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Recombinant endo-inulinases: determination the activation and deactivation energies and optimum temperatures in inulin hydrolysis

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Abstract
The aim of paper was to determine the activation, deactivation energies and optimum temperatures for recombinant endo-inulinases of various origins, including also recombinant endo-inulinases from *Aspergillus niger*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. The activity recombinant endo-inulinases of various origins vs. temperature curves were analyzed. A mathematical model describing the effect of temperature on recombinant endo-inulinase activity was used. Based on the analysis, values of the activation energies E were in the range from 22.08 ± 13.94 kJ mol^{-1} to 62.62 ± 17.24 kJ mol^{-1} and in the range from 29.80 ± 8.83 kJ mol^{-1} to 92.69 ± 15.31 kJ mol^{-1} for recombinant endo-inulinase *A. niger* and various origins, respectively. The deactivation energies E_D were from the range from 146.80 ± 20.31 kJ mol^{-1} to 301.95 ± 95.81 kJ mol^{-1} and in the range from 159.96 ± 14.80 kJ mol^{-1} to 289.43 ± 21.18 kJ mol^{-1} for recombinant endo-inulinase *A. niger* and various origins, respectively. The optimum temperatures T_{opt} were obtained in the range from 328.67 ± 1.32 K to 335.94 ± 1.22 K and in the range from 319.41 ± 0.85 K to 338.53 ± 0.45 K for recombinant endo-inulinase *A. niger* and various origins, respectively.

Keywords Recombinant endo-inulinases · Activation energy · Deactivation energy · Optimum temperature

Introduction

The activity of the enzyme changes with temperature. In the first stage, the activity of the enzyme increases with increasing temperature. At a certain temperature, referred to as T_{opt}, the activity of the enzyme is maximal. When the T_{opt} is exceeded, the activity of the enzyme decreases. The knowledge of the optimum temperature T_{opt} and the activation energy E and deactivation energy E_D of the enzyme enables the bioprocess optimization with the reduction of its costs at the same time. The T_{opt} value is mostly determined experimentally. The values of the activation energies E and E_D can be determined from the curves Arrhenius of the dependence of the logarithm of the reaction rate (ln$v$) on the reciprocal of temperature (T^{-1}) [1]. It has been shown that the determined values of E and E_D by application of the Arrhenius relationship is burdened with an error [2, 3].

Inulin consists of linear chains of 2,1-β-D-fructofuranose molecules terminated with a glucose residue at the reducing end. Inulin is a reserve carbohydrate (0.5 – 22%) in many plant tubers, roots or leaves [4–6]. Inulin can be used for the production of many bio-based products, such as high-fructose syrups, bioethanol, fructose and glucose inulo-oligosaccharides [5, 7, 8].

Inulinases (2,1-β-D-fructan fructanohydrolase) are hydrolysed, catalyzing the inulin hydrolysis of about 90 – 95% converse of inulin [9]. Inulinases can be classified into endo-inulinases (EC 3.2.1.153) and exo-inulinases (EC 3.2.1.80). Exo-inulinases remove terminal fructose residues from the non-reducing end of inulin, and endo-inulinases act on the internal bonds of the inulin molecule to produce inulotrioses, inulotetraoses and fructo-oligosaccharides [7, 10, 11]. Inulinases occur inter alia in plants, fungi *Penicillium* sp., *Aspergillus niger* and bacteria *Pseudomonas mucidolens*, *Arthrobacter* sp. S37 can to produce endo-inulinase [12–21].

Endo-inulinases obtained from them are characterized by high activity and high thermostability [12]. The optimum

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temperatures for endo-inulinases are around 40 °C [2, 3]. They are higher for commercial endo-inulinases [14, 21] even above 60 °C [15, 17] and 65 °C [16, 20] for recombinant endo-inulinases.

Formation of the recombinant endo-inulinases associated with their industrial use. Commercial of preparations endo-inulinase should have economic and technical advantages including low price, high activity and stability under industrial reaction conditions [16].

There have been many publications in the literature on *A. niger* recombinant endo-inulinase [13–17] used for high-performance engineered endo-inulinanse for fructooligosaccharides production from inulin. There are also publications on recombinant endo-inulinase of various microbial origins, including *Pseudomonas mucidolens* into *Saccharomyces cerevisiae* EBY 100 [19], endo *Arthrobacter* sp. S37 in *Yarrowia lipolytica* Po1h [18] and *Penicillium restrictum* A191 in *Escherichia coli* [20]. However, processes involving recombinant endo-inulinase of various origins purposes can not be designed and optimized without knowing the values of the activation energy E and deactivation energy E_D and optimum temperature T_{opt}. Additionally, the parameters E, E_D and T_{opt} for recombinant endo-inulinases *A. niger* [13–17] were determined.

Determination the parameters E, E_D, T_{opt} for recombinant endo-inulinase activity

The activity of recombinant endo-inulinase in the inulin hydrolysis reaction changes with temperature. At temperatures below the optimum temperature T_{opt}, the endo-inulinase activity increases with increasing temperature. After exceeding the optimum temperature T_{opt}, the enzyme activity decreases with increasing temperature. Both recombinant endo-inulinase activity increase or decrease are described by the first-order equations due to the enzyme concentration, like in recombinant exo-inulinase [23, 24].

In particular studying recombinant endo-inulinase activity during the inulin hydrolysis, it is assumed that the change in substrate concentration C_S during reaction time t describes by the first-order equations

$$\frac{dC_S}{dt} = -kC_E \tag{1}$$

where k is the kinetic constant of the enzymatic reaction (min^{-1}) and C_E is the concentration of the active enzyme (M).

Next, the change in recombinant endo-inulinase dimensionless activity A it is also assumed that by the first-order kinetics [23–25] with the following equation

$$\frac{dA}{dt} = -k_D A \tag{2}$$

where k_D is the deactivation process kinetic constants (min^{-1}). Dimensionless activity of enzyme A is expressed by the equation

$$A = \frac{C_E}{C_{E0}} \tag{3}$$

where C_{E0} is initial concentration of the active enzyme (M).

The kinetic constants k and also, the deactivation constants k_D depend on temperature T according the equation Arrhenius. The initial assumptions described by equations Eqs. 1, 2 and 3 allow to present the final form of the solution Eq. (4), which was presented in greater detail in the earlier work [26]. The Eq.(4) describes the change in the absolute activity of the enzyme A vs. temperature T

$$A = \frac{\exp\left(\frac{(T_{opt}-T)E_D B}{RTT_{opt}(\exp B-1)}\right)\left(1-\exp\left(-B\exp\left(\frac{(T-T_{opt})E_D}{RTT_{opt}}\right)\right)\right)}{1-\exp(-B)} \tag{4}$$

where T_{opt} is the optimum temperature at which recombinant endo-inulinase activity shows maximum activity (K), T is temperature (K), R is gas constant (8.314 J mol^{-1} K^{-1}), E_D is the activation energy of the deactivation process (J mol^{-1}) and dimensionless parameter B is determined from the relationship

$$B = k_{D0} t \exp\left(-\frac{E_D}{RT_{opt}}\right) \tag{5}$$

where t is an inulin hydrolysis time of recombinant endo-inulinases (min) and k_{D0} is a pre-exponential factor the kinetic constant of the deactivation process of recombinant endo-inulinase (min^{-1}).

When the value of the deactivation energy E_D and the parameter B were known, the activation energy E is calculated from the equation

$$E = E_D - \frac{E_D B}{\exp B - 1} \tag{6}$$

Based on equations Eq. (4) and Eq. (6), the values of deactivation energy E_D, optimum temperatures T_{opt} and parameter B were determined in SigmaPlot 14.5 by using a method of nonlinear estimation [24, 26–31] in which the residual sum of squares RSS (Eq. (7)) was minimized

$$RSS(E_D, B, T_{opt}) = \sum_{i=0}^{n} \frac{1}{A^2}\left(A - A_{cal}(E_D, B, T_{opt})\right)^2 \tag{7}$$

where A - recombinant endo-inulinase of various origins activity determined experimentally, $A_{cal}(E_D, B, T_{opt})$

- recombinant endo-inulinase of various origins activity calculated from Eq. (4).

Equations from Eq. (4) to Eq. (6) are used to determine the parameters E, E_D and T_{opt} of inulin hydrolysis by endo-inulinase *Aspergillus niger* [24], by exo-inulinases *A. niger* [27] and by recombinanat exo-inulinases *A. niger* [28], olive oil hydrolysis by lipase [3] and hydrolysis of starch by α-amylase *Bacillus* spp. [26] and porcine pancreas [29].

Results and discussion

The previously study presented the values optimum temperatures T_{opt}, the activation energies E and deactivation energies E_D for inulin hydrolysis by endo-inulinases *A. niger* [24]. The main aim of this study was to determine the parameters T_{opt}, E, E_D for inulin hydrolysis by recombinant endo-inulinases *A. niger* [13–17]. Additionally, these parameters were analyzed for recombinant endo-inulinases of various origins [18–20]. Table 1 shows the conditions for measuring recombinant endo-inulinases activity, such as concentration of inulin, pH buffer and type, and time measurement. Measurements of endo-inulinase activity were determined using the Nelson-Somogyi method at 610 nm [13, 16] and dinitrosalicylic acid at 540 nm [15, 17–20].

Volkov et al. [13] analyzed the recombinant endo-inulinase obtained from gene *A. niger* strain into *Penicillium canescens* A3. Used strains have been obtained from the Laboratory of Enzyme Biotechnology in Bach Institute of Biochemistry in Russian Academy of Sciences.

Endo-inulinase gene from *A. niger* sp. F4 was loned and expressed in *Yarrowia lipolytica* Po1h by Liu et al. [15]. Used strains has been obtained from the Ocean University of China. Recombinant endo-inulinases *A. niger* degraded inulin with a high degree of polymerization.

High-yield endo-inulinase for fructooligosaccharides production from inulin was constructed by Mao et al. [16]. Used strains have been obtained also from the Ocean University of China. An inulin binding module (IBM) from a cycloinulinooligosaccharide fructanotransferase of *Bacillus macerans* CFC1 was fused into either N-(IBM-Endo) or C-(Endo-IBM) terminal of an endo-inulinase *A. niger* was fused.

Gen endo-inulinase from *A. niger* CICIM F0620 has been cloned and expressed in *Pichia pastoris* by He et al. [17]. Used strains have been obtained from the State Key Laboratory of Food Science and Technology at Jiangnan University in China. The recombinant endo-inulinase activity was 4.18 times that observed using the native gene.

Endo-inulinase gene from *Arthrobacter* sp. S37 was cloned and expressed in *Yarrowia lipolytica* Po1h by Li et al. [18]. Endo *Arthrobacter* sp. S37 strain (collection number 2E001892) and *Y. lipolytica* Po1h strain (collection number 2E0189) were used from the Marine Microorganisms Culture Collection of China (MCCC). The endo-inulinase activity produced by the recombinant *Y. lipolytica* was very high. The optimum pH of the recombinant endo-inulinase *Y. lipolytica* was 4 while, the native endo-inulinase *Arthrobacter* sp. was optimum in pH at 7.

Endo-inulinase gen from *Pseudomonas mucidolens* was cloned and expressed into *S. cerevisiae* EBY 100 by Hyun-Chul et al. [19]. Used strains have been obtained from own the Laboratory of Department of Biotechnology and Bioengineering, Dong-Eui University, Korea. The recombinant endo-inulinase *S. cerevisiae* was achieved the highest yield 71.2 of oligosaccharides from inulin after 30 h of reaction.

Endo-inulinase gene from *Penicillium restrictum* A191 was cloned and expressed in *E. coli* by Puratos [20]. Used strains by Puratos [20] has been obtained from the BCCM/MUCL Agro-food and Environmental Fungal Collection,

Table 1 Conditions for measuring of recombinant endo-inulinases activity

Concentration of inulin	pH buffer	*t*/min	Inulinase source	Ref.
Aspergillus niger				
1%	5.0 sodium acetate	5	*A. niger* to *P. canescens* A3	[13]
1%	4.5 sodium acetate	5	recombinant *A. niger* (Megazyme)	[14]
2%	4.5 phosphate	10	*A. niger* F4 in *Yarrowia* lipolytica Po1h	[15]
2%	4.5 sodium acetate	10	*Bacillus macerans* CFC1 in *A. niger* F4	[16]
6%	6.0 sodium acetate	10	*A. niger* CICIM F0620 in *Pichia pastoris* KM71	[17]
Various origins				
2%	4.0 citrate-NaH_2PO_4	15	*Arthrobacter* sp. S37 in *Yarrowia lipolytica* Po1h	[18]
5%	7.0 phosphate	20	*Pseudomonas mucidolens* into *S. cerevisiae* EBY 100	[19]
5%	5.0 citrate and arboxymethylcellulose	15	*Penicillium restrictum* A191 in *E. coli*	[20]

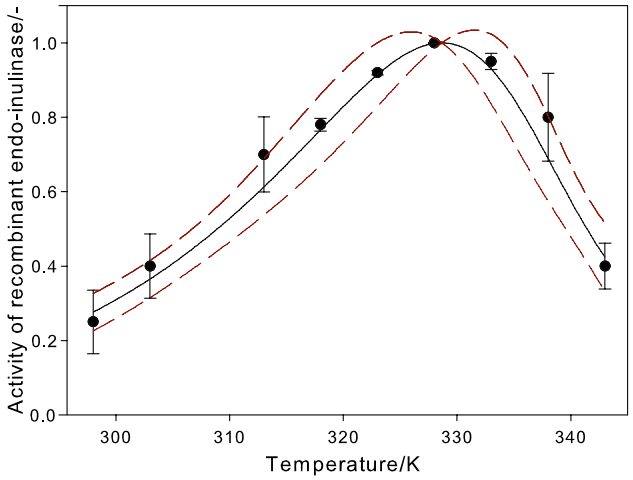

Fig. 1 The activity of recombinant endo-inulinase *A. niger*: (•) by measurements Volkov et al. [13], (–) from Eq. (4), (−−) 95% confidence bond

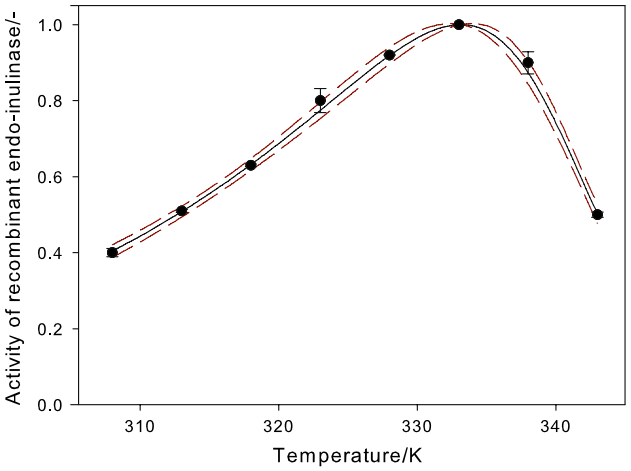

Fig. 3 The activity of recombinant endo-inulinase *A. niger* F4: (•) by measurements Liu et al. [15], (–) from Eq. (4), (−−) 95% confidence bond

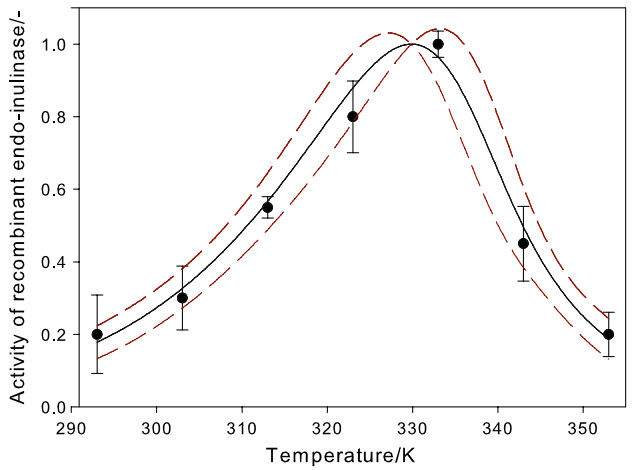

Fig. 2 The activity of recombinant endo-inulinase *A. niger*: (•) by measurements Megazyme [14], (–) from Eq. (4), (−−) 95% confidence bond

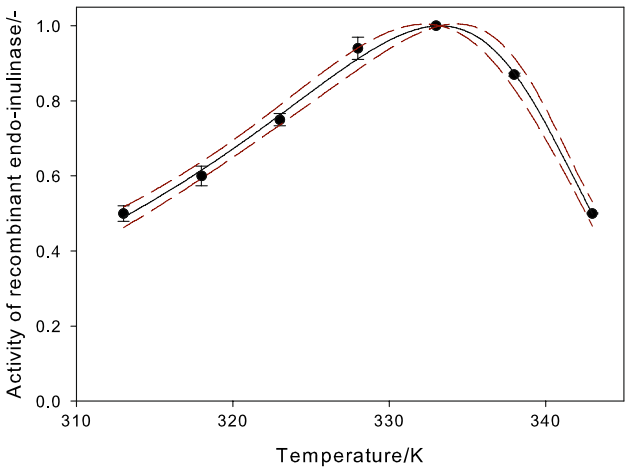

Fig. 4 The activity of recombinant endo-inulinase *A. niger* F4 (IBM-Endo): (•) by measurements Mao et al. [16], (–) from Eq. (4), (−−) 95% confidence bond

Université Catholique de Louvain, Belgium, the number MUCL 42612. The optimum temperature of the recombinant endo-inulinase *E. coli* was 65 ℃.

The effect of temperature on the recombinant endo-inulinases activity

Based on experimental data on the change in the activity of recombinant endo-inulinase from *A. niger* [13–17] and of recombinant endo-inulinase of various origins [18–20] values of deactivation energy E_D, *B* parameter and optimum temperature T_{opt} are determined from Eq. (4) and listed in Table 2. Figures 1–9 show the experimental data on the activity of recombinant endo-inulinases.

Knowing the deactivation energy E_D and the parameter *B* values, the activation energy *E* is calculated from Eq. (6). The obtained results T_{opt}, E_D, *B* and *E* for recombinant endo-inulinases *A. niger* and different origins activity were shown and presented according to the increasing values T_{opt} in Table 2.

Table 3 shows the statistical data calculated for obtained the parameters E_D, T_{opt} and *B* of recombinant endo-inulinases.

The residual sum of squares *RSS* were obtained below 0.153, the high regression coefficients R^2 were above 0.95 for recombinant endo-inulinase *A. niger*. *F*-Fisher test values were in the range from 53.92 to 932.77 with a low probability value *P* were below 0.0013.

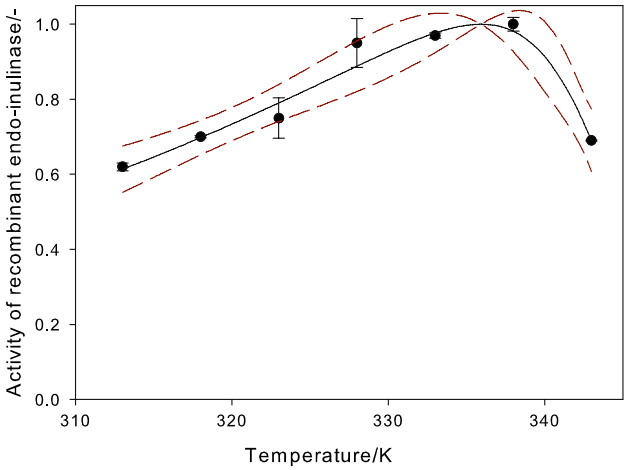

Fig. 5 The activity of recombinant endo-inulinase *A. niger* F4 (Endo-IBM): (•) by measurements Mao et al. [16], (–) from Eq. (4), (––) 95% confidence bond

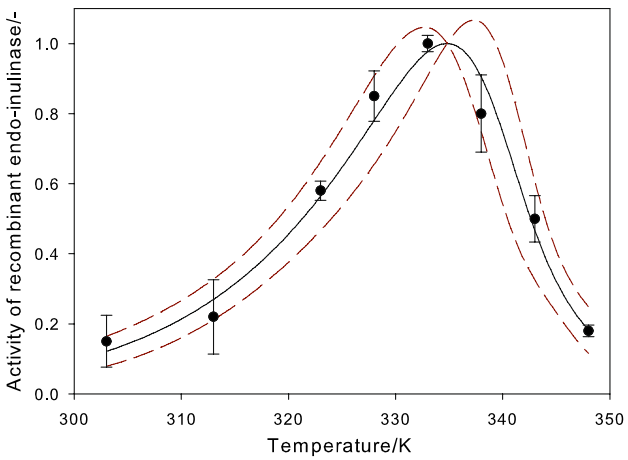

Fig. 6 The activity of recombinant endo-inulinase *A. niger* CICIM F0620: (•) by measurements He et al. [17], (–) from Eq. (4), (––) 95% confidence bond

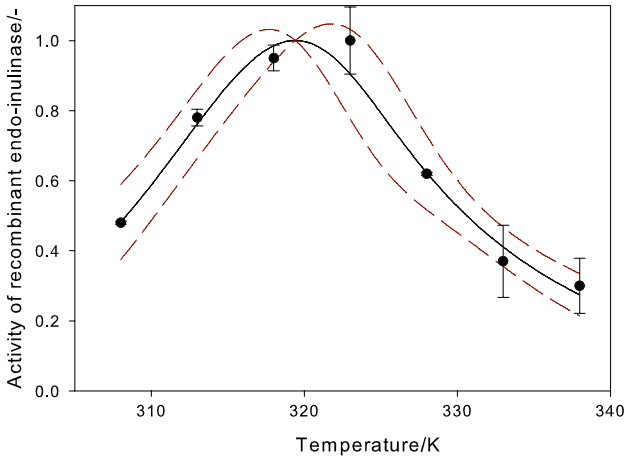

Fig. 7 The activity of recombinant endo-inulinase *Yarrowia lipolytica* Po1h: (•) by measurements Li et al. [18], (–) from Eq. (4), (––) 95% confidence bond

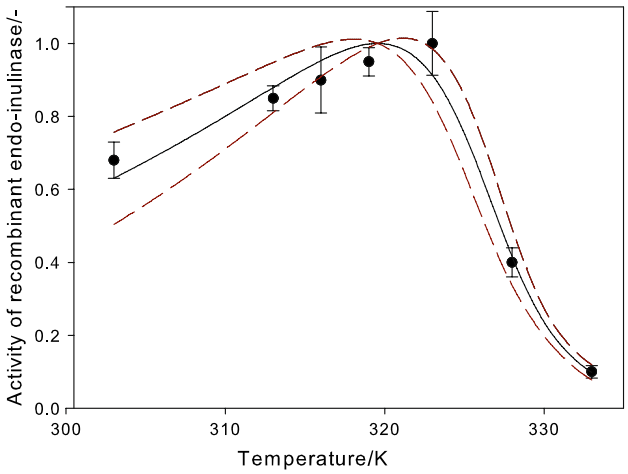

Fig. 8 The activity of recombinant endo-inulinase *Saccharomyces cerevisiae* EBY 100: (•) by measurements Hyun-Chul et al. [19], (–) from Eq. (4), (––) 95% confidence bond

For recombinant endo-inulinase of various origins the residual sum of squares *RSS* were obtained below 0.088, the high regression coefficients R^2 were above 0.97. The statistical data in Table 3 confirmed that it was appropriate to apply Eq.(4) when determining the parameters E_D, T_{opt} and *B*. *F*-Fisher test values were in the range from 72.07 to 349.48 with a low probability value *P* below 0.0007.

Also, Figs. 1-9 present standard deviation errors for experimental points, with the 95% confidence bands for the obtained curves.

The values of activation energy *E*

The valeues of activation energies *E* were obtained in the range from 22.08 ± 13.94 kJ mol^{-1} to 62.62 ± 17.24 kJ mol^{-1} and in the range from 27.21 ± 6.95 kJ mol^{-1} to 92.69 ± 15.31 kJ mol^{-1} for recombinant endo-inulinase *A. niger* and various origins, respectively.

Table 2 shows, that the values *E* estimated for recombinant endo-inulinases from *A. niger* F4 (Endo-IBM) - Fig. 5, was lowest about 60% than recombinant endo-inulinases from *A. niger* F4 (IBM-Endo) - Fig. 4 [16].

Table 2 The values of parameters estimated recombinant endo-inulinases from various origin

Fig.	T_{opt} /K	E /kJ mol^{-1}	E_D /kJ mol^{-1}	B	E_D/E	Ref.
Aspergillus niger						
1	328.91 ± 1.32	42.00 ± 14.59	146.80 ± 20.31	0.64 ± 0.17	3.50	[13]
2	329.88 ± 1.23	44.88 ± 8.85	143.24 ± 9.87	0.71 ± 0.11	3.20	[14]
3	333.16 ± 0.36	36.14 ± 4.68	213.59 ± 11.84	0.36 ± 0.03	5.91	[15]
4	333.26 ± 0.38	38.25 ± 11.66	214.87 ± 15.48	0.38 ± 0.09	5.61	[16]
5	335.94 ± 1.22	22.08 ± 13.94	301.95 ± 95.81	0.15 ± 0.07	13.67	[16]
6	334.81 ± 1.08	62.03 ± 17.24	250.58 ± 28.02	0.55 ± 0.11	4.00	[17]
Various origins						
7	319.41 ± 0.85	92.69 ± 15.31	168.58 ± 7.78	1.43 ± 0.21	1.82	[18]
8	319.57 ± 0.57	27.21 ± 6.95	295.80 ± 18.78	0.19 ± 0.04	10.87	[19]
9	338.53 ± 0.45	59.02 ± 12.45	289.43 ± 21.18	0.45 ± 0.07	4.91	[20]

T_{opt} is the temperature at which recombinant endo-inulinase shows maximum activity, B is parameter determines Eq. (5), E_D is the deactivation energy recombinant endo-inulinase, E is the activation energy recombinant endo-inulinase

Table 3 The statistical data for the parameters E_D, T_{opt} and B of recombinant endo-inulinases

	Aspergillus niger						various origins		
Fig.	1	2	3	4	5	6	7	8	9
RSS	0.0980	0.1070	0.0203	0.0238	0.0437	0.1526	0.0875	0.0754	0.0472
R^2	0.9710	0.9722	0.9973	0.9953	0.9642	0.9560	0.9730	0.9943	0.9936
F	100.48	69.90	932.77	426.24	53.92	54.31	72.07	349.48	233.60
P	<0.0001	0.0008	<0.0001	<0.0001	0.0013	0.0004	0.0007	<0.0001	0.0005
Ref.	[13]	[14]	[15]	[16]	[16]	[17]	[18]	[19]	[20]

RSS is the sum of squared errors, R^2 is regression coefficients, F-Fisher test value, P-value is probability value for value parameters E_D, T_{opt} and B

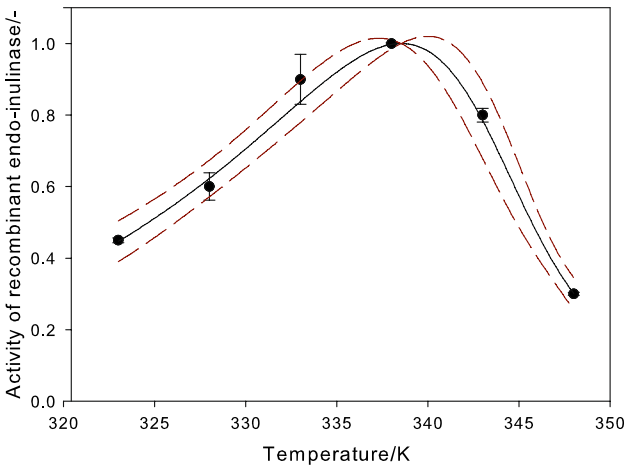

Fig. 9 The activity of recombinant endo-inulinase *Escherichia coli*: (•) by measurements Puratos [20], (–) from Eq. (4), (−−) 95% confidence bond

For the commercial endo-inulinases *A. niger* in previous work [24], it was found that E is in the range from 23.53 ± 3.20 kJ mol^{-1} to 50.66 ± 3.61 kJ mol^{-1}.

The endo-inulinase *Arthrobacter* sp. S37 in *Yarrowia lipolytica* Po1h [18] is characterized by the highest the value E of among analyzed cases.

The values of deactivation energy E_D

The values of deactivation energies E_D of recombinant endo-inulinase *A. niger* were obtained in the range from 143.24 ± 9.87 kJ mol^{-1} to 301.95 ± 95.81 kJ mol^{-1} (Table 2). These values E_D are higher than the values obtained for non-recombinant endo-inulinases *A. niger* shown in previous work [24] which the values E_D were in the range from 88.42 ± 5.03 kJ mol^{-1} to 142.87 ± 2.75 kJ mol^{-1}.

The values E_D of recombinant endo-inulinases from *A. niger* were higher about 40% (IBM-Endo) and 100% (Endo-IBM) [16] than the values E_D obtained for non-recombinant endo-inulinase *A. niger* equals 150.89 ± 40.72 kJ mol^{-1} calculated from Eq. (4).

The deactivation energy E_D of the commercial inulinase of *Aspergillus niger* (Fructozyme LTM, Novozymes, Denmark) for marked k_D Ricca et al. [22] was calculated from the Arrhenius equation as equal to 313.47 kJ mol^{-1}.

The values of deactivation energies E_D for recombinant endo-inulinases of various origins were obtained ine the range from 168.58 ± 7.78 kJ mol^{-1} to 295.80 ± 18.78 kJ mol^{-1} (Table 2).

In most of analyzed cases, the recombinant endo-inulinases *A. niger* have higher values of E_D, so they are more thermally stable than, that no-recombinant endo-inulinases *A. niger* [24].

When the E_D/E relationship is known for an *A. niger* and of various origins recombinant endo-inulinases, one can choose the enzyme with the highest thermal stability. The higher the E_D/E value of a given recombinant endo-inulinases, the more stable the enzyme is. The better activity, thermostability was obtained for the C-terminal fusion (Endo-IBM) recombinant endo-inulinase *A. niger* [16]. In this work, the value of the E_D/E is equal to 13.67 but also this value is almost 2.5 yields higher than form for the N-terminal fusion (IBM-Endo) recombinant endo-inulinase *A. niger* [16]. Among recombinant endo-inulinase of various origins, the most stable is *Pseudomonas mucidolens* into *S. cerevisiae* [19] with the value E_D/E equals 10.87, but with low value T_{opt} equals 319.57 ± 0.57 K.

The values of optimum temperature T_{opt}

The values of optimum temperature T_{opt} for recombinant endo-inulinase from *A. niger* were determined in the range from 328.67 ± 1.32 K to 335.94 ± 1.22 K [13–17]. This values T_{opt} are higher even 18 °C than determined for no-recombinant endo-inulinases from *A. niger* [24]. The estimated values of the optimum temperatures T_{opt} for recombinant endo-inulinase of various origins [18–20] were found to be in the range from 319.41 ± 0.85 K to 338.53 ± 0.45 K.

Based on the analyses, it was observed that recombinant endo-inulinase *A. niger* at Fig. 1, Fig. 3, Fig. 4 and recombinant endo-inulinase *E. coli* at Fig. 9, are characterized by the determined optimal temperature values T_{opt}, correspond to the optimum temperature determined experimentally. Additionally, the statistical parameters (*F*–Fisher test values and probability value *P*) for these measurements are the highest. Whereas for recombinant endo-inulinase *Aspergillus niger* (Endo-IBM) at Fig. 5, recombinant endo-inulinase *Yarrowia lipolytica* at Fig. 7 and recombinant endo-inulinase *S. cerevisiae* at Fig. 8, estimated optimum temperatures T_{opt} are about 3.5 °C lower than the values determined experimentally. In the applied method of determining the parameters E_D, T_{opt}, *B* with Eq. (4) all measuring points that contribute to the final T_{opt} result are included.

The highest optimum temperature T_{opt} has been determined for the recombinant endo-inulinase *Penicillium restrictum* A191 in *E. coli* by measurements Puratos [20]. That value T_{opt} is most highest than those described in the available literature. Higher values T_{opt} obtained for recombinant endo-inulinases *Aspergillus niger*. The maximal activities of recombinant endo-inulinase *A. niger* F4 by expressed *Bacillus macerans* CFC1 [16] and *A. niger* CICIM F0620 in *Pichia pastoris* [17] are at temperatures 335.94 ± 1.22 K and 334.81 ± 1.08 K, respectively.

Conclusions

The values of parameters the activation energies *E* and the deactivation energies E_D and the optimum temperatures T_{opt} of inulin hydrolysis by recombinant endo-inulinases from *Aspergillus niger* and from various origins were determined. The differences in the estimated values of the activation energy *E* are equal to about 40 kJ mol^{-1} and about 70 kJ mol^{-1} for recombinant endo-inulinases from *A. niger* and from various origins, respectively. The differences in the estimated values of deactivation energy E_D are equal to about 160 kJ mol^{-1} and about 130 kJ mol^{-1} for recombinant endo-inulinase *A. niger* and from various origins. For the optimum temperatures T_{opt}, the difference between the obtained values is about 7.5 °C and about 19 °C for recombinant endo-inulinases from *A. niger* and from various origins. The recombinant endo-inulinases *A. niger* were characterized by the higher values of optimum temperature T_{opt} as well as higher values of energy E_D compare to the values of recombinant endo-inulinases from various origins and also no-recombinant endo-inulinases *A. niger*. Knowledge of parameters *E*, E_D, T_{opt} for recombinant endo-inulinase from a various origin of yeast and bacteria strains will allow design, modeling and optimization of inulin hydrolysis.

Declarations

Conflict of interest The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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