Effects of temperature and time on oil extraction from some Nigerian indigenous fresh water microalgae species

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Abstract: This study determined the effects of temperature and time on oil extraction from indigenous freshwater microalgae–*Dictyosphaerium*, *Chlorella*, *Desmodesmus* and *Cosmarium* species, cultured in fifteen 2-litre column photobioreactors (PBR) (three per specie). Growth and specific growth rates for the mixotrophic cultivation of the species were greater than that of autotrophic. Oil was extracted from the dried microalgae species at temperatures ranging from 40°C to 120°C at 20°C intervals and times ranging from 30 to 210 minutes at 30 minutes' intervals, by accelerating solvent extraction method. Extraction temperature, time and type of microalgae species had significant effect (p<0.05) on oil yield (temperature > time > type of species). As extraction temperature and time increased, *Desmodesmus armatus* gave the optimum oil yield (72.6% at 92.5°C), whereas, *Cosmarium* spp. produced the least (45.5% at 91.7°C). Optimal oil yield and temperatures of *Desmodesmus subspicatus*, *Chlorella lewinii and Dictyosphaerium* spp. were 68.2% and 92.5°C; 72.3% and 91.9°C; and 66.7% and 92.5°C respectively. The optimization result showed that oil extraction from microalgae should be conducted at about 80°C and at the first 30 minutes of heating for oil extraction. These findings reduce extraction wastages of time, cost, energy, resources and chemicals.
Keywords: microalgae, cultivation, photobioreactor, growth rate, time-temperature optimization, oil yield

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1 Introduction

Four most serious environmental problems confronting humanity today are climate change, green house effect, global warming and environmental degradation caused primarily by the heavy use of non-renewable fossil resources for energy purposes (Chisti and Yan, 2011). As a result of these anthropogenic activities, carbon dioxide (CO_2) and other harmful compounds like particulate matter, unburnt hydrocarbon, nitrogen oxides, carbon monoxide, and smoke are released by power plants, industries and automobiles which can be sequestered by chemical absorption technology using microscopic plants like microalgae (Nabi et al., 2009; Du et al., 2016; Kwaka et al., 2017). Microalgae cultivation is also capable of being used for a large number of applications, such as biofuel production (Slade and Bauen, 2013, waste water treatment (Chisti, 2007), food supplement (Luiten et al., 2003; Soontornchaiboon et al., 2012), fish and animal feed (Spolaore et al., 2006; Bishop and Zubeck, 2012), pharmaceutical (Ashraf-Khorassani et al., 1999), phytochemical fields (Benthin et al., 1999) and the production of some bioactive compounds (Chen et al., 2009; Harun et al., 2010). Nowadays, there has been an increasing interest in non-food feedstock for biofuel production in order to avoid the food-fuel conflict. The first and second generation feedstocks have fundamental drawbacks. The third generation feedstock like microalgae are seen as non-food feedstock and promising candidates for the industrial production of biofuel because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops (Demirbas, 2009).

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One of the main obstacles to fully taking advantage of oil-producing biomaterials especially microalgae biomass, is the ability to successfully and efficiently extract lipid/oil from it. Thus, many methods can be employed in extracting oil from microalgae and other oil-bearing seeds of vegetable origin. Accelerated solvent extraction (ASE) using organic solvent and supercritical fluid extraction are the two most commonly used methods in microalgae oil extraction that involves high temperature extraction. Even though ASE requires relatively high pressure, ASE using organic solvent like n-hexane has the benefits of inexpensive and less solvents consumption (Pawliszyn, 1993), higher lipid recovery yield (Demirbas, 2009; Du et al., 2016), automatic procedure for simultaneous extraction of multiple samples and short sample preparation time.

Optimum oil extraction temperature and time vary from species to species. At present, there are few literatures on the effect of manipulating the extraction temperature and time for various microalgae species on the oil yield. Oil extraction at the optimum temperature has been known to offer several advantages for oil bearing feedstocks. These advantages include increased oil yield, better oil properties and production of bioactive compounds for chemical and pharmaceutical purposes (Leesing et al., 2011). According to Wu et al. (2017), oil extraction represents one of the first critical steps in biofuel production from microalgae. The quality and quantity of oil extraction from biomaterials is a function of time and extraction temperature. However, oil extraction varies in quantity because of inadequate knowledge of operating extraction conditions such as extraction temperature and time. As a result, oil extraction from microalgae is often carried out at suboptimal conditions. Islam et al. (2014) had earlier indicated that biofuel from microalgae could be positively manipulated by selecting process extraction conditions that favour extraction of oils over optimal extraction conditions. Oil extraction from microalgae is dynamic in nature and complex. The oil bearing-cells metabolic responses to heat application during oil extraction process need to be studied for optimal oil yield, using appropriate models. Studying the key variables that affect oil yield during microalgae oil extraction is expedient in order to boost yield which will enhance and increase biofuel production and profitability on investment.

Therefore, the present study was conducted to determine the effects of temperature and time on oil extraction from some Nigerian indigenous fresh water microalgae species using ASE.

2 Materials and method

2.1 Materials

The fresh water microalgae species used in the experimental study were *Dictyosphaerium* spp., *Desmodesmus subspicatus*, *Chlorella lewinii*, *Desmodesmus armatus* and *Cosmarium* spp. (Nsukka, Nigerian strains). The samples were harvested from stagnant water bodies within Nsukka environments, that indicated greenish colour as evidence of microalgae growth. The collection was conducted between April and November, 2014. The samples were transported to the Department of Plant Science and Biotechnology laboratory, University of Nigeria, Nsukka for identification, isolation and sub culturing. Fifty 2-liter conical flasks, one hundred test tubes, one hundred beakers, fifty measuring cylinders and sixty each 5 cm^3 and 10 cm^3 syringes used for the study were properly washed with detergent in lukewarm water, sterilized by autoclaving in an 18-liter capacity autoclave (Model: TT-280A), at 121^oC for 15 minutes. 70% alcohol was used to swab the work table area to prevent contamination. The instruments were dried in a hot air oven at temperature of 105^oC for eight hours then preserved in a storage closet. Fifteen (15) 2-litre column photobioreactors (PBR), three for each of the species were used for the cultivation of the microalgae species. The PBRs with a working volume of 1.5-litre was equipped with an aquarium air pump and six (6) external light sources (15W, 150 V fluorescent light), mounted by the sides, set at 20 cm from each of the PBRs. The lighting source used for the PBR was constructed with florescent lamps with continuous illumination of 129 μmol photons m^{-2} s^{-1}, determined using conversion and calibration factors of 800 lumens and 0.0135 respectively per lamp.

2.2 Preparation of medium used in the cultivation of indigenous fresh water microalgae species

The medium used for the isolation and growth of the

isolates was BG-11 medium prepared following the procedure of Rippka et al. (1979). The medium was sterilized by autoclaving at a pressure of 15 Pa and temperature of 121°C for 15 minutes while the pH was adjusted to 7.4 using 0.1 N hydrochloric acid (HCl) and measured with a pH meter (Choi, 2015).

2.3 Isolation, purification, identification and cultivation of microalgae species

The isolation and pre culture of the microalgae strain was carried out according to the methods by Bekirogullari et al. (2017), Choi (2015) and Ogbonna et al. (1997) with a slight modification. This involves streaking and successive serial dilution until a pure, axenic culture was attained.

Purification was firstly by successive decantation of the upper growing layer into a freshly prepared BG-11 between 10.00 am and 12.00 pm in order to avoid photo inhibition of the cells. This was followed by the purification in the isolation media solidified with 1.5% agar-agar allowed to grow on a laboratory bench for 21 days. The microalgae colony were transferred into a freshly prepared BG-11 medium beefed up with 4.0 g L^{-1} of glucose in 15 constructed PBRs. These were grown for 15 days and harvested. Further sub culturing was done three times to produce pure cultures of the test organism. The colonies were transferred on a fresh sterile BG-11 medium beefed up with 4.0 g L^{-1} of glucose in 15 constructed PBRs. The bubble column 2-litre PBRs (three for each of the species), constructed with glass vessels, having a working volume each of 1.5-litre was used for the cultivation of the microalgae species. The PBRs were equipped with an aquarium air pump and six external light sources (15 W, 150 V fluorescent light) mounted by the sides and set at 20 cm from each other. The microalgae species were grown for 15 days, harvested and used for the subsequent studies.

Species were identified under an objective microscope (Olympus, USA) on the basis of cell morphology and colonial characteristics. The species were identified using identification keys by Edmondson (1959) and Van Vuuren et al. (2006). Incubation was near the transparent window of the laboratory, for adequate sun light at room temperature (30°C±2°C) and pH 7.4. Agitation was done manually every 12 hours and mechanically for three hours per day by the help of an aquarium air pump. Atmospheric carbon dioxide (CO_2) was diffused, by aerationg into the culture through Polyurethane foam used to plug/cork the mouth of the bottle of the microalgae species.

2.4 Determination of the growth rate and specific growth rate of microalgae cell species

Cell growth rate is usually expressed as an increase in cell concentration over a given period of time. Growth rate (g cm^{-3} day^{-1}) and specific growth rate (μ) (day^{-1}) were determined using the expressions (Equations (1) and (2) respectively) by Ogbonna (2013).

$$\frac{dX}{dt} = \frac{X_2 - X_1}{t_2 - t_1} \quad (1)$$

$$\mu = \frac{X_2 - X_1}{t_2 - t_1} \times \frac{1}{\chi} \quad (2)$$

where, X_1 and X_2 which are cell concentrations (g cm^{-3}) were extrapolated from the standard curve (SC); t_1 and t_2 were the days of cultivation (day) while χ is average cell concentration (g cm^{-3}) between t_1 and t_2, which was computed using Equation (3)

$$\chi = \frac{X_1 + X_2}{2} \quad (3)$$

2.5 Dewatering and recovery of microalgae biomass

Flocculation using 4 g cm^{-3} of aluminum sulfate, supported by centrifugation (Kenley, Model: centr04-fba-us) at 3500 rpm for 15 minutes and filtering was employed in the microalgae harvesting (Al Hattab et al., 2015). The supernatant was discarded and the residue collected and sun dried for oil extraction.

2.6 Lipid/oil extraction from microalgae biomass

Twenty-five grams (25 g) of dried and mechanically-grinded microalgae biomass obtained from each of the cultivated species by several replications, were subjected to solvent extraction using n-hexane by ASE method using an ASE 200 accelerated solvent extractor [Dionex (UK), Camberley, chrysene) Surrey] (Figure 1) according to Saima et al. (1997). Oil was extracted from the dried microalgae species at high pressure and temperature ranging from 40°C to 120°C at 20°C intervals and time ranging from 30 to 210 minutes at 30 minutes intervals. The oil yield (OY % by wt) was calculated using Equation (4)

$$OY = 100\left(\frac{M_O}{M_{MA}}\right) \quad (4)$$

where, M_O is mass of oil in grams and M_{MA} is mass of microalgae biomass in grams.

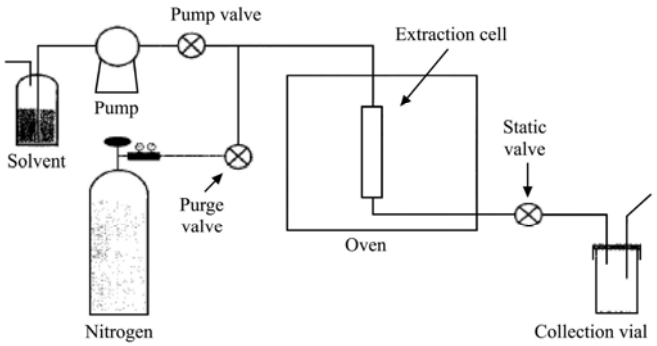

Figure 1 Schematic diagram of accelerated solvent extraction (ASE) system

2.7 Statistical analyses of data

During cultivation, an experimental design in completely randomized design (CRD) with a total of 30 observations (5 levels of microalgae species × 2 levels of cultivation × 3 replications) was conducted. During oil extraction, a 5×7×5 factorial in CRD with a total of 175 observations (5 levels of extraction temperature × 7 levels of extraction time × 5 species) was also conducted. Data was analyzed using descriptive and inferential statistics with SPSS, version 21; Excel package, Windows 10; PrismGraph 6 and Minitab 16. Analysis of variance (ANOVA) and F-test were carried out and results presented in Duncan Multiple Range format. For each of the microalgae species, mathematical models were developed to predict the microalgae oil yield at various oil extraction temperatures and duration using Prism Graph 6.

3 Results and discussion

3.1 Growth rate and specific growth rate

Values for the growth rate (g cm^{-3} day^{-1}) of the five different cultivated microalgae species are shown in Table 1 while Table 2 presents values for the specific growth rate (day^{-1}) for the same microalgae species. Growth and specific growth rate for the mixotrophic cultivation (i.e with glucose) were higher than that of the autotrophic (i.e without glucose) for the five different species of microalgae cultivated. ANOVA results showed that only glucose had significant effect on microalgae growth rate ($p \leq 0.05$). Microalgae species and the interaction of species and glucose was not significant ($p \geq 0.05$). It is not advisable to extract oil at very high extraction times (Shao et al., 2012; Muruganandam et al., 2017; Chaouche et al., 2017) as much of the oil from the biomass cells would have either been exhausted or the solvent completely evaporated at these times thereby leading to wastage of time, energy and resources.

Table 1 Growth rate for the five different cultivated microalgae species (g cm^{-3} day^{-1})

Days of cultivation	*Desmodesmus armatus* A1		*Dictyosphaerium* spp. A5		*Desmodesmus subspicatus* A7		*Chlorella lewinii A9*		*Cosmarium* spp.*ISO*	
	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose
2^{nd}- 4^{th}	0.16715	0.03570	0.03689	0.070671	0.182674	0.005175	0.087159	0.006081	0.022912	0.14613
4^{t} -6^{th}	0.01656	0.05174	0.14134	0.111723	0.02484	0.021217	0.006081	0.028377	0.004582	0.176171
6^{th}-8^{th}	0.00155	0.02432	0.03949	0.045729	0.013972	0.019665	0.04814	0.030404	0.010692	0.013747
8^{th}-10^{th}	0.01190	0.04605	0.04832	0.022864	0.00414	0.053819	0.000507	0.104388	0.1222	0.028513
10^{th}-12^{th}	0.00517	0.13247	0.00155	0.104968	0.085904	0.00414	0.010135	0.025844	0.360489	0.116599
12^{th}-14^{th}	0.50093	0.44038	0.109125	0.33465	0.253053	0.615815	0.050167	0.452518	0.055499	0.323829
Standard Dev.	0.198437	0.160729	0.051943	0.112792	0.102459	0.243576	0.033934	0.172165	0.136571	0.113006

Table 2 Specific growth rate for different cultivated microalgae species (day^{-1})

Days of cultivation	*Desmodesmus armatus* A1		*Dictyosphaerium* spp. A5		*Desmodesmus subspicatus* A7		Chlorella lewinii A9		*Cosmarium* spp. ISO	
	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose	Glucose	Non glucose
2^{nd}- 4^{th}	0.219877	0.09732	0.031968	0.126866	0.17536	0.00947	0.059147	0.015152	0.031142	0.373212
4^{th} -6^{th}	0.017544	0.113895	0.106084	0.151089	0.019884	0.037037	0.003881	0.065116	0.006004	0.24679
6^{th}-8^{th}	0.001614	0.045854	0.026099	0.050985	0.010848	0.03204	0.029697	0.061475	0.013734	0.015211
8^{th}-10^{th}	0.012202	0.076658	0.030185	0.023681	0.00317	0.078313	0.000303	0.165862	0.134078	0.03014
10^{th}-12^{th}	0.005214	0.169987	0.000944	0.096008	0.061527	0.005556	0.006031	0.034023	0.258583	0.10686
12^{th}-14^{th}	0.334254	0.325679	0.061947	0.218305	0.14584	0.451099	0.028821	0.365534	0.030661	0.211436
Standard Dev.	0.14311	0.100733	0.036548	0.070462	0.074026	0.172863	0.022525	0.13205	0.099497	0.138705

3.2 Effects of extraction temperature, time and microalgae species on oil yield

The mean values of oil yield under different extraction temperatures (^{o}C) and time (minutes) for the different species of microalgae studied is presented in Tables 3 using Duncan Multiple Range Test (DMRT) format for separation of the mean where differences are significant.

Table 3 Mean values of oil yield (%) under varying temperature (^{o}C) and time (minutes) for *Desmodesmus armatus, Desmodesmus subspicatus, Chlorella lewinii, Dictyosphaerium* spp. and *Cosmarium* spp. in DMRT format

Microalgae Species	Extraction temperature (^{o}C)	Microalgae oil yield (%)						
		Time (Minutes)						
		30	60	90	120	150	180	210
A1	40	0.92^{a}	1.2^{a}	2.1^{b}	2.2^{b}	6.3^{c}	29.6^{d}	0.7^{a}
	60	49.4^{bc}	49.6^{c}	49.6^{c}	49.1^{b}	49.8^{c}	50.2^{d}	1.4^{a}
	80	56.7^{d}	55.0^{b}	55.4^{bc}	55.9^{c}	57.2^{d}	57.8^{e}	1.5^{a}
	100	52.7_{b}	53.2^{c}	53.1^{bc}	55.3^{d}	55.4^{d}	55.3^{d}	2.5^{a}
	120	52.3^{c}	52.4^{c}	52.8^{c}	50.1^{b}	52.7^{c}	53.5^{d}	1.3^{a}
A7	40	0.6^{a}	1.5^{b}	1.8^{b}	1.9^{b}	3.1^{c}	26.4^{d}	0.8^{a}
	60	46.1^{bc}	46.4^{bc}	46.3^{bc}	45.9^{b}	46.5^{c}	47.0^{d}	1.1^{a}
	80	53.4^{e}	51.7^{b}	52.1^{c}	52.6^{d}	53.9^{f}	54.5^{g}	0.4^{a}
	100	49.4^{b}	49.9^{c}	49.9^{c}	52.0^{d}	52.1^{d}	52.0^{d}	0.3^{a}
	120	49.0^{c}	49.1^{c}	49.4^{c}	46.8^{b}	49.3^{c}	50.9^{d}	1.3^{a}
A9	40	1.0^{a}	1.7^{a}	2.2^{a}	2.4^{a}	6.3^{b}	26.0^{c}	0.7^{a}
	60	49.4^{b}	48.6^{b}	49.6^{b}	49.1^{b}	47.6^{b}	50.0^{b}	1.4^{a}
	80	56.8^{cd}	54.0^{b}	55.0^{bc}	55.9^{cd}	56.0^{cd}	57.0^{d}	1.3^{a}
	100	52.7^{b}	53.2^{bc}	53.1^{bc}	54.7^{cd}	55.6^{d}	52.7^{b}	2.5^{a}
	120	51.7^{c}	52.4^{cd}	52.8^{d}	50.1^{b}	52.5^{cd}	52.5^{cd}	1.6^{a}
A5	40	0.2^{a}	0.8^{ab}	1.5^{bc}	1.7^{c}	5.6^{d}	26.1^{e}	0.1^{a}
	60	44.7_{bc}	44.9^{bc}	44.9^{bc}	44.4^{b}	45.1^{cd}	45.5^{d}	0.8^{a}
	80	51.9_{d}	50.3^{b}	50.7^{bc}	51.2^{c}	52.4^{d}	53.1^{e}	1.5^{a}
	100	48.0_{b}	48.5^{b}	48.4^{b}	50.5^{c}	50.7^{c}	50.6^{c}	1.9^{a}
	120	47.7^{c}	47.8^{c}	48.1^{c}	45.5^{b}	48.0^{c}	48.8^{d}	1.5^{a}
ISO	40	0.8^{a}	1.0^{a}	1.7^{a}	1.8^{a}	4.8^{b}	9.2^{c}	0.6^{a}
	60	28.7^{bc}	29.0^{bcd}	28.9^{bc}	28.5^{b}	29.1^{cd}	29.5^{d}	0.3^{a}
	80	36.1^{e}	34.4^{b}	34.9^{c}	35.4^{d}	36.7^{f}	37.3^{g}	0.2^{a}
	100	32.1^{b}	32.6^{c}	32.5^{c}	34.6^{d}	34.8^{d}	34.7^{d}	0.2^{a}
	120	31.7^{c}	31.9^{cd}	32.2^{d}	39.5^{b}	32.1^{cd}	32.9^{e}	0.1^{a}

Note: Mean values with different letters in the same column are significantly different ($p \leq 0.05$); mean values with the same letters in the same column are not significantly different. A1, A7, A9, A5 and ISO are *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp. respectively.

3.2.1 Effects of extraction temperature and time on the oil yield of *Desmodesmus armatus*

Oil yield was found to vary with extraction temperature and time for the *Desmodesmus armatus*. The highest oil yield was produced by *Desmodesmus armatus* at 80^{o}C after 180 minutes of extraction (57.824%). *Desmodesmus armatus* maximum oil yield of 57.8% was higher than the yield of 56.3% for *Chrysophy*, far higher than the oil yield of 45.6% from *Chlorella protothecoides* reported by Wang et al. (2016) and better than the results of extracted oil of 15.5% (% dry weight) for *C. minutissima*, 40.3% for *T. fluviatilis* and 39.5% for *T. pseudonana*, presented by Neto et al. (2013) using conventional solvent extraction methods. *Desmodesmus armatus* oil yield was also far higher than both the predicted and experimental oil yield of 45.9% and 45.5% respectively from *Nannochloropsis* after extraction time of 25.05 minutes. However, *Desmodesmus armatus* highest oil yield was slightly lower than the yield of 63.8% reported for *Chrysophy* and *Chlorella* sp. (Zhou et al., 2017). The minimum oil yield was attained at the time of 210 minutes and the extraction temperature was 40^{o}C.

3.2.2 Effects of extraction temperature and time on the oil yield of *Desmodesmus subspicatus* (A7)

Oil yield also varied with extraction temperature and time for *Desmodemus subspicatus*. As extraction temperature and time increased from 40°C-120°C and 30-210 minutes respectively, oil yield for *Desmodemus subspicatus* also varied. The maximum value of oil yield attained at 80°C after 180 minutes was 54.5%. The minimum value of oil yield attained at 40°C was 0.6% after 30 minutes. The maximum oil yield of *Desmodemus subspicatus* was three times higher than the maximum oil extraction yield of 18.8% obtained after extraction time of 120 minutes, and extraction temperature of 60°C for microalgae using solvent extraction method (Wu et al., 2017). The yield was also slightly better than the *Chlorella vulgariss* oil yield of 52.5% reported by Araujo et al. (2013) and three times far better than the optimum yield of 17.7% of oil for *Chlorella vulgaris* using supercritical carbondioxide ($SCCO_2$) extraction method (Bahadar et al., 2015). However, the maximum oil yield from *Desmodesmus subspicatus* was slightly lower than the value of 65.2% recorded for *Chlorella protothecoides* (Chen et al., 2012).

3.2.3 Effects of extraction temperature and time on the oil yield of *Chlorella lewinii*

There was also a variation for *Chlorella lewinii* oil yield with extraction temperature and time. The minimum and maximum values of oil yield for 60°C, 80°C, 100°C and 120°C were 1.4% and 50.0%; 1.3% and 57.0%; 2.5% and 55.6%; and 1.6% and 52.5% after times of 210 and 180 minutes; 210 and 180 minutes; 210 and 150 minutes; and 210 and 180 minutes respectively. The maximum oil yield of *Chlorella lewinii* (57.0%) at 80°C after extraction time of 180 minutes was clearly within the range of values of total recovered oil (14%-8%) for four different species of microalgae (*Isochrysis galbana*, *Nannochloropsis gaditana*, *Nannochloropsis* sp. and *Phaeodactylum tricornutum*) earlier reported by Ryckebosch et al. (2014), depending on the type of species and solvent used. The oil yield for *Chlorella lewinii* obtained in this study was above that reported by Seo et al. (2014) for microalgae enhanced with florescent-painted lighting source and that of Drira et al. (2017) for microalgae cultivated in a PBR subjected to osmotic stress. It is not advisable to extract oil at very high extraction times (Shao et al., 2012; Muruganandam et al., 2017; Chaouche et al., 2017) as much of the oil from the biomass cells would have either been exhausted or the solvent completely evaporated at these times thereby leading to wastage of time, energy and resources.

3.2.4 Effects of extraction temperature and time on the oil yield of *Dictyosphaerium* spp. (A5)

The trend of oil yield and extraction temperature with time for *Dictyosphaerium* spp. was virtually the same with that of other species earlier studied. *Dictyosphaerium* spp. had a maximum oil yield of 53.108% at temperature of 80°C after extraction time of 180 minute which was very high when compared to Shankar et al. (2017) who had earlier reported a 1.9 and 1.7 folds higher oil yield for *Chlorella* and *Chlorococcum* sp. respectively, using protic ionic liquid assisted cell method as against the conventional (traditional) solvent extraction method. This value was also higher than the oil yield value of 33.9% for microalgae at 40°C and 35 MPa reported by Tang et al. (2011) using $SCCO_2$ extraction.

3.2.5 Effects of extraction temperature and time on *the* oil yield of *Cosmarium* spp.

A maximum value of oil yield (39.544%) was recorded after 120 minutes extraction and at 120°C while a minimum value (0.6%) was attained after 210 minutes at 40°C for *Cosmarium* spp. Despite the generally low oil content of *Cosmarium* spp. compared to the four other species studied, the maximum oil yield of 39.5% obtained at 120°C after 120 minutes of extraction time was clearly higher than the average oil yield of 5.8% reported by Pohndorf et al. (2016) for *Spirulina* sp. and also higher than the value of 0.03% reported for *Nannochloropsis* sp. by Pradana et al. (2017).

3.3 Statistical analyses

ANOVA results showed that extraction temperature, time and type of microalgae species had significant effects (at 5% level of probability, $P<0.05$) on *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp. oil yield, with temperature having more significant effect, followed by time and type of species. This is clearly in good agreement with the findings of Millao and Uquiche (2006). The interaction of temperature and time

had more significant effect, followed by temperature and species; and species and time. The interaction of species, temperature and time had the least effect on microalgae oil yield (Table 4).

Table 4 ANOVA result on the effect of temperature, time and species on oil yield.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	411360.389[a]	174	2364.140	2.760E3	0.000
Intercept	985419.368	1	985419.368	1.150E6	0.000
Organism	26398.366	4	6599.591	7.704E3	0.000
Time	157296.216	6	26216.036	3.060E4	0.000
Temperature	181517.249	4	45379.312	5.297E4	0.000
Species * Time	3985.592	24	166.066	193.861	0.000
Species * Temperature	4158.129	16	259.883	303.380	0.000
Time * Temperature	36485.635	24	1520.235	1.775E3	0.000
Species * Time * Temperature	1519.204	96	15.825	18.474	0.000
Error	599.638	700	.857		
Total	1397379.395	875			
Corrected Total	411960.027	874			

R Squared = 0.999 (Adjusted R Squared = 0.998)

Pair wise comparison of oil yield for *Desmodesmus armatus* at different levels of temperature and time using DMRT (Table 3), showed that at extraction temperature of 80°C, oil yield at 210 minutes; 60 minutes; 120 minutes; 150 minutes; and 30 minutes and 180 minutes were significantly different (P<0.05). For extraction temperature of 40°C, oil yield at 30 minutes, 60 minutes and 210 minutes were not significant. Oil yield at 90 minutes and 120 minutes were also not significantly different whereas oil yield at 30, 60 and 210 minutes; and 90 and 120 minutes were all found to be significantly different from those obtained at 150 and 180 minutes, when extracted under normal room temperature (about 40°C).

For the extraction at 40°C, oil yield of *Desmodesmus subspicatus* at 30 minutes was significant with that at 150 and 180 respectively, but not significant with the oil yield at 210 minutes. For extraction temperature of 80°C, oil yield were significant for all the levels of extraction time. For C*hlorella lewinii*, at extraction temperature of 40°C, which is almost a room temperature, oil yield at times of 30, 60, 90, 120 and 210 minutes were not significant. But oil yield at the same levels of extraction times were significantly different from those recorded at 150 and 180 minutes' time. Oil yield at extraction time of 30 minutes, 90 minutes, 120 minutes and 150 minutes were not significant at extraction temperature of 80°C. However, they were significant from oil yield recorded at 60, 180 and 210 minutes respectively.

Extraction at room temperature (40°C) for *Dictyosphaerium* spp., pair wise comparison, using DMRT format, oil yield for different extraction times showed that oil yield for time of 30 minutes and 210 minutes were not significant. Equally, oil yield for 90 and 120 minutes were also not significant. However, oil yield for extraction times of 120, 150 and 180 minutes were significantly different (P<0.05). At 80°C of oil extraction temperature for *Dictyosphaerium* spp., oil yield after 30 minutes and 150 minutes were not significant whereas were significantly different from values recorded after times of 60, 120 180 and 210 minutes.

At 120°C of extraction temperature for *Cosmarium* spp., oil yield at times of 60 and 150 minutes were not significant. Oil yield in 210, 180, 120 and 90 minutes were significantly different. Oil yield in 30, 60, and 150 were not significantly different. Equally, oil yield at extraction times of 60, 90, and 150 were also not significantly different (P<0.05). At 80°C of extraction temperature for *Cosmarium* spp., oil yield in all the seven levels of extraction times (30-210 minutes) were significantly different.

3.4 Models for predicting oil yield at varying extraction temperature and time

The relationship between oil yield, extraction temperature and time for *Desmodesmus armatus, Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp. gave a second-order polynomial (quadratic) equation (Equation (5)), Where, coefficients A, B, and C in Equation (5) are quadratic functions of time having the general form, $\beta=\beta_0+\beta_1 t+\beta_2 t^2$ as clearly shown by the plots of the coefficients against time. In this case, β represents A, B and C. These models are in perfect agreement with the model earlier developed by Bahadar et al. (2015) for *Chlorella vulgaris*.

$$Y=A+BT+CT^2 \quad (5)$$

The plots of coefficients against time are as presented in Figure 2 for the different microalgae species.

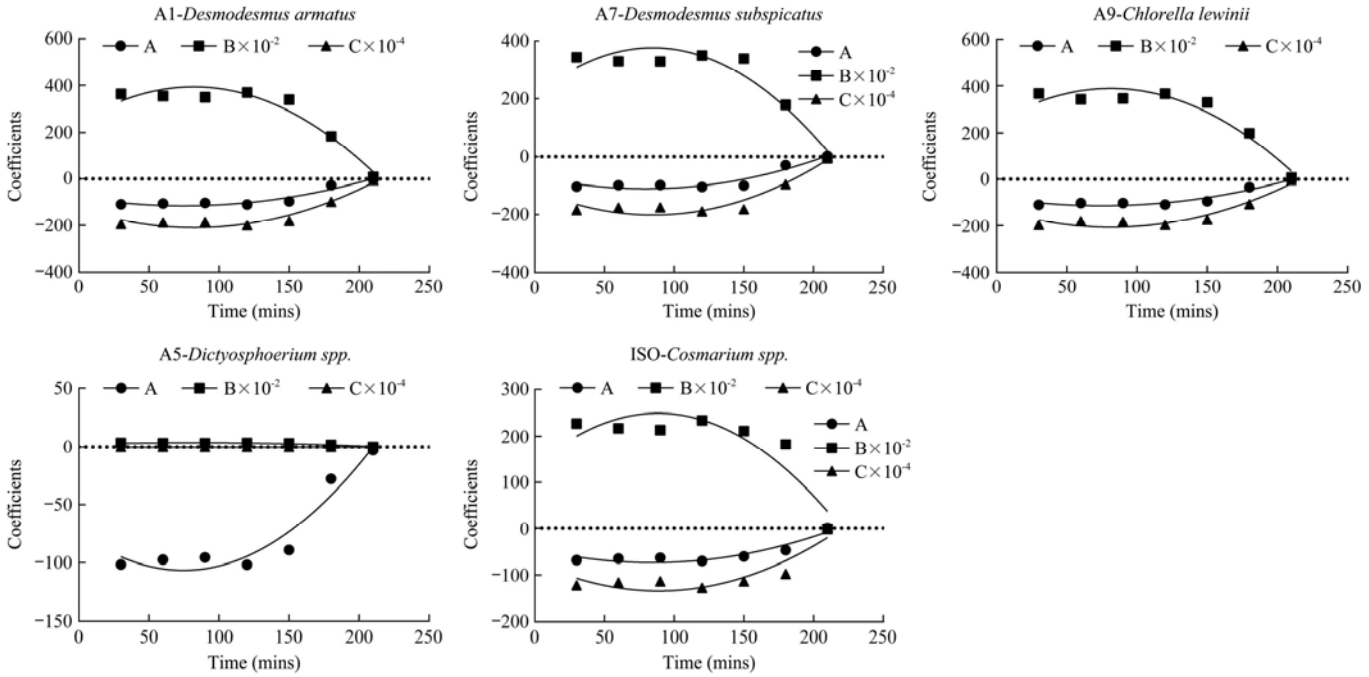

Figure 2 Graph of the coefficients with time for *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp.

By substituting the values of coefficients (Figure 2) in Equation (5), then the model developed for predicting oil yield for *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarius* spp. at various temperatures and times are developed as presented in Equations (6), (7), (8), (9) and (10) respectively, with their various R^2 values showing high levels of correlation.

$$Y = -79.12 - 0.9595t + 0.0064t^2 + (2.439 + 0.03644t - 0.0002221t^2)T + (-0.01291 - 0.0001964t + 0.000001191t^2)T^2 \quad (R^2 = 0.8325) \quad (6)$$

$$Y = -68.28 - 1.067t + 0.006701t^2 + (2.138 + 0.03842t - 0.0002267t^2)T + (-0.01131 - 0.0002075t + 0.00000122t^2)T^2 \quad (R^2 = 0.83868) \quad (7)$$

$$Y = -79.02 - 0.9254t + 0.006175t^2 + (2.459 + 0.03498t - 0.0002145t^2)T + (-0.01311 - 0.0001863t + 0.000001142t^2)T^2 \quad (R^2 = 0.82859) \quad (8)$$

$$Y = -74.17 - 0.8673t + 0.005805t^2 + (2.259 + 0.03326t - 0.0002028t^2)T + (-0.01194 - 0.00018t^2 + 0.000001091t^2)T^2 \quad (R^2 = 0.8242) \quad (9)$$

$$Y = -43.8 - 0.6995t + 0.004144t^2 + (1.369 + 0.02541t - 0.0001436t^2)T + (-0.007221 - 0.0001377t + 0.0000007739t^2)T^2 \quad (R^2 = 0.81216) \quad (10)$$

where, Y is the oil yield (%); t is extraction time (mins) and T is the extraction temperature (^oC)

3.5 Process optimization using experimental data

Table 5 is a two-factor randomized factorial design used for the optimization.

Table 5 Two factors randomized factorial design for analysis of the oil yield (%) using MINITAB

Temperaure (^oC)	Time (mins)	A1	A7	A9	A5	1SO
40	210	0.7	0.8	0.7	0.1	0.6
120	30	52.3	49.0	51.7	47.7	31.7
80	120	55.9	52.6	55.9	51.2	35.4
40	30	0.9	0.6	1.0	0.2	0.8
120	210	1.3	1.3	1.6	1.5	0.1

Note: A1, A7, A9, A5 and ISO are *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp. respectively.

After process optimization analysis of the experimental data using MINITAB, the optimum value generated for the oil yield, extraction temperature and extraction time is summarized and presented in Figure 3. Maximum oil yield did not necessarily amount to the optimum values for oil yield, temperature and time. It was obvious from the optimization result that oil extraction from microalgae, should be done at high temperature (above 80^oC) and at a lower time (about 30 minutes). This will help to prevent wastage of time, energy, resources and chemicals for the extraction which are obviously very costly (Chen et al., 2013).

Desmodesmus armatus gave the highest optimum oil yield (72.6%) at a temperature of 92.53°C. *Cosmarium* spp. produced the least optimum oil yield (45.5%) at a temperature of 91.7°C. The oil yield and extraction temperatures for *Desmodesmus subspicatus, Chlorella lewinii* and *Dictyosphaerium* spp. were 68.154% and 92.5°C; 72.3 and 91.7°C; and 66.7% and 92.5°C respectively. All the microalgae species were maintained at optimum extraction time of 30 minutes.

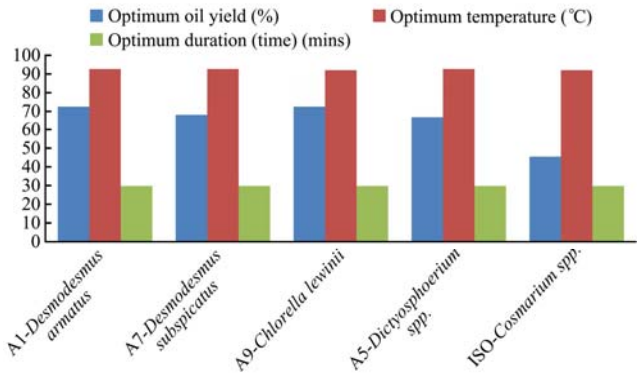

Figure 3 Bar-chart of optimum oil yield, extraction temperature and time for five microalgae species.

4 Conclusions

In conclusion, significant quantity of oil can be extracted from *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp., which can be affected by extraction temperature and time. Growth and specific growth rate for the mixotrophic cultivation (i.e with glucose) were higher than that of the autotrophic (i.e without glucose) for the five different species of microalgae species cultivated. All the microalgae species maintained an optimum extraction time of 30 minutes. The optimization result showed that oil extraction from microalgae, should be done at higher temperature (above 80°C) and at lower time (30 minutes). This will help to prevent wastage of time, energy, resources and chemicals for the extraction which are obviously very costly. A two-order polynomial equation was most appropriate for the prediction of oil yield against extraction temperature and time for *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and *Cosmarium* spp. The models developed for the prediction of oil yield for *Desmodesmus armatus*, *Desmodesmus subspicatus*, *Chlorella lewinii*, *Dictyosphaerium* spp. and Cosmarium spp. gave a coefficient of determination, R^2 values of 0.8325, 0.8387, 0.8286, 0.8242 and 0.8122 respectively. This study enriched the literature by providing optimum extraction temperature and time of oil yield for some indigenous microalgae species which are useful in the emerging bio-fuel industry.

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