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Determination of effective diffusion coefficient of acetophenone in κ-carrageenan and asymmetric bioreduction in packed bed reactor

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ABSTRACT

The effective diffusion coefficient and bioreduction of acetophenone in 1% κ-carrageenan gel containing *Lactobacillus kefir* NRRL B-1839 were investigated experimentally. Both free and immobilized cells in 0.3 cm and 0.4 cm diameter particles were used and the results were analyzed according to Michaelis–Menten kinetics and the values of the constants were determined as: r_{max} = 0.18 mmol L^{-1} min^{-1}, K_{M0} = 0.37 mmol L^{-1}, K_{M1} = 1.68 mmol L^{-1}, and K_{M2} = 2.66 mmol L^{-1}. Effective diffusion coefficient for acetophenone in κ-carrageenan was determined as $D_e = 3.0 \times 10^{-7}$ cm^2/s. Using immobilized *Lactobacillus kefir* in an up-flow packed bed reactor, continuous production of (*R*)-1-phenylethanol through asymmetric bioreduction of acetophenone was carried out using glucose as an energy source. The effects of the residence time and concentrations of substrate and glucose on conversion were investigated at pH 8 and 35 °C. Enantiomerically pure (*R*)-1-phenylethanol (ee > 99%) was produced with a conversion of 79% and productivity of 2.50 mmol L^{-1} h^{-1} at 15 mmol L^{-1} substrate and 0.02 g/mL glucose concentration.

1. Introduction

Biocatalysis has become a key focus area in biotechnology and new approaches for the utilization of biocatalysts have continued to increase over the past year. Chiral compounds, particularly chiral alcohols are very important intermediates in the synthesis of enantiomerically pure pharmaceuticals. For example, the conversion of substituted acetophenones to their corresponding optically active alcohols (phenylethanols) is one of the most common reactions in organic chemistry. (*R*)-1-Phenylethanol or (*S*)-1-phenylethanol have a number of potential applications and used as building blocks for the synthesis of bioactive compounds such as pharmaceuticals, agrochemicals and natural products [1–6].

Enzymatic or microbial production of the chiral alcohols is the best method of preparation of them from the corresponding ketones. Alcohol dehydrogenases (ADHs) catalyze the enantioselective reduction of ketones for the synthesis of chiral alcohols with the help of nicotinamide cofactors (NADH or NADPH) [7]. Whole cells rather than isolated enzymes were used preferentially to avoid enzyme purification and cofactor addition or the requirement for an associate system for cofactor regeneration, since bioreduction using isolated ADHs often require stoichiometric amounts of NADH or NADPH [8]. The most important advantage of using whole cells is that the whole reaction system for cofactor regeneration is present within the cell themselves. Simple and cheap compounds, such as glucose or ethanol, can be used as an energy source for cofactor regeneration when whole cells are used. Above all, whole cells are easier to be obtained and cheaper than isolated enzymes [9,10].

Living cell catalyzed bioreduction of ketones proceeds effectively in suspended (free) cell systems. However, the reuse or recycling of the biocatalyst is very difficult. Immobilization by entrapment is a widely used and simple technique. Immobilized cell systems offer several unique advantages over conventional suspended (free) cell systems. Immobilization facilitates the easy separation of the cells from the products, resistance to the chemical environment, repeated use in many processes, high cell density per reactor volume and higher productivity. The immobilized cells can also be used in packed columns or fluidized bed reactors. Additionally, cell immobilization is considered to be beneficial for stabilizing the whole cell biocatalyst [11,12].

A variety of different microorganisms have been used as free cells [13–18] and immobilized cells [6,19,20] in calcium alginate for bioreduction of acetophenone. Kurbanoglu et al. [6] investigated asymmetric reductions of acetophenone and its analogues using calcium alginate immobilized *Rhodotorula glutinis* cells in a batch system. The beads were suspended in buffer containing glucose (energy source for cofactor regeneration) and 1 mM acetophenone. (*S*)-1-Phenylethanol was obtained with high enantiomeric excess (>99%). After 15 times repeated use of immobilized cells in batch processes, overall yield was still of 77% [6].

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For further study, Kurbanoglu et al. [19] used a specially designed reactor for continuous production of (*S*)-1-phenylethanol by immobilized cells of *Rhodotorula glutinis* in calcium alginate gel. A cylindrical designed column with a 1 L working volume reactor was set up horizontally. Initial acetophenone concentration was investigated from 0.06 to 0.3 mM. In this study, the obtained conversion, yield, and enantiomeric excess were of 100%, 75%, and higher than 99%, respectively [19].

Hasegawa et al. [20] investigated the bioreduction of acetophenone in calcium alginate immobilized *Hansenula capsulata* cells using a packed bed reactor. The performances of batch reactor (conversion 42%) and packed bed reactor (conversion 77%) were compared. Continuous production of (*S*)-1-phenylethanol was accomplished at higher than 99% enantiomeric excess. Above 100 min residence time, the conversion did not change significantly [20].

One of the disadvantages of immobilization is the mass transfer restriction caused by the additional resistance of the gel. When immobilized cells are used, effectiveness factor and diffusion of substrate are of primary importance in the design of bioreactors [21–23]. In a reaction involving a porous catalyst, diffusional resistance is encountered. A common method of verifying the pore diffusion effect is to compare the reaction rates for different sizes of catalyst supports. Therefore, the particle size is a critical parameter. Mehmetoglu [24] was investigated the effective diffusion coefficient of sucrose in calcium alginate gel for two different particle sizes. Effective diffusion coefficient for sucrose was estimated at the value of 4.05×10^{-6} cm^2/s [24]. The determination of effective diffusion coefficients of acetophenone for the studied system is important. In literature, effective diffusion coefficient of acetophenone was calculated in calcium alginate [25] but not for κ-carrageenan.

The R-specific ADHs from microorganisms such as *Lactobacillus kefir* or *Lactobacillus brevis* are particulary advantageous for the reduction of ketones, leading enantioselectively to the corresponding R-compounds [7]. Production of (*R*)-1-phenylethanol using immobilized *Lactobacillus kefir* cells did not studied so far with up-flow continuous packed bed reactor. Previously reported studies used S-specific microorganisms and involved the production of (*S*)-1-phenylethanol in a batch system or horizontally operated packed bed reactor [6,19,20]. However, these studies have different kinds of microorganisms using a different entrapment matrix. Continuous processes have a number of advantages over batch processes, including maximum reaction rates, minimum nutrient depletion and product inhibition, higher space-time yields, low down-times for the reactor, operation under steady-state conditions [11,12].

The aim of this paper is to study the bioreduction of acetophenone in an up-flow packed bed reactor by using R-specific cultures of *Lactobacillus kefir* immobilized in κ-carrageenan. Another objective is to study the operating conditions to obtain a high conversion and productivity at a high substrate concentration. The mass transfer effects have also been investigated in terms of effectiveness factors, and effective diffusion coefficients of acetophenone in κ-carrageenan gel was determined.

2. Theoretical analysis

It is a well-known fact that the apparent reaction rate decreases due to diffusion limitations in porous media. To model such an effect, an effectiveness factor (η) is defined for coupled intraparticle diffusion-reaction, given by [21,26].

$$\eta = \frac{\text{apparent reaction rate with intraparticle mass transfer resistance}}{\text{reaction rate without mass transfer resistance}} \quad (1)$$

The rate of reaction that takes place in a porous particle containing immobilized biocatalysis can be evaluated based on the following assumptions: (1) microorganisms act as an enzyme stores, (2) microorganism is distributed uniformly throughout the particle, (3) transport of substrate through the catalyst is described by the Fick' s law form relating the diffusive flux to the substrate concentration gradient, and the effective diffusivity of substrate is constant, (4) the reaction is isothermal and involves no change of pH, (4) electrostatic effects are negligible, and (5) the reaction kinetics are expressed by Michaelis–Menten kinetics of the general form [21,26].

$$\text{Michaelis–Menten kinetics}: r = \frac{r_{\max} C}{K_M + C} \quad (2)$$

Here, $r_{\max}$ and K_M are maximum reaction rate and Michaelis–Menten constant, respectively. Then, the mass balance equation written for a spherical porous particle indicates that diffusion rate is equal to the reaction rate at steady state [21,26], i.e.:

$$D_e \left(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr} \right) = \frac{r_{\max} C}{K_M + C} \quad (3)$$

By solving this nonlinear equation numerically, with boundary conditions $C = C_o$ at $r = R$ and $dC/dr = 0$ at $r = 0$, the relationship between effectiveness factor (η) and observable modulus (Φ) is obtained, given in a graphical form for a spherical porous immobilized particle by Blanch and Clark [26].

According to this graphical form, when effectiveness factor is known, observable modulus can be obtained with the help of dimensionless Michaelis–Menten constant (β). Observable modulus is defined analogously to the square of the Thiele modulus. The definition of observable modulus (Φ) is given for Michaelis–Menten kinetics by Eq. (4) [26].

$$\Phi = \frac{r_{app}}{D_e C_o} \left(\frac{R}{3} \right)^2 \quad (4)$$

Here, r_{app}, D_e, C_o and R are the apparent reaction rate, the effective diffusivity of substrate, bulk concentration of substrate and particle radius, respectively [26]. In this study, it is assumed that no external resistance to the diffusion exits.

3. Materials and methods

3.1. Strain and chemicals

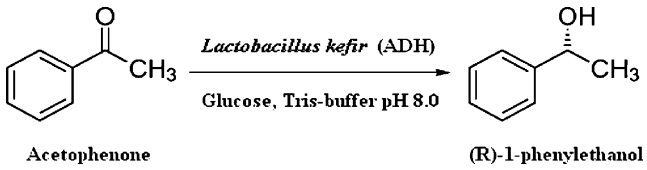
The strain used *Lactobacillus kefir* NRRL B-1839 was kindly supplied from U.S. Department of Agriculture, National Center for Agricultural Utilization Research (U.S.). Glucose (Merck) was used as cofactor regeneration. Acetophenone, (*R*)-1-phenylethanol, (*S*)-1-phenylethanol and other chemical reagents were purchased from Sigma Co. All solutions were prepared with distilled water.

3.2. Media and culture conditions

Culture medium for cell growth was glucose 22 g L^{-1}, casein peptone 10 g L^{-1}, meat extract 10 g L^{-1}, yeast extract 5 g L^{-1}, sodium acetate 5 g L^{-1}, K_2HPO_4 2 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.2 g L^{-1}, $MnSO_4 \cdot H_2O$ 0.05 g L^{-1}, Tween 80 1 mL L^{-1}. *Lactobacillus kefir* was cultivated in 2 L Erlenmeyer flask at 35 °C and pH 6.9 for 24 h in culture medium for cell growth. The cell suspensions were collected in sterile tubes, and centrifuged at 9500 rpm for 15 min at 4 °C.

3.3. Immobilization of Lactobacillus kefir in κ-carrageenan

The *Lactobacillus kefir* cells were immobilized in κ-carrageenan gel beads by entrapment method. Wet cells (16 g) were thoroughly suspended in 30 mL of sterile deionized water and made up to a total volume of 50 mL with sterile deionized water. κ-carrageenan solution (2%, w/v) was prepared by dissolving κ-carrageenan in



Scheme 1. Asymmetric bioreduction of acetophenone using *Lactobacillus kefir*.

sterile deionized water at 50 °C. The cell suspension (50 mL) was mixed with an equal volume (1:1, v/v) of κ-carrageenan solution and stirred for 5 min. The mixture was taken for the preparation of beads and dropped into a well-stirred sterile KCl solution (1 M) using a syringe. Each mixture drop solidified upon contact with KCl and formed beads that encapsulated the *Lactobacillus kefir* cells. The beads were left to harden for 1 h min at room temperature after which they were washed with sterile KCl solution (0.05 M) to remove un-encapsulated cells. The beads obtained were used at once for bioreduction reactions. All experiments were carried out twice and the averaged values are presented in this study.

3.4. Determination of the kinetic parameters and the effective diffusion coefficient

For the determination of the Michaelis–Menten constants, bioreduction of acetophenone was carried out in a batch system. The experiments were performed with different initial acetophenone concentrations between 2 and 10 mmol L^{-1} in 50 mL Erlenmeyer flasks containing 25 mL working volume. The reaction rate values at 150 rpm, 175 rpm and 200 rpm were approximately same. The mixing rate was chosen as 200 rpm to eliminate the external film resistance with an orbital shaker and temperature was kept constant at 35 °C. Into separate 25 mL reaction media, 0.36 g.d.c (grams dry cell) of free cells and κ-carrageenan beads of two sizes (0.3 cm and 0.4 cm) containing the same amount of cells were placed, separately. The reaction media include 2% (w/v) glucose and acetophenone dissolved in dimetlysulfoxide in 50 mM Tris–HCl buffer at pH 8. Taken samples at certain reaction time were extracted with methyl tertiary butyl ether. The reaction rate is expressed by Michaelis–Menten kinetics described in Section 2. Using both free and immobilized cells, produced (*R*)-1-phenylethanol concentration versus time was obtained. Initial reaction rates were calculated using the slopes of the initial production rates. A Lineweaver–Burk (LB) plot was used to calculate the kinetic constants of the Michaelis–Menten kinetics. Calculated kinetics constants for free cells are intrinsic values of r_{max} and K_M whereas calculated kinetics constants for immobilized cells are considered apparent values of $r_{max,\ app}$ and $K_{M,\ app}$. Using these kinetics constants, apparent reaction rate and reaction rate without mass transfer resistance were calculated for 15 mM substrate concentration. The substrate concentration was much more higher than K_M (0.37 mM) values. This means that an apparent zero-order kinetics can approximate the Michaelis–Menten expression on this operating domain. Effectiveness factors (η) were calculated using apparent reaction rate and reaction rate without mass transfer resistance (Eq. (1)). Knowing the effectiveness factor and with the assistance of figure given by Blanch and Clark [26] using zero-order kinetics ($\beta \to 0$) curve, observable modulus was obtained. Then, from Eq. (4), effective diffusion coefficient of acetophenone was calculated.

3.5. Continuous bioreduction system

Lactobacillus kefir has an intracellular NADP-dependent ADH [7] and is used as an enzyme source in this study. Asymmetric bioreduction of acetophenone (Scheme 1) with immobilized *Lactobacillus kefir* was investigated in packed bed reactor. A pyrex glass column reactor, of 27 cm in length and 1.7 cm inner diameter, with a water cooling jacket was employed. Immobilized cells were packed into the reactor and washed with Tris–HCl buffer (pH 8). The substrate solution includes glucose and acetophenone dissolved in dimetlysulfoxide in 50 mM Tris–HCl buffer at pH 8. It was pumped by a peristaltic pump at the different flow rates. The aliquots (1 mL) of outlet stream were taken at 30 min intervals, during 9 h. Filtered aliquots of samples were extracted with methyl tertiary butyl ether and analyzed by high pressure liquid chromatography.

3.6. Analytical methods

The concentration of acetophenone, (*R*)-1-phenylethanol and (*S*)-1-phenylethanol was determined by high-pressure liquid chromatography (Thermo Finnigan Spectra System) with 4.6 mm × 50 mm Chiralcel-OB column (Daicel Chemical Ind. Ltd. France) using eluent n-hexane–iso-propanol, 95:5, flow rate of 0.9 mL/min, detection monitored by UV absorption at 254 nm with diode array detector.

The conversion rate (c) was determined from the ratio of reacted substrate concentration (C_o–C) to its initial substrate concentration (C_o). In Eq. (5), C is the substrate concentration in a certain reaction time.

$$c\% = \frac{C_o - C}{C_o} \times 100 \quad (5)$$

The enantiomeric excess (ee) of (*R*)-1-phenylethanol was calculated as follows:

$$ee\% = \frac{[C_{(R)-1-PE}] - [C_{(S)-1-PE}]}{[C_{(R)-1-PE}] + [C_{(S)-1-PE}]} \times 100 \quad (6)$$

Here, $C_{(R)-1-PE}$ and $C_{(S)-1-PE}$ are the concentrations of (*R*)-1-phenylethanol and (*S*)-1-phenylethanol, respectively.

4. Results and discussion

4.1. Effective diffusion coefficient of acetophenone in κ-carrageenan gel

Bioreduction reactions were performed with free and for two sizes of immobilized microorganisms in a batch system as described in Section 3.4. The concentration increase of R-1-phenylethanol with time at 2 mM, 4 mM, 8 mM and 10 mM initial acetophenone concentrations was obtained for both free and immobilized cells. Under these conditions, the reaction rate of the free microorganisms can be assumed to be the rate without mass transfer resistance. Apparent reaction rate was the rate when immobilized microorganisms was used in the experiments [26]. For an initial acetophenone concentration of 8 mM, the produced R-1-phenylethanol concentration versus time is displayed in Fig. 1. For other studied acetophenone concentrations, similar data were also obtained. For determining the initial rates, these data were used and the best fit lines were plotted by Excel programme. The reaction rates were calculated using the slopes of these curves. Assuming that the microorganisms, which act as enzyme stores, cause the reaction to follow the Michaelis–Menten kinetics, a Lineweaver–Burk (LB) plot was used to calculate the kinetic constants (Fig. 2). The K_M and r_{max} values were determined from the slopes and from the intercepts in Fig. 2, respectively. For the free and immobilized cells, intrinsic and apparent Michaelis–Menten constants are given in Table 1.

Knowing kinetics constants, the values of apparent reaction rate and reaction rate without mass tranfer resistance were calculated. Using these rates, the effectiveness factors (η_1 and η_2) were obtained as 0.17 and 0.12, respectively from Eq. (1) for the two

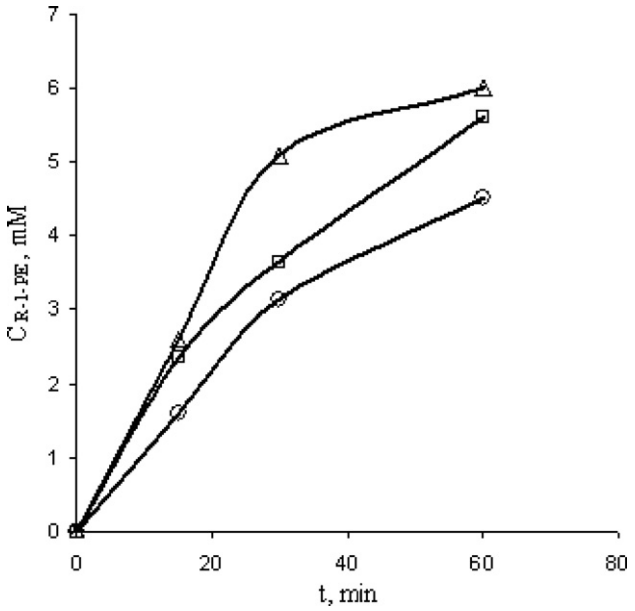


Fig. 1. Product (R-1-PE) observed kinetic curve by using free microorganisms (—△—), or by using different radius immobilized microorganisms ((—□—), D_1 = 0.3 cm and (—○—), D_2 = 0.4 cm), C_{so} = 8 mM, 2% (w/v) glucose, pH 8 Tris–HCl buffer, 200 rpm, 35 °C.

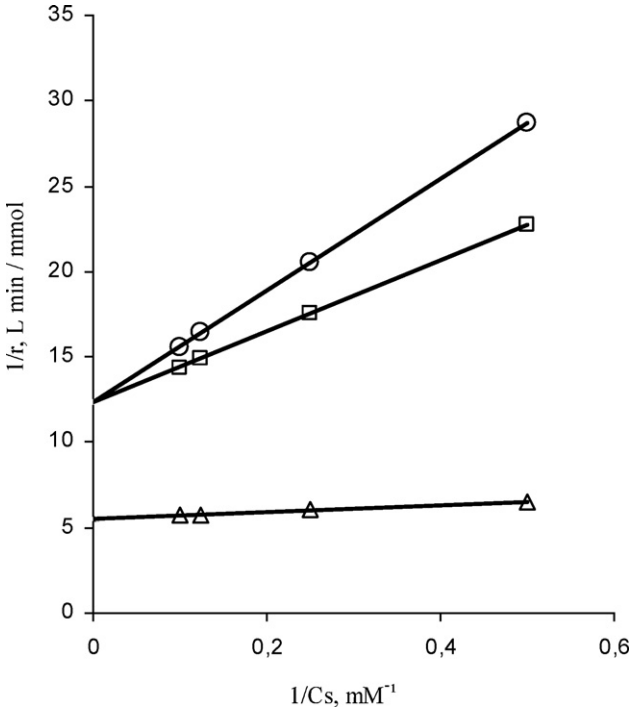


Fig. 2. $(1/r)$ vs $(1/C_s)$ values for free microorganisms (—△—) and different radius immobilized microorganisms ((—□—) D_1 = 0.3 cm and (—○—) D_2 = 0.4 cm).

Table 1
Intrinsic and apparent Michaelis–Menten constants for free and immobilized microorganism.

	K_M (mmol L^{-1})	r_{max} (mmol L^{-1} min^{-1})
Free microorganism	0.37	0.18
Immobilized microorganism	$K_{M,app}$ (mmol L^{-1})	$r_{max,app}$ (mmol L^{-1} min^{-1})
D_1 = 0.3 cm	1.68	0.08
D_2 = 0.4 cm	2.66	0.08

Table 2
Effectiveness factors and effective diffusion coefficients for different conditions.

	η	D_e (cm^2/s)
D_1 = 0.3 cm	0.17	2.5×10^{-7}
D_2 = 0.4 cm	0.12	3.5×10^{-7}

used diameters involved. When the value of the effectiveness factor is much smaller than 1, this means that the diffusion effects are significantly important. The observable modulus was obtained using the effectiveness factor with the assistance of figure given by Blanch and Clark [26] for spherical porous immobilized particle using $\beta \to 0$ curve on graphical form. For high substrate concentrations ($\gg K_M$), Michaelis–Menten kinetic model approaches zero order and dimensionless Michaelis–Menten constant ($\beta = K_M/C$) converges to zero ($\beta \to 0$). This graphical form demonstrates a general approach between observable modulus (Φ) and effectiveness factor (η) for two limiting forms of Michaelis–Menten model (zero-order kinetics, $\beta \to 0$ and first-order kinetics, $\beta \to \infty$). For using, $\beta \to 0$ curves on graphical form, the values of observable modulus corresponding to η_1 and η_2 were determined as $\Phi_1 = 10$ and $\Phi_2 = 11.8$, respectively. From definition of observable modulus from Eq. (4) and knowing apparent reaction rate, substrate concentration (15 mM) and particle radius, the effective diffusion coefficients were calculated and given in Table 2.

Entrapment in polymeric gels, such as alginate and κ-carrageenan, is a very effective and popular way of both enzyme or whole cell immobilization [27]. The effective diffusion coefficient of acetophenone in calcium alginate gel was determined as 3.24×10^{-6} cm^2/s [25]. However, there is no study on the effective diffusion coefficient of acetophenone in κ-carrageenan. In our study, for two sized involved, the values of the effective diffusion coefficients were close in range (Table 2). Due to $\beta \to 0$ curve was used to determine the observable modulus on graphical form, the small difference between the values of effective diffusion coefficients occurred. Looking at Table 2, it means that the precision in determining D_e by this method is of ca. 0.5×10^{-7} cm^2/s (standard deviation), i.e. 16% (relative). The arithmetic average of these values, that is, 3×10^{-7} cm^2/s, can be considered as the effective diffusion coefficient of acetophenone in κ-carrageenan. Because κ-carrageenan forms strong and brittle gels than alginate, the value of the diffusivity of acetophenone in κ-carrageenan is smaller than that for the alginate support. Also the Wilke–Chang correlation predicts a value of diffusivity of 9.21×10^{-6} cm^2/s for acetophenone in water [28]. As expected, the diffusivity of acetophenone in different matrices was much more smaller than that of in water. In addition, the effectiveness factor decreases with increasing particle diameter [21]. In this work, it was also observed that the particle size of 0.3 cm gave higher reaction rate. For this reason it was used for further experiments with the packed bed reactor.

4.2. Asymmetric bioreduction in a packed bed reactor using immobilized Lactobacillus kefir cells

4.2.1. Effect of substrate concentration

Several levels of substrate concentrations from 15 to 40 mM were presented to determine their effects on the conversion and enantiomeric excess and concentration of (*R*)-1-phenylethanol. Fig. 3 shows the variation of the (*R*)-1-phenylethanol concentration for different initial acetophenone concentrations, and over 9 h of reaction time. At all studied substrate concentrations, steady-state was reached approximately after 7 h.

Concentration of (*R*)-1-phenylethanol increased with increasing substrate concentrations. At 4.5 h residence time (residence time (τ) is the ratio between the packed-bed volume and the volumetric inlet flow rate), 12.8 mM (*R*)-1-phenylethanol

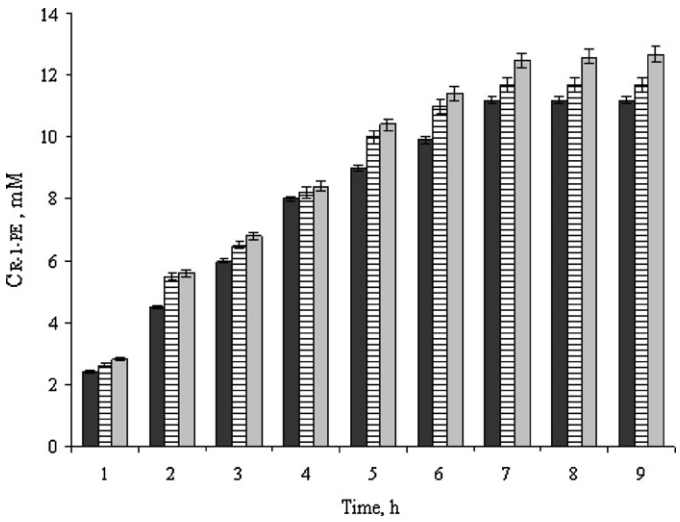


Fig. 3. The effect of different acetophenone concentration on (*R*)-1-phenylethanol concentration, $C_{glucose}$ = 0.02 g/mL, T = 35 °C, pH 8, τ = 4.5 h ((■) C_{so} = 15 mM; (▤) C_{so} = 25 mM; (▩) C_{so} = 40 mM).

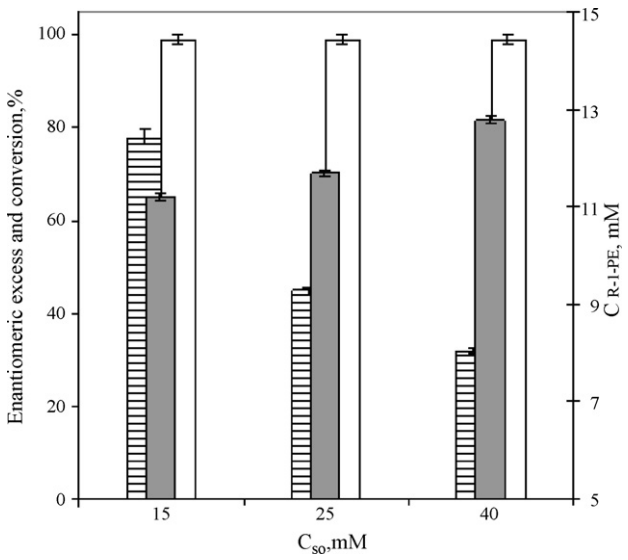


Fig. 5. The effect of different acetophenone concentrations on conversion, enantiomeric excess and (*R*)-1-phenylethanol concentration, $C_{glucose}$ = 0.02 g/mL, T = 35 °C, pH 8, τ = 4.5 h ((▤) conversion, %; (□) enantiomeric excess, %; (▩) (*R*)-1-PE concentration, mM).

was obtained at 40 mM initial substrate concentration, whereas 11.2 mM (*R*)-1-phenylethanol was produced at 15 mM initial substrate concentration. For industrial applications of asymmetric bioreduction, high product concentration and high productivity are important. In our study, continuous production of (*R*)-1-phenylethanol was carried out in packed bed reactor at much higher substrate concentrations (15 mM, 25 mM and 40 mM), comparatively to the experiments of Kurbanoglu et al. [19] and Hasegawa et al. [20] as 0.3 mM and 20 mM, respectively.

The change of conversion and enantiomeric excess of product can be seen in Fig. 4. In terms of conversion, 15 mM initial acetophenone concentration gave the highest conversion as 79%. For studied acetophenone initial concentrations, the obtained enantiomeric excess was more than 99%. When acetophenone initial concentration was higher than 15 mM, the conversion decreased significantly.

The summary of conversion, enantiomeric excess and product concentration at steady-state are shown in Fig. 5. Product concentration increased with increasing substrate concentration. In related to (*R*)-1-phenylethanol concentration, the productivity increased. The productivity was 2.82 mmol L^{-1} h^{-1} at 40 mM acetophenone concentration whereas it was 2.50 mmol L^{-1} h^{-1} at 15 mM acetophenone concentration. That means higher productivity was obtained with increasing substrate concentration. On the contrary, conversion decreased. However, enantiomeric excess did not change with substrate concentration.

4.2.2. Effect of glucose concentration

The enantioselective reduction of carbonyl compounds using isolated enzymes often require stoichiometric amounts of NADH or NADPH. Since these cofactors are so expensive, their use in stoichiometric amounts is not economically feasible. Therefore whole cells rather than isolated enzymes were used to avoid cofactor addition. During the whole cell bioreduction, cofactor regeneration can be accomplished by fermentable carbohydrate within the cells themselves. Simple sugars, mostly glucose, was used as an energy source

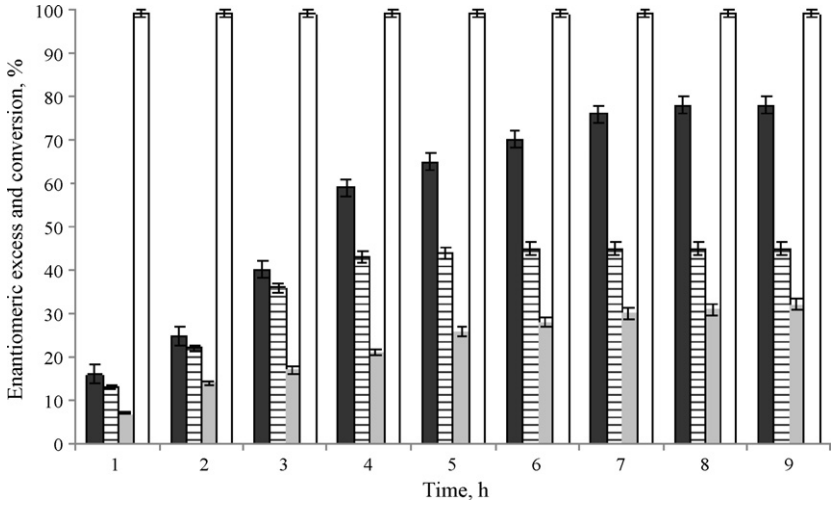


Fig. 4. The effect of different acetophenone concentration on conversion and enantiomeric excess, $C_{glucose}$ = 0.02 g/mL, T = 35 °C, pH 8, τ = 4.5 h ((■) C_{so} = 15 mM; (▤) C_{so} = 25 mM; (▩) C_{so} = 40 mM; (□) enantiomeric excess %).

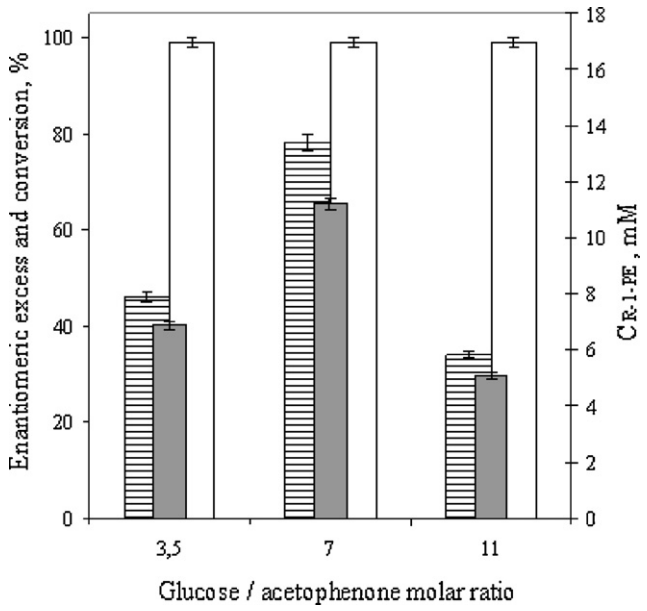


Fig. 6. The effect of glucose to acetophenone molar ratios on conversion, enantiomeric excess and (*R*)-1-phenylethanol concentration, C_{so} = 15 mmol L^{-1}, T = 35 °C, pH 8, τ = 4.5 h ((▤) conversion, %; (□) enantiomeric excess, %; (▩) (*R*)-1-PE concentration, mM).

for cofactor regeneration when whole cells are used. Lack of glucose during asymmetric reduction causes low conversion and low product concentration because glucose supplies the regeneration of cofactor. In our study, glucose was also used during asymmetric bioreduction of acetophenone. The effect of glucose concentration on conversion and enantiomeric excess and concentration of (*R*)-1-phenylethanol is shown in Fig. 6. Asymmetric bioreduction was investigated at three different glucose concentrations, 0.01, 0.02 and 0.03 g/mL. In this cases, the molar ratios of glucose to acetophenone were obtained as 3.5, 7 and 11, respectively. As can be seen from Fig. 6, when molar ratios were increased from 3.5 to 7, the concentration of product increased. The highest (*R*)-1-phenylethanol concentration and conversion was obtained at glucose to acetophenone molar ratio 7 (0.02 g glucose/mL). When molar ratio was 11, product concentration and conversion decreased significantly. It means that higher glucose concentration caused inhibition on bioreduction. However, enantiomeric excess did not change with glucose concentration.

The effect of glucose concentration on bioreduction was investigated by several researchers for production of chiral compounds such as *R*-(−)-mandelic acid or ethyl-(*S*)-3-hydroxybutanoate [29,30]. They reported that when glucose concentration was not enough for the consumption by cells, the biocatalytic activity would be inhibited. Hasegawa et al. [20] studied at 20 mM initial substrate concentration and the molar ratio of glucose to acetophenone was as 0.1, 0.2, 0.5, 1, 2, 3, 5 and 10 in a batch system. At lower molar ratios (0.1–0.5), exhaustion of the glucose caused low concentration of (*S*)-1-phenylethanol. They observed that molar ratios of glucose to acetophenone should be above 1 for enhancing product concentration [20]. They obtained 77% conversion in a packed bed reactor. In our study, the results show that when molar ratio of glucose to acetophenone was 7, the highest conversion (79%) was reached. In our study, lower molar ratios did not give satisfactory conversion. The differences of molar ratio between the studies possibly come from the use of different type microorganisms.

4.2.3. Effect of residence time

To study the effect of the residence time on bioreduction, the substrate solution was pumped by peristaltic pump at different flow rates to the column.

For studied residence times, the obtained enantiomeric excess was more than 99%. Fig. 7 shows that conversion increased with increasing residence time at steady-state conditions. When residence time increased, high (*R*)-1-phenylethanol concentration was obtained. This causes a rise in both conversion and product concentration because of the much more reaction time for substrate with the biocatalysts in the column. However, productivity decreased at the same conditions. When final conversions were compared between 4.5 h and 9 h residence time, the values of conversions were close in range. Because of that experiments did not carried out above 9 h residence time. On the other hand, to obtain both high productivity and high concentration, 3.75 h was economically feasible residence time.

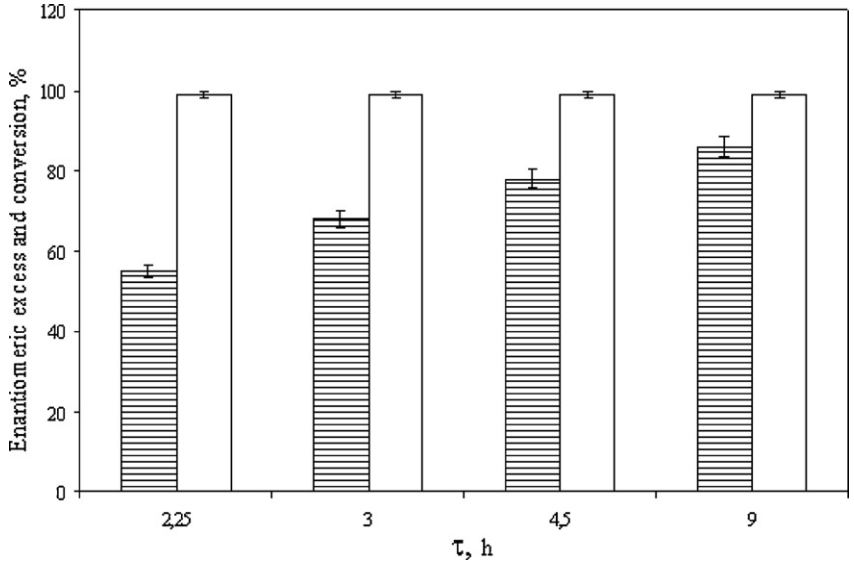


Fig. 7. Effect of residence time on conversion and enantiomeric excess (C_{so} = 15 mM, $C_{glucose}$ = 0.02 g/mL, T = 35 °C, pH 8).

5. Conclusions

Asymmetric bioreduction of acetophenone is accomplished by immobilized cells in this study. The use of immobilized whole cells in industrial processes has attracted considerable attention due to various advantages, such as an increase of conversion and cellular stability, and a decrease of procedure expenses due to the easy cell recovery and reutilization. Immobilization provides high cell concentrations and cell reuse. However, mass transfer limitations are important when immobilized cells were used. To verify the effect of mass transfer limitations, effectiveness factor and observable modulus were obtained experimentally. The results show that mass transfer resistance is important. The value of effective diffusion coefficient of acetophenone in κ-carrageenan was obtained as 3×10^{-7} cm^2/s. In this context, the diffusivity of acetophenone in κ-carrageenan fills the space of the literature.

Continuous production of (*R*)-1-phenylethanol was accomplished with the highest conversion (79%) in a packed bed reactor, the obtained conversion (77%) being comparable with those obtained in the literature [20]. Also at higher residence times (9 h), much higher conversion (86%) was obtained in this study. However, not only conversion, but also productivity is important for industrial application. To reach both high productivity and high concentration, a residence time of 3.75 h was economically feasible. Our results show that production of (*R*)-1-phenylethanol conducted in a packed bed reactor leads to a good conversion and productivity.

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