Biodegradation: Selection of Suitable Model

A. Četkauskaitė, U. Grigonis, and J. Beržinskenė

Department of Biochemistry and Biophysics, Faculty Nature Sciences, Vilnius University, Čiurlionio Str. 21, 2009 Vilnius, Lithuania

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Biodegradation of five herbicides, two acetanilides (propanil and propachlor), and three phenylureas (diuron, monuron, and fenuron) was analyzed in samples of river water during a period of 6–8 weeks. Concentrations of 0.2–5.0 mg/liter of the herbicides were used for biodegradation. Two types of river water samples with different numbers of microorganisms were collected from the Neris River: upstream and downstream of the city of Vilnius. The initial concentration of microorganisms varied from $4.7 \times 10^5$ to $2.7 \times 10^6$ cells/liter and from $1.4 \times 10^4$ to $5.3 \times 10^6$ cells/liter in water samples from the Neris River upstream and downstream of Vilnius, respectively. Chemical analysis was performed by the HPLC technique, using standards of herbicides and likely degradation products. Chemical parameters of different river water samples used in biodegradation experiments were analyzed. A second-order reaction rate model was used for the analysis of biodegradation data. Values of the first-order rate constants ($K_b$) revealed the following decrease in the biodegradation rate of herbicides: propanil > diuron > monuron > propachlor > fenuron. This sequence was constant for all water samples analyzed. The set of decreasing value of second-order biodegradation rate constants ($K_{b*}$) differed from the set of first-order constants ($K_b$) because the total number of bacteria in the water samples varied by up to two orders of magnitude, and this variation influenced the calculated values of $K_{b*}$. Thus, different sets of $K_{b*}$ values were obtained for the water samples from the river upstream and downstream of the city. Schemes of a variety of biodegradation models are presented, and the suitability of the second-order reaction rate model for the description of biodegradation of xenobiotics is discussed.

INTRODUCTION

The history of kinetic research in analytical biodegradation of xenobiotics goes back more than 40 years. A variety of kinetic models were developed by technologists and analytical researchers for different purposes or endpoints, mainly bioremediative or prognostic. The following scheme presents some examples of kinetic models in biodegradation (Fig. 1). It illustrates different analytical endpoints in biodegradation research and different results obtained for the risk assessment. The BOD$_5$/COD ratio, or biochemical index, is one of the earliest and most popular (among wastewater treatment technologists) evaluations of wastewater discharges (WWD). It helps to characterize the biodegradability of industrial wastewaters and the quality of processes in wastewater treatment plants. On the basis of the different rates of reactions and different data application in biotechnology or pure modeling, the first-order and second-order kinetic models were used by various scientists (Larson and Cowan, 1995; Novick and Alexander, 1985; Paris and Rogers, 1986) to describe the biodegradation processes. Relatively expensive standard methods of chemical analysis were and still are used for biodegradation evaluation in the aforementioned technological and kinetic approaches. Searching for cost-effective-analysis methods in biodegradation led to the application of toxicological evaluation of biodegradation endpoints; that is, to the use of acceptable/functional biodegradation (see Fig. 1, positions IV and VII).

Experiments on the biodegradation of phenols, dichlorophenoxyacetic acid derivatives, aniline derivatives, and acetanilides by surface water microorganisms in nonstandard conditions were performed by various groups of researchers (Paris and Rogers, 1986; Paris and Wolfe, 1987; Paris et al., 1981, 1983; Steen and Collete, 1989; Vasilyeva et al., 1989) during the period 1981–1990.

Primary microbial transformation of chlorinated herbicides was described by using the second-order reaction rate equation by Paris et al., (1981):

$$\frac{dc}{dt} = K_{b*} B \cdot C,$$

where $K_{b*}$ is the second-order reaction rate constant, $B$ is bacterial biomass, $C$ is the concentration of the substance in water, $t$ is time, and $Kb* / B = K$— that is, the first-order rate constant $K$ (on the basis of this concept, $K$ linearly depends on microbial biomass).

This equation describes the dependence of the biodegradation rate of the xenobiotic on both its concentration and its bacterial biomass (i.e., first-order in both chemical and bacterial concentration) and provides some insight into the effects of the chemical structure of xenobiotics within the...
same class of chemicals (Paris et al., 1981). The reasons why authors used the second-order rate constants are:

1. Exposure Analysis Modeling System (EXAMS II) type models, describing the fate/transformation of xenobiotics in the aquatic environment (chemical degradation, biodegradation, sorption, volatilization, etc.), were created by using second-order reaction rate constants ($K$);

2. Second-order microbial degradation constants, $K_a$, obtained in experiments with pure or mixed microbial populations from surface waters, were found to have positive correlation with appropriate physicochemical parameters of molecules of chemical congeners.

For example, positive correlation of $K_a$ was obtained with:

- (i) Van der Waals radius, $\gamma_{vdw}$, for chlor-, brom-, methyl-, nitro-, cyano-monosubstituted phenol and aniline derivatives (Paris et al., 1982, 1983; Paris and Wolfe, 1987);
- (ii) octanol/water partition coefficient, $P_{oct/w}$, for 2,4-dichlorophenoxy acetic acid ester derivatives (Paris et al., 1984);
- (iii) $P_{oct/w}$ and Hammett constant, $\sigma$, for mono-, di-, tri-, and tetrachloro-substituted derivatives of phenoxy acetic acid esters (Paris et al., 1984);
- (iv) frequency of the C=O peak infrared spectrum, for acetanilides (Steen and Collete, 1989).

Many experiments were performed by using mixed bacterial cultures from surface waters; some of them (Steen and Collete, 1987; Vasilyeva et al., 1989, Paris and Wolfe, 1987, Paris and Rogers, 1986) aimed at comparing biodegradation rates of the same compound by using microflora from different sources of surface water.

Concentrations of test compounds were relatively low (0.1–1.0 mg/liter) in these experiments where the
second-order reaction rate model was used to describe biodegradation. Meanwhile, standard tests (ISO and OECD) of biodegradation recommend the use of much higher concentrations of test compounds—namely, 5–40 and 100 mg/liter (ISO, 1994; Kitano, 1992). Thus, it was important to analyze biodegradation of chlorogenic compounds in surface waters where:

(a) higher concentrations of chemicals tested are present (more than 0.1–1.0 mg/liter) that are comparable with COD or BOD$_5$ values usually characteristic of surface waters;

(b) significant difference in bacterial biomass exists in different water samples;

(c) significant difference in nutrient (N and P) concentration exists in different water samples.

Phenylurea and acetanilides are chlororganic herbicides of older classes. They were intensively used in agriculture in Lithuania until 1990 (Suksyte, 1990). Some of them, such as propanil and pronamide, are still used in mixtures (basagran, etc.) or together with new-generation herbicides such as sulfonylureas (Valionis et al., 1992). Thus, analyses was made of biodegradation of phenylurea and acetanilide herbicides in a higher concentration range—namely, 1–5 mg/liter. The microbial transformation kinetics for two acetanilide (propanil and propachlor) and three phenylurea (fenuron, monuron, and diuron) herbicides was measured in surface water samples from the Neris River that differed in total numbers of bacteria up to two orders of magnitude.

MATERIALS AND METHODS

Test Samples

Samples were collected in the Neris River upstream and downstream of the city of Vilnius at the sampling points used for water monitoring by chemical laboratories of the Joint Research Center of the Environment Protection Ministry of Lithuania during joint expeditions in the summer periods of 1991–1994. Two types of river water samples with different numbers of microorganisms were collected from the Neris River upstream and downstream of Vilnius (city’s wastewater treatment plant). The initial concentration of microorganisms varied in those waters by up to a factor of 100. Some water samples were collected in the Vilnelė River, tributary of the Neris River. Water samples were transported to the laboratory in a few hours and stored at +2°C, and the experiment was started on the next day.

Biodegradation Experiments

Control samples (50 ml) for biodegradation analysis of the herbicides were autoclaved, and, after cooling, the herbicides were added to all samples; triplicate samples in flasks (each 50 ml) were incubated on the water-bath shakers as described in Četkauskaitė et al. (1994).

Biodegradation experiments lasted as follows: (1) with propanil, 200 h (1 week and 1 day); (2) with propachlor, 1000 h (6 weeks); (3) with fenuron, 1200 h (7 weeks); (4) with monuron, 1400 h (8 weeks); (5) with diuron, 1400 h (8 weeks). Samples for chemical and microbiological analysis were taken from each flask aseptically every 2 days (for propanil samples) or within 3–4 days (for other herbicides) during the biodegradation experiment.

Chemical Analysis

The chemicals used were: herbicides propanil and propachlor (99.9% purity, reference standards, EPA-Research Triangle Park, USA), 3,4-dichloraniline (EPA, technical grade), and acetanilide (HPLC grade, Merck, Germany). Fenuron, monuron, and diuron were from Aldrich (rare chemicals, analytical grade).

Chemical analysis was performed by the HPLC technique by using Waters chromatograph: solvent delivery system, Model 6000 A, and UV detector, Model 450. The aforementioned herbicides and some of their degradation products were used as reference standards.

The conditions for HPLC analysis of acetanilides were: (1) reversed phase (C18) Ultrasphere ODS column 4.6 mm × 25 cm (Beckman Instruments); (2) UV detector with absorption $\lambda_{max} = 225$ nm for propanil and 3,4-dichloraniline, $\lambda_{max} = 238$ nm for propachlor; (3) mobile phase—acetonitrile:water = 75:25 for propanil and 80:20 for propachlor, (4) flow rate 1 ml/min$^1$. The chemical parameters of different river water samples (COD, BOD, ammonia, nitrate, phosphate concentrations, etc.) were determined by chemical laboratories of the Joint Research Center of Lithuanian Environmental Protection Ministry.

Microbiological Analysis

Changes in microbial biomass (bacterial biomass, number of cells/liter) during the biodegradation experiment were determined by direct plate count. Nutrient agar medium, containing 17 g/liter sprat hydrolysate, 11.2 g/liter agar, 5.9 g/liter sodium chloride, and glucose, 0.5%, pH 7.2–7.4, was used for microbiological analysis in the laboratory. The standard microbiological parameters of different river water samples (numbers of psychrophiles and mezophiles, and total number of microorganisms) were determined by the methods published in the official document of the Environment Protection Ministry of Lithuania (LAND-595/M-01, 1995).

Results Analysis

Statistical and regressive analysis of the data from biodegradation experiments was based on the least-square method and equations of linear regression. The half-life of
the herbicide biodegradation reaction and first-order reaction rate constants were calculated according to the equations (Metsler, 1980). Statistical and regresional parameters, offered by scientists from the Department of Microbiology of the U.S.A EPA Environmental Research Laboratory in Athens, Georgia (Steen and Collete, 1989; Paris et al., 1981), were used in this work.

RESULTS

Biodegradation data obtained with river water samples are presented in Figs. 2–4 and Table 1. The highest biodegradation rate was found for acetonilide propanil. Results of these experiments revealed that propanil was readily biodegraded to 3,4-dichloraniline in different water samples. Figure 2 presents examples of primary biodegradation of propanil in water samples from the Neris River (Fig. 2A) and propanil biodegradation with simultaneous formation of 3,4-dichloraniline in water from the Vilnest River (Fig. 2B). No appreciable enhancement of bacterial biomass in the biodegradation of the acetonilides propanil or propachlor during the time period from 2 to 6 weeks was observed (see Figs. 3A and 3B, respectively). A slow decrease in bacterial biomass occurred during experiments on the biodegradation of phenylurea herbicides (data not provided). Biodegradation of phenylurea herbicides and the acetonilide propachlor (having an isopropyl radical attached to the nitrogen of the anilide bond) was negligible in water samples from the Neris River downstream of the city (see Fig. 4) compared with the control (sterile) samples. Experiments with other concentrations and water samples from different sampling places exhibited the same negligible biodegradation of phenylurea herbicides and propachlor during the

FIG. 2. Biodegradation of propanil in water samples from the Neris River (A) and the Vilnest River (B). (A) 1, initial concentration of propanil was 1 mg/liter; 2, initial concentration of propanil was 0.2 mg/liter; 3, control (autoclaved water + 1 mg/liter propanil); (B) 1, concentration of propanil; 2, concentration of 3,4-dichloraniline. For design and conditions of experiment, see Materials and Methods section. Abbreviation: ppm, parts per million, or mg/liter.

FIG. 3. Changes in bacterial biomass (number) of water samples from the Neris River during biodegradation experiment with propanil (A) and propachlor (B). (A and B) 1, water samples from the Neris River upstream of the city + appropriate compound; 2, water samples from the Neris River downstream of the city + appropriate compound. For design and conditions of experiment, see Materials and Methods section.
FIG. 4. Changes in concentration of monuron (A), diuron (B), fenuron (C), and propachlor (D) in water samples from the Neris River downstream of the city (A–D) 1, control (autoclaved river water + appropriate compound); 2, sample (river water + appropriate compound). For design and conditions of experiment, see Materials and Methods section.

time period up to 1400 h (8 weeks; data not provided). According to the second-order reaction rate equation, the biodegradation rate increases when the total bacterial biomass increases (see the preceding formula). Data on chemical and microbiological analysis summarized in Table 1 do not indicate such correlation: the biodegradation rate

\[ \text{Note. Abbreviations: } t_{1/2}, \text{ biodegradation half-life}; r^2, \text{ squared correlation coefficient}; K_s, \text{ first-order biodegradation rate constant}; \text{ Bact. biomass avg., average of bacterial biomass in samples during biodegradation experiment}; K_b, \text{ second-order biodegradation rate constant.} \]

<table>
<thead>
<tr>
<th>Substance</th>
<th>Initial conc (ppm)</th>
<th>( t_{1/2} ) (h)</th>
<th>( r^2 )</th>
<th>( K_s ) (L/h)</th>
<th>( K_b ) (liter/N h)</th>
<th>( t_{1/2} ) (h)</th>
<th>( r^2 )</th>
<th>( K_s ) (L/h)</th>
<th>( K_b ) (liter/N h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propanil</td>
<td>0.2</td>
<td>95</td>
<td>0.77</td>
<td>( 7.29 \times 10^{-3} )</td>
<td>( 1.5 \times 10^6 )</td>
<td>( 4.8 \times 10^{-9} )</td>
<td>76</td>
<td>0.94</td>
<td>( 9.08 \times 10^{-3} )</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>82</td>
<td>0.73</td>
<td>( 8.45 \times 10^{-3} )</td>
<td>( 1.2 \times 10^6 )</td>
<td>( 7.0 \times 10^{-9} )</td>
<td>85</td>
<td>0.9</td>
<td>( 8.19 \times 10^{-3} )</td>
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<tr>
<td>Propachlor</td>
<td>2</td>
<td>4107</td>
<td>0.73</td>
<td>( 1.69 \times 10^{-4} )</td>
<td>( 1.6 \times 10^6 )</td>
<td>( 1.0 \times 10^{-10} )</td>
<td>2240</td>
<td>0.74</td>
<td>( 3.09 \times 10^{-4} )</td>
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<tr>
<td></td>
<td>5</td>
<td>5706</td>
<td>0.54</td>
<td>( 1.21 \times 10^{-4} )</td>
<td>( 3.5 \times 10^6 )</td>
<td>( 3.5 \times 10^{-11} )</td>
<td>3803</td>
<td>0.82</td>
<td>( 1.82 \times 10^{-4} )</td>
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<tr>
<td>Fenuron</td>
<td>1</td>
<td>17047</td>
<td>0.04</td>
<td>( 4.06 \times 10^{-5} )</td>
<td>( 1.7 \times 10^6 )</td>
<td>( 2.4 \times 10^{-11} )</td>
<td>8280</td>
<td>0.13</td>
<td>( 8.37 \times 10^{-5} )</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>77691</td>
<td>0.03</td>
<td>( 8.92 \times 10^{-6} )</td>
<td>( 4.4 \times 10^6 )</td>
<td>( 2.0 \times 10^{-12} )</td>
<td>n.d.</td>
<td></td>
<td></td>
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<tr>
<td>Monuron</td>
<td>1</td>
<td>3868</td>
<td>0.8</td>
<td>( 1.79 \times 10^{-4} )</td>
<td>( 3.8 \times 10^5 )</td>
<td>( 4.7 \times 10^{-10} )</td>
<td>3487</td>
<td>0.84</td>
<td>( 1.99 \times 10^{-4} )</td>
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<td></td>
<td>5</td>
<td>3899</td>
<td>0.52</td>
<td>( 1.78 \times 10^{-4} )</td>
<td>( 6.7 \times 10^5 )</td>
<td>( 2.7 \times 10^{-10} )</td>
<td>3888</td>
<td>0.69</td>
<td>( 1.78 \times 10^{-4} )</td>
</tr>
<tr>
<td>Diuron</td>
<td>1</td>
<td>1920</td>
<td>0.55</td>
<td>( 3.61 \times 10^{-4} )</td>
<td>( 2.1 \times 10^6 )</td>
<td>( 1.7 \times 10^{-11} )</td>
<td>2041</td>
<td>0.52</td>
<td>( 3.39 \times 10^{-4} )</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1595</td>
<td>0.62</td>
<td>( 4.34 \times 10^{-4} )</td>
<td>( 3.6 \times 10^7 )</td>
<td>( 1.2 \times 10^{-11} )</td>
<td>1885</td>
<td>0.57</td>
<td>( 3.68 \times 10^{-4} )</td>
</tr>
</tbody>
</table>
of substances does not increase in water samples from different sampling places (Neris upstream and downstream of the city) that differed in bacterial biomass (number) by a factor of 100 or less (see samples with propanil or monuron, Table 1). The first- and second-order rate constants presented in Table 1 fit the following sets of decreasing values:

(1) For first-order ($K_1$): propanil > diuron > monuron = propachlor > fenuron (in samples from the Neris River upstream and downstream of Vilnius);

(2) For second-order ($K_2$): propanil > monuron > propachlor > fenuron > diuron (in samples from the Neris River upstream of Vilnius) and propanil > diuron > fenuron > propachlor = monuron (in samples from the Neris downstream of Vilnius; both with lower concentrations of herbicides).

In summary,

(1) Acetanilide propanil was readily biodegraded in various water samples; degradation was confirmed by calculating both first- and second-order biodegradation rate constants.

(2) The values of first-order biodegradation rate constants ($K_1$) revealed the following sequence of decreasing transformation rates of the compounds tested in different water samples: propanil > diuron > propachlor = monuron > fenuron.

(3) The set of decreasing values of second-order rate constants ($K_2$) marked more clearly in the range of values of first-order constants ($K_1$) and for the water samples from the river upstream and down stream of the city. Therefore, study was made to determine if there was any correlation of biodegradation rates with chemical or microbiological parameters of water samples used in the experiments.

Comparison of main water quality parameters in two groups of water samples from the Neris River upstream and downstream of Vilnius is given in Table 2. It can be seen that N and P content in river water samples downstream of the city increased from three to eight times (for $\text{NH}_4^+$) and from four to eight times (for $\text{PO}_4^{3-}$), respectively. COD and BOD$_5$ values in river water samples downstream of the city increased by a factor of 1.3–2.2 and 1.6–7.0 respectively. As indicated earlier, the biodegradation of acetanilide and phenylurea herbicides was not faster in river water samples downstream of the city (see data presented in Table 1). On the other hand, concentrations of N and P compounds in the river water samples downstream of the city reached up to 2 and 110–120 $\mu$g/liter, respectively. These concentrations are not high and can be the reason for long acclimation periods of appropriate species of microorganisms. For example, $K_1$ for P compounds varies from 0.4 to 500 $\mu$g/liter for different microorganisms. Additionally, high concentrations of P compounds are needed for effective biodegradation at alkaline pH values—for example, pH 8.0 and higher as noted by Alexander (1994). It is important to note that even lower concentrations of nutrients did not limit propanil biodegradation. As presented in Table 3, two types of river water samples collected from the Neris River—upstream of Vilnius and downstream of the city wastewater treatment plant—and had different numbers of microorganisms. The reason for selecting these sites was that, during this period, the wastewater treatment plant of Vilnius had only mechanical treatment (flocculation and sedimentation, etc.) and no biological treatment facilities of wastewater. The initial concentration of microorganisms varied from $4.7 \times 10^5$ to $2.7 \times 10^6$ cells/liter and from $1.4 \times 10^8$ to $5.3 \times 10^8$ cells/liter for water samples from the Neris River upstream of Vilnius and downstream of the Vilnius wastewater treatment plant, respectively. So, the total numbers of bacteria in different water samples varied by up to two

<table>
<thead>
<tr>
<th>Date of beginning of the experiment</th>
<th>COD$^a$</th>
<th>BOD$_5^a$</th>
<th>$[\text{O}_2]^\gamma$</th>
<th>pH</th>
<th>$\text{NH}_4^b$</th>
<th>$\text{NO}_3^b$</th>
<th>$\text{PO}_4^{3-}$</th>
<th>COD$^a$</th>
<th>BOD$_5^a$</th>
<th>$[\text{O}_2]^\gamma$</th>
<th>pH</th>
<th>$\text{NH}_4^b$</th>
<th>$\text{NO}_3^b$</th>
<th>$\text{PO}_4^{3-}$</th>
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</thead>
<tbody>
<tr>
<td>August 6, 1991</td>
<td>27.2</td>
<td>6.3</td>
<td>10.68</td>
<td>8.75</td>
<td>0.24</td>
<td>0.251</td>
<td>0.007</td>
<td>36.2</td>
<td>13.4</td>
<td>11.3</td>
<td>8.75</td>
<td>0.8</td>
<td>0.2</td>
<td>0.058</td>
</tr>
<tr>
<td>September 9, 1991</td>
<td>24.2</td>
<td>2.0</td>
<td>8.38</td>
<td>8.35</td>
<td>0.25</td>
<td>0.56</td>
<td>0.029</td>
<td>29.1</td>
<td>14.1</td>
<td>7.92</td>
<td>8.32</td>
<td>1.71</td>
<td>0.47</td>
<td>0.121</td>
</tr>
<tr>
<td>October 13, 1993</td>
<td>n.d.</td>
<td>1.44</td>
<td>11.8</td>
<td>8.15</td>
<td>0.11</td>
<td>0.5</td>
<td>0.02</td>
<td>n.d.</td>
<td>5.76</td>
<td>11.4</td>
<td>8.12</td>
<td>0.9</td>
<td>0.55</td>
<td>0.11</td>
</tr>
<tr>
<td>June 2, 1994</td>
<td>14.7</td>
<td>4.5</td>
<td>8.9</td>
<td>8.43</td>
<td>0.09</td>
<td>0.5</td>
<td>0.046</td>
<td>32.3</td>
<td>7.8</td>
<td>9</td>
<td>8.05</td>
<td>0.24</td>
<td>0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

| Note. The following substances were tested for biodegradation in appropriate water samples (w.s): Propanil in w.s. of August 6, 1991; propachlor in w.s. of September 5, 1991; diuron in w.s. of July 21, 1993; monuron in w.s. of October 13, 1993; fenuron in w.s. of June 2, 1994. n.d., not determined. |
|-----------------------------------|--------|----------|----------------|----|-------------|------------|--------------|--------|----------|----------------|----|-------------|------------|--------------|
| $^a$In mg $\text{O}_2$/liter. |
| $^b$In mg N/liter. |
| $^c$In mg/liter. |
### TABLE 3
Some Microbiological Water Quality Parameters of Water Samples from the Neris River

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Neris upstream of Vilnius</th>
<th>Neris downstream of Vilnius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date of beginning of experiment</td>
<td>Psychrophiles (10^6/liter)</td>
</tr>
<tr>
<td>August 6, 1991</td>
<td>0.81</td>
<td>0.32</td>
</tr>
<tr>
<td>September 9, 1991</td>
<td>1.4</td>
<td>0.078</td>
</tr>
<tr>
<td>October 13, 1993</td>
<td>1.3</td>
<td>0.023</td>
</tr>
<tr>
<td>June 2, 1994</td>
<td>0.7</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Note. n.d., not determined.

orders of magnitude at the beginning of the biodegradation experiment. There were observed differences in temperature and initial bacterial biomass in river water samples. Temperature varied from 11 to 21°C water samples upstream of Vilnius) and from 12 to 23°C (water samples downstream of Vilnius). The initial number of bacteria varied from 0.75 to 2.13 \times 10^6/liter and from 197 to 294.5 \times 10^6/liter, respectively. The high number of bacteria was observed even at lower temperatures in the water samples from both sampling places (Table 3; water samples of September 9, 1991, or July 21, 1993). It means that differences in temperature did not strongly affect the initial (total) number of bacteria in the water of the appropriate sampling place.

It is important to note that:
1. no significant increase in first-order reaction rate constants in water samples downstream Vilnius was observed despite the increase in COD and BOD₅ values, nutrient concentration, and total bacterial number;
2. Owing to the increase in the total number of bacteria in the water samples from the Neris River downstream of the city, the second-order reaction rate constants for these samples had lower values compared with the samples from the river upstreams of the city.

**DISCUSSION**

The role of specific microbiological and physicochemical factors in different or unique biodegradation rates of the appropriate compound in a certain environment is obvious. But it is important to note that some insight into this phenomenon can be obtained from experiments:

1. Novick and Alexander (1985) found cometabolic biodegradation of chloraniline herbicides such as propachlor and monuron in lake waters and wastewaters. It means that an increase in bacterial biomass is not necessarily connected with the biodegradation of such compounds.
2. Biodegradation of propachlor (like some other acetanilide and phenylurea herbicides) in lake waters was described by using first-order and even zero-order reaction kinetics (Novick and Alexander, 1985).
3. According to Paris et al. (1981), if substrate concentration is very low (lower than Kₕ in Monod expression), the Monod equation can be reduced to the second-order reaction equation. Still very little is known about the dependence of the biodegradation rate on “high” or “low” substrate concentrations or their threshold values. For example: (i) different concentrations were reported to be suitable for monuron cometabolism and mineralization (10 mg/liter and 10 μg/liter, respectively), and (ii) high concentrations of phenylurea herbicide linuron (namely, 50 μM or 12.35 mg/liter) were found to induce an amidase activity (Alexander, 1994).
4. Absence of fast biodegradation of phenylurea herbicides in surface waters may depend on long acclimatization periods: that is, induction of specific enzyme systems and growth rate of specialized bacterial strains. Data on biodegradation of various pesticides including monuron demonstrated different acclimatization periods up to a few months duration (Alexander, 1994).
5. Encirclement of the anilide bond strictly influences the biodegradation of 3,4-dichloraniline derivatives: karsil, dicryl or monuron, and fenuron having a nitrogen atom or a longer, unsaturated, branched aliphatic chain at the carboxylic side, are not hydrolyzed by acylamidases of the fungus Fusarium solani (Rotmistrov et al., 1975). Data on biodegradation of acetanilide herbicides in pure Pseudomonas cultures confirmed fast biodegradation of propanil and slow biodegradation of propachlor (Četkauskaitė et al., 1994).
Thus, it is clear that: (1) biodegradation of chloraniline herbicides can occur in cometabolizing microorganisms; (2) it can be induced by high concentrations of compounds, and, in this case, reduction of the Monod equation to the second-order reaction equation is questionable; (3) structural changes in proximity of cleavable bonds of these compounds can strictly inhibit the activity of widespread hydrolytic enzymes.

On the other hand, the description of biodegradation data in terms of “second-order” reaction kinetics has problems that can be answered as in Table 4.

Finally, the decrease in enzymatic activity of biodegradative microorganisms can be represented by competitive and noncompetitive inhibition mechanisms, as was done for the inhibition of urease activity by phenylurea herbicides (fenuron, monuron, diuron, siduron, linuron, and neburon) in soil by Schaffer (1993). An illustration of existing kinetic models for the description of the biodegradation rate is presented in Fig. 5. This scheme was compiled according to the description of the variety of models of biodegradation kinetics presented by Martin Alexander in his comprehensive book Biodegradation and Bioremediation, Chapter 6, “Kinetics” (Alexander, 1994). According to the material presented in this chapter and this scheme, the “second order” reaction rate model really is the conventional concept based on the kinetics of the first-order biodegradation reaction.

**CONCLUSIONS**

1. The biodegradation rate of two acetanilide herbicides (propanil and propachlor) and three phenylurea herbicides (fenuron, monuron, and diuron) in surface waters can be described by first-order kinetic constants.

2. The following set of decreasing values of the first-order constants ($K_r$) can be revealed for the herbicides tested: propanil $\geq$ diuron $\geq$ monuron = propachlor $\geq$ fenuron.

3. No appreciable increase in the first-order reaction rate constants in the river water samples downstream of the Vilnius wastewater treatment plant was observed despite the increase in COD and BOD$_5$ parameters (up to 2.2 and 7.0 times, respectively), biogen concentration (up to 8 times for NH$_4^+$ and PO$_4^{3-}$, and total bacterial number (up to a factor of 100).

4. The “second order” biodegradation reaction rate model is really the conventional concept based on the kinetics of the first-order biodegradation reaction.

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**TABLE 4**

<table>
<thead>
<tr>
<th>Concepts of the “second order” reaction</th>
<th>Results of current experiments and observation from literature sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Biodegradation of xenobiotic is accompanied by enhancement of total bacterial biomass (or number of bacteria).</td>
<td>(a) No appreciable constant enhancement of bacterial biomass was observed in biodegradation of acetanilides propanil or propachlor during the time period up to 6 weeks.</td>
</tr>
<tr>
<td>2. Biodegradation rate increases when the total bacterial biomass increases (see formula).</td>
<td>(b) Normal cases of cometabolism, first-order, Monod, Michaelis-Menten, or even zero-order type kinetics can equally describe biodegradation by nongrowing microorganisms (Alexander, 1994).</td>
</tr>
<tr>
<td>3. Changes in biodegradation rate can be described by second-order kinetic equations because there is a multiplication of two reactive substances.</td>
<td>(a) No such correlation was observed in the current experiments: biodegradation rate of substances did not increase when it was analyzed in water samples from different sampling places with difference in bacterial biomass by up to a factor of 100.</td>
</tr>
<tr>
<td>4. Addition of nutrients (N and P) to surface water samples enhances biodegradation of chemical substances because it helps to increase the total bacterial biomass or the mass of specialized bacterial strains or both.</td>
<td>(b) The assumption that the amount of total bacterial biomass correlates with activity of specialized bacterial strains may be right only for low-substrate-specificity hydrolytic bacterial enzymes, but it does not fit highly specific biodegradation reactions of complex organic compounds (Vasilyeva et al., 1989).</td>
</tr>
<tr>
<td></td>
<td>(c) Such a description can be applied only conditionally (upon an agreement) because it contravenes the understanding of chemical kinetics. In fact, it is first-order biodegradation kinetics [the first multiplicand is reactive chemical concentration and the second one is catalyst (enzyme) concentration instead of the second reactive substance] (Metzler, 1980).</td>
</tr>
<tr>
<td></td>
<td>(d) Data of current experiments revealed that increases in N and P content in river water samples from three-fold to eight-fold did not increase the biodegradation of acetanilide herbicides;</td>
</tr>
<tr>
<td></td>
<td>(e) The addition of nutrients cannot be helpful if another cofactor is required for the growth of specialized bacterial strains (Alexander, 1994).</td>
</tr>
<tr>
<td></td>
<td>(f) The addition of nutrients to the natural waters does not satisfy natural conditions of bacterial starvation in surface waters.</td>
</tr>
</tbody>
</table>
FIG. 5. Kinetic analysis of microbial biodegradation: a variety of models for growing and nongrowing microorganisms, and dependence of their application on the substrate concentration in the environment. Abbreviations: [S] substrate concentration in the environment; $S_0$, initial concentration of substrate; $K_m$, Michaelis constant (substrate concentration at which the rate of enzymatic reaction is half the maximum rate); $K_4$, Monod constant (substrate concentration at which the rate of growth is half the maximum rate).

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REFERENCES


