

7 DETERMINATION OF TOXICITY THRESHOLDS OF INDUSTRIAL WASTESTREAMS TO ACTIVATED SLUDGE PROCESS USING FED BATCH REACTOR

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INTRODUCTION

Upsets of activated sludge processes at Publicly Owned Treatment Works (POTWs) can be caused by toxic wastestreams discharged by industrial users. A mild case of toxicity can manifest itself in depression of nitrification rates (if applicable) or in killing the protozoan population with a resulting increase in the effluent turbidity. These two classes of the activated sludge population (i.e., nitrifiers and protozoa) are more vulnerable to toxins than are chemoorganotrophic bacteria. In more serious toxicity cases, POTWs can suffer loss of treatment capacity leading to a prolonged recovery period and, in extreme cases, causing need for reseeded the plant. Toxic upsets are sometimes followed by a filamentous bulking episode. A POTW upset may result in failure to meet discharge permit limits and can have a detrimental effect on the receiving stream.

In order to reduce the chances of a toxic upset, some POTWs with the voluntary cooperation of major industrial users, have implemented a program of screening industrial wastestreams for potential toxicity to the activated sludge. Such programs are particularly desirable and effective when industrial discharges contribute a significant part of the POTW's load and when the characteristics of the industrial discharges change significantly and frequently (such as those associated with specialty organic chemical plants).

If the composition of a wastestream is known, its potential impact on a biological treatment process can be evaluated from literature data or by using toxicity estimation methods based on molecular structure of the subject compounds. The lack of an adequate data base and the potential presence of synergistic/antagonistic effects limit application of this method, however.

Experimental methods of assessing toxicity to biological treatment can be divided into three categories: 1) respirometric methods, 2) methods based on measurement of specific bacterial cell constituents, 3) methods measuring inhibition of substrate removal.

Respirometric methods include direct, short-term measurement of oxygen uptake rate inhibition;^{1,2} inhibition of BOD₅ measurement;³ and cumulative measurements of oxygen consumption in the Warburg apparatus or modifications thereof.^{4,5} The major problem with the respirometric methods is that interpretation of the results is difficult for streams containing biodegradable constituents. In addition, some classes of organic toxicants (i.e., substituted phenols) have been found to stimulate oxygen consumption without an accompanying substrate removal at less than the threshold toxicant concentration. This effect has been attributed to uncoupling of oxidative phosphorylation, resulting in loss of respiratory control and oxidation of intracellular constituents with loss of biomass.^{6,7}

Several methods for evaluating toxic effects by measurement of specific intracellular constituents have been developed. Determination of the energy storing compound, adenosine triphosphate (ATP), has been shown to be sensitive to the effects of uncouplers, but not to heavy metals.⁸ Another method in this category is measurement of dehydrogenase activity.⁹

Assays using cultures or organisms other than heterogenous activated sludge are sometimes used to evaluate potential toxicity to activated sludge. The Microtox[®] test, during which inhibition of *Photobacterium phosphoreum* light production is measured, is perhaps the best known of these surrogate toxicity assays.¹⁰ While this specific strain of bacteria has been found to be a sensitive toxicity

indicator, the direct applicability of Microtox[®] results to toxicity to heterogenous activated sludge microorganisms is still open to question.⁸

More direct methods of assessing toxicity to biological treatment are measurements of inhibition of its primary function—substrate removal. Substrate removal inhibition can be measured in continuous flow reactors^{9,11} or in batch tests.¹² There are trade-offs between these two approaches. Continuous flow tests are expensive and time-consuming, particularly if a range of different toxicant concentrations is to be tested. On the other hand, batch tests typically neglect the effects of acclimation and do not detect chronic effects. Both collective (TOC, COD) and compound-specific analytical methods can be used for substrate removal monitoring in these toxicity assessment tests. Sludge concentration, biomass acclimation and age, presence of complexing agents, and potential toxicant sorption on biomass, among other factors, can influence test results and need to be considered, regardless of the test protocol. For a comprehensive review of these factors, refer to Schneider.⁸

The fed batch reactor (FBR) procedure is one of the methods employing measurement of substrate removal. The FBR test was originally developed by Williamson and McCarty¹³ for rapid determination of nitrification kinetic coefficients. Philbrook and Grady¹⁴ used FBR techniques to evaluate biodegradation kinetics for priority pollutants (2-chlorophenol). Watkin⁷ adapted the FBR procedure for determination of inhibitory and non-inhibitory responses of 2,4-dichlorophenol and glucose.

In the study reported herein, a fed batch reactor procedure was investigated as a method to determine toxicity of industrial discharges and individual chemicals to activated sludge processes. The method is based on continuous addition of a high strength feed containing a potential toxicant to a batch activated sludge reactor for a period of two to three hours. In this way, sludge response can be obtained over a large toxicant concentration range in a single test.

EXPERIMENTAL PROCEDURES

A schematic diagram of the fed batch reactor (FBR) configuration used is shown in Figure 1. The reactor consisted of a two-liter aeration tank into which high strength wastewater was fed at a flow rate of 0.1 to 0.2 L/hr. The initial FBR volume and feed flow rate were selected so the feed volume added to the FBR during the test was small compared to the initial volume. Consequently, the sludge concentration in the vessel and the substrate addition rate did not change appreciably during the test. Moreover, the withdrawal of reactor contents for the sampling approximately compensated for the increase in volume due to the substrate addition. The sample withdrawals also compensated for the build-up of biomass concentration due to growth.

During the two- to three-hour testing period, oxygen uptake rate, TOC or COD, and pH were determined at time intervals of 15 to 30 min, together with other measurements required for a specific application. Initial and final mixed liquor volatile suspended solids (MLVSS), total dissolved solids (TDS), reactor volume, and influent flow rates were also determined. If necessary, adequate inorganic nutrients were added to the wastewater sample being tested, and the feed was neutralized.

The sludge samples tested in the FBR were usually taken from full-scale activated sludge systems, typically a POTW. In this way the complicating effects of sorption and acclimation should be minimized. In the time between the sludge sample collection and test initiation, the mixed liquor was maintained by batch or continuous feeding with POTW wastewater (without the tested stream component) or with readily degradable organic substrate. Prior to testing, the sludge was washed with deionized water and the salinity readjusted with NaCl. The reactor was usually operated at an initial MLVSS of approximately 2,000 mg/L.

The discharges evaluated by the FBR test fell into two general categories: samples of actual process wastewater of a mixed or unknown composition and, less frequently, specific compounds. For an actual wastewater stream, the sample was first analyzed for organic and inorganic strength, and a similar characterization was performed on the control influent to the biological treatment system. In preparing the FBR feed, the tested wastestream was diluted with the control influent such that the concentration of the tested wastestream in the reactor at the end of the test would be equal to 2-5 times that expected in the full-scale application. If the strength of the prepared feed was less than 500 mg/L TOC, it was supplemented with additional substrate (glucose or nutrient broth), in order to obtain an appreciable TOC increase in the reactor during the FBR run.

When warranted, the FBR test results were processed using a simple computer program which introduced corrections due to the changes in the reactor's volume during the test, accounted for the sludge and substrate withdrawn with samples and calculated substrate removal and oxygen uptake rates in specific terms.

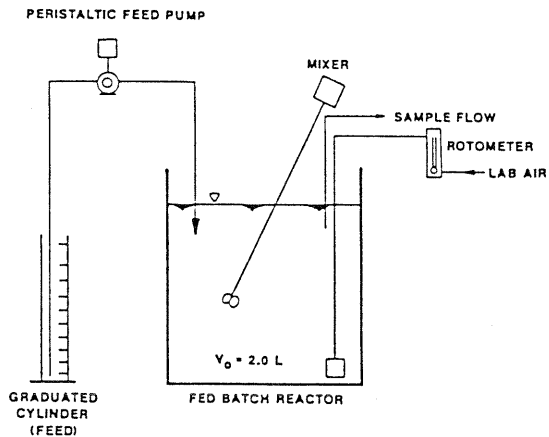


Figure 1. Fed batch reactor configuration.

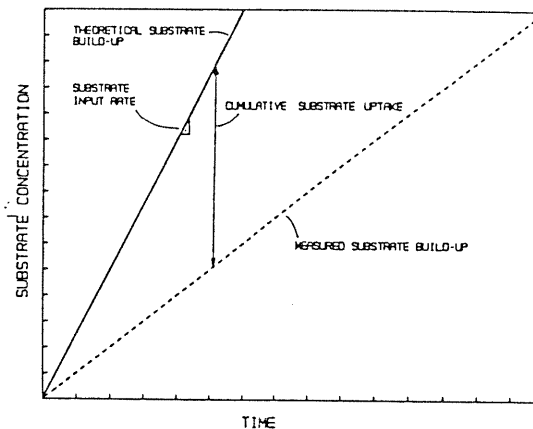


Figure 2. Theoretical response of a fed batch reactor.

THEORETICAL RESPONSE OF A FED BATCH REACTOR

Figure 2 depicts an idealized response of a fed batch reactor when no toxic effects are present. The measured substrate concentration increases at a constant rate throughout the test, and the difference between the slope of this line and the theoretical substrate build-up curve is equal to the maximum reaction rate. It is assumed that, shortly after the start of the test, the substrate concentration in the reactor becomes much higher than the saturation constant (K_s), and therefore the reaction proceeds at its maximum rate from the onset of the test. The oxygen uptake rate initially rapidly increases from the endogenous respiration rate to a maximum specific value and remains constant throughout the test.

The expected response of a fed batch reactor in terms of organic substrate removal inhibition can be modeled using several inhibition models from the literature.

Competitive Inhibition Model

$$R_r = R_m S / (S + K_s (1 + I/K_i)) \quad (1)$$

where R_r = reaction rate
 R_m = maximum reaction rate
 S = organic substrate concentration
 K_s = saturation constant
 I = inhibitor concentration
 K_i = inhibition constant

Figure 3 presents results of FBR response modeling based on Equation 1 for different rates of toxicant concentration increase. Mathematical analysis of Equation 1 and review of Figure 3 demonstrate that, for substrate concentrations much greater than K_s which is usually the case in an FBR test, the reaction rate is constant throughout the test, resulting in a linear substrate build-up curve. This is a consequence of parallel and proportional increases in the substrate and toxicant concentrations as the test progresses. Inhibition can be detected if a parallel control test, without inhibitors present, can be performed.

Noncompetitive Inhibition Model

$$R_r = R'_m S / ((S + K_s)(1 + I/K_i)) \quad (2)$$

Equation 2 predicts a gradual decrease in the reaction rate, as illustrated in Figure 4. At the beginning of the test, the substrate build-up is the same as in the uninhibited control. At some time into the test, which depends on the ratio of inhibitor addition rate to K_i , inhibition of the substrate removal rate starts to manifest itself as a gradual, upward deflection of the substrate build-up curve. Eventually, the substrate build-up curve becomes parallel to the calculated feed input line, indicating complete inhibition of the substrate removal.

The Haldane equation¹⁵ is a variation of the noncompetitive model and predicts a similar FBR response.

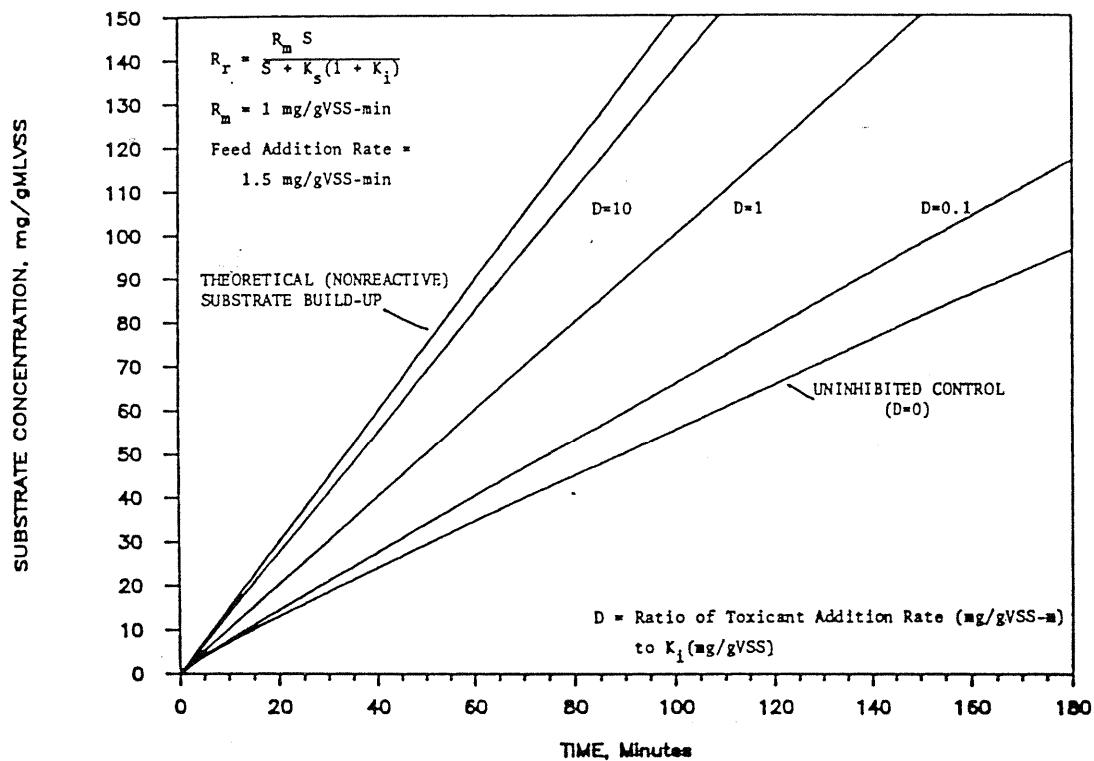


Figure 3. Theoretical FBR response – competitive inhibition.

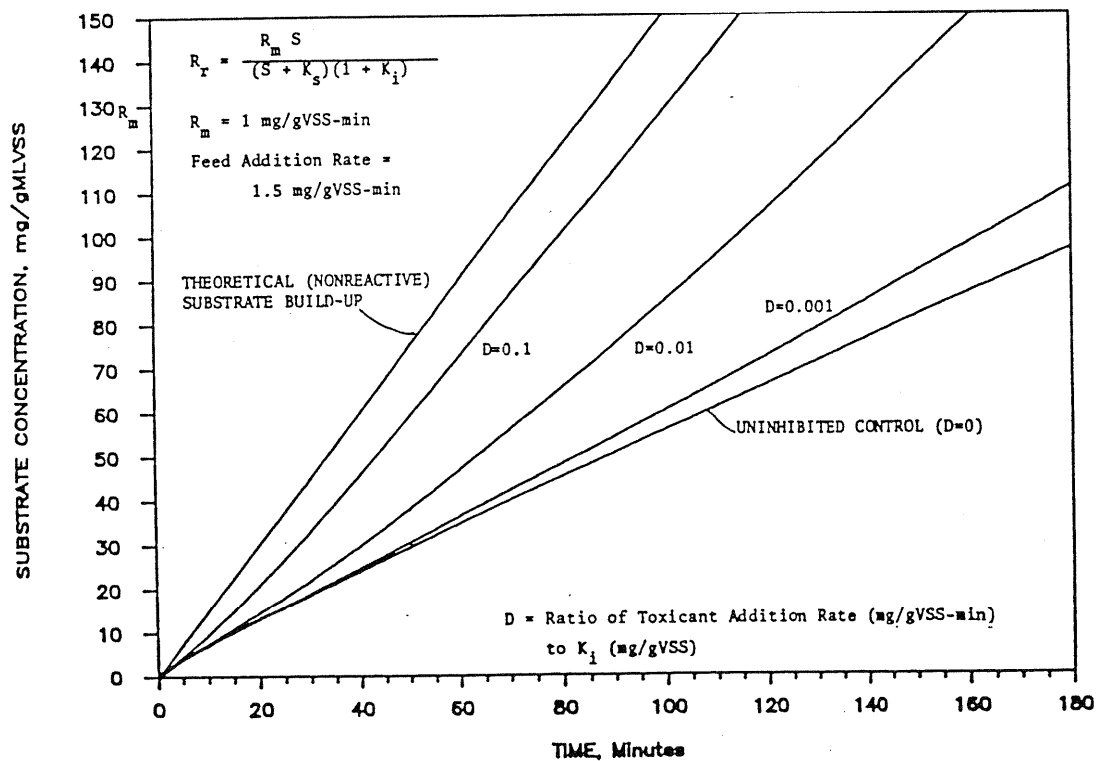


Figure 4. Theoretical FBR response – noncompetitive inhibition.

Other Inhibition Models

Several other inhibition models proposed in the literature use an exponential term in the denominator of the rate expression. An example is the following equation by Watkin.⁷

$$R_r = R_m S / ((S + K_s)(1 + (I/K_i)^n)) \quad (3)$$

where: $n = \text{constant}$

Exponential inhibition models predict that at some point into the test, an upward deflection of the substrate build-up curve will occur, marking the threshold inhibitor concentration (Figure 5). The sharpness of the deflection depends on the value of the constant, n .

Apart from the effect on the substrate removal rate, inhibition would also be manifested during the test by a declining oxygen uptake rate (OUR), provided that respiration rates are not impaired by uncoupling, as discussed in the previous section.

RESULTS

Application of the FBR procedure to determine inhibitory effects is illustrated in this section, with results from several tests on actual and synthesized wastestreams.

Figure 6 presents an FBR test response to an actual chemical plant wastestream. The substrate build-up curve was linear throughout test, without signs of upward deflection. Congruently, the oxygen uptake rate, after increasing from 20 to 50 mg O₂/L-hr during the first 20 minutes, remained stable and actually increased slightly throughout the test.

Similar responses were obtained in parallel FBR tests in which a synthetic feed, based on nutrient broth, was used (Figure 7). The parallel tests were performed under identical conditions, except mercuric chloride was added to the nutrient broth feed in one of the tests. As expected, the substrate build-up curve deflected upward as the test progressed and, after the first 100 minutes, it became parallel to the substrate input line—indicating complete inhibition of the biological reaction (Figure 8). The OUR, after an initial increase, gradually declined, eventually to less than the initial endogenous rate. In a subsequent test, the mercuric chloride was added to the feed at a higher concentration than in the preceding test at 90 minutes into the test. The FBR response (Figure 9) initially closely paralleled that of the uninhibited control (compare with Figure 7). Shortly after the toxicant addition to the feed, the OUR sharply declined, and the substrate build-up curve essentially paralleled that of the feed input. The results of these three tests are summarized in Figure 10, where the cumulative

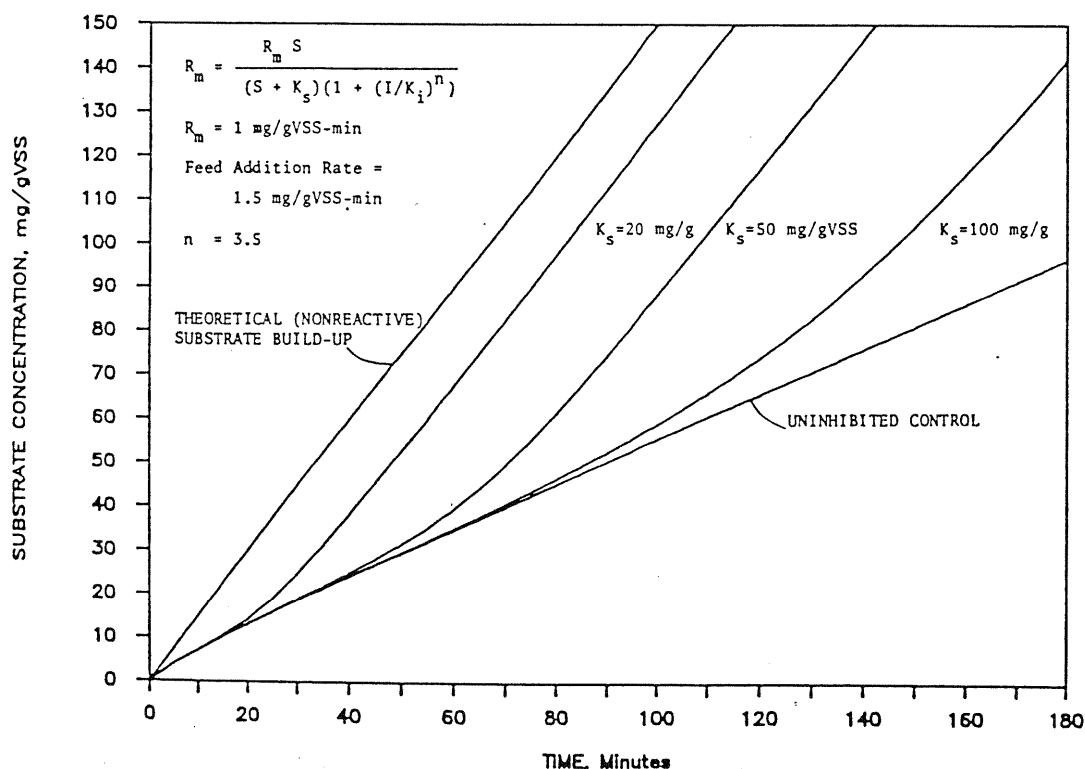


Figure 5. Theoretical FBR response—noncompetitive inhibition, type II.

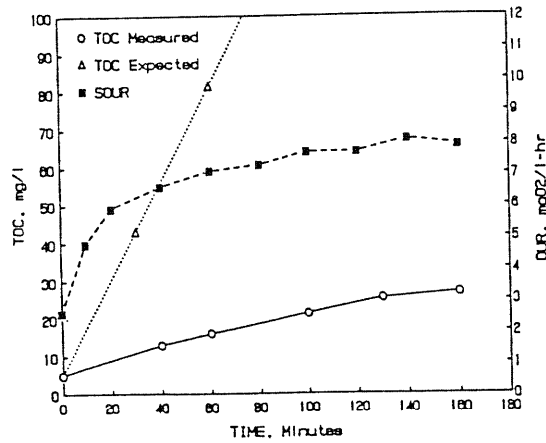


Figure 6. Results of FBR test with chemical waste stream (A23).

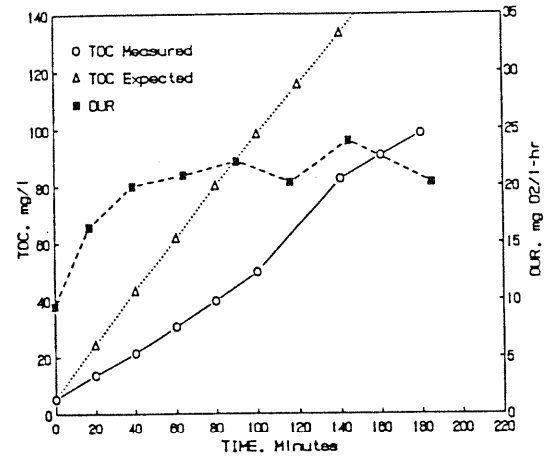


Figure 7. FBR test results. Nutrient broth control.

specific substrate removal is plotted. From Figure 10, in both tests with mercuric chloride, the sludge's ability to remove the substrate was completely impaired at mercuric chloride concentrations of about 3 mg/L. This result is consistent with toxicity data obtained by other methods, as reported in the literature.¹²

In another FBR test, dichlorophenol (DCP) was added to the nutrient broth based feed at 90 minutes into the test. A rapid decline in the OUR resulted (Figure 11). The DCP addition increased the TOC concentration of the feed; however, the cumulative TOC removal plot (Figure 12) illustrates that the substrate removal rate declined as the DCP concentration increased.

The results of two FBR tests in which inhibition was observed using actual industrial wastestreams are presented in Figures 13 and 14. In the first example (Figure 13), an upward deflection of the substrate build-up curve indicated progressing inhibition and was accompanied by a gradual decline in the OUR. The rapid increase in the OUR after 40 minutes, followed by a gradual decline, indicated the uncoupling effect. A similar OUR response was recorded in the second test (Figure 14). The upward deflection of the substrate build-up curve in this test was almost complete, and no substrate removal occurred after the first 90 minutes.

More than 100 wastestream samples, originating from three different chemical plants, were tested using FBR procedures. Whenever inhibition to biological treatment was indicated, a safe procedure for treatment of the particular wastestream was determined. Based on the threshold inhibition concentration determined from the FBR test, a safe discharge rate for the inhibitory wastestream was calculated. Alternative methods of handling inhibitory wastestreams included pretreatment and off-site disposal.

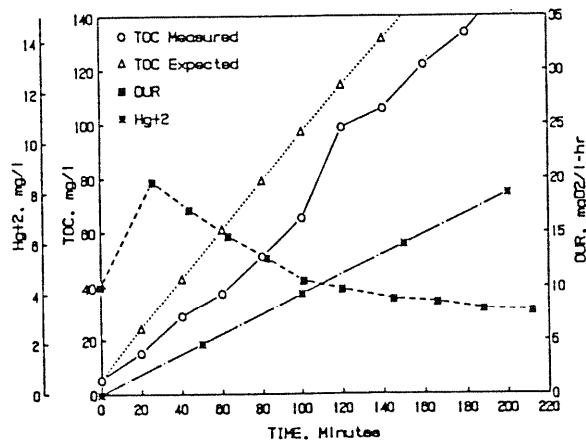


Figure 8. FBR test results. Nutrient broth with mercuric added to the feed at time = 0 minutes.

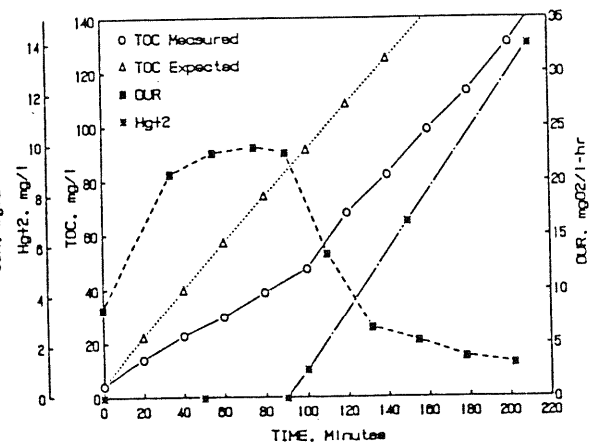


Figure 9. FBR test results. Nutrient broth with mercuric added at time = 90 minutes.

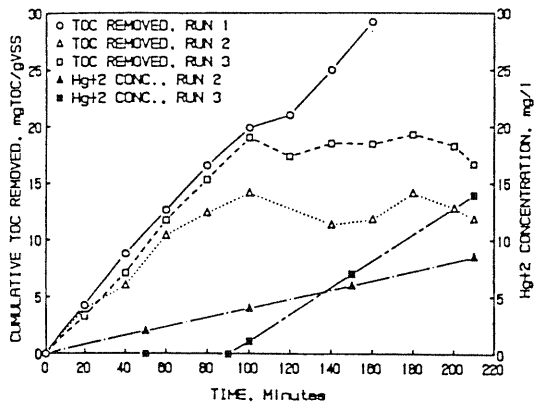


Figure 10. Summary of FBR tests with mercuric. Plot of cumulative substrate removed. Run 1 = control; Run 2 = Hg added at time 0; Run 3 = Hg added at time 90 minutes.

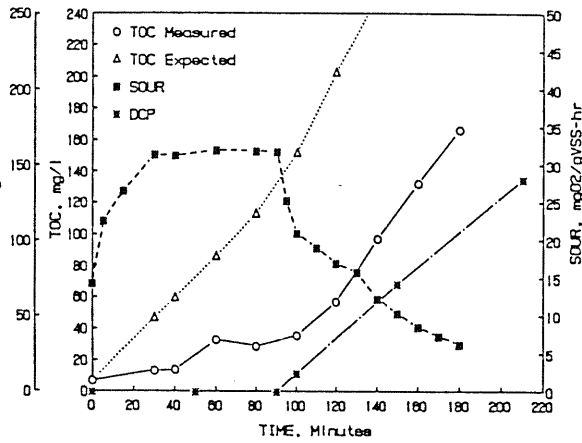


Figure 11. Results of FBR test with nutrient broth and DCP added to feed at time = 90 minutes.

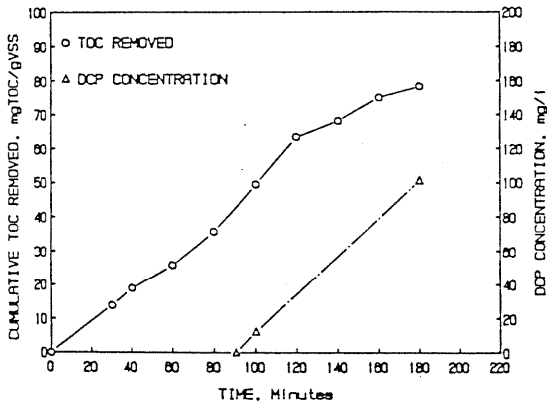


Figure 12. Plot of cumulative substrate removed in FBR test with DCP added at T = 90 minutes.

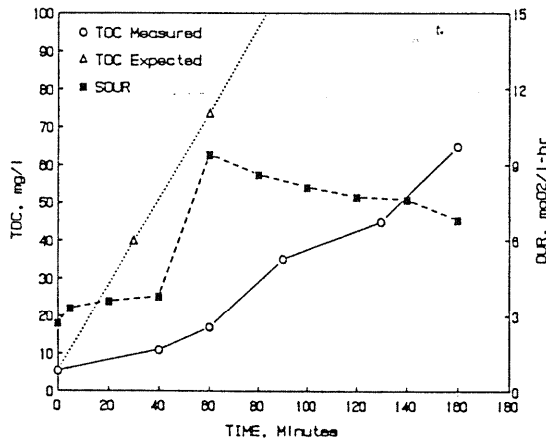


Figure 13. Results of FBR test with chemical waste stream (CS1).

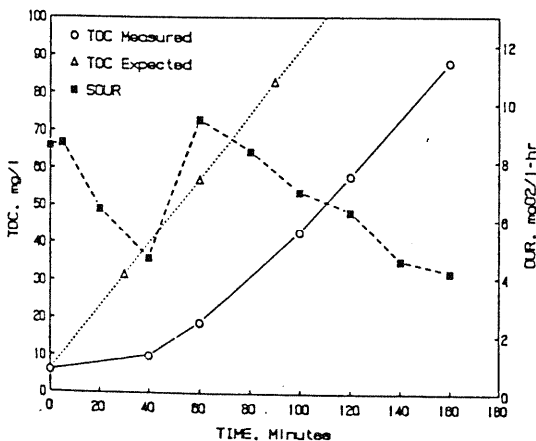


Figure 14. Results of FBR test with chemical waste stream (CS2).

DISCUSSION

The interpretation of data obtained from a single FBR test depends on factors such as type of toxicant (organic or inorganic), composition of the suspect stream (specific compound or unknown), presence of organic substrate in the stream, and type of toxicant response (inhibition model). The interrelationship of these factors is discussed below.

Specific Compounds

Inorganic Compounds. When using the FBR procedure to evaluate the inhibition effects of specific inorganic compounds (e.g., heavy metals), a control FBR test can easily be performed using a sample of the influent to the biological treatment system under consideration. If the influent TOC is less than 500 mg/L, supplemental substrate addition (e.g., glucose or nutrient broth) may be required in order to obtain a measurable TOC increase during the FBR test. The tested compound's concentration in the FBR feed is under the experimenter's control and is selected so the TOC concentration in the test reactor, at the test end, is 2-5 times greater than the maximum concentration expected in the full-scale application. Regardless of the inhibition model used, comparison with the control FBR test enables not only determination of toxic inhibitory effects, but also of the approximate threshold concentration. If the competitive inhibition model applies, the threshold concentration can be estimated by comparing the slope of the tested compound's build-up curve with the slopes of the uninhibited control build-up and input curves (e.g., Figure 3). For the other models, the tested compound's concentration at the time that upward deflection of the build-up curve is observed corresponds to the threshold value.

Organic Compounds. If the specific compound being tested is at least partially biodegradable, interpretation of the FBR test results is more difficult. The build-up of organic matter in the reactor as measured by collective parameters, such as TOC or COD, interferes with accurate calculation of the toxicant concentration at which inhibition occurs; only a rough estimation is possible. The usefulness of an uninhibited control test is limited in this situation, because additional substrate (the specific compound being tested) in the influent does not allow direct comparison of the substrate removal rates and OURs.

Unknown Compounds

In tests with actual wastestreams, specific compounds suspected of biological treatment inhibition are frequently unknown. In these cases, the experimenter is limited to selecting the volumetric ratio of the tested wastestream to the full-scale influent in the FBR feed.

If little or no organic matter is present in the tested wastestream, a control FBR test (influent without the tested wastestream) can provide useful information, particularly when the competitive inhibition model applies.

If organic substrate is present in the tested wastestream, the usefulness of the control FBR test is limited, for the same reasons as described previously for specific organic compounds. Where the competitive inhibition model applies, substrate build-up data can give a qualitative answer if inhibition is present at the selected feed composition. When other models are applicable, the upward deflection of the substrate build-up curve enables estimation of the threshold concentration based on volumetric ratio.

OUR Aspects

Besides the information obtained from the substrate build-up data, analysis of OUR provides additional, and on many occasions critical, information on the toxicity of the tested wastestream. Immediately after the test start, the OUR invariably increases to a value corresponding to a maximum respiration rate for the given sludge and substrate mixture. If that initial OUR increase is followed by a sustained decline, inhibition is clearly present. Varying, uncontrolled OUR indicates uncoupling of oxidative phosphorylation which is characteristic of some toxicants.

SUMMARY AND CONCLUSIONS

The FBR test was found to be a simple and inexpensive test for rapid screening of wastestreams for acute inhibition/toxicity to activated sludge. The test has been used to help determine if newly generated wastestreams will impair biological treatment performance at POTW's and industrial activated sludge plants at the expected rates of discharge. Under favorable conditions, a threshold concentration (or rate of discharge) can be established. The test is performed using samples of the full-

scale biological treatment sludge, under conditions which more closely resemble the actual, continuous flow situation than do standard batch tests with a step input of the tested wastestream.

Disadvantages of the FBR test include some drawbacks inherent to all batch-type tests including, most importantly, lack of definition of chronic effects. Furthermore, biological sludge used in the FBR test does not have the benefit of acclimation to the tested wastestream. Interpretation of the substrate removal rates may also be complicated by substrate biosorption, i.e., rapid initial substrate uptake onto the biological floc sorptive mechanisms. Some sludges, particularly in combination with simple, carbohydrate-based substrates, exhibit biosorption. In an FBR test, following the rapid initial substrate uptake, a decrease in the apparent removal rate occurs as manifested by an upward deflection of the substrate build-up curve.¹⁶ This can be mistaken for inhibition. Evaluation of the OUR data and, when appropriate, results from control FBR tests help in proper data interpretation.

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