limited to suitable growth rate and cost saving from purchase of synthetic fertilizers [18].

Most research on algae lipid as biodiesel feedstock has focused on improving growth rate and lipid productivity with limited research on biodiesel production and the quality of this potential microalgae biodiesel [21, 22]. The main aim of this work was to produce biodiesel from microalgae feedstock cultivated using nutrients in FFW and to determine the quality of biodiesel thereafter and also to compare the quality of biodiesel obtained from microalgae species cultivated in FFW to quality of biodiesel of same species cultivated in a microalgae standard

growth media. This will in turn, establish the suitability of nutrients in FFW as cheap alternative growth media for growing algae biomass feedstock for biodiesel production. Finding a suitable cheap alternative microalgae growth media to purchased synthetic growth medium nutrients will eliminate the cost of purchased culture nutrients, thereby reducing the cost of biodiesel production by providing cheaper feedstock.

2 Methodology

All experiments were performed in triplicate, and all reagents purchased from Sigma-Aldrich (South Africa). FFW was collected and prepared for microalgae cultivation. The next step involved utilizing prepared FFW for microalgae cultivation. After cultivation, harvested algal biomass was used for biodiesel production, and produced biodiesel properties were calculated.

2.1 Preparation of fish farm wastewater

Fish farm wastewater was collected from David Fincham Aquaculture, Johannesburg, South Africa. *Oreochromis Niloticus* (tilapia) was farmed in a recirculating system using 10,000-L fish tanks, containing about 200 kg of fish, fed daily with a 32% protein fish feed at 4 wt%. The system ran a backwash to remove debris from the recirculating water once a day. Collected wastewater was filtered using a vacuum pump and a 509ws filter paper and sterilized in an autoclave at 120 °C for 20 min. pH was measured using a Ohaus Starter 3100 m, while nutrients (ammonia, nitrate and phosphate) were measured using Hach ammonia nitrogen test kit model N1-8, nitrate test kit model N1-11 and orthophosphate test DOC326.98.00023, respectively. Distilled water was used to modify nutrient load in sterilized FFW, while sterilized FFW was used in the original form in unmodified FFW cultivation. The characteristics of the FFW is shown in Table 1.

2.2 Microalgae cultivation

Cultivation of algae was done in batch using three species of microalgae (*Tetradismus obliquus*, *Heterochlorella luteoviridis* and *Chlamydomonas reinhardtii*) obtained from the South African National Phycology Culture Collection, Evolution Studies Institute, University of the Witwatersrand. The microalgae species were cultivated in a 10 L perspex (plexi glass) photobioreactor with a height of 100 cm and an inside diameter of 14.5 cm. The reactor included a diffuser at the base and a 12/12-h light/dark regime, with an artificial light intensity of 2388 lx. An air flotation method was used for continuous supply of air at 7 mL/min and

Table 1 Characteristics of fish farm wastewater

Characteristic	Value	Unit
Unmodified fish waste water		
NH ₄	4.6 ± 0.16	mg/l
NO ₃	140 ± 6.55	mg/l
PO ₄	15 ± 2.09	mg/l
pH	7.3 ± 0.72	
Modified fish farm wastewater		
NH ₄	2.3 ± 0.08	mg/l
NO ₃	70 ± 3.27	mg/l
PO ₄	7.5 ± 3.27	mg/l
pH	7.3 ± 0.72	

for CO₂ at 2 mL/min for 1 h a day. A Brooks Instrument 0154 mass flow meter was used to measure flow rate of gases. Lights were supplied from the sides of the reactors using eight fluorescence lamps (Osram L58 Watts/640 cool white) with protective lamp cover. Light intensity was measured with a MAJOR Tech MT940 light meter. Optical density at 680 nm was used to determine the daily growth rate of the algae species. Microalgae was harvested in the lag phase using a 0.45-μ cellulose acetate filter paper in a pressure filter, washed with distilled water and oven-dried at 28 °C for 48 h. Microalgae cultivation is shown in Fig. 1.

2.3 Biodiesel production

Biodiesel was made by direct transesterification of algal biomass as adapted from Johnson and Wen [23]. Fifty milligrams of dried and crushed algal biomass was weighed into a 15-mL glass bottle with a silicon screw cap. The sample was mixed with 2 mL of methanol and 600 μL 95% sulphuric acid and vortexed for roughly 30 s. The mixture was then heated for 60 min at 300 rpm at 90 °C and allowed to cool to room temperature. After cooling, 2 mL of hexane was added to the reaction mixture and mixed, followed by the addition of 1 mL of distilled water to separate the mixture into two phases. The tubes were centrifuged for 5 min at 4000 rpm, with the solvent layer containing the FAME separated and collected for characterization.

2.4 FAME characterization

A GC/MS was used to measure the FAME composition. One microlitre of FAME extract was injected into a GC/MS with Rtx5 column (30 m × 320 mm × 1.0 μm film thickness) (Supelco, USA). Standard split injection was used with a split of 100. An injection temperature of 180 °C and column oven temperature of 140 °C was used, increased to 250 °C at 4 °C/min, with the detector at 220 °C with a

Fig. 1 Microalgae growing in photobioreactors

carrier gas flow rate of 50 mL/min. A Supelco C14:0 to C22:0 FAME mixture was used as standard. Peak areas were used to quantify the FAME.

2.5 Calculation of fuel properties

The FAME composition of biodiesel produced were used to calculate biodiesel properties: cetane number (CN), Iodine value (IV), cold filter pour point (CFPP, kinematic viscosity (ν), density (ρ), higher heating value (HHV), long chain saturated factor (LCSF), degree of unsaturation (DU) and saponification value (SF). CN, CFPP and LCSF [21, 24], and SV, IV, DU, ν , ρ and HHV were calculated as in literature [10].

3 Results and discussion

3.1 Characterization of FFW

Wastewater was collected from the recirculatory system, used without modification (unmodified FFW) and modified (modified FFW) with distilled water to dilute the nutrient load to provide a more nutrient stress growth media. FFW was analysed for nitrates, ammonia and phosphates required for the growth of microalgae. The characteristics (ammonia, nitrogen, phosphorus and pH) of both unmodified and modified FFW is shown in Table 1. The nutrient composition of the FFW used in

this study is in the range of some FFW previously used for microalgae cultivation for different purpose [25–33]. This indicates that the FFW in this study has required nutrients to support microalgae growth and accumulation of biochemicals.

3.2 Microalgae growth rate

Microalgae growth rate was monitored daily using an optical density of 680 nm using a Shimadzu UV-1800. Similar growth pattern was observed in the three different species cultivated in the three growth media. In every species, cultivation in modified FFW growth media had the highest growth rate followed by cultivation in unmodified FFW and least growth rate in the TAP media (Figs. 2, 3, 4). Algae in nitrate as nitrogen source attains higher growth rate than algae cultivated in ammonia-based media as a nitrogen source [31]. FFW contains nitrate in addition to

Fig. 2 Growth curve of *Tetrademus obliquus* cultivated in the three different growth media

Fig. 3 Growth curve of *Heterochlorella luteoviridis* cultivated in the three different growth media

Fig. 4 Growth curve of *Chlamydomonas reinhardtii* cultivated in the three different growth media

Fig. 7 Percentage FAME of *Chlamydomonas reinhardtii* cultivated in the three culture media

Fig. 5 Percentage FAME of *Tetrademus obliquus* cultivated in the three culture media

Fig. 6 Percentage FAME of *Heterochlorella luteoviridis* cultivated in the three culture media

ammonia (Table 1). This could be the reason for higher growth rates obtained in species cultivated in both FFW.

3.3 Composition of FAME

As can be seen in Figs. 5, 6, 7, all species cultivated in the three different growth media accumulated less than 1% C12:0, C14:0, C16:1, C17:0, C20:2 and C22:0 and accumulated between 1 and 2% of C14:1 and C20:0 FAME except for all species cultivated in modified FFW that had values of C14:1 less than 1% and C20:0 in *Heterochlorella luteoviridis* in unmodified FFW above 2%. C18:0 was generally above 4% but less than 10% in all samples except in *Heterochlorella luteoviridis* in modified FFW with 13.09% and absent in *Chlamydomonas reinhardtii* in unmodified FFW and *Chlamydomonas reinhardtii* in modified FFW. C18:2 was above 20% in species cultivated in TAP standard growth media and unmodified FFW (11.20–13.59%) in all species cultivated in modified FFW. C16:0 was the second most abundant FA in all samples cultivated. Generally, most samples contained above 24% of C16:0, having highest accumulation of 34.54% in *Tetrademus obliquus* in modified FFW and lowest of 19.41% in *Chlamydomonas reinhardtii* in unmodified FFW. The most abundant FA in all samples is C18:1(x) with about 40% in all samples cultivated. *Chlamydomonas reinhardtii* in unmodified FFW had 22.27% of SFA, the lowest concentration of SFA in all samples, about twice lesser than *Tetrademus obliquus* in modified FFW with the highest SFA concentration of 46.60%. Concentration of MSFA was above 41% in all the samples with 59.01% as the highest concentration in *Chlamydomonas reinhardtii* in modified FFW and 41.36 as the lowest concentration in *Heterochlorella luteoviridis* in tris-acetate-phosphate standard. Concentration of PUFA were generally lower in modified

FFW samples nearly twice lower in concentration than in species cultivated in both TAP and unmodified.

The main fatty acid components of all species were C16 to C18 comprising over 95% (Figs. 2, 3, 4, 5). Saturated fatty acids were above 25% in all the samples except in *Chlamydomonas reinhardtii* in FFW with 22.27% while unsaturated fatty acids were above 60% in all cultivations. This is in agreement with studies by Malibari et al. [26] and Wu and Miao [34] that obtained mostly C16 to C18 fatty acids, above 25% saturated fatty acid and above 60% unsaturated fatty acids. Wu and Miao [34] suggested that effects of nitrogen starvation is dependent on the species. In this study, the lowest composition of saturated fatty acid in *Chlamydomonas reinhardtii* in each media can be attributed to this fact. Studies by Huerliman et al. [9] and Malibari et al. [26] suggest that composition of microalgae fatty acids is dependent on growth media and duration of cultivation. Since research by Wu and Miao [34] suggest that fatty acid composition are species dependent, the lower percentage composition of saturated fatty acids observed in *Chlamydomonas reinhardtii* cultivated in both unmodified and unmodified FFW about 10% lower than in same species cultivated in TAP. On the other hand, the lower poly unsaturated fatty acids observed in all species cultivated in modified FFW can be attributed to the impact of media on fatty acid composition as suggested by Huerlimann et al. and Malibari [9, 26].

3.4 Properties of biodiesel

Experimental procedures used to determine the fuel properties of biodiesel are difficult, long, expensive [35, 36] and usually have insufficient microalgae to supply the amount of oil required [36]. Simple and reliable calculation methods are used instead of experimental procedures to estimate biodiesel fuel properties [35, 36]. As a theoretical work, established reliable calculations (equations 1–12) for estimating fuel properties of biodiesel from the composition of FAME were used to estimate biodiesel properties in this study.

3.4.1 Cetane number (CN)

The CN deals with the ignition properties of fuels. It is associated with ignition delay time, combustion smoothness, knock time, stability, efficiency of combustion and release of carbon monoxide, nitrous oxides and hydrocarbons [37]. Generally, ignition properties get better with increasing CN [38]. The higher the CN, the lesser the ignition delay time [39]. CN increases with an increase in molecular weight of the FAs [37]. All the samples had CN above 59 which is higher than both the ASTM D6751-02 and EN 14214 standards limits of ≥ 47 and ≥ 51 , respectively. The

Fig. 8 Cetane number of the three microalgae species cultivated in the different growth media

CN of this study (Fig. 8) is comparable to the CN in studies by Wu and Miao [34] that obtained a CN above 58 in different concentrations of nitrate. In this study, *Heterochlorella luteoviridis* in unmodified FFW had the highest CN of 64.39, while *Chlamydomonas reinhardtii* in modified FFW had the least CN of 59.17. Inasmuch as *Heterochlorella luteoviridis* in unmodified FFW biodiesel will have the least ignition delay time than others and highest ignition delay time with *Chlamydomonas reinhardtii* in unmodified FFW, all fuel samples have short ignition delay time which is a good quality and will generally run smoothly with lesser emissions. This is attributed to the fact that CN obtained in all samples satisfied the CN in both EN 14214 and the ASTM D6751 standards and the fact that CN properties get better with increasing CN values.

3.4.2 Kinematic viscosity

Kinematic viscosity directly affects atomization process during combustion. Viscosity increases with increasing molecular weight of FAs and reduces with increasing number of double bonds [37]. High kinematic viscosity can lead to certain operation problems like engine deposits. Most issues associated with high viscosity are noticed during low ambient temperatures and during cold start engine conditions. Kinematic viscosity is also used to monitor the quality of stored biodiesel as it increases with reducing biodiesel quality [39]. All calculated viscosity (Fig. 9) fall within the range of both ASTM D6751-02 and EN 14214 standard of 1.9–6.0 mm²/s and 3.5–5.0 mm²/s, respectively, with most of the calculated values falling within the upper limit of the EN 14214 standard but within the mean range of ASTM D6751-02. All samples of *Chlamydomonas reinhardtii* had lowest kinematic viscosity values even though the difference in value did not vary so much with other

Fig. 9 Kinematic viscosity of the three microalgae species cultivated in the different growth media

species. The biodiesel obtained from all samples will lead to less operational problem to the engine due to proper atomization characteristic of the biodiesel produced. Biodiesel obtained from all samples of *Chlamydomonas reinhardtii* have the best viscosity because they had the least values when compared to values obtained from other species. Of all the samples, *Chlamydomonas reinhardtii* samples had the lowest composition of saturated FA and more unsaturated FA. This is in agreement with studies by Ramirez-Verduzco et al. [37] that kinematic viscosity reduces with increasing number of double bonds. The values obtained in this study may not have varied widely from each other because all samples accumulated similar FAs and had similar molecular weight. The variations must have been from level of unsaturation of each sample.

Fig. 10 Density of the three microalgae species cultivated in the different growth media

3.4.3 Density

Density also known as specific gravity decreases with increasing molecular weight and increases with increasing number of double bonds [37]. It is a major parameter that affects engine performance. Fuel density determines the mass of fuel injected and also influences the energy content and air fuel ratio within the combustion chamber [22]. All densities (Fig. 10) fall within the EN 14214 standard of 0.86–0.90 g/cm³. All densities had similar values for each sample cultivated in the different media with samples of all *Chlamydomonas reinhardtii* slightly higher. Similar density can be associated with accumulation of similar molecular weight FAs. The slight difference in fuel density obtained can be associated with lower levels of double bonds in all samples of *Chlamydomonas reinhardtii*. This is also in agreement with studies that density increases with increasing number of double bonds [37].

3.4.4 Higher heating value (HHV)

HHV is the amount of heat produced when 1 g of fuel is combusted to produce CO₂ and H₂O at its original temperature. HHV reduces with increasing number of double bonds [37]. Biodiesel has HHV of 39–41 MJ/kg [2] and about 10% lesser than petrodiesel that is ~46 MJ/kg [40]. There is no stipulated HHV limit for both ASTM D6751-02 and EN 14214 standards, but according Demirbas [2], the HHV for biodiesel is between 39 and 41 MJ/kg. Biodiesel from all samples (Fig. 11) had HHV values between 39.68 and 39.92 MJ/kg and are within the biodiesel range of 39–41 MJ/kg given in Demirbas, 2009 [2]. *Chlamydomonas reinhardtii* in unmodified FFW had the lowest HHV and can be related to higher composition of unsaturated FAs.

Fig. 11 HHV of the three microalgae species cultivated in the different growth media

Fig. 12 Iodine value of the three microalgae species cultivated in the different growth media

3.4.5 Iodine value

Iodine value is the parameter used in measuring rate of saturation of the fuel [37]. All samples (Fig. 12) had IV with the ≤ 120 of EN 14214 with *Chlamydomonas reinhardtii* in unmodified FFW having the highest IV of 94 $I_2/100g$ and *Tetradesmus obliquus* in modified FFW with the lowest IV of 54 $I_2/100g$. Generally, samples cultivated in modified FFW had lower IV while samples in TAP and unmodified FFW had similar value per species except for *Chlamydomonas reinhardtii* in unmodified FFW which had highest value overall. As a measure of saturation, highest values obtained in *Chlamydomonas reinhardtii* in unmodified FFW show least saturation while lowest value obtained in *Tetradesmus obliquus* in modified FFW show high saturation. The iodine values as measure of saturation also corresponds to the highest values obtained from calculated degree of unsaturation. For the same reason, samples cultivated in modified FFW had lower iodine especially *Tetradesmus obliquus* in modified FFW and *Heterochlorella luteoviridis* in modified FFW.

3.4.6 Cold filter plugging point (CFPP)

The cold filter plugging point (CFPP) of a biodiesel determines its performance during the cold weather. Lower temperatures can cause the fuel to thicken and reduce the flow rate of the fuel, thereby impacting on the performance of the fuel pipes, pumps and injectors [38]. The CFPP reduces with increasing unsaturation [24]. This is in agreement with the results of this study as can be seen in (Fig. 13), Species of *Chlamydomonas reinhardtii* in both FFW had lowest CFPP values. Most of the samples exceeded the CFPP limits of EN 14214 standard of $\leq 5/\leq -20$ °C with only all the samples of *Chlamydomonas reinhardtii* in the three different growth

Fig. 13 CFPP of the three microalgae species cultivated in the different growth media

media falling within the limit with 4.45 °C, -4.5 °C and -4.02 °C, respectively. The remaining samples had CFPP values more than 5 °C which exceeds the upper limit of the EN 14214 range. This means biodiesel from *Chlamydomonas reinhardtii* generally have good CFPP although with better flow from samples cultivated in FFW which would not thicken during cold temperatures and affect fuel delivery.

3.4.7 Saponification value

Chlamydomonas reinhardtii in unmodified FFW had the highest SV of 194 mg KOH/g, while *Chlamydomonas reinhardtii* in modified FFW had the lowest SV of 188 mg KOH/g. The range of SV (Fig. 14) is not stated in both

Fig. 14 Saponification value of the three microalgae species cultivated in the different growth media

ASTM D6751-02 and EN 14214 but values obtained fall with the same range as the samples measured in literature [10].

4 Conclusion

FFW nutrients supported the accumulation of desirable biodiesel fatty acids. C16 and C18 were the main fatty acid component in all samples making up to 95%. All samples had saturated fatty acids above 22% and over 60% unsaturated fatty acids. Desirable biodiesel properties (Cetane number, kinematic viscosity, density, higher heating, cold filter plugging point and iodine value) were obtained. Similar biodiesel properties were obtained from all microalgae species cultivated in both fish farm waste media when compared to the TAP standard growth media. *Chlamydomonas reinhardtii*, in both FFW media, had the most suitable biodiesel properties, especially the CFPP. This makes biodiesel obtained from *Chlamydomonas reinhardtii* in FFW most suitable for all regions, including cold temperature regions. Biodiesel from other algal species can only be suitable for use in hot regions or only during summer in temperate regions due to a poor CFPP. Most important biodiesel parameters of all samples were within the range of both ASTM D6751-02 and EN 14214 biodiesel standards. This indicates that nutrients in FFW are as suitable as the TAP standard growth media for microalgae biodiesel feedstock production for *Tetrademus obliquus*, *Heterochlorella luteoviridis* but more suitable for biodiesel production from *Chlamydomonas reinhardtii* species cultivated in FFW. This can be attributed to the inhibition of PUFA accumulation in *Chlamydomonas reinhardtii* cultivated in both FFW.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Atadashi IM, Aroua MK, Aziz AA (2010) High quality biodiesel and its diesel engine application: a review. *Renew Sustain Energy Rev* 14:1999–2008. <https://doi.org/10.1016/j.rser.2010.03.020>
- Demirbas A (2009) Progress and recent trends in biodiesel fuels. *Energy Convers Manag* 50:14–34. <https://doi.org/10.1016/j.enconman.2008.09.001>
- Ma F, Hanna MA (1999) Biodiesel production: a review. *Bioresour Technol* 70:1–15. [https://doi.org/10.1016/S0960-8524\(99\)00025-5](https://doi.org/10.1016/S0960-8524(99)00025-5)
- Van Gerpen J (2005) Biodiesel processing and production. *Fuel Process Technol* 86:1097–1107. <https://doi.org/10.1016/j.fuproc.2004.11.005>
- Vasudevan PT, Briggs M (2008) Biodiesel production—current state of the art and challenges. *J Ind Microbiol Biotechnol* 35:421. <https://doi.org/10.1007/s10295-008-0312-2>
- Demirbas A (2007) Progress and recent trends in biofuels. *Prog Energy Combust Sci* 33:1–18. <https://doi.org/10.1016/j.pecs.2006.06.001>
- Agarwal AK (2007) Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Prog Energy Combust Sci* 33:233–271. <https://doi.org/10.1016/j.pecs.2006.08.003>
- Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, Hadavand Mirzaei H, Mirzajanzadeh M, Malekzadeh Shafaroudi S, Bakhtiari S (2013) Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. *Algal Res* 2:258–267. <https://doi.org/10.1016/j.algal.2013.04.003>
- Huerlimann R, de Nys R, Heimann K (2010) Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnol Bioeng* 107:245–257. <https://doi.org/10.1002/bit.22809>
- Islam MA, Magnusson M, Brown RJ, Ayoko GA, Nabi MN, Heimann K (2013) Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles. *Energies* 6:5676–5702. <https://doi.org/10.3390/en6115676>
- Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technol* 86:1059–1070. <https://doi.org/10.1016/j.fuproc.2004.11.002>
- Le Williams PJB, Laurens LML (2010) Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. *Energy Environ Sci* 3:554. <https://doi.org/10.1039/b924978h>
- Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J* 54:621–639. <https://doi.org/10.1111/j.1365-3113X.2008.03492.x>
- Meher LC, VidyaSagar D, Naik SN (2006) Technical aspects of biodiesel production by transesterification—a review. *Renew Sustain Energy Rev*. <https://doi.org/10.1016/j.rser.2004.09.002>
- Chisti Y, Yusuf C, Chisti Y (2007) Biodiesel from microalgae. *Biotechnol Adv* 25:294–306. <https://doi.org/10.1016/j.biotechadv.2007.02.001>
- Hu Z, Lee JW, Chandran K, Kim S, Sharma K, Khanal SK (2014) Influence of carbohydrate addition on nitrogen transformations and greenhouse gas emissions of intensive aquaculture system. *Sci Total Environ* 470–471:193–200. <https://doi.org/10.1016/j.scitotenv.2013.09.050>
- Troell M, Rönnbäck P, Halling C, Kautsky N, Buschmann A (1999) Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. *J Appl Phycol* 11:89–97. <https://doi.org/10.1023/A:1008070400208>
- Heidari SA, Farhadian O, Soofiani NM (2011) Biomass production and ammonia and nitrite removal from fish farm effluent by *Scenedesmus quadricauda* culture. *J. Environ. Stud.* 37:15–28
- FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome, 200 pp
- Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and

- wastewaters: a review. *Appl Energy* 88:3389–3401. <https://doi.org/10.1016/j.apenergy.2010.12.042>
21. Stansell GR, Gray VM, Sym SD (2012) Microalgal fatty acid composition: implications for biodiesel quality. *J Appl Phycol* 24:791–801. <https://doi.org/10.1007/s10811-011-9696-x>
 22. Hoekman SK, Broch A, Robbins C, Ceniceros E, Natarajan M (2012) Review of biodiesel composition, properties, and specifications. *Renew Sustain Energy Rev* 16:143–169. <https://doi.org/10.1016/j.rser.2011.07.143>
 23. Johnson MB, Wen Z (2009) Production of biodiesel fuel from the microalga *schizochytrium limacinum* by direct transesterification of algal biomass. *Energy Fuels* 23:5179–5183. <https://doi.org/10.1021/ef900704h>
 24. Álvarez-Díaz PD, Ruiz J, Arbib Z, Barragán J, Garrido-Pérez MC, Perales JA (2015) Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. *Bioresour Technol* 181:90–96. <https://doi.org/10.1016/J.BIORTECH.2015.01.018>
 25. Gao F, Li C, Yang ZH, Zeng GM, Feng LJ, Zhi Liu J, Liu M, Wen Cai H (2016) Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. *Ecol Eng* 92:55–61. <https://doi.org/10.1016/j.ecoleng.2016.03.046>
 26. Malibari R, Sayegh F, Elazzazy AM, Baeshen MN, Dourou M, Aggelis G (2018) Reuse of shrimp farm wastewater as growth medium for marine microalgae isolated from Red Sea—Jeddah. *J Clean Prod* 198:160–169. <https://doi.org/10.1016/J.JCLEPRO.2018.07.037>
 27. Nasir NM, Bakar NSA, Lananan F, AbdulHamid SH, Lam SS, Jusoh A (2015) Treatment of African catfish, *Clarias gariepinus* wastewater utilizing phytoremediation of microalgae, *Chlorella* sp. with *Aspergillus niger* bio-harvesting. *Bioresour Technol* 190:492–498. <https://doi.org/10.1016/J.BIORTECH.2015.03.023>
 28. Egloff S, Tschudi F, Schmautz Z, Refardt D (2018) High-density cultivation of microalgae continuously fed with unfiltered water from a recirculating aquaculture system. *Algal Res* 34:68–74. <https://doi.org/10.1016/J.ALGal.2018.07.004>
 29. Halfhide T, Åkerstrøm A, Lekang OI, Gislerød HR, Ergas SJ (2014) Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions. *Algal Res* 6:152–159. <https://doi.org/10.1016/J.ALGal.2014.10.009>
 30. Michels MHAA, Vaskoska M, Vermuë MH, Wijffels RH (2014) Growth of *Tetraselmis suecica* in a tubular photobioreactor on wastewater from a fish farm. *Water Res* 65:290–296. <https://doi.org/10.1016/j.watres.2014.07.017>
 31. Guerrero-Cabrera L, Rueda JA, García-Lozano H, Navarro AK (2014) Cultivation of *Monoraphidium* sp., *Chlorella* sp. and *Scenedesmus* sp. algae in Batch culture using Nile tilapia effluent. *Bioresour Technol* 161:455–460. <https://doi.org/10.1016/J.BIORTECH.2014.03.127>
 32. Ansari FA, Singh P, Guldhe A, Bux F (2017) Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. *Algal Res* 21:169–177. <https://doi.org/10.1016/j.algal.2016.11.015>
 33. Guo Z, Liu Y, Guo H, Yan S, Mu J (2013) Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. *J Environ Sci* 25:S85–S88. [https://doi.org/10.1016/S1001-0742\(14\)60632-X](https://doi.org/10.1016/S1001-0742(14)60632-X)
 34. Wu H, Miao X (2014) Biodiesel quality and biochemical changes of microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* in response to nitrate levels. *Bioresour Technol*. <https://doi.org/10.1016/j.biortech.2014.08.017>
 35. Islam MA, Ayoko GA, Brown R, Stuart D, Heimann K (2013) Influence of fatty acid structure on fuel properties of algae derived biodiesel. *Procedia Eng* 56:591–596. <https://doi.org/10.1016/J.PROENG.2013.03.164>
 36. Talebi AF, Tabatabaei M, Chisti Y (2015) Biodiesel analyzer: a user-friendly software for predicting the properties of prospective biodiesel. *Biofuel Res J* 2:55–57. <https://doi.org/10.18331/brj2015.1.2.4>
 37. Ramírez-Verduzco LF, Rodríguez-Rodríguez JE, del Jaramillo-Jacob AR (2012) Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition. *Fuel* 91:102–111. <https://doi.org/10.1016/J.FUEL.2011.06.070>
 38. Meher LC, VidyaSagar D, Naik SN (2006) Technical aspects of biodiesel production by transesterification—a review. *Renew Sustain Energy Rev* 10:248–268. <https://doi.org/10.1016/J.RSER.2004.09.002>
 39. Knothe G (2006) Analyzing biodiesel: standards and other methods. *J Am Oil Chem Soc JAOCS*. <https://doi.org/10.1007/s11746-006-5033-y>
 40. Refaat AA (2009) Correlation between the chemical structure of biodiesel and its physical properties. *Int J Environ Sci Technol* 6:677–694. <https://doi.org/10.1007/BF03326109>

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