Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2021, 12, 257-274
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Potential of *Dictyosphaerium* sp. LC172264 Concomitant Remediation of Cassava Wastewater and Accumulation of Lipids for Biodiesel Production

Innocent Okonkwo Ogbonna[1*], Joseph Ikwebe[2], Otsai Otsima Okpozu[3], Chijioke Nwoye Eze[4], James Chukwuma Ogbonna[5]

[1]Department of Microbiology, Federal University of Agriculture, Makurdi, Nigeria
[2]Department of Biochemistry, Federal University Wukari, Nigeria
[3]Environmental Science Unit, Federal University of Agriculture, Makurdi, Nigeria
[4]Department of Science Laboratory Technology, University of Nigeria, Nsukka, Nigeria
[5]Department of Microbiology, University of Nigeria, Nsukka, Nigeria
Email: *innocentia09@yahoo.com, i.joseph@fuwukari.edu.ng, otsimaokpozu@gmail.com, nwoye.chijioke@unn.edu.ng, james.ogbonna@unn.edu.ng

How to cite this paper: Ogbonna, I.O., Ikwebe, J., Okpozu, O.O., Eze, C.N. and Ogbonna, J.C. (2021) Potential of *Dictyosphaerium* sp. LC172264 Concomitant Remediation of Cassava Wastewater and Accumulation of Lipids for Biodiesel Production. *Advances in Bioscience and Biotechnology*, **12**, 257-274.
https://doi.org/10.4236/abb.2021.128016

Received: June 13, 2021
Accepted: August 17, 2021
Published: August 20, 2021

Abstract

As a way of making algal feedstock feasible for biofuel production, simultaneous utilization of microalga *Dictyosphaerium* sp. LC172264 for cassava wastewater remediation and accumulation of lipids for biodiesel production was investigated. The algal biomass, lipid contents and composition were measured from the autotrophic, heterotrophic and mixotrophic cultured algal cells. Physicochemical parameters of the cassava wastewater and bioremediation potentials were measured. Biodiesel properties were deduced and compared with the standards. The results showed that mixotrophic culture was the best for both biomass accumulation (1.022 g/L) and lipid contents (24.53%). Irrespective of the culture condition, the predominant fatty acids were similar and included 11-Octadecenoic acid (vaccenic acid ($C_{19}H_{36}O_2$), oleic acid ($C_{18}H_{34}O_2$) and 14-methyl pentadecanoic acid (isopalmitic acid ($C_{17}H_{34}O_2$). The percentage reduction of total dissolved solids was 79.32% and 89.78% for heterotrophy and mixotrophy respectively. Biochemical oxygen demand was 72.95% and 89.35%, chemical oxygen demand was 72.19% and 84.03% whereas cyanide contents reduced from the initial value of 450 mg/L to 93.105 (79.31%) and 85.365 mg/L (81.03%) respectively. *Dictyosphaerium* sp. showed good growth and lipid production under mixotrophic condition and produced good quality biodiesel under the three cultivation modes. Even though both mixotrophic and heterotrophic conditions had good promise of

cassava wastewater remediation by *Dictyosphaerium* sp., mixotrophy showed superiority.

Keywords

Biodiesel Production, Cassava Wastewater, *Dictyosphaerium* sp., Fatty Acid Profile

1. Introduction

The massive irreversible environmental degradation arising from the combustion of fossil fuels to meet the energy needs of man places a huge moral burden on society. The concomitant release of greenhouse gases into the atmosphere does almost incalculable damage to the protective ozone layer, consequently leading to global warming and other climate change issues with an undesirable negative impact on our wellbeing and environment [1]. If fossil fuel combustion is not stopped by 2100, the damage to the environment would be severe, pervasive and irreversible, causing long-lasting changes in all components of the climate system [2]. With the unrelenting increase in anthropogenic activities and the projected explosion of the human population by 2050, it is now no longer sustainable to continue to combust petroleum hydrocarbons at current rates. It has therefore never been more urgent to replace fossil fuels with greener, renewable and cheaper energy alternative sources.

Amongst several strategies and new methods for sustainable energy production from renewable carbon sources is the implementation of a "waste to wealth" strategy where potentially damaging wastes sources are converted to useful products and in the process eliminating the waste. This strategy also eliminates the potential competition for food sources that may affect global food security. The use of wastes for bioenergy production has been severally reported. These include municipal wastewater [3], industrial wastewater [4], agricultural wastewater [5], etc. The processing of cassava generates a lot of cassava wastewater (CWW) which is toxic, odoriferous and undesirable [5]. Cassava wastewater is a problematic acidic waste and a source of environmental concern because of its typically high hydrogen cyanide content (>400 $mg{\cdot}L^{-1}$) and biological oxygen demand (BOD) of 25,000 - 50,000 $mg{\cdot}L^{-1}$ [6]. Nevertheless, it is a rich source of organic and inorganic nutrients capable of supporting the growth of organisms like microorganisms. Microalgae have the capability of using effluent as substrate to produce biomass from which oil can be extracted and subsequently used as precursor for products that substitute hydrocarbon fossil fuel.

Accordingly, Neves *et al.* [7] obtained total nitrogen (g/L) and a total phosphorous (g/L) of 0.25 ± 0.01 and 0.16 ± 0.01 respectively from cassava wastewater. They also obtained from the wastewater, a carbon to nitrogen ratio of 96.0 ± 4.24 and nitrogen to phosphorous ratio of 1.50 ± 0.071. These characteristics

make the cassava wastewater suitable for the cultivation of algal biomasses, the promising alternative renewable sources of energy feedstock with neutral CO_2 emissions. In addition, cassava starch hydrolysate has been used as culture medium for the production of high *Ankistrodesmus* sp. cell density and enhanced lipid content in a heterotrophic culture [8]. Nigeria, which is the highest producer of cassava in the world and with production accounting for about 20% of total world production [9], unfortunately, has no policy of handling and safely disposing of this hazardous wastewater, as the daily practices have been to recklessly and inappropriately discharge this effluent into the environment without caution into roadside ditches or fields and allow to flow freely, settling in shallow depressions, eventually percolating into the subsoil or flow into streams [5] [6]. Other strategies were bacterial-algal co-culture in wastewater for enhancing remediation and biofuel production. Leong *et al.* [10] in a quest to make algal biofuel production economically feasible and not compromising environmental prospects, co-cultured microalgae and bacteria alongside enhancing lipid-based biofuel production and municipal wastewater bioremediation.

Previous studies had recently demonstrated the use of cassava wastewater as substrate for microalgal biomass production and the harvesting of lipid for biodiesel production. Neves *et al.* [7] evaluated biodiesel production potentials of a microalga *Phormidium autumnale* using cassava wastewater as substrate under heterotrophic condition and obtained good quality biodiesel, high biomass density and high lipid contents. More recently, Ogbonna *et al.* [5] and Okpozu *et al.* [11] had demonstrated the use of *Desmodesmus subspicatus* LC172266 and *Desmodesmus armatus* respectively for the production of biomasses, lipids and biodiesel using cassava wastewater as substrate. They also demonstrated high quality bioremediation of the cassava wastewater by the algae. In a like manner, Kumar *et al.* [12] in a review demonstrated that there is a potential synergy of biofuel production with waste remediation along with value-added co-products recovery using membrane-based system for algal biorefinery. These are all in line with the recommendation of Chisti [13] that for algal biofuel production to be feasible in order to mitigate the high cultivation cost, incorporation of biofuel production with other co-product formation or processes is required.

Dictyosphaerium sp. has been shown to be a high lipid producer and its applicability in biodiesel production has also been demonstrated [14]. Jiang *et al.* [15] used a high-lipid producing *Dictyosphaerium ehrenbergianum* grown in struvite precipitated liquid digestate for biodiesel production, with lipid and biomass productivities being higher than when cultured in the standard BG-11 medium. The microalga *Dictyosphaerium ehrenbergianum* Nageli has been reported to be useful in biodiesel production with oleic, palmitic and linoleic acids as the predominant fatty acids [16]. They also demonstrated that the qualities of the biodiesel produced with this organism as conforming to the standard qualities and therefore recommended *Dictyosphaerium ehrenbergianum* Nageli to be good feedstock for biodiesel production.

In the present study, we evaluated the use of cassava wastewater as a medium for the cultivation of microalga *Dictyosphaerium* sp., as a means of bioremediating the wastewater and elaboration of lipids for biodiesel production. In addition to measuring the physicochemical parameters of the cassava wastewater and bioremediation potentials, algal biomass quantities, lipid contents and fatty acid profile were also measured from the alga cultivated under autotrophic, heterotrophic and mixotrophic culture conditions. Biodiesel properties were deduced and compared.

2. Materials and Methods

2.1. Cassava Wastewater Collection and Analysis

The cassava wastewater used in this study was collected from local cassava milling plants in Benue State, Nigeria. The initial physicochemical parameters including the pH were measured. The cassava wastewater was prepared basically according to the method described by Ogbonna *et al.* [5]. Briefly, the wastewater was filtered through a typical sand filter as adapted using the improvised funnel, mesh, wire gauze and sand column [5]. A 50 mL quantity of one-molar tetra oxo sulphate (VI) acid was added to a ten-liter volume of the cassava wastewater filtrate. The mixture was heated with stirring until gelatinization occurred which eventually turned milky with further heating. The heated milky solution was mixed with 32.67 mL of 60% sodium hydroxide solution on a liter basis with further stirring to neutralize the mixture. According to Ogbonna *et al.* [5] and Okpozu *et al.* [11], to every millilitre volume of the cassava wastewater was added a 10 mL volume of the BBM was added. The pH of the CWW was adjusted to 7.0 using dilute HCl and 1% NaOH. This was thereafter sterilized by autoclaving at 121˚C for 10 min with subsequent addition of ampicillin and ketoconazole (250 and 200 mg respectively) upon cooling.

2.2. Microalga, Culture Media and Inoculation

Dictyosphaerium sp. used for this study was previously isolated and identified with 18S rRNA sequencing using
18S forward-(ITS-1)'TTTCTGCCCTATCAACTTTCGATG' and
18S reverse-(ITS-4)'TACAAAGGGCAGGGACGTAAT' primers [14] [17] [18]. The organism was deposited in the GenBank under an Accession Code LC172264. The microalga was subsequently maintained in Bold Basal Medium (BBM) [5]. The BBM was composed of stock solutions 1 to 10. Stock solutions 1 - 6 was each prepared per 400 mL and included: 10.0 g $NaNO_3$, 3.0 g $MgSO_4{\cdot}7H_2O$, 3.0 g K_2HPO_4, 7.0 g KH_2PO_4, 1.0 g $CaCl_2$ and 1.0 g NaCl respectively. The stock solution 7 which was composed of the trace elements solution included 8.82 g $ZnSO_4{\cdot}7H_2O$, 1.44 g $MnCl_2{\cdot}4H_2O$, 0.71 g MoO_3, 1.57 g $CuSO_4{\cdot}5H_2O$ and 0.49 g of $Co(NO_3)_2{\cdot}6H_2O$ per liter of distilled water. Stock solution 8 was made up of 11.42 g of H_3BO_3 per liter of water. Stock solution 9 was made up of 50 g EDTA and 31 g of KOH per liter of water. Finally, solution 10 was composed of 4.98 g

of $FeSO_4 \cdot 7H_2O$ and 1.0 mL H_2SO_4 (conc). Each of the stock solution was autoclave-sterilized at 121˚C for 15 min. To make BBM, in a liter of volumetric flask, 10 mL each of the sterile stock solutions 1 - 6 were respectively added, followed by the addition of 1 mL each of stock solutions 7 - 10. This was made up to a 1.0 L volume with sterile distilled water. The BBM was used for photoautotrophic cultivation whereas the heterotrophic and mixotrophic cultures were done using BBM medium supplemented with cassava wastewater (CWW) as described above. Each cultivation was done in a one-liter Erlenmeyer flask containing 500 mL of appropriate medium as adapted from Ogbonna *et al.* [5] and Okpozu *et al.* [11]. The inoculation of each set-up was done with 20% (v/v) of the isolate seed culture (approximately 10^6 CFU/mL of *Dictyosphaerium* sp. in exponential phase in BBM). The photoautotrophic cultures were exposed to approximately 12 hours of sunlight daily [average light intensity ~2000 LX (Digital light meter, model LX-1000, Japan)] at a temperature of 28˚C ± 2˚C. The set-up was agitated 2-times daily (vigorous hand-shaking for 2 min) during the hours of sunlight. For heterotrophic and mixotrophic cultivations, the seed cultures were grown in darkness for 72 h for acclimatization before 20% v/v inoculation described previously. After inoculation as described above, the conical flasks for the heterotrophic cultures were wrapped in aluminum foil sheets and incubated in dark cupboards at a temperature of 28˚C ± 2˚C for the duration of its growth. For mixotrophic cultures, the acclimatized microalgal seed culture was inoculated into the 500 mL medium in two replicates. The set-up was exposed to sunlight for six hours daily from 7:00 am to 1:00 pm after which it was returned to stand in the dark. The experiments were done in two replicates for each growth mode.

2.3. Determination of Growth, Lipid Content and Composition

Determination of growth rates for each culture mode was done using spectrophotometer at Optical Density 680 nm (HACH, Loveland, Colorado, USA). A growth curve was generated based on the Optical Density (OD_{680}) readings and a standard curve was used to convert to cell dry weight. The dried cell (DC) (0.5 g) was used for the lipid extraction. Lipid was extracted from the DC by the method of Bligh and Dyer [19] in chloroform: methanol: water solvent system. The extracted lipid was expressed as percentage (%).

GC-MS chromatographic determination of fatty acids was run in a Shimadzu plus machine (Shimadzu, Japan, GCMS-QP2010 Plus). The chromatogram was equipped with a micro-bore capillary column Db. 30.0 with a Helium gas as a gas carrier at a flow rate of 1.8 mL/min and a total flow of 40.8 mL. The oven temperature was set at 70˚C whereas the interface and source temperatures were 250˚C and 200˚C respectively. The velocity control was a linear one and the pressure was set at 116.9 kPa. The start and end time of the chromatogram were 3 and 24 min respectively [17]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library to ascertain the molecular name and molecular weight.

2.4. Determination of Fatty Acid Methyl Ester (Biodiesel) Property

Fatty acid methyl ester property was determined by means of a Biodiesel Analyzer software [20].

2.5. Measurement of the Physicochemical Properties of the Cassava Wastewater

2.5.1. Measurement of Electrical Conductivity (EC)

Using Ademoroti [21] recommended protocol, the electrical conductivity (EC) was measured by using a conductivity probe (Hanna combo pH/EC meter HI 98129). The electrical conductance (micro second per centimeter (μS/cm)) of the CWW was determined by inserting the probe into the wastewater and taking reading after 3 min.

2.5.2. Measurement of Total Dissolved Solids (TDS) of the CWW

The total dissolved solid (TDS) of the CWW was measured using filtration method of American Standard Test Methods [22]. In brief, a 50 mL CWW sample added into an evaporating dish was completely dried by heating at 180˚C. Upon dryness to constant weight, the TDS was calculated using the equation below in Equation (1).

$$\mathrm{TDS}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right)=\frac{\text{mg residue}\times 1000}{\text{mL of sample}} \tag{1}$$

2.5.3. Measurement of Dissolved Oxygen and Biochemical Oxygen Demand

The Dissolved Oxygen (DO) was measured using a modification of Winkler-Azide protocol while the Biochemical Oxygen Demand (BOD) was measured as the difference between DO of samples immediately after collection and DO of samples after incubation at 20˚C for five days [22] as adapted from Okpozu *et al.* [11].

2.5.4. Measurement of Chemical Oxygen Demand (COD)

The COD was measured using a method adapted from Ademoroti [23] by a titrimetric method with Ferroin indicator as presented recently by Okpozu *et al.* [11].

2.5.5. Measurement of pH of CWW Solution

The CWW sample pH was measured by a means of a calibrated pH meter (Hanna Instrument C-99-USA). The calibration was done using buffers at pH 4, 7 and 10 [21].

2.5.6. Measurement of Hydrogen Cyanide (HCN) Content of the Solution

Hydrogen cyanide content of the CWW was measured using the method adapted from Ezeh *et al.* [24] and Sawyerr *et al.* [25] with modification. This was determined by spectrometric method using alkaline picarate solution. The sand-filtered CWW was further filtered using Whatman No. 1 filter paper. Alkaline picarate

was prepared by dissolving 1 g of picric acid and 2 g of anhydrous sodium carbonate (Na_2CO_3) in a small volume of warm water and making up to a 100 mL volume. This was stored in an amber-colored bottle in a refrigerator (4˚C) until used. In the measurement of HCN content, 4 mL of alkaline picarate was added to a 5 mL quantity of the filtered CWW. This was warmed for 5 min in a water bath (55˚C) for brown color development and was allowed to cool and read against a blank at 490 nm (UV-Spectrophotometer). The blank was prepared by using distilled water in the place of CWW. The HCN content was expressed as mg/L and was extrapolated from a standard curve.

2.6. Statistical Analysis

The data obtained from this study were analyzed using Statistical Package for Social Science (SPSS version 20.0).

3. Results and Discussion

3.1. Pre-Treatment of Cassava Wastewater

This study focused on the evaluation of *Dictyosphaerium* sp. LC172264 for dual cassava wastewater remediation and concomitant accumulation of lipids for biodiesel production. Before the wastewater was used, it was pre-treated via sand filtration, acid hydrolysis, alkali neutralization and subsequently autoclave-sterilized. The sand filtration was done to simulate natural water filtration taking place in the soil and this resulted in the removal of large particulate matter in the wastewater with the CWW appearing ash to colorless. Pre-treatment processes have been customary in wastewater treatment by microalgae. Hence, Rui *et al.* [26] used anaerobic digestion of the wastewater by microorganisms before the cultivation of microalgae on the wastewater. The present sand filtration has been recently employed in the bioremediation of cassava wastewater by other species of microalgae in previous studies [5] [11]. Cassava processing wastewater was recently demonstrated to be an ideal platform for third generation biodiesel production [7] because of the nutrients it contains.

3.2. Growth, Lipid Content and Composition *Dictyosphaerium* sp. LC172264

The growth pattern of *Dictyosphaerium* species under autotrophic, heterotrophic and mixotrophic cultivation modes are presented in **Figure 1**. The mixotrophic culture had the highest biomass content with a maximum cell dry weight value of 1.022 g/L equivalent to optical density value of 2.842 ± 0.12 on the day 8 of incubation. Heterotrophic culture accumulated its highest biomass concentration of 0.63 g/L (OD value equivalent to 1.981 ± 0.70, on day 10 of incubation) while autotrophic growth was the lowest of the three with its highest biomass concentration of 0.238 g/L (OD value equivalent to 1.477 ± 0.51, day 10 of incubation). Cells presented in g/L were extrapolated from the standard curve of the plot of cell dry weight (g/L) against the optical density readings (OD_{680}) with R^2 = 0.8982. The three growth curves did not show any pronounced lag period and

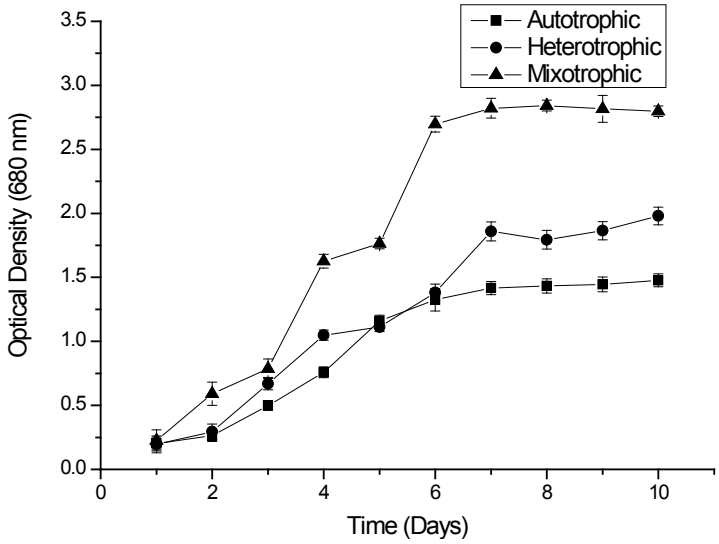

Figure 1. Time course of *Dictyosphaerium* sp. growth under different cultivation conditions.

attained stationary growth from the 6th day of incubation. From the results, whereas under heterotrophic cultivation, *Dictyosphaerium* sp. LC172264 accumulated approximately half the biomass obtained under mixotrophic condition, photoautotrophic cultured cells were only able to get 1/6th approximated equivalence. This therefore implies that the organism prefers mixotrophic metabolism needing both light energy and an organic carbon substrate.

Evidence supporting superiority of mixotrophic cultivation of some microalgae in organic carbon substrates had been recorded [27] [28]. In a previous study with the microalga, Ogbonna and Ogbonna [14] had demonstrated the heterotrophic and mixotrophic growth ability of the isolate using glucose and glycerol as substrates. However, using glucose and glycerol as substrates, higher biomass content was recorded than the biomass of the present study.

The percentage lipid content of *Dictyosphaerium* sp. and final biomass concentration under the different cultivation conditions is presented in **Figure 2**. The *Dictyosphaerium* sp. grown in mixotrophic condition had more biomass concentration and accumulated more lipids than the other cultivation modes. The highest lipid content (%) for autotrophic, heterotrophic and mixotrophic culture modes were 15.32, 16.54 and 21.16 respectively whereas the biomass contents were 0.238, 0.63 and 1.022 g/L respectively. Using CWW as carbon and additional nitrogen source gave lower percentage lipid content than what was previously reported (42.3% ± 1.33%) where glucose and glycerol were the carbon sources [14]. The reason for the disparity could be the differences in medium compositions since glucose as a carbon source is more easily assimilated than hydrolyzed cassava starch.

3.3. Fatty Acid Composition of *Dictyosphaerium* sp. LC172264 under Different Cultivation Modes

In order to ascertain the suitability of use of *Dictyosphaerium* sp. LC172264 for

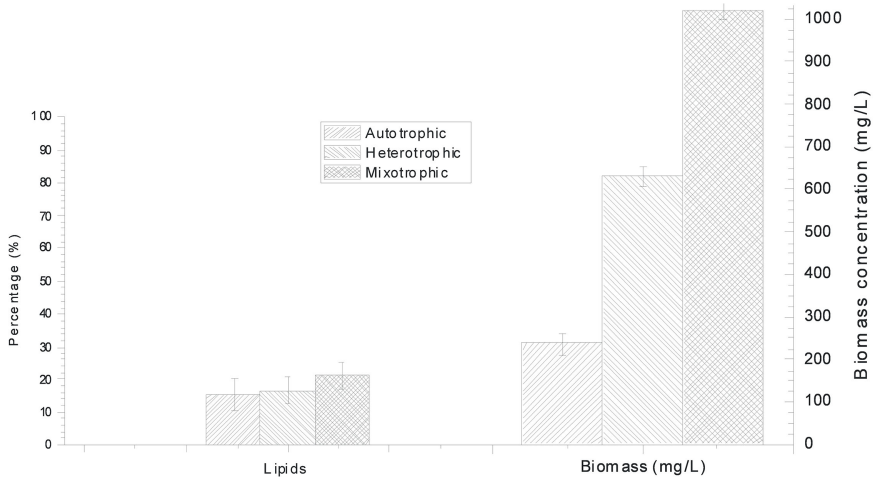

Figure 2. Final biomass content (mg/L) and % Lipid content of *Dictyosphaerium* sp. grown under different conditions.

biodiesel production and under what cultivation modes, we assessed the lipid class and composition (the determinants of biodiesel quality [29] produced under autotrophic, heterotrophic and mixotrophic cultivation conditions. The fatty acid compositions of the microalga cultivated under autotrophic, heterotrophic and mixotrophic culture conditions showed some slight variations in the chemistry and quantities of the eluted compounds. The GC-MS profiles of the total ion chromatogram of lipids of *Dictyosphaerium* sp. grown under the different cultivation conditions are presented in **Figures 3-5**. The most abundant compounds in the extracts of autotrophically cultivated cells included 11-Octadecenoic acid (Vaccenic acid ($C_{19}H_{36}O_2$) (35.06%), oleic acid (27.70%), 14-methyl pentadecanoic acid (isopalmitic acid ($C_{17}H_{34}O_2$) (10.94%) (**Table 1**). The other compounds were Nopinene (2.05%), Ethyl-2-hexanal (0.72%), 1,5-Heptadiene, 6-methyl-2-(4-methyl-3-cyclohexene) (0.98%), Cis-Ocimene (0.60%), Isothujol (0.24%), Hexadecanoic acid (6.32%), Octadecanoic acid (5.91%), Ricinoleic acid (1.04%), Eicosanoic acid (0.49%) (**Table 1**). For heterotrophic cultivated *Dictyosphaerium* sp. cells, the major fatty acids eluted included 11-Octadecenoic acid (Vaccenic acid ($C_{19}H_{36}O_2$) (49.16%) and Pentadecanoic acid (15.07%) (**Table 2**). Others were Oleic acid (9.89%), Octadecanoic acid (7.31%), 2-Ethyl-2-hexenal (1.29%) 7-hexadecanoate (0.81%), Hexadecanoic acid (1.94%), Linolelaidic acid (0.11%), Stearic acid (7.06%), Ricinoleic acid (1.90%), Eicosanoate (0.81%), Stigmasterol (4.67%) (**Table 2**). Mixotrophically cultured cells of *Dictyosphaerium* sp. also had 11-Octadecenoic acid (Vaccenic acid ($C_{19}H_{36}O_2$) (43.23%) as the predominant fatty acid in the extract. The other major fatty acids are Oleic acid (21.04%) and 14-methylpentadecanoic acid (14.10%) (**Table 3**). The others included 2-Ethyl-2-Hexanal (0.89%), Hexadecanoic acid (4.65%), Octadecanoic acid (9.09%), Stearic acid (5.25%), Ricinoleic acid, (1.31%) Methyl isoheptadecanoate (0.44%) (**Table 3**). Therefore, from the three culture modes, 11-Octadecenoic acid, Oleic acid, Pentadecanoic acid and Octadecanoic acid were the predominant fatty acids. From the list of the eluted compounds, *Dictyosphaerium* sp.

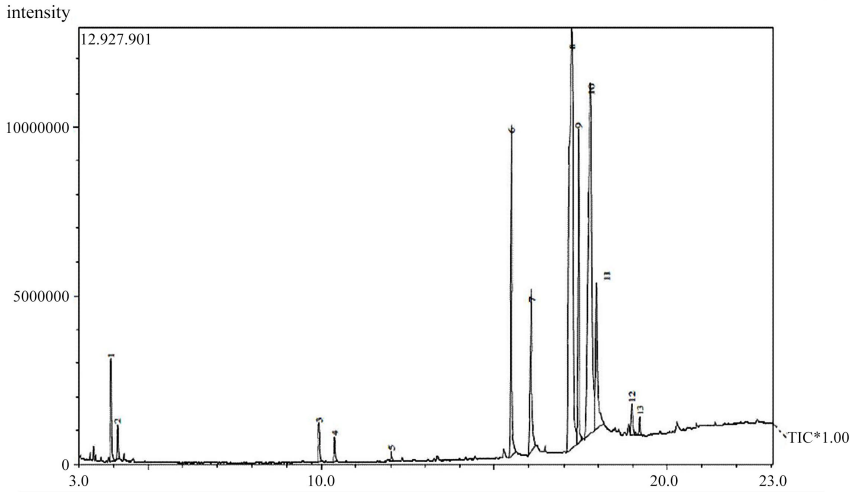

Figure 3. Total ion chromatogram of lipid of *Dictyosphaerium* sp. grown under autotrophic condition.

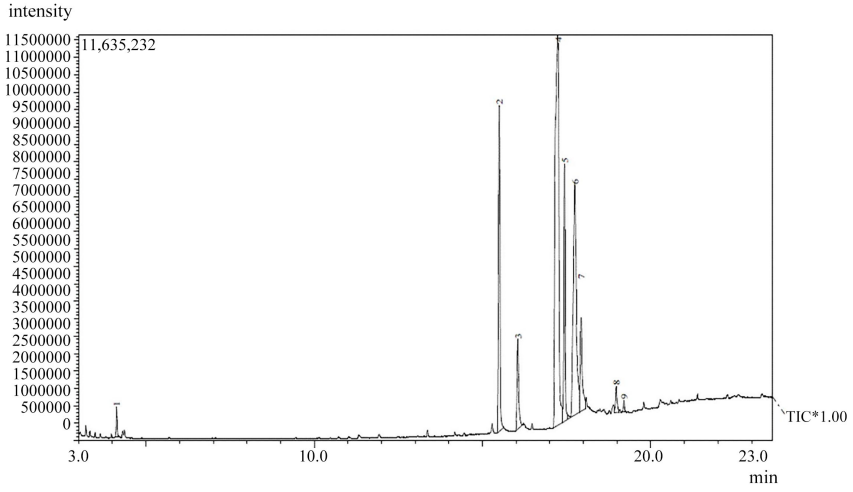

Figure 4. Total ion chromatogram of lipid of *Dictyosphaerium* sp. under mixotrophic growth.

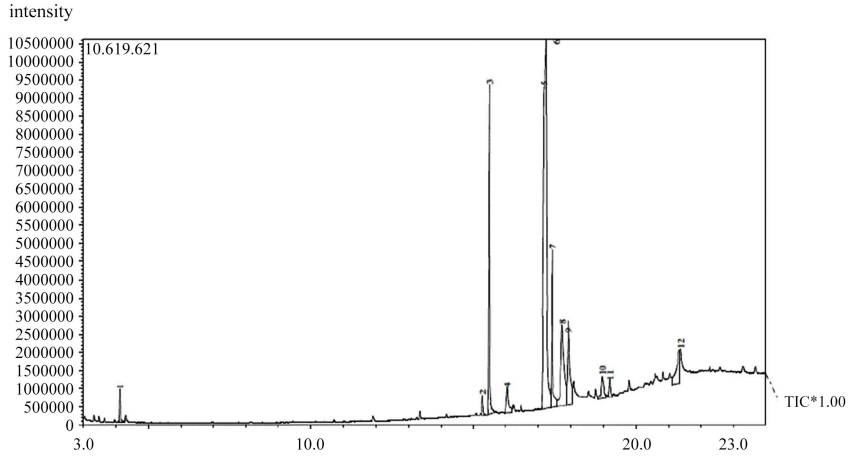

Figure 5. Total ion chromatogram of lipid of *Dictyosphaerium* sp. under heterotrophic culture.

Table 1. Chemical profile of lipid from *Dictyosphaerium* sp. LC172264 grown in autotrophic mode.

Peak	Retention Time	Compound	Area (%)	Molecular Weight	Molecular Formula
1	3.929	Nopinene	2.05	136	$C_{10}H_{16}$
2	4.119	Ethyl-2-hexanal	0.72	126	$C_8H_{14}O$
3	9.939	1,5-Heptadiene, 6-methyl-2-4-methyl-3-cyclohexene	0.98	204	$C_{15}H_{24}$
4	10.386	Cis-Ocimene	0.60	136	$C_{10}H_{16}$
5	12.024	Isothujol	0.24	154	$C_{10}H1O$
6	15.497	Methyl 14-methyl pentadecanoate	10.94	270	$C_{17}H_{34}O_2$
7	16.067	Hexadecanoic acid	6.32	256	$C_{16}H_{32}O_2$
8	17.236	11-Octadecenoic acid, methyl ester	35.06	296	$C_{19}H_{36}O_2$
9	17.435	Stearic acid, methyl ester	7.96	298	$C_{19}H_{38}O_2$
10	17.780	Oleic acid	27.70	282	$C_{18}H_{34}O_2$
11	17.953	Octadecanoic acid	5.91	284	$C_{18}H_{36}O_2$
12	18.969	Ricinoleic acid, methyl ester	1.04	312	$C_{21}H_{36}O_3$
13	19.198	Eicosanoic acid, methyl ester	0.49	326	$C_{21}H_{42}O_2$

Table 2. Chemical profile of lipid from *Dictyosphaerium* sp. LC172264 grown in heterotrophic mode.

Peak	Retention Time	Compound	Area (%)	Molecular Weight	Molecular Formula
1	4.119	2-Ethyl-2-hexenal	1.29	126	$C_8H_{14}O$
2	15.277	7-hexadecanoate, methyl ester	0.81	268	$C_{17}H_{32}O_2$
3	15.491	Pentadecanoic acid, methyl ester	15.07	270	$C_{17}H_{34}O_2$
4	16.038	Hexadecanoic acid	1.94	256	$C_{16}H_{32}O_2$
5	17.039	Linolelaidic acid, methyl ester	0.11	294	$C_{19}H_{34}O_2$
6	17.246	11-Octadecenoic acid, methyl ester	49.16	296	$C_{19}H_{36}O_2$
7	17.425	Stearic acid, methyl ester	7.06	298	$C_{19}H_{38}O_2$
8	17.730	Oleic acid	9.89	282	$C_{18}H_{34}O_2$
9	17.923	Octadecanoic acid	7.31	284	$C_{18}H_{36}O_2$
10	18.967	Ricinoleic acid, methyl ester	1.90	312	$C_{19}H_{36}O_3$
11	19.199	Eicosanoate, methyl ester	0.81	326	$C_{21}H_{42}O_2$
12	21.342	Stigmasterol	4.67	414	$C_{29}H_{50}O$

Table 3. Chemical profile of lipid from *Dictyosphaerium* sp. LC172264 under mixotrophic cultivation.

Peak	Retention Time	Compound	Area (%)	Molecular Weight	Molecular Formula
1	4.119	2-Ethyl-2-Hexanal	0.89	126	$C_8H_{14}O$
2	15.496	14-methyl pentadecanoic acid,	14.10	270	$C_{17}H_{34}O_2$
3	16.045	Hexadecanoic acid	4.65	256	$C_{16}H_{32}O_2$
4	17.224	11-Octadecenoic acid, methyl ester	43.23	296	$C_{19}H_{36}O_2$
5	17.430	Octadecanoic acid, methyl ester	9.09	298	$C_{19}H_{38}O_2$
6	17.736	Oleic acid	21.04	282	$C_{18}H_{34}O_2$
7	17.927	Stearic acid	5.25	284	$C_{18}H_{36}O_2$
8	18.964	Ricinoleic acid, methyl ester	1.31	312	$C_{19}H_{36}O_3$
9	19.197	Methyl isoheptadecanoate	0.44	284	$C_{18}H_{36}O_2$

is an ideal feedstock for biodiesel production under the present different cultivation modes in CWW. It produces predominantly, irrespective of the cultivation condition, monounsaturated (MUFA) and saturated fatty acids (SFA). These species of fatty acids are the recommended fatty acids for biodiesel production [30]. Similar results of fatty acid class and proportion had recently been reported in *D. subpitacus* and *Desmodesmus armatus* when they were cultivated in cassava wastewater for biofuels [5] [11]. The fatty acid methyl ester production by microalga *Dictyosphaerium ehrenbergianum* Nageli showed that oleic, palmitic and linoleic acids were the predominant fatty acids which were closely related to the acids obtained in the present study [16].

The predominant fatty acid obtained with *Dictyosphaerium* sp. grown on glucose and glycerol carbon substrates [14] were similar to the present study implying that irrespective of the growth condition, the fatty acid composition of microalgae may be species dependent.

3.4. Properties of the Fatty Acid Methyl Esters (Biodiesel)

To ascertain the qualities of these fatty acids obtained under autotrophic, heterotrophic and mixotrophic cultivation modes for use as biodiesel feedstock, biodiesel property analyses (BiodieselAnalyzer software, [20] were carried out and whose values were presented in **Table 4**. The iodine value ($gI_2/100g$), the cetane number, and kinematic viscosity were within the recommended limits (EN 14214 and ASTM D6751) for biodiesel obtained from *Dictyosphaerium* sp. grown under the three cultivation conditions. This could signify the suitability of use of *Dictyosphaerium* sp. as a feedstock for biodiesel production using CWW as a substrate. The density of biodiesel produced via heterotrophic metabolism was 0.816 g/cm^2 and was therefore lower than the EN 14214 set standard. However, biodiesel from *Dictyosphaerium* sp. cultured under autotrophic and mixotrophic

Table 4. Comparison of qualities of the fatty acid methyl esters of *Dictyosphaerium* sp. under different cultivation modes with biodiesel standards.

Biodiesel attributes	Aut	Het	Mixo	ASTM D6751	EN 14214	DSME	DAME	PME
Saponification Value (mg/g)	206.64	190.64	203.28	N	N	117.40	132.00	-
Iodine Value (g I_2/100g)	57.69	55.32	58.87	N	120.00 (max)	7.20	57.00	49.56
Cetane number	59.73	62.48	59.91	47.00 (min)	51.00 (min)	91.10	75.00	61.00
Long Chain Saturated Factor	8.66	8.81	9.27	N	N	8.85	15.47	-
Cold Filter Plugging Point (˚C)	10.73	11.19	12.63	N	≤5/≤ −20.00	−8.30	−12.50	13.00
Kinematic Viscosity (mm^2/s)	3.98	3.75	4.10	1.90 - 6.00	3.50 - 5.00	2.28	3.80	4.53
Density (g/cm^2)	0.87	0.82	0.87	N	0.86 - 0.90	0.49	0.65	0.87

max = maximum, min = minimum, n = no limit assigned, - = not reported, Aut = autotrophic, Het = heterotrophic, Mixo = mixotrophic, DSME = *Desmodesmus subspitcatus* oil methyl ester, DAME = *Desmodesmus armatus* oil methyl ester, PME = plant oil methyl ester, ASTM D6751 and EN 14214 vehicular biodiesel standards (DSME, DAME and PME were adapted from Ogbonna *et al.* [5], Okpozu *et al.* [11] and Arora *et al.* [4] respectively).

conditions had densities within recommended standards. As a general rule, the qualities of the fatty acid methyl esters from the different cultivation modes were similar even though the quantities slightly varied. This is probably because the same substrate and microalga were used, thereby implying that the cultivation conditions (autotrophic, heterotrophic and mixotrophic) had no remarkable influence in biodiesel quality. It is also remarkable to note that most of the parameters assessed were within the ASTM D6751 and EN 14214 recommended values. Compared to the values reported for *Desmondesmus subspitacus* and *Desmondesmus armatus*, and plant oil methyl esters, the results of the present study had slight variations with saponification values of *Dictyosphaerium* sp. methyl ester (present study) being higher than *Desmodesmus subspitcatus* oil methyl ester and *Desmodesmus armatus* oil methyl ester (**Table 4**).

3.5. Bioremediation Potential of Microalga *Dictyosphaerium* Species Grown on Cassava Wastewater

What could make the production of biofuel from CWW attractive is the ability to combine the fuel production with bioremediation. Cassava wastewater is an odoriferous, high insect (mosquito) breeding liquid pollutant. Concomitant utilization of microalgae for biomass production for sustainable biofuels and phytoremediation had been demonstrated [5] [7] [11] [31]. In the very recent times, several attempts have also been made to bioremediate different categories of wastewater with a concomitant production of algal based biofuel. For instance, whereas Nguyen *et al.* [32] investigated the relationship between bacteria growth and lipid production by cultivation of microalgae *Chlorella vulgaris* in seafood wastewater, Leong *et al.* [33] compared the performances of microalgal-bacterial co-cultivation to bioremediate synthetic and municipal wastewaters whilst producing biodiesel sustainably. Rosli *et al.* [34] modelled to enhance attached microalgal biomass growth onto fluidized beds packed in nutrients-rich wastewater whilst simultaneously biofixing CO_2 into lipid for biodiesel. For a microalga to be a proper candidate for bioremediation, it must show good growth qualities on the medium. The microalga *Dictyosphaerium* species LC172264 utilized in the

present study grew under autotrophic (BBM), heterotrophic, and mixotrophic conditions using CWW plus BBM as substrates.

There was a gradual decrease in the electrical conductivity (EC) ($\mu s \cdot cm^{-1}$) as the incubation time increased with mixotrophic cultures showing lower EC than the heterotrophic counterpart. The electrical conductivity is used to determine the quantity of ions in the water which implies that in the present study, *Dictyosphaerium* species LC172264 grown under mixotrophic condition in CWW removed compounds containing these ions more than under heterotrophic condition. The value of the total dissolved solids (TDS) of the cassava wastewater samples upon the growth of *Dictyosphaerium* sp. under heterotrophic and mixotrophic conditions showed an inverse relationship between TDS and incubation time. The biochemical oxygen demand and chemical oxygen demand (COD) also progressively decreased with the increasing time of incubation for both the heterotrophic and mixotrophic cultures. For these physicochemical properties, there existed higher reduction in the mixotrophic cultures than the heterotrophic counterpart. Wastewater poses a serious threat to the environment and to the health of life forms when its EC, TDS, BOD, COD and HCN are very high as they are related to the organic matter and chemical contents of the wastewater. The marked reduction in the values of this parameter signifies the usefulness of *Dictyosphaerium* sp. in bioremediation.

The percentage reduction in the physicochemical parameters of the cassava wastewater CWW culture media during the period of the growth of *Dictyosphaerium* species is shown in **Figure 6**. Total dissolved solids (TDS) were better reduced (89.78%) in the mixotrophic mode than in the heterotrophic growth culture (79.32%). The electrical conductivity (EC) % reduction value was 86.77% under both growth modes. The implication of the high % reduction in EC and TDS is that the microalga is a good candidate for bioremediation and the higher the %, the better bioremediation. The percentage reduction in the BOD under mixotrophic culture was 89.35% whereas it was 72.95% under in the heterotrophic growth mode. For COD, the percentage reduction in mixotrophic culture was 84.03% whereas it was 72.19% in the heterotrophic culture mode. A major component of the CWW with both environmental and health implications is the hydrogen cyanide (HCN) content. In order to determine the effect of cultivation condition on the cyanide concentration, cyanide content was measured at the beginning and the 5th day of cultivation. The cyanide (HCN) contents reduced from the initial 450 mg/L to 93.105 (79.31%) and 85.365 mg/L (81.03%) for heterotrophic and mixotrophic culture modes respectively. These results indicate that, TDS, EC, BOD. COD and HCN were better reduced in mixotrophic cultures. This goes further to support the earlier result that mixotrophically cultured cells of microalga *Dictyosphaerium* species LC172264 had better growth and lipid contents. This likewise further strengthens the fact that the ability of an organism to grow well on wastewater conferred better bioremediation ability because of the depletion of the available nutrients.

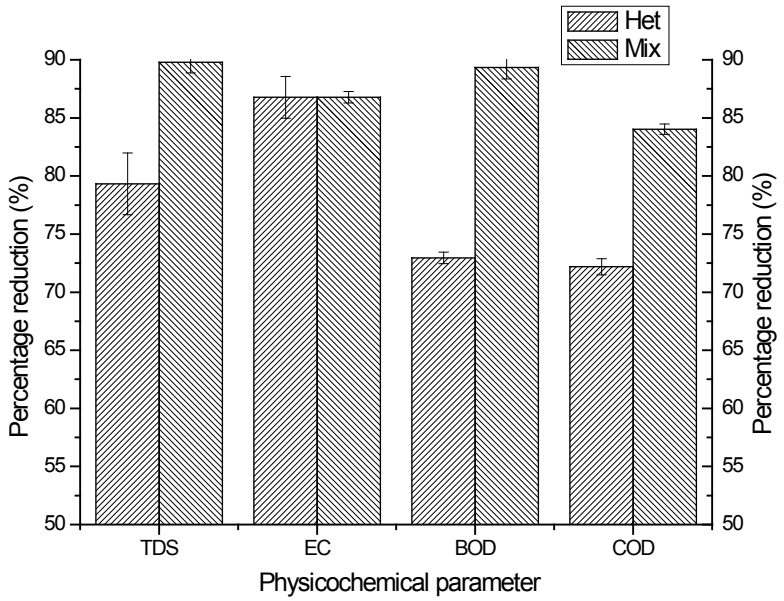

Figure 6. Percentage reduction (%) in the physicochemical parameters of cassava wastewater under heterotrophic and mixotrophic cultures.

Characteristically, CWW has been described to be high in hydrogen cyanide content (>400 mg·L^{-1}) and biological oxygen demand (BOD) (25,000 - 50,000 mg·L^{-1}) [6]. High quantities of these two environmental parameters (hydrogen cyanide and BOD) in water indicate high pollution. In remediating CWW, Neves *et al.* [7] obtained a total nitrogen (g/L) and a total phosphorous (g/L) of 0.25 ± 0.01 and 0.16 ± 0.01 respectively from cassava wastewater. They also obtained from the wastewater, a carbon to nitrogen ratio of 96.0 ± 4.24 and nitrogen to phosphorous ratio of 1.50 ± 0.071. Whereas Leong *et al.* [10] and Leong *et al.* [35] focused on nitrogen removal efficiency, the present work reports cyanide removal from the culture medium. Cyanide is however a nitrogen-rich compound and its measurement alongside biofuel production and bioremediation could stand as a novel approach and a major contribution. The cyanide removal of the present study was efficient.

4. Conclusion

Dictyosphaerium species LC172264 under the two cultivation modes showed good remediation potentials; however, the mixotrophic cultures had more biomass and lipid contents than the heterotrophic and autotrophic counterparts. Overall, the *Dictyosphaerium* species of the present study is ideal for concomitant cassava wastewater remediation and biodiesel production under mixotrophic condition.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Montgomery, H. (2017) Preventing the Progression of Climate Change: One Drug or Polypill? *Biofuel Research Journal*, **13**, 536.

[2] United Nations Panel on Climate Change (UNIPCC) (2014) Climate Change Synthesis Report Summary for Policy-Makers. United Nations Panel on Climate Change, Geneva, 1-32. https://www.ipcc.ch/report/ar5/

[3] Marella, T.K., Datta, A., Patil, M.D., Dixit, S. and Tiwari, A. (2019) Biodiesel Production through Algal Cultivation in Urban Wastewater Using Algal Floway. *Bioresource Technology*, **280**, 222-228. https://doi.org/10.1016/j.biortech.2019.02.031

[4] Arora, N., Patel, A., Sartaj, K.M., Pruthi, P.A. and Pruthi, V. (2016) Bioremediation of Domestic and Industrial Wastewaters Integrated with Enhanced Biodiesel Production Using Novel Oleaginous Microalgae. *Environmental Science and Pollution Research*, **23**, 20997-21007.

[5] Ogbonna, I.O., Okpozu, O.O., Ikwebe, J. and Ogbonna. J.C. (2018) Utilisation of *Desmodesmus subspicatus* LC172266 for Simultaneous Remediation of Cassava Wastewater and Accumulation Of lipids for Biodiesel Production. *Biofuels*, **10**, 657-664. https://doi.org/10.1080/17597269.2018.1426164

[6] Food and Agriculture Organization (International Fund for Agricultural Development) (2001) Strategic Environmental Assessment: An Assessment of the Impact of Cassava Production and Processing on the Environment and Biodiversity. *Proceedings of the Validation Forum on the Global Cassava Development Strategy*, Vol. 5, Rome, 26-28 April 2000.

[7] Neves, C., Maroneze, M.M., dos Santos, A.M., Francisco, E.G., Wagner, R. and Zekpa Jacob-Lopes, E. (2015) Cassava Processing Wastewater as a Platform for Third Generation Biodiesel Production. *Scientia Agricola*, **73**, 412-416. https://doi.org/10.1590/0103-9016-2015-0302

[8] Salim, M.A. (2013) Heterotrophic Growth of *Ankistrodesmus* sp. for Lipid Production Using Cassava Starch Hydrolysate as a Carbon Source. *The International Journal of Biotechnology*, **2**, 42-51.

[9] Kuiper, L., Ekmekci, B., Hamelinck, C., Hettinga, W., Meyer, S. and Koop, S. (2007) *Bio*-Ethanol from Cassava. Ecofys Netherlands BV, Utrecht.

[10] Leong, W.H. Zaine, S.N.A., Ho, Y.C., Uemura, Y., Lam, M.K., Khoo, K.S., Kiatkittipong, W., Cheng, C.K., Show, P.L. and Lim, J.W. (2019) Impact of Various Microalgal-Bacterial Populations on Municipal Wastewater Bioremediation and Its Energy Feasibility for Lipid-Based Biofuel Population. *Journal of Environmental Management*, **249**, Article ID: 109384. https://doi.org/10.1016/j.jenvman.2019.109384

[11] Okpozu, O.O., Ogbonna, I.O., Ikwebe, J. and Ogbonna, J.C. (2019) Phycoremediation of Cassava Wastewater by *Desmodesmus armatus* and the Concomitant Accumulation of Lipids for Biodiesel Production. *Bioresource Technology Reports*, **7**, Article ID: 100255. https://doi.org/10.1016/j.biteb.2019.100255

[12] Kumar, R., Ghosh, A.K. and Pal, P. (2020) Synergy of Biofuel Production with Waste Remediation along with Value Added Co-Products Recovery through Microalgae Cultivation: A Review of Membrane-Integrated Green Approach. *Science of the Total Environment*, **698**, Article ID: 134169. https://doi.org/10.1016/j.scitotenv.2019.134169

[13] Chisti, Y. (2007) Biodiesel from Microalgae. *Biotechnology Advances*, **25**, 294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

[14] Ogbonna, I.O. and Ogbonna, J.C. (2018) Effects of Carbon Source on Growth Cha-

racteristics and Lipid Accumulation by Microalga *Dictyosphaerium* sp. with Potential for Biodiesel Production. *Energy and Power Engineering*, **10**, 29-42. https://doi.org/10.4236/epe.2018.102003

[15] Jiang, Y., Pu, X., Zheng, D., Zhu, T., Wang, S., Deng, L. and Wang, W. (2018) Cultivation of Lipid-Producing Microalgae in Struvite-Precipitated Liquid Digestate for Biodiesel Production. *Biotechnology for Biofuels*, **11**, Article No. 101. https://doi.org/10.1186/s13068-018-1102-3

[16] Nayana, B., Venkatesh, H.N., Sudharshana, T.N., Manjunath, K. and Mohana, D.C. (2020) Isolation and Utilization of *Dictyosphaerium ehrenbergianum* Nageli for Biodiesel Production: Lipid Extraction and Biodiesel Property Analysis. *Biofuels*, **11**, 885-892. https://doi.org/10.1080/17597269.2018.1432270

[17] Ogbonna, I.O. and Ogbonna, J.C. (2015) Isolation of Microalgae Species from Arid Environments of Northern Nigeria and Evaluation of Their Potentials for Biodiesel Production. *African Journal of Biotechnology*, **14**, 1596-1604. https://doi.org/10.5897/AJB2014.14327

[18] Eze, C.N., Ogbonna, J.C., Ogbonna, I.O. and Aoyagi, H. (2017) A Novel Flat Plate Air-Lift Photobioreactor Installed with Inclined Reflective Broth Circulation Guide for Improved Biomass and Lipid Productivity by *Desmodesmus subspicatus* LC172266. *Journal of Applied Phycology*, **29**, 2745-2754. https://doi.org/10.1007/s10811-017-1153-z

[19] Bligh, E.G. and Dyer, W.J. (1959) A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911-917. https://doi.org/10.1139/o59-099

[20] Talebi, A.F. Tabatabaei, M. and Chisti, Y. (2014) Biodiesel Analyzer: A User-Friendly Software for Predicting the Properties of Prospective Biodiesel. *Biofuel Research Journal*, **1**, 55-57.

[21] Ademoroti, C.M.A. (1996) Environmental Chemistry and Toxicology. Foludex Press Ltd., Ibadan.

[22] American Public Health Association Part 10000 (1998) Biological Examinations, Section 1020. In: Clesceri, L.S., Greenberg, A.E. and Eaton, A.D., Eds., *Standards Methods for the Examination of Water and Wastewater*, 20th Edition, American Public Health Association, Washington DC.

[23] Ademoroti, C.M.A. (1996) Standard Methods for Water and Effluents Analysis. Foludex Press Ltd., Ibadan.

[24] Ezeh, E., Okeke, O., Aburu, C.M. and Anya, O.U. (2018) Comparative Evaluation of the Cyanide and Heavy Metal Levels in Traditionally Processed Cassava Meal Products Sold within Enugu Metropolis. *International Journal of Environmental Sciences and Natural Resources*, **12**, 46-52.

[25] Sawyerr, O.H., Odipe, O.E., Olalekan, R.M. and Ogungbemi, O.H. (2018) Assessment of Cyanide and Some Heavy Metals Concentration in Consumable Cassava Flour "Lafun" Acrsoo Osogbo Metropolis, Nigeria. *MOJ Ecology and Environmental Sciences*, **3**, 369-372. https://doi.org/10.15406/mojes.2018.03.00115

[26] Rui, C.B., Rong, L.C., Lauren, D.A., Yan, L.A.R., Jan, S.B. and Wei, L.A. (2012) Freshwater Algal Cultivation with Animal Waste for Nutrient Removal and Biomass Production. *Biomass and Bioenergy*, **39**, 128-138. https://doi.org/10.1016/j.biombioe.2011.12.045

[27] Xu, H., Miao, X.L. and Wu, Q.Y. (2006) High Quality Biodiesel Production from a Microalga *Chlorella protothecoides* by Heterotrophic Growth in Fermenters. *Journal of Biotechnology*, 126, 499-507. https://doi.org/10.1016/j.jbiotec.2006.05.002

[28] Cheng, Y., Lu, Y., Gao, C.F. and Wu, Q.Y. (2009) Alga-Based Biodiesel Production and Optimization Using Sugar Cane as the Feedstock. *Energy and Fuels*, **23**, 4166-4173. https://doi.org/10.1021/ef9003818

[29] Griffiths, J.M. and Harrison, T.L. (2009) Lipid Productivity as a Key Characteristic for Choosing Algal Species for Biodiesel Production. *Journal of Applied Phycology*, **21**, 493-507. https://doi.org/10.1007/s10811-008-9392-7

[30] Knothe, G. (2008) "Designer" Biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties. *Energy & Fuels*, **22**, 1358-1364. https://doi.org/10.1021/ef700639e

[31] Bhatt, N.C., Panwar, A., Bisht, T.S. and Tamta, S. (2014) Coupling of Algal Biofuel Production with Wastewater. *The Scientific World Journal*, **2014**, Article ID: 210504.

[32] Nguyen, T.D.P., Nguyen, D.H., Lim, J.W., Chang, C.K., Leong, H.Y., Tran, T.N.T., Vu, T.B.H., Nguyen, T.T.C. and Show, P.L. (2019) Investigation of the Relationship between Bacteria Growth and Lipid Production Cultivating of Microalgae *Chlorella vulgaris* in Seafood Wastewater. *Energies*, **12**, Article No. 2282. https://doi.org/10.3390/en12122282

[33] Leong, W.H., Kiatkittipong, K., Kiatkittipong, W., Cheng, Y.W., Lam, M.K., Shamsuddin, R., Mohamad, M. and Lim, J.W. (2020) Comparative Performances of Microalgal-Bacterial Co-Cultivation to Bioremediate Synthetic and Municipal Wastewaters Whilst Producing Biodiesel Sustainably. *Processes*, **8**, Article No. 1427. https://doi.org/10.3390/pr8111427

[34] Rosli, S.S., Lim, J.W., Jumbri, K., Lam, M.K., Uemura, Y., Ho, C.D., Tan, W.N., Cheng, C.K. and Kadi, W.N.A. (2019) Modeling to Enhance Attached Microalgal Biomass Growth onto Fluidized Beds Packed in Nutrients-Rich Wastewater Whilst Simultaneously Biofixing CO_2 into Lipid for Biodiesel Production. *Energy Conversion and Management*, **185**, 1-10. https://doi.org/10.1016/j.enconman.2019.01.077

[35] Leong, W.H., Lim, J.W., Lam, M.K., Lam, S.M., Sin, J.C. and Samson, A. (2021) Novel Sequential Flow Baffled Microalgae-Bacterial Photobioreactor for Enhancing Nitrogen Assimilation into Microalgal Biomass whilst Bioremediating Nutrient-Rich Wastewater Simultaneously. *Journal of Hazardous Materials*, **409**, Article ID: 124455. https://doi.org/10.1016/j.jhazmat.2020.124455