Effect of pH Condition on the Growth and Lipid Content of Microalgae Chlorella vulgaris & Chroococcus minor

Abrar T. Al-Safaar, Ghaidaa H. Al-Rubiaee and Suaad K. Salman

Abstract: Two locally isolated microalgae (green algae Chlorella vulgaris and blue green algae Chroococcus minor) were used in the current study to test their ability to produce biodiesel through stimulated in different pH levels treatments (pH 5, pH 9) and effect of pH level on the quantity of protein, carbohydrate. Showed that the accumulation of lipids in C. vulgaris more efficient than C. minor, The treatment pH 9 was recorded C. vulgaris the highest lipid content from 8% at control to 32% as well as highest carbohydrate content from 51% to 31%. The treatment pH 9 was recorded in C. minor was recorded the highest lipid content from 5% at control to 12% as well as highest carbohydrate content from 15% at control to 18%, but showed decreased protein content from 40% to 30%. The results revealed that Stearic acid and Oleic acid content increased content for both algae at pH 9 levels.

Key words: microalgae, pH level, lipid content, biodiesel

1 Introduction

THE basic sources of energy are fossil fuels, natural gas, and coal, hydro electrical and nuclear. The need of energy is increasing continuously due to the increase in population and industrialization. These resources of energy are limited [1] and their combustion will lead to generation of the energy-related emissions of greenhouse gases (GHG) such as carbon dioxide, sulfur dioxide, methane and nitrogen oxides [2]. During the last few decades, global atmospheric gases (GHG) such as carbon dioxide, methane, and nitrogen oxides [2]. During the last few decades, global atmospheric concentrations of GHG have considerably raised in growth rate of CO2 emissions that main cause of global warming [3]. Biodiesel is one of the better choices among varieties of bioenergy, and microalgae are claimed to be the best crop for biodiesel production [4, 5]. Renewable and cleaner biofuels from microalgae have attracted widespread attention in recent years [6, 7]. The most common biofuel from microalgae is to produce biodiesel from algal lipids (oil) through transesterification. The most important compounds are Triacylglycerides [4, 8].

Microalgae are unicellular photosynthetic organisms that use light energy and carbon dioxide, with higher photosynthetic efficiency than plants for the production of biomass [9]. In particular, microalgae could be used for the production of lipids. Which can be cultured throughout the year, have a simple reproducing system, ability to grow in wastewater/seawater/brackish water, non-interference of food chain, and high-lipid productivity, use water most effectively and do not need rich soil for growth [8, 10]. Most green microalgae produce starch under normal conditions. When exposed to stress under abnormal conditions will start to synthesize lipids especially triacylglycerol which is stored in their oil bodies [11]. Several studies have shown that the quantity and quality of lipids within the cell can vary as a result of changes in growth conditions, such as pH variation, temperature and light intensity, nutrient media characteristics, concentration of nitrogen, phosphates and iron [12, 13].

Many reports study fluctuations of the pH in the medium also have been found to alter the lipid composition of microalgae [14, 15, 16 and 17]. The effects of pH on the lipid and FA composition of a Chlamydomonas sp. and Chlamydomonas reinhardtii, and the unidentified Chlamydomonas sp. FAs of polar lipids were more saturated than those in C. reinhardtii, grown at pH 1 than that in the cells cultivated at higher pH. The increase in saturation of fatty acids represent an adaptive reaction at low pH to decrease membrane lipid fluidity [18].

The present study aimed to examine the ability of some isolated local algae to produce lipids in different pH, also to determine the lipids quality that uses in biodiesel.

2 Materials and Methods:

2.1 Sampling and Collection of Microalgae
Fresh water samples of algae were collected from the ponds in Al-Mustansiriya University. The samples were collected by sterile container (100 ml) which was marked with date and location of sampling then transported to laboratory immediately to be incubated under suitable condition (268 µE/m²/s, ,16:8 light: dark and 25±2 C˚).

2.2 Algae Isolation and Purification
According to [19, 20] two methods were used for isolation and purification: streaking on plate agar, Chu-10 media solution solidified by 1.5 % agar-agar and sterilized by autoclave, after sterilization Chu-10 with 45-50 C˚ was poured. Into petri-dishes which 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 µE/m²/s, 25±2 C˚ and 16:8 lights: a dark period of 10 -14 days. The same way again to the media culture for BG-11.

2.3 Preparation and Sterilization of Media
Modified Chu-10 was used for the green algal growth [21, BG-11 culture media for cyanobacteria [22] and described their components in tables1 and 2, respectively, as it was prepared Stock solutions of each salt for macronutrients and stock solutions for micronutrients combined as follows:

- Abrar Thamer Al-Safaar
- Ghaidaa Hussein Al-Rubiaee
- Suaad Kadim. Salman

Al-Mustansiriya University/College of Science Biology department / Botany

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TABLE 1: Stock Solutions Component of Chu-10

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Adjusted pH for media Chu-10 and BG-11 to 6.4 and 7.5, respectively.

$$K = \frac{c}{t} \times 3.322 \quad \text{[24]}$$

**TABLE 2: stock Solutions Component of BG-11**

<table>
<thead>
<tr>
<th>Macronutrient salt</th>
<th>Concentration g/L</th>
<th>Micronutrient salt</th>
<th>Concentration g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>150</td>
<td>H₂BO₃</td>
<td>2.86</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>30</td>
<td>MnCl₂.4H₂O</td>
<td>1.18</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>75</td>
<td>ZnSO₄.7H₂O</td>
<td>0.222</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>27.181</td>
<td>(NH₄)MoO₄.2H₂O</td>
<td>0.0124</td>
</tr>
<tr>
<td>Citric acid</td>
<td>6</td>
<td>CuSO₄.5H₂O</td>
<td>0.072</td>
</tr>
<tr>
<td>Ferric ammonium</td>
<td>6</td>
<td>CO(NO₃)₂.6H₂O</td>
<td>0.048</td>
</tr>
<tr>
<td>Citrate</td>
<td>EDTA.Na₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.5 Algae Cultivation for Biomass

A100 ml suitable media for both isolated algae and transfer 10 ml of isolated algae then incubated for 14 days, also transfer this culture A100 ml suitable media for both isolated algae and transfer 10 ml of 2.5 Algae Cultivation for Biomass (0.01N) then sterilized in autoclave except K₂HPO₄ at 121°C, 1.5 hr for 15min.

2.6 Determination of the Growth Curve

Growth curve was determined for the purpose of identifying growth phases. Then the deposition cultures at the beginning of stationary phase, on the Chlorella vulgaris harvested in the twelve day but Chroococcus minor in the ten day. Microalgae concentration was determined daily by optical density (OD) measurement at 540 nm by UV-Vis spectrophotometer, all measurements of the study were triplicates. The growth rate (K) and doubling time (G) were calculated according to the following equation:

$$K = \frac{(\log OD_t - \log OD_0)}{t} \times 3.322 \quad \text{[24]}$$
In pH 9 treatment spent in lag phase four days and then logarithmic phase began until nine days entered stationary phase which lasted until the fourteenth day and then observed the beginning of a decline in the number of cells and color change from green to yellowish green figure (2).

In pH 5 treatment spent in lag phase three days and then logarithmic phase began until the ten day and then entered stationary phase until the seventeen day and then observed the beginning of a decline in the number of cells figure (6).

The results shown the effect of different pH on growth rate (K) and doubling time (G) for *C. vulgaris* showed a decrease in growth rates but in contrast increased doubling time of all treatment, except control treatment. The highest value of growth rat (k) was 0.33 and lowest doubling time 1 day at control treatment while the lowest value of (k) was 0.13 in pH 9 treatment and doubling time is record 3.9, but at pH 5 treatment the (k) value and doubling time reached 0.20 and 2.2 day respectively. Table (3), Figure (4 and 5).

### TABLE 3 Effect of Different pH Level on Growth Rate (K) and Doubling Time for Both Algae of *C. vulgaris* and *C. minor*

<table>
<thead>
<tr>
<th>pH Treatment</th>
<th>Growth Rate</th>
<th>Doubling Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. vulgaris</td>
<td>C. minor</td>
<td>C. vulgaris</td>
</tr>
<tr>
<td>Control</td>
<td>0.33±0.021</td>
<td>0.030±0.25</td>
</tr>
<tr>
<td>pH 9</td>
<td>0.13±0.026</td>
<td>0.09±0.026</td>
</tr>
<tr>
<td>pH 5</td>
<td>0.20 ±0.026</td>
<td>0.12±0.026</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the P< 0.05 level*
Finally, in pH 5 treatment spent in lag phase six days and then logarithmic phase began until eight day then observed the beginning of a decline in the number of cells and change color media in the eighth day figure (8).

One of the major factors in the cultivation of algae is pH because it determines the solubility and availability of CO$_2$ and essential nutrients, and it can have a significant effect on the metabolism of algae [30]. Due to the absorption of inorganic carbon from algae, and can pH significantly higher in cultures of algae [31].

Different growth is observed for each alga isolate in all treatments. The effect of different levels of pH in *C.vulgaris* and *C.minor* biomass growth was significant between (pH5 and pH9). The results showed that increase in pH value to (9) stimulated lipid production by *C.vulgaris* and *C.minor*, in comparison to lower lipid accumulation at 5 pH treatment.

Somchai *et al.* [32] mentioned that the pH levels less than 7.5 and higher than 9 caused an adverse effect on growth, at pH 6 the filament of blue green alga was broken up into smaller filaments. These results indicated that pH levels at lower and higher than 5 pH could inhibit photosynthesis and affect the morphology of alga. Also, pH values in the range of 8 pH to 9 pH were important for determining the free CO$_2$ concentrations in the medium. With higher pH values, additional pH effects were observed involving a decrease in the relative high affinity of low CO$_2$ adapted algae to free CO$_2$[33].

### 3.3 The effect of pH on lipids accumulation

The extracted lipids content of the early of stationary phase for both algae studies, Table (4) show the result lipids content for *C.vulgaris* was increased from 8% at control treatment to 32% at 9 pH treatment, and its was 21% at treatment pH 5. The same trend was shown for *C.minor* the lipid content increased from 5% at control treatment to 22%, 12% at pH 9, pH 5 treatment respectively, (figure 11 and 12).
Rai et al. [34] observed that for Chlorella sp. maximum lipid production of 0.1995 g L⁻¹ with lipid accumulation of 23% at pH 8 and 24 h photoperiod.

Rodolfi, et al. [7] noticed better growth of C. vulgaris at pH 6.5 and 7.0, and accumulated lipid at pH 7 and 8.5, so optimal for growth and lipid accumulation of C. vulgaris was at pH 7.0. Alkaline pH increases the flexibility of the cell wall of mother cells, which prevents its rupture and inhibits autospore release, thus increasing the time for cell cycle completion [14].

Moheimani (2013) [35] found pH 7 and 7.5 ideal for accumulation of lipid in Tetrasielmis suecica and Chlorella sp. While we found no significant effect of pH change on lipid accumulation, the treatment with a pH change to 8 exhibited the greatest overall accumulation (averaging 24.75% by mass). In this study we observed the effects of pH on the biochemical composition of both microalgae C. vulgaris and C. minor. It found that increased carbohydrate and decrease in protein content for C. vulgaris better than C. minor per cell for all treatment.

Carbohydrate content for C. vulgaris was increased from 18% at control treatment to 25% at pH 9 treatment, and it was 21% at pH 5 treatment. While Carbohydrate content for C. minor was increased from 15% at control treatment to 22% at pH 5 treatment, and it was 18% at pH 5 treatment.

Finally, protein content for C. vulgaris decreased for all treatment when compared with 51% control treatment, and it ranged from 17% to 32% at pH 9 and 5 pH treatment respectively. Protein content for C. minor also decreased for all treatment, and it ranged from 40% to 15, 30% pH at 9 and 5 pH treatment respectively (table 4).

<table>
<thead>
<tr>
<th>pH treatment</th>
<th>C. vulgaris</th>
<th>C. minor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>Control</td>
<td>51</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>21</td>
</tr>
</tbody>
</table>

Two fatty acids were analyzed using the gas Chromatography (GC). Stearic acid and Oleic acid under stress condition of pH (pH9) two fatty acids (oleic, stearic and) appeared, This agreed with study of Thompson, (1996) [38] fatty acids found in Chlorophyceae, C16:0, C18:1, C18:0 were reported as the most common type.

<table>
<thead>
<tr>
<th>TABLE5 Oleic and Acid Stearic Acid Count in Both Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>C. vulgaris</td>
</tr>
<tr>
<td>C. minor</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>pH 9</td>
</tr>
<tr>
<td>pH 5</td>
</tr>
</tbody>
</table>

Table 5: Oleic and acid stearic acid count in both algae

References


