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REACTION OF THE HERBICIDE ETHOFUMESATE WITH ACTIVATED SLUDGE

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Biochemical decomposition of the water solution of the herbicide Ethofumesate (EFS) with the activated sludge (AS) adapted on EFS was studied. The EFS initial concentration was in the range $S_0 = 0.05 - 0.2$ *mmol* dm^{-3}, *the AS initial concentration (expressed as the weight of dry biomass in the volume unit of the reaction mixture) was* $X_0 = 0.4 - 2.2$ *g* dm^{-3}. *Experiments were carried out in a batch isothermal stirred reactor at temperatures of 15 – 40 °C. It was found out that EFS is decomposed by means of AS very slowly. At given conditions, the initial velocity of the EFS decomposition was* $v = 3$ *to 30 mg EFS* h^{-1} g^{-1} *AS. Concentrations of AS and dissolved oxygen in the reaction mixture were practically constant during the whole reaction time in all the experiments. The value of v decreased at 20 – 25 °C with increasing ratio of initial AS and EFS concentrations* $p = X_0/S_0$. *At temperatures of 30 and 35 °C, an inductive period of ca two hours took place before EFS decomposition. In the liquid phase of the reaction mixture an unknown substance was found beside EFS, which is not a decomposition product of EFS but originates probably from the microorganisms*

of AS. All the analyses carried out indicate a complicated reaction mechanism (probably dictated by physical rather then chemical steps) and the fact that EFS is a species hard to decompose biologically.

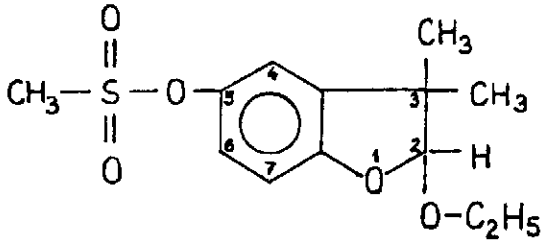
Introduction

Ethofumesate (EFS) is the main component of a herbicide Nortron 20 BC which is used against weeds. The kinetics and mechanism of its decomposition in aqueous solution by means of a mixed culture of microorganisms called activated sludge (AS) were studied. The research was carried out in the cooperation with the Environment Department of the chemical factory Synthesia Pardubice a.s., CZ, which is producing Nortron 20 BC above-mentioned.

Experimental

Characterisation of EFS [1]

EFS is an ester of the methanesulphonic acid: 2-ethoxy-2,3-dihydro-3,3-dimethyl-benzofuran-5-yl methansulphonate. Its formula is $C_{13}H_{18}O_5S$ and its structure is



(1)

EFS has molar mass 284.54 g mol^{-1}, melting point 70 – 72 °C and no boiling point at normal pressure. The tension of its vapour at 25 °C is 8.717×10^{-4} Pa, density 1.14 g cm^{-3}. The rate of EFS hydrolysis in water solution is: pH 7 – stable, 0.1 M KOH – halftime ≈10 days, 0.1 M HCl – halftime ≈30 days. Solubilities of EFS: water (0.11 g dm^{-3}, i.e. 3.84×10^{-4} M), ethanol (≈100 g dm^{-3}), glycerol (≈250 g dm^{-3}), acetone, benzene, chloroform and dioxane (≈400 g dm^{-3}). Pure EFS is a white crystalline powder with a low biological toxicity.

Adaptation of AS to EFS

The basic AS, fed with the peptone concentrate, was obtained as described in [2]. Due to the small solubility of EFS in water the original pepton nutrient (12 cm^3 of pepton concentrate in 1 dm^3 of water) was gradually substituted by a mixture containing in 1 dm^3 water saturated with EFS (i.e. 0.384 mM at 25 °C) not only 0.45 g of $(NH_4)_2HPO_4$ (as N and P source) but also 2 cm^3 of the peptone concentrate. The adaptation apparatus was daily checked by measuring the feed of the nutrient mixture (5.5 $dm^3\,day^{-1}$), pH (7 – 8), temperature (21 – 25 °C), concentration of dissolved oxygen (ca 90 % of the oxygen concentration in distilled water saturated with the air at the same temperature), concentration of AS (approximately by the Imhoff test, more exactly by turbidimetry [3]), X value (kept between 0.5 and 1 g dm^{-3}), qualitative microscopic picture of AS and concentration of EFS in the feed and reactor (UV spectroscopy and coupling method).

Determination of EFS

The UV spectrophotometry was based on the use of the characteristic spectrum of the solution of EFS in water, consisting of two bell-shaped curves with maxima at 225 and 277 nm. The following relations between EFS concentration (S) in distilled water and absorbancy (A) at room temperature were obtained

$$S\,[\text{mol dm}^{-3}] = 1.432\times10^{-4}A + 1.490\times10^{-6} \quad (225\text{ nm}) \qquad (2)$$

$$S = 1.356\times10^{-3}A + 3.198\times10^{-6} \quad (277\text{ nm}) \qquad (3)$$

They are valid for optical path of 1 cm in a quartz cell and water as the reference liquid. This method is very fast and exact, but only in pure solutions of EFS and water. It could be used in the experiments only for EFS determination in the feed solutions for AS adaptation apparatus before the peptone concentrate was added. In the filtrates of the samples of the reaction mixture, one or more components absorb beside EFS in this wavelenght region.

The coupling method is described in [4]. It is based on an alcaline substitution of the CH_3SO_3 group for the OH group at position 5 of the EFS molecule. This derivate can (at suitable pH) react with diazotized suphanilic acid, giving a red azo dye measurable spectrophotometrically in visible region.

In two kinetic experiments the HPLC method of EFS determination was used simultaneously with the coupling method described. The direct feeding of

concentrated sample and elution with 60% w/w of methanol in water were used. For details see Ref. [5].

The reactor and experimental measurements were described in [2]. In addition, the electromotive force of the element Pt/saturated calomel electrode was measured during every experiment, and every gravimetric determination of the X value in concentrated AS suspensions was supported by the determination of the weight loss during annealing of dry biomass X_v.

Determination of X_v

A sample of AS was dried to constant weight in a porcelain crucible. After flameless combustion over a gas lamp, the crucible was annealed in the muffle furnace to constant weight at 550 °C 2 hours. After cooling in dessiccator it was weighed and from the weight decrease in comparison with X the value X_v [% w/w] was calculated. The X_v value represents the combustible, i.e. organic part of dry AS.

Results and Discussion

Fourteen kinetic measurements were carried out with the initial concentrations of AS in the range $X_0 = 0.4 - 2.2$ g dm^{-3}, those of EFS in the interval $S_0 = 0.5\times10^{-4} - 2\times10^{-4}$ mol dm^{-3} and the reaction temperature 15 – 35 °C. The ratio of AS to EFS was $p = X_0/S_0 = 2.7 - 19.6$ g AS mmol^{-1} EFS.

Dependences S – time (t)

Immediately after mixing of AS in EFS suspesion an intense and fast decrease in the EFS concentration took place up to 60 – 55% of the S_0 value (see Fig.1), probably according to the adsorption of EFS on the solid particles of AS. The following decrease in S caused by its biochemical reaction consists of two straight lines in the experiments carried out at 15 – 25 °C . The first line is steeper and, after 3 – 4 hours, changes into the second one with substantially smaller slope (Fig.1). At temperatures 30 and 35 °C an induction period of ca 2 hours preceded the biochemical decomposition of EFS, at temperatures higher than 40 °C the irreversible denaturation of AS took place. In 6 – 7 hours of the reaction time the concentration of EFS dropped approximately to the value $0.3\times S_0$ in the experiments at 15 – 25 °C, whereas at higher temperatures (30 and 35 °C) only ca to $0.6\times S_0$.

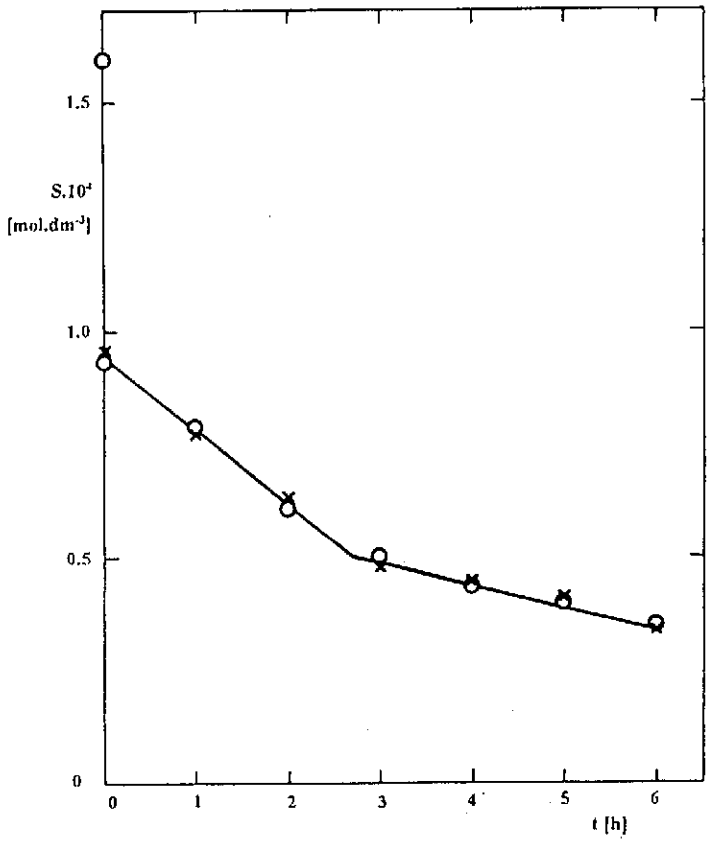


Fig. 1 Example of $S - t$ dependence, experiment 14: 20 °C, S_0 = 0.1595 mmol dm^{-3}, X_0 = 0.93 g dm^{-3}. o – HPLC values; x – spectrophotometry

Dependence $X - t$

There was practically no change in the value of X in the reaction mixture during the reaction. The measured changes in X are at the limit of experimental error of the turbidimetric method; the maximum difference was 6% w/w of the average X value, but in most experiments much less, ca 2%. The amount of X remains then constant during the reaction course. The average value of X_v from all experiments was 84.4% w/w of X with the standard deviation value 4.2% of X_v. This result indicates a practically constant organic component in AS used in the experiments.

Time Dependence of Concentration of Dissolved Oxygen (O) – t

The value of O in the reaction mixture was measured by means of a Clark oxygen probe with continuous graphic record. Both the O values at different reaction times of every experiment and the respiration rate (i.e. the decrease of O in the samples of the reaction mixture from these times under anaerobic conditions) were measured. It was found out that the O value oscillates during the reaction over about ±5% of the initial value (O_0), which is ca 90% of the oxygen concentration in distilled water at the temperature of the experiment. All $O - t$ dependences were linear until $O \leq 0.2O_0$. Therefore, in the experiments the dissolved oxygen decreases by reaction of zero order in oxygen, and the kinetics of the biochemical decomposition of EFS is independent of its concentration.

Time Dependence of Electromotive force (EMF) – t

Three types of EMF – t dependences were found: 1) EFS ≈ 200 mV, no change during the experiment, 2) permanent EMF increase (from 120 – 140 mV to 180 – 220 mV) during the experiment, 3) EFS decreased at first (cca 20 mV), then it increased above the initial value (from ca 150 to 230 mV in most experiments). No relation between the experimental conditions, decomposition of EFS and the EMF – t dependences was found.

pH – *t Dependence*

In all the experiments a mild decrease in pH took place during the reaction from pH 8 to 7.5 – 7.1.

Kinetics of the Biochemical Reaction between EFS and AS

The velocity (v) of the biochemical decomposition of EFS by means of adapted AS generally depends on the concentrations of EFS (S), AS (X), dissolved oxygen (O), anorganic nutrients (Z) and temperature (T).

$$v = -\frac{dS}{dt} = f(S, X, O, Z, T) \tag{4}$$

According to the results mentioned above this v is independent of O and the X, Z and T values are constant in every experiment carried out. The course of the expe-

Table I Reaction conditions of the experiments and the initial reaction rate (v) of the biochemical decomposition of EFS with adapted AS

Exp. No.	T °C	S_0 mmol dm^{-3}	X_0 g dm^{-3}	p g X_0 mmol^{-1} S_0	$v(t=0)\times10^{-4}$ mol S h^{-1} g^{-1} X_0
1	25	0.0532	0.377	7.1	0.119
2	25	0.1952	0.819	4.2	0.223
3	25	0.1113	1.023	9.2	0.156
4	25	0.0907	0.974	10.7	0.083
5	25	0.1501	1.116	7.4	0.096
6	25	0.1538	1.270	8.3	0.110
7	25	0.1507	1.279	8.5	0.0938
8	25	0.1488	1.209	8.1	0.119
9	35	0.1491	1.057	7.1	0.160
10	30	0.1432	1.370	9.6	0.0876
11	15	0.1484	0.978	6.6	0.261
12	25	0.1129	2.215	19.6	0.039
13	25	0.2150	0.573	2.7	0.366
14	20	0.1595	0.930	5.8	0.177

rimental $S-t$ dependences (2 lines and an induction period at $T > 30$ °C) indicate a complicated reaction mechanism and exclude the differential rate equation (4) as a simple power function of S multiplied by a rate constant (i.e. $v = kS^n$). Table I gives (beside data about T, S_0 and X_0), therefore, only initial velocities of the EFS decrease $v(t=0)$, expressed as the number of moles of EFS decomposed per 1 hour, related to 1 g of dry AS in 1 dm^3 of the reaction mixture.

$$v(t=0) = \frac{\Delta S_0}{tX_0} \quad [\text{mol h}^{-1}\,\text{g}^{-1}] \tag{5}$$

By means of $v(t=0)$ it is possible to compare the kinetic experiments carried out.

It is interesting that $v(t=0)$ decreases in the temperature range of 20 – 25 °C with the increasing ratio $p = X_0/S_0$ until to $p \approx 10$ g AS mmol^{-1} EFS. For $p > 10$ $v(t=0)$ decreases already very slowly (see Fig. 2). Mostly with growing X_0/S_0 ratio the substrates decrease more quickly.

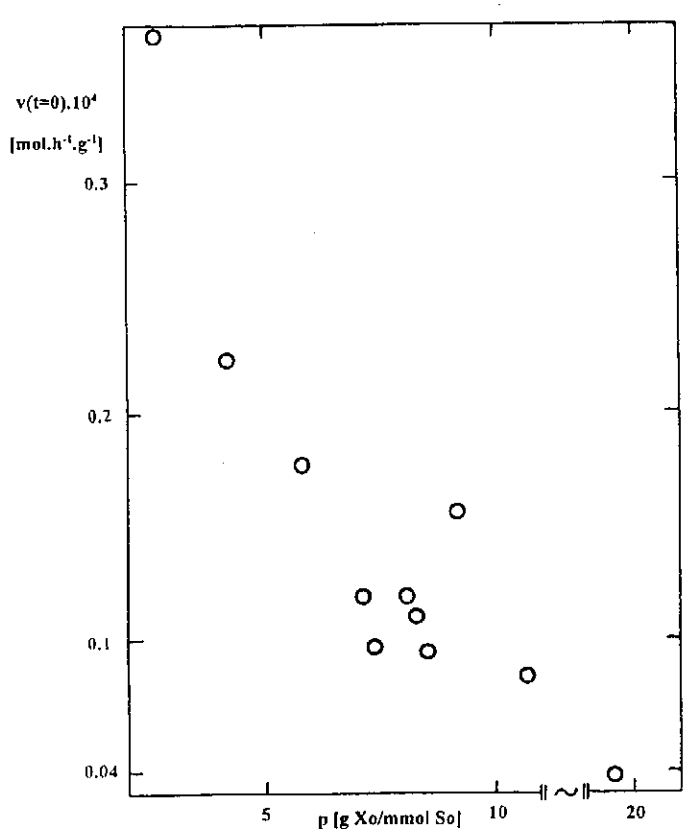


Fig. 2 Dependence of the initial rate of biochemical destruction of EFS, $v(t = 0)$ [mol S h^{-1} g^{-1} Xo], on the ratio of initial concentrations of AS and EFS $p = X_0/S_0$ [g $mmol^{-1}$] according to Table I for 20 – 25 °C

Furthermore, it is remarkable that for nearly the same p but at a 10 ° lower temperature, the $v(t = 0)$ value is twice as high greater (compare e.g. experiments 1 and 11). On the other hand, higher temperature does not increase the velocity of the EFS decomposition (experiment 11 at 15 and 9 at 35 °C). The dependence $v(t = 0) - T$ is thus not one of Arrhenius type. This fact together with the other experimental results indicate that the biochemical reaction between EFS and AS has a complicated kinetics and mechanism dictated by physical rather than chemical steps. The actual activity of AS (which cannot exactly be determined in advance) is playing surely an important role, too.

The velocity of EFS decomposition can be indicated as very slow; its value in the experiments carried out is in order of 10^{-2} to 10^{-3} milimol (i.e. 30 - 3 mg) of removed EFS per hour and per 1 g of dry AS. If we take into account the following substantial reduction of EFS decrease after the first 3 - 4 hours of the

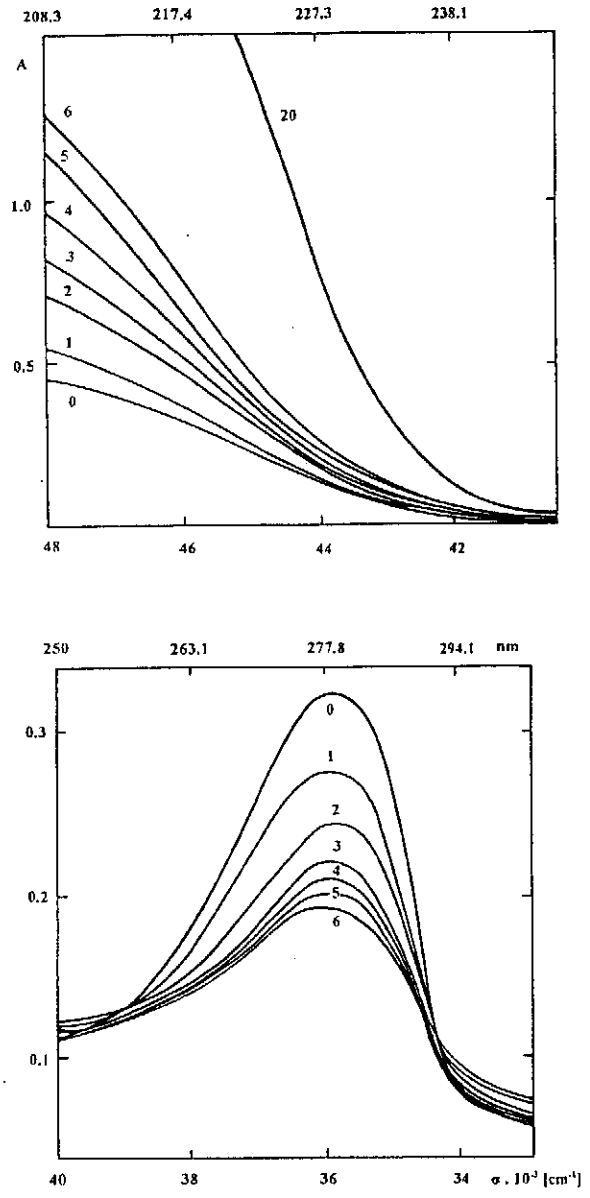


Fig. 3 UV absorption spectra of filtrates of samples from the experiment 14. a) 208 – 250 nm: optical length 1 mm, b) 250 – 303 nm , 10 mm. Apparatus Specord M 40 (Zeiss, Jena, BRD), the reference liquid – distilled water. Numbers at individual curves are times of sample taking in hours

reaction, EFS can be defined as a species very hard to decompose biologically. This had to be, of course, expected according to its structure.

Composition of the Reaction Mixture

was studied by means of UV absorption spectrometry and HPLC methods.

UV absorption spectra of the filtrates of the samples of reaction mixture show broad bands over the whole area measured 208 – 250 nm (Fig. 3a). The value of absorbance (A) increases at these wavelenghts with time (even when the concentration of EFS decreases) so that the dependences A (at constant wavelenght) – time are linear. The velocity of the increase in A depends on the initial concentration of AS. The absorption spectra measured in the interval of 250 – 303 nm (Fig. 3b) are bell-shaped with A maximum at 277 nm corresponding to the maximum of absorbance of water solutions of EFS. The whole spectral band decreases with increasing reaction time, but its decrease does not quantitatively correspond with the decrease of EFS concentration in the reaction mixture. The forms of both sides of spectra are changed at the same time. These parts have at longer reaction times higher A values in comparison with the values expected according to the decomposition of EFS. In some experiments the complete spectra had higher absorbances at longer than at shorter reaction times even when the EFS concentration permanently decreased. It was found (on the basis of factor analysis [6] of the series of spectra of samples from the given experiment, measured in the region of 250 – 303 nm) that these filtrates contain two kinds of species absorbing in this UV region.

The HPLC spectrum of such filtrates is always composed of the peak of EFS and peak of a unknown substance, according to its peak position much more polar than EFS. This substance cannot be considered an intermediate of the EFS decomposition because the magnitude of its signal increases linearly with time even when EFS no longer decreases or is completely decomposed. Probably, this species is extracted from the particles of AS in the course of the experiment.

The experimental results described show that the biochemical decomposition of EF probably does not take place on the surface of the AS microorganisms by means of exoenzymes, but EFS has to permeate into the interior of the cells to be decomposed. In this way the primary – faster – decrease of EFS from the liquid phase of the reaction mixture, determined by its transport velocity into the microorganisms, can be explained. After saturation of all microorganisms with EFS during first 3 – 4 hours the secondary – slower – decrease of EFS in the solution, dictated by the consecutive slow EFS decomposition in the interior of the cells, takes place.

References

[1] FBC, British Patent, 1 271 659 (1972).

[2] Komers K.: *Reaction of 2-naphthol with Activated Sludge*, This Journal.

[3] Komers K.: Sb. Věd. Prací Vys. Škola. Chem. Technol., Pardubice **54**, 55 (1990).

[4] Komers K.: Analyst **115**, 467 (1990).

[5] Jandera P.: University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, personal comunication

[6] Meloun M., Havel J., Högfeldt E.: *Computation of Solution Equilibria*, Ellis Horwood Ltd, Chichester 1988.