Microbiology, Vol. 70, No. 3, 2001, pp. 277–284. Translated from Mikrobiologiya, Vol. 70, No. 3, 2001, pp. 329–336.
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EXPERIMENTAL ARTICLES

Microbial Degradation of 3,4-Dichloroaniline Sorbed by Activated Carbon

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Received July 25, 2000; in final form, October 5, 2000

Abstract—The availability of 3,4-dichloroaniline (DCA) sorbed by activated carbon to degradative microorganisms was studied. A *Paracoccus denitrificans* strain capable of growing on medium with DCA as the sole source of energy, carbon, and nitrogen was used in the experiment. The high sorption capacity of all the carbons studied (powdered RS and SKT-6A and granular AG-3) in relation to DCA (350 to 360, 480 to 520, and 540 to 580 mg/g, respectively) was demonstrated. The sorptive capacity correlated positively with the specific surface area and the total volume of the sorbent micropores. The bulk of the DCA was reversibly sorbed and amenable to microbial degradation; however, the degradation rates significantly differed. When RS, SKT, and Agrosorb preliminarily saturated with DCA were incubated in a culture of *P. denitrificans*, the bulk of the reversibly sorbed DCA was decomposed (in the absence of the other carbon sources) in 2, 5, and 10 weeks, respectively, after which the process slowed down. At the end of the experiment (29 weeks), 81 to 87% of the DCA underwent full mineralization, which was accompanied by the release of chlorine ions. A small fraction of the xenobiotic (0.8 to 1.9%) remained a reversibly sorbed fraction (extractable with acetone) and 12 to 17% of the initial DCA seemed to have been chemically transformed and bound by carbon. The studied carbons may be used in biological decontamination of chloroaniline-polluted soils to decrease the toxic effect of chloroanilines on microorganisms.

Key words: 3,4-dichloroaniline, degradative microorganisms, bioremediation, sorbents.

Since 1929, activated carbon, along with other sorbents, has been used to solve the problems related with environmental pollution. It has been widely used in the purification of drinking water chlorine and organic pollutants [4, 10]. The addition of this sorbent to activated sludge substantially accelerated the decontamination of sewage in the purifying units of enterprises which produce different chemicals, plastics, and dyes [12]. The introduction of activated carbon into soil decreased the penetration of pesticides and polychlorinatedbiphenyls (PCB) into the ground water and superficial reservoirs, as well as prevented these pollutants from accumulating in fish [13, 15]. The application of small doses of activated carbon on farming lands (50 to 400 kg/ha) or its use in seed coating decreased the aftereffects of a number of persistent herbicides, which favored the crops and allowed ecologically pure products to be obtained on contaminated soils [6]. Earlier, we proposed the method of adsorptive bioremediation for the decontamination of soils heavily contaminated with toxic organic chemicals. The method is based on the simultaneous introduction into contaminated soil of activated carbon and, if necessary, specific degradative microorganisms. Activated carbon performs the function of a buffer that maintains the concentration of pollutants in the soil solution at a level that is low-toxic for degradative microorganisms. The effectiveness of this method was demonstrated when the consequences of the accidental spill of 17 tons of propanide (3,4-dichloropropioanilide) in the Krasnodar krai were liquidated. Four months after treating the soil with activated carbon in a mixture with the microorganisms degrading chloroanilines, the total concentration of the herbicide and its main metabolite, 3,4-dichloroaniline (DCA), decreased from 15 to 0.5 g/kg, and one or two years later, their content in the soil approached the permissible level, constituting 5 to 10 mg/kg [1, 17]. Activated carbon was also shown to accelerate the detoxification of soil heavily contaminated with the explosive 2,4,6-trinitrotoluene (2 g/kg) [18].

The practical application of this method on a large scale may significantly extend the application of microorganisms for bioremediation of soils contaminated with various organic chemicals. In so doing, however, the potential danger of the conservation of the adsorbed xenobiotic and the secondary pollution of the environment should be considered. Therefore, the wide-scale

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Table 1. Characteristics of activated carbons used in the experiments

Conventional name	Production name (and place)	Starting material	Activation method	Specific surface area, m^2/g	Pore volume, cm^3/g (%)			Ash content, wt %	Fe content, wt %
					total	mesopores + macropores	micropores		
Agrosorb	AG-13 (Dzerzhinsk)	Coal	Steam treatment	963	0.55	0.19 (35)	0.36(65)	28.2	1.4
SKT	SKT-6A (Elektrostal')	Peat	Chemical treatment	1028	0.53	0.13(29)	0.40(75)	14.1	0.65
RS	RS (Krasnodar)	Rice straw	Steam treatment	410	0.50	0.4(80)	0.1(20)	13.0	0.59

use of sorbents for biological soil decontamination should be preceded by a comprehensive study of the mechanisms of sorption of chemicals by activated carbon and of the accessibility of sorbed pollutants to degradative microbes. In several works, the feasibility of microbial degradation of organic substances sorbed by activated carbon has been demonstrated [4, 9, 11, 14]. However, information regarding this problem is very limited and contradictory.

DCA is one of the most toxic and persistent chloroanilines. It usually accumulates in soils that are treated with its derivative arylamide herbicides (propanide, linuron, diuron, etc.). Earlier, a number of microorganisms capable of growing on medium with mono- and dichloroanilines as the sole sources of energy, carbon, and nitrogen were isolated from the soil of the Kuban rice fields regularly treated with propanide [7, 8]. Among the strains isolated, a *P. denitrificans* strain was the most resistant to DCA. DCA decomposition by this and other degradative bacteria follows the pathway of its oxidative deamination, yielding 4,5-dichloropyrocatechol, with the subsequent cleavage of the aromatic ring via the *ortho*- or *meta*-pathway and the complete degradation of the molecule.

This work was designed to study the capacity of different varieties of activated carbon for DCA sorption and to determine the accessibility of the sorbed DCA to *P. denitrificans*.

MATERIALS AND METHODS

Growing of microorganisms. The *Paracoccus denitrificans* strain was grown in minimal liquid medium of the following composition (g/l): K_2HPO_4, 7, KH_2PO_4, 3, $MgSO_4 \cdot 7H_2O$, 0.1; 4-chloroaniline, 0.2; trace elements, according to [8]; distilled water.

Characteristics of activated carbons. Three varieties of activated carbons were used in the experiments, including the granular AG-3 (hereinafter Agrosorb) and the powdered SKT-6A (hereinafter SKT) and RS. The micropore structure parameters were calculated according to the Dubinin–Radushkevich equation from the adsorption isotherms of benzene vapors, which were determined by the standard methods using McBen balance at 20°C. Analysis of the physicochemical and adsorption properties of the carbons was performed according to the methods adopted in the technology of activated carbons [3, 5]. The ash and iron contents were determined according to GOST (State Standard) 4453-74. The carbon characteristics determined are shown in Table 1.

DCA sorption isotherms. The isotherms of DCA sorption by activated carbons were obtained according to the standard methods in a static mode [4, 10]. To do this, suspensions of activated carbon (0.05 g) in 100 ml of DCA solutions (0, 30, 60, 100, 150, and 300 mg/l) were shaken in Erlenmeyer flasks on a shaker at room temperature. After the sorption equilibrium was established (48 h for Agrosorb and 30 min for SKT and RS, as determined in a preliminary experiment), the liquid phase was separated by filtration through a dense paper filter (the blue tape), and the equilibrium DCA concentration in the solution was determined. The amount of the DCA sorbed was calculated from the difference between the initial and equilibrium concentrations.

Preparation of activated carbon saturated with DCA. Activated carbon (0.2 g) was placed in a 1-cm glass column and saturated with DCA by continuous washing with a DCA solution (300 mg/l), performed until the concentration of the chemical in the eluate was 25% of the initial level. For further experiments with microorganisms, activated carbon was dried in the air, and a mixed sample of the carbons saturated on several columns was prepared and analyzed for the content of reversibly sorbed and bound hydrolyzable DCA. The experiments were carried out under sterile conditions to prevent the samples from infection with microorganisms degrading chloroanilines.

DCA desorption. DCA desorption from activated carbon was studied in the dynamic mode immediately after its saturation on the column. Fractionated DCA extraction was carried out. At first, activated carbon in the column was washed with distilled water until the concentration of DCA in the eluate stabilized at a value of less than 10 mg/l. Residual DCA molecules that were more firmly sorbed by the carbon were then desorbed with acetone. Activated carbon was transferred into Erlenmeyer flasks and shaken an additional three or four times for 1 h with 100 ml of acetone in order for

the reversibly sorbed DCA to be extracted more completely. The content of the bound hydrolyzable DCA remaining in the carbon was determined by alkaline hydrolysis of the sample (1-h boiling with 100 ml of 5 N NaOH) and subsequent distillation of the liberated DCA with vapor and trapping in 6 N HCl. The concentration of DCA and possible metabolites in solutions and extracts was determined by high-pressure liquid chromatography. The amount of desorbed DCA was determined from the difference between the total amount of DCA in the inlet and outlet solutions. The amount of the desorbed xenobiotic was assessed from the total amount of DCA detected in the aqueous eluate, acetone eluate, and extracts.

Availability of sorbed DCA to *P. denitrificans*. A weighed portion of activated carbon saturated with DCA (0.12 g) was placed in a flask with 100 ml of mineral medium (pH 7) containing (g/l of distilled water) K_2HPO_4, 7, KH_2PO_4, 3, $MgSO_4 \cdot 7H_2O$, 0.1, and inoculated with *P. denitrificans* cells (2×10^5 cells/ml). Control samples were not inoculated. To study the influence of activated carbon on *P. denitrificans*, control samples were prepared without activated carbon. In these control experiments, the dynamics of DCA decomposition (60 mg/ml) were studied in the medium of the above composition inoculated with *P. denitrificans* (2×10^5 cells/ml). All flasks were incubated under stationary conditions in the dark at 28°C. The dissolved and sorbed DCA content in the samples was determined at regular time intervals (3 to 10 days). The biodegradation rate was judged from the release of chlorine ions into the solution. This was based on the earlier established virtually stoichiometric release of chlorine ions during DCA utilization by *P. denitrificans* [8], as well as on the fact that the sorption capacity of activated carbons with respect to chlorine ions is insignificant [4]. For this purpose, 2 ml of the solution was periodically withdrawn from each of the samples and analyzed for the chlorine ion content.

Chemical analysis of the samples. For separate determination of dissolved and sorbed DCA in the suspension of activated carbon, all the samples were filtered through the paper filter with a blue tape, and the DCA concentration in the filtrate was measured. The carbon was then placed together with the filter into conical flasks, and DCA was extracted three times by shaking with acetone (100 ml) for 1 h. When studying the accessibility of the sorbed DCA to *P. denitrificans*, fractionated extraction of DCA in several samples was carried out with water and then by acetone. For this purpose, the sample was shaken with 100 ml of water to remove the bulk of the easily extractable xenobiotic, after which the remaining DCA was extracted with acetone.

Analysis of the filtrates and extracts for the DCA content was carried out by high-pressure liquid chromatography using a Liquohrome chromatograph (Radelkis) equipped with a UV-VIS detector and a Nucleosil chromatographic column (Keystone Scientific, Inc., Bellefonte, PA USA). Elution was carried out with a mixture of solution A (1.38 g of sodium acetate and 20 ml of glacial acetic acid per 1 l of water) and solution B (1.38 g of sodium acetate and 20 ml of gla-

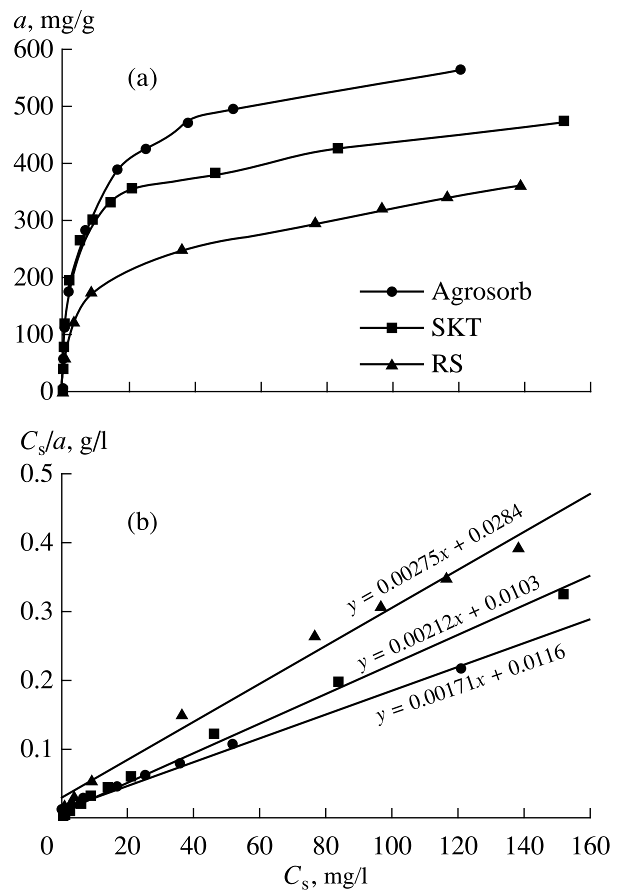

Fig. 1. Isotherms of DCA sorption by activated carbons plotted (a) in the ordinary coordinates and (b) in the Langmuir equation coordinates.

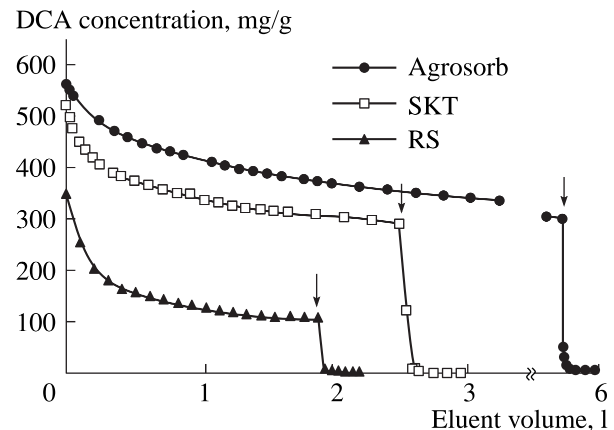

Fig. 2. Residual content of DCA in activated carbons after its desorption with water and then with acetone from the three varieties of activated carbons. The arrows show the beginning of washing with acetone.

Table 2. Transformation of DCA sorbed by different varieties of carbons after a 200-day incubation period in medium with *P. denitrificans* as compared to sterile control

Conventional name of the carbon	Initial DCA content in the carbon, mg/g	Inoculation	DCA detected, %			Notdetected, %
			decomposed with the evolution of Cl^-	extracted with acetone	total	
Agrosorb	563	*P. denitrificans*	81*	1.7	82.7	18.3
		Control	0	80.7	80.7	19.3
SKT	473	*P. denitrificans*	83	1.9	84.9	15.1
		Control	0	79.1	79.1	21.9
RS	348	*P. denitrificans*	87	0.8	87.8	12.2
		Control	0	63.6	63.6	36.4

* The relative determination error did not exceed 5%.

cial acetic acid per 1 l of methanol), with the A to B ratio of 30 : 70 (pH 3.3); the elution rate was 1 ml/min. The DCA concentration in the aqueous and acetone solutions was determined from the absorption at 254 nm. Before analysis, the sample was additionally filtered through the Millipore filter using a syringe with a nozzle for filtration.

The chlorine ion concentration was determined potentiometrically using an OP-Cl-7113D ion-selective membrane electrode in a pair with an OP-7203 comparison electrode and an OP-264 potentiometer (Radelkis, Hungary). The sensitivity of the method with respect to chlorine ions is 2 mg/l, which corresponds to the complete decomposition of 5 mg DCA/l. The relative determination error is 5% at the complete decomposition of 50 mg DCA/l.

RESULTS

DCA sorption isotherms. The isotherms of DCA sorption by the three varieties of activated carbons are shown in Fig. 1. All of the sorption isotherms belong to the *L*-type and, according to Jills' classification [4, 10], are satisfactorily described with the Langmuir equation

$$\frac{C_s}{a} = \frac{1}{a_m b} + \frac{1}{a_m} C_s,$$

where C_s is the equilibrium sorbate concentration in the solution (mg/l); a is specific sorption (mg/g); a_m is maximal sorption (mg/g); and b is a constant. In this equation's coordinates, the isotherms are plotted as straight lines whose slope can be used to calculate the maximal DCA sorption values, which constituted 583 ± 25, 480 ± 21, and 364 ± 19 mg/g for Agrosorb, SKT, and RS, respectively.

DCA desorption. Proceeding from the supposition that the sorbed DCA extractable with water and the sorbed DCA extractable with acetone differ in their accessibility, step-wise DCA desorption from the carbons was carried out. In all the three variants, the elution of the xenobiotic by water decreased with time (Fig. 2). At first, the eluent concentration of the chemical reached 100 mg/l and more, but after its concentration had decreased to 4–6 mg/l, it remained at this level for a long period of time. As a result, 42, 44, and 66% of the xenobiotic was eluted with water from the DCA-saturated carbons Agrosorb, SKT, and RS, respectively.

The remaining reversibly sorbed DCA was comparatively readily desorbed by acetone, mostly when the carbon was passively washed in the column. Only an insignificant amount of DCA (1%) was extracted during the additional extraction with shaking. The total amount of the reversibly sorbed xenobiotic detected in Agrosorb, SKT, and RS as a result of the sequential extraction with water and acetone was 540 ± 28, 521 ± 25, and 334 ± 17 mg DCA/g, respectively, which was close to the values of the maximal DCA sorption by the carbons calculated from the adsorption isotherms. Only a small DCA portion (0.5%) was extracted from activated carbon during alkaline hydrolysis. Thus, the bulk of DCA absorbed by the carbons was sorbed reversibly (i.e., due to van der Waals forces and hydrogen bonds).

Availability of sorbed DCA to *P. denitrificans*. The results of the experiments on the availability of carbon-sorbed DCA to *P. denitrificans* are shown in Fig. 3 and in Table 1. A decrease in the concentration of DCA extracted from the carbons with acetone occurred throughout the incubation period (200 days). RS-sorbed DCA was degraded at a maximum rate, while the degradation of the xenobiotic sorbed by SKT and, especially, Agrosorb occurred slowly (Fig. 3a). The process of decomposition of DCA sorbed by Agrosorb, SKT, and RS occurred mainly in the first 10, 5, and 2 weeks, respectively. However, the amount of the remaining extractable DCA in all these carbons was insignificant: 13.1, 14. 3, and 3.8 mg DCA/g sorbent (or 1.7, 1.9, and 0.8%), respectively. After a 200-day incubation period, the control (not inoculated) samples retained 80.7, 79.1, and 63.3% of the initial DCA, respectively. The

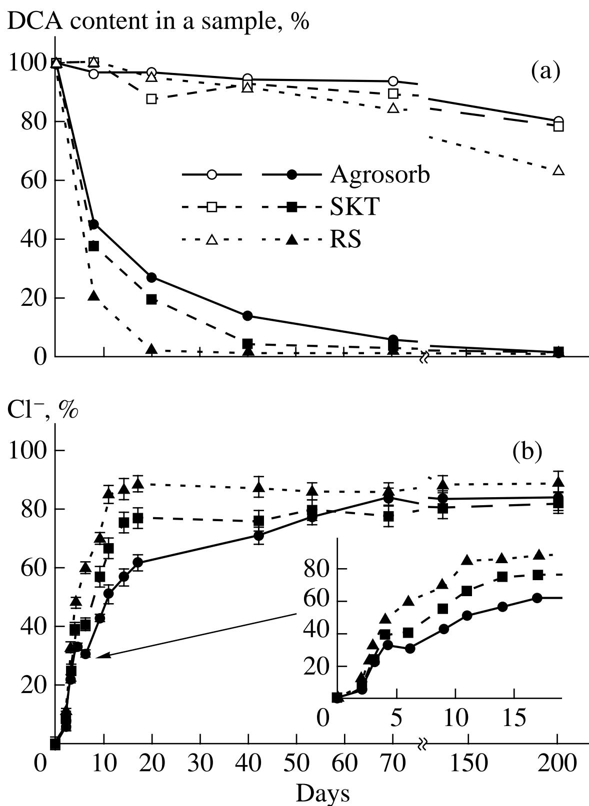

Fig. 3. (a) Changes in the total sample content of DCA (dissolved and acetone-extractable) and (b) release of chlorine ions during the incubation of the suspensions of three varieties of DCA-saturated carbons in medium with *P. denitrificans* (filled symbols) and in the sterile control (open symbols).

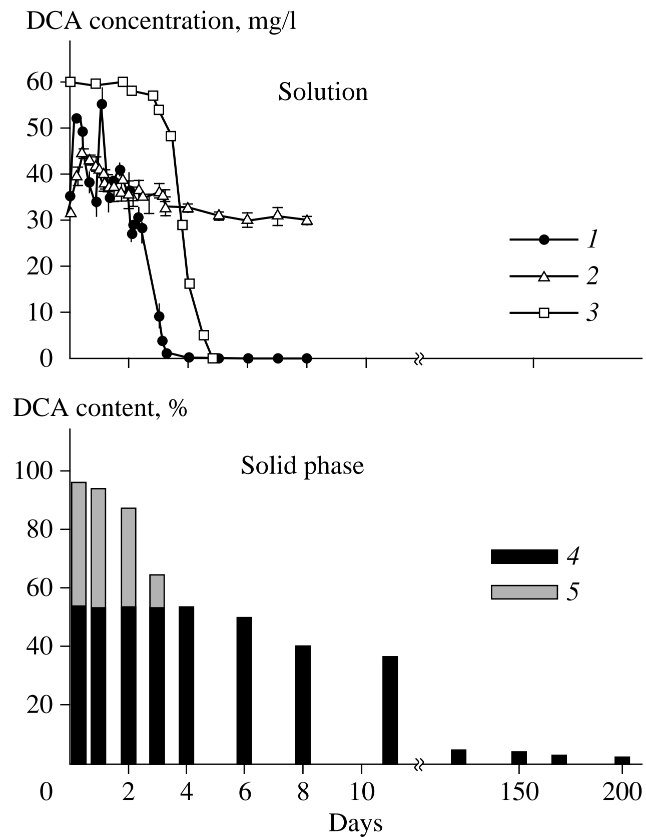

Fig. 4. Decomposition of (*1, 4, 5*) Agrosorb-sorbed DCA under the action of *P. denitrificans* in comparison with DCA decomposition in (*2*) the sterile control and (*3*) carbon-free culture medium. The DCA content in the supernatant (*1, 2, 3*) and in the solid phase sequentially extracted with (*5*) water and (*4*) acetone.

control samples remained sterile throughout the experiment.

In the test samples with microorganisms, the accumulation of chlorine ions in the solution occurred in parallel with the disappearance of DCA (Fig. 3b), whereas in the control uninoculated samples, chlorine ions did not accumulate. The dechlorination curves allowed us to reveal the two-step form of the curves of DCA decomposition by microorganisms. At the first stage, DCA was decomposed at the maximum rate that was virtually the same for all the carbons. At this stage, 35 to 40% of the xenobiotic adsorbed by Agrosorb and SKT and 65 to 80% of that adsorbed by RS underwent dechlorination. On the contrary, the DCA mineralization rates at the second stage were substantially different; the DCA mineralization pattern was a mirror image of the disappearance pattern of the extractable xenobiotic; DCA dechlorination occurred at the highest rate in the RS suspension and at the lowest rate in the Agrosorb suspension. However, in all variants, a significant amount of the xenobiotic (81 to 87% of the initial value) underwent microbial decomposition at the end of incubation. The calculations of the DCA balance at the end of the experiment, taking into account the DCA completely decomposed with the release of chlorine ions and the DCA remaining on the carbon in the reversibly sorbed (extractable by solvent) form and in the bound hydrolyzable form, allowed 83 to 88% of the initial sorbate to be detected.

The results of one more experiment in which the dissolved DCA content was analyzed and fractionation analysis of the solid phase was carried out confirmed the two-stage nature of the sorbate biodegradation process. At the beginning of the incubation of DCA-saturated Agrosorb, SKT, and RS, the xenobiotic concentrations in the aqueous phase was 30, 43, and 75 mg/l, respectively. The dissolved and water-extractable DCA decomposed almost simultaneously (over three to four days), which was accompanied by a virtually stoichiometric release of chlorine ions. Figure 4 shows the results obtained with Agrosorb. It should be noted that the decomposition of the dissolved and water-extractable DCA was characterized by rates and curves similar to the characteristic of DCA decomposition by *P. denitrificans* in sorbent-free medium at an initial DCA concentration of 60 mg/l. Although the content of the extractable DCA in the carbons continued decreasing at the next stage, the sorbed DCA decomposition rate,

Table 3. Comparison of the sizes of DCA molecule, activated carbon pores, and bacterial cells [4, 10]

	Size, nm
Activated carbon macropores	>500
Activated carbon mesopores	2–500
Activated carbon micropores	<2
P. denitrificans cells	500
3,4-Dichloroaniline molecules	0.35×1.0

however, slowed down. The supernatant concentration of the chemical remained low (0.01 to 0.05 mg/l). The DCA decomposition at the second stage was also accompanied by the release of chlorine ions; however, the amount of completely mineralized DCA appeared to be by 16 to 19% less than the amount of decomposed xenobiotic calculated from its disappearance.

DISCUSSION

The results of our experiments show that activated carbons possess a high sorption capacity with respect to DCA and are capable of considerably decreasing its concentration in an aqueous solution. The different time of establishment of the equilibrium between the dissolved and sorbed DCA in the suspensions of the powdered SKT and RS carbons (0.5 h) and the granular Agrosorb carbon (48 h) reflects the prolonged process of the penetration of DCA molecules into the micropores of the granules [4]. The convex shape of the DCA adsorption isotherms, satisfactorily described by the Langmuir equation, is indicative of the predominantly monolayer filling of the sorptive surface of the activated carbons [4, 10].

The calculated and experimentally obtained sorptive capacities of the carbons with respect to DCA were close. For Agrosorb, SKT, and RS, they varied within 540–580, 480–520, and 350–360 mg/l, respectively. These values satisfactorily correlate with the specific area of the carbon surface (963, 1028, and 310 m^2/g, respectively), as well as with the total micropore volume (0.36, 0.40, and 0.1 cm^3/g, respectively).

The regularities revealed agree perfectly with the modern concept of the mechanism of the sorption of organic compounds by carbons. Activated carbon is a carbon sorbent in which slit-like micropores whose width is less than 2 nm occupy a significant volume. The increased surface hydrophobicity ensures the high sorptive ability of activated carbon with respect to many hydrophobic organic molecules, particularly DCA, whose molecular size ($0.35 \times 1\ nm^2$) allow them to penetrate into most of the micropores. The main surface area of activated carbons is usually accounted for by micropores; therefore, the relative content of micropores determines the sorption properties of carbons. As a result, fine-pore activated carbons such as Agrosorb and SKT, obtained from coal and peat, significantly exceed in their sorption properties the coarse-pored carbon RS, obtained on the basis of raw plant material. Somewhat better sorption characteristics of Agrosorb, having a somewhat lesser specific surface area and a lesser micropore volume compared with SKT, may be related to an increased content of iron and other ash elements that normally increase the chemosorption of many chemically active organic compounds. Catalytic oxidation, polymerization, and the binding of certain substituted anilines and phenols by activated carbons were reported in a number of papers [16, 19, 20].

A significant part of the DCA was comparatively easily washed out from the carbons with water, but, after attaining a certain level, the process dramatically slowed down. As a result of prolonged washing of Agrosorb, SKT, and RS with water under kinetic conditions, the sorbed DCA fraction extractable with water constituted 42, 44, and 65 to 80%, respectively. The rest of the reversibly sorbed DCA was easily washed out with acetone. The size of the water-extractable DCA fraction in the carbons correlates well with the total volume of meso- and macropores (29, 35, and 80%, respectively); on the contrary, the size of the acetone-extractable DCA fraction correlates with the micropore content (71, 65, and 20%, respectively). This fact gives indirect evidence of the localization of the water-extractable fraction of the sorbed DCA molecules predominantly in macro- and mesopores and of the localization of the acetone-extractable fraction in the micropores. Desorption of physically sorbed organic molecules in solutions is known to occur due to diffusion toward a decrease in the chemical concentration gradient. The cause for the nonuniform desorption of DCA from the activated carbons is the decelerated diffusion of molecules from micropores due to frequent collisions with their surfaces [4, 10]. After the washout of DCA molecules from the macro- and mesopores, the release of DCA into the solution continues mostly due to the molecules present in the micropores. However, the DCA removal rate decelerates, since it is limited by the surface diffusion rate inside the micropores. With the passage of time, the DCA desorption rate becomes negligible. This occurs because of a dramatic increase in the molecular adsorption energy due to partial overlapping of the adsorption forces exerted by the opposite walls of the finest micropores, which are commensurable in width with the size of the adsorbate molecule. Organic solvents (acetone, etc.) whose energy of sorption by the activated carbon surface is close to that of DCA are able to readily dislodge the sorbed xenobiotic molecules from the micropores.

The results of our experiments with *P. denitrificans* showed that degradative microorganisms using DCA as the substrate are capable of utilizing both water-extractable and most of the acetone-extractable DCA. The comparison of the sizes of DCA molecules pores of the activated carbons, and bacterial cells (Table 3) suggests

the ability of the bacteria to penetrate only into macropores; hence, for DCA to be utilized by microorganisms, its desorption from the pore space to the outer surface of the carbon particles is necessary. The comparison of the rates and the degrees of completeness of the microbial decomposition of different DCA fractions suggests that the DCA molecules sorbed in macro- and mesopores are readily extractable with water and at the same time, are easily accessible to the degradative microorganisms. This conclusion is supported by the virtually simultaneous decomposition of the dissolved and water-extractable xenobiotic in the suspensions of activated carbons. The similarity between the dynamics of the disappearance of these two fractions and the dynamics of the DCA decomposition in the culture of *P. denitrificans* testifies to the absence of any inhibitory effect of the carbon on bacterial growth. Moreover, activated carbon stimulates bacterial growth, since it serves as a sort of a buffer decreasing the toxic effect of the high DCA concentrations on microorganisms. In the presence of activated carbon, microorganisms utilize a significantly greater amount of DCA than it is possible in the culture medium without the sorbent. Calculations show that if all the DCA initially present in the suspension with Agrosorb were in the solution, its concentration (approximately 275 mg/l) would substantially exceed the maximum concentration of this substrate at which the growth of *P. denitrificans* is possible (100–150 mg/l) [8].

The degradation rate of the acetone-extractable fraction is substantially less than that of the water-extractable fraction. Nevertheless, at the end of the experiment (200 days), the bulk of DCA sorbed by carbons (79 to 83%) was utilized by microorganisms with the release of chlorine ions. Easier desorption and, accordingly, quicker decomposition of RS-sorbed DCA are likely to be determined by a lesser micropore volume in this carbon, as compared with Agrosorb and SKT. Decelerated DCA dechlorination in the suspension of the granular carbon Agrosorb can also be explained by a significantly greater distance between the bacterial cells and DCA molecules sorbed by carbon granules, as compared with the molecules sorbed by the finer powdered particles.

Despite prolonged incubation, a small portion of carbon-sorbed DCA (0.7 to 1.8% of the initial value) remained unavailable to the microorganisms. Apparently, this is the xenobiotic fraction sorbed by the finest pores.

Thus, *P. denitrificans* cells, penetrating only into the macropores, are also able to degrade the xenobiotic present in the mesopores and in most micropores. The bacteria seem to accelerate the xenobiotic desorption from the carbon pores due to cell excretions either acting as surface-active compounds or acidifying the medium in the microzones on the surface of the carbons. Balance calculations allow the detection of only 83 to 88% of the initial DCA; the remaining 12 to 17% may be accounted for by the chemical transformation of DCA on the surface of the activated carbons. Evidently, more significant chemical DCA transformation in the sterile variants (15 to 36%) occurs due to a higher DCA concentration in the medium throughout the incubation period.

Thus, if sorbents are used during the bioremediation of soil contaminated with DCA and its derivative herbicides, there is a certain danger of the retention of residual amounts of reversibly sorbed xenobiotic. However, the low accessibility of this DCA fraction to the degradative microorganisms suggests its low toxicity to other soil flora and fauna. Moreover, as this portion of the xenobiotic is gradually released into the soil solution, the DCA molecules should be quickly utilized by degradative microorganisms. Prolonged (no less than seven years) retention of such microorganisms introduced into the soil was demonstrated by us earlier [2]. Additionally, by selecting a sorbent characterized by the character of interaction with the sorbate, ensuring a high sorption capacity on the one hand, and the most complete desorption of the latter on the other hand, it will be possible to considerably lower the risk of the retention of residual amounts of the xenobiotic in soil.

ACKNOWLEDGMENTS

We are grateful to M.S. Sokolov for helpful discussions and V.S. Belousov for providing samples of activated carbon.

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