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KINETIC OF ALCOHOL DEHYDROGENASE FROM *THERMOANAEROBIUM BROCKII* USING ALCOHOLS AND CARBONYL COMPOUNDS AS SUBSTRATES

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ABSTRACT

Alcohol dehydrogenase (ADH) is a versatile enzyme because it is capable of catalyzing alcohols to carbonyl derivatives and vice-versa by changing the pH of the reaction medium. This work was focused on the evaluation of basic kinetic parameters (activation energy, V_{max}, K_M, etc.) regarding the action of ADH from *Thermoanaerobium brockii* (ADH_{tb}) on alcohols (2-propanol, 2-hexanol and 1-phenylethanol) and carbonyl compounds (acetaldehyde, acetone, 2-hexanone and acetophenone). Moreover, the use of the membrane reactor (MB) for the continuous conversion of a pro-chiralic ketone (2-hexanone) to a chiral alcohol (2-hexanol) was evaluated. A conversion yield of about 42% was attained by feeding the MR alternately with 2-hexanone (10mM) and 2-propanol (10mM) under the conditions as follows: 30°C, feeding rate = 2mL/h, UF-membrane = 500Da, agitation = 100 rpm, pH 8.8 (2-propanol solution) and pH 4.0 (2-hexanone solution).

KEYWORDS: Alcohol dehydrogenase, *Thermoanaerobium brockii*, alcohols, carbonyl compounds.

INTRODUCTION

Alcohol dehydrogenase (ADH) is a common designation of a group of enzymes which catalyses the conversion of alcohols into carbonylic compounds and vice-versa depending on

the pH of the reaction medium. At alkaline pH, the oxidation (alcohol to carbonyl compound) prevails, whereas at acidic pH, the reduction (carbonyl compound to alcohol) is favored.

Dehydrogenases, in general, require coenzymes (NAD, NADH, etc.) for their catalytic activity. As coenzymes are very expensive substrates, they must be regenerated during the reaction in order to make the process applicable for biotransformation. The coenzyme regeneration is so important that only few processes are effectively used, although about 30% of all currently known enzymes are oxi-red type catalysts.[1]

In a previous work[2] the conversion of NAD into NADH or NADP into NADPH was successfully attained by using a coupled enzyme system (for example, glutamate dehydrogenase/glucose 6-phosphate dehydrogenase), in which one enzyme required the coenzyme in oxidized form, whereas the other was in the reduced form. However, as previously demonstrated[3] NAD(H)-dependent alcohol dehydrogenase from *Saccharomyces cerevisiae* (ADH_{sc}) successfully converted acetaldehyde into ethanol concomitantly to NAD/NADH recycling through a continuous process carried out in a membrane reactor. Moreover, it was also shown that the conversion of ketones into alcohols (chiral types included) simultaneously with NAD/NADH recycling would also be possible to attain through the continuous process.[4]

Alcohol dehydrogenases (E.C. 1.1.1.11) are largely distributed throughout earthly living beings, mainly among microorganisms. Each enzyme belonging to this group must be kinetically evaluated because each one has a particular characteristic not only related to the type of the coenzyme required but also to the molecular feature of its substrate which can enter into the active site and suffer either oxidation or reduction.[5]

Beside ADH_{sc}, horse liver ADH and *Thermoanareobium brockii* (ADH_{tb}) are other alcohol dehydrogenases largely employed as enzyme immunoassay markers and in organic syntheses.[6] The ADH_{tb} differently from ADH_{sc} is a NADP(H)-dependent alcohol dehydrogenase.[6]

The present work focuses on the kinetic behavior of ADH_{tb} against alcohols (2-propanol, 2-hexanol and 1-phenylethanol) and carbonyl compounds (acetaldehyde, acetone, 2-hexanone and acetophenone) as substrates as well as its use in a membrane reactor for the continuous

conversion of 2-hexanone to 2-hexanol with the concomitant NADP/NADPH recycling in the presence of 2-propanol as a co-substrate.

MATERIAL AND METHODS

MATERIAL

Alcohol dehydrogenase (ADH_{tb} – E.C. 1.1.1.2) from *Thermoanaerobium brockii* and the cofactors (β-NADP and β-NADPH) were purchased from Sigma Chemical Co (St. Louis, MO, USA). The UF-membrane (cut-off of 500 Da) was kindly donated by Bioengineering® AG (Wald, Switzerland). All of the other chemicals used were also of the highest analytical grade.

METHODS

Enzyme Assay

ADH_{tb} activity was assayed spectrophotometrically at 25°C by measuring the change in absorbance of NADPH at 340nm using a Beckman DU 640 spectrophotometer equipped with a Peltier effect-controlled temperature cuvette holder. The standard assay for the oxidation reaction was performed adding 250µL of TRIS-HCl buffer 100mM (pH 8.0), 500µL of substrate (100mM 2-propanol, 25mM 2-hexanol or 50mM 1-phenylethanol), 200µL of β-NADP 10mM, 10µL of deionized water and 10µL of ADH_{tb} (1mg/mL) into a 3mL-quartz cuvette (light path = 1cm). The blank tube, used for calibrating the spectrometer, was made substituting the ADH_{tb} by 20µL of deionized water.

The standard assay for the reduction reaction was performed adding 200µL of buffer 100mM, 400µL of substrate (20mM acetaldehyde, 50mM acetophenone and 2-hexanone or 100mM acetone), 100µL of β-NADPH 2mM, 100µL of deionized water and 200µL of ADH_{tb} (0.1mg/mL) into a 3mL-quartz cuvette (light path = 1cm). The blank tube, used for calibrating the spectrometer, was made by substituting the ADH_{tb} for 300µL of deionized water. The buffers employed differed according to the carbonyl compound used as a substrate, i.e., TRIS-HCl for acetaldehyde (pH 7.0), phosphate for acetophenone (pH 6.0) and for 2-hexanone (pH 5.0) and acetate (pH 3.0) for acetone.

One unit of ADH_{tb} (U) represented 1mmol of β-NADPH produced or consumed per minute at the adequate temperature for each substrate used (10°C: 2-hexanol and 1-phenylethanol; 30°C: acetaldehyde, acetophenone, 2-hexanone and acetone; 40°C: 2-propanol), on a basis of an absorption coefficient of 6.22 $mM^{-1}.cm^{-1}$ for β-NADPH at 340nm.

The enzyme activity (EA), expressed as U/mL, was calculated through the equation:

$EA = [(\Delta Abs/min).V] \div (V_{adh} .6.22)$ (Eq. 1)

Where V = reaction volume and V_{adh} = volume of the enzyme solution.

Effect of pH, Temperature and Initial Substrate Concentration on the Enzyme Activity

The activity of ADH_{tb} against the pH variation was evaluated by carrying out the oxidation and reduction reactions at pH intervals of 6.0-9.5 and 2.5-7.5, respectively. The effect of the temperature, in turn, was evaluated in all cases by changing it from 30°C (303K) to 50°C (323K).

The effect of the initial substrate concentration on ADH_{tb} activity was made by changing its concentration at different intervals according to the type of substrate evaluated. Thereby, the intervals were: 2-propanol (from 5 to 50mM), 2-hexanol and acetone (from 5 to 100mM), 1-phenylethanol (from 2 to 100mM), acetaldehyde (from 1 to 100mM), acetophenone and 2-hexanone (from 0.25 to 20mM).

Membrane Reactor Test

The membrane reactor (MR) was operated in the continuous mode, the coenzyme (β-NADPH, 5mM) and ADH_{tb} (150U) being introduced into the MB which was coupled to a 500 Da-ultrafiltration membrane. The reactor was fed with a solution of 2-propanol (50mM) or 2-hexanone (20mM) continuously. Each substrate was prepared with the correspondent buffer previously mentioned (100mM TRIS-HCl buffer, pH 8.0 for 2-propanol and100mM phosphate buffer, pH 3.5 for 2-hexanone). The temperature and feeding rate were set at 30°C and 2mL/h, respectively. The concentration of the substrate or the product was measured from the outlet samples collected every 3h. The test was made in triplicate.

The MR employed was purchased from Bioengineering® AG (Wald Germany). The 10mL-reactor is a 316L-stainless steel cylinder, presenting on its bottom part, an inlet and an outlet for the external water bath for the temperature control. The diameter of the UF-membrane used was 63mm. The reactor can be sterilized (autoclave up to 134°C for 30 min) and resist high temperatures (up to 150°C) and corrosion by most substances (except strong acids, pH < 1.0; and alkalies, pH>12.0). The flux throughout the reactor is from the bottom (substrate inlet) to the top (product outlet).

Analytical Techniques

Determination of alcohol

The concentration of alcohols (2-propanol and 2-hexanol) in collected samples was determined by the dichromate method previously described by Isarankura-Na-Ayudhya et al.[7]

Protein determination

Protein, eventually released from the MR and presented into the outlet fluid, was determined based on the difference between UV absorbance measured at 215nm and 225nm, using bovine serum albumin (BSA from Sigma) as a standard.[8]

Cofactor detection

The eventual release of the cofactors (β-NADP and β-NADPH) from the MR was made by taking samples from the outlet fluid and reading its absorbance at 260nm and 340nm through a spectrophotometer.

RESULTS

The ADH_{tb} activity against different alcohols and carbonyl compounds was measured by the formation or consumption of β-NADPH at time intervals during which the linearity was maintained (Figures 1 and 2).

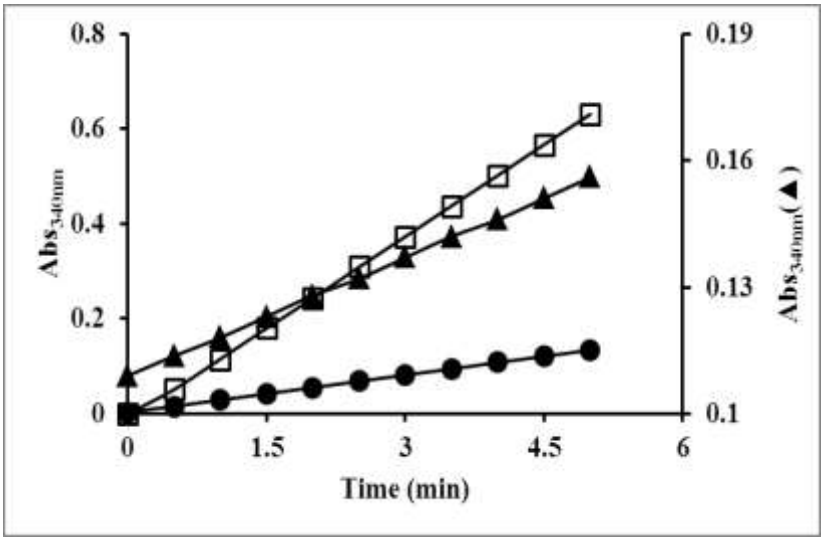

Figure 1. Variation of β-NADPH during the oxidation of 2-propanol (•), 2-hexanol (□) and 1-phenylethanol (▲) to the correspondent ketones catalyzed by ADH_{tb}.

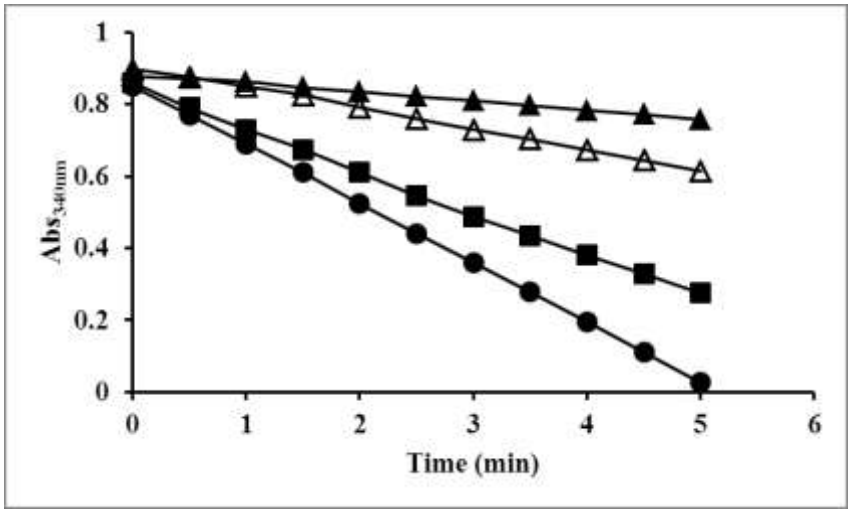

Figure 2. Variation of β-NADPH during the reduction of acetaldehyde (Δ), acetone (•), 2-hexanone (▲) and acetophenone (■) to the correspondent alcohols catalyzed by ADH_{tb}.

As alcohol dehydrogenases without exception have their activity strongly dependent on the pH of the medium, their activity varies with the pH in the presence of different substrates. When the substrate is an alcohol, the high activity is observed at pH over 7.0 (Figure 3), whereas the high activity for carbonyl compounds occurs at pH below 7.0 (Figure 4). This distinction is quite important when the enzyme is employed in organic synthesis, mainly if the desired product is a chiral alcohol attained from a carbonyl derivative.

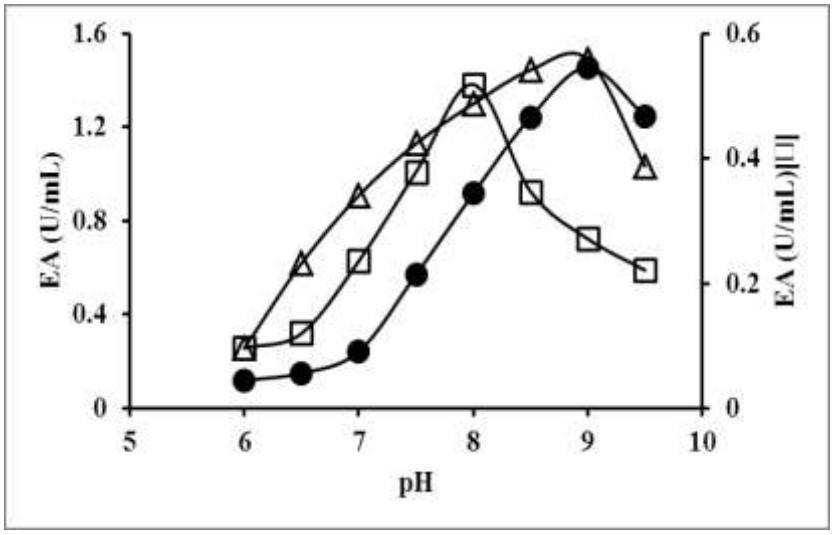

Figure 3. Profile of ADH_{tb} activity variation against pH. 2-propanol (•), 2-hexanol (□) and 1-phenylethanol (Δ) employed as its substrates. The activity values regarding 1-phenylethanol must be divided by ten.

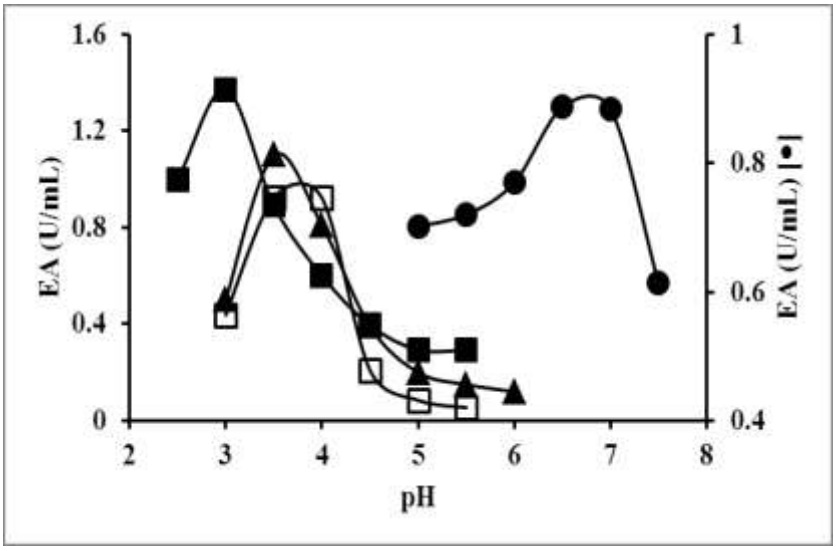

Figure 4. Profile of ADH_{tb} activity variation against pH. Acetaldehyde (•), acetone (■), 2-hexanone (▲) and acetophenone (□) were employed as its substrates.

The activity of ADH_{tb} was studied at temperatures varying from 30°C (303K) to 50°C (323K). For this study and further ones related to the effect of coenzyme concentration on the enzyme activity and the stability of the enzyme, only the 2-propanol and the carbonyl compounds were used as its substrates. This choice resulted from the fact that in the organic synthesis, the reduction of carbonyl compounds by alcohol dehydrogenase is more valuable than the reverse reaction.[9]

Figures 5 and 6 present the variation of ADH_{tb} activity against the temperature.

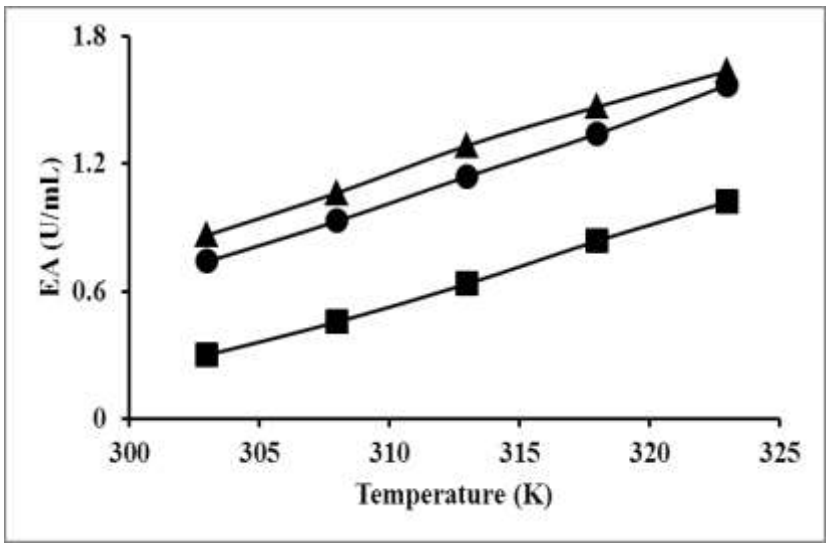

Figure 5. Variation of ADH_{tb} activity as the temperature was increased from 303K to 323K. The substrates employed were: 2-propanol (•), acetaldehyde (■) and acetone (▲).

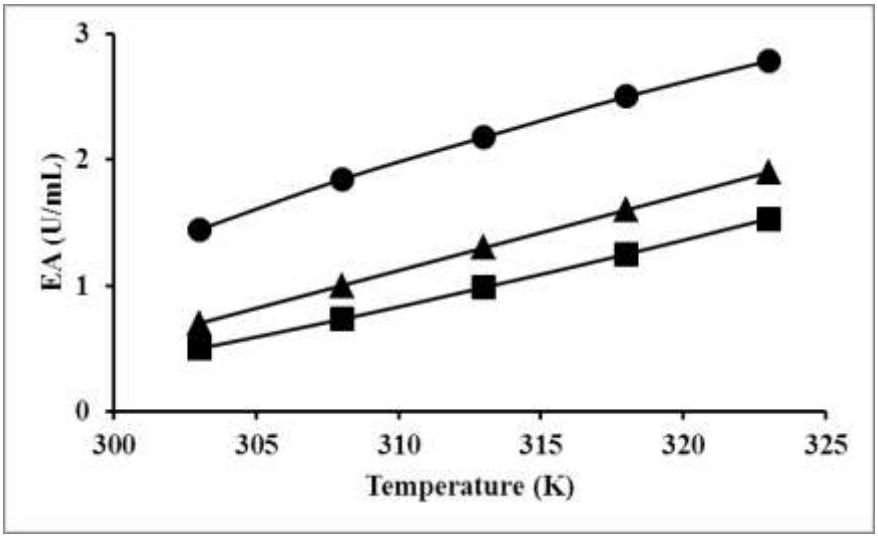

Figure 6. Variation of ADH_{tb} activity as the temperature was increased from 303K to 323K. The substrates employed were: 2-hexanone [pH 3.0] (●), 2-hexanone [pH 4.0] (■) and acetophenone (▲). The data related to acetophenone must be divided by ten.

By applying the conventional Arrhenius' approach the following minimum square regression equations were attained.

[2-propanol] Log (EA) = - 1554.(1/T) + 5.0 (r = - 0.9993) (Eq. 2)

[Acetaldehyde] Log (EA) = - 2608.(1/T) + 8.11 (r = - 0.9995) (Eq. 3)

[Acetophenone] Log (EA) = - 3114.(1/T) + 8.98 (r= - 0.998) (Eq. 4)

[2-hexanone] $Log\ (EA)_{pH\ 3.0}$ = - 1376.(1/T) + 4.72 (r = - 0.998) (Eq. 5)

$Log\ (EA)_{pH\ 4.0}$ = - 2369.(1/T) + 7.53 (r = - 0.994) (Eq. 6)

[Acetone] Log(EA) = - 1364.5.(1/T) + 4.45 (r = - 0.992) (Eq. 7)

The correspondent activation energy values calculated from equations 2-7 for each substrate are presented in Table 2.

The effect of the substrate concentration on the ADH_{tb} activity was presented in Figure 7 (alcohols) and 8 (carbonyl compounds).

As can be seen from Figures 7 and 8 for all substrates, the ADH_{tb} catalytic activity followed the Michaelis-Menten's pattern. By taking the inverse of the substrate concentration and the ADH_{tb} activity, the kinetic constants (V_{max} and K_M) through the conventional Lineweaver-Burk's method[10] were calculated. Their values are presented in Table 2. The minimum square regression equations were.

[2-propanol]:	(1/EA) = 6.88.(1/S) + 0.945	(r = 0.9998)	(Eq. 8)
[2-hexanol]:	(1/EA) = 13.91.(1/S) + 1.543	(r = 0.9992)	(Eq. 9)
[1-phenylethanol]:	(1/EA) = 8.7.(1/S) + 6.5	(r = 0.9998)	(Eq. 10)
[Acetaldehyde]:	(1/EA) = 1.251.(1/S) + 0.976	(r = 0.9990)	(Eq. 11)
[Acetophenone]:	(1/EA) = 1.67.(1/S) + 7.98	(r = 0.997)	(Eq. 12)
[2-hexanone]:	(1/EA) = 3.29.(1/S) + 3.55	(r = 0.998)	(Eq. 13)
[Acetone]:	(1/EA) = 3.43.(1/S) + 0.31	(r = 0.9990)	(Eq. 14)

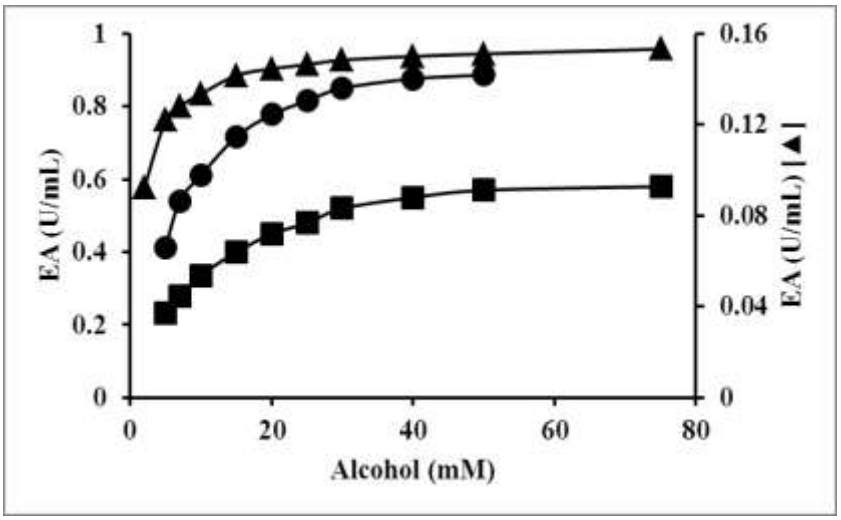

Figure 7. Effect of alcohol concentration on the ADH_{tb} activity. (•) 2-propanol, (■) 2-hexanol and (▲) 1-phenylethanol.

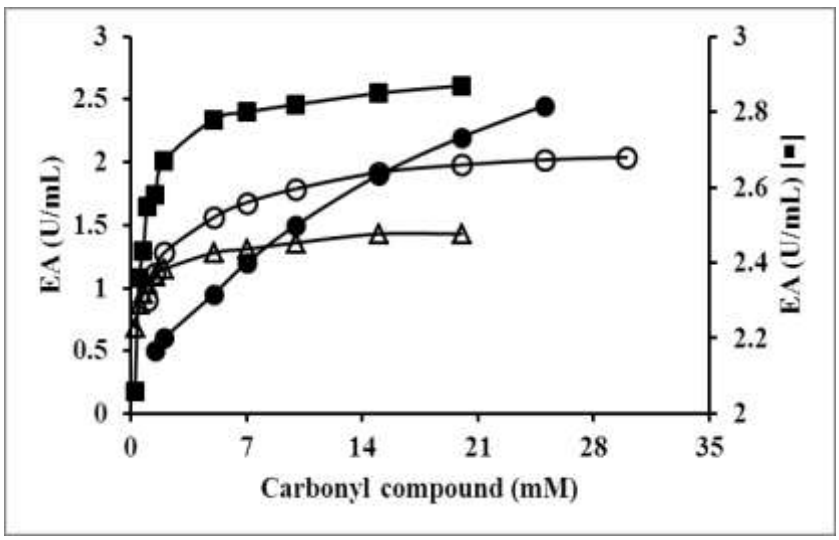

Figure 8. Effect of carbonyl compounds concentrations on the ADH_{tb} activity. (o) Acetaldehyde, (■) 2-hexanone, (Δ) acetophenone and (•) acetone. The data regarding acetaldehyde, 2-hexanone and acetophenone must be divided by two, one hundred and ten, respectively.

As ADH_{tb} is a NADP(H)-dependent alcohol dehydrogenase, the effect of the coenzyme concentration on its activity was also evaluated. The study was made by using 2-propanol and all of the carbonyl compounds as substrates. The data is presented in Figure 9.

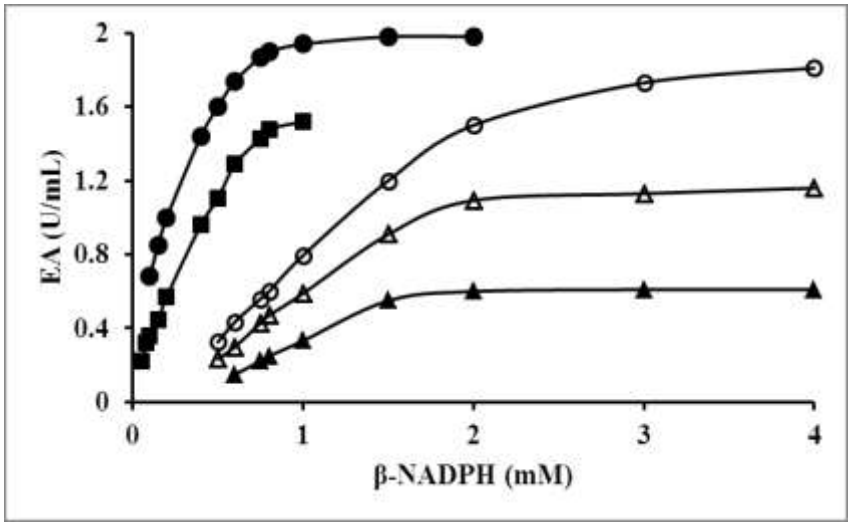

Figure 9. Effect of β-NADPH concentration on the ADH_{tb} activity in the presence of 2-propanol (•), acetaldehyde (■), 2-hexanone (Δ), acetophenone (▲) and acetone (o). The data regarding 2-propanol, acetaldehyde and acetophenone must be divided by two, four and five, respectively.

Undoubtedly, from Figure 9, the Michaelis-Menten pattern occurred in all cases. By applying the Lineweaver-Burk's method, the following minimum square regression equations were attained:

[2-propanol]: (1/EA) = 0.217.(1/β-NADPH) + 0.831 (r = 0.996) (Eq. 15)

[Acetaldehyde]: (1/EA) = 0.82. (1/β-NADPH) + 1.99 (r = 0.998) (Eq. 16)

[Acetophenone]: (1/EA) = 10.17. (1/β-NADPH) + 3.36 (r = 0.998) (Eq. 17)

[2-hexanone]: (1/EA) = 1.72. (1/β-NADPH) + 0.526 (r = 0.990) (Eq. 18)

[Acetone]: (1/EA) = 1.47. (1/β-NADPH) + 0.97 (r = 0.994) (Eq. 19)

The correspondent values of the kinetic constants are shown in Table 2.

Figure 10 corresponds to the stability of ADH_{tb} whose residual activity has been measured by using 2-propanol and 2-hexanone as its substrates. The conversion of 2-hexanone into 2-hexanol (chiralic alcohol) was carried out in the membrane reactor in which 2-propanol was used as the co-substrate – its conversion into acetone restored the β-NADPH which, in turn, was reused by the enzyme for reducing more of 2-hexanone. The choice of those substrates

was based on the fact that the enzyme activity had been kept at 0.42 U/mL for both compounds (Table 1).

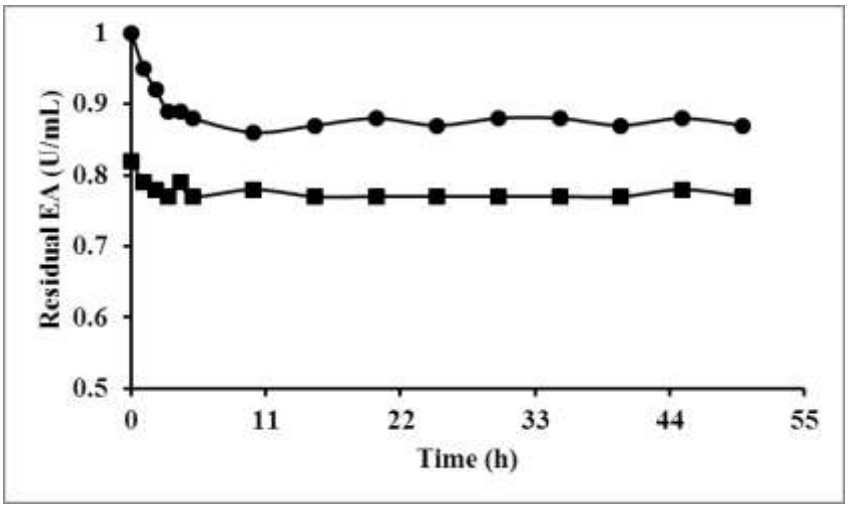

Figure 10. Stability of ADH_{tb} maintained at pH 8.0 (■) and pH 3.5 (●) for 50h before measuring its residual activity against 2-propanol and 2-hexanone, respectively. In both cases the temperature was set at 40°C.

DISCUSSION

From Table 1, the ADH_{tb} activity varied significantly according to the chemical nature of the substrate. The highest enzyme activities occurred with acetone > 2-hexanol > acetophenone. In Table 1, the useful information related to the ADH_{tb} activities against the substrates are presented [2-propanol (co-substrate) and 2-hexanone and acetophenone as the main substrates, leading to the chiral alcohols 2-hexanol and 1-phenylethanol, respectively]. Taking into account that the activity ratios of acetophenone /2-propanol and 2-hexanone/2-propanol were about 4.5 times and 1.0 time, respectively, 2-hexanone was chosen as the main substrate. Its conversion into 2-hexanol by the enzyme would follow a rate of the same magnitude as when compared to 2-propanol (a co-substrate). Such a condition should reach a steady-state faster when the reaction is conducted inside a membrane reactor (MR), which is intended to be operated under a continuous regimen. In addition, from Table 2, the activation energy for 2-propanol oxidation (29.8 kJ/mol.K) and 2-hexanone reduction (26.3 kJ/mol.K) by ADH_{tb} differed only about 12%; the operational temperature of the MR may remain unchanged (30°C, for example), regardless of the type of substrate fed into the reactor. It should be remembered that the co-substrate (2-propanol) and the main substrate (2-hexanone) was alternately fed into the membrane reactor.

TABLE 1. The square minimum regression lines (showed in Figures 1 and 2) and the enzyme activity regarding alcohols and carbonyl compounds as its substrates. The absorbance reading and time of reaction were represented as *y* and *t*, respectively.

Substrate	Straight line equations	r	EA (U/mL)
2-Propanol	y = 0.0262.t + 0.0029	0.9997	0.421
2-Hexanol	y = 0.129.t - 0.013	0.9992	2.07
1-Phenylethanol	y = 0.0093.t + 0.109	0.9990	0.15
Acetaldehyde	y = - 0.0589.t + 0.909	0.9997	0.947
Acetophenone	y = - 0.115.t + 0.854	0.9994	1.85
2-Hexanone	y = - 0.0248.t + 0.883	0.9993	0.420
Acetone	y = - 0.1691.t + 0.872	0.9990	2.72

By comparing the activity of ADH_{tb} against 2-hexanol (2.1 U/mL), 2-propanol (0.42 U/mL) and 1-phenylethanol (0.15 U/mL), it is clear that the worst and the best substrates were 1-phenylethanol and 2-hexanol, respectively. Perhaps these extremes are due to the difference in the molecular structure of both compounds, i.e., an alcohol molecule having an aromatic ring presents lower affinity to the active site of the enzyme than an aliphatic one. Between the aliphatic alcohols, the chain length seems to favor the longer one. However, the opposite occurs when ketones are used as substrates of the ADH_{tb}.

As can be seen from Figures 3-4, the $pH_{optimum}$ for ADH_{tb} varied between 8.0 and 9.0 when alcohols (2-propanol, 2-hexanol and 1-phenylethanol) were used as its substrates; whereas in the case of the carbonyl compounds as its substrates (acetaldehyde, acetone, 2-hexanone and acetophenone), it varied from 3.0 to 6.5. Considering only the ketones as the ADH_{tb} substrates, the $pH_{optimum}$ would vary from 3.0 to 4.0 (Table 2). Taking into account that the isoelectric point of ADH_{tb} is around 6.5[11], the enzyme has its alcohol oxidation capability enhanced at pH above this value (preferentially in alkaline medium), in which the net charge of a whole molecule should be negative. Conversely, when the net molecule charge is positive, which occurs at pH below 6.5, the reduction capability of the ADH_{tb} is favored.

Regarding the effect of the temperature on the ADH_{tb} activity (EA) from Figures 5 and 6, a linear correlation between enzyme activity and temperature increasing can be seen. This result points to the fact that the denaturing temperature is over 323K, which corroborates the thermal stability presented by the enzyme as already described in the literature.[6] Moreover, it must be stressed that an interaction between pH and temperature can occur – as shown in Figure 6 when 2-hexanone was the substrate and the reaction carried out at pH 3.0 and 4.0 -, when ADH_{tb} is the catalyst used for reducing carbonyl compounds to the correspondent

alcohols. As ADH_{tb} belongs to the class of enzymes having a quaternary structure level[12], so each protein chain (four in the case of ADH_{tb}) can react differently as the pH and temperature are simultaneously changed. Considering the reduction of 2-hexanone by ADH_{tb} carried out at pH 3.0 and pH 4.0 the activation energy was equal to 26.3 kJ/mol.K and 45.4 kJ/mol.K, respectively (Table 2). Thereby, the interaction ADH_{tb}-2-hexanone (enzyme-substrate complex) requires 42% less energy at pH 3.0 than pH 4.0, i.e., the reacting molecules at pH 3.0 can reach high collision effectiveness with less kinetic energy. According to GODFREY and WEST[13] it is always valuable to verify if pH/temperature interaction would occur in enzyme catalysis in general.

TABLE 2. Activation energy, $pH_{optimum}$ and kinetic constants regarding the ADH_{tb} activity measured against several substrates.

Substrate	E_a, (kJ/mol.K)	V_{max}, (U/mL)	K_M, (mM)	$pH_{optimum}$
2-propanol	29.8	*1.1/1.2	*7.3/0.26	9.0
2-hexanol	-	0.65	9.0	7.8
1-phenylethanol	-	0.15	1.34	9.0
Acetaldehyde	50.0	*1.02/0.50	*1.28/0.41	6.5
Acetone	26.1	*3.23/1.03	*11.1/1.52	3.0
2-hexanone	**26.3/45.4	*0.28/1.9	*0.93/3.27	3.5
Acetophenone	59.6	*0.13/0.30	*0.21/3.03	4.0

*The first and second values refer to the kinetic constants determined by varying the substrate and coenzyme concentration, respectively. **The activation energy (E_a) measured for the 2-hexanone reduction at pH 3.0 and 4.0, respectively.

In our previous work, the possibility of recycling the coenzyme (β-NADH/β-NAD) by employing two enzymes – one catalyzing the reduction of coenzyme (glucose 6-phosphate dehydrogenase) and the other, (glutamate dehydrogenase) the reverse reaction were demonstrated.[2] After that, the coenzyme recycling was achieved by using the substrate-coupled procedure catalyzed by the yeast NAD(H)-dependent alcohol dehydrogenase (ADH), since the substrates – ethanol and acetaldehyde, in that study – were alternately fed into the membrane reactor.[3]

Thereby, the conversion of 2-hexanone into chiral 2-hexanol catalyzed by ADH_{tb} in the presence of β-NADPH using a membrane reactor was made by feeding the reactor alternately with 2-hexanone and 2-propanol (co-substrate). The main result is presented in Figure 11. The 2-hexanone/2-hexanol conversion yield can be estimated to around 42% (Figure 11). This result indicates that the process is a possible approach and that the process deserves to

be improved. When 2-propanol is fed into the reactor, a small volume of the outlet solution contains both acetone and chiral alcohol (washed-out as 2-propanol is fed into the reactor). However, the separation of the acetone and the alcohol is easily made by fractionated precipitation, a unit operation well known at an industrial level.

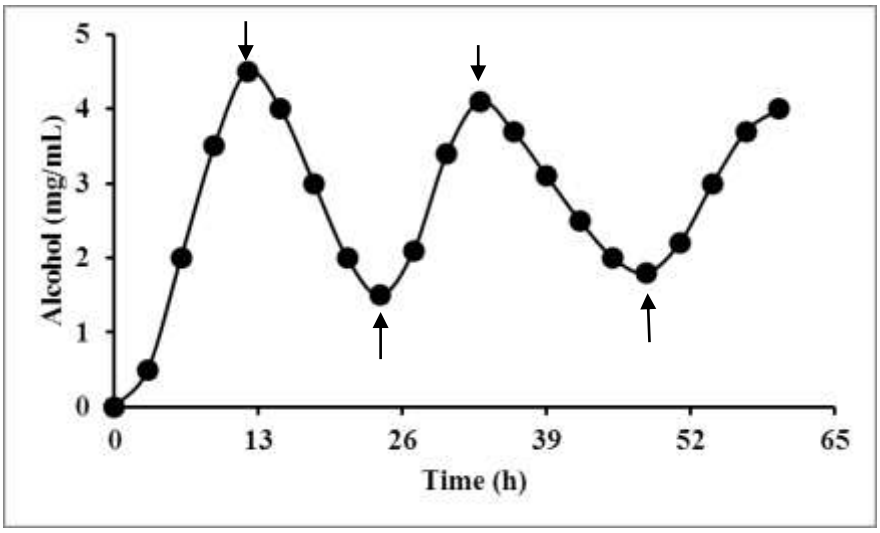

Figure 11. Profile on the continuous conversion in the membrane reactor fed with 10mM 2-hexanone, using 2-propanol (10mM) as the co-substrate. Conditions of reaction: 30°C, feeding rate = 2mL/h, UF-membrane = 500Da, 100rpm and 50mM acetate buffer [pH 4.0 (2-hexanone solution) and pH 8.8 (2-propanol solution)]. The up-directed arrows indicate 2-hexanone addition, whereas down-directed arrows indicate addition of 2-propanol.

CONCLUSION

The presented data led to the conclusion that ADH_{tb} catalyses effectively the oxidation and reduction of alcohols and carbonyl compounds, since the pH of the reaction medium is changed adequately. As a general rule, the pH of the medium for oxidation and reduction reactions must be, respectively, alkaline and acidic. The enzyme activity, nonetheless, depends on the nature of the main substrate, the carbonyl compounds being its preferred substrates. In spite of ADH_{tb} having a reaction rate varying with the initial concentration of all substrates used in this work according to the Michaelis-Menten's model, the kinetic constants associated with each substrate differed significantly, pointing to the fact that the complex ADH_{tb}, its molecular structure, has different affinities for alcohols and carbonyl compounds. Finally, the conversion of a pro-chiral ketone to a chiral alcohol is possible

through the use of a membrane reactor fed alternately with the ketone and a cheap alcohol (2-propanol in the present study) acting as an auxiliary substrate.

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