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The starch hydrolysis by *α*-amylase *Bacillus* spp.: an estimation of the optimum temperatures, the activation and deactivation energies

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Abstract
Amylases have potential application, inter alia, in processes with starch hydrolysis. The present paper reports the estimation of the optimum temperatures, the activation and deactivation energies of starch hydrolysis by *α*-amylase *Bacillus* spp. The literature activity of *α*-amylase *Bacillus* spp. versus temperature curves was analyzed. The mathematical model presented the activity of *α*-amylase *Bacillus* spp. and the starch hydrolysis. Both the starch hydrolysis and the deactivation process of *α*-amylase were analyzed by the first-order equations according to the enzyme concentration. Determined optimum temperatures T_{opt} were in the range from 323.67 ± 1.48 K to 354.00 ± 2.27 K, activation energies E_r were in the range from 18.01 ± 7.22 kJ mol^{-1} to 102.85 ± 20.53 kJ mol^{-1}, and the values of deactivation energies E_d were in the range from 79.76 ± 8.77 kJ mol^{-1} to 162.85 ± 32.23 kJ mol^{-1}. The present study is related to the starch hydrolysis by *α*-amylase *Bacillus* spp. The obtained results might find application in the industry hydrolysis of starch.

Keywords Deactivation energy · Activation energy · Optimum temperature · *α*-amylase *Bacillus* spp

Introduction

Amylases are hydrolytic enzymes that hydrolyze the glycosidic bonds present in starch molecules and produce dextrins and oligosaccharides [1]. Generally, amylases are classified into the following three subtypes: *α*, *β* and *γ*. The enzyme *α* -amylase (E.C. 3.2.1.1) catalyzes the hydrolysis (biodegradation) of *α*-1,4-glycosidic bonds present in starch, glycogen and other related carbohydrates to low molecular weight products, such as glucose, maltose and maltotriose [2]. The optimum pH for *α*-amylase is found to be 7.0. *β*-Amylase (EC 3.2.1.2) catalyzes the hydrolysis of the non-reducing *α* -1,4-glycosidic linkages to yield successive maltose units. *β*-Amylase has a maximally active a range of 4.0–5.5 pH. In turn, *γ*-amylase (EC 3.2.1.3) catalyzes the hydrolysis *α* -1,6-glycosidic bonds, unlike other amylases and also hydrolyze the amylose and amylopectin non-reducing *α*-1,4-glycosidic linkages and produces glucose [1, 2]. The optimum pH of *γ*-amylase is equal to 3 [3]. *α*-Amylase will be discussed later in this paper.

α-Amylase can be isolated from microorganisms, plants and animals [4] and has extensive applications in industry in textiles, detergent, fermentation and the food industry. Moreover, it is used in baking, brewing [4–13] and medicine [14–16]. The activity of *α*-amylase is important in each of the mentioned branches and particular in the industrial hydrolysis of starch by *α*-amylase.

Bacillus spp. are a source of enzymes characterized by wide availability, work safety and ease of cultivation, obtaining an economic enzyme in production. Among the bacterial species, the most widely used source for commercial production of *α*-amylases are *B. amyloliquefaciens* and *B. licheniformis*. It has been reported that these *α*-amylases are stable at extreme thermal conditions [1].

An important point which should be noted is that with the discovery of new bacterial strains, it is necessary to determine the optimum temperature T_{opt}, the activation energy E_r and the deactivation energy E_d for *α*-amylase *Bacillus* spp. Importantly, based on literature review, it can be concluded

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that activation energy E_r and the deactivation energy E_d for α-amylase *Bacillus* spp. were presented in previous studies [17–19] for α-amylase *Bacillus licheniformis*.

Starch hydrolysis by α-amylase *Bacillus* spp. is usually carried out at optimum temperatures higher than 50 °C [5–12] and even 100 °C [20, 21]; thus, a significant deactivation of the enzyme may occur.

The study aimed to determine parameters of the optimum temperatures T_{opt}, the activation energies E_r and the deactivation energies E_d of starch hydrolysis by α-amylase *Bacillus* spp. such as α-amylases from *B. subtilis*, *B. amyloliquefaciens* and *B. licheniformis*. The obtained values can be used in industrial design process and modeling of starch hydrolysis.

Methods

Measurement of α-amylase *Bacillus* spp. activity

Literature data [5–12] for α-amylase *Bacillus* spp. from different origins were analyzed. α-Amylase *Bacillus* spp. activity is most often determined by Bernfeld [5, 7–10, 12, 22]. According to this method determination of the α-amylase activity, the reaction mixture containing 1% (v/v) starch and buffer solutions was prepared. After adding the appropriate amount of enzyme, the reaction solution should be incubated for different times (min) at 90 °C. The reaction was stopped by the addition of a 3, 5-dinitrosalicylate acid (DNS). During the breakdown of starch by α-amylase, maltose is formed, the amount of which was determined spectrophotometrically. The unit of α-amylase was defined as the amount of enzyme which produced 1 μmol of reducing sugar as glucose in 1 min under specified conditions. The quantity of reducing sugar was measured spectrophotometrically at 540 nm. Also to determination of activity α-amylase is used Fuwa's colorimetric method [23] of iodine-starch color reaction [6, 11]. One unit of α-amylase activity was defined as the amount of enzyme that decreased the absorbance of 660 nm in 10 min.

Parameters: Optimum temperatures T_{opt}, activation energies E_r and the deactivation energies E_d of starch hydrolysis by α-amylase *Bacillus* spp. were estimated from the activity change curves at temperature effect [5–12].

α-amylase *Bacillus* spp. activity versus temperature

The values of activation energies E_r and E_d can be determined of the dependence of the logarithm of the reaction rate ($\ln v$) on the reciprocal of temperature ($1/T$), the so-called Arrhenius dependence [17, 18]. It has been shown that the determined values of E_r and E_d by application of the Arrhenius relationship is burdened with an error [19, 24–26].

When studying the starch hydrolysis by α-amylase *Bacillus* spp., it is assumed that the change a substrate concentration C_S during reaction time t and change dimensionless activity a [17, 19] are described by the first-order equations

$$\frac{dC_S}{dt} = -k_r C_E \tag{1}$$

$$\frac{da}{dt} = -k_d a \tag{2}$$

where k_r, k_d are the enzymatic reaction and deactivation process kinetic constants, respectively (min^{-1}) and C_E is the concentration of the active enzyme (M). Dimensionless activity of enzyme a is expressed by the equation

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

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

Considering equation describing the dimensionless activity of enzyme a and Eq. (1) in Eq. (2), it was obtained

$$\frac{dC_S}{dt} = -k_r C_{E0} \exp\left(-k_d t\right) \tag{4}$$

Kinetic constants k_r and k_d are dependent on temperature T according to the Arrhenius equations in general form

$$k = k_{i0} \exp\left(-\frac{E_i}{RT}\right) \tag{5}$$

where i is equal to r or d, depending on whether the enzymatic reaction or the deactivation process is analyzed, E_r is the activation energy for the enzymatic reaction (kJ mol^{-1}), while E_d is the activation energy of the deactivation process (kJ mol^{-1}), R is the gas constant equals (8.315 J mol^{-1} K^{-1}, and T is the temperature (K).

Substituting Eq. (5) into Eq. (4) leads to

$$\frac{dC_S}{dt} = -k_{r0} \exp\left(-\frac{E_r}{RT}\right) C_{E0} \exp\left(-k_{d0} \exp\left(-\frac{E_d}{RT}\right) t\right) \tag{6}$$

Integration of Eq. (6) leads to the following relation

$$\int_0^{C_S} dC_S = -k_{r0} \exp\left(-\frac{E_r}{RT}\right) C_{E0} \int_0^t \exp\left(-k_{d0} \exp\left(-\frac{E_d}{RT}\right) t\right) dt \tag{7}$$

for the bonds condition $C_S\,(t = 0) = 0$ and $C_S\,(t) = C_S$.

The substrate concentration C_S is calculated after integrating Eq. (7)

$$C_S = -\frac{k_{r0}}{k_{d0}} \exp\left(\frac{E_d - E_r}{RT}\right) C_{E0} \left(\exp\left(-k_{d0} \exp\left(-\frac{E_d}{RT}\right) t\right) - 1\right) \tag{8}$$

It is well known that 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 dimensionless enzyme activity a can be described as follows:

$$a(T) = \frac{C_S(T)}{C_S(T_{opt})} \tag{9}$$

Dependence of the change in the dimensionless activity of the enzyme versus the temperature measurement T is presented in the following

$$a(T) = \exp\left(\frac{(T_{opt} - T)(E_d - E_r)}{RTT_{opt}}\right)\frac{\left(\exp\left(-k_{d0}\exp\left(\frac{E_d}{RT}\right)t\right) - 1\right)}{\left(\exp\left(-k_{d0}\exp\left(\frac{E_d}{RT_{opt}}\right)t\right) - 1\right)} \tag{10}$$

The maximum activity is determined by calculate the necessary condition, i.e.

$$\frac{da(T)}{dT} = 0 \tag{11}$$

Considering account the described assumption Eq. (11), the effect of temperature on the dimensionless activity a of the enzymes describes the equation:

$$a = \frac{\exp\left(\frac{(T_{opt}-T)E_d\beta}{RTT_{opt}(\exp\beta-1)}\right)\left(1 - \exp\left(-\beta\exp\left(\frac{(T-T_{opt})E_d}{RTT_{opt}}\right)\right)\right)}{1 - \exp(-\beta)} \tag{12}$$

where T_{opt} is the optimum temperature for α-amylase *Bacillus* spp. and dimensionless parameter β is determined by the equation

$$\beta = t_a k_{d0} \exp\left(-\frac{E_d}{RT_{opt}}\right) = t_a k_d(T_{opt}) \tag{13}$$

where t_a is time of assay α-amylase *Bacillus* spp. activity (min).

The transformation of Eq. (13) allows to determine the value of the parameter deactivation constant k_d at optimum temperature T_{opt}

$$k_d(T_{opt}) = \frac{\beta}{t_a}. \tag{14}$$

With the values of the dimensionless parameter β and the deactivation process energy E_d, it is possible to calculate the value of the activation energy E_r with the following relationship

$$E_r = E_d - \frac{\beta E_d}{\exp\beta - 1}. \tag{15}$$

Based on Eq. (12), the T_{opt}, β and E_d parameters were estimated by the Levenberg−−Marquardt procedure [26–30], calculated in SigmaPlot 14.5 the minimum sum of squared errors SSE defined by the equation

$$\mathrm{SSE}(T_{opt}, E_d, \beta) = \sum_{i=0}^{n} \frac{1}{(a_{exp})^2}\left(a_{exp} - a(T_{opt}, E_d, \beta)\right)^2 = \min \tag{16}$$

where a_{exp} is α-amylase *Bacillus* spp. dimensionless activity determined experimentally and $a(T_{opt}, E_d, \beta)$ is α-amylase *Bacillus* spp. activity calculated from Eq. (12).

Equations from Eq. (12) to Eq. (15) were used to determine optimum temperatures and the activation energies inter alia of starch hydrolysis by α-amylase *Bacillus licheniformis* [23], α-amylase from porcine pancreas [27], inulin hydrolysis by exo-inulinases *Aspergillus niger* [28] and recombinant exo-inulinases [29] and olive oil hydrolysis by porcine pancreas lipase [26].

Results

Literature data [5–12] for α-amylase *Bacillus* spp. from different origins were analyzed. Table 1 presents the conditions for measuring α-amylase activity during the hydrolysis of starch with the various buffer pH and the various measurement times [5–12]. The activity of α-amylase *Bacillus* spp. at a specified temperature was determined in the pH range from 6.5 to 7.2.

α-Amylase *Bacillus* sp. B-10 used by Singh et al. [5] was purified from bacterial strains isolated from soil samples. These were collected from different agricultural farms, with

Table 1 Measurement conditions of α-amylase *Bacillus* spp. activity used to starch hydrolysis

Source α-amylase *Bacillus* spp.	pH phosphate buffer	*t*/min	References
Bacillus sp. B-10	7.2	30	[5]
Bacillus sp. PS-7	6.5	10[a]	[6]
B. subtilis	7.0	3	[7]
B. amyloliquifaciens BH072	7.0	3	[8]
B. amyloliquifaciens TSWK1 − 1	7.0	20	[9]
B. licheniformis SKB4	6.5	5	[10]
B. licheniformis AI20	7.0	10[a]	[11]
Bacillus sp. 12B	7.0	30	[12]

[t] *t* is the reaction time of α-amylase *Bacillus* spp. activity

[a] method of iodine-starch (λ equals 660 nm)

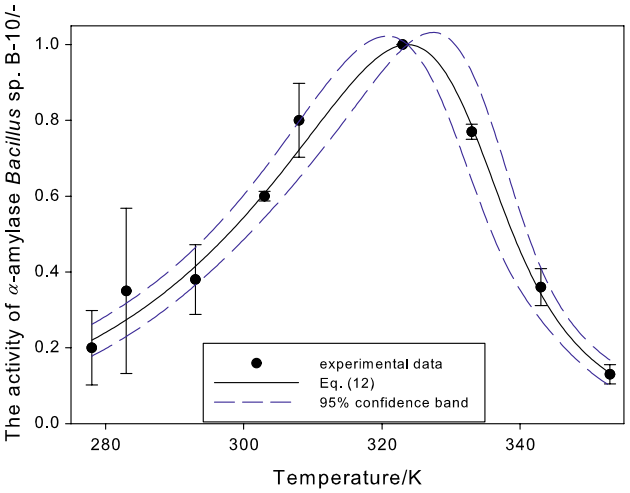

Fig. 1 The activity of α-amylase *Bacillus* sp. B-10 by Singh et al. [5]

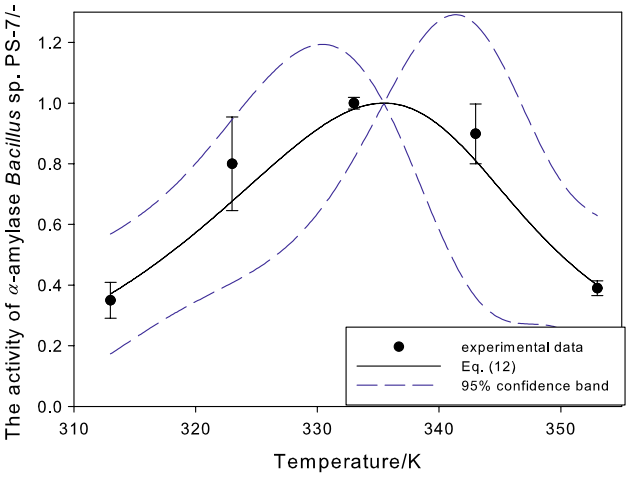

Fig. 2 The activity of α-amylase *Bacillus* sp. PS-7 by Sadhi et al. [6]

kitchen waste and compost from Bijnor (U.P.), India, and which were mixed properly. The next amylolytic bacterial strains named *Bacillus* sp. PS-7 was isolated from a hot spring of Manikaran, HP, India [6]. *B. subtilis* isolated from fermented banana waste was selected by Shula and Kar for α-amylase production [7]. *B. amyloliquefaciens* BH072 was isolated from honey [8]. *B. amyloliquifaciens* TSWK1 − 1 was collected from the hot water reservoir at Tulsi Shyam, Gujarat, India [9]. α-Amylase *B. licheniformis* SKB4 studied by Samanta et al. [10] was purified from bacterial strains isolated from soil isolate. The bacterial strain used in the work Abdel-Fattah et al. [11] named *B. licheniformis* AI20 was isolated from garden soil samples collected from Indonesia. In turn, α-amylase *Bacillus* sp. 12B presented by Božić et al. [12] was isolated from wild-type strains of *Bacillus* sp. from some regions of Serbia.

Based on experimental data showing the change in the activity of α-amylase *Bacillus* spp. [5–12] in function of temperature, values of the optimum temperatures T_{opt}, deactivation energies E_d and β parameters were determined from Eq. (12). Figures 1–8 present the experimental data of α-amylase activity as a function of temperature and the activity curves plotted by Eq. (12) for the values estimated parameters T_{opt}, E_d and β presented in Table 2.

Table 2 presents the value of parameters T_{opt}, E_d and β for α-amylase *Bacillus* spp. by the increasing value of optimum temperatures. The next step was to calculate deactivation constants k_d at optimum temperature T_{opt} and the activation energy parameter E_r values based on Eq. (14) and Eq. (15), respectively. The calculated $k_d(T_{opt})$ and E_r values are placed in Table 2.

Table 3 presents statistical data calculated for the estimated the parameters of α-amylase *Bacillus* spp. High values of regression coefficient (R^2 above 0.95) in most of the

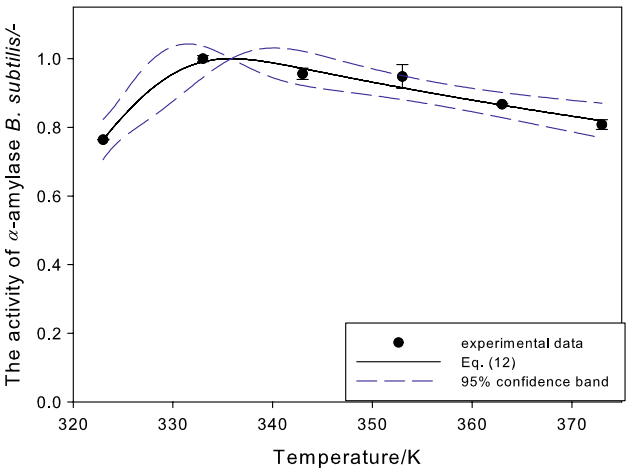

Fig. 3 The activity of α-amylase *Bacillus subtilis* by Shula and Kar [7]

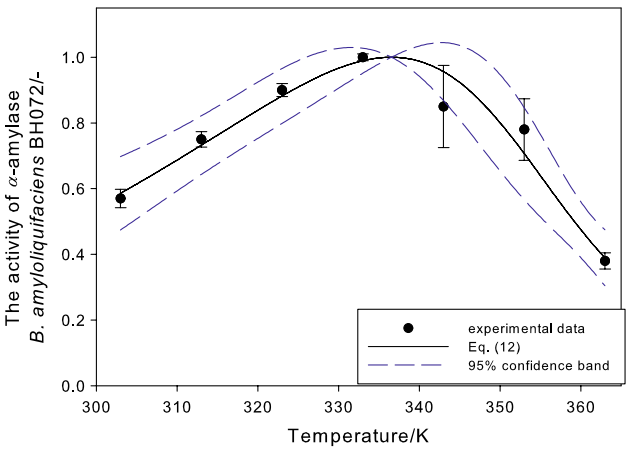

Fig. 4 The activity of α-amylase *Bacillus amyloliquifaciens* BH072 by Du et al. [8]

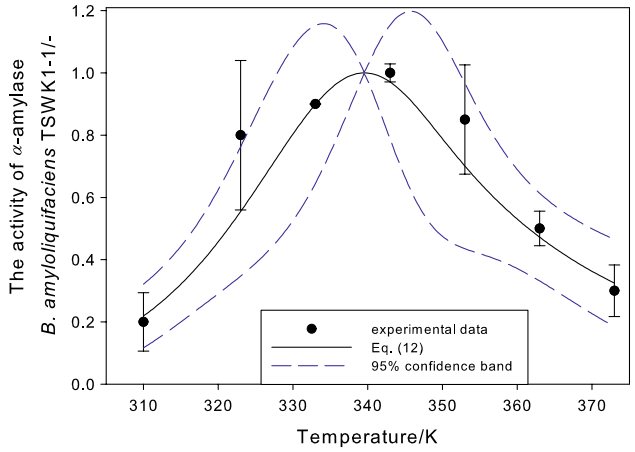

Fig. 5 The activity of α-amylase *Bacillus amyloliquifaciens* TSWK1 − 1 by Kikani and Singh [9]

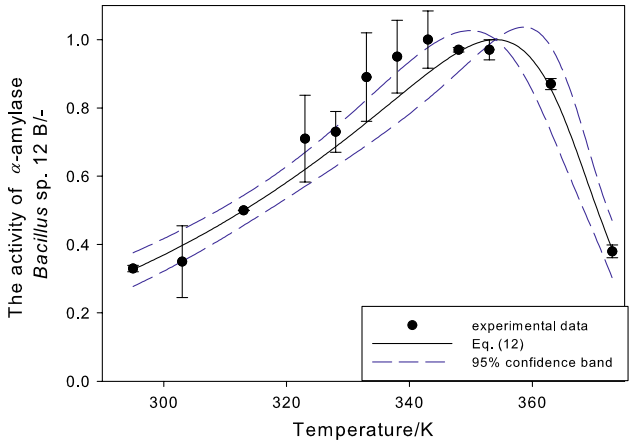

Fig. 8 The activity of α-amylase *Bacillus* sp. 12 B by Božić et al. [12]

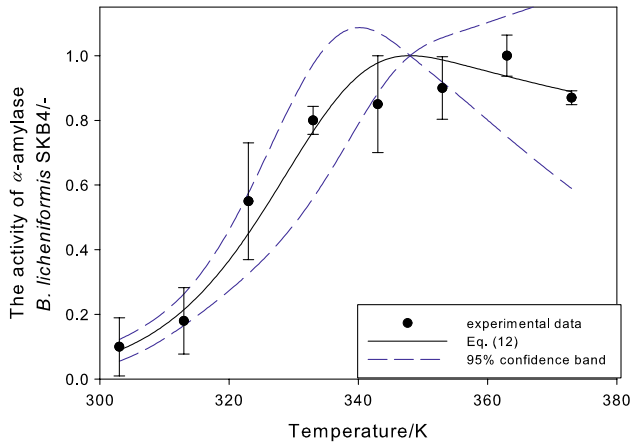

Fig. 6 The activity of α-amylase *Bacillus licheniformis* SKB4 by Samanta et al. [10]

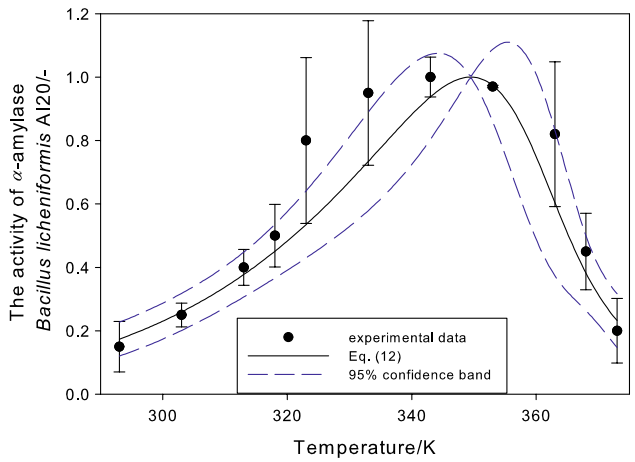

Fig. 7 The activity of α-amylase *Bacillus licheniformis* AI20 by Abdel-Fattah et al. [11]

analyzed cases were noted. The sum of squared errors SSE below 0.20 was obtained. The *F*-Fisher test values and low probability value were calculated. The statistical data confirmed the accuracy of the estimated values parameters.

Additionally, Figs. 1–8 present standard deviation errors for experimental data with the 95% confidence bands. The statistical data confirmed that the application Eq. (12) when determining parameters is justified.

Discussion

This work aimed to identify the values of the activation energy E_r and the deactivation energy E_d and the optimum temperature of starch hydrolysis by T_{opt} of starch hydrolysis α-amylase *Bacillus* spp. based on the literature activity versus temperature. The obtained values can be used in works focused on industrial designed and modeling of the process starch hydrolysis by α-amylase *Bacillus* spp.

The values of optimum temperature T_{opt}

The determined values of the optimum temperature T_{opt} of starch hydrolysis by α-amylase *Bacillus* spp. were in the range from 323.67 ± 1.48 K to 354.00 ± 2.27 K (Table 2) and are different by about thirty degrees. The highest value of T_{opt} was calculated for α-amylase *Bacillus* sp. 12B, with a long 30 min measurement time. It is worth noting that in an earlier work [19], a T_{opt} of starch hydrolysis (pH 8.9) by α -amylase *Bacillus licheniformis* EMS-6 was determined and equal to 339.76 ± 0.95 K for the measurements presented by Haq et al. [17]. The presented values of the optimum temperature T_{opt} in Table 2 are acceptable, when we know that optimum temperatures could be even 100 °C [20, 21].

Table 2 The value of parameters for α-amylase *Bacillus* spp

Fig.	t /min	T_{opt} /K	β	E_d /kJ mol^{-1}	$k_d(T_{opt})$ /min^{-1}	E_r /kJ mol^{-1}	Ref.
1	30	323.67 ± 1.48	0.51 ± 0.08	123.91 ± 9.04	0.02 ± 0.003	28.83 ± 6.19	[5]
2	10	335.47 ± 2.57	1.01 ± 0.44	132.94 ± 16.86	0.10 ± 0.04	56.02 ± 25.99	[6]
3	3	335.85 ± 2.46	4.11 ± 0.48	88.11 ± 19.82	1.37 ± 0.16	102.85 ± 20.53	[7]
4	3	336.53 ± 2.40	0.41 ± 0.16	96.04 ± 16.92	0.14 ± 0.05	18.35 ± 8.87	[8]
5	20	339.57 ± 3.81	1.65 ± 0.44	107.62 ± 7.32	0.08 ± 0.02	65.41 ± 15.62	[9]
6	5	348.02 ± 4.48	4.01 ± 1.36	79.76 ± 8.77	0.80 ± 0.27	73.85 ± 12.45	[10]
7	10	349.48 ± 3.36	0.44 ± 0.15	144.20 ± 30.24	0.04 ± 0.02	29.41 ± 13.68	[11]
8	30	354.00 ± 2.27	0.23 ± 0.06	162.85 ± 32.23	0.01 ± 0.002	18.01 ± 7.22	[12]

T_{opt} is the temperature at which α-amylase shows maximum activity, β is dimensionless parameter determines Eq. (13), E_d is the deactivation energy α-amylase *Bacillus* spp., $k_d(T_{opt})$ deactivation constant at optimum temperature T_{opt}, E_r is the activation energy of starch hydrolysis by α-amylase *Bacillus* spp

Table 3 The statistical data for α-amylase *Bacillus* spp.

Fig.	SSE	R^2	p			F	P	Ref.
			E_d	T_{opt}	β			
1	0.1304	0.9657	< 0.0001	< 0.0001	0.0009	84.58	< 0.0001	[5]
2	0.1378	0.9547	0.0157	< 0.0001	0.1473	21.07	0.0453	[6]
3	0.0242	0.9961	0.0212	< 0.0001	0.0035	47.01	0.0054	[7]
4	0.0820	0.9887	0.0048	< 0.0001	0.0661	156.58	0.0012	[8]
5	0.1902	0.9316	0.0001	< 0.0001	0.0199	27.26	0.0047	[9]
6	0.1548	0.9716	0.0003	< 0.0001	0.0317	85.81	< 0.0001	[10]
7	0.2009	0.9226	0.0014	< 0.0001	0.0213	41.76	< 0.0001	[11]
8	0.0986	0.9573	0.0007	< 0.0001	0.0079	101.00	0.0001	[12]

SSE 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 β; T_{opt} is the temperature at which α-amylase shows maximum activity, β is dimensionless parameter determines Eq. (13), E_d is the deactivation energy α-amylase *Bacillus* spp

The values of activation energy E_r

Results obtained in this work have demonstrated that the values of the activation energy E_r of starch hydrolysis by α-amylase *Bacillus* spp. are in the range from 18.01 ± 7.22 kJ mol^{-1} to 102.85 ± 20.53 kJ mol^{-1}. It should be noted that the lowest value was obtained for the α-amylase *Bacillus* sp. 12B, with a long 30 min measurement time [12]. This fact, together with a high T_{opt} value, proves the very good parameters of α-amylase *Bacillus* sp. 12B.

The value of the activation energy E_r determined in an earlier paper [19] for the hydrolysis of starch by an α-amylase *Bacillus licheniformis* EMS-6 was within the range of values reported in Table 2 and amounted to 27.16 ± 6.89 kJ mol^{-1}. In turn, the value of the activation energy E_r determined by Samanta et. al. [10] for the hydrolysis of starch (pH 8.9) by α-amylase *Bacillus* sp. was 31.53 kJ mol^{-1} and this value is over two twice lower than the calculated value from Eq. (15) and shown in Table 2. According to the calculations for the measurement of Božić et al. [12], the energy activation value E_r for *Bacillus* sp. 12B was four times lower compared to the E_r values obtained for α-amylase *B. licheniformis* SKB4 by Samanta et al. [10]. The observed difference may be due to the different times in which the α-amylase activity is determined.

Comparing the value of activation energy E_r for α-amylase *Bacillus* spp. of different origins for the starch hydrolysis time and equal to 30 minutes, the value of E_r for α-amylase *Bacillus* sp. B-10 is higher about 60% than the value of E_r for α-amylase *Bacillus* sp. 12B. On the other hand, when comparing the values of E_r for α-amylase *B. licheniformis* of different origins, the value of E_r for α-amylase *B. licheniformis* AI20 [11] is lower about 60% than the value of E_r for α-amylase *B. licheniformis* SKB4 [10].

The values of deactivation energy E_d

The obtained values of the deactivation energy were in the range from 79.76 ± 8.77 kJ mol^{-1} to 162.85 ± 32.23 kJ mol^{-1}

(Table 2). Short measuring times result in lower E_d values. In an earlier work [19], the E_d value of hydrolysis of starch by α-amylase *Bacillus licheniformis* EMS-6 was found as equal to 143.54 ± 13.31 kJ mol^{-1}.

The difference in the obtained the activation energy of the deactivation process E_d can be used by the different times in which the α-amylase activity is determined.

Comparing the value of deactivation energy E_d for α -amylase *Bacillus* spp. of different origins and thus the starch hydrolysis time of 30 minutes, the value of E_d for α -amylase *Bacillus* sp. B-10 is lower about 30% than the value of E_d for α-amylase *Bacillus* sp. 12B. On the other hand, when comparing the values of E_d for α-amylase *B. licheniformis* of different origins, the value of E_d for α-amylase *B. licheniformis* AI20 [11] is higher about 40% than the value of E_d for α-amylase *B. licheniformis* SKB4 [10]. The reason for the differences in the obtained values E_d may be due to the longer measurement time for *B. licheniformis* AI20 [11], and then process deactivation was apparent.

The values of deactivation constant $k_d(T_{opt})$

The calculated from Eq. (14) values of the deactivation constant k_d at optimum temperature T_{opt} were in the range from 0.01 ± 0.003 min^{-1} to 1.37 ± 0.16 min^{-1} (Table 2). The highest $k_d(T_{opt})$ value, proving the thermostability of α-amylase, was obtained for α-amylase *Bacillus subtilis* at a temperature equal to 335.85 ± 2.46 K, while the lowest $k_d(T_{opt})$ value was obtained for α-amylase *Bacillus* sp. 12B at a temperature equal to 354.00 ± 2.27 K.

The knowledge the values $k_d(T_{opt})$ and transform Eq. (5) allows to calculate the values of k_{d0}.

Conclusions

The study aimed to identify a parameter for α-amylase *Bacillus* spp., which has never been determined by other researchers before, i.e., the energy deactivation E_d. Additionally, the parameters of the optimum temperatures T_{opt} and activation energies E_r of starch hydrolysis by α-amylase from the different origins of *Bacillus* spp. the family were determined.

The lower deactivation energy values E_d were obtained for those α-amylases for which the measurement time was shorter, i.e., up to 10 minutes. The exception is amylase α -amylase *Bacillus* sp. 12B. Also, shorter measurement times resulted in higher values of T_{opt} in α-amylases *Bacillus* from a given genus.

The differences in the obtained values E_r, E_d and T_{opt} are, above all, different origins of *Bacillus* spp. The noted differences in values of parameters can be caused by the various duration of the α-amylase *Bacillus* spp. activity assay.

To sum up, it should be pointed out that the obtained values of the E_r, E_d and T_{opt} can be used to design and optimize starch hydrolysis by α-amylase *Bacillus* spp. in the industry where the saccharification of the processed starch was used.

Authors' contributions JM was involved in the conception and design of the study. JM and JL were involved in the review literature. JM and JL were involved in analysis and interpretation of data. JM was involved in drafting the article. JM was involved in the final approval of the version to be submitted. All authors read and approved the final manuscript.

Declarations

Conflict of interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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