Wat. Res. Vol. 21, No. 2, pp. 147–153, 1987
Printed in Great Britain

0043-1354/87 $3.00 + 0.00
Pergamon Journals Ltd

BEHAVIOUR OF BIOLOGICAL REACTORS IN THE PRESENCE OF TOXIC COMPOUNDS

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(*Received June 1985*)

Abstract—A model of the influence of toxic compounds on the biological processes in waste water treatment reactors has been developed. The model predicts the behaviour of reactors influenced by toxic compounds acting as non-competitive inhibitors. The effects of a toxic compound on a process is quantified in terms of the inhibition coefficient K_i for the compound and the reactor resistance to inhibition values. The proposed model was utilized for the analysis of data obtained in a packed bed reactor for denitrification in the presence of chromium Cr^{6+}. The inhibition coefficient for chromium was found to be $1.2\,mg\,l^{-1}\,Cr^{6+}$ and the reactor resistance to inhibition was $2.9\,mg\,l^{-1}\,Cr^{6+}$.

Key words—inhibition, biological reactors design

INTRODUCTION

Toxic compounds are commonly removed from waste water before biological treatment to avoid process deterioration due to biological inhibition. This procedure is especially feasible when the concentration of toxic compounds is constant and relatively high, or when the compound itself is a valuable resource. Frequently, however, the presence of toxic compounds in biologically treated wastewater is sporadic and the treatment processes can be seriously impaired.

The increasing trend of combining industrial and municipal waste water for treatment in sewage plants increases the possibility of introducing toxic materials into the plant's influent. The deleterious effects of toxic compounds on biological processes are complex. Toxins can slow down microorganisms respiration rate which influences the substrate conversion rate. Prolonged exposure to toxic compounds can slow down microorganisms growth rate and even leads to changes in the composition of microbial population. Designing procedures for biological waste water treatment plants generally, do not account for the possible effects of toxic compounds present in the waste water. The need for design procedures taking into account the effects of biochemical reaction inhibitors in biologically treated waste water is increasing.

The influence of inhibitors on biochemical reactions with pure enzymes is fairly well understood (Bailey and Ollis, 1977; Atkinson and Mavituna, 1983). Equations describing biochemical reaction velocities as functions of inhibitor concentration have, however, only limited application in analysis of biological waste water treatment reactors because influence of toxic compounds on living microorganisms are more complex than the influence on pure enzymes. Effects of toxic compounds on operation parameters of biological reactors have been observed by numerous authors. Sujarittanonta and Sherrard (1981), who investigated the influence of nickel ions on activated sludge nitrification, stated that the nickel toxicity to nitrifiers is a function of the toxic mass-to-biomass ratio rather than the concentration of toxic materials. This conclusion was supported by investigations by Randall and Buth (1984). Similar conclusions have been stated regarding influence of copper ions on the activated sludge process (Directo and Multon, 1962; McDermott *et al.*, 1963; Ayres *et al.*, 1965; Lamb and Tollefson, 1973). These literature results can be analysed and explained in terms of the concept presented in this paper.

THEORETICAL CONSIDERATIONS

Toxic compounds in biologically treated waste water can cause inhibition of enzyme catalysed reaction rates due to reactions between the enzymes and inhibitors. The influence of the inhibitor on the biochemical reaction may be described as an inhibition rate (I) according to the equation:

$$I = \frac{V_0 - V}{V_0} = \frac{k_0 - k}{k_0} \tag{1}$$

where

V_0 = reaction rate in the absence of inhibitor, $mg\,l^{-1}\,h^{-1}$
V = reaction rate in the presence of inhibitor, $mg\,l^{-1}\,h^{-1}$
k_0 = specific reaction rate in the absence of inhibitor (maximum specific rate), h^{-1}

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k = specific reaction rate in the presence of inhibitor, h^{-1}.

The relationship between reaction velocity and inhibitor concentration for non-competitive inhibition can be described by the same equation for pure enzyme inhibition (Aiba *et al.*, 1973) and for biomass of living microorganisms (Pirt, 1975):

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

where

V = velocity of reaction, $mg\, l^{-1}\, h^{-1}$
V_{max} = maximum velocity of an enzyme catalysed reaction when saturated with substrate, $mg\, l^{-1}\, h^{-1}$
s = substrate concentration, $mg\, l^{-1}\, h^{-1}$
i = inhibitor concentration, $mg\, l^{-1}\, h^{-1}$
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 velocity to one-half of the maximum reaction rate. When the concentration of substrate within the reactor is much greater than the Michaelis constant ($s \gg K_m$) equation (2) may be reduced to the form:

$$V = \frac{V_{max} \cdot K_i}{K_i + i}. \quad (3)$$

Since the maximum reaction rate takes place in the absence of inhibitor:

$$V_0 = V_{max}.$$

Graphical determination of K_i by linearization of equation (3) was proposed by Dixon and Webb (1964).

$$\frac{1}{V} = \frac{1}{V_0} + \frac{i}{V_0 \cdot K_i} \quad (4)$$

which may also be expressed as:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{i}{k_0 \cdot K_i}. \quad (5)$$

The theoretical line representing equations (4) and (5) is presented in Fig. 1. The baseline intercept ($1/V = 0$) gives $-K_i$. From this figure it can be seen that:

$$\frac{k}{k_0} = \frac{V}{V_0} = \frac{K_i}{K_i + i}. \quad (6)$$

The dependency of inhibition rate on the inhibitor concentration can be determined by combining equations (1) and (6), which yield:

$$I = 1 - \frac{K_i}{K_i + i} \quad (7)$$

or

$$I = \frac{i}{K_i + i}. \quad (8)$$

Biological reactors can be operated with full efficiency despite the presence of inhibitor in the influent. This phenomena was called "the reactor resistance to inhibition" (Lewandowski, 1985). The reactor resistance to inhibition (RRI) value was defined as the smallest concentration of inhibitor in the influent at which the process efficiency is impaired. The existence of positive RRI value is due to the difference between the time needed for the process performance and the reactor detention time. The excess detention time over that required leads to a "reserve" in reaction rate. In fact the reactor could be operated at a rate which is smaller than existing substrate conversion rate with the same efficiency. The reserve in reaction rate may be reduced by inhibitor action without deterioration of the obtained results. Introduction of toxic compounds in the influent of biological reactors can cause decrease in substrate conversion rate. The measured decrease in

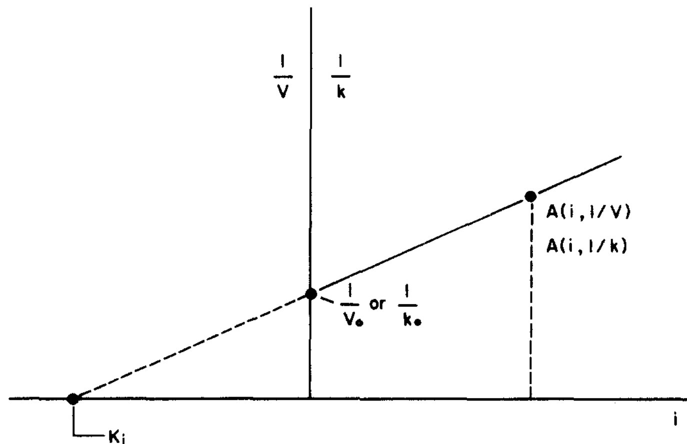

Fig. 1. Theoretical plot of the reciprocal of the reaction rate ($1/V$) and specific reaction rate ($1/k$) as a function of inhibitor concentration.

substrate conversion rate is not, however, equivalent with the inhibition rate which describes the slow down in reaction rate. The influence of inhibition rate on substrate conversion rate depends on the operation parameters of the biological reactor.

The behaviour of the biological reactor can be characterized by substrate conversion rate (P):

$$P = \frac{s_i - s_e}{s_i} \tag{9}$$

where

s_i = influent substrate concentration, mg l^{-1}
s_e = effluent substrate concentration, mg l^{-1}.

The concentration of substrate converted during the process (S) is:

$$S = s_i - s_e. \tag{10}$$

The potential substrate concentration converted in the presence of inhibitor (S_p):

$$S_p = t \cdot V \tag{11}$$

where

t = liquid detention time, h.

The maximum potential substrate converted in the absence of inhibitor (S_{p0}):

$$S_{p0} = t \cdot V_0. \tag{12}$$

The potential substrate conversion rate, in the presence of inhibitor (P_p):

$$P_p = \frac{S_p}{s_i} = \frac{t \cdot V}{s_i}. \tag{13}$$

The maximum potential substrate removal rate in the absence of inhibitor (P_{p0}):

$$P_{p0} = \frac{S_{p0}}{s_i} = \frac{t \cdot V_0}{s_i}. \tag{14}$$

Substituting in equation (3) for V and V_0 from equations (13) and (14), respectively, the substrate conversion rate (P) can be expressed as:

$$P = P_{p0} \cdot \frac{K_i}{K_i + i} \tag{15}$$

which can be rearranged to a linear equation for inverse conversion rate, $1/P$, as a function of inhibitor concentration, i:

$$\frac{1}{P} = \frac{1}{P_{p0}} + \frac{1}{P_{p0} \cdot K_i} \cdot i. \tag{16}$$

To obtain complete substrate conversion in a biological reactor the operation parameters must be set according to the existing specific reaction rate (k). Negative effects of toxic compounds on substrate conversion rate can be compensated for by increasing biomass concentration and/or liquid detention time.

Effect of biomass concentration

Biomass concentration (x_0) required for complete substrate conversion in the absence of inhibitor is:

$$x_0 = \frac{s_i}{k_0 \cdot t}. \tag{17}$$

Biomass concentration (x) required for complete substrate conversion in the presence of inhibitor is:

$$x = \frac{s_i}{k \cdot t}. \tag{18}$$

The increase in biomass concentration required to compensate for inhibitor presence therefore equals:

$$x - x_0 = \frac{s_i}{k \cdot t} \cdot \frac{k_0 - k}{k_0} \tag{19}$$

which by substituting from equation (18), yields:

$$x = x_0 \cdot \frac{k_0}{k}. \tag{20}$$

By combining equations (6) and (20) the following linear equation is obtained:

$$x = x_0 \left(1 + \frac{i}{K_i}\right). \tag{21}$$

If the biological reactor does not perform the complete substrate conversion which means that there is no reserve in reaction rate the changes in biomass concentration cause the changes in the observed inhibition rate. The increase in biomass concentration required to compensate for the presence of inhibitor concentration equal i can be calculated by combining equations (1), (18) and (19):

$$x - x_0 = x \cdot I. \tag{22}$$

If the inhibitor concentration remains constant, the increase in biomass concentration required to compensate for the inhibitor influence remains constant:

$$x \cdot I = \text{const.} \tag{23}$$

Equation (23) means, that under the same conditions, a constant concentration of inhibitor inhibits a constant concentration of biomass, independent of the total biomass concentration. The constant in equation (23) equals the concentration of biomass being inactivated by inhibitor action and can be calculated by combining equations (8) and (21):

$$x \cdot I = x_0 \cdot \frac{i}{K_i}. \tag{24}$$

The biomass concentration calculated from equation (24) is inactive in the substrate removal process. The concentration of biomass being active in the process (x_r) is then:

$$x_r = x - x \cdot I = x(1 - I). \tag{25}$$

Effect of liquid detention time

The liquid detention time (t) required for a defined substrate conversion in a biological reactor in the presence of inhibitor depends on substrate concentration and reaction rate:

$$t = \frac{s_i}{k \cdot x}. \tag{26}$$

Table 1. Results of the theoretical analysis

i (mg l^{-1})	V [equation (3)] (mg l^{-1} h^{-1})	S_p [equation (11)] (mg l^{-1})	s_e (mg l^{-1})	P [equation (9)]	$1/P$
0	$20.0 = V_0$	$80.0 = S_{p0}$	0	1	1
1	18.2	$72.7 > s_i$	0	1	1
2	16.7	$66.7 > s_i$	0	1	1
3	15.4	$61.5 > s_i$	0	1	1
4	14.3	57.1	2.9	0.95	1.05
5	13.3	53.3	6.7	0.89	1.12
6	12.5	50.0	10.0	0.83	1.20
7	11.8	47.1	12.9	0.79	1.27
8	11.1	44.4	15.6	0.74	1.35

Combining equations (6) and (26), yield:

$$t = \frac{s_i}{k_0 \cdot x} \cdot \frac{K_i + i}{K_i}. \tag{27}$$

The liquid detention time needed for complete substrate conversion in the absence of inhibitor (t_0) equals:

$$t_0 = \frac{s_i}{k_0 \cdot x}. \tag{28}$$

Equation (27) can be written as:

$$t = t_0 \cdot \frac{K_i + i}{K_i} \tag{29}$$

which leads to the following linear relationship between t and i;

$$t = t_0\left(1 + \frac{i}{K_i}\right). \tag{30}$$

THEORETICAL EXAMPLE AND PRACTICAL APPLICATIONS

A theoretical example was analysed to illustrate a reactor response to the introduction of a toxic compound:

A continuous flow stirred tank reactor (CFSTR) is operated as follows:

influent substrate concentration, $s_i = 60$ mg l^{-1},
detention time, $t = 4$ h,
maximum reaction rate in the absence of inhibitor,

$$V_0 = 20 \text{ mg l}^{-1} \text{ h}^{-1},$$

inhibition constant of the dosed inhibitor, $K_i = 10$ mg l^{-1}.

Hypothetical substrate conversion rates obtained at various influent inhibitor concentrations are presented in Table 1. The results of the analysis are presented by the line drawn in Fig. 2. From this graph it is seen that inhibitor concentrations less than the RRI value do not influence the substrate conversion rate. The intercept with the x-axis ($1/P = 0$) determines the inhibition constant value, $K_i = 10$ mg l^{-1}. The intercept with the theoretical line ($1/P = 1$) determines the highest concentration of inhibitor by which complete substrate conversion is still possible, i.e. the reactor resistance to inhibition value. The RRI value was found to be 3.3 mg l^{-1}.

The theoretical example presented in Fig. 2 allows prediction of substrate conversion rate at any liquid detention time. From the data presented in this figure

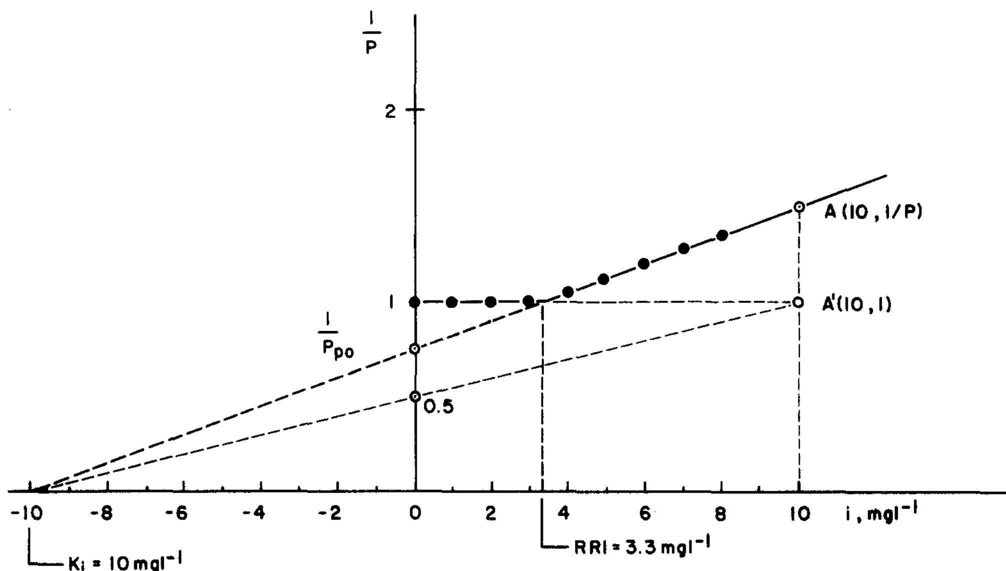

Fig. 2. Theoretical response of a biological reactor to the addition of toxic compound.

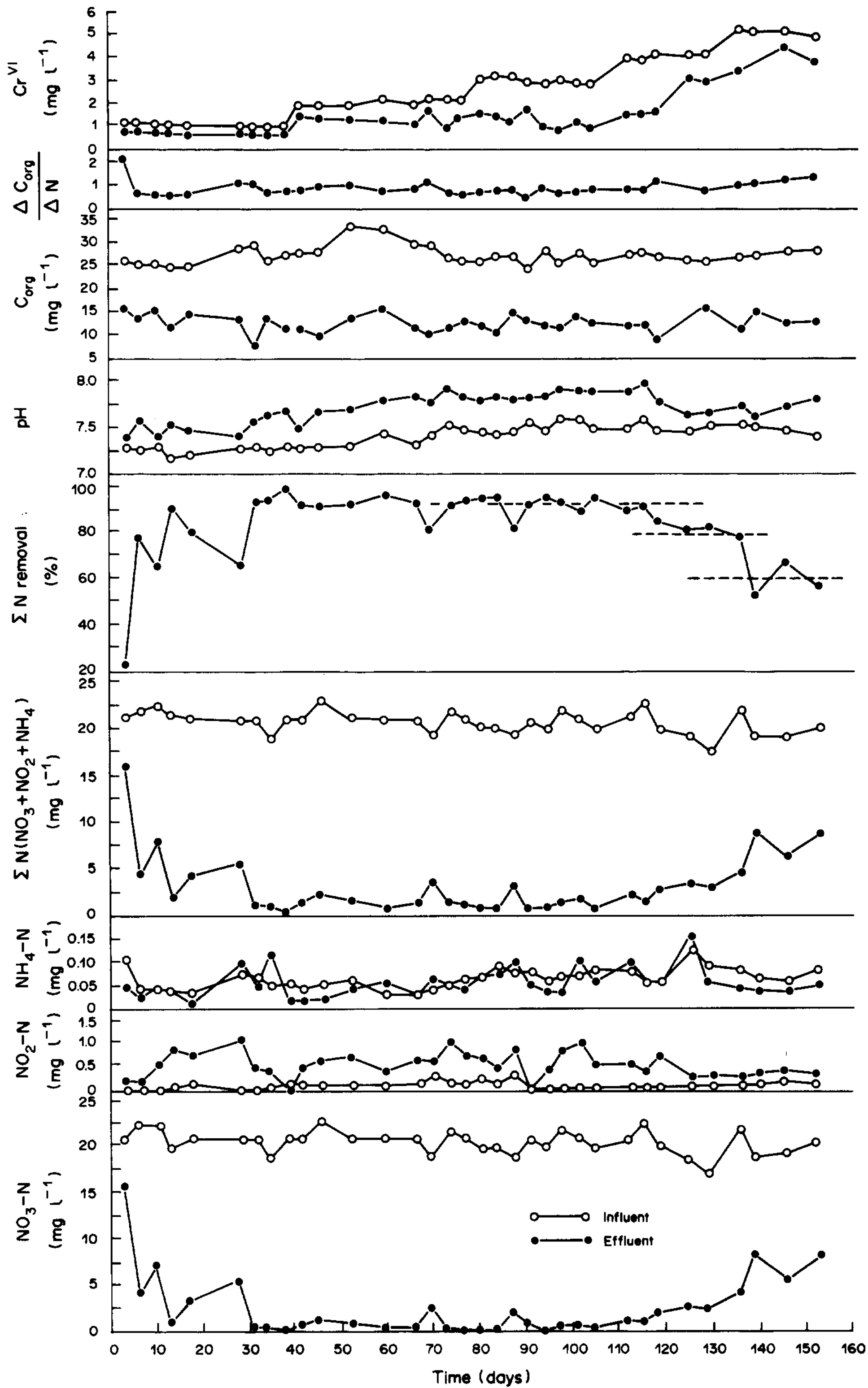

Fig. 3. The performance of the packed bed reactor for denitrification in the presence of chromium Cr^{6+}.

it is also possible to determine the liquid detention time required for complete substrate conversion for any given concentration of inhibitor. As an example the liquid detention time, which allows complete substrate conversion at inhibitor concentration of $10\,mg\,l^{-1}$, was graphically determined in Fig. 2. To obtain complete substrate conversion in the reactor, substrate conversion rate must equal $1/P = P = 1$ for the given inhibitor concentration. Graphically (Fig. 2) it is then determined that the inverse maximum

potential substrate conversion rate, $1/P_{p0} = 0.5$, and $P_{p0} = 2$. The required liquid detention time is then found from equation (14):

$$t = \frac{P_{p0} \cdot s_i}{V_0} = \frac{2 \cdot 60}{20} = 6\text{ h}.$$

For experimental confirmation of the theoretical considerations a packed bed reactor (PBR) operated for denitrification in the presence of chromium Cr^{6+} was analysed. The data analysed have previously been published by the author (Lewandowski, 1985). Iso-butanol as the sole carbon and energy source for denitrifiers was used. Liquid detention time was 1 h. Influent chromium concentration was increased stepwise in small increments. The results of the PBR operation are presented in Fig. 3. The results show that, following the relatively long initial adaptation period, the increase in chromium concentration in the influent decreased substrate removal (conversion) rate. At influent chromium concentration of 3 mg l^{-1} Cr^{6+} the substrate conversion rate was $P = 0.95$. After increasing the chromium concentration to 4 mg l^{-1} Cr^{6+}, substrate conversion rate decreased to $P = 0.80$. At chromium concentration of 5 mg l^{-1} Cr^{6+}, substrate conversion rate was found to be $P = 0.65$. These data were plotted in Fig. 4 to determine the influence of chromium on reactor performance.

The linear equation fitted to the data in Fig. 4, describing the influence of chromium on substrate conversion was:

$$1/P = 0.245(i) + 0.3. \qquad (31)$$

The values of the Reactor Resistance to Inhibition

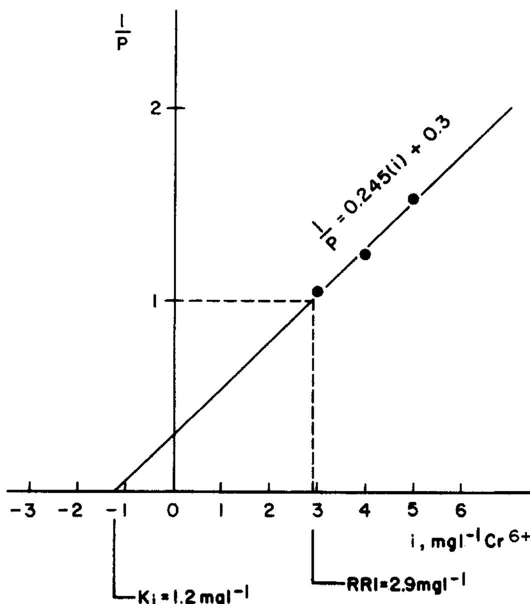

Fig. 4. Determination of the inhibition coefficient (K_i) and reactor resistance to inhibition (RRI) value for denitrification by the packed bed reactor in the presence of chromium Cr^{6+}.

(RRI) and inhibition coefficient K_i were determined from equation (31):

$$K_i = 1.2\text{ mg l}^{-1}\, Cr^{6+} \quad (\text{at } 1/P = 0)$$

$$\text{RRI} = 2.9\text{ mg l}^{-1}\, Cr^{6+} \quad (\text{at } 1/P = 1).$$

A significantly different K_i values for chromium Cr^{6+} inhibition were found for denitrification in the packed bed reactor and in the fresh activated sludge. The inhibition coefficient K_i for the denitrification process in fresh activated sludge was found to be 84.4 mg l^{-1} Cr^{6+} (Lewandowski, 1985), which indicates that the denitrification process in fresh activated sludge is much less sensitive to the presence of chromium than the same process in the packed bed reactor. The large difference may have been caused by different microbial composition of the two systems.

The resistance to inhibition concept may be supported by recent literature results. Neufeld *et al.* (1984) describes the "shoulder effect" for nitrification inhibition. They stated that low levels of inhibitor had no influence on rates of biological nitrification, but higher levels had profound effects. This effect as well as the other results obtained in this study, can be fully predicted on the base of the reactor resistance to inhibition concept. The reactor resistance to inhibition concept predicts that the highest concentration of inhibitor at which complete substrate conversion is possible depends on the reactor biomass concentration which, for activated sludge nitrification, is proportional to the sludge age. This relationship between sludge age and substrate conversion rate in the presence of selected toxic compounds was observed in the study by Neufeld *et al.* and explained as an effect of bacterial system properties. The reactor resistance to inhibition concept, however, demonstrates that this relationship is a direct consequence of the reactors operating parameters such as liquid detention time, biomass concentration (sludge age), electron donor utilized and temperature.

CONCLUSIONS

(1) Biological reactors for waste water treatment operated in the presence of toxic compounds acting as non-competitive inhibitors can perform complete substrate conversion when the toxic compound concentration does not exceed the reactor resistance to inhibition (RRI) value.

(2) Reactors response to the introduction of toxic compounds in terms of substrate conversion rate can be described by a linear equation.

(3) The proposed data analysis allows for determination of the inhibition constant (K_i) and the reactor resistance to inhibition (RRI) value.

(4) Substrate conversion rate in biological reactors exposed to toxic compounds can be predicted based on the proposed equations.

Acknowledgements—The work was supported by the Research Programme 10.2. of The Polish Academy of Sciences.

The author is grateful to Dr Rune Bakke, Institute for Biological and Chemical Process Analysis, Montana State University, for his review and critical comments on the paper.

REFERENCES

Aiba S., Humprey A. E. and Mills N. F. (1973) *Biochemical Engineering*, 2nd edition, p. 97. Academic Press, New York.

Atkinson B. and Mavituna F. (1983) *Biochemical Engineering and Biotechnology Handbook*, pp. 492–497. Macmillan, London.

Ayres K. C., Shumate K. S. and Hanna G. P. (1965) Toxicity of copper to activated sludge. *Proc. 20th Ind. Waste Conf. Purdue Univ.*, pp. 516–524.

Bailey J. and Ollis D. F. (1977) *Biochemical Engineering Fundamentals*, pp. 122–127. McGraw-Hill, New York.

Directo L. S. and Moulton E. Q. (1962) Some effects of copper on the activated sludge process. *Proc. 17th Ind. Waste Conf. Purdue Univ.*, pp. 95–104.

Dixon M. and Webb E. C. (1964) *Enzymes*, p. 328. Longmans, Green, London.

Lamb A. and Tollefson E. L. (1973) Toxic effect of cupric, chromate and chromium ions on biological oxidation. *Wat. Res.* **7**, 599–613.

Lewandowski Z. (1985) Denitrification by packed bed reactors in the presence of chromium(VI)—resistance to inhibition. *Wat. Res.* **19**, 589–596.

McDermott G. N., Moore W. A., Post M. A. and Ettinger M. B. (1963) Effects of copper on aerobic biological sewage treatment. *J. Wat. Pollut. Control Fed.* **35**, 226–241.

Neufeld R. D., Greenfield J. H., Hill A. J., Rieder C. B. and Adekoya D. O. (1984) Nitrification inhibition biokinetics. EPA Project Summary. EPA-600/S2-83-111.

Pirt J. S. (1975) *Principles of Microbe and Cell Cultivation*, p. 174. Wiley, New York.

Randall C. W. and Buth D. (1984) Nitrite build-up in activated sludge resulting from combined temperature and toxicity effects. *J. Wat. Pollut. Control Fed.* **56**, 1045–1049.

Sujarittanonta S. and Sherrard J. H. (1981) Activated sludge nickel toxicity studies. *J. Wat. Pollut. Control Fed.* **53**, 1314–1322.