

BIOLOGICAL DENITRIFICATION IN THE PRESENCE OF CYANIDE

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Abstract—The influence of cyanide presence on the denitrification process using methanol, propanol, sodium acetate, glucose and acetone as the energy and carbon sources were described. It was shown that during denitrification the carbonyl compounds used as the sole energy and carbon sources for denitrifiers can form cyanohydrins with the cyanide ion. The product of cyanohydrin hydrolysis is biodegradable serving as electron donors for denitrifying bacteria. The simultaneous denitrification and cyanide removal was proved.

Key words—wastewater treatment, denitrification, cyanide, cyanohydrin formation, hydrolysis

INTRODUCTION

Cyanide ions adversely affect the biological processes by inactivating heavy metal catalysts and forming very stable complexes with the metal. Since the cytochrome oxidase system involves catalysis by iron and copper, cyanide is often referred to as the respiratory poisons. However, cyanide is a very unspecific inhibitor, inhibiting a large number of enzymes which do not involve metal catalysis.

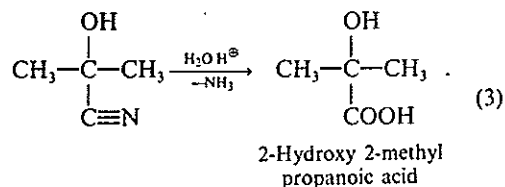
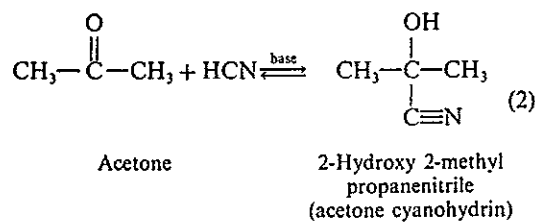
The biological wastewater treatment processes deteriorates as a result of cyanide presence in wastewater which are to be treated is well known. Gurnham (1955) stated the influence of $2 \text{ mg}^{-1} \text{ CN}^-$ on the unacclimated trickling filters and that there was a possibility of acclimating the system up to $200 \text{ mg}^{-1} \text{ CN}^-$. According to Ludzak and Schafer (1960) the activated sludge system could tolerate $50 \text{ mg}^{-1} \text{ CN}^-$ when fed with sewage.

The tolerance limits of bacterial systems due to the presence of inhibitors depend on many factors. In this work the effect of the kind of substrate oxidized during the denitrification process has been investigated. The process used Packed Bed Reactors (PBR) units on synthetic wastewater in anoxic conditions, using methanol, propanol, acetone, sodium acetate or glucose as the energy and carbon sources and nitrate as the terminal electron acceptor. General discussion of denitrification has been published in many papers. This work will limit discussion to the impact of the cyanide presence on the course of the process and the possible reactions among the compounds used as the electron donors in the process and the cyanide.

It is well known in organic chemistry that hydrogen cyanide added to the carbonyl compounds forms, under certain circumstances hydroxynitriles, usually called "cyanohydrins" (Roberts and Caserio, 1979). Cyanohydrin synthesis can be presented according to the following scheme:



Glucose and acetone under certain conditions can form cyanohydrins. Glucose is an aldohexose, which means that it is a six-carbon sugar with a terminal aldehyde group. Cyanide reacts with the open chain forms of aldoses to form cyanohydrins which then hydrolyse to the corresponding aldonic acid and ammonium ion. In the case of acetone the cyanohydrin formation is followed by hydrolyse to the corresponding hydroxy acid and ammonium ion. Formation and hydrolyse of acetone cyanohydrin could be shown as follows:



Cyanohydrin formation requires a basic catalyst. It is an equilibrium reaction. In the absence of catalyst the equilibration is very slow. Effective catalysts are substances with an alkaline reaction, such as potassium cyanide for example. For cyanohydrin synthesis the carbonyl compound is treated with an alkali cyanide and a strong acid. The literature descriptions of cyanohydrin synthesis strongly underlines the presence of an acid in the reaction vessel because of hydrogen cyanide formation (Vogel, 1964). That is why the work by Raef *et al.* (1977) is somewhat

confusing as the greatest velocity of the cyanide-glucose reaction in sealed ampoules was obtained by using a pH value equal to 11.

In this work it was attempted to show the process of biological denitrification in the presence of cyanide in the common pH value range. From the five compounds used in the investigations as the exogenous carbon and energy sources, two of them—glucose and acetone—could form cyanohydrins as well as serving as the electron donors for denitrifying bacteria. The investigations should show if the cyanohydrin formation and hydrolyse are possible during the biological process course and hence the possibility of simultaneous nitrate and cyanide removal.

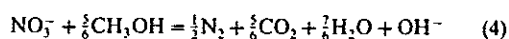
EXPERIMENTAL PROCEDURE

The laboratory-scale submerged filters consisted of a plastic column 45 mm i.d. and 0.8 m high. Columns were placed in thermostatic bath and the temperature of 25°C was maintained during the whole experimental period. Reactor packing consisted of ceramic Raschig rings 15 mm long, 16 mm o.d. and 10 mm i.d. The PBR were filled with the packing material up to a height of 0.5 m. Total surface of the packing was 0.27 m². The porosity of the reactors were 0.625, total volume 0.8 l. and liquid volumes 0.5 l. Detention time, based on the liquid volumes was 1 h. The wastes to be treated were supplied to the columns with the use of peristaltic pumps and entered at the bottom part. The effluent ports were placed at the top parts of the reactors. The packing material was submerged during the exploitation.

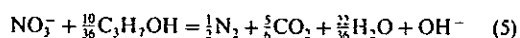
The synthetic waste was prepared and stored in plastic containers, feed solutions were changed twice a week. The nitrate concentration in the influent was adjusted to 20 mg l⁻¹ NO₃-N (as KNO₃) and phosphate to 2 mg l⁻¹ PO₄-P (as Na₂HPO₄). As microelements, the following compounds were added in the following concentrations: KBr—0.0625 mg l⁻¹; H₃BO₃—0.625 mg l⁻¹; KI—0.0625 mg l⁻¹; ZnSO₄—0.0250 mg l⁻¹. The organic compounds: methanol CH₃OH, propanol C₃H₇OH, sodium acetate CH₃COONa, acetone (CH₃)₂CO and glucose C₆H₁₂O₆ were added to the individual containers in the concentrations stated in Figs 1–5 which of about 100% greater from stoichiometric requirements according to equations (4)–(8) given below. The cyanide concentration CN⁻ (added as KCN) increased during the investigation period according to experimental programme from 1 mg l⁻¹ CN⁻ at the very beginning of the process up to the constant deterioration of the obtained results in terms of denitrification efficiency. The feed solutions were deoxygenated by means of sodium sulphite (Na₂SO₃) by stoichiometric addition according to the dissolved oxygen concentration. All the reagents used were laboratory grade.

The stoichiometric quantities of the compounds used as electron donors were obtained according to the following equations:

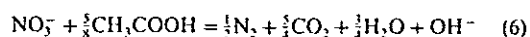
For methanol:



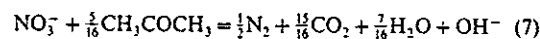
For propanol:



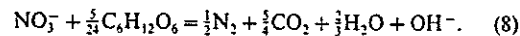
For acetic acid:



For acetone:



For glucose:



The reactor seeding was accomplished by means of acclimated activated sludge to the anoxic conditions and to the other parameters of the experiment's environmental conditions. The activated sludge taken from The Municipal Treatment Plant was held under anoxic conditions during 24 h. To the sludge, 100 mg l⁻¹ NO₃-N, 2 mg l⁻¹ PO₄-P, the appropriate amount of the organic carbon compound and 1 mg l⁻¹ CN⁻ was added. The procedure was made for five used carbon compounds. After 24 h the inoculum was poured into the individual filters and the experimental programme was started. Synthetic wastes of concentration 1 mg l⁻¹ CN⁻ were pumped through the filters by means of peristaltic pumps. Samples were collected twice a week. After obtaining good results, the nitrate conversion to nitrogen gas was about 90% and the cyanide ion concentration in influent was increased to the next level and so on up to the constant deterioration of obtained results.

Measurements for pH were made by means of pH meter, for ammonium by means of an ion selective electrode. The nitrate, nitrite and cyanide ions concentration were measured by following the procedures outlined in *Standard Methods for Examination of Water and Wastewater* (APHA, 1965). Organic carbon concentration was analysed by means of Beckman's Organic Carbon Analyzer. Methanol concentration was measured by the procedure outlined in *Standard Methods of Chemical Analysis* (Welcher, 1963). Acetate was measured after sample acidification as acetic acid according to the procedure described by Hermanowicz *et al.* (1976). In the case of methanol and acetate the organic carbon concentration in the sample was calculated from the measured compound concentration. In all the other cases the organic carbon concentration was measured indirectly. All the colorimetric determinations were made by means of spectrophotometer u.v.-VIS Perkin-Elmer-Hitachi 2000.

RESULTS

The results of the denitrification process using methanol as the sole carbon and energy source is presented in Fig. 1. The submerged filter was exploited during 140 days. No adaptation to the cyanide presence was observed during this period. At the very beginning cyanide ion concentration in influent was maintained at 1 mg l⁻¹ CN⁻, but the results obtained were very poor. Denitrification efficiency was at the level of about 40% and did not show any tendency to improve. After decreasing the cyanide concentration in influent to 0.1 mg l⁻¹ CN⁻, a sudden improvement of the process efficiency was observed, up to almost 100% denitrification. The next increase of cyanide concentration (0.5 mg l⁻¹ CN⁻) did not show any effect on the process course but a further increase of up to 1 mg l⁻¹ CN⁻ strongly affected the process course and deteriorated the results. Raising the CN⁻ concentration up to 2 mg l⁻¹ resulted in a decrease of the denitrification efficiency to 40%. The pH value in influent during the whole investigation period was in the range of 7.6–7.8 and in the effluent in the range of 7.8–8.1, depending on the denitrification efficiency. The cyanide concen-

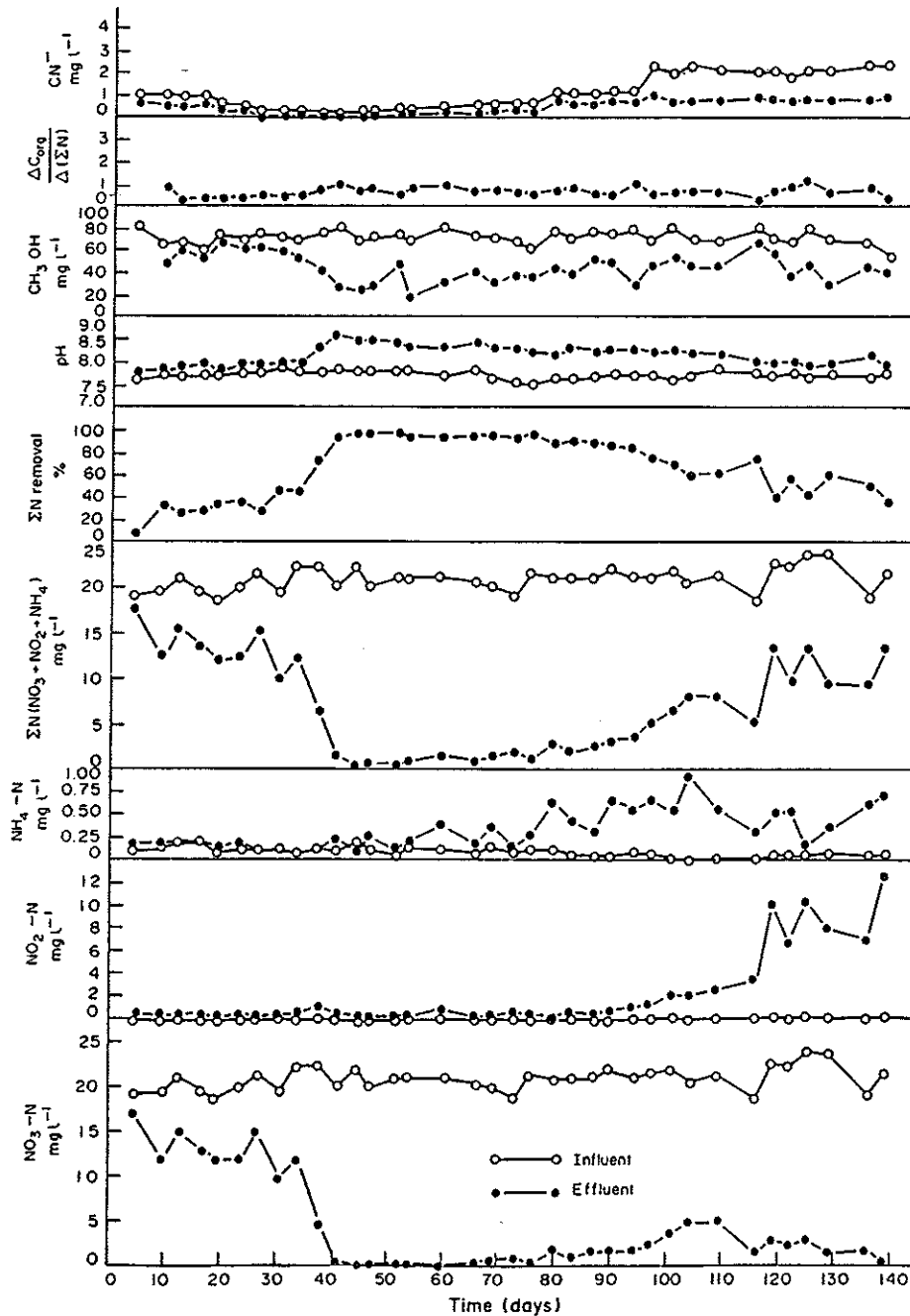


Fig. 1. The exploitation course of the PBR using methanol as the electron donor.

tration in effluent was constantly detected smaller than in influent which could be a result of cyanide stripping and complexing with the metabolites.

The results of denitrification using propanol as the carbon and energy source was presented in Fig. 2. The filter was exploited during 105 days. No adaptation to the cyanide presence was observed during this period. The cyanide concentration during the start-up procedure was maintained at the level of $1 \text{ mg l}^{-1} \text{ CN}^-$. The rate of nitrate removal was slowly

raised to almost 95% after 2 months of exploitation. The raising of the cyanide concentration up to $5 \text{ mg l}^{-1} \text{ CN}^-$ affected the process course and slowly deteriorated the obtained results. By the end of investigation when the cyanide concentration in influent was $5 \text{ mg l}^{-1} \text{ CN}^-$ the denitrification efficiency dropped to 65%. The pH value in influent during the whole investigation period was in the range 7.4–7.6 and in effluent 7.8–8.1 depending on the denitrification efficiency. The cyanide concentration

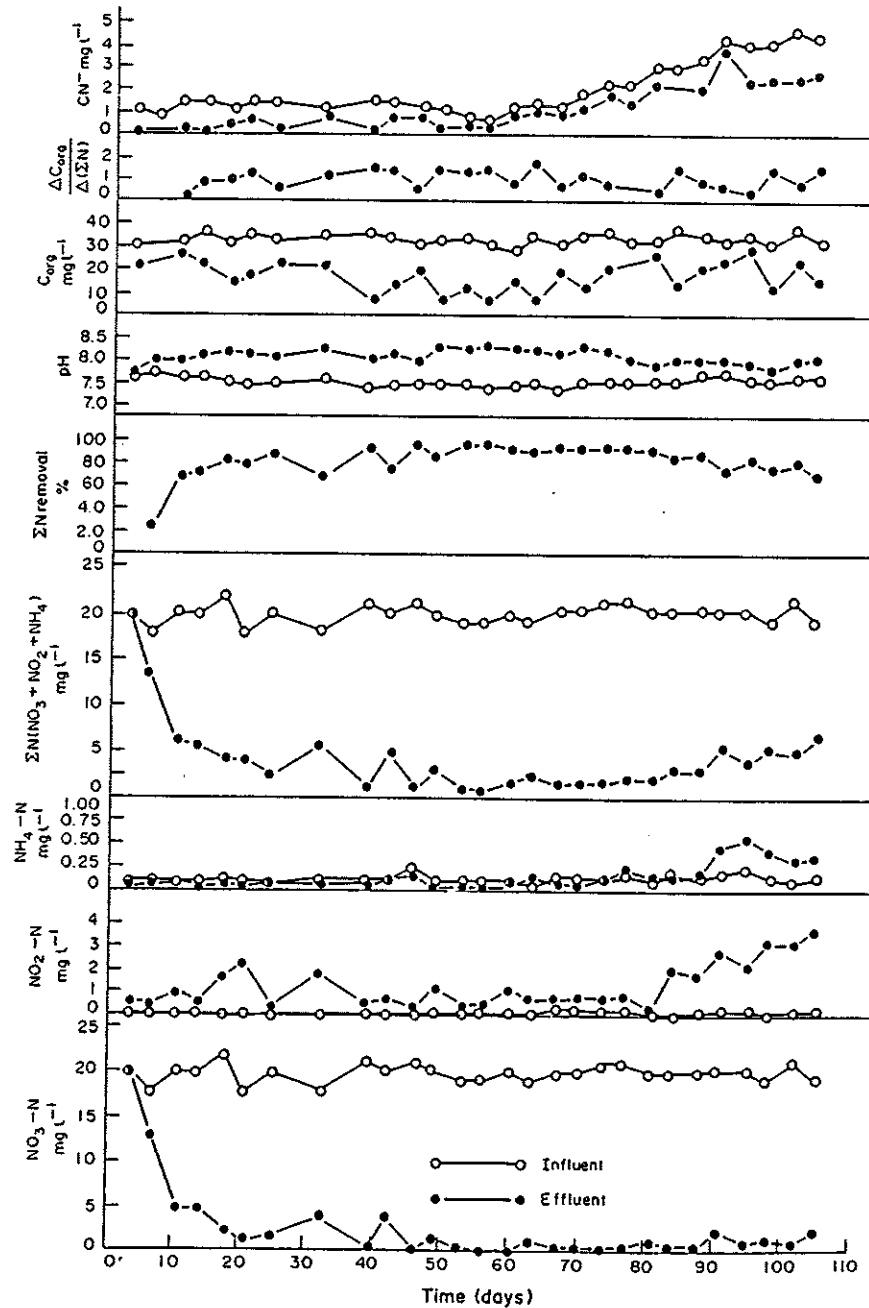


Fig. 2. The exploitation course of the PBR using propanol as the electron donor.

in effluent was, probably from the same reasons as in the case of methanol, constantly detected smaller than in influent.

The results of the process using acetate as an electron donor is presented in Fig. 3. The submerged filter was exploited during 175 days. The cyanide concentration was raised in this period from $1 \text{ mg l}^{-1} \text{ CN}^{-}$ at the very beginning of the process to $7 \text{ mg l}^{-1} \text{ CN}^{-}$ by the end.

The results in terms of denitrification efficiency deteriorated constantly, down to 75% of nitrate nitrogen removal and did not show any tendency to

improve. During the whole investigation time the nitrite nitrogen was detected in the feed container which proves that the denitrification process had started before the wastewater was introduced into the filter. The pH value in influent during the whole investigation period was in the range 7.5–7.8 in effluent 8.5–9.1 depending on the process efficiency.

The results of the process using glucose as an energy and carbon source is presented in Fig. 4. The submerged filter was exploited during 85 days. In this time the cyanide concentration was slowly raised from 1 mg l^{-1} up to $50 \text{ mg l}^{-1} \text{ CN}^{-}$. After the short

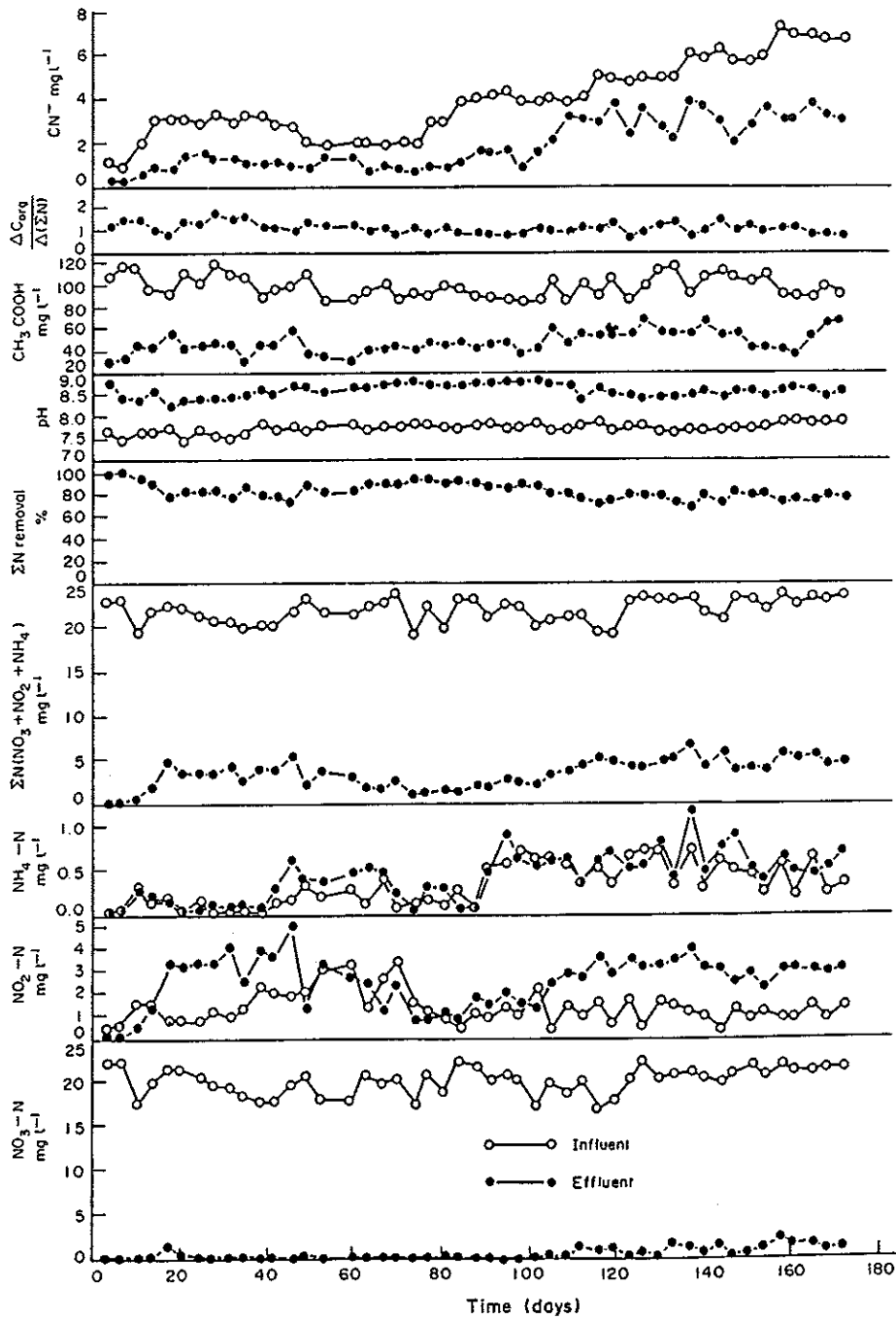


Fig. 3. The exploitation course of the PBR using sodium acetate as the electron donor.

start-up procedure the nitrate conversion to nitrogen gas was raised to almost 100%, measured as the difference between the nitrate concentration in the influent and the sum of nitrate and nitrite in the effluent. Also, an increase of ammonia nitrogen concentration in effluent was observed and cyanide disappeared from the effluent. When the cyanide concentration in influent was equal to 27.5 mg l⁻¹ CN⁻ ammonia nitrogen in effluent was raised to 15 mg l⁻¹ NH₄-N. The cyanide concentration CN⁻

could be expressed as "cyanide nitrogen" CN-N per analoga to "ammonia nitrogen" NH₄-N. In this way 27.5 mg l⁻¹ CN⁻ in influent corresponded with 14.8 mg l⁻¹ CN-N. Therefore it could be stated that all of cyanide nitrogen 14.8 mg l⁻¹ CN-N in influent was converted to 15.0 mg l⁻¹ of ammonia nitrogen NH₄-N detected in effluent. The difference, 0.2 mg l⁻¹ NH₄-N, is almost equal to the ammonia nitrogen concentration in influent which passed through the filter. Because of conversion of cyanide

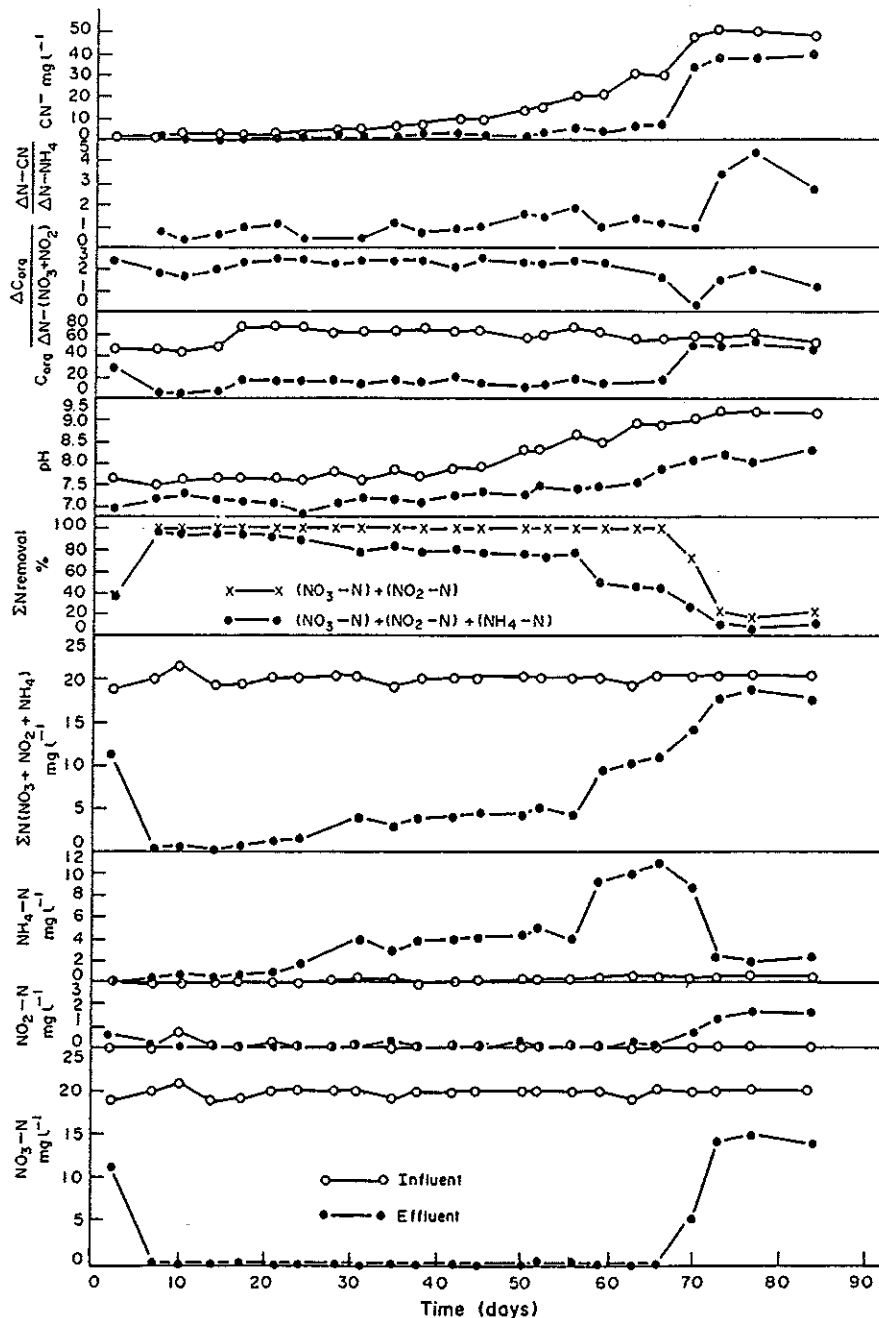


Fig. 4. The exploitation course of the PBR using glucose as the electron donor.

nitrogen to ammonia nitrogen the total rate of nitrogen removal expressed as the sum of ammonia, nitrate and nitrite nitrogen is erroneous. In Fig. 4, two curves presented the efficiency of the nitrogen removal. The first one is the difference between the sum of nitrite and nitrate in influent and effluent, representing in fact the denitrification efficiency, and the second one presented the difference between the sum of nitrate, nitrite and ammonia nitrogen. As a result of the denitrification process a decrease in the pH value was observed. The experimental data did not fit to the stoichiometric equation (8) according to

which OH^- ions are released during the process course and pH value should be increased. The difference between the expected and the obtained results could be explained. The glucose concentration in influent was about 100% greater than the amount required for denitrification, according to the stoichiometric equation. The denitrification process in the presence of glucose was performed in a time shorter than the residence time in the PBR. The absence of an exogenous terminal oxidant forced the bacteria to utilize organic compounds formed within the cells as the terminal electron acceptor. During the glucose

fermentation process a considerable amount of formic acid, acetic acid, lactic acid, succinic acid and other organic acids are produced (Busch, 1971) which resulted in a decrease in the pH value. The ratio of organic carbon removed to the sum of nitrate and nitrite removed was about 2.5 during the exploitation period. The same ratio in the case of the other described filters was about 1.0. This supported the thesis that part of glucose was used for denitrification purposes.

The denitrification process runs with the efficiency close to 100% up to a concentration of $30 \text{ mg l}^{-1} \text{ CN}^{-}$ in influent. When the concentration of cyanide in the influent was raised to over $30 \text{ mg l}^{-1} \text{ CN}^{-}$ the denitrification efficiency suddenly decreased to 20% when the cyanide concentration attained $50 \text{ mg l}^{-1} \text{ CN}^{-}$. Accordingly to the increase of cyanide concentration in influent the pH value increased respectively as the potassium cyanide consisted of a salt with a strong basis. The feeding solution was not buffered during the process and it is possible that the sudden decrease in denitrification efficiency was a result of increasing the pH value of the influent to over 9. According to equation (1) the cyanohydrin formation is the reaction between the carbonyl compound and the hydrogen cyanide. Cyanide and hydrogen cyanide in water find themselves in equilibrium strongly affected by pH. The increase in pH value resulted in a decrease in the hydrogen cyanide concentration. Lack of hydrogen cyanide caused the decrease in cyanohydrin formation velocity and an increase in cyanide concentration. The cyanide inhibits the biochemical reaction which effected in the break-down of the denitrification. The ratio of cyanide nitrogen removed to ammonium nitrogen formed was constantly about 1 when the process was stable.

The results of the denitrification process using acetone as an energy and carbon source is presented in Fig. 5. The submerged filter was exploited during 165 days. During the first 30 days of exploitation the cyanide concentration in influent increased from 1 to $2 \text{ mg l}^{-1} \text{ CN}^{-}$ which resulted in a decrease of denitrification efficiency to 60%.

No adaptation of the bacteria system to the cyanide presence was observed during this time. No increase in ammonium concentration of the effluent was observed which proved that cyanohydrin formation inside the filter did not occur. The explanation could be that there was a lack of hydrogen cyanide in the filter because of too high pH value of the feeding solution ($\text{pH} = 7.6$). The pH value of influent was, from the 30th day, artificially decreased by means of an addition of sulfuric acid which fixed the level to pH 6.5. The decrease of the pH value resulted in a sudden improvement of the obtained results. The denitrification efficiency attained 100% in 40 days of exploitation and fixed on this level during the rest of investigation period. The cyanide concentration in influent was slowly raised to

$30 \text{ mg l}^{-1} \text{ CN}^{-}$. Cyanide concentration in effluent dropped down below 1 mg l^{-1} independently from the cyanide concentration in influent. The ratios of cyanide nitrogen removed to ammonia nitrogen formed was about 1 during the whole investigation period.

DISCUSSION

The results of the cyanide presence on the denitrification process course was observed using five different carbon compounds as the sole exogeneous carbon and energy sources for denitrifiers metabolism. The results pointed at the two possible mechanisms of cyanide influence. The first one observed in the case of denitrification using methanol, propanol and acetate as the electron donors was the simple inhibition of the reaction course. No adaptation of denitrifiers to the cyanide presence was observed. A concentration of about $5 \text{ mg l}^{-1} \text{ CN}^{-}$ affected the process course and deteriorated the denitrification efficiency. In the case of denitrification using glucose and acetone as the electron donors the reaction between those compounds and cyanide strongly influenced the obtained results. Both glucose and acetone were able to form cyanohydrin under the conditions of the investigation. The products of cyanohydrin hydrolysis are ammonium and the corresponding hydroxy acid. As the hydroxy acids are biodegradable the intermediary products of reaction are difficult to detect. Anyhow the second product of hydrolyse i.e. ammonium proved that the process occurred. The cyanide disappearance in effluent was connected with the increase of ammonia nitrogen concentration. The ratios of cyanide nitrogen CN-N removed to the ammonia nitrogen $\text{NH}_4\text{-N}$ formed were presented in Figs 4 and 5. Both glucose and acetone, when the process was stable the calculated ratios were close to 1, proved that all of the cyanide nitrogen was converted to ammonia nitrogen.

During the work an important problem was revealed—the influence of the pH value of the cyanohydrin formation and hydrolyse. In the case of glucose the filter filling material was seeded in presence of $1 \text{ mg l}^{-1} \text{ CN}^{-}$ and the start-up procedure was relatively short—in 8 days 100% of denitrification efficiency was obtained. As a result of organic acids formation inside the filter, the pH value dropped from 7.6 to 7.0 at the very beginning of the process (Fig. 4). In the case of acetone when the pH value increased during the process as the result of OH^{-} ions releasing from 7.6 to 7.9, the start-up procedure failed during the first 30 days. When the pH value of the influent was artificially decreased to 6.5, a 100% efficiency of denitrification was obtained during the next 8 days. The observations could be explained by the fact that cyanohydrin formation needs the presence of a strong acid which is underlined in preparative chemistry. The obtained results were however different from the ones obtained by Raef *et al.* (1977).

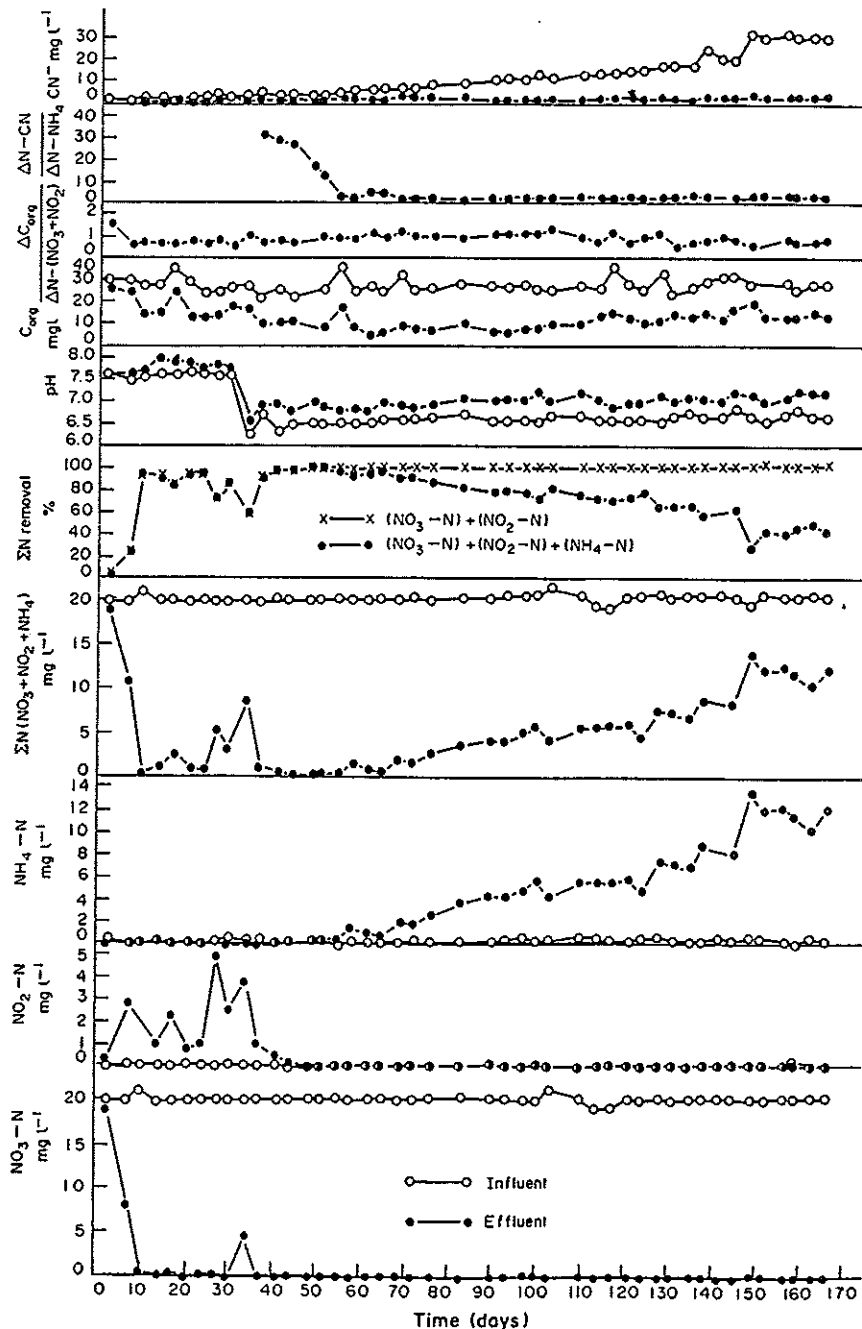


Fig. 5. The exploitation course of the PBR using acetone as the electron donor.

In this work the reaction rates of cyanide-glucose reaction in sealed ampoules at 30°C are as follows: in pH 7.0, $k = 0.5 \times 10^{-3} \text{ mg CN}^{-1} \text{ h}^{-1}$ and in pH 11, $k = 30.5 \times 10^{-3} \text{ mg CN}^{-1} \text{ h}^{-1}$. The pH value of 11 was recognized as optimum for glucose-cyanide reaction. In the investigations described in this work the process broke down when the pH value of the effluent reached 9 (Fig. 4). The reaction described by Raef *et al.* had another nature than the cyanohydrin formation. Raef proposed combining the two waste streams containing an aldose carbohydrate and cy-

anide at high pH as a pretreatment step prior to biological oxidation. The investigations described in this work showed the possibility of biological decomposition of the wastewater in the commonly met range of pH values.

CONCLUSIONS

(1) The research results clearly indicated the inhibitory effect of cyanide on denitrification process

when methanol, propanol and acetate was used as electron donors.

(2) The denitrification process in presence of cyanide is possible using acetone and glucose as the electron donors. The toxic effect of cyanide is removed as a result of acetone-cyanide and glucose-cyanide reactions.

(3) The investigations showed the possibility of simultaneous denitrification and cyanide removal by utilizing the cyanohydrin reaction within the Packed Bed Reactors. The cyanohydrins were formed by the pH values close to neutral, nevertheless the organic chemistry literature recommended for cyanohydrins synthesis the conditions by which the microorganisms would not be able to survive. The presence of metabolism products of bacteria inside the biological filter had probably the catalytic effect on the reaction because in the feed containers no cyanohydrin formation was observed.

(4) Results obtained in the Packed Bed Reactor during denitrification does not limit the applying of the method. Further research is needed to verify the possibility of cyanohydrin formation during the other biological wastewater treatment processes, first within the activated sludge. This could lead to the development of the new treatment process which will

enable us to treat wastewater containing cyanide.

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