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Response surface methodology as an optimization strategy for the light-controlled asymmetric hydrogenation of 4-(trimethylsilyl)-3-butyn-2-one by photosynthetic bacteria

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ABSTRACT

Enantiomerically pure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol {(*S*)-TMSBL} is a key intermediate for the synthesis of many biologically and structurally interesting compounds and pharmaceuticals. Herein we propose a new light-controlled asymmetric hydrogenation of 4-(trimethylsilyl)-3-butyn-2-one (TMSBO) to enantiopure (*S*)-TMSBL by photosynthetic bacteria *Rhodobacter sphaeroides*, which is a newly isolated photosynthetic bacteria strain that has the capacity to capture light energy and to generate NADPH through photosynthetic electron-transfer reactions; no oxygen or other metabolic intermediates were used. Response Surface Methodology (RSM) was used to investigate the effects of substrate concentration, pH, and temperature on the reaction yield. A 3^3 factorial design was performed to optimize the production of (*S*)-TMSBL. The optimum conditions were: cell concentration (200 g/L), shaking speed (140 rpm), pH (6.9), substrate concentration (14.4 mmol/L), and temperature (33.6 °C). This optimization strategy led to an increase of the yield from 88.9% to 94.5%.

1. Introduction

Organosilicon compounds are unnatural compounds with carbon–silicon bonds which endow them with some unique chemical and physical characteristics when compared to conventional organic compounds. Enantiomerically pure organosilicon compounds such as 3-butyn-2-ol not only play an important part in asymmetric synthesis and functional materials, but also many of them are bioactive and can be applied as silicon-containing drugs,[1,2] such as (*S*)-3-butyn-2-ol while its derivative (*S*)-4-(trimethylsilyl)-3-butyn-2-ol {(*S*)-TMSBL} is a crucial intermediate for the synthesis of 5-lipoxygenase inhibitors.[3]

These compounds can be synthesized in enantiomerically pure form from silicon-containing ketones either biologically, by using a biocatalytic system, or chemically via stereoselective reduction using either a catalytic system or a stoichiometric amount of a reducing agent.[4] Biocatalytic synthesis is usually preferred to chemical synthesis due to a number of advantages including outstanding enantioselectivity, mild reaction conditions, environmental friendliness, and the regeneration of cofactor [i.e., nicotinamide adenine dinucleotide phosphate, NADPH] in situ in whole cells.[5–7]

Among the various biological approaches, there has been much interest in the whole cell-catalyzed biocatalytic enantioselective reduction of prochiral ketones to give enantiopure chiral alcohols because of the high theoretical yield of this reaction. For example, horse liver alcohol dehydrogenase, *Rhodotorula* sp. AS2.2241 cells and *Saccharomyces cerevisiae* cells were successfully used as biocatalysts for the synthesis of enantiomerically pure (*S*)-1-trimethylsilylethanol.[8,9] In comparison to isolated enzymes, whole microbial cells are preferred in order to avoid the need for enzyme purification and addition or complicated extra regeneration of the coenzyme. Among the various microorganisms, the great potential of the yeast cell as a practical biocatalyst has been well recognized due to its high bioavailability, ease of use, low environmental pollution, cost-effectiveness, high efficiency, and mild reaction conditions.[9] In addition, the biocatalytic reduction of 4-(trimethylsilyl)-3-butyn-2-one to give enantiopure (*R*)-4-(trimethylsilyl)-3-butyn-2-ol was successfully conducted with high enantioselectivity by using immobilized whole cells of a novel strain *Acetobacter* sp. CCTCC M209061 or immobilized *Candida parapsilosis* CCTCC M203011 cells.[10–12]

Although those microorganisms are inexpensive, readily available as biocatalysts and their enzymes are well characterized, their utilization for actual production is very low. Since NADPH may be regenerated in situ by using a second redox-reaction to allow it to re-enter the reaction cycle, formate, glucose, and simple alcohols are used to drive the oxidized form of the coenzyme to the reduced forms. Multiple reductases with opposite stereoselectivities toward carbonyl compounds in one microorganism sometimes lower the enantiomer purity of chiral alcohols. The use of plant cells is also problematic with regard to the reproducibility of experiments,

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since the activity of these biocatalysts depends on the places they originate and is easily changeable throughout the year. Thus, for each substrate, a special catalyst needs to be found.

Herein we report on the efficient synthesis of enantiopure (*S*)-TMSBL, which is a crucial intermediate for the synthesis of 5-lipoxygenase inhibitors through the light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *Rhodobacter sphaeroides* (Fig. 1), which is a newly isolated photosynthetic bacteria strain with the capacity of capturing light energy to generate NADPH through photosynthetic electron-transfer reactions; no oxygen or other metabolic intermediates were used, which makes it easy to keep higher activities of redoxase and to separate the reduced product. The reducing power of NADPH generated through photosynthesis can also be used in the reduction of exogenous substrates. We have illustrated a possible mechanism for the light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria (see Fig. 1). The photosynthetic process is driven by the light energy captured by the light-harvesting complexes of photosystem I (PSI) and photosystem II (PSII). The chromatophore molecule in the thylakoid membrane was excited by light energy, the electrons passed along the photosynthetic electron-transport chain via plastoquinone (PQ), the cytochrome, plastocyanin (PC), photosystem I (PSI), and ferredoxin (Fd). The ferredoxin-$NADP^+$ oxidoreductase then transfers the electrons to $NADP^+$ to give NADPH. When the microbes were incubated under illumination, (*S*)-TMSBL alcohol was obtained with high ee and the selectivity was considerably higher than that observed for the reaction completed in the dark; the configuration of the product was (*R*)-TMSBL. These results suggest that NADPH is usually considered to freely bind in, and dissociate from, an active site of (*S*)-redox enzymes [the enzymes that selectively produce (*S*)-TMSBL] or (*R*)-redox enzymes [the enzymes that selectively produce (*R*)-TMSBL]; there are some patterns that enable the transfer of the hydride from the coenzyme NADPH.

We next applied Response Surface Methodology (RSM) to select the best combination of experimental variables (reaction system pH, substrate concentration, and temperature) in order to optimize the light-controlled asymmetric hydrogenation of TMSBO to enantiopure (*S*)-TMSBL by photosynthetic bacteria *R. sphaeroides*.

2. Results and discussion

2.1. Screening of anoxygenic phototrophic bacteria

The anoxygenic phototrophic bacteria capable of reducing TMSBO to (*S*)-TMSBL were screened, using absolute configuration, stereoselectivity, and yield as benchmarks. A total of 51 anoxygenic

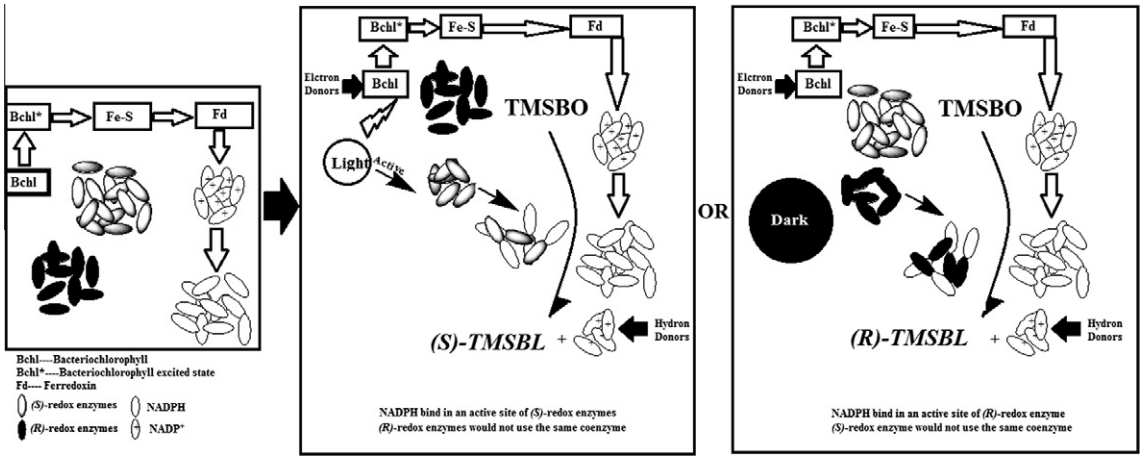

Figure 1. The mechanism of the light-controlled asymmetry hydrogenation of TMSBO by photosynthetic bacteria.

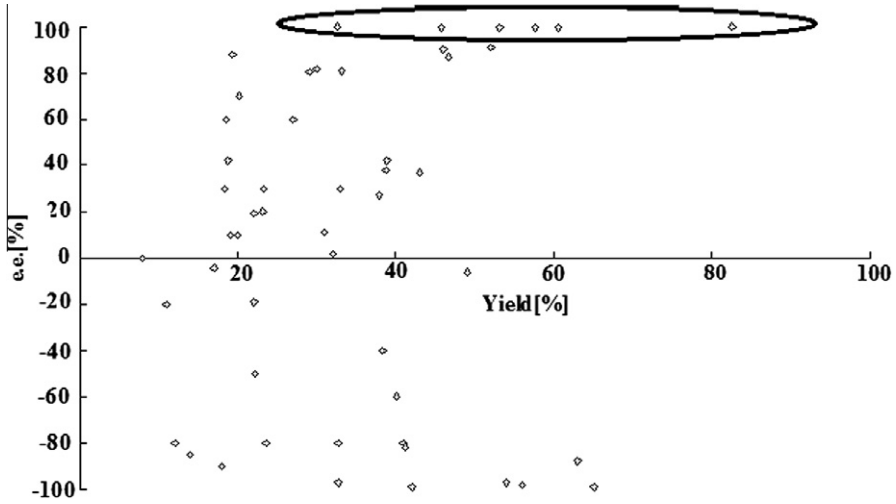

Figure 2. 17 mmol/L TMSBO and 0.2 g/mL wet cells were added to the above mentioned asymmetric reduction system and were inoculated into a 200-mL bubble column photobioreactor containing 100 mL Tris–HCl (50 mmol/L, pH 7.0) The reaction mixture was shaken at 140 rpm and 30 °C under continuous illumination with a fluorescent lamp (daylight type;0–53.6 (μmol photons m^2 s^{-1}). A 5% CO_2 gas (v/v, mixed with air): prodded by a gas cylinder which was aerated from the photobioreactor bottom at rate of 0.1 v/v min^{-1} (volume gas per volume broth per minute).

phototrophic bacteria strains were tested. As shown in Figure 2, six strains were found to be potentially useful for the preparation of (*S*)-TMSBL, with the desired ee ranging from >99% and moderate to high yields (circled). We screened further for the most suitable microbe. The results showed that photosynthetic bacteria *R. sphaeroides* exhibited the capacity to capture light energy to generate NADPH through photosynthetic electron-transfer reactions; no oxygen or other metabolic intermediates were used, which made it easy to obtain higher activities of the redoxase and to separate the reduced product. The reducing power of NADPH generated through the photosynthesis can also be used in the reduction of TMSBO to produce (*S*)-TMSBL, and so was chosen for further study.

2.2. Effect of illumination on the asymmetric reduction of TMSBO by photosynthetic bacteria *Rhodobacter sphaeroides*

Since the microbe we used is a type of photosynthetic bacteria that grows under illumination, we supposed that the relative enzymatic activities were largely influenced by the amount of light in the environment. To confirm the regeneration efficiency of the cofactor NADPH by a light reaction during photosynthesis, we investigated the effect of illumination on the light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria. The results are shown in Table 1. Illumination not only improved the chemical yield (86.7%), but also the enantiomeric purity. Exposed to illumination, (*S*)-TMSBL was obtained with 99.2% ee, where the selectivity was considerably higher than that observed for the reaction completed in the dark; the configuration of the product (*R*)-TMSBL. From this result it is evident that the relative activities of the enzymes that conduct the light-controlled asymmetric hydrogenation of TMSBO may also vary and reactions in the light may afford different stereoselectivities from those in the dark. Thus, we have found that NADPH is usually considered to freely bind in and dissociate from an active site of (*S*)-redox enzymes [the enzymes that selectively produce (*S*)-TMSBL] or (*R*)-redox enzymes (the enzymes that selectively produce (*R*)-TMSBL), and that there are some patterns that enable the transfer of the hydride from the coenzyme NADPH. Under illumination, only the (*S*)-redox enzymes are activated, the (*S*)-redox enzymes thus generated use NADPH selectively while the (*R*)-redox enzymes would not use the same coenzyme. Under darkness, the (*R*)-redox enzymes preferentially use the thus generated reduced form of the coenzyme. The (*S*)-redox enzymes can also participate in the reduction in the dark and consequently, both (*R*)- and (*S*)-TMSBL are produced.

Table 1
Light-controlled asymmetric hydrogenation of 4-(trimethylsilyl)-3-butyn-2-one by various photosynthetic bacteria

Entry	Light	Time (h)	Yield (%)	ee (%)	Configuration
1	On	24	86.7	99.2	(*S*)
2	Off	24	39.7	66.9	(*R*)

17 mmol/L of TMSBO was added to a suspension of 0.2 g/mL cells (wet weight) in 100 mL Tris–HCl (50 mmol/L, pH 7.0). The reaction mixture was shaken at 140 rpm at 30 °C under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}). 5% CO_2 gas (v/v, mixed with air), provided by a gas cylinder, was aerated from the photobioreactor bottom at a rate of 0.1 v/v min^{-1} (volume gas per volume broth per minute).

2.3. Effect of independent variables on the light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *R. sphaeroides*

2.3.1. Effect of pH on the reaction

The buffer pH influenced not only the enzymatic enantioselectivity and activity but also the regeneration of the coenzymes, which in turn affects the reaction rates.[8] Thus, the effect of pH was also investigated.

From the results shown in Figure 3, the optimum buffer pH was determined at 30 °C using four different buffers of various pH values: acetate buffer (pH 4–6), potassium phosphate buffer (pH 6–8), Tris–HCl buffer (pH 8–9), and carbonate buffer (pH 9–10). The best performances were observed under neutral pH conditions (pH 6.0–7.2), particularly at pH 6.6 for the potassium phosphate buffer. A lower or higher pH caused a decrease in the yield especially in an acidic environment (pH <5), i.e. from 84.2% to 10.0%. The enantiopurity remained stable over the pH range 5 to 10, and then decreased dramatically to 70.0% at pH 4. These results indicated that the enzyme system responsible for the reduction reaction was sensitive to a strongly acidic environment, but stayed active over a wide pH range (5–10). As a result, pH 6.6 was chosen as the favorable pH.

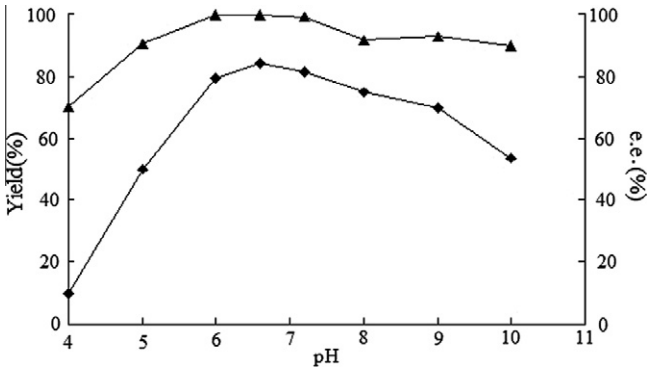

Figure 3. Effect of pH on light-controlled asymmetric hydrogenation of TMSBO. The product ee (▲) and yield (♦) were assayed under various pH conditions. Reaction conditions: 0.2 s/mL wet cells, 17 mmol/L TMSBO in 100 mL buffer 30 °C and 140 rpm under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}).

2.3.2. Effect of substrate inhibition on the reaction

Microbial cells, when employed in organic synthesis as biocatalysts, can always be seriously repressed by synthetic substrates, especially in the presence of a high concentration of these substrates.[13] The results are shown in Figure 4.

Increasing the substrate concentration caused the ee value and yield to continuously decrease, due to the known inhibitory effect on the cells. The ee value and yield reached their highest values (99.1% and 87.4% respectively) at a substrate concentration of 17 mmol/L; both of them decreased dramatically to their minima at 60 mmol/L of TMSBO. The mechanism of the substrate inhibition is generally expressed by the non-competitive inhibition as shown in Figure 5.[14–16]

In Figure 5, S, P, E, ES, and ES_2 are substrate, product, enzyme, enzyme–substrate complex, and enzyme-substrate-substrate complex, respectively. Moreover, K_m, K_p, and K_i represent Michaelis–Menten constant, catalytic rate constant, and inhibition constant, respectively. The dependence of the reaction rate on the substrate concentration can be derived as:

$$V = K_p[\mathrm{ES}] = \frac{V_m[S]}{K_m + [S] + ([S]^2/K_i)}$$

where V_m is the maximum reaction rate. The [ES] and [*S*] are concentrations of the enzyme–substrate complex and the substrate respectively.

2.3.3. Effect of cell concentration on the reaction

Figure 6 shows that the conversion increased with the concentration of cells used in the reduction. Higher conversion rates can

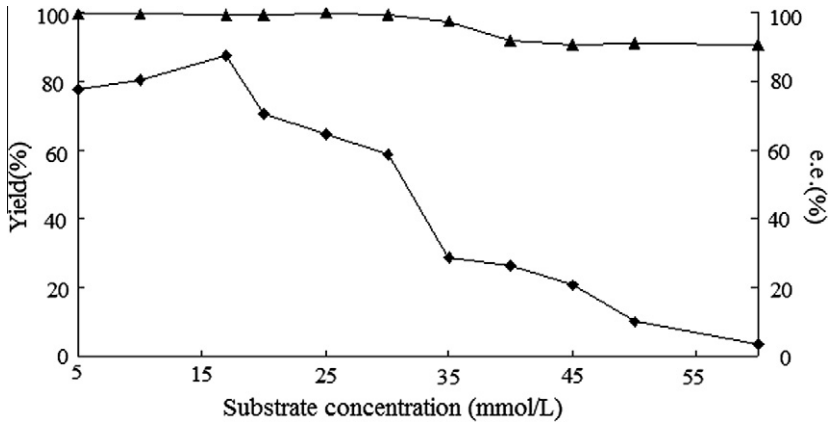

Figure 4. Effect of substrate concentration on light-controlled asymmetric hydrogenation of TMSBO. The product ee (▲) and yield (♦) were assayed at different substrate concentrations. Reaction conditions: 0.2 g/mL wet cells, 100 mL potassium phosphate buffer (0.1 mml/L, pH 6.6), 30 °C and 140 rpm under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}), 48 h.

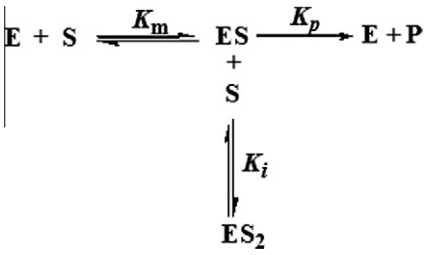

Figure 5. The mechanism of substrate inhibition on light-controlled asymmetric hydrogenation of TMSBO.

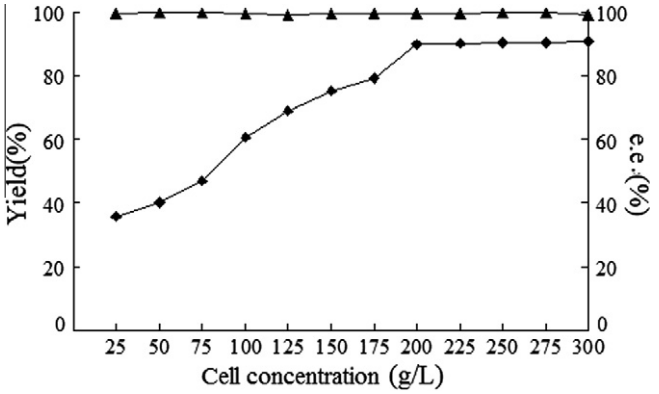

Figure 6. Effect of cell concentration on light-controlled asymmetric hydrogenation of TMSBO. Reaction conditions: 100 mL potassium phosphate buffer (0.1 mmol/L, pH 6.6), 30 °C and 140 rpm under continuous illumination with a fluorescent lamp (daylight type 0–53.6 μmol photons m^2 s^{-1}) 48 h.

be obtained with more biomass because of the large amounts of reductase and co-enzyme NADPH in these cells. The optimum cell concentration was found to be 200 g L^{-1}. The mixture was too thick to be stirred well when the cell concentration was higher than 200 g L^{-1}. The ee of (*S*)-TMSBL remained steady at >99% and did not vary with cell concentration.

2.3.4. Effect of shaking speed on the reaction

The shaking speed influences the diffusion and partition of the substrate and the product. Herein it was found that the initial reaction rate and the molar conversion increased while the enantiomeric purity of the product was maintained at 96.8–99.7% ee when increasing the shaking speed from 25 to 200 rpm. A slight change in the initial reaction rate, the molar conversion, and the enantiomeric purity of the product were observed with a further increase in shaking speed above 140 rpm. Therefore, the optimal shaking speed was considered to be 140 rpm (see Fig. 7).

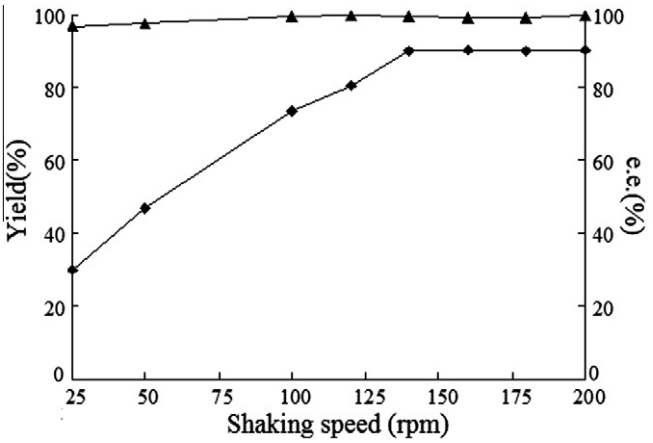

Figure 7. Effect of shaking speed on light-controlled asymmetric hydrogenation of TMSBO. The product ee (▲) and yield (♦) were assayed at different shaking speed. Reaction conditions: 0.2 g/mL wet cells and 17 mmol/L TMSBO in 100 mL potassium phosphate buffer (0.1 mmol/L, pH 6.6% 30 °C under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}), 48 h.

2.3.5. Effect of temperature on the reaction

It is well known that temperature has a significant effect on the activity and stability of a biocatalyst in addition to the equilibrium of a reaction. The ee values and yields of the product at different temperatures are shown in Figure 8. The yield did not change greatly when the temperature was between 20 and 35 °C. Maximum levels of 99.7% ee and 87.3% yield were obtained at 30 °C. As the temperature reached 40 °C, the yield decreased sharply. The main reason for this was that the deactivation rate of the enzyme was accelerated at higher temperatures. The enantiometric excess of (*S*)-TMSBL remained at >99% ee at different temperatures.

2.4. Response Surface Methodology (RSM) applied to the optimization of light-controlled asymmetric hydrogenation of 4-(trimethylsilyl)-3-butyn-2-one by photosynthetic bacteria

Several preliminary tests were performed in order to evaluate the effects of the parameters on the light-controlled asymmetric

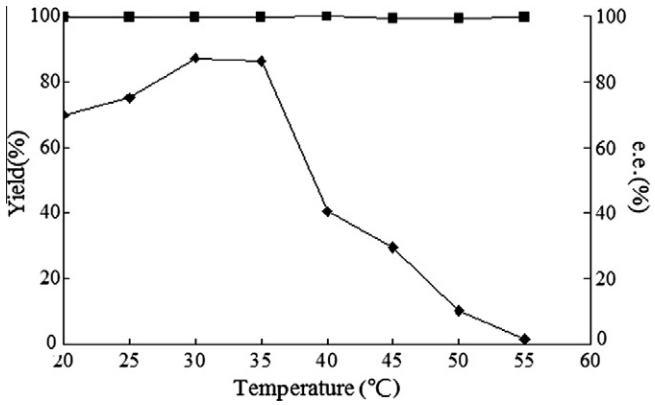

Figure 8. Effect of reaction temperature on light-controlled asymmetric hydrogenation of TMSBO. The product ee (▲) and yield (♦). Reaction conditions: 0.2 g/mL wet cells, 17 mmol/L TMSBO in 100 mL potassium phosphate buffer (0.1 mml/L, pH 6.6), 30 °C and 140 rpm under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}), 48 h.

hydrogenation of 4-(trimethylsilyl)-3-butyn-2-one by photosynthetic bacteria; the pH (X_3), substrate concentration (X_4), and temperature (X_5) were regarded as the most important factors. Two sequential full factorial designs (3^3) with three variables varying at three levels were carried out in order to obtain the optimal conditions for (*S*)-TMSBL preparation. The variables and their coded and uncoded values are presented in Table 2 for the first design. Table 3 shows the 27 experiments of each design. The runs were performed in a random order, but are presented in a standard order.

On the basis of these preliminary experiments, the effect of pH, substrate concentration, and temperature on the yield of (*S*)-TMSBL was studied with RSM, which led to the following regression equation which is an empirical relationship between the yield of (*S*)-TMSBL and the test variables in a coded unit. The following was the second order polynomial equation showing the fitted response surface:

$$Y = -920.0547 + 138.3963X_3 + 25.5384X_4 + 20.8673X_5 + 0.1930X_3X_4 + 0.4367X_3X_5 + 0.0458X_4X_5 - 11.2444X_3^2 - 0.9889X_4^2 - 0.3658X_5^2$$

where Y is the response, i.e. the yield of (*S*)-TMSBL, and X_3, X_4, and X_5 are coded values of the test values of pH, substrate concentration, and temperature, respectively. The vector STATS contains the R-square statistic along with the F and p values for the regression (0.7375, 5.3063, and 0.0016).

The regression model developed can be represented in response surfaces and contour plots to help understand the interactions among the three variables and to determine the optimum level of each variable for the maximum response. Response surfaces with contour (at the base) plot show the interaction of two independent variables when another variable is fixed at zero. Figs. 9–11 present the response surface curves established for the optimization of the light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria. The symmetrical shapes indicate that there was a significant interaction between each of the parameters (pH, substrate concentration, and temperature; Figures 9–11). As shown in the surface plots, there was interaction between each pair of variables. All of the interactions between the selected three variables were significant. According to the quadratic regression analysis, the results predicted by the model showed that the maximum yield could be achieved when the initial pH,

Table 2
Levels of the factors considered in the experimental design

Variable	Name	Coded level		
		−1	0	1
X_1	Cell concentration		200 g mL^{-1}	
X_2	Shaking speed		140 rpm	
X_3	pH	6	7	8
X_4	Substrate concentration	10 mmol/L	17 mmol/L	20 mmol/L
X_5	Temperature	30 °C	35 °C	40 °C

Table 3
Batch experiments for each normalized level of the three considered

Run	X_3	X_4	X_5	Yield (%)	ee (%)	Residual vector	R_{int}
1	−1	−1	−1	73.1	99.6	10.863	−2.9518, 23.1245
2	−1	−1	0	68.3	99.8	4.4353	−12.1049, 20.9754
3	−1	−1	1	41.8	99.2	−4.6269	−18.4957, 9.2418
4	−1	0	−1	52.2	99.4	−20.5853	−33.4005, −7.7702
5	−1	0	0	84.7	99.6	9.4590	−7.7476, 26.6657
6	−1	0	+1	49.9	99.5	−9.5077	−25.5380, 6.5226
7	−1	+1	−1	56.7	99.2	9.3934	−5.1184, 23.9053
8	−1	+1	0	41.8	99.3	−8.6498	−25.2828, 7.9832
9	−1	+1	+1	45.3	99.7	9.9958	−4.4037, 24.3953
10	0	−1	−1	60.2	99.6	−10.1026	−25.9347, 5.7295
11	0	−1	0	75.5	99.4	2.1630	−15.7846, 20.1105
12	0	−1	+1	53.9	99.1	−4.1826	−20.7410, 12.3759
13	0	0	−1	86.6	99.5	5.1464	−12.5622, 22.8549
14	0	0	0	88.9	99.2	2.8074	−15.1153, 20.7301
15	0	0	+1	80.2	99.6	7.7573	−9.6845, 25.1992
16	0	+1	−1	55.6	99.6	−0.9660	−18.2058, 16.2739
17	0	+1	0	62.7	99.8	0.8074	−17.1713, 18.7862
18	0	+1	+1	45.5	99.8	−3.4303	−20.5825, 13.7219
19	+1	−1	−1	56.8	99.6	1.6973	−12.3560, 15.7506
20	+1	−1	0	51.6	99.9	−8.7204	−24.7795, 7.3386
21	+1	−1	+1	56.5	99.3	9.2507	−3.9588, 22.4602
22	+1	0	−1	71.7	99.5	4.0670	−12.5919, 20.7259
23	+1	0	0	80.8	99.6	6.3447	−11.2554, 23.9447
24	+1	0	+1	57.5	99.4	−5.4887	−22.0338, 11.0563
25	+1	+1	−1	44.6	99.8	1.2635	−14.0564, 16.5834
26	+1	+1	0	42.2	99.6	−8.6465	−25.2799, 7.9870
27	+1	+1	+1	40.3	99.5	0.2325	−15.1014, 15.5663

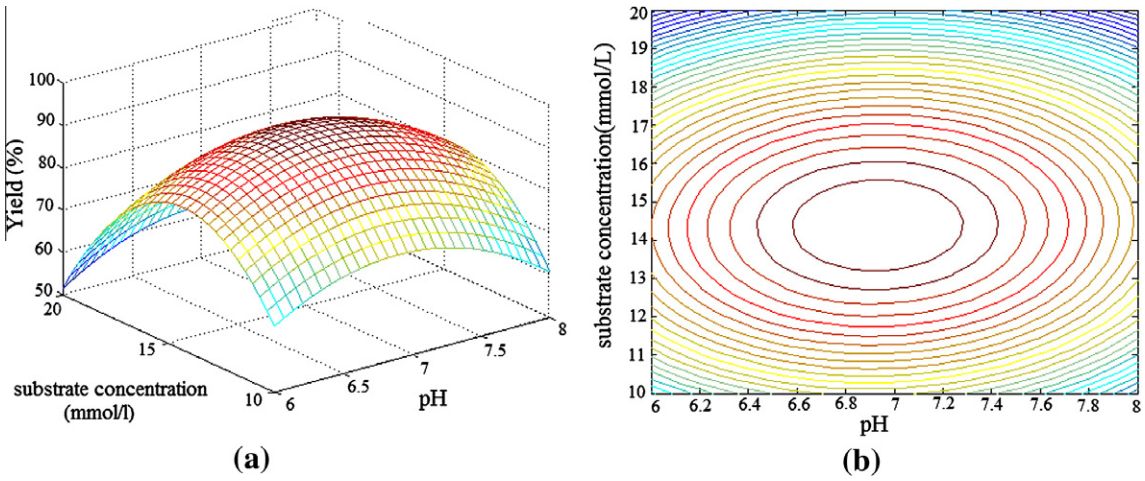

Figure 9. Response surface plots (a) and the contour graphic lines (b) on light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *Rhodobacter sphaeroides* optimization of variables. The interaction between substrate concentration and pH. Reaction conditions: 0.2 g/mL wet cells and TMSBO were added to the above mentioned asymmetric reduction system and were inoculated into a 200-mL bubble photobioreactor containing 100 mL buffers. The reaction mixture was shaken at 140 rpm under continuous illumination with a fluorescent lamp (daylight type, Q-53.6 μmol photons m^{-2} s^{-1}).

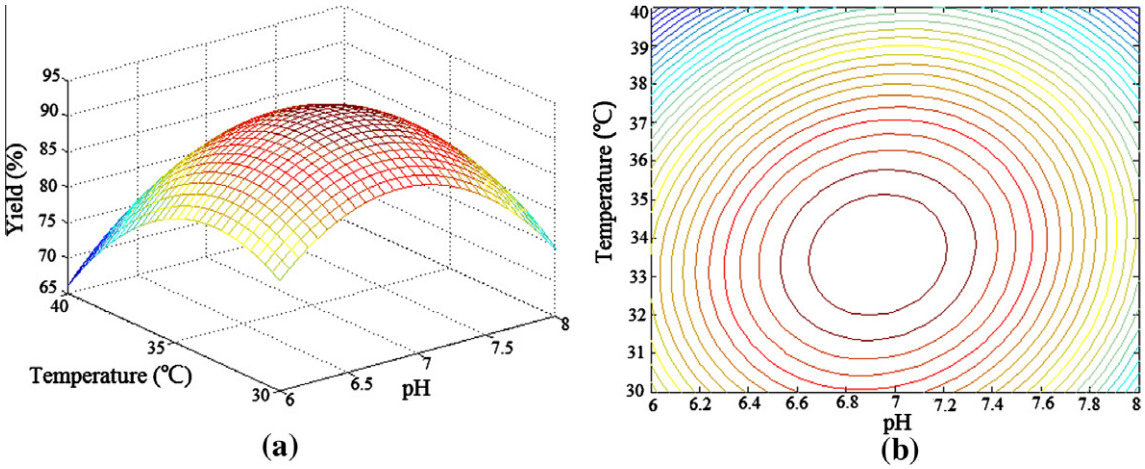

Figure 10. Response surface plots (a) and the contour graphic lines (b) on light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *Rhodobacter sphaeroides* optimization of variables. The interaction between temperature and pH. Reaction conditions: 0.2 g/mL wet cells and TMSBO were added to the above mentioned asymmetric reduction system and were inoculated into a 200-mL bubble photobioreactor containing 100 mL buffers. The reaction mixture was shaken at 140 rpm under continuous illumination with a fluorescent lamp (daylight type. 0–53.6 μmol photons m^{-2} s^{-1}).

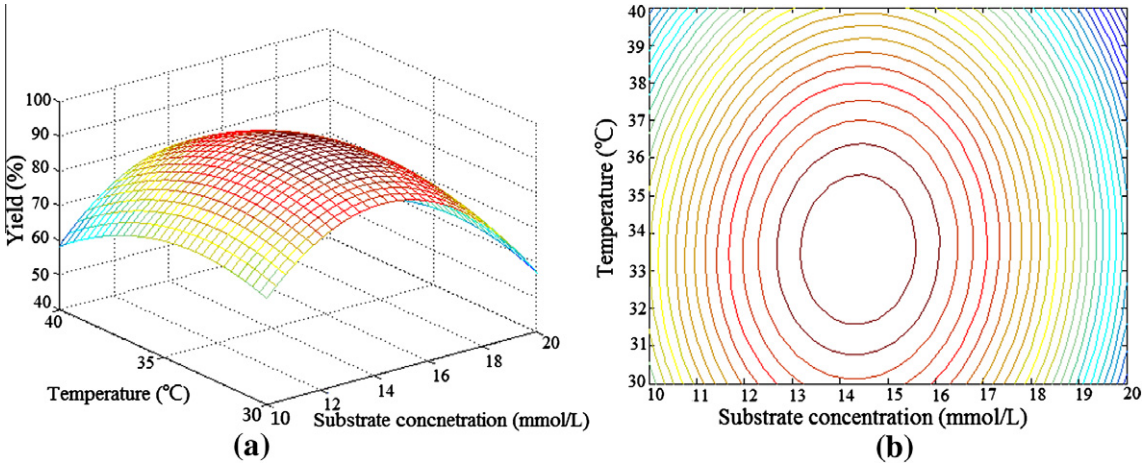

Figure 11. Response surface plots (a) and the contour graphic lines (b) on light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *Rhodobacter sphaeroides* optimization of variables. The interaction between substrate concentration and temperature. Reaction condition: 0.2 g/mL wet cells and TMSBO were added to the above mentioned asymmetric reduction system and were inoculated into a 200-mL bubble photobioreactor containing 100 mL buffers. The reaction mixture was shaken at 140 rpm under continuous illumination with a fluorescent lamp (daylight type. 0–53.6 μmol photons m^{-2} s^{-1}).

substrate concentration, and temperature were set at 6.9, 14.4 mmol/L and 33.6 °C, respectively. The maximum predicted yield was 93.4%.

In order to confirm the optimization results, the suggested conditions were performed in triplicate. Under these suggested conditions, the mean value of the (*S*)-TMSBL yield was 94.5%, which was in agreement with the predicted value. This optimization strategy led to an enhancement of the yield from 88.9% to 94.5%. The models developed were considered to be accurate and reliable for predicting the production of (*S*)-TMSBL by photosynthetic bacteria *R. sphaeroides*.

3. Conclusion

The light-controlled asymmetric hydrogenation of TMSBO by photosynthetic bacteria *R. sphaeroides* is a very promising technology for the production of high-quality (*S*)-TMSBL. The results herein clearly indicate that RSM is an effective method for the optimization of the reaction conditions for maximizing the yield of (*S*)-TMSBL. The values of the three main variables, pH (6.9), substrate concentration (14.4 mmol/L), and temperature (33.6 °C), were found to be optimum for the production of (*S*)-TMSBL with high levels of activity and stability.

4. Experimental

4.1. Materials and microorganism

4-(Trimethylsilyl)-3-butyn-2-one (97% purity), 4-(trimethylsilyl)-3-butyn-2-ol (97% purity), and *n*-decane (>99% purity) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were from commercial sources and were of reagent grade or better. All photosynthetic bacteria were preserved in our laboratory (Institute of Applied Chemistry, Shanxi University).

4.2. Cell culture and medium

Photosynthetic bacteria *R. sphaeroides* was cultivated in medium containing (per liter) 0.5 g of KH_2PO_4, 0.6 g of K_2HPO_4, 1.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.2 g of NaCl, 0.05 g of $CaCl_2{\cdot}2H_2O$, 0.1 g of yeast extract, 4 g of malic acid, and 0.2 mL of trace element solution [2 g of ethylenediaminetetraacetic acid disodium salt, 2 g of green vitriol, 0.1 g of boric acid, 0.1 g of cobalt chloride, 0.1 g of zinc chloride, 0.1 g of manganese(II) chloride tetrahydrate, 0.02 g of sodium molybdate, 0.02 g of nickelous chloride, 0.01 g of cupric chloride, and 0.001 g of sodium selenite].

The medium was adjusted to pH 7.2 with 2 M NaOH and autoclaved for 30 min. A pre-culture was prepared by inoculation of 100 mL of the complex medium with fresh cells from an agar plate (Swab of inoculation loop). Incubation was performed in a 1000-mL Erlenmeyer shaking flask. These were grown in the medium filled with nitrogen under continuous illumination provided by fluorescence lamps (200 W) at 30 °C. After five days, the cultured bacteria (OD_{680} was approximately 1.0, where OD_{680} is the optical density at 680 nm, used to indicate the microalgal biomass density based on turbidimetry) (Ultrospec® 3300 pro Amersham Biosciences Co., Ltd) were collected by centrifugation (5000 rpm, 20 min), washed twice with phosphate buffer (0.2 mmol/L, pH 7.0) and separated from the aqueous medium by centrifugation to give a cell wet mass of 2–5 g per 400 mL batch. The ratio 'cell wet mass'/'cell dry mass' of approximately 4.0 was determined by lyophilization of the samples of wet cells. The wet cells were used directly for this reaction.[9]

4.3. General procedure for the light-controlled asymmetric hydrogenation system

An appropriate substrate was added to the above mentioned asymmetric reduction system and then inoculated into a 200-mL bubble column photobioreactor containing 100 mL of the 0.1 M potassium phosphate buffer (KPB, pH 7.0).The reaction mixture was shaken at 140 rpm at 30 °C under continuous illumination with a fluorescent lamp (daylight type, 0–53.6 μmol photons m^{-2} s^{-1}). The 5% CO_2 gas (v/v, mixed with air) was provided by a gas cylinder, which was aerated from the photobioreactor bottom at a rate of 0.1 v/v min^{-1} (volume gas per volume broth per minute). Finally, the reaction mixture was extracted with ethyl acetate and the organic phase was dried over anhydrous Na_2SO_4. The concentrations of product and substrate were determined, and the chemical yield and enantiomeric excess (ee) were evaluated.

4.4. Experimental design and data analysis

The experimental design and analysis of results were carried out using MATLAB 6.5.0 (Mathworks, USA). Response Surface Methodology (RSM) was used to investigate the effects of substrate concentration, pH, and temperature on the reaction yield. A 3^3 factorial design was performed in order to optimize the production of (*S*)-TMSBL.

The following equation describes the regression model utilized in the factorial planning, including the interaction terms:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \mp \sum \beta_{ij} X_i X_j$$

where Y is the predicted response variable {the yield of (*S*)-TMSBL}, β_0 is the intercept coefficient, β_i is the coefficient of the linear effects, β_{ij} is the coefficient of interaction and X_i and X_j are the independent variables.

4.5. Analysis

Gas chromatographic analysis was performed using a SHIMADZU model 7900 gas chromatograph (GC) equipped with a GP CHIRASIL-DEX (25 m × 0.25 mm; Agilent Technologies Co., Ltd) and flame ionization detector. The split ratio was 100:1. The injector and the detector were both kept at 250 °C. The column temperature was held at 71 °C for 1 min, then programed to increase at 1 °C/min to 100 °C, and maintained for 2 min at this temperature. The carrier gas was nitrogen and its flow rate in the column was 2.5 ml/min. Quantitative data were obtained after integration on an HP LaserJet 5200L integrator. An internal standard method was used for the calculations. The retention times for ATMS, *n*-nonane, (*R*)-1-TMSBL, and (*S*)-1-TMSBL were 3.374, 5.202, 5.417, and 5.747 min, respectively. JASCO DIP-378 polarimeter was used for the determination of the enantiomeric excesses (ee). The products were identified by ICT GC-MS analyses (model 2010, SHIMADZU). The structures were confirmed by comparison with the mass spectroscopic database.

The reaction degree and enantioselectivity are indicated by yield (chemical yield (%)) and ee (%), respectively, defined as

$$\text{yield} = \frac{C_p}{C_o} \times 100$$

$$\text{ee} = \frac{C_s - C_R}{C_s + C_R} \times 100$$

where C_o is the initial substrate concentration, C_p is the final product concentration, C_s is the final (*S*)-form product concentration, and C_R is the final (*R*)-form product concentration.

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References

1. Kawamoto, T.; Yamanaka, H.; Tanaka, A. *Appl. Biochem. Biotech.* **2000**, *88*, 17–22.
2. Tacke, R. *Recent Results in Bioorganosilicon Chemistry: Novel Sila-drugs and Microbial Transformations of Organosilicon Compounds*; Chichester: Ellis Horwooppd, 1985. pp 251–262.
3. Stampfer, K.; Visser, R.; Kanzian, C. *International Journal of Forest Engineering* **2006**, *17*, 71–77.
4. Wills, M. *Chem. Soc. Rev.* **1995**, *24*, 177–179.
5. Ema, T.; Yagasaki, H.; Okita, N.; Nishikawa, K.; Korenage, T.; Sakai, T. *Tetrahedron: Asymmetry* **2005**, *16*, 1075–1078.
6. Zymanĭczyk-Duda, E.; Brzezinĭska-Rodak, M.; Klimek-Ochab, M.; Lejczak, B. *Curr. Microbiol.* **2010**, *62*, 1168–1172.
7. Kuo, T. M.; Huang, J. K.; Labeda, D.; Wen, L.; Kenothe, G. *Curr. Microbiol.* **2008**, *57*, 437–441.
8. Di-heng, Luo; Min-Hua, Zong; Jian-He, Xu *J. Mol. Catal. B-Enzym.* **2003**, *24*, 83–88.
9. Wen-Yong, Lou; Min-Hua, Zong; Yuan-Yuan, Zhang; Hong, Wu *Enzyme Microb. Tech.* **2004**, *35*, 190–196.
10. Xiao, Zi-Jun; Zong, Min-Hua; Lou, Wen-Yong *Bioresource Technol.* **2009**, *100*, 5560–5565.
11. Zhang, Bo-Bo; Lou, Wen-Yong; Zong, Min-Hua; Hong, Wu *J. Mol. Catal. B-Enzym.* **2008**, *54*, 122–129.
12. Zhang, Bo-Bo; Lou, Wen-Yong; Chen, W. J.; Zong, Min-Hua *PLoS One* **2012**, *7*, e37641.
13. Carvalho, C. C. C. R.; Poretti, A.; Fonseca, M. M. R. *Appl. Microbiol. Biot.* **2005**, *69*, 268–275.
14. Evans, C. T.; Hanna, K.; Payne, C.; Conrad, D. W.; Misawa, M. *Enzyme Microb. Tech.* **1987**, *9*, 417–421.
15. Lortie, R.; Andre, G. *Enzyme Microb. Tech.* **1991**, *13*, 960–963.
16. Wang, J.; Araki, T.; Ogawa, T.; Matsuoka, M.; Fukuda, H. *Biotechnol. Bioeng.* **1999**, *62*, 402–411.