ISSN 0023-1584, Kinetics and Catalysis, 2016, Vol. 57, No. 3, pp. 394–403. © Pleiades Publishing, Ltd., 2016.
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Synthesis of Nanostructured Carbon on Ni Catalysts Supported on Mesoporous Silica, Preparation of Carbon-Containing Adsorbents, and Preparation and Study of Lipase-Active Biocatalysts

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Received September 15, 2015

Abstract—This work continues a series of our studies on the synthesis of nanostructured carbon (NSC) by the pyrolysis of $H_2 + C_3–C_4$ alkane mixtures on nickel and cobalt metal catalysts supported on chemically diverse inorganic materials (aluminosilicates, alumina, carbon) having different textural characteristics (mesoporous and macroporous supports) and shapes (granules, foamed materials, and honeycomb monoliths). Here, we consider Ni catalysts supported on granular mesoporous silica (SiO_2). It has been elucidated how the yield of synthesized carbon depends on the Ni/SiO_2 catalyst preparation method (homogeneous precipitation or impregnation) and on the composition of the impregnating solution, including the molar ratio of its components—nickel nitrate and urea. The morphology of catalytic NSC and Ni distribution in the silica granule have been investigated using a scanning electron microscope with an EDX analyzer. Carbon-containing composite supports (NSC/SiO_2) have been employed as adsorbents for immobilizing microbial lipase. The enzymatic activity and stability of the resulting biocatalysts have been estimated in transesterification reactions of vegetable (sunflower and linseed) oils involving methyl or ethyl acetate, esterification, and synthesis of capric acid–isoamyl alcohol esters in nonaqueous media.

Keywords: supported Ni catalysts, nanostructured carbon, lipase adsorption, heterogeneous biocatalysts, esterification, transesterification

DOI: 10.1134/S002315841603006X

INTRODUCTION

The synthesis of nanostructured carbon (NSC) on inorganic matrices differing in their chemical nature (metal oxides, silica, graphite) and in textural properties and shape (granules, rods, honeycomb monoliths) provides means to obtain a wide variety of unique composite supports and materials, including those usable in heterogeneous biocatalysis. Containing nanostructured surface carbon, these supports can efficiently adsorb enzymatically active substances, such as protein molecules of enzymes. Heterogeneous biocatalysts prepared by enzyme adsorption on carbon-containing supports can accelerate many practically important processes, for example, esterification reactions yielding an ester linkage between acid and alcohol residues. These reactions provide a basis for obtaining aroma compounds for the food, fragrance, and cosmetic industries. By varying the composition of the initial substrates (alcohol and acid), it is possible to synthesize aroma compounds having various odors: for example, isoamyl butyrate smells like a pear [1, 2]. There have been reports on the enzymatic esterification of organic (capric, palmitic, lauric, salicylic) acids with aliphatic (isoamyl, cetyl, ethyl) alcohols in nonaqueous media in the presence of lipase [2–7].

Nanostructured carbon is synthesized by hydrocarbon pyrolysis on metallic catalysts (Ni, Cu, Fe, and their alloys) supported on an inorganic material [8, 9]. In particular, Ni catalysts are commonly supported on SiO_2 [10–23]. The following methods are used to prepare silica-supported Ni catalysts: (1) impregnation of a support with a nickel salt solution (Impr) [12, 14, 15], (2) intercalation of nickel compounds into a silicate matrix using sol–gel processing [12, 16, 17], and (3) homogeneous precipitation (HP) [18–23].

The HP method is based on urea decomposition in aqueous solutions of salts at $85 \pm 5°C$:

$$CO(NH_2)_2 + 3H_2O \rightarrow 2OH^- + 2NH_4^+ + CO_2 \text{ [23]}.$$

The hydroxide ion reacts with Ni^{2+} to yield colloidal α-$Ni(OH)_2$ particles (since the solubility product of nickel hydroxide is $SP < 10^{-15}$), and these particles precipitate on the surface of an inorganic support [20, 21, 23]. The pyrolysis of alkanes (C_1, $C_3–C_4$) or ethyl-

Table 1. Textural characteristics of the adsorbents and initial silica

Sample	Preparation method	Supported nickel content, wt %	Carbon content*, wt %	S_{BET}, m^2/g	S_{micro}, m^2/g	V_{Σ}, cm^3/g	V_{micro}, cm^3/g	D_{av}, nm
Ni/SiO_2	HP (0.1 mol/L $Ni(NO_3)_2$, 1 mol/L urea)	3.5	5	145	14.1	0.67	0.008	18.4
	Impr (0.05 mol/L $Ni(NO_3)_2$)	0.8	4	130	1.8	0.74	0.002	22.5
	Impr (0.5 mol/L $Ni(NO_3)_2$)	1.7	16	140	6.1	0.54	0.004	15.2
KSK-G**	–	0	0	150	5.4	0.75	0.004	19.7

* According to thermoanalytical data.

** Initial support for the Ni catalysts.

ene (C_2) on the Ni/SiO_2 catalyst yields carbon as filamentous structures (nanofibers) [23–25]. Supports, including silicates, that have an NSC layer on their surface are employed in heterogeneous biocatalysis for adsorptive immobilization of enzymatically active substances, namely, microbial cells and enzymes [17, 26].

This work continues a series of our studies on the synthesis of catalytic filamentous carbon by the pyrolysis of C_3–C_4 alkanes on supported Ni and Co catalysts. The support for the nickel catalysts prepared in this study by homogeneous precipitation and impregnation was mesoporous silica. We studied how the activity of the catalysts in the pyrolysis of a hydrogen + C_3–C_4 alkane mixture and the NSC yield depend on the Ni/SiO_2 preparation conditions, primarily on the composition of the impregnating solution. The resulting $NSC/Ni/SiO_2$ composite carbon-containing adsorbents were used to immobilize the enzyme lipase and to prepare heterogeneous biocatalysts for conversion of vegetable oil triglycerides. The enzymatic activity and stability of these biocatalysts was studied in triglyceride transesterification and fatty acid esterification in nonaqueous media.

EXPERIMENTAL

The support for the Ni catalysts was KSK-G silica gel (Russia) with a granule size of 2—5 mm. The textural characteristics of supports and adsorbents were determined by nitrogen porosimetry using AUTOPORE 9200 and ASAP 2400 V3.07 devices (Micromeritics Instrument Corporation, United States). The porosimetry data are presented in Table 1. The specific surface area (S_{BET}) of the initial silica was 150 m^2/g, its total pore volume (V_{Σ}) was 0.75 cm^3/g, its average pore diameter (D) was 19.7 nm, its micropore volume (V_{micro}) was 0.004 cm^3/g, and the specific micropore surface area (S_{micro}) was 5.4 cm^2/g.

Supported Ni catalysts for the pyrolysis of the H_2 + C_3–C_4 mixture were prepared by the HP and impregnation methods. The preparation conditions are specified in an earlier work [23]. The nickel nitrate concentration in the solution was varied between 0.005 and 0.1 mol/L; the urea concentration, between 0.1 and 2 mol/L. The molar ratio of nickel nitrate to urea was varied between 1 : 2 and 1 : 200.

The morphology of the supported catalysts and carbon deposits were studied by scanning electron microscopy (SEM) on a JSM 6460 LV microscope (JEOL, Japan) fitted with an EDX spectrometer (Oxford INCAEnergy).

The catalytic pyrolysis of the C_3–C_4 alkanes + hydrogen (8 : 1 v/v) gas mixture was performed in a fixed-bed reactor at 500°C for 1 h, as was described in earlier works [17, 23]. The amount of synthesized carbon was determined by thermal analysis (TA) using an STA-449 C Jupiter system (Netzsch, Germany) and was also calculated from the change in the catalyst weight measured after the pyrolysis run (500°C, 1 h) or after annealing (800°C, 4 h). In the calculation of the carbon content, we took into account the moisture content of the initial silica (4.8%), which was measured by drying the material at 110°C for 4 h, and also the weight lost by the initial silica subjected to heat treatment at 500 and 800°C (5.4 and 7.1%, respectively). The weight loss of the Ni catalysts ($-\Delta$) at 500°C in the presence of H_2 and in the absence of C_3–C_4 alkanes (when there was no pyrolysis) and the weight loss as a result of annealing at 800°C were, on average, 5.7 and 9.6%, respectively. The $-\Delta$ value depended weakly on the supported Ni content in the wide range from 0.5 to 6.0% Ni, indicating that the metal was possibly carried away at high temperatures. Taking into account all $-\Delta$ values enabled us to estimate the amount of synthesized carbon and to calculate the NSC yield (Y) in terms of the amount of carbon per unit weight of supported Ni (g/g). Many replica experiments were made, and the scatter of experimental data was the same as in the study of commercial supports.

The $NSC/Ni/SiO_2$ supports were used to adsorb microbial lipase from *Thermomyces lanuginosus* (SIGMA). For comparison, the same operation was

carried out with the initial silica. Adsorption was performed for 2 days at 20–22°C while occasionally stirring a support sample (3 g) and lipase in a buffer solution (6 mL, pH 7.0). The initial concentration of the protein was 12.5 mg/mL. Next, lipase was adsorbed again under the same conditions but at its initial concentration of 8.5 mg/mL. The total amount of lipase adsorbed was calculated in terms of amount of lipase (mg) per gram of support.

The enzymatic activity of the biocatalysts was determined in a stirred batch reactor at 20 and 40°C. The reactor was placed in a KT-104 temperature-controlled shaker reciprocating at a rate of 90 min^{-1}. The duration of one reaction cycle was 5 min to 24 h. The following reactions were investigated: hydrolysis of tributyrin (I) under the conditions described in our earlier study [17], esterification of capric acid with isoamyl alcohol (II), and transesterification of sunflower oil triglycerides involving an acyl group donor (methyl acetate) or acceptor (dimethyl carbonate), and transesterification of linseed oil triglycerides involving ethyl acetate (III). The hydrolysis of emulsified tributyrin (reaction (I)) was carried out in a buffer solution at pH 7.0, and reactions (II) and (III) were performed in organic nonaqueous solvents (hexane or a hexane–diethyl ether mixture).

To carry out reactions (II) and (III), a biocatalyst sample (0.1–0.5 g) predried in air at 22°C for 3 days was placed in the reactor, and the reaction medium (2–5 mL) was poured thereto. In reaction (II), the reaction medium consisted of 0.1 mol/L capric acid, 0.4 mol/L isoamyl alcohol, and a hexane–diethyl ether (1 : 2) mixture as the solvent. In reaction (III), the reaction medium consisted of 0.1 mol/L vegetable (sunflower or linseed) oil, 1.85 mol/L methyl acetate or dimethyl carbonate or 2.3 mol/L ethyl acetate, and a solvent (hexane). Transesterification products were identified by thin-layer chromatography (TLC), as was described in our earlier publication [27]. The tributyrin hydrolysis and ester synthesis rates were determined by acid–base titration from the increase and decrease in the concentration of butyric acid and capric acid, respectively. The activity of biocatalysts was estimated in international Units of enzyme activity (U), that is, in terms of substrate conversion rate in μmol/min per gram of biocatalyst. The specific activity of adsorbed lipase was expressed as U per milligram of protein. Capric acid conversion was calculated via the formula $x = \frac{C_0 - C_t}{C_0}$, where C_0 is the initial concentration of capric acid and C_t is its concentration at the point in time t (in mol/L).

The stability of biocatalysts in reactions (I)–(III) was estimated under the above-specified conditions. At the beginning of each reaction cycle, we measured the initial reaction rate or substrate (capric acid) conversion for 5–60 min; next, the biocatalyst was left in the reaction medium for 1–3 days. After 500-h-long operation, the biocatalyst was washed with the solvent mixture for 2 days and its initial activity was then measured again.

RESULTS AND DISCUSSION

Preparation of Supported Ni Catalysts and Carbon-Containing Adsorbents

We carried out a comparative study of the Ni/SiO_2 catalysts prepared by the HP and Impr methods and investigated the effect of the composition of the impregnating solution on the physicochemical properties of the catalysts themselves and on those of carbon-containing adsorbents.

In the HP method, the molar ratio of the components of the impregnating solution— $Ni(NO_3)_2$ and urea—were varied in two ways.

In the first series of experiments, the urea concentration was varied between 0.1 and 2 mol/L at a fixed nickel nitrate concentration of 0.01 or 0.02 mol/L. Electron microscopic examinations demonstrated that, as the urea concentration is increased, the particle size of the precipitated nickel hydroxide decreases (Fig. 1, images a1, b1, c1). The resulting carbon is in the form of nanofibers (Fig. 1, image b2). As the $Ni(NO_3)_2$: urea molar ratio is changed from 1 : 10 to 1 : 100, the supported Ni content decreases by 25–29%: for example, at a $Ni(NO_3)_2$ concentration of 0.02 mol/L, the nickel content decreases from 0.7 to 0.5%. As a consequence, the amount of the resulting carbon decreases by one order of magnitude, namely, from 2–3 to less than 0.1%.

In the other series of experiments, the nickel nitrate concentration was varied at a fixed urea concentration of 1 mol/L. In this case, as the $Ni(NO_3)_2$ concentration is decreased, the particles size of precipitated nickel hydroxide increases (Fig. 2, images a1–e1). According to earlier data [23], the urea conversion at 85°C in 3 h (as in this study) is 10% and, at a component molar ratio of 1 : 10, there are two OH^- ions per Ni^{2+} ion; that is, the process yields nickel hydroxide $Ni(OH)_2$. When the component ratio is below 1 : 10, for example, at a nickel nitrate concentration higher than 0.1 mol/L and a fixed urea concentration of 1 mol/L, supported Ni forms both from precipitated nickel hydroxide and from nickel(II) nitrate. At reactant ratios of 1 : 2 and 1 : 5, small Ni-containing particles on the silica surface form aggregates 0.1–0.5 μm in diameter (Fig. 2, images a1, b1). The carbon resulting from catalytic pyrolysis has a well-defined filamentous structure (Fig. 2, images a2–e2). It can clearly be seen that, when the ratio between the components of the impregnating solution is 1 : 10 to 1 : 50, the Ni/SiO_2 catalyst and $NSC/Ni/SiO_2$ adsorbents have a "crust" structure. Chemical and gravimetric analyses demonstrated that, as the initial nickel nitrate concentration is raised under the conditions examined, the supported nickel content increases nonlinearly to 5.6%

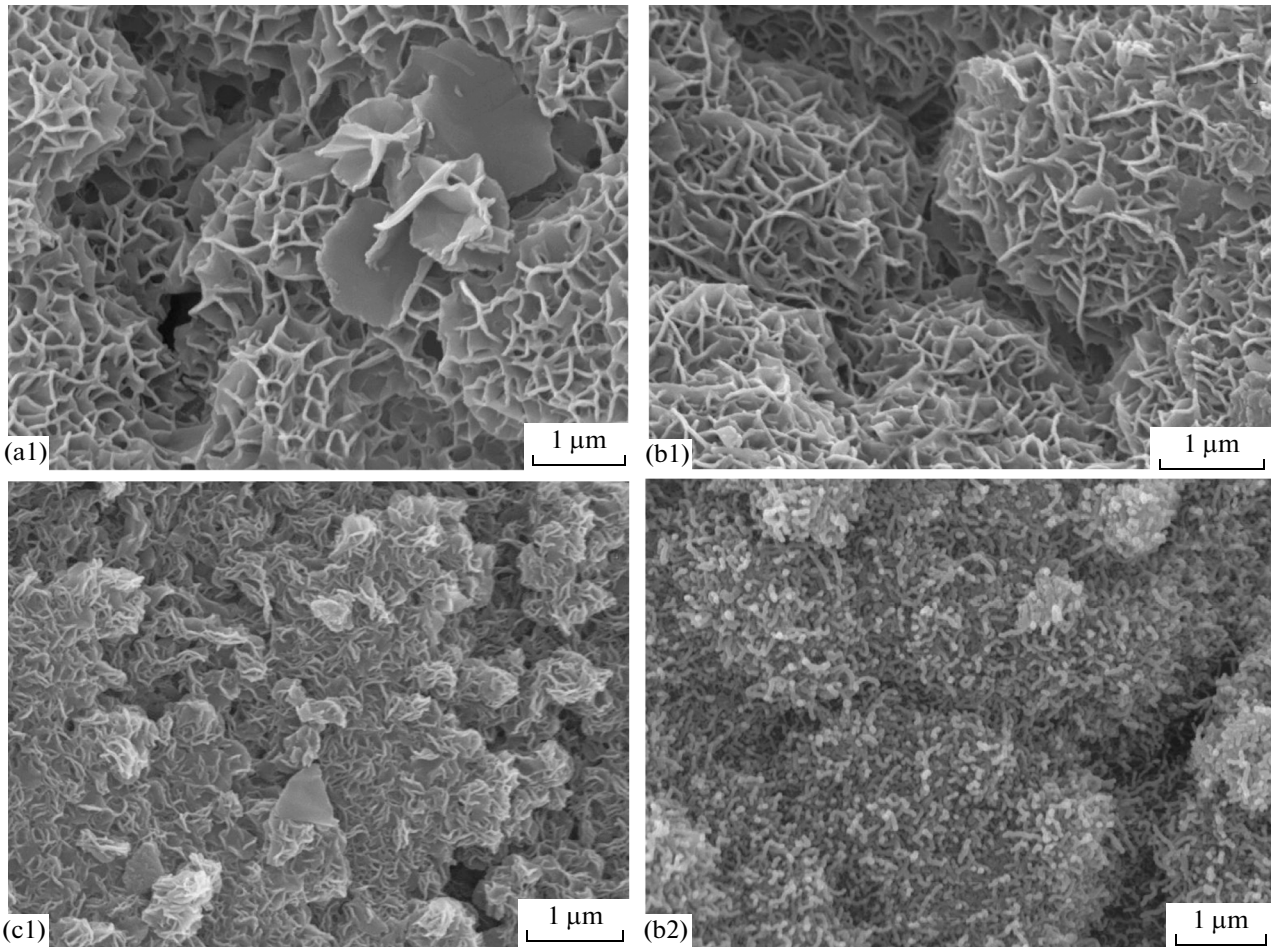

Fig. 1. SEM images of supported Ni catalysts and an NSC-containing adsorbent synthesized at an initial nickel nitrate concentration of 0.02 mol/L in the impregnating solution and nickel nitrate-to-urea molar ratio of (a1) 1 : 10 (0.72% Ni), (b1) 1 : 50 (0.52% Ni), and (c1) 1 : 100 (0.50% Ni). (b2) Nanostructured carbon on catalyst b1 (1% C).

(Fig. 3a, curve *1*). The amount of synthesized carbon also increases nonlinearly, the maximum amount of NSC being 8% (Fig. 3b, curve *1*).

In the case of the Impr method, the supported nickel content increases almost linearly to 2.5% with an increasing initial nickel nitrate concentration in the impregnating solution (Fig. 3a, curve *2*). As the silica-supported nickel content increases, the amount of synthesized carbon increases linearly, the maximum NSC content being 27% (Fig. 3b, curve *2*).

The maximum supported nickel content of the catalysts prepared by the HP method is approximately 3 times higher than that of the samples prepared by the Impr method (Fig. 3a). At the same time, the amount of carbon forming on the catalysts prepared by impregnation is approximately 5 times larger than the amount of carbon forming on the catalysts prepared by the HP method (Fig. 3b). Calculations demonstrated that the carbon yield Y on the Ni/SiO_2 catalysts obtained by the HP and Impr methods is practically independent of the amount of supported Ni and is, on average, 1.3 g/g (at most 1.9 g/g) and 6.2 g/g (at most 8.7 g/g), respectively. A comparison of the highest carbon yields obtained under fixed pyrolysis conditions on all HP catalysts hitherto studied by the authors demonstrates that the $Y \approx 1$ g/g value, observed in this work, is about 30 times smaller than the same value for Ni/foam glass catalysts ($Y \approx 29$ g/g) [23]. Obviously, this is due to the effect of the porous structure of the support on the distribution of catalytically active nickel-containing particles. For example, foam glass ($S_{BET} = 0.4$ m^2/g) is a macroporous support, whereas silica ($S_{BET} = 150$ m^2/g) is a mesoporous one (Table 1). Owing to the macroporous structure of foam glass, nickel hydroxide particles are uniformly distributed inside the glass granule [23], while they are practically absent inside the mesoporous silica granule, and this leads to the formation of a crust-type carbon-containing adsorbent.

In cleaved granules of the carbon-containing adsorbents obtained on HP catalysts, the naked eye can see a black carbon crust and a light inner part of

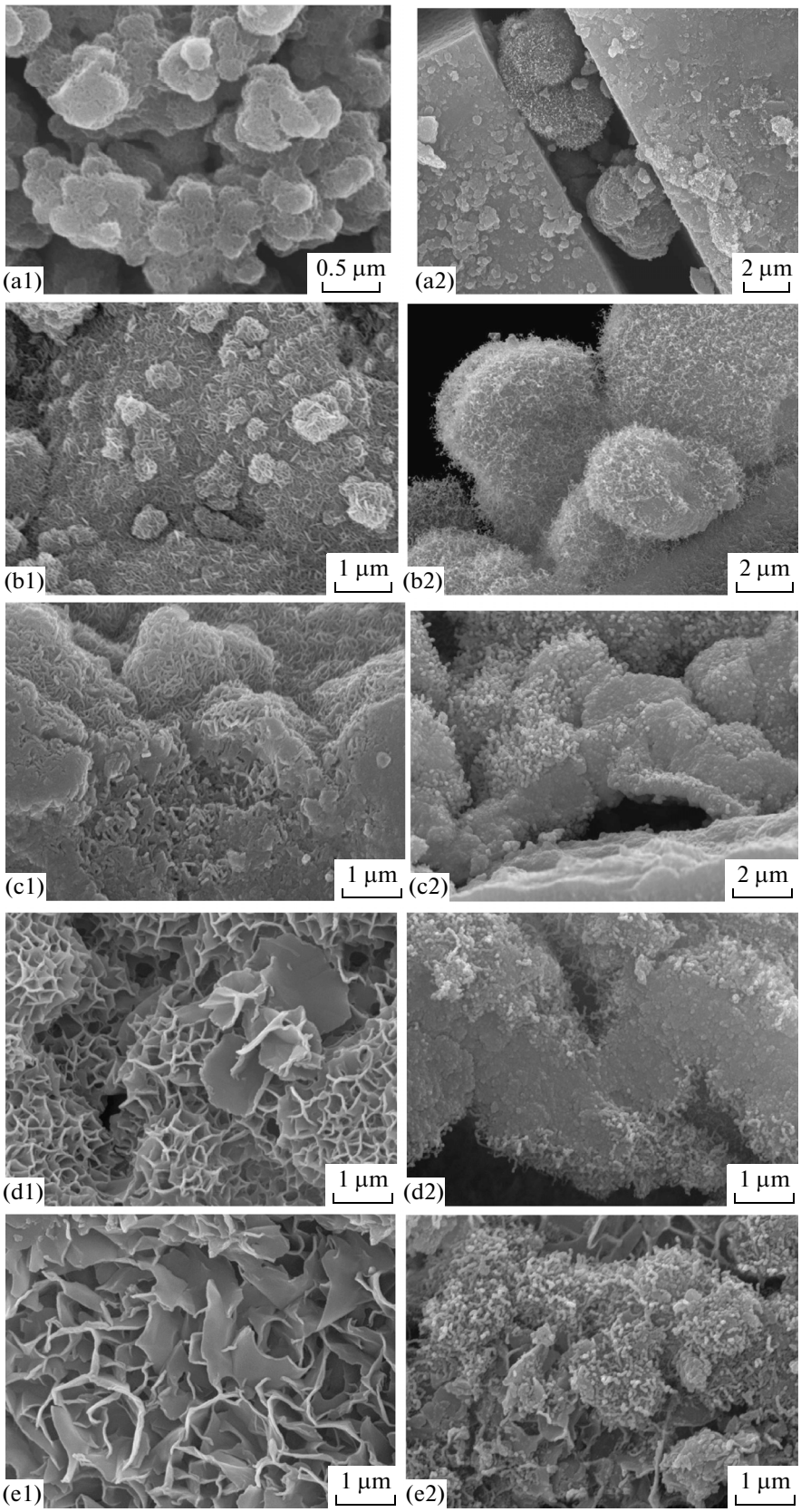

Fig. 2. SEM images of (a1–e1) supported Ni catalysts prepared at a fixed initial urea concentration of 1 mol/L in the impregnating solution and nickel nitrate-to-urea molar ratios of (a1) 1 : 2 (5.68% Ni), (b1) 1 : 5 (3.93% Ni), (c1) 1 : 10 (2.76% Ni), (d1) 1 : 50 (0.52% Ni), and (e1) 1 : 200 (0.15% Ni). (a2–e2) NSC formed on catalysts a1–e1; the carbon content of the adsorbents is (a2) 4, (b2) 4, (c2) 2, (d2) 1, and (e2) <0.1%.

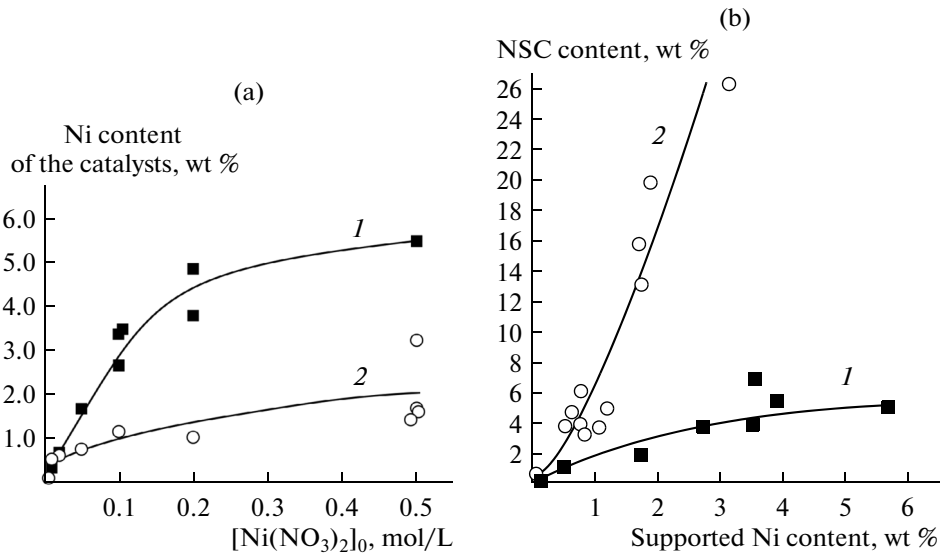

Fig. 3. (a) Supported nickel content on silica as a function of the initial $Ni(NO_3)_2$ concentration in the impregnating solution in the (*1*) HP and (*2*) Impr methods. The urea concentration is 1 mol/L. (b) Amount of synthesized carbon on Ni/SiO_2 catalysts prepared by the (*1*) HP and (*2*) Impr methods.

silica; that is, carbon synthesis does not take place inside the granules. Electron microscopic examinations with EDX analyses confirmed this observation. Indeed, as is clear from Fig. 4, a well-defined crust-type distribution of Ni is typical of the Ni/SiO_2 catalysts prepared by the HP method (Fig. 4a), while nickel in the catalysts prepared by impregnation is more uniformly distributed in the granule (Fig. 4b).

A TA study of the synthesized carbon-containing adsorbents demonstrated that carbon is burned away above 450°C. The shape of the thermogravimetric profiles of the adsorbents depends on the Ni catalyst preparation method (Fig. 5). Carbon on the catalysts prepared by the HP method is burned away in a narrower temperature range (Fig. 5a) than carbon on the catalysts prepared by impregnation (Figs. 5b, 5c). This fact is further evidence of crust-type carbon distribution, in which carbon is relatively highly accessible to oxygen and, as a consequence, is readily oxidized (burned away). It follows from Fig. 5 that NSC with a well-defined filamentous structure forms on the nickel-rich catalysts. The carbon deposits on the catalysts that contain <1% Ni and were prepared by impregnation do not have a distinct filamentous structure (Fig. 5b).

Thus, in this study we prepare supported Ni catalysts by using different preparation methods and obtained different types of $NSC/Ni/SiO_2$ composite adsorbents by the pyrolysis of $H_2 + C_3–C_4$ alkanes. The carbon-containing adsorbents on the Ni catalysts synthesized by the HP method have a crust-type structure. The carbon deposited on the Ni catalysts obtained by impregnation is relatively uniformly distributed inside the SiO_2 granules.

Preparation of Lipase-Active Biocatalysts

For preparing biocatalysts by the adsorptive immobilization of lipase, we chose $NSC/Ni/SiO_2$ adsorbents, whose characteristics are presented in Table 1 and in Fig. 5. The initial silica was also used for comparison. The adsorbents that were selected for lipase adsorption differed in their carbon content, carbon deposit morphology, and carbon distribution inside the granule. The crust-type (5% NSC)/Ni/SiO_2 (HP) adsorbents are hereafter designated **I**; the adsorbents that are characterized by a uniform carbon distribution and have no well-defined filamentous structure, (4% NSC)/Ni/SiO_2 (Impr), are designated **II**; the adsorbents with a uniform distribution of nanofibers, (16% NSC)/Ni/SiO_2 (Impr), are designated **III**.

It was discovered that lipase adsorption depends on the $NSC/Ni/SiO_2$ adsorbent type and structure. The amount of lipase adsorbed on the crust-type adsorbent **I** was 3–5 times as small as the amount of lipase adsorbed on adsorbents **II** or **III** or on the initial silica (Table 2). This finding cannot be explained in terms of textural distinctions between the adsorbents, because the porous structures of all of the samples examined here have similar parameters (Table 1). The adsorbents differ slightly in micropores specific surface area and micropore volume. For the crust-type adsorbent, these parameters are approximately 2 times larger than for the others (Table 1). Micropores are inaccessible to the enzyme being adsorbed, because the size of the lipase molecule is ~10 nm. Since the micropores account for <1% of the total pore volume (Table 1), the multifold decrease in the amount of lipase adsorbed on the crust-type adsorbent **I** cannot be attributed to this specific feature of the porous struc-

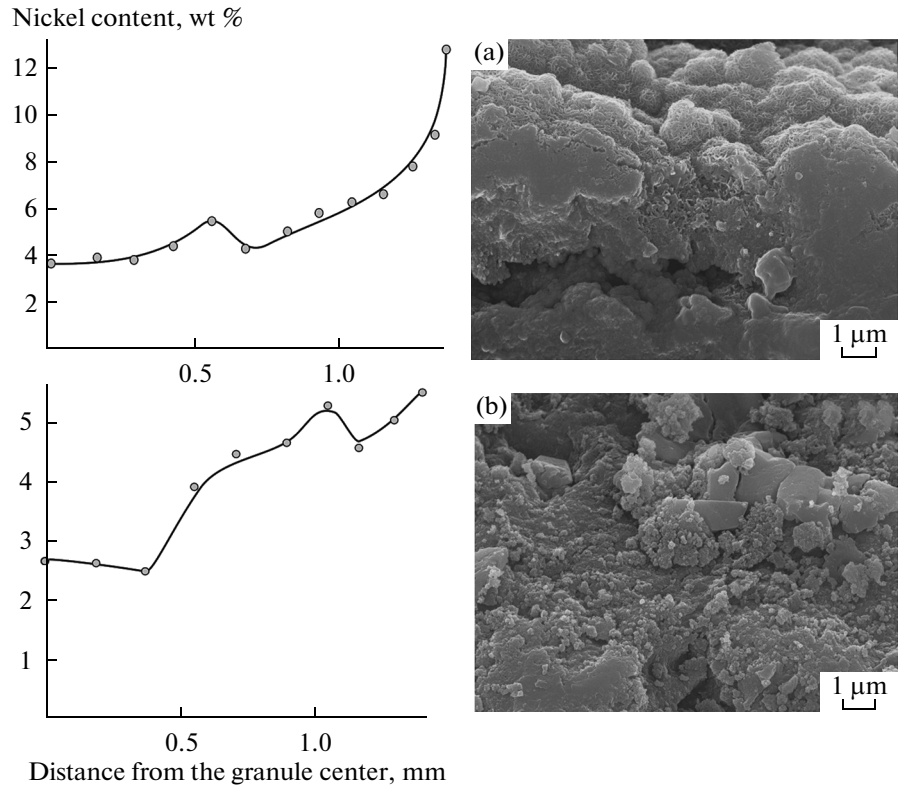

Fig. 4. EDX curves illustrating the distribution of nickel in the cut section of a granule (left column) and electron micrographs of supported Ni catalysts cracks (right column): (a) catalyst prepared by the HP method (Ni content of 3.52%, initial nickel nitrate concentration of 0.1 mol/L, initial urea concentration of 1 mol/L); (b) catalyst prepared by the Impr method (Ni content of 1.72%, initial nickel nitrate concentration of 0.5 mol/L).

ture. It is possible that the carbon crust hampers the transport of protein molecules into the granule bulk.

The activity of the biocatalysts was studied in the hydrolysis of emulsified tributyrine in an aqueous medium at pH 7.0. The biocatalysts rapidly lose their enzymatic activity, irrespective of the NSC/Ni/SiO_2 adsorbent type (Fig. 6a). Earlier [27, 28], we demonstrated that one of the causes of the inactivation of biocatalysts in reactions involving "oil" substrates is the dehydration of adsorbed lipase as a result of triglycerides displacing "essential" water molecules from the microenvironment of the enzyme. For verifying this hypothetical inactivation mechanism, the biocatalysts were also examined in an esterification reaction, whose product is water. Under the isoamyl caprinate synthesis conditions, the water molecules forming in the organic solvent medium can accumulate in the silica matrix in the immediate vicinity of adsorbed lipase. Calculations showed that, under the given conditions, the total conversion of capric acid yields 9 μL of water, which can be strongly bound by KSK-G silica gel, since the pore volume of this support is one order of magnitude larger than the volume of the resulting water.

Table 2 lists data characterizing the activity of the biocatalysts and the specific activity of adsorbed lipase

Table 2. Properties of the prepared biocatalysts in esterification

Adsorbent for lipase immobilization	Amount of lipase adsorbed, mg/g	Initial activity*, EA/g	Specific activity of adsorbed lipase, EA/mg	Time to 85–90% conversion, min
I	5.4	6.1	1.1	120
II	32.6	45.4	1.4	20
III	20.1	36.4	1.8	30
KSK-G	17.3	45.4	2.6	30

* The activity of the biocatalysts was determined at 20°C in a reaction medium containing 0.1 mol/L capric acid, 0.4 mol/L isoamyl alcohol, and 10 wt % biocatalyst.

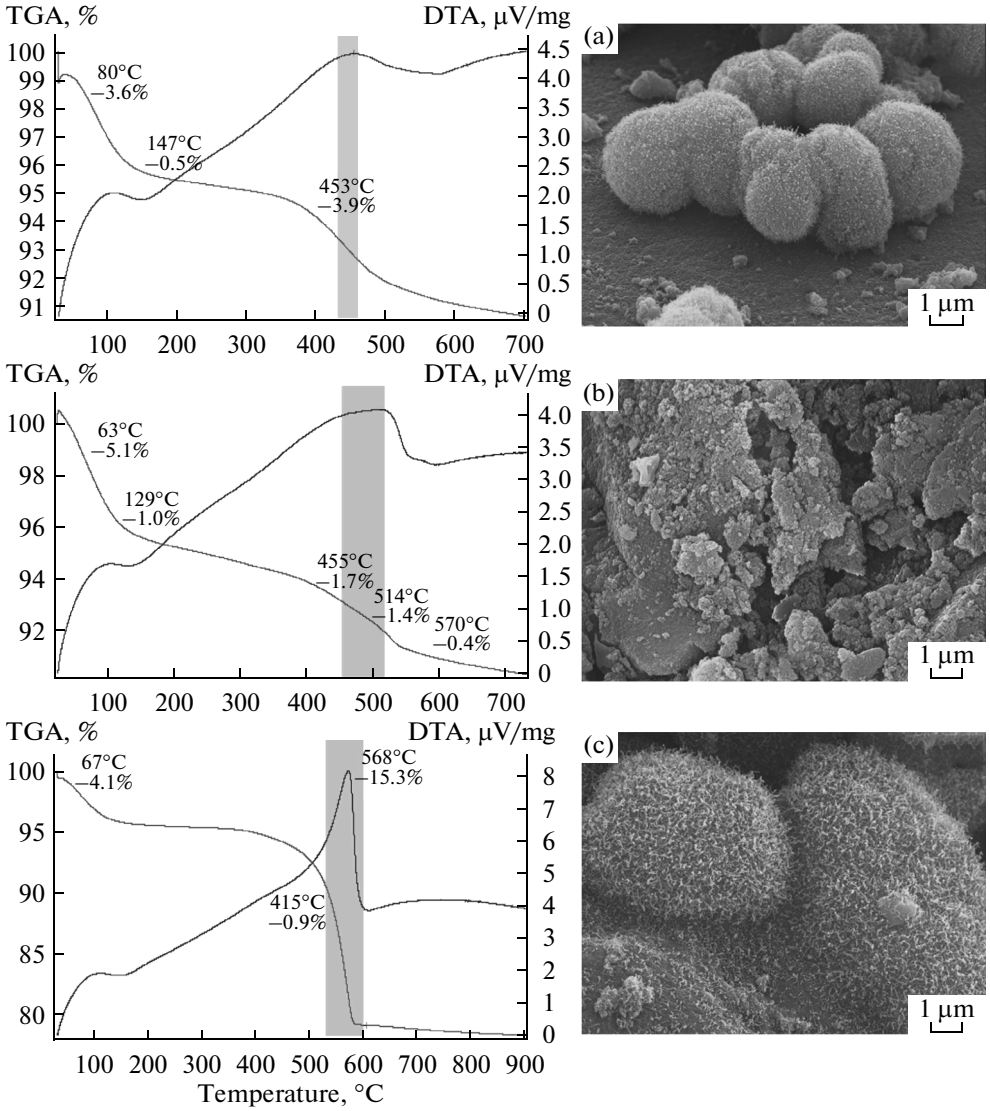

Fig. 5. Thermoanalytical curves (left column) and SEM images (right column) of NSC/Ni/SiO_2 adsorbents: (a) adsorbent containing 5% NSC, based on the 3.52% Ni catalyst prepared by the HP method; (b) adsorbent containing 4% NSC, based on the 0.8% Ni catalyst prepared by the Impr method; (c) adsorbent containing 16% NSC, based on the 1.72% Ni catalyst prepared by the Impr method.

in the esterification reaction. The specific activity of lipase adsorbed on the NSC-containing supports is 1.4–2 times lower than that of lipase adsorbed on the initial silica. The lowest specific activity of lipase was observed with the crust-type adsorbent. The adverse effect of the carbon surface on the activity of adsorbed lipase was also reported in our previous works [17, 29]. It was hypothesized in those works that, in lipase adsorption on hydrophobic carbon, there can be a "wrong" orientation of lipase on the surface. In other words, the active site of the enzyme can be "blocked" (screened) by the carbon surface and its accessibility to the substrate can thus be diminished. On the hydrophilic surface of the initial (carbon-free) silica, lipase is "rightly" oriented toward the substrate, maximizing the activity of the biocatalyst. All of the carbon-containing biocatalysts show an increased activity in the second or third reaction cycles (after 150-h-long operation), as is clear from Fig. 6b, and this is possibly evidence of the formation of a favorable, aqueous microenvironment around adsorbed lipase. A comparison between inactivation curves *2* and *4* in Fig. 6b demonstrates that the higher the NSC content of the adsorbent the lower the stability of the biocatalyst. Note that the on-stream stability of the biocatalysts in the isoamyl caprinate synthesis medium is fairly high. On being operated for over 500 h, the biocatalysts retain more than 70% of their initial activity (Fig. 6b). The activity of the biocatalysts does not change as they are stored in the hexane–diethyl ether mixture. The abrupt changes in the activity of the biocatalysts observed on passing from one reaction cycle to

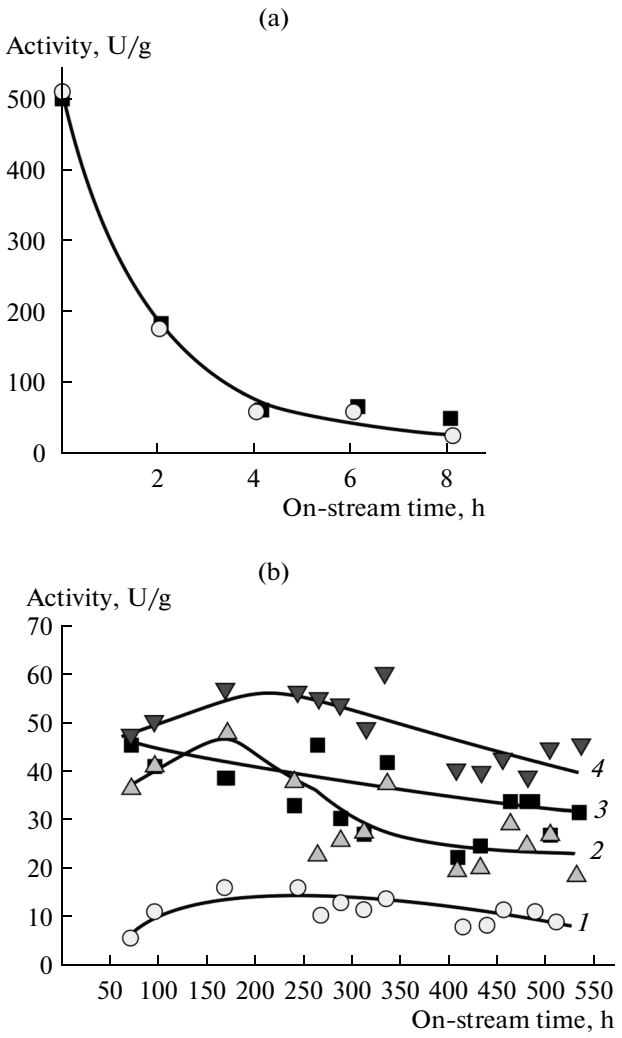

Fig. 6. Activity and stability of biocatalysts containing immobilized lipase: (a) tributyrin hydrolysis over the (■) initial SiO_2 and (○) (3%NSC)/Ni/SiO_2 (HP) adsorbent; (b) esterification reaction (isoamyl caprinate synthesis) over the (*1*) (5%NSC)/Ni/SiO_2 (HP) adsorbent, (*2*) (16%NSC)/Ni/SiO_2 (Impr) adsorbent, (*3*) initial SiO_2, and (*4*) (4%NSC)/Ni/SiO_2 (Impr) adsorbent.

another might be due to uncontrolled changes in reaction conditions inside prepared biocatalysts. We are planning to optimize the ester synthesis conditions, including by improving the reactor design.

The activity of the biocatalysts was also studied in the transesterification of vegetable oils with methyl or ethyl acetate or dimethyl carbonate. It turned out that the methyl or ethyl esters of fatty acids form only in the presence of methyl or ethyl acetate. The reaction rates in the presence of these acyl group donors are the same. The formation of fatty acid esters involving dimethyl carbonate proceeds very slowly. Noticeable amounts of the product were detected by TLC as late as 144 h after the beginning of catalyst operation. This result is at variance with data of Erzheng Su et al. [30], who carried out the enzymatic transesterification of an inedible oil from *Pistacia cinensis* in dimethyl or diethyl carbonate, which was used as the solvent and acyl donor. In that study, the yield of fatty acid esters was as high as 97.6%.

We focused on linseed oil transesterification with ethyl acetate. The mixture of the ethyl esters of essential ω-unsaturated acids from linseed oil (linoleic (18 : 2) and linolenic (18 : 3) acids), called vitamin F, is used in cosmetics, pharmaceuticals, and diets. In the transesterification reaction, as in esterification, the highest activity (~2 U/g) is shown by the biocatalyst based on adsorbent **II**; the lowest activity (~0.5 U/g), by the biocatalyst based on the crust-type adsorbent **I**. The full conversion of linseed oil triglycerides is achieved in 24 h and does not change throughout ten reaction cycles (biocatalyst on-stream time of 330 h). The biocatalysts were tested in linseed oil transesterification with ethyl acetate for 1000 h. Their half-inactivation time was determined to ~500 h at 40°C.

CONCLUSIONS

Different types of NSC/Ni/SiO_2 carbon-containing adsorbents were obtained by the pyrolysis of H_2 + C_3–C_4 alkane mixtures on supported Ni catalysts prepared by homogeneous precipitation or by impregnation of mesoporous silica. On the catalysts prepared by homogeneous precipitation, NSC appeared as carbon nanofibers localized near the granule surface, and these adsorbents had a crust-type structure. In the case of the catalysts prepared by impregnation, NSC synthesis took place inside SiO_2 granules, carbon was relatively uniformly distributed in the granules, and, at a supported Ni content of <1%, the carbon deposits did not have a well-defined filamentous structure.

Biocatalysts possessing a lipase activity were prepared by lipase adsorption on the resulting NSC/Ni/SiO_2 composites. The study of lipase adsorption and of the activity and stability of the adsorbed enzyme demonstrated that the optimal supports are the adsorbents obtained using Ni/SiO_2 catalysts prepared by impregnation. The amount of lipase adsorbed on these adsorbents, was 20–30 mg/g. These biocatalysts showed the highest activity and stability in hydrolysis, transesterification, and esterification reactions. With crust-type supports, both the amount of lipase adsorbed and the activity of the biocatalysts were many times lower. The stability of the biocatalysts prepared was estimated by performing batch esterification and transesterification processes for 500–1000 h. The half-inactivation time of the biocatalysts in linseed oil transesterification with ethyl acetate was determined to be ~500 h at 40°C.

ACKNOWLEDGMENTS

This study was supported by the Russian Academy of Sciences and Federal Agency for Scientific Organizations.

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Translated by D. Zvukov