SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
6 (2000)

REACTION OF 2-NAPHTHOL WITH ACTIVATED SLUDGE

Karel KOMERS
Department of Physical Chemistry, University of Pardubice,
CZ–532 10 Pardubice

Received January 21, 2000

Dedicated to the 50th anniversary of university education in Pardubice

In a batch stirred isothermal reactor the biochemical reaction has been studied of water solution of 2-naphthol (BN) and activated sludge (AS) adapted to BN at aerobic conditions in the BN concentration range $S_0 = 0.5 - 2$ *mmol* dm^{-3}, *AS concentration range (expressed as the weight of dry biomass in the unit of the reaction volume)* $X_0 = 0.1 - 4$ *g* dm^{-3} *and temperature range* $11 - 40\,^{\circ}C$.

The decomposition of BN in the reaction mixture can be described as a reaction of zero order in BN and probably 1st order in AS if the ratio of initial concentrations $p = X_0/S_0$ *is greater than 0.6 g AS per mmol BN. At lower p values the reaction order in BN fluctuated between 0 and 2. The concentration of AS was practically constant during every experiment, the concentration of oxygen dissolved in the reaction mixture decreased by a reaction of zero order.*

In addition to the decomposition of BN a red dinaphthol derivative is formed as a side product. Its concentration increases at higher temperature and smaller p. The rate constant of the BN decomposition increased from $k = 0.23$ *at* $11\,^{\circ}C$ *to* $k = 0.68$ *mmol* $S\,g^{-1}\,X\,h^{-1}$ *at* $30\,^{\circ}C$. *In this temperature interval the*

Arrhenius equation between k and temperature is fulfilled.

The temperature above 40 °C denaturates AS and stops the decomposition of BN.

Introduction

2-naphthol (BN) is produced in Synthesia Pardubice a.s. in large quantities and therefore its residue are contained in the waste water from this chemical factory. One advantageous method of removing is its biochemical decomposition by a mixed culture of microorganisms called activated sludge (AS) adapted to BN. This paper is a contribution to the understanding of the mechanism and kinetics of this biochemical reaction and it resulted from the cooperation with the Environment Department of Synthesia Pardubice.

Experimental

Adaptation of AS to BN

AS was obtained in an air-saturated stirred flow reactor (volume 5 dm^3) connected to a sedimentation cone (volume 1.5 dm^3) and equipped with the suspension recycling (3 $dm^3\ h^{-1}$). At first AS was continually fed with the pepton suspension (cca 3 $dm^3\ day^{-1}$). The peptone suspension was prepared by dispersing the basic peptone mixture (peptone 48 g, gelatine 16 g, starch 32 g, Na_2HPO_4 8 g, KCl 1.12 g, $MgSO_4$ 0.8 g, $FeSO_4$ 0.8 g in 1 dm^3 water) in lukewarm drinking water (10 – 12 cm^3 mixture in 1 dm^3 water). After producing enough concentrated peptonic AS (X = 2 g dm^{-3}) the peptone suspension was gradually replaced by aqueous solution of BN (2 mmol BN and 10 mmol $(NH_4)_2HPO_4$ in 1 dm^3 water). After 6 weeks of adaptation, the AS was fed with only the aqueous solution of BN (5 – 10 dm^3 daily). The retention time of this AS adapted to BN was 12 – 14 hours. This AS was used in the kinetic experiments. The condition of the AS in the described adaptation apparatus was controlled by everyday measurement of the feed, temperature (18 – 25 °C), pH (7 – 8), concentration of dissolved oxygen (100 – 80% of the equilibrium amount of O_2 in the distilled water at the same temperature), concentration of BN in the feed and in the reactor, concentration and microscopic picture of AS. The feeding was realized with a glass piston pump from a 20 dm^3 tank. Concentration of the dissolved oxygen was measured by the Clark oxygen probe with the continual registration of the signal. The BN concentration in the feed and reactor (after centrifugation of AS) was determined (after coupling of BN with diazotized sulphanilic acid) by means of absorption photometry.

Azo coupling method: Into a 50 cm^3 calibrated flask such a volume of the

sample was added (with a pipette) as to reach the BN concentration of 0.01 – 0.03 mmol dm^{-3} in the flask after final filling it to the mark. To this sample 10 cm^3 of the Sörensen buffer pH 8 were added and, by mixing, the coupling of BN was carried out with 0.2 cm^3 0.01 M solution of the diazotized sulphanilic acid in 0.01 M aqueous solution of HCl. This mixture was diluted to 50 cm^3 with distilled water, carefully homogenized, and its absorbance (A) at 490 nm was measured in a cell by means of a Specol 11 apparatus (Zeiss, Jena, BRD). A linear dependence was found between the BN concentration (S) and the value of A

$$S\,[\mathrm{mol\,dm^{-3}}] = (4.7496\times10^{-5}A - 8.29\times10^{-7})\frac{50}{x}$$

where x is the volume of the sample in cm^3.

The pH value was measured by means of the electrochemical cell glass electrode/saturated AgCl electrode with a pH meter Radelkis, Hungary.

Determination of AS in the adaptation apparatus: The AS concentration was checked by the height of its layer after 30 min sedimentation of the suspension in a 100 cm^3 glas cylinder of 2 cm diameter. If this height was greater than 10 cm (which corresponded to $X = 2$ g dm^{-3}) the amount of AS was reduced approximately to one half.

The microscopic picture of AS was evaluated qualitatively at the magnification of 1:10 to 1:45 according to the individual dominant higher organisms (rotators, vorticellas, ciliates) present and their vitality.

Reactor and Kinetic Measurements

The kinetic measurements were carried out in an apparatus demonstrated in Fig. 1, similar to that described in Ref. [1]. The basis was a batch reactor 1 with the volume of 800 cm^3 of the reaction mixture. The reaction mixture was stirred with propeller 2 and heated to the chosen temperature with the water from thermostat 12. The mixture was continually circulated by means of the pump 6 through the respiration vessel 5 kept at the same temperature. At any movement it was possible to stop this circulation, switch on electromagnetic stirrer 4 in 5, first to determine the actual concentration of the oxygen dissolved in the reaction mixture and then to measure its decrease with time under anaerobic conditions, all with the help of the oxygen probe 8.

Before each experiment, the calculated amounts of distilled water, inorganic nutrients in the form of solid $(NH_4)_2HPO_4$ and water solution of BN were dosed. After heating to chosen temperature and sufficient saturating of this mixture with air the experiment was started by adding such a volume of concentrated and homoge-

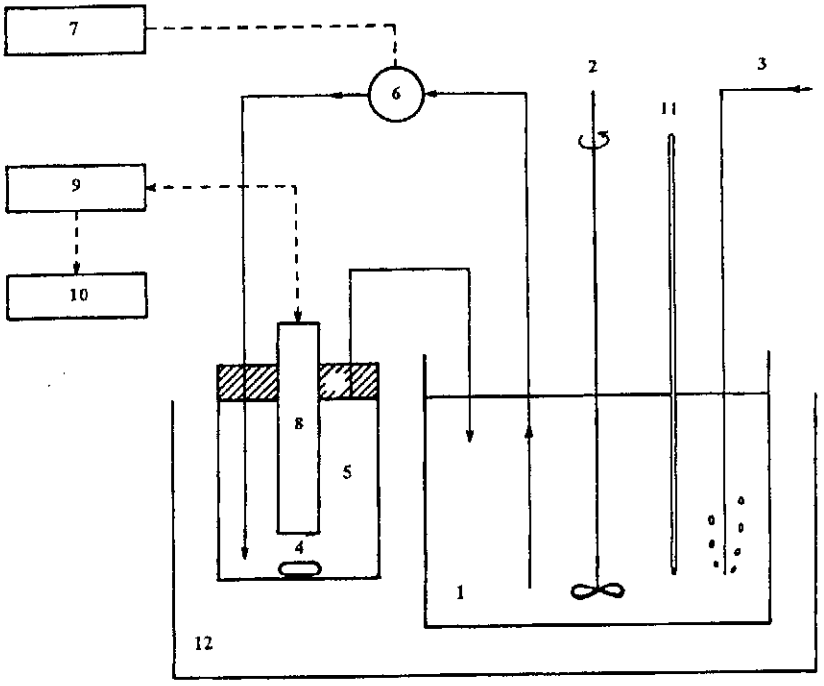

Fig. 1 Scheme of reactor and apparatus used for measuring respiration velocities: 1 – reactor, 2 – propeller stirrer in reactor, 3 – air saturation in reactor, 4 – electromagnetic stirrer in respiration vessel 5, 6 – peristaltic pumps, 7 – control of start and stop of the pump, 8 – Clark oxygen probe, 9 – charging and measuring the probe signal

nized suspension of AS (obtained from the adapted AS by sedimentation and, as the case may be, after decanting with distilled water) which corresponded to the chosen X value. At the chosen time intervals the samples were taken from the reaction mixture to determine the concentrations of AS and BN, pH value and absorption spectra of the liquid part of the suspension in the UV and VIS regions. Beside it, concentration of the dissolved oxygen in the reaction mixture and the respiration rate were measured in different reaction phases.

The approximate AS concentration in the concentrated suspension was determined by the fast turbidimetric method [3] according to the calibration curve from the previous experiment. The exact AS concentration in the concentrated suspension was determined gravimetrically [2]: 8 cm^3 of intensively homogenized AS suspension was centrifuged (centrifuge Janetzki T 24, MLW, BRD) 10 min at 20000 rpm and the sediment was dried at 105 °C to constant weight. The result was the value of the weight of dry biomass in the unit volume of the concentrated suspension [g dm^{-3}]. This determination made it possible to find 1) the exact X

value in the initial reaction mixture, 2) the actual turbidimetric calibration curve turbidance $T(520\,\text{nm}) - X[\text{g dm}^{-3}]$ for a given AS [3]. Then, during the experiment, the *actual* X value was measured using the turbidimetric method, which is much faster than the gravimetric one.

The BN concentration and pH value were determined in the same way as in the adaptation apparatus.

The UV-VIS absorption spectra of the filtrates of the reaction mixtures at different reaction times were measured by means of the spectrophotometer Specord 40 M (Zeiss, Jena, BRD) in 0.2 – 5 cm quartz cells.

Results and Discussion

By the method described altogether 55 experiments were carried out with the concentration of BN in the range S = 2 to 5 mmol dm^{-3} and of AS in the range X = 0.1 to 4 g dm^{-3}, at temperatures from 11 to 40 °C, in the medium of water solution of 0.01 M $(NH_4)_2HPO_4$.

The results of the above described analyses allow the following conclusions:

- The reaction between BN and AS starts with the very fast (ad)sorption of BN on the particles (flakes) of AS. The intense initial decrease in the BN concentration demonstrates this conclusion.
- Afterwards, monotonic decrease of BN in the reaction mixture takes place until its total decomposition into non-aromatic products – see the spectra of reaction mixtures, Fig. 3.
- The form of the isothermal decrease in BN concentration vs. time $S(t)$ is given by the starting ratio $p = X_0/S_0$, temperature and activity of AS. On the other hand, $S(t)$ does not depend on the concentration of the oxygen dissolved in the reaction mixture (O). The dependence $O(t)$ fulfils the kinetic equation of zero order.
- The AS concentration, expressed by means of X values, changes in the first 6 hours of the reaction in all the experiments only within the limits of experimental error of X determination, i.e. ±5 % w/w. Therefore, in each experiment, the X value could be taken as constant in this time interval.
- The pH value decreases during the experiment mildly from 8 to 7.2.
- All dependences $S(t)$ obtained can be described with the differential kinetic equation

$$-\frac{dS}{dt} = kS^n \qquad (1)$$

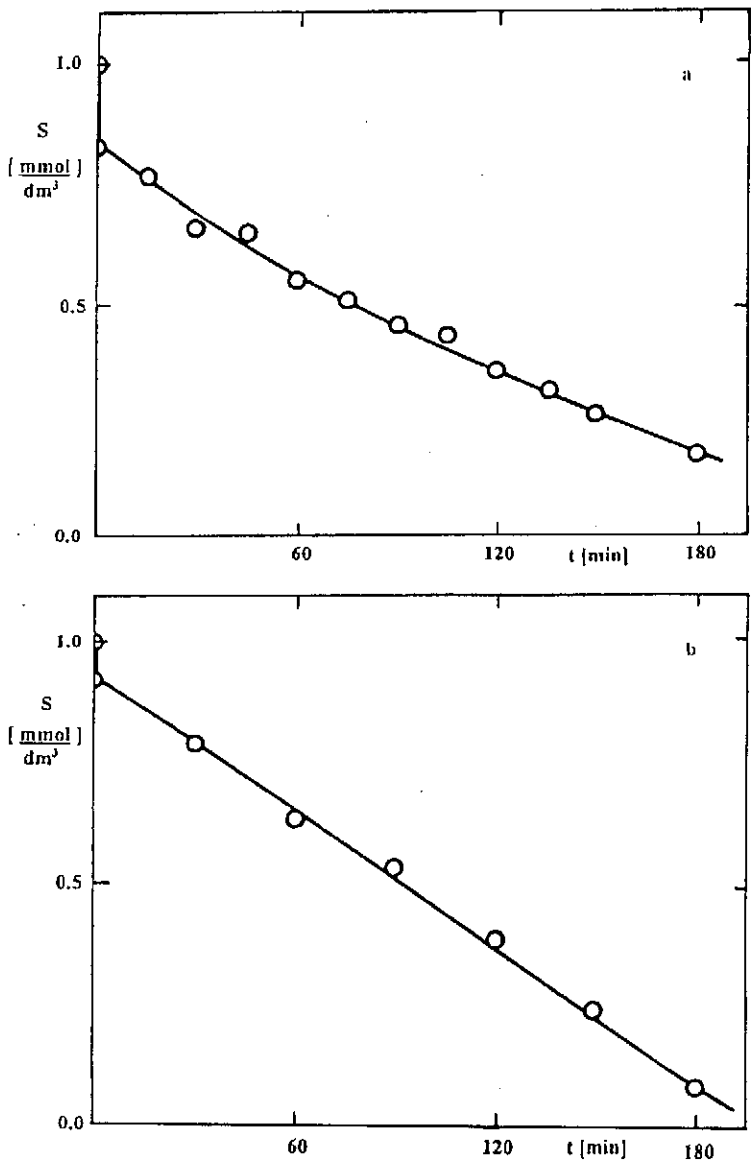

Fig. 2 Two characteristic dependences of concentration of BN (S) in the reaction mixture vs time (t). a) 20 °C, p = 0.591 g X mmol^{-1} S, n = 0.6, k' = 0.36 h^{-1}; b) 20 °C, p = 0.783 g X mmol^{-1} S, n = 0, k' = 0.306 h^{-1}

with a suitable value of the reaction order n (in S) and a velocity constant k. The constant k includes the dependence on temperature (T) and concentrations of AS (X), dissolved oxygen (O) and inorganic nutrients (Z)

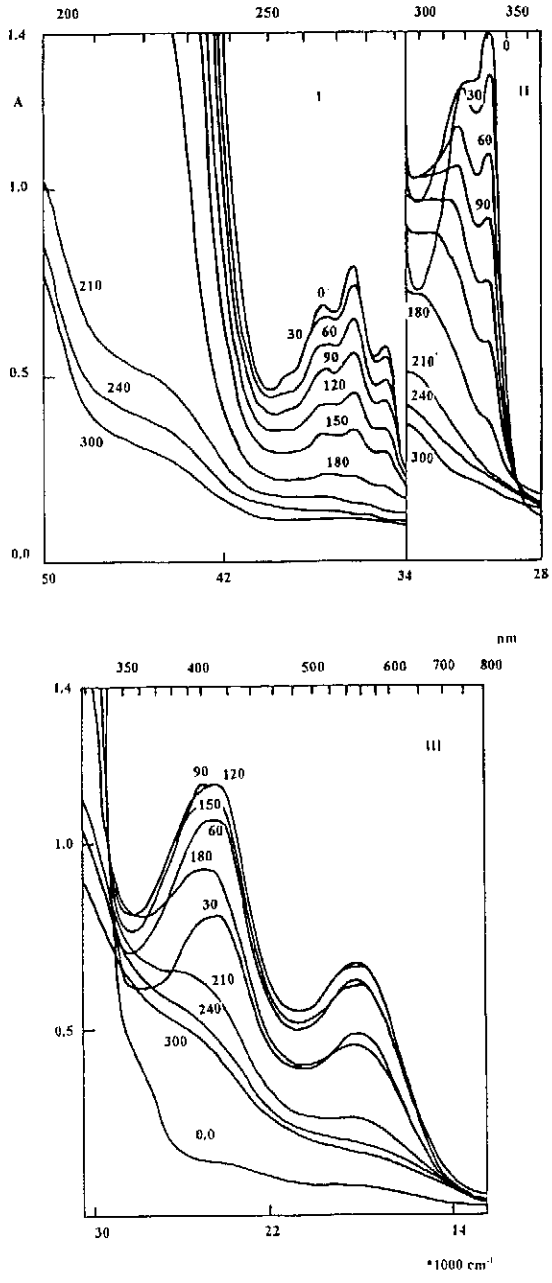

Fig. 3 Absorption spectra of filtrates of samples from the experiment in Fig. 2b. Numbers at the curves are reaction times in minutes. Part I – optical path of cell 2 mm, II – 10 mm, III – 50 mm, the reference solution – water

$$k = k(T, X^x, O^o, Z^y)$$

where x, o, z are the reaction orders in the respective reactants. In our experiments T, X and Z are constant, o is zero.

If the ratio $p > 0.6$ g X mmol^{-1} S, then also $n = 0$ (see Fig. 2b). The reaction is of zero order in BN and the velocity of the BN decrease does not depend on the BN concentration

$$-\frac{dS}{dt} = k \tag{2}$$

If we in Eq. (1) express the BN concentration in relative form $c = S/S_0$, where S_0 is the initial BN concentration, we have

$$-\frac{dc}{dt} = k' c^n \tag{3}$$

where $k' = kSo^{n-1}$ and for $n = 0$ it is

$$-\frac{dc}{dt} = k' = \frac{k}{S_0} \tag{4}$$

The dependence k' [t^{-1}] vs p at 25 °C and $n = 0$ for the experiments carried out is given in Fig. 4 and approximately forms a line without intercept. The relative large dispersion of the k' values for the most frequently measured ratio $p \approx 1$ can be explained by a change in the AS biological activity in decomposing BN. This activity is not expressed by the X value exactly enough.

The $k'(p)$ linearity can be explained by Eq. (5)

$$k' = k^* \frac{X_0}{S_0} = k^* p \tag{5}$$

The reaction is also of the 1st order in AS if $p > 0.6$ g AS mmol^{-1} BN and $n = 0$.

At $p < 0.6$ g AS mmol^{-1} BN the reaction order in BN fluctuates within the interval $n = 0$ to 2 (for an example see Fig. 2a), i.e. the reaction velocity is controlled by another mechanism.

The course of the reaction is strongly affected by the temperature (T). The

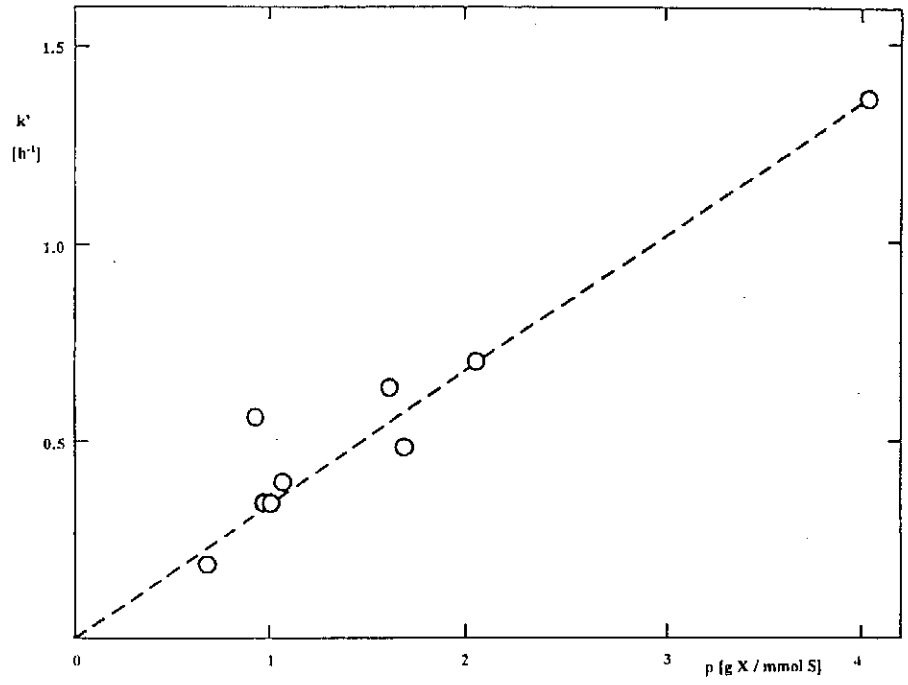

Fig. 4 Dependence of rate constant k' [h^{-1}] for experiments with $n = 0$ and 25 °C vs ratio of initial concentrations of AS and BN $p = X_0/S_0$

Table I Average values of rate constants $k^* = k'/p$ of the reaction of BN with AS. In all the experiments: $n = 0$; $p < 0.6$ g AS $mmol^{-1}$ BN; $S_0 = 0.5 - 2$ mmol dm^{-3}; $X_0 = 0.1 - 4$ g dm^{-3}

Temperature, °C	Number of experiments	k^*, mmol g^{-1} h^{-1}
11	1	0.2295
20	6	0.4034 ± 0.0601
25	9	0.5123 ± 0.0320
30	2	0.6811 ± 0.1137

velocity of the BN decomposition increases with increasing T – see Table I. But the value of $T = 40$ °C is the limit because of irreversible denaturation of AS above this T. In Fig. 5 the dependence $\log k^*$ vs. $1/T$ is shown. It is linear in the given temperature interval, the $\Delta T = 10$ °C changes the k^* value ca 1.68 times. The magnitude of this change demonstrates that the rate-controlling step in the complicated mechanism of this heterogeneous reaction is probably of chemical nature.

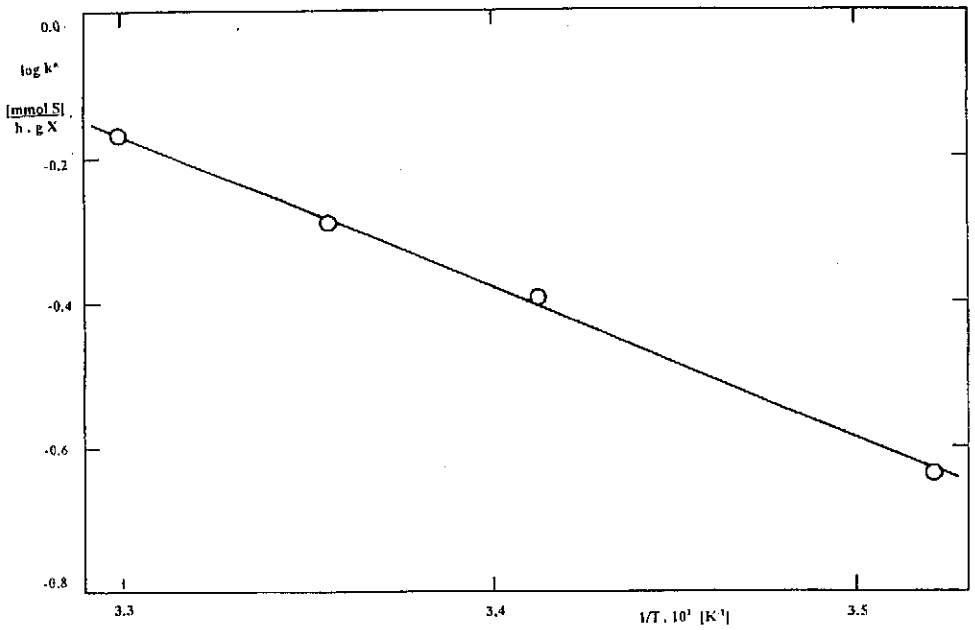

Fig. 5 Demonstration of validity of Arrhenius dependence of k^* on temperature T from Table I

Visually and also according to the UV–VIS absorption spectra of the samples from the individual experiments (for an example see Fig. 3, part III) it can be indicated that beside decomposition of BN, a red intermediate I is formed and then decomposed in the actual reaction mixture. It was demonstrated by means of the factor analysis [4,5,6] of these series of spectra that in the samples only two *soluble* species absorbing the radiation above 250 nm are present. The presence of these two substances (BN and I) in the filtrates is also confirmed by the isosbestic point at 340 nm (see Fig. 3, part III).

The rate of formation and the maximum concentration of I depend on ratio p, T and probably also on the biological activity of AS. I is formed faster and in higher concentration at smaller p and higher T, its formation slows down at higher AS activity to BN. However, the concentration of I in the reaction mixture is very low; the maximum value found was ca 5×10^{-5} M. After decomposition of BN, I is also decomposed by cells of AS in ca one third of the time necessary for the decomposition of BN. The intermediate I was isolated and its constitution determined by means of the combined interpretation of its H^1, C^{13} NMR, MS and IR spectra [7]. It is a dinaphthol derivative: 4-(2′-dihydroxy-1-naphthyl)-6-hydroxy-1,2-naphthoquinone.

By means of the UV–VIS spectra of water solution of I at different pH values and 25 °C the dissociation constants of both acid hydrogens K_1 = 3.47×10^{-7} and

K_2 = 3.8×10^{-10} were determined, without possibility to tell which H is split off as first. The non-dissociated form of I is yellow, both dissociated forms (i.e. I^- and I^{2-}) are dark red. Thus, the substance I is an acid-base indicator.

According to the small concentration of I in the reaction mixtures and to the dependence of this concentration on *p*, *T* and AS activity we can assume that the formation of I is a side, parasitic and retarding process to the main reaction – decomposition of BN.

References

[1] Pitter P., Tuček T., Chudoba J., Žáček L.: *Laboratory Methods in Technology of Water* (in Czech), Prague 1982.

[2] Sedláček M.: *Analytical Methods of Sludges and Solid Wastes* (in Czech), Státní zemědělské nakladatelství, Prague 1978.

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

[4] Havel J., Meloun M.: Talanta **32/3**, 171 (1985).

[5] Bernštejn I.J., Kaminskij J.L.: *Spectrophotometric Analysis in Organic Chemistry* (in Russian), Izdatělstvo "Chimia", Moscow 1986..

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

[7] Komers K., Lyčka A., Jirman J., Kolb I.: Collect. Czech. Chem. Commun. **53**, 1954 (1988).