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Preparation and Characterization of Supports with a Synthesized Layer of Catalytic Filamentous Carbon: IV. Synthesis of Carbon Nanofibers on a Co/Al_2O_3 Catalyst

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Abstract—A comparative study of the synthesis of carbon layers, including catalytic filamentous carbon, on the surface of various alumina modifications was made. The synthesis was performed by the pyrolysis of alkanes (a propane–butane mixture) on Co/Al_2O_3 supported catalysts. The texture characteristics (specific surface area and pore structure) of the starting supports and adsorbents with a synthesized carbon layer were studied. The surface morphology of Co/Al_2O_3 catalysts and the synthesized carbon deposits was studied by scanning electron microscopy. It was found that carbon nanofibers were formed only on the catalysts prepared by the homogeneous precipitation of Co compounds onto the surface of macroporous α-Al_2O_3, whereas carbon deposits on mesoporous aluminum oxides did not exhibit a pronounced fibrous structure. The applicability of $C/Co/Al_2O_3$ carbon-containing adsorbents to the immobilization of the nitrile hydratase enzyme and the preparation of a biocatalyst for acrylonitrile hydration to acrylamide was considered.

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INTRODUCTION

Because of the intense development of nanotechnologies, the synthesis of unique carbon filaments—nanotubes and nanofibers—is extensively studied worldwide. Reviews on this subject matter contain hundreds of references [1, 2]. The major portion of publications was devoted to electron-microscopic studies and nearly artistic descriptions of the variety of individual deposited carbon structures (fishbone, card batch, bamboo, nanobells, etc.) and their conglomerates (bundles, granose aggregates, bird's nest, etc.). For the synthesis of these unique carbon filaments, the sublimation of graphite followed by desublimation (electric arc and laser thermal synthesis) or the pyrolysis of saturated and unsaturated (more rarely, aromatic) hydrocarbons is mainly used. It is a prevalent opinion that the catalytic pyrolysis of hydrocarbons is the most promising process for the large-scale commercial production of nanotubes and nanofibers. This process can be performed at comparatively low temperatures and atmospheric pressure on simple equipment. As for now, the longest filamentous nanotube aggregates and macroscopic carbon ribbons [2], as well as granulated supports with grain sizes of 1 mm or greater and specific surface areas higher than 200 m^2/g formed by the chaotic interweaving and condensation of carbon nanofibers [3–6], were obtained with the use of the pyrolytic method. The technology of the synthesis of catalytic filamentous carbon (CFC) makes it possible to manufacture it in kilogram amounts, and supports based on bulk CFC are of interest as efficient adsorbents for various compounds, in particular, for enzymatically active substances (individual enzymes, cell compartments, and whole microorganisms) [7].

From the scientific and practical standpoint, the synthesis of thin carbon layers (1–2 μm) on the surfaces of macrostructured inorganic supports (honeycomb monoliths and foam materials) with the retention of their complicated geometric shapes and mechanical strengths is of special interest. For example, ceramic honeycomb monoliths with a synthesized CFC layer, which can be used as new composite adsorbents for the immobilization of enzymes (lactase, lipase, and glucoamylase) and simultaneously serve as reactors for enzymatic reactions, were prepared and characterized [8–12].

It is well known that iron subgroup metals (cobalt and nickel) are the most active catalysts for the synthesis of carbon nanofibers [1, 2]. Nickel-containing catalysts are most frequently used for the pyrolysis of hydrocarbons; these catalysts are prepared by various methods. For example, Ni catalysts for the preparation of the above granulated carbon supports (bulk CFC) were prepared by either the coprecipitation of

Table 1. Hygroscopicity of supports and adsorbents

Starting supports		Adsorbents	
designation	weight loss, wt %	designation	weight loss, wt %
Al_2O_3-I	<0.1	$C/Co/Al_2O_3$-I	<0.1
Al_2O_3-II	0.3–0.4	$C/Co/Al_2O_3$-II	0.1
Al_2O_3-II	4.5–5.1	$C/Co/Al_2O_3$-III	1.6

nickel and aluminum hydroxides [3–5] or the mechanochemical activation of corresponding metal oxide and hydroxide powders [13, 14]. The homogeneous precipitation of metal compounds from the aqueous solutions of metal salts in the presence of urea is a less commonly used method, which is based on the relatively slow decomposition of urea at an elevated temperature (≥85°C) with the formation of a precipitant as OH^- ions and metal hydroxide colloid particles [15, 16].

The productivity of the synthesis of carbon nanofibers is characterized by the yield of carbon ((g synthesized carbon)/(g metal)). The yield depends on both the nature of the metal catalyst and support and synthesis conditions, and it can vary over a wide range. For example, the yields of synthesized carbon in the pyrolysis of methane on Ni supported on SiO_2, TiO_2, and zeolite at 700°C were 7.8, 10.8, and 66.0 g/(g Ni), respectively [17]. The maximum yield of carbon described in the literature is as high as 375–392 g/(g Ni) [6].

The synthesis of carbon nanofibers on Co-containing catalysts was considered in a much smaller number of publications. It was found that two-, three-, and four-layered nanotubes with conical caps can be prepared by the pyrolysis of methane at 900°C on a catalyst containing Mo, Co, and Mg in a ratio of 1 : 5 : 94 [18].

It is well known that carboxylic acid amides, such as acrylamide, which are commercial products, are used in various chemical processes, primarily, for the manufacture of various polymers. The biocatalytic process of acrylamide production with the participation of the enzyme nitrile hydratase is greatly superior to the traditional chemical process in main parameters (conversion and selectivity), and toxic by-products are not formed in the former process. Large-scale plants for the production of bio acrylamide with an annual output of more than 100000 t are in operation in Japan, the United States, and France. In Russia, the largest plant with an annual output of 14000 t is in operation at the SP MSP (Perm); at this plant, a 30–50% solution of bio acrylamide is produced with the participation of suspended cells of the *R. rhodochrous* M8 strain. The *Rhodococcus ruber* gt1 producer strain was obtained by selection and isolated in the Laboratory of Chemical Mutagenesis at the Institute of Ecology and Genetics of Microorganisms, Ural Division, Russian Academy of Sciences [19]. Cobalt ions are the constituents of the active center of this enzyme, and they are responsible for the exhibition of enzymatic activity [20].

The aims of this work were the following: (1) to study the synthesis of a carbon layer by the pyrolysis of a propane–butane mixture in the presence of hydrogen on supported Co-containing catalysts prepared by the homogeneous precipitation of hydroxo Co compounds in aluminum oxides with various pore structures, (2) to study the texture characteristics of Co/Al_2O_3 catalysts and $C/Co/Al_2O_3$ carbon-containing adsorbents, (3) to study the morphology of supported cobalt compounds and a surface carbon layer by scanning electron microscopy, (4) to study the adsorptive immobilization of the enzyme nitrile hydratase on $C/Co/Al_2O_3$ adsorbents, and (5) to study the properties (activity and stability) of thus prepared heterogeneous biocatalysts in the reaction of acrylonitrile hydration to acrylamide.

EXPERIMENTAL

The following commercial supports based on alumina (from OAO Katalizator, Novosibirsk) were used as starting supports for the preparation of catalysts for the pyrolysis of a propane–butane mixture: (1) KN-21 support (S_{BET} = 0.5 m^2/g; henceforth, referred to as Al_2O_3-I) as rings 5–6 mm in height and 7 and 2 mm in inner and outer diameters, respectively; (2) AOK-63-31 support (S_{BET} = 13 m^2/g; Al_2O_3-II) as rods 12 mm in diameter and 12 mm in length; and (3) AOK-63-11 S support (S_{BET} = 246 m^2/g; Al_2O_3-III) as granules (balls) 0.63–1.25 mm in diameter.

The Co/Al_2O_3 catalysts were prepared by the homogeneous precipitation of bivalent cobalt compounds onto the surfaces of the starting supports in the presence of urea. The preparation conditions were described in more detail elsewhere [16].

The synthesis of CFC was performed by the pyrolysis of a propane–butane mixture in the presence of hydrogen at 600°C in a catalytic fixed-bed reactor (see [8, 9] for details).

The total amount of carbon synthesized on a support C_Σ (wt %) was determined gravimetrically as described previously [8, 9]. An increase in the weight of the support as a result of carbon synthesis during pyrolysis on Co/Al_2O_3-I was consistent with the weight loss upon the annealing of the $C/Co/Al_2O_3$ composition within the limits of experimental error (1–2%). In the calculations of the carbon contents of catalysts prepared with the use of Al_2O_3-II and Al_2O_3-III supports, the hygroscopicity of supports (which was evaluated upon drying the starting support at 200°C for 4 h to constant weight) and the weight losses of the starting supports under conditions of annealing (800°C, 4 h) were taken into consideration. Table 1 indicates that the Al_2O_3-III starting support exhibited a comparatively high hygroscopicity (≥5%); for this

reason, it is industrially used as a drying adsorbent. After depositing carbon, the hygroscopicity of adsorbents based on Al_2O_3-II and Al_2O_3-III decreased.

The efficiency of the synthesis of CFC was evaluated by the yield of carbon, which is equal to the weight (g) of CFC synthesized on 1 g of supported Co.

The specific surface areas of the starting supports (Al_2O_3), catalysts (Co/Al_2O_3), and adsorbents with synthesized carbon layers ($C/Co/Al_2O_3$) were measured by the BET method using the thermal desorption of argon on a SORBI-M instrument (ZAO Meta, Russia). The pore-size distribution was determined by mercury porosimetry on an AUTOPORE 9200 Instrument (Micromeritics, USA). The X-ray diffraction analysis of Al_2O_3 and Co/Al_2O_3 samples was performed on an HZG 4 diffractometer (Siemens, Germany). The electron-microscopic studies of the surface morphology of catalysts and adsorbents were performed with the use of JSM 6460 LV (JEOL, Japan) and LEO 1430 (Germany) scanning electron microscopes.

The $C/Co/Al_2O_3$ carbon-containing adsorbents were used for the immobilization of nitrile hydratase as a constituent of an enzyme preparation, which exhibited catalytic activity in the reaction of acrylonitrile hydration to acrylamide. This preparation was produced from the cells of the nitrile-utilizing *Rhodococcus ruber* gt1 producer strain, which was obtained, as noted above, by selection in the Laboratory of Chemical Mutagenesis at the Institute of Ecology and Genetics of Microorganisms, Ural Division, Russian Academy of Sciences [19]. The biomass of *R. ruber* gt1 was initially disintegrated six times (30 s each) by ultrasound at a frequency of 22 kHz at 0–4°C and then centrifuged. The supernatant liquid contained nitrile hydratase, which was isolated by salting out the protein with ammonium sulfate by increasing the concentration of $NH_4(SO_4)_2$ to 45%. The enzyme content of the preparation was 72% on a total protein basis.

Nitrile hydratase was adsorbed on the starting supports and the prepared adsorbents from a solution of the enzyme preparation under batch conditions at 4–6°C for 24–216 h. The adsorption of nitrile hydratase was determined by comparing the protein contents before, during, and after adsorption and expressed in terms of mg of protein adsorbed on 1 g of adsorbent. The protein amount in solution was determined by the standard Bradford method using the Coomassie G-250 dye.

Nitrile hydratase activity was measured at 22°C and pH 7.5 in a 0.01 M phosphate buffer solution. Acrylonitrile at a concentration of 0.59 mol/l was used as a substrate. The specific enzymatic activity was expressed in terms of μmol of product (acrylamide) formed in 1 min per 1 mg of protein. The concentrations of the enzymatic reaction substrate (acrylonitrile) and product (acrylamide) were determined on a Shimadzu HPLC chromatograph (Japan). The specific enzymatic activity in an acrylonitrile solution was 300 $\mu mol\ min^{-1}\ mg^{-1}$. The experimental error was no higher than 15%.

In the calculations of biocatalyst activities, we took into consideration the adsorption of the enzymatic reaction substrate and product on the starting supports and the prepared carbon-containing adsorbents. To measure the adsorption, we determined the decrease in the acrylonitrile content of an initial 2% acrylonitrile solution after a 1-h contact with an adsorbent. Analogously, we found the adsorption of acrylamide from its solution with an initial concentration of 10 mmol/l.

To evaluate the stability of biocatalysts prepared by the immobilization of nitrile hydratase on $C/Co/Al_2O_3$, a number of consecutive 20-min reaction cycles were performed on these biocatalysts. After the next cycle, the biocatalysts were washed with a 0.01 M phosphate buffer solution with pH 7.5.

RESULTS AND DISCUSSION

X-ray diffraction analysis demonstrated that starting supports I and II were α-alumina; the θ-Al_2O_3 phase was present in them only in trace amounts. Support III was a mixture of γ- and χ-Al_2O_3 phases, whose concentrations were 67 and 33%, respectively.

The pyrolysis of a propane–butane mixture in the presence of hydrogen occurred on the Co/Al_2O_3 catalysts with Co contents of 0.1–0.2 wt % prepared by the homogeneous precipitation of bivalent cobalt compounds onto the surfaces of various aluminum oxides. Thus, the $C/Co/Al_2O_3$ cobalt-containing adsorbents were obtained.

The most significant increase (by an order of magnitude) in the specific surface area and a decrease in the average pore diameter on the surface (d_{av}) were observed in the synthesis of a carbon layer on macroporous Co/Al_2O_3-I (Table 2). From Table 2, it also follows that the greater the amount of carbon deposited on α-Al_2O_3, the smaller the pore size. In the pore structures of supports II and III, mesopores occupied about 40 and 30% of the total pore volume, respectively. The texture characteristics of carbon-containing adsorbents based on these supports were almost the same as the corresponding characteristics of Al_2O_3-II and Al_2O_3-III (Table 2).

Comparative studies demonstrated that the amount and yield of synthesized carbon considerably depended on the crystallographic form of the starting alumina. As can be seen in Table 3, the average concentration of synthesized carbon in Co/α-Al_2O_3 catalyst granules, that is, catalysts on supports I and II as rings or rods, was no higher than 1 wt %, whereas it was 2–4 wt % on Co/γ-Al_2O_3 granules. Starting supports I and II (α-Al_2O_3 containing no Co) did almost not undergo carbonization in the course of the pyrolysis of an H_2–propane–butane mixture (the carbon content was no higher than 0.1%), whereas intense carbon deposition on starting support III (γ- and χ-Al_2O_3)

Table 2. Texture characteristics of catalysts and adsorbents

Support	Al_2O_3		Co/Al_2O_3	$C/Co/Al_2O_3$		
	S_{BET}, m^2/g	d_{av}, nm	S_{BET}, m^2/g	C_Σ, wt %	S_{BET}, m^2/g	d_{av}, nm
I (rings)	0.5	1400	1	0.2	1	518
				3.1	9	23
II (rods)	13	51	13	0.5	15	54
III (balls)	246	8	221	2.5	206	8

was observed (the carbon content was 2.6–2.7 wt %). Thus, we can conclude that the activity of Co/Al_2O_3-I and Co/Al_2O_3-II in the course of alkane pyrolysis depended on only supported cobalt compounds, whereas the activity of Co/Al_2O_3-III was total, and this catalyst underwent carbonization as a result of propane and butane pyrolysis on both the starting support and the supported Co. This circumstance was taken into account in the calculation of the yield of synthesized filamentous carbon. The yield of carbon decreased in the order of supports I > II > III, and a maximum yield was observed on Al_2O_3-I (α modification) (Table 3).

Note that shell-type adsorbents were formed on all of the test supports: carbon was deposited near the granule surface, whereas the inner region remained uncarbonized (Fig. 1). Evidently, this was due to the fact that the catalytically active particles of hydroxo cobalt compounds were mainly deposited onto the outer surface of a granule as a ring, rod, or ball in shape.

The electron-microscopic study revealed differences in the surface morphology of the test Co-containing catalysts. Comparatively small flake particles of hydroxo cobalt compounds of size ≤1 μm were detected on the surface of Co/Al_2O_3-I (Fig. 2, image a1). Not only small particles but also particle aggregates were present on the surface of Co/Al_2O_3-II (Fig. 2, image a2), whereas mainly coarse flake particles of size 1–2 μm occurred on Co/Al_2O_3-III (Fig. 2, image a3). It is likely that these latter were formed as a result of a surface redistribution of the hydroxo compounds of Co in the course of catalyst drying; Fig. 2 also shows unoccupied surface regions (image a3).

Table 3. Physicochemical characteristics of Co/Al_2O_3 catalysts

Support (modification)	Concentration, wt %		Yield of carbon*, g/(g Co)
	Co	C_Σ	
Al_2O_3-I (α)	0.12	0.32	2.5
Al_2O_3-II (α)	0.23	0.44	1.8
Al_2O_3-III (γ)	0.16	2.8	1.2

* With consideration for the activity of the starting support (with no supported cobalt) in the pyrolysis of an H_2–propane–butane mixture.

The carbon deposits prepared by the catalytic pyrolysis of an H_2–propane–butane mixture also had different morphology. Deposits with a pronounced carbon nanofiber structure were formed on Co/α-Al_2O_3-I catalyst (Fig. 2, image b1). Rounded deposits as knobs similar to carbon fiber growth points were observed on Co/Al_2O_3-II catalysts (Fig. 2, image b2). Deposits of this kind can also be clearly seen on the surface of coarse flake particles of cobalt compounds on Co/γ-Al_2O_3-III catalyst (Fig. 2, image b3).

As mentioned above, the annealing of adsorbents at 800°C for 4 h was performed to determine the amount of synthesized carbon. The annealed Co/Al_2O_3 catalysts had different colors depending on alumina modifications. Thus, $C/Co/Al_2O_3$-I catalysts acquired a dark gray color, which indicated that cobalt occurred as the Co_3O_4 compound. The bright blue color of annealed $C/Co/Al_2O_3$-III adsorbents suggested the formation of cobalt aluminates $CoAlO_4$. However, X-ray diffraction analysis did not reveal reliable differences in the crystallographic forms of both the starting supports and catalysts (freshly prepared and annealed). This may be explained by a low concentration of supported cobalt (≤0.2 wt %). The repeated carbonization of annealed catalysts occurred at a lower intensity: the amount of newly synthesized carbon decreased by a factor of about 2 and did not exceed 0.1 wt %. Consequently, we can conclude that the resulting carbon-containing adsorbents cannot be regenerated by annealing and the repeated synthesis of a surface carbon layer.

Thus, the synthesis of carbon nanofibers occurred on Co supported on the surface of macroporous α-alumina, and the yield of carbon was 3–4 g/(g Co). Previously [21], it was found that the yield of carbon on an analogous Ni/Al_2O_3-I catalyst was as high as 76 g/(g Ni). It is also known from the literature that Fe/zeolite and Ni/zeolite supported catalysts are considerably different in activity in the reaction of methane pyrolysis at 700°C, and the yields of carbon on them were 3.0 and 66.0 g/(g metal), respectively [17]. An analysis of the experimental results obtained in this work and published data demonstrated that Co supported on alumina exhibited a comparatively low

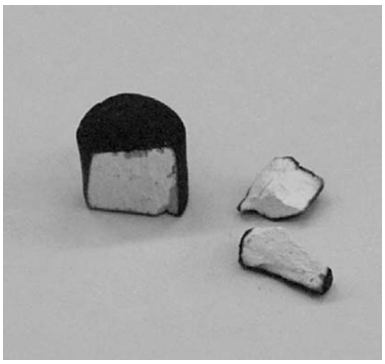

Fig. 1. Photograph of $C/Co/Al_2O_3$-II adsorbent rod cleavages (average carbon content of 0.5%).

activity in the synthesis of CFC. A comparison between the morphology of CFC layers synthesized on Co/α-Al_2O_3 (this work) and Ni/α-Al_2O_3 [21] suggests that carbon fibers with different thicknesses were formed in these cases. Thus, the nanofiber thickness in $C/Co/Al_2O_3$-I was greater than that in $C/Ni/Al_2O_3$-I by a factor of 1.5–2 (Fig. 3, b1 and b2). This is evidently due to differences in the sizes of supported particles of hydroxo Co and Ni compounds (Fig. 3, a1 and a2), which were 1–2 and 0.1–0.3 μm, respectively. For the same reason, all of the prepared $C/Co/Al_2O_3$ adsorbents exhibited a shell structure, whereas the CFC layer on the Ni/α-Al_2O_3 catalyst was uniformly distributed across the granule (ring-shaped) [21]. Indeed, α-Al_2O_3-I is a macroporous support (according to mercury porosimetry data, d_{av} = 3 μm), and smaller nickel hydroxide particles can be precipitated on the entire granule surface including the inner pore space, whereas coarser particles of the hydroxo compounds of cobalt were mainly precipitated on the outer surface.

EXAMPLE OF THE PRACTICAL APPLICATION OF $C/Co/Al_2O_3$ ADSORBENTS

We studied the applicability of the prepared $C/Co/Al_2O_3$ carbon-containing adsorbents to the immobilization of the enzyme nitrile hydratase, which was isolated from the *R. ruber* gt1 producer strain, and to the preparation of heterogeneous biocatalysts for the conversion of nitriles into amides. For comparison, the following commercial mesoporous supports were additionally used: SUMS (C/γ-Al_2O_3) and Sibunit.

The study of adsorptive immobilization processes demonstrated that the amount of protein adsorbed on $C/Co/Al_2O_3$-I and $C/Co/Al_2O_3$-III, as well as on the SUMS and Sibunit supports, monotonically increased as the duration of adsorption was increased (Fig. 4). This can be a consequence of the slow diffusion of the enzyme into the granules of mesoporous adsorbents or a result of the interaction of protein molecules in an adsorption layer. Because the absorption monotonically increased with protein concentration in solution (Fig. 5, curves *2* and *3*), the latter reason is most probable. A concave shape of the adsorption isotherm of nitrile hydratase on $C/Co/Al_2O_3$-II (Fig. 5, curve *1*) also suggests that the interaction of protein molecules with each other plays an important role, and it is stronger than the interaction of these molecules with the adsorbent surface. It is likely that the low adsorption of the enzyme on this support was due to the shell struc-

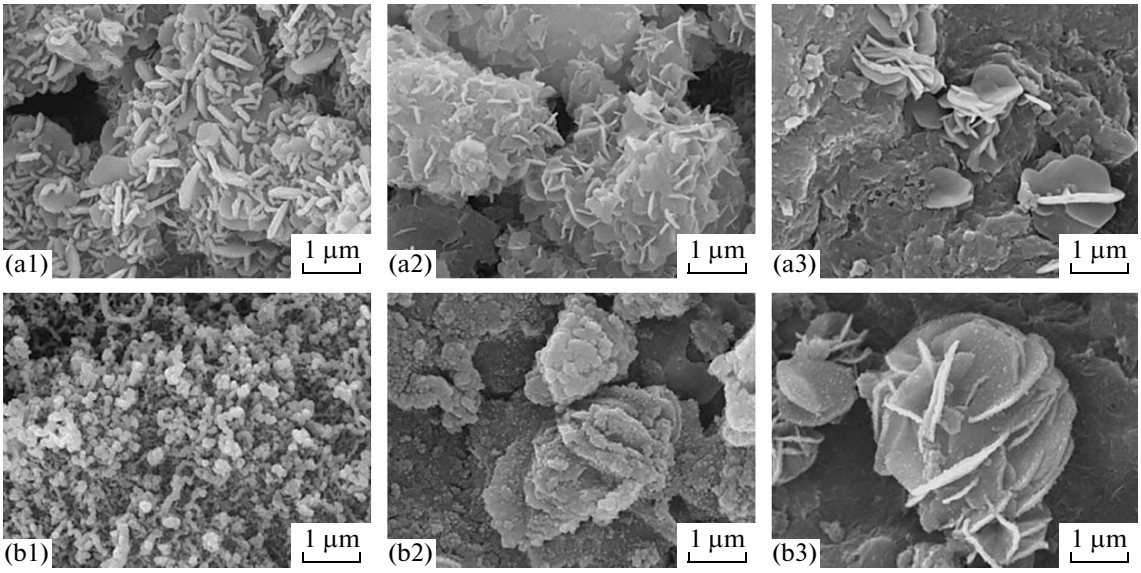

Fig. 2. Electron-microscopic images of the surfaces of (a1–a3) catalysts and (b1–b3) adsorbents containing 2–3 wt % carbon: (a1) Co/Al_2O_3-I (0.10% Co), (a2) Co/Al_2O_3-II (0.20% Co), (a3) Co/Al_2O_3-III (0.16% Co), (b1) $C/Co/Al_2O_3$-I, (b2) $C/Co/Al_2O_3$-II, and (b3) $C/Co/Al_2O_3$-III.

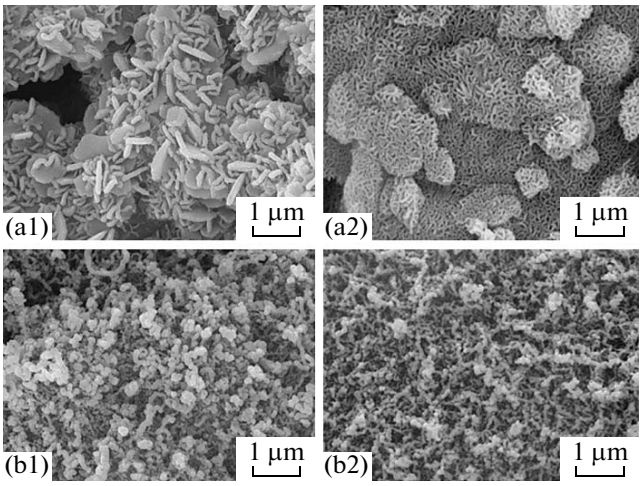

Fig. 3. Electron micrographs of the surfaces of catalysts and carbon-containing adsorbents: (a1) Co/Al_2O_3-I (0.10% Co), (a2) Ni/Al_2O_3-I (0.11% Ni), (b1) $C/Co/Al_2O_3$-I (3.1 wt % C), and (b2) $C/Ni/Al_2O_3$-I (8.4 wt % C).

ture of the granule; as demonstrated above (Fig. 1), the major portion of the granule volume was occupied by uncarbonized alumina. Indeed, additional studies showed that the adsorption of nitrile hydratase on uncarbonized initial Al_2O_3 samples was much smaller (by a factor of 6–8 on supports I and III) than on carbon-containing adsorbents.

To compare the activities of the resulting biocatalysts, it was necessary to evaluate the adsorption capacities of supports for the enzymatic reaction substrate (acrylonitrile) and product (acrylamide). It was found that the adsorption of acrylonitrile on all of the supports was greater than the adsorption of acrylamide by more than one order of magnitude (Fig. 4). Thus, the substrate concentration was relatively high in the microenvironment of immobilized nitrile hydratase.

The activity of nitrile hydratase after immobilization on any $C/Co/Al_2O_3$ carbon-containing adsorbents was no higher than 2% of the initial nitrile hydratase activity in solution regardless of the morphology of carbon deposits. The activity of the enzyme immobilized on supports with no carbon was higher than that on carbonized supports by a factor of 3–9 (Table 4). This can result from various reasons: first, a less dense structure of an adsorption layer because the adsorption on initial aluminum oxides was several times smaller, as noted above; second, a less significant deformation of the enzyme molecule upon the interaction with the polar hydrophilic surface of Al_2O_3; and, third, a regular orientation of the enzyme molecule on the support at which the active center of the enzyme was oriented to a substrate solution and not shielded by the adsorbent surface. Indeed, the specific activity of nitrile hydratase immobilized on the surface of Sibunit, which is more hydrophobic, was lower than the biocatalyst activity on the SUMS support (Table 4). Thus, we assume that the enzyme molecule was less deformed on a hydrophilic surface than on a hydrophobic one. Note that the occurrence of cobalt as a constituent of the adsorbent did not increase, as it would be expected, the specific activity of immobilized Co-dependent nitrile hydratase. It is likely that Co compounds can interact with the active center of the enzyme to cause an inappropriate orientation of its adsorbed molecule to result in a decrease in the activity of the immobilized enzyme. Indeed, the activity of nitrile hydratase immobilized on Sibunit and SUMS supports containing no cobalt was higher than the activity of the same enzyme immobilized on $C/Co/Al_2O_3$ (Table 4).

This study did not allow us to find a correlation between the stability of biocatalysts and the physico-

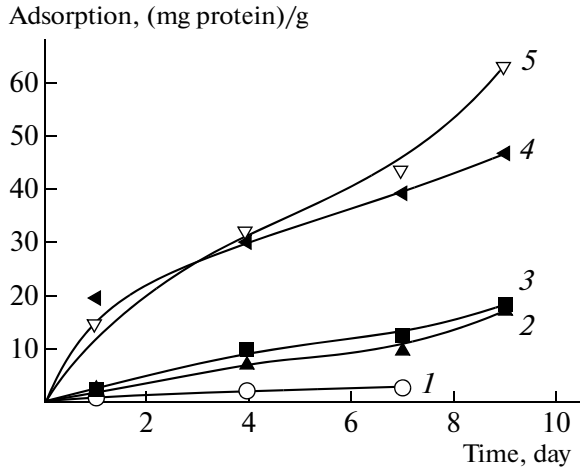

Fig. 4. Adsorption of nitrile hydratase on carbon-containing adsorbents: (*1*) $C/Co/Al_2O_3$-II, (*2*) $C/Co/Al_2O_3$-III, (*3*) $C/Co/Al_2O_3$-I, (*4*) Sibunit, and (*5*) SUMS.

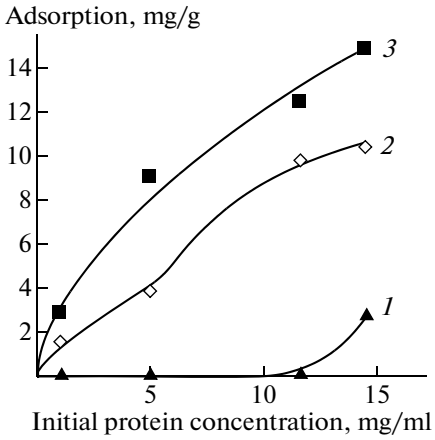

Fig. 5. Dependence of the adsorption of nitrile hydratase on (*1*) $C/Co/Al_2O_3$-II, (*2*) $C/Co/Al_2O_3$-III, or (*3*) $C/Co/Al_2O_3$-I upon the initial protein concentration in solution. The adsorption time is 168 h.

Table 4. Adsorption of acrylonitrile and acrylamide on the starting supports and carbon-containing adsorbents and the specific activity of immobilized nitrile hydratase

Adsorbent	C_Σ, wt %	Adsorption of acrylonitrile, mg/g	Adsorption of acrylamide, mg/g	Specific activity of the immobilized enzyme*, μmol min^{-1} (mg protein)$^{-1}$
Al_2O_3-I	0	30.0	0.07	29
C/Co/ Al_2O_3-I	3.1	29.2	0.33	7
Al_2O_3-II	0	2.6	0.04	30
C/Co/Al_2O_3-II	0.5	2.6	0.05	8
Al_2O_3-III	0	58.0	0.23	18
C/Co/Al_2O_3-III	2.5	18.8	0	2
SUMS	20	22.9	0.76	30
Sibunit	99	93.0	3.34	10

* With the adsorption of acrylamide on supports taken into account.

chemical parameters of the supports used (Fig. 6). Nitrile hydratase immobilized on the C/Co/Al_2O_3-II adsorbent exhibited a maximum stability. In the third reaction cycle, this biocatalyst retained to 80% of its initial activity (Fig. 6). Other biocatalysts halved their activity in two or three reaction cycles.

Thus, this study demonstrated that Co/Al_2O_3 catalysts prepared by the homogeneous precipitation of bivalent cobalt compounds on various aluminum oxides exhibited catalytic activity in the pyrolysis of a propane–butane mixture in the presence of hydrogen with the formation of carbon deposits of different morphology. Carbon nanofibers were synthesized only on a catalyst prepared based on macroporous α-alumina; in this case, the specific surface area of a sample considerably increased (by a factor of 2–8).

Carbonized Co/Al_2O_3 catalysts can be used as adsorbents for the immobilization of the enzyme nitrile hydratase, and the adsorption of this enzyme increased in the presence of a carbon layer. The activity of nitrile hydratase after adsorptive immobilization considerably decreased (by an order of magnitude), as compared with the activity of this enzyme in solution. The stability of immobilized biocatalysts was also considerably low; in the majority of cases, their activity halved after two or three reaction cycles.

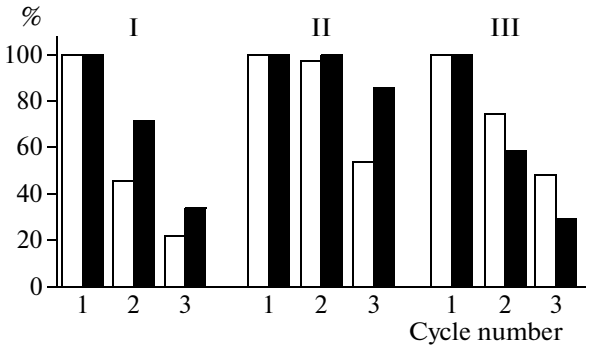

Fig. 6. Stability of biocatalysts prepared by the adsorption of nitrile hydratase on starting supports I, II, and III (white bars) and on carbon-containing adsorbents based on these supports (black bars).

Of course, a further search for effective adsorbents for enzyme immobilization and the preparation of highly stable and active heterogeneous biocatalysts a is promising area of science and technology.

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