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JAN DOJLIDO*, ANDRZEJ STOJDA*, ELŻBIETA GANTZ*, JAN KOWALSKI*

# BIODEGRADABILITY OF P-NITROPHENOL

Results of studies on p-nitrophenol (PNP) biodegradation in a river are presented. It has been stated that after a certain adaptation time of microorganisms the rate of biodegradation process and its efficiency are high. It has been stated, moreover, that the presence of PNP in municipal sewage does not affect the total treatment efficiency.

## 1. INTRODUCTION

The p-nitrophenol (PNP) is used in chemical industry, mainly to produce pesticides and azo-dyes. It is employed also for wood impregnation. In several chemical plants in Poland, producing or using PNP, its concentration in raw wastewaters reaches several hundred mg/dm$^3$. Since PNP in such concentrations is toxic for activated sludge microorganisms, the preliminary chemical treatment of wastewaters is indispensable.

The research upon PNP biodegradability was carried out by PITTER [2] who observed the effect of PNP contents on wastewater treatment by use of activated sludge in static conditions. In his research neither respirometry nor PNP biodegradation in river water was dealt with.

The purpose of the extensive PNP biodegradability tests carried out in the Institute of Meteorology and Water Management was to determine:

stoichiometry of biochemical process of PNP decomposition,

kinetics of PNP biodegradation in river water,

effect of PNP presence in domestic sewage on the overall efficiency of activated sludge treatment,

efficiency of PNP removal from wastewaters by activated sludge treatment.

## 2. EXPERIMENTAL

### 2.1. ANALYTICAL PROCEDURE

PNP concentrations were determined spectrophotometrically. The quinone form of PNP reacts with $Na^+$ ions at hight pH, giving yellow coloured *quasi*-salt. The absorbance was measured at the wavelength of 405 nm.

* Institute of Meteorology and Water Management, 01-673 Warszawa, ul. Podleśna 61, Poland.

### 2.2. RESPIROMETRIC TESTS

The tests were carried out with the use of Sapromat apparatus. Synthetic wastewaters (yeast extract or glucose solution) with addition of the known amount of PNP were inoculated with domestic sewage and placed in a closed flask of Sapromat. The oxygen uptake was measured every hour during the whole test lasting for a dozen days. After the test had been completed, the PNP, ammonia, nitrites, and nitrates contents were determined [1].

### 2.3. TEST OF PNP BIODEGRADATION IN RIVER WATER

The river water, sampled from Vistula river with the addition of a known amount of PNP, was poured into the 10 $dm^3$ bottles and stirred to obtain a turbulent movement similar to that in the river. The fluorescence light of 1000 lux simulated daylight. The concentration of PNP was measured during several days of illumination.

### 2.4. TEST OF TREATMENT OF PNP-CONTAINING WASTEWATERS BY ACTIVATED SLUDGE METHOD

The investigations were conducted in a laboratory unit of a continuous flow mode of operation. The unit consisted of 1 $dm^3$ aeration chamber and 0.5 $dm^3$ settling tank separated by the wall with a chink at the bottom [1]. The simulated domestic sewage with a known amount of PNP was pumped into the unit by peristaltic pump.

Of four chambers operating in parallel three were fed with sewage with an addition of PNP and one (control chamber) without PNP. During the tests lasting ca forty days the following parameters were determined: PNP contents, COD and MLSS (dry weight). The retention time was checked by volumetric measurement.

## 3. RESULTS AND DISCUSSION

### 3.1. RESPIROMETRIC DATA

The measurements were performed in seven series [1], including 3–6 samples in each. The initial concentrations of PNP ranging from 0 to 100 mg/$dm^3$ were increased consecutively in order to determine the toxic concentration at which the vital activity of microorganisms is inhibited and biochemical processes are stopped. The changes in oxygen uptake and PNP concentrations obtained for one series are presented in fig. 1.

The final carbonaceous BOD (denoted by $L_c$) was read out. The dependence of $L_c$ on initial PNP concentration $c$ (fig. 2) was determined from the measurements of BOD of samples with different initial concentrations of PNP. In this figure the slope of straight

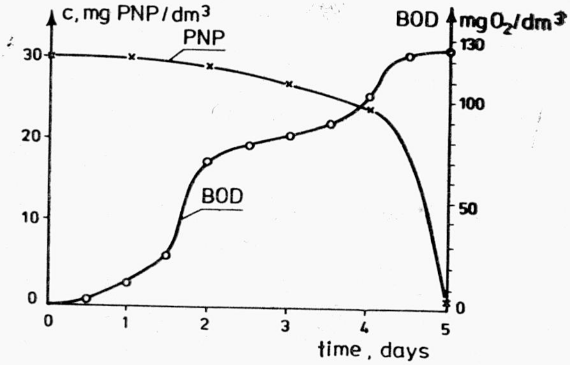


Fig. 1. BOD values and PNP content during the respirometric test
Rys. 1. Wartości BZT i zawartość PNP podczas testu respirometrycznego

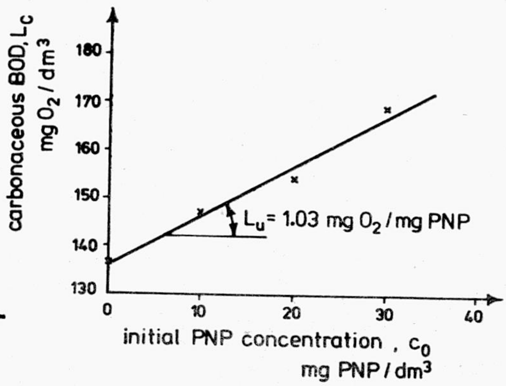


Fig. 2. Final carbonaceous BOD ($L_c$) as a function of initial PNP content ($c_0$)
Rys. 2. Końcowe BZT węglowe ($L_c$) jako funkcja początkowej zawartości PNP ($c_0$)

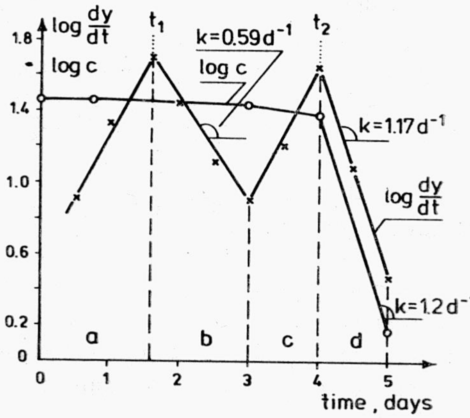


Fig. 3. Curves from fig. 1 after linearization
Rys. 3. Krzywe z rys. 1 po linearyzacji

line obtained expresses the unit oxygen uptake $L_u$, and its intersection with the ordinate indicates the BOD of sewage without PNP.

The mean unit oxygen uptake $L_u$, calculated by linear regression, was:

$$L_u = 1.03 \pm 0.12 \text{ mgO}_2/\text{mg PNP} \qquad (1)$$

(the precision given at 90% confidence level).

By determining nitrogen compounds we could state that about one half of nitrogen bound in PNP molecule is converted into ammonia, and next into nitrates, and one half is assimilated by microorganisms. So biodegradation of PNP is described by the following stoichiometric formula:

$$HO \cdot C_6H_4 \cdot NO_2 + 5O_2 \longrightarrow 6CO_2 + H_2O + NH_3.$$

The value of $L_u$ calculated from the above equation amounts to 1.15 mg $O_2$/mg PNP.

The kinetics of PNP biodegradation can be determined easier using the graphs in linearized form, presented in fig. 3. Graphic representations of BOD and PNP decreases show that the biodegradation process follows four subsequent phases:

phase a — phase of intense bacterial growth due to decomposition of simulated wastewater constituents,

phase b — oxygen uptake decrease due to depletion of organic compounds in simulated wastewater,

phase c — second growth phase in which the number of bacteria capable of PNP degradation increases,

phase d — decrease of the oxygen uptake rate due to depletion of PNP contents.

From analysis of BOD curve the following parameters may be determined:

initial delay of simulated wastewater degradation ($\Delta t_1$),

delay of PNP degradation ($\Delta t_2$),

coefficients of simulated wartewater $k_w$ and PNP $k_{PNP}$ biodegradation, provided that the process follows the monomolecular reaction described by Streeter-Phelphs equation.

The delay $\Delta t_1$ is obtained from the formula:

$$\Delta t_1 = t_{1,c} - t_{1,0} \tag{2}$$

where:

$t_{1,c}$ — time of the maximum oxygen uptake for the test sample with PNP at the concentration $c$ (fig. 3),

$t_{1,0}$ — time of the maximum oxygen uptake for the control sample.

Analogically, the delay $\Delta t_2$ can be calculated as follows:

$$\Delta t_2 = t_{2,c} - t_{1,0} \tag{3}$$

where:

$t_{2,c}$ — time corresponding to the maximum rate of PNP biodegradation in the sample containing PNP at the concentration $c$,

$t_{1,0}$ — time of the maximum oxygen uptake for the control sample.

The results obtained represent graphically the effect of PNP concentration upon the delay of biochemical processes (fig. 4).

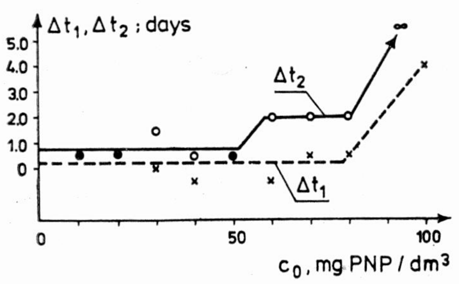


Fig. 4. Inhibition time of glucose decomposition ($\Delta t_1$) and of PNP decomposition ($\Delta t_2$) as a function of the PNP initial content ($c_0$)

Rys. 4. Czas inhibicji rozkładu glukozy ($\Delta t_1$) i PNP ($\Delta t_2$) jako funkcja początkowej zawartości PNP ($c_0$)

From the data obtained the following conclusions may be formulated:

At PNP concentrations up to 80 $mg/dm^3$ the delay of the sewage biodegradation is not significant, $\Delta t_1$ is near zero. At higher concentrations of PNP the delay increases and at $c = 100$ mg $PNP/dm^3$ equals four days.

At PNP concentrations up to 50 $mg/dm^3$ its biodegradation occurs simultaneously with the biodegradation of all others sewage constituents. Within the range of 50 to 80 mg $PNP/dm^3$ the delay $\Delta t_2$ is equal two days. At the PNP concentration equal to 100 $mg/dm^3$ and higher, it is practically resistant to biodegradation.

At lower concentrations of PNP its biodegradation rate is high, i.e. the Streeter-Phelphs coefficient $k_{PNP}$ is equal ot higher than the coefficient $k_w$ corresponding to the biodegradation of simulated sewage. At higher PNP concentrations its decomposition rate decreases and at 100 mg $PNP/dm^3$ $k_{PNP}$ drops to zero.

## 3.2. THE TESTS OF BIODEGRADATION IN THE RIVER WATER

Four series of measurements were carried out, using three parallelly operating installations. After the PNP concentration had dropped to zero, the next dose of PNP was added. The results allow us to determine the time $t_0$ of microorganism adaptation for the PNP biodegradation. Its rate $v$ was also calculated. The exemplary curves of PNP biodegradation obtained for the series is presented in fig. 5. The mean values of $t_0$ and $v$ given in

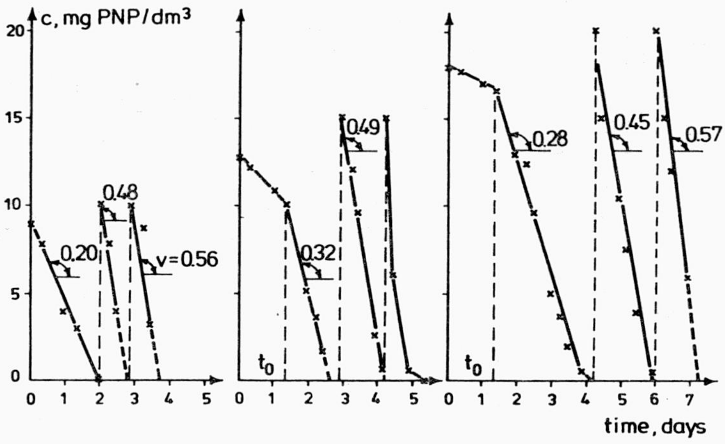


Fig. 5. Decomposition of PNP in river water

Rys. 5. Rozkład PNP w rzece

tab. 1 show that PNP is easily biodegraded in river water, but requires a certain time for the adaptation of the microorganisms which at the highest concentrations of PNP tested, i. e. 20 $mg/dm^3$, is equal to 1.4 day. If PNP is added after the microorganisms are adapted, the biodegradation proceeds without delay. Its decomposition rate was high, but it should be emphasized that at lower initial concentrations it dropped significantly.

Table

Kinetic parameters of p-nitrophenol biodegradation
Parametry kinetyczne rozkładu p-nitrofenolu

| Initial PNP concentration $C_0$ mg/dm³ | Not adapted microorganisms (the first PNP addition) | | Repeated addition of PNP | | Subsequent addition of PNP | |
|---|---|---|---|---|---|---|
| | $V$ mg/dm³ · h | $t_0$ days | $V$ mg/dm³ · h | $t_0$ days | $V$ mg/dm³ · h | $t_0$ days |
| 5 | 0.1 | 0 | 0.16 | 0 | 0.28 | 0 |
| 10 | 0.18 | 0.6 | 0.43 | 0 | 0.53 | 0 |
| 15 | 0.35 | 1.3 | 0.48 | 0 | 0.50 | 0 |
| 20 | 0.35 | 1.4 | 0.45 | 0 | 0.48 | 0 |

### 3.3. TREATMENT BY ACTIVATED SLUDGE PROCESS

The aeration chambers containing activated sludges were fed initially with simulated domestic sewage (BOD was equal to ca 360 mg $O_2$/dm³ and COD — to ca 420 mg $O_2$/dm³) to which the dose of PNP was added to obtain 5 mg PNP/dm³ in the inflow. Gradually the PNP contents $c_0$ in the inflow was rised up to 40 mg/dm³. The measurements of PNP concentrations $c_e$ in the effluent, i.e. in the treated sewage, are presented in fig. 6. The curves show that efficiency of PNP removal by not adapted activated sludge is low. After ca two weeks of adaptation the PNP removal was very high, so that only its traces were found in the effluent.

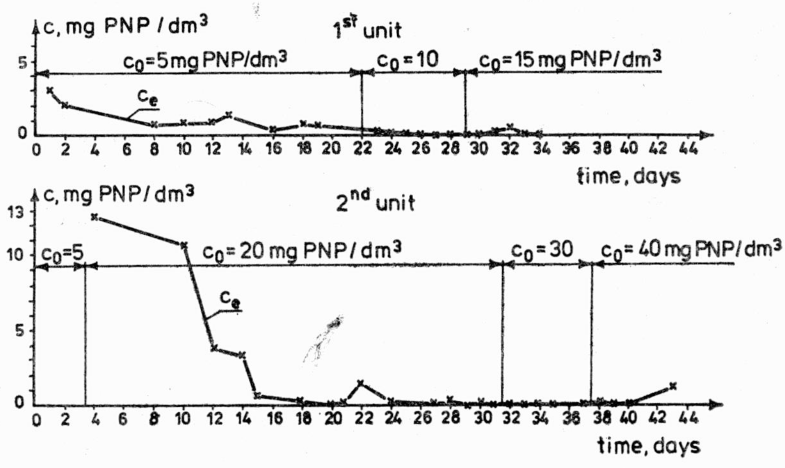


Fig. 6. PNP removal by the activated sludge
Rys. 6. Usuwanie PNP przez osad czynny

The results of COD determinations in the inflow and effluent allow us to determine the effect of PNP on overall treatment efficiency. The kinetics of treatment can be described by the equation of the second order reaction

$$dC/dt = KCm \tag{4}$$

where:

$C$ — COD of sewage (mg $O_2/dm^3$),
$m$ — dry mass of activated sludge ($g/dm^3$),
$t$ — time (h),
$K$ — kinetic coefficient.

Assuming the above we can characterize the efficiency of sewage treatment by the value $K$. This value can be calculated from the equation describing the continuous flow treatment:

$$K = \frac{C_0 - C_e}{C_e} \times \frac{1}{mt_r} \tag{5}$$

where:

$C_0$ and $C_e$ — COD of the inflow and the effluent, respectively,
$t_r$ — retention time (h).

The results of calculations show that the values of $K$ observed in the experiments with the use of sewage containing PNP were near the same as those characterizing the treatment of sewage without PNP (in control chambers). Thus, we can state that PNP at the concentrations up to 40 $mg/dm^3$ does not affect the efficiency of the treatment of domestic sewage by activated sludge.

## 4. CONCLUSIONS

p-Nitrophenol is easily biodegradable at its concentrations up to ca 50 $mg/dm^3$. At higher concentrations its biodegradation proceeds slower, and at 100 $mg/dm^3$ or above the biochemical processes are almost completely inhibited.

In river water the biodegradation of PNP needs a certain time of initial adaptation of microorganisms, what implies that the sudden discharge of PNP to the river in the case of heavy misoperation of the wastewaters treatment plant in the factory producing PNP can pollute the river within a long distance from the discharge point. If the river biocenosis is adapted to the PNP, the rate of its biodegradation is high.

The efficiency of PNP removal from wastewaters treated with activated sludge is high. The presence of PNP in domestic sewage does not affect the overal efficiency of the sewage treatment.

## REFERENCES

[1] *Biodegradation, toxicity and methods of determination of selected organics* (typescript), Institute of Meteorology and Water Management, Warszawa, 1979.

[2] Pitter P., *Determination of biological degradability of organic substances*, Water Res., 10, 231 (1976).

## BIODEGRADACJA P-NITROFENOLU

Przedstawiono wyniki badań nad biodegradacją p-nitrofenolu (PNP) w rzece. Stwierdzono, że po upływie określonego czasu adaptacji mikroorganizmów szybkość procesu biodegradacji jest znaczna, zaś jego efektywność duża. Stwierdzono ponadto, że obecność PNP w ściekach komunalnych nie ma wpływu na całkowitą wydajność procesu oczyszczania tych ścieków.

## BIOLOGISCHER ABBAU DES P-NITROPHENOLS

Es wurden die Untersuchungsergebnisse von biologischem Abbau des P-Nitrophenols (PNP) in einem Fluss dargestellt. Es wurde festgestellt, daß nach gewisser Mikroorganismenadaptationszeit die Geschwindigkeit des biologischen Abbaus beträchtlich ist und ihre Effektivität groß ist. Außerdem wurde festgestellt, daß die Anwesenheit des PNP in häuslichen Abwässern keinen Einfluß auf die gesamte Leistung des Abwasserreinigungsprozesses hat.

## БИОДЕГРАДАЦИЯ Р-НИТРОФЕНОЛА

Обсуждены результаты исследований по биодеградации р-нитрофенола в реке. Выявлено, что после истечения определённого времени адаптации микроорганизмов скорость процесса биодеградации значительна, а его эффективность высока. Кроме того выявлено, что присутствие р-нитрофенола в коммунальных сточных водах не оказывает влияния на полный выход процесса очистки сточных вод.