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TECHNICAL NOTE

INHIBITION COEFFICIENT (K_i) DETERMINATION IN ACTIVATED SLUDGE

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Abstract—A method of measurement of the inhibition coefficient, K_i, of chemicals within the activated sludge has been proposed. It makes possible the objective determination of the influence of non-competitive inhibitors on the reaction rate by means of simple respiration rate measurements with the application of a dissolved oxygen meter. The values of K_i for chromium Cr^{6+}, for cyanide CN^- and for two pesticides—DDVP and des-methyl DDVP was determined.

Key words—activated sludge, inhibitors, inhibition constant

INTRODUCTION

For research and practical purposes there is strong need for the investigation of inhibitor effects on the biological wastewater treatment processes. The biochemical literature gives several models for the effects of inhibitors. These models are, however, constructed for the single, pure enzyme investigations and sometimes have limited application to the conditions existing in biological reactors with the mixed microorganisms involved in the treatment process. In biological reactors for wastewater treatment there exist many various microorganisms producing a lot of enzymes inhibited in different ways by the toxic compounds. The observed effect is the total influence of the toxic compounds on the biological system rather than the pure inhibition of a single enzyme. If enzyme kinetics are included in or justified for treatment process models consideration should be given to reactions which are more complex than the single substrate Michaelis–Menten type.

Enzymes are inhibited by many groups of chemical reagents as well as the physical factors, violent mechanical agitation, irradiation and other factors which lead to denaturing of the enzyme protein. Enzyme activity can be blocked by molecules similar in structure to the reaction substrate. In some cases enzyme inhibitor complexes are reversible and the inhibition is directly related to the ratio of inhibitor and substrate concentrations and their adsorption coefficients. In other cases, the enzyme–inhibitor complex is not reversible and the enzyme is permanently deactivated. Some inhibitors are adsorbed near the active site but are large enough physically to block access by substrate molecules. Many protein-precipitants inactivate enzymes. There is a group of inhibitors which forms insoluble salts which precipitate by virtue of their heavy positively-charged ions. This type of enzyme inhibitor is non-competitive and includes the ions of heavy metals. The other example of non-competitive inhibitor is cyanide which acts by inactivating heavy metal catalysts and forming very stable complexes with the metal. The non-competitive type of inhibitor is the matter of investigation in this work.

The relationship between the reaction velocity and inhibitor concentration for this type of inhibition may be illustrated as in Fig. 1. The curve is a graphical solution of the kinetic equation of non-competitive inhibition (Aiba *et al.*, 1973):

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

where:

V = velocity of a reaction (mg l^{-1} h^{-1})
V_{max} = maximum velocity of enzyme 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, that concentration of substrate giving half maximum rate (mg l^{-1})
K_i = inhibition coefficient, that concentration of inhibitor giving half maximum inhibition (mg l^{-1}).

An exogeneous electron acceptor such as oxygen is reduced in the final reaction of the respiration chain. The substrate oxidation rate is then equal to the oxygen reduction rate:

$$V = \frac{-ds}{dt} = \frac{-d(O_2)}{dt}. \quad (2)$$

The change in concentration of dissolved oxygen in a closed system may be easily measured by means of

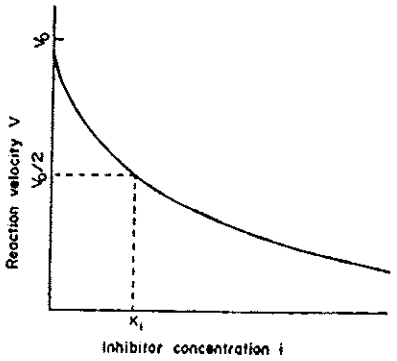

Fig. 1. Influence of the inhibitor concentration on the enzyme catalyzed reaction rate.

an oxygen electrode. In this work the measurement of the respiration rate was chosen for assessing the substrate utilization rate.

The velocity of the biochemical reaction within the activated sludge may be described by Michaelis–Menten equation:

$$V = \frac{-ds}{dt} = \frac{V_{max} \cdot s}{K_m + s}. \quad (3)$$

When oxygen was considered to be the substrate the rough estimation of the Michaelis constant value for the compounds oxidized in this work was found to be about 0.1 mg l^{-1} O_2. Since the concentration of dissolved oxygen within the activated sludge samples was close to saturation it can be stated that $s \gg K_m$. Equation (3) can be then reduced to:

$$V = \frac{-d(O_2)}{dt} = V_{max}. \quad (4)$$

On the same basis in the presence of non-competitive inhibitor, equation (1) can be reduced to:

$$V = \frac{V_{max} \cdot K_i}{K_i + i}. \quad (5)$$

A simple graphical method which gives K_i was described by Dixon and Webb (1964). If the reaction velocity is determined with a series of inhibitor concentrations, keeping the substrate concentrations constant, a straight line is obtained on plotting $1/V$ against i. The point of intersection gives $-K_i$ directly. This is evident from linearization of the equation (5) to the form:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{i}{V_{max} \cdot K_i}. \quad (6)$$

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

To verify the method of the K_i determination the following measurements in the activated sludge were made. As inhibitors chromium Cr^{6+}, cyanide and two pesticides—*O,O*-dimethyl-*O*-(2,2 dichlorovinyl)-phosphate (DDVP) and *O*-methyl-*O*-(2,2 dichlorovinyl) phosphate-sodium salt (des-methyl DDVP), were chosen. As the compounds oxidized methanol and propanol were utilized.

MATERIALS AND METHODS

Activated sludge taken from the Municipal Treatment Plant was aerated continuously during 24 h for oxidation of the organic carbon compounds adsorbed on the flocs and dissolved in the sludge liquor. After this the temperature of the sludge was raised to 25°C which was chosen as the temperature for the measurements. The sludge was still continuously aerated.

The measurements of the respiration rate were done in the apparatus shown in Fig. 2. The apparatus consisted of the reactor tank, dissolved oxygen meter and recorder. The reactor tank was of 140 ml volume and was thermostatted at 25°C. In the upper part the tank was stoppered by means of a conical-shaped plastic stopper. Through the stopper to the reaction tank the oxygen electrode, thermometer and the injection needle were introduced and fixed. During the measurements the activated sludge was continuously mixed by means of a magnetic stirrer. The sludge, previously vigorously aerated, was poured into the reaction tank which was carefully stoppered to remove the air bubbles from the sludge liquor. The stirrer was switched on and the apparatus was ready for use. The first phase of measurement involved the stabilization of conditions and determination of the basic respiration rate, i.e. the rate of respiration without addition of substrate. After the aeration of the sludge for 24 h and oxidizing practically all of the carbon compounds present in the sludge liquor, the basic respiration rate can be identified with the endogeneous respiration rate. After

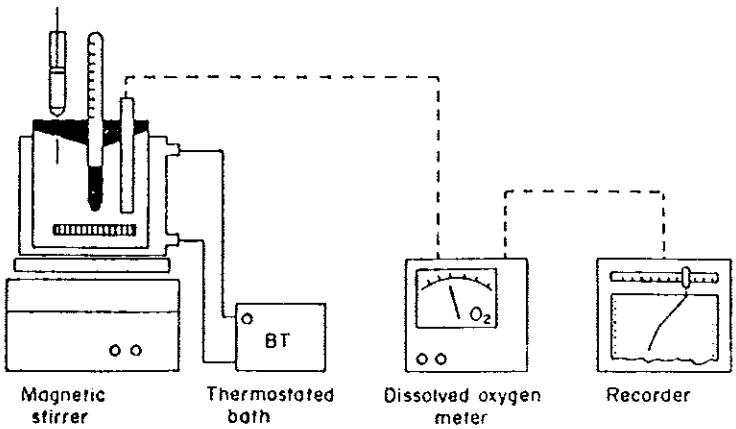

Fig. 2. Laboratory apparatus.

stabilization of conditions and obtaining the straight line on the recorder the substrate (one of the alcohols diluted in water) was injected. The respiration rate increased, being now the maximum substrate respiration rate. Next, the inhibitor at known concentration was injected into the reaction chamber. The respiration rate decreased as a result of inhibition, being now the inhibited respiration rate. The measurements were repeated for several concentrations of inhibitor to cover the region between the maximum substrate respiration rate and the basic respiration rate. The results obtained in this way were plotted in the axis $(1/V, i)$ to determine the K_i value. The intercept was determined analytically with the application of the least squares method.

Dilutions of alcohols injected into the reaction chamber was of 10% and the concentrations of alcohols in the sample of 200 mg l^{-1}. The dilution of chromium solution injected into the reaction chamber was of 10%. As the chromium compounds potassium dichromate $K_2Cr_2O_7$ was used. The dilution of cyanide (as potassium cyanide) injected into the reaction chamber was of 50 mg l^{-1} CN^-. The two pesticides used as the reaction inhibitors were both injected in the form of the concentrated solutions. The compounds used in the work were: *O,O*-dimethyl-*O*-(2,2 dichlorovinyl)phosphate (DDVP) and *O*-methyl-*O*-(2,2 dichlorovinyl)phosphate–sodium salt (des-methyl DDVP). In the cases of chromium and cyanide, methanol was used as substrate and in the case of both pesticides—propanol. During all the measurements the pH value was constantly at 7.5.

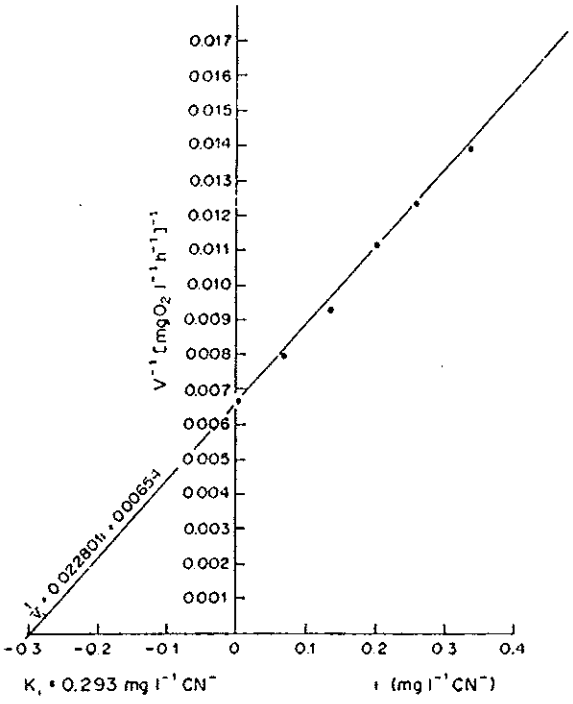

Fig. 4. Determination of the K_i value for cyanide CN^-.

RESULTS

The influence of the biomass concentration on the K_i value is presented in Fig. 3. The measurements were made using methanol as the substrate oxidized and chromium Cr^{6+} as the inhibitor by the activated sludge at suspended solids concentrations of 5.6, 3.7 and 2.3 g l^{-1}. The measured K_i values were equal 87, 85 and 89 mg l^{-1} Cr^{6+} respectively. It can be seen that the biomass concentration had no influence on the K_i value, which had the mean value of 87 mg l^{-1} Cr^{6+}.

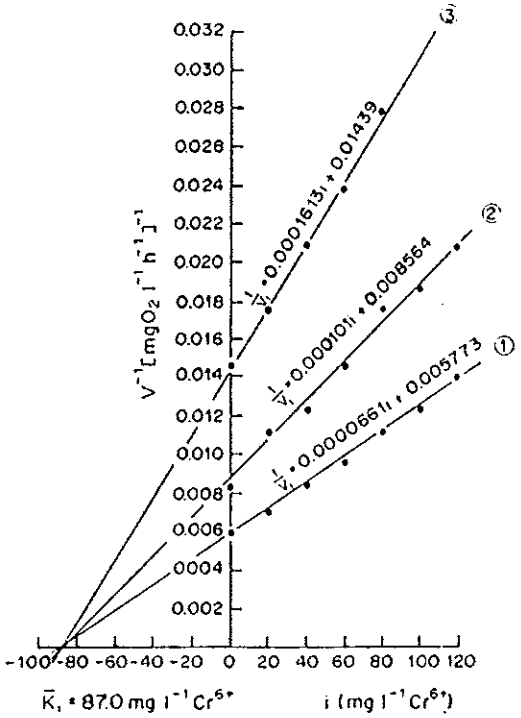

Fig. 3. The influence of the biomass concentration on the K_i value for chromium Cr^{6+}. 1—TSS = 5.6 g l^{-1}, K_i = 87 mg l^{-1} Cr^{6+}; 2—TSS = 3.7 g l^{-1}, K_i = 85 mg l^{-1} Cr^{6+}; 3—TSS = 2.3 g l^{-1}, K_i = 89 mg l^{-1} Cr^{6+}.

Inhibition coefficient K_i measured for cyanide using methanol as the substrate oxidized was 0.293 mg l^{-1} CN^- (Fig. 4). Inhibition coefficients K_i measured for the two pesticides using propanol as the substrate oxidized were equal to 2220 mg l^{-1} DDVP and 13,800 mg l^{-1} des-methyl DDVP. The results are presented in Fig. 5.

DISCUSSION

The toxic effect of some compounds, especially these of heavy metals, has been investigated many times. The results presented in the literature show clearly the toxic influence of some chemical compounds on the biological wastewater treatment processes. The authors utilize the variety of methods, for example BOD test (Mowat, 1976), the lethal concentration LC_{50} determination (Mearns *et al.*, 1976) or determination of % reduction in substrate conversion (Lamb and Tollefson, 1973). All of them give the description of the behaviour of the microbial systems being inhibited. The comparison of results is, however, practically impossible. The toxic effect of inhibitor on the bacterial system is complex and involves both the influence on the chemical reaction rate and the growth of microorganisms. That is why the comparison of results presented in various papers

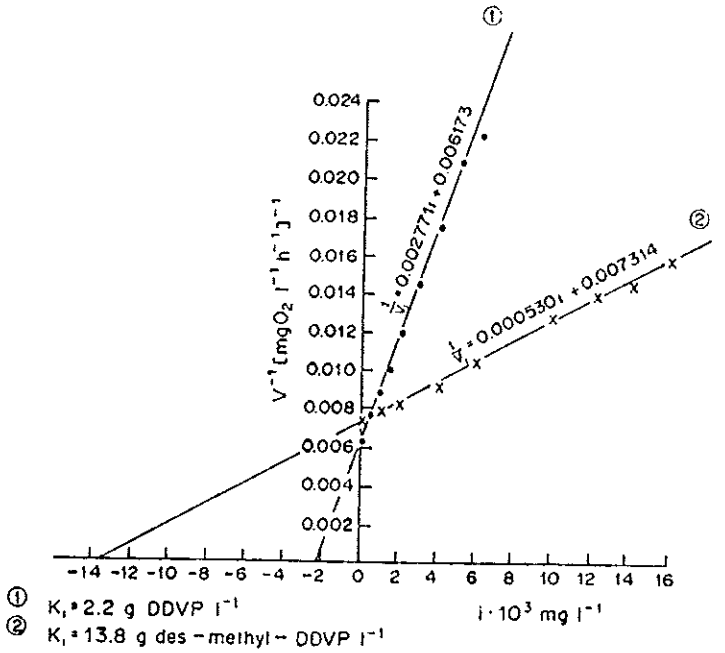

Fig. 5. Determination of the K_i value for DDVP and des-methyl DDVP.

is difficult. Some of them insist on the inhibition of the chemical reaction rate or both on the growth and chemical reaction rate (BOD test) the others on the growth or survival parameters (LC_{50}).

As it was mentioned earlier, the K_i coefficient is implicated in the biochemical models describing inhibition. These models have been principally constructed for systems involving a single enzyme, single substrate and single inhibitor. The presented work proves the possibility of the K_i coefficient measurement within bacterial systems. This measurement does not replace the other methods but can be considered as a useful tool for the quick assessment of the inhibitory potential of the individual compound. The advantage of being able to assess the toxic effect of a particular inhibitor within about 2 h makes the described measurement attractive both from the research and practical point of view.

CONCLUSIONS

(1) The possibility of determining the inhibition coefficient K_i in activated sludge systems by means of respiration rate measurements by the application of a dissolved oxygen meter have been proved.

(2) The measured values of K_i were equal to:

87 mg l^{-1} of chromium Cr^{6+};
0.293 mg l^{-1} of cyanide CN^-;
2220 mg l^{-1} of DDVP;
13,800 mg l^{-1} of des-methyl DDVP.

(3) The applied method of measurement of the inhibition coefficient is simple and time-saving.

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