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# 浮游藻类叶绿素荧光产量与光合活性关系实验研究

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**摘要** 以蛋白核小球藻为研究对象，通过毒性胁迫、光照胁迫和温度改变蛋白核小球藻的光合活性，研究蛋白核小球藻叶绿素荧光产量与光合活性参数$F_v/F_m$的变化关系。结果表明：3种不同生长环境下，蛋白核小球藻的叶绿素荧光产量随着$F_v/F_m$改变而发生较为明显变化，最大变化范围为235～668 $(\mu g \cdot L^{-1})^{-1}$；$F_v/F_m$与叶绿素荧光产量之间具有明显负线性相关性，线性优度$R^2$超过0.91。该研究结果为发展更为准确的藻类叶绿素a质量浓度活体荧光检测方法提供了重要依据。

**关键词** 光谱学；浮游藻类；活体荧光法；叶绿素荧光产量；光合活性；浓度检测

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## 1 引 言

浮游藻类是水生态系统的重要初级生产者，其通过光合作用为地球生命系统提供基础的能量来源，在物质循环和能量转化过程中起着重要作用[1]，在水生态系统中具有重要的生态功能，同时有毒藻类的暴发也会威胁水生态安全[2]，因此浮游藻类浓度监测对维护水生态系统正常发展至关重要。传统的浮游藻类浓度检测方法有显微计数法、流式细胞仪计数法、分光光度法、高效液相色谱法、遥感法和活体荧光法等。其中活体荧光法是通过测量活体藻类细胞中叶绿素a分子受激辐射产生的荧光强度来反演叶绿素a的浓度，具有灵敏度高、测量速度快、无需样品预处理、无污染等优点，已成为目前藻类叶绿素a浓度现场快速监测的重要手段。但该方法使用的前提是活体藻类叶绿素荧光产量（单位叶绿素a浓度在单位光强激发下所释放的荧光强度）恒定。众多研究已表明，活体藻类的叶绿素荧光产量是变化的，进而导致叶绿素a浓度的测量存在较大误差。Jakob等[3]研究发现，在相同条件下蓝藻和绿藻的叶绿素荧光产量相差40倍甚至更高；Laisk等[4]研究表明，实验室培养藻类的叶绿素荧光产量与自然河流中藻类的差异较大，实验室校准的荧光仪在测量自然河流藻类叶绿素a浓度时，测量误差达46%；Biswal等[5]研究表明，藻类叶绿素荧光产量随环境的不同而变化显著（相差高达15倍）。藻类活体荧光是细胞捕光色素吸收光能激发叶绿素a分子释放的，其叶绿素荧光产量与活体细胞光合作用过程中光能的吸收、传递和释放效率密切相关[6-7]，叶绿素荧光产量变化可能受叶绿素荧光参数的影响。Pérez等[8]指出藻类活体荧光可能受藻细胞自身光学特性、光合活性等影响。因此，藻类光合活性可能影响叶绿素荧光产量。目前，藻类光合活性对叶绿素荧光产量影响的程度和规律尚不清晰。

本文以蛋白核小球藻为实验藻种，选择最大光化学量子产率$F_v/F_m$（$F_v$通过$F_m-F_0$计算得到，$F_0$为暗适应状态下测得的瞬时荧光强度值，$F_m$为给予饱和光照时测得的最大荧光强度值）作为藻类光合活性表征参数，通过毒性胁迫、光照胁迫和温度来改变蛋白核小球藻的光合活性，研究蛋白核小球藻叶绿素荧光产量与$F_v/F_m$的变化关系，探索活体荧光法测量藻类叶绿素a质量浓度的误差来源，为后续发展准确的藻类叶绿素a质量浓度活体荧光检测技术提供依据。

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## 2　材料与方法

### 2.1　藻种选择与培养

实验所用藻种为蛋白核小球藻（*Chlorella pyrenoidosa*），它是淡水湖库的常见绿藻，在适宜的培养条件下生长状态良好，藻细胞分布均匀，其纯种培养体购于上海光语生物科技有限公司。采用BG11培养基接种藻种，培养过程在恒温摇床（MQD-S3R）中进行，光源为白色冷荧光灯管，设置培养条件如下：温度为（25±1）℃，光照强度为8000 lx，光暗时间比为12 h∶12 h。后续实验研究需要保证藻液培养3天以上，以达到较好的活性状态[9]。

### 2.2　藻类叶绿素荧光产量测量

藻类叶绿素荧光产量是单位叶绿素a质量浓度在单位光强激发下所释放的荧光强度。为了获得准确的叶绿素荧光产量，参照《水质 叶绿素a的测定 分光光度法》（HJ 897—2017）测量样品藻类的叶绿素a质量浓度 $c_{\mathrm{Chla}}$；使用荧光分光光度计（F7000，日本日立公司）测量蛋白核小球藻的三维荧光光谱，如图1（a）所示；选择蛋白核小球藻特征荧光光谱区（激发波长范围为380～500 nm，发射波长范围为660～750 nm），采用荧光区域积分法得到活体藻类的荧光强度 $F$［图1（b）］。由于荧光光谱测量过程中激发条件相同，激发光强度为常数，因此，利用式（1）计算藻类叶绿素荧光产量 $\eta$。实验均配制3组平行样，取平均值作为最终测试结果。

$$\eta=\frac{\int_{660}^{750}\int_{380}^{500}F(\lambda_{\mathrm{ex}}\lambda_{\mathrm{em}})\mathrm{d}(\lambda_{\mathrm{ex}}\lambda_{\mathrm{em}})}{c_{\mathrm{Chla}}E},\tag{1}$$

式中：$E$ 为激发光强；$\lambda_{\mathrm{ex}}$ 为激发波长；$\lambda_{\mathrm{em}}$ 为发射波长。

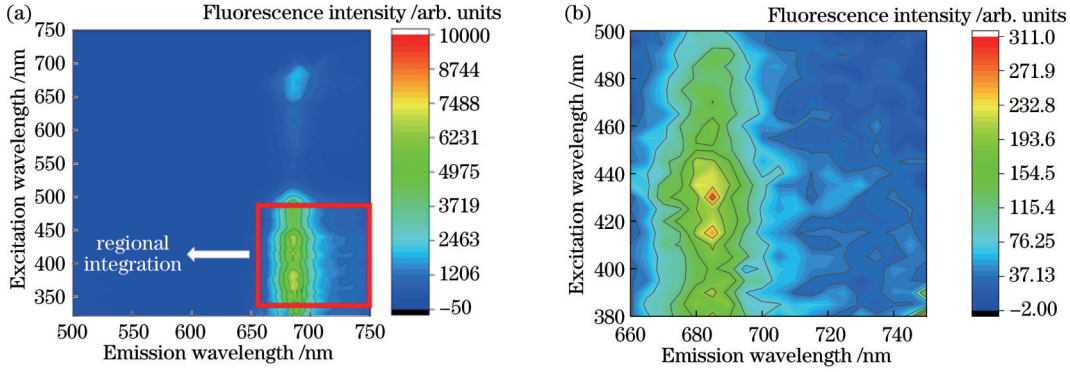


图1　蛋白核小球藻三维荧光光谱。（a）三维荧光光谱；（b）积分区域三维荧光光谱

Fig. 1　Three dimensional fluorescence spectrum of *Chlorella pyrenoidosa*. (a) Three dimensional fluorescence spectrum; (b) three dimensional fluorescence spectrum in integral region

### 2.3　藻类光合活性参数测量

选择最大光化学量子产率 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 作为藻类光合活性表征参数，该参数表征光合反应中心的光能转换效率，是目前使用最多的光合荧光参数[9]。采用ACT2&FastOcean FRRF藻类荧光仪（英国CTG公司）测量 $F_{\mathrm{v}}/F_{\mathrm{m}}$，将藻样品进行15 min暗适应，确保藻样品光合反应中心处于完全开放状态，选择450 nm（蛋白核小球藻的特征激发波长）激发光源，测量叶绿素荧光诱导曲线，利用曲线获得基本参数初始荧光强度 $F_0$ 和最大荧光强度 $F_{\mathrm{m}}$，并代入式（2）计算得到 $F_{\mathrm{v}}/F_{\mathrm{m}}$。实验均配制3组平行样，取平均值作为 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 最终测试结果。生长状态良好的藻 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 值通常在0.6以上。

$$\frac{F_{\mathrm{v}}}{F_{\mathrm{m}}}=\frac{F_{\mathrm{m}}-F_0}{F_{\mathrm{m}}}。\tag{2}$$

## 3　结果与讨论

通过添加毒性胁迫物质，以及控制光照和温度，模拟获得3种不同生长环境下蛋白核小球藻样品，利用上述方法同步测量样品的 $\eta$ 和 $F_{\mathrm{v}}/F_{\mathrm{m}}$。在此基础上，分析蛋白核小球藻叶绿素荧光产量与 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 的变化关系。

### 3.1　毒性胁迫实验

敌草隆（DCMU）是典型的藻类光合作用过程电子抑制剂[10]。将蛋白核小球藻培养液与DCMU标准液按一定比例混合，用去离子水稀释，配制成DCMU质量浓度分别为0、0.57、1.14、4.56、6.84、9.12、11.4、15.96、34.2 μg/L的9个待测藻类样品，在胁迫15 min后测量样品中蛋白核小球藻的荧光强度、叶绿素a质量浓度和 $F_{\mathrm{v}}/F_{\mathrm{m}}$，获得浮游藻类 $\eta$ 和 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 随DCMU质量浓度的变化关系（图2）。3组重复实验中藻的质量浓度分别为71.8、78.9、93.1 μg/L。

图2中阴影部分表示重复实验组的测量结果，实线部分为多组重复实验对应的蛋白核小球藻 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 和 $\eta$ 平均值。可以看出：不同质量浓度蛋白核小球藻在DCMU胁迫下，$\eta$ 与 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 的变化趋势相反；随着DCMU质量浓度增加，$F_{\mathrm{v}}/F_{\mathrm{m}}$ 呈现逐渐下降的趋势，由0.605变化到0.229，下降幅度达62%；叶绿素荧光产量 $\eta$ 呈现逐渐上升的趋势，由245 $(\mu g\cdot L^{-1})^{-1}$ 变化到678 $(\mu g\cdot L^{-1})^{-1}$，上升幅度为177%。此外，3次不同藻

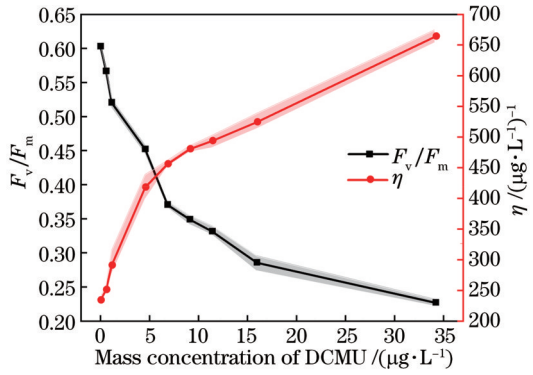


图 2 蛋白核小球藻叶绿素荧光产量和 $F_v/F_m$ 随 DCMU 质量浓度的变化

Fig. 2 Change of chlorophyll fluorescence yield and photosynthetic activity $F_v/F_m$ of *Chlorella pyrenoidosa* with DCMU mass concentration

质量浓度的实验结果变化趋势相同，重复性高，数据波动范围不超过 5%。

将每组实验测得的 $F_v/F_m$ 与叶绿素荧光产量 $\eta$ 进行线性拟合，拟合结果如图 3 所示。可以看出，通过 DCMU 胁迫改变蛋白核小球藻的 $F_v/F_m$，$F_v/F_m$ 与 $\eta$ 之间具有明显的负线性相关性，线性优度 $R^2$ 为 0.9614。

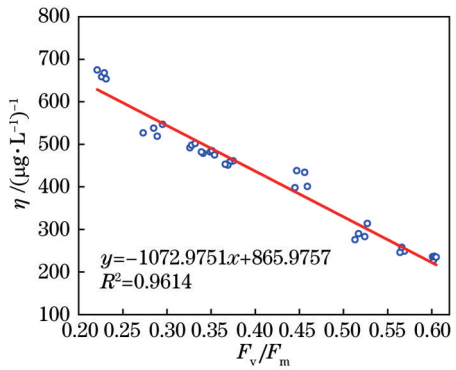


图 3 DCMU 胁迫下蛋白核小球藻叶绿素荧光产量和 $F_v/F_m$ 的相关性

Fig. 3 Correlation between chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* under DCMU stress

### 3.2 光照胁迫实验

使用 MC 1000-8D 藻类在线培养与检测系统（北京易科泰生态技术有限公司）控制藻类培养光照。实验室正常藻类培养光照强度在 5600 lx 左右，设置 5600、11200、16800、22400、28000、33600、39200、44800 lx 8 个培养光照强度，每个样品培养 2 h 后分别重复测量蛋白核小球藻的叶绿素 a 质量浓度、荧光强度和 $F_v/F_m$ 3 次，计算时取 3 次测量结果的平均值。3 组重复实验中藻的质量浓度分别为 71.8、78.9、93.1 μg/L。浮游藻类叶绿素荧光产量 $\eta$ 和 $F_v/F_m$ 随光照强度的变化关系如图 4 所示。从图 4 可以看出：不同质量浓度的蛋白核小球藻在不同培养光照胁迫下培养 2 h，其 $\eta$ 与 $F_v/F_m$ 呈现相反的变化趋势；随着光照强度增加，$F_v/F_m$ 值逐渐下降，$\eta$ 值逐渐上升，且该变化趋势与藻的质量浓度无关；当培养光照强度从 5600 lx 增加到 44800 lx 时，$F_v/F_m$ 由 0.563 降至 0.388，降低了 26% 左右，$\eta$ 由 241 (μg·L$^{-1}$)$^{-1}$ 升至 453 (μg·L$^{-1}$)$^{-1}$，增加了 80%。每组实验结果的变化趋势相同，可重复性高，数据波动范围在 10% 以内。

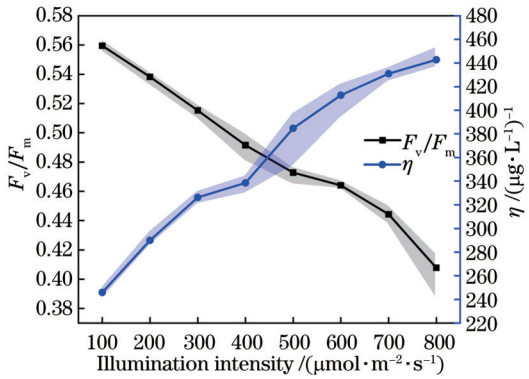


图 4 蛋白核小球藻叶绿素荧光产量和 $F_v/F_m$ 随光照强度的变化

Fig. 4 Chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* changed with illumination intensity

进一步对每组培养光照胁迫实验中蛋白核小球藻的 $F_v/F_m$ 值与 $\eta$ 值进行线性拟合，结果如图 5 所示。可以看出，光照胁迫实验中 $F_v/F_m$ 与 $\eta$ 之间呈现明显的负线性相关关系，线性优度 $R^2$ 为 0.9195。

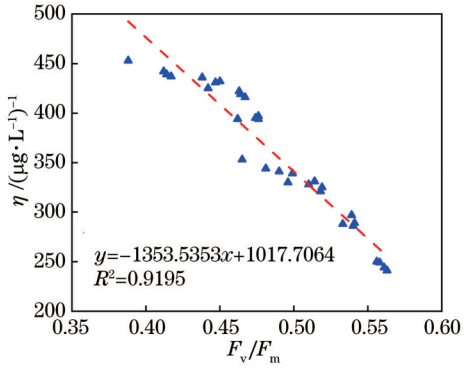


图 5 光照实验中蛋白核小球藻叶绿素荧光产量和 $F_v/F_m$ 的相关性

Fig. 5 Correlation between chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* in illumination experiment

### 3.3 温度实验

实验使用水浴锅来控制藻液培养温度，设置 5、10、15、20、25、30、35、40、45、50 ℃ 10 个温度梯度，每个样品重复测量 3 次，当温度达到设置温度时立即测量蛋白核小球藻的叶绿素 a 质量浓度、荧光强度和 $F_v/F_m$，计算时取 3 次测量结果的平均值。3 组重复实验中

藻的质量浓度分别为71.8、78.9、93.1 μg/L。浮游藻类叶绿素荧光产量$\eta$和$F_v/F_m$随温度的变化关系如图6所示。从图6可以看出，不同质量浓度的蛋白核小球藻样品的叶绿素荧光产量与$F_v/F_m$同样呈现相反的变化趋势，且每组重复实验的数据波动范围在8%以内。随着温度增加，$F_v/F_m$先缓慢上升再快速下降，而$\eta$先缓慢下降再快速上升，该变化趋势与藻的质量浓度无关。在5～25 ℃温度范围内，$F_v/F_m$从0.566升高至0.605，上升了7%，$\eta$由253 $(\mu g\cdot L^{-1})^{-1}$降至284 $(\mu g\cdot L^{-1})^{-1}$，下降了2%；在25～50 ℃温度范围内，$F_v/F_m$由0.605降至0.376，下降了38%，而$\eta$由284 $(\mu g\cdot L^{-1})^{-1}$升至473 $(\mu g\cdot L^{-1})^{-1}$，上升了91%。

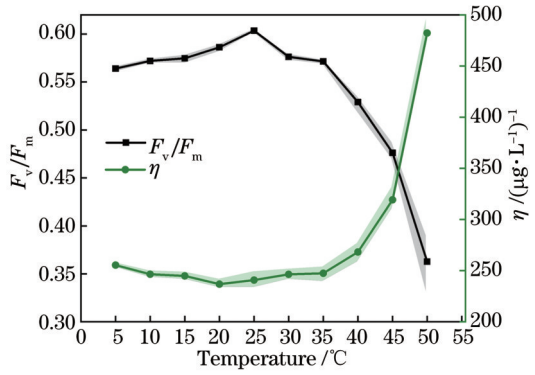


图6 蛋白核小球藻叶绿素荧光产量和$F_v/F_m$随温度的变化

Fig. 6 Chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* changed with temperature

进一步对每组温度实验中蛋白核小球藻的$F_v/F_m$与$\eta$进行线性拟合，结果如图7所示。可以看出，温度实验中$F_v/F_m$与叶绿素荧光产量$\eta$之间也呈现良好的负线性相关关系，线性优度$R^2$为0.9439。

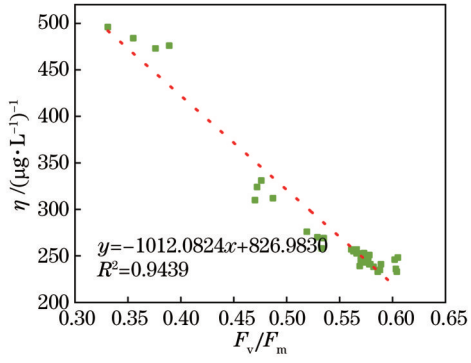


图7 温度实验中蛋白核小球藻叶绿素荧光产量和$F_v/F_m$的相关性

Fig. 7 Correlation between chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* in temperature experiment

将3种类型实验的$F_v/F_m$与叶绿素荧光产量对应关系以及线性拟合结果进行对比分析，如图8所示。可以明显看出：3种生长环境下，$F_v/F_m$与叶绿素荧光产量之间均呈现明显的线性负相关关系，线性优度$R^2$均在0.91以上；3种生长环境下，叶绿素荧光产量随$F_v/F_m$的变化率接近，拟合直线的斜率范围和直线截距基本一致，相对标准偏差分别为15.9%和11.2%。由此可见，藻类叶绿素荧光产量和$F_v/F_m$之间呈现明显的负线性相关关系，且与生长环境无关。表1为3种环境下蛋白核小球藻叶绿素荧光产量和$F_v/F_m$的线性拟合参数。

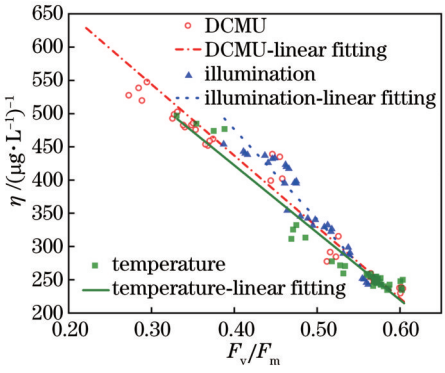


图8 3组实验中蛋白核小球藻叶绿素荧光产量和$F_v/F_m$的相关性

Fig. 8 Correlation between chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* in three experiments

表1 3组实验中蛋白核小球藻叶绿素荧光产量和$F_v/F_m$的线性拟合参数

Table 1 Parameters after linear fitting of photosynthetic activity and fluorescence yield of *Chlorella pyrenoidosa* in three experiments

| Experimental condition | Slope | Intercept | Linearity |
|---|---|---|---|
| Toxic stress | −1073 | 866 | 0.9614 |
| Temperature | −1012 | 1018 | 0.9195 |
| Illumination | −1354 | 827 | 0.9439 |

## 4 结 论

通过毒性胁迫、光照胁迫和温度来改变蛋白核小球藻的光合活性，模拟3种不同生长环境，研究蛋白核小球藻叶绿素荧光产量与光合活性参数$F_v/F_m$的变化关系。结果表明：3种生长环境下，藻类叶绿素荧光产量是变化的，且随着$F_v/F_m$增加，叶绿素荧光产量呈现下降趋势，二者具有明显的负线性相关性，线性优度$R^2$均在0.91以上；在每组实验中，在对不同质量浓度藻样品的3次重复测量结果中，$F_v/F_m$和叶绿素荧光产量数据波动幅度较小，均不超过10%，由此可见，$F_v/F_m$与叶绿素荧光产量之间的负相关性与藻类质量浓度无关；对比分析毒性胁迫、温度和光照胁迫3种类型实验的结果发现，3组实验中叶绿素荧光产量随$F_v/F_m$的变化率基本一致，即叶绿素荧光产量随$F_v/F_m$的线

性变化关系与藻类生长环境无关。综上可见，活体荧光法的前提假设“藻类叶绿素荧光产量恒定”是不可靠的，$F_v/F_m$是叶绿素荧光产量的重要影响因素，也是活体荧光法测量叶绿素a质量浓度的主要误差来源。本研究结果可为后续发展更为准确的藻类叶绿素a质量浓度活体荧光检测方法提供重要依据。

## 参 考 文 献


[1] 段亚丽，苏荣国，石晓勇，等. 基于小波高频分量的浮游植物活体荧光识别技术研究[J]. 中国激光，2012，39(7)：0715003.
Duan Y L，Su R G，Shi X Y，et al. Differentiation of phytoplankton populations by-*in vivo*-fluorescence based on high-frequency component of wavelet[J]. Chinese Journal of Lasers，2012，39(7)：0715003.

[2] 张宛宛，谢玉为，杨江华，等. DNA宏条形码(metabarcoding)技术在浮游植物群落监测研究中的应用[J]. 生态毒理学报，2017，12(1)：15-24.
Zhang W W，Xie Y W，Yang J H，et al. Applications and prospects of metabarcoding in environmental monitoring of phytoplankton community[J]. Asian Journal of Ecotoxicology，2017，12(1)：15-24.

[3] Jakob T，Schreiber U，Kirchesch V，et al. Estimation of chlorophyll content and daily primary production of the major algal groups by means of multiwavelength-excitation PAM chlorophyll fluorometry：performance and methodological limits[J]. Photosynthesis Research，2005，83(3)：343-361.

[4] Laisk A，Oja V. Variable fluorescence of closed photochemical reaction centers[J]. Photosynthesis Research，2020，143(3)：335-346.

[5] Biswal A K，Dilnawaz F，David K A V，et al. Increase in the intensity of thermoluminescence *Q*-band during leaf ageing is due to a block in the electron transfer from $Q_A$ to $Q_B$[J]. Luminescence，2001，16(5)：309-313.

[6] 杜胜蓝，黄岁樑，臧常娟，等. 浮游植物现存量表征指标间相关性研究Ⅰ：叶绿素a与生物量[J]. 水资源与水工程学报，2011，22(1)：40-44.
Du S L，Huang S L，Zang C J，et al. Correlation research between the indicators of phytoplankton standing stock Ⅰ：chlorophyll a and biomass[J]. Journal of Water Resources and Water Engineering，2011，22(1)：40-44.

[7] First M R，Robbins-Wamsley S H，Riley S C，et al. Assessment of variable fluorescence fluorometry as an approach for rapidly detecting living photoautotrophs in ballast water[J]. Journal of Sea Research，2018，133：53-59.

[8] Pérez G L，Galí M，Royer S J，et al. Variability of phytoplankton light absorption in stratified waters of the NW Mediterranean Sea：the interplay between pigment composition and the packaging effect[J]. Deep Sea Research Part I，2021，169：103460.

[9] 华卉，殷高方，赵南京，等. 基于叶绿素荧光动力学的压载水活体藻细胞数表征参数选择研究[J]. 光学学报，2021，41(6)：0617001.
Hua H，Yin G F，Zhao N J，et al. Study on selecting characterization parameters of viable algae cells number in ballast water based on chlorophyll fluorescence kinetics[J]. Acta Optica Sinica，2021，41(6)：0617001.

[10] Chen W，Westerhoff P，Leenheer J A，et al. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter[J]. Environmental Science & Technology，2003，37(24)：5701-5710.


# Experimental Study on Relationship Between Chlorophyll Fluorescence Yield and Photosynthetic Activity in Planktonic Algae


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## Abstract

**Objective** We aim to study the influence of photosynthetic activity change on chlorophyll fluorescence yield and explore the error source of measuring algae chlorophyll a concentration by living fluorescence method, so as to provide an important basis for the subsequent development of on-site accurate detection method of algae chlorophyll concentration in water based on living fluorescence method.

**Methods** We use ACT2&FastOcean FRRF algal fluorescence meter (CTG Company, UK) to measure the photosynthetic activity parameter $F_v/F_m$. After 15 min dark adaptation of the algal sample (the photosynthetic reaction center of the algal sample is fully open), we selecte an excitation light source of 450 nm (the characteristic excitation wavelength of *Chlorella pyrenoidosa*) and measure the chlorophyll fluorescence induction curve. According to the basic

parameters of the curve, the maximum photochemical quantum yield $F_v/F_m$ is calculated. We use HJ897-2017 water quality determination of chlorophyll a spectrophotometry to measure the concentration of chlorophyll a of sample algae. In the experiment, the Hitach7000 fluorescence spectrophotometer is used to measure the three-dimensional fluorescence spectrum of *Chlorella pyrenoidosa*, as shown in Fig. 1(a). The characteristic fluorescence spectrum region of *Chlorella pyrenoidosa* (excitation wavelength range of 380-500 nm and emission wavelength range of 660-750 nm) is selected, and the fluorescence intensity of living algae is obtained by fluorescence region integration method. The fluorescence yield, namely the fluorescence intensity per unit of chlorophyll a, is further obtained. Three groups of parallel samples are allocated for the experiment, and the final test results are taken as the average value, so as to study the change rule between photosynthetic activity and fluorescence yield of *Chlorella pyrenoidosa*.

**Results and Discussions** Under the DCMU toxicity stress, the chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* with different mass concentrations have the opposite trend under the DCMU stress. With the increase in DCMU mass concentration, $F_v/F_m$ value changes from 0.605 to 0.229, showing a gradual downward trend, with a decline of 62%; $\eta$ value changes from 245 $(\mu g\cdot L^{-1})^{-1}$ to 678 $(\mu g\cdot L^{-1})^{-1}$, showing a gradual upward trend, with an increase of 177%. The variation trend of each group of experiments is the same; the repeatability is high, and the data fluctuation range is within 5%. The variation relationship between photosynthetic activity and fluorescence yield is independent of algae mass concentration. Therefore, there is a significant negative correlation between photosynthetic activity and fluorescence yield. Chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* cultured in different mass concentrations for two hours under different light intensities show a reverse trend. With the increase in light intensity, $F_v/F_m$ value gradually increases, and $\eta$ is gradually decreasing. The changing trend has nothing to do with the algae mass concentration. When the light intensity increases from 5600 to 44800 lx, the photosynthetic activity value decreases from 0.563 to 0.388, with an average decrease of about 26%. The chlorophyll fluorescence yield increases from 241 $(\mu g\cdot L^{-1})^{-1}$ to 453 $(\mu g\cdot L^{-1})^{-1}$, with an average increase of 80%. In addition, the changing trend of each group of experiments is the same, with high repeatability, and the data fluctuation range is within 10%. In the temperature experiment, the chlorophyll fluorescence yield and photosynthetic activity of *Chlorella pyrenoidosa* samples with different mass concentrations also show the opposite trend, and the fluctuation range of each group of repeated experimental data is within 3%. With the increase in temperature, $F_v/F_m$ value rises slowly and then decreases rapidly, and $\eta$ trend of change has nothing to do with the mass concentration of algae. At 5-25 ℃, $F_v/F_m$ increases from 0.566 to 0.605, with an average increase of 7%, and $\eta$ changes from 253 $(\mu g\cdot L^{-1})^{-1}$ to 284 $(\mu g\cdot L^{-1})^{-1}$, an average decrease of 2%. At 25-50 ℃, $F_v/F_m$ decreases from 0.605 to 0.376, with an average decrease of 38%, and $\eta$ rises from 284 $(\mu g\cdot L^{-1})^{-1}$ to 473 $(\mu g\cdot L^{-1})^{-1}$, an average increase of 91%. By simulating three environmental conditions to change the photosynthetic activity of *Chlorella pyrenoidosa*, the effect of the change of photosynthetic activity on the change of fluorescence yield is studied. The results show that the change range of photosynthetic activity of *Chlorella pyrenoidosa*a is 0.229-0.605, and the change range of fluorescence yield is 235-668 $(\mu g\cdot L^{-1})^{-1}$. There is a negative correlation between photosynthetic activity and fluorescence yield of *Chlorella pyrenoidosa*. The linear fitting results between photosynthetic activity and fluorescence yield measured under three environmental conditions are $y=-1073x+866$, $y=-1012x+1018$, and $y=-1354x+827$, and the linear goodness $R^2$ between the two is above 0.91. It can be seen that the change of photosynthetic activity is an important factor affecting the change of fluorescence yield, and it is the error source of inaccurate measurement of *in vivo* fluorescence method.

**Conclusions** The photosynthetic activity of *Chlorella pyrenoidosa* is changed by toxic stress, illumination, and temperature control, and three different growth environments are simulated to study the influence of changes in the photosynthetic activity of algae on the change of chlorophyll fluorescence yield. The results show that in three different growth environments, with the change of photosynthetic activity, the chlorophyll fluorescence yield of algae changes. The photosynthetic activity of algae is an important factor affecting the yield of chlorophyll fluorescence. With the increase in photosynthetic activity, the yield of chlorophyll fluorescence shows a downward trend. There is an obvious negative correlation between the two, and the correlation coefficient $R^2$ can reach more than 0.91. This study is an *in vivo* fluorescence method, which can provide an experimental basis for the subsequent improvement of the measurement accuracy of the *in vivo* fluorescence method.

**Key words** spectroscopy; planktonic algae; *in vivo* fluorescence method; fluorescence yield of chlorophyll; photosynthetic activity; concentration detection