Plant Archives Volume 20 No. 2, 2020 pp. 4035-4042 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ESTIMATION OF LIPIDS CONTENT FOR SOME ALGAL SPECIES ISOLATED FROM DIFFERENT REGIONS IN AL-NAJAF CITY UNDER DIFFERENT CONDITIONS

Dalal Abdul Hussein Kadhim*, Hameedah Abd Noor Abbood, Ali Yasser Al-Essawi, Athraa Jabar Mardan and Hanan Fadhel Kadhim

Department of Biology, Faculty of Education for Girls, University of Kufa, Iraq.

Abstract

The current study is an attempt to high light the possibility of stimulating the amount of total lipids of three species of local algae, *Prymnesium parvum, Botryococcus braunii and Chlorella vulgaris* were isolated, for the purpose of studying its contents of lipids and their potential is stimulated to produce lipids. In the present study, algae samples were collected from various areas of Najaf city, including, Low sea of Najaf, Shat AL- Kufa and AL- Abbasiyah regions. Identified and isolated several species of algae used in extracting some fatty compounds studies of which: *Gloeocapsa sp., Prymnesium parvum, S. Chlorella, Botryococcus braunii, Cylindrocapsa, Spirulina sp., Chlorella sp., Oscillotoria sp., Strigeoclonium sp., Neochloris sp., Microcystis, Chlorella pyrenoidosa, Scenedesmus dimorphus, Prymnesium parvum* attended a pure culture of the *Prymnesium parvum, Botryococcus braunii, Chlorella vulgaris* and three cultures were selected for growth and culture, BG11, Chu13, Chu10 under standard conditions (2 ± 25°C and 3000 l Lux and 16:8 light : dark as light system). The experiments included the study of the effect of the quality of the medium on fats. The second factor of agriculture medium with a number of transactions remove the nitrogen from the medium, add NaCl at a concentration of 2g/L. The results of the present study showed that the highest total lipids was recorded when nitrogen was removed from the Chu13 medium for algae *Prymnesium parvum*. was 319.8mg/g dry weight and the lowest total fat in the *Chlorella vulgaris* in the medium of control Chu10 (88.1). Also recorded the highest percentage of total lipids recorded at the genus *Prymnesium parvum* in the medium BG11 after removal of nitrogen and reached 80.48%. The lowest percentage of total lipids the was recorded at *Chlorella vulgaris* genus in the medium of control Chu10 and reached 31.49%.

Key words: Estimation, Lipids, Algal species, Regions, *Prymnesium parvum, Botryococcus braunii and Chlorella vulgaris.*

Introduction

The world faces a major problem of high consumption of all types of energy, for the steady increase in the number of human society as well as civilizational development, which depends heavily on civilian life consumed by nature for energy of all kinds, which prompted scientists to think and search for alternative sources of energy at low cost and usable in several areas (Sivasubramanian, 2009). Biofuels are a promising alternative to fossil fuels and as a friend this fuel is produced from chemical reactions of vegetable oils or animal fats with alcohol in the presence of acid as an adjunct to the fatty acids called Fatty Acids Methyl Esters, which is called biofuel (Knothe, 2010). The process of producing biofuels has gone on for several generations, it was based on oils and animal residues as well agricultural crops of economic importance such as corn and the sun flower to be a source of production led this way to the consumption of large quantities of these crops which adversely affected the as a source of food in the countries that depend on it, so was thought of another source, representing some non-plants the economic alternative to fats and oils used for the same purpose, but researchers faced another problem is the length of time the growth of many of these plants and large areas required for cultivation, and here emerged attempts to use farms algae as a viable alternative to the production of biofuels, has been successful success in the scientific circles and because of the characteristic of properties, including their ability to install dual CO_2 and convert it into sugars, fats and proteins through processes the phylogeny that the

***Author for correspondence :** E-mail : dalala.alesawi@uokufa.edu.iq

algal cell can do, and its breeding and culture requires limited space (Nigam and Singh, 2010). In recent decades, environmental studies have tended to look for ways and techniques to develop biomass production for algae, especially micro-algae, it is believed that algal cells do not have high levels of nutrient growth and absorption compared with other plant organisms, they are the most efficient living organisms to produce biomass, as is for their cells because of the excellent structure and the efficiency of the contents made the a good resistance to environmental variables to the middle of their presence as well as their ability to compete in access to sources of food and energy with other living organisms so it was able from adaptation and diffusion in various environments and even from extreme ones), (Jayanta *et al.,* 2012; Patil *et al.,* 2007; Wolk, 1973; Shao *et al.,* 2015). Because algae have ability to produce proteins, carbohydrates, lipids and fatty acids, there have been many attempts to use algae mixtures in food diets for fish, poultry and others the order to determine the importance of the type and concentration of acid and its physiological effects and benefits to the organisms fed (Lopez *et al.,* 2015). That the fat produced from the cells of these algae can be used in the production of bio- energy, especially biofuels, which will reduce pollution levels (Croft *et al*., 2005). Research has also shown that zooplankton fed on planters is good content from fatty acids have been characterized by a large biomass (Grant *et al.,* 2014). For the purpose of achieving the economic and productive objectives of the use of these algae, environmental studies have been conducted in recent years have led to the application of so called algal cultures with their different types and experimentation with different types and the application of programs to support these cultures with different growth drivers have given these applications important results in increase biomass and stimulate levels of total (fat, proteins, carbohydrates, etc.) (Shao *et al*., 2015) to their roles in making changes a task at the level of growth, physiology and biochemistry of various algae species. The aims of the study to estimation of lipids content for some species of algae, (*Prymnesium parvum, Botryococcus braunii, Chlorella vulgaris*) locally isolated from different regions in Al-Najaf City under different conditions.

Materials and Methods

Collection of samples

Samples were collected from low sea of AL- Najaf, Shat AL- Kufa and AL- Abbasiyah regions in 2016, The samples collected using the netting system with 20 micrometer in diameter. The samples were kept in sealed plastic bottles and sealed with a fixed date and area combine each sample.

Preparation of cultures medium

The synthetic breeding circles were Chu-10, Chu-13 and BG-11 in the form of solutions stoke solution sterilized by the autoclave at 121˚C for 15 min under pressure 1.5. except for phosphate-containing solutions, K_2HPO_4, K_2PO_4, which has been sterilized at (0.45) microns in order to avoid deposition of phosphate salts on the walls of the preparation bottles during sterilization by autoclave (AL-Arajy ,1996). All solutions were left treasury to cool at room temperature and then kept in the refrigerator until use. Solid mediums were prepared by adding a gar at concentration 1.5-2% to sterile liquid mediums and dissolving them with indirect heating at a temperature of 45-50˚C (inside a water bath) and sterilized again.

Examination and preservation of samples

The samples were tested using a photovoltaic microscope mounted on an 40x and 100x magnification force for the purpose of determination algae species which collected during the research. And for the purpose of classification and identification of the test species in the study using a camera for the composite microscope to compare with the existing forms in the approved classification books, the samples with small concentrations were concentrated by centrifuge at 2000 cycles for 5 minutes, some samples were examined spatially and kept in irradiated media, as was done add drops of the Lugal solution Iodine: Potassium iodide by (10:20)g dissolved in ice acetic acid and the size complete to 200ml for the purpose of classification of algae, the sources of classification were used (Prescot, 1982; Bellinger and Sigee, 2010).

Isolation and purification of algae

Algae were isolated using the dilution method (Hoff and Snell, 1999) and method streaking method (Belcher and Swale, 1982) by using semi-solid media by adding 1.5% to 2% to liquid media. The plates and tubes were tested after incubation at a temperature of 2 ± 25˚C and the lighting system 18˚C (Lighting of darkness) and intensity of lighting - 3000 lux for 7 days with the daily shake of the tubes until one type of algae is obtained for all samples. For the purpose of isolates Purification method was followed (Patterson, 1983) was used to purify the isolates access to (pure) axenic culture for test algae through the use of isolated obtained by method of dilution and to reach an appropriate size to the purification process 250-500 ml by eliminating the fungi and bacteria that may be associated with the algal species take 50 ml of isolated genus and put in the dark for 24 hours, pulling 10 ml of

the medium after incubation in the dark mediated sterile pipette and add to it the same liquid medium in which isolation was developed with add drops of nutrient broth and incubate again in the dark (2–3) hour and then the use of the centrifuge device at the speed of 3000 cycles/ minutes for two minutes, after which was retained by depositing the leachate and washing the sediment by of the sterile medium and repeated the washing process from 12-15 times after which part of each sample of the residue was planted on the medium of the nutrient agar of the bacterial growth test by dissolving 15g of nutritious per liter of distilled water.

Effect of the quality of the culture medium

The three communities BG11, Chu 13, Chu 10 and their efficacy in the development of algae were present for 49 days (seven weeks) isolated three algae were selected for the purpose of examining the effect of different activation on them *(Prymnesium parvum, Botryococcus braunii, Chlorella vulgaris)*.

Effect of nitrogen stress

He nitrogen stress test was performed by preparing free media by removing its source (the stored solution for nitrogen) in the three medium as development was carried out in laboratory conditions 2 ± 25 and lighting system 18 - 6 hours dark and intense lighting 3000 lux for seven weeks with three replicates to test the first test size 150 ml for each culture and four replicates of 750 ml per culture for the purpose of production for each treatment in the test culture medium.

Effect of NaCl Addition

Prepare a storage solution of NaCl and thaw 30g/L in an appropriate amount of distilled water and complete the volume to 1 liter and infestation by means of the sterilizer and kept at 4˚C until use repeat to reach a concentration of 2 g/L) Add 10 mL of sodium chloride solution to the farm with a final size of 150 ml and 30 ml for a farm with a final size of 750 ml) and cultivar plant grown under the same conditions (2 + 25˚C and 18˚C - 6 hours dark lighting and - 3000 lux as light intensity) by 4 replicates per treatment for the three populations of tested species.

Design of Photobioreactor

The tubular photobioreactor tubular development system (W.I.T.H.1) was designed using locally available materials (steel structure, pipes) plastic Capacities 3, Plastic connections of various PVC type sizes (3,2,1,0.5) and as needed, water pumps of 1000 and 2000 l / h, cooling system and heating system with digital control A/C control system type QD-UO8C A multi-parameter water quality monitor of pHt-026 was also used to determine pH, temperature and electrical conductivity And quantity salts. As well as the use of a speed measuring device to determine the speed required for testing the first two basins (100 × 33 × 50 cm) and the second (50 × 50 × 36) cm for the conservation of the algal culture.

Harvest of algae

For the purpose of harvesting the algae under study, Centrifugation was used at 3000 cycles/minute for 10 minutes. The leachate was removed and the precipitate was taken. It contains the micro algae. The samples were dried in a 40˚C heat oven after which it was weighed and stored for use according to each transaction (Jawad, 1982; Eppley *et al*., 1997).

Determination of total lipids

Total lipid was estimated based on the Bligh (Yadavalli *et al.,* 2012) by weighing 1g of dry algae, adding methanol alcohol: Chloroform (1:2) and stored for 18 hours at 25˚C inside closed test tubes, after incubation the mixture was stirred for 2 minutes mediated by the Vortex, and then added 1 ml of chloroform and reagent sample again for 1 min after shaking for the second time add 1 ml of distilled water and then again for 1-2 minutes later the samples are placed in the centrifuge for 10 minutes at a speed of 2000 cycles /accurate. The lower fat-containing layer was then collected by a pipette that was transferred to pre weighed test tubes by means of the sensitive balance (W1). The sample was dried in an oven at 50˚C and for 40 minutes. The sample was weighed after drying (W2).

The total lipid product was calculated based on the equation :

$P = (C \times DCW) \div t$

P = total fat (g / L-1 / day)

C = fatty content of algal cells (g / g)

DCW = dry weight of g / l cells

T = harvest time (days).

Test the optical reactor system

Genus *Chlorella vulgaris* was selected depending on the data obtained for the purpose of production of the most algae. 120 l of the algae culture in standard conditions (2 ± 25˚C and 18-6 hours illumination dark and intense lighting 3000 lux and 400 l / h speed with medium(Chu-13 center promise) manual shaking (for one day per day) as the first control medium and the treatment at 400 l / h as control medium second. The harvest was done 25 days after treatment.

Statistical analysis

The results were analyzed and differences were

found using the one-way analysis of variance test and the T-test as appropriate for experiment design using the statistical analysis (SPSS V.15.0) statistical package for the social sciences and tested the lowest significant difference was (L.S.D) below the probability level $P \leq 0.05$.

Results and Discussion

The studied algae species

Three species of local algae, *Prymnesium parvum, Botryococcus braunii, Chlorella vulgaris* were isolated, for the purpose of studying its contents of fats and their potential is stimulated to produce lipids.

Effect of total chemical content of tested algae

The results of algae development under laboratory conditions showed different responses when changing the type of genus well as various treatments of nitrogen removal or addition of sodium chloride or vitamins as well about changing the speed of current that was as follows: -

Effect of changing the quality of the medium

The results in the table 1 were followed to change in the mean of the three species of total lipids production note that the algae *Botryococcus braunii* (117.18, 185.36, 168.4) mg/g dry weight, has been recorded respectively.

Table 1: Concentrations of the chemical components of the culture medium * Chu-10.

Sequence	Materials	Concentration of solution storage g/L	Added volume to prepare the middle liter ml	Final concentration Mg/L
1	K2HPO4	4.000	2.5	10
2	NaNO3	8.000	2.5	20
3	MgSo4.7H2O	10	2.5	25
4	CaCl2	16.000	2.5	40
5	Na2CO3	8.000	2.5	20
6	FeCl3	0.320	2.5	0.8
7	EDTA-NA	4.000	2.5	10
8	NaCl	30.000	2.5	75
9	NaSiO3	5.700	2.5	14.25
10	a.HBO3	0.288	2.5	0.72
	b.MnCl2.4H2O	0.020	2.5	0.05
	c.ZnSO4.7H2O	0.224	2.5	0.56
	d.CoCl2.6H2O	0.004	2.5	0.01
	E.CuSO4.5H2o	0.080	2.5	0.20
	F.(NH4)6Mo7O24.4H2O	0.028	2.5	0.07

* Chu10 = modified by (Kassim *et al*., 1999).

As for the *Prymnesium parvum* genus has been a recorded (119.0, 177.38, 185.38) mg/g dry weight. That the *Chlorella vulgaris* genus had recorded (175.6, 197.2, 87.19) mg/g dry weight in the medium BG11, Chu13, Chu10, respectively. It is up to the results above to compare the three tested algae note that the highest amount of total fat is recorded in *Chlorella vulgaris* when developed in the medium Chu13 amounted to 198.2 mg/g (dry weight) while the least amount of total fat it was recorded for *Chlorella vulgaris* genus and reached 97.1 mg/g at the medium of Chu10 and had differences in the algae *Botryococcus braunii* and *Chlorella vulgaris* in the center Chu10 while the differences were significant in the three algae tested at Chu13 while the differences were significant between the three algae in the BG11 medium. The results of the percentage of total fat showed that genus *Botryococcus braunii sp.,* has recorded (59.7, 29.7 and 48.4)% the record (65.7, 38.4 and 44.6)% as well as the *Chlorella vulgaris* genus had a record of (52.5, 36.4 and 31.49)% In the medium BG11, Chu13, Chu10, respectively. The differences were significant among the ratios percent of total fat production in Chu10 and Chu13 in the three algae tested, as well all medium recorded significant differences when comparing the areas within the single genus. The highest percentage Percent of total lipids when the mean change was recorded at the development of *Prymnesium parvum* genus in the middle BG11 was 65.79% and the lowest percentage of total fat was recorded in Chu10 during

Table 2: Concentrations of the chemical components of the medium Chu-13 .*

Sequence	Materials	Concentration of solution treasures g/L	Concentration of solution treasures g/L	Concentration of solution treasures g/L
1	Kkno3	40	10	400
2	K2PO4	8	10	80
3	CaCl2	10.7	10	107
4	MgSO4.7H20	20	10	200
5	Citric acid	10	10	100
6	Ferric citrate	2	10	20
7	Micro element		1	
8	a. H3BO3	5.720		5.72
	b. CoCl2	0.02		0.02
	c.ZnSO4.7H2O	0.44		0.44
	d. CuSO4.5H2O	0.16		0.16
	e. Na2MoO4	0.084		0.084
	f. MnCl2	0.362		3.62

(Yamaguchi *et al*., 1987)

development *Chlorella vulgaris* genus amounted to 31.49% table. Significant changes were recorded in the total amount of lipids recorded or the percentage when changing medium table 4 the highest total fat content was (197.2 mg/g dry weight) at the medium of Chu13 for algae *Chlorella vulgaris* either the least amount was recorded at the same genus but when the development in the medium of Chu10 amounted to (87.19 mg/g dry weight), but when you follow the percentage we find different results with the highest percentage of fat of the kidneys at *Prymnesium parvum* at the culture medium of BG11 was 65.79% and the lowest percentage was recorded at *Chlorella vulgaris* genus at the medium of Chu13 and 36.4% reported that the difference in total lipids was dependent on the type of genus and the type of medium in which it is grown and this is consistent (Kirrolia *et al*., 2012) when studying for *Chlorella vulgaris*.

Effect of removal of nitrogen from the culture medium.

The results presented are shown in a table 5 remove that of nitrogen from the three tested culture medium, the genus was recorded *Botryococcus braunii* (144.7, 215.6 and 165.6) mg/g (dry weight) and genus record *Prymnesium parvum* (207.3, 319.8, 225.7 and 207.3) mg/g dry weight as well as genus *C. Humicola* has recorded

Table 3: Concentration of the chemical components of BG11 medium.

Sequence	Materials	Concentration of solution treasures g/L	Added size to prepare the liter of thecult-ure medium/ml	Final concentration Mg/L
1	NaNO3	150	10	1500
2	K2HPO4.3H2O	4.0	10	40
3	MgSO4.7H2O	7.5	10	75
4	CaCl2.2H2O	3.6	10	36
5	Citric acid	0.6	10	6
6	Ferric citrate	0.6	10	6
7	EDTA-Na	0.1	10	1
8	Na2 CO3	2.0	10	2
9	Micronutrient Solution	Mg/1	1	0.061
	a. H3BO3	61.0		0.169
	b. MnSO4.H2O	169.0		0.287
	c. Zn SO47H2O	287.0		0.0025
	d. Cu SO4.5H2O	2.5		0.0125
	e. (NH4) 6Mo7O24.4H2	12.5		

BG11 = (Rippka & Herdman, 1992)

(235.9, 219.7 and 190.5) mg/g (175.6, 97.2, 88.1) mg/g dry weight in the medium BG11, Chu13, Chu10 respectively and from observation results we find that the highest amount of fat the appeared at *Prymnesium parvum* after its development in the free Chu13 medium nitrogen was (319.83 mg/g) dry weight and the lowest total fat was recorded in genus *Botryococcus braunii* at BG11 was nitrogen-free and reached (144.7 mg/g) significant differences between the medium after removal of nitrogen for all three medium and algae tested at comparing them with the nitrogen containing classes and when comparing the tested algae with the free of charge medium nitrogen itself showed that the three tested algae showed significant differences in the BG11, Chu13 nitrogen-free mediums, while *Prymnesium parvum* recorded significant differences from the algae others in the medium are nitrogen free Chu13.

The results are shown in a table 6 she showed the percentage of total lipids when removing nitrogen from the three medium we find that the mole *Botryococcus braunii* recorded (53.4, 50.47 and 60.0)% as either *Prymnesium parvum* record (80.4, 72.4 and 80.3)% while the genus *Chlorella vulgaris* recorded (67.1, 40.0 and 63.2)% at its development in the medium BG11, Chu13, Chu10 respectively. These results indicate that the highest percentage was recorded at *Prymnesium parvum* genus and the nitrogen-free Chu10 medium was reached 80.30%, while the lowest percentage was recorded at the genus *Botryococcus braunii* when developing in the medium chu13nitrogen-free was 50.4% and the differences were significant between percentages of total lipids in all tested nitrogen-free culture medium when compared with non-nitrogen-containing control medium BG11 N free in the genus *Chlorella vulgaris*. Removal of nitrogen from the culture medium the results of the study showed a significant increase in the removal of nitrogen from the medium by its quantity and percentage of the total lipids of algae tested. *Prymnesium parvum* in the medium was nitrogen-free Chu13 while the least amount was genus *Botryococcus braunii* and reached 144.7% of the percentage recorded when the *Prymnesium parvum* genus reached 81.1%, while the lowest percentage was *Chlorella vulgaris*, which reached (40%). The increase in total fat from the removal of nitrogen from the plant species to the fact that the nitrogen of the elements essential for the building of proteins and amino acids, so the process of masturbation tends to build lipids for carbohydrates instead of building proteins and that the removal of nitrogen led to the formation of fats and acids reported that the highest total differences production was obtained when the nitrogen

Table 4: Chemical content: mean and percentage of total lipids when changing the culture medium.

Treatment	mg/gm Control Standarderror ± average			percentage %Standard error ± average		
T. L	Chu 10	Chu 13	BG11	Chu 10	Chu 13	BG11
Botryococcus braunii	117.1 ± 0.93A	185.3 ± 1.58 A	168.4 ±1.84a	48.41±0.39 A	29.70±0.26 A	59.7 ±0.63 a
Prymnesium parvum	119 ± 1.06 a	177.3 ± 1.02 A	185.3 ±5.73 a	44.69±0.41 A	38.46±0.22 A	65.79 ±2.05 a
Chlorella vulgaris	87.19 ± 0.75 A	197.2 ± 1.83 A	175.6 ±25.0a	31.49±0.27 A	36.4 ±0.35 A	52.5 ±7.58 a
LSD	1.55	3.20	39.68	0.98	0.77	11.51

A= represents the significant differences P ≤ 0.05 between the tested genus
a= represents the significant differences P ≤ 0.05 when changing the plant medium
TL = total fat

Table 5: Chemical content: mean of lipids (mg/g) dry weight when removing nitrogen from cultures medium of the tested algae.

Treatment	Control Standard error ± average			Zero N standard error ± average		
T .L	Chu 10	Chu 13	BG11	Chu10	Chu 13	BG11
Botryococcusbraunii .	119.1±0.9 A	185.3±1.5A	168.4±1.84 a	165.6±2.Aa	215.6±7.4a	144.7±4.75Aa
Prymnesium parvum	119 ± 1.06a	177.3±1.0A	185.3±5.73 a	225.7±2.3a	319.83±2.a	207.3±5.04Aa
Chlorella vulgaris	88.1 ±0.75A	97.2 ±1.83A	175.6±25.03a	190.5±5.4a	219.7±4.7a	235.9 ±4.56Aa
L .S .D	1.55	3.20	39.68	9.20	13.42	12.17

A= represents the significant differences P ≤ 0.05 between the tested genus
a = represents the significant differences P ≤ 0.05 when removing the nitrogen from the cultures medium of the tested algae.

Table 6: Percentage mean of total lipids when removing nitrogen from the three genus of tested algae .

Treatment ratio	Control Standard Error ± Medium			Zero N standard error ± average		
T. L.	Chu 10	Chu 13	BG11	Chu 10	Chu 13	BG11
Botryococcus braunii	48.41±0.3A	29.70±0.2A	59.7 ±0.63 a	60.0 ±0.92a	50.47±1.76Aa	53.4±1.76Aa
Prymnesium parvum	47.69±0.4A	38.46±0.2A	65.7 ±2.05 a	80.3±0.85a	72.4 ± 0.48Aa	80.4 ±2.03Aa
Chlorella vulgaris	31.49±0.2A	36.4 ±0.35A	52.5 ±7.58 a	63.2 ±1.81a	40.0 ± 0.88Aa	67.1 ±1.31A
LSD	0.98	0.77	11.51	2.51	2.232	3.76

A= represents the significant differences P ≤ 0.05 between the tested genus
A = represents the significant differences P ≤ 0.05 when removing the nitrogen from the cultures medium of the tested algae.

Table 7: chemical content mean of total lipids mg / g (dry weight) for tested algae when sodium chloride NaCl when added to the culture medium.

Treatment	Control Standard error ± average			NaCl 2 g / L standard error ± average		
TL	Chu 10	Chu 13	BG11	Chu10	Chu 13	BG11
Botryococcusbraunii	117.1±0.9A	185.3 ±1.58A	168.4±1.84a	131.7±3.54a	214.7±2.2a	141.3±1.57Aa
Prymnesium parvum	119 ± 1.06A	177.3 ±1.02A	185.3±5.73a	229.6 ±5.3Aa	208.4±5.5a	224.1±1.58Aa
Chlorella vulgaris	89.1 ±0.75A	198.2 ±1.83A	176.6±25.3a	105.3±3.14a	243.5±6.1a	200.3 ±1.0A
L.S.D	1.55	3.20	39.68	10.29	12.61	2.88

A= represents significant differences P ≤ 0.05 is the difference between tested genus
a =represents significant differences P ≤ 0.05 when sodium chloride is added.

Table 8: Percentage mean of total lipids of algae tested when Sodium chloride is added to the culture medium.

Treatment percentage	Control Standard error ± average			NaCl Shock Standard Error Average		
TL	Chu 10	Chu 13	BG11	Chu10	Chu 13	BG11
Botryococcus braunii	48.41±0.39A	29.70±0.26A	59.7±0.63	64.4±1.74a	49.6±0.53a	39.4±0.44Aa
Prymnesium parvum	44.69±0.41A	38.46±0.22A	65.7±2.05	60.6±1.42a	47.1±1.28a	67.2 ±0.47A
Chlorella vulgaris	31.49±0.27A	36.4 ±0.35A	52.5±7.58	36.5±1.11Aa	44.2±1.12Aa	57.82±0.29A
L.S.D	0.98	0.77	11.51	1.99	1.84	0.14

A= represents significant differences P ≤ 0.05 is the difference between tested genus
a =represents significant differences P ≤ 0.05 when sodium chloride is added

was removed from the plant medium may return which found an increase in the enzyme Carboxylase acutely CoA which represents the key to increasing the rate of fatty acid build-up, also represents a control factor in lipid structure public (Hu *et al*., 2008).

Treatment of culture medium by sodium chloride

Reference the results in a table 7 the effect of adding sodium chloride at a concentration of (2g/L) in the total lipids volume of algae was shown the laboratory notes that the algae *Botryococcus braunii* recorded (141.3, 214.7 and 131.7 mg/g) weight dry as recorded *Prymnesium parvum genus* (224.1, 208.4 and 229.6) mg/g (dry weight) while the genus *Chlorella vulgaris* recorder (200.3, 243.5 and 105.3) mg/g dry weight in BG11, Chu13, Chu10 respectively. The highest total lipids intake was recorded at development of *Prymnesium parvum* genus in the Chu10 medium after sodium chloride treatment amounted to (229.61 mg/g) dry weight. The lowest value was recorded at Chu 10 *Chlorella vulgaris* and reached (105.34 mg/g) dry weight when developing in the Chu10 medium and there were significant differences between the medium after add sodium chloride in the three algae tested in BG11, Chu10, while they were mean differences in the Chu13 medium after adding sodium chloride only in *Chlorella vulgaris* genus when comparing the medium of the single algae. When comparing the control and the medium after the addition all differences were significant.

The results presented are shown in a table 8 as for the percentage of total lipids we find that the genus *Botryococcus braunii* while the genus *Prymnesium parvum* recorded (67.2, 47.1 and 60.6)% as well as *Chlorella vulgaris* which recorded (57.8, 44.2 and 36.5)% in the BG11, Chu13 and Chu10 respectively. Therefore, the highest percentage total fat was recorded *Prymnesium parvum* at BG11 and the lowest percentage was lost the *Chlorella vulgaris* was recorded after its development in the Chu13 medium after the addition of sodium chloride and (36.5%). She was significant differences in the three algae tested in BG11 were still significant the addition of sodium chloride, while the significant differences were in Chu13, Chu10 genus comparison between the three algae when compared within the same one after the addition, while comparison among the medium and their treatment within the single genus, we find that the differences in the genus *Botryococcus braunii* in the three medium and the medium Chu13, Chu10 S. *Prymnesium parvum* and *Chlorella vulgaris*. The results of the addition of sodium chloride to the studied cultivars studied in the present study showed a significant effect for the three tested strains compared to NaCl control was the highest amount recorded at genus was *Chlorella vulgaris* in the medium Chu13 Nacl and amounted to 243.5 mg/g or the highest percentage *Prymnesium parvum* was recorded in the BG11 NaCl medium and the lowest total fat was recorded at *Chlorella vulgaris* in the medium Chu10 NaCl was 105 mg/g and 36% higher than control. The stimulation effect of the presence of sodium chloride in the culture medium is due to the exposed algae Salinity stress works to increase the total lipid composition of the effect of osmosis and prevent or neutralize the transmission water from inside the cell to the outside is a defensive state by the algal cells to counter salinity change in (Rao *et al*., 2007) and other studies have confirmed that exposed algal cells salinity stress. The construction of small particles acting as osmosis organizations such as glycerol and other compounds as a response to osmotic pressure change when salinity increases (Hu, 2004). This is a mechanical process this is what (Dean *et al*., 2012) noted when studying *cholorella vulgaris* sp., with total fat increase reaching 66.2%, (Takagi *et al*., 2006).

References

Al-Aarajy, M. (1996). Studies on the mass culture of some microalgae as food for fish larvae. Ph. D. thesis, Univ. Basrah, Pp: 107.

Bellinger, E.G. and D.O. Sigee (2010). Aky to more frequently occurring freshwater algae. Freshwater algae .john. Wiley and Soncitd.

Belcher, H. and E. Swale (1982). Culturing algae .the Burlington priss.cambridage.pp: 25.

Croft, M.T., A.D. Lawrence, E. Deery, M. Warren and M.J. Smith (2005). Algae acquire vitamin B12 through a symbiotic relationships with bacteria, *Nature,* **(438):** 90-93.

Dean, A., J. Nicholson and D. sigee (2012). Changing paterns of carbon allocation in lake phytoplankton: *an FTIR analysis hydrobiologia,* **(684):** 109-127.

Grant, M., E. Kazamia, P. Cicuta and A. Smith (2014). Direct exchange of Vitamin B12 is demonstrated by modeling the growth dynamic of algal-bacterial cocultures. *The ISME J.,* **1(10):** 1-10.

Hu, Q. (2004). Environmental Effects on cell composition, in Richmond A. ed. Handbook of microalgal culture , Bitechno. Appl. Phycol. Blackwell Sci., Victoria, 83-93.

Hoff, F.H. and T.W. Snell (1999). Plankton culture manual. 5thed., horida aqua. Tarms, Inc. Florida.

Jayanta, T., M. Mohan Chandra and G. Goswami Bhabesh Chandra (2012). Growth, Total Lipid content and Fatty Acid Profile of a Native Strain of the Freshwater Oleaginous Microalgae *Ankistrodesmus falcatus* (Ralf) grown under Salt Stress Condition. *Int. Res. J. Biological Sci.,* **1(8):** 27-

35.

Kassim, T.I., AL-saadih and N.A. Salman (1999). Production of some phytoplankton and zooplankton and their use as live food for fish larva. Iraqi. *J. Agric proc. of 2nd sa.confer.,* **4(5):** 188-201.

Kirrolia, A., N. Bishnoi and R. Singh (2012). Effect of shaking in cubation temperature, salinity and media composition on growth rates of green microalgae chlorococcum sp. *J. Algal Biomass Utln.,* **3(3):** 46-53.

Knothe, G. (2010). Biodiesel and renewable diesel A comparison. *Progress in Energy and Combustion Science,* **36(3):** 364-373.

Lopes, V.G., C.C. Garcia, G.A. Ferande, G.S. Basto, Y. chisti and M.F. Sevilla (2010). *Protein measurements of algal and cyanobacterial biomass Bioresource Technology,* **101:** 7587-7591.

Mahapatra, D.M. and T.V. Ramachandra (2013). Algal biofuel : bountiful lipid from *Chlorococcum* sp. proliferating in municipal wastewater. *Current sci.,* **105(1):** 47-55.

Ndruleviciute, V., V. Makareviciene, V. Skorupskaite and M. Gumbyte (2014). Biomass and oil content of *Chlorella* sp., *Haomatococcus* sp., *Nannochlopsis* sp. and *Scendesmus* sp. Under Mixotrophic Growth Conditions in the presence of technical Glycerol. *J. Appl. phyco.,* **(26):** 83-90.

Nigam, P.S. and A. Singh (2010). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science*. In press.

Patil, K., T. Lqvist, E. Olsen, G. Vogt and O. Gisler (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed, *J. Inter. Aquacult.,* **(15):** 1-9.

Patterson, G. (1983). Effect of heavy metals on fresh water chlorophyta. PhD. thesiss, Univ. Durham.

Prescot, G.W. (1982). Algae of the Western Great Lake Area William¼ C. Brown CO., Publishers, Dubuque, lowa. pp:977.

Provasoli, L. and F. Carlucci (1974). Vitamins and growth regulators. In: Stewart, W.D.P., ed. Algal physiology and biochemistry. Blackwell scientific, UK, pp. 741-787.

Rao, A.R., C. Daynanda, R. Sarada, T.R. Shamala and G.A. Ravishankar (2007). Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresour. Technol.,* **98(3):** 560-564. *Applications.* **2(3):** 2446-2453.

Shao-Yi, Y. and Hand Shiuh-Kuang (2015). Enhancing Algal Growth by Stimulation with LED Lighting and Ultrasound. *J. Nanomaterials,* **10:** ID 531352, 11-18.

Sivasubramanian, V. (2009). Current status of Research on algal bio-fuels in India. *J. Algal Biomass Utln.,* **1(1):** 1-4.

Takagi, M., Y. Karseno and T. Yoshida (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglycride in marine microalgae *Dunaliella* cells. *J. Biosci. Bioeng.,* **101(3):** 223-226.

Wolk, C.P. (1973). Physiology and Cytological Chemistry of Blue-green Algae. *Bacteril. Reviwes,* **37(1):** 32-101. USA.

Yadavalli, R., R.S. Rao and C.S. Rao (2012). Lipid accumulation studies in *Chlorella pyrenoidosa* using customized photo bioreactor-effect of nitrogen source, Light intensity and mode of operation. *(IJERA), International Journal of engineering research and applications*, **2(3):** 2446-2453.

Yamaguchi, K., H. Nakano, M. Murakami, S. Kansu, O. Nakayama, M. Kanda, A. Nakmura and H. Iwamoto (1987). Lipid composition of green algae *Botryoccoccus branrii, agric. biol. chem.,* **51(2):** 493-498.