# 响应面法优化雨生红球藻培养基

# Optimization the Culture Medium of *Haematococcus pluvialis* by Response Surface Methodology

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## Introduction

*Haematococcus pluvialis* is an excellent algae species for production natural astaxanthin. Optimizing the nutrients composition of the culture medium is important to improve the density of algal cells and astaxanthin content. A single-factor test was conducted to screen the optimal concentration range of $NaNO_3$, $MgSO_4 \cdot 7H_2O$, $K_2HPO_4 \cdot 3H_2O$ and $Na_2CO_3$, and the medium was optimized using the response surface methodology and validation tests.

## Data

We showed that:

1）The results showed that the mass concentration of $NaNO_3$, $MgSO_4 \cdot 7H_2O$, $K_2HPO_4 \cdot 3H_2O$ and $Na_2CO_3$, were in the ranges of 1500-2000, 80-640, 20-60 and 20-80 mg·L$^{-1}$, respectively. The most promoting effects on algal cell density were in the order of $NaNO_3$, $Na_2CO_3$, $K_2HPO_4 \cdot 3H_2O$ and $MgSO_4 \cdot 7H_2O$.

2）The optimal nutrient concentrations respectively were 1556.42 mg·L$^{-1}$, 51.43 mg·L$^{-1}$, 42.18 mg·L$^{-1}$ and 358.59 mg·L$^{-1}$. The interaction between $K_2HPO_4 \cdot 3H_2O$ and $MgSO_4 \cdot 7H_2O$ was significant($P$<0.05).

3）Cultured *H. pluvialis* for 8 days in the optimized medium, the *Fv/Fm* of PSII and *Chl-a* content of the algae cells were 0.69 and 7268.97 μg·L$^{-1}$, respectively, which were both significantly higher than the that in the control group, and the cell density was 3.53×10$^5$ mL$^{-1}$, which was significantly higher than the control by 20.48 %($P$<0.05).

## Methods

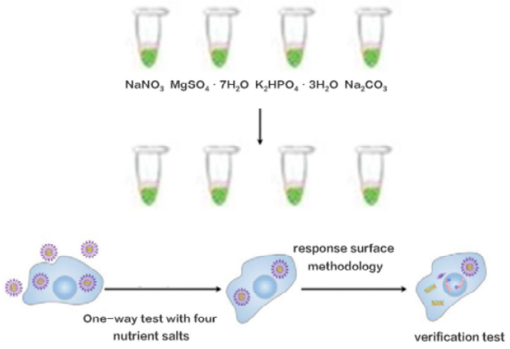



Table 1 Concentrations of nutrients

| 营养盐 Nutrients | 质量浓度 Mass concentration/(mg·L$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $NaNO_3$ | 0 | 750 | 1500 | 3000 | 4500 | 6000 |
| $MgSO_4 \cdot 7H_2O$ | 0 | 40 | 80 | 160 | 320 | 640 |
| $K_2HPO_4 \cdot 3H_2O$ | 0 | 20 | 40 | 80 | 160 | 320 |
| $Na_2CO_3$ | 0 | 10 | 20 | 40 | 80 | 160 |

Table 2 Box-Behnken experimental factor level（mg·L$^{-1}$）

| 水平 Levels | 因素 Factors | | | |
|---|---|---|---|---|
| | A | B | C | D |
| -1 | 1000 | 80 | 20 | 20 |
| 0 | 1500 | 360 | 40 | 50 |
| 1 | 2000 | 640 | 60 | 80 |

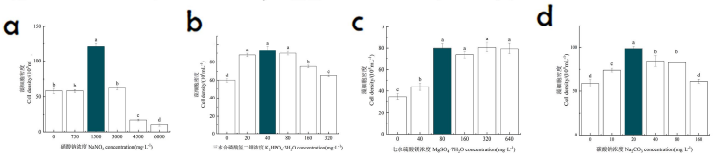
1) 1.00×10$^5$ mL$^{-1}$，培养温度为 21±1 ℃，光照强度为 2500 lux，光照周期是 12 h/12 h（光/暗），培养规模在 250 mL 三角瓶中盛装100 mL 培养基。定时摇瓶 5 次，随机调换位置，尽可能使处理组的光照一致。每个试验做 3 瓶平行对照，每瓶每次取 2 个样测定参数。

2) 经单因素试验法筛选$NaNO_3$, $MgSO_4 \cdot 7H_2O$, $K_2HPO_4 \cdot 3H_2O$ 和 $Na_2CO_3$ 的适宜质量浓度，分别以A、B、C和D代表上述4种营养盐，进行4因素3水平的Box-Behnken设计试验。

3) 验证试验。

## Results

不同营养盐浓度对雨生红球藻生长的影响如图所示。随营养盐浓度升高，细胞密度均呈先上升后下降的趋势。不同$NaNO_3$浓度组间细胞密度存在显著差异（$P$<0.05），1500 mg·L$^{-1}$时细胞密度最高，为1.21×10$^6$ mL$^{-1}$。$MgSO_4 \cdot 7H_2O$浓度为80 mg·L$^{-1}$，细胞密度达到最高，为8.07×10$^5$ mL$^{-1}$，但与其他浓度组无显著差异（$P$>0.05）；$K_2HPO_4 \cdot 3H_2O$和$Na_2CO_3$浓度分别为40 mg·L$^{-1}$和20 mg·L$^{-1}$时，细胞密度最大，分别为9.27×10$^5$ mL$^{-1}$和9.87×10$^5$ mL$^{-1}$，均显著高于其他浓度组（$P$<0.05）。




Note: Different lowercase letters indicate significant differences between different concentrations of the same nutrient（$P$<0.05）.the same below.

Fig.1 Effects of different concentrations of nutrients on the growth of *Haematococcus pluvialis*

e

Table 3 Analysis of variance of regression model

| 变异来源 Source | 平方和 Sum of squares | 自由度 Dy | 均方 Mean square | F value | P value |
|---|---|---|---|---|---|
| 模型 Model | 1032.38 | 14 | 73.74 | 25.62 | <0.0001** |
| A | 21.33 | 1 | 21.33 | 7.41 | 0.0165* |
| B | 0.34 | 1 | 0.34 | 0.12 | 0.7374 |
| C | 5.32 | 1 | 5.32 | 1.85 | 0.1955 |
| D | 13.40 | 1 | 13.40 | 4.65 | 0.0488* |
| AB | 0.45 | 1 | 0.45 | 0.16 | 0.6989 |
| AC | 1.00 | 1 | 1.00 | 0.35 | 0.5650 |
| AD | 1.00 | 1 | 1.00 | 0.35 | 0.5650 |
| BC | 15.96 | 1 | 15.96 | 5.54 | 0.0337* |
| BD | 5.43 | 1 | 5.43 | 1.89 | 0.1913 |
| CD | 2.79 | 1 | 2.79 | 0.97 | 0.3417 |
| $A^2$ | 237.90 | 1 | 237.90 | 82.64 | <0.0001** |
| $B^2$ | 93.90 | 1 | 93.90 | 32.62 | <0.0001** |
| $C^2$ | 82.08 | 1 | 82.08 | 28.52 | 0.0001** |
| $D^2$ | 853.90 | 1 | 853.90 | 296.64 | <0.0001** |
| 残差 Residual | 40.30 | 14 | 2.88 | | |
| 失拟项 Lack of fit | 34.10 | 10 | 3.41 | 2.20 | 0.2328 |
| 纯误差 Pure error | 6.20 | 4 | 1.55 | | |
| 总和 Cor Total | 1072.66 | 28 | | | |

上述模型的方差分析结果如表4所示。该回归方程F值为25.62，模型极显著（$P$ < 0.01）。由显著性水平检验可知A、D、$A^2$、$B^2$、$C^2$、$D^2$和BC对细胞密度有显著影响（$P$ < 0.05）。如表5所示，决定系数$R^2$ = 0.9624，矫正系数$RAdj^2$ = 0.9249。

f

Table 4 Analysis of variance of regression model

| 标准偏差 Standard deviation | 均值 Average value | 置信度 Confidence level | 决定系数 Decision factor | 矫正系数 Correction factor | 精密度值 Precision value |
|---|---|---|---|---|---|
| 1.70 | 25.70 | 6.60 | 0.9624 | 0.9249 | 16.374 |

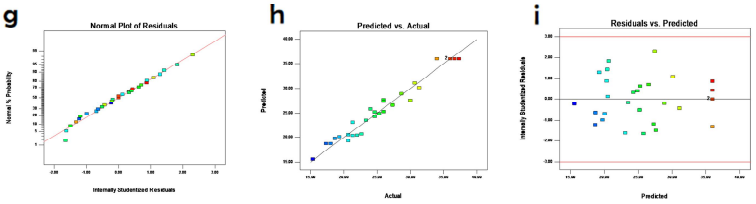



Fig. 2 Distribution of residual normal probability, and residual error and predicted value of the equation, and distribution of predicted and actual value in experiment

通过图2残差分析，残差的正态概率分布图与试验实际值的分布图可得出各散点都靠近同一条直线，说明$NaNO_3$、$MgSO_4 \cdot 7H_2O$、$K_2HPO_4 \cdot 3H_2O$和$Na_2CO_3$拟合性较好。

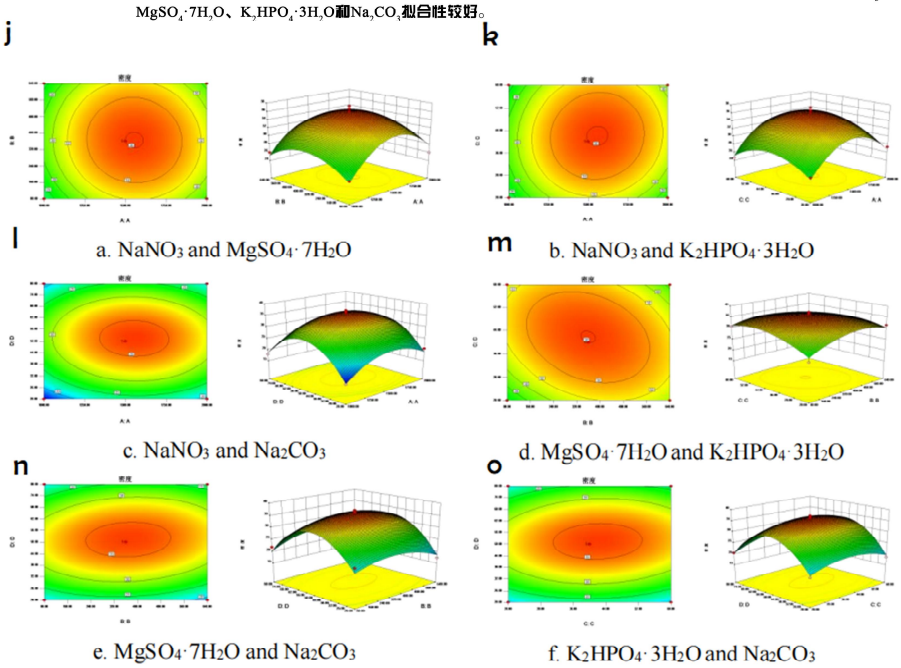



Fig. 3 Contour line and response surface of interaction of various factors on cell density

使用优化后培养基培养雨生红球藻8 d后，细胞密度、*Fv/Fm*和*Chl-a*浓度均极显著高于对照组（$P$ < 0.01），优化组藻细胞密度为3.53×10$^5$ mL$^{-1}$，比对照组提高了20.48 %（对照组为2.93 ×10$^5$ mL$^{-1}$）。*Fv/Fm*和*Chl-a*含量均在第8 d时达到最高，分别为0.69和7268.97 μg·L$^{-1}$，极显著高于对照组（0.65和6244.16 μg·L$^{-1}$）（$P$<0.01）。

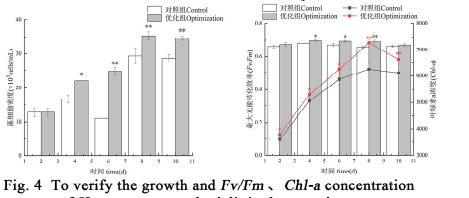



Fig. 4 To verify the growth and *Fv/Fm*、*Chl-a* concentration of *Haematococcus pluvialis* in the experiment

## Conclusion

本研究拟合4因素与响应值之间的函数关系，通过对回归方程分析，优化培养基。考虑多种营养盐交互作用的影响，由点及面更加系统的考虑雨生红球藻最佳生长浓度，结合残差分析、方差分析和等高线图得到各营养盐间存在交互作用，进而得出4因素对提升雨生红球藻细胞密度的最优值，提高雨生红球藻生长速率。

## Referances

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GL, YANG XW, CHENG JY, 2016. Effects of Ammonia Concentrations and Temperatures on *Haematococcus pluvialis* Growth[J]. Journal of Food Science and Biotechnology, 35(02): 136-143.

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