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Occurrence of fungi degrading aniline and its derivatives in biocenoses of wastewater treatment systems

TOMASZ SŁOMCZYŃSKI, ANNA GRABIŃSKA-ŁONIEWSKA

Institute of Environmental Engineering Systems, Warsaw University of Technology,
Nowowiejska 20, 00-653 Warsaw, Poland

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It has been found that numerous yeast-like microorganisms from the genera *Geotrichum, Trichosporon, Candida, Rhodotorula* and *Sporobolomyces* occurring in wastewater treatment systems biocenoses are able to utilize aniline, p-nitroaniline and acetanilide as a sole C-source. High active in this respect were strains belonging to the species of *Geotrichum candidum, G. sericeum* and *Candida boidinii.*

Key words: yeast-like microorganisms, biodegradation, aromatic amines.

INTRODUCTION

Aromatic amines such as aniline ($C_6H_5NH_2$), p-nitroaniline ($NO_2C_6H_4NH_2$) and acetanilide ($CH_3CONHC_6H_5$) are the components of wastewaters from the production of organic dyes, photographic reagents, aromatic substances and drugs. These chemicals belong to nonreadily biodegradable compounds. It is manifested by a low values of maximum specific substrate removal rate (q_{max}) given by C h u d o b a and P i t t e r (1990), amounted (mg g^{-1} h^{-1}) 19.0 for aniline and 14.7 for acetanilide[1]. Therefore, they can accumulate in the environment and thus impair natural circulation of elements and cause a restriction of self-purification process in surface waters.

These hindrances could be eliminated by biological degradation of above pollutants in wastewater treatment plants. The highly efficient technologies applying acclimatized and selected microorganisms should be recommended for this purpose. This study tends to the selection of highly active strains from the wasterwater treatment units mycoflora which could be used in enhancing the removal of aniline, p-nitroaniline and acetanilide during wastewaters purification.

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[1] For very readily biodegradable compounds e.g. glucose q_{max} is equal 180.0 and phenol – 90.0 mg g^{-1} h^{-1}.

MATERIALS AND METHODS

The isolates assayed in this study originated from the biocenoses of different wastewater treatment systems (Grabińska-Łoniewska, Sláviková, 1990; Grabińska-Łoniewska et al., 1993).

Their ability of utilization of the tested aromatics as a sole C-source for growth was examined according to method given in our previous paper (Grabińska-Łoniewska et al., 1995). The concentrations (mgl^{-1}) in a basal medium were: aniline-150, acetanilide-100 and p-nitroaniline-50. These were demonstrated earlier by Furmańska (1990) as a readily biodegradable by activated sludge microorganisms.

RESULTS AND DISCUSSION

Actually no data concerning the biodegradation of aniline, p-nitroaniline and acetanilide by fungi are available. Studies of Furmańska (1990) were devoted to the biodegradation of these compounds by mixed cultures of activated sludge microorganisms. The main component of these biocenoses were bacteria. It was evidenced by Grabińska-Łoniewska et al. (1993) who used the same activated sludge samples and proved that quantity of microscopic fungi in these biocenoses didn't exceed 4-8 CFU mg^{-1}. The findings of Furmańska (1990) has led to establishing the following order of these compounds according to their decreasing biodegradability rate: aniline > acetanilide > p-nitroaniline. It corresponds with the values of theoretical oxygen demand (TOD) for these compounds equal to 3.01; 2.54 and 1.97 mg O_2, respectively.

The importance of the elimination of the above compounds from industrial discharges is confirmed by the fact that aniline being the lowest toxic compound among these aromatics, exhibit the growth and multiplication fishes already at concentration of 100 mgl^{-1}, crustaceans *Daphnia* sp. – 0.4 mgl^{-1}, and algae *Scenedesmus* sp. – 10 mgl^{-1}. The nitrification process occurring in natural waters is restricted at the concentration of 8 mgl^{-1}. It follows that the concentration of aniline in wastewaters discharged to water reservoirs should not exceed 0.1 mgl^{-1} (Grady, Dang, 1989).

In our study we tested for their ability to degrade aniline, p-nitroaniline and acetanilide overall 36 of isolates originated both from activated sludges being grown by Furmańska (1990)[2] and another ones (Grabińska-Łoniewska, Sláviková, 1990; Grabińska-Łoniewska et al., 1993).

It has been found that among the 25 tested isolates, 19 were able to utilize aniline as a sole C-source (Table 1, Fig. 1). The best growth on this compound was noted for *Geotrichum candidum* strains A2D1 and NA2D2 (cell yield after 7 days of incubation, comparing with control cultures of 816 and 300 %, respectively). *G. sericeum* strain P5D5 (cell yield – 377 %) as well as *Candida boidinii* strains M3D3, M18D3 and M4D3 (560, 495 and 239 %, respectively).

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[2] Designated by the letters A, NA, and AC

Table 1

Growth response of various yeast-like microorganisms to aniline
(evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name		Number of CFU 10^6 ml^{-1} after 7 days of incubation at 26°C	
		control cultures (growth medium without C-source)	growth medium with aniline as a C-source
Geotrichum candidum Link	strain A2D1	0.62	5.68
	strain A2D3	1.52	2.10
	strain AC2D3	1.52	2.50
	strain NA2D2	2.75	3.86
	strain NA2D5	1.20	4.80
	strain O7D1	1.48	2.06
Geotrichum klebahnii (Stautz) Morenz	strain P6D6	3.62	4.06
Geotrichum sericeum (Pelaez et Ramirez) von Arx	strain P5D5	1.70	8.12
Trichosporon cutaneum (de Beurmann, Gougerot et Vaucher) Ota	strain M5D5	2.64	5.60
Candida boidinii Ramirez	strain M3D3	0.80	5.28
	strain M4D3	1.14	3.87
	strain M18D3	0.60	3.57
Candida famata (Harrison) Meyer et Yarrow	strain DCZ-1	4.60	5.20
	strain O5D4	0.90	1.97
	strain M17D6	3.97	4.20
Candida lambica (Lindner et Genoud) van Uden et Buckley	strain O5D5	2.50	7.82
	strain O7D7	4.90	8.00
	strain O14D7	6.20	10.57
Candida tropicalis (Castellani) Berkhout	strain O11D8	4.40	9.20
Candida inconspicua (Lodder et Kreger – van Rij) Meyer et Yarrow	strain O9D7	4.10	7.82
Candida maltosa Komagata, Nakase et Katsuya	strain M6D7	5.88	6.46
Sporobolomyces lactosus Slávikova et Grabińska-Łoniewska	strain P3D2	1.26	1.74
Rhodotorula rubra (Demme) Lodder	strain M11D4	1.57	1.85
	strain Ml8D3	2.26	5.60

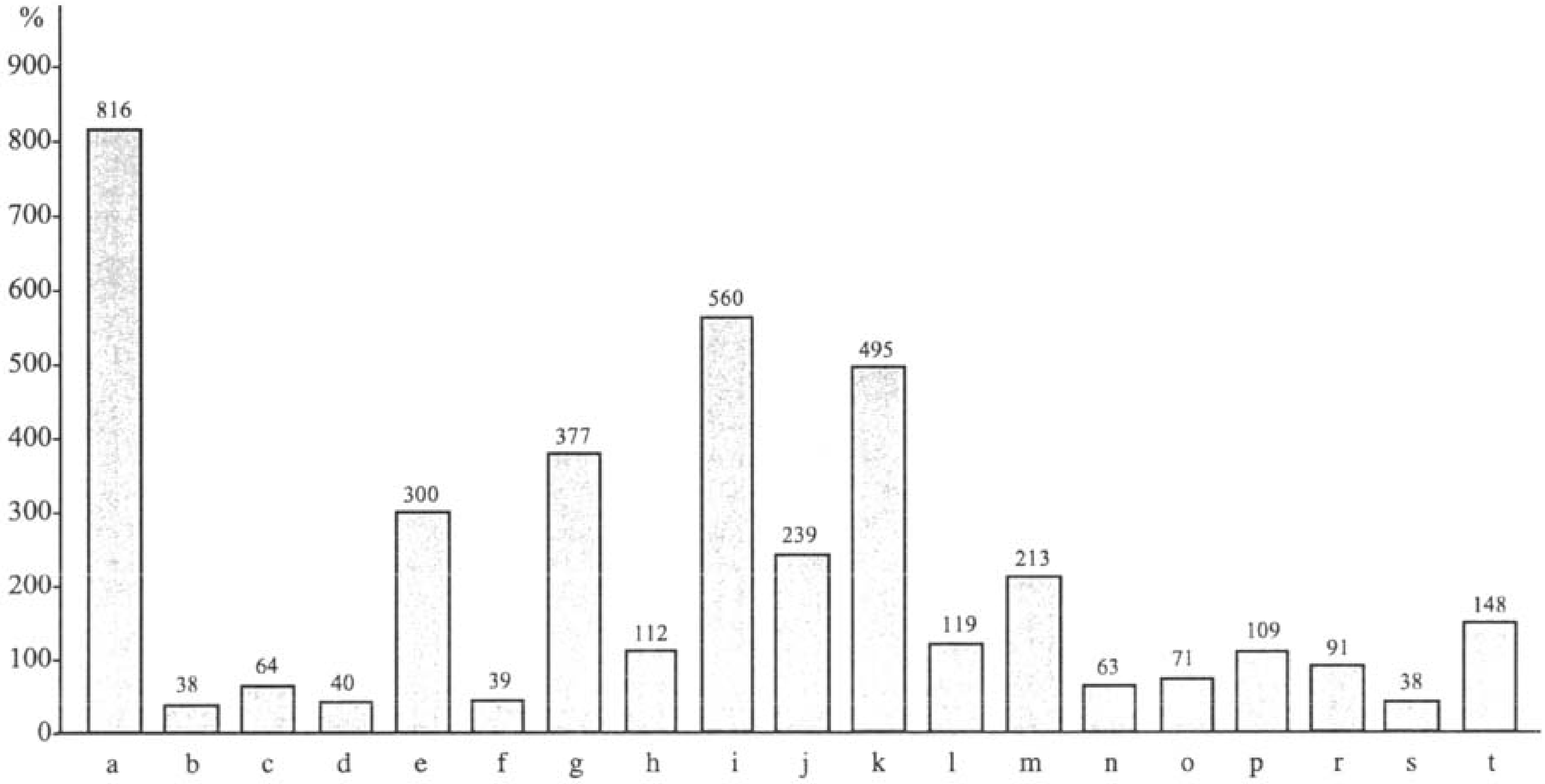

Fig. 1. Comparison of the intensity of growth of different yeast-like strains on aniline as a sole C-source
(A percentage CFU yield in the growth medium comparing with control cultures)

Geotrichum candidum strain A2D1 (a), strain A2D3 (b), strain AC2D3 (c), strain NA2D2 (d), strain NA2 D5 (e), strain O7G1 (f); *G. sericeum* strain P5D5 (g); *Trichosporo cutaneum* strain M5D5 (h); *Candida boidinii* strain M3D3 (i), strain M4D3 (j), strain M18D3 (k); *Candida famata* strain O5D4 (l); *Candida lambica* strain O5D5 (m), strai O7D7 (n), strain O14D7 (o); *Candida tropicalis* strain O11D7 (p); *Candida inconspicua strain* O9D7 (r); *Sporobolomyces lactosus* strain P3D2 (s); *Rhodotorula rubr* strain M18D3 (t)

Table 2

Growth response of various yeast-like microorganisms to acetanilide
(evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name		Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C	
		control cultures (growth medium without C-source)	growth medium with acetanilide as a C-source
Geotrichum candidum Link	strain A2D1	0.62	3.57
	strain A2D2	0.76	1.15
	strain A3D4	2.26	2.74
	strain NA2D5	2.75	4.12
	strain NA2D5	1.20	1.86
Geotrichum klebahnii (Stautz) Morenz			
	strain P6D6	3.62	9.12
Geotrichum sericeum (Pelaez et			
Ramirez) von Arx	strain P2D2	2.60	10.80
	strain P5D4	5.68	7.64
	strain P5D5	1.70	10.72
Candida boidinii Ramirez	strain M3D3	0.80	3.18
	strain M4D3	1.14	6.16
	strain M18D3	0.60	6.16
Candida famata (Harrison)			
Meyer et Yarrow	strain DCZ-1	4.60	5.20
	strain O5D4	0.90	2.35
	strain M17D6	3.97	4.37
Candida lambica (Lindner et Genoud)			
van Uden et Buckley	strain O5D5	2.50	4.02
	strain O14D7	6.20	7.44
Candida inconspicua (Lodder et Kreger			
– van Rij) Meyer et Yarrow	strain O9D7	4.10	4.32
Candida maltosa Komagata,			
Nakase et Katsuya	strain M6D7	5.88	8.10
Sporobolomyces lactosus Slávikova			
et Grabińska-Łoniewska	strain P3D2	1.26	2.60
Rhodotorula rubra (Demme) Lodder			
	strain M11D4	1.57	1.72
	strain Ml8D3	2.26	2.64
Hansenula californica (Lodder)			
Wickerham	strain Ml6D3	1.42	2.10

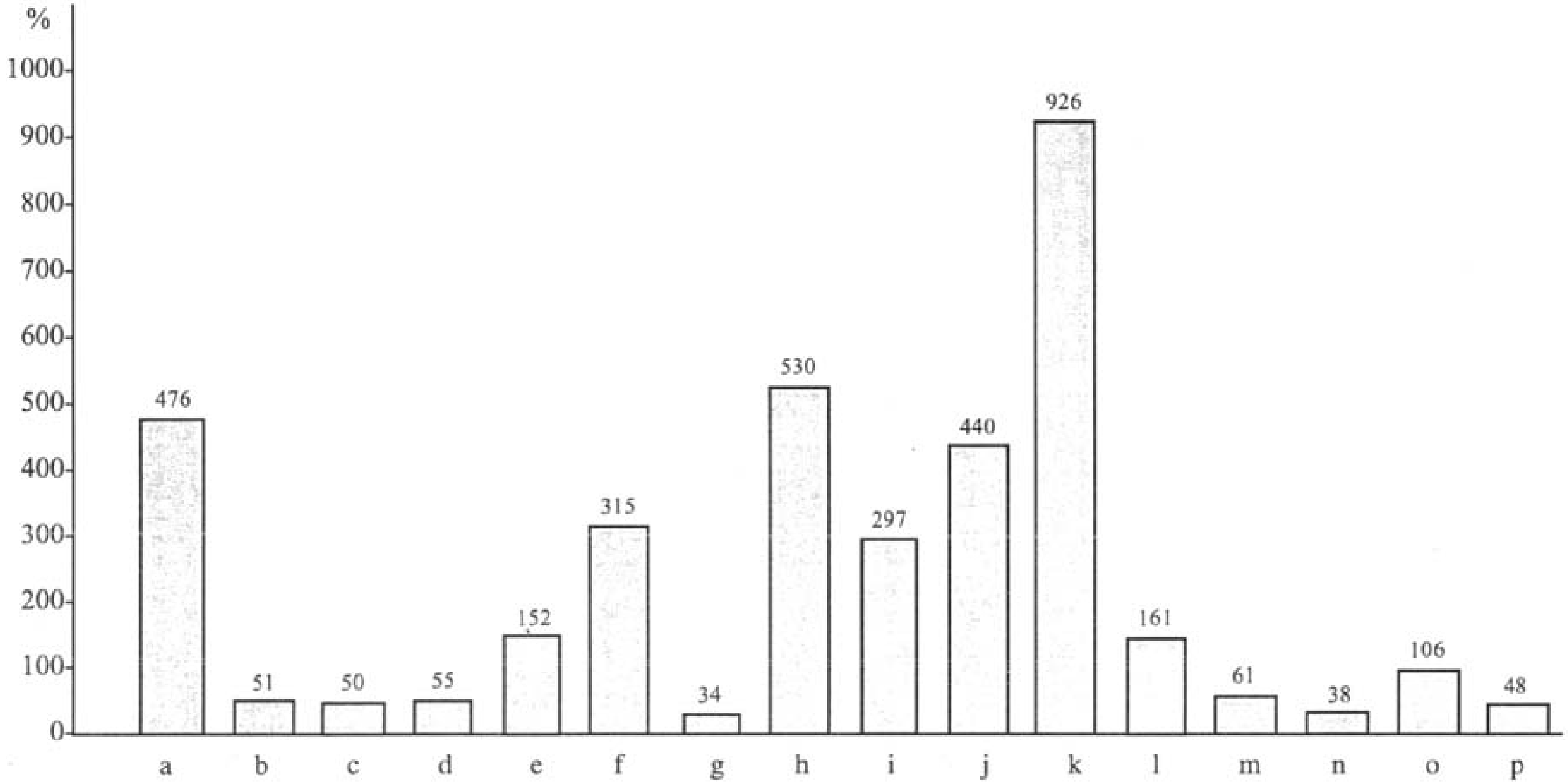

Fig. 2. Comparison of the intensity of growth of different yeast-like strains on acetanilide as a sole C-source
(A percentage CFU yield in the growth medium comparing with control cultures)

Geotrichum candidum strain A2D1 (a), strain A2D2 (b), strain NA2D2 (c), strain NA2 D5 (d); *Geotrichum klebahnii* strain P6D6 (e); *Geotrichum sericeum* strain P2D2 (f), strain P5D4 (g), strain P5D5 (h); *Candida boidinii* strain M3D3 (i), strain M4D3 (j), strain M18D3 (k); *Candida famata* strain O5D4 (l); *Candida lambica* strain O5D5 (m); *Candida maltosa* strain M6D7 (n); *Sporobolomyces lactosus* strain P3D2 (o); *Hansenula californica* strain M16D3 (p)

Table 3

Growth response of various yeast-like microorganisms to p-nitroaniline
(evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name		Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C	
		control cultures (growth medium without C-source)	growth medium with p-nitroaniline as a C-source
Geotrichum candidum Link	strain A2D1	0.62	1.90
	strain A2D2	0.76	1.10
	strain NA2D2	2.75	7.64
	strain NA2D5	1.20	2.20
	strain AC2D3	1.52	1.86
Geotrichum sericeum (Pelaez et Ramirez) von Arx	strain P5D5	1.70	11.30
Trichosporon cutaneum (de Beurmann, Gougerot et Vaucher) Ota	strain M5D5	2.64	4.20
Candida boidinii Ramirez	strain M3D3	0.80	2.80
	strain M4D3	1.14	1.56
	strain M18D3	0.60	2.88
Candida famata (Harrison) Meyer et Yarrow	strain DCZ-1	4.60	5.28
	strain M17D6	3.97	8.12
Candida lambica (Lindner et Genoud) van Uden et Buckley	strain O5D5	2.50	4.06
	strain O7D7	4.90	9.12
	strain O14D7	6.20	6.84
Candida tropicalis (Castellani) Berkhout	strain O11D7	4.40	10.40
Candida maltosa Komagata, Nakase et Katsuya	strain M18D7	3.41	3.62
Rhodotorula rubra (Demme) Lodder	strain M4D4	3.92	4.03
	strain M11D4	1.57	2.26
	strain Ml8D3	2.26	4.20
Hansenula californica (Lodder) Wickerham	strain M18D5	4.90	5.42

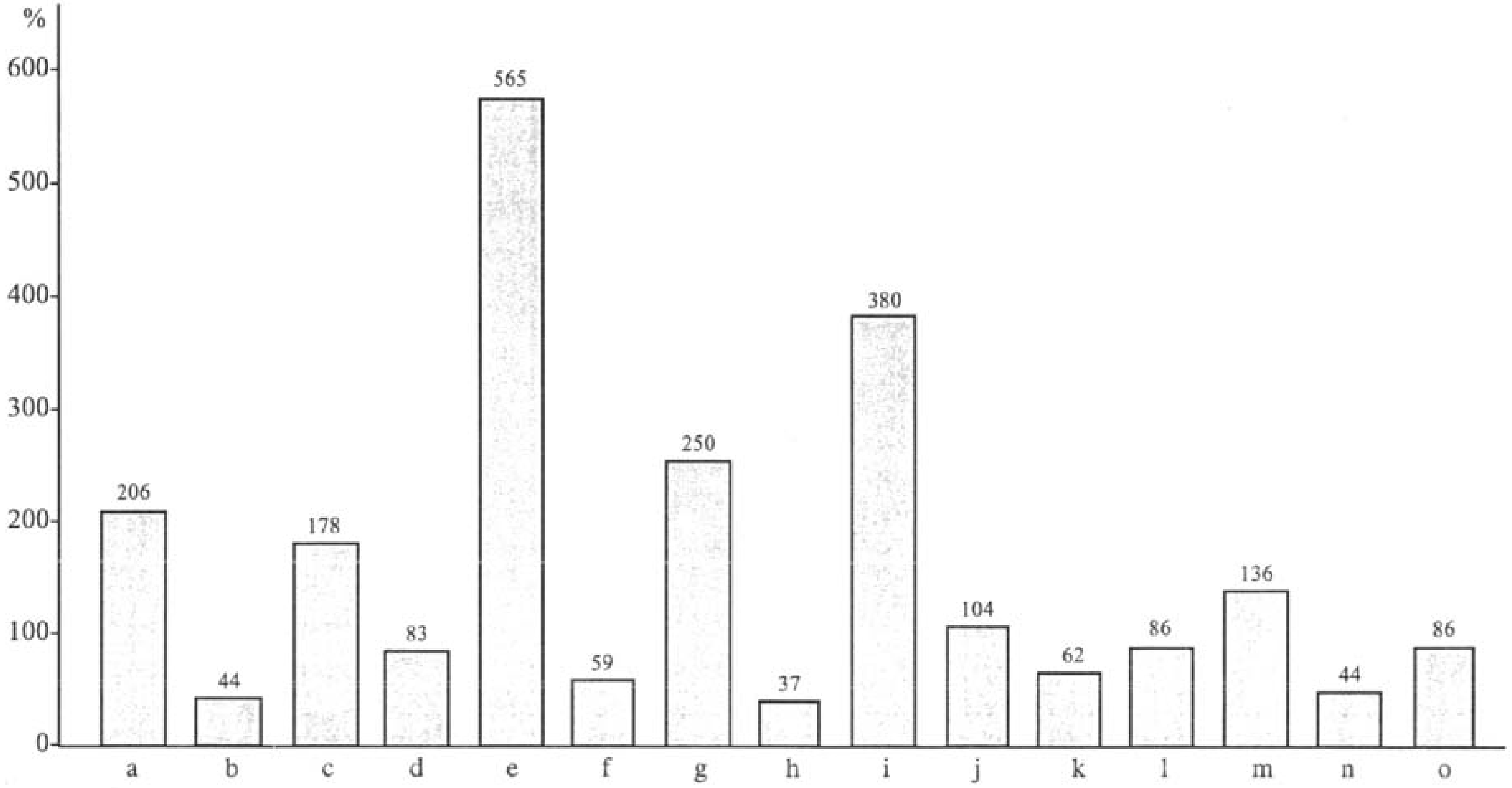

Fig. 3. Comparison of the intensity of growth of different yeast-like strains on p-nitroaniline as a sole C-source
(A percentage CFU yield in the growth medium comparing with control cultures)

Geotrichum candidum strain A2D1 (a), strain A2D2 (b), strain NA2D2 (c), strain NA2 D5 (d); *Geotrichum sericeum* strain P5D5 (e); *Trichosporon cutaneum* strain M5D5 (f); *Candida boidinii* strain M3D3 (g), strain M4D3 (h), strain M18D3 (i); *Candida famata* strain M17D6 (j); *Candida lambica* strain O5D5 (k), strain O7D7 (l) *Candida tropicalis* strain O11D7 (m); *Rhodotorula rubra* strain M11D4 (n), strain M18D3 (o)

A similar intensity of growth was observed on acetanilide. Among the 16 of acetanilide-utilizers, the most active were: *Geotrichum candidum* strain A2D1, *G. sericeum* strains P5D5 and P2D2 (cell yield 476, 530 and 315 %, respectively) and *Candida boidinii* strains M18D3, M4D3 and M3D3 (926, 440 and 297 %, respectively) (Table 2, Fig 2.)

It was found that the same species namely, *Geotrichum candidum, G. sericeum* and *Candida boidinii* intensively metabolized p-nitroaniline. But it is also noteworthly, that the cell yield on this substrate was considerably less than on the aniline and acetanilide. This confirms the assumption of F u r m a ń s k a (1990) regarding the susceptibility of these aromatics on biodegradation. Utilization of p-nitroaniline was attributed to 15 of all 21 tested isolates (Table 3, Fig. 3). The best growth was found for *Geotrichum sericeum* strain P5D5 (cell yield 565 %), *G. candidum* strains A2D1 and NA2D2 (206 and 178 %, respectively) as well as *Candida boidinii* strains M18D3 and M3D3 (380 and 250 %, respectively). It is interesting that the most active of the tested aromatics-utilizers belonging to the genus *Geotrichum* were those isolated from activated sludges treating the wastewaters containing these pollutants (F u r m a ń s k a, 1990) or petrochemical wastes (G r a b i ń s k a et al., 1993) while representatives of species *Candida boidinii* originated from anaerobic denitrifying biocenoses (G r a b i ń s k a - Ł o n i e w s k a, S l á v i k o v á, 1990).

Basing on the achieved results the following strains can be recommended in wastewater treatment process for enhancing the removal of:

- aniline: *Geotrichum candidum* strain A2D1, *Candida boidinii* strains M3D3 and M18D3;
- acetanilide: *Geotrichum candidum* A2D1, *G. sericeum* strain P5D5, *Candida boidinii* strains M4D3 and M18D3;
- p-nitroaniline: *Geotrichum sericeum* strain P5D5 and *Candida boidinii* strain M18D3.

The studies on this topic are currently in progress.

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Występowanie grzybów rozkładających anilinę i jej pochodne w biocenozach urządzeń do oczyszczania ścieków

Streszczenie

Wykazano, że w biocenozach oczyszczających ścieki występują liczne grzyby z rodzajów *Geotrichum*, *Trichosporon*, *Candida*, *Rhodotorula* i *Sporobolomyces* zdolne do wykorzystywania aniliny, p-nitroaniliny i acetanilidu jako jedynego źródła C. Najbardziej aktywne pod tym względem były gatunki *Geotrichum candidum*, *G. sericeum* i *Candida boidinii*.