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Studies on the decomposition of keratin wastes by saprotrophic microfungi. I. Criteria for evaluating keratinolytic activity

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The abilities and keratinolytic activity of saprotrophic microfungi (37 strains) have been investigated. Uniform criteria for the evaluation of this activity have been established.

Key words: keratinolytic activity, decomposition of keratin.

INTRODUCTION

Waste feathers are a valuable organic waste due to their high keratin protein contents. When feathers comprise 10% the total mass of raw material (Statistical Year-book 1992) the annual production of waste feathers from broilers only in Poland amounts to 40 000 tons. The main difficulty in their processing is the insolubility of feathers caused by the presence of keratin, i.e. protein belonging to the scleroprotein group. Keratin resistance to chemical agents and enzymatic lysis (proteases) is mainly attributed to the presence of numerous disulfite bonds. So far the main direction in feather utilization has been the production of poultry fodder. However, when compared to other protein fodder types, feather meal obtained by thermohydrolysis is characterised by the lowest index of digestibility of amino acid for poultry (Normy Żywienia Drobiu, 1992). A considerable amount of feathers, difficult to estimate as it comes from small slaughter farms (without any waste utilization facilities), is totally wasted in the waste removal areas or is ploughed into fields. This latter practice is dangerous for the soil environment and

both human and animal health. It is well known that during uncontrolled keratin decomposition, especially by anaerobic bacteria, large quantities of toxic substances, among others, hydrogen sulfite are released.

In the light of these facts the problem of searching for alternative methods of utilization and neutralisation of feathers and other by-products from animal breeding, processing and handling (Klasyfikacja odpadów. JOŚ, 1995) is very important. In the last few years such methods as microbiological processing of waste with high keratin content have aroused a lot of interest. This can be seen from the research done on the use of some keratinolytic *Procaryota*, w.e. proper bacteria and actinomycetes (Elmayergi, Smith 1971; Martin, So 1979; Daler, Neitcher 1981; Wiliams, Shih 1988; Williams et al. 1990; Kałużewska, Wawrzkiewicz, Łobarzewski 1991; Lin et al. 1992). Less interest in this respect is given to fungi (Malviya et al. 1982; Nigam, Kushwaha 1989; Wawrzkiewicz, Wolski, Łobarzewski 1987), even though these organisms are capable of decomposing complex organic matter. The ability to use raw keratin as a full-value substrate is shown by the so-called keratinophilic fungi — a specialized group that comprises both animal and human parasites and saprophytic species found in the soil environment.

The aim of the present work proceeded by some preliminary studies (Korniłłowicz 1994) was to evaluate the abilities and keratinolytic activities of the saprotrophic micromycetes. In the course of studies some products of hydrolysis and mineralization of feather protein were determined, together with metabolic and proteolytic activity of the fungi. At the same time an attempt to determine uniform criteria for the evaluation of keratinolytic activity of these microorganisms was undertaken.

MATERIALS AND STUDY METHODS

Strains of fungi. In the present study the author's collection of fungi obtained from various cultivated soils (Central Poland) and lake bottom deposits (Pojezierze Łęczyńsko-Włodawskie) has been used. The environments, isolation methods, methods used to obtain pure cultures and their identification were described in earlier works (Korniłłowicz 1991/1992, 1993a, b). Isolated strains were stored at +4°C in the Sabouraud's scarves. Taxonomic characteristics of the strain collection are presented in Table 1.

Four randomly chosen strains representing the most frequently isolated species were chosen for further studies: 2-3 isolates from the common taxa which were less frequent and 1 strain from the species which were seldom isolated. In total, 37 strains of keratinophilic fungi belonging to 16 species, counting perfect and imperfect forms separately, were chosen for the

Table 1
Characteristics of the some keratinophilic fungi

Species	Total number of strains total,	from soil	from bottom sediments	Taxonomic position*	Ecological group
Arthroderma quadrifidum Dawson et Gentles, tel.	67	50	17	*A. Onygenales*	Geophilic dermatophytes
Trichophyton terrestre Durie et Frey complex. an. *A. quadrifidum*	107	66	41	*D. Moniliales*	
Arthroderma uncinatum Dawson et Gentles, tel.	15	15	0	*A. Onygenales*	
Trichophyton ajelloi (Vanbr.). Ajello, an. *A. unicinatum*	261	249	12	*D. Moniliales*	
Microsporum cookei Ajello, an.	49	40	9	*D. Moniliales*	
Anixiopsis stercoraria Hansen, tel. *Ch. pruinosum*	17	0	17	*A. Onygenales*	*Chrysosporium* group
Arthroderma curreyi Berk., tel.	21	0	21	*A. Onygenales*	
Chrysosporium sp., an. *Arthroderma curreyi* Berk.	30	3	27	*D. Moniliales*	
Chrysosporium asperatum Carm, an. [= *Myceliophtora vellerea* (Sacc. et Speg.) van Oorschot]	188	183	5	*D. Moniliales*	
Chrysosporium europae Singler, Guarro et Punsola, an.	2	0	2	*D. Moniliales*	
Chrysosporium keratinophilum (Frey) Carm., an.	28	4	24	*D. Moniliales*	
Chrysosporium kreiselii Dominik, an.	18	18	0	*D. Moniliales*	
Chrysosporium pannicola (Corda) van Oorschot et Stalpers, an.	26	19	7	*D. Moniliales*	
Chrysosporium pruinosum (Gilman et Abbott) Carm., an.	47	13	34	*D. Moniliales*	
Chrysosporium tropicum Carm., an.	2	2	0	*D. Moniliales*	
Chrysosporium tuberculatum (Kuehn) Dominik, an. [= *Myceliophtora*, an. *Arthroderma tuberculatum* Kuehn]	56	51	5	*D. Moniliales*	
Chrysosporium serratum (Eidam) Dominik, an. [= *Myceliophtora*, an *Ctenomyces serratus* Eidam]	104	94	10	*D. Moniliales*	
Ctenomyces serratus Eidam, tel.	60	60	0	*A. Onygenales*	

Explanations: tel. = teleomorph; an. = anamorph; *A = *Ascomycetes; D = Deuteromycetes*

Table 2
Origin of strains used for investigations

Strains of fungus	Soils				Bottom sediments	
	A	B	C	D	E	F
A. quadrifidum, teleomorph + *T. terrestre*, anamorph				-1* -2 -1 -2		
M. cookei			-1			-2
M. ajelloi	-3 -4	-2	-1			
A. stercoraria, telemorph + *Ch. pruinosum* sp., anamorph	-2				-1	-1
A. curreyi, telemorph + *Chrysosporium* sp., anamorph						-1 -2 -1
Ch. asperatum	-1 -3 -4	-2				
Ch. europae						-1
Ch. keratinophilum			-2		-1	
Ch. kreiselii				-1		
Ch. pannicola	-1 -2					
Ch. tropicum						-1
Ch. tuberculatum				-1 -2		
Ct. serratus		-1		-4		-2 -3
G. catenulatum			-1			
P. lilacinus	-1					
V. lecanii			-1			
V. psalliotae		-1				

Explanations: ▭ – geophilic dermatophytes; ▥ – *Chrysosporium* sp. group; ■ – non-keratinophilic fungi; * – number of strains; + – common taxa; A – sandy; B – loamy; C – chernozem; D – black soil; Bottom sediments: E sandy; F – gytija. (Characteristics of soil and bottom sediments were presented in earlier articles)

experiments (Table 2). The collection created that way increased by 4 isolates of the so-called non-keratinophilic fungi which were commonly found on keratin refuse placed in the soil (K o r n i ł ł o w i c z 1991/1992, 1993a, 1996).

S u b s t r a t e s. White wast hen feathers (Leghorn chickens) obtained from the Poultry Processing Plant (Zakłady Drobiarskie) in Lublin were used in the present research. All the experiments were carried out on the same batch of material. Feather preparation methods that do not change keratin structure were described earlier (K o r n i ł ł o w i c z 1994). Characteristics of the chemical composition of the substrate used, which is of basic importance for the present study are presented in Table 3. The analysis of contens of biogenic elements and sulphur was carried out by means of methods of elemental analysis. Total protein was determined by Kjedahl's method using an automatic Kiel-Foss analyser (type Tecator Kjeltec Auto 1030) and applying factor 6.25.

F u n g i c u l t u r e s. Individual experiments were carried out in liquid cultures using native feathers as the only sources of C, N, and S (medium I) or C and N (medium II).

T a b l e 3
Chemical composition of chicken feathers

Contents* (%)			
C org.	N total	S total	protein total
49,09	14.72	3.67	87.94

* — mean values from 3 repetitions

Medium I containing 0.5 g of ground feathers in 100 cm^3 of mineral salt solution with pH 6.5 was used to determine substrate decomposition, analyses of its products, respiration activity and general proteases activities. The composition of the mineral medium (II) had been described in the introductory work (K o r n i ł ł o w i c z 1994). Induction of the keratinolytic enzymes was carried out in medium II with mineral salt composition according to N i c k e r s o n, N o v a l and R o b i n s o n (1963) using 0.5 g of feathers for 50 cm^3 of mineral nuttrient medium with pH of 7.8. One cm^3 of spore suspension with the density of 10^3 (medium I) was used as inoculum and 10^6 for medium II. The control constituted mineral-keratin base (feathers) which was inoculated with 1 cm^3 of mineral nutrient medium instead of the inoculum.

Fungi cultures and controls were incubated at 20 +/−2°C under the conditions of medium volume standardization applying, depending on the

experiment, 70 to 126 days of incubation. On the individual study dates 3 to 5 repetitions were prepared. Detailed explanations of the dates of analyses were given below or in the documentation material.

Degree of substrate utilization was determined by Chesters an Mathinson's method (1963) after after-culture liquids had been separated.

Direct biomass measurements of mycelium were not possible because of the feather mass overgrowth through fungi hyphae. Approximate evaluation of its size was made after feathers were dissolved in 50 cm^3 of 10% NaOH and the index of mycelium loss determined for each of fungi strains bred on Sabouraud's bullion separately was taken into consideration (Chesters, Mathinson 1963).

Biochemical analyses of fungi cultures. $N\text{-}NH_4^+$ content in the after-culture filtrates was determined by Nessler's method, $S\text{-}SO_4^{2-}$ niepheleometirically at the wavce length of 490 nm. Hydrogen sulfite contents were detected by a paper slip saturated with lead acetate. The pH of the after-culture liquids was determined potentiometrically directly after substrate and mycelium filtration.

The amount of peptide substances in the filtrates was determined by means of Lowry's et al. method (1951) using bovine serum albumine as a standard. The level of amine groups was analysed according to Baileys (1962) using the standard curve plotted for leucine.

Respiration activity was determined by Rühling and Tyler's method (1973) on the basis of CO_2 liberation. Determination of total proteolytic activity was carried out according to the method used in the preliminary studies (Korniłłowicz 1994) in relation to casein as a substrate.

Detection of keratinolytic enzymes was carried out in the cell-free after--culture filtrates by means of Yu et al's method as modified by Takiuchi et al. (1982) using chicken feathers as a substrate instead of guinea pig hair (Korniłłowicz 1994).

The so-obtained data were evaluated statistically. The analyses comprised characteristics of the variation of the above-mentioned features on the consecutive study dates as expressed by the values of the variation index Cv, which describes variability in the percentage of the mean value in relation to the mean value and coefficient of interclass variability h^2 (called heredibility index in genetics). It denotes what percentage of total variability of a given feature results from the differentiation in the study object (i.e. fungi strains):

C_v and h^2 are calculated according to the following formula:

where S_d is standard deviation; $\bar{x}$ is arithmetical mean; $S_x^2 = S_a^2 + S_e^2$, whereas S_a^2 and S_e^2 are variation components which determine, total variation, object variation (strain) and interobject variation (random) respectively. The mutual relations among the features studied and the behaviour of individual features

$$C_v = \frac{S_d}{\bar{x}} \cdot 100\%; \qquad h_2 = \frac{S_a^2}{S_x^2} \cdot 100\%$$

during the whole study period were analysed by means of the correlation and regression methods calculating correlation coefficients r and determination coefficients R^2.

Regression functions are polynominals of the second or third degree; the extent to which they math experimental data ais measured by determination coefficients (square of the coefficient curveline correlation expressed in %).

RESULTS

Abilities of saprotrophic fungi to decompose native keratin

The abilities of saprotrophic fungi to decompose of native keratin were evaluated on the basis of the degree of utilisation of chicken feathers, which was earlier accepted as a basic index of keratinolytic activity of these microorganisms.

Even though all the studied strains (37 isolates) used feathers as the only source of C, N and S, only representatives of the geophilic dermatophytes and *Chrysosoporium* carried out total lysis of this substrate. The remaining fungi (4 isolates), which were called non-keratinophilic in the beginning, decomposed only about 20-30% of the keratin waste mass that was used (Table 4).

Among keratinophilic fungi species such as: *Arthroderma quadrifidum* and *A. curreyi* together with anamorphs *Trichophyton terrestre* and *Chrysosporium* sp., and some isolates of *T. ajelloi*, *Ch. keratinophilium*, *Ch. pruinosum* and *Ch. pannicola* they appeared to be the most active destroyers of keratin in feathers. The dynamics of decomposition of raw feathers by fungi was expressed by quick loss of this substrate in the first 3 weeks (especially) between the 7th and 14th day), then the lysis slowed down between the 3rd and 6th week, and accerelated again but to lesser degree in the remaining period. This is clearly illustrated in Fig. 1 showing statistically elaborated relation between substrate mass loss and the time of culture.

Macroscopic and microscopic observations of mycelium showed that the decrease in biodegradation rate was parallel to spore production of micromycetes.

Table 4

Degree of utilization of native feather keratin (in %) by fungi (means from 3 repetitions)

Strain of fungus		Time of cultivation (days)						Total lysis of feathers (days)
		3	7	14	21	42	70	
Geophilic dermatophytes								
A. quadrifidum	1	8,0	22,4	71,5	78.8	82.2	94.0	98
	2	4.6	24.0	51.0	77.0	80.0	91.0	98
M. cookei	1	3.0	21.0	32.0	45.0	49.0	57.0	126
	2	5.0	15.1	46.0	54.4	62.0	67.0	126
T. ajelloi	1	7.6	28.0	58.6	65.0	66.0	68.0	126
	2	3.0	27.0	33.0	76.0	80.0	89.0	98
	3	3.9	18.2	43.9	48.0	68.0	72.6	126
	4	3.5	16.0	40.0	50.0	57.0	63.5	126
T. terrestre	1	6.0	25.5	52.2	64.9	69.0	74.0	26
	2	3.1	5.5	47.5	64.7	69.0	85.5	126
Chrysosporium group								
A. stercoraria		2.0	5.0	13.5	26.0	73.0	86.0	126
A. currevi	1	4.0	15.0	56.0	75.0	82.0	96.0	98
	2	2.6	14.2	55.6	77.0	84.0	96.0	98
Chrysosporium sp.		4.5	31.0	58.0	70.4	81.0	84.0	98
Ch. asperatum	1	5.2	32.0	51.0	60.0	69.5	76.4	126
	2	3.1	33.0	60.4	66.0	80.0	93.0	98
	3	4.9	31.0	55.5	61.0	73.2	83.0	98
	4	4.0	20.0	49.0	65.0	65.0	75.0	126
Ch. europae		2.7	11.0	27.0	56.2	76.0	83.0	98
Ch. keratinophilum	1	4.3	12.5	28.0	51.0	84.5	96.0	98
	2	3.0	12.0	60.0	79.0	90.0	92.0	98
Ch. kreiselii		2,5	5.6	41.0	51.0	65.0	75.0	126
Ch. pannicola	1	4.8	9.0	51.0	74.0	91.0	96.0	98
	2	4.0	22,5	57,0	69,0	89.0	96.5	98
Ch. pruinosum	1	1.2	17.2	49.0	86.0	90.0	100.0	70
	2	4.4	22,0	51,0	66,4	88.0	90.0	98
Ch. tropicum		2,0	23.0	41.0	62.0	81.0	82.0	126
Ch. tuberculatum	1	5.7	12.0	31.0	39.0	43.0	62.0	126
	2	6.0	20,0	40,0	57,0	64.5	74.0	126
Ct. serratus	1	2.0	6.0	51.0	54.0	83.0	86.0	98
	2	6.2	7.0	43.0	55.0	73.0	82.0	98
	3	5.2	25.0	46.0	58.0	77.0	95.0	98
	4	5.6	33.0	44.0	63.0	74.0	76.0	98
Non-keratinophilic fungi								
G. catenulatum		1.2	14.4	17.0	22.0	24.5	30.2	not observed
P. lilacinus		2.2	3.0	29.0	29.5	33.0	33.0	
V. lecanii		4.0	8.3	11.0	14.0	21.0	23.0	
V. psalliotae		2.6	8.2	13.0	25.0	26.0	28.5	

Products of feather protein catabolism in fungi cultures

Detection of degradation products we conducted parallely to the observation of substrate disappearance from the fungi culture. The aim of former was not only to learn about basic products of lysis and mineralization of raw feathers, but also to capture mutual relations between catabolites produced, as well as to combine the dynamics of their release with the degree of substrate usage by fungi.

Peptide substrate release. Earlier preliminary studies (Korniłłowicz 1994) showed that the release of peptide bindings in fungi cultures which decompose raw chicken feathers can be used as an index

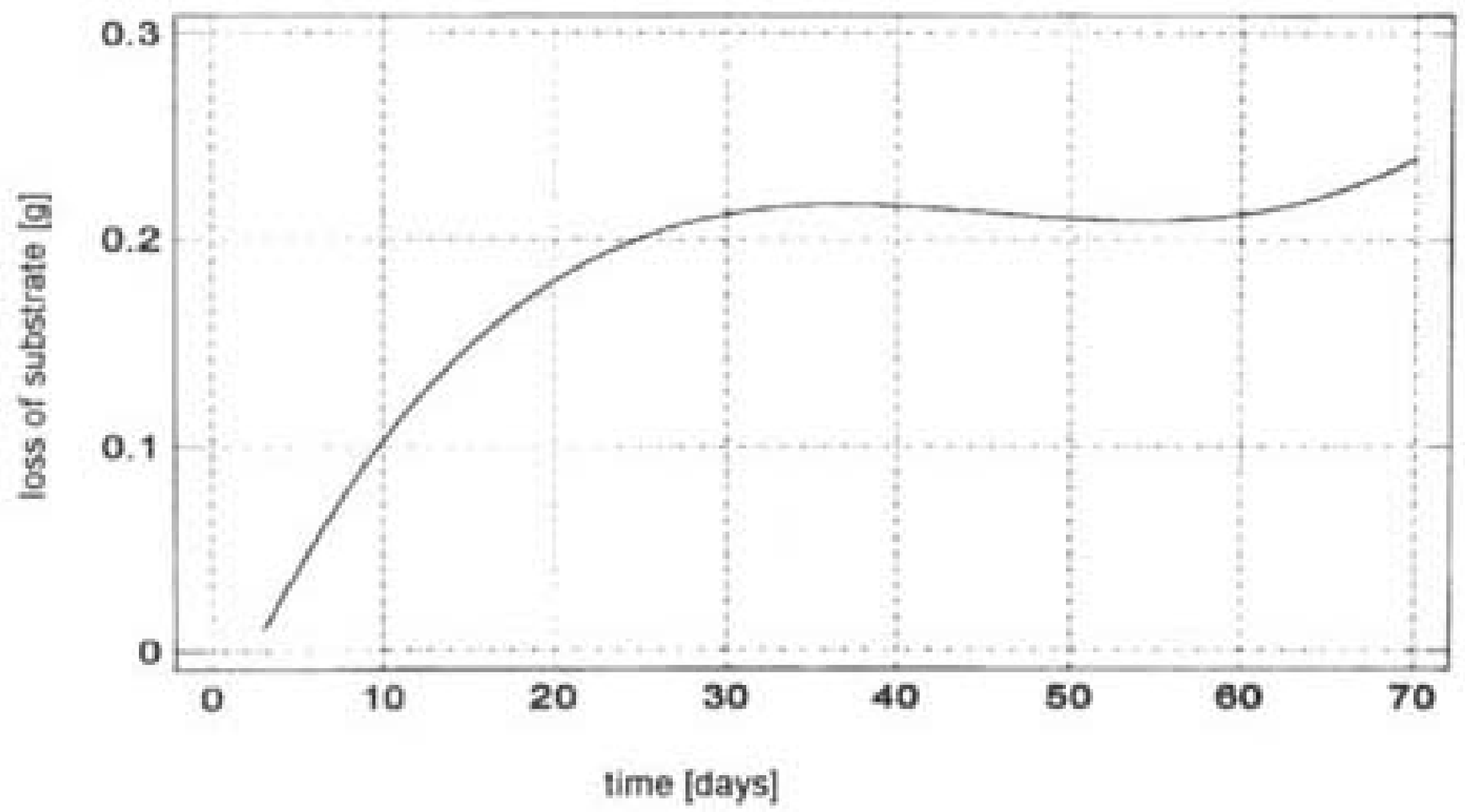


Fig. 1. Dynamics of the loss of waste feather mass in fungi cultures ($R^2 = 56.3\%$)

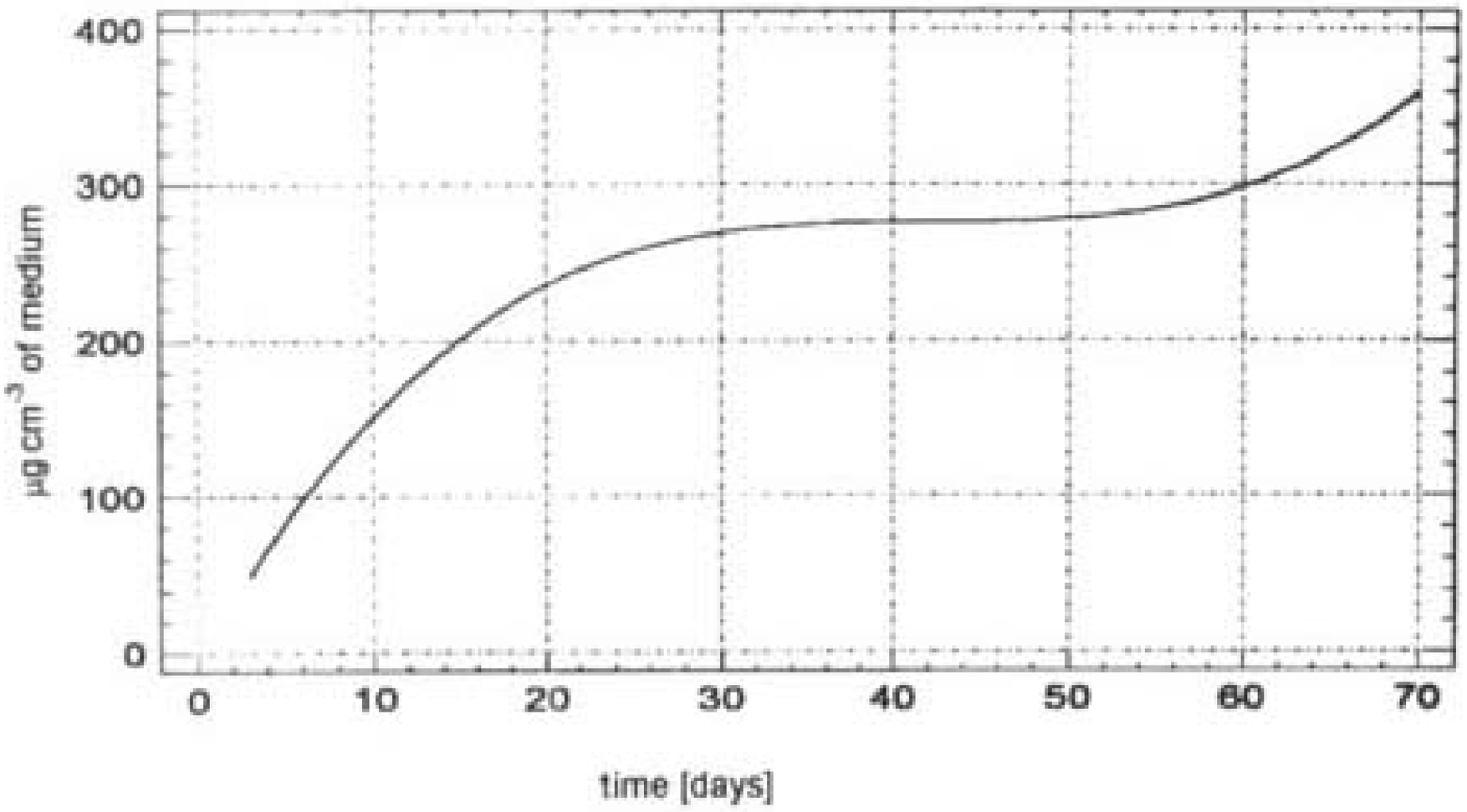


Fig. 2. Release of peptides in fungi cultures decomposing waste feathers ($R^2 = 60\%$)

Table 5
Correlation coefficient (r) between the studied properties of fungi

Feature	1	2	3	4	5	6	7	8
	Time (days)	Peptide release	N-NH_2 release	NH_4^+ production	SO_4^{2-} production	Exoprotease activity	pH of medium	CO_2 excretion
1. Loss of substrate	7	0.59^{xx}	0.47^{xx}	0.38^{xx}	0.38^{xx}	0.46^{xx}	0.66^{xx}	0.42^{x}
	14	0.41^{xx}	0.36^{xx}	0.70^{xx}	0.22^{xx}	0.27^{xx}	0.59^{xx}	0.43^{x}
	21	0.36^{xx}	0.03	0.69^{xx}	0.25^{xx}	−0.04	0.51^{xx}	0.10
	42	0.42^{xx}	0.02	0.51^{xx}	0.33^{xx}	0.03	0.54^{xx}	0.30
	70	0.73^{xx}	0.26^{xx}	0.67^{xx}	0.16	0.13	0.16	−0.19
								0.28
2. Peptide release	7		0.46^{xx}	0.30^{xx}	0.03	0.47^{xx}	0.36^{xx}	0.02
	14		0.63^{xx}	0.36^{xx}	0.24^{x}	0.10	0.21^{x}	0.45^{x}
	21	x	−0.13	0.17	0.30^{xx}	−0.07	−0.05	0.56^{xx}
	42		0.12	0.27^{xx}	0.24^{x}	0.13	0.31^{xx}	−0.53^{xx}
	70		−0.26^{xx}	0.46^{xx}	0.20	0.17	−0.02	0.48^{xx}
								−0,17
3. N-NH_3 release	7			0.22^{xx}	0.68^{xx}	0.11	0.21^{x}	0.40
	14			0.36^{xx}	0.66^{xx}	−013	0,21^{x}	0,63^{xx}
	21		x	−0.47	−0.09	−0.02	0.25^{xx}	0,44^{xx}
	42			0.13	0.08	0.01	−0.12	0,04
	70			0.21^{x}	0.20^{x}	−0.24^{x}	−0,04	−0,17
								0.42^{x}
4. NH_4^+ production	7				−0.18	0.29^{xx}	0.44^{xx}	0.19
	14				0.18	0.26^{xx}	0.70^{xx}	0.25
	21			x	0.23^{x}	−0.02	0.34^{xx}	−0.03
	42				0.50^{xx}	0.02	0.06	0.52^{xx}
	70				0.28^{xx}	0.11	−0.04	−0.40
								−0.17

cont. 5

	1	2	3	4	5	6	7	8
5. SO_4^{2-} production	7					−0,12	0,18	0,30
	14					0.25	0.25^{xx}	0.40
	21				x	0.14	0.03	−0.01
	42					−0.25^{xx}	−0.01	−0.51^{x}
	70					−0.32^{xx}	−0.07	−0.43^{x}
								−0.61^{xx}
6. Exoprotease activity	7						0.32^{xx}	−0.16
	14						0.16	0.36
	21					x	0.10	0.46^{x}
	42						0.30^{xx}	−0.10
	70						−0.17	0.23
								−0.46
7. pH of medium	7							0.52^{xx}
	14						x	0.17
	21							0.41^{x}
	42							
	70							
								0.13
								0.43^{x}
								0.31

^{x}r − significance of the coefficient at p = 0.05; ^{xx}r − significance of the coefficient at p = 0.01

Table 6
Release of peptide substances in fungi cultures decomposing waste feathers

Strain of fungus	Range of release					
	days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1-2	2.4-9.0	24.8-30.8	37.6-38.4	53.8-62.0	58.2-67.6	73.0-81.4
M. cookei str. 1-2	3.6-7.4	9.2-17.6	43.2-51.6	50.0-98.0	48.2-67.0	80.0-87.2
T. ajelloi str. 1-4	3.8-13.2	14.0-45.4	29.4-75.0	55.4-82.2	51.0-106.0	65.2-94.6
T. terrestre str. 1-2	4.6-13.2	9.8-21.8	38.0-45.4	44.8-58.6	66.6-90.0	58.4-88.0
Chrysosporium group						
A. stercoraria	4.0	5.4	10.2	21.0	55.4	59.4
A. curreyi str. 1-2	5.6-9.0	11.0-13.4	31.8-40.4	39.4-47.6	52.8-67.6	63.0-74.4
Chrysosporium sp.	11.0	25.2	41.8	53.0	48.8	74.2
Ch. asperatum str. 1-4	7.4-12.2	23.6-35.0	43.6-55.0	40.6-59.4	46.6-70.0	73.4-89.0
Ch. europae	8.2	27.6	32.6	29.8	27.8	43.4
Ch. keratinophilum str. 1-2	3.8-7.4	5.8-10.0	31.6-33.2	39.8-59.0	47.4-80.0	67.8-89.8
Ch. kreiselii	9.4	8.6	32.0	51.4	44.4	72.2
Ch. pannicola str. 1-2	6.0-7.2	13.4-37.8	36.2-58.6	46.8-47.0	61.4-72.6	74.8-89.6
Ch. pruinosum str. 1-2	3.0-4.8	16.4-24.8	43.4-52.4	34.6-78.0	54.,6-91.2	64.2-106.0
Ch. tropicum	3.0	16.4	3.4	32.2	54.8	65.0
Ch. tuberculatum str. 1-2	2.2-9.8	16.6-25.0	28.0-79.8	24.0-3.2	29.0-39.12	49.0-64.0
Ct. serratus str. 1-4	4.0-15.6	5.8-33.6	31.4-42.6	34.0-56.2	41.0-63.8	73.8-95.0
Non-keratinophilic fungi						
G. catenulatum	10.8	10.0	27.0	41.0	29.2	38.0
P. lilacinus	9.6	26.8	33.0	42.0	27.2	23.8
V. lecanii	11.4	8.2	23.6	29.6	11.6	30.6
V. psalliotae	19.0	14.2	26.0	38.2	19.0	26.2

Explanation: mg g^{-1} of substrate – mean values from 3 repetitions

of keratinolytic activity of these microorganisms. This was confirmed by the present study conducted on numerous strains of material. A positive correlation between the level of peptides in the medium and the rate of decomposition of this substrate was observed (Table 5). Both fungi groups showed abilities for splitting of peptides. However, maximum amount of these bindings in the non-keratinophilic fungi cultures rarely exceeded 40 mg g^{-1} of feathers, whereas in the keratinophilic fungi cultures it was always higher (Table 6). In the case of keratinophilic fungi it was usually 50% of the total amount of these substances. In the later period between the (3rd and 6th week) the process of peptidolysis slowed down and was activated again. This is illustrated very clearly on the graph of the regression curve for this feature (Fig. 2).

Table 7

Changes in the amine N content in the after-culture medium of fungi decomposing waste feathers

Strain of fungus		Time of cultivation					
		days					
		3	7	14	21	42	70
Geophilic dermatophytes							
A. quadrifidum	1	0.1	0.3	1.0	1.2	1.3	0.4
	2	0.2	0.5	0.9	1.1	0,5	0.5
M. cookei	1	0.1	0.2	0,5	1.3	0.7	0.8
	2	0.1	0.3	1.4	2.2	1.0	0.7
T. ajelloi	1	0.05	0.7	1.7	1.2	2.2	1.2
	2	0.06	1.3	1.3	1.4	0.4	0.5
	3	0.02	0.4	0.8	0.7	1.0	0.4
	4	0.02	0.4	0.7	0.7	0.8	0.2
T. terrestre	1	0.04	0.7	0.8	1.3	0.9	0.8
	2	0.06	0.3	1.2	1.2	1.0	0.5
Chrysosporium group							
A. stercoraria		0.06	0.3	0.6	0.2	1.4	0.4
A. curreyi	1	0.06	0.9	1.4	1.6	0.6	0.6
	2	0.02	0.2	0.7	1.7	0.6	0.6
Chrysosporium sp.		0.02	0.5	1.0	0.7	1.3	0.6
Ch. asperatum	1	0.02	1.0	2.0	1.0	2.3	0.4
	2	0.01	0.5	1.6	2.3	1.0	0.8
	3	0.0	0.4	1.6	2.3	1.1	0.6
	4	0.02	0.7	0.8	0.7	0.5	0.5
Ch. europae		0.0	0.3	1.0	2.2	1.0	0.7
Ch. keratinophilum	1	0.05	0.2	2.0	1.1	2.3	0.3
	2	0.02	0.1	0.8	2.4	1.2	0.7
Ch. kreiselii		0.01	0.1	0.5	0.6	1.3	0.5
Ch. pannicola	1	0.02	0.5	0.6	1.3	1.4	0.3
	2	0.01	0.4	1.3	2.1	1.3	0.8
Ch. pruinosum	1	0.05	0.6	2.5	1.0	2,2	0,3
	2	0,07	0.6	0.4	1.0	1.0	0.3
Ch. tropicum		0.01	0.2	1.3	0.6	0.8	0.7
Ch. tuberculatum	1	0.02	0.4	0.4	0.8	1.2	0.3
	2	0.01	0.4	2.4	2.2	0.9	0.5
Ct. serratus	1	0.5	0.2	1.8	1.0	2.4	0.3
	2	0.1	0.5	0.5	0.8	1.2	0.3
	3	0.02	0.4	1.4	2.1	1.2	0.8
	4	0.3	0.6	1.3	0.9	1.3	0.9
Non-keratinophilic fungi							
G. catermulatum		0.09	0.2	0.2	0.09	0.02	0.02
P. lilacinus		0.02	0.6	0.8	0.7	0.8	0.7
V. lecanii		0.1	0.2	0.1	0.2	0.2	0.2
V. psalliotae		0.2	0.3	0.3	0.3	0.4	0.3

Explanation: see Table 6

The content of N amine in the after-culture filtrates. This study aimed at a preliminary evaluation of the total content of amino acids in the medium of after-culture fungi. Transformation of amino acids into a digestible form is important, among others, in the utilization of waste feather in fodder production.

The maximum of N amine release in the keratinophilic fungi cultures exceeded 1 mg g^{-1} of the substrate reaching 1.2 to 2.0 mg g^{-1} of feathers (Table 7). In cultures of non-keratinophilic fungi the content of amino groups was 0.4-0.8 mg $N\text{-}NH_2$ g^{-1} of the susbstrate studied. Assuming, after Wolski that the content of N amine in the chicken feather keratin is on the average 0.45 mg per 100 mg of dry mass, it has been estimated that in the keratinophilic fungi cultures 25-60% of $N\text{-}NH_2$ from this substrate changes into a soluble form, and in the non-keratinophilic fungi cultures only 5 to 23%.

The dynamics of amine bindings release in the individual strain cultures only in the first 2-3 weeks was similar (an increase of contents). In the later period considerable changes in the level of these compounds were observed depending on the fungi strain. The made the determination of a common for all the strains regression function impossible.

Accumulation of $N\text{-}NH_4$ in the medium. When the present study was planned out, it was assumed that a low ratio C:N in feathers would be favourable for the mineralization of nitrogen.

The experiments carried out showed that during the process of decomposition of feathers fungi periodically accumulated 400-500 μg $N\text{-}NH_4^+$ cm^{-3} of medium, i.e. 80-100 mg g^{-1} of substrate (Table 8). It was calculated that 30-60% of N from feathers changed into ammonia form in the presence of keratinophilic fungi. In the case of non-keratinophilic fungi amonification took place in the 15-17% of the substrate only. The fungi studied did not produce oxidated nitrogen forms, i.e. $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$.

Statistically elaborated relation between the content of $N\text{-}NH_4^+$ in the medium and the culture showed that dissemination was most intensive in the first 3 weeks of fungi growth on feathers (especially between the 7th and 14th day). In the later period the accumulation of $N\text{-}NH_4^+$ was weaker. At the same time the production of NH_4^+ showed a clear correlation with the loss of substrate mass and release of peptide substances (Table 5).

Sulfate release. Taking into account high sulphur content in the feather keratin (Table 3) it was justifiable to determine basic products of transformation of this component as well. In the preliminary studies (Korniłłowicz 1994) it was found that the main fungi metabolite was sulfate. The various species of keratinophilic fungi in the peat of organic S mineralization period released 12-18 mg $S\text{-}SO_4^{-2}$ g^{-1} of substrate, i.e.

Table 8

$N\text{-}NH_4^+$ production during the decomposition of waste feathers by fungi

Strain of fungus	Range of release					
	days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1-2	1.0-1.5	29.4-77.8	86.8-92.0	76.4-107.0	51.0-57.4	45.6-54.8
M. cookei str. 1-2	0,5-2.1	6.4-20.0	38.8-74.6	62.4-88.2	64.5-75.6	31.2-80.2
T. ajelloi str. 1-4	1.9-9,0	12.1-21.4	38.8-75.2	52.6-109.2	31.8-66.2	43.6-59.0
T. terrestre str. 1-2	7.2-32.0	18.5-40.4	45.6-97.2	64.6-84.4	47.4-85.6	42.2-65.8
Chrysosporium group						
A. stercoraria	2,0	25.8	27.6	51.6	63.2	68.2
A. curreyi str. 1-2	0.5-2.6	14.5	62.4-107.0	71.0-93.6	40.4-61.3	52.8-53.2
Chrysosporium sp.	1.6	3.4	62.4	91.4	68.8	72.8
Ch. asperatum str. 1-4	3.1-4.5	33.6-76.4	75.2-101.2	65,6-95.0	60.2-106.8	454-83.6
Ch. europae	3,5	12,0	52.2	83.2	76.0	76.8
Ch. keratinophilum str. 1-2	0.5-1.0	12.4-14.3	21.5-107.0	98.8-106.6	39.4-87.0	50.8-84.0
Ch. kreiselii	0,98	2.6	44,0	46.8	38.2	37.5
Ch. pannicola str. 1-2	0.9-1.6	31.8-53.2	96.4-100.6	89.4-104.2	56.6-96.0	50.9-83.6
Ch. pruinosum str. 1-2	0.6-1.1	17.2-81.0	59.0-98.8	84.0-87.8	60.2-96.4	50.8-64.4
Ch. tropicum	1.15	17.1	58.8	87.6	95.6	58.8
Ch. tuberculatum str. 1-2	0.8-1.2	30.4-39.6	81.8-90.6	56.0-67.2	37.0-57.8	45.8-67.0
Ct. serratus str. 1-4	1.1-3.9	6.7-44.6	57.0-77.6	63.6-97.6	47.4-95.6	45.6-81.8
Non-keratinophilic fungi						
G. catenulatum	0.98	13.9	27.8	39.2	27.6	17.6
P. lilacinus	6.3	15.0	266	22.6	35.8	30.4
V. lecanii	4.1	14.5	29.4	55.0	30.6	12.6
V. psalliotae	1.8	5.2	16.2	33.0	16.5	16.6

Explanations: see Table 6

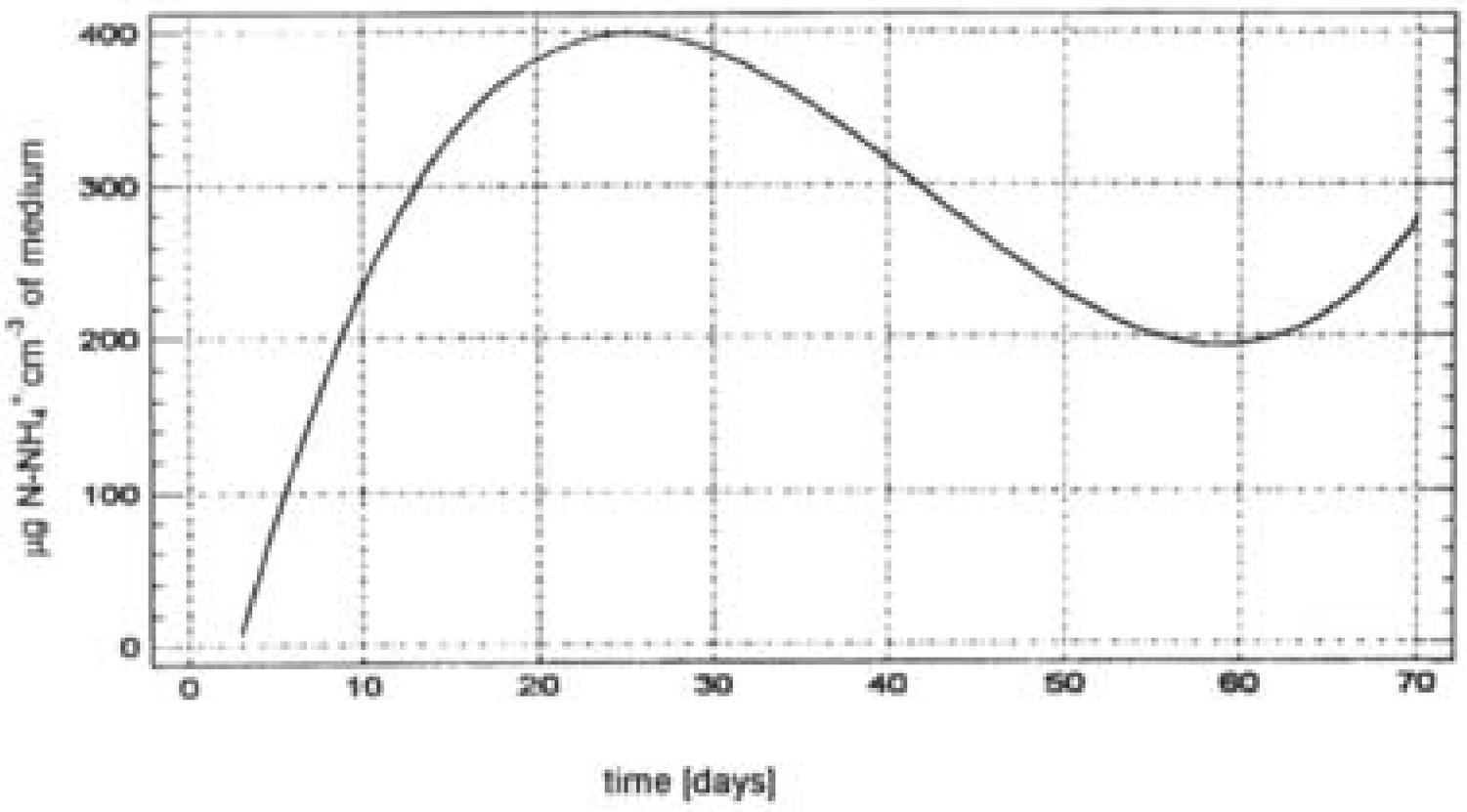


Fig. 3. $N\text{-}NH_4^+$ accumulation in the after-culture substrates of fungi decomposing feathers ($R^2 = 60.1\%$)

Table 9
Sulfate release during the feathers decomposition of feathers

Strain of fungus	Range of production of $S\text{-}SO_4^{2-}$					
	days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1-2	0.18-0.24	0.74-1.85	3.9-5.1	4.6-5.3	7.4-13.0	5.2-6.4
M. cookei str. 1-2	0.10-0.34	1.5-1.7	3.9-4.9	5.9-9.0	9.9-10.3	11.0-18.0
T. ajelloi str. 1-4	0.19-3.12	0.72-6.26	2.70-7.05	4.6-13.0	6.8-14.1	6.5-9.7
T. terrestre str. 1-2	0.38-1.92	0.39-4.42	2.70-4.80	5.40-8.50	6.9-11.3	6.2-18.4
Chrysosporium group						
A. stercoraria	0.12	0,58	3.1	5.16	7.12	6.3
A. curreyi str. 1-2	0.30-0.32	2.06-3.22	4.0-8.35	6.7-9.1	7.12-11.8	6.6-12.4
Chrysosporium sp.	0.33	3.42	5.43	10.3	13.4	7.1
Ch. asperatum str. 1-4	0.15-1.42	1.84-5.08	3.8-8.5	3.4-12.5	7.8-12.5	5.2-12.4
Ch. europae	0,12	0.30	3.6	6.0	9.26	12.7
Ch. keratinophilum str. 1-2	0.15-1.4	0.48-1.69	1.02-3.88	5.6-11.8	13.7-18.5	10.1-15.7
Ch. kreiselii	0.17	0.90	4.0	5.2	12.6	5.7
Ch. pannicola str. 1-2	0.31-0.73	0.34-1.06	3.90-4.15	4.06-8.30	7.35-11.60	6.7-10.3
Ch. pruinosum str. 1-2	0.11-0.44	0.24-3.98	3.2-10.2	5.6-19.4	8.0-18.8	6.0-13.0
Ch. tropicum	1.44	4.26	10.2	19.6	18.8	13.04
Ch. tuberculatum str. 1-2	0.15-0.56	0.53-2.26	3.1-6.2	5.1-6.4	7.2-8.1	7.2-9.1
Ct. serratus str. 1-4	0.12-1.36	0.53-2.03	4.0-10.2	5.5-11.2	7.2-11.8	5.0-12.4
Non-keratinophilic fungi						
G. catenulatum	0.29	0.94	2.1	9.2	4.04	3.0
P. lilacinus	0.06	0.08	0.94	4.7	6.3	3.06
V. lecanii	0.34	0.42	1.74	4.14	4.17	4.56
V. psalliotae	0.38	1.17	1.58	4.5	5.45	4.12

Explanations: see Table 6

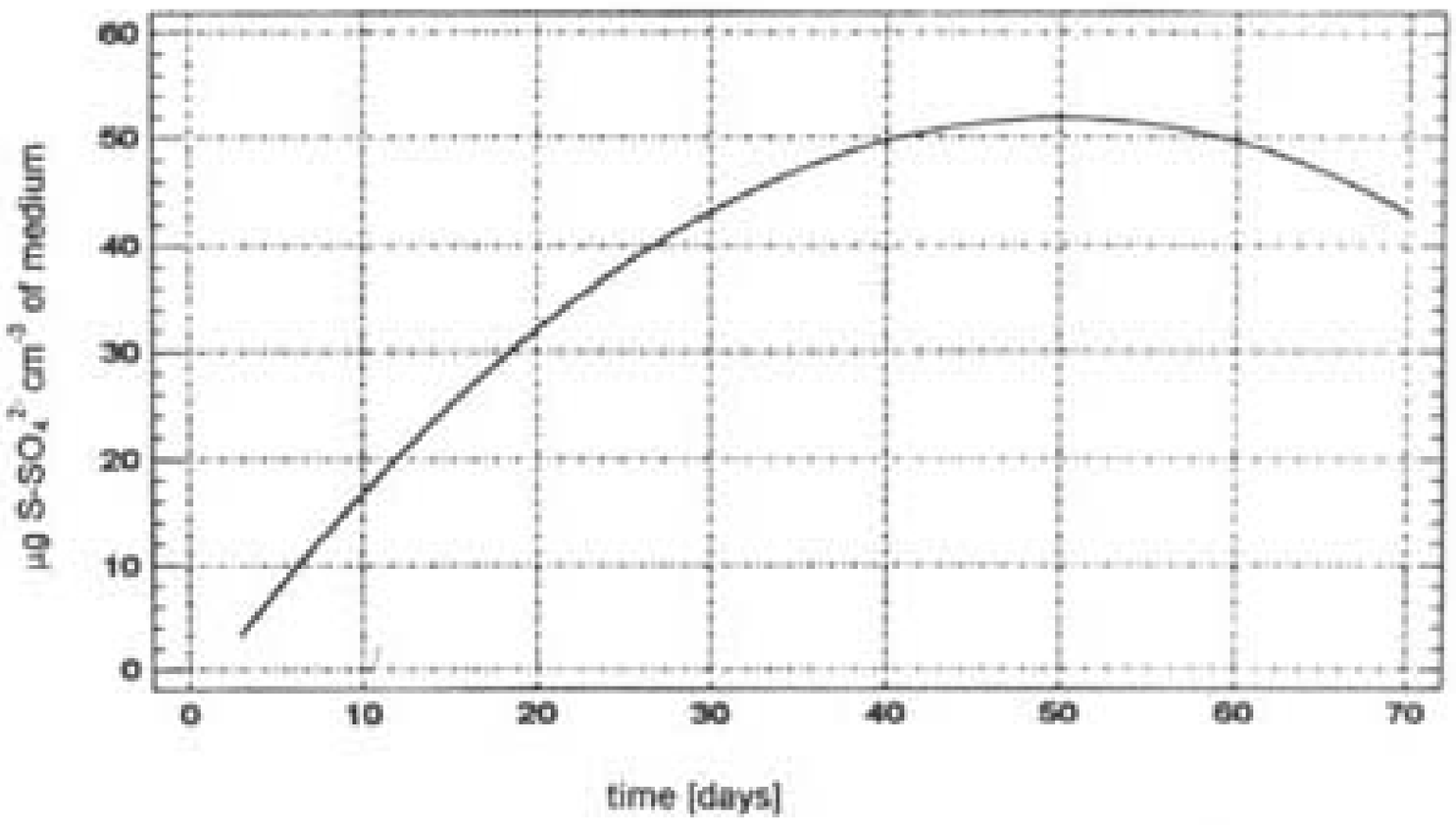


Fig. 4. Sulfate release in fungi cultures decomposing waste feathers ($R^2 = 54.8\%$)

about 30-50% of this component (Table 9), whereas non-keratinophilic fungi produced 2.8-4.0 mg $S\text{-}SO_4^{-2}$ g^{-1} of feathers, which constituted 8-11% of sulphur substrate.

It should be stressed that sulfate production was clearly visible only in the 2nd week of culture (Table 9, Fig. 4), i.e. in the period when the process of proteolysis and amonification were very advanced. In the later period sulfate release also quickly increased showing slight weakening at the end of the experiment. These observations were confirmed by the values of correlation coefficients between sulfate accumulation and peptide and N-ammonia accumulation (Table 5). Data presented in these Table show that there is a the relationship between sulfate release and keratin substrate mass loss.

C h a n g e s i n t h e pH of substrates in the fungi cultures decomposing waste feathers. The studies conducted showed the degradation of raw feathers by all the fungi strains studied was accompanied by alkalization of medium (Table 10, Fig. 5). As it was expected, alkalization of the medium was stronger in the keratinophilic fungi cultures (pH maximum 8.5-9.0) than in the non-keratinophylic ones (8.0-8.5) — Table 10. The highest increase in pH value of the substrate was noted in the phase of quick lysis, especially between the 7th and 14th day (Fig. 5)' and showed high positive correlation with the production of $N\text{-}NH_4$ (Table 5). In the next 3 weeks the pH of the medium underwent slight variations and then decreased slowly (Fig. 5), which was undoubtedly caused by the neutralization of NH_4^+ by sulfate and the escape of NH_3 (unpublished data). Changes in the pH values were significantly correlated with the decrease of feather mass (Table 5).

G e n e r a l m e t a b o l i c activity of keratinophilic fungi. General metabolic activity of these fungi (selected strains of dermatophytes and *Chrysosoporium*) was evaluated on the basis of their respiration activity and biomass growth in the medium containing feathers as the only source of C, N and S. It was found that fungi respiration as measured by the amount of CO_2 released was the strongest in the first 2 weeks of cultivation (Fig. 6). Activation of the respirometric activity was significantly correlated with the loss of substrate mass and peptide release (Table 5). It was calculated that in the period of increased respiration activity fungi oxidated from 8 to 16 mg of organic C in the feathers in 24 hours. In total, these microorganism transformed 70% of this component into mineral form and incorporated about 20% of organic C into mycelium. It was observed that the maximum of mycelium growth (Table 11) appeared in the period of the strongest lysis, deamination, and decarboxilation of the keratin substrate, i.e. between the 7th and 21th day of culture.

Table 10

Changes in the of pH medium in fungi cultures decomposing waste feathers (mean values from 3 repetitions)

Strain of fungus	Range					
	Days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1-2	6.7	7.1-8.0	8.8-9.0	8.6-9.1	8.6-9.1	8,4-8.9
M. cookei str. 1-2	6.0-6.7	7.1-7.5	8.1-8.5	8.3-8.5	8.0-8.5	7.8-8.2
T. ajelloi str. 1-4	6.7-6.8	7.1-7.9	8.5-8.7	8.5-8.7	8.4-8.7	8.3-8.5
T. terrestre str. 1-2	6.5-6.9	7.9-8.0	8.4-8.9	8.3-9.0	8.1-9.0	7.9-9.0
Chrysosporium group						
A. stercoraria	6.1	6.8	8.6	8.9	9.1	9.1
A. curreyi str. 1-2	6.7-6.9	7.6-7.7	8.1-8.6	8.3-8.7	8.1-8.7	7.9-8.9
Chrysosporium sp.	6.7	8.3	8.8	8.7	8.4	8.4
Ch. asperatum str. 1-4	6.9-7.1	7.1-8.8	8.6-8.8	8.6-8.7	8.4	8.4-8.5
Ch. europae	6,0	6.4	8.2	8.6	8.6	8.4
Ch. keratinophilum str. 1-2	6.6-6.9	7.1-7.5	8.7-8.8	8.6-8.7	8.7-8.8	8.3-8.4
Ch. kreiselii	6.7	7.0	8.6	8.3	8.6	8.5
Ch. pannicola str. 1-2	6.2-6.8	7.0-7.5	8.6-8.8	8.6-9.0	8.7-9.2	8.3-9.1
Ch. pruinosum str. 1-2	6.6-6.7	7.7-8.6	8.7-8.9	8.7-9.0	8.7-9.1	8.5-9.1
Ch. tropicum	6.7	7.7	8.7	8.7	8.7	8.4
Ch. tuberculatum str. 1-2	6.6-6.9	7.1-7.7	8.6-8.8	8.4-9.0	8.5-9.1	8.4-9.1
*Ct. serratus*str. 1-4	6.6-6.8	7.1-8.3	8.5-8.8	8.1-9.0	7.9-9.06	7.1-8.9
Non-keratinophilic fungi						
G. catenulatum	6.6	7.4	8.0	8.2	8.2	8.2
P. lilacinus	6.7	6.9	7.6	7.9	7.5	7.7
V. lecanii	6.6	7.0	8.0	8.0	8.2	8.2
V. psalliotae	6.6	7.3	8.0	8.2	8.4	8.2

Explanations: see Table 6

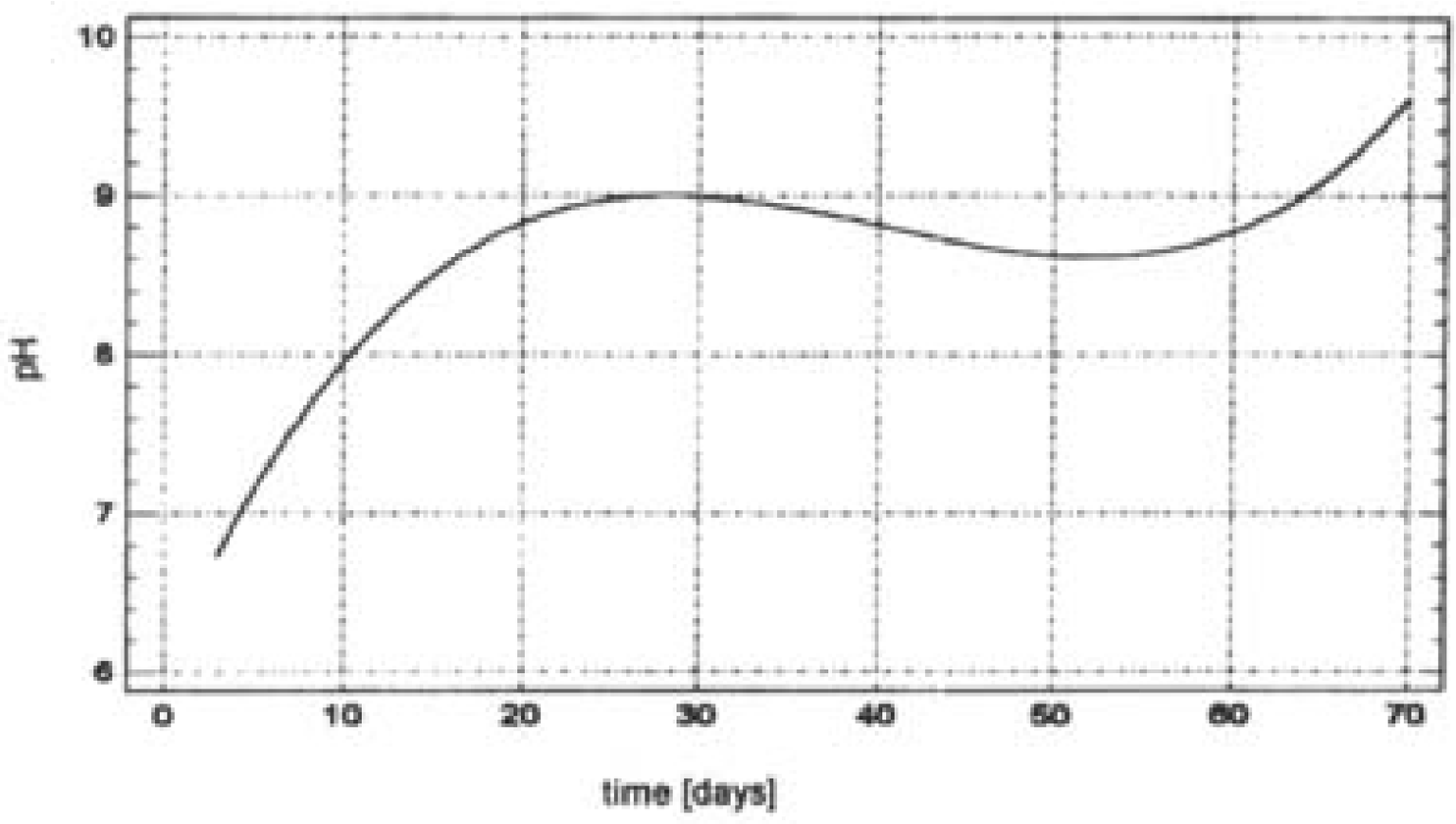
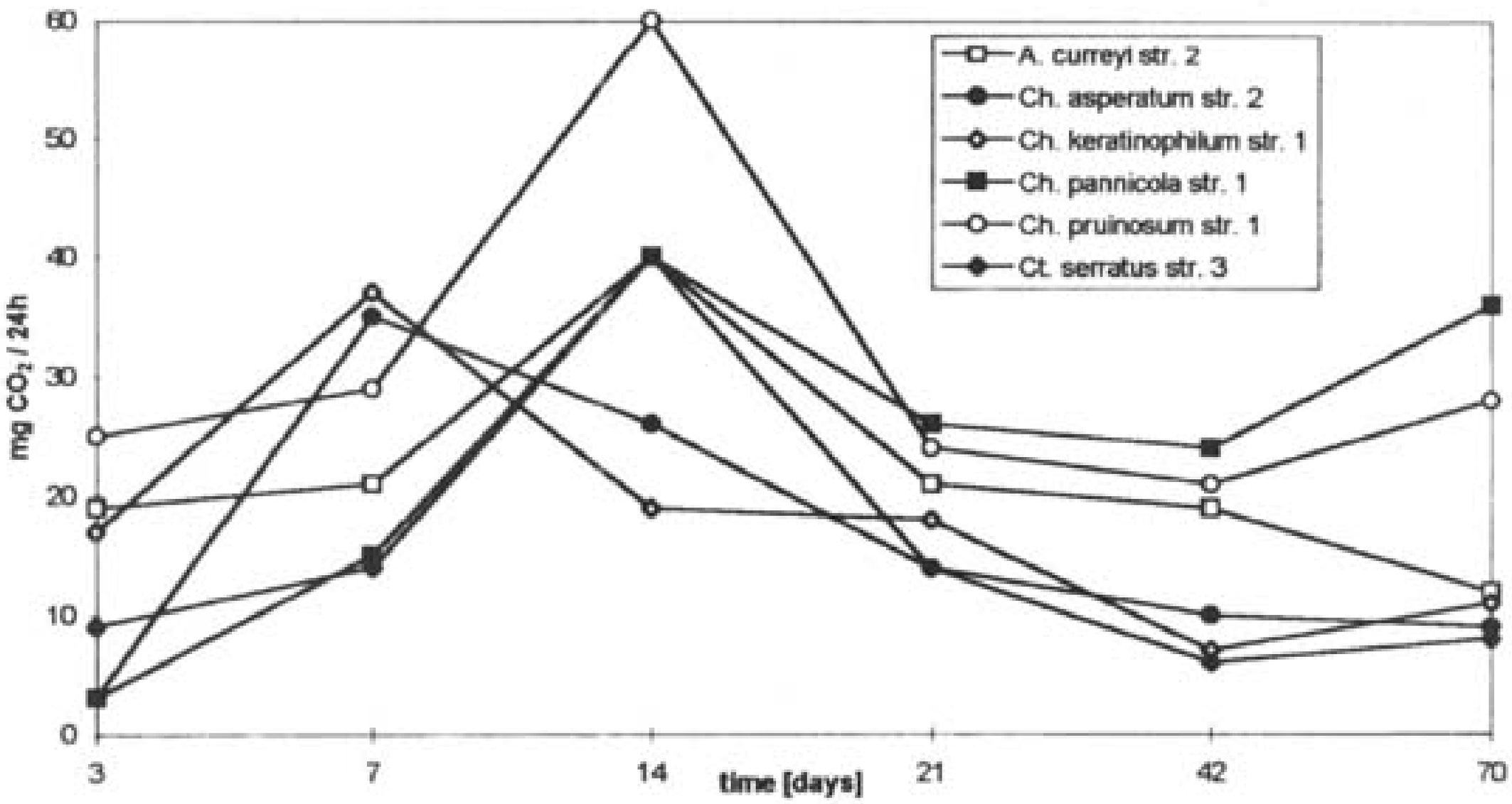


Fig. 5. Changes in the pH of medium in fungi cultures decomposing waste feathers ($R^2 = 51.8\%$)



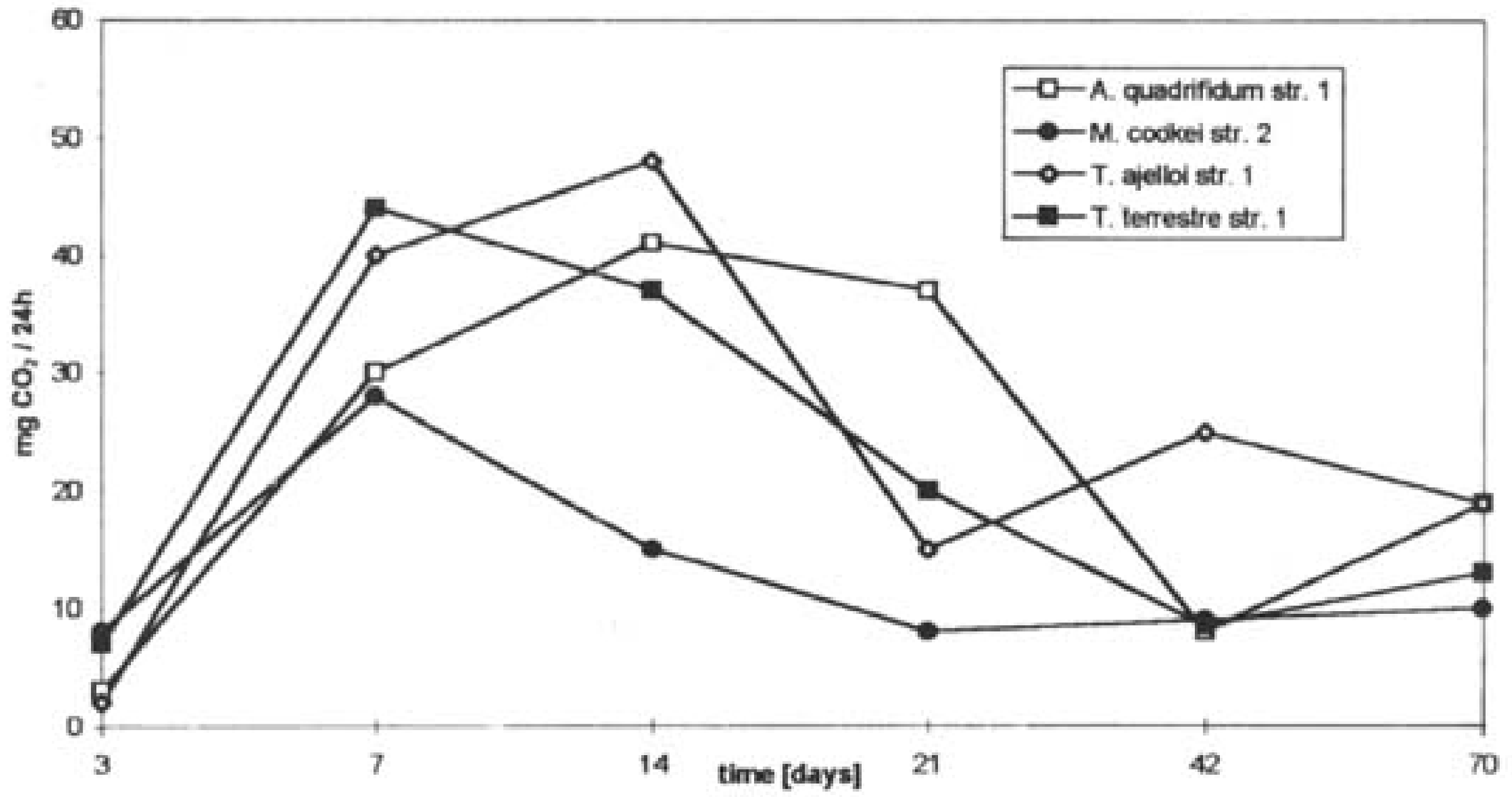


Fig. 6. Release of CO_2 in fungi cultures decomposing waste feathers

Table 11

Changes of mycelium biomass (mg d.m.) during decomposition of waste feathers

Strain of fungus	Range					
	Days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1	43	68	78	131	82	70
M. cookei str. 2	0	13	40	50	45	40
T. ajelloi str. 1	23	83	147	126	116	115
T. terrestre str. 1	11	37	86	121	91	82
Chrysosporium group						
A. curreyi str. 2	10	19	178	250	162	153
Ch. asperatum str. 2	9.5	30	40	60	63	162
Ch. keratinophilum str. 1	8	36	54	270	95	70
Ch. pannicola str. 1	8.5	11	27	103	95	83
Ch. pruinosum str. 1	11.5	39	53	153	138	123
Ct. serratus str. 3	10	26	61	67	87	150

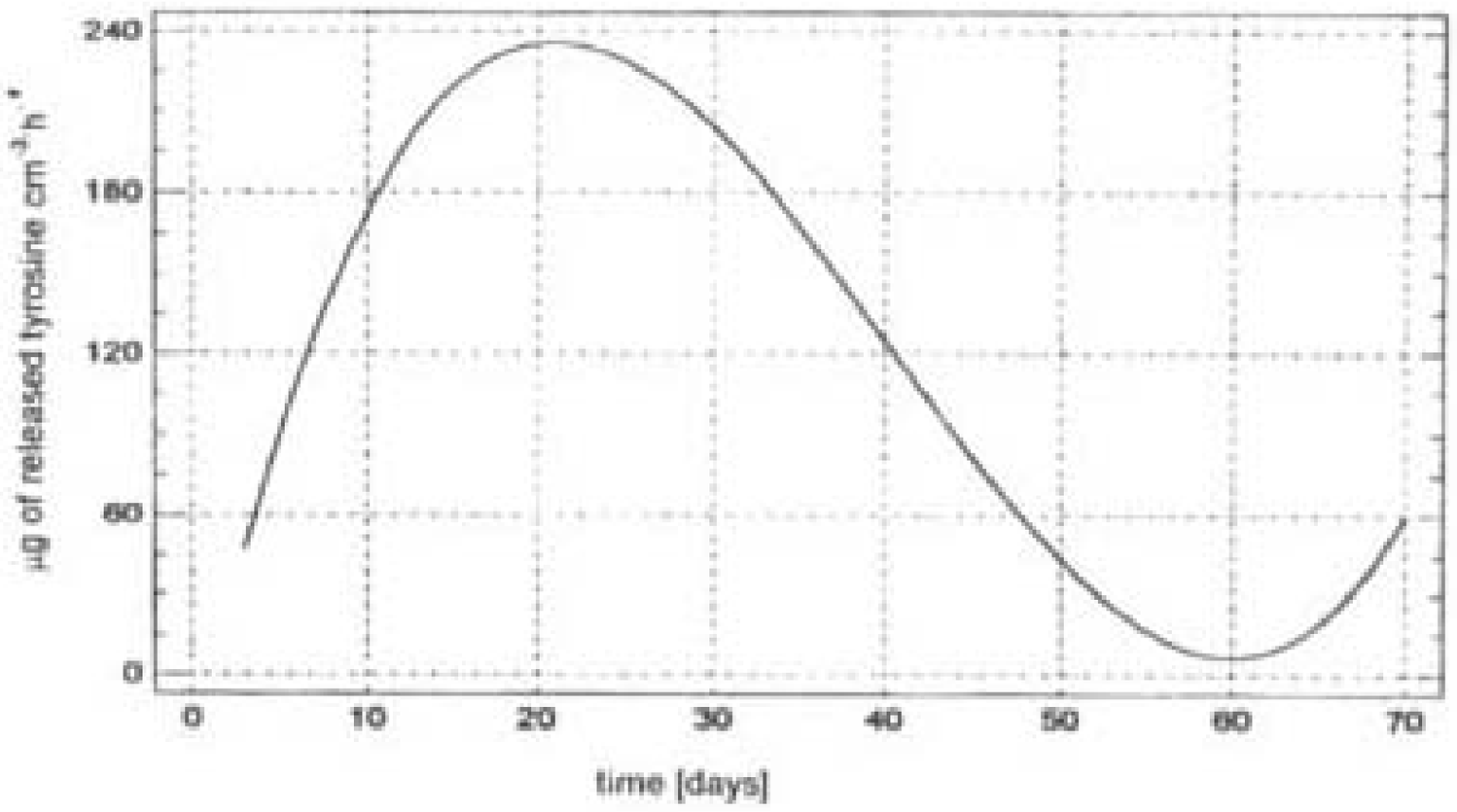


Fig. 7. Exoprotease activity of fungi decomposing waste feathers ($R^2 = 43.8\%$)

Proteolytic activity of after-culture filtrates of fungi decomposing waste feathers. The aim of this study was to determine the relationship between the dynamics of proteolytic activity changes and the rate of feather solubilization as measured by the release of peptide substances and substrate mass loss.

In the raw after-culture filtrates of all the strains studied the presence of intercellular proteases which were active in relation to casein was noted (Table 12; Fig. 7). The maximum of enzymatic activity was observed in the 3rd week of

Table 12

Protease activity (casein as a substrate) in after-culture fluids of fungi (in μg of released tyrosine cm^{-3} $hour^{-1}$ — mean values from 3 repetitions)

Strain of fungus	Range of activity					
	Days					
	3	7	14	21	42	70
Geophilic dermatophytes						
A. quadrifidum str. 1-2	21-60	74-157	70-166	170-206	66-88	59-70
M. cookei str. 1-2	19	69-84	114-147	66.7-91.0	42-54	24-96
T. ajelloi str. 1-4	21-96.7	55.7-177.0	101-367	201-323	78-130	8.35-111.0
T. terrestre str. 1-2	33.3-58.0	57-96	203-280	126-163	60-108	42-107
Chrysosporium group						
A. stercoraria	32	38	131	207	198	50
A. curreyi str. 1-2	18-34	52-131	178-372	178-227	126-186	100-111
Chrysosporium sp.	53,7	211	382	58	97	70
Ch. asperatum str. 1-4	29.3-62.0	91-311	153-449	65-383	86-134	73-126
Ch. europae	—	47.6	100	59	30	23
Ch. keratinophilum str. 1-2	—	24-30	63-209	261-267	84-130	62-86
Ch. kreiselii	2.9	25	381	371	188	25
Ch. pannicola str. 1-2	72-108	182-270	340-419	276-280	126.0-176.7	70.0-83.3
Ch. pruinosum str. 1-2	21	47.3-56.0	141-210	182-389	104-200	49-102
Ch. tropicum	—	50	210	388	190	85
Ch. tuberculatum str. 1-2	47	232-310	185-297	270-375	129-150	127-169
Ct. serratus str. 1-4	24-70	32-222	170-347	119-236	67-180	69-169
Non-keratinophilic fungi						
G. catenulatum	42	150	248	378	205	115
P. lilacinus	65	191	180	97	52	16
V. lecanii	55	108	97	114	58	51
V. psalliotae	137	243	357	326	160	63

culture when the loss of feather mass was also quickly increasing (Table 4). After this period in most of the cultures a rapid decrease of proteolytic activity appeared and discrepancies between this activity and the solubilization rate were discovered (Table 5).

Properties of keratinolytic after-culture filtrates of some *Chrysosporium* species and geophilic dermatophytes. These studies aimed at the evaluation of keratinolytic activity of fungi proteases and determination whether the secretion of these enzymes shows any relation to the dynamics of the keratin substrate decomposition. The above study was carried out on some strains which were grown in the substrate containing raw feathers as the only source of C and N.

Table 13

Activity of keratinase of after-culture fluids in enzymatic units (U) c^{-3} (mean values from 3 repetitions)

Strain of fungus	Time of cultivation					
	Days					
	3	7	14	21	42	70
Geophillic dermatophytes						
A. quadrifidum str. 1	1.0	4.0	2.0	3.0	3.0	1.5
M. cookei str. 2	0.1	1.0	0.5	0.3	0.6	2.0
T. ajelloi str. 2	1.0	3.0	1.5	1.4	0.2	2.0
T. terrestre str. 1	10	6.0	0.3	0.4	1.0	0.1
Chrysosporium group						
A. curreyi str. 2	1.0	2.0	1.4	10	2.0	1.0
Chrysosporium sp.	2.0	3.0	2.4	2.0	0.5	0.1
Ch. asperatum str. 2	1.0	1.4	2.0	0.2	2.0	0.1
Ch. keratinophilum str. 2	1.0	1.0	0.2	2.0	2.0	3.0
Ch. kreiselii	0.5	4.0	3.0	0.2	0.1	0.1
Ch. pannicola str. 1	2.0	1.3	1.0	3.0	1.5	1.0
Ch. pruinosum str. 1	0.5	4.0	2.0	3.0	1.0	0.3
Ch. tuberculatum str. 2	1.0	3,5	1.0	0.5	1.0	0.1
Ct. serratus str. 1	1.0	3.0	1.4	1.0	2.3	0.3

All the fungi studied secreted enzymes which catalysed „digestion" of feather proteins in the free-cells system (Table 13). The above fact was interpreted as an indirect proof of the existence of keratinolytic proteases in the after-culture fluids, called by various authors keratinase. Higher values of the so-called keratinase were mainly noted in the phase of rapid feather lysis, especially in the 7th day of their decomposition (Table 13).

Statistical evaluation of the interrelations with other physiological features showed the lack of significant correlation between exokeratinase activity and biodegradation of native feather keratin.

Statistical evaluation of the stability of feather decomposition parameters and fungi activity. It was thought advisable to determine the spectrum of variation of the physiological features for the evaluation of the parameters of fungi keratinolytic activity.

Individual parameters of substrate decomposition and biochemical activity of fungi showed high total variability (Table 14). Low values of the variability coefficients Cv (4-7%) were noted only in relation to the changes in the pH of substrate. In the remaining cases Cv values were high and often reached up to 50%. This was expressed by the higher value of Cv coefficients in the older

Table 14
Statistical evaluation of the parameter stability of feather decomposition and fungi activity

Days of cultivation	Parameter	Loss of substrate	Release of				Exoprotease activity	pH of medium
			peptides	$N\text{-}NH_2$	$N\text{-}NH_4^+$	$S\text{-}SO_4^{2-}$		
3	$\bar{X}$	0.014	38.93	0.423	9.82	3.29	41.9	6.66
	Cv	43	50	87	87	122	75	4
	h^2	70	95	96	97	99	97	93
7	$\bar{X}$	0.057	99.55	2.15	137.3	10.02	129.5	7.53
	Cv	56	49	61	71	96	69	7
	h^2	96	98	97	98	99	98	95
14	$\bar{X}$	0.143	199.30	5.29	340.1	24.4	228.3	8.55
	Cv	37	32	58	38	58	49	4
	h^2	96	91	98	99	99	98	90
21	$\bar{X}$	0.179	239.30	16.13	368.0	37.76	220.3	8.57
	Cv	32	33	38	34	56	47	3
	h^2	96	97	99	91	98	98	98
42	$\bar{X}$	1.227	275.0	5.52	294.3	50.4	114.5	8.57
	Cv	32	36	51	40	35	44	4
	h^2	98	98	99	82	98	98	99
70	$\bar{X}$	0.263	358.0	12.02	275.5	47.8	77.8	8.79
	Cv	28	26	46. 6	33	41	50	56
	h^2	98	98	99	96	98	97	99

Explanations: $\bar{X}$ – arithmetic mean; Cv – variation index in %; h^2 – coefficient h^2 in %

cultures, i.e. at least 21 days old. At the same time statistical calculations showed that the variability of individual features was caused by the differentiation of strains, which was confirmed by very high values of coefficient h^2 reaching as high as 95%.

DISCUSSION

In earlier studies (Korniłłowicz 1989, 1991/92) attention was drawn to the different interpretations found in literature of such phenomena as keratinophilness and keratinolyticism. Due to the lack of uniform criteria for evaluation of keratinolytic abilities and activities of saprotrophic micromycetes an attempt was made to select such criteria (Korniłłowicz 1994). According to some authors (Vries 1962; English 1963, 1965, 1969; Griffin 1960; Safranek, Goos 1982) keratinophilness is an ecological equivalent of keratinolyticism. While considering this problem, the present author took into consideration Mathinson's (1964) point of

view, who distinguished truly keratinolytic fungi from potentially keratinolytic ones. The latter are only characterised by strong proteolytic properties.

According to the adopted nomenclature all the species of *Chrysosporium* and geophilic dermatophytes selected for the experiment represented keratinolytic forms (total lysis of feathers). Non-keratinophilic fungi of which the lack the ability to complete lysis of feather keratin but clearly display abilities for the hydrolysis of ordinary proteins, were asumed to be potentially keratinolytic. The ability to utilize only "soft" keratin by these microorganisms enable their classification as poorly keratinolytic (E n g l i s h 1965). Fungi that are potentially keratinolytic can play a significant role in the transformation of these protein fractions which contain a lower level of disulfite groups ("soft" keratin) than "hard" keratin as they are well spread in the soil and other natural environments.

The indices obtained for measuring fungi keratinolytic activities confirmed that the most reliable ones were: the loss in mass of the keratin substrate, and coupled with it, the increase of content of peptides in the medium. This was demonstrated by the almost identical graph of the curvilinear regression function of both these features and a positive correlation between them observed during the whole study period. Another index that was correlated with keratin substrate decomposition was their production of ammonia and changes in the pH of medium. Moreover, a significant correlation, though rather low, with the utulization of native keratin was shown by the sulfate production. Correlation of the feature discussed with other indices, such as the content of amino residues and exoproteases activity, was less documented. In all the cases the values of correlation coefficients were differentiated in relation to the time of measurement. The latter factor determined the stability of the features studied. Statistical calculations disclosed that the variability of almost all the individual parameters of keratin substrate decomposition was caused by the strain differentiation. Without any doubt, physiological and morphological differences between species and strains, such as a germination rate, substrate overgrowth, structure of the eroding mycelium, and stages in the fungus development, played a decisive role in this respect. This hypothesis is based on the observation that feather lysis took place in a more intensive way in the quickly growing species than in the slow growers. Also the perfect stages were more active than the imperfect ones. E n g l i s h (1969) indicates that fungi which produced the so-called perforating organs (dermatophytes) were stronger de stroyers of the native keratin. Fungi with morphologically simpler structures had as a rule poorer keratinolytic properties. From the ecological point of view, an observation on the differentiation of keratinolytic activity of strains in relation to their origin is very interesting. Higher activity was shown by the strains selected from the neutral and slightly alkaline soils, than from the acidic soils. This latter observation could well explain the reason for the antagonism

in the populations of soil keratinomycetes, which was reported earlier by the present author (K o r n i ł ł o w i c z 1993a). The information gathered showed that while looking for the keratinolytically active fungi one must concentrate on the environments with the pH level favouring keratinolysis.

In the course of the present study it was found that the degradation of keratin waste under the influence of fungi leads to the release of significant amounts of easily hydrolysing peptide bundings. Despite the fact that fungi feather lysates are surely a better proposition than the commercial meal of feathers as far as digestible amino acids are concerned, their high ammonia content makes them impossible to used as fodder.

In the experimental setting the ammonia production reached 50-70% of feather N-NH_3. As far as productivity was concerned, microorganisms the studied resembled strongly keratinolytic actinomycetes *Streptomyces fradiae* (N i c k e r s o n, N o v a l, R o b i n s o n 1963). Accumulation of N-NH_4^+ in the after-culture medium of the studied fungi caused its alkalization.

On the basis of the studies conducted it also follows that the lysates obtained by the fungi transformation of waste feathers are characterised by a considerable content of sulphur forms that are available to plants. The keratinophilic fungi studied transformed into sulfate from 30-50% of S from organic feathers. No toxic release of hydrogen sulfite was observed.

Intensive mineralization of nitrogen and sulphur and alkaline reaction of lysate indicate the utilization of keratinolytically active fungi in the processing of waste feathers through the composting together with plant material as the carbon component. Since keratin from feathers is mainly colonized by the saprophytic fungi (K o r n i ł ł o w i c z 1991/92, 1993, 1995) additional introduction of active strains should limit the development of forms that are pathogenic to animals and humans. Confirmation of the above assumption would require additional studies and observations on the course of waste feather mass decomposition under the condition of composting.

In the present experiment it was found that the changes in the raw keratin waste in the saprotrophic fungi cultures were connected with the synthesis of proteolytic enzymes released extracellularly. Rapid hydrolysis of casein by the cells-free after-culture filtrates pointed to the high activity of these enzymes in relation to ordinary protein. Cells-free after-culture filtrates were characterised by the low activity in relation to native feather keratin. In this respect the studied saprotrophes closely resemble fungi that cause skin disease (C h a t t a w a y, E l l i s, B a r l o w 1963); W a w r z k i e w i c z, Ł o b a r z e w s k i, W o l s k i 1987; G r z y w n o w i c z et al. 1989). On the other hand this feature differentiated them from the keratinolytic actinomycetes (G a l a s, K a ł u ż e w s k a 1989; K a ł u ż e w s k a, W a w r z k i e w i c z, Ł o b a r z e w s k i 1991) whose afer-culture filtrates totally dissolved feathers, hair, wool and other horny products of the skin. The above

differences can be explained by the different keratinolytic mechanisms of actinomycetes and fungi; which was stressed by K u n e r t (1989). N i c k e r s o n et al. (1989) showed that actinomycetes decompose native keratin (wool) exclusively by means of enzymes. Biodegradation of native keratin by fungi (wool and hair) however, involves the mechanical destruction of substrate in the presence of the mycelium eroding complex and production of inorganic sulphite which takes part in the destruction of disulfite bonds (E n g l i s h 1963, 1965; P a g e, S t o c k 1974; K u n e r t 1972, 1973, 1976; R u f f i n et al. 1976; W a w r z k i e w i c z, Ł o b a r z e w s k i, W o l s k i 1987).

The lack of clear correlation between the fungi enzymatic activity and decomposition degree of the native keratin point to the role of non-enzymatic factors in fungi keratinolysis (feather keratin).

The lysis of feathers in fungi cultures is initiated by the enzymatic digestion of simple proteins, which was proved by the positive correlation between proteases activity (measured in relation to casein) and peptide release, with a simultaneously negative interrelation of this feature and sulfate production that was observed in the first days of decomposition. Also the quick increase of respiration activity pointed to the utilization of more accessible sources of carbon and energy in this period. It seems that the assimilation of food which is available more easily by fungi spores germinating on the surface of feathers enabled the production of the structures specialised in the decomposition of native keratin. This is in agreement with the findings of P a g e and S t o c k (1974). The mentioned authors indicated that *Microsporum gypseum* during the process of hair decomposition first hydrolysed the protein of cell membrane, which exposed disulfite bonds of the intercellurally located keratin and induced its sulfitolysis (hydrolysis of the S-S bridges). This process took place with the eroding complex of mycelium (K u n e r t 1972). The process of sulfitolysis undoubtedly leads to the loosening of the structure of keratin substrate which, in turn, enables further penetration of substrte by mycelium. Other studies author (K o r n i ł ł o w i c z 1996) showed that extracellular proteases of fungi take over digestion functions of the denatured keratin. The fact that the changes recorded concerned keratin component was confirmed by the stimulation of sulfate release and peptide accumulation correlated with it.

A rapid decrease of proteases activity was probably due to the inhibition of the enzyme activity caused by the accumulation of the decomposition products. C o h e n (1972; 1973a, b) indicated that the products of protein proteolysis and ammonification were inhibitors of fungi proteases, also of the fungi which took part in the process of natural keratin degradation. This was confirmed by the present results indicating that the inhibition of the proteolytic fungi activity was generally coupled with a strong decrease in their respiration activity. This points to the stage of transformation of the culture from the phase of intensive growth and quick substrate degradation into the stationary

phase characterised by lower down decomposition rate. Further changes of feather keratin probably required modifications of the enzymatic apparatus of fungi adapted to the changes taking place in the substrate. This can account for the reoccurring acceleration of keratinolysis in the later period of fungi growth.

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Badania nad rozkładem odpadów keratynowych przez saprotroficzne mikromycetes. I.

S t r e s z c z e n i e

Celem prezentowanej pracy jest ocena uzdolnień i aktywności keratynolitycznej saprotroficznych grzybów glebowych z jednoczesnym ustaleniem jednolitych kryteriów oceny tej aktywności.

W badaniach wykorzystano własną kolekcję grzybów (37 szczepów) wyodrębnionych głównie z gleb uprawnych. Doświadczenia prowadzono w hodowlach płynnych stosując keratynę odpadową (pióra kurcząt) jako jedyne źródło C, N i S. Okresowe analizy hodowli grzybów obejmowały: określenie stopnia wykorzystania substratu, oznaczenie w przesączach pohodowlanych ilości substancji peptydowych, grup aminowych, N-amonowego i siarczanów oraz pH podłoża. Ponadto określono ogólną aktywność metaboliczną hodowli, biomasę wyrosłej grzybni oraz aktywność proteo- i keratynolityczną przesączy pohodowlanych. Uzyskane dane poddano ocenie statystycznej.

Stwierdzono, że saprotroficzne grzyby glebowe wykorzystujące natywną keratynę jako źródło C, N i S i energii można podzielić na typowo keratynolityczne, tj. całkowicie rozkładające te skleroproteiny oraz potencjalnie lub słabo keratynolityczne czyli niezdolne do kompletnej solubilizacji tych substratów. Wyznaczone jednolite kryteria aktywności keratynolitycznej grzybów obejmowały: ubytek masy substratu, uwalnianie substancji peptydowych i amoniaku, wydzielanie siarczanów i alkalizację podłoża. Stwierdzono, że degradacja odpadów keratynowych w stacjonarnych hodowlach grzybów przebiega dwufazowo. W fazie I określonej jako szybkiej lizy upłynnieniu ulegało 50% substratu. Faza II nazwana powolną rozpoczynała się zahamowaniem tempa rozkładu substratu, a następnie wyraźnym przyspieszeniem przyczyniającym się do kompletnej solubilizacji natywnej keratyny.