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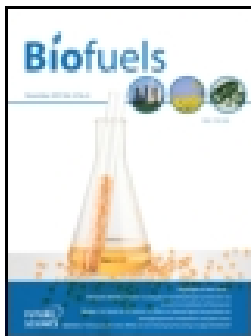119 PUBLICATIONS   2,306 CITATIONS

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# Utilisation of *Desmodesmus subspicatus* LC172266 for simultaneous remediation of cassava wastewater and accumulation of lipids for biodiesel production

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## ABSTRACT

In view of the current demand for bioenergy and environmental sustainability, utilisation of *Desmodesmus subspicatus* LC172266 for simultaneous remediation of cassava wastewater and accumulation of lipids for biodiesel production was investigated. The microalga was grown under photoautotrophic, heterotrophic and mixotrophic culture conditions using Bold's Basal Medium (BBM) and cassava wastewater as substrates. Results showed that biomass accumulation under unoptimised autotrophic, heterotrophic and mixotrophic growths conditions were 0.217, 0.63 and 1.042 g/L, respectively. The lipid contents were 15.86, 21.40 and 24.70%, respectively. Fatty acid profiles revealed that although the most abundant compounds under autotrophic conditions were those recommended for biodiesel production, oleic acid eluted under mixotrophic (19.61%) and heterotrophic (10.25%) conditions exhibited more acceptable biodiesel quality. The physical properties of the fatty acid methyl esters (FAMEs) also meet the required biodiesel standards. The percentage reduction in total dissolved solids (TDS, 84.04), electrical conductivity (EC, 82.31), biochemical oxygen demand (BOD, 85.85) and chemical oxygen demand (COD, 89.04) under heterotrophic cultivation were more favourable than their respective mixotrophic values (80.56, 74.63, 62.22, and 51.39%). Although the lipid accumulation of the microalga under mixotrophic conditions was greater, bioremediation potential of cassava wastewater was more favourable under heterotrophy.

## Introduction

The inexorably increasing anthropogenic activities and energy needs for heating and transportation are currently met mostly by fossil fuels, the combustion of which inevitably leads to increase in atmospheric carbon compounds. The methods of energy generation nowadays are not compatible with the environment as the use of sequestered hydrocarbon fossil fuels comes at a huge cost to the environment which is threatening the existence of mankind on earth with pollution and global warming. This is also more worrisome when there is a consideration that conventional petroleum is essentially non-renewable. Intertwined with this practical impediment is an apparent moral dilemma of environmental pollution arising from its very usage [1].

Fossil fuels pose serious threats to the environment due to the production of greenhouse gases (GHGs), including carbon dioxide [2]. The role of GHGs in warming our planet, and the associated reality of human-driven climate change, are fully accepted [3]. Concerns about climate change and global warming, coupled with increasing global demand for oil and the necessity of substituting natural fuel for energy production, require the implementation of new methods for

energy production by using natural and renewable carbon resources [4]. Recently, the United Nations' Intergovernmental Panel on Climate Change said that the damage to the environment would be severe, pervasive and irreversible, causing long-lasting changes in all components of the climate system if fossil fuel combustion is not stopped by 2100 [5]. To continue to combust fossil fuels at current rates without regard for the environment is no longer sustainable, particularly in the face of the projected 9 billion people and 2 billion cars on planet Earth by 2050 [6].

The use of renewable carbon sources for energy production fulfils two objectives: reduction in carbon emission from fossil fuels and use of carbon from compounds that if left in the environment constitute an environmental nuisance [7]. In this regard, intensive research has been on-going to explore suitable alternative energy sources that could potentially be substituted for fossil fuels. Biofuels are attractive replacements for fossil fuel-derived fuels and they are recognised for their potential role in reducing GHG emissions. Switching from fossil fuels to biofuels will reduce the world's reliance on oil [8].

Chisti [7] described microalgae as sunlight-driven cell factories. They have the ability to use sunlight and

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available nutrients to produce biomass, which can in turn be used to produce a wide range of products ranging from cosmetics to pharmaceuticals to biofuels. Biomass from microalgae gives promise as an alternative energy source since it is renewable and produces carbon-neutral emissions. Depending on the species of microalgae, growth can be in a medium containing the required nutrients and a carbon source, being either atmospheric carbon dioxide or carbon compounds, within the medium in the presence or absence of sunlight [9]. Although microalgae lipids are potential feedstocks for biodiesel production due to their higher biomass productivities and lower requirement for cultivation area than currently used plant crops for biodiesel production [10], to generate 1 kg of algal biodiesel, 3726 kg of fresh water, in addition to other significant macro- and micronutrient inputs, is needed [11,12]. This, consequently, makes algal biodiesel production economically less viable [13]. However, coupling wastewater treatment with biofuel production is a very attractive option for energy and fresh water. Wastewater as a substrate provides an effective nutrient base for algal cultivation since it contains much of the macronutrient and trace element requirements for algal cultivation with the enormous benefit of being a source of water for cultivation [12]. Bux [12] adds that cultivation of microalgae able to undergo mixotrophic growth provides a significant advantage in the removal of organic carbon from wastewater in addition to nitrogen and phosphorus enabling nutrient recovery and conversion of $CO_2$ into biologically bound carbon. As it is a 'freely available' resource, the use of wastewater for algal cultivation has the potential to significantly improve the economics of biofuel production.

Many industrial processes produce wastes in quantities that are intolerable to the environment. One such process is cassava processing, which produces copious amounts of wastewater containing some compounds which are toxic to life and the environment particularly when disposed of inappropriately [14]. Cassava is a root crop grown mainly for its starch content. Almost all the varieties of cassava contain varying quantities of toxic compounds, thus making its consumption without proper processing dangerous. Cassava is processed into many non-toxic products for consumption, one of which is fried grated cassava flakes called garri whose production produces large amounts of liquid effluent. Nigeria is the largest producer of cassava, accounting for about 18% (42 billion kg) of world production [15], with the most advanced cassava transformation processes in Africa [14]. During cassava processing in the country, a huge amount of liquid effluent is concomitantly generated – through washing or squeezing of the grated pulp – and naturally discharged into the environment, almost always with no caution. Cassava wastewater, if released directly into the environment without proper treatment, is a source of pollution. In many areas where traditional processing is practised, it is normally discharged beyond the 'factory' wall into roadside ditches or fields and allowed to flow freely, settling in shallow depressions, eventually percolating into the subsoil or flow into streams [16]. Water released from cassava during squeezing especially can have potentially harmful effects on the environment, particularly if generated in large amounts, because of its high biological oxygen demand (BOD) of 25,000–50,000 mg/L and a typical cyanide concentration of more than 400 mg/L [16]. Although this liquid effluent is acidic and contains a number of contaminating substances, it is a rich source of organic matter (soluble carbohydrates and proteins) and suspended solids (lipids and non-soluble carbohydrates – starch or cellulose fibres) [16] – a complete mixture of nutrients in solution and suspension. Microalgae can grow and produce biomass on this effluent, which in turn can serve as a precursor for the production of many products [17]. This serves as a way of mitigating the environmental pollution in addition to the conversion of potential industrial waste into useful products, such as biofuel. The use of microalgae for simultaneous wastewater treatment and accumulation of lipids for biodiesel production has previously been espoused [11,18]. The present study therefore aimed at investigating the utilisation of *Desmodesmus subspicatus* LC172266, a microalga, for simultaneous remediation of cassava wastewater and accumulation of lipids for biodiesel production.

## Materials and methods

### Cassava wastewater

Cassava wastewater was obtained from a local cassava mill, and the initial pH of the solution and other physicochemical parameters were measured. To simulate the filterability of the wastewater by the soil, a bespoke apparatus was designed thus: a funnel 30 cm long and 2.5 cm in diameter was fitted with wire gauze (mesh size = 0.1 mm) just before the mouth of the funnel was constructed. Coarse sandy soil from a river, in the amount of 98.19 cm$^3$, was poured into the funnel, making a sand column 5 cm high. The cassava wastewater was filtered through the sand column to simulate the natural filtration activity of soil. A molar concentration of $H_2SO_4$ (50 mL) was added to 10 L of the filtrate and heated for 1 h while stirring continuously until the wastewater gelatinised. The heating was continued until the milky gelatinised liquid became watery (total heating time was 1 h). After heating, 32.67 mL of 60% NaOH solution was added to 1 L of the heated mixture and stirred vigorously to neutralise and stabilise the pH. Each litre of the mixture was supplemented with 10.0 mL of the stock solutions of sterilised Bold's Basal Medium (BBM) and 1.0 mL each of the trace metal

stocks before stirring vigorously to mix. This was then sterilised by autoclaving at 121°C for 10 min. Thereafter, 200 mg of ketoconazole and 250 mg of ampicillin was added to the sterile medium.

### The microalga and growth conditions

*Desmodesmus subspicatus* LC172266 isolated from a water body in North East Nigeria [19,20] was used for this study. The preliminary growth of the pure culture of the alga was done in 1000-mL Erlenmeyer flasks containing 500 mL of sterilised BBM for the photoautotrophic cultures. Cassava wastewater enriched with BBM was used for the heterotrophic and mixotrophic growth cultures. Seed culture was grown in BBM up to the exponential growth phase for the purpose of acclimatisation. The seed culture for mixotrophic and heterotrophic growth phases was further acclimatised to grow in the dark for 72 h. All inoculations were done aseptically and in replicates. In determining the autotrophic growth, 50 mL of the seed culture grown in BBM was inoculated into 500 mL of BBM (20% v/v inoculum ratio). The flasks were then exposed to approximately 12 h of sunlight daily (intensity $\sim$ 2000 lux) at room temperature (30 $\pm$ 2°C) for 14 days. The flasks were mildly agitated at 3-h intervals during the exposure to sunlight. For heterotrophic and mixotrophic cultures, the cassava wastewater enriched with BBM was used. The heterotrophic seed culture was inoculated into 500 mL of the medium at a 20% v/v inoculum ratio. Whereas the conical flasks were incubated in the dark for heterotrophic culture, the mixotrophic cultures were exposed to sunlight for 4 h daily between 9:00 am and 1:00 pm, after which they were incubated in the dark. Other growth conditions were as stated above. The growth rates of all cultures were measured spectrophotometrically at 680 nm [21] using a Hach DR 5000 spectrophotometer (HACH, Loveland, Colorado, USA). A growth curve was generated based on the optical density (OD) readings and a standard curve was used to convert to cell dry weight.

### Determination of dissolved oxygen (DO) and biochemical oxygen demand (BOD)

The modified Winkler–Azide method [22] was used to analyse cassava wastewater samples for dissolved oxygen (DO), while biochemical oxygen demand (BOD5) was determined by the difference between DO of samples immediately after collection and DO of samples after incubation at 20°C for 5 days [23]. For the DO content, the wastewater was dispensed into two 300-mL bottles labelled Day 1 and Day 5. To the Day 1 sample, 2 mL each of $MnSO_4$ and alkali-iodide-azide reagent were added well below the surface of the liquid and bottles were stoppered with care to avoid the formation of bubbles. The solution was mixed by inverting

the bottle a number of times until a clear supernatant was obtained. The solution was allowed to settle for 2 min and then the DO content of the sample was determined immediately, after which 2 mL of concentrated $H_2SO_4$ was added by allowing the acid to run down the neck of the bottle. The bottle was stoppered again and mixed by inverting gently until dissolution was completed. When the iodine was uniformly distributed, 2 mL of starch solution was added to 203 mL of the sample and then titrated with 0.0125 M sodium thiosulphate ($Na_2S_2O_3$. $5H_2O$) solution to a colourless solution. The other sample, labelled Day 5, was kept in an incubator at 20°C for 5 days before determination of the DO content. The BOD (mg/L) was obtained by finding the difference in DO between Day 1 and Day 5.

### Determination of chemical oxygen demand (COD)

Chemical oxygen demand (COD) was determined using the titrimetric method described by Ademoroti [22]. Anti-bumping granules were introduced into reflux flasks, to which 20 mL each of the samples was added followed by 2 mL of 20% (w/v) mercuric sulphate solution before swirling. To these, 10 mL of 0.021 M potassium dichromate ($K_2Cr_2O_7$) was added to each mixture. Then, using a dispensing pipette, 30 mL of 1% (w/v) silver sulphate ($Ag_2SO_4$) was also added to each flask. Each flask was fitted to a condenser and the contents boiled gently for 2 h, after which the flask was removed and allowed to cool for approximately 10 min. The condenser was washed with distilled water and then the contents of the flask diluted to 150 mL. To the flasks, 2 drops of Ferroin indicator was added and the residual dichromate titrated with standardised ferrous ammonium sulphate (($NH_4)_2$ $Fe(SO_4)_2$ $6H_2O$). The COD was obtained using Equation (1):

$$COD \ (mg \ / \ L) = (V_b - V_a) \times 16000 \times \frac{M}{volume \ of \ sample}$$

(1)

where

- $V_b$ = average number of millilitres of ferrous ammonium sulphate used in titrating the appropriate blank;
- $V_a$ = number of millilitres of ferrous ammonium sulphate used in titrating the sample; and
- M = molarity of standard ferrous ammonium sulphate solution.

### Determination of electrical conductivity (EC) and total dissolved solids (TDS)

The electrical conductivity (EC) was determined by placing a conductivity probe (Hanna combo pH/EC meter HI 98 129) into the cassava wastewater sample in a 250-mL conical flask. The electrical conductance

obtained in microseconds per centimetre ($\mu$s/cm) was recorded directly from the meter after 3 min [24].

Total dissolved solids (TDS) was determined by the filtration method (American Society of Testing and Materials) (ASTM). Fifty millilitres of the sample was transferred into a weighed evaporating dish and evaporated to dryness at 180°C. The increase in weight over the empty dish represented the TDS [23]. The TDS was calculated using Equation (2):

$$TDS \ (mg \ / \ L) \ = \frac{mg \ residue \ \times \ 1000}{mL \ of \ sample} \qquad (2)$$

### Lipid extraction and determination of lipid composition (GC-MS analysis)

Lipid extraction was performed in accordance with Bligh and Dyer's [25] protocol using chloroform, methanol and water as solvents. The extracted lipids were analysed by Gas Chromatography Mass Spectrometry (GC-MS) (Shimadzu, Japan, Model GCMS-QP2010 Plus, with a Db 30.0 capillary column). The initial oven temperature was programmed at 70°C (isothermal for 5 min), with an increase of 10°C/min, to 250°C, then 5°C/min to 280°C, and finally ending with a 24-min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s and scan range of 40–1000 m/z. Helium was used as the carrier gas, at a flow rate of 1.8 mL /min. The injected sample volume and the inlet pressure were 1.0 $\mu$L and 116.9 kPa, respectively. The injection temperature was 250°C and injection mode was split. The GC program ion source temperature was 200°C, interface temperature was 250°C, solvent cut time was 2.50 min, detector gain mode was relative, and detector gain was 0.00 kV while the threshold was 2000.
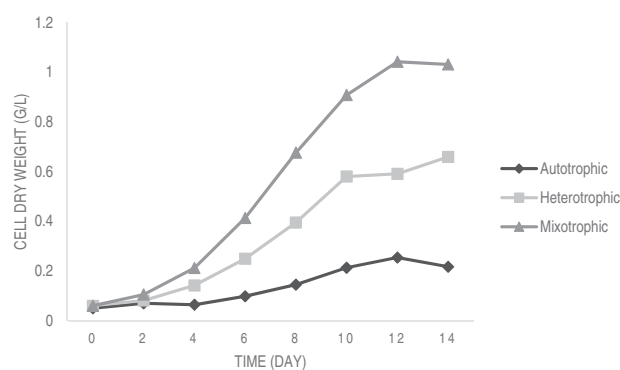
## Results

### Physicochemical properties of cassava wastewater

The baseline physicochemical properties of the fresh cassava wastewater (FCW), hydrolysed cassava wastewater (HCW) and hydrolysed cassava wastewater plus Bold's Basal Medium (HCW+BBM) used for the growth



**Figure 1.** Time course of *Desmodesmus subspicatus* LC172266 growth under the three cultivation conditions.
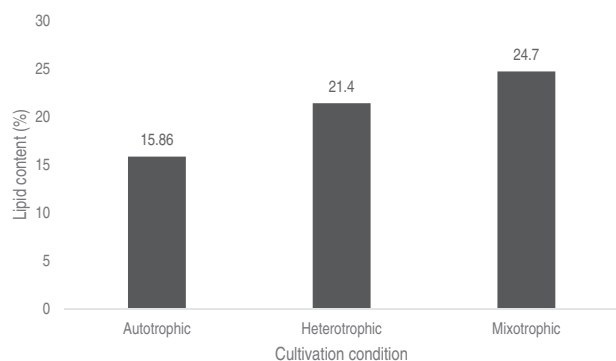
of the microalga species in mixotrophic and heterotrophic growth conditions are shown in Table 1. FCW had an EC of 1090 s/m, TDS of 510.00 mg/L, BOD of 577.48 mg/L, COD of 1570.00 mg/L and pH of 2.13. A marginal decrease in EC was observed in the HCW (1070 s/m), TDS increased appreciably to 890.00 mg/L, there was a minimal reduction in BOD (546.48 mg/L), COD remained unchanged (1571.00 mg/L) and there was a reduction in pH (1.47) compared to FCW. A tripling of the EC (3680.00 s/m) compared to FCW was observed in the HCW+BBM, TDS more than doubled (1960.00 mg/L), BOD was 562.35 mg/L, COD was 1571.00 mg/L and a significant rise in pH (5.7) was observed compared to FCW. The % reduction in TDS (84.04), EC (82.31), BOD (85.85) and COD (89.04) under heterotrophic cultivation was more considerable than the respective reduction values (80.56, 74.63, 62.22 and 51.39%) obtained in mixotrophic cultures.

The growth curves of the alga under the different conditions are presented in Figure 1. The mixotrophic growth mode was the highest, with a maximum cell dry weight value of 1.042 g/L. Heterotrophic growth accumulated a biomass of 0.659 g/L, while autotrophic growth was the lowest with a biomass of 0.217 g/L after 14 d of growth. Similarly, the lipid content (%) of the microalga after incubation shows the autotrophic growth mode had the lowest value, 15.86%, while heterotrophic and mixotrophic growth conditions had 21.4% and 24.7% lipid contents, respectively (Figure 2).

### GC-MS profiles showing fatty acid composition of the alga under different cultivation conditions, and biodiesel quality analysis

Table 2 describes the identity of the compounds in the GC-MS analysis of the samples under autotrophic conditions. Of the eight compounds found, the most abundant were 9,15-octadecadienoic acid (44.94%), methyl 14-methyl pentadecanoate (15.17%), 1,9-tetraddecadiene (16.69%), stearic acid (12.18%), palmitic acid (3.75%), octadecanoic acid (4.46%), methyl ricinoleate (2.12%) and behenic acid methyl acid (0.71%).

**Table 1.** The physicochemical properties of the cassava wastewater.

| Parameter | Value | | |
| --- | --- | --- | --- |
| | Fresh Casava Wastewater (FCW) | Hydrolysed Casava Wastewater (HCW) | HCW + Bold's Basal Medium (BBM) |
| Conductivity (s/m) | 1090.00 | 1970.00 | 3680.00 |
| TDS (mg/L) | 510.00 | 890.00 | 1960.00 |
| BOD (mg/L) | 577.48 | 546.34 | 562.35 |
| COD (mg/L) | 1570.00 | 1571.00 | 1570.00 |
| pH | 2.13 | 1.47 | 5.70 |

Note: FCW = fresh cassava wastewater; HCW = hydrolysed cassava wastewater; HCW + BBM = hydrolysed cassava wastewater supplemented with Bold's Basal Medium.

**Figure 2.** Lipid content (%) of the microalga cultivated under different culture conditions.

Under heterotrophic growth conditions for *Desmodesmus subspicatus*, 16 compounds were identified (Table 3). They included 11-octadecanoic acid (24.88%), oleic acid (19.61%) and methyl 5-(2-undecyclo propyl) pentanoate. Other compounds present were 2-ethyl 2-hexenal (0.92%), 3,4-dimethyl-3-hexen-2-one (0.24%), octanoic acid (1.86%), methyl caprate (1.81%), undecanoic acid (3.90%), tridecanoic acid (6.15%), palmitic acid (2.37%), methyl palmitoleate (1.04%), methyl 14-methyl pentadecanoate (8.42%), hexadecanoic acid (7.20%), octadecanoic acid (4.77%) and eicosanoic acid (0.25%).

The chemical composition of the lipid extract from *Desmodesmus subspicatus* LC172266 grown in mixotrophic mode is presented in Table 4. The major compounds identified were linolelaidic acid (22.93%), 11-octadecenoic acid (18.16%), methyl 14-methyl pentadecanoate (18.05%) and oleic acid (10.25%). Other compounds present were 2-ethyl-2-hexenal (1.09%),

capric acid (0.65%), methyl tridecanoate (0.35%), palmitic acid (2.46%) 7-hexadecenoic acid (1.94%), stearic acid (5.91%), cis-oleic acid (3.71%), hexadecane (2.25%), methyl-dihydrosterculate (3.48%), linoleoyl chloride (1.21%), 2-methyl nonadecane (1.70%), eicosanoic acid (1.58%), eicosane (1.11%), octadecane (0.62%), behenic acid (0.38%), icosane (0.88%), pentadecane (0.56%) and heptadecane (0.71%).

The biodiesel quality potentials of the fatty acids were analysed using the software BiodieselAnalyzer© [26], and the physical properties compared with commercially used plant oil methyl esters (jatropha, palm) and ASTM D6751 and European Committee for Standarsation (EN) 14214 vehicular biodiesel standards as reported by Arora *et al.* [11]. Results of the biodiesel properties (Table 5) show that the physical properties of the fatty acid methyl esters (FAMEs) were in consonance with acceptable standards and compare favourably with those described in the literature. The parameters included, for example, cetane number (which measures the combustibility and cold start properties of the fuel), which was quite high (91), and kinematic viscosity, which was found to be 2.28 mm$^2$/s.

## Discussion

This study focused on the simultaneous removal of carbon compounds as well as other nutrients from cassava wastewater by *Desmodesmus subspicatus* LC172266 and its accumulation of lipids for biodiesel production. Sand filtration as a pre-treatment procedure was done to remove the large particulate matter in the wastewater, but other methods such as

**Table 2.** Chemical composition of *Desmodesmus subspicatus* LC172266 extract grown in autotrophic mode.

| Peak | Retention time (min) | Compound | Area (%) | Molecular weight | Molecular fomula |
|---|---|---|---|---|---|
| 1 | 15.475 | Methyl 14-methyl pentadecanoate | 15.17 | 270 | $C_{17}H_{34}O_2$ |
| 2 | 16.027 | Palmitic acid | 3.75 | 256 | $C_{16}H_{32}O_2$ |
| 3 | 17.178 | 9,15-octadecadienoic acid methyl ester | 44.94 | 294 | $C_{19}H_{34}O_2$ |
| 4 | 17.412 | Stearic acid methyl ester | 12.18 | 298 | $C_{19}H_{38}O_2$ |
| 5 | 17.704 | 1,9-tetraddecadiene | 16.69 | 194 | $C_{14}H_{26}$ |
| 6 | 17.903 | Octadecanoic acid | 4.46 | 284 | $C_{18}H_{36}O_2$ |
| 7 | 18.955 | Methyl ricinoleate | 2.12 | 312 | $C_{19}H_{36}O_3$ |
| 8 | 19.182 | Behenic acid methyl acid | 0.71 | 354 | $C_{23}H_{46}O_2$ |

**Table 3.** Chemical composition of lipid from *Desmodesmus subspicatus* LC172266 grown in heterotrophic mode.

| Peak | Retention time (min) | Compound | Area (%) | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 4.117 | 2-Ethyl-2-hexenal | 0.92 | 126 | $C_8H_{14}O$ |
| 2 | 4.296 | 3,4-Dimethyl-3-hexen-2-one | 0.24 | 126 | $C_8H_{14}O$ |
| 3 | 5.669 | Octanoic acid methyl ester | 1.86 | 158 | $C_9H_{18}O_2$ |
| 4 | 8.416 | Methyl caprate | 1.81 | 186 | $C_{11}H_{22}O_2$ |
| 5 | 11.032 | Methyl 5-(2-undecyclopropyl) pentanoate | 11.80 | 310 | $C_{20}H_{38}O_2$ |
| 6 | 11.787 | Undecanoic acid | 3.90 | 186 | $C_{11}H_{22}O_2$ |
| 7 | 13.359 | Tridecanoic acid methyl ester | 6.15 | 228 | $C_{14}H_{28}O_2$ |
| 8 | 13.990 | Palmitic acid | 2.37 | 256 | $C_{16}H_{32}O_2$ |
| 9 | 15.274 | Methyl palmitoleate | 1.04 | 268 | $C_{17}H_{32}O_2$ |
| 10 | 15.497 | Methyl 14-methyl pentadecanoate | 8.42 | 270 | $C_{17}H_{34}O_2$ |
| 11 | 16.081 | Hexadecanoic acid | 7.20 | 256 | $C_{16}H_{32}O_2$ |
| 12 | 17.259 | 11-Octadecenoic acid methyl ester | 24.88 | 296 | $C_{19}H_{36}O_2$ |
| 13 | 17.430 | Stearic acid methyl ester | 4.78 | 298 | $C_{19}H_{38}O_2$ |
| 14 | 17.810 | Oleic acid | 19.61 | 282 | $C_{18}H_{34}O_2$ |
| 15 | 17.952 | Octadecanoic acid | 4.77 | 284 | $C_{18}H_{36}O_2$ |
| 16 | 19.191 | Eicosanoic acid methyl ester | 0.25 | 326 | $C_{21}H_{42}O_2$ |

**Table 4.** Chemical composition of lipid extract from *Desmodesmus subspicatus* LC172266 grown in mixotrophic mode.

| Peak | Retention time (min) | Compound | Area (%) | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1 | 4.121 | 2-Ethyl-2-hexenal | 1.09 | 126 | $C_8H_{14}O$ |
| 2 | 11.850 | Capric acid ethyl ester | 0.65 | 200 | $C_{12}H_{24}O_2$ |
| 3 | 13.356 | Methyl tridecanoate | 0.35 | 228 | $C_{14}H_{28}O_2$ |
| 4 | 15.497 | Methyl 14-methyl pentadecanoate | 18.05 | 270 | $C_{17}H_{34}O_2$ |
| 5 | 16.036 | Palmitic acid | 2.46 | 256 | $C_{16}H_{32}O_2$ |
| 6 | 16.236 | 7-Hexadecenoic acid methyl ester | 1.94 | 268 | $C_{17}H_{32}O_2$ |
| 7 | 17.173 | Linolelaidic acid methyl ester | 22.93 | 294 | $C_{19}H_{34}O_2$ |
| 8 | 17.224 | 11-Octadecenoic acid methyl ester | 18.16 | 296 | $C_{19}H_{36}O_2$ |
| 9 | 17.421 | Stearic acid methyl ester | 5.91 | 298 | $C_{19}H_{38}O_2$ |
| 10 | 17.729 | Oleic acid | 10.25 | 282 | $C_{18}H_{34}O_2$ |
| 11 | 17.926 | Cis-Oleic acid | 3.71 | 282 | $C_{18}H_{34}O_2$ |
| 12 | 18.073 | Hexadecane | 2.25 | 226 | $C_{16}H_{34}$ |
| 13 | 18.203 | Methyl di hydro sterculate | 3.48 | 310 | $C_{20}H_{38}O_2$ |
| 14 | 18.810 | Linoleoyl chloride | 1.21 | 298 | $C_{18}H_{31}ClO$ |
| 15 | 18.954 | 2-Methyl nonadecane | 1.70 | 282 | $C_{20}H_{42}$ |
| 16 | 19.198 | Eicosanoic acid methyl ester | 1.58 | 326 | $C_{21}H_{42}O_2$ |
| 17 | 19.785 | Eicosane | 1.11 | 282 | $C_{20}H_{42}$ |
| 18 | 20.591 | Octadecane | 0.62 | 254 | $C_{18}H38$ |
| 19 | 20.836 | Behenic acid methyl ester | 0.38 | 354 | $C_{23}H_{46}O_2$ |
| 20 | 21.383 | Icosane | 0.88 | 282 | $C_{20}H_{42}$ |
| 21 | 22.270 | Pentadecane | 0.56 | 212 | $C_{15}H_{32}$ |
| 22 | 23.307 | Heptadecane | 0.71 | 240 | $C_{17}H_{36}$ |

**Table 5.** Comparison of biodiesel properties of *Desmodesmus subspicatus* LC172266 with plant oil methyl esters (palm oil methyl ester (PME), jatropha oil methyl esters (JME)), ASTM D6751 and EN 14214 vehicular biodiesel standards (Adapted from [11]).

| Quality parameters | | | Lipids (this study) | Plant oil methyl esters [11] | |
|---|---|---|---|---|---|
| Biodiesel properties | ASTM D6751 | EN 14214 | FAME | PME | JME |
| Iodine value (g $I_2$/100 g) | z | 120 (max) | 7.20 | 49.56 | 96.55 |
| Saponification value (mg/g) | z | z | 117.40 | - | - |
| Cetane value | 47 (min) | 51 (min) | 91.10 | 61 | 54 |
| High heating value (MJ/kg) | z | z | 22.31 | - | - |
| Long chain saturation factor (% wt) | z | z | 8.85 | - | - |
| Cold filter plugging point (°C) | z | $\leq 5/\leq -20$ | −8.30 | 13 | −2 |
| Kinematic viscosity (mm²/s) | 1.9–6.0 | 3.5–5.0 | 2.28 | 4.53 | 4.33 |
| Density (g/cm³) | z | 0.86–0.90 | 0.49 | 0.87 | 0.88 |

min = minimum value set; max = maximum value set.
z No limit designated for the physical property.

anaerobic digestion of the wastewater by microorganisms before the cultivation of microalgae [27] have been practised. Sand filtration was used because it is cheap, easy and efficient.

The microalga was grown under autotrophic, mixotrophic and heterotrophic conditions. The biomass concentration achieved under these three conditions varied as autotrophic growth produced significantly the lowest biomass. In the main, mixotrophic growth produced more cell dry weight than the other two growth regimes. Previous studies [11,28–30] have shown that mixotrophic mode especially produced more biomass than heterotrophic, which in turn yielded more biomass than the corresponding autotrophic conditions, in *Chlorella* and *Chlamydomonas*.

Similarly, the lipid contents of the microalga under autotrophic mode were lower than those obtained under heterotrophic and mixotrophic cultivations, corroborating the reports that heterotrophically cultivated microalgae produce higher amounts of lipids than those obtained under photoautotrophic conditions [28,31–35]. Specifically, heterotrophic cultivation of *Chlorella vulgaris* as obtained by Wu *et al*. [36], led to an increase of about 30% in lipid concentration over that of the photoautotrophic culture. In the same way, Jimènez *et al*. [37], comparing photoautotrophic

cultures with heterotrophic cultivation of *Chlorella* sp., obtained about 8 times more lipids under heterotrophic conditions. Liu *et al*. [38] also obtained higher oil content (9 times) when algae were cultured heterotrophically in glucose medium compared with their photoautotrophic counterpart.

Biodiesel production is dependent on lipid class and composition. The microalga in the present study produced fatty acids that are indicative of potentially good-quality biodiesel. The predominant fatty acids obtained were those recommended for good-quality biodiesel [39]. These include 11-octadecenoic acid, oleic acid, pentadecanoic acid, stearic acid, linolelaidic acid, hexadecanoic acid and palmitic acid in relatively good percentages of abundance. These fatty acids belong to the carbon skeletons between $C_{14}$ and $C_{20}$, which form the key compounds in biodiesel production. The excellent physical properties of the FAMEs indicate the generally acceptable quality of the resulting biodiesel after transesterification. This is in agreement with the ASTM D6751 and EN 14214 standards, and with the reports of Arora *et al*. [11] using *Chlamydomonas* sp. and Nayak *et al*. [18] using the microalga *Scenedesmus* sp. Although the three cultivation modes yielded key compounds for biodiesel production, the presence of oleic acid in the heterotrophic and

mixotrophic cultures could mean that without further treatment, the two modes would be better than autotrophic in terms of biodiesel quality.

Furthermore, the bioremediation potential of *Desmodesmus subspicatus* LC172266 was established after determining the differences in the physicochemical parameters before and after the algal growth on the wastewater. The pH of the FCW was very acidic (2.13), and this could potentially have far-reaching consequences for the soil and the aquatic ecosystem if disposed directly into the environment without treatment. After 14 days of growth of the microalga in the wastewater, the nutrients were removed to varying extents by the organism. There was no statistically significant difference ($p > 0.05$) between the reduction potential of the nutrients by the organism under the heterotrophic and mixotrophic growth conditions.

A side-by-side consideration of the EC and TDS revealed a near-direct relationship existing between them. The level of EC of a water body is dependent on its TDS and salinity [38]. The TDS value of 510 mg/L found in the FCW in the present study exceeds the maximum permissible limit of the discharge value of 35 mg/L for water and 45 mg/L for land. This could have inimical effects upon discharge into water bodies, as has previously been reported [14]. The efficiency of removal by the microalga of TDS was shown by the average residual TDS, 419.61 mg/L for heterotrophic and 308.18 mg/L for mixotrophic growth modes. The reduction of EC of the wastewater from 3672.57 $\mu$S/cm to an average residual of 710.26 $\mu$S/cm for heterotrophic growth mode and 636.20 $\mu$S/cm for mixotrophic growth mode is indicative of the greater effectiveness of mixotrophic growth in terms of the removal of ions.

The initial BOD of 577.48 mg/L in the cassava wastewater was too high for its disposal directly into water bodies without treatment. The depletion of nutrients by the microalga was greater under heterotrophic than mixotrophic growth mode. The residual BOD average values found in the present study support that previously reported [40,41], but the COD value of 1570.00 mg/L obtained for cassava wastewater was much higher than the maximum acceptable COD of wastewater dischargeable into water (120 mg/L). The cellulosic component of the wastewater could not be utilised by the microalgae, and hence remained in the wastewater and was measured by the COD. The microalga reclaimed the wastewater, as shown by the difference between the residual COD values and the initial COD values. Haiying *et al.* [42] and Su *et al.* [43] reported a COD removal rate of 88.0% and above, which is in agreement with the results in the present study.

## Conclusions

The phycoremediation potential of *Desmodesmus subspicatus* LC172266 for cassava wastewater was high in both the heterotrophic and mixotrophic cultivation conditions although more effective under heterotrophic cultivation. However, lipid production was higher under mixotrophic than heterotrophic and autotrophic growth modes. The lipid composition of the alga grown under the three conditions was good for biodiesel production, giving excellent physical properties. However, in terms of quality, fatty acids eluted under mixotrophic and heterotrophic modes were more acceptable for biodiesel production than their photoautotrophic counterpart.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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