INFLUENCE OF SALINITY IN FATTY ACID PRODUCTION OF *Dunaliella* sp. AS FEEDSTOCK FOR BIODIESEL

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Abstract: *Dunaliella* sp. is a unicellular, eukaryotic, photosynthetic, and halophilic microalga. It is one of the prospective microalgae being utilized for its significant amounts of valuable chemical matters such as carotenoid, glycerol, and lipids. *Dunaliella* has a potential as feedstock in the production of biodiesel but there is only limited data and information available as most studies are focused to commercial production of high-value products such as beta-carotene. Therefore, it is necessary to evaluate the culture condition of *Dunaliella* sp. that promotes higher lipid production. *Dunaliella* sp. was cultivated at varying salinity conditions (45 g/L, 50 g/L, 55 g/L, 60 g/L, 65 g/L, and 70 g/L) using Conway medium (CM). Algal biomass for each culture condition was harvested at the end of logarithmic phase [Period 1 (P1) or day 10] and onset of decline phase [Period 2 (P2) or day 13] of cultivation. At P1, the highest biomass was obtained from CM50 (1.152 ± 0.120 g/L) while CM45 (2.109 ± 0.168 g/L) for P2. Lipid/oil was extracted from algal biomass cultivated at different salinity concentrations by Bligh and Dyer method using solvent mixture of chloroform and methanol (1:2). CM60 has the highest oil yield in both periods, P1 (1.494 ± 0.190 %) and in P2 (1.636 ± 0.173%). Lipid/Oil from top three (3) oil producing treatments were transesterified to produce the Fatty Acid Methyl Ester (FAME) and subjected to Gas Chromatography - Mass Spectrometry (GC-MS) for fatty acid composition analysis. There are 22 fatty acids identified in P1 while 28 fatty acids in P2. However, only 11 fatty acids identified in all treatments were desirable for biodiesel production namely myristic acid, methyl pentadecanoate acid, palmitic acid, stearic acid, palmitoleic acid, methyl palmitoleate acid, valeric acid, elaidic acid, linoleic acid, oleic acid, and petroselinic acid. Among algal cultures, only CM55 exhibited ideal mix ratio of fatty acid's palmitoleic (16:1), oleic acid (18:1), and myristic acid (14:0) with good biodiesel property (5:3:1). Therefore, *Dunaliella* sp. has a potential as feedstock for biodiesel production with decent amount biomass and lipid/oil using Conway medium that can also exhibit good fuel properties when cultivated at 55g/L of salinity.

Keywords: *microalgae, Dunaliella* sp., *lipid, fatty acid methyl ester, biodiesel*

1. INTRODUCTION

Globally, an economic growth combined with a rising population has led to the steady increase in energy demands. If the governments around the world stick to current policies, the global energy need will increase to almost 60% in 2030 (IEA, 2007; Patil et al., 2008). Fossil fuels are recognized as the major energy source, but depletion of supply resulted to unsustainable economy (IEA, 2007). Moreover, the continued use of fossil fuels for energy production has contributed to environmental pollution, ecological degradation, and climate change (Hallenbeck & Benemann, 2002; IEA, 2007; Milano et al., 2016). Thus, the modern research community is continuously developing clean and viable energy sources and technologies that are not harmful to the environment (Hallenbeck & Benemann, 2002). Researchers enticed interests to alternative sources for renewable and sustainable energy with high potential for biofuel production such as

microalgae because of higher productivity as compared to land plants as well as its capability to enormously increase its biomass at shorter time (Hu et al., 2008). First generation biofuels are produced from agricultural feedstock that can also be used for food or feed purposes (Mata et al., 2010). The possible competition between food and fuel demand makes it impossible to produce enough biofuel to offset significant percentage of fuel consumption for transportation (Mata et al., 2010; Milano et al., 2016; Peng et al., 2020). Whilst algae appear to be a good candidate as feedstock for biofuel as they can avoid this problem. Also, they lack heavy supporting structures and anchorage organs that can pose technical limitations to their harvesting (Rasoul-Amini, 2014; Peng et al., 2020).

Dunaliella species are green microalgae present in marine environment. Under stress condition, this microalga can accumulate considerable number of valuable metabolites such as carotenoid (Tafreshi & Shariati, 2006), glycerol (Hadi et al., 2008), vitamin and protein (Ghoshal et al., 2002). Moreover, *Dunaliella* sp. offers immense potential in the production of biodiesel, but there is only limited published data and information available as most studies are focused on commercial production of high-value products such as beta-carotene (Tafreshi & Shariati, 2006; Scott et al., 2010; Ahmed et al., 2017). Therefore, it is necessary to evaluate the culture condition of *Dunaliella* sp. that promotes higher lipid production. In this study, *Dunaliella* sp. was cultivated in varying saline concentrations to determine optimal growth condition for biomass and to correlate it with lipid/oil production. Lipid produced by *Dunaliella* sp. cultivated in different saline conditions was characterized by its fatty acid composition profile to provide a reasonable foundation to its potential as biofuel source.

2. METHODOLOGY

2.1 Growth performance of Dunaliella sp.

Pure culture of *Dunaliella* sp. was acquired from the Bureau of Fisheries and Aquatic Resources (BFAR) Regional Field Office 1, Alaminos City, Pangasinan and maintained at Phycology Laboratory-Research Institute for Science and Technology (RIST). Prior the experiment, *Dunaliella* was acclimated at different saline concentrations of Conway medium (45g/L to 70 g/L). And then, *Dunaliella* was cultivated at different saline concentrations of Conway medium (CM45=45 g/L, CM50=50 g/L, CM55=55 g/L, CM60=60 g/L, CM65=65 g/L, and CM70=70 g/L) with initial cell density ranges from 1.18×10^5 cells/mL to 1.27×10^5 cells/mL in three (3) replicates. The algal cultures were provided with 24h illumination and continuous aeration. The daily cell density and growth rate were estimated by direct counting using hemocytometer for 13 consecutive days (Martinez-Goss et al., 1975). The total chlorophyll content (TCC) of the cells of each culture was determined on period 1 (P1; Day 10) and period 2 (P2; Day 13) by extracting the chlorophyll using methanol. The absorbance of the extracts was determined using spectrophotometer at wavelength of 650 nm and 665 nm (Martinez-Goss & Dionisio-Sese, 2001).

2.2 Algal wet biomass and lipid extraction

Wet algal biomass from various saline concentrations was harvested by vacuum microfiltration and subsequently centrifuged to obtain the algal pellet. Lipid was

extracted in approximately 2 g of algal pellet using 4.5 mL methanol-chloroform (2:1 v/v). After 2 hours, 1.5 mL of chloroform and deionized water was added, mixed using a vortex mixer, and then centrifuged at 3500 rpm for 10 minutes. The bottom layer was collected and removed the solvent by evaporation to obtain the crude lipid (Bligh & Dyer, 1959).

2.3 Fatty acid profile and characterization

Crude oil extracted from the top three (3) lipid producing cultures was transesterified to produce fatty acid methyl ester (FAME). And then, the fatty acids present were identified by Gas Chromatography Mass Spectrometry (GC-MS) at the National Chemistry Instrumentation Center (NCIC), Ateneo De Manila University. The resulting chromatogram was analyzed and grouped by its chain length (short chain, medium chain, long chain, and very long chain) and degree of saturation (saturated and unsaturated).

2.4 Statistical analyses

All the data gathered for the growth rate, biomass, and lipid production from *Dunaliella* sp. cultivated in varying saline conditions were evaluated using one-way analysis of variance (ANOVA) and Post-hoc Tukey (HSD) using Statistical Program for Social Science (SPSS) version 20 to determine the differences among the treatments. Moreover, correlation between biomass, oil, and salinity concentration was evaluated.

3. RESULTS AND DISCUSSION

3.1 Growth performance of Dunaliella sp.

The established growth curve of *Dunaliella* sp. at different salinities is presented in Figure 1. The growth rate (μ^{-1}) of *Dunaliella* sp. cells served as the basis in establishing the phases of cell growth namely lag phase, log or exponential phase, stationary phase, and decline phase. Among the salinity concentrations during log phase (Day 2 - Day 8), CM45 achieved the highest cell density and growth rate at 1.9×10^5 cells/mL and 0.211 μ^{-1}, respectively. At stationary phase, CM70 obtained the highest cell density and growth rate at 4.28×10^5 cells/mL and 0.10 μ^{-1}, respectively. At the beginning of decline phase, CM60 has the highest cell density and growth rate at 2.84×10^5 cells/mL and 0.054 μ^{-1}, respectively.

All the phases of growth are present in all salinity concentrations and observed within the same time frame. *Dunaliella* sp. can survive in a wide range of salinities (Ben-Amotz & Avron, 1992; Tafreshi & Shariati, 2006; Scott et al., 2010; Ahmed et al., 2017). However, rate of cell proliferation and density differs among saline concentrations. The ability of *Dunaliella* sp. to maintain and enhance cell growth varies depending on the salinity. The preferred salinity was higher than 45 g/L or ppt to achieve optimal growth (Abu-Rezq et al., 2010), which is comparable with the result obtained in this study. It was also reported that the optimum salinity condition for *D. salina* and *D. viridis* is 1.0 M NaCl, which is equivalent to 58 g/L (Jimenez & Niell, 1991; Mishra et al., 2008; Hounslow, 2010; Rad et al., 2011). This salinity concentration is within the range of CM

55 and CM 60 used in this study. Moreover, the growth rate of *Dunaliella* sp. is not significantly different among treatments ($p > \alpha_{0.05}$).

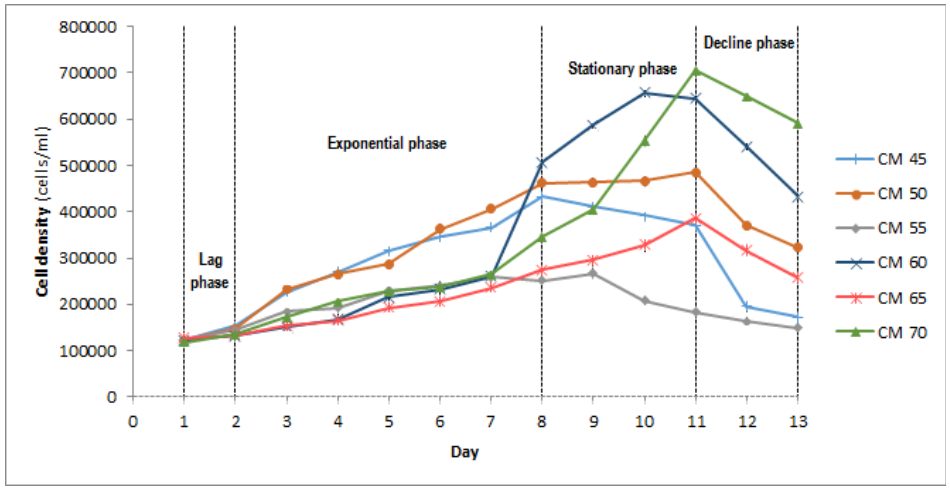

Figure 1. Growth curve of *Dunaliella* sp. at different salt concentrations during 13 days of cultivation lag phase (Day 1), exponential phase (Day 2 to Day 8), stationary phase (Day 8 to Day 11), and decline phase (Day 11 to Day 13).

In Figure 2, the summary of the total chlorophyll content (TCC), chlorophyll *a*, and chlorophyll *b* was determined at different salt concentrations (P1 and P2). The highest chlorophyll content was observed during P1, specifically in CM55 (5.82 ±0.87 µg/mL), followed by CM60 (5.76 ±0.5 µg/mL) and CM70 (4.35 ±0.17 µg/mL). Moreover, CM55 also has the highest TCC (4.88 ±0.71 µg/mL) at period 2 while CM70 has the lowest (3.8 ±0.78 µg/mL). It is evident that chlorophyll production of *Dunaliella* sp. is inversely related with increasing salinity and with the cultivation period. Additionally, it was observed that chlorophyll *b* is the prominent pigment found in the strain of *Dunaliella* sp. Salinity stress induces change in growth rate, pigmentation, chloroplast structure, and lipid composition in *Dunaliella salina* thus its mortality. It was observed that at higher saline concentration, the decrease in chlorophyll content may be related to chloroplast degradation (Al-Hassan et al., 1987) or low cell growth and proliferation. Salinity is one of the variable factors that changes the yield and composition of algal biomass in *Dunaliella viridis*, due to oxidative stress build-up (Talebi et al., 2013; Hamed et al., 2018). But most of *Dunaliella* species have the capability of adjusting to oxidative stresses by elevating carotene to chlorophyll ratio, which functions as compensatory mechanism. Implying that chlorophyll plays a major role in damage repair processes under stressful conditions (Heraud & Beardall, 2000). This could lead us to the results that chlorophyll content decreased at higher salinity but still capable of proliferating due to its fast adaptation and by altering its chlorophyll composition.

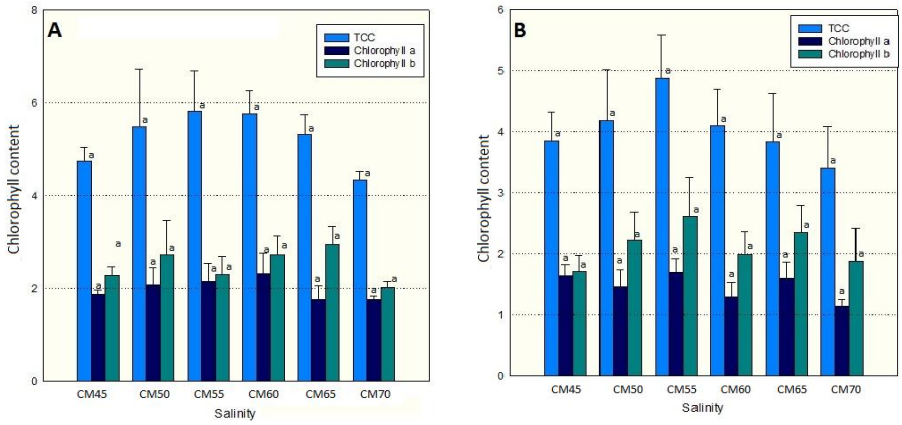

Figure 2. Production of chlorophyll *a*, chlorophyll *b* and total chlorophyll content of *Dunaliella* from different salt concentrations during **(A)** Period 1 (Day 10) and **(B)** Period 2 (Day 13).

3.2 Algal wet biomass and lipid production

The summary of total wet algal biomass and percent oil yield for P1 and P2 is presented in Figure 3. Results showed that P2 has the higher values for algal biomass than P1 indicating that proliferation of cells is apparent at P2. In P1, the highest wet biomass was obtained from CM50 (1.152 ± 0.120 g/L). On the other hand, CM45 (2.109 ± 0.168 g/L) got the highest wet biomass in P2. ANOVA revealed no significant differences ($\alpha_{0.05} < 0.942$) among different salinity concentrations in P1. Likewise, there is no significant difference ($\alpha_{0.05} < 0.694$) among different salinity concentrations in P2. This result coincided with the report of Abu-Rezq et al. (2010) that *D. salina* is best grown in 45 ppt. And this study revealed that growth performance of *Dunaliella* sp. is lower in the culture medium with salinity higher than 45 ppt. On the other hand, the highest percent oil yield was obtained from CM65 of P1 (1.494 ± 0.190 %) and P2 (1.636 ± 0.173 %) but there is no significant difference ($\alpha_{0.05} < 0.650$) in P1. Meanwhile in P2, significant difference ($\alpha_{0.05} > 0.030$) between CM60 and CM70 was observed. These results agreed with the findings of Takagi & Karseno (2005) that the optimum starting salinity to produce lipid is around 1.0 M (58 ppt).

Furthermore, there is no significant correlation between wet biomass from P1 (r = 0.250, $\alpha_{0.05} < 0.071$) and P2 (r = 0.135, $\alpha_{0.05} < 0.335$) with % oil yield from P1 and P2, respectively. CM60 (0.976 ± 0.085 g/L) has the lowest wet biomass among different saline concentrations during P1 but obtained the highest % oil yield (1.494 ± 0.190 %). This implied that *Dunaliella* sp. induces lipid production at high salt concentration although exhibiting lower algal biomass (Takagi & Karseno, 2006; Sonnekus, 2010; Hounslow, 2010). Varying salinity conditions demonstrate the production of lipid from algal cells which is more apparent in moderately high salinities by altering the composition of fatty acids (Sharma et al., 2012; Davis et al., 2015).

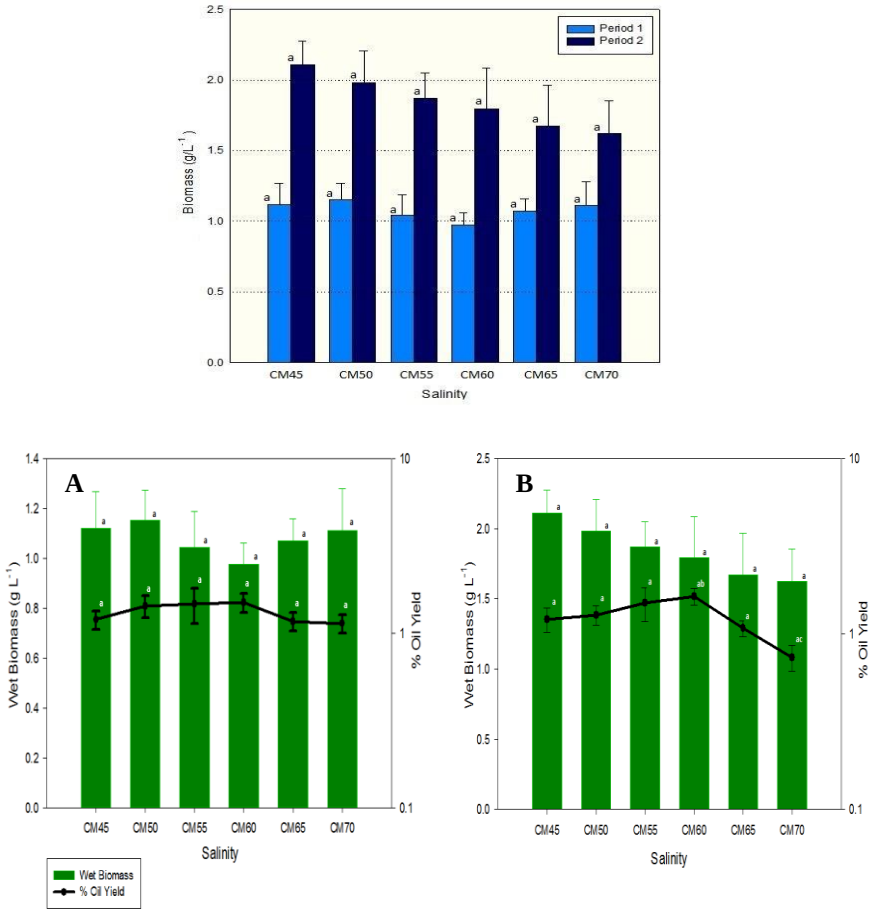

Figure 3. Summary of the total wet biomass of *Dunaliella* sp. obtained from various salinity conditions of the culture medium at P1 (10th day) and P2 (13th day) (left chart); Summary and relationship of total wet algal biomass and percent oil yield from *Dunaliella* sp. **(A)** Period 1 (Day 10) and **(B)** Period 2 (Day 13) (right chart).

3.3 Fatty acid profile and characterization

3.3.1 Fatty acid composition

CM50, CM55, and CM60 produced the highest lipid content and were used in the characterization of fatty acids (summarized in Table 1). Based on the results, the total number of fatty acids of CM50 decreases from P1 to P2. For CM55, the total SFA increased from 4 SFA in P1 to 7 SFA in P2, along with a decreased in UFA content from 7 UFA in P1 to 5 UFA in P2. For CM60, decreased in total SFA from 7 SFA in P1 to 4 SFA in P2 was observed and an increased in UFA from 16 UFA in P1 to 19 UFA in P2.

Table 1. Fatty Acid Methyl Ester (FAME) profile of *Dunaliella* sp. at P1 and P2 from top producing cultures.

FAME	Common Name	Lipid No.	Period 1			Period 2		
Saturated			CM50	CM55	CM60	CM50	CM55	CM60
Methyl tetradecanoate	Myristic acid	C14:0	+	+	+	+	+	-
Pentadecanoic acid, methyl ester	Methyl pentadecanoate	C15:0	+	+	+	+	+	-
Hexadecanoic acid, methyl ester	Palmitic acid	C16:0	+	+	+	+	+	+
Valeric acid, undec-2-enyl ester	Methyl heptadecanoate	C17:0	-	-	+	-	-	-
Methyl stearate	Stearic acid	C18:0	+	+	+	+	+	+
n-Nonadecanol-1	Nonadecanol	C19:0	-	-	-	-	+	-
cis-11-Eicosenoic acid, methyl ester	Eicosenoic acid	C20:0	+	-	+	-	+	-
n-tetracosanol-1	Lignocerol	C24:0	-	-	-	-	-	+
Tetrahydropyranyl ether of citronellol	Tetrahydropyran	C40:0	+	-	+	-	+	+
Total saturated:			6	4	7	4	7	4
Unsaturated								
Methyl 9-methyltetradecanoate	Methyl tetradecanoate	C14:1 (9)	-	-	-	-	-	+
2-Pentadecanone, 6,10,14-trimethyl-	Pentadecanone	C15:1 (2)	-	-	+	-	-	+
9-Hexadecenoic acid, methyl ester, (Z)-	Palmitoleic acid	C16:2 (9)	+	+	+	+	+	+
Methyl hexadec-9-enoate	Methyl palmitoleate	C16:1 (9)	+	+	+	+	-	+
9-Octadecenoic acid (Z)-, methyl ester	Valeric acid	C16:1 (cis-9)	+	+	+	+	-	-
(R)-(-)-Methyl-8-Hexadecyn-1-ol	Phytol	C16:1	-	-	-	-	+	-
E, E-10,12-Hexadecadien-1-ol acetate	Bombykol	C16:2	-	-	-	-	+	-
7,10- Hexadecadienoic acid, methyl ester	Hexadecadienoic acid	C16;2 (7,10)	-	-	-	-	-	+
4,7,10-Hexadecatrienoic acid, methyl ester	Hexadecatrienoic	C16:3	+	+	+	-	-	+
Methyl 4,7,10,13-hexadecatetraenoate	Hexadecatetraenoate	C16:4	-	-	+	+	-	+
cis-11-Eicosenoic acid, methyl ester	Heptadecanol	C17:1	+	-	+	-	-	-
6-Octadecenoic acid methyl ester, (Z)-	Petroselinic acid	C18:1 (6)		-	+	-	-	+
9-Octadecen-1-ol, (Z)-	Oleyl alcohol	C18:1 (9-ol)	-	-	-	-	+	-
9-Octadecenoic acid, methyl ester, (E)	Elaidic acid	C18:1(9)	-	-	-	+	+	-
9,12-octadecadienoic acid (Z, Z)-, methyl ester	Linoleic acid	C18:2 (9-12)	+	-	+	+	-	+
n-Heptadecanol-1	Heptadecanol	C18:2	-	+	-	-	-	-
8,11 -octadecadienoic methyl ester	Vaccenic acid	C18:1 (8-11)	-	+	-	-	-	-
8- Octadecenoic acid, methyl ester	Oleic	C18:1 (8)	-	+	-	-	-	+
5,8,11,14-Eicosapentaenoic acid, methyl ester	Gondoic	C20:1 (5,8,11,14)	+	-	-	-	-	+
(Z)-14-Tricosenyl formate	Tricosenyl	C23:1	-	-	-	-	-	+
5,8,11,14,17-Eicosapentaenoic acid, methyl ester	Omega-3	C25:5	+	-	-	-	-	+
2H-pyran, Tetrahydro-2-(12-pentadecynyloxy)	Narasin	C42:2	+	-	-	-	-	-
11,13-Dihydroxy-tetradec-5-enoic acid,methy	Tetredecenoic	N/A	+	-	-	-	-	-
Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)	Nonane	N/A	-	-	-	+	-	+
1H-2-Benzothiopyran, octahydro-, cis-	Benzothiopyran	N/A	-	-	-	-	-	+
		Total unsaturated	11	7	9	7	5	15
		Total fatty acids:	17	11	16	11	12	19
		Total fatty acid per period:	**22 Fatty acids**			**28 fatty acids**		

The number of fatty acids in each sample varied from each period and salt concentrations. Result showed that in total, there are 22 fatty acids identified in Period 1 while 28 fatty acids in Period 2. However, only 11 fatty acids are desirable for biodiesel production namely myristic acid, methyl pentadecanoate acid, palmitic acid, stearic acid, palmitoleic acid, methyl palmitoleate acid, valeric acid, elaidic acid, linoleic acid, oleic acid and petroselinic acid. These fatty acids were further clustered into 4 major groups according to its chain length specifically in number of carbon present namely myristic acid group (C14:0), Methyl pentadecanoate group (C15:0), hexadic group (C16: n), and octadic group (C18: n). The relative value to the % oil yield (RV) and group percentage value compared to overall area (GPV) was determined (Table 2). Myristic acid (C14:0), Methyl pentadecanoate, (C15:0) and Hexadic group (C16: n) were relatively high in terms of RV and GPV (Table 2).

Table 2. Summary of computed relative value (RV) and group percentage value (GPV) of top producing cultures from P1 and P2.

Major Groups	**Lipid no.**		P1			P2		
			CM50	CM55	CM60	CM50	CM55	CM60
Myristic acid	C14:0	RV	0.072	0.062	0.064	0.054	0.071	-
		GPV	5.06%	4.25%	4.3%	4.23%	4.73%	-
Methyl Pentadecanoate	C15:0	RV	0.014	0.026	0.009	0.014	0.022	0.009
		GPV	0.96%	1.77%	0.58%	1.1%	1.46%	0.59%
Hexadic Group	C16:n	RV	1.03	1.274	0.967	0.913	0.687	1.079
		GPV	72.55%	86.8%	64.76%	71.08%	74.85%	65.94%
Octadic Group	C18:n	RV	0.306	0.017	0.453	0.301	0.285	0.548
		GPV	21.42%	7.18%	30.35%	23.6%	18.97%	33.48%

Likewise, Octadic group (C18: n) is relatively high at CM60 (0.548 and 33.48%) in P2. The result implied that at higher salinity the accumulation of octadic (C18: n) in the cell increases (Zhila et al., 2011). High salinity changes the lipid biosynthetic pathway of the cell and stored in the form of FA and resulted to changes in the fatty acid profile of the cell. Findings in *Botryococcus braunii* and *Nannochloropsis oculata* showed that during high saline stress condition, they undergo elongation of palmitic acid (C16:0) into oleic acid (C18:1), which increases the number of C18 fatty acids in the plasma membrane and promotes desaturation to lessen the permeability of NaCl, preventing the destruction of cells (Kuiper, 1985; Peeler et al., 1989; Fuji et al., 2001; Azachi et al., 2003; Gu et al., 2012). These findings coincided with *Dunaliella* sp., which CM60 got the highest GPV for C18 fatty acids in P1 (30.35%) and in P2 (33.48%). Alongside, CM60 also got the lowest GPV for C16 fatty acids in P1 (64.76%) and P2 (65.94%), respectively.

3.3.2 FAME characteristics

FAME quality of biodiesel is characterized by its chain length and degree of saturation since the composition and amount of FA varied. Hence, it is important to determine the mixture that will promote high quality fuel. It was proposed that the ideal mix ratio of fatty acids at palmitoleic acid (16:1): oleic acid (18:1): myristic acid (14:0) is 5:4:1 (Shenk et al., 2008). This FA ratio gave low oxidative potential, good cold plugging point rating and good cetane number in biodiesel properties. Table 3 presents the computed ratio of FA based on GPV and a close to ideal mix ratio was obtained from CM55 (5:3:1) in P2 which has the same ratio obtained by Fakhry and El Maghraby (2013) from *D. salina*.

Table 3. Summary of computed ratio of fatty acids based on GPV from different salinity in P1 and P2.

Salinity (ppt)		Fatty Acids		
		C16:1	**C18:1**	**C14:0**
P1	**CM50**	15.99	15.12	5.06
	ratio	**3**	**3**	**1**
	CM55	11.43	6.04	4.25
	ratio	**2**	**1**	**1**
	CM60	15.15	14.73	4.30
	ratio	**3**	**3**	**1**
P2	**CM50**	17.96	21.49	4.23
	ratio	**4**	**5**	**1**
	CM55	22.50	12.44	4.73
	ratio	**5**	**3**	**1**
	CM60	11.07	14.06	n.a.
	ratio	-	-	-

In P2, CM55 obtained a moderately high GPV of SFA (4.73%) specifically with myristic acid (C14:0) and UFA (22.50%) specifically with palmitoleate acid (C16:1), which described to have good cold plugging point rating and good cetane number. Cold plugging point rating of biodiesel determined by the amount of SFA that has higher melting point than UFA wherein SFA crystallizes at higher temperature compared to UFA. Thus, high SFA promotes higher cold flow and pour point that lessen rapid formation of crystals clogging fuel lines and filters that might cause major operation problems. On the other hand, cetane number is the prime indicator of fuel quality in state-run of diesel engines which is influenced by increasing amount of SFA. Moreover, a low GPV of oleic acid (C18:1) (12.44%) described to have low oxidation. Oxidation stability of biodiesel is determined by the number and position of double bonds in fatty acids. Elevated levels of UFA might result to higher oxidation that will cause fuel to deteriorate. Also, it might pose a critical problem in the storing capacity, but small amount has no reasonable effect on the fuel performance (Lakshminarayanan & Aghav, 2009; Knothe, 2005).

4. CONCLUSIONS

Dunaliella sp. is a green, unicellular, eukaryotic, photosynthetic, and halophilic microalga. It has a potential as feedstock in the production of biodiesel but due to limited published data and information available, development of its commercial production is still dormant. This necessitates the evaluation of the culture condition of *Dunaliella* sp. that promotes higher lipid production with good fuel quality. It is also a highly important undertaking in the development of clean and feasible energy source and technologies that are not harmful to the environment. Thus, the current study determined the optimal

salinity of the culture medium for *Dunaliella* sp. under laboratory condition that produces high yield of lipid with good fuel quality based on its fatty acid composition.

Based on the results, we concluded that *Dunaliella* sp. can grow at different salinities as high as 70 g/L. However, high amount of wet biomass yield was obtained from culture medium with salinity condition of 50 g/L in Period 1 and 45 g/L in Period 2, hence can be considered as the optimum salinity concentrations per period specific for biomass production. On the other hand, total chlorophyll content decreases as salinity concentration increases wherein chlorophyll *b* is more prominent. Chlorophyll plays a major role in damage repair processes under stressful conditions. So, increase in the amount of *chl b* is the probable adaptations of *Dunaliella* sp. to higher saline condition, which can be used in future studies to evaluate the physiological effect of salinity in the growth of *Dunaliella* sp. And then, oil production and fatty acid synthesis of *Dunaliella* sp. varied upon exposure to different saline conditions. The oil production of *Dunaliella* sp. increases as the salinity concentration increases and the optimal salinity concentration specifically for lipid production is 60 g/L. Lastly, 55 g/L salinity (CM55) exhibited good fuel quality using the ideal mix ratio of palmitoleic (16:1), oleic acid (18:1), and myristic acid (14:0) at 5:3:1 ratio. Therefore, *Dunaliella* sp. has a potential as feedstock for biodiesel production with decent amount biomass and lipid/oil using Conway medium that can also exhibit a good fuel property when cultivated at 55g/L of salinity. Research on *Dunaliella* sp. can be enriched by considering other factors that affect the lipid/oil production such as nutrient deprivation, light intensity, and photoperiod. Furthermore, development of new extraction method for higher recovery rate of lipid production is recommended.

5. ACKNOWLEDGEMENT

We would like to express our deepest appreciation to Mr. Gerard C. Khonghun of Salinas Foods, Inc. for funding the materials used for this study. To Antonietta D. Evangelista of BFAR Regional Office 1, Alaminos, Pangasinan, for providing the mother culture used in this study and for the techniques in isolation and cultivation of *Dunaliella* sp. Lastly, to Dr. Elvis Chua of National Chemistry Instrumentation Center, Ateneo de Manila University, for imparting his knowledge, time, and effort with the GC-MS analysis.

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