A FED-BATCH TECHNIQUE TO EVALUATE BIODEGRADATION RATES OF INHIBITORY COMPOUNDS WITH ANAEROBIC BIOFILMS ATTACHED TO GRANULAR ACTIVATED CARBON

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ABSTRACT

Adsorption kinetics render measurements of substrate utilization rates in biofilms attached to adsorbing materials difficult. A fed-batch technique which maintains a constant concentration of substrate in the presence of biomass attached to granular activated carbon (GAC) was developed to measure biological kinetics independent of adsorption kinetics. The fed-batch technique used pre-adsorbed substrate on GAC as a source of substrate. During the experiment, the mass of substrate that is biodegraded is negligible when compared to the large mass of adsorbed substrate. Near equilibrium adsorption phenomena maintains the concentration of substrate constant throughout the experiment and eliminates mass transport resistance within the biofilms. Substrate utilization rates were measured at a specific substrate concentration by monitoring methane gas production rates. Samples of GAC with attached biomass were removed from two expanded-bed anaerobic reactors. GAC samples from a 3-ethylphenol (3-ep) fed reactor were used in batch experiments with 3-ep as the substrate and GAC samples from an orthochlorophenol (OCP) fed reactor were used in batch experiments with OCP as the substrate. Data were observed to fit Haldane kinetics and predictions from the Haldane kinetic parameters were in agreement with transient behavior from the 3-ep fed reactor. This technique was useful in predicting threshold inhibitory levels for continuous treatment of inhibitory wastewaters in expanded-bed GAC reactors. The fed-batch technique might also be applied to measure substrate utilization rates of biomass attached to other adsorbable materials such as soils.

KEY WORDS

Adsorption, Inhibition; Fed-Batch Technique; Kinetics; Biofilms; Granular Activated Carbon.

INTRODUCTION

A variety of biologically inhibitory organic compounds, in particular phenolic compounds, have been successfully treated in continuous flow, anaerobic granular activated carbon (GAC) expanded-bed reactors (Nakhla et al., 1988). One important operational feature of the GAC reactor is maintenance of the concentration of inhibitory compounds below the "threshold" inhibitory level (Fox et al., 1988). Knowledge of the kinetics of inhibitory substrates could be used to predict safe operating concentrations and this information could be applied to reactor operation and design. Measurement of biological kinetics in biofilms attached to GAC particles presents a severe problem when the substrates to be tested are readily adsorbed. The same problem in the measurement of biological kinetics exists for biomass attached to other adsorbable materials such as soils. During batch tests, simultaneous removal of substrate by biological utilization and adsorption makes analysis of the biological kinetics difficult if not impossible.

Most measurements of biodegradation rates of inhibitory substrate have been done with batch tests using suspended biomass. Steady-state operation of chemostats at high concentrations
of inhibitory substrates is impossible due to system instability (Calvin and Rozich, 1986). The Haldane inhibition model has been successfully used to describe the microbial degradation of several phenolic compounds. Batch test data on the aerobic biodegradation of phenol, 2,4-dinitrophenol, orthochlorophenol, and pentachlorophenol were used to fit the Haldane inhibition model (Haldane, 1965, Klecka and Maier, 1985, Calvin and Rozich, 1986, Gaudy et al., 1988). Suidan et al. (1988) used the Haldane inhibition model to describe the anaerobic biodegradation of phenol using data obtained from both a chemostat and batch tests. Thus, batch tests are a proven technique for the measurement of biodegradation rates of inhibitory compounds. However, separation of anaerobic biofilms from attachment surfaces to obtain suspended biomass for batch tests or chemostat studies could lead to erroneous results. Culture changes between suspended growth and attached growth are likely to occur and symbiotic relationships essential to anaerobic microcosms present in biofilms might be disrupted.

In this study, a fed-batch technique was developed to measure biodegradation rates in biofilms attached to GAC. Fed-batch techniques typically maintain a constant substrate concentration by the semi-continuous addition of substrate at a rate equivalent to the rate of biological utilization. In the technique discussed herein, a large mass of substrate is added to the system by the addition of GAC with pre-adsorbed substrate. The large mass of adsorbed substrate is much greater than the mass of substrate utilized during the test. Substrate is desorbed from the GAC at a rate comparable to the rate of biological substrate utilization. Since a negligible change in the total mass of substrate occurs, the substrate concentration is maintained constant. Thus, the GAC with adsorbed substrate acts as both a source of substrate and as a buffer to maintain a constant substrate concentration. The technique has the important advantage that batch tests are performed directly with attached biomass samples and substrate utilization rates are measured at a specific substrate concentration. A critical disadvantage of the technique is that substrate utilization rates must be indirectly measured as the rate of product formation, i.e., methane.

MATERIALS AND METHODS

GAC samples were removed from two 11 liter anaerobic GAC expanded-bed reactors which were temperature controlled at 35°C. Both reactors contained 20x30 U.S. standard mesh size, day = 0.7 mm, Calgon F-400 GAC. One reactor was a two-stage reactor with a 1 liter expanded-bed GAC (side) reactor attached to the recycle line of the main reactor. The main reactor contained 1.35 kg GAC while the side reactor contained 0.15 kg GAC and the primary purpose of the side reactor was for GAC adsorption. The two-stage reactor was fed a mixture of acetate and 3-ethylphenol (3-ep) at influent concentrations of 5 g/l and 1.875-2.5 g/l, respectively, at the time GAC samples were removed from the reactor. The influent flowrate was maintained constant at 10 liters/d. Details of the operation of the two-stage reactor are presented elsewhere (Fox et al., submitted). The second GAC reactor was fed a mixture of acetate, phenol, and orthochlorophenol (OCP) at influent concentrations of 2 g/l, 1 g/l, and 4 g/l, respectively, at the time GAC samples were removed. The influent flowrate was maintained at 3 liters/d and the reactor contained 1.5 kg GAC.

Batch experiments were performed entirely in 160 ml serum bottles. Three grams of virgin GAC were used in each sample and 65-155 ml of concentrated 3-ep solution (5-6.5 g 3-ep/l) or OCP solution (6.5-9 g OCP/l) was added to serum bottles containing the virgin GAC. Typically, serum bottles were prepared in duplicate. Serum bottle headspace was purged with nitrogen and serum bottles were sealed with butyl rubber stoppers and placed on a shaker in a temperature control room (35°C). After 2-3 days of contact time, the liquid solution was decanted from the GAC. The serum bottles containing GAC samples loaded with substrate were then filled with 90-95 ml of reactor effluent and 4 ml of phosphate buffer (0.1 M, pH = 7.0). After the headspace was purged with nitrogen, serum bottles containing GAC loaded with substrate and reactor effluent were sealed and placed on a shaker in the temperature control room for one day. This allowed substrate to desorb from the GAC into the effluent and for equilibrium to be approached between the adsorbed mass of substrate and liquid concentration of substrate. Finally, GAC media samples (1.0-1.8 g) from the appropriate reactor were added to the serum bottles containing reactor effluent and GAC loaded with substrate. GAC media samples were removed from the reactors by lowering a 20 cm long, 1 cm ID glass tube with a cork stopper on the bottom into the center of the expanded-bed. Media was allowed to gently fall into the glass tube. After the glass tube was filled with media, the media was then transferred to the serum bottles by removing the cork from the glass tube and allowing the media to rapidly fall into the effluent in the serum bottle while the headspace was being purged with nitrogen. The total volume occupied in the bottles was adjusted to 100 ml with reactor effluent and the serum bottles were sealed with a butyl rubber stopper.
Technique to evaluate biodegradation rates

All serum bottles were placed in a temperature control room (35°C) for twenty minutes prior to the beginning of the experiment to ensure that the temperature of the samples was equilibrated. Following equilibration, gas was released from the serum bottles with a syringe to equilibrate the pressure inside the serum bottles with atmospheric pressure. Serum bottles were placed on a shaker table to provide mixing and the experiment was begun immediately since substrate was already present. Methane gas production was monitored with time using the syringe technique as described by Owen et al. (1979). Gas samples were analyzed for methane content. One ml liquid samples were also taken with a syringe and immediately acidified with H3PO4 at the same time that methane gas production was monitored. Liquid samples were analyzed with gas chromatography for substrate concentrations and possible intermediates such as phenol and or volatile acids. After completion of the test, the pH was analyzed for and media samples were dried at 104°C and weighed. The weight of GAC media samples was corrected for the mass of substrate adsorbed and for the mass of virgin GAC.

Total methane production was calculated by combining the methane content, total gas produced, gas pressure, and gas temperature. Methane gas production was corrected for water vapor and Henry's Law was used to estimate the volume of dissolved methane in the liquid phase. Changes in volume due to the withdrawal of liquid samples during serum bottle batch tests were corrected for. All calculations were done with a LOTUS spreadsheet (Lotus Development Corp., U.S.A.).

RESULTS AND DISCUSSION

Four independent sets of batch experiments were performed with GAC samples with 3-ep as the substrate and GAC withdrawn from the main reactor of the two stage reactor and one set of batch experiments was performed with GCP as the substrate with GAC samples from the second reactor. All four sets of batch experiments with 3-ep as the substrate were done with GAC samples removed from the two-stage reactor at steady-state or pseudo-steady-state conditions. A summary of reactor performance at the time GAC samples were removed and the concentration ranges of 3-ep used in each set of batch tests are presented in Table 1. The concentrations used during the first three experiments ranged from 10 to 110 mg 3-ep/l while experiment 4 was designed to cover a range of 3-ep concentrations from 3 mg/l to 180 mg/l. A wide range of 3-ep concentrations was necessary to search for the parameters in the Haldane equation.

\[ \frac{dS}{dt} = \frac{kX}{K_s + S + S^2/K_i} \]  

Where V is the reactor volume, S is the substrate concentration, and kX is a measure of the maximum substrate utilization rate. Low 3-ep concentrations were necessary to characterize Ks, the Half-Velocity Constant, and high 3-ep concentrations were needed to characterize the inhibition constant, Ki, the Haldane inhibition constant.

<table>
<thead>
<tr>
<th>Batch Test Experiment No.</th>
<th>Reactor Effluent 3-ep, mg/l</th>
<th>Rate of 3-ep Biodegradation in Reactor g 3-ep/day</th>
<th>Concentration Range of 3-ep Used in Batch Test, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.38</td>
<td>12.0</td>
<td>10.4-83.</td>
</tr>
<tr>
<td>2</td>
<td>2.50</td>
<td>12.5</td>
<td>7.5-41.7</td>
</tr>
<tr>
<td>3</td>
<td>4.06</td>
<td>15.8</td>
<td>12.9-108</td>
</tr>
<tr>
<td>4</td>
<td>19.8</td>
<td>25.5</td>
<td>3.0-181</td>
</tr>
</tbody>
</table>

An example of results from duplicate experiments with 3-ep as the substrate are presented in Figure 1. The 3-ep concentration was virtually constant throughout the experiment. Methane gas production was linear with respect to time and correlation coefficients greater than 0.99 resulted from linear regression analysis. Methane gas production rates were measured as the slope of methane production versus time. Some fed-batch experiments did exhibit a decrease in substrate concentration as the experiments progressed, however, the decreases in substrate concentration were generally less than five percent. The substrate concentration for each experiment was calculated as the average of measured substrate concentrations. No intermediates were detected during the experiments indicating that the rate-limiting step in the conversion of 3-ep to methane was the initial attack on 3-ep.

An assumption critical to the analysis of experimental data is the assumption of fully penetrated biofilms during the experiments. This assumption allows for the results to be
Fig. 1. A sample of fed-batch experiment results with 3-ep as the substrate.

Treated as a completely mixed batch reactor. If equilibrium between the solid phase concentration of substrate and the liquid phase of substrate exists, then a concentration gradient in the biofilm separating the two phases must be negligible. Isotherm experiments were performed to measure the equilibrium relationship for 3-ep at 35°C under anaerobic conditions (Fox, 1989). A Freundlich isotherm fits the results well. The two-stage reactor was operated at equilibrium or near equilibrium conditions indicating that the biofilm within the reactor was fully penetrated with respect to 3-ep. The equilibrium liquid phase concentration expected during batch tests may be calculated using the Freundlich isotherm based on the total mass of 3-ep and total mass of GAC used. The total mass of 3-ep per total mass of GAC used and the measured and theoretical 3-ep concentrations for batch tests covering a wide range of liquid phase concentrations are presented in Table 2.

<table>
<thead>
<tr>
<th>Adsorbed Phase Concentration q, g 3-ep/gGAC</th>
<th>Predicted Equilibrium Liquid Phase Concentration mg 3-ep/l</th>
<th>Measured Liquid Phase Concentration mg 3-ep/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>.122</td>
<td>2.7</td>
<td>3.1-4.0</td>
</tr>
<tr>
<td>.150</td>
<td>7.5</td>
<td>5.5-6.5</td>
</tr>
<tr>
<td>.169</td>
<td>13.3</td>
<td>11-12</td>
</tr>
<tr>
<td>.188</td>
<td>22.0</td>
<td>16-18</td>
</tr>
<tr>
<td>.206</td>
<td>34.5</td>
<td>24-30</td>
</tr>
<tr>
<td>.225</td>
<td>52.7</td>
<td>44-46</td>
</tr>
<tr>
<td>.248</td>
<td>84.2</td>
<td>96-101</td>
</tr>
<tr>
<td>.274</td>
<td>144.</td>
<td>159-161</td>
</tr>
<tr>
<td>.290</td>
<td>179.</td>
<td>186-190</td>
</tr>
</tbody>
</table>

In general, theoretical equilibrium liquid phase concentrations are in agreement with measured liquid phase concentrations. At high 3-ep concentrations, the measured values tend to exceed equilibrium values while at lower 3-ep concentrations, equilibrium values tend to exceed measured values. This is a consequence of adsorption-desorption phenomena whereby 3-ep is desorbed from the 3 grams of virgin GAC to the GAC sample or vice-versa. In either
Technique to evaluate biodegradation rates

The four data sets obtained from the fed batch experiments with 3-ep were simultaneously used to search for the kinetic parameters in the Haldane equation. The fed batch experiments measured the rate of 3-ep biodegradation at a specific 3-ep concentration and three parameters in the Haldane equation were unknown, $K_s$, $K_i$, and $k_X$. The parameter, $k_X$, is treated as a measure of the biological activity and a homogeneous distribution of biomass on the GAC samples was assumed. Growth during the batch experiments was assumed negligible since a large sample of biomass was used in comparison to the mass of substrate utilized. Since the biological activity was different for each data set, the data sets were normalized to the observed biological activity in the reactor at the time the test was performed. Normalization was done by using the rate of 3-ep biodegradation and the effluent 3-ep concentration in the two-stage reactor at the time when GAC samples were removed. This increased the number of data points used during the search and reduced the number of unknown parameters. Normalization also eliminated errors associated with biomass measurements. Since a mixed culture was present, any biomass measurement would not specifically measure the 3-ep utilizing consortia. All four data sets were combined to search for the kinetic parameters, $K_s$ and $K_i$. Non-linear regression was used to search for the parameters by minimizing the error in the substrate utilization rate. A $K_s$ of 5.01 mg 3-ep/l and a $K_i$ of 143 mg 3-ep/l resulted from the search technique.

Model predictions for each data set based on the parameters found by non-linear regression are presented in Figure 2. Batch test data are plotted along with the point corresponding to the performance of the two-stage reactor at the time of each batch test. Since the levels of biological activity were normalized to results from the two-stage reactor, the model predictions were essentially fit to the reactor results and all model predictions fit the point corresponding to the performance of the reactor exactly. The values of $k_X$ presented in Figure 2 were calculated for each set of batch tests based on the results of the parameter search. Values of $k_X$ vary less than six percent indicating that the concentration of 3-ep utilizers in the reactor was constant during the period in which the fed-batch experiments were performed. In general, the Haldane equation predicts the observed trends in the data sets very well.

![Fig. 2. Fed-batch experiment results with Haldane model fit.](https://iwaponline.com/wst/article-pdf/23/7-9/1337/101810/1337.pdf)
Throughout the operation of the two-stage reactor, observed trends in 3-ep biodegradation agreed well with results from the fed-batch experiments. Biodegradation rates of 3-ep increased rapidly when the effluent 3-ep concentration increased from 2 mg/l to 12 mg/l. The Haldane model predicts that 3-ep biodegradation rates increase rapidly when the 3-ep concentration is increased from 0 to 15 mg/l and that the 3-ep biodegradation rate decreases at 3-ep concentrations greater than 30 mg/l. For short term transient experiments where growth may be assumed to be negligible, Haldane kinetics may be used to predict 3-ep utilization based on one biomass level. A variable loading experiment performed with the two-stage reactor is an example of such a short term experiment. During the variable loading experiment, the influent loading was tripled and returned to normal several times. The final time the loading was tripled, the effluent 3-ep concentration increased until severe inhibition was observed. The effluent 3-ep concentration was then decreased by replacement of GAC with virgin GAC in the side reactor during hours 153-160. Model prediction and experimental results are presented in Figure 3 for the variable loading experiment. Utilization rates of 3-ep were calculated based on effluent 3-ep concentrations and results are presented in terms of methane gas production. It was assumed that all the influent acetate was converted to methane since over 99% removal of acetate was observed during the experiment and acetate is weakly adsorbed on GAC. Although the experiment did last longer than five days, high shearing rates during the experiment likely prevented any accumulation of biomass. The biomass level (kX) used to estimate the methane gas production was based on the fed-batch test performed on GAC samples (Experiment 4) removed from the two-stage reactor during the variable loading experiment (Hour 46). Considering the nature of the variable loading experiment in which the influent loading was tripled and reduced to normal several times and effluent 3-ep concentrations ranged from 8 to 207 mg/l, the haldane model predicts observed trends in methane gas production well. This experiment also demonstrates that the fed-batch technique could be used to predict reactor failure and operational control of the reactor could be based on fed-batch experimental results to prevent reactor failure.

![Predicted Methane Gas Production vs Observed Methane Gas Production](https://iwaponline.com/wst/article-pdf/23/7-9/1337/101810/1337.pdf)

**Fig. 3.** Variable loading experiment and Haldane model prediction based on kinetic parameters from the fed-batch experiments.

A set of batch experiments was performed to characterize 3-ep biodegradation with suspended biomass. The goal of the experiments was to verify results obtained in the fed-batch experiments and identify intermediates in the biodegradation of 3-ep. Details of the experimental technique are presented by Fox (1989). One liter of biomass was suspended in reactor effluent by insonating 50 g of GAC. Aliquots of 100 ml of the suspended biomass mixture were placed in serum bottles. Batch tests were done with nominal initial 3-ep concentrations ranging from 15 mg/l to 205 mg/l. One serum bottle was spiked with phenol and another serum bottle was spiked with m-cresol, both suspected intermediates in 3-ep biodegradation, at nominal initial concentrations of 100 mg/l, respectively.
With only one exception, a lag phase was observed in the biodegradation of 3-ep. The exception was the sample which was not spiked with 3-ep and contained the 3-ep concentration of 15 mg/l present in the original effluent. Lag phases increased in duration with increasing initial nominal 3-ep concentration. With an assigned $kX$ value, the kinetic parameters obtained from the fed-batch experiments fit the results from the batch test with a nominal initial 3-ep concentration of 15.8 mg/l well (Figure 4). The lag phases prevent direct comparison of results from suspended biomass batch tests with the fed-batch tests performed with attached biomass. Phenol was detected at concentrations over 1 mg/l in several samples indicating that phenol is an intermediate in the biodegradation of 3-ep.

![Fig. 4. Haldane model fit of batch experiment with 3-ep as the substrate using kinetic parameters from the fed-batch technique.](https://iwaponline.com/wst/article-pdf/23/7-9/1337/101810/1337.pdf)

Results from the batch tests which were spiked with phenol and m-cresol were very similar to one another. Lag phases in the biodegradation of phenol and m-cresol were of similar duration and in both tests sequential substrate utilization was observed in both cases. Biodegradation of 3-ep did not begin until the concentration of phenol or m-cresol was below 50 mg/l even though 3-ep was the substrate that the microorganisms were acclimated to. Sequential substrate utilization in batch tests with phenolic compounds was also observed by Arvin et al. (1989) and Namkoong et al. (1989).

Meier et al. (submitted) observed that lag phases increased in duration with increasing initial concentrations of pentachlorophenol during batch tests with suspended biomass. It was proposed by Meier that lag phases were the result of toxicity and increasing concentrations of pentachlorophenol killed an increasing percentage of the pentachlorophenol utilizing organisms. Meier et al. (submitted) also found that the duration of lag phases decreased if the microorganisms were attached to sand particles prior to exposure to high concentrations of pentachlorophenol. The results of Meier are consistent with results observed with the biodegradation of 3-ep. The duration of lag phases in batch tests with suspended biomass increased with increasing initial concentrations of 3-ep. Fed-batch experiments with attached biomass did not exhibit any lag phase even at 3-ep concentrations as high as 180 mg/l. The mechanism whereby protection of attached microorganisms to toxicity was not clear since mass transport limitations were not significant in either set of experiments.

Results and model predictions from fed-batch experiments performed with orthochlorophenol (OCP) are presented in Figure 5. Since GAC samples were taken from the GAC reactor during a non-steady-state period, the actual performance of the reactor at the time was uncertain. Therefore, the normalization technique used to reduce the number of unknown kinetic parameters in analyzing the rate of 3-ep biodegradation was not applicable to the analysis of OCP biodegradation. Experimental problems were also apparent in the analysis of OCP biodegradation rates. At the time of the experiment, the reactor effluent OCP concentration...
was 75 mg/l. Measurement of OCP biodegradation rates at low OCP concentrations required time for equilibrium to be approached due to a large amount of desorption from the GAC samples removed from the reactor. Methane gas production rates were based on measurements after the OCP concentration had stabilized approximately 8-10 hours after initiation of the experiments. At OCP concentrations greater than 320 mg/l, phenol was detected in the samples at concentrations ranging from 1 to 5 mg/l. Since phenol was fed to the reactor, a significant mass of adsorbed phenol was present in the GAC samples. Phenol was either displaced by the more strongly adsorbed OCP or produced as an intermediate in OCP biodegradation (Boyd and Shelton, 1984). Thus, the observed methane gas production rates might be a measure of the combined biodegradation rates of phenol and OCP and the actual OCP biodegradation rate might be more inhibited at higher OCP concentrations than the data indicate.

The data were fit to both the Haldane model and the Monod model (Monod, 1949) by minimizing the error in substrate utilization rate. Due to the flat area of the curve (Figure 5) for OCP concentrations ranging from 130-420 mg/l, the possibility that Monod kinetics might fit the data as well as Haldane kinetics was investigated. The parameter, kX, was an unknown search parameter in both cases and results are presented in Table 3. The Haldane model fit significantly reduced the residual error in comparison to the Monod model. This experiment demonstrates that the fed-batch experimental technique may be used for compounds other than 3-ep, however, the data must be carefully scrutinized in each case. More reliable results from the fed-batch experiments with OCP should be obtained if the reactor is at steady-state with lower effluent phenol and OCP concentrations.

**TABLE 3 Summary of Kinetic Parameters for OCP Biodegradation with the Monod and Haldane Models**

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>Monod Model</th>
<th>Haldane Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>kX, ml CH4/gGAC-hr</td>
<td>.152</td>
<td>.187</td>
</tr>
<tr>
<td>Ks, mg OCP/l</td>
<td>23.9</td>
<td>37.4</td>
</tr>
<tr>
<td>K1, mg OCP/l</td>
<td>----</td>
<td>1450</td>
</tr>
<tr>
<td>Summation of Errors2</td>
<td>.00179</td>
<td>.00129</td>
</tr>
</tbody>
</table>
CONCLUDING REMARKS

The fed-batch technique developed herein provides a method of measuring biodegradation rates with biofilms attached to adsorbable materials. No other conventional technique will allow for the measurement of biodegradation rates independent of adsorption kinetics. The fed-batch technique is also useful with inhibitory substrates at high concentrations. This technique might be applied in other methods than those discussed and used in this paper. Substrates adsorbed onto GAC or other adsorbable materials might be added to soils or adsorbable materials with attached biomass. Although near equilibrium adsorption phenomena might not exist between different adsorbable materials, the large mass of substrate should still maintain a constant substrate concentration and mass transport limitations should be minimized. Testing soil samples or samples from aquifers that were inoculated would be useful to verify that inoculation was successful and to locate microorganisms in aquifers. Many other possible applications of the fed-batch technique exist.

In all cases, the technique will depend upon the measurement of product formation. Radiolabeled substrates might be used for measurements under aerobic conditions, however, there is a practical limit to the mass of radio-labeled compounds that can be used. The rate of chloride ion production during the biodegradation of chlorinated substrates might be useful in particular cases. This would have been useful in the OCP biodegradation experiment discussed above had the reactor effluent not already contained a high concentration of chloride ions. Caution must always be exercised to ensure that utilization of the substrate is the rate-limiting step in the production of the measured product. The presence of other adsorbable substrates which might also provide a source of product must also be considered as the presence of phenol during the OCP experiment might have increased methane gas production.

CONCLUSIONS

1. The fed-batch technique provided a method for measuring biodegradation rates of inhibitory substrates with biofilms attached to GAC.
2. Results from the fed-batch technique were successfully described with Haldane inhibition kinetics.
3. Results from the fed-batch technique were consistent with results from the continuous operation of a GAC reactor. Fed-Batch techniques might also aid in the design and operational control strategy of the GAC reactors.

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