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The Effect of pH on the Accumulation of Astaxanthin in Haematococcus Pluvialis in the Induction Stage

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Abstract: In order to explore the influence of Haematococcus pluvialis on the accumulation of astaxanthin in the medium pH environment during the induction phase, two algae species bank algae liquids were selected (the algae species bank established by Shanghai Guangyu Biotechnology Co, Freshwater Algae Species Bank of Chinese Academy of Sciences) through the method of comparative experiment to explore the medium composition (modified BG11) in the same ratio, the light intensity is $240\mu mol \cdot m^{-2} \cdot s^{-1}$, the temperature is 15~25°C, through citric acid and hydroxide Sodium solution is used to adjust the algae environment with a small gradient of pH 6.5, 7, and 7.5 respectively. Regularly take the algae fluid to detect and record the absorbance value and the average cell radius of Haematococcus pluvialis cells, observe the color of the algae fluid, the induction effect, and record the red blood cell data generated by Haematococcus pluvialis, and then use the least squares curve to simulate Collect data to show the accumulation of astaxanthin and subsequent experimental verification. It is concluded that the astaxanthin accumulation effect of the freshwater algae species bank with a pH of 6.5 under this comparative environment is better than that of the other two pH environments, and the freshwater algae species bank algae fluid has a better astaxanthin accumulation effect in this environment Guangyu's conclusion of the species library algae liquid.

Keywords: Haematococcus Pluvialis, Astaxanthin, pH

1. Introduction

Haematococcus pluvialis is a kind of ubiquitous green algae belonging to the order of Volvox, Haematococcus family. It is a freshwater single-celled green algae that can synthesize and rapidly accumulate astaxanthin under various stress conditions. At present, in order to increase the yield of astaxanthin produced by Haematococcus pluvialis, the stress induction method is mainly used. A large number of studies have shown that the environmental factors affecting the growth of Haematococcus pluvialis and the accumulation of astaxanthin include light intensity, culture temperature, pH value of the algae solution, nutrients in the culture medium, and reactor type, among which pH value is one of the key factors [1-2]. Through preliminary preparation experiments and consulting relevant literature, it is known that Haematococcus pluvialis is conducive to the production of natural astaxanthin in the environment of pH 6~8 [3-5]. By studying the effects of three different pH media on the growth and astaxanthin accumulation of Haematococcus pluvialis, this paper explores which pH media under these three experimental conditions can quickly enter the astaxanthin accumulation stage and finally obtain higher cell density and astaxanthin accumulation.

2. Experimental Design

2.1. Materials and Reagents

2.1.1. Algae Species

Two types of Haematococcus pluvialis species were used in the experiment: one was the algae species bank established by Shanghai Guangyu Biotechnology Co, Ltd. (abbreviated as the species bank of Guangyu algae), and the other was the freshwater algae species provided by the Institute of Aquatic

Biology, Chinese Academy of Sciences. (Referred to as freshwater algae species bank). The culture of algae liquid is divided into two stages: one is the expansion stage of algae cells, and the other is the induction stage of algae cells.

2.1.2. Medium Composition

The medium used for cultivating Haematococcus pluvialis is a modified BG11 culture formula. The ingredients are shown in Table 1:

Table 1. Medium composition table.

chemical composition	Solution concentration
$NaNO_3$	1500gl/L
$K_2HPO_4 \cdot 3H_2O$	40gl/L
$MgSO_4 \cdot 7H_2O$	75mg/L
$Cacl_2 \cdot 2H_2O$	36mg/L
$C_6H_8FeNO_7$	6mg/L
$C_7H_8O_7$	6mg/L
EDTA	1mg/L
Na_2CO_3	20mg/L
A5+Co	1ml/L

This is a normal medium. The experiment uses the induction method of reducing nitrogen and adding salt. The medium used in the astaxanthin accumulation stage does not add sodium nitrate solution, and the added sodium chloride concentration is 1g/L. The reagents used to adjust the pH of the medium (measured with an electronic pH meter) are: sodium hydroxide solution and citric acid solution.

2.2. Induction Protocol

The induction experiment was conducted twice, and each experiment took three sets of the same samples to prevent interference from special cases of the experiment. The measured data was also the average value of the three same samples: the first time the algae solution and freshwater The algae species library algae liquid was induced separately, and 12 500mL conical flasks were taken, and each group took three 150mL bottles of algae liquid in the expansion stage, according to the ratio of algae liquid and culture medium (BG11 without sodium nitrate solution) 1:1 Three bottles of 300mL algae solution were prepared by induction, and the pH environment of the three bottles of algae solution were 6.5, 7, and 7.5 respectively. The second experiment used the phosphorus species and freshwater algae species as variable setting control experiments. Add appropriate citric acid or sodium hydroxide solution to adjust the two kinds of algae species pools to adjust the pH of each group to 6.5, 7, and 7.5 respectively. A total of 6 bottles of 300 mL of Haematococcus pluvialis liquid in the induction stage.

The conical flask was placed on the photobiological culture frame (Figure 1), and the light intensity was set to $240 \mu mol \cdot m^{-2} \cdot s^{-1}$. The culture was carried out at 15~25°C [6]. In order to prevent the precipitation and adherence of algae, it was placed on a shaker with a given frequency of 100 r/min [7]. On average, 3~5 mL algae solution was taken from each conical flask every two days for data recording, and the change of algae solution was observed [8].
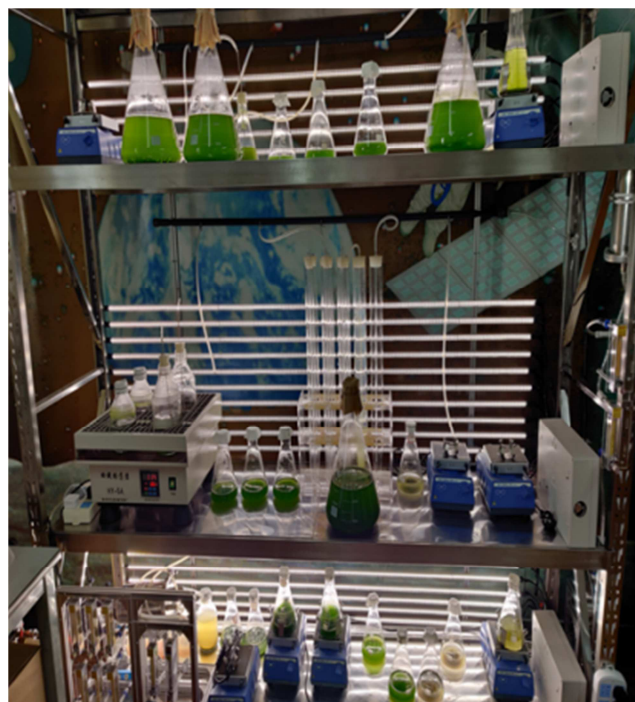

Figure 1. Photobiological culture rack.

2.3. Detection Method

1. Record the absorbance value of the algae liquid, and measure the astaxanthin content of Haematococcus pluvialis cells in the induction stage with a grating spectrophotometer. The grating spectrophotometer is highly practical and accurate. It is performed in the visible light region. Standard instrument for general chemical colorimetric analysis [9-10].
2. Record the radius of the algae cells, use an inverted microscope to observe the cell morphology, cell density and cell size of Haematococcus pluvialis during the induction phase. The visual field image can be displayed on the computer screen, which is convenient for recording the changes of the algae cells in each algae liquid.

2.4. Algae Liquid pH Control Method

The pH value of the control solution is controlled by a single-chip microcomputer as the main controller. The current pH value of the solution is detected by a pH probe and compared with the set value. The pH value of the solution is controlled by programming the PID control of the single-chip microcomputer. The control flow chart is shown in Figure 2 Show:

By comparing the set value with the pH value of the solution measured by the sensor, when the error is greater than 0.1, the pH value needs to be corrected. Since the pH value of the solution needs a stable time, the detection frequency is set to detect once every 3 hours, so as to realize Haematococcus pluvialis The pH value of the solution is stable.
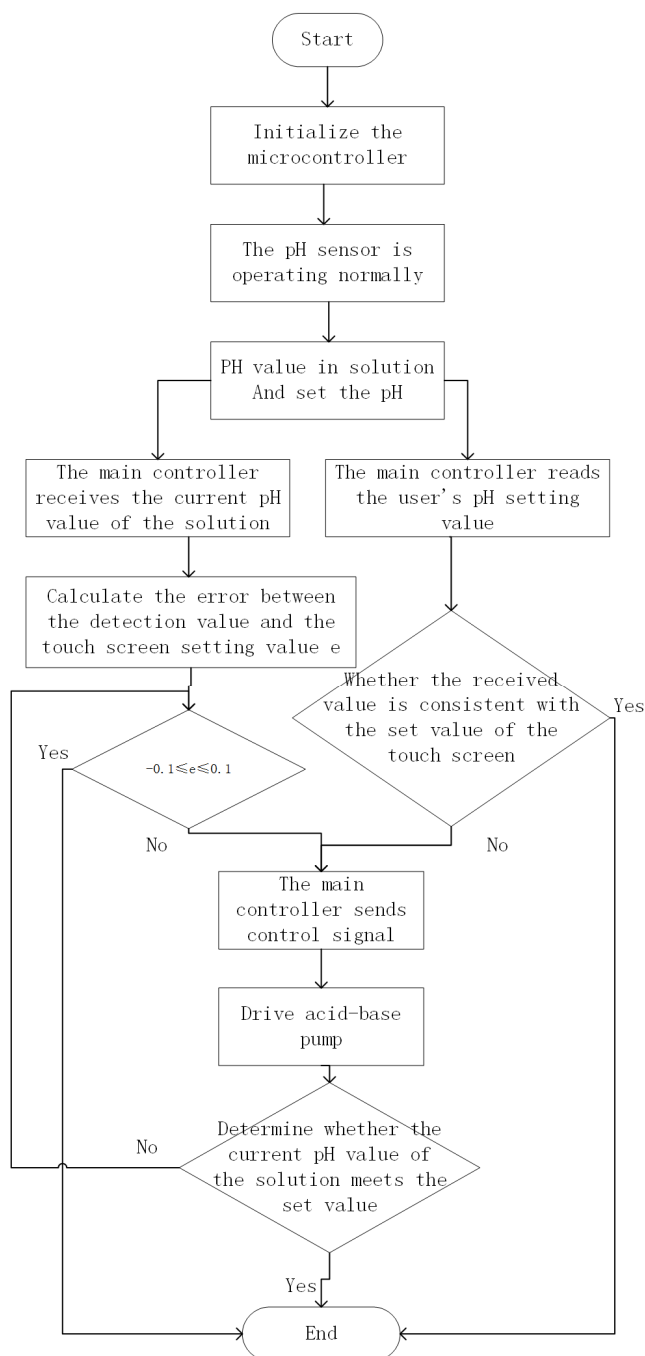
Figure 2. *Flow chart of solution pH control.*

of 681nm, which is the absorption peak of chlorophyll in Haematococcus pluvialis. The two satisfies a linear relationship between the optical density 0.1~1.1: OD681=2×10-6N+0.0327, where N represents the density of Haematococcus pluvialis swimming cells (units/mL). The minimum variance R^2=0.9891, the correlation is significant, so the OD value of the culture can be used to determine the cell density [11]. The following trend chart 3 was obtained by measuring the OD values of six types of algae liquids over a period of twenty days.

For the freshwater algae species bank, the early absorbance value of the algae fluid with a pH of 6.5 increased faster than others, but on the 25th day of the experiment, the freshwater algae species bank with a pH of 7 reached an ideal absorbance. Value, the other two bottles stop rising when the wavelength reaches 3.01. It can be seen that the growth state of the freshwater algae species bank algae solution is obviously better than that of the other two flask algae cells when the pH value is 7 in the induction phase.

It can be seen from Figure 7 that the pH value of 7 is obviously better than the pH values of 7.5 and 6.5 for the algae liquid of the Species library of Spectacles Species. The absorbance values of the three algae liquids with different pH values began to increase synchronously on the ninth day of the experiment and reached a steady level of 3.214.

3.2. Data Compilation of Algae Red Blood Cell Radius

The average cell radius in Haematococcus pluvialis liquid can best reflect the cell growth and state, and the average cell radius of each algae liquid for forty days is recorded.

The method of calculating the average radius in the experiment: shake the culture flask, take 1~3mL of algae liquid and observe under an inverted microscope, and display the field of view image on the computer screen, select a relatively uniform viewing angle and record eight to twelve cell radii And calculate the average radius of the cell. The selected cells should meet the conditions that are as clear as possible and are normal in size and not dense, so as to avoid special cases that affect the direction of experimental data [12].

By observing the change in the average radius of red blood cells produced by Haematococcus pluvialis in 40 days: the overall change trend of the cell radius of the six bottle algae sap is similar, and the general trend is that the cell radius of the cells that have just undergone the induction experiment has decreased after about ten days. After more than twenty days, it tends to a relatively stable average cell radius. By testing the cell density of Haematococcus pluvialis, it can be seen that the number of cells in each algae fluid changes with time. Small, the cell radius gradually tends to a stable value in the later stage. The accumulation effect of astaxanthin mainly depends on the number and size of astaxanthin-containing red blood cells produced during the induction phase. The changes in red blood cell radius data can be obtained through a period of recording. Figure 4:
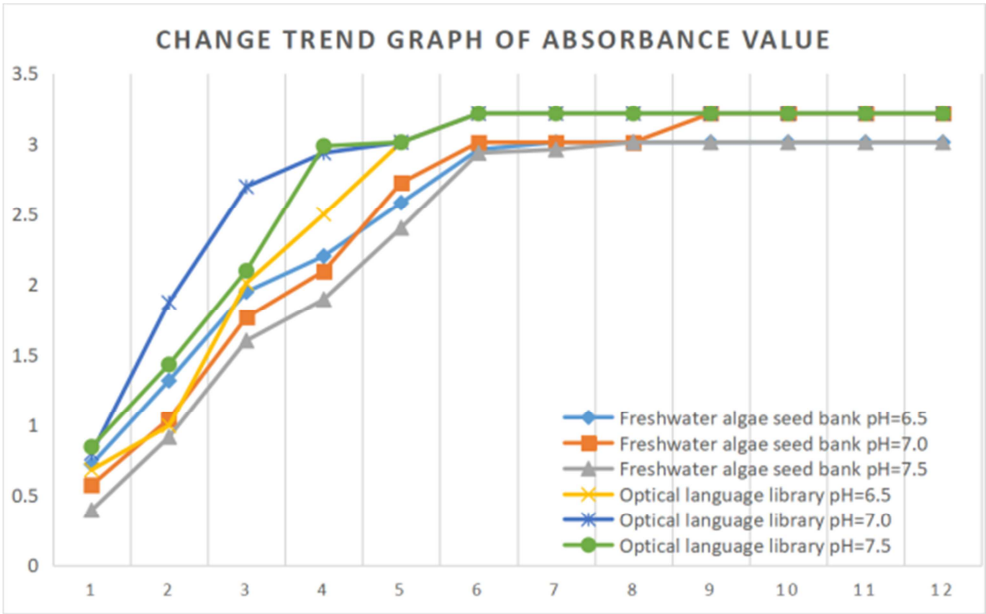
3. Experimental Data Detection

3.1. Data Record of Absorbance Value

For the growth determination of Haematococcus pluvialis, the absorbance value (OD value) is the degree of absorption of light of the corresponding wavelength. The algae can absorb the absorbance of the corresponding wavelength. The logarithmic growth culture of algae cells is fully shaken and mixed, and then placed in CARY UV- Scanning the visible spectrum absorption curve on the visible spectrophotometer, only an obvious absorption peak was found at the wavelength

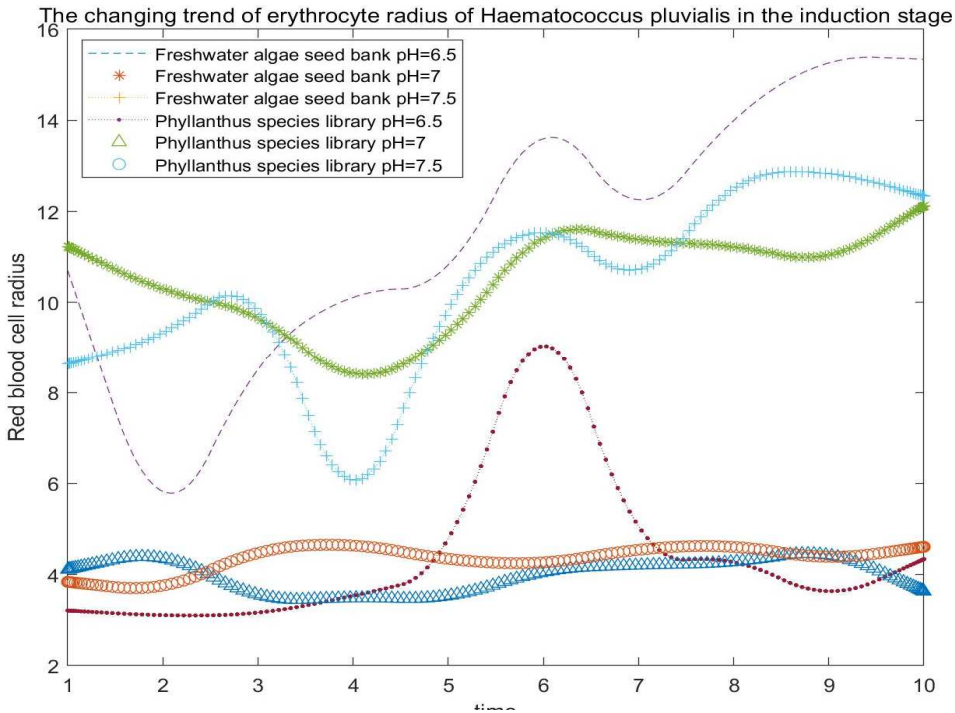**Figure 3.** *Change trend graph of absorbance value.*

Figure 4. *Chart of the average radius of red blood cells of Haematococcus pluvialis.*

Figure 8 is a graph showing the trend of the radius of astaxanthin-containing red blood cells produced by six bottles of Haematococcus pluvialis algae liquid. Six bottles of Haematococcus pluvialis were observed to contain astaxanthin only on the tenth day after the completion of the induction phase. Haematococcus pluvialis, by observing the cell state of the algae liquid and sorting the red blood cell radius trend chart in the algae liquid, and comparing the number of red blood cells or the number of red blood cells in the astaxanthin accumulation stage of the algae liquid of the phytococcus under the same environment during the experiment The erythrocyte radius is generally not as good as

that of freshwater algae species bank astaxanthin in the accumulation stage of red blood cells.

Comparison of the red blood cell parts of the three bottles of algae fluid in the middle of the experiment: it can be seen that the average radius of the red blood cells produced after 20 days of the freshwater algae seed bank algae fluid induction experiment reached 10μm or more, but through the data comparison method, under this condition, the fresh water The number or average radius of the red blood cells produced by the algae pool algae solution under pH=6.5 environment is slightly better than that of the other two pH environments.
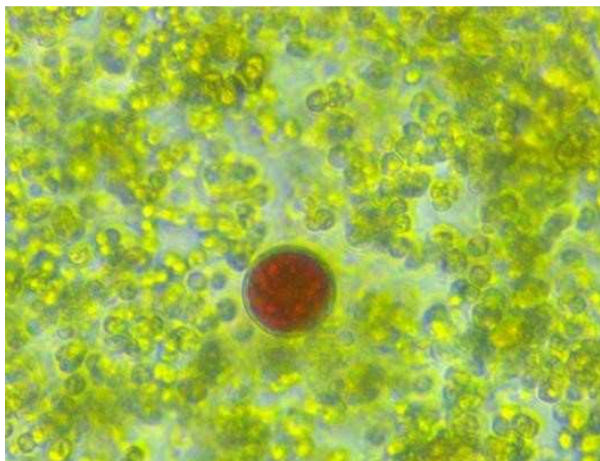
Figure 5. Freshwater algae seed bank pH=6.5 algae liquid.

4. Least Squares Curve Fitting

4.1. Choice of Fitting Algorithm

The experiment is a short-term prediction. After sorting and analyzing the previous data, it is found that the red blood cell radius growth trend can be approximated as a curve in the short term, and the least squares method is used for curve fitting through comparison.

4.2. Principle of Least Squares Curve Fitting

Curve fitting: Given the horizontal and vertical coordinates of a set of points, it is necessary to draw a curve (or straight line) as close to these points as possible for further processing or analysis of the relationship between two variables. The process of obtaining this curve equation is curve fitting. The fitting curve is selected according to the principle of the smallest sum of squares of deviations, and

the binomial equation is adopted as the method of fitting the curve, which is called the least squares method [13].

Give data points $pi(xi, yi), i = 1, 2, …, m$. Find the approximate curve y=φ(x). And minimize the deviation of the approximate curve from y=f(x). The deviation of the approximate curve at point pi is

$$\delta i = \varphi(xi) - y, \quad i = 1, 2, …, m.$$

Fit the polynomial by setting is

$$y = a_0 + a_1 x + a_2 x^2 + … + a_k x^k.$$

By using the following least square difference fitting method:

$$\min_{\varphi} \sum_{i=1}^{m} \delta_i^2 = \sum_{i=1}^{m} (\varphi(x_i) - y_i)^2$$

Expand available:

$$R^2 = \sum_{i=1}^{n} [y_i - (a_0 + a_1 x + … + a_k x^k)]^2$$

The red blood cell radius in the algae liquid of the freshwater algae species bank with the three pH values is curve-fitted by the least square method, and the red blood cell change trend in a period of time in the future is predicted, and the discrete data and the graphical trend of the fitting function are drawn using MATLAB. The prediction effect map of Figure 6 is obtained by fitting the data multiple times.

The curve of multiple fittings is compared with the original data graph curve. When 90% of the output is the same, the last 20% of the data is basically in line with the actual situation. It can be seen from the predicted curve that the freshwater algae species under the same conditions Under the condition of pH=6.5 in the algae solution, both the current data and the future forecast trend are relatively good.
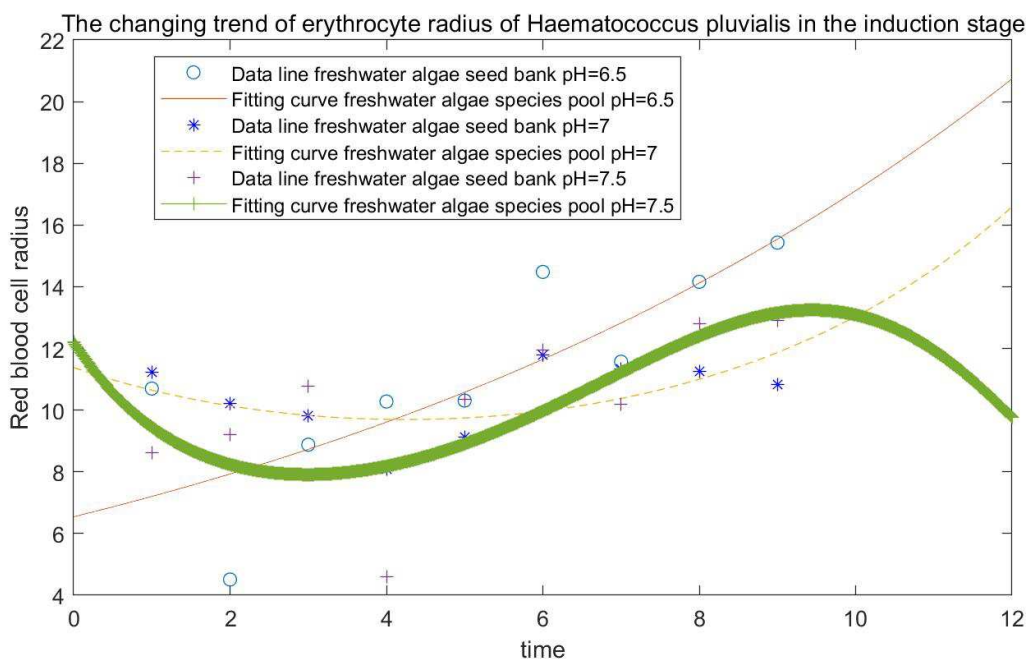

Figure 6. Trends of prediction of erythrocyte radius of algae liquid in freshwater algae species bank.

Integrate and predict the average red blood cell radius data of the freshwater algae species bank, and then observe the cell radius data and the state of the algae fluid state of the freshwater algae species bank algae fluid and the algae fluid in the light whisper algae under the same pH=7 environment.

When the induction phase lasted for more than forty days, the freshwater algae species bank algae fluid had turned yellow-brown, but the whole of the species bank algae fluid was still green. Moreover, the total number of red blood cells and the average radius of the algae liquid of the species library are far less than the red blood cells of the freshwater algae species library, which shows from the side that the freshwater algae species library has a better red blood cell accumulation effect under this condition.
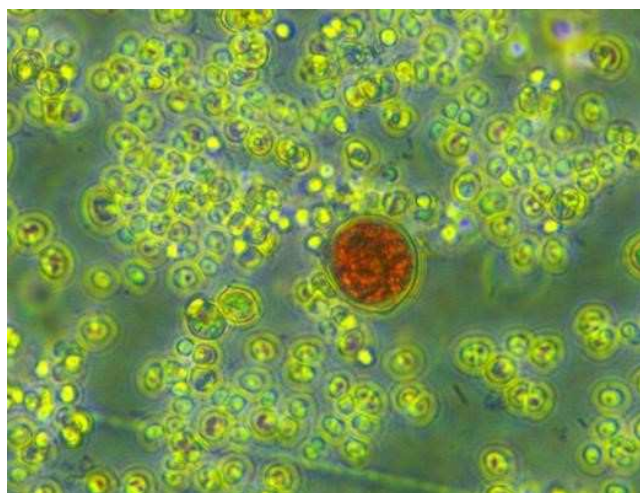

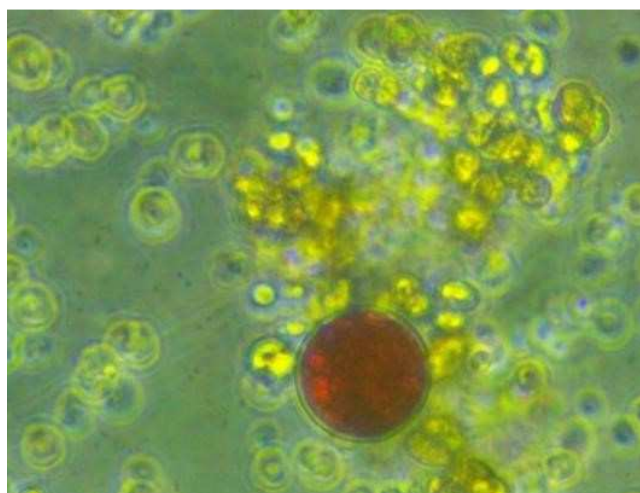*Figure 7. pH value of freshwater solution = 7.5.*

Figure 8. pH value of freshwater solution = 6.5.

In order to verify the accuracy of the prediction fitting results, the freshwater algae species bank algae solution was sampled again after 60 days. Under the same environment, the freshwater algae species bank algae solution with a pH of 6.5 Haematococcus pluvialis produces red blood cell radius better than others. Figure 7 and Figure 8 show the effect diagrams of the algae liquid under two pH environments.

5. Conclusion and Summary

Under the conditions of light intensity $240\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperature of 15~25°C in the culture environment, the algae solution and freshwater algae species and culture medium (BG11 are not included). (Containing sodium nitrate) 1:1 ratio method for nitrogen reduction and salt induction, and the induced algae solution is adjusted with sodium hydroxide solution or citric acid solution to adjust the pH value, so that the two sets of pH are 6.5 and 7 respectively. 7.5 freshwater algae species library algae liquid and light language algae species library algae liquid.

Data such as the absorbance value of the algae fluid, the average radius of the algae fluid cells, and the average radius of the red blood cells containing astaxanthin were sorted into corresponding trend graphs for comparison and analysis. The following conclusions can be drawn under the above experimental environment:

1. Under the same conditions of freshwater algae seed bank algae liquid in the way of nitrogen reduction and salt addition, in the algae liquid pH value of 6.5, 7, 7.5 three different environments, the pH environment of 6.5 freshwater algae seed bank algae liquid astaxanthin accumulation effect is better than the other two pH environments.

2. For the fresh-water algae liquid and the photoalgae liquid, under other same conditions, the induction was conducted according to the ratio of raw liquid to BG11 medium (without sodium nitrate) of 1: 1 and the addition of sodium chloride concentration of 1 g/L. The accumulation effect of astaxanthin induced by fresh-water algae liquid was significantly better than that induced by photoalgae liquid.

In summary, Haematococcus pluvialis was cultured at a light intensity of $240\mu mol \cdot m^{-2} \cdot s^{-1}$ and a temperature of 15~25°C. Under the induction conditions of nitrogen reduction and salt addition, astaxanthin accumulation was carried out in the algal cells of Haematococcus pluvialis in the environment of pH 6.5.

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