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INHIBITION OF NITRIFICATION IN THE PACKED BED REACTORS BY SELECTED ORGANIC COMPOUNDS

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Abstract—The effect of methanol, acetone, formalin and glucose on the nitrification process in the packed bed reactors has been investigated. For the utilized compounds the inhibition constant K_i was determined according to Dixon's method. The determined values were as follows: methanol $K_i = 116.0\ mg\ l^{-1}$; acetone $K_i = 804.2\ mg\ l^{-1}$; formalin $K_i = 61.5\ mg\ l^{-1}$. The value of K_i for glucose has not been determined because glucose in applied concentration up to $11{,}325\ mg\ l^{-1}$ had no effect on the nitrification course.

Key words—nitrification, inhibition, K_i measurement

INTRODUCTION

Inhibition of biological treatment processes in the continuously exploited reactors involves both the inhibition of the substrate conversion and the inhibition of growth. Inhibition of the substrate conversion is described in enzymology by a sequence of equations depending on the kind of inhibition: competitive, non-competitive, mixed (Bailey and Ollis, 1977). The rate of the inhibition in these equations reflects the inhibition constant K_i. The equations have been principally constructed for the investigations with pure enzymes. That is why the application of these equations to the description of the bacterial system being inhibited is troublesome. The present work proved the possibility of determining the inhibition constant K_i within packed bed reactors applied for nitrification process performance.

As inhibitors, the following organic compounds were used: methanol, acetone, formalin and glucose. It is generally accepted that organic compounds can inhibit the nitrifiers' activity but the mechanism of this inhibition is not clear (Painter, 1970). It is suggested that the toxic effect of organic compounds may be due to dissolved oxygen concentration limitation (Kiff, 1972). The literature data (Hockenbury and Grady, 1977) describe the toxic influence of methanol as 50% inhibition caused by $160\ mg\ l^{-1}$ of methanol (Hooper and Terry, 1973) and that of acetone as 75% inhibition caused by $2000\ mg\ l^{-1}$ of acetone (Tomlison *et al.*, 1966).

MATERIALS AND METHODS

Four laboratory scale submerged filters, shown in Fig. 1, were constructed. Each filter consisted of a plexiglas column 50 mm i.d. and 1.1 m tall. The waste to be treated entered at the bottom, as well as the air for aeration, and flowed upward. The effluent ports were placed at the top of the filters. Columns were packed to a depth of 1 m with marble 3–8 mm in dia crushed and previously treated at 850°C. The reactors were designed as packed bed reactors with chemically active beds according to the concept described by Kowalski and Lewandowski (1983). The neutralization reaction between hydrogen ions generated during nitrification and the packing material prevented the pH value decreasing during the course of the process. The detention time based on the liquid volumes was 2 h in all cases. Each of the filters operated with an air flow rate $0.5\ l\ min^{-1}$ which maintained the relative constant dissolved oxygen concentration in the effluent equal to about $6\ mg\ l^{-1}\ O_2$. The synthetic wastes were prepared in tap water. Before the treatment process the water characteristic was changed by dissolving $5\ mg\ l^{-1}$ PO_4–P (as Na_2HPO_4) and $100\ mg\ l^{-1}$ NH_4–N (as NH_4Cl). As the seeding material activated sludge containing the active nitrifiers was used. The inoculum composition was as follows: $100\ mg\ l^{-1}$ NH_4–N; $5\ mg\ l^{-1}$ PO_4–P; 100 ml of thickened activated sludge; 1 l. of tap water. The inoculum was poured into the filters, retained and aerated for 5 days. After this the start-up procedure was considered complete and the feed solution was pumped through the filters. After the next 2 weeks the reactors attained an efficiency close to 100% in terms of ammonia nitrogen oxidation. Since the pH value of the feed solution was about 7.5 the loss of ammonia due to stripping was found to be negligible. All of the ammonia introduced to the reactors was oxidized to nitrate with an efficiency close to 100%.

The investigations involved the influence of the presence of four organic compounds: methanol, acetone, formalin and glucose in the course of the nitrification process. Every compound was introduced to the individual reactor under shock-load conditions. Each of the four reactors served for the investigation of one compound. The measurements of the toxic influence were made twice a week. In the meantime the reactors were supplied with the feed solution without a toxic compound. During the measurements the reactors were fed with the feed solutions containing the investigated organic compounds for 4 h (doubled retention time). After this the influent and effluent of every reactor was analyzed for pH, ammonia, nitrate, nitrite and organic carbon concentration. The feed containers were changed for the others without a toxic compound. The concentration of the tested toxic compound was chosen randomly for the individual measurements. Calculation of the nitrification rate has been made according to the following equation:

$$V = \frac{[NO_3\text{–}N]\text{effluent} - [NO_3\text{–}N]\text{influent}}{RT}\ mg\ l^{-1}\ h^{-1}. \quad (1)$$

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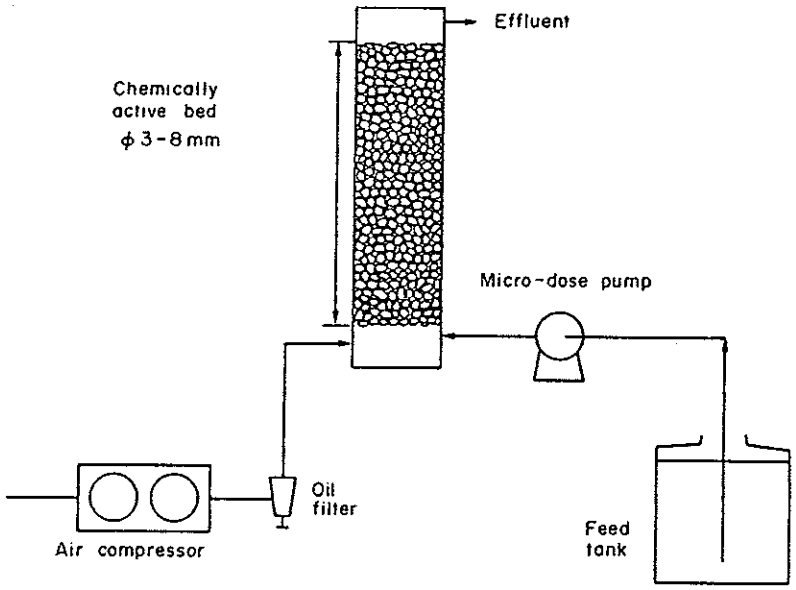

Fig. 1. Laboratory apparatus.

In all of the investigated cases no nitrite accumulation was observed during the course of the investigation. No clogging of the beds was observed. The reactors did not need re-inoculation. Three days after the test they showed an efficiency of nitrification close to 100% which allowed the next measurement to be made according to the described procedure. Measurements for pH were made by means of a pH-meter and for ammonium ions concentration by means of an ion-selective electrode. The concentrations of nitrite and nitrate were determined by following the procedures outlined in *Standard Methods* (APHA, 1975). Organic carbon concentration was measured with the use of the Beckman's Organic Carbon Analyzer. Inhibition of nitrification caused by the utilized compounds was that of non-competitive kind. The relationship between reaction velocity and inhibitor concentration for this kind of inhibition can be described by the following equation (Aiba *et al.*, 1973):

$$V = \frac{V_{max} \cdot s \cdot K_i}{(K_m + s) \cdot (K_i + i)} \tag{2}$$

where

V = velocity of reaction (mg l^{-1} h^{-1})
V_{max} = maximum velocity of an enzyme catalyzed reaction when saturated with substrate (mg l^{-1} h^{-1})
s = substrate concentration (mg l^{-1})
i = inhibitor concentration (mg l^{-1})
K_m = Michaelis constant (mg l^{-1})
K_i = inhibition constant (mg l^{-1}).

The value of K_i is equal to the concentration of inhibitor which causes a decrease in reaction rate to half of the maximum. A simple graphical method which gives K_i was given by Dixon (Dixon and Webb, 1964). If the reaction velocity is determined with a series of inhibitor concentrations a linear relationship is obtained on plotting $1/V$ against i. The point of intersection gives $-K_i$. Since the concentration of substrate is usually much greater than Michaelis constant $s \gg K_m$ the equation (2) can be reduced to the linear form:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{i}{V_{max} \cdot K_i}. \tag{3}$$

By putting $1/V = 0$ the intersection point with the base-line gives $-K_i$.

The values of K_i for the investigated compounds were calculated with the utilization of results of reaction rate measurements according to equation (3). Because the organic compounds used as inhibitors were partially utilized by heterotrophs the organic carbon concentration determined in the effluent was taken into account. The consumption of organic carbon reached 25% of the amount introduced into the filters.

RESULTS AND DISCUSSION

The results of the measurements and calculations have been presented in Figs 2–5. From the equations describing the inhibitor influence on the reaction

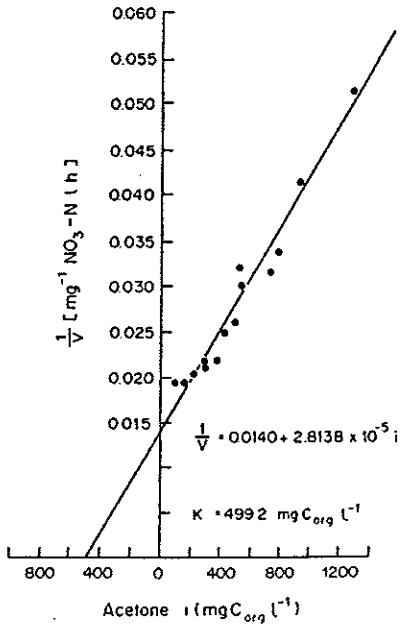

Fig. 2. The results of the measurements using acetone as the inhibitor.

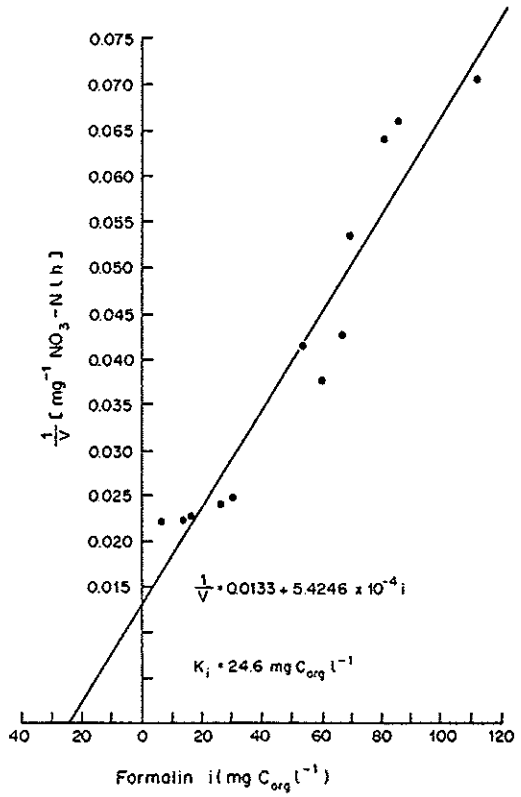

Fig. 3. The results of the measurements using formalin as the inhibitor.

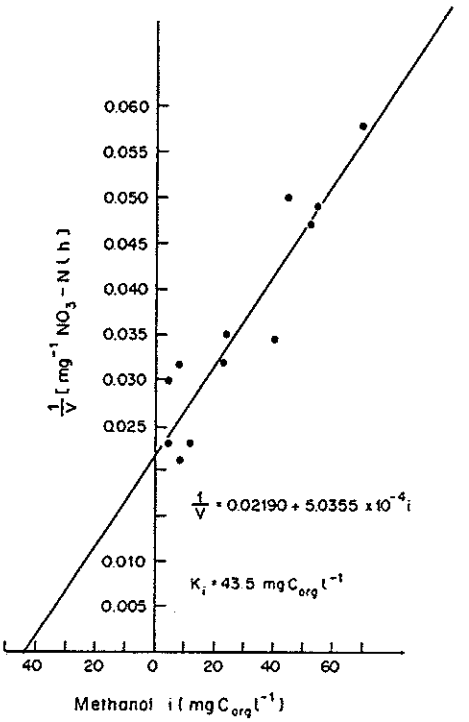

Fig. 4. The results of the measurements using methanol as the inhibitor.

velocity the inhibition coefficients have been calculated. The respective equations and the calculated values were as follows:

Acetone (Fig. 2):

$$1/V = 0.0140 + 2.8138 \cdot 10^{-5}(i) \qquad (4)$$

$K_i = 499.2$ mg l^{-1} C_{org} or $K_i = 804.2$ mg l^{-1} of acetone.

Formalin (Fig. 3):

$$1/V = 0.0133 + 5.4246 \cdot 10^{-4}(i) \qquad (5)$$

$K_i = 24.6$ mg l^{-1} C_{org} or $K_i = 61.5$ mg l^{-1} of formalin.

Methanol (Fig. 4):

$$1/V = 0.02190 + 5.0355 \cdot 10^{-4}(i) \qquad (6)$$

$K_i = 43.5$ mg l^{-1} C_{org} or $K_i = 116.0$ mg l^{-1} of methanol.

Glucose (Fig. 5):
glucose in the applied concentration up to 4530 mg l^{-1} C_{org}, i.e. 11,325 mg l^{-1} of glucose, had no effect on the nitrification course.

The results give the following sequence of the inhibition potential of the utilized compounds: the most potent was formalin, next methanol and acetone. Glucose had no effect on the nitrification process. The results in general fit to the literature data—methanol is a more potent inhibitor than acetone (Hooper and Terry, 1973; Tomlinson *et al.*, 1966). The K_i value which in fact is the concentration of inhibitor which causes the 50% reduction in substrate conversion rate has been determined for methanol as equal to 116 mg l^{-1}. This value is of 27.5% smaller than the value of 160 mg l^{-1} reported by Hooper and Terry. In the case of acetone the valued of 8100 mg l^{-1} determined by Hooper and Terry is 10 times greater than the value of 804 mg l^{-1} determined in this work. The extrapolation of the line drawn in

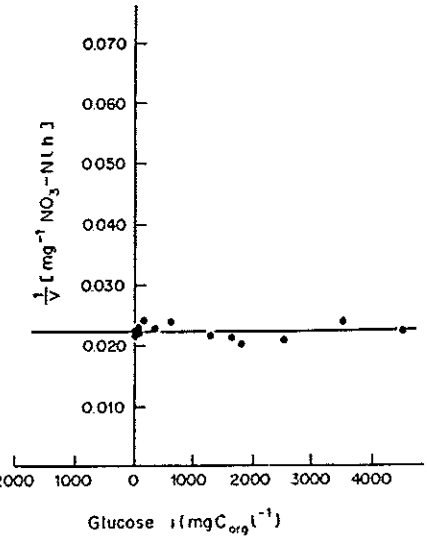

Fig. 5. The results of the measurements using glucose as the inhibitor.

Fig. 2 shows the concentration of acetone which causes the 75% inhibition in nitrification equal to 2488 mg l^{-1}. This value is 19.6% greater than the value of 2000 mg l^{-1} reported by Tomlinson *et al.*

Introduction of glucose did not cause any decrease in the nitrification velocity. The lack of inhibition proved that the inhibition of nitrification was not caused by the dissolved oxygen limitation within the filters but reflected the properties of the individual organic compounds.

CONCLUSIONS

(1) Some organic compounds can inhibit the process of nitrification.

(2) For the tested compounds the following inhibition coefficients K_i have been determined:

$K_i = 116.0$ mg l^{-1} of methanol;
$K_i = 61.5$ mg l^{-1} of formalin;
$K_i = 804.2$ mg l^{-1} of acetone.

(3) Glucose in applied concentration up to 11,325 mg l^{-1} had no effect on the nitrification course.

(4) The packed bed reactors with a chemically active bed enabled an estimation of the inhibition coefficients for nitrification to be made.

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