Effect of Magnetic Field on Protein and Oxygen-production of Chlorella Vulgaris

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Abstract

Chlorella will be affected by the magnetic field during its growth. A gradient method is applied to compare effects of the magnetic field on the growth of chlorella cultured primarily. Magnetic treatment of chlorella indicates that chlorella is affected by the magnetic field with weaker strength to some extent, for example, protein content of chlorella increases as well as oxygen dissolution in chlorella solution. With increasing of the magnetic field strength, the effect decreases. During the treatment , if the strength is 0.24T, protein and oxygen are produced properly; if it is 0-0.18T, protein content in chlorella trends to increase; if it is 0.24-0.30T, maximum content of protein appears. Relationship between incremental differences of oxygen content in chlorella solution and magnetic field strengths is y=-63.889x2+30.988x-1.6507. Research indicates that when magnetizing time is 15-20 minutes, the growth of chlorella is affected positively by the magnetic field.
Key Words: chlorella, magnetic field, protein, oxygen dissolution.

1 Introduction

1.1 Purpose of research

Chlorella spp is a unicellular alga of chlorophyta chorella which appeared on the earth as earlier as 2 billion years ago. It appeared in a spherical or elliptical shape with diameter of 3～12 μ m. There are various kinds of chlorellas which appear both in fresh water and sea water and are also grown in an artificial culture medium. There are about 15 kinds of known chlorellas in the world now and about more than one hundred with its variations. Common kinds appeared in our country are chlorella vulgaris, chlorella ellipsoidea and chlorella py-renoidosa. As chlorella appears extensively and grows easily and rapidly, it's a good material for biotechnological research. And it's nutritious as a good source of single cell protein (SCP). Therefore, chlorella can be applied widely and its development forms a microalgal biotechnology combined with the development of spirulina, salty algal and corallina---a new branch of biotechnology field[1-2].

1.2 Research and development

Wang Haiying[3] from Food and Biological Engineering Institute of South China University of Technology cultured chlorella with a circulating magnetic treatment in a steady electromagnetic field, studied changes of its growth, colorant and element accumulation and discussed the possible mechanism of electromagnetic field effect of chlorella. Research indicated that different strengths of electromagnetic field have different effects on the growth of chlorella, the magnetic treatment has no effect on the colorant composition of chlorella cell but only changes its content, functional group of chlorella cell doesn't change and the magnetic treatment has greater effect on the accumulation of microelements in chlorella, especially metallic ions. Li Zhiyong[4] cultured spirulina with a self-developed strengthening light reactor of magnetic field and found that proper magnetic field treatment could stimulate the growth of spirulina, culturing period of spirulina shortened 2 to 3 days, protein content increased and microelements of Sr,Ni,Cu,Mn and Zn all increased significantly. Wang Depei[5] cultured spirulina at 0.1T of magnetic induction density and found that amylose content increased. And research by Hirano[6] also indicated that magnetic field could accelerate the growth of spirulina and sugar content and algae protein increased with the light.

Impacts of magnetic field on organisms are very complicated. Material molecules are composed of a large number of atoms and electronics. Under an external constant magnetic field, a magnetic force, electric effect and magnetic energy level split will be applied on the material in the magnetic field. In the magnetic field, some energy levels can be split to form more sublevels. Therefore, energy level distribution, biochemical reaction and electron transfer are affected. The split torque of magnetic energy level is directly proportional to the magnetic field strength[7].All these magnetic force, magnetic force torque, magnetic resonance and electric effect above will cause metabolization in the biological cells and movements of electrons and ions, activities of free radicals and protein and enzyme of magnetic atoms and permeation of biofilms as well as changes of cell growth.

2 Experimental materials and testing methods

2.1 Experimental materials

Chlorella for experiments: ordinary sea water chlorella pyrenoidosa provided by the aquatic organism laboratory of Dalian Ocean University.

Water for experiments: tap water, pure water and sea water are applied. Pure water is Wahaha pure water, tap water is from laboratory domestic water and sea water is from sand filtered water in Heishijiao.

Experimental drugs: Conway nutrient, $MnSO_4$ solution, KI—NaOH solution, concentrated H_2SO_4, Bovine Serum Albumin (BSA), Coomassie Brilliant Blue G—250, etc.

Magnetic environment equipments for experiments: electromagnet constant current power, recirculated water refrigeration units.

Others: OPTIZEN2120UV spectrophotometer, ultrasonic processor and centrifugal machine.

2.2 Culturing of algae for experiments

Sand filtered sea water is filtered by an acetyl cellulose filtering membrane of 0.45m and is high-pressure sterilized. Sea water is mixed with Conway nutrient in the proportion of 1000:1 at room temperature, shaked for 4 times every day and extended-cultured periodically.

2.3 Testing methods

During the experiment, algal solution（3million/ml）to be treated is prepared as per the required density and extracted by the siphon to avoid chlorella with precipitation entering and causing errors of experimental data. 75ml of it is extracted into the small mouth bottle each time and treated in the magnetic field.

Changes of different magnetic fields at the same time: chlorella solution for culturing is placed in the magnetic field and cultured at different magnetic field strengths of 0.12T, 0.18T, 0.24T, 0.30T, 0.36T, 0.42T and 0.48T. Their dissolved oxygen and protein contents are measured respectively. Space between two magnets is 10cm and the time is 15 minutes.

Changes of different times at the same magnetic field: chlorella solution for culturing is placed in the magnetic field (0.24T) for the times of 15 minutes, 30 minutes, 45 minutes and 60 minutes. Their dissolved oxygen and protein contents are measured respectively. Space between two magnets is 10cm and the strength is 0.24T.

2.3.1 Measuring methods of protein content of chlorella solution

Coomassie Brilliant Blue Method is applied to measure protein content of chlorella solution. Coomassie Brilliant Blue G-250 can interact with protein through Van der Waals' adsorption to form a compound of protein and Coomassie Brilliant Blue---a kind of blue solution. The position of maximum adsorption λmax of this dye is moved and it is reached at 595nm. Therefore, measuring sensitivity of protein can be improved greatly. Differences of solution colors are applied to measure colors. Coomassie Brilliant Blue G-250 has the features of stable colors and high sensitivity. Minimum content of protein is about 1υm.

Testing procedures:

1. Plotting standard protein curves

(1) Prepare 13 tubes. One is taken as a backup and others are divided into two groups, in which samples, water and agents are added in order. That is 0.1mg/ml protein solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0ml are added into the tubes respectively and deionized water is added to 1.0ml respectively. Coomassie Brilliant Blue G-250 of 4.0ml is added into the tubes respectively. Each tube should be shaked evenly at once after adding (to avoid more gas bubbles being formed).

(2) After adding agents for 2 to 5 minutes, a color plate is applied. Light adsorption values of samples at 595nm measured by the spectrophotometer are A595. The blank is for the first tube, that is 4.0mlG－250 is added into 1.0ml of H_2O.

(3) Take the standard protein quality (mg) as the X-axis and absorbance value A595 as the Y-axis to plot a standard curve. From this standard curve, in terms of measured value A595 of chlorella, protein contents of unknown samples can be determined. Graphic matching equation is: $y=0.3833x$

2. Measuring protein content of chlorella

(1) Cultured chlorella solution is sucked into 12 tubes by use of a siphon with each tube of 10ml. They are put into the centrifugal machine for centrifuging of 20 minutes (2000r/min).

(2) Dehydrated chlorella is collected with 10ml of deionized water (pure water) added and wall-broken with an ultrasonic processor for 20 minutes.

(3) Chlorella solution with wall broken is centrifuged for 20 minutes (2000r/min).

(4) 1.0ml supernatant of centrifuged chlorella solution is taken out. 4.0ml Coomassie Brilliant Blue G-250 is added into the supernatant to measure its A595 value and its protein content in terms of the standard curve.

2.3.2 Measuring methods of dissolved oxygen of chlorella solution

Iodimetry method is applied to measure dissolved oxygen. $MnSO_4$ solution and KI—NaOH solution are added into treated chlorella solution and dissolved oxygen in the solution is conversed into the brown precipitation of trivalent manganese compound quantitatively. With starch as the indicator, I_2 produced during the above reaction is titrated standardly with $Na_2S_2O_3$ and dissolved oxygen in water is calculated, that is $nNa_2S_2O_3:nO_2=4:1$

Testing procedures:

1. $MnSO_4$ solution of 1ml and KI—NaOH solution of 1ml are respectively added into a bottle of chlorella solution (about 75ml)with known volume of V_1. After covering with a cap(without air), there is even precipitation at the bottom of the bottle.

2.1ml of concentrated H_2SO_4 is added to precipitate and dissolve. All of it is poured into an iodimetry bottle and titrated with $Na_2S_2O_3$ to light yellow. Then 1ml of starch is added till the liquid is titrated to no color and volume of V is derived.

3. Content of dissolved oxygen in chlorella solution is calculated.

Equation of dissolved oxygen in water:

$$DO(mg/L) = (8.000 \times 1000CV)/V_1 \qquad (1)$$

C is the concentration of $Na_2S_2O_3$ (mol/L) .

3 Effect of magnetic field on chlorella

3.1 Effect of magnetic field on chlorella protein

3.1.1 Effect of changes of magnetic field on chlorella protein as the time is the same

Chlorella is treated at different magnetic fields for the same time(30min). Absorbance of its algal solution is measured. Protein content of chlorella is calculated with the standard protein curve. X-axis is regarded as the added magnetic field strength which also stands for changes of the magnetic field and Y-axis is regarded as the protein content, shown as Fig.1.

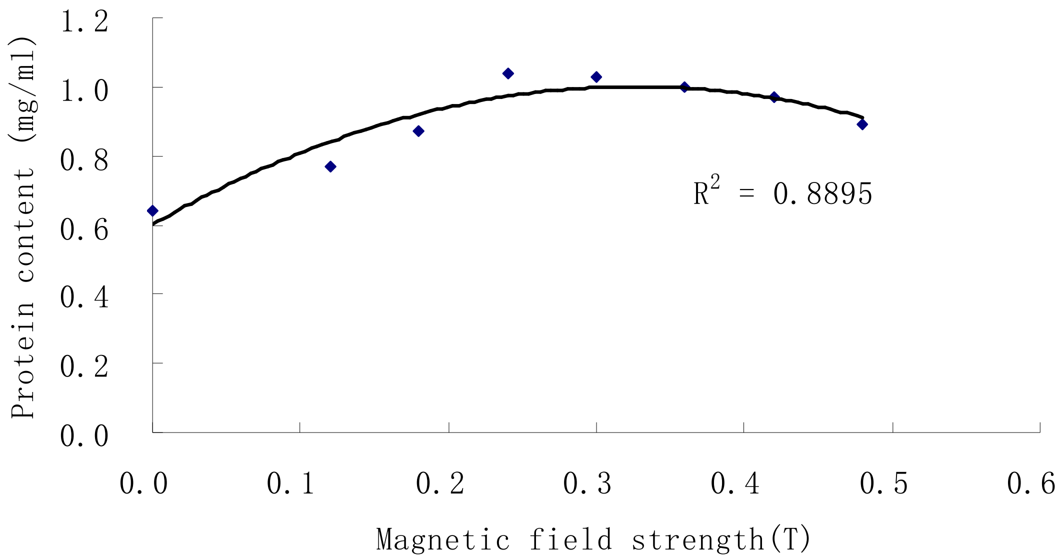

Fig.1 Effect of changes of the magnetic field on chlorella protein as the time is the same

Fig.1 matches the mathematical model of related coefficient R^2 = 0.8895, $y = -3.7533x^2 + 2.4474x + 0.6017$, it shows that when the magnetic field strength is 0~0.24T, protein content tends to increase and when the magnetic field strength is 0.24~0.30T, maximum protein content of chlorella is reached. That means weak magnetic field strength tends to increase protein content, however, when the magnetic field strength increases to a certain value, protein content of chlorella won't be affected greatly.

3.1.2 Effect of time changes on chlorella protein at the same magnetic field strength

Chlorella is placed in the magnetic field (0.24T). Absorbance of its algal solution is measured for a period of time (15minutes) and its protein content is calculated with a standard protein curve. X-axis represents the added magnetizing time while Y-axis represents protein content, shown as Fig.2.

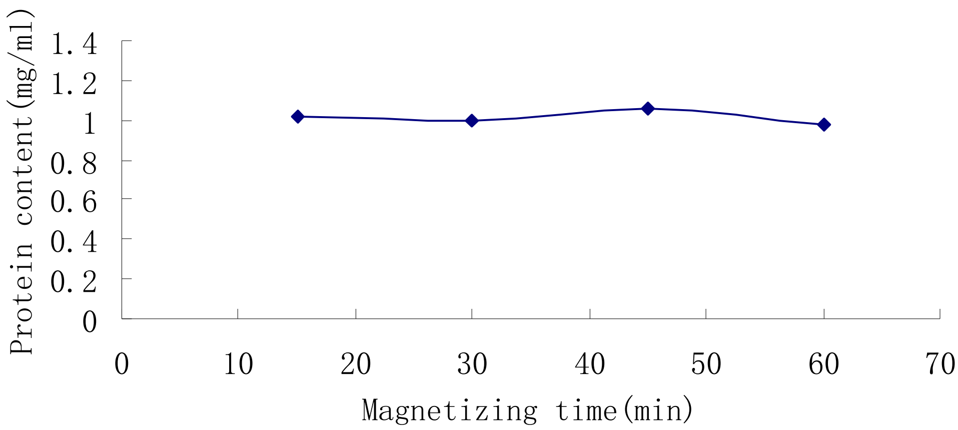

Fig.2 Effect of time changes on protein content of chlorella at the same magnetic field strength

Fig.2 indicate that protein content of chlorella still remains about 1.0mg/ml no matter what the magnetizing time is. Therefore, protein content of chlorella has nothing to do with the magnetizing time.

The above indicates that weak magnetic field strength tends to increase protein content, however, when the magnetic field strength increases to a certain value, protein content of chlorella won't be affected greatly and the magnetizing time has no impact on protein content in algal solution. Therefore, treatment of chlorella solution by magnetic field has no great effect on protein content.

3.2 Effect of magnetic field on dissolved oxygen of chlorella

Oxygen production of plant reflects its photosynthesis directly. Dissolved oxygen (Do) is the content of oxygen dissolved in water, in terms of milligrams of oxygen per liter water. Content of dissolved oxygen in water is affected by many factors, such as water temperature, dissolved ions and organisms. Well, dissolved oxygen in nutritious water is mainly controlled by the biological process. Therefore, as the number of algae increases to a certain order of magnitude, its number and its life activities must control the change of dissolved oxygen in water. Dissolved oxygen is one of the important factors for water purification. High content of dissolved oxygen is helpful for degradation of various contaminants in water to purify water faster, however, if the content of dissolved oxygen is lower, contaminants in water will be degraded more slowly.

3.2.1 Effect of change of magnetic field on dissolved oxygen of chlorella as the time is the same

Chlorella is placed in different magnetic fields for the same time (2h) to measure dissolved oxygen of its solution. Testing results are shown as Fig.3.

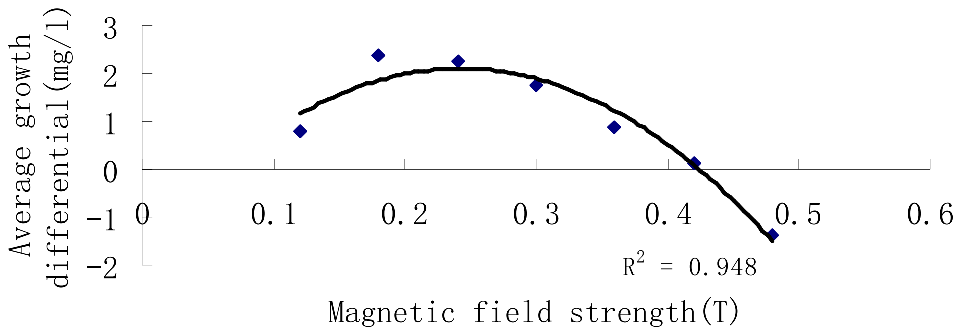

Fig.3 Relationship between magnetic field and dissolved oxygen of chlorella as the time is the same

The above shows that whether chlorella is treated or not, after 2 hours, at a certain ambient condition, dissolved oxygen tends to increase. But, if magnetic field strengths of treating are different, growth differential of dissolved oxygen are different, tending to increase from positive growth to negative growth. Take X-axis as the current added to the two ends of the magnetic field to represent changes of the magnetic field and Y-axis as the growth differential of dissolved oxygen compared with comparing groups. Plot curves of growth differential of dissolved oxygen and magnetic field strengths, shown as Fig.3. It matches the mathematical model of related coefficient R^2=0.948, y=-63.889x^2+30.988x-1.6507.

3.2.2 Impact of time changes on dissolved oxygen of chlorella at the same magnetic field

Chlorella is placed in the magnetic field(0.24T) to measure dissolved oxygen in it every 15 minutes. Testing results shows: For both treated groups and untreated groups, dissolved oxygen tends to increase after 2 hours at a certain ambient condition. However, if treating time is different, growth differential of dissolved oxygen changes differently without evident regularity. Take y as the growth differential of dissolved oxygen compared with comparing groups and x as the treating time. Plot the curves of the growth differential of dissolved oxygen and the treating time, shown as Fig.4.

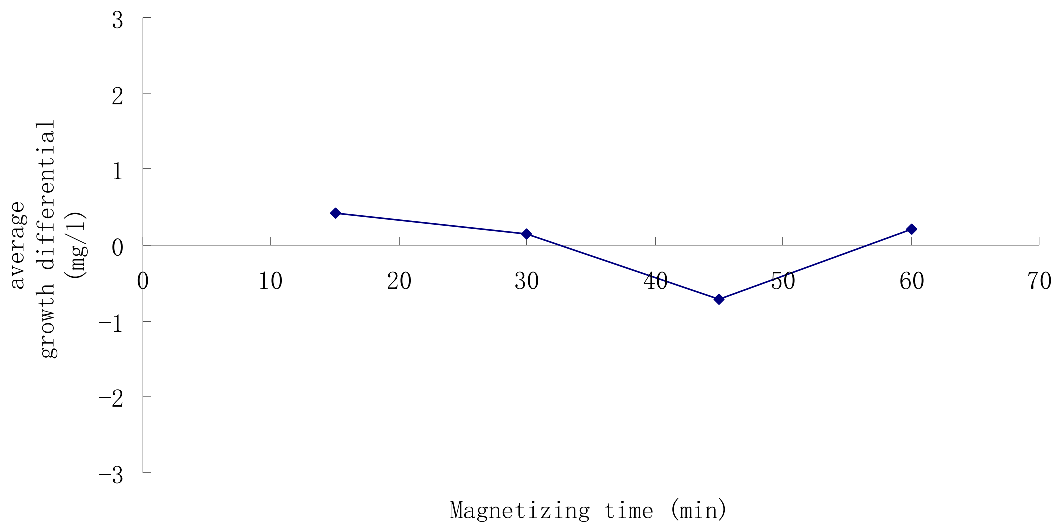

Fig.4 Curve of treating time and dissolved oxygen of chlorella at the same magnetic field

If the number of chlorella changes evidently per unit water, dissolved oxygen of water will change after chlorella solution is impacted by a certain magnetic field strength. For algal solution in a sealed container, oxygen in water can only come from photosynthesis of chlorella. Chlorella is a kind of "photoautotrophy" organism which can converse water, CO2 and inorganic nutrient salt into organic matter required by its metabolism through absorbing light energy and emit oxygen . Photosynthesis reaction formula of algae is as follows: [8]

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ + \text{light energy}$$
$$\longrightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$$

The curve of Fig.3 shows: as the magnetic field strength is about 0.24T, maximum dissolved oxygen of chlorella solution is reached, that means its oxygen produces best at this time.

4 Conclusions

The paper discusses chlorella pyrenoidosa which is treated with the magnetic field to measure changes of chlorella.

We come to the following conclusions through summary and analysis of testing results.

1. Magnetic field has a certain effect on protein content of chlorella, especially weak magnetic field can speed up production of protein in chlorella. When the magnetic field is 0-0.24, protein of chlorella tends to increase and when the magnetic field is 0.24-0.3T, maximum protein of chlorella is reached. However, when the magnetizing time is 15-20min, the magnetic field has no impact on protein of chlorella. Impact of the magnetic field on chlorella protein doesn't increase with the magnetizing time.

2. Dissolved oxygen of chlorella solution is affected by the magnetic field to some extent. At 0-0.24T of magnetic field strength, the magnetic field tends to increase dissolved oxygen, at about 0.24T of magnetic field strength, the magnetic field tends to stimulate dissolved oxygen and at more than 0.24T, the magnetic field tends to inhibit dissolved oxygen. When the magnetizing time is 15-30min, the magnetic field has some impact on dissolved oxygen of chlorella. Impact of the magnetic field on dissolved oxygen of chlorella doesn't increase with the magnetizing time.

The above indicates that magnetic field has some effect on chlorella growth. At weaker magnetic field strength, the magnetic field can accelerate production of protein and oxygen. At about 0.24T of magnetic field strength, maximum protein and oxygen appears. With increasing of magnetic field strength, the magnetic field has less impact on chlorella growth, even inhibiting its growth. Therefore, we get a conclusion that at 0.24T of magnetic field strength, chlorella grows best and at 15-30min of magnetizing time, the magnetic field is ensured to stimulate chlorella growth.

Analysis indicates that weaker magnetic induction density can speed up chlorella growth, increase nutritional value of chlorella and improve health function of chlorella furtherly with less energy. As a new microalgae culturing and enhancing technology, it is promising in its future application.

References

[1]Chen Ying, Li Wenbing, Sun Yongru: The current status and prospect on the research and application of chlorella's biological technology, *J. Biological Engineering Development*, **18**(6)(1998), 12-16.

[2]Li Yonghan: Aquatic Feeding Attractant Biology, Dalian Press, 2000.

[3]Wang Haiying, Guo Siyuan, Zheng Bisheng: Accumulating nutrient in chlorella vulaaris enhanced by magnetic-treatment, *J. Food Science and Technology*, **8**(2004),7-11.

[4]Li Zhiyong, Guo Siyuan, Li Lin: Effects of magnetic field on the nutrition of spirulina platensis and mechanisms analysis, *J. Acta Biophysica Sinica*, **17**(3)(2001), 587-591.

[5]Wang Depei, Gao Daxiong: Preliminary research of the effect of magnetic field on suger produced by spirulina platensis, *J. Microbiology Bulletin*, **1**(25)(1998),17-19.

[6]HiranoM, OhtaA, AbeK: Magnetic field effects on photosynthesis and growth of the cyano bacterium Spirulina platensis,*J. Journal of Fermentation and Bioengineering*, **86**(3)(1998)313-316.

[7]Wang Haiying, Guo Siyuan, Zheng Bisheng: Accumulating nutrient in chlorella vulaaris enhanced by magnetic-treatment, *J. Food Science and Technology*, **8**(2004),7-11.

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