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The influence of wavelength of light on cyanobacterial asymmetric reduction of ketone

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ABSTRACT

Asymmetric reduction of ketone by a microalga, *Synechocystis* sp. PCC 6803, smoothly afforded to the corresponding (*S*)-alcohol in excellent enantiomeric excess by the aid of illumination of orange and red LED lights which are more effective than other LEDs such as blue and green lights. The condition under minimum energy flux (1.0 W/m^2) of orange-red LEDs is enough for the reduction of ketone, and it seems that orange-red light rather effectively forwarded the regeneration of coenzyme.

Microalgae are useful microorganisms; they produce approximately half of the atmospheric oxygen and use simultaneously carbon dioxide (CO_2) to grow photoautotrophically. Furthermore, microalgae can work as biocatalysts and have the potential for producing valuable substances.[1] Usually, microalgae are used as the sources of fine chemicals and biofuels.[2] *Synechocystis* sp. PCC 6803 is a unicellular cyanobacteria and is used as a model microalga by scientists around the world because it was the first photosynthetic organism for which the entire genome sequence was determined.[3]

We have developed the use of microalgae as biocatalysts for converting artificial substrates to useful products. Chiral alcohols were obtained by asymmetric reduction of ketones using microalgae such as cyanobacteria and chlorella under fluorescent light illumination.[4] In the reduction, ketones were converted into the corresponding optically active alcohols by NADPH generated in the chlorophyll of microalgae using light energy.[4b] Moreover, since this reaction was conducted by the living cells of algae, a consumed NADPH was regenerated by light energy and H_2O in the microalgae body.

Herein, we would like to report the asymmetric reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone **1** into the corresponding fluorinated chiral alcohol **2** using *Synechocystis* sp. PCC 6803 under LED illumination (Scheme 1). In this Letter, we studied the relationship between the yield of reductive product and both wavelength and intensity of LED light.

Photomicroorganisms require light for growing and also as an energy source to carry out reduction of artificial ketones.[4] In microalgae, photons of light are one of the major energy sources for the growth of cells. Therefore, both wavelength and intensity of applied light are known to contribute to the growth of microalgae,[5] and are thought to also affect bioconversion of artificial substrates. Hence, aiming to develop a more efficient bioconversion system that works with less energy consumption, we have studied the effect of such factors on the bioconversion with microalgae by using light-emitting diodes (LEDs) as a light source. Since LEDs are highly monochromatic efficient light sources and are now available to emit each of several colors from red through purple, LEDs are suitable for our present study. All irradiation intensities were determined by the radiometer (Delta OHM: HD2302.2 with LP471RAD probe) and were adjusted to give certain energy fluxes (0, 1.0, 2.5, 5, 12, 22 and 60 W/m^2) by both the number of LEDs used and shading the light by using the appropriate number of copier paper.

The substrate, 2′,3′,4′,5′,6′-pentafluoroacetophenone **1**, was reacted in the suspension culture of *Synechocystis* sp. PCC 6803 under red LED illumination (660 nm),[6] and the effect of the light intensity on the yield of the product alcohol was investigated. The results are shown in Figure 1.

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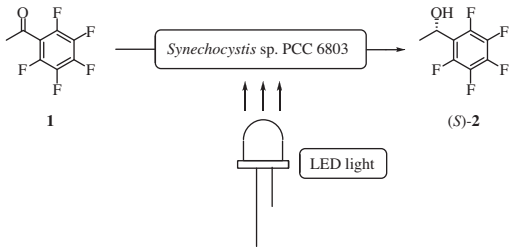

Scheme 1. Asymmetric reduction of 2′,3′,4′,5′,6′-pentafluoroacetophenone using *Synechocystis* sp. PCC 6803 under LED illumination.

As a result, the microalga reduced the ketone into the corresponding alcohol **2** (>99% ee) under red LED (660 nm). The reaction scarcely proceeded under dark conditions while irradiation of faint lights can activate the reduction of ketone (70% yield at 1 W/m^2 for 4 days) and the yield of the product increased according to the increase of irradiation intensities. While strong light effectively drove the reaction, it was harmful to the microalgae; in the case of 1 day processing under 60 W/m^2, the reaction was quenched and the green suspension of the reaction mixture was turned into white that indicates the algae cells were annihilated. Thus, the results indicate that minimum energy flux, 1.0 W/m^2, is enough for bioconversions in the present system.

To investigate the effect of light precisely, the effect of the color of LED used on the chemical yields of the reduction was tested at constant energy flux and irradiation period; the reactions under irradiation of wavelengths, 470 nm (blue), 535 nm (green), 595 nm (yellow), 612 nm (orange), 620 nm, (red) and 660 nm (red) were tested under minimum energy flux, 1.0 W/m^2. The results are shown in Figure 2.

Although the reactions under blue and green LED gave the product only at 29% and 41% yields, respectively, the reaction using orange and red LEDs afforded the corresponding (*S*)-**2** in about 70% yield. Differences in the yields among the colors of LEDs applied were little observed when irradiation of high flux (over 3.5 W/m^2) was used.

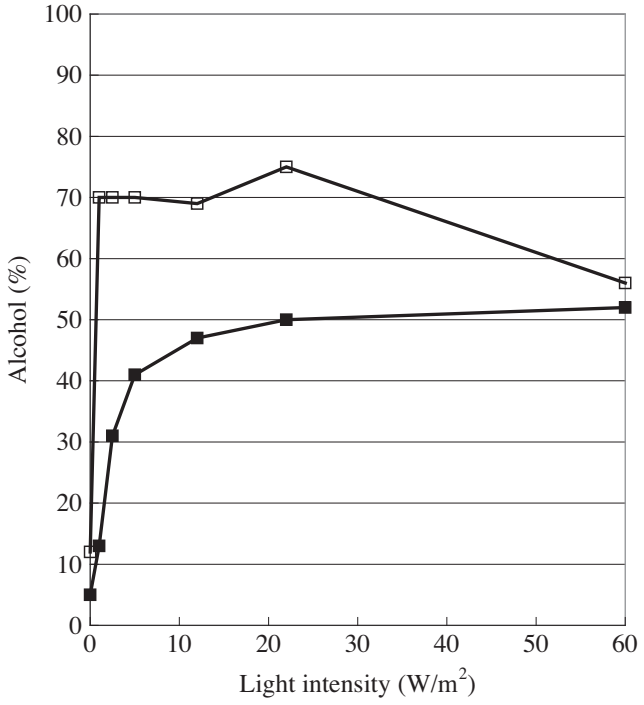

Figure 1. The reaction of **1** using *Synechocystis* sp. PCC 6803 under red LED light (660 nm). Reaction time: ■ (1 day), □ (4 days).

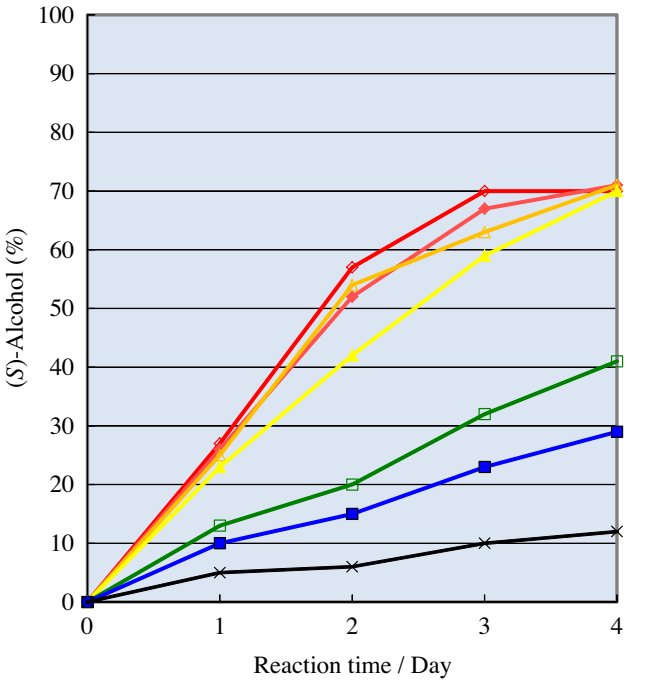

Figure 2. The reaction under 1.0 W/m^2 of light intensity ■: 470 nm, □: 535 nm, ▲: 595 nm, △: 612 nm, ♦: 620 nm, ◊: 660 nm, x: in the dark.

In addition, we tried the reduction of 4-trifluoromethylacetophenone **3** using LED illumination (1.0 W/m^2). As a result, the microalgae reduced the ketone into the corresponding alcohol (*S*)-**4** (>99% ee), and the reactions for 4 days using blue, green, yellow, orange and red (620 and 660 nm) LED gave the products in 47, 59, 63, 65, 67, and 67% yields, respectively. Here also the use of red LED was more effective than that of blue LED.

It was reported that the use of red LED irradiation gave the most effective performance for the photoautotrophic cultivation.[5b,7] In general, algae photosynthesize the precursors of biomass from CO_2 using NADPH and ATP in the Calvin cycle.[8] The present results showed the same tendency in the growth and reductive reactivity of cyanobacteria. In biocatalytic reduction of ketones using microalgae, the oxidized form of the coenzyme produced from reducing the ketones is recycled by light energy. Therefore, present results suggest that orange-red light rather effectively drives the regeneration of coenzyme.

In conclusion, we have found in the first time, the effect of color light in LED irradiation on chemical yields of the biotransformation. The condition under minimum energy flux (1.0 W/m^2) of orange-red LEDs is enough for the reduction of the ketone, our reduction system requires less electric power and conducts ecological system for biotransformation. Since the present reaction system is highly sensitive for intensity and wavelength of applied light and its yield shows good reproducibility, it could be utilized as a novel probe to elucidate regeneration mechanism of coenzyme by light. The use of LED lights for bioconversion of the other reactions is now being investigated in our laboratories.

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6. The cultivation of *Synechocystis* sp. PCC 6803 was performed in 200 mL of BG-11 liquid medium under continuous illumination provided by fluorescent lamps (2000 lux) including incubator with air-bubbling at 25 °C. The cultivated suspension culture of *Synechocystis* sp. PCC 6803 for one week (OD_{750} = 4) and BG-11 medium were mixed to make the suspension culture (OD_{750} = 2). 0.5 mg of 2′,3′,4′,5′,6′-pentafluoroacetophenone in DMSO (50 μL) was added to 6 mL of the suspension culture. The mixture was shaken at 120 rpm at 25 °C under red LED illumination. The light intensity of LED was determined by the radiometer (Delta OHM: HD2302.2 with LP471RAD probe). After the reaction, 10 μL of *n*-dodecane was added. The resulting mixture was extracted with 5 mL of ethyl acetate. The extract was washed with saturated aqueous sodium chloride (2 mL). After drying with anhydrous sodium sulfate, the chemical yield was determined by GC equipped DB-1 column (25 m) analysis using *n*-dodecane as the internal hydrocarbon standard. The enantiomeric purities were determined by GC equipped CP-cyclodextrin-B-2,3,6-M-19 (CPCD, 25 m) analysis based on GC area ratio. The retention times of ketone, (*R*)-alcohol, and (*S*)-alcohol in GC analysis were referred to the literature.[9]
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