

Spaceflight Microgravity Reduced Photosynthetic Electron Transport and Altered Energy Distribution in *Euglena gracilis**

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Abstract The effects of 17-day spaceflight microgravity on the photosynthetic activity of *Euglena gracilis* were investigated during the SIMBOX mission on board the Chinese Shenzhou-8 spacecraft. We found that microgravity decreased F_v/F_m , while chlorophyll a and carotenoid contents were increased. The fluorescence yields were clearly reduced by microgravity, but the shape of fluorescence transients (O-J-I-P) was not changed. The quantum yield of primary photochemistry (ϕ_{Po}), the quantum yield of electron transport (ϕ_{Eo}) and the performance index of PSII (PI_{ABS} and PI_{CS}) in microgravity group were decreased, while ABS/RC and DIO/RC were increased. 77K fluorescence emission spectra indicated that microgravity altered energy distribution between PSII and PSI and there are redshifts under microgravity. These results suggested that microgravity may impair photosynthesis by inhibition of acceptor sides of PSII in electron transfer pathway and alter PSI structure to cause a reduction of energy transfer to PSI in *Euglena gracilis*.

Key words microgravity, photosynthesis, chlorophyll a fluorescence, *Euglena gracilis*, electron transport

DOI: 10.16476/j.pibb.2016.0196

Algae are regarded as promising producers in establishing controlled ecological life support system (CELSS) in long-term space exploration^[1]. Various alga strains are easy to cultivate, grow fast and are resistant to changes in environmental conditions. In addition, they recycle inorganic and/or organic materials, produce oxygen and provide nutritious, protein- and vitamin-rich food for the crew^[2]. Photosynthesis of phototrophs is the basis in CELSS since it can convert the light energy to reduce oxidized molecules under simultaneous supply of oxygen and food for humans during long-duration space missions^[3-4]. Many studies showed that simulated microgravity inhibited photosynthetic activity of plants, including reductions of net photosynthetic rate^[5], photosynthetic electron transport rate^[6-10] and excitation energy transfer rate^[11]. Similar results were

also found in plants from spaceflight, but partly due to environmental confines such as lack of ventilation and ethylene scrubbers in closed chambers. Algae grows in medium, nutrition utilization and gas exchange are through water, which made them suffer little from outer environmental conditions and more suitable for photosynthesis research in space. Until now there is limited information about algae photosynthesis in real microgravity. A better understanding of microgravity

*This work was supported by grants from The Chinese Natural Science Foundation (30970688), State Key Laboratory of Freshwater Ecology and Biotechnology (2016FBZ07), and the Chinese Manned Spaceflight Project.

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Received: June 15, 2016 Accepted: August 29, 2016

on algal photosynthesis is important to maintain and optimize the algae productivity in space, and further constitution of the material and energy recycle in CELSS.

Chlorophyll fluorescence in living cells have been regarded as very useful tools to assess the performance of the photosynthetic apparatus^[12]. Recent improvements in detecting fluorescence kinetics by direct, time-resolved fluorescence measurements, can be applied to various oxygenic photosynthetic material *e.g.* for determination of impacts of different stressors on photosynthesis^[13-16]. The fluorescence transients (O-J-I-P) polyphasic transients have been found to change its shape according to different environmental conditions, which can be used to elucidate the inhibition sites in photosystems in detail^[17-18]. In addition, 77K low temperature fluorescence spectroscopy can help to sort out energy distribution between PS I and PS II during energy transfer in photosystem^[19-20].

In this study, we employed the green fresh-water flagellate *Euglena gracilis* as an oxygen producer in establishing of the CELSS during SZ-8 mission spaceflight since *Euglena gracilis* is a suitable model organism for photosynthesis research and space biology^[10, 21]. Recent research demonstrated that gravitaxis in *Euglena gracilis* is mainly based on a physiological mechanism^[22] and alignment of the axis of motile and immobilised cells was compared at different accelerations during a short-term sounding rocket experiment (Texus 40)^[23]. In addition, *Euglena* has been testified to provide sufficient oxygen for fish in small closed systems on board the Foton M-2 satellite on a 12-day mission^[24]. However, there are a few reports about the effect of real microgravity on photosynthesis of *Euglena*, especially in the investigation about inhibition sites and energy distribution of microgravity. In this study, this issue is addressed in more detail during SZ-8 mission spaceflight and several biochemical and physiological parameters were examined to the effect of real microgravity on photosynthesis, which include below: (1) determination of chlorophyll fluorescence after spaceflight to identify the inhibition sites of photosynthesis system; (2) investigation of photosynthetic pigment composition of *Euglena gracilis* after spaceflight; (3) measuring an 77K assay (low temperature fluorescence) to study effects of spaceflight on energy distribution between PS I and

PS II during energy transfer.

1 Materials and methods

1.1 Organisms and culture

Euglena gracilis was provided by University of Erlangen (Erlangen, Germany) and cultured in D1 medium with a little modification^[10, 21]. $(\text{NH}_4)_2\text{HPO}_4$ was added into the medium with a final concentration of 200 mg/L. *Chlorella pyrenoidosa* was provided by the FACHB (Freshwater Algae Culture Collection of Institute of Hydrobiology, the Chinese Academy of Sciences) and cultured in 1/2 strength Bold's Basal Medium (BBM)^[25]. *Bulinus australianus* was cultured in our laboratory^[2].

1.2 Experiment hardware

The experimental hardware was designed and constructed by EADS (Astrium, Friedrichshafen, Germany) on behalf of DLR, the Germany Space Administration (Bonn, Germany). Figure 1 showed the draft of the experiment hardware, which is composed of two main parts: the upper chamber with volume of about 15 ml and lower chamber of about 45 ml. They were separated by an AeroSeal membrane. The AeroSeal membrane allows exchange of small molecules and gases but is not permeable for algae. Two LED-arrays, each with 6 lights (665 nm), were located along one side of the lower compartment. One group (day lights) has light cycle of 12 h : 12 h. Another (night lights) was switched on all the time. Light intensity was about 15 ~ 250 $\mu\text{E}/\text{m}^2\cdot\text{s}$ when the day lights were switched on (depending on the position in the chamber), and the average intensity was 82.4 $\mu\text{E}/(\text{m}^2\cdot\text{s})$. The night lights intensity was about 4 ~ 9 $\mu\text{E}/(\text{m}^2\cdot\text{s})$. Two identically containers were employed in the experiment, a flight module (FM) for μg -exposure and a 1g-ground reference (ground module: GM). After sample loading, the flight module

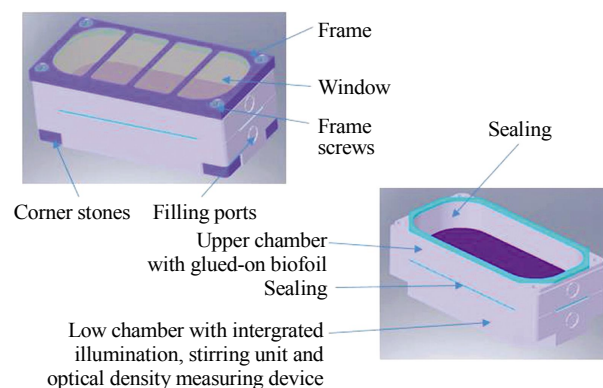


Fig. 1 Draft of the Joint container

(FM) was mounted on a μ g-slot into the BIOBOX experiment incubator, which was designed for space applications. The GM was kept under the same light and temperature conditions as the FM and all procedures were kept the same as flight samples.

1.3 Load and retrieve procedure

Flight samples loading were started 12 h before launch. Each part of the container was strictly sterilized before use. *Euglena* was diluted to 4×10^4 cells per milliliter with fresh D1 medium and added into lower chamber. As a secondary oxygen producer and food provider, *Chlorella* was filled into the upper chamber together with 3 *Bulinus australianus* snails. The initial concentration of *Chlorella* was adjusted to 3.2×10^5 cells/ml with 1/2 BBM medium. The spacecraft SZ-8 was launched in Jiuquan Satellite Launch Center on Nov. 1, 2011 and reentry landed on Nov. 17, 2011. Power was turned on after launch and turned off before retrieval. The first and last cycles were light on (day lights and night lights on). Microgravity level of most time in SZ-8 mission is below 0.001g. After landing, SIMBOX were transported to Beijing within 8 h for further sampling processing.

1.4 Measurement of fluorescence transients

Fluorescence transients (O-J-I-P) measurements were conducted immediately after the descent of payload. The samples were measured at 25 °C using a Plant Efficiency Analyzer (Hansatech, UK) according to Strasser *et al.*^[26]. Illumination (650 nm) was provided by an LED array and was focused onto the sample to provide a homogeneous irradiance. The fluorescence signals were detected by a high performance PIN photodiode detector. All samples were dark-adapted for 15 min prior to measurements. The fluorescence intensities at 50 μ s, 2 ms (J-step) and 30 ms (I-step) were denoted as F_0 , F_J and F_I , respectively. The specific parameters were calculated according to the J-I-P-test^[16,26].

1.5 Measurement of low temperature fluorescence spectroscopy

77K fluorescence spectroscopy used Mullineaux's method^[20], with small modifications^[4, 19]. Algae cells were rinsed and re-suspended in sucrose/phosphate/citrate medium to Chl a concentration of 100 μ mol/L. A few drops of suspension were filled in a hard quartz tubule and rapidly frozen in liquid N₂. 77K fluorescence spectra were recorded through excitation at 436 and 480 nm on a Fluorescence Spectrometer

F4500 (Hitachi, Japan).

1.6 Measurement of photosynthetic pigments

Pigments were measured and calculated according to standard method as Winterma and Demots^[27]. 2 ml cultures were centrifuged and extracted in 95% (v/v) ethanol for less than 24 h at 4 °C. The absorbances of the supernatants were determined at 665, 649 and 470 nm with a UV/Vis spectrophotometer (TU-1810, Purkinje General Instrument Co. Ltd, Beijing, China). Chlorophyll content was normalized by cell number.

1.7 Statistics

The results were analyzed according to the *t*-test or one-way ANOVA followed by least significant difference (LSD) test. The confidence level was set at 95% ($P < 0.05$).

2 Results

2.1 Microgravity reduced cell growth and photosynthesis activity

The color of FM culture was pale green, differing from the GM of dark green. The total number of *Euglena gracilis* cells in both chambers increased from initially 1.8×10^6 cells to 1.06×10^7 cells in the FM and 1.4×10^7 cells in the GM. The F_v/F_m values are frequently considered as an effective indicator in determination photosynthesis efficiency *in vivo*. Analysis showed that the value of F_v/F_m in FM was significantly lower than the control samples (Figure 2). The value of F_v/F_m in FM was only 70% of GM (The F_v/F_m in FM and GM was (0.3236 ± 0.0194) and (0.4632 ± 0.0447) respectively). These findings indicate that spaceflight inhibit the photosynthesis activity in algae.

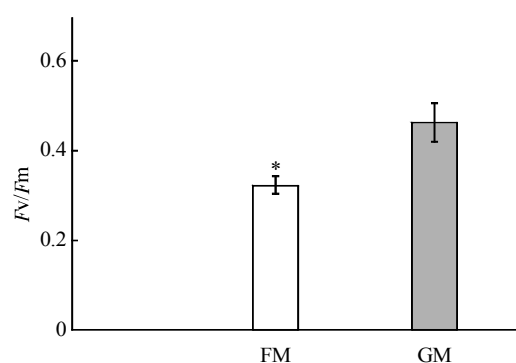


Fig. 2 Changes in photosynthetic activity (F_v/F_m) in *Euglena* under spaceflight microgravity (FM) and ground control (GM)

*There were significant differences between the FM and GM ($P < 0.05$).

□: FM; ■: GM.

2.2 Microgravity altered the contents of photosynthetic pigments

As shown in Table 1, the contents of Chl *a* and carotene in the flight samples (FM) increased remarkably than the control (GM). Chl *b* was not detected in FM group. These results indicate that spaceflight microgravity may induce synthesis of Chl *a* and carotene in algae cells.

Table 1 Chlorophyll a (Chl a) content, Chlorophyll b (Chl b) content and carotenoid content (Car) per cells in *Euglena* under spaceflight microgravity (FM) and ground control (GM)

Treatments	Chl <i>a</i> (mg/10 ⁵ cells)	Chl <i>b</i> (mg/10 ⁵ cells)	Car (mg/10 ⁵ cells)
FM	1.114±0.013*	0*	0.258±0.034*
GM	0.214±0.008	0.030±0.003	0.061±0.012

*There were significant differences between the FM and GM ($P < 0.05$).

2.3 Changes of chlorophyll fluorescence transients of *Euglena* under microgravity

The effect of spaceflight on the electron transport of PS II was investigated by measuring the fast Chlorophyll *a* fluorescence transients. A typical polyphasic rise of fluorescence induction (O-J-I-P) was found in all groups (Figure 3). A significant shape change in the original fluorescence after exposure to spaceflight microgravity was not found, while the fluorescence yield at phase J, I, P considerably decreased (Figure 2). V_J and V_k values were not changed compared to the control (data not shown). These data also reveal that spaceflight may decrease fluorescence yield, which then affect photosynthesis efficiency in algae cells.

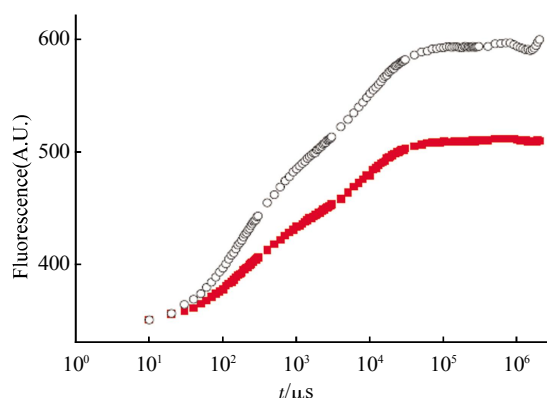


Fig. 3 Changes in the polyphasic chlorophyll fluorescence transients (OJIP) in *Euglena* under spaceflight microgravity (FM) and ground control (GM)

■ : FM; ○ : GM.

2.4 Inhibition sites induced by microgravity in the electron transport chain

Based on the analysis data from chlorophyll *a* fluorescence transient, the “JIP-test” has been developed to monitor photosynthesis process under environment stress. “JIP-test” results can be presented as a spider-plot to present that the energy fluxes transfer though per reaction center (RC). In the FM, significant impairments of chlorophyll *a* fluorescence parameters in *Euglena gracilis* were found (Figure 4). Compared to the control group (GM), DIO/RC, ABS/RC and $\phi D0$ of microgravity groups were obviously increased, while PI_{CS} , PI_{ABS} , $\phi P0$, $\phi E0$ were significantly decreased (Figure 4). These results showed that microgravity probably inhibits electron transport at the acceptor side of PS II in the tested algae.

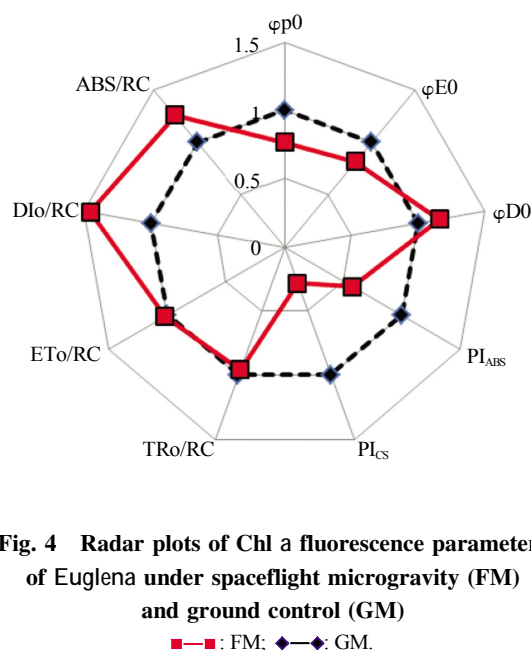


Fig. 4 Radar plots of Chl *a* fluorescence parameters of *Euglena* under spaceflight microgravity (FM) and ground control (GM)

■ : FM; ◆ : GM.

2.5 Energy distribution between PSI and PSII under microgravity

The 77K fluorescence emission spectra excited at 436 nm (the peak of the absorption band for Chl *a*) or at 480 nm (the peak of absorption band for Chl *b*), yielded two major peaks: one peak at about 698 nm from core complex of PS II and another peak at about 730 nm from PS I (Figure 5). In addition, redshifts in 77K fluorescence emission spectra of the FM compared to the GM were found.

Also a reduced emission peak of PS I and PS II

at an excitation of 480 nm fluorescence (the peak of absorption band for Chl *b*) compared to the 436 nm excitation (the peak of the absorption band for Chl *a*) was detected. The ratio of peak fluorescence of PS II to PS I in the microgravity group was higher than that

in the control, suggesting that more energy was transferred to PS II than to PS I in the FM and the energy distribution within the two photosystems was changed.

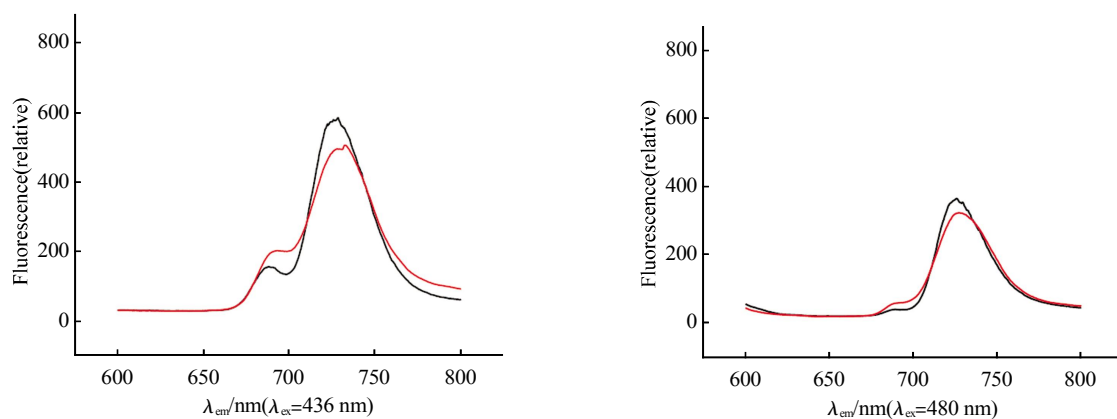


Fig. 5 Changes in 77K fluorescence emission spectral of *Euglena* under spaceflight microgravity (FM) and ground control (GM)

— : GM; — : FM.

3 Discussion

Space environment affects photosynthesis activities in many aspects. The total biomass of *Chlorella pyrenoidosa* decreased in a 7-day spaceflight mission^[2]. Partial volumes of stromal thylakoids, starch grains and plastoglobuli increased while thylakoid membrane stacking decreased in *Brassica rapa* grown aboard the space shuttle Columbia (STS-87)^[11]. A reduction in whole chain electron transport happened in dwarf wheat onboard the International Space Station^[28-29]. Temperatures and net photosynthetic rates of plant leaves were affected during parabolic airplane flight^[5]. Photochemical activity and excitation energy transfer rate inhibited in spaceflight plant^[11].

Fv/Fm reflects the potential opening degree of the PS II reaction center and photosynthesis activity^[17]. Our data of Fv/Fm are in coincidence with those results that photosynthetic efficiency (Fv/Fm) was inhibited under space conditions (Figure 2). Besides, compared to the open cultures, the ground control group in its closed container showed also a decrease compared to open cultures, but was far less affected compared to the cells which were exposed to microgravity. The value of Fv/Fm decreased 14.8% in closed container on ground, but decreased 42.8% in FM group compared to the open culture (unpublished

data). The photosynthetic inhibition was also found in plant growing in close chamber or module under microgravity conditions with low air velocity^[30-31]. In our systems, closure without outside gas exchange triggered the oxygen saturation and low pressure stresses during the light switched on/ off stages, which probably caused the decrease of photosynthesis capacity in closed container. Our data support the hypothesis that the μ g-effects exceeded the effects of closed culture conditions.

“JIP-test” can provide information about the energy fluxes per reaction center and disclose the potential inhibition sites in the electron transport chain in more details. It is well-known that a fast fluorescence rise consisting of a sequence of phases, labeled as O, J, I and P. The minimal value of fluorescence signal is denoted as O (for origin, corresponds to minimal fluorescence intensity F_0 , which reflects the fluorescence yield at full photochemical quenching in the absence of photo-electrochemical stimulation, *i.e.* under dark-adapted conditions). The J step represents the accumulation of the Q_A-Q_B form, as demonstrated by experimental results and theoretical simulations^[15, 18]. Thus, the I step has been suggested to reflect an accumulation of the $Q_A-Q_B^-$ form, whereas the P step represents an accumulation of the $Q_A-Q_B^{2-}$ form^[17-18].

This O-J-I-P polyphasic transient is found to change its shape in different environmental conditions and such changes can present the actual description of a given physiological state^[32]. In this study, a typical polyphasic rise of fluorescence induction (O-J-I-P) was found in all groups (Figure 3), while the fluorescence yields of FM at phase J, I, P were considerably decreased. No significant differences of V_J and V_K between FM and GM were found in the recovery course, which suggested that microgravity does not inhibit electron transport at the donor side of PS II. At the same time, the quantum yield of primary photochemistry (ϕ_{Po}) and the quantum yield of electron transport (ϕ_{Eo}) were also decreased during spaceflight. The result implies that microgravity probably inhibits electron transport at the acceptor side of PS II in the FM algae.

As for the parameters of energy flux (per reaction center, RC) for absorption (ABS/RC), trapping (TRo/RC), electron transport (ETo/RC) and dissipation (DIO/RC)^[16], it was found that spaceflight increased ABS/RC and DIO/RC significantly, but did not impair TRo/RC and ETo/RC (Figure 4). These results indicate that the energy absorption and dissipation are increased, while energy trapping and transport are not changed, and the whole energy flux rate is reduced under microgravity. The data about energy flux are in agreement with the effect of microgravity on PI_{ABS} . The increased ABS/RC-ratio in microgravity group (FM), probably resulted from a decreased number of reaction centers or an increase in the antenna size^[13-14, 16]. In this study, we found that Chl *a* content was higher, while the Chl *b* content was lower in FM compared to GM (Table 1). Since all of the Chl *b*-molecules and most of Chl *a*-molecules act as antenna pigments, it is possible that high ABS/RC-ratios in the FM were due to high Chl *a* level in the cells. The high DIO/RC in FM can probably be explained with decreased photosynthetic efficiency (limited demand of ATP and NADPH₂, because of reduced photochemistry) but increased heat dissipation due to non-photochemical quenching (protection of the photosynthetic apparatus from over-excitation). In the GM, the trend was different: ABS/RC and DIO/RC were decrease, and TRo/RC and ETo/RC were increased. These results suggested that the whole energy flux rate of the ground control was high and photosystem maintained under normal levels during experimental period.

The 77K fluorescence emission analysis provided

more direct information about energy transfer and distribution to different parts of photosystems^[19, 33]. Evidence showed that under stress situation, such as low temperature and high light, the plant cells would reduce energy transfer and yield, fluorescence intensity from the photosystems, electron transport rates and diminished effective quantum yields^[33-34]. In our 77K fluorescence spectral experiment (Figure 5), the ratio of the fluorescence peak of PS II to PS I in FM group was higher than that in the control, suggesting that excitation energy tended to transfer to PS II rather than to PS I. Besides, we also found that the PS I peak at 730 nm in the control thylakoids shifted to 733 nm in the microgravity group. These results about red shift and reduction of PS I fluorescence emission peak indicated that microgravity possibly altered PS I structure and caused less energy transfer to PS I. A similar phenomenon was also found in high plants under clinotation^[4, 7, 35], that simulated microgravity reduced energy distribution to PS I and changed PS I fluorescence emission peak. In addition, we also found that there is also a reduced fluorescence emission level at an excitation at 480 nm (the peak of absorption band for Chl *b*) compared to 436 nm-excitation (the peak of the absorption band for Chl *a*), a possible reason for this is that there is far lower Chl *b* content than Chl *a* in *Euglena* (the ratio of Chl *a* to Chl *b* is higher than 6 in the wild type)^[36], and we even could not detect Chl *b* of *Euglena* cells in FM groups, so there are low Chl *b*-levels and fluorescence emission level excited at Chl *b* absorption wavelength is about half of values excited at Chl *a* absorption wavelength.

In summary, the 17-day spaceflight decreased the photosynthetic activity in *Euglena gracilis* and also reduced the quantum yield of primary photochemistry (ϕ_{Po}), the quantum yield of electron transport (ϕ_{Eo}) and the performance index of PS II (PI_{ABS} and PI_{CS}), while ABS/RC and DIO/RC were increased in microgravity. Microgravity decreased fluorescence yields, but the shape of fluorescence transients (O-J-I-P) was not changed. Microgravity also altered energy distribution between PS II and PS I and caused redshifts of energy shifting to PS II. These results implied that the inhibition site of microgravity on the photosynthesis of *Euglena gracilis* may locate at the acceptor sides of PS II and changed antenna size of PS II, and microgravity may also change the PS I structure which led to a reduction of energy transfer to PS I.

Acknowledgements The authors thank Airbus D&S (Astrium), DLR (Germany) and GESSA (China), for hardware development, training and experiment support. The authors are also grateful to Prof. WEN Xiao-Gang and Dr. CHEN Bo-Ya for their technical support in 77K fluorescence spectral experiment.

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空间飞行微重力降低裸藻光合电子传递 并改变光合能量分配*

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摘要 利用神舟 8 号飞船的 SIMBOX 发射机会, 对真实微重力影响裸藻光合作用活性进行了研究. 我们发现, 微重力降低了光合活性(F_v/F_m), 提高了细胞内叶绿素 *a* 和胡萝卜素含量. 快速叶绿素荧光动力学研究显示微重力降低了叶绿素荧光强度, 但快速叶绿素荧光动力学曲线的形状(O-J-I-P)没有改变. 在微重力处理下裸藻的最大光化学效率(ϕ_{Po})、用于电子传递的量子产额(ϕ_{Eo})和光合作用性能指数(PIABS and PICS)都明显降低, 但单位反应中心吸收的光能(ABS/RC)和单位反应中心耗散的能量(DIo/RC)都明显升高. 77K 低温荧光光谱实现微重力改变了能量在PS I 和PS II 之间的分配并出现了红移现象. 这些结果表明真实微重力降低光合作用的活性有可能通过两个途径, 即抑制裸藻抑制光合电子传递中PS II 的受体端和改变PS I 的结构从而引起流向PS I 的能量传递减少.

关键词 微重力, 光合作用, 叶绿素荧光, 纤细裸藻, 空间飞行

学科分类号 Q256, Q949.23

DOI: 10.16476/j.pibb.2016.0196

* 国家载人航天工程、国家自然科学基金(30970688)和淡水生态和生物技术国家重点实验室(2016FBZ07)资助项目.

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收稿日期: 2016-06-15, 接受日期: 2016-08-29