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BIOLOGICAL REACTOR RESISTANCE TO INHIBITION

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Abstract—The phenomenon of complete substrate conversion within biological reactors in the presence of toxic compounds, called the Reactor Resistance to Inhibition (RRI), has been investigated. It was theoretically demonstrated that the RRI value, which means the highest concentration of inhibitor by which complete substrate conversion is possible, depends on liquid detention time. The excess detention time over that required for complete conversion, imply the existence of a "reserve" reaction potential. The value of this "reserve" can be reduced by inhibitor action with no influence on the observed substrate conversion rate. The influence of detention time on biological denitrification in Packed Bed Reactors in the presence of chromium Cr^{6+} was investigated. The RRI value for 1 h detention time was 1.5 mg l^{-1} Cr^{6+}. For 3 h detention time the RRI value was 22 mg l^{-1} Cr^{6+}. The relationship between the RRI value and detention time was linear.

Key words—denitrification, inhibition, biological reactor design, chromium Cr^{6+}

INTRODUCTION AND THEORETICAL CONSIDERATIONS

The phenomenon of full conversion of substrate within biological reactors in the presence of a toxic compound is called the Reactor Resistance to Inhibition (RRI), (Lewandowski, 1985). The value of the RRI is equal to the lowest concentration of inhibitor in the influent at which process efficiency is reduced. Inhibitor concentration lower than the RRI value does not influence the process efficiency. The reason for this tolerance of inhibitors is the existence of "reserve" reaction potential when the reactors liquid detention time is greater than the time required for complete substrate conversion.

The relationship between reaction velocity and inhibitor concentration for non-competitive inhibition can be described by the following equation (Aiba *et al.*, 1973):

$$V = \frac{V_{\max} \cdot s \cdot K_i}{(K_m + s) \cdot (K_i + i)} \tag{1}$$

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 of 1/2 of the maximum reaction rate. The velocity of the biochemical reactions may be described by the Michaelis–Menten equation:

$$V = \frac{V_{\max} \cdot s}{K_m + s}. \tag{2}$$

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:

$$V = V_{\max}. \tag{3}$$

On the same basis equation (1) can be reduced to:

$$V = \frac{V_{\max} \cdot K_i}{K_i + i}. \tag{4}$$

K_i can be determined graphically by linearization of equation (4), (Dixon and Webb, 1964).

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{i}{V_{\max} \cdot K_i}. \tag{5}$$

The baseline interception ($1/V = 0$) gives $-K_i$. The maximum reaction velocity takes place in the absence of inhibitor. Defining:

$$V_{\max} = k_0 \cdot x$$

and

$$V = k \cdot x$$

where

k_0 = specific reaction rate in the absence of inhibitor (maximum specific rate), h^{-1}
k = specific reaction rate in the presence of inhibitor, h^{-1}
x = biomass concentration, mg l^{-1}

equation (5) can be expressed as:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{i}{k_0 \cdot K_i}. \tag{6}$$

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Based on equation (6) a Dixon's plot using the specific reaction rate, which is independent of the biomass concentration, can be constructed. This theoretical plot is presented in Fig. 1.

The time needed for the complete substrate conversion in the biological reactor, (T), depends on the substrate concentration and the rate of reaction:

$$T = \frac{s}{V} = \frac{s}{k \cdot x}. \tag{7}$$

From Fig. 1:

$$\frac{k}{k_0} = \frac{K_i}{K_i + i}. \tag{8}$$

Combining equations (7) and (8), yield:

$$T = \frac{s}{k_0 \cdot x} \cdot \frac{K_i + i}{K_i}. \tag{9}$$

The time needed for complete substrate conversion in the absence of inhibitor (t_0) is equal to:

$$t_0 = \frac{s}{k_0 \cdot x} \tag{10}$$

so equation (9) can be rewritten as:

$$T = t_0 \cdot \frac{K_i + i}{K_i} \tag{11}$$

or

$$T = t_0 + t_0 \cdot \frac{i}{K_i}. \tag{12}$$

Detention time required for complete substrate removal in the presence of inhibitor concentration i can be calculated from equation (12). The Reactor Resistance to Inhibition (RRI) value has been defined as the smallest concentration of inhibitor at which process efficiency was deteriorated. In terms of RRI equation (12) can be written as:

$$T = t_0 + t_0 \cdot \frac{\mathrm{RRI}}{K_i}. \tag{13}$$

The detention time given by this equation is equal to that required for the complete substrate conversion in the presence of inhibitor concentration equal to the RRI value. RRI value may be determined from equation (13):

$$\mathrm{RRI} = \frac{T - t_0}{t_0} \cdot K_i. \tag{14}$$

According to equation (14) the RRI value depends on the difference between the liquid detention time accepted for operation and detention time required for complete substrate removal in the absence of inhibitor. The goal of this study was to determine the influence of liquid detection time in a Packed Bed Reactor during denitrification in the presence of chromium Cr^{6+} on the value of the Reactor Resistance to Inhibition.

MATERIALS AND METHODS

The Packed Bed Reactor (PBR) used in this study consisted of a plastic column 45 mm i.d. and 0.8 m tall. The column was placed in a thermostatic bath and temperature of 25°C was maintained during the experimental period. Reactor packing consisted of ceramic Raschig rings 15 mm long, outer and inner diameters of 16 and 10 mm, respectively. The PBR was filled with the packing material up to 0.5 m height. Total surface of the packing was 0.27 m^2. The porosity of the reactor was 0.625, total volume 0.8 l. and liquid volume 0.5 l. Liquid detention time was changed according to the experimental procedure. The wastewater to be treated was supplied to the bottom of the column by peristaltic pumps. The effluent port was at the top of the reactor. The packing material was submerged during the experiment. The synthetic waste was prepared using tap water and stored in plastic containers. The feed solution was changed twice a week. The nitrate concentration in the influent was 20 mg l^{-1} NO_3-N (as KNO_3), phosphate 2 mg l^{-1} (as Na_2HPO_4). Methanol was used as the sole carbon and energy source for denitrifiers in concentration of about 100% greater than stoichiometric requirements. The chromium concentration Cr^{6+} (added as $K_2Cr_2O_7$) was 1 mg l^{-1} Cr^{6+} at the beginning of the process and was raised during the experiment until progressive deterioration of the results in terms of denitrification efficiency was observed. The liquid detention time at the beginning of the filler operation was 1 h. The concentration of chromium in the influent was slowly raised observing the denitrification efficiency. The concentration of chromium which caused a decrease in the denitrification efficiency below 95% was accepted as the value of the Reactor Resistance to Inhibition. When the denitrification efficiency dropped below 95%, the detention time was increased to 2 h which caused an improvement in denitrification efficiency. At the new detention time the concentration of chromium in the influent was again increased to obtain decrease in denitrification efficiency below 95%. The procedure was repeated for detention times equal to 1, 2 and 3 h.

The feed solution was deoxygenated by means of sodium sulphite Na_2SO_4 by stoichiometric addition according to the dissolved oxygen concentration. All the reagents used were laboratory grade. The stoichiometric quantities of methanol were obtained from the following equation:

$$NO_3 + 5/6CH_3OH = 1/2N_2 + 5/6CO_2 + 7/6H_2O + OH^-. \tag{15}$$

The reactor was seeded by activated sludge acclimated to the experimental conditions. The activated sludge taken from a municipal treatment plant was held under anoxic conditions for 24 h. To the sludge 100 mg l^{-1} NO_3-N,

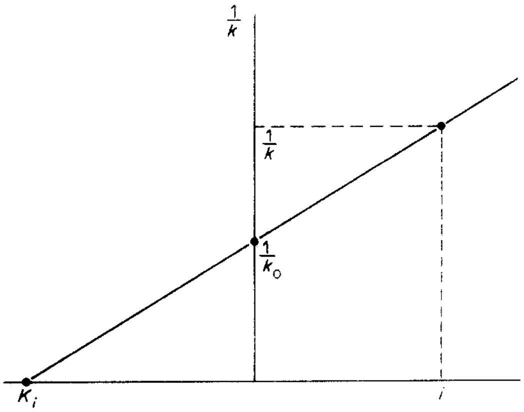

Fig. 1. Theoretical plot of the reciprocal of the specific reaction rate vs inhibitor concentration.

2 mg l^{-1} PO_4-P, methanol and 1 mg l^{-1} chromium Cr^{6+} were added. After 24 h, considered as the adaptation period, the inoculum was poured into the filter and the experiment was started. Synthetic wastes based on tap water containing 1 mg l^{-1} Cr^{6+} was pumped through the filter by means of peristaltic pumps. Samples were collected twice a week. After obtaining good results, which means nitrate conversion to nitrogen gas of more than 95%, the chromium concentration in the influent was increased to the next level and so on until deterioration of denitrification efficiency below 95% was observed.

Chromium concentration was measured by means of the AAS spectrometer. Measurements for pH were made by means of a pH meter, for ammonium by means of an ion selective electrode. The nitrate and nitrite were measured by following the procedures outlined in *Standard Methods* (APHA, 1975). Organic carbon concentration was analysed with a Beckman Organic Carbon Analyzer. All the colorimetric determinations were made by means of spectrophotometer UV-VIS Perkin–Elmer–Hitachi 200.

RESULTS AND DISCUSSION

The results of the operation of the Packed Bed Reactor are presented in Fig. 2. The concentration of chromium in the influent was increased continuously during the operation period. The data presented in Fig. 2 show that with 1 h detention time the decrease in the denitrification efficiency below the 95% criterion occurred at influent chromium concentration of

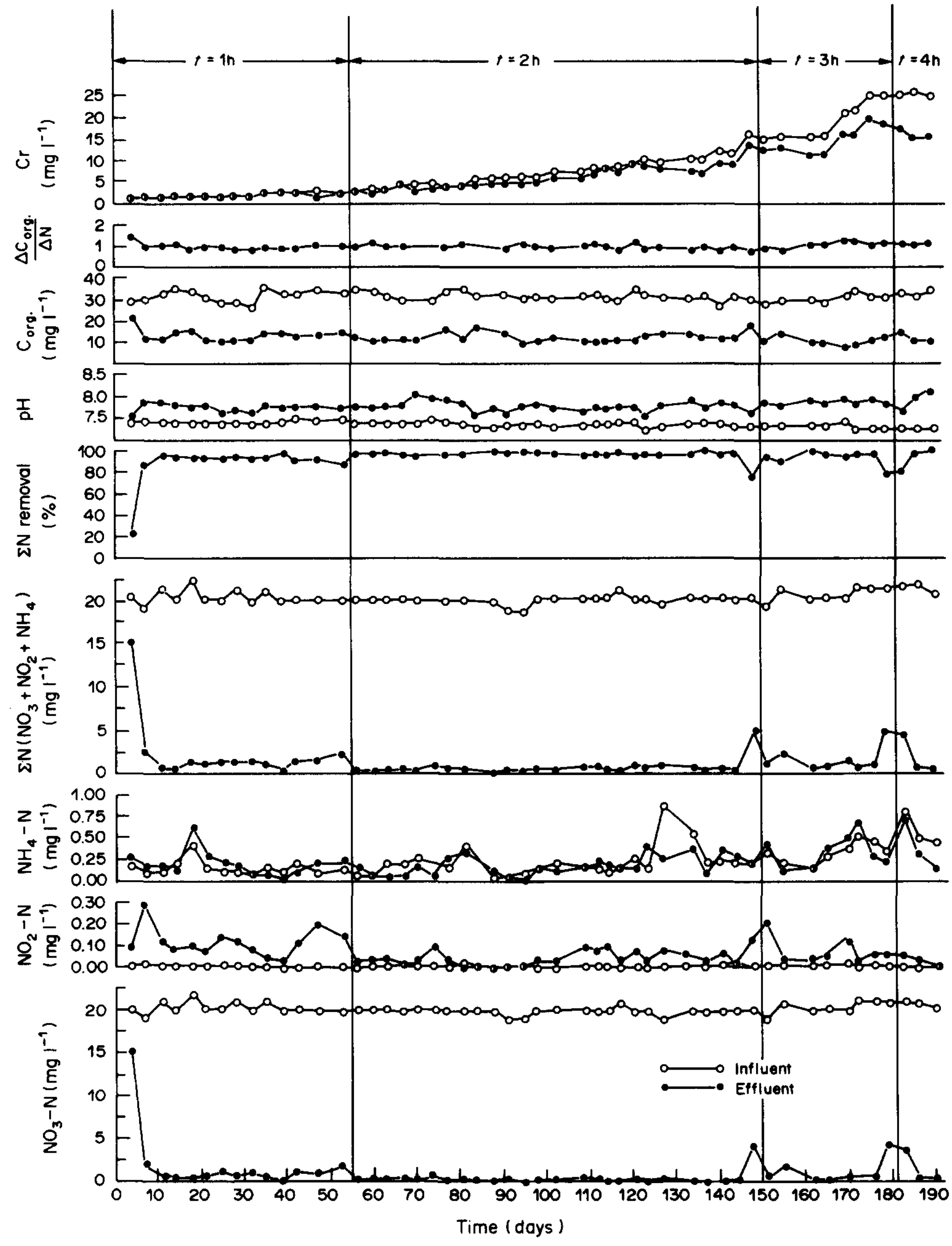

Fig. 2. The performance of the Packed Bed Reactor.

1.5 mg l^{-1} Cr^{6+}. The following increase in detention time to 2 h improved the process efficiency. At detention time of 2 h, the decrease in the process efficiency below 95% was observed at influent chromium concentration of 11 mg l^{-1} Cr^{6+}. The following increase in the detention time to 3 h improved the denitrification efficiency again. With detention time 3 h the decrease in denitrification efficiency below 95% has been caused by the chromium concentration in the influent equal to 22 mg l^{-1} Cr^{6+}. The increase in detention time to 4 h caused an increase in the process efficiency to over 95%.

According to the data presented in Fig. 2 the concentration of chromium detected in the effluent was consistently smaller than in influent. The reason for this was probably the reduction of chromium Cr^{6+} to chromium Cr^{3+} by bacterially produced hydrogen sulphide. Possibility of such a reduction has been described by Smillie *et al.* (1981). Chromium Cr^{3+} is practically insoluble in water.

The concentration of soluble Cr^{3+} in equilibrium with $Cr(OH)_3$ is about 10^{-10} mol l^{-1} (Elderfield, 1970). In the water environment considerable quantities of the Cr^{3+} are deposited into the sediments as oxides and hydroxides and possibly adsorbed to particulates as suggested by Curl *et al.* (1965). Inside the reactor a green sludge of chromium hydroxide was found which demonstrated that part of chromium had been reduced to Cr^{3+}.

The relation between the liquid detention time and the Reactor Resistance to Inhibition value was plotted in Fig. 3 and described by the following equation:

$$RRI = 10.25(t) - 9 \qquad (16)$$

where

t = detention time in the Packed Bed Reactor, h.

The results presented in Figs 2 and 3 as well as equation (16) support the theoretical considerations; detention time did influence the Reactor Resistance to Inhibition value. Equation (16) predicts the behaviour of the investigated reactor when supplied with wastewater containing various concentrations of chromium Cr^{6+}. For example, 95% efficient denitrification in a reactor exposed to 20 mg l^{-1} chromium Cr^{6+} influent concentration can be obtained at detention time:

$$t = \frac{20 + 9}{10.25} = 2.8 \text{ h}.$$

The resulting detention time may be, for practical reasons, too long. There are, however, other means to control the RRI value—the kind of substrate oxidized, temperature and biomass concentration (Lewandowski, 1985). In the described case we could, for example, change the kind of compound serving as the electron donor for another which gives greater reaction rate in the absence of inhibitor and, therefore, increases the RRI value without changing the detention time.

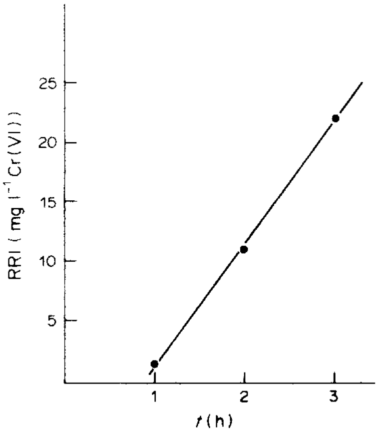

Fig. 3. Reactor Resistance to Inhibition (RRI) value vs detention time for the investigated Packed Bed Reactor.

CONCLUSIONS

(1) The possibility to control the Reactor Resistance to Inhibition value by changing the liquid detention time has been demonstrated.

(2) The value of the Reactor Resistance to Inhibition depends on the difference between the detention time accepted for operation and detention time required for the process performance in the absence of inhibitor. The greater the difference, the greater the RRI value.

(3) The plot of detention time vs the Reactor Resistance to Inhibition value allows prediction of the detention time required for the process performance in the presence of inhibitor.

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