

TYMOTEUSZ JAROSZYŃSKI, EDWARD GOMÓLKA*

BIODEGRADABILITY OF BENZOIC ACID IN ACTIVATED SLUDGE PROCESS

A part of results from the investigations on biodegradation of benzoic acid under static conditions and the effect of its concentrations on the treatment of synthetic wastewater with activated sludge under dynamic conditions are described [4].

1. CHARACTERISTICS OF BENZOIC ACID

Benzoic acid (C_6H_5COOH) is the simplest aromatic carboxylic acid. It is a solid crystalline forming colourless glossy silky plates or needles, its melting temperature is 394.5 K [7], its solubility in water increases with the temperature being equal to 2.9 g/dm^3 at 293 K [5]. Small amounts of benzoic acid are present in domestic sewage to which it passes with the urine. High concentrations of this acid are present in wastewater from chemical industry, being e.g. a side product in benzaldehyde production. It appears also in wastewater from dye, textile and pharmaceutical industries [4].

Benzoic acid is strongly toxic for fish and other water organisms. Its bacteriocidal and bacteriostatic activities depend on the pH of the medium. In experiments conducted with fishes *Gambusia affinis* no toxic effects have been observed at concentrations lower than 56 mg/dm^3 . For most fishes, however, a ten times higher concentration appeared to be lethal [6]. Investigations conducted in Soviet Union have shown that the concentration of 200 mg/dm^3 deteriorates the quality of the treated wastewater and that for this compound the oxidation possibility of the digesters amounts to $750\text{--}800 \text{ g/m}^3\text{d}$ [8].

Organic acids are easily mineralized biologically, being intermediate products in biodegradation of a large number of organic compounds, e.g. bacteria: *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas fluorescens* oxidize aromatic compounds through carboxylic acids to pyrokatechol [1], [9].

The mechanism of biological oxidation of benzoic acid is much complicated, consisting of a number of intermediate enzymatic reactions catalysed by appropriate enzymes produced

* Institute of Environment Protection Engineering, Technical University of Wrocław, Wrocław, Poland.

in living bacterial cells. This process is shown in fig. 1 for *Pseudomonas arvilla* mt-2. The metabolism of benzoic acid may run in meta or ortho (β -ketoaldypine) pathway. The first process yields pyruvic acid and acetic aldehyde, the product of the latter being acetylo-coenzyme A and succinylcoenzyme A. These products are the energy sources in cytric acid cycle [2], [9].

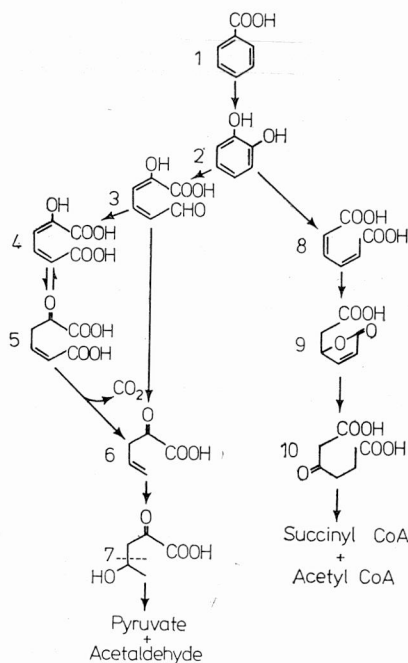


Fig. 1. Metabolism of benzoic acid in presence of *Pseudomonas arvilla* mt-2

1 - benzoic acid; 2 - pyrocatechol; 3 - 2-hydroxymuconic semialdehyde; 4 - oxalocrotonate (enol); 5 - 4-oxalocrotonate (ketol); 6 - 2-oxopent-4-enolate; 7 - 4-hydroxy-2-oxovalerate; 8 - cis, cis-muconate; 9 - (+)-muconolactone; 10 - β -ketoaldypic acid

Rys. 1. Metabolizm kwasu benzoowego przez *Pseudomonas arvilla* mt-2

1 - kwas benzoowy; 2 - pirokatechyna; 3 - aldehyd 2-hydroksy semimukonowy; 4 - kwas 4-oksalkrotonowy (enol); 5 - kwas 4-oksalkrotonowy (ketol); 6 - kwas 2-okso-4-pentenowy; 7 - kwas 4-hydroksy-2-oksowalerianowy; 8 - kwas cis, cis-mukonowy; 9 - (+)-mukonolak ton; 10 - kwas β -ketoaldypinowy

2. EXPERIMENTAL

2.1. PURPOSE AND METHODS OF INVESTIGATIONS

The purpose of the investigations was to examine the biodegradability of benzoic acid in activated sludge process and to determine its susceptibility to chemical oxidation. The laboratory-scale experiments were conducted under static and dynamic conditions. The concentration of benzoic acid in wastewater was examined by extraction with cyclohexane and UV spectrophotographic method using the apparatus Spectromom 202, at the wavelength of 232 nm [4].

Biodegradation of benzoic acid under static conditions was conducted on municipal wastewater from Wrocław and synthetic wastewater, while the experiment under dynamic conditions comprised only a model synthetic wastewater [4].

Static experiments aimed at determining the influence of different concentrations of benzoic acid on mineralization of organic compounds in wastewater of the same composition were conducted in parallel. To this end several respirometric flasks (of a large volume each) were used (fig. 2).

Dynamic investigations were performed in the system consisting of two identical reactors, used to aerate the mixture of wastewater with aerated sludge (fig. 3).

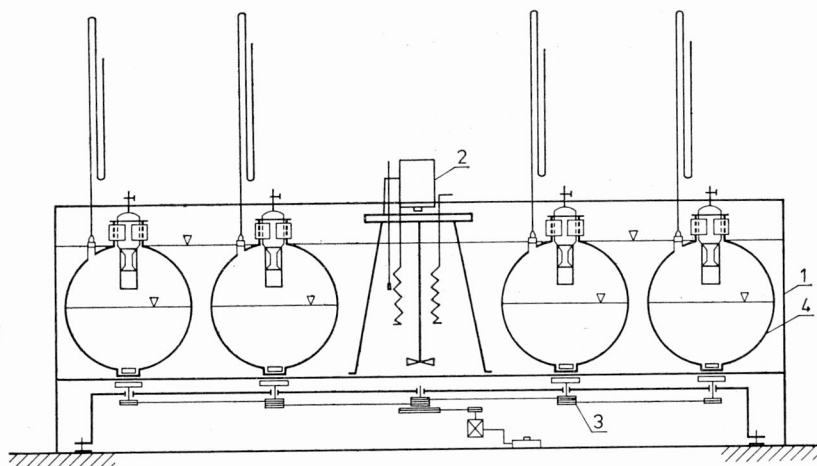


Fig. 2. Respirometer

1 — thermostatic vessel; 2 — thermostat; 3 — magnetic stirrer; 4 — respirometric flask

Rys. 2. Respiometr

1 — naczynie termostaticzne; 2 — termostat; 3 — mieszadło magnetyczne; 4 — kolba respirometryczna

In such a two stage system the following procedure is used: wastewater in tank 9 is conveyed by means of the peristaltic pump 10, to the supply pipe 6, it flows through the pipe and immediately before the jet pump 3 it joins the mixture of wastewater with the wastewater sucked in from the reactor. Thereupon the aerated mixture flows along the central pipe 2 to reach the further part of the cylindrical vessel 1. The flocks of activated sludge deposited there are sucked again into the jet pump, so that the circulation of the activated sludge is closed. In the series arrangement the outlet from one reactor was at the same time the inlet of the second reactor which was additionally supplied with the crude wastewater from the tank 9.

The experiments were conducted at 293 K, using solid benzoic acid added to wastewater. Its content in aerated wastewater was systematically controlled by measuring the amounts of oxygen consumed and carbon dioxide produced. Additionally, the basic

physicochemical parameters of the wastewater were examined, and the biological composition of the activated sludge, produced during the aeration of wastewater, was determined after completion of each experimental series.

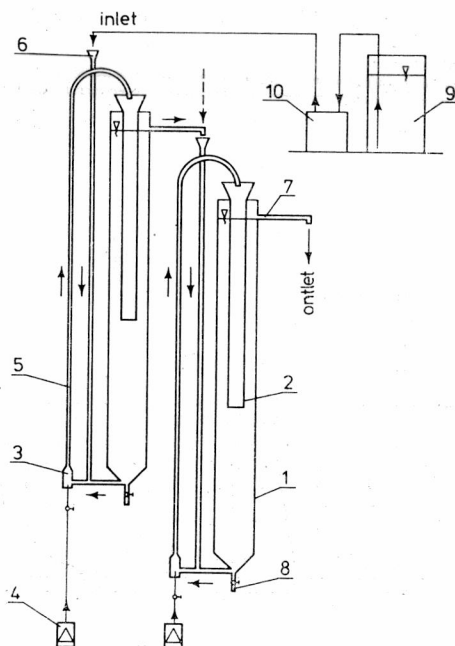


Fig. 3. Scheme of apparatus for two stage wastewater treatment

1 — cylindrical vessel; 2 — central pipe; 3 — jet pump; 4 — compressor of "Skalar" type; 5 — delivery pipe; 6 — supply pump (wastewater inlet); 7 — wastewater outlet; 8 — draining valve; 9 — tank with wastewater; 10 — peristaltic pump, type 315

Rys. 3. Schemat aparatury badawczej do dwustopniowego oczyszczania ścieków

1 — naczynie cylindryczne; 2 — rura środkowa; 3 — strumienica; 4 — sprężarka typu „Skalar”; 5 — rura tłoczna; 6 — rura zasilająca (dopływ ścieków); 7 — odpływ ścieków; 8 — zawór spustowy; 9 — zbiornik ze ściekami; 10 — pompa perystaltyczna, typ 315

2.2. RESULTS

2.2.1. SUSCEPTIBILITY OF BENZOIC ACID TO CHEMICAL OXIDATION AND BIODEGRADATION AT THE DETERMINED VALUES OF COD AND BOD [3]

The data obtained are given in fig. 4. From the investigations performed it follows that for oxygen consumption determined experimentally the oxidation of benzoic acid is weak, and with the increasing concentration its degree drops from 7.6% to 0.2%. On the other hand, at the COD value determined experimentally the oxidation degree reaches 98%.

Biodegradability of benzoic acid is presented in fig. 5. It appears that BOD_5 constitutes about 76% of the theoretical oxygen demand (1.5 g of O_2/g of benzoic acid).

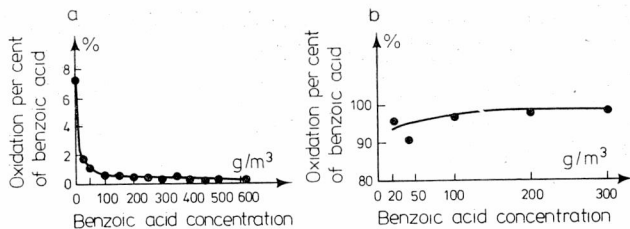


Fig. 4. Oxidation of benzoic acid vs. its concentration for the COD and oxygen consumption values determined experimentally in per cent

a — permanganate COD; b — dichromate COD

Rys. 4. Zależność procentu utleniania kwasu benzoowego od jego stężenia w próbce, w warunkach oznaczania

a — utlenialności; b — ChZT

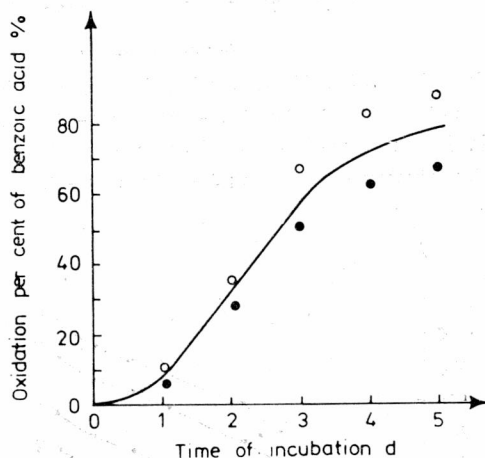


Fig. 5. BOD for benzoic acid

Rys. 5. Przebieg krzywej BZT dla kwasu benzoowego

2.2.2. RESULTS OF RESPIROMETRIC INVESTIGATIONS CONDUCTED UNDER STATIC CONDITIONS

Respirometric investigations under static conditions were performed after the preliminary investigations had been completed. In the first series of experiment initial concentrations of benzoic acid in municipal wastewater ranged from 0 (control) to 100–500 g/m³. The curves representing the removal of benzoic acid from municipal wastewater show (fig. 6) that a complete biodegradation of benzoic acid takes place not later than after only several hours of aeration and is accompanied with a simultaneous degradation of organic pollutants. At the initial concentrations of benzoic acid, amounting to 400 g/m³ and 500 g/m³, the BOD was strongly inhibited during the first 44 h (fig. 7, curves 5 and 6).

Initial concentrations of benzoic acid in the second series were higher, ranging from 500 to 1200 g/m³. The curves illustrating the oxygen demand versus aeration time of

wastewater with different concentrations of benzoic acid (fig. 8) show distinct stagnation and adaptation period (curves 4, 5, 6 for the initial concentrations of 700, 900 and 1200 g/m³, respectively) which was the longer the higher was the concentration of benzoic acid. Similar relationship was also found for concentrations of benzoic acid versus the aeration time of municipal wastewater (fig. 9).

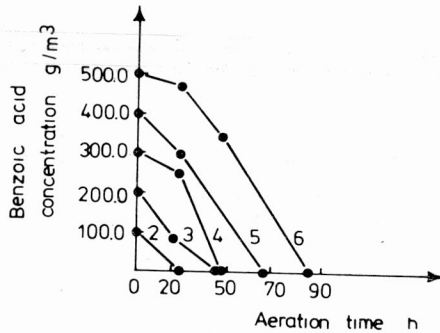


Fig. 6. Concentration of benzoic acid vs. aeration time of municipal wastewater

2 – wastewater at initial concentration of benzoic acid 100 g/m³; 3 – 200 g/m³; 4 – 300 g/m³; 5 – 400 g/m³; 6 – 500 g/m³

Rys. 6. Zależność stężenia kwasu benzooesowego od czasu napowietrzania ścieków miejskich

2 – ścieki o początkowej zawartości kwasu benzooesowego 100 g/m³; 3 – 200 g/m³; 4 – 300 g/m³; 5 – 400 g/m³; 6 – 500 g/m³

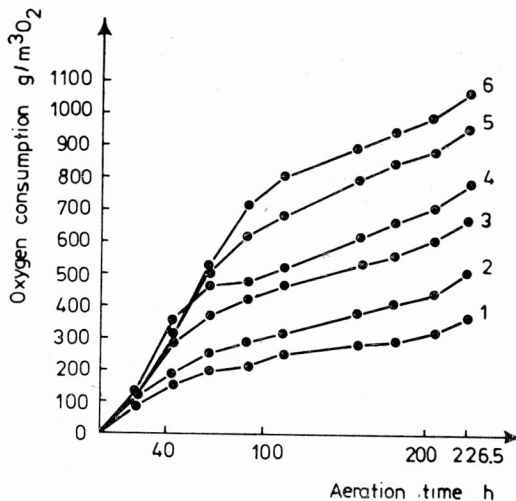


Fig. 7. Oxygen consumption vs. aeration time of wastewater with different initial concentrations of benzoic acid

1 – without benzoic acid; 2 – initial concentration of benzoic acid 100 g/m³; 3 – 200 g/m³; 4 – 300 g/m³; 5 – 400 g/m³; 6 – 500 g/m³

Rys. 7. Zależność zużytego tlenu od czasu napowietrzania ścieków, zawierających różne początkowe stężenia kwasu benzooesowego

1 – ścieki bez kwasu benzooesowego; 2 – ścieki o początkowej zawartości kwasu benzooesowego 100 g/m³; 3 – 200 g/m³; 4 – 300 g/m³; 5 – 400 g/m³; 6 – 500 g/m³

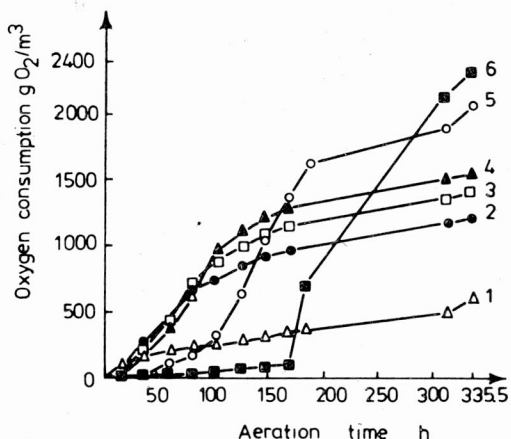


Fig. 8. Oxygen consumption vs. aeration time of wastewaters with different initial concentration of benzoic acid

1 – without benzoic acid; 2 – initial concentration of benzoic acid 500 g/m³; 3 – 600 g/m³; 4 – 700 g/m³; 5 – 900 g/m³; 6 – 1200 g/m³

Rys. 8. Zależność zużytego tlenu od czasu napowietrzania ścieków, zawierających różne początkowe stężenia kwasu benzooesowego

1 – ścieki bez kwasu benzooesowego; 2 – ścieki o początkowej zawartości kwasu benzooesowego 500 g/m³; 3 – 600 g/m³; 4 – 700 g/m³; 5 – 900 g/m³; 6 – 1200 g/m³

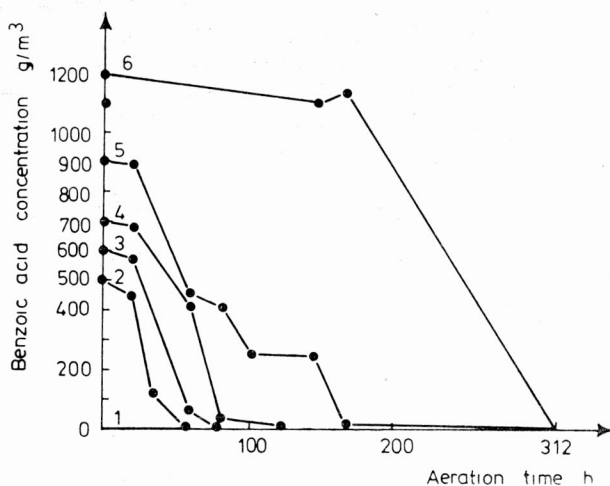


Fig. 9 Concentration of benzoic acid vs. aeration time of municipal wastewater

1 – without benzoic acid; 2 – with initial concentration of benzoic acid 500 g/m³; 3 – 600 g/m³; 4 – 700 g/m³; 5 – 900 g/m³; 6 – 1200 g/m³

Rys. 9. Zależność stężenia kwasu benzooesowego od czasu napowietrzania ścieków miejskich

1 – ścieki bez kwasu benzooesowego; 2 – ścieki o początkowej zawartości kwasu benzooesowego 500 g/m³; 3 – 600 g/m³; 4 – 700 g/m³; 5 – 900 g/m³; 6 – 1200 g/m³

From the results obtained it follows that the natural alkalinity of crude wastewater does not suffice to neutralize 900 g/m³ and higher concentrations of benzoic acid. This is manifested in the decrease of the pH value (the values of pH in experiments 5 and 6 being 4.4 and 4.1, respectively) and in the prolongation of the lag time (from 35 h at 900 g/m³ to 168 h at 1200 g/m³). That is why in the third series of experiment benzoic acid was neutralized with KHCO₃ bringing about a considerably reduction of its bacteriostatic effect. Consequently, the shape of BOD curves was changed (see fig. 10) and for the initial concentrations of benzoic acid equal to 1200 g/m³ the lag and adaptation period was shortened, being however still noticed at higher concentrations (fig. 11). The last series

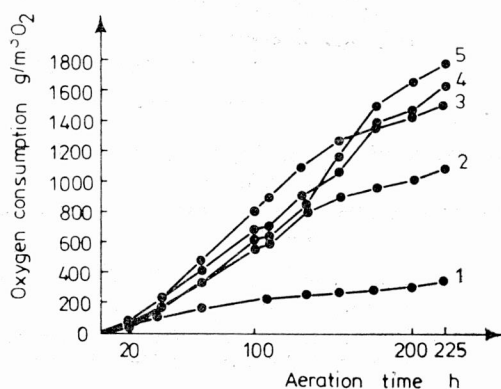


Fig. 10. Oxygen consumption vs. aeration time of wastewaters with different concentration of benzoic acid and potassium bicarbonate

1 — without benzoic acid and potassium bicarbonate; 2 — initial concentration of benzoic acid and potassium bicarbonate — 600 and 492 g/m³, respectively; 3 — 800 and 656 g/m³; 4 — 100 and 820 g/m³; 5 — 1200 and 984 g/m³

Rys. 10. Zależność zużytego tlenu od czasu napowietrzania ścieków, zawierających różne początkowe stężenia kwasu benzoowego i wodorowęglanu potasowego

1 — ścieki bez kwasu benzoowego i wodorowęglanu potasowego; 2 — ścieki o początkowej zawartości kwasu benzoowego 600 g/m³ i wodorowęglanu potasowego 492 g/m³; 3 — 800 i 656 g/m³; 4 — 100 i 820 g/m³; 5 — 1200 i 984 g/m³

of experiments comprised the BOD tests of the activated sludge adapted to 800 g/m³ concentration of benzoic acid, performed in medium containing only mineral salts with benzoic acid as the only source of carbon. The latter was neutralized only with KHCO₃. Initial concentrations of benzoic acid ranged in this series from 600 to 800 g/m³. From the results obtained it follows that under optimal conditions of the process (supply of biogenic compounds, alkalinity and pH) the biodegradation rate of benzoic acid is constant for its different concentrations. The loss of benzoic acid indicates also a high rate of mineralization process.

2.2.3. RESULTS OF INVESTIGATIONS CONDUCTED UNDER DYNAMIC CONDITIONS

Throughout the experiment the inflow intensity, aeration time and hydraulic loading of the both chambers were kept constant. In the first reactor the inflow intensity amounted to 0.1 dm³/h, aeration time was close to 11 h and hydraulic loading of the chamber equaled

2.2 m³/d. In the second reactor the values of the corresponding parameters amounted to 0.2 dm³/h, circa 6 h and 4.12 m³/m³d, respectively. In the initial period of experiment the activated sludge was adapted to benzoic acid by addition of the latter to synthetic wastewater: 25 g/m³ — for the first 3 days and 30 g/m³ for the next 3 days. The final concentration of benzoic acid in adaptation period equaled 100 g/m³. Thereupon the following doses of benzoic acid were added consecutively: 150 g/m³ (4 days), 200 g/m³ (7 days), 300 g/m³ (9 days), 500 g/m³ (10 days), 600 g/m³ (20 days) and finally 800 g/m³.

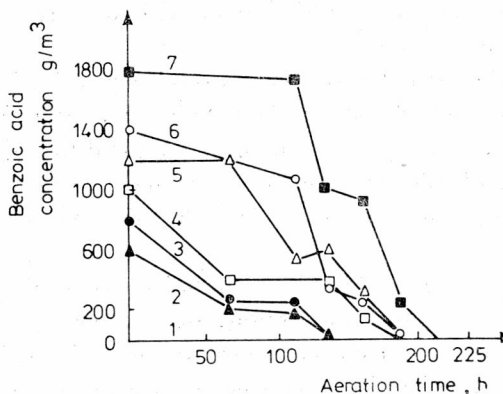


Fig. 11. Concentration of benzoic acid vs. time of municipal wastewaters

1 — without benzoic acid and without potassium bicarbonate; 2 — with initial concentration of benzoic acid and potassium bicarbonate 600 and 492 g/m³, respectively; 3 — 800 and 656 g/m³; 4 — 1000 and 820 g/m³; 5 — 1200 and 984 g/m³; 6 — 1400 and 1148 g/m³; 7 — 1800 and 1476 g/m³

Rys. 11. Zależność stężenia kwasu benzoowego od czasu napowietrzania ścieków miejskich

1 — ścieki bez kwasu benzoowego i wodorowęglanu potasowego; 2 — ścieki o początkowej zawartości kwasu benzoowego 600 g/m³ i wodorowęglanu potasowego 492 g/m³; 3 — 800 i 656 g/m³; 4 — 1000 i 820 g/m³; 5 — 1200 i 984 g/m³; 6 — 1400 i 1148 g/m³; 7 — 1800 i 1476 g/m³

3. CONCLUSIONS

For the experiment conducted under dynamic conditions the following conclusions are drawn:

1. Benzoic acid is the compound to which activated sludge can be adapted.
2. Within the examined range of benzoic acid concentrations in crude wastewater (i.e. up to 800 g/m³) the adapted activated sludge is not sensitive to rapid changes in concentrations of this acid.
3. In high concentrations of benzoic acid activated sludge has a high sedimentation index, ranging from 118 to 280 cm³/g. It was nevertheless characterized by a large area of flocules which eventually influenced a distinct separation of the deposit from the liquid.
4. The loading of activated sludge with BOD₅ ranging from 0.3 to 1.5 g/g dm d the decrease of benzoic acid BOD₅, dichromate and permanganate COD amounted to 100%, 98%, 80% and 90%, respectively. For the same pollution indices the same effects have

been obtained for the loading of digester with BOD_5 from 700 to 3200 $g O_2/m^3 d$ and for the operation of the activated sludge within the range of 1700 to 25000 gh/m^3 .

5. From the investigations performed it follows that the oxidizing potential of digester for benzoic acid reaches 1760 $g/m^3 d$.

Respirometric experiments conducted in static conditions allow to formulate the following conclusions:

1. Benzoic acid is biodegradable under aerobic conditions and can be removed from the wastewater by aerobic biological method.

2. Up to the concentration of 200 g/m^3 the acid is quickly biodegraded by bacteria present in crude municipal wastewater.

3. A lag and adaptation period in BOD process takes place at the initial concentrations of benzoic acid ranging within 900–1200 g/m^3 . After adaptation the biodegradation rate of benzoic acid increases, reaches natural its maximum and then decreases.

4. Because of sufficiently high alkalinity of the municipal wastewater the neutralization of benzoic acid initial concentrations up to 700 g/m^3 does not accelerate the process of its biodegradation.

5. The neutralization of benzoic acid at initial concentrations of 1000–1800 g/m^3 in crude wastewater does not stop the prolongation of the lag and adaptation period.

6. Biodegradation of benzoic acid in concentrations up to 1800 g/m^3 is however possible under optimal conditions of the process and when the activate sludge is adapted to benzoic acid.

7. Benzoic acid in concentration of 500 g/m^3 inhibits the process of nitrification.

8. Colour compounds formed during biodegradation of benzoic acid give the wastewater a specific yellowishgreen colour, the intensity of which depends on the amount of benzoic acid being biodegraded and on the aeration time of wastewater. Due to various biochemical reactions of these compounds taking place during aeration the specific colour disappears.

REFERENCES

- CHAKRABARTY A. M., *Genetic basis of the biodegradation of salicylate in Pseudomonas*, Journal of Bacteriology, Vol. 112 (1972), p. 815.
- [2] FEIST C. F., HEGEMAN G. D., *Phenol and benzoate metabolism by Pseudomonas putida: regulation of tangential pathways*, Journal of Bacteriology, Vol. 100 (1969), pp. 869–877.
- [3] HERMANOWICZ W., DOZAŃSKA W., DOJLIDO J., *Fizyczno-chemiczne badanie wody i ścieków*, Arkady, Warszawa 1976.
- [4] JAROSZYŃSKI T., *Wpływ kwasu benzoowego na przebieg procesu BZT i oczyszczanie ścieków osadem czynnym* (unpublished), Typescript in Library of Institute of Environment Protection Engineering, Technical University of Wrocław, Wrocław 1977.
- [5] KIRK OTHMER, *Encyclopedia of Chemical Technology*, Second completely revised edition, 1964.
- [6] MEINCK F., STOOFF H., KOHLSCHÜTTER H., *Ścieki przemysłowe*, Arkady, Warszawa 1975.
- [7] *Poradnik fizykochemiczny*, WNT, Warszawa 1974.
- [8] STASIAK M., *Wpływ substancji mineralnych i organicznych na procesy biologiczne oczyszczania ścieków*, Wodociągi i kanalizacja, Nowa Technika w Inżynierii Sanitarnej, No. 5, Arkady, Warszawa 1975.

- [9] WILLIAMS P. A., MURRAY K., *Metabolism of benzoate and the methylbenzoates by Pseudomonas putida arvilla mt-2: evidence for the existence of a TOL plasmid*, Journal of Bacteriology, Vol. 120 (1974), pp. 416-423.

PODATNOŚĆ KWASU BENZOESOWEGO NA BIOCHEMICZNY ROZKŁAD W PROCESIE OSADU CZYNNEGO

Przedstawiono wyniki badań nad chemicznym utlenianiem i biochemicznym rozkładem kwasu benzoowego. Wykazano, że kwas benzoowy jest oporny na chemiczne utlenianie za pomocą KMnO_4 , natomiast w 98% jest utleniany w warunkach oznaczania ChZT. W warunkach oznaczania BZT₅ metodą rozcieńczeń rozkład kwasu benzoowego wynosi około 76% teoretycznego zapotrzebowania tlenu. Badania nad biochemicznym rozkładem kwasu benzoowego w warunkach statycznych (w respirometrach) w środowisku ścieków miejskich i wodnej pożywki mineralnej wykazały, że neutralizacja kwasu benzoowego przy jego początkowych stężeniach do 700 g/m^3 nie wpływa na przyspieszenie czasu jego rozkładu. Przy zaadaptowanym osadzie czynnym i optymalnych warunkach prowadzenia procesu kwas benzoowy do stężenia 1800 g/m^3 może być usuwany ze ścieków. Zawartość kwasu benzoowego powyżej 500 g/m^3 hamuje proces nitrifikacji. Badania w warunkach dynamicznych wykazały, że kwas benzoowy może być praktycznie w całości usunięty ze ścieków przez zaadaptowany osad czynny przy stężeniach do 800 g/m^3 .

DIE ABBAUBARKEIT DER BENZOESÄURE IM BELEBTSCHLAMMVERFAHREN

Der vorliegende Beitrag berichtet über die chemische Oxydation und den biochemischen Abbau der Benzoessäure. Diese Säure ist gegenüber dem Kaliumpermanganat beständig, sie wird jedoch durch das Kaliumdichromat im Verfahren der CSB-Bestimmung zu 98% chemisch oxydiert. Im BSB₅-Verdünnungsverfahren, beträgt der Abbau der Benzoessäure etwa 76% des theoretischen Sauerstoffbedarfs.

Versuche im Batch-Prozeß (in Respirometern) sowohl in Vermischung mit kommunalem Abwasser wie mit einer mineralen Nährstofflösung haben erwiesen, daß die Neutralisation der Benzoessäure bei Startkonzentrationen bis zu $700 \text{ g C}_6\text{H}_5\text{COOH/m}^3$ praktisch keinen Einfluß auf die Abbaubeschleunigung ausübt. Wird Belebtschlamm für den Abbau dieses spezifischen Substrats adaptiert, kann er Konzentrationen bis zu 1800 g/m^3 gut vertragen. Konzentrationen von mehr als $500 \text{ g C}_6\text{H}_5\text{COOH/m}^3$ hemmen jedoch die Nitrifikation.

Im Durchflußverfahren, wird die Benzoessäure durch adaptierten Belebtschlamm voll abgebaut, wenn die Konzentration von 800 g/m^3 nicht überschritten wird.

СКЛОННОСТЬ БЕНЗОЙНОЙ КИСЛОТЫ К БИОХИМИЧЕСКОМУ РАЗЛОЖЕНИЮ В ПРОЦЕССЕ АКТИВНОГО ИЛА

В статье приведены результаты исследований по химическому окислению и биохимическому разложению бензойной кислоты. Доказано, что бензойная кислота устойчива к химическому окислению с помощью KMnO_4 , но в 98% окисляется в условиях определения ХПК. В условиях определения БПК₅ методом разбавлений, разложение бензойной кислоты составляет около 76% теоретической потребности в кислороде. Исследования по биологическому разложению бензойной кислоты в статических условиях (в respirometрах) в среде городских сточных вод и водной

минеральной питательной среды показали, что нейтрализация бензойной кислоты при его начальных концентрациях до 700 г/м^3 не влияет на ускорение её разложения. При адаптированном активном иле и в оптимальных условиях проведения процесса, бензойная кислота до концентрации 1800 г/м^3 может удаляться из сточных вод. Содержание бензойной кислоты выше 500 г/м^3 тормозит процесс нитрификации.

Исследования в динамических условиях показали, что бензойная кислота практически может быть полностью удалена из сточных вод адаптированным активным илом, при концентрациях до 800 г/м^3 .