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DEGRADATION OF 4-METHYLPYRIDINE BY *ARTHROBACTER* SP.

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Absrtact - In the process of degradation of 4-methylpyridine by bacterium *Àrthrobacter* sp. KM-4MP were isolated two metabolites and identified as 4-methylpyridin-2-ol and 8-hydroxy-4-methylidene-1H-cyclopenta[1,2-b:3,4-c']dipyridine-2,3,5,7(4H,6H)-tetrone.

INTRODUCTION

Development of the chemical industry led to the contamination of the biosphere by the hazardous substances. Very harmful pollutants are heterocyclic organic compounds. Derivatives of pyridine are an important class of heterocyclic compounds. They contained in wastewater of chemical plants, plants of the production of synthetic rubber, plastics, dyes (Dobson *et al.*, 1985; Pereira *et al.*, 1988; Rogers *et al.*, 1985). Pure pyridines are widely used as reactants in the production of agricultural chemicals, such as herbicides and also pharmaceuticals (Kaiser *et al.*, 1996; Khasaeva and Parshikov. 2015; Parshikov and Khasaeva, 2015; Parshikov, 2015a,b).

MATERIALS AND METHODS

The object of the researches served the strain of the bacterium *Àrthrobacter* sp. KM-4MP obtained from the collection of microorganisms of the Department of Microbiology, Moscow State University.

To study of the degradation of 4-methylpyridine (I) was used the synthetic medium having the following composition (g/L): Na_2HPO_4 – 4.26; KH_2PO_4 – 2.65; $MgSO_4 \cdot 7H_2O$ – 0.2; $FeSO_4 \cdot 7H_2O$ – 0.01; $CaCl_2 \cdot 2H_2O$ – 0.02; $MnSO_4 \cdot H_2O$ – 0.002; Na_2MoO_4 – 0.001; deionised water – 1 L; pH 7.0 – 7.2. Cultivation was carried out in flasks (750 ml) with 200 ml of a medium on a shaker (200 rpm/min) at 28-30°C.

As the source of carbon and nitrogen in the liquid medium was added 1.5 g/L 4-methylpyridine. The degradation process was performed for 36 hours.

Degradation products were extracted with chloroform and after evaporation were dissolved in 0.5-1.0 ml of ethanol and had been conducting separating on chromatographic plates of "Silufol UV-254" (DC-Alufolies Kieselgel 60 F254, Merck, Germany). For chromatography were used the following solvent systems:

1. Chloroform - methanol (20:3);
2. Chloroform - acetone - ethanol (7:2:2);
3. Ethanol - ammonia - water (20:1:4);
4. Ethyl acetate - petroleum ether (5:1).

Chromatograms were visualized in UV light or iodine vapors. For the preparative isolation of individual products was used column chromatography (Silicagel L 40/100, Chemapol, Czech Republic) in a solvent system 3, and preparative thin layer chromatography in solvent systems 2, 3 and 4. Electron ionization (EI) mass spectrometry was performed at an electron energy of 70 eV on the instrument Finigan MAT-4615. ^1H nuclear magnetic resonance (NMR) spectral analyses were performed at 60 MHz Tesla BS-467 (Czech Republic) NMR spectrometer operating at 28°C. Compounds were dissolved in $CDCl_3$.

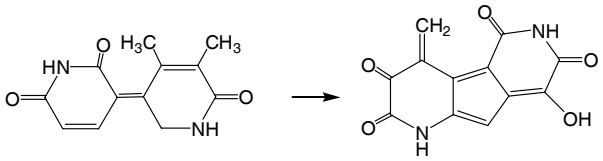
RESULTS

As a result, bioconversion 4-methylpyridine (**I**) was isolated substance **II** and identified as 4-methylpyridin-2-ol (Table 1). Compound **II** has been accumulating in the log phase (18-20 hours) and decreases in the stationary phase (36 hours).

Compound **II** is completely disappear from the incubation liquid to 36 hours of incubation. Also, was isolated the blue pigment (**III**) but only in amounts sufficient for mass spectral analysis. In the ^{1}H NMR spectrum of compound **II** was observed doublet of proton H-6 with a chemical shift of 6.37 ppm ($J_{5,6}$ = 6.6 Hz), broad singlet of proton H-3 with a chemical shift of 6.37 ppm, doublet of doublets of the proton H-5 with a chemical shift of 6.12 ppm ($J_{3,5}$ = 1.7 Hz, $J_{5,6}$ = 6.6 Hz) and three protons of the methyl group with a chemical shift of 2.23 ppm.

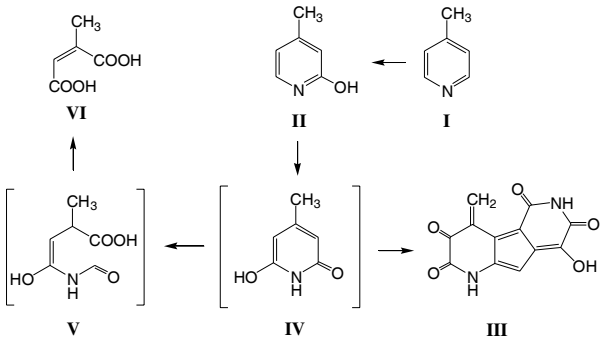
Based on mass spectral analysis the blue pigment had the structure of substituted diazafluorene (**III**), which is possibly was formed from the diazaquinone (**IIIa**) as a result of dehydration reaction (Table 2, Fig. 1).

Figure 2 shows alleged the mechanism of the degradation of 4-methylpyridine by bacterium *Arthrobacter* sp. KM-4MP.



IIIa **III**

Fig. 1. The structure of the blue pigment which was synthesized by *Arthrobacter* sp. **KM-4 MP**, in time of growing on 4-methylpyridine. IIIa - diazaquinone; III – diazafluorene.



VI **II** **I**

V **IV** **III**

Fig. 2. The catabolism of 4-methylpyridine (**I**) by the strain of *Àrthrobacter* sp. KM-4MP; **II** - 4-methylpyridin-2-ol; **III**-8-hydroxy-4-methylidene-1H-cyclopenta[1,2-b:3,4-c′] dipyridine-2,3,5,7(4H,6H)-tetrone (blue pigment); **IV** and **V** - possible intermediates; **VI** - methylmaleic acid.

DISCUSSION

Typically, such processes of biodegradation ending by accumulation of dicarboxylic acids (such as methylmaleic acids) in incubation fluid, but we were not able to isolate these substances in sufficient quantities for analysis (Kost *et al.*, 1977).

The literature contains information about pigment formation in the oxidation of pyridine derivatives. The common name for that is azaquinones. Formation of blue pigment was observed in the metabolism of nicotinic acid by

Table 1. Mass spectrum of 4-methylpyridin-2-ol (II)

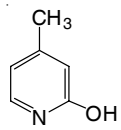
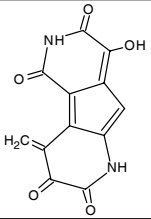
Structure of compound II	*m/z*	Relative abundance, %	The formation of fragments
	109	100	M^+
	81	10	M^+ - CO
	80	54	M^+ - HCO
	53	17	M^+ - HCO–HCN

Table 2. Mass spectrum of diazafluorene (8-hydroxy-4-methylidene-1H-cyclopenta[1,2-b:3,4-c′]dipyridine-2,3,5,7(4H,6H)-tetrone)

Structure of compound **III**	*m/z*	Relative abundance, %	The formation of fragments
	259	100	M^+
	242	15	M^+ - OH
	224	4	M^+ - OH-HOH
	215	5	M^+ - H_2 - NCO
	268	72	M^+ - OH - HOH 2CO
	162	18	M^+ - CH_3 - CO-CO-NH

microorganisms of the genus *Bacillus* (Ensign *et al.*, 1965), of nicotine, or of 2-hydroxypyridine by bacteria *A. crystallopoietes*, or by strains of *Arhtrobacter* (Kolenbrander, 1977).

Thus, the degradation of 4-methylpyridine by bacteria *Àrthrobacter* sp. KM-4MP happening thru the initial hydroxylation of the pyridine ring.

It is known that during degradation of 2- and 4-ethylpyridine by a mixed culture of bacteria from soil initially happening hydroxylation then cleavage of ring (Feng *et al.*, 1994).

It was also found that the process of degradation of 2-, 3-, and 4-methylpyridines by fungi stops at the formation of the corresponding hydroxymethyl pyridines (Modyanova *et al.*, 1990).

It was established that hydroxylation of 4-hydroxypyridine by strain *Agrobacterium* sp. 35S to 3,4-dihydroxypyridine catalyzes enzyme - monooxygenase. (Houghton *et al.*, 1972).

We can assume that the source of oxygen in the oxidation of the heterocyclic ring of 4-methylpyridine by strain Arthrobacter sp. KM-4MP is molecular oxygen, and the enzyme that catalyzes this reaction are a class of monooxygenases.

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