The Combined Effect of pH and Radiation on The Biochemical Content of Asterococcus sp. Algae

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Abstract

This study aimed to determine the interactive effect of radiation and pH on the chemical content, represented by protein, lipids, carbohydrates, fatty acids, chlorophyll, mineral elements, and ash, in the alga *Asterococcus sp.* Algal samples were collected from different areas of the Euphrates River in the city of Nasiriyah, southern Iraq. They were purified and isolated using appropriate techniques and conditions, and then cultured in appropriate media according to different parameters of radiation and pH. The results showed a significant decrease in the percentage of protein, carbohydrates, fats, calories, chlorophyll, fatty acids, mineral elements, and ash in the treatments exposed to radiation and different pH levels. We conclude that radiation and pH have a clear and significant effect on the nutritional components of the *Asterococcus* algae due to their effect on the chlorophyll pigment, which is the source of energy in the algae.

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Introduction

One of the methods for studying the nutritional value of microalgae is to study the chemical composition (1). Through chemical composition data, we can identify the nutritional value of the algae, especially since algae cultivation is characterized by its exposure to various influences that alter the components of the individual cell and consequently change some components of the biomass such as the effect of heavy metals and pesticides (2). Therefore, its nutritional value varies widely depending on the cultivation conditions such as pH, light (3), temperature, and composition of the culture medium (4). Through information about the chemical composition of algae, it is possible to determine which of them has the highest nutritional value and thus benefit from it as a food source. Many researchers have conducted numerous studies on the interaction between environmental factors on the chemical content and nutritional value of algae in general and their effect on fats and fatty acids in particular. It has been observed that algae exist in environments where they are under the influence of more than one factor, which has drawn attention to the overlapping effects between these factors, whether the effect is synergistic or antagonistic. The pH is one of the important factors that influence many aspects, including its effect on the growth of organisms, especially on the growth of algae. Acidic and basic conditions may lead to the decomposition of some cellular components or the destruction of some enzymes (5), thus affecting some chemical compounds, carbohydrates, lipids and some important processes that affect the chemical content and nutritional value of algae. The pH is also an important factor in the characteristics of aquatic environment communities and the distribution of organisms in them. Algae have been observed to exist in environments with a pH close to neutral between 7.5-7. However, there are algae genera that exist in environments with high or low pH. For example, it has been observed that the genus Spirulina prefers to grow in environments with a basic pH 8.5-9 in some lakes around the world. Fats were among the chemical components affected by changes in pH values, especially for the fatty acids that make up fats. In a study conducted by (6), they noticed that when *Parphyridium cruentum* was grown at an ideal pH for its growth 8.5, it caused an increase in arachidronic and linolenic fatty acids and a decrease in linoleic fatty acid compared to the effect of other environmental factors such as light intensity and temperature. The reason was attributed to the effect of pH on the metabolism and accumulation of many chemical components, including fatty acids. This agrees with what (7) concluded regarding the effect of pH on the accumulation of unsaturated fatty acids, which in turn affected the chemical content and nutritional value directly or indirectly. There are many types of radiation within the visible spectrum, some of which are dangerous and some of which are less dangerous. The most prominent of these is ultraviolet (UV) radiation. These are invisible electromagnetic radiations characterized by different wavelengths and emitted with sunlight. The impact of UV radiation on biological and chemical aspects has become an important issue over the past three decades, especially following changes in the ozone layer. This layer covers the atmosphere and protects living organisms from harmful UV rays, especially UV-B (8). There are many effects of UV radiation, some of which are beneficial and others harmful. Many researchers have studied the impact of these rays on living organisms, and algae have occupied a significant portion of these studies due to their importance in the aquatic environment. Most research and studies have focused on the impact of UV-B radiation, as it is the radiation most widely reaching the Earth's surface, especially when the ozone layer is damaged (9).

Materials and Methods

Collecting algal samples

Water samples were collected from different areas of the Euphrates River to investigate algal species. They were collected in sealed plastic bottles. The samples were brought directly to the laboratory to investigate the algal species to be isolated. The samples were then dried using a LAB ConCo18 Freez drier and stored in clean, sterile, sealed glass bottles at -18°C until use (10) (Epply, 1977).

Algal isolation and identification

To obtain unialgal cultures, the dilution and plotting methods were used (11)
To identify the algae, the following taxonomic sources were used: (12-14)
Division: **Chlorophyta**
Class: **Chlorophyceae**
Order: **Tetraspiranes**
Family: **Gloeocystaceae**
Genus: ***Asterococcus***
Species: ***As. sp***.

Algal purification

The unialgal cultures were purified of bacteria according to the method of (15) and its details are explained in (16) (Anderson, (2005) which includes washing with sterile distilled water several times and then sedimentation by centrifugation. It was possible to separate the germs from the algae by centrifuging the sample at a speed of 3000 rpm for (90) seconds, after which the filtrate was removed and the sediment (algal cells) was washed again in sterile distilled water, and this process was repeated at least 12 times. To ensure purity, it was planted on the nutrient agar medium as explained in (11) as it was incubated at a temperature of 37^0C for 18 hours in the culture cabinet and this process was repeated several times to ensure that the culture was free of germs by observing that the algal cultures were free of any growth and thus pure algal isolates (axenic cultures) were obtained.

Algal cultivation

The Chu-10 culture medium modified by (17) was used to grow the algal isolates (Table 1). The culture medium was prepared in the form of standard stock solutions and stored at4°C in the refrigerator without sterilization until use. 1 ml of each was mixed when preparing the culture medium, then the volume was completed with distilled water according to the required volume and the pH was adjusted to 7.4 when culturing the algae by adding drops of 0.2 N sodium hydroxide solution. The culture media were then sterilized using a Webeco autoclave at a temperature of 121°C and a pressure of 1.5 pounds per inch for 20 minutes. Phosphorus was added after sterilization by filtering using filter paper with holes of 0.2 micrometers in diameter to prevent phosphate precipitation during sterilization. The isolated and identified species were transferred from the liquid culture medium using. The sterile pipette was pipetted into a number of sterile glass beakers with a volume of 100 cm3 containing 70 cm3 of sterile culture medium. The mouths of the beakers were closed with clean cotton and incubated at a temperature of 2±25 °C and lighting of 150 micro Einsteins/2 m/s in a growth cabinet with a system of 8:16 hours of darkness: light, taking into account the continuous shaking of the samples until the desired growth is achieved (18).

Table 1: Chemical composition of the Chu-10 culture medium modified by (Al-Aaragy 1996)

Concentration g/L	**Chemical compound**	**Concentration g/L**	**Chemical compound**
0.045	$MnCl_2.4H_2O$	53.3	$NaNO_3$
0.007	$(NH4)_6Mo_7O_{24}.4H_2O$	10	K_2HPO_4
0.056	$ZnSO_4.7H_2O$	25	$MgSO_4.7H_2O$
0.02	$CuSO_4.5H_2O$	40	$CaCl_2.2H_2O$
0.01	$CoCl_2.6H_2O$	1.46	$FeCl_3.6H_2O$
0.72	H_3BO_3	6.2	$Na_2SiO_3.9H_2O$
7.3	pH	31.8	Na_2.EDTA

Propagation of algae isolates

: To obtain a biomass of the isolated algae for laboratory experiments, the isolates were propagated using glass flasks with a capacity of 1000 cm3. The culture medium was added at a rate of 700 L cm3 to each flask, and each flask was inoculated with a volume of 70 cm3 of Storage farms are grown under optimal planting conditions to obtain the required quantity.

The effect of the interaction of pH and radiation

A series of experiments were conducted to demonstrate the interaction of two of the studied factors on the Chemical components and fatty acid content of *Asterococcus* algae. This alga was exposed to two different factors: pH and radiation. The values and concentrations were taken, and samples of the studied algae were exposed to them (pH0 + 0 radiation, (pH1 + 6.5 + 2-wm radiation), (pH9 + 2 + 2-wm radiation), and (pH10 + 3 + 2-wm radiation). Three replicates were performed for each concentration and value. All samples were incubated under other standard conditions, and samples were taken for chemical analysis (19).

Estimating the chemical content of the studied algae

Estimating protein concentration

The amount of protein was estimated according to the method of (20), by reading the protein concentration at a wavelength of 280 nanometers (the wavelength of maximum absorbance for proteins) and reading the absorbance at a wavelength of 260 nanometers (the wavelength of maximum absorbance for nucleic acids). The following equation was then applied to calculate the protein concentration.

Protein concentration = $1.55*A_{280} - 0.77 *A_{260}$

Absorbance at a specific wavelength

A volume of 10 cm3 is taken from the algae culture and centrifuged at a rate of 3000 rpm for 15 minutes. After that, the precipitate is taken and made up to 10 cm3 with distilled water. The centrifugation process is then repeated. After that, a specific volume of the filtrate is placed in the spectrophotometer cell and the absorbance is measured at wavelengths 280 and 260 nanometers. The equation mentioned above is applied.

Estimating the quantity of carbohydrates

The total quantity of carbohydrates in the algae samples was estimated using the Phenol-Sulpheric Acid method, according to (21), as described in the following steps:

1- Prepare a series of dilutions of standard solutions of stored glucose to obtain the following concentrations: 10, 20, 40, 60, 80, and 100 µg/cm3.

2-Take a volume of 0.5 cm3 from the series of standard solutions prepared above and 0.5 cm3 from the sample in which the amount of carbohydrates is to be estimated.

3- Add 0.5 cm3 of 5% phenol reagent, which was prepared by adding 5 grams of pure phenol to a specific volume of distilled water. Then, the volume is completed to 100 cm3 with distilled water in each test tube and mix well.

4- Add 2.5 cm3 of pure concentrated sulfuric acid (98%) to each tube, then leave it to cool at room temperature until a brown color appears.

5- The absorbance was measured at a wavelength of 488 nanometers, and distilled water was used as a blank solution.

6- Create a standard curve for the relationship between glucose concentration and absorbance, then calculate the glucose concentration of the samples used after comparing them with the glucose concentrations in the standard curve. Prepare a standard glucose solution with a concentration of 100 micrograms/cm3, which is prepared by dissolving 100 mg of glucose in a volume of 1 liter of distilled water.

Estimating the amount of fat

For fat content, the (22) was followed to extract total fats using a Soxhlet apparatus. The solvent used was hexane, following the following steps:

1- 0.5 g of algal samples were taken.

2- Lipids were extracted using a soxhelt device using hexane as a solvent.

3- After extraction, the samples were completely dried using a rotary evaporator.

Gross energy

The total energy in algae was calculated using the following equation, as reported in (23)

)GE (kcal/kg) = 5.72 * (% protein) + 9.5 * (% lipids) + 4.03 * (% carbohydrate

Chlorophyll concentration was calculated using the Lorenzen equation described in (24) and following the following steps:

100cm3 of a liquid algal culture was filtered using a GF/F (Glass fiber) filter paper, taking care to add 1 cm3 of a saturated MgCo3 solution during filtration. The paper was stored frozen in a clean glass bottle in a dark place until it was ground. When grinding, it must be taken into account that the process is carried out in the dark, as the paper is ground in a ceramic mill using 8 cm3 acetone at a concentration of (V/V%) 80, and left at room temperature for 18 hours in the dark, after which it is centrifuged at a speed of (5000 rpm) for 15 minutes to get rid of impurities. Then the filtrate containing the chlorophyll extract is taken and the volume is completed to 10 cm3 with acetone (V/V%) 80. The optical density of the sample is measured using a spectrophotometer at a wavelength of 650 nanometers, and it is left for 10 minutes. The optical density measurement process of the sample is repeated after adding diluted hydrochloric acid with a standard of (0.2N) and at the same wavelength. Acetone (80%) was used as a blank solution, and the amount of chlorophyll (a) in the sample was calculated according to the equation:

µg cha per sample = 11.9/2.43(Da – Db)/V/L

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µg cha is the amount of chlorophyll-a in micrograms in the sample

Da: Optical density before adding the acid

Db: Optical density after adding the acid

V: Volume of acetone used in the extraction in milliliters

L: Length of the photocell in centimeters

Estimation of fatty acid content

The lipid content of fatty acids was estimated according to the method ndescribed by Mahdi et al., 1996. The extracted lipids were converted to volatile derivatives, then (1) microliters were injected into a gas

chromatography (GC) device (FTER-8400s "Japan"). The results were received as a graph, and the percentage was calculated and expressed as (%) total lipids.

Estimation of Mineral Elements:

The mineral elements in the studied alga were estimated using the sample digestion method according to the method of (25) as described in the following steps:

1- A weight of (0.5) g was taken from the alga sample and placed in a thermal oven at a temperature of 200°C for one hour until dryness.

2- The sample was gradually dissolved in 10cm³ of concentrated hydrochloric acid, and then 5cm³ of concentrated nitric acid was gradually added to it, and the mixture was heated until dissolved.

3- The sample was dried in a boiling water bath (LDS "Kottenmann") after being placed in a ceramic dish until complete dryness.

4- The remaining solution was dissolved in the smallest amount of hydrochloric acid 5cm³, and then the mineral elements were estimated using an atomic absorption spectrophotometer (M410 "Corning).

Statistical analysis

The results were analyzed statistically using one way-ANOVA to determine the significance of the effect of the various coefficients using SPSS var. 25. The significance of the differences from the means was tested using the Duncan test 0.05.

Results and Discussion

Table 2: Percentage of chemical compounds

Chemical compounds	Dry weight %
Proteins	60.10
Carbohydrates	10.21
Fat	5.2
Total energy (kcal/kg)	460.110
Chlorophyll mg/100 cm	3 1.2

Table 3: The effect of the interaction of pH and radiation period on the chemical components of algae

Treatments (Radiation+ pH)	Proteins %	Carbohydrates %	Fat %	Total energy kcal/kg	Chlorophyll mg/100 cm3
Control	60.10 a	10.21 a	5.20 a	460.11a	1.20 a
1 +6.5	32.80 b	9.35 b	2.13 b	200.10 b	0.10 b
2 + 9	0.00 c	7.20 c	0.00 c	29.20 c	0.00 c
3 + 10	0.00 c	0.00 d	0.00 c	0.00 c	0.00 c

*a–d different letters within each column indicate significant difference ($p < 0.05$)

Table 4: Effect of the interaction of pH and radiation period on the content of fatty acids in algae.

Treatments (Radiation + pH)	Fatty Acids					
	Palmitic	Stearic	Palmitoleic	Oleic	Linoleic	Linolenic
Control	25.80 a	1.12 a	13.40 a	8.20 a	10.80 a	20.70 a
1+6.5	6.30 b	0.00 b	2.70 b	2.10 b	0.00 b	3.30 b
2 + 9	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
3 + 10	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c

*a–cdifferent letters within each column indicate significant difference ($p < 0.05$)

Table 5: Effect of the interaction of pH and radiation period on the content of mineral elements in algae.

Treatments (Radiation + pH)	mineral elements					
	Nitrogen	phosphorus	potassium	magnesium	iron	ash
Control	10.12	1.3	1.4	0.80	0.29	8.1
1+6.5	3.1	0.2	0.1	0.2	0.2	3.8
2 + 9	-	-	-	-	-	-
3 + 10	-	-	-	-	-	-

Protein content

The chemical composition of the algae, as detailed in Table 3, was demonstrably influenced by both pH and radiation levels. The highest protein concentration, representing 32.80% of dry weight, was observed at a pH of 6.5 and a UV exposure of 1 wm-2. This value was lower than the control sample, which showed 60.10% dry weight. An increase in concentration to 10 and an increase in the irradiation period to 3 wm-2 resulted in a reduction of proteins in the sample. The algae were exposed to two pollutants: pH and radiation. The chemical composition of the algae cells was largely impacted, including the protein content. The highest protein concentration was observed at 6.5 + wm-2 1 radiation, followed by a decrease in protein. This could be attributed to the algae's limited tolerance to prolonged exposure to high levels of pesticides and radiation. Another possibility is that the protein synthesis mechanisms failed because of damage to the organelles and enzymes involved, which affected the protein content. The algae's incapacity to produce organic molecules may be the cause of its slow demise as a result of its incapacity to withstand harsh environments and extreme pollution. This is in line with the findings of (26), who verified that the majority of algae, including spirulina, are susceptible to the harmful effects of radiation and pesticides, the two most potent contaminants.

Carbohydrate content

At (6.5 + 1) wm-2 radiation, the maximum concentration of carbohydrates found was (9.35%) dry weight. Following that, it was noted that as pH concentration and radiation exposure time (10 + 3) wm-2 increased, carbohydrates vanished (Table 3). When exposed to radiation and pH, carbohydrates were more resilient than proteins. The findings show that when pesticide concentrations and radiation times increase, the amount of carbs decreases until they completely vanish at (10 + 3 wm-2 radiation). Carbohydrate tolerance may result from algal cells producing carbs as a means of adapting to novel environments. However, when concentrations rise, the majority of the enzymes involved in carbohydrate synthesis suffer damage, which eventually causes them to vanish. Alternatively, the process of photosynthesis could be the cause.

It has demonstrated a higher endurance to adverse environments, but as pH and radiation
levels rise, the optical systems completely deteriorate, stopping this process. In addition to the effect of radiation, which works to destroy most of the internal chemical components and is reflected in the content of the algae and its incapacity to communicate, the reason could also be the algae's incapacity to continue growing and building its cells as a result of exposure to high concentrations of toxic substances.

Fat content

The greatest fat concentration was found at a concentration of (6.5) and an exposure period of 1wm-2, reaching (2.13%) dry weight, which is less than that found in the control sample (5.2%) dry weight, according to the results of estimating the fat concentration in the algae (Table3). No fat was produced when the concentration and duration of the radiation exposure to 9+2wm-2 radiation were increased. According to the aforementioned, at a concentration of 10 and an irradiation time of 3wm-2, no total energy value was observed, and the greatest total energy value was 200.1kcal/kg. Fats behaved similarly to the other components, showing a drop in fat content at 6.5+1 wm-2 radiation, followed by the loss of fatty materials at high concentrations and times. The failure of cells to continue producing the majority of the organic components required for their continuity, which results in the death of the algae, is one of the most notable causes. Alternatively, the lack of any kind of fatty acids at these levels and concentrations could be the cause, which results in a shortage of one of the most crucial components required for the production of fats, or the cause may be the breakdown of the majority of enzymes and the damage of all the structures required for the production of fatty materials, in addition to the detrimental effects of radiation, particularly with the lengthening of the exposure period, which results in the absence of both fatty materials and fatty acids (this is what was observed through the results of the current study). The most well-known proponents of this viewpoint are (27). The overall energy value acquired from exposing the algae to the parameters of pH and radiation is naturally extremely tiny, reaching (200.1) kilocalories/kg, according to the results that emerged. due to the algae's low concentration of vital organic molecules and brief radiation duration.

Chlorophyll content

Chlorophyll had a pattern similar to that of other chemical components. It recorded its highest content at low concentrations associated with short periods of radiation 6.5+1 wm-2 radiation), recording a concentration of 0.1mg/100 cm3. However, when the concentration was increased to 9 wm-2 and the radiation period increased to (2) wm-2, chlorophyll disappeared and was not observed (Table 3). The results obtained indicate that the chlorophyll content also suffered from the effects of pH and radiation, which led to a decrease in its content with increasing pH concentrations and radiation period. The reason, as previously mentioned, may be due to the failure of the algal cells to continue performing their vital functions, including chlorophyll synthesis. Alternatively, the reason may be damage to the enzymes

responsible for producing this pigment. Alternatively, it may be due to a shortage of the raw materials necessary for chlorophyll synthesis, including magnesium, which leads to a decrease in the quantity of these materials. Primary and thus low pigment production, (8) confirmed that if magnesium deficiency occurs due to the effect of radiation, it leads to a decrease in the amount of chlorophyll produced. While (27) supported the opinion that explains that most of the structures are due to the effect resulting from treating the algae with radiation in addition to the toxic effect due to the pH, which leads to weakening of the cells and thus their death.

Fatty acid content

Radiation and pH have different effects on the lipid content of fatty acids. Palmitic and linoleic acids, which reached 5.2% and 3.1% of total lipids, had the highest levels at low concentrations and brief radiation exposure times (6.5 wm-2 radiation). At the same amounts, other fatty acids vanished. However, all fatty acids, both saturated and unsaturated (Table 4), show a decrease in fatty acid content at low concentrations and short radiation durations when pH and radiation duration are increased to 10 wm-2. Afterwards, as pH levels rose and radiation exposure times increased, all fatty acids vanished. The damage to the algae's internal structure and its incapacity to produce any chemical components could be the cause, as could the fact that these two factors inhibit all the enzymes involved in the biosynthesis processes of the organic components and compounds, or the algae's incapacity to withstand the detrimental effects of these two factors working together.

Mineral elements content

Radiation and pH had the same effects on mineral elements as they did on the other parts of the algal cell. The majority of mineral elements had the highest values in the current data at (6.5 pH + 1 wm-2 radiation). As pH and radiation levels increased, all mineral elements were shown to vanish. This is explained by the alga's inability to carry on with cell production and by the failure and termination of all other processes, including absorption capacity. Alternatively, the algae may perish when exposed to the previously indicated quantities and times. In line with the decline in the alga's mineral content, the alga's ash content also clearly decreased. The low proportion of ash collected may potentially be impacted by the lack of a cell wall, which is in line with the results of (28-29).

CONCLUSION

Proteins, carbohydrates, fats, fatty acids, and mineral elements are among the chemical components of Asterococcus sp. algae. The results of the study of the interaction of two factors together (pH and radiation) revealed that some factors had a positive (synergistic) interaction effect on the chemical content, which resulted in a decrease in the concentrations of these chemical components, particularly when the pH and the duration of radiation exposure increased to the highest value (10+3 wm-).

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