

Valorization of digestates and vermicompost as alternative culture media for *Chlorella vulgaris*: biomass production, biochemical composition and biodiesel quality

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Abstract

Environmentally friendly and sustainable alternative systems for the valorization of organic residues are increasingly explored to obtain high value bioproducts such as proteins, pigments, lipids, and carbohydrates through microalgal biorefineries. However, the large-scale application of microalgae is often limited by the high cost of synthetic culture media. In this study, the use of vermicompost (VR), vermicompost leachate (LX), and digestates from cow manure (DB), swine manure (DP), a 1:1 mixture of both (DM), and mixed livestock digestate (DC) were evaluated for the cultivation of *Chlorella vulgaris*, in comparison with the synthetic Bold Basal medium (MC). The DM medium produced 43% more biomass, 155% more proteins, 159% more lipids, and 150% more carbohydrates than MC. All media, except DB, resulted in higher lipid concentration than MC, with DC showing the highest value, exceeding the synthetic medium by 172%. Additionally, in DM were identified 9 out of the 10 Fatty acids methyl esters (FAMES) detected, compared to only 4 in MC; oleic and palmitic acids were produced in all treatments. Biodiesel from lipids obtained using LX, VR, DB, and DP met the five evaluated quality parameters, whereas biodiesel from MC met four of them. These results demonstrate the potential of digestates and vermicompost based media as low cost and sustainable alternatives for microalgal biomass production and biodiesel oriented biorefineries.

1. Introduction

Raw materials for pigments, proteins, carbohydrates, and lipids production are generally attained from biological sources whose availability is limited by the large amounts of land and water required for their production, thereby compromising food security. Microalgae represent an alternative, and their use has gained increasing importance in recent years across various industries, due to their potential for producing industrially relevant compounds [1,2] having application in food, pharmacy, and cosmetics; for example, protein content in microalgae biomass range from 23% to 63% on dry biomass basis depending upon the genera and species [3]. Besides, microalgae have higher surface area productivity and photosynthetic efficiency compared to terrestrial crop plants. Additionally, microalgae provide a dual benefit by reducing greenhouse gas emissions through photosynthesis while generating biomass rich in carbohydrates, pigments, lipids, and proteins [4].

Chlorella vulgaris is a model microalga due to its high photosynthetic efficiency and its ability to adapt to different cultivation conditions [5]. It has been widely studied for its high content of important biomolecules such as amino acids, vitamins, carotenoids, lipids (e.g., omega-3 fatty acids), carbohydrates (e.g., β -glucans), and proteins [6,7]; it has been widely used as human food supplements. *Chlorella vulgaris* has good-quality protein with amino acids rich in methionine, lysine and alanine, important components for fish dietary; inclusion of 2.5–10% algae into fish diets enhanced growth [8]. The microalgae studies have found also important applications in the field of biofuels, they produce desirable fatty acids methyl esters (FAME) for biodiesel production, including palmitic acid, stearic acid, and eicosatrienoic acids [9]. The lipids and carbohydrates they produce can be converted into biodiesel, bioethanol, biohydrogen, and biomethane through thermochemical and biochemical processes [10,11];

thereby reducing some of the disadvantages associated with conventional energy production. For example, diesel production involves the exploitation of non-renewable resources, air pollution, and health risks [12] as well as the emission of pollutants such as nitrates, sulfates, and fine particulate matter [13]. Besides, oil crops cannot sustainably provide sufficient biodiesel [14]. Ethanol production generates significant environmental impacts, including high water demand [15]. Hydrogen produced from natural gas and coal results in high CO₂ emissions [16]. Despite the advantages offered by microalgae, large-scale biofuel production using these organisms requires low-cost culture media for the process to be economically feasible [17].

One of the main challenges in the industrial-scale utilization of microalgae cultivation lies in the selection and optimization of the culture medium, as this factor directly influences the biochemical composition of the biomass, production costs, and consequently, the quality of the derived products and application of them [18]. Various organic culture media derived from agro-industrial residues or effluents have been evaluated as a sustainable strategy to reduce costs and improve the biochemical profile of microalgae [19,20]. For example, using wastewater from sugar industries increased in 59%, 83.82%, and 110.66% in microalgal biomass, protein, and lipid production, respectively, compared to the synthetic BG11 medium [21]. Using 20% aquaculture wastewater, lipid content increased by 11.81%, compared to more concentrated wastewater treatments (40, 60, 80 and 100%), due to nutrient deficiency in the residual water [22]. The use of digestates has been shown to increase biomass production and improve lipid composition, particularly in terms of polyunsaturated fatty acid (PUFA) production [23]. These findings are consistent with previous studies demonstrating the feasibility of using alternative media for microalgal cultivation [24]. The use of these materials also promotes waste valorization, aligning with the principles of the circular bioeconomy [3].

The aim of this study was to evaluate the use of vermicompost, digestates from mono-digestion and co-digestion of different manures, as culture media for *Chlorella vulgaris*, for carbohydrate, protein, lipid, and pigment production, as well as the lipids profile and its application in biodiesel production; thereby providing an alternative use for these waste-derived materials.

2. Materials and Methods

2.1 Microalgae

The microalga used was *Chlorella vulgaris*, provided by the Bioprocess Laboratory of the Unidad Profesional Interdisciplinaria de Biotecnología of the Instituto Politécnico Nacional.

2.2 Culture media preparation

Digestates were obtained from the anaerobic digestion of cow manure (DB), swine manure (DP), and a 1:1 mixture of both manures (DM). The anaerobic digestion was performed in 250 mL serum bottles, with 5% total solids at 30 °C, for a total fermentation time of 32 days [25].

Additionally, a digestate from the Universidad Autónoma de Chapingo, Mexico (DC) was used to compare with the laboratory-produced digestate under controlled conditions. This digestate was collected from the University digester, where manure from all livestock is processed.

All digestates were filtered through porous cloth, and the filtrate was diluted with distilled water to adjust the initial total nitrogen concentration to 40 mg L⁻¹, corresponding to that of the modified synthetic Bold Basal medium (MC).

Vermicompost and vermicompost leachate were obtained from GreenForest®. 200 g of vermicompost (VR) were mixed with 1 L of warm water, stirred, and maintained at room temperature for 3 days. The mixture was then filtered and diluted with distilled water to reach a total nitrogen concentration of 40 mg L⁻¹. Additionally, 10 mL of commercial vermicompost leachate (LX) was diluted with distilled water to achieve a total nitrogen concentration of 40 mg L⁻¹.

The modified Bold Basal medium (MC) was used as control to compare with the alternative media. It was prepared as follows (g L⁻¹): NaNO₃, 0.250; MgSO₄·7H₂O, 0.075; CaCl₂·2H₂O, 0.025; NaCl, 0.025; K₂HPO₄, 0.070; KH₂PO₄, 0.175; and FeSO₄·7H₂O, 0.004. The pH was adjusted to 6.8 [26].

2.3 Microalgae cultivation

A total of 15 mL of inoculum, previously adjusted to an optical density (OD₆₀₀) of 0.5, was transferred into 250 mL flasks containing 150 mL of each culture medium adjusted to 40 mg L⁻¹ of total nitrogen. The flasks were kept at 24 °C, under a light intensity of 3000 lux, with a 12:12 h light/dark photoperiod. Continuous aeration was provided using Elite 799 air pumps (19 L).

After 12 days, the procedure was repeated, and the biomass obtained from the second cultivation was used for subsequent analyses. All measurements were performed in triplicate.

2.4 Analytical methods

2.4.1 Characterization of culture media

To characterize the culture media, Chemical Oxygen Demand (COD), total nitrogen (N), total phosphorus (P), and total potassium (K) were quantified using Hach® kits and a Hach DR 2800 spectrophotometer, following the manufacturer's methodology. Total organic carbon (TOC) was calculated. These analyses were performed at the beginning and at the end of the cultivation period [27].

2.4.2 Biomass, pigments, proteins, carbohydrates, and lipids quantification

After 12 days of cultivation, total pigment content (sum of chlorophyll *a*, chlorophyll *b*, and carotenoids), proteins, carbohydrates, and lipids were quantified from the harvested biomass.

For biomass determination, 20 mL of microalgal suspension was centrifuged (Hettich centrifuge) at 13,000 rpm for 5 minutes. The cell pellet was separated, and the biomass was directly measured using

an ADAM AMB 50 thermobalance.

For pigment quantification, 1 mL of the microalgal suspension was centrifuged as described above. The cell pellet was resuspended in 1 mL of methanol and stored for 24 h at 4 °C in darkness. The sample was then centrifuged at 13,000 rpm for 5 minutes, the supernatant was recovered and measured the absorbance using a Thermo Scientific® Multiskan GO UV–Vis spectrophotometer at 470, 653, and 666 nm for the determination of carotenoids, chlorophyll *b*, and chlorophyll *a*, respectively. The sum of them was the total pigments [28].

Proteins were quantified using the Bradford method. One milliliter of the cell suspension was centrifuged as previously described, added 1 mL of 1 N NaOH, and placed in a boiling water bath for 2 hours to achieve hydrolysis. After that 100 µL was mixed with 1 mL of Bradford reagent and after 10 minutes, measured the absorbance at 595 nm. The calibration curve was prepared using bovine serum albumin [29].

For total carbohydrate quantification, 1 mL of the cell suspension was centrifuged as described above, mixed with 1 mL of 2 N HCl, and maintained in a boiling water bath for 2 hours for hydrolysis. Subsequently, 200 µL of 5% phenol and 1 mL of concentrated H₂SO₄ were added. After 10 minutes, the sample was vortexed, and after 30 minutes, measured the absorbance at 490 nm. The calibration curve was prepared using glucose [30].

For total lipid determination, 30 mL of the microalgal suspension was centrifuged at 6000 rpm for 15 minutes. The cell pellet was treated with 5 mL of 0.4 N HCl at 90 °C. After cooling, 10 mL of isopropanol was added, and the mixture was agitated at 250 rpm for one hour. Then, mixed with 10 mL hexane shaken, and allowed to stand for phase separation. The organic phase was dried with anhydrous sodium sulfate and transferred to a pre-weighed vial. Hexane was evaporated at 45 °C and the vial was allowed to cool. Total lipid content was determined by weight difference [26].

2.4.3 Biodiesel production

For the transesterification reaction, 1 mL of hexane and 1 mL of 0.5 N methanolic HCl were added to the vials previously used for lipid determination. The vials were capped and vortexed, then placed in a thermoblock at 80 °C for 2 hours. Subsequently, added 200 µL of distilled water and vortexed again and mixed with 1 ml HPLC-grade hexane, after 10 minutes, the organic phase was filtered through a 0.22 µm PTFE filter and transferred to a vial for chromatographic analysis [26]. The chromatographic analysis, was performed in an Agilent gas chromatograph (Technologies 7890B; Santa Clara, CA, USA) coupled to a mass spectrometer (GC/MSD Agilent Technologies 5977A Series; Santa Clara, CA, USA) with a VF-MAXms column (30 m × 0.25 mm, 0.50 µm), with helium as the carrier gas at a flow rate of 2.4 mL·min⁻¹. The injector temperature was maintained at 230 °C. The oven temperature was initially set at 140 °C for 5 minutes and then increased at a rate of 8 °C per minute up to 250 °C, where it was held for 15 minutes. The transfer line (MSD) temperature was maintained at 180 °C. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively [31].

To predict the quality of the obtained biodiesel, the iodine value (IV), cetane number (CN), higher heating value (HHV), density (D), kinematic viscosity (KV), and degree of unsaturation (UD) were calculated using the corresponding equations [32] and compared with the parameters specified in the EN 14213 [33] and ASTM D6751 standards [34].

3. Results and Discussion

3.1 Culture media assay

According to the initial characterization, the culture media showed significant variations in pH and composition compared to the synthetic medium (Table 1). These differences may have influenced the growth and metabolism of *Chlorella vulgaris*, since nutrient availability is critical for its development.

The initial pH of the alternative culture media was slightly alkaline and higher than that of the synthetic medium (MC). The pH of digestates generally ranges from 7.5 to 8.4 [35] due to their ammonia and phosphate content, which confers alkalinity to the culture medium [36]. This condition is desirable for microalgal cultivation because alkaline media favor the availability of dissolved CO₂, which is essential for photosynthesis [37].

Table 1. Characterization of the different culture media used for microalgal cultivation.

Treatments	Initial pH	Total organic carbon (mgL ⁻¹)	P total (mgL ⁻¹)	K total (mgL ⁻¹)
MC	6.75 ± 0.03	-	139.67 ±1.53	50.67 ±2.52
LX	7.96 ± 0.01	144.93 ±1.33	15.10 ±0.20	38.50 ±1.50
VR	8.16 ± 0.05	179.04 ±0.84	31.17 ±0.15	151.67 ±1.53
DP	7.06 ± 0.01	289.82 ±0.19	55.80 ±0.30	19.50 ±0.80
DB	7.25 ± 0.01	621.04 ±1.17	101.53 ±0.25	102.67 ±1.53
DM	6.88 ± 0.01	409.38 ±0.84	78.20 ±0.40	35.50 ±3.50
DC	8.27 ± 0.01	61.38 ±0.19	16.30 ±0.40	56.17 ±1.04

Values shown are the mean of three replicates (n = 3) ± standard error (SE). Identical letters within a column indicate no statistically significant difference (P < 0.05).

The P, K, and total organic carbon (TOC) contents differed among the digestates. The synthetic medium has the highest content of P, surprising between 38.14 to 124.57 g to the alternative culture media. The DM medium showed intermediate TOC, P, and K contents between DB and DP. Besides DM and DC were both from a co-digestion process the amount of nutrients were different, DM had 6.7 times more TOC and almost 5 times more P than DC, it has 1.6 times more K than DM. DB showed the highest TOC and K,

however, P was 37.5% lower than MC. These results are consistent with those reported by [38], who indicated that digestate obtained from cow manure digestion generally contains higher amounts of organic matter compared to digestate derived from swine manure. Lee et al [39] reported that bovine manure digestate contained 150 mg L⁻¹ of K and 60 mg L⁻¹ of P.

3.2 Biomass, carbohydrate, proteins, pigments, and lipids content

According to Table 2, the DM culture medium resulted in the highest amounts of biomass, proteins, and carbohydrates, exceeding the synthetic medium by 43%, 155%, and 150% respectively. Biomass obtained with DM was even higher than values reported by Koutra et al [40], who indicated a value of 1.24 g L⁻¹, like those obtained with VR, DP, and DC. Initial pH had effect on biomass, according to Panahi et al [6] *Chlorella vulgaris* grow better at pH 6 than 8, it tolerates better low pH than higher. The highest biomass production was from DM, MC and DP which had low initial pH values. DB reported the low biomass and had the highest initial pH.

Except for DB, which was statistically similar to MC, all other media produced more lipids than MC, the improvement was from 33 (DB) to 172% (DC). This could be explained because lipid production is affected besides N deficiency, by low illumination, causing stress conditions and in consequence the accumulation of energy in form of lipids [41]; all the vermicomposta and digestate based media had a dark color.

The highest pigment content was obtained with MC, which was 46% higher than DM, 42% higher than DP, 91% higher than DB, and 58% higher than DC. The amount of carbohydrate was higher in DM, followed by DC, VR and DP; the other media (LX and DB) were statistically equal then the synthetic medium. LX had low TOC this could explain the low carbohydrate production.

It is noteworthy that although DB contained the highest concentrations of TOC, P, and K, it resulted in the lowest biomass and lowest content of the measured compounds of interest, pigments, proteins, lipids, and carbohydrates. This may be attributed to its high darker coloration, which could have interfered with light penetration and, consequently, with photosynthesis and microalgal growth.

The increases in biomass, lipids and carbohydrates shown by some of the alternative media, may be attributed to their mixotrophic nature, where microalgae can also utilize organic carbon present in the culture medium. This observation is consistent with Peng et al [42], who reported that *C. vulgaris* cultivated under mixotrophic conditions produced higher amounts of biomass and lipids compared to photoautotrophic conditions.

Results are like other authors, as Azcárraga-Salinas et al [24], who found that in a *Scenedesmus obliquus* culture using digestates, vermicompost tea, and vermicompost leachate showed an improved in biomass (23-95%), lipid (70-110%), and carbohydrate (238-489%) using different digestates as culture media compared to synthetic medium.

Table 2. Composition and biomass of *Chlorella vulgaris* cultivated in different alternative media and in the synthetic medium.

Culture media	Biomass (gL ⁻¹)	Total Pigments (mgL ⁻¹)	Protens (mgL ⁻¹)	Lipids (mgL ⁻¹)	Carbohidrates (mgL ⁻¹)
MC	1.70 ± 0.20 ^{ab}	25.36 ± 1.99 ^a	139.36 ± 3.59 ^b	167.78 ± 2.94 ^f	561.36 ± 9.06 ^d
LX	0.78 ± 0.15 ^c	18.12 ± 1.48 ^b	96.00 ± 2.71 ^{cd}	223.33 ± 5.09 ^e	560.61 ± 9.11 ^d
VR	1.55 ± 0.14 ^b	11.48 ± 0.36 ^c	101.64 ± 5.43 ^c	284.44 ± 4.84 ^d	800.84 ± 1.22 ^c
DP	1.58 ± 0.16 ^b	14.52 ± 1.76 ^{bc}	126.10 ± 0.49 ^b	342.22 ± 4.00 ^c	784.39 ± 9.98 ^c
DB	0.62 ± 0.15 ^c	2.16 ± 0.60 ^d	84.51 ± 2.33 ^d	173.33 ± 3.85 ^f	441.65 ± 3.60 ^e
DM	2.43 ± 0.14 ^a	13.47 ± 0.71 ^{bc}	355.23 ± 2.00 ^a	435.56 ± 4.84 ^b	1403.43 ± 19.79 ^a
DC	1.55 ± 0.13 ^b	10.46 ± 0.33 ^c	131.38 ± 1.12 ^b	456.67 ± 3.85 ^a	1196.99 ± 18.58 ^b

Values shown are the mean of three replicates (n = 3) ± standard error (SE). Identical letters within a column indicate no statistically significant difference (P < 0.05).

The amounts of biomass, proteins, lipids, and carbohydrates were higher in the digestate obtained from the mixture of manures (DM) compared to those produced using a single type of manure (DP or DB). Besides, DC resulted in the third-highest protein concentration, the highest lipid content, and the second-highest carbohydrate content. Both DM and DC digestate were derived from anaerobic co-digestion of mixtures of manures, indicating that the co-digestion of manures from different animal sources provides characteristics that favor the growth and composition of *C. vulgaris*. Co-digestion, by combining the properties and characteristics of more than one type of manure, provides a better C/N ratio and improved biodegradability compared to mono-digestion, demonstrating a synergistic effect [43]. Furthermore, the co-digestion of swine and bovine manure prevents inhibition caused by ammonia and volatile fatty acids (Wi et al and Pulgarín-Muñoz et al) [44,45] reported that the co-digestion of sewage sludge with agro-industrial fruit and vegetable residues increased the biodegradable organic content. Hilgert et al [46] indicated that the degradability of cow and swine manure differs due to the animals' diets, being higher in swine manure because of grain-rich feeding and slower in cow manure due to

pasture-based feeding. Azcárraga-Salinas et al [24] reported similar behavior, medium with digestate from the co-digestion of swine and cow manure improved the biomass, pigments, lipids, and carbohydrates in 13%, 10%, 8%, and 22% respectively compared to using a digestate from swine manure digestion; and improvements of 20%, 114%, 8%, and 45% respectively compared to digestate from cow manure anaerobic digestion.

FAMES produced in the culture medium

Ten different fatty acids methyl esters (FAMES) were identified across the treatments (Table 3). Palmitic acid and oleic acid were produced in all culture media. Palmitic acid was the most abundant FAME in all media, which is consistent with reports indicating that this FAME is the most abundant in *Chlorella* sp. [47]. The most common FAMES in microalgae are palmitic, stearic, oleic, linoleic and linolenic acids [48,49]. The composition of lipids as well as the proportion of unsaturated, mono and polysaturated acids depends on culture media, microalgae species, and growth conditions [23].

In the synthetic medium MC and in the LX, VR, and DP media, were identified four FAMES. Three of these FAMES have been previously reported in *C. vulgaris* cultivated in three different synthetic media, where their proportions varied, emphasizing that the selection of the culture medium is crucial for FAME production and composition [50]. In the DB medium, were identified only palmitic and oleic acids, whereas in the DM medium, 9 of the 10 detected FAMES were produced, except for bicyclopropyl hexyl-2-octanoic acid. In DC, five FAMES were identified. This confirms a synergistic effect in the DM and DC media, which were yielded by different manures co-digestion. This effect was reflected not only in the content of value-added compounds (table 3) but also in a greater diversity of fatty acids.

The percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated acids (PUFAs) could determine the microalgae potential applications. For example, a higher proportion of SFAs and MUFAs is recommended for biofuel production [51] because a high PUFAs proportion decreased the biodiesel stability causing poor oxidative stability [52]. In all culture media, more than 50% SFAs and MUFAs were attained, highlighting DB, which reached 100%, followed by the vermicompost-based media: 72.74% with VR and 66.61% with LX. The remaining media showed values like MC. The lipidic acids produced in the synthetic media MC and using digestates produced more than 40% PUFAs compared to treatments with vermicompost based media that reached lower than 33.5%. These results are consistent with those reported by Arora and Philippidis [51] who observed more than 50% SFAs and MUFAs in three *Chlorella* strains cultivated under mixotrophic conditions. González-Falfán et al [31], reported for *Scenedesmus* sp. cultivated with different no synthetic media, 60%–69% SFA proportion, and unsaturated in the range of 31% to 38%. PUFAs in microalgae allow them to remain metabolically active and survive the environmental stress conditions [53,54]. Ermis et al [22] tested wild-type microalgae cultivated on digestate and three synthetic media, with digestate improved the lipid production as well as saturated and polyunsaturated fatty acids proportion.

Table 3. Lipid profile of fatty acids produced in each treatment by *Chlorella vulgaris* and percentage of saturated and unsaturated fatty acids.

Treatment	Fatty acids	% Saturation
MC	Palmitic acid (C16:0)	SFAs: 42.48 ± 2.23
	Oleic acid (C18:1)	MUFAs: 11.69 ± 0.65
	Bi cyclopropyl hexil-2-octanoic acid (C21:0)	PUFAs: 45.83 ± 2.13
	Linolenic acid (C18:3)	
LX	Palmitic acid (C16:0)	SFAs: 47.99 ± 1.29
	Oleic acid (C18:1)	MUFAs: 18.82 ± 1.51
	Bi cyclopropyl hexil-2-octanoic acid (C21:0)	PUFAs: 33.20 ± 1.31
	Linolenic acid (C18:3)	
VR	Palmitic acid (C16:0)	SFAs: 40.76 ± 1.29
	Oleic acid (C18:1)	MUFAs: 31.98 ± 1.75
	Bi cyclopropyl hexil-2-octanoic acid (C21:0)	PUFAs: 27.26 ± 0.51
	Araquidonic acid (C20:4)	
DP	Palmitic acid (C16:0)	SFAs: 25.68 ± 2.32
	Oleic acid (C18:1)	MUFAs: 28.56 ± 0.97
	Linoleic acid (C18:2)	PUFAs: 45.76 ± 1.13
	Linolenic acid (C18:3)	
DB	Palmitic acid (C16:0)	SFAs: 51.17 ± 2.77
	Oleic acid (C18:1)	MUFAs: 48.83 ± 2.77
DM	Palmitic acid (C16:0)	
	7-hexadecenoic acid (C16:1)	SFAs: 16.33 ± 0.26
	7,10-hexadecadienoic acid (C16:2)	MUFAs: 40.09 ± 0.80
	7,10,13-hexadecatrienoic acid (C16:3)	PUFAs: 43.58 ± 0.39

	9,12-octadecadienoic acid (C21:2)	
	16-methyl heptadecanoic acid (C17:1)	
	Oleic acid (C18:1)	
	Linoleic acid (C18:2)	
	Linolenic acid (C18:3)	
	Palmitic acid (C16:0)	SFAs:: 21.92 ± 0.46
DC	Oleic acid (C18:1)	MUFAs: 29.54 ± 1.36
	12,15-Octadecadienoic acid (C18:2)	PUFAs: 48.55 ± 0.49
	Linolenic acid (C18:3)	
	Araquidonic acid (C20:4)	

The letter C refers to the carbon chain length, and the number after the colon indicates the number of unsaturations of the fatty acid methyl ester in each treatment. Values shown are the average of three replicates ($n = 3$) ± standard error (SE).

Theoretical determination of biodiesel quality

Based on the lipid profile and the degree of unsaturation of the fatty acids, it is possible to predict the biodiesel quality for each treatment. According to Table 4, biodiesel obtained from the lipids generated using the vermicompost-based media (LX and VR), as well as from digestates derived from a single type of manure digestion (DB and DP), meets the five evaluated quality parameters established by the international standards EN 14213 [33] and ASTM D6751[34]. In contrast, biodiesel from lipids generated by MC and DM did not meet the iodine index value requirement, while DC failed to meet both the iodine index value and cetane number criteria.

The SFA content is inversely related to the iodine index value; higher SFA percentages result in lower iodine index values. Low iodine index can improve the oxidative stability of biodiesel but may also increase its cloud point, thereby limiting its use in cold climates [55]. Low iodine index values, such as that observed for DB (41.83), are ideal for biodiesel intended for use in cold environments, since saturated fatty acids (C16:0) prevent polymerization [56].

Biodiesel with high cetane number values improves ignition quality [36]. This parameter is associated with high proportions of SFAs and MUFAs, which should be present in balanced ratios, as observed in DP and DB. An unbalanced proportion may lead to incomplete combustion [51], as in the case of DM, which showed more than two times the percentage of MUFAs relative to SFAs, and MC and LX, which also exhibited more than two times the MUFA content compared to SFAs. High PUFA content tends to reduce the cetane number [57].

In terms of kinematic viscosity and higher calorific value, biodiesel obtained from lipids from all culture media complied with the established standards. Viscosity is an important parameter because high viscosity can cause injector clogging whereas low viscosity improves fuel flow under cold conditions [58]. The higher calorific value indicates whether biodiesel is suitable for industrial applications [51].

The biochemical composition of the microalgae allows the suggestion of potential applications. DM and DC media for its high carbohydrates production could have potential use in biorefineries for bioenergy conversion. Microalgae with high amounts and diversity of PUFAs have been used as feed to enhance the fatty acid content of oily fish [59,60] besides carotenoids and PUFAs reduce mortality, enhance stress response and aquatic animals environmental adaptation [3] linolenic, linoleic, and palmitic acids are highly valued in aquaculture [61]. Therefore, MC, DP, DM, and DC media could be suitable for this purpose, as their PUFA percentages exceeded 43%. The DB treatment met all five biodiesel quality parameters and showed the highest cetane number and the lowest iodine index value, making it the most suitable for biodiesel production.

Table 4. Predicted biodiesel quality parameters obtained for lipids from each treatment.

Tratament	Iodine index (IV)	Cetane number (CN)	High calorific value (MJ/kg) (HHV)	Kinematic Viscosity (mm ² /s) (KV)	Density (g/cm ³) (D)	Insaturation grade (UD)
MC	129.61 ± 5.56 ^b	51.84 ± 1.65 ^{cd}	39.79 ± 0.040 ^b	4.18 ± 0.092 ^{bc}	0.88 ± 0.008 ^b	103.36 ± 4.27 ^b
LX	102.74 ± 2.21 ^c	56.98 ± 0.77 ^b	39.79 ± 0.023 ^b	4.31 ± 0.05 ^{abc}	0.88 ± 0.003 ^d	85.21 ± 1.25 ^c
VR	114.36 ± 3.04 ^c	54.88 ± 0.60 ^{bc}	39.90 ± 0.007 ^a	4.38 ± 0.026 ^{ab}	0.88 ± 0.004 ^{bc}	86.51 ± 2.70 ^c
DP	103.76 ± 3.28 ^c	55.25 ± 0.59 ^{bc}	39.71 ± 0.005 ^{cd}	4.13 ± 0.008 ^{cd}	0.88 ± 0.004 ^{cd}	120.08 ± 3.82 ^a
DB	41.83 ± 2.37 ^d	67.95 ± 0.34 ^a	39.74 ± 0.010 ^{bc}	4.49 ± 0.006 ^a	0.87 ± 0.003 ^e	48.83 ± 2.77 ^d
DM	134.22 ± 1.59 ^{ab}	48.51 ± 0.38 ^{de}	39.61 ± 0.007 ^d	3.93 ± 0.055 ^{de}	0.88 ± 0.002 ^{ab}	120.79 ± 2.48 ^a
DC	145.26 ± 2.25 ^a	46.08 ± 0.52 ^e	39.67 ± 0.006 ^{cd}	3.88 ± 0.018 ^e	0.88 ± 0.003 ^a	126.62 ± 1.29 ^a

ASTM D6751	≤120	≥47	≥35	1.9-6.0	0.86-0.90	NA
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EN 14213: European Committee for Standardization (CEN). ASTM D6751: American Society for Testing and Materials (ASTM). Values shown are the mean of three replicates ($n = 3$) $\pm$ standard error (SE).

Conclusions

The use of digestates and vermicompost represents effective alternative culture media for *Chlorella vulgaris*. Biodiesel potentially obtained from vermicompost-based media and from digestates derived from cow or swine manure meet all the evaluated quality parameters. Digestates obtained through co-digestion of manures (DM and DC) increased the protein, lipid, and carbohydrate contents compared to monodigestion, and promoted a greater diversity of FAMES. The digestates exerted different effects on microalgal composition, suggesting different potential applications. DB is recommended for biodiesel production, whereas DM and DC are suitable for bioenergy applications such as bioethanol production due to their high carbohydrate content. MC, DP, DM, and DC are suitable for aquaculture feed because of their high PUFA content.

Declarations

Data availability Not applicable.

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