Optimization of asymmetric bio-oxidation with resting cells for preparation of (S)-omeprazole in the chloroform–water biphasic systems using response surface methodology

Yuanyuan Zhang[a,b*], Kuiying Lv[a,d], Yashan Deng[a,d], Huiling Li[a,c], Zhiyong Wang[a,d], Depeng Li[a,b], Xin Gao[a,c*], Fanye Wang [a,†]

Abstract: (S)-Omeprazole is a very effective anti-ulcer medicine, and it is a significant challenge to prepare it by whole cells and to substantially increase the substrate concentration. In the chloroform–water biphasic system, resting cells of the mutant of *Rhodococcus rhodochrous*(*R. rhodochrous*)ATCC 4276 were employed to catalyze the bio-oxidation of the omeprazole sulfide for preparation of (S)-omeprazole. At a high substrate concentration(180 mM) and cell concentration(100 g/L), the bio-oxidation was optimized using response surface methodology(RSM), and the optimal yield of (S)-omeprazole obtained was 92.9% with enantiomeric excess(e.e.) (>99%), and no sulfone product was detected under the optimal conditions: the reaction temperature was 37°C, pH of phosphate buffer, 7.3 and the reaction time, 43h respectively. A quadratic polynomial model was established, which predicts the experimental data with very high accuracy according to R^2 of 0.9990. The chloroform–water biphasic system may mainly contribute the significant improvement of substrate tolerance because almost all substrates may partitioned in the organic phase (water solubility of omeprazole sulfide is only about 0.5 mg/ml), resulting in little damage and inhibition to cells by substrates. The mutant of *R. rhodochrous* ATCC 4276 exhibited a high enantioselective, activity and substrate and product tolerance. The aerated flask provides enough oxygen for a high concentration of cells. Accordingly, the bio-oxidation is thus more promising for efficient preparation of chiral sulfoxides.

Keywords: (S)-omeprazole; resting cells; response surface methodology; organic–aqueous biphasic systems; asymmetric sulfoxidation.

1 Introduction

Chiral sulfoxides belong to the class of chiral organic sulfur compounds, including various

*Corresponding author at: Department of Pharmaceutics, Qingdao University of Science and Technology, 53 Zhengzhou Road, 266042 Qingdao, China.

*E-mail:zyy800928@126.com (Y. Zhang); zljh06@163.com(J. Liu)

a State Key Laboratory Base for Eco-Chemical Engineering in College of Chemical Engineering, QingdaoUniversity of Science and Technology, Mail box 70, 53 Zhengzhou Road, Qingdao 266042, China

b William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, 151 West Woodruff Avenue, Columbus, Ohio 43210,USA

c Kekulé-Institutfür Organische Chemie und Biochemie der Rheinischen Friedrich-Wilhelms-Universität Bonn Gerhard-Domagk-Str. 153121 Bonn Germany

d Department of Pharmaceutical Engineering, College of Chemical Engineering, Qingdao University of Science and Technology, Mail box 70, 53 Zhengzhou Road, Qingdao 266042, China

†

chemical and biologically active molecules, especially medicines, such as the anti-ulcer drug proton pump inhibitors(PPI). Single enantiomer (S)-omeprazole is one of chiral sulfoxides, having better therapeutic effect than racemates, own to the enantioselective pharmacokinetics of human body(Andersson and Weidolf, 2008; Pai and Pai, 2007). Chiral sulfoxides are mainly prepared by the asymmetric oxidation of sulfides with metal complex catalysts(Adam et al., 1998; Delamare et al., 2009; Dembitsky, 2003; Maitro et al., 2010) and some by enzymatic catalysts, such as monooxygenases(Kamerbeek et al., 2003; Zambianchi et al., 2007), horseradish peroxidases(Adam et al., 2002; Colonna et al., 1999; Van Deurzen et al., 1997; Dzyuba and Klibanov, 2003), hemoglobin myoglobin(Ozaki et al., 1997; Ozaki et al., 1999) and cytochrome(Akasaka et al., 1993), however, there are many defects including environmental damage, requirement of a cofactor cycling system and cost-expensive. While chiral sulfoxides may also be formed by whole cell bio-oxidation of prochiral sulfides, which has many benefits, such as cost-effective and no requirement of expensive cofactor regeneration. In recent years, whole cell bio-oxidations of prochiral sulfides in single water phase system have attracted a lot of attentions(Borges et al., 2009; Carballeira et al., 2009; Elkin et al., 2013; Holland et al., 1997; Holland et al., 2003; Li et al., 2009; Li et al., 2011b; Li et al., 2011a; Olivo et al., 2005; Pinedo-Rivilla et al., 2007; Ricci et al., 2005).

More especially, proton pump inhibitors are prepared by whole cell bio-oxidations of prochiral sulfides. The chiral bio-oxidation of omeprazole sulfides was conducted with 15 strains with e.e. of 17% to 99% of the (R) form(Holt et al., 1998). A fungal strain *Cunninghamella echinulata* MK40 was employed to prepare rabeprazole with 99% e.e. of the (S) form, and also formation of omeprazole and lansoprazole with the yield of 49% and 0.6% respectively(Yoshida et al., 2001). With fed-batch culture in a stirred bioreactor, the asymmetric bio-oxidation of the omeprazole sulfide was carried out to prepare (S)-omeprazole with conversion of 77%. Above works were performed in single water phase systems and the substrate concentration was very low (0.08-1.5g/L), and conversions reduced substantially with the increase of the substrate concentration owing to strong substrate and product inhibition on the cells(Li et al., 2011b).

A high concentration of substrate and product is of great significance to improve the yield per unit volume, i.e. operating strength of a reactor, reducing the production cost effectively. Therefore, many scientists are committed to improving the concentration of substrate and product. In the whole cell

chiral bio-oxidation of organic sulfides for the formation of enantiomeric sulfoxides were carried out in organic–aqueous biphasic systems, such as the isooctane–aqueous biphasic system, to significantly improve the tolerance of cells to substrate(Gong and Xu, 2005; He et al., 2006; Kansal and Banerjee, 2009). However, so far, no studies have been done on the whole cell bio-sulfoxidation of the omeprazole sulfide in an organic–water biphasic system.

In this study in order to significantly improve the substrate concentration, organic–aqueous biphasic systems were used to prepare enantiomeric (S)-omeprazole through the whole cell bio-sulfoxidation of the omeprazole sulfide, in which a mutant of *R. rhodochrous* ATCC 4276 was employed. The yield and e.e. of (S)-omeprazole were markedly improved by optimization the process conditions using response surface methodology (RSM) with significant less tests.

2. Materials and methods

2.1. Chemicals and microbial strain

(S)-omeprazole, was bought from Suzhou Vita Chemical Co., Ltd, KH_2PO_4 and K_2HPO_4, Qingdao Huadong Chemical Reagent and Glass Instrument Co., Ltd., omeprazole from Shandong Shouguang Fukang Pharmaceutical Co., Ltd, chloroform and acetonitrile, from Qingdao Hailitan Chemical Reagent Co., Ltd and omeprazole sulphide from Jinan Wald Chemical Co., Ltd, respectively. All other chemicals were commercially available with analytical grade purity and were used with no any treatment. A mutant of *R. rhodochrous* ATCC 4276 used in the present study was obtained with a complex mutagenesis by using 0.025 moL/L $NaNO_2$ for 20 min and UV irradiation with the distance 20 cm for 30 seconds, and the resulted mutant had a better bio-oxidation activity and tolerance to substrates than the wild strain.

2.2 Preparation of resting cell biocatalysts

Strains of a mutant of *Rhodococcus rhodochrous* ATCC 4276 were inoculated 1-L Erlenmeyer flasks containing 200mL of a medium composed of potassium nitrate(1g), potassium dihydrogen phosphate(1g), potassium dihydrogen phosphate(1g), sodium chloride(1g), magnesium sulfate heptahydrate(0.2 g), calcium chloride dehydrate (0.02g), ferric chloride (0.001g), yeast powder(1g), n-hexadecane(1ml) per litre of distilled water, adjusted pH to 6.8-7.0 with 1moL/L sodium hydroxide solution. Incubation was carried out at 30°C for 62h on a rotary shaker at 160 rpm. The cells were then

harvested by centrifugation at 5000 rpm for 15 min and directly used as biocatalysts to catalyze asymmetric sulfur oxidation of the omeprazole thioether to synthesize esomeprazole.

2.3 Bio-oxidation of omeprazole sulfide catalyzed by resting cells in an organic–aqueous biphasic system to form (S)-omeprazole

The bio-oxidation was performed in a flask (1L volume) in a water bath shaker at 150 rpm and 34-40°C for 35-45 h. The flask was equipped with a stainless steel pipe (3 mm inner diameter) which was connected to clean air source by silicone rubber hose, to achieve aeration rate of 0.2vvm. The organic and aqueous phase was chloroform and phosphate buffer (pH 6.3-8.3, glucose 2g/L), respectively. Omeprazole sulfide was dissolved in chloroform, leading to a final concentration of 100-200mM (32.9-65.8g/L), and both the substrate and product concentrations were only on account of the volume of chloroform unless otherwise specified. The final concentration was 10-20mM (3.29-6.58g/L), on the other hand, when the concentration was on account of the total volume of reaction media including water and chloroform. The omeprazole sulfide-chloroform solution, the phosphate buffer and harvested resting cells of a mutant of *R. rhodochrous* were added into the flask in 0.3-0.4 volume of the flask, leading to the final concentration of 60-100 wet cell g/L and 1/9 ratio of chloroform/phosphate buffer. Samples were taken from the reaction mix at specific time intervals and phase separation was carried out by centrifugation at 4000×g for 6 min to obtained resting cells, chloroform and water, respectively. Omeprazole was extracted twice from water phase with chloroform, and then the resulted chloroform layers containing omeprazole obtained from two extractions were combined. The obtained organic phase was used to HPLC analysis for the enantiomeric purity and conversion. Both the ^{1}H and ^{13}C NMR data obtained for (S)-omeprazole agree with the literature values(Seenivasaperumal et al., 2010).

2.4 HPLC analysis

The conversion of omeprazole sulfide, enantiomeric excess(e.e), the yields of (S)-omeprazole and (R)-omeprazole were analyzed using a chiral HPLC (Agilent 1200 LC, Agilent Technologies, Inc., and Santa Clara USA), which is equipped with a diode array detector worked at 302 nm. The used column was a chiral column Amylose-SA with 250×4.6mm, 5um (YMC, Japan) worked at 30°C. 20μL of the sample was used for HPLC analysis. A mixture of 15:85 (v/v) acetonitrile: phosphate buffer (pH6.0) was employed for a mobile phase at a flow rate of 0.6 mL min^{-1}. The retention times for (S)-omeprazole

and (R)-omeprazole were 5.8 and 6.9 min, respectively.

2.5 NMR spectrum

Both the ^{1}H and ^{13}C NMR spectra were recorded in CDCl$_3$ on Bruker AV-500, 500MHz for ^{1}H and 125MHz for ^{13}C, respectively, in which CHCl$_3$ was used as internal standard (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.36 ppm).

2.6 Experimental design and statistical analysis

The bio-oxidation of the omeprazole sulfide catalyzed by resting cells of a mutant of *R. rhodochrous* in organic–aqueous biphasic systems to prepare(S)-omeprazole, is a complex process involving many variables including the reaction time, the concentration of resting cells, ratio of chloroform to phosphate buffer, pH of phosphate buffer and the reaction temperature, which can affect the yield and e.e. of (S)-omeprazole. According to the single factor preliminary experiments, three independent variables were selected as follows: the reaction temperature (A), pH of phosphate buffer (B) and the reaction time (C), while the yield of (S)-omeprazole was chosen as a response variable. For three factors and three levels all seventeen experimental points were thus designed using Design Expert 8.0.5, and the central point experiment was performed five times. The experimental design of variables and levels was shown in Table 1.

3. Results and discussion

3.1 Preparation of (S)-omeprazole via the bio-oxidation of the omeprazole sulfide catalyzed by resting cells of the mutant of *R. rhodochrous*

As seen from Table 2, the highest yield of (S)-omeprazole was 92.13% (entry 10) and all of e.e.>99%, and no sulfone formation was assayed during the bio-oxidation of the omeprazole sulfide catalyzes by resting cells of the mutant of *R. rhodochrous*. It is worth noting that the concentration of the substrate omeprazole sulfide was 180mM (59.22 g/L) on account of only the volume of organic phase, or 5.92g/L on account of the total volume of reaction media including chloroform and phosphate buffer, which is much higher than other research works: 0.5-1.5g/L on account of the total volume of reaction media including aqueous and organic phase(Aguirre-Pranzoni et al., 2015; El'kin et al., 2010; Grishko et al., 2013; Holland et al., 1991; Holland et al., 1992; Tarasova et al., 2017; Yoshida et al., 2001), indicating that the mutant of *R. rhodochrous* used in the study are provided with very good

substrate and product tolerance. Moreover, the function of chloroform–phosphate buffer biphasic system is far superior to that of the single water phase system, which substantially enhances the tolerance of both the substrate and product.

In the study, a high yield, e.e. and substrate concentration were achieved, to which several factors can contribute as follows. Firstly, the mutant of *R. rhodochrous* ATCC 4276 possesses a high enantioselectivity, catalytic activity as well as substrate and product tolerance, and no consecutive bio-oxidation of the sulfoxide to sulfone was detected. The mutant was a very good intact cell biocatalyst used in bio-oxidation of prochiral sulfides to prepare chiral sulfoxides. There are many microorganisms used for the preparation of chiral sulfoxides by bio-oxidation of prochiral sulfides with a high enantiopurity, including *Trametes species, H. specie, M. isabellina, Rhizopus specie, Trichaptum specie, B. cinerea, T. viride* and *E. lata*(Holland et al., 1991; Holland et al., 1995; Mascotti et al., 2012; Pinedo-Rivilla et al., 2007; Ricci et al., 2005), however, the tolerance and yields obtained from those strains in bio-oxidation reaction are both less than those obtained from our strain. Secondly, the employed flask was aerated, thus, oxygen was supplied enough to support the bio-oxidation of the omeprazole sulfide catalyzed by whole cells. A high concentration of resting cells (100 g wet cell/L) was achieved, which is more beneficial to the bio-oxidation process. For flask incubation the cell concentration is usually not too high because of the limitation of oxygen supply, such as 20-40 wet cell g/L(Li et al., 2011b),70-100 wet cell g/L(He et al., 2013), and 6.6 lyophilized yeast g/L(Aguirre-Pranzoni et al., 2015). Thirdly, the biggest contribution to the remarkable improvement of substrate tolerance may come from adopting the chloroform–phosphate buffer biphasic system because almost all substrates may partitioned in the organic phase (water solubility of omeprazole sulfide is only about 0.5 mg/ml), resulting in little damage and inhibition to cells by substrates. Adopting organic–aqueous biphasic systems can enhance substantiality both the substrate tolerance and enantioselectivity(Gong and Xu, 2005; He et al., 2006; Kansal and Banerjee, 2009; Yoshida et al., 2001). For the aqueous-organic biphasic system the ratio of water to organic solvent affects not only the reaction interfacial areas of microbial cells to substrates but also the influence of organic solvents on microbial cells, therefore resulting in markedly influences on the bio-catalytic activity of microbial cells(Gong and Xu, 2005; He et al., 2012; He et al., 2013; Li et al., 2011b).It is usually considered that organic solvents have markable effect on enzyme activity(He et al., 2012; Kansal and Banerjee, 2009), and bio-

oxidation activities positively correlate with solvent log P, e.g. the relative activities of cells were 33.5% in ethyl acetate (log P 0.68) and 161%inchloroform (log P 1.97), respectively(He et al., 2013). A solvent with low log P, for instance, log P<1,is called the polar solvent, and it is generally believed that the polar solvent can breakdown cell membranes, leading to cell activity reducing(Yoshida et al., 2001).

The catalytic activity of cells may be markedly enhanced by an organic solvent with good biocompatibility, accordingly, chloroform was employed as an organic phase herein. The single variable preliminary experiments has been carried out (data not shown), and the optimal ratio of phosphate buffer to chloroform was determined as 9:1(v/v). Because the solubility of omeprazole thioether in chloroform is greater than 0.15g/mL, for the chloroform-phosphate buffer biphasic system the substrate omeprazole sulfide and product (S)-omeprazole are almost distributed in chloroform, by contrary, cells entirely partition in phosphate buffer phase. We ponder that the omeprazole sulfide and (S)-omeprazole distributed in chloroform may result in little or no damage of resting cells, thus little or no inhibition to the bio-catalytic activity of cells. It may be explained that the adsorption of substrates by cells in a single aqueous system can occur, resulting in the enrichment of substrates on cells. However, only a small part of the adsorbed substrate is involved in the bio-oxidation reaction to form sulfoxides catalyzed by resting cells, while other adsorbed substrates are not involved in the reaction, conversely, those adsorbed substrates might damnify cells, inhibiting the bio-catalytic activity of resting cells. Different from a single aqueous system, however, in an organic–aqueous biphasic system the substrate is almost entirely distributed in the organic solvent, not in the aqueous phase and on enrichment in cells. The adsorbed substrate in resting cells may thus be significantly decreased, accordingly, the adsorbed substrate by cells right might primarily involve in bio-oxidation and not harm resting cells and inhibit the bio-catalytic activity of cells. He et al. also observed the adsorption of substrates by cells and they extracted the adsorbed substrate from cells with ethyl acetate(He et al., 2013).

3.2 Optimization of asymmetric bio-oxidation of the omeprazole sulfide catalyzed by resting cells of the mutant of *R. rhodochrous* by RSM

To investigate the effect of independent variables on experimental results, RSM is designed and analyzed, determining optimal experimental conditions, probing the interaction between experimental

variables and establishing the optimal fitting model. Table 2 displays test results of bio-oxidation of the omeprazole sulfide for preparation of (S)-omeprazole bio-catalyzed by resting cells. As seen in Table 3, analysis of variance for the fitted model regression analysis was carried out with the Design Expert 8.0.5 package to determine the effects of the reaction temperature (A), pH of phosphate buffer (B) and the reaction time (C) on the yield of (S)-omeprazole. Using the Design Expert 8.0.5 package, a model, such a quadratic polynomial equation was developed as follows:

$$Y = 91.76 + 0.57A + 0.96B + 4.91C + 0.63AB - 1.23AC - 1.09BC - 10.15A^2 - 10.49B^2 - 4.15C^2 \quad (1)$$

A positive effect will impose to the yield of (S)-omeprazole which is positively correlated to an independent variable when the coefficient of the independent variable in eq1 is positive, in other words, the yield increases with the increase of the independent variable. By contrary, a negative coefficient of the independent variable imposes a negative effect on the yield. As seen in eq1 that linear terms A, B, C and interaction term AB are provided with positive coefficients, signifying that those independent variables elevate the yield of (S)-omeprazole. On the contrary, with a negative coefficient, interaction terms AB and AC, and all quadratic terms decline the yield of (S)-omeprazole.

As shown in Table 3, the correlative coefficient R^2 is 0.9990, demonstrating that 99.90% of the variation of the yield of (S)-omeprazole may be expounded by the quadratic polynomial equation, based on R^2 of 0.9990, the simulated data of the model correlate the experimental data very well. According to the adjusted R^2 of 0.9976, only 0.0014 different from the measured coefficient R^2, it is further proved that the observed responses are correlated the simulated very well. It is generally believed that the smaller the p-value, the greater the significance of the correlative coefficient. By contrary, the larger the F-value, the greater the significance of the corresponding coefficient. In the present work, the F-value of the model, i.e. eq1 was 755.28 with a low probability value (p<0.0001), confirming further that the model predicts the yield of (S)-omeprazole very accurately. With the Prob>0.1, the insignificant lack-of-Fit F-value of 3.3 further demonstrates that the observed data can be well explicated by the model, which is thus sufficient for predicting the yield of (S)-omeprazole within the range of experimental variables. As shown in Table 3 and eq1, according to the F-value, all of the linear, interaction and quadratic terms have a very significant effect on the yield of (S)-omeprazole (p<0.0001-0.0073) except interaction term AB(0.0229), specially B, C and A^2, etc. The reaction temperature (A), buffer pH (B) and the reaction time (C) have a greater effect on the bio-oxidation of

prochiral sulfides to form (S)-omeprazole.

As seen in Figs 1 and 2, the yield of (S)-omeprazole increases with the reaction temperature ascending from 34 to about 37°C, however, the yield decreases with further ascending of the reaction temperature from 37 to 40 °C, demonstrating that the optimal temperature for the *R. rhodochrous* was 37°C, and when the reaction temperature diverges this optimal point, the bio-catalytic activity of *R. rhodochrous* will decrease. In general, biocatalysts are very sensitive to temperature, thus, the reaction temperature is a very important factor that has marked effect on the bio-catalytic activity and stability of both enzymes and cells(Kim and Nicell, 2006; Mathpati et al., 2016; de Miranda et al., 2015; Waghmare et al., 2017).

As seen in Figs 1 and 3, the yield of (S)-omeprazole increases with the elevating in buffer pH from 6.3 up to about 7.3, however, the yield of (S)-omeprazole reduces with further elevating of buffer pH up to 8.3. The bio-catalytic activity of *R. rhodochrous* cells is effected by buffer pH, and pH can affect the ionic state of substrates and enzymes, resulting in effect on the yield and e.e. of the product catalyzed by enzymes(Luo et al., 2003) and whole cells(Tarasova et al., 2017).

As seen in Figs 2 and 3, the yield of (S)-omeprazole increases rapidly with the extending in the reaction time from 35 to about 43 h, however, the yield improved little although the reaction time prolongs from 43 to 45 h. The optimal reaction time may be about 43 h, and the yield increases little, but time-cost increases more when reaction time further prolongs too long.

3.3 Interactive effects of independent factors on the bio-oxidation of the omeprazole sulfide catalyzed by resting cells of the mutant of *R. rhodochrous*

The interactive effects of independent variables including the reaction temperature (A), pH of phosphate buffer (B) and the reaction time (C) on the response variable, the yield of (S)-omeprazole were interpreted based on response surface and contour plots, which intuitively expose the effect of the interaction of variables on the yield. Usually, elliptical contour plots proclaim that the mutual interaction between factors may be marked, while circular contour plots express that the interaction may be not marked. As shown in Figs 1-3, the contour plots are elliptical with p<0.0029, 0.0008 and 0.0015, respectively, expressing that the mutual interactions between A, B and C are marked, especially, the mutual interaction between the reaction temperature (A) and the reaction time (C) is very significant, indicating that it has the highest influence on the yield of (S)-omeprazole. Eq1 shows

that the interactions of AC, AB and BC are synergistic due to their positive coefficient. Fig. 1 shows the effects of A and B on the yield of (S)-omeprazole at constant C of 40 h, in which the yield of (S)-omeprazole is sensitive to a minor variation of the experimental factors A and B. Temperature, pH and reaction time have been confirmed to have important influence on the asymmetric oxidation of sulfide by whole cells in previous studies(Babiak et al., 2011).However, the interactive effects of these factors have not been reported in these literatures.

3.4 Determining and verifying of optimal conditions for the bio-oxidation of the omeprazole sulfide catalyzed by resting cells of the mutant of *R. rhodochrous*

The highest yield can be obtained under the optimal conditions, thus, optimal conditions of the asymmetric bio-oxidation in a chloroform-phosphate buffer biphasic system were defined using RSM. The effect of the reaction temperature (A), pH of phosphate buffer (B) and the reaction time (C)on the yield of (S)-omeprazole expressed well by both the model, regression equation 1 and the response surface analysis and contour plots, with which optimal conditions of the asymmetric bio-oxidation were established and confirmed experimentally. The optimal parameters obtained are as follows: the reaction temperature was 37°C, pH of phosphate buffer, 7.3 and the reaction time, 43h, respectively. Based on the results of three repeated tests under 100g/L of resting cells and 180mM of omeprazole sulfide, the optimal yield of (S)-omeprazole was 92.9% which is in very good agreement with the estimated results (93.2%), and the corresponding e.e. was >99%, while no sulfone product was detected. This confirms that in the chloroform–water biphasic system, the asymmetric bio-oxidation of the omeprazole sulfide catalyzed by resting cells of the mutant of *R. rhodochrous* is therefore more promising for the efficient asymmetric bio-oxidation for preparation of chiral sulfoxides.

Conclusions

In the chloroform–water biphasic system, the resting cells of the mutant of *R. rhodochrous* ATCC 4276 were employed to catalyze the bio-oxidation of the omeprazole sulfide for preparation of (S)-omeprazole. At a high substrate concentration(180mM) and cell concentration(100g/L), the bio-oxidation was optimized using RSM, and the optimal yield of (S)-omeprazole obtained was 92.9% with e.e.(>99%), and no sulfone product was detected under the optimal conditions: the reaction temperature was 37°C, pH of phosphate buffer, 7.3 and the reaction time, 43h respectively. A quadratic polynomial model was established, which predicts the experimental data with very high accuracy according to R^2

of 0.9990. The chloroform–water biphasic system may mainly contribute the significant improvement of substrate tolerance because almost all substrates may partitioned in the organic phase, resulting in little damage and inhibition to cells by substrates. The mutant of *R. rhodochrous* ATCC 4276 exhibited a high enantioselective, activity and substrate and product tolerance, the bio-oxidation is thus more promising for efficient preparation of chiral sulfoxides.

Acknowledgements

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Table 1. Variables and levels of Box-Behnken test design

Factors	Code	Levels		
		-1	**0**	**1**
Temperature (℃)	A	34	37	40
pH	B	6.3	7.3	8.3
Time (h)	C	35	40	45

Table 2. Box–Behnken design and results of RSM for the yield [1]

Run	Temperature(°C)	pH	Time(h)	Yield(%)
	A	B	C	Y
1	1	1	0	73.68
2	-1	0	-1	71.17
3	0	0	0	92.03
4	-1	0	1	83.24
5	0	0	0	91.42
6	0	0	0	91.64
7	0	1	1	81.93
8	1	0	-1	74.15
9	-1	-1	0	69.82
10	0	0	0	92.13
11	1	-1	0	70.34
12	0	0	0	91.56
13	0	-1	-1	70.12

14	1	0	1	81.29
15	0	-1	1	82.34
16	0	1	-1	74.08
17	-1	1	0	70.64

1 No sulfone formation was assayed during the bio-oxidation of the omeprazole sulfide and all of e.e.>99%atthesubstrate concentration, 180mM.

Table 3 Analysis of variance for the fitted model

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob>F
Model	1280.65	9	142.29	755.28	<0.0001**
A	2.63	1	2.63	13.98	0.0073**
B	7.43	1	7.43	39.44	0.0004**
C	192.86	1	192.86	1023.70	<0.0001**
AB	1.59	1	1.59	8.43	0.0229*
AC	6.08	1	6.08	32.25	0.0008**
BC	4.77	1	4.77	25.34	0.0015**
A^2	433.39	1	433.39	2300.40	<0.0001**
B^2	463.37	1	463.37	2459.5	<0.0001**
C^2	72.45	1	72.45	384.53	<0.0001**
Residual	1.32	7	0.19		
Lack of Fit	0.94	3	0.31	3.30	0.1396
Pure Error	0.38	4	0.095		
Cor Total	1281.96	16			

R^2 0.9990 R^2_{Adj} 0.9976 R^2_{Pred} 0.9878

*Significant at $p<0.05$.   **Significant at $p<0.01$.
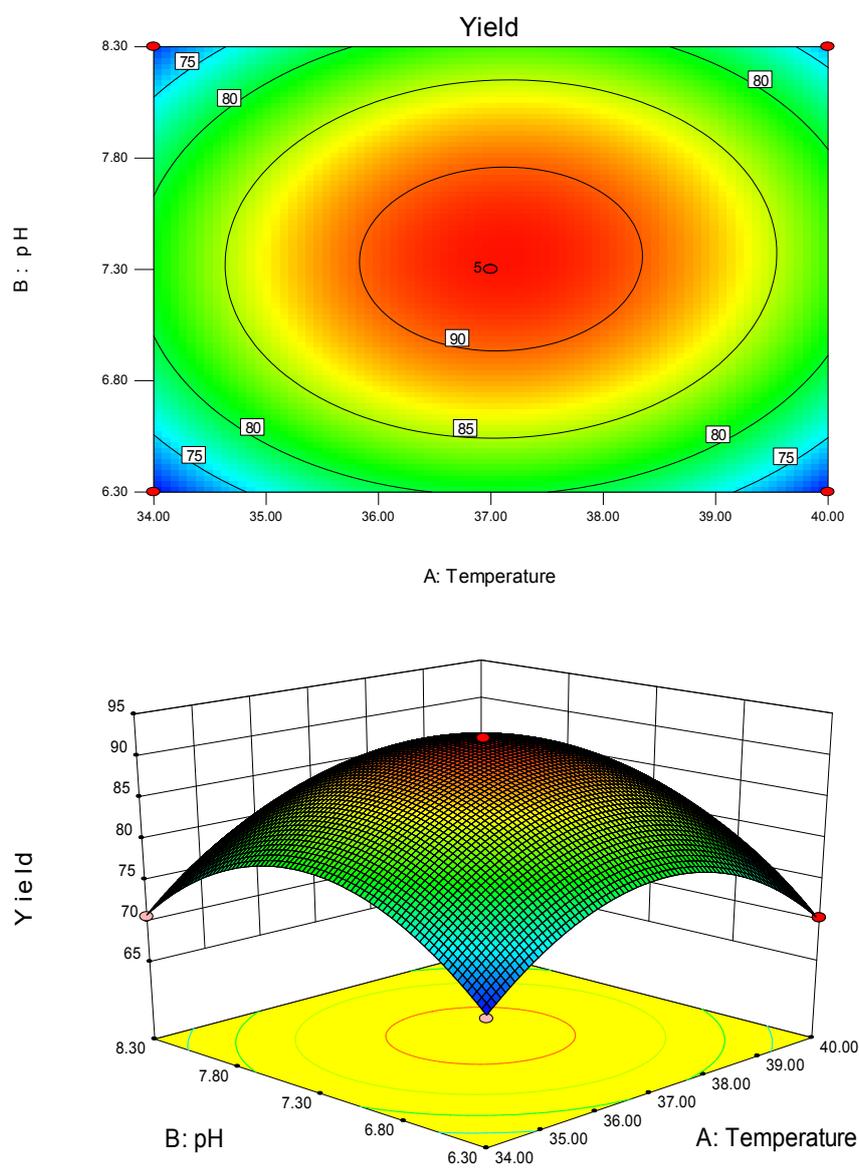
Figure 1 Response surface and profile plots of the effect of the reaction temperature (A)/pH(B) on the yield of (S)-omeprazole
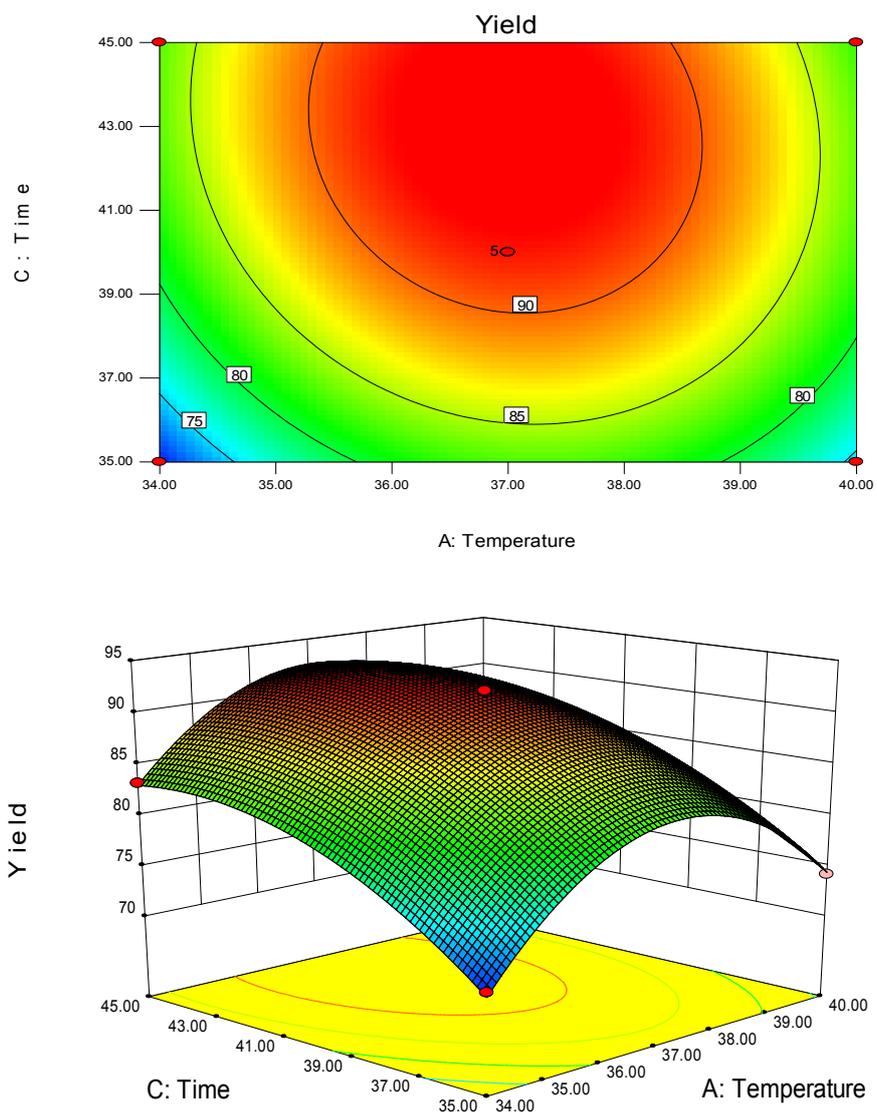
Fig. 2 Response surface and profile plots of the effect of the reaction temperature(A)/the reaction time(C) on the yield of (S)-omeprazole.
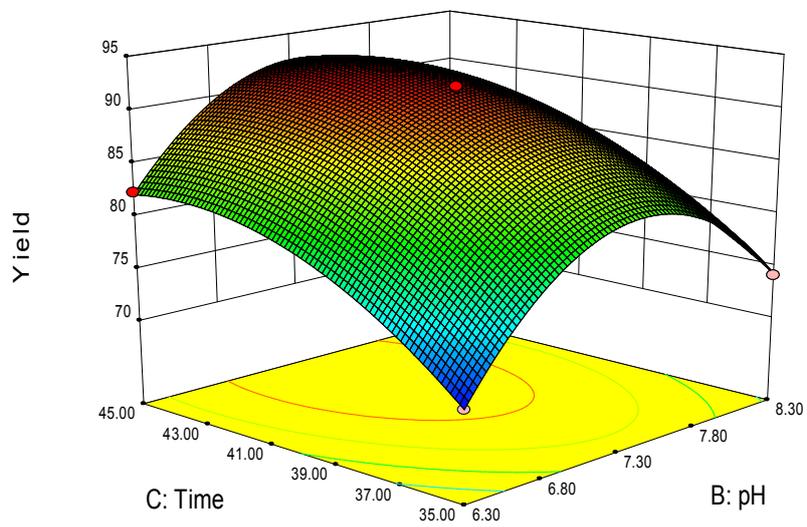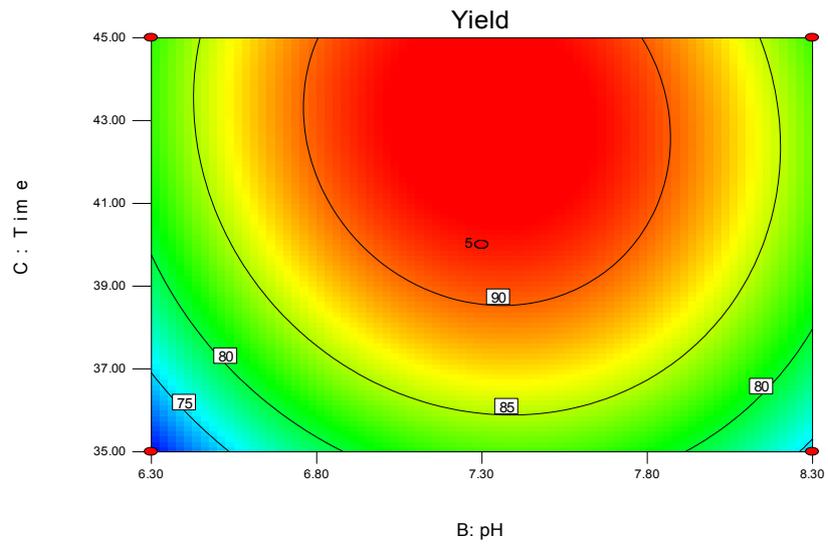
Figure 3 Response surface and profile plots of the effect of buffer pH (B)/the reaction time(C) on the yield of (S)-omeprazole.