STUDY OF THE KINETICS OF BIODEGRADATION OF TETRAETHYLENE GLYCOL MONO-*n*-OCTYL ETHER AND TETRAETHYLENE GLYCOL AND SIMULATION OF THE FORMATION OF SOME INTERMEDIATES

Vladimír DLASK[a1], Karel KOMÁREK[b] and Jiří PLOCEK[c]
[a]Department of Physical Chemistry, [b]Department of Analytical Chemistry,
[c]Department of Process Control and Computer Techniques,
The University of Pardubice,
CZ–532 10 Pardubice

The biodegradation courses of the pure tetraethylene glycol mono-n-octyl ether and tetraethylene glycol using the mixed microbial culture under the static conditions are described. The formation of main intermediates in dependence on time was simulated by chosen kinetic models, differential equations and worked out programme. Both the decreases in the concentration of initial compounds and the increases in the concentration of formed metabolites in dependence on time were determined by means of analytical method. The analytical results in graphic form were compared with the data found by simulation of the biodegradation.

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[1] To whom correspondence should be addressed.

Introduction

Alkylphenyl polyethylene glycol ethers (APPGE), alkyl polyethylene glycol ethers (APGE) and polyethylene glycols (PEG) are compounds that contaminate environment, especially surface water. They get into water from various industrial media, in which they are present, such as heat-conducting liquids or hydraulic liquids. Lower PEGs are formed by biodegradation of APPGE or APGE [1–8]. Thanks to their good biodegradability, APGEs are most frequently used. However, some intermediates are slowly biodegraded. That is why both the monitoring of these compounds in water and studies of biodegradability course are very important.

Tetraethylene glycol and its *n*-monooctyl ether are hydrolytically degraded to lower glycols [8], aldehydes [8] and various carboxylic acids [4]. However, the studied compounds are degraded as well, so some of the intermediates formed, e.g. ethylene glycol (or its mono-*n*-octyl ether) are cumulated. In the last step of degradation, CO_2 and H_2O are formed and biomass grows.

In this work we tried to describe the formation and decomposition of tetraethylene glycol and its *n*-monooctyl ether by the means of kinetic differential equations on the basis of experimental data of dependences of concentrations vs. time. We determined the rate constants of these equations by the means of the program called „The Integrated Environment"[9,10].
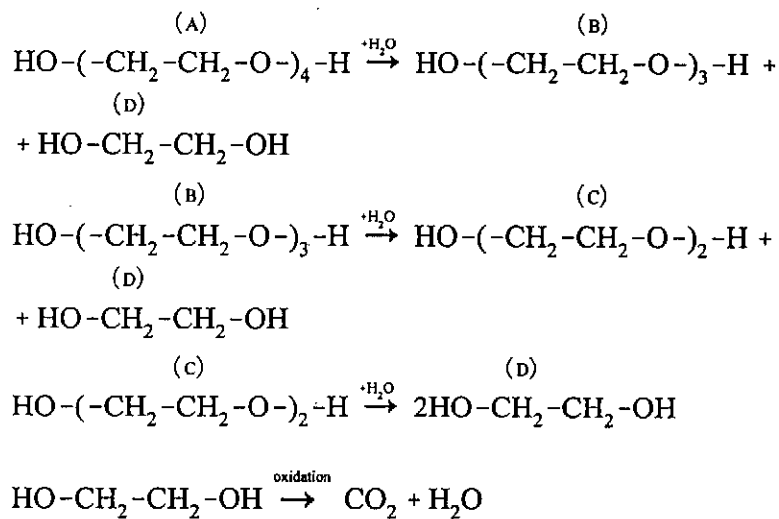
Experimental

Testing of Biodegradability

We used a modified single kinetic test for the determination of the biodegradability of tetraethylene glycol and and its *n*-monooctyl ether [8]. The initial concentration of the tested compounds was 1000 $mg\,dm^{-3}$, the concentration of inoculum was decreased and a nonadapted sludge was used. The activated sludge from the waste water treatment plant of Malenovice was used as the source of the mixed culture. The supernatant prepared by sedimentation of sludge (dry solids 10 $g\,dm^{-3}$) was used for the inoculation. This sample of sludge water contained 10^5 micro-organisms per 1 cm^3. 100 ml samples were taken out at chosen time intervals and the chemical demand of oxygen (COD) was determined. Then, the presence of oligomers and intermediates was determined by the means of gas chromatography and mass spectrometry [11].

Theory

Biodegradation of Tetraethylene Glycol

The oxyethylene groups are hydrolytically split off and lower glycol ethers are formed

$$
\overset{(A)}{HO-(-CH_2-CH_2-O-)_4-H} \xoverset{+H_2O}{\rightarrow} \overset{(B)}{HO-(-CH_2-CH_2-O-)_3-H} +
$$

$$
\overset{(D)}{+HO-CH_2-CH_2-OH}
$$

$$
\overset{(B)}{HO-(-CH_2-CH_2-O-)_3-H} \xoverset{+H_2O}{\rightarrow} \overset{(C)}{HO-(-CH_2-CH_2-O-)_2-H} +
$$

$$
\overset{(D)}{+HO-CH_2-CH_2-OH}
$$

$$
\overset{(C)}{HO-(-CH_2-CH_2-O-)_2-H} \xoverset{+H_2O}{\rightarrow} \overset{(D)}{2HO-CH_2-CH_2-OH}
$$

$$
HO-CH_2-CH_2-OH \xoverset{oxidation}{\rightarrow} CO_2 + H_2O
$$

Scheme I

This scheme of reaction is only one of the possible kinds of the biodegradation and it was experimentally confirmed. It can be written in the following form

$$
A \xrightarrow{k_i} B + D \qquad C \xrightarrow{k_{iii}} 2D
$$

$$
B \xrightarrow{k_{ii}} C + D \qquad D \xrightarrow{k_{iv}} CO_2 + H_2O
$$
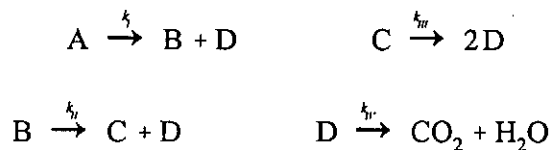
Scheme II

The decrease in concentration of tetraethylene glycol (compound A) in time by means of biodegradation can be expressed by Monod's equation [12]

$$-\frac{d[A]}{dt} = \frac{\mu_{max}}{Y} \frac{[A]}{K_A + [A]} \left[X_0 + Y\left([A]_0 - [A]\right)\right] \qquad (1)$$

where μ_{max} — maximum specific growth rate
Y — growth yield
K_A — half-velocity constant
$[A]_0$ — initial concentration of substrate
X_0 — initial concentration of biomass

Using the association of constant of μ_{max}, Y, K_A, $[A]_0$, X_0, the equation (1) can be rewritten in the form

$$\frac{d[A]}{dt} = \frac{k_1'[A]\left(k_2'[A] - 1\right)}{k_3' + [A]} \qquad (2)$$

The decrease in concentration of A cannot be described by the integrated form of (2) for any constants k_1', k_2', k_3'. Monod's model does not describe the rate of growth of microorganisms, as mentioned in [13]. It comes to the conclusion that creation of complicated kinetic models is not acceptable, because the models contain many parameters and the estimation is always difficult. In addition, the biodegradability of organic compounds is changeable, there take place physiological changes in activated sludge and in this way the whole system is influenced. Thus, the calculated parameters possess negligible meaning from the point of view of physical chemistry.

The following model resulted from our calculations: it describes the rise and extinction of particular components

$$\frac{d[A]}{dt} = \left(k_1 + k_2[A]\right)\left(k_3[A] - 1\right) \qquad [A]_0 = 5.21 \ \text{mmol dm}^{-3} \qquad (3)$$

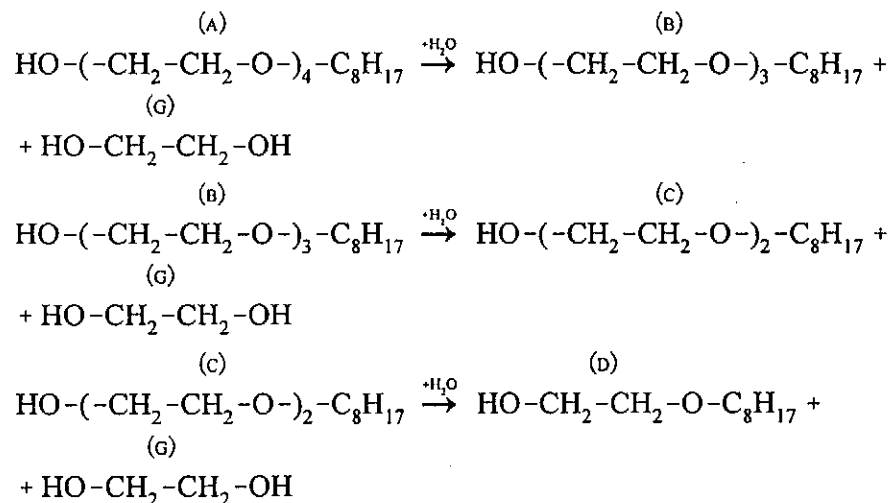$$\frac{d[B]}{dt} = k_{13}[A] + k_4[B]\frac{k_6[B] - 1}{k_5 + [B]} \qquad (4)$$

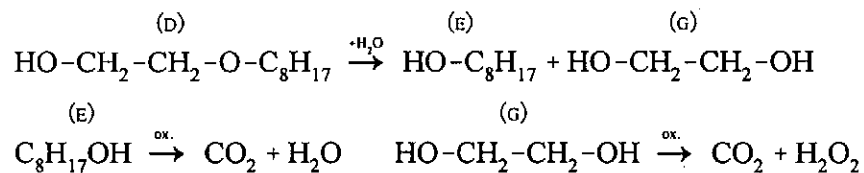$$\frac{d[C]}{dt} = k_{10}[B] + \frac{k_8[C]\big(k_9[C] - 1\big)}{k_7 + [C]} \tag{5}$$

$$\frac{d[D]}{dt} = k_{14} - k_{15}\big([A]_0 - [A]\big) - \frac{k_{17}[D]}{k_{18} + [D]} + \frac{k_{19}[C]}{k_{20} + [C]} \tag{6}$$

Equation (3) is derived from equation (2) by neglecting [A] in the denominator and by extending numerator with absolute member $k_1 + k_2[A]$. Equations (4) and (5) express loss in the concentration of compounds according to Monod's theory. The equation (6) was a very hard task to formulate. Compound D (ethylene glycol) is produced in all reactions and degraded in one reaction only. The estimated values of constants were substituted into equations (3) – (6) and calculated concentration ~ time dependences were compared with the experimental ones. The results are given in Figs 1 – 4.

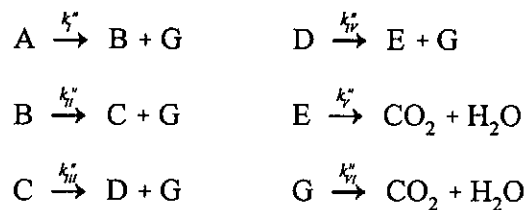Biodegradation of the Tetraethylene Glycol Mono-*n*-octyl Ether

In this case, oxyethylene groups are hydrolytically split off and lower oligomers and ethylene glycol are formed

(A)   (B)
$$HO-(-CH_2-CH_2-O-)_4-C_8H_{17} \xrightarrow{\cdot H_2O} HO-(-CH_2-CH_2-O-)_3-C_8H_{17} +$$
(G)
$$+ HO-CH_2-CH_2-OH$$

(B)   (C)
$$HO-(-CH_2-CH_2-O-)_3-C_8H_{17} \xrightarrow{\cdot H_2O} HO-(-CH_2-CH_2-O-)_2-C_8H_{17} +$$
(G)
$$+ HO-CH_2-CH_2-OH$$

(C)   (D)
$$HO-(-CH_2-CH_2-O-)_2-C_8H_{17} \xrightarrow{\cdot H_2O} HO-CH_2-CH_2-O-C_8H_{17} +$$
(G)
$$+ HO-CH_2-CH_2-OH$$

$$\underset{(D)}{HO-CH_2-CH_2-O-C_8H_{17}} \xrightarrow{+H_2O} \underset{(E)}{HO-C_8H_{17}} + \underset{(G)}{HO-CH_2-CH_2-OH}$$

$$\underset{(E)}{C_8H_{17}OH} \xrightarrow{ox.} CO_2 + H_2O \qquad \underset{(G)}{HO-CH_2-CH_2-OH} \xrightarrow{ox.} CO_2 + H_2O_2$$

Scheme III

The scheme can be simply rewritten as follows

$$A \xrightarrow{k_I''} B + G \qquad D \xrightarrow{k_{IV}''} E + G$$

$$B \xrightarrow{k_{II}''} C + G \qquad E \xrightarrow{k_V''} CO_2 + H_2O$$

$$C \xrightarrow{k_{III}''} D + G \qquad G \xrightarrow{k_{VI}''} CO_2 + H_2O$$

Scheme IV

The best model describing the concentration ~ time dependences, derived from our calculations, reads as follows

$$-\frac{d[A]}{dt} = k_1''[A]\left(k_2'' + [A]_0 - [A]\right) \qquad [A]_0 = 3.01 \text{ mmol dm}^{-3} \qquad (7)$$

$$\frac{d[B]}{dt} = k_3'' - k_4''\left([A]_0 - [A]\right) - \frac{k_5''[B]}{k_6'' + [B]} + \frac{k_7''\left([A]_0 - [A]\right)}{k_8'' + \left([A]_0 - [A]\right)} \qquad (8)$$

$$\frac{d[\mathrm{C}]}{dt} = -k_9'' + k_{10}''[\mathrm{B}] + \frac{k_{11}''[\mathrm{C}]}{k_{12}'' + [\mathrm{C}]} - \frac{k_{13}''[\mathrm{B}]}{k_{14}'' + [\mathrm{B}]} \tag{9}$$

$$\frac{d[\mathrm{D}]}{dt} = k_{15}'' - k_{16}''\big([\mathrm{A}]_0 - [\mathrm{A}]\big) - \frac{k_{17}''[\mathrm{D}]}{k_{18}'' + [\mathrm{D}]} + \frac{k_{19}''[\mathrm{C}]}{k_{20}'' + [\mathrm{C}]} \tag{10}$$

$$\frac{d[\mathrm{E}]}{dt} = k_{21}'' - k_{22}''\big([\mathrm{A}]_0 - [\mathrm{A}]\big) - \frac{k_{23}''[\mathrm{E}]}{k_{24}'' + [\mathrm{E}]} + \frac{k_{25}''[\mathrm{D}]}{k_{26}'' + [\mathrm{D}]} \tag{11}$$

We can observe a relation between equations (2) and (7). The forms of right-hand sides of equations (8) – (11) were selected on the basis of experience from equations (3) – (6). The values of constants ($k_1'' - k_{26}''$) determined by the means of „Integrated Environment" were substituted into equations (7) – (11) and the calculated concentration ~ time dependences were compared with the experimental ones. The results are given in Figs 5 – 9.

Determination of Mathematical Model of Reaction Including Constants in Rate Equations

There are no accurate data concerning the mechanism of this biochemical reaction, and so this is a very difficult task that has not been solved yet. We were referred to the hypothesis, which is expressed by scheme I and scheme III.

For determination of the constants in equations (3) – (6) and (7) – (11) we used a program [9,10], which forms the „Integrated Environment" for the simulation of chemical reactions. In this program, there are stored experimental data, and a mathematical model is transformed into „simulation model", which is then edited quickly and easily to modifications, which represent following scrutinizing alternatives of the mathematical model.

The equations occurring in the mathematical model are differential equations (generally non-linear) with the starting condition. The solutions were carried out by the Runge–Kutta method of the 2nd or 4th order with the possibility to choose the integration step.

The constants of these equations were determined by minimizing the criterion
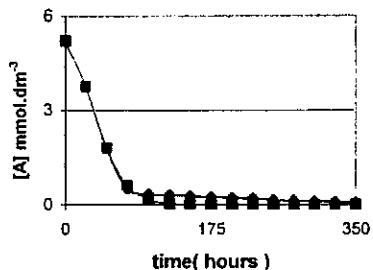
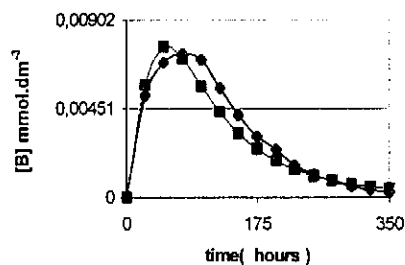Fig. 1 Dependence of concentration of compound A on time

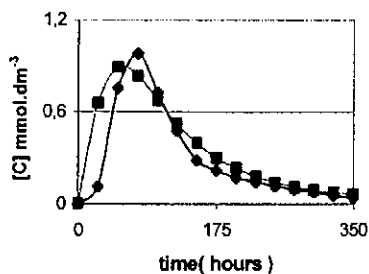

Fig. 2 Dependence of concentration of compound B on time

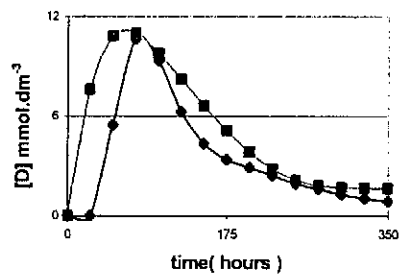

Fig. 3 Dependence of concentration of compound C on time

Fig. 4 Dependence of concentration of compound D on time

$$\text{crit.} = \sum_{from}^{to} \left(\frac{|[A_t]_m - [A_t]_V|}{[A_t]_m} + \frac{|[C_t]_m - [C_t]_V|}{[C_t]_m} + \frac{|[D_t]_m - [D_t]_V|}{[D_t]_m} + \frac{|[B_t]_m - [B_t]_V|}{[B_t]_m} \right) 100$$

where from – the starting point of comparison .... 25 hours
to – the ending point of comparison ....350 hours
t – time of measurements (0; 25; 50 .... 325; 350)
m – experimental data
v – calculated data

This criterion depends upon the constants (k_1,...) and chosen mathematical model. In addition to, we examined several models.

The comparison between experimental and calculated data of compounds A, B, C, D is presented graphically.

Results and Discussion

The determination of constants in rate equations was carried out by means of the „Integrated Environment"[9,10]. There was employed the record of concentrations of compounds A, B, C, D in time. The concentration data were obtained at equidistant intervals.

The experimental data were connected in such a way to form smooth curve. We read the concentrations in equidistant time intervals from this curve. We used this because the following procedure was the most progressive one.

The values of constants in rate equations of biodegradation of tetraethylene glycol were determined as follows

Constant	Value	Dimension
k_1	0.0368	$mmol\ dm^{-3}\ h^{-1}$
k_2	0.00725	h^{-1}
k_3	1.41	$mmol^{-1}\ dm^3$
k_4	0.0006	$mmol\ dm^{-3}\ h^{-1}$
k_5	0.0388	$mmol^{-1}\ dm^3$
k_6	0.00001	$mmol^{-1}\ dm^3$
k_7	25.00	$mmol^{-1}\ dm^3$
k_8	0.631	$mmol\ dm^{-3}\ h^{-1}$
k_9	0.487	$mmol^{-1}\ dm^3$
k_{10}	9.32	h^{-1}
k_{13}	0.000065	h^{-1}
k_{14}	1.44	$mmol\ dm^{-3}\ h^{-1}$
k_{15}	0.0712	h^{-1}
k_{17}	1.16	$mmol\ dm^{-3}\ h^{-1}$
k_{18}	0.0729	$mmol^{-1}\ dm^3$
k_{19}	0.00027	$mmol\ dm^{-3}\ h^{-1}$
k_{20}	0.594	$mmol^{-1}\ dm^3$

where $[A]_0 = 5.21$ mmol dm^{-3}

Graphical representation of solving of equations (3) – (6) and comparison of calculated concentrations with experimental data is given in Fig. 1 – 4 (calculated: ■; experimental:◆).

The values of the constants in rate equations of biodegradation of tetraethylene glycol mono-*n*-octyl ether were determined as follows

Constant	Value	Dimension
k_1''	0.0160	mmol dm^{-3} h^{-1}
k_2''	0.0501	mmol dm^{-3}
k_3''	1.15	mmol dm^{-3} h^{-1}
k_4''	2.48	h^{-1}
k_5''	65.0	mmol dm^{-3} h^{-1}
k_6''	15	mmol dm^{-3}
k_7''	1822	mmol dm^{-3} h^{-1}
k_8''	34.2	mmol dm^{-3}
k_9''	5.23×10^{-3}	mmol dm^{-3} h^{-1}
k_{10}''	0.0125	h^{-1}
k_{11}''	2.50×10^{-8}	mmol dm^{-3} h^{-1}
k_{12}''	30.0	mmol dm^{-3}
k_{13}''	2.74×10^{-7}	mmol dm^{-3} h^{-1}
k_{14}''	0.390	mmol dm^{-3}
k_{15}''	0.0727	mmol dm^{-3} h^{-1}
k_{16}''	0.0241	h^{-1}
k_{17}''	1.300	mmol dm^{-3} h^{-1}
k_{18}''	0.517	mmol dm^{-3}
k_{19}''	5.04×10^{-4}	mmol dm^{-3} h^{-1}
k_{20}''	0.103	mmol dm^{-3}
k_{21}''	0.224	mmol dm^{-3} h^{-1}
k_{22}''	0.207	h^{-1}
k_{23}''	1.21	mmol dm^{-3} h^{-1}

Constant	Value	Dimension
k_{24}''	0.505	$\mathrm{mmol\ dm^{-3}}$
k_{25}''	3.40×10^{-8}	$\mathrm{mmol\ dm^{-3}\ h^{-1}}$
k_{26}''	0.800	$\mathrm{mmol\ dm^{-3}}$

where $[A]_0 = 3.01\ \mathrm{mmol\ dm^{-3}}$

Graphical representation of solving of equation (7) – (11) and comparison of calculated concentrations with experimental data is given in Figs 5 – 9 (calculated: ■; experimental: ♦).
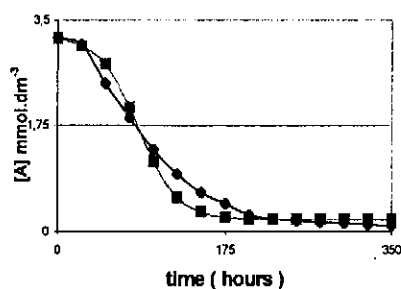

Fig. 5 Dependence of concentration of compound A on time
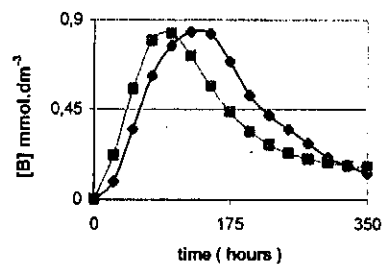
Fig. 6 Dependence of concentration of compound B on time

Conclusion

The graphical results correspond very well and it can be assumed that the proposed reaction scheme is in accordance with the reaction and that the procedure of estimation of constants is also correct. The applicability of the „Integrated environment" was also confirmed. Advantages of this method are simplicity and speed of calculation.

The right-hand sides of equations (2) – (11) are complicated. Their forms had to be used from the point of view of description of concentration ~ time dependences. We assumed that we have dealt with a multicomponent substrate. From the point of view of sewage purification, the program „Integrated Environment" could be involved in the control of the process of activation, whose aim is liquidation of defined substrate in the shortest time.
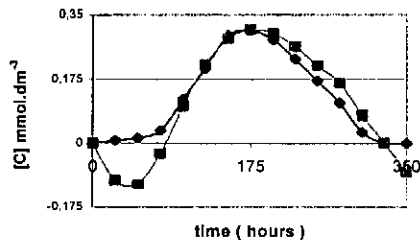
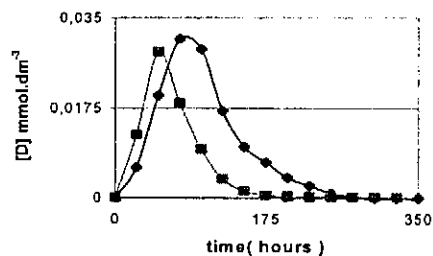Fig. 7 Dependence of concentration
of compound C on time

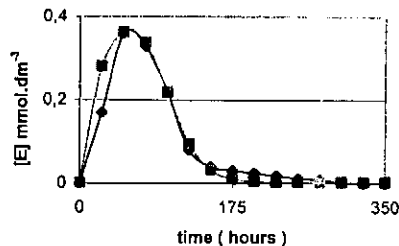Fig. 8 Dependence of concentration
of compound D on time

Fig. 9 Dependence of concentration
of compound E on time

Acknowledgements

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