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ISOLATION OF α-AMYLASE FROM ACTIVATED SLUDGE, ITS TREATMENT AND SOME PROPERTIES

The activities of amylase, proteinase, lipase and phosphatase have been investigated in activated sludge from the wastewater treatment plant (Ciernie, Wałbrzych district). It has been stated that the sludge is characterized by a relatively high activity of amylase. An attempt has been made to isolate this enzyme from the sludge. To this end the previously homogenized sludge was fractioned with ammonia sulphate, dialized to calcium acetate and fractioned again with acetone. Finally, column chromatography was applied using molecular sieves. This method gave two separate peaks corresponding to different activities of amylase in acid (pH = 5) and neutral (pH = 7.2) media. It has been stated that amylase acting in acid medium is obtained in the form of crystals which are of proteine nature since the maximum absorption in UV occurs at 275 nm.

1. INTRODUCTION

α-amylase catalyzes hydrolysis of α-1, 4-glycoside bonds in α-glucanes, such as amylase, amylopectines and glycogens. α-amylase is an endoenzyme, i.e. it hydrolyses inner α-1,4 glycoside bond in a polysaccharide molecule into maltose and maltodecstrines. This enzyme is present in animals, plants and bacteria. This of bacterial origin has a relatively most numerous literature [3], [4], [11], [14], [20], [24], [25].

The so far conducted investigations on bacterial amylases have been aimed at growing bacterial strains characterized by two main features: a high thermoresistivity and a high yield of the enzyme since then its production becomes

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profitable. There are already known some strains, the amylases of which withstand successfully the temperatures higher than 363 K. Some efforts have also been made to reveal the structure of amylase (insufficiently known yet) which is expected to provide with a clude to its much valuable properties, i.e. resistivity to high temperatures and to proteases. Microbes produce two kinds of amylases, i.e. the liquifying and saccharifying ones [25]. The first amylase detaches from its non-reducing end maltooligosaccharides (mainly the maltohexol and maltopentysol units), whereas the second one detaches maltotriosyl units.

According to the newest investigations, bacterial α-amylases occur in two forms: of zinc containing tetramer of a molecular weight of 9 600 daltons [24], and of a diamer of a molecular weight of 48 000 daltons [11], [14]. MITCHEL et al. [20] have observed the reduction of molecular weight to 24 000 daltons in 6 M guanidine hydrochloride and this seems to be the smallest subunit of the enzyme molecule. ROBYT et al. [24] have stated that the tetramer is very stable in diluted solution and it is the most active form of the enzyme.

Many papers published recently indicate more and more common utilization of enzymes in wastewater treatment technology. OVERBACK et al. [22] and TEUBER [26] have shown that the degradation of some organic compounds present in wastewater may be substantially accelerated by addition of isolated enzymes. According to the author's own investigations [12], [13] as well as those of other authors, enzymes (amylase and proteinase) introduced into activated sludge markedly accelerate the degradation of starch and proteins contained in food industry wastewater. MÜNNECKE [21] used immobilized enzymes to prevent the environment pollution and to remove pesticide residue. Enzymatic preparation, called a "drain cleaner" and used for liquification of sewerage congestions, has been produced since several years by the Miles Chemical Co. This preparation contains not only aerobic and anaerobic bacteria but also three enzymes (amylase, proteinase and lipase) and is applied in the form of water solution.

Amylase finds also its applications in food industry (production of glucose and starch syrups), in breweries (reduction of the amounts of maltose) and in textile industry (desizing raw cloth). Despite the interest shown by various branches of industry in Poland this enzyme is not produced yet.

The author used activated sludge, commonly applied to biodegradation of wastewater, as a substrate for isolation of α-amylase. Growth of activated sludge biomass during the wastewater treatment is very inconvenient, for the costly disposal of the excessive sludge must be applied. The utilization of activated sludge for the production of the enzyme would therefore solve simultaneously this difficult technological problem and faced by wastewater treatment plants.

The purpose of the study presented in this paper is to find out the method of α-amylase isolation from the excessive activated sludge for its application in the industry or for a possible intensification of the wastewater treatment process.

2. MATERIAL AND METHODS

2.1. CHARACTERISTICS OF THE SUBSTRATE FOR THE ISOLATION OF α-AMYLASE

The substrate for α-amylase isolation was activated sludge taken periodically from the wastewater treatment plant at Ciernie, province Wałbrzych. Its biological activity was estimated by measuring the activities of dehydrogenases and by microscopic examinations. The species of microorganisms found in the activated sludge are described below.

In biocenosis of the activated sludge, apart from bacteria, the dominant species was *Opercularia coarctata* Elap. et Lahm. which in the period of investigations amounted to 80–90 % of the total population of protozoan. This species being characteristic of the rapid reduction of organic compounds indicated that the treatment process ran properly. The presence of *Rhabdostyla ovum* (from 20 to 39 thousand individuals in cm^3 of activated sludge) was stated in the course of the investigations. The following species were, moreover, stated: *Voritcella convellaria* Ndm., *Vorticella campanula* Ehr., *Vorticella microstoma* Ehr. and *Linotus fasciola* Ehr. The mentioned above species were not numerous and grew periodically. The presence of *Zooglea ramigera* Itagis, *Amoeba proteus* Pall. and *Zooflagellate* spec. fiv. was stated sporadically.

Dry weight of the activated sludge ranged within 2000–3000 mg/dm^3, 82 % of which fell to organic dry weight. Index of the activated sludge varied from 60 to 100 cm^3/g. BOD_5 load oscillated from 0,4 to 0.7 g per 1 g of dry weight of activated sludge.

2.2. MEASUREMENT TECHNIQUE

2.2.1. Measurement of the enzyme activity

The activity of α-amylase was determined from the total amount of oligo-, di- and polysaccharides formed during enzymatic hydrolysis of starch. Saccharides were determined using anthrone reagent, according to HEWIT [8]. The mixture, subject to incubation, consisted of 1.0 cm^3 of 0.1 M acetate buffer of pH 5.0 (activity of acid amylase) or 1.0 cm^3 of 0.1 M Veronal buffer of pH 7

(activity of neutral amylase) with the addition of 0.01 M calcium acetate, 0.2 cm^3 of enzyme containing 20 μg of protein and 10 mg of starch as a substrate. The mixture was incubated at 323 K for 1 h. Enzymatic reaction was interrupted by adding 96 % etanol (two volumes of the mixture) due to which the enzyme was denaturated and the excess of non-degradated substrate precipitated. The quantity of enzyme which — under the above conditions — released 1 micromole of saccharides (in terms of glucose) was assumed to be the unit of activity. The specific activity of the enzyme was, however, expressed in micromoles of glucose referred to 1 mg of protein. Protein was determined by the LOWRY method [17] using a crystalline albumiane of bovine serum (Koch–Light product) as a standard.

2.2.2. Paper chromatography

The products of enzymatic hydrolysis of starch were identified by paper chromatography, using Whatman No. 3 filter paper and a set of solvents composed of n-propanol, ethyle acetate and water (6:1:3) [15]. The separation was conducted by ascending technique during 24 h, chromatograms were developed by basic solution of silver nitrate.

2.2.3. Electrophoresis in polyacrylamid gel

Electrophoresis was conducted in 14 cm long glass pipes of diameter 3 mm. Gel was prepared according to the method of DAVIS [5]. Solution of the enzyme containing 3 mg of protein was mixed with 40 % solution of saccharose, in such a ratio that the final concentration of protein be 150–200 μg. Then 0.1 cm^3 of this mixture was introduced into each pipe. Electrophoresis, conducted in Tris-glycine buffer of pH 8.3 at 275 K and 40 V/cm, lasted for 3 h. Thereupon gel was removed from the pipes and divided longitudinally in two parts. One part was put in a test-tube, adding amide black, while the second one was cut into 5 mm long fragments and put into test-tubes, adding 0.5 cm^3 of 0.1 M acetic buffer of pH 5.0, 0.2 cm^3 of 1 % solution of starch, 0.1 cm^3 of 0.01 M calcium acetate and one drop of toluene, and finally making up to 2.0 cm^3. Incubation at 323 K lasted for 16 h. The products of enzymatic hydrolysis of starch were identified by using paper chromatography.

2.3. METHODS OF ENZYME ISOLATION AND ITS PURIFICATION

2.3.1. Step I — preparation of the crude extract

Biomass taken from the activated sludge chamber was subject to 2 hour sedimentation. The obtained 5 dm^3 of sediment free from supernatant was suspendent in 0.14 M NaCl in 2:1 ratio. 250 cm^3 portions of the suspension

were homogenized in an Unipan homogenizer, at 10,000–12,000 rpm. Homogenates were left at 275 K for 4 h and then centrifuged for 15 min at 3,000 rpm. The sediment was rejected adding to 1 dm^3 of the supernant 50 cm^3 of 2 M calcium acetate. The pH of the mixture was adjustated to 6.5 by addition of 2 M NaOH and the mixture was left for 2 h at 275 K. Thereupon the mixture was centrifuged at 4,000 rpm for 20 min, the sediment was rejected and pH of the supernatant was reduced to 6.2 by adding 1 M acetic acid. After heating the supernatant at 328 K for 30 min the precipitated protein was centrifuged at 4,000 rpm for 20 min. The sediment was again rejected and supernatant used for further steps of preparation.

2.3.2. Step II — salting out with ammonium sulphate

The supernatant obtained in step I was adjusted to pH 6.5 by means of 1 M NaOH. Then solid ammonium sulphate (400 g per 1 dm^3 of the liquid) was batched at a continuous mechanical stirring. The solution was left at 275 K for 16 h and then centrifuged at 2,500 rpm for 20 min. Supernatant was rejected and the sediment suspended in 0.1 M acetic buffer of pH 5.0.

2.3.3. Step III — dialysis

Suspended deposit obtained in step II was subject to 48 hour dialysis in presence of 0.01 M solution of calcium acetate at 275 K. Fresh solution of calcium acetate was given every 5 h. During dialysis colour substance and some proteins that disturbe further steps of the enzyme purification were precipitated. Dialysate was centrifuged at 3,000 rpm for 10 min. Sediment was rejected.

2.3.4. Step IV — precipitation with acetone

Supernatant obtained in step III was supplied with acetone frozen to 253 K and given in 2:1 volume ratio, left at 275 K for 2 h and then centrifuged at 3,500 rpm for 15 min. Supernatant was rejected and the sediment suspended in 0.01 M calcium acetate.

2.3.5. Step V — column chromatography

The process was conducted in a 2.5×100 cm column filled with sephdex G–100 (40–120 mesh). The column was balanced with 0.1 M acetic buffer of pH 5.0 mixed with 0.1 M calcium acetate. Solution of enzyme containing 50 mg of protein was put on the column. Elution of proteins was conducted using 0.1 acetic buffer of pH 5.0 with the addition of 0.01 M calcium acetate. 5 cm^3 fractions were collected by means of an automatic fraction collector. Elution of protein was controlled spectrophotometrically at 275 nm. At the same time the activity of α-amylase was measured.

2.3.6. Step VI — crystallization

To the mixture of active fractions obtained in step V small amounts of acetone frozen to 253 K was added, crystals appeared after 8 h.

2.3.7. Step VII — recrystallization

Crystals obtained in step VI were separated by centrifugation at 6,000 rpm during 20 min. Supernatant was removed and the crystalls suspended in 0.01 M calcium acetate. pH of the suspension was adjusted to 10.5 by means of 0.02 M calcium hydroxide. Consequently, the crystals were dissolved. Insoluble remnant was separated by centrifugation at 6,000 rpm during 20 min. Sediment was rejected and supernatant adjusted to pH 8.0 by means of 0.1 M acetic acid, left for 5 h at 275 K. Adding afterwards again 0.1 M acetic acid, pH of the enzyme solution was adjusted to 6.0 and the solution was left at 275 K for 2–3 days. Crystals of enzyme were separated by centrifugation at 6,000 rpm for 25 min and five times washed 30 % aqueous solution of acetone. Crystalline preparation of the enzyme obtained in this way was used for further studies on its properties.

2.4. DETERMINING THE EFFECT OF THE SUBSTRATE CONCENTRATION ON THE RATE OF HYDROLYSIS

The effect of the substrate concentration on the rate of hydrolysis was investigated according to the following procedure: The increasing molar concentrations of substrates (starch, glycogen, amylase, amylopectin) were made up to the volume of 9 cm^3 by adding 0.1 M acetic buffer of pH 5.0. The mixture was preincubated at 323 K for 30 min, thereupon 0.1 cm^3 of enzyme (containing 11.2 μg of protein) was added and incubated for 1 h. The reaction was interrupted by the addition of 2 volumes of 96 % ethanol. After centrifugation the total sugar in the supernatant was determined using anthrone reagent.

3. RESULTS AND DISCUSSIONS

The separate steps of the enzyme purification are given in tab. 1. As it follows from the data presented by applying thermal denaturation followed by fractioning with ammonium sulphate, dialysis and precipitation with acetone we have achieved a high degree of enzyme purification — the specific activity of the enzyme being 4.7 enzymatic units. Column chromatography with the application of molecular sieves employed in further steps of purification allowed us to state that in the mixture of proteins being separated there exist two amylases the optimum pH of which are different (fig 1.). One amylase flowing

Table 1

Stages of separation of α-amylase from activated sludge

Etapy wydzielania α-amylazy z osadu czynnego

Stage	Protein content g	Activity		Degree of purification	Efficiency %
		Glucose μmole	Specific μmole/mg of protein		
Raw extract	119.99	9.255	0.077	—	100
Sulphate ammonium fractionation	10.10	5.250	0.519	6.74	56.72
Dialysis	2.40	2.770	1.154	14.98	29.92
Acetone fractionation	0.50	2.350	4.700	61.00	25.39
Chromatographic separation on Sephadex G–100	0.10	1.920	19.200	249.45	20.74
Crystallization	0.05	1.200	24.00	312.00	12.96
Recrystallization	0.03	1.000	33.330	432.85	10.80

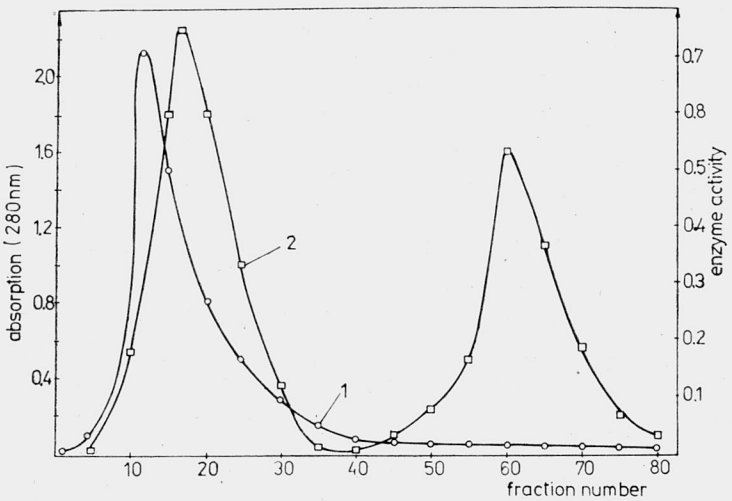

Fig. 1. Chromatographic separation of α-amylase isolated from the activated sludge on Sephadex G–100

1 – protein, *2* – activity

Rys. 1. Rozdział chromatograficzny α-amylazy wydzielonej z osadu czynnego na Sephadex G–100

1 – białko, *2* – aktywność

out of the column in the first separation phase (peak I) actes in acid (pH 5.0), while the second one in neutral (pH 7.0) media.

Active fractions taken from each of the two peaks were collected separately and 0.1 volume of acetone (253 K) per 1 volume of the enzyme was added.

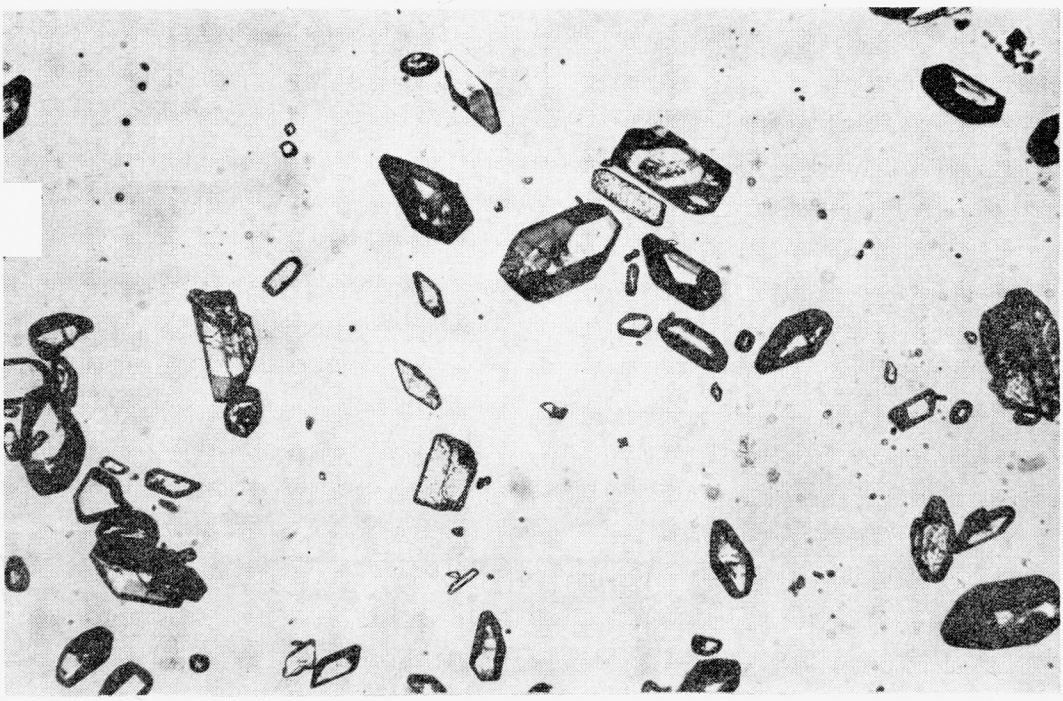

Fig. 2. α-amylase crystals isolated from activated sludge, magn. 200 ×

Rys. 2. Obraz mikroskopowy kryształów α-amylazy wydzielonej z osadu czynnego, powiększenie 200 ×

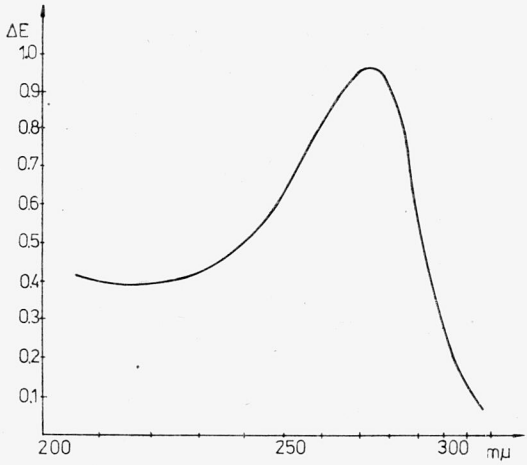

Fig. 3. Spectral analysis of α-amylase crystals isolated from activated sludge. Absorption spectrum od crystals in UV was made at 200–300 nm in spectrophotometer Specord UV VIS (produced in East Germany)

Rys. 3. Analiza widmowa kryształów α-amylazy wydzielonej z osadu czynnego. Spektrum absorpcji w ultrafiolecie kryształów enzymu wykonano w zakresie długości fali 200–300 nm w spektrofotometrze Spekord UV VIS (produkcji NRD)

After 2 days first crystals of enzyme appeared solely in the collected fractions of the first peak (acid amylase). Microscopic picture of crystals is given in fig. 2.

The fact that enzymatic protein has been obtained in crystalline form is of a great importance, having especially in mind that general methods of protein crystallization have not been developed yet. The tests with biuret and Folin–Giocalteau reagents which stated the presence of peptide bonds have confirmed protein nature of the crystals obtained by us. Further evidence has been provided by UV spectrum taken at the wavelengths ranging within 200–300 nm (fig. 3). As it follows from the diagram, the maximum absorption falls to 275 nm, which is characteristic of the proteins of higher contents of aromatic amino acids such as thyrosine, phenylalanine and tryptofan.

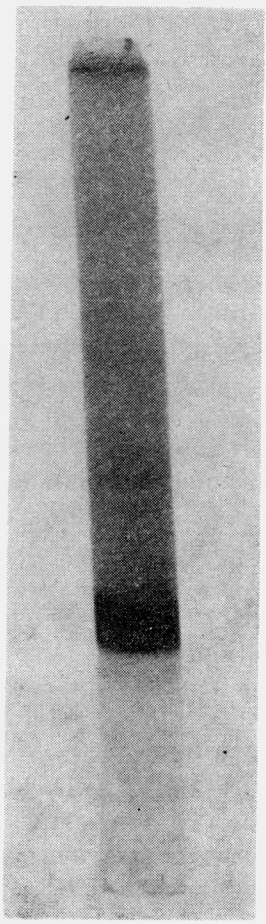

Fig. 4. Electrophoresis of α-amylase isolated from activated sludge in polyacrylamid gel

Rys. 4. Rozdział elektroforetyczny α-amylazy wydzielonej z osadu czynnego w żelu poliakrylamidowym

One of the criteria of the purity of the obtained enzymatic preparation is their homogeneity, which is determined by a number of analytical methods. In our conditions, the obtained preparation has been subject to electrophoretic separation on polyacrylamid gel in Tris-glycine buffer of pH 8.3. Results of electrophoresis are presented in fig. 4. The obtained distinct band of protein migrating toward anode showed the activity of α-amylase.

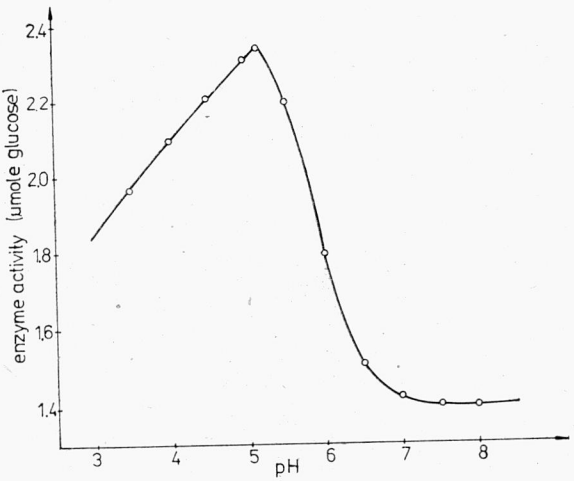

Fig. 5. Effect of pH on amylum hydrolysis by α-amylase isolated from activated sludge using 0.1 M acetate buffer of pH 3.6–5.5 and 0.1 M Veronal buffer of pH 5.6–10

Rys. 5. Wpływ pH na hydrolizę skrobii przez α-amylazę wydzieloną z osadu czynnego. Używano 0.1 M bufor octanowy o pH 3,6–5,5 oraz bufor weronalowy o pH 5,6–10

The effect of pH on the activity of α-amylase is shown in fig. 5. The enzyme reveals the highest activity in acid environment (pH 5). The fact that only one peak of activity has been obtained allows us to infer that the cystalline protein separated by us shows only one catalytic activity characteristic of α-amylase.

As it is well known bacterial amylases are characterized by relatively high tolerance to temperature variations [2]. In our investigations we have stated that 323 K is the optimal temperature for catalytic processes conducted by α-amylase from activated sludge (fig. 6).

Investigations of kinetics of hydrolysis of various substrates, performed by the methods presented in section 2.4, have allowed us to determine the following parameters of the kinetic equation of reaction (referred to 1 mg of the enzymatic protein):

for glycogen

$$K_m = 0.1 \times 10^{-4} \text{ mg/dm}^3,$$

$$V_{\max} = 0.33 \text{ mg/min};$$

for amylase

$$K_m = 2 \times 10^{-4} \text{ mg/dm}^3,$$

$$V_{\max} = 0.55 \text{ mg/min};$$

for amylopectine

$$K_m = 0.1 \times 10^{-4} \text{ mg/dm}^3,$$

$$V_{\max} = 0.2 \text{ mg/min}.$$

It is well known that α-amylases act on inner chains of substrates by hydrolysing 1,4- and 1,6-glycoside bonds, and that the reaction yields initially high molecular dextrines which eventually become the low-molecular ones. A long-lasting hydrolysis of polysaccharides in the presence of α-amylase yield mainly glucose, maltose and maltotriose. From the presented kinetic data obtains some relationships, namely: for the substrates of glycogen type and amylopectine the values of K_m and $V_{\max}$ are very similar. Analogical resemblance in the above values are observed for starch and amylose. This is probably due to the fact that the affinity of α-amylase obtained from activated sludge toward different substrates is different depending on their spatial structure [18], [23].

The influence of some activators and inhibitors on the activity of the obtain-

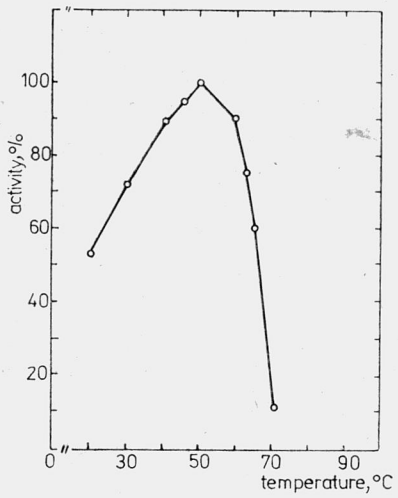

Fig. 6. Effect of temperature on the activity of α-amylase isolated from activated sludge
Enzyme was first heated from 20 to 70° C for 30 min and then quickly cooled. Its activity was measured under conditions described in the text

Rys. 6. Wpływ temperatury na aktywność α-amylazy wydzielonej z osadu czynnego
Enzym ogrzewano przez 30 min w temp. 20–70° C, następnie szybko chłodzono i mierzono jego aktywność w warunkach podanych w tekście

ed enzyme has been also studied. It has been stated that Ca^{2+} ions exert a particularly stimulating effect. As it follows from the data by FISHER et al. [6] the number of Ca^{2+} cations and their bond strength of a protein molecule differ depending on the kind of α-amylases, e.g. one molecule of α-amylse separated from *Aspergillus oryzae* binds permanently 10 Ca^{2+} ions [1], while that from

Table 2

Effects of various compounds on the activity of α-amylase from the activated sludge

Wpływ różnych związków na aktywność α-amylazy wydzielonej z osadu czynnego

Compound	Concentration M	Activity referred to control sample %
Control sample	—	100
$CaCl_2$	2×10^{-2}	150
NaCl	2×10^{-2}	115
NaF	2×10^{-2}	100
Na_2SO_4	2×10^{-2}	100
Na_2HPO_4	2×10^{-2}	80
EDTA	2×10^{-2}	40
PCMB	2×10^{-2}	80
$CuCl_2$	2×10^{-2}	0.00
$HgCl_2$	2×10^{-2}	0.00

Bacillus subtilis only 3–5 ions [6]. It has been stated that α-amylase separated from *Bacillus acidocaldarius* is equally activated by Ca^{2+} and Mg^{2+} ions [7]. Stimulating effect of Ca^{2+} ions on amylases proved by treating them with metal-binding agents depends on the bond strength of Ca^{2+} ions in catalytic centre and on the treatment time and concentration of agents binding Ca^{2+} ions. Since in the presence of the latter ions this enzyme is less sensitive to denaturating agent, thus it is its spatial configuration that is affected by these cations. As it follows from the data given in tab. 2, EDTA — as an agent binding bivalent ions — is responsible for about 60 % inhibition of the enzyme activity. From the data given by many authors [1], [9], [10], it follows that almost all the amylases become inactive after removal of Ca^{2+} ions. Inhibitory effect of phosphates consists probably in precipitation of Ca^{2+} ions in form of calcium phosphate. Inhibitory effect of PCMB would, however, indicate the presence of SH groups or disulphides in the catalytic centre. α-amylase from *Aspergillus oryzae* is an example of α-amylase in which one disulphide bond participates in catalytic centrum [19], [27]. Inhibitory effect of heavy metals (copper, mercury) results from the fact that they denaturate enzymatic protein.

The proof that the obtained enzyme belongs to hydrolases catalyzing the decomposition of polysaccharides is given by chromatographic data resulting

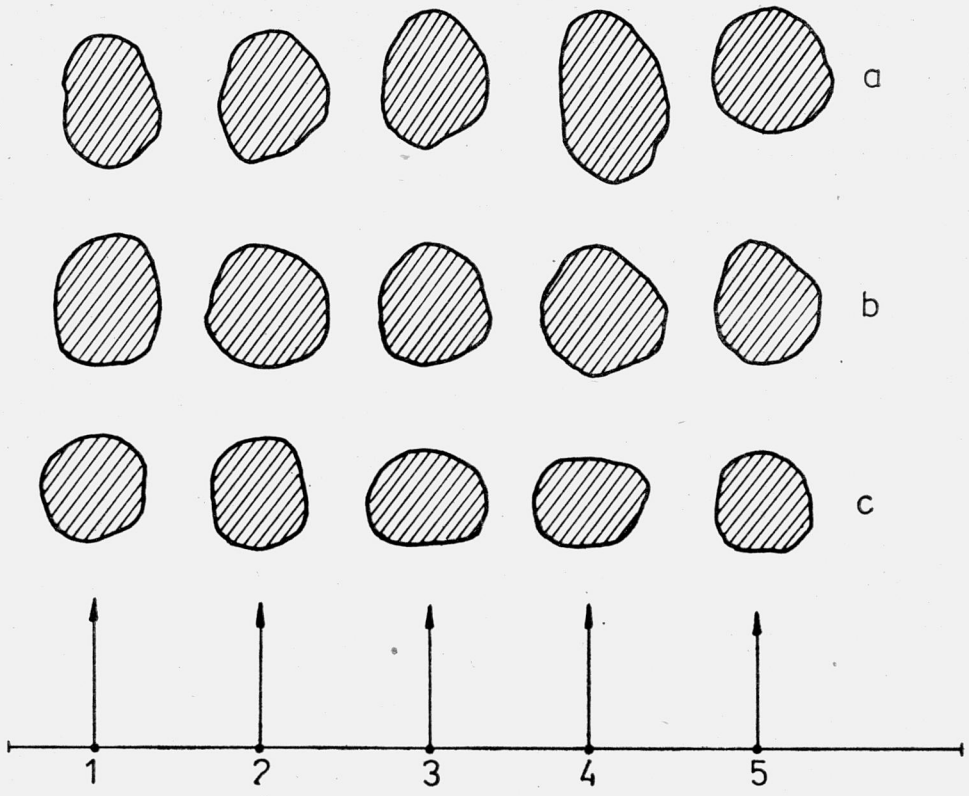

Fig. 7. Chromatograms of the products of amylum hadrolysis by α-amylase isolated from activated sludge in the particular separation phases

1 – crude extract, *2* – fractions after precipitation with ammonia sulphate, *3* – fraction after precipitation with acetone, *4* – fraction after chromatographic separation on Sephadex G–100, *5* – standard: a) glucose b) maltose, c) maltotriose

Rys. 7. Obraz chromatograficzny produktów hydrolizy skrobii przez α-amylazę wydzieloną z osadu czynnego podczas poszczególnych etapów wydzielania

1 – ekstrakt surowy, *2* – frakcja po wytrąceniu siarczanem amonu, *3* – frakcja po wytrąceniu acetonem, *4* – frakcja po rozdziale chromatograficznym na Sephadex G–100, *5* – standary: a) glukoza, b) maltoza, c) maltotrioza

from hydrolysis of starch. Not only each stage of separation and purification of the enzyme was controlled by means of its activity and the quality of enzymatic protein, but also the appearence of products of hydrolysis of the used substrates (figs. 7 and 8). As we see in the included chromatograms, after preliminary stages of the enzyme purification (fractioning with ammonium sulphate, precipitation with acetone and column chromatography) and after incubation with starch, at each stage there appear such products of a hydrolysis like glucose, maltose and maltriose, whereas after the incubation of crystalline enzyme preparation there appears an additional spot lying on the maltotetrose level.

It is well known that α-amylases — regardless their origin — are always slightly contaminated with an enzyme known as glucoamylase which catalyses hydrolysis of low-molecluar glucose polymers. Most probably crystallization results in separation of α-amylase from glucoamylase and then there appears an additional product — maltotetrose which is not hydrolyzed by α-amylase.

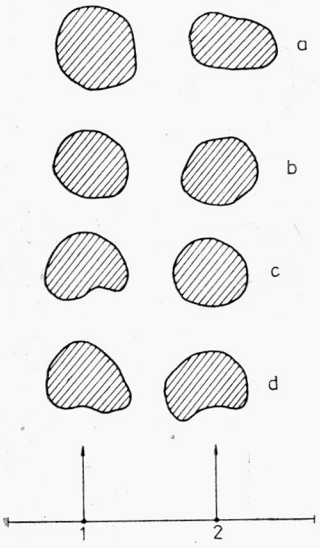

Fig. 8. Chromatograms of the products of amylum hydrolysis by crystalline α-amylase isolated from activated sludge

1 — crystalline enzyme, *2* — standards: a) glucose, b) maltose, c) maltotriose, d) maltotetrose

Rys. 8. Obraz chromatograficzny produktów hydrolizy skrobii przez krystaliczną α-amylazę wydzieloną z osadu czynnego

1 — krystaliczny enzym, *2* — standardy: a) glukoza, b) maltoza, c) maltotrioza, d) maltotetroza

Crystalline preparation of the enzyme obtained by us is characterized by a high stability, since kept for several months at 275 K in 0.01 M solution of calcium acetate it lost solely 5–10 % of its former activity.

On the ground of the quoted results it seems that separation and purification processes were conducted properly and in the desired directions. Further investigations will deal with composition of amino acids, and determining molecular weight of α-amylase separated from activated sludge in order to compare it with α-amylases of bacterial or animal origin.

4. CONCLUSIONS

1. The applied technique of the enzyme purification allowed us to obtain it in crystalline form.

2. The obtained preparation of the enzyme shows a high stability with respect to temperature as well as to pH of the medium. When stored frozen or lyophilized it does not loose its activity for several months.

3. The enzyme hydrolyses α-1,4-glycosyde bonds in glycogen, strach, amy-

lose and amylopectine. It is inhibited by heavy metal ions and activated by calcium ions.

4. The enzyme was isolated from a waste raw material from which many other enzymes, important both in food industry and wastewater treatment technology, may be isolated.

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WYDZIELANIE, OCZYSZCZANIE I NIEKTÓRE WŁAŚCIWOŚCI α-AMYLAZY Z OSADU CZYNNEGO

W osadzie czynnym pochodzącym z oczyszczalni ścieków (Ciernie, województwo wałbrzyskie) przebadano aktywność amylazy, proteinazy, lipazy i fosfatazy. Stwierdzono, że osad cechuje stosunkowo duża aktywność amylazowa i podjęto próbę wydzielenia tego enzymu z osadu czynnego. Aby wydzielić i oczyścić enzym, osad czynny poddawano homogenizacji, a następnie frakcjonowano siarczanem amonowym, dializowano do octanu wapniowego i ponownie frakcjonowano acetonem. W końcowym etapie oczyszczania zastosowano technikę chromatografii kolumnowej z wykorzystaniem sit molekularnych. W wyniku rozdziału chromatograficznego otrzymano dwa odrębne szczyty aktywności, odpowiadające amylazie działającej w środowisku kwaśnym (pH = 5,0) i obojętnym (pH = 7,2). Enzym działający w środowisku kwaśnym wydzielono w postaci krystalicznej. Stwierdzono, że otrzymane kryształy enzymu mają charakter białkowy, ponieważ maksimum adsorpcji w nadfiolecie przypada na 275 nm.

DIE AUSSCHEIDUNG, REINIGUNG UND EINIGE EIGENSCHAFTEN DER α-AMYLASE DES BELEBTSCHLAMMS

Im Belebtschlamm der Abwasserreinigungsanlage Ciernie wurde die Aktivität folgender Enzyme untersucht: der Amylase, Proteinase, Lipase und der Phosphatase. Festgestellt wurde eine verhältnismäßig hohe Amylaseaktivität und es wurde versucht diese aus dem Belebtschlamm abzusondern. Zu diesem Zweck wurde der Belbtschlamm homogenisiert, mit Ammoniumsuflat fraktioniert, anschliessend zum Kalziumacetat dialisiert und erneut mit Azeton fraktioniert. Als abschliessende Reinigung wurde die Kolonnen-Chromatografie unter Ausnutzung der molekularen Siebe angewandt. Als Resultat der chromatografischen Trennung ergaben sich zwei Peaks die der Amylase-Aktivität im sauren (pH = 5,0)

und im neutralen Bereich (pH = 7,2) entsprachen. Der Enzym konnte aus dem sauren Millieu in kristalliner Form gewonnen werden. Die Kristalle haben eine Eiweißstruktur mit einem Adsorptionsmaximum im Ultraviolett bei 275 nm.

ВЫДЕЛЕНИЕ, ОЧИСТКА И НЕКОТОРЫЕ СВОЙСТВА α-АМИЛАЗЫ ИЗ АКТИВНОГО ИЛА

Активный ил, от очистной станции сточных вод (Терне, валбжиское воеводство) был исследован под углом активности следующих энзимов: амилазы, протеиназы, липазы и фосфатазы. Отмечено, что он обладает сравнительно высокой алмазной активностью и была предпринята попытка выделения этого энзима из активного ила. Для выделения и очистки энзима была принята следующая методика: активный ил подвергался гомогенизации, а затем фракционировался сульфатом аммония, диализовался до ацетата кальция и снова фракционировался ацетоном. В конечном этапе очистки была применена техника колоночной хроматографии с использованием молекулярных сит. В результате хроматографического разделения были получены два отдельных пика активности, соответствующие алмазу, действующему в кислой среде (pH = 5,0) и в нейтральной среде (pH = 7,2). Энзим, действующий в кислой среде, был выделен в кристаллическом виде. Выявлено, что полученные кристаллы энзима имеют белковый характер, так как максимум абсорбции в ультрафиолете приходится на 275 нм.