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Light-Emitting Diode Power Conversion Capability and CO_2 Fixation Rate of Microalgae Biofilm Cultured Under Different Light Spectra

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Received: 30 January 2020; Accepted: 19 March 2020; Published: 25 March 2020

Abstract: Microalgae biofilm-based culture has attracted much interest due to its high harvest efficiency and low energy requirements. Using light-emitting diodes (LEDs) as light source for microalgae culture has been considered as a promising choice to enhance the economic feasibility of microalgae-based commodities. In this work, the LED power conversion capability and CO_2 fixation rate of microalgae biofilms (*Chlorella ellipsoidea* and *Chlorella pyrenoidosa*) cultured under different light spectra (white, blue, green and red) were studied. The results indicated that the power-to-biomass conversion capabilities of these two microalgae biofilms cultured under blue and white LEDs were much higher than those under green and red LEDs (*C. ellipsoidea*: 32%–33% higher, *C. pyrenoidosa*: 34%–46% higher), and their power-to-lipid conversion capabilities cultured under blue LEDs were 61%–66% higher than those under green LEDs. The CO_2 fixation rates of these two biofilms cultured under blue LEDs were 13% and 31% higher, respectively, than those under green LEDs. The results of this study have important implications for selecting the optimal energy-efficient LEDs using in microalgae biofilm-based culture systems.

Keywords: microalga; biofilm-based cultivation; light spectrum; power conversion capability; CO_2 fixation rate

1. Introduction

Microalgae are photosynthetic microorganisms that can convert light, CO_2 and nutrients into biomass [1-3]. Due to the high growth rates, high lipid contents and ability to mitigate CO_2 emissions, microalgae have potential to be promising biological sources to produce high-value biomolecules and biofuels [4-6]. However, the success of microalgae-based commodities is dependent on the biomass productivity and production cost [7-9]. Recently, some researchers reported that cultivating microalgae as biofilm (i.e., cells are attached to solid surface) can enhance the economic feasibility of microalgae-based commodities, due to its lower water consumption, higher volumetric productivity, higher harvest efficiency and reduced energy requirements compared with suspended systems [10-12]. Therefore, developing more efficient biofilm-based microalgae culture system has attracted much attention recently [13-15].

To date, various microalgae biofilm-based reactors have been developed to enhance their performance, such as horizontal [16], flow lane [17], twin-layer [18], and rotating biofilm reactors [19].

Extensive studies have reported that many factors can affect the performance of microalgae biofilm-based systems, such as the light condition, the amount of CO_2 supplementation, nutrient type and concentration [12,20,21]. Among these factors, light directly affects the photosynthesis of microalgae, thus their growth and cellular composition [22-25]. Generally, sunlight and artificial illumination are often used as light sources for microalgae culture. The sunlight is cost-effective, but has the disadvantages of changes in weather and season, and day and night cycles. Artificial illuminations, such as fluorescent lamps and light-emitting diode (LED) lights, are more manageable and adjustable, and are of growing interest.

Recently, using LEDs in microalgae culture has been considered as a promising choice due to its advantages of small size, low power consumption, narrow wavelength band and reduced heat release [26-29]. Some researchers have studied the LED power conversion efficiency of microalgae cultured in suspended systems [30,31]. For example, Ma et al. studied LED power-to-biomass efficiency for *Nannochloropsis* cultured in suspended systems, and reported that using LED as light source in suspended microalgae culture was more energy efficient [30]. Ajayan et al. found that LEDs provided more light penetration in a column photobioreactor compared with the fluorescent lights, and improved the cell concentration, specific growth rate, total pigments and lipid contents of *C. reinhardtii* [31]. Maroneze et al. evaluated the role of LED photoperiods on the growth and lipid content of *S. obliquus* cultured in a bubble column photobioreactor, and found that the photoperiods was an effective strategy to reduce the production cost of microalgae biomass [32]. These above studies indicated that the LEDs might be promising using in suspended microalgae culture systems. It should be noted that, for biofilm-based culture systems, the transmission and refraction of light are totally different from that in suspended systems. This difference may affect the light conversion capability of microalgae [33]. However, to date, little research has studied the LED power conversion capability of microalgae cultured in biofilm-based systems.

To address this gap, this study explored the LED power conversion capability and CO_2 fixation rate of two widely used microalgae biofilms cultured under different light spectra (white, blue, green and red). The conversion capabilities of LED power-to-biomass, power-to-lipid, power-to-protein, and power-to-carbohydrate, as well as the CO_2 fixation rate for two widely used microalgae biofilms cultured under different LEDs were determined. This study would provide guidance in selecting suitable light spectra and reducing energy consumption for developing more efficient microalgae biofilm-based culture systems.

2. Materials and Methods

2.1. Microalgae Biofilm Culture

The microalgae used in this study were *Chlorella ellipsoidea* (FACHB–40) and *Chlorella pyrenoidosa* (FACHB–9). Before inoculation of biofilm culture, both microalgae strains were pre-cultivated in 500 mL flasks with 100 mL inoculum at 25 ± 1 °C under continuous irradiance of 100 μmol photons m^{-2} s^{-1} for 6 days. Afterwards, these preprepared cell suspensions were evenly vacuum filtered onto some filtration membranes to form microalgae biofilm with an initial inoculum density of 4.0 ± 0.1 g m^{-2}. Then, these filtration membranes were put into the biofilm culture bioreactors (polymethyl methacrylate chambers: 400 × 200 × 200 mm), containing the BG–11 culture medium solidified with 1% agar (see supporting information (SI) Table S1 and S2 for the details) [34]. As shown in Figure 1, these bioreactors were placed into an incubator to maintain a proper temperature (25 ± 1 °C) for microalgae growth [35]. The inside of these bioreactors was aerated with compressed air enriched with 1% CO_2 (vol/vol) at a rate of 0.1 VVM (volume of air per volume of culture per minute).

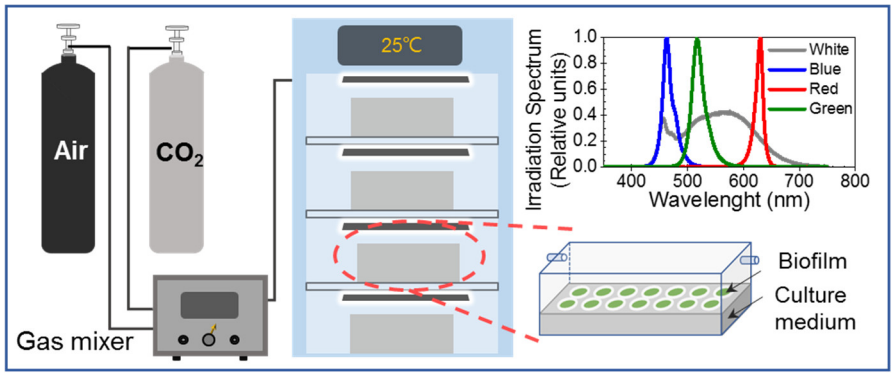

Figure 1. Schematic diagram of the microalgae biofilm culture system.

During cultivation, four kinds of LEDs (J&K Photoelectric Technology, Shanghai, China), including white (JK–W300200, 400–750 nm), blue (JK–B300200, 440–500 nm), green (JK–G300200, 500–550 nm), and red (JK–R300200, 610–650 nm) LEDs were fixed above the bioreactors as the light sources for microalgae cultivation. All the LEDs in the biofilm cultivation were continuous lighting. The photon flux densities of these LEDs at the biofilm surfaces were set to be approximately 100 μmol photons m^{-2} s^{-1}, which were measured with a 4π quantum scalar sensor (QSL 2100, Biospherical Instruments Inc., San Diego, CA, USA). The light spectra of these four LEDs were characterized with a fiber spectrometer (USB4000, Ocean Optics Inc., Dunedin, FL, USA) between 350 and 750 nm, as shown in Figure 2(a). Additionally, the absorption spectra of the *C. ellipsoidea* and *C. pyrenoidosa* were characterized by a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA), as shown in Figure 2(b).

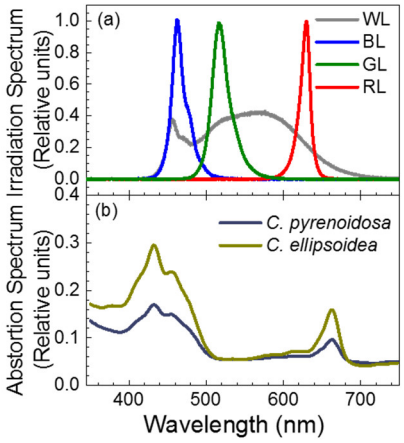

Figure 2. Irradiation spectra of different light-emitting diodes (LEDs) (a) and absorption spectra of the *C. ellipsoidea* and *C. pyrenoidosa* (b).

2.2. Determining the Biomass and Chlorophyll Contents

To evaluate microalgae growth, microalgae biofilm were harvested after 6 days culture. The collected biofilm were resuspended with deionized water to remove the soluble nutrients, followed by centrifuging and drying to a constant weight at 105 °C. After cooling in a desiccator, the microalgae biomass was weighed by an analytical balance (XS105, METTLER TOLEDO, Switzerland).

The chlorophyll contents of microalgae cells cultured under white, blue, green and red LEDs were determined according to the methods described by Wellburn [36]. In detail, the chlorophyll was extracted with 80 vol% acetone. The absorbance of chlorophyll solvent was measured at 646 and 663 nm with a visible spectrophotometer (721, INESA, Shanghai, China). The chlorophyll *a* (chl–*a*), chlorophyll *b* (chl–*b*) concentrations (mg L^{-1}) were calculated by:

$$\text{chl–}a = 12.21 \times OD_{663nm} - 2.81 \times OD_{646nm} \quad (1)$$

$$\text{chl–}b = 20.13 \times OD_{663nm} - 5.03 \times OD_{646nm} \quad (2)$$

The measurements for biomass and chlorophyll contents were repeated three times. The results were shown as mean ± standard deviation.

2.3. Determining Cellular Composition

The cellular compositions of microalgae are critical evaluation parameters in various microalgae-based commodities. Generally, the main organic components of microalgae are lipids, proteins, and carbohydrates, which represent approximately 80% of the microalgal dry biomass. To investigate the cellular compositions of microalgae biofilm cultured under different LEDs, the harvested cells were frozen at –70 °C and lyophilized. The total lipids were measured according to the methods described by Bligh and Dyer [37], in which chloroform was used to extract the lipid and evaporated at 60 °C. The protein content was measured by a colorimetric method [38,39], in which microalgae biomass was pretreated with thermal alkaline and the standard sample was bovine serum albumin (see Figure S1). The carbohydrate content was determined by the phenol-sulfuric method [40,41], the standard sample was glucose (see Figure S2). All the experiments were repeated in triplicate and the results are shown in mean ± standard deviation.

2.4. LED Power Conversion Capability

We determined the conversion capability of LED power-to-biomass, power-to-lipid, power-to-protein, and power-to-carbohydrate for these microalgae biofilms cultured under different LEDs, which were calculated by [30]:

$$\text{LED power conversion capability} = \frac{C_t\text{-}C_0}{t \times P} \quad (3)$$

where C_t is the accumulation of biomass, lipid, protein and carbohydrate (g m^{-2}) at time t, C_0 is the accumulation of biomass, lipid, protein and carbohydrate (g m^{-2}) at time t_0 (at the beginning of inoculation), P is the power consumption of different LED units.

2.5. Determining CO_2 Fixation Rate

The CO_2 fixation rate of microalgae biofilms cultured under white, blue, green and red lights were determined by [42]:

$$CO_2 \text{ fixation rate} = \frac{X_t\text{-}X_0}{t} \times C\% \times \frac{44}{12} \quad (4)$$

where X_t is microalgae biomass (g m^{-2}) at time t, X_0 is microalgae biomass (g m^{-2}) at time t_0 (at the beginning of inoculation), C% is the carbon content of the biomass, which was determined by an elemental analysis (vario EL cube, Elementar, Germany). The experiments were repeated at least three times. The results were shown as mean ± standard deviation.

3. Results and Discussion

3.1. Growth of Microalgae Biofilms Cultured Under Different LEDs

The dry biomass yields and chlorophyll contents (including chl–a and chl–b) of *C. ellipsoidea* and *C. pyrenoidosa* biofilms were determined to evaluate their growth. Figure 3 indicates that the microalgae biomasses cultivated under different light spectra were obviously different. In general, for both microalgae, the biomass cultured under the blue LED was much higher than those under the white, green and red LEDs. The total chlorophyll contents of microalgae cultured under the blue and white LEDs were much higher than those under the green and red LEDs.

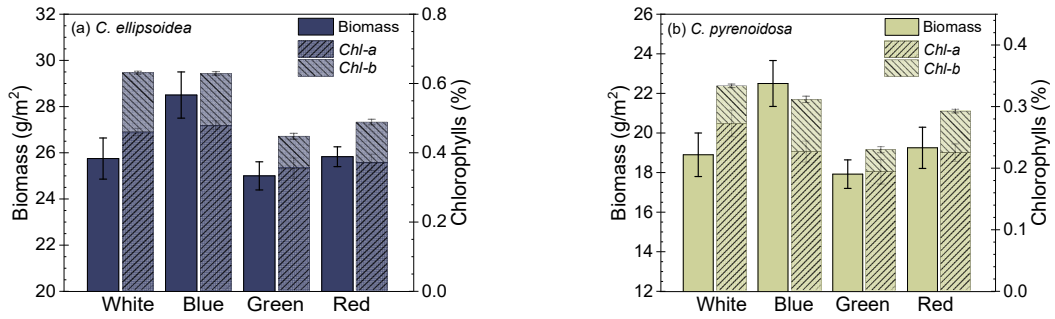

Figure 3. Dry biomass yields and chlorophyll contents (including chl–*a* and chl–*b*) in *C. ellipsoidea* (a) and C. *pyrenoidosa* (b) cultured under different LEDs.

Previously, many studies reported that both the blue and red light can promote the growth of green algae in suspended culture system, because the green algae can absorb the blue light and red light more efficiently [43]. In this work, Figure 3(a) indicates that the biomass of *C. ellipsoidea* was ~14% higher under the blue light than that under the green light. Similarly, Figure 3(b) shows that the biomass of *C. pyrenoidosa* was ~26% higher under the blue light than that under the green light. Evidently, the above results suggested that the blue LED was efficient in enhancing cell growth, whereas, we found that the red LED had little influence on cell growth. We think that it may be related to the characteristics of microalgae pigments. On one hand, as shown in Figure 2(b), the irradiation spectra of blue LEDs match with the absorption peaks of *C. ellipsoidea* and *C. pyrenoidosa* at the light spectra of 420–480 nm (blue), whereas, the irradiation spectra of red LEDs do not match well with the microalgal absorption peaks at the light spectra of 620–680 nm (red). On the other hand, we found that the chlorophyll contents of these two microalgae cultivated under red LEDs were much lower than those cultured under blue LEDs.

3.2. The LED Power Conversion Capability of Microalgae Biofilm

The LED power conversion capability is an important factor in the assessment of microalgae biofilm cultivation. In this work, we determined the conversion capabilities of LED power-to-biomass, power-to-lipid, power-to-protein, and power-to-carbohydrate. Figure 4 shows the LED power-to-biomass conversion capability for these two microalgae biofilms cultivated under different light spectra, which were calculated based on the biomass accumulation and power consumption of different LEDs (right axis in Figure 4). The results indicate that the power-to-biomass conversion capability of *C. ellipsoidea* and *C. pyrenoidosa* cultured under different LEDs ranged from 862 to 1147 mg/kW·h, and from 618 to 905 mg/kW·h, respectively. The LED power-to-biomass conversion capabilities for these two microalgae cultured under white and blue lights were much higher than those cultured under green and red lights, indicating that these microalgae cells utilized blue and white lights more effectively. Particularly, for *C. ellipsoidea*, the power-to-biomass conversion capability of cells cultured under blue and white lights increased by 32%–33% compared with those under green light. Similarly, for *C. pyrenoidosa*, the power-to-biomass conversion capability of cells cultured under blue and white lights increased by 34%–46% compared with those under green light.

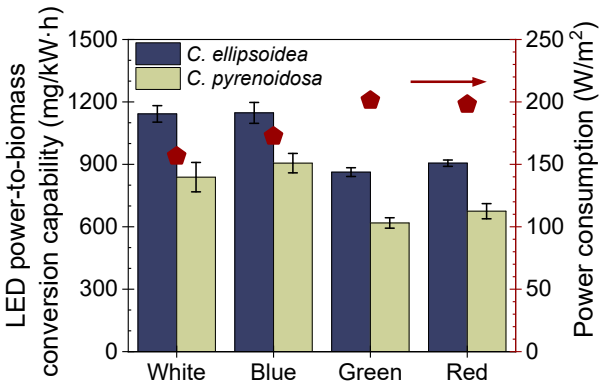

Figure 4. The LED power-to-biomass conversion capability of microalgae cultured under different LEDs (blue and drab column), and power consumption of different LEDs (red pentagon ⬟).

Furthermore, cellular compositions of microalgae are generally critical evaluation parameters in various applications. Figure S3 shows the lipids, proteins and carbohydrates contents for the *C. ellipsoidea* and *C. pyrenoidosa* cultured under different LEDs. It was found that the main organic components in the microalgae represented approximately 80% of microalgae dry biomass, which were consistent with the literature [35]. Based on the cellular composition and power consumption of different LEDs, we determined the conversion capabilities of LED power-to-lipid, power-to-protein, and power-to-carbohydrate. Figure 5 indicates that the power-to-carbohydrate conversion capabilities for both microalgae under white LEDs were the highest. The power-to-protein conversion capabilities for both microalgae cultured under white and blue LEDs were higher than those under green and red lights. Moreover, we found that both microalgae cultured under blue LED showed the highest power-to-lipid conversion capability. In particular, the power-to-lipid conversion capability for *C. ellipsoidea* and *C. pyrenoidosa* were 12.6% and 14.8% higher, respectively, under blue light than under white light. The power-to-lipid conversion capability for *C. ellipsoidea* and *C. pyrenoidosa* were 60.7% and 66.3% higher, respectively, under blue light than under green light. Similar results have been reported for the suspended culture system. Ra et al. evaluated the effects of LED wavelength on the lipid production of *Picochlorum atomus* with two-phase suspended cultivation, and found that the lipid accumulation of *P. atomus* was 329% higher under blue light than that under green light [44]. Kang et al. determined the effect of using wastewater and wavelength filters on microalgal productivity and lipid accumulation with open raceway ponds, and found that the lipid productivity was highest under blue wavelength, at least 46.8% higher than that under white wavelength [45].This may be because blue light can promote the synthesis of lipids [46,47] and has low energy consumption.

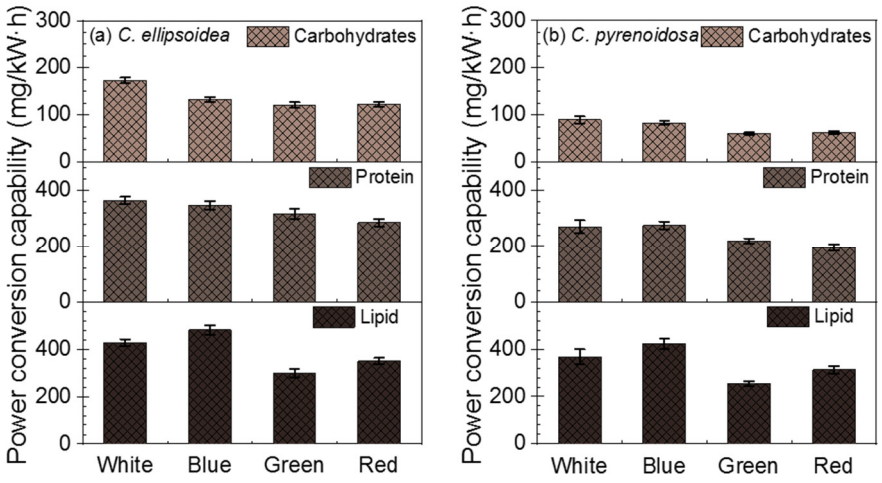

Figure 5. The conversion capabilities of power-to-carbohydrates, power-to-protein and power-to-lipid for *C. ellipsoidea* (a) and *C. pyrenoidosa* (b).

Overall, these results suggested that these two microalgae biofilms cultured under blue and white LEDs showed higher conversion capabilities of power-to-biomass and power-to-protein compared with those under green and red LEDs. These two microalgae cultured under blue LEDs possessed the highest power-to-lipid conversion capabilities. The results revealed that it is feasible to induce the synthesis of different chemical components in cells by adjusting the light spectrum in microalgae biofilm-based culture systems.

3.3. The CO_2 Fixation Rate of Microalgae Biofilm

The CO_2 fixation rates of microalgae biofilms cultivated under different LEDs were determined by evaluating the difference in the carbon content (C%) of microalgae between the inoculation and harvest (see Table S3). Figure 6 indicates that the CO_2 fixation rates for the *C. ellipsoidea* and *C. pyrenoidosa* cultured under blue LEDs were ~13% and ~31% higher, respectively, than those under green light. This may be attributed to the higher photosynthetic performance of microalgae. Additionally, previous study reported that, in suspended culture system, white light was the most effective light for CO_2 fixation compared with the blue, red and yellow lights [48]. Evidently, this study indicated that the influence of the light spectra on the CO_2 fixation rate of microalgae would be different between the biofilm cultured system and the suspended culture system. This may be due to the different characteristics of light transmission and refraction in these two microalgae culture systems. Further studies should be conducted to understand the light transfer phenomena in microalgae biofilm.

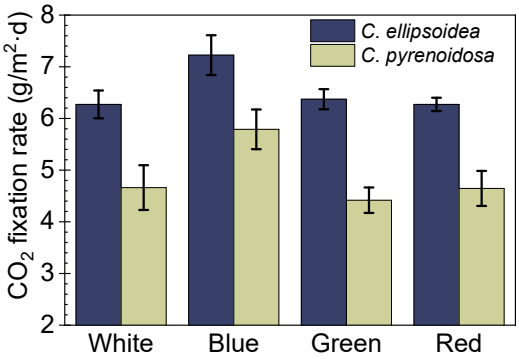

Figure 6. The CO_2 fixation rate for *C. ellipsoidea* and *C. pyrenoidosa* cultured under different LEDs.

3.4 Implications of LED Selection in Biofilm-Based Microalgae Cultivation

Previous studies reported that using LEDs with specific narrow bands in suspended microalgae culture systems may be more economical than the fluorescent lamp and filament lamp [29,49]. The results of this study suggest that the LEDs are also promising in biofilm-based microalgae culture systems. Furthermore, the study also indicates that the light spectra of LEDs can significantly affect the power conversion capability and CO_2 fixation rate of microalgae biofilms. For the *C. ellipsoidea* and *C. pyrenoidosa* biofilms, the power-to-biomass conversion capabilities and power-to-protein conversion capabilities were much higher for cells cultured under blue and white LEDs than those under green and red LEDs. Meanwhile, the power-to-lipid conversion capabilities and CO_2 fixation rate were the highest for these two microalgae biofilms cultured under blue LEDs. Moreover, considering the simultaneous improvement of power-to-biomass conversion capabilities, power-to-lipid conversion capabilities and CO_2 fixation rate, blue LEDs may have great potential using in microalgae biofilm cultivation for the biofuel production and CO_2 mitigation. The results of this study will have important implications for selecting the optimal energy-efficient LEDs to use in microalgae biofilm-based culture systems.

4. Conclusions

The results of this study indicated that the LED power conversion capability and CO_2 fixation rate of *C. ellipsoidea* and *C. pyrenoidosa* biofilms cultured under white, blue, green and red LEDs were significantly different. These two microalgae biofilms cultured under white and blue LEDs showed higher power-to-biomass conversion capabilities and power-to-protein conversion capabilities than those under green and red LEDs. The power-to-lipid conversion capabilities and CO_2 fixation rate were the highest for these two microalgae biofilms cultured under blue LEDs. This study would provide guidance in selection of suitable LEDs and reducing energy consumption for developing more efficient microalgae biofilm-based culture systems.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1: **Table S1 and S2.** The culture medium for microalgae. **Figure S1**. The relationship between the content of bovine serum albumin and the optical density of solution. **Figure S2.** The relationship between the content of glucose and the optical density of solution. **Figure S3**. Biochemical composition for the *C. ellipsoidea* (a) and *C. pyrenoidosa* (b) under different LEDs. **Table S3.** The contents of carbon, nitrogen, hydrogen, and oxygen in microalgae determined with an elemental analyzer.

Author Contributions: H.Y. and X.R.Z. carried out the experiments, analyzed the data, and drafted the manuscript. Y.W., Y.A.M.X., and X.Y.W. carried out part of experiments. X.R.Z., Z.Y.J. and X.X.Z. designed the study, and revised the manuscript. All authors read and approved the final manuscript.

Funding: This work is supported by the National Science Foundation of China (No. 51706019), and the Fundamental Research Fund for the Central Universities (No. FRF–AS–17–001 and No. FRF–BD–18–015A).

Conflicts of Interest: The authors declare no conflicts of interest.

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