

Enhancement of biodiesel production from local isolates of microalgae

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Abstract

Ten microalgae isolates were isolated from some ponds and Tigris River in Iraq, and screened for growth and lipid production. The results showed that the isolates *Chroococcus dispersus*, *Anabaena augstumalis* and *Chlorella vulgaris* exhibited higher growth rate and lipid production and reached (0.95, 1.9 and 1.17) g/l, respectively. The selected isolates stimulated by studying the effect of different levels of pH and salinity on growth and lipid production. The results showed higher lipid production by *C. vulgaris* reached maximum (3.45g/l) at lower pH 6, followed by *A. augstumalis* (2.75g/l at pH11) and *C. dispersus* (2.68 g/l at pH9). The results also showed that lipid production by *C. dispersus* and *A. augstumalis* was stimulated by decreasing salinity concentration and reached maximum (2.94 and 2.5 g/l), respectively at 0.4 g/l of NaCl. While, maximum lipid production by *C. vulgaris* was (1.72 g/l) obtained at salt concentration of 1g/l. The combined effect of pH and salinity on lipid production studied. Maximum lipid production by *C. dispersus* (8.43 g/l) observed at (pH9 + salt 0.4g/l). In case of *C. vulgaris*, total lipid production reached 4.43g/l which obtained at (pH6 + salt 0.75g/l). While, lipid production in microalgae of *A. augstumalis* was favored by alkaline condition, and maximum lipid production (4.42 g/l) observed at (pH11 + salt 0.75g/l). Higher oil content was observed in *C. vulgaris* and reached 33.2% (0.332 g of oil / g of dry algal biomass) when the microalgae cultivated at (pH9 + salt 0.4 g/l). In comparison, to lower oil content (%) observed with *A. augstumalis* and *C. dispersus* reached to 16 % and 13.8% when the isolates stimulated at (pH11 + salt 0.4 g/l) and (pH 6 + salt 0.75 g/l), respectively. Analysis of lipid content by GC technique had shown that the lipid content of microalgae *C. dispersus* contained only stearic acid. While, oil content in *C. vulgaris* and *A. augstumalis* contained only stearic acid, but palmitic acid and oleic acid were detected in control and stimulus conditions.

Keywords; Biodiesel production; Microalgae; pH effect; Salinity effect; Lipid Content.

Introduction

Most of industrial biodiesel is made from oil (triglycerides) of raw materials (rapeseed, sunflower, soybean, etc.). The raw materials are also necessary to feed humans and animals. A large demand for raw materials to produce biodiesel could thus increase their price. Moreover, the culture of conventional vegetable material requires an important amount of water, chemical fertilizers and pesticides, which have a negative impact on the environment [1].

To overcome these problems, researchers are currently exploring a way of producing biodiesel-using microalgae. During their growth, photoautotrophic microalgae consume inorganic carbon (CO₂) through the photosynthesis process [2].

Biodiesel has many environmental benefits over other fuels that help to reduce the human footprint on the natural world. Biodiesel used immediately to replace conventional diesel in the transportation market. The Biodiesel is a suitable alternative in that it can be produced from many different sources such as animal fats, cooking oil, plant oils, and algae [3].

Algae are photosynthetic organisms which produce large amounts of O₂. In which it contributes the oxygen concentration in an atmosphere which enhances life on our biosphere [4]. Microalgae characterized by fast growing and highly efficiency of photosynthesis [5] and a very effective feedstock for biodiesel production. Chisti, [6] mentioned that some algal species have been found to have very high oil contents, ranging up to and sometimes beyond 50% dry weight. Some species of microalgae can produce more than 50% of their dry mass as triacylglycerides or long chain hydrocarbons that can be converted to biodiesel and jet fuel. About 20-75% of lipids can be accumulated as part of their dry mass in algae. Niehaus *et al.*, [7] mentioned that most algae species produce triacylglycerides and alkenes from what are known as the fatty acid biosynthetic pathway.

One of the most important compounds, which are converted into biodiesel *via* trans esterification reactions are triacylglycerides (TAGs). TAGs are used in algal cells as a source of energy [8 and 9]. TAGs consist of glycerol and three fatty acids (FAs). Exchange of glycerol with small alcohols (Transesterification reaction) produced biodiesel. The quantity and quality of lipids and fatty acids differ depending on the culture conditions. The limitation of nutrient affects is in the lipid content of algae, and in eukaryotic algae. There is inversely related between both carbohydrate, and protein with lipid contents when the stationary phase began [10]. The decrease in nutrient concentrations during the growth phases enhanced to produce much lipid content.

The environmental stress stimulated the lipid production and accumulation in algae. Algae responses accompanied by changing in different growth parameters, and the morphological and developmental pattern.

A few studies were conducted for the effect of pH on biodiesel production from algae [11 and 12]. Gardner *et al.*, [13] found that *Scenedesmus* sp. produced higher lipid production at pH > 9.

The current work aimed to study the effect of different values of pH and salinity on lipid production of locally isolated algae from some aquatic systems in Iraq.

Materials and Methods

Algae were collected by a phytoplankton net (mesh pore 0.2 μ) from different ponds and artificial canal in northern Iraq, as well as from ponds and Tigris river within Baghdad city. Samples were transported to

sterile container (100 ml) which was marked with the date and location of sampling, and then transported to the laboratory immediately to be incubated under suitable and controlled conditions for algal growth at 268 $\mu\text{E}/\text{m}^2/\text{s}$, and 16:8 lights: dark and $25 \pm 2 \text{ C}^\circ$.

Isolation of algae:

Two techniques were used for algae isolation, serial dilution method and streaking on plate agar [14].

Serial dilution method

This method was used to get the isolation of pure algae. Ten test tubes were prepared each one contains 9 ml Chu-10 nutrient solution, 1ml of algal sample was added to the first tube and shook carefully, then 1ml from the first tube transported to the second tube and so on then incubated for two weeks. This process was repeated with examining of each dilution with a compound microscope until one species of algae were obtained. After the target dilution was microscopically examined several times and confirmed as unialgal culture then (2 ml) was transferred into (20 ml) of the Chu-10 enhancement solution, then incubated under suitable conditions for algal growth till the culture turned into greenish color [15]. Isolated algae were identified according to Prescott [16], Desikachary [17] and Bellinger & Sigeo, [18].

Streaking on plate Agar

Chu-10 media solution solidified by 1.5 % agar-agar and sterilized by autoclave, after the sterilization Chu-10 medium was poured in Petri-dishes, which were left to solidify; sterile loop was used for streaking straight line. Then the plates were kept in a cooled illuminated incubator with light intensity about 268 $\mu\text{E}/\text{m}^2/\text{s}$, at $25 \pm 2 \text{ C}^\circ$ and 16:8 lights: dark period of 10 -14 days. Aggregated colonies were observed on the surface of the plates. Part of these colonies was streaked on another plate. Each subculture was examined by using a compound microscope, this method was repeated until unialgal culture had been gained [19].

A small part of the confirmed unialgal culture was transferred which into Chu-10 medium solution within a 250 ml sterile flask and incubated for two weeks to get appropriate growth. In order to examine the viability of the unialgal growth, these cultures were renewed every two weeks by sub culturing into another Chu-10 nutrient solution [16].

Screening of isolated algae

Ten algae isolates were obtained, these isolates were tested for growth and total lipid contents and incubated in light intensity 268 $\mu\text{E}/\text{m}^2/\text{s}$, temperature $25 \pm 2 \text{ C}^\circ$ and 16:8 light: dark period.

All isolates were incubated in illuminator at $25 \pm 2 \text{ C}^\circ$ for 21 days. Microalgae growth and total lipid contents were determined daily. The growth rate (K) and doubling time (G) were calculated according to Huang *et al.*, [20].

Stimulation treatments

Three isolates showed highest growth and lipid production. These selected algae were undergone in different treatments of two factors (pH and salinity) individually and combined. Six different levels of pH (5, 6, 7, 8, 9, and 11), in each 250 ml flasks containing (90 ml of Chu₁₀) were added and the pH was adjusted, then 10 ml of the old culture of each isolated alga (*C. vulgaris*, *C. dispersus* and *A. augustumalis*) was added to each flask. The flasks were incubated at light intensity 268 $\mu\text{E}/\text{m}^2/\text{s}$, $25 \pm 2^\circ\text{C}$ and 16:8 lights: dark period. Microalgae growth and total lipid were determined daily.

Seven concentrations of NaCl were used (zero, 0.2, 0.4, 0.6, 0.8, 1 and 1.5) g/l to test the viability of these algae to the growth and lipid production under the circumstances of the stimulus. In each 250 ml, flasks containing 90 ml of Chu₁₀ from different concentrations of NaCl were added, and then 10 ml of the each old culture of alga (*C. vulgaris*, *C. dispersus* and *A. augustumalis*) was added to each flask. The flasks were incubated at light intensity 268 $\mu\text{E}/\text{m}^2/\text{s}$, $25 \pm 2^\circ\text{C}$ and 16:8 lights: dark period. Microalgae growth and total lipid were determined daily.

The biodiesel production of isolated algae was carried out at optimum condition of pH and salinity obtained from a previous study in 7.5 l glass pools. The dimensions of the glass pools were 50 cm length, 50 cm width and 30 cm high. For each algal isolates; four glass pools containing (5 L of Chu₁₀ and 500 ml alga) were conducted at optimum condition of pH and salinity. One of these ponds considered as a control (pH 7 and salinity 0.075g/l) for each isolate. The algal isolates were incubated at about $25 \pm 2^\circ\text{C}$ for 15 days, after the end of the incubation period the algal isolates were harvested for determination of dry weight (biomass), total lipid, and protein and carbohydrate contents.

Harvesting of Algae

Microalgae isolates harvested from glass pools at the beginning of the stationary phase, under stimulation after fifteen days of incubation for all algal isolates. All isolated algal cultures were treated with cooled centrifuge and dried the precipitated material (algae) as explained by Hassan et al. [21] and [22, 23]. The results of these processes were used for analysis the study parameter.

2.7. Determination of Total Lipids

Total lipids were determined by colorimetric method (with sulfo-phosphovanillic mixture). This was carried out commercially purchased kit [24].

2.8. Growth parameters (Protein and Carbohydrate)

Bradford [25] and Dubois et al. [26] methods were used to determine protein and carbohydrate, respectively.

Lipid Extraction

Lipid extraction has conducted a series of repeated digestion with 200 ml of methanol and hexane (1:1) for each one gram dried weight (DW) by using soxhlet. After the process taken 3-4 hours, the color solvents in the cylinder will change from green to colorless. Rotary evaporator at 40°C dried the extracted samples for a few minutes. The samples were poured out to clean plates and left at room temperature at 25°C overnight, then the samples transported to testing tubes to be analyzed for lipid content [27 and 28].

Lipid Analysis

Methyl esterified samples were diluted (40µl FAME sample + 960µl hexane) in the clean vial with micropipettes. The sample vials were put in auto-injector vial tray. The sample (1µl) was injected into the gas chromatograph (GC-Packard, 438A, U.S.A) by an auto injector and capillary column (SE/30, 3m, 1/8" diam, 0.25 µm film thickness). The elution's were detected on a flame ionization detector. Reagent user FID and the temperature of reagent 325°C. The oven temperature was 100 C°→300 C° and increased to 10 C° per min. The injector temperature was kept at 300C°. The flow rate of carrier gas (He) was 30 ml per min. The amplified signals were transferred and recorded in a computer with GC-solutions software. The quantitative method was followed with external standard mixtures of fatty acids (C6-C24, Sigma, USA) and was run earlier under similar conditions. The data of total lipids were statistically analyzed and expressed as mean ± standard deviation [29].

Statistical Analyses

Analysis of the study data was performed by using completely randomized design. A statistical analysis system software used to explain the interactions between the investigated parameters and lipid production [30]. For each treatment at least a significant differences at level $P \leq 0.05$ were used.

Results

Isolate algae were collected from some ponds and Tigris river in Iraq. The isolated algae were *C. Dispersals*, *C. minor*, *Oscillatoria amoena*, *Nostoc linka*, *Anabaena variabilis*, *A. augstumalis*, *C. vulgaris*, *Nitzschia palea*, *Microcyst aeruginosa* and *Westiellopsisprolifca*.

These isolated algae was screened for biomass and total lipid production. Among the results of screened isolates, five isolates (*C. dispersus*, *A. augstumalis*, *C. vulgaris*, *N. palea* and *O. amoena*) showed higher biomass and lipid production.

Different growth curves and growth rates (K) were observed from each algal isolates, and the harvesting time varied from one to another. The stationary phase of *C. dispersus* and *A. augstumalis*, started in 13 days while in *C. vulgaris* in 14 days and *N. palea* in 12 days and finally in *O. amoena* in 15 days (Figure 1).

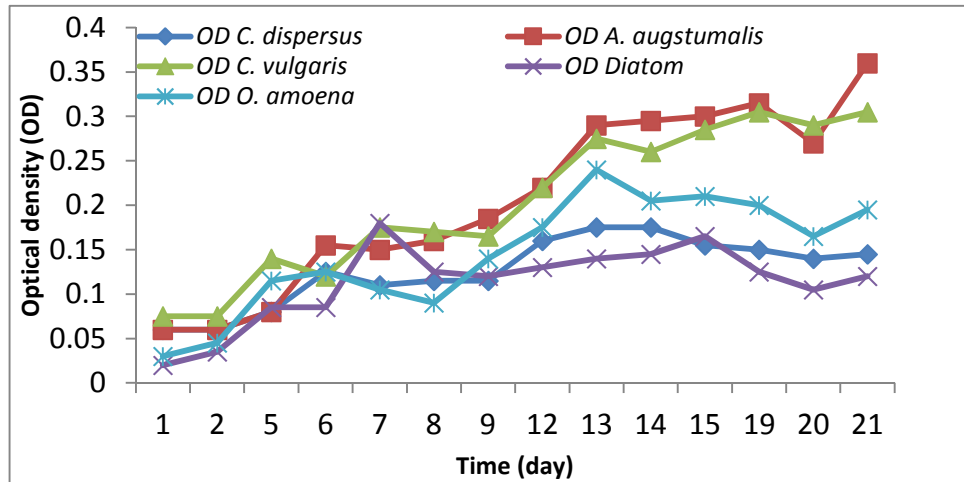


Fig. 1: Growth curve of five isolated algae at (25C°, 268 µE/m²/s) at pH 7 and for a period of 21 days of incubation.

Algae density was monitored by measuring the turbidity, and oil concentration. Figure 2 showed that the isolates *A. augstumalis*, *C. vulgaris*, *C. disperse* exhibited higher biomass and lipid production. The total lipid production observed after 15 days of incubation were 1.9, 1.17 and 0.95g/l for *A. augstumalis*, *C. vulgaris*, *C. disperses*, respectively. Therefore, these isolates were selected for the purpose of biodiesel production due to the high lipid production and shorter time required to reach stationary phase.

Biodiesel can be produced from microalgae because it contains considerable amount of lipid contents [31]. The results in the current study were agreeing with Mulumba and Farag, [32] they screened 7 algal isolates for growth and lipid production. The results were evaluated as the isolates variation in growth and total lipid production. In addition, they showed that the cell growth reached stationary phase after 12 days incubation.

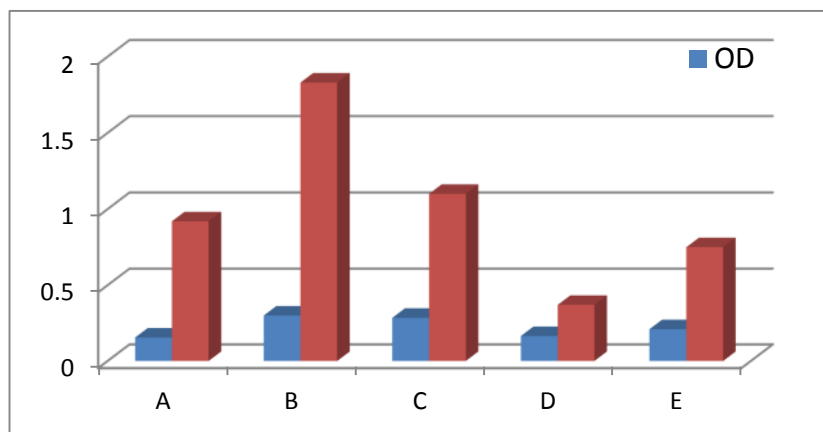


Fig. 2: Growth and lipid concentration of different algae isolate (A: *C. dispersus*, B: *A. augstumalis*, C: *C. vulgaris*, D: Diatom, E: *O. amoena*) at 25 °C, 268 µE/m²/s, after 15 days of incubation.

Effect of pH on biodiesel production:

Different response to pH experiments were illustrated in figure 3, which showed variation in lipid production. Higher lipid production by *C. dispersus* reached to 2.68 g/l when pH increased to 9, in comparison to lower lipid concentration (1.78 g/l) at pH 5 after 15 days of incubation. The lipid production by *C. vulgaris* reached maximum 3.45 g/l at treatment pH 6, while minimum lipid production was at pH 8, after 10 days of incubation (Figure 3). Higher lipid production (2.75 g/l) by *A. augstumalis* was recorded at treatment pH 11 also after 10 days of incubation.

The results showed that an increase in treatment pH 9 stimulated lipid production by *C. dispersus*, in comparison to lower lipid concentration observed in treatment pH 5. The results also showed that the increase in pH value did not stimulate the lipid production in *C. Vulgaris* and higher lipid production obtained at treatment pH 6, in comparison to decrease in lipid production when pH values increased above pH 6. While, in case of *A. augstumalis* the total lipid production was stimulated when pH value increased, and higher lipid concentration (2.75 g/l) observed at treatment pH11, while lower lipid concentration (1.68 g/l) was at pH6.

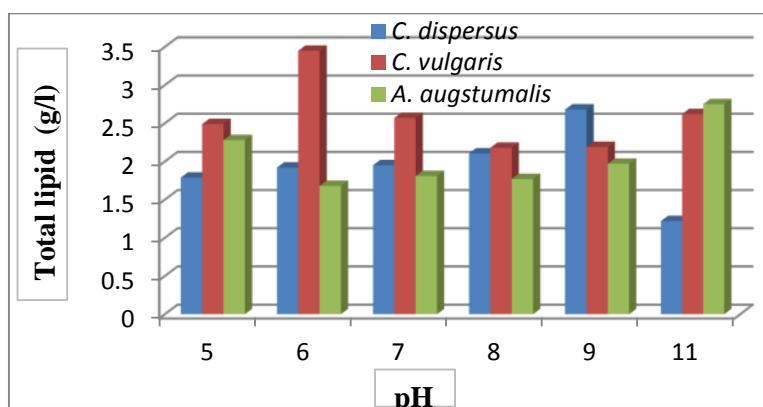


Fig. 3: Total lipid production of *C. vulgaris* and *A. Augstumalis* and *C. disperses* at different values of pH at $25 \pm 2 \text{ }^\circ\text{C}$ and $268 \text{ } \mu\text{E}/\text{m}^2/\text{s}$ after 10 and 15 days of incubation, respectively.

Somchai *et. al.*, [33] mentioned that at pH levels was affected on the growth of cyanophyceae (filamentous algae) and the pH level less than 6 caused broken of the filament algae, while they ranged ($< 7.5 - 9 <$) and caused an impact on growth. These results indicated that pH levels at lower and higher than pH5 could be inhibited photosynthesis and effect on the morphology of alga. William, [34] observed better growth of *C. vulgaris* at pH 6.5 and 7.0, while lipid production increased at pH 7. The stress of higher pH for *Chlorell* caused an accumulation of triglyceride [35]. This stress increased the time of completion of cell cycle due to inhibit autospore.

Effect of Salinity on Total Lipid Production:

Salinity was playing an important role in the production of various biofuels. Salinity was considered as one of the most significant ecological factors affecting the growth and metabolic activities of plants and microorganisms [36]. The results in Figure 4, illustrated the effect of different concentrations of salt on

growth and total lipid production. The results showed that lipid production by *C. dispersus* and *A. augstumalis* was stimulated at lower salinity concentration and reached the maximum 2.94 and 2.5 g/l, respectively at treatment salinity 0.4 g/l after 15 days of incubation, above this concentration total lipid production decreased sharply. The results in figure 4 also, showed that the isolate *C. Vulgaris* was able to tolerate the salt concentration up to 1.5 g/l. Maximum lipid production (1.72 g/l) was observed at treatment salinity 1 g/l after 15 days, above or lower this concentration the total lipid production was decreased.

Microalgae differ in their adaptability to salinity and other stress conditions. Many authors [37, 38 and 39] studied the osmotic stress and their impact on the growth of algae. Mus *et al.*, [40] showed that the *Chlorella* sp. biomass was affected by salinity and the biomass growth decreased with salinity increases. Many studies have revealed that the highest number of cells was observed on day 15th with 30 g/l salinity [41, 42 and 39].

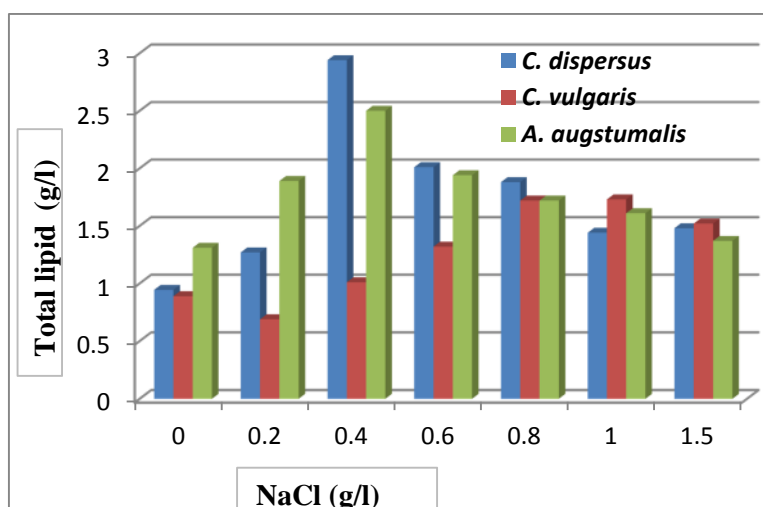


Fig. 4: Total lipid production of *C. dispersus*, *C. vulgaris* and *A. augstumalis* at different concentrations of salt at 25 ± 2 C° and $268 \mu\text{E}/\text{m}^2/\text{s}$ after 15 days.

The results of Sanchez *et al.*, [43] suggested that low to medium salinity concentrations, between 0 and 2 g/l NaCl, were appropriate for the promotion of growth rate. The alga *Scenedesmus* has been reported as salt tolerance [44]. *S. Almeriensis* showed tolerance to medium salt concentrations, and recorded higher biomass productivities at treatment NaCl (4 g/l) in comparison with that grown in fresh water media [43]. The same results were observed for *S. obliquus*, *S. armatus*, and *S. bernadii* in a study of Kaewkannetra *et al.*, [44]. While in another study increasing of the salinity, from 0 to 0.2 g/l, in the nutrient medium led to an increase of (1.6) fold biomass yield of cyanobacterium. This suggested that salinity stimulates growth in this algal strain.

The Effect of combined treatments (pH and Salinity) on the Total Lipid Production

Stimulation of biodiesel production, carbohydrate and protein were performed with the different pH and salinity values in optimum conditions of pH and salinity for algae isolates after 15 days of incubation. To study the effect of combining (pH and salinity) conditions to stimulate the lipid production. The large-

scale production was prepared in glass pools in 7.5 l and the algae isolates were cultivated under optimum conditions of pH and salinity for 15 days.

The result in figure 5 showed that the total lipid, carbohydrate and protein concentration of *C. dispersus* were stimulated at treatment (pH9 and NaCl, 0.4g/l) and reached 8.43, 0.525 and 0.018 g/l, respectively. While the minimum concentration of lipid, carbohydrate and protein productions were observed in treated (pH7 and salinity 0.75g/l) and reached 2.76, 0.067 and 0.009 g/l, respectively. The results also suggested that the synergistic action of pH and salinity did not stimulate biodiesel production in microalgae, and stimulation of lipid production resulted from an increase in pH value rather than salinity.

In case of *C. vulgaris*, the total lipid and protein productions were stimulated at lower pH value with moderate salt concentration (pH6 and salt 0.75g/l) and reached 4.43g/l and 0.032 g/l, respectively. In comparison to *C. vulgaris*, lower lipid and protein concentrations (1.85 and 0.001 g/l) obtained at treatment (pH7 and salt 0.8g/l). Also carbohydrate concentration increased in lower pH value, but with slightly increases in salinity concentration and reached maximum 1.837 g/l at treatment (pH6 salt 0.8 g/l) (Figure 6). The results obviously showed that the lipid production in *C. vulgaris* was stimulated at lower pH and moderate salinity concentration. While an increase in pH value and salinity concentration, affects adversely lipid, carbohydrate and protein productions.

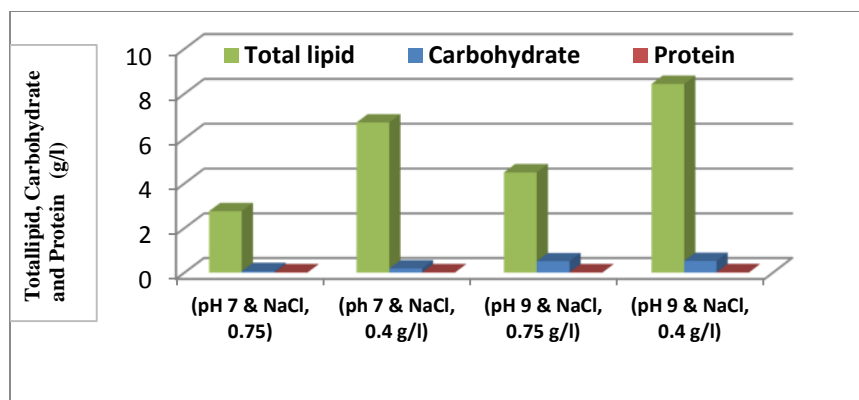


Fig. 5: Total lipid, carbohydrate and protein concentrations of *C. dispersus* in various pHs and salinity concentration at 25 ± 2 C° and $268 \mu\text{E}/\text{m}^2/\text{s}$ after 15 days.

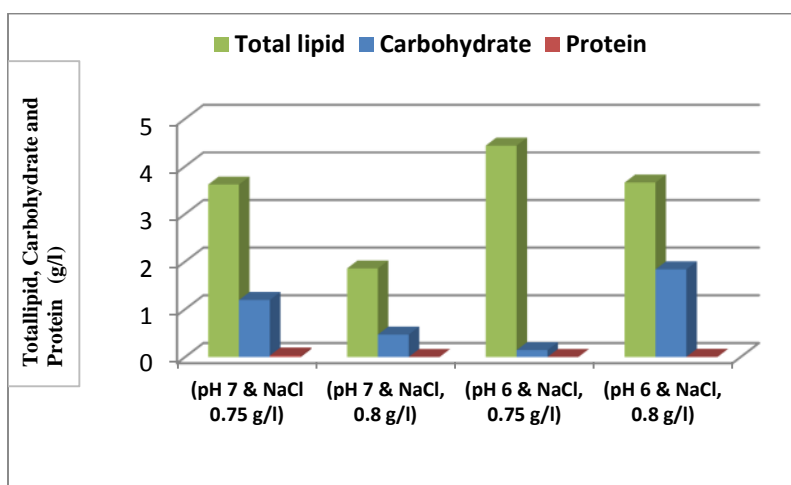


Fig. 6: Total lipid, carbohydrate and protein concentrations of *C.vulgaris* in various pH and salinity concentrations at 25 ± 2 C° and $268 \mu\text{E}/\text{m}^2/\text{s}$ after 15 days.

Total lipid production in microalgae *A. augstumalis* was stimulated at alkaline condition and in moderate salinity concentration (Figure 7). Maximum lipid production observed in treatment (pH11 and salt 0.75 g/l) and reached to 4.42 g/l. While minimum lipid production (2.84 g/l) obtained at neutral pH and moderate salinity concentration (pH7 and salt 0.75g/l). These results suggested that lipid production by *A. augstumalis* was stimulated at higher pH values and moderate salt concentration (pH11 salt 0.075g/l).

Duan *et al.*, [45] mentioned the positive response of salinity (NaCl) effect on lipid production. This effect was noticed on intracellular lipid accumulation, while the cell growth was inhibited.

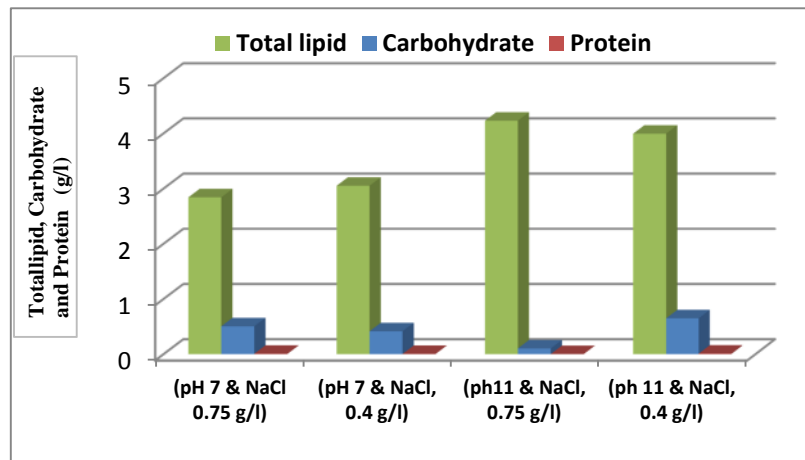


Fig. 7: Total lipid, carbohydrate and protein of *A. augstumalis* at various pH and salinity concentrations at 25 ± 2 C° and $268 \mu\text{E}/\text{m}^2/\text{s}$ after 15 days.

The explanation for the above situation might be that salinity can stimulate microalgae to accumulate intracellular lipid. Although, the salt stress could induce osmotic stress and as a result stimulates lipid accumulation. The result also showed that under stress conditions (pH and salinity) the carbohydrate and protein content decreased, this agreed to study of Norman *et al.*, [46]. The protein production was affected by the growth phase in all salinity levels evaluated; significant differences ($P < 0.05$) were observed among growth phases. Clear tendency in which the increase of salinity caused a decrease in protein content.

Microalgae oil content:

All content presented as g oil / dry weight of age. The results of oil content (%) for algal treatment were varied according to treatment (figure 8). Higher oil content was observed in *C. vulgaris* and reached 33.2% (0.332 g of oil / g of dry algal biomass) at treatment (pH6, and salt 0.75g/l), in comparison, to lower oil content (6%) at control treatment (pH7 and salt 0.75 g/l). Lower oil content was observed by *A. augstumalis* and *C. dispersus* (16 % and 13.8%) at treatments (pH11 and salt 0.75 g/l) and (pH 9 and salt 0.4 g/l), respectively. Compared to lower oil content of 5.1 and 3.30 g/l respectively with the control treatment (pH7 and salt 0.75 g/l). From these results, it can be concluding that the lipid production by *A. augstumalis* and *C. dispersus* stimulated by elevation in pH values rather than salt concentration. In case of *C. vulgaris* the lipid production was stimulated by lowering pH values and increase in salt concentration.

Nagle *et al.*, [47] observed oil content (%) in eight microalgae species varied from 3 to 13% maximum lipid content of (11.3, 10.95 and 9.8%) was observed with *Oscillatoria sp*, *Phormidium tenne* and *Spirulina major*, respectively. Lohery [48] revealed that neutral and polar solvent (Hexane and ethanol) was prepared to extract as crude oil from algae. In another study, the alga *Hapalosiphon sp.* showed a highest lipid content of $9.95 \pm 0.34\%$ obtained by cultivation in salinity 10 ppt, but did not stimulate lipid accumulation in Cyanobacterial strain. Possibly, the Cyanobacterial cells may provide more energy for the extrusion of Na^+ in cells to maintain osmotic balance [49], and then it decreased the amount of storage product (lipid). Another study showed that a maximum lipid percentage ($31.09 \pm 0.58\%$ of dry weight.) was observed by *Spirulina* strain ws-41 at (0.5 M NaCl) and (pH 9) [50].

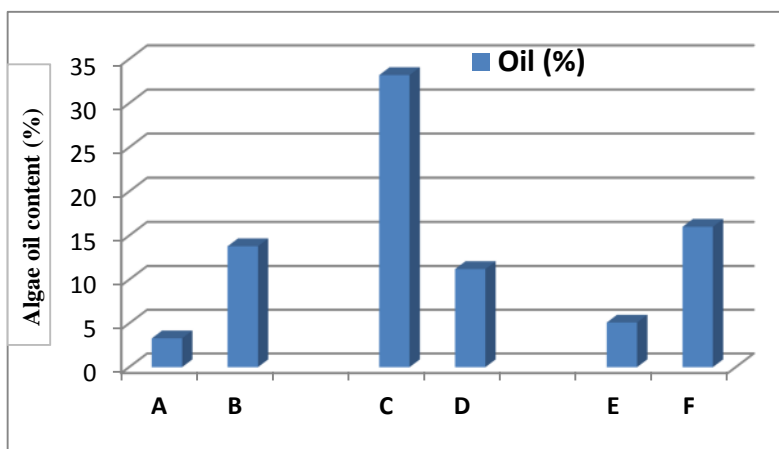


Fig. 8: Algae oil content (%) after harvesting and extraction by hexane (A: *C. dispersus* (pH7 and NaCl, 0.75 g/l), B: *C. dispersus* (pH9 and NaCl, 0.4g/l), C: *C. vulgaris* (pH7 and NaCl, 0.75 g/l), D: *C. vulgaris* (pH7 and NaCl, 0.75 g/l), E: *A. augstumalis* (pH7 and NaCl, 0.75g/l), F: *A. augstumalis* (pH11 and NaCl, 0.75 g/).

Analysis of lipid content of microalgae:

Six treatments including control and stimulated condition by pH and salinity were used to determine lipid compositions of algae isolates according to standard fatty acids (Figure 9), using GC techniques. The results in Figure 10, showed that the control treatment (pH7 and NaCl, 0.75 g/l) of *C. dispersus* contained only stearic acid (1.09 g/l) while, no lipid was detected when the algae were stimulated at higher pH and lower salinity concentration (pH9 and NaCl, 0.4g/l).

In *C. vulgaris* only stearic acid (9.78 g/l) was found in control treatment (pH 7 and NaCl, 0.75) g/l while, in treatment (pH 6 and NaCl, 0.75 g/l) oleic acid (1.15 g/l), stearic acid (1.26 g/l) and palmitic acid (0.12 g/l) were observed (Figure 11).

In comparison to *A. agustumalis* lipid content included, oleic acid (1.1201g/l), stearic acid (0.1713 g/l) and palmitic acid (0.08 g/l) were found in control treatment (pH7 and NaCl, 0.75) g/l while, only stearic acid (3.98 g/l) was detected when the algae was stimulated at treatment (pH11 and NaCl, 0.4 g/l) (Figure 12). The analysis of fatty acids by GC technique revealed presence of stearic acid only in *C. vulgaris* under the natural condition (pH 7 and NaCl, 0.75 g/l). While, under stress conditions of pH and salinity (pH6 salt 0.75g/l), three fatty acids (oleic, stearic and palmitic acids) appeared. The results agreed with the study of Kirrolia *et al.*, [51]. The percentage of saturated fatty acids in microalgae decreased as the concentration of NaCl increased, while the percentage of highly unsaturated fatty acid increased.

The results also agreed with the study of Thompson, [52]. The fatty acid found in Chlorophyceae, C16:0, C18:1, C18:0 were reported as the most common type. The results were in accordance with the study of Liu *et al.*, [53].

The results of fatty acids for *C. dispersus* under stimulated conditions, showed only stearic acid existence in natural condition. These results also were recorded in the study of Samah *et al.*, [49] that revealed that the amounts of most FA increased steadily until the 15th days at (30 g/l). However, palmitic acid decreased slightly on the 10th day of culture, except for stearic acid whose amount remained constant during the entire period, while the amount of all fatty acid increased steadily until the 10th day except for stearic, palmitic and oleic acids beyond (10 days), and FA quantities declined.

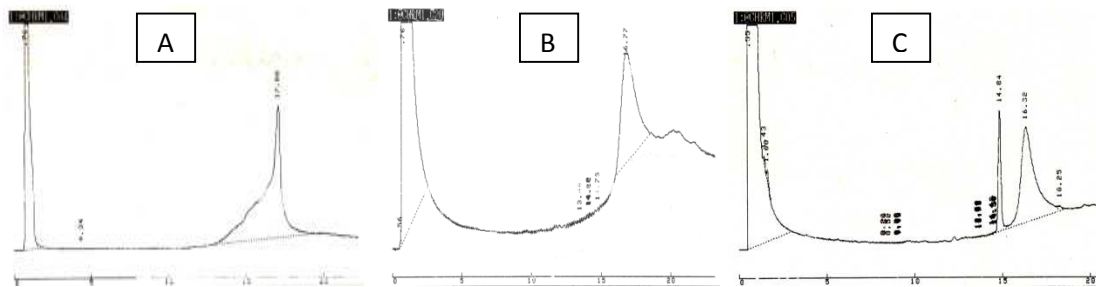


Fig. 9: Standards of Oleic acid (A), Stearic acid (B) and Palmitic acid (C) analyzed by GC technique.

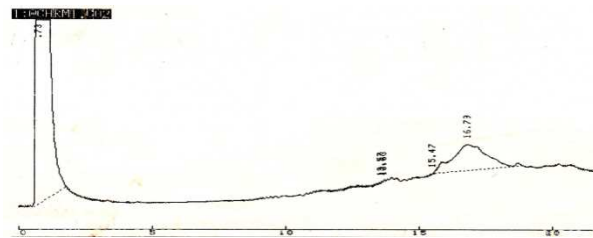


Fig. 10: Chromatogram of GC for control (pH7 and NaCl, 0.75 g/l) of *C. dispersus*.

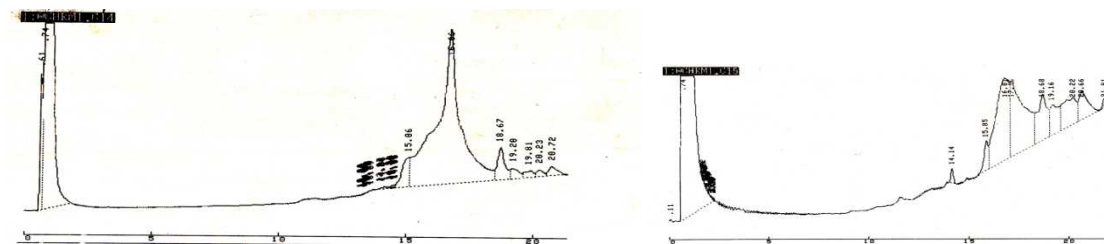


Fig. 11: Chromatogram of GC for control and treatment at (pH6 and NaCl, 0.75 g/l) of *C. vulgaris*.

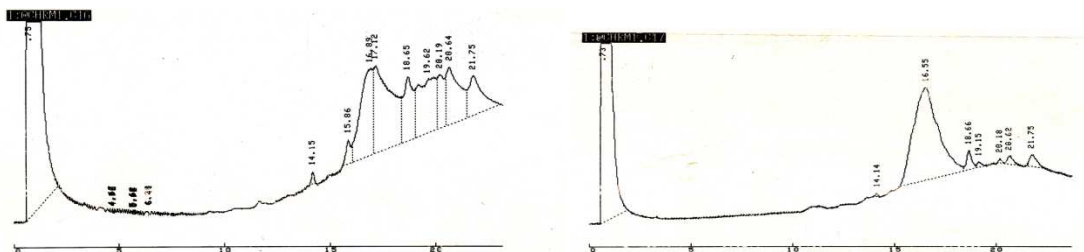


Fig. 12: Chromatogram of GC for control and treatment at (pH11 NaCl, 0.75 g/l) of *A. augstumalis*.

Conclusion

Microalgae could be an economical choice for biodiesel production, due to its availability and low cost and environmental friendly properties. The combined effect of pH and salinity were stimulated lipid production in microalgae. Among the three microalgae tested for biodiesel production, the dry algal biomass of *C. vulgaris* contained higher lipid concentrations of 33.2 % in mass. The lipid content of selected microalgae contained stearic acid, palmtic acid and oleic acid under stimulus condition.

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References

- [1] Smith, V.H., Sturm, B.S.M., deNoyelles, F.J. and Billings, S.A. The ecology of algal biodiesel production. Trends in Ecology and Evolution, Vol.25, No.5, pp. 301-309.2009.
- [2] Chen, C., Yeh, K., Aisyah, R., Lee, D., and Chang, J. Cultivation photo bioreactor design and harvesting of microalgae for biodiesel production: A critical review, Biores. Technol. Vol.102, No.1, pp. 71-81.2011.
- [3] Robert, K. Benefits of Biodiesel. National Biodiesel Board, Biodiesel, org, Oct. Web. 17 May. 2009.
- [4] Field, C.B., Behrenfeld, M.J., Randerson, J.T., and Falkowski, P. Primary production of the biosphere, integrating terrestrial and oceanic components, Sci. Vol.281, pp. 237-240.1998.
- [5] Darzins, A., Philip,P., and Edye, L. Current Status and Potential for Algal Biofuels Production, A report to IEA Bioenergy, task 39.T39-T2.2010.
- [6] Chisti, Y. Biodiesel from microalgae, Biotechnol, Advances. Vol.25, No.3, pp.294-306.2007.
- [7] Niehaus, T.D., Okada, S., Devarenne, T.P., Watt, D.S., Sviripa, V., and Chappel, J. Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii* Proc Natl Acad Sci U S A, Vol.108, No. 30,pp. 12260-12265.2011.
- [8] Fukuda, H., Kondo, A., and Noda, H. Biodiesel fuel production by transesterification of oils, J. Biosci Bioeng. Vol.92, No.5, pp.405-416. 2001.
- [9] Chisti, Y. Biodiesel from microalgae beats bioethanol, Trends Biotech. Vol.26, No.3, pp.126-131.2008.

- [10] **Borowitzka, M. A.**, 1988. Fats, oil, and hydrocarbons, In *Microalgae Biotechnology*, Cambridge, United Kingdom. Cambridge University Press, pp.257-287.1988.
- [11] **Molina, Grima, E. E. H., Belarbi, F. G., Acién Fernandex, A., Robles Medina, Yusuf C.** Recovery of microalgae biomass and metabolites, process options and economics, *Biotechnology Advances* Vol.20, pp.491–505.2003.
- [12] **Gutierrez, C. C.** The Effect of Salinity and pH on Growth and Lipid Content of Algae ,The University of Texas at Austin in Partial Fulfillment, Master of Arts, August, 1-45.2009.
- [13] **Gardner, R., Peters, P., Peyton, B., Cooksey, K E.**, Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of Chlorophyta. *J. Appl. Phycol.* Vol.26, pp.1005-1016. 2011.
- [14] **Guillard, R.R.L.** Purification methods for microalgae. In: *Algal Culturing Techniques*, Andersen, R.A. (ed.), Elsevier Academic Press, and Amsterdam, pp. Vol.117- 132.2005.
- [15] **Largeau, C., Casadevall, E., Berkloff, C., and Dhamelincourt, P.** Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *J. Photochemistry.* Vol.19, No.6, pp.1043-1051.1980.
- [16] **Prescott, G.W.** *Algae of the Western Great Lakes Areas*.Koenigstien, Germany, Ottokaeltz Science Puplichers, pp.237-488.1982.
- [17] **Desikachary, T.V.** *Cyanophyta*. Indian Council of Agricultural Research New Delhi. pp. 686. 1959.
- [18] **Bellinger, E. G., and Sigee, D. C.** *Algae as Bioindicators*, in *Freshwater Algae, Identification and Use as Bioindicators*, John Wiley & Sons, Ltd, Chichester, UK,1-150.2010.
- [19] **Sinigalliano, C.D., Win shell, J., Guerrero, M.A., Scorzetti, G., Fell, J.W., Eaton, R.W., Brand, L., and Rein, K.S.**, 2009. Viable cell sorting of dinoflagellates by multiparametric flow cytometry, *Phycologia*. Vol.48, No.4, pp.249-257.2009.
- [20] **uang, X., Li, C., Liu, C., and Zeng, D.** Studies on the Ecological Factors of *Oocystis borgei*. *J. Zhanjiang Ocean Univer.*Vol. 22, No. 3, pp. 8-12.2002.
- [21] **Hassan, F. M., Aljbory, I. F., Jumaily, E. F.** Stimulation of biodiesel production from two algae: *Chlorella vulgaris* Berjerinck and *Nitzschia palea* (Kütz.) Smith, and study their some growth parameters under different light intensity. *IOSR-JESTFT*. Vol.6, No. 2, pp.31-42. 2013.
- [22] **Eppley, R.W., Harrison, W.G., Chisholm, S.W., and Stewart, E.** Particulate organic matter in surface waters off Southern California and its relationship to phytoplankton. *J. Mar. Res.*Vol.34, pp. 671-696.1977.
- [23] **Jawad, A.M.** Interaction between Cyanobacteria and other microorganisms. Ph.D. Thesis. Liverpool University. England.1982.
- [24] **Derven, B., and Schmit,J. M.**, La reaction Sulfo-vanilligue dansletude deslipids sorigues Applications Biochemigues, Cliniques et. Pharmacologiques. *Bull. Trav. Soc. Pharm. Lyon*, Vol.8, pp.173-178.1964.
- [25] **Bradford, M.** A rapid and sensitive method for the quantization of microgram quantities of protein using the principle of protein dye binding. *Anal. Biochem.* Vol.72, pp.248-254. 1976.
- [26] **Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A., and Smith, F.** Colorimetric method for determination of sugars and substances. *Anal. Chem.* Vol.28, No.3, pp.350-356.1956.
- [27] **Fajardo, A. R., Cerdan, L. E., Medina, A. R., Fenandez, F. G. A., Moreno, P. A. G., and Grima, E. M.** Lipid extraction from the microalgae *Pheodactylum tricorutum*. *Eur. J. Lipid Sci. Technol.* Vol.109, No. 2, pp. 120-126.2007.
- [28] **AOAC “Association of Official Analytical Chemists”.** Official Methods of Analysis, 16th Edition. AOAC International, Gaithersburg, M.D.1995.

- [29] **Kodandoor, S., and Madaiah, R.** Total lipid and Fatty Acid Composition in Some Freshwater Cyanobacteria, Journal of algae biomass utilization, department of Biosciences, Mangalore University, Karnataka, India Vol.2, No.2, pp.83-97.2011.
- [30] **SAS (Statistical Analysis System).** User's Guide. Statistical. Version 7th ed. Inc. Cary. N.C. USA.2004.
- [31] **Hassan, F.M., Hadi, R.A., Kassim, T.I., and Al-Hassany, J.S.** Systematic study of epiphytic algae after restoration of Al-Hawizah marshes, southern of Iraq. Int. J. Aqua. Sci. Vol.3, No.1, pp.37-57.2012.
- [32] **Mulumba, N., and Farag, I. H.** Tubular Photobioreactor for Microalgae Biodiesel Production. Inter. J. Engineer. Technol. (IJEST). Vol.4, No.2, PP.703-709.2012.
- [33] **Somchai, W., Chalor, L., and Niti, C.h.** Effect of pH on the blue green algae isolated from pacific white shrimp ponds, Kasetstar Uni. Fisheries Res.Bulletin. Vol.32.1, pp. 1-9.2008.
- [34] **William, M., Rowley, 2010.** Nitrogen and Phosphorus Biomass- Kinetic Model for *Chlorella Vulgaris* in A biofuel Production Scheme. In Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Engineering and Science, University Dayton Research Institute, 111pp.
- [35] **Guckert, J.B., and Cooksey, K.E.** Triglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high pH induced cell cycle inhibition. J. Phycol. Vol. 26, No.1, pp.72–79.1990.
- [36] **Carrieri, D., Momot, D., Brasg, I.A., Ananyev, G.;; Lenz, O.B., and Bryant, D.A.** Boosting to fermentation rates and product yields with sodium stress cycling, application to renewable fuel production by Cyanobacteria. Appl. Environ. Microbial. Vol.76, No.19, pp. 6455–6462.2010.
- [37] **Asulabh, K. S., Supriya, G., and Ramachandra, T. V.** Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp., and *Chaetoceros* sp. Energy and Wetlands Research Group Center for Ecological Sciences, Indian Institute of Science, Bangalore, pp. 1-7.2012.
- [38] **Wang, S., Li, L., Zhang, D., and Pan, X.** Effect of NaCl and Cd on photosystem II (PS II) activity of *Chlorella pyrenoidosa*. Chinese J. Appl. Environm. Biol. Vol. 17, No. 6, pp. 839-846. 2011.
- [39] **Rao, A. R., Dayananda, C., Sarada, R., Shamala, T. R., and Ravishankar, G. A.** Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresource Technology. Vol.98, No. 3, Pp.560-564.2007.
- [40] **Mus, F., Dubini, A., Seibert, M., Posewitz, M.C., and Grossman, A.R.** Anaerobic adaptation in *Chlamydomonas reinhardtii*, anoxic gene expression, hydrogenase induction and metabolic pathways. J. Biol. Chem. Vol. 282, pp. 25475-25486.2007.
- [41] **Jiang, Y., and Chen, F.** Effects of salinity on cell growth and docosahexaenoic acid content of the heterotrophic marine microalgae *Cryptocodinium cohnii*. J. Indus. Microbiol. . Biotechnol. Vol.23, No.6, pp508-513.1999.
- [42] **Sen, B., Alp, M. T., and Kocer, M. A. T.** Studies on growth of marine microalgae in batch, cultures. I. *Chlorella vulgaris* (Chlorophyta). Asian J. Plant Sci. Vol.4, No.6, pp. 636-638.2005.
- [43] **Sanchez, F. J. M., Fernandez, F. G., Acien, A., Rueda, J., Perez- Parra and E., Molina.** Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Proc. Biochem. Vol. 43, pp. 398-405.2008.
- [44] **Kaewkannetra, P., Enmak, P., and Chiu, T.** The Effect of CO₂ and Salinity on the Cultivation of *Scenedesmus obliquus* for Biodiesel Production. Biotechnol. Biopro. Engin. Vol.17, pp. 591-597.2012.
- [45] **Duan ,X., Ren, G. Y., Liu, L. L., and Zhu, W. X.** Salt-induced osmotic stress for lipid overproduction in batch culture of *Chlorella vulgaris*. African J. Biotechnol. Vol.11, no.27, pp.7072-7078.2012.

- [46] Norman, G., Jose, A. L., Anselmo, M., Marcel, M., Nolberta, H., and Antonio, G. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. Department de Investigations Scientifics Technological, Universidad de Sonora Blvd. Mexico. Vol. 40, No.2, pp.435-440.2012.
- [47] Nagle, V.L., Mhalsekar, N.M., and Jagtap, T.G. Isolation, optimization and characterization of selected cyanophycean members. Indian J.Mar. Sci. Vol. 39, No.2, PP. 312-318.2010.
- [48] Lohrey, C. Biodiesel Production from Microalgae: Co-Location with Sugar Mills. Master Thesis in Biological Department of Biological and Agricultural Engineering. University of Idaho, pp.1-80.2008.
- [49] Ayachi, S. A., El Abed, Wissal, D. and Marzouk, B. Chlorophylls, Proteins and Fatty Acids Amounts of *Arthrospira platensis* Growing under Saline Conditions. Pak. J. Bio. Sci Vol.10, No.14, pp. 2286-2291.2010.
- [50] Parveen, K., Sushil, K. S., and Pradeep, K. S. Isolation of producing Spirulina strain ws-41 and effect of various cultural factor on their lipid accumulation, Discovery Science. Vol.9, No.21, pp. 41-49. 2014.
- [51] Kirrolia, A., Bishnoia, R.N., and Singh, N. Salinity as a factor affecting the physiological and biochemical traits of *Scenedesmus quadricauda*. J. Algal Biomass Util., Vol. 2, No. 4, pp.28-34.2011.
- [52] Thompson, G.A. Lipids and membrane function in green algae. Biochim. Biophys. Acta. Vol. 1302, pp.17-45.1996.
- [53] Liu, J., Huang, J., Fan, K.W., Jiang, Y., Zhong, Y., Sun, Z., and Chen, F. Production potential of *Chlorella zofingienensis* as a feedstock for biodiesel, *Biores. Technol.* Vol.101, No.22, pp.8658- 8663.2010.