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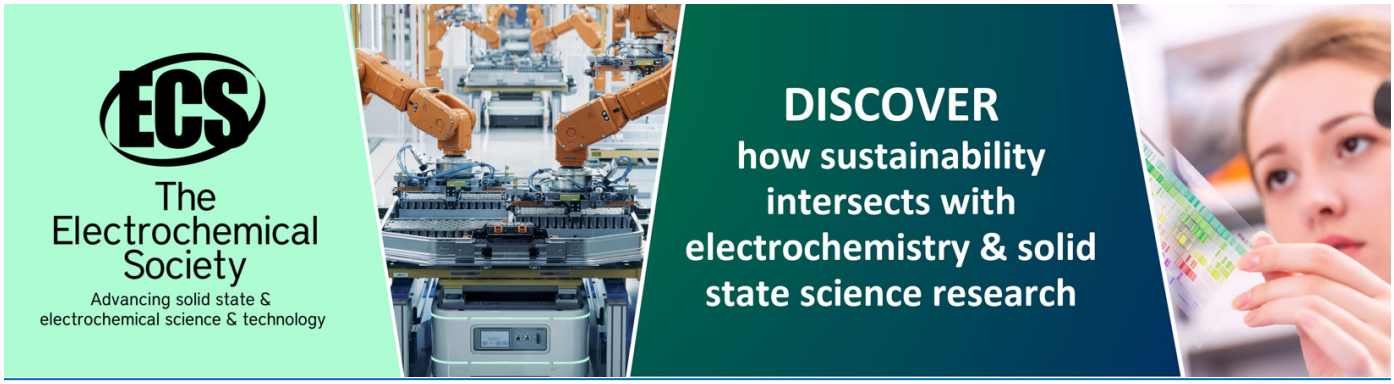

Grey relational analysis to explore the culture factors of Haematococcus pluvialis

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Abstract. Astaxanthin has superior antioxidant capacity, and Haematococcus pluvialis is recognized as the most powerful organism to accumulate natural astaxanthin in nature. In artificial culture, environmental factors can affect the yield of astaxanthin. In order to reduce blindness in the cultivation of Haematococcus pluvialis and effectively increase astaxanthin production, grey relational analysis was used to calculate the correlation between the culture factors and the growth condition of Haematococcus pluvialis, and advantage analysis was performed to investigate the influence degree of different culture factors on the growth and astaxanthin accumulation of Haematococcus pluvialis. The results showed that the effects of light intensity and temperature were greatest during the proliferative culture stage, while the effects of temperature and light duration were greatest during the astaxanthin induction stage. The application of grey relational analysis also provides a convenient reference tool for the design of research to improve the yield of astaxanthin induced by Haematococcus pluvialis, and other culture factors affecting astaxanthin yield can be analyzed and compared using this method, providing more ideas for optimizing culture methods and developing culture devices.

1. Introduction

Astaxanthin is a kind of red ketone carotenoid with super antioxidant activity. It is often used as an additive in aquaculture feeds for fish and shrimp[1]. In recent years, its application in food, pharmaceuticals, cosmetics and advanced nutritional supplements is also on the rise[2]. The research on the artificial culture and extraction of astaxanthin from Haematococcus pluvialis, which is recognized as the organism with the highest natural astaxanthin content in nature, is of far-reaching significance. It is also a hot topic in the field of natural astaxanthin production in the world in recent years[3,4]. It is very important to study the technology of culturing high quality Haematococcus pluvialis and producing astaxanthin efficiently. The analysis of the influence degree of culturing factors can reduce the blindness of culturing Haematococcus pluvialis and provide a reference for improving the yield of astaxanthin efficiently. The high value of Haematococcus pluvialis is accompanied by the difficult cultivation and production technology. Only a few companies have actually mastered the technology of mass production of astaxanthin by Haematococcus pluvialis. At present, about 10 companies in the world, mainly from China, the United States, Israel, Japan and India, have achieved commercial cultivation of Haematococcus pluvialis, while the rest of the countries and enterprises are mostly in the research and development stage. Natural astaxanthin is in short supply in the market.

Haematococcus pluvialis has special biological characteristics. Under favorable growth conditions, the cells are green, mobile cells that divide and grow continuously; when the growth environment is harsh, the cells gradually stop swimming while generating a thick cell wall and begin to accumulate astaxanthin inside the cell, becoming a large red cell state. In production, the "two-step method" is generally adopted for cultivation[5]. It has been shown that culture factors such as temperature, light intensity, pH, and photoperiod affect the growth, astaxanthin accumulation and the ability to resist oxidation has a significant impact[6,7]. The analysis of the effects of each factor provides a basis for further research, but the extent of the respective effects of multiple culture factors when they act together is not yet known. Insufficient reference information is available for practical studies such as quantifying the cultivation index of Haematococcus pluvialis and increasing the yield of astaxanthin.

Based on previous studies, this paper sets up multiple environmental factor variables for experimentation, and uses grey relational analysis to explore the influence of different culture factors on the growth of Haematococcus pluvialis and the accumulation of astaxanthin. It provides directions for studying the growth of Haematococcus pluvialis and increasing the production of astaxanthin, and reduces the blindness of designing and improving the cultivation scheme.

2. Grey relational analysis

The system of uncertainty where part of the information is known and part of it is unknown is called "grey system". The grey system theory, founded by Chinese scholar Prof. Julong Deng, is based on the analysis, modeling, prediction, decision-making and control of abstract systems[8]. At present, it has been successfully applied in engineering control and complex ecosystem. For the growth of Haematococcus pluvialis, the experimental results show that changing the culture environment will affect the culture effect, but the complex change process is not clear when cells are affected. So the system between the growth environment and the growth status of Haematococcus pluvialis belongs to the gray system.

In order to study the extent of environmental influence on the growth of Haematococcus pluvialis, factor analysis of their growth systems is required. The common methods include principal component analysis, regression analysis and other factor analysis. However, these methods will have many shortcomings, such as the need to provide a large number of data, low accuracy of calculation and possible abnormal interference. In gray systems theory, relational analysis is used to measure the degree of association between factors based on the degree of similarity or dissimilarity in their developmental dynamics. The method is based on trends in the data, so there is no need to provide a large number of sample data, the selection of samples is not too restrictive, the calculation is small and the stability of the analysis results is high. Therefore, this paper uses the grey relational analysis method to analyze the growth environment and growth status of Haematococcus pluvialis, so as to explore the influence degree of each factor.

According to the grey relational axiom and calculation method proposed by Prof. Julong Deng, when analyzing the correlation of gray system data, it is necessary to select the characteristic behavior series as a reference and the relevant factor series as a comparison. Assuming that m sets of data were collected, each with n subseries, the characteristic behavior sequence x_0 and the correlated factor sequence x_i are represented by equation (1) and equation (2), respectively.

$$x_0=\{x_0(1),x_0(2),\cdots,x_0(m)\} \tag{1}$$

$$x_{\mathrm{i}}=\{x_i(1),x_i(2),\cdots,x_i(m)\},i=1,2,\cdots,n \tag{2}$$

The correlation coefficient between the correlated factor sequence x_i and the characteristic behavior sequence x_0 reflected in the k-th group of data can be calculated by equation (3).

$$\xi(x_0,x_i)=\frac{\min\limits_i\min\limits_k|x_0(k)-x_i(k)|+\rho\max\limits_i\max\limits_k|x_0(k)-x_i(k)|}{|x_0(k)-x_i(k)|+\rho\max\limits_i\max\limits_k|x_0(k)-x_i(k)|} \tag{3}$$

Among them, $\max\limits_i\max\limits_k|x_0(k)-x_i(k)|$ and $\min\limits_i\min\limits_k|x_0(k)-x_i(k)|$ are the maximum and minimum range respectively. $\rho\in[0,1]$ is the resolution coefficient, which reflects the magnitude of the resolution.

According to the relational coefficient of each sequence, the gray relational degree r_i of each sequence x_i to sequence x_0 can be calculated by equation (4).

$$r_i = \frac{1}{n}\sum_{k=1}^{n}\xi_i(k) \tag{4}$$

Using the results of grey correlations, information that is otherwise not easily accessible can be synthesized and processed in a unified way, so that the pros and cons of the various factors in the system can be analyzed.

3. Experimental method

The seeds of Haematococcus pluvialis used in the experiment were purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology. They were cultured in Tianjin Key Laboratory of Information Sensing and Intelligent Control as laboratory culture materials.

3.1. Schematic design

Control groups were set up with four culture conditions: temperature, light intensity, pH and daily light duration, and all 480 experimental samples were cultured in a constant light incubator. The corresponding BG11 medium was used for the two culture stages of Haematococcus pluvialis. Before adding algal solution to the medium, the pH value of the medium was adjusted with standard solutions of HCl and NaOH. Use a digital pH meter to measure and label the pH of the medium for each vial of samples. Each vial of samples was prepared as a 30 mL mixture based on a 1:1 ratio of Haematococcus pluvialis solution to medium.

Temperature conditions were set between 18°C and 32°C with 2°C intervals for the control. The constant temperature light incubator is equipped with a DC-driven LED light, which can effectively prevent the growth of Haematococcus pluvialis from being affected by pulsed light such as PWM drive. The samples were divided into four light duration control groups, which were set to provide 8 hours, 12 hours, 16 hours and 24 hours of light every day as the control conditions of light duration. The astaxanthin induction phase requires the use of high-intensity light for induction, so a control range of light intensities with different intervals was used for the two phases. The light intensity conditions were set between 10-40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a control every 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the proliferative culture phase and between 170-240 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a control every 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the astaxanthin induction phase.

3.2. Sample data collection

Results were sampled in an ultra-clean workbench up to day 10 of the culture to avoid interference with algal cell growth by sampling midway through the process. Take 15mL each from the experimental sample for data measurement. The proliferation culture experiment samples measured algal cell dry weight and average radius data. Induction experiment samples measured algal cell dry weight and astaxanthin content data.

4. Results and analysis

4.1. Experimental results

From the measurement results, 8 groups of samples corresponding to different temperatures, light intensity, pH and daily light duration are selected uniformly so that the influence of each environmental variable can be expressed. The data selected for the two cultivation stages of Haematococcus pluvialis are shown in Table 1 and Table 2 below.

Table 1. Selection of data for proliferative culture stage.

	Dry Weight ($g \cdot L^{-1}$)	Average Radius (μm)	Temperature (°C)	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH	Light Duration (h)
Sample 1	0.22	1.76	18	10	7.5	8

Sample 2	0.36	2.41	22	15	7	12
Sample 3	0.47	2.92	26	20	8.2	16
Sample 4	0.56	3.34	30	25	6.7	24
Sample 5	0.43	2.53	20	30	7.3	8
Sample 6	0.62	3.26	24	35	8.1	12
Sample 7	0.77	3.44	28	40	6.5	16
Sample 8	0.39	2.81	32	15	7.7	24

Table 2. Selection of data for astaxanthin induction stage.

	Dry Weight ($g \cdot L^{-1}$)	Astaxanthin accumulation (mg)	Temperature (℃)	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH	Light Duration (h)
Sample 1	0.52	19.33	18	170	8.1	8
Sample 2	0.62	23.21	22	180	7.3	12
Sample 3	0.85	26.67	26	190	6.8	16
Sample 4	1.24	33.23	30	200	7.4	24
Sample 5	0.64	24.46	20	210	6.5	8
Sample 6	0.83	27.53	24	220	7.1	12
Sample 7	1.32	34.95	28	230	6.5	16
Sample 8	1.78	36.69	32	240	8.3	24

4.2. Grey relational analysis

In the data collected during the proliferative culture stage of Haematococcus pluvialis, the characteristic behavior sequences is the sequences of average radius and dry weight; during the astaxanthin induction phase, the characteristic behavior sequences is the sequences of astaxanthin accumulation and dry weight. Sequences of correlates for the two phases of data were temperature, light intensity, pH, and light duration. Since the sequences have different magnitudes, they should be normalized when calculating the correlation coefficients in order to reduce the influence of large differences in the data on the calculation results. For sequence $x=\{x(1),x(2),\cdots,x(n)\}$, sequence $\overline{x}$ calculated according to equation (5) is the result of dimensionless initialization.

$$\overline{x}=\left(1,\frac{x(2)}{x(1)},\cdots,\frac{x(n)}{x(1)}\right) \tag{5}$$

According to the basic idea of grey relational analysis, the similarity degree between the shapes of the series curves is compared to determine whether the factors are closely related to each other. The point line diagram after data processing in the two stages is shown in Figure 1 and Figure 2 respectively. From Figure 1, it can be seen that the curve shape of the dry weight at the proliferation culture stage is more similar to the curve shape of light intensity and light duration, and the curve shape of the average radius is more similar to the curve shape of temperature and light duration. As can be seen in Figure 2, the curve shape of the dry weight during the astaxanthin induction phase is more similar to the curve shape of temperature and daily light duration, and the curve shape of astaxanthin accumulation is more similar to the curve shape of temperature and light intensity.

Substituting the processed sequence into equation (3) and equation (4), the relevance degree of each sequence in the two culture stages of Haematococcus pluvialis is calculated as shown in Table 3 and Table 4 respectively. Ranking the correlations in the table gives the degree of influence of each factor on the culture status.

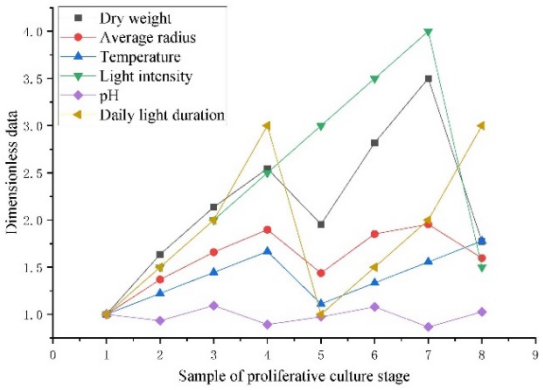

Figure 1. Dimensionless data point line diagram of proliferative culture stage.

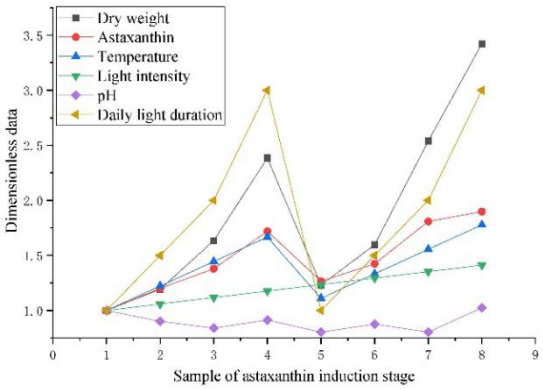

Figure 2. Dimensionless data point line diagram of astaxanthin induction stage.

Table 3. Relevance degree of proliferative culture stage.

	Temperature	Light Intensity	pH	Light Duration
Dry Weight	0.6869	0.8185	0.5786	0.7025
Average Radius	0.8134	0.6615	0.6542	0.7426

Table 4. Relevance degree of astaxanthin induction stage.

	Temperature	Light Intensity	pH	Light Duration
Dry Weight	0.6869	0.8185	0.5786	0.7025
Average Radius	0.8134	0.6615	0.6542	0.7426

4.3. Advantage analysis

There is more than one characteristic behavior sequence and related factor sequence of the data from two cultivation stages of Haematococcus pluvialis, and the superiority analysis is needed. The correlation matrix R_1 of the growth and culture stage of Haematococcus pluvialis and the correlation matrix R_2 of astaxanthin induction stage are shown in equation (6) and equation (7), respectively.

$$R_1=\begin{bmatrix}0.6869 & 0.8185 & 0.5786 & 0.7025\\ 0.8134 & 0.6615 & 0.6542 & 0.7426\end{bmatrix} \tag{6}$$

$$R_2=\begin{bmatrix}0.7706 & 0.7210 & 0.6198 & 0.8021\\ 0.8801 & 0.7511 & 0.5748 & 0.6576\end{bmatrix} \tag{7}$$

From the correlation matrix R_1, it can be seen that r_{12}=0.8185 is maximal at the proliferation culture stage, indicating that light intensity has a significant impact on dry weight. And r_{21}=0.8134 is next to r_{12}, indicating that the temperature has the greatest influence on the cell radius. Therefore, the light intensity and temperature are better than the light duration and pH in the proliferative culture stage. Similarly, from the correlation matrix R_2, it can be concluded that in the astaxanthin induction stage, the factors of temperature and light duration are better than those of light intensity and pH.

In the two correlation matrices, the sum of the elements in the first column is greater than the sum of the elements in the other columns, followed by the sum of elements in the second column. Therefore, the culture of Haematococcus pluvialis and the induction process of astaxanthin are both influenced by a combination of environmental factors, with temperature being the paramount factor, followed by light intensity.

5. Conclusion

In this paper, the grey relational analysis was used to calculate and analyze the correlation between the growth environment and growth status of the two cultivation stages of Haematococcus pluvialis, and to explore the influencing factors of the growth and astaxanthin accumulation of Haematococcus

pluvialis. After several experimental measurements and selection of data for analysis, the results of the analysis were consistent. Therefore, in the experiment and research of culturing Haematococcus pluvialis and increasing astaxanthin production, the research of light intensity and temperature reasonable setting should be increased in the proliferation culture stage, and astaxanthin induction stage should focus on temperature and daily light duration, so that the experimental research is more targeted.

The culture cycle of Haematococcus pluvialis is long, and it has strict environmental requirements. Natural astaxanthin products in the market are in short supply. How to improve the astaxanthin yield is a pressing challenge. Through the analysis of the correlation between various factors and characteristics, it provides a simple and feasible tool for the research and design of astaxanthin production. It also reduces the blindness in the research process of cultivation of Haematococcus pluvialis, and is more conducive to make new progress and breakthrough in the batch production of natural astaxanthin.

Acknowledgments
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