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硕 士 学 位 论 文

# 高产虾青素的雨生红球藻培养基及培养模式的若干研究

# Studies of cultures medium and cultures modes of *Haematococcus pluvialis* for enhanced production of astaxanthin

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# 中文摘要

虾青素以其卓越的生理功能，在国际上已被广泛应用于水产养殖、食品及医药业中。雨生红球藻被公认为自然界中生产天然虾青素的最佳生物来源，因此利用雨生红球藻获取虾青素，无疑具有广阔的发展前景。

本研究建立了一种雨生红球藻中的虾青素酯皂化进行分离测定的反相高效液相色谱方法。虾青素在 15min 内得到了较好的分离，其中虾青素的检出限为 0.049mg/L，在 1-15mg/L 范围内虾青素的含量与峰面积具有良好的线性关系；该方法的精密度（RSD）为 3.27%，回收率为 100.7%。研究首次获得 HPLC 法与 Jensen 分光光度法的关系式 y = 8.496x - 12.237（r=0.9991）。

研究开展了雨生红球藻 BBM 培养基的优化改良。考察了维生素 $B_1$、$B_{12}$、不同形态铁及醋酸钠等多种微量组分对雨生红球藻生长及虾青素积累的影响。研究结果表明：适量维生素、铁等微量组分的加入，具有促进雨生红球藻生长、提高胁迫条件下的微藻存活率以及促进虾青素积累等作用。在此基础上对 BBM 培养基进行正交实验改良，无铁的 BBM 配方基础上添加 1g/L NaAc、0.05ppm 维生素 $B_{12}$ 和 18μmol/L EDTA-$Fe^{3+}$，获得新型培养基命名为 CBBM。此培养基配方培养雨生红球藻的生长指数可达 0.5，虾青素产率为 22.58mg/L（20.43mg/g）。

在国内首次开展了雨生红球藻的半连续培养模式与一次培养模式的比较研究，发现雨生红球藻的半连续培养可使培养第一阶段的生物量增加 2-3 倍，虾青素的总产量至少增加 3 倍左右，藻粉内的虾青素含量亦增加 66%以上。所获藻粉的质量及重量显著提高。此外，藻液内孢子比例大幅下降，普遍低于 40%。

在半连续培养模式下，CBBM 培养基相对原始 BBM 培养基具有提高雨生红球藻藻密度、存活率、藻粉和虾青素产量的效果。其中 10%更新率组存活率为 44%，虾青素产率为 30.1mg/L，虾青素含量为 25.7mg/g，分别较较传统一次培养模式下采用 BBM 培养基对照组提高了 1.2 倍、2 倍和 1 倍；虾青素产率和含量较半连续培养模式下采用 BBM 培养基对照组提高了 1.3 倍和 60%。

本研究还针对几种胁迫方式开展研究发现：高盐度时，雨生红球藻会积累虾青素，同时藻细胞会大量死亡；氮缺乏条件下，可以促进雨生红球藻积累虾青素，同时降低了其孢子比率；蓝光可以促进雨生红球藻生长和虾青素累积，3 000 lx

为蓝光最佳胁迫光强。

关键词：虾青素；雨生红球藻；培养基；培养模式；胁迫条件

# Abstract

Astaxanthin is the main carotenoid pigment in aquatic animals and is widespread in nature world. The fresh-water green unicellular alga *Haematococcus pluvialis* (Chlorophyceae) has become a focus due to its ability to accumulate the high-value pigment astaxanthin. It is generally accepted that *Haematococcus pluvialis* can accumulate the highest levels of astaxanthin (1-3% by dry weight) among the natural creatures and has gained acceptance in aquaculture and other markets as a "concentrated" form of natural astaxanthin. Therefor, the natural carotenoid pigment astaxanthin has become commercially available in aquaculture, poultry feeds and in the worldwide nutraceutical market.

A reversed-phase high performance liquid chromatograph (RP-HPLC) method was developed for the separation of astaxanthin ester that had been saponified from the *Haematococcus pluvialis*. Separation and identification of astaxanthin was completed within 15min.The detection limit and the linear range of astaxanthin was 0.049mg/L and 1-15mg/l, The method was rapid, simple, precite and accurate (precision of the method (RSD) 3.27%, average recovery 100.7%). There is a good linear relationship between the concentration of astaxanthin by HPLC and that by spectrophotometer (y = 8.496x - 12.237, r=0.9991).

The medium improving was studied. By the test experimental, the effect of the Vitamin $B_1$, Vitamin$B_{12}$, different species of iron and NaAc on the two culture stage of *Haematococcus plvialis* was studied. The results showed that: the growth rate and the survival, the static cell ratio, the astaxanthin production under stress condition were improved when the medium (BBM) were enriched with Vitamin $B_1$, Vitamin$B_{12}$ and iron. The medium was improved by adding a few components. When 1g/L NaAc, 0.05ppm Vitamin$B_{12}$ and 18μmol/L EDTA-$Fe^{3+}$ were added in the BBM medium, the growth rate can be achieved 0.5 and astaxanthin concentration was 22.58mg/L( 20.43mg/g). The new medium was named CBBM.

Semi-continuous cultures were compared with batch culture for the production of

astaxanthin and *Haematococcus pluvialis*. In a first stage of semi-continuous cultures obtained a 2 to 3-fold higher biomass of green vegetative cells. Besides, the production and the content of astaxanthin increased over 3 times and 66% in semi-continuous cultures. Furthermore, the quality and the avoirdupois of products were obviously improved. It was of outstanding significance that the ratio of aplanospore was sharply decreased. In the semi-continuous cultures, the ratio of aplanospore was lower than 40%.

In the semi-continuous cultures, the growth rate and the survival, the biomass, the astaxanthin production under stress condition in CBBM were higher than that in BBM.

Effects of stress conditions on astaxanthin accumulation of *Haematococcus pluvialis* were studied. The production of astaxanthin under high salinity was improved, but the survival would fall remarkably. The efficiency of the accumulation of astaxanthin under nitrogen deficiency had apparent predominance. The ratio of aplanospore was sharply decreased. The algae cultured and stressed by blue light accumulated most biomass and astaxanthin compared with other wavelength illumination and blue light is the most suitable stress condition. 3 000 lx is the most suitable stress conditions for astaxanthin accumulation by blue light.

**Key Words**: *Haematococcus pluvialis*; Astaxanthin; Cultures medium; Cultures mode; Stress conditions

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