

**A Preliminary Analysis of Benzene Fate in Industrial Wastewater Treatment
Plants: Implications for the EU Existing Substances Risk Assessment**

**Prepared by
Tom Parkerton**

On behalf of the CEFIC Aromatics Producers Association and CONCAWE

August 25, 2001

Background

In the latest draft risk assessment report, benzene is categorized as a readily biodegradable substance (UBA, 2001). In order to assess the fate of benzene during wastewater treatment, a biodegradation rate of 1 hr^{-1} has been selected as input to the SIMPLETREAT model. While this value correctly reflects the default input that is specified in the EU Technical Guidance Document (TDG) for readily biodegradable substances, this assumption may significantly understate the true role that biodegradation plays in benzene removal at industrial WWTPs which have acclimated microbial populations. The available evidence supporting this view is provided below.

Lab Studies

Cano & Wilcox (1998) performed laboratory tests to determine the biodegradation kinetics of benzene with activated sludge from an industrial (i.e. refinery) WWTP. This study used both direct benzene measurements (BOX tests) and respirometry methods (EKR tests) to characterize benzene biodegradation kinetics. Both test methods were designed in such a way that experimental results were not confounded by potential volatilization losses. Two sets of experiments were conducted to assess the influence of influent concentration on biodegradation kinetics. Further details of this study are provided in Appendix I.

Results from both tests indicated that the experimental data conformed well to the Monod kinetic model which is described by two constants: the maximum substrate removal rate (Q_m) and the half saturation constant (K_s). These parameters can be combined to estimate the first-order biodegradation rate (i.e. $K_{bio} = Q_m / K_s$) in units of liters per gram of volatile suspended solids per hour (L/gVSS/hr). Table 1 summarizes the first-order rate constant derived from this work. Both test methods gave comparable estimates and biodegradation rates were shown to increase in direct proportion to the influent concentration. The authors concluded that results support the "specialist" concept, i.e. as benzene concentration increases the competent biomass available to utilize this substrate concurrently increases.

Table 1. First-order biodegradation rates obtained in laboratory tests with industrial activated sludge.

Benzene influent concentration (mg/l)	First-order biodegradation rate (L /gVSS /h)	
	Box test	EKR test
1.3	17 $\pm$ 2	37 $\pm$ 17
14	532 $\pm$ 85	519 $\pm$ 3

Two earlier studies have also reported biodegradation rates for benzene in simulation tests reflecting acclimated conditions. Namkung & Ritmman (1987) report a benzene biodegradation rate of 13.3 L/gVSS/hr for the laboratory-scale activated sludge system described by Weber & Jones (1984). In another independent study, electrolytic respirometry was used to investigate benzene biodegradation by acclimated sludge (Naziruddin et al. 1995). These authors report a first order biodegradation rate for

benzene of 218 L/gVSS/hr. Thus results obtained in these earlier studies are in good agreement with biodegradation rates reported by Cano & Wilson (1998).

The available simulation tests indicate that the first order biodegradation rate for benzene is in the order of 10-100 L/gVSS/ hr. Based on a default suspended solids (SS) concentration of 4g/L as assumed in the SimpleTreat 3.0 model (Struijs, 1996), and a VSS/SS ratio of 0.8, this translates to estimated biodegradation rates in WWTPs of 32 - 320 hr⁻¹. Therefore, this analysis suggests that actual WWTP biodegradation rates for benzene are more than one to two orders of magnitude higher than the TDG default assumption.

To illustrate the importance of correctly specifying this key model input parameter, a sensitivity analysis with SimpleTreat 3.0 was performed using both the TDG default assumption ($K_{bio} = 1 \text{ hr}^{-1}$) and the lower-bound estimate derived from simulation tests ($K_{bio} = 32 \text{ hr}^{-1}$). Two aeration (surface vs. bubble) and primary sedimentation (with or without) scenarios were also considered. Model predictions describing benzene WWTP fate are summarized in Table 2.

Table 2. Sensitivity Analysis to Benzene Biodegradation Rate Using SimpleTreat 3.0

with primary sedimentation

Surface aeration

Removal	Biodegradation Rate	
	$K_{bio}=1/\text{hr}$ (%)	$K_{bio}=32/\text{hr}$ (%)
To air	46.1	7.0
to water	5.7	0.3
via primary sludge	1.2	1.2
via surplus sludge	0.0	0.0
Degraded	47.0	91.5
Total	100.0	100.0

Overall removal

94.3 99.7

without primary sedimentation

Surface aeration

Removal	Biodegradation Rate	
	$K_{bio}=1/\text{hr}$ (%)	$K_{bio}=32/\text{hr}$ (%)
To air	44.8	2.7
to water	4.0	0.2
via surplus sludge	0.1	0.0
Degraded	51.1	97.1
Total	100.0	100.0

Overall removal

96.0 99.8

with primary sedimentation

bubble aeration

Removal	Biodegradation Rate	
	$K_{bio}=1/\text{hr}$ (%)	$K_{bio}=32/\text{hr}$ (%)
To air	23.0	5.3
to water	8.2	0.4
via primary sludge	1.2	1.2
via surplus sludge	0.0	0.0
Degraded	67.5	93.2
Total	100.0	100.0

Overall removal

91.8 99.6

without primary sedimentation

bubble aeration

Removal	Biodegradation Rate	
	$K_{bio}=1/\text{hr}$ (%)	$K_{bio}=32/\text{hr}$ (%)
To air	14.8	0.6
to water	6.2	0.2
via surplus sludge	0.1	0.0
Degraded	79.0	99.2
Total	100.0	100.0

Overall removal

93.8 99.8

Based upon the different scenarios investigated, SimpleTreat predicts total removal from WWTPs between 92-96% if the TDG default biodegradation rate is used for model calibration. In contrast, >99% removal is predicted for all scenarios if model calibration is based on the biodegradation rate derived from simulation tests. Furthermore, model predictions that are based on the TGD default assumption appear to significantly underestimate removal via biodegradation while overestimating losses due to volatilization. For example, model predictions based on the TGD default indicates that from ca. 15-46% of the benzene is lost via air stripping. In contrast, SimpleTreat predicts <1 to 7% is lost as a result of volatilization if a more realistic biodegradation rate is adopted.

In summary, depending on the biodegradation rate selected for model calibration, WWTP fate predictions differ in terms of both the magnitude of overall removal as well as the relative importance of different removal mechanisms. To determine which calculation provides a better appraisal of the true WWTP fate of benzene available monitoring data were compiled and reviewed.

WWTP Monitoring Data

Paired influent/effluent measurements for benzene in 10 German refineries have been published by DGMK (1984, 1991). Similar data have been reported for two U.S. refineries (API, 1981). Influent/effluent measurements for benzene have also been reported in the continuous-flow activated sludge pilot plant reported by Kincannon et al. 1983. In Figure 1, benzene influent concentration is plotted against the corresponding effluent concentration for all the above studies. Individual WWTP facilities are denoted by different plotting symbols. Reference lines indicating 90, 99 and 99.9% removal are also included. Influent benzene concentrations range over five orders of magnitude and bracket the proposed PNEC (denoted as solid brown line). Visual inspection of Figure 1 suggests overall removals in the range of 90-99.9% for cases in which the influent concentration is below the PNEC. With the exception of WWTP 3, observed removals are usually >99% when the influent concentration is above the PNEC.

This trend is more obvious by plotting the mean removal for each facility as a function of the average influent concentration as shown in Figure 2. Additional data from two municipal WWTPs reported by Namkung & Rittman (1987) are also included in this plot. Observed removals are generally lower and more variable when influent concentrations are below the PNEC. However, consistent removals >99% are demonstrated for WWTPs receiving higher influent concentrations. Again the exception is WWTP 3 which exhibits large error bars reflecting variable removals. However, it should be pointed out that the removal statistics for this facility are highly influenced by a single influent/effluent pair which, for example, could have been collected during a temporary plant upset. If this data point is excluded, then the average removal for this WWTP is $98\% \pm 4\%$.

Discussion

As discussed in the literature review by Temmik (2001), the lower limit of applicability of the Monod model corresponds to a substrate concentration of about 100 ug/l which coincidentally corresponds approximately to the PNEC for benzene (UBA, 2001). Below this concentration threshold, sufficient energy is not available to support bacterial growth on the substance and biodegradation is mediated by a secondary utilization mechanism. Therefore, the trend shown in Figure 2 is not unexpected since WWTPs with influent

concentrations below the PNEC cannot support specialized bacterial populations that are adapted to utilize benzene as an energy source. However, the opposite applies for influent concentrations that are much greater than the PNEC. In such situations microbial populations with benzene specific degraders can serve to rapidly utilize this substrate for growth resulting in much higher biodegradation rates than specified by TGD default assumptions. Similar results have also been recently reported for toluene in pilot plant studies (Temminck, 2001). At influent concentrations of ca. 100 ug/l removals were variable and ranged from 96-99%. However at influent concentrations between 730-1880 ug/l removals were consistently 99.9%.

Ready biodegradation tests involve a high substrate to biomass ratio and are based on an ultimate rather than primary biodegradation endpoint. Moreover, these tests rely upon an unacclimated inoculum. Therefore, extrapolation of such tests to define biodegradation kinetics under actual operating conditions of a WWTP provides a "worst case" scenario for readily biodegradable substances.

Calibration of the biodegradation rate used in SimpleTreat model based on simulation tests designed to more accurately reflect WWTPs conditions yielded predicted removals that were in closer agreement with monitoring data for WWTPs where Monod kinetics were expected to apply (i.e. influent concentration > PNEC). While lower removals may occur when influent concentrations are below the PNEC this case has little relevance for risk assessment. More importantly, available laboratory and field data indicate WWTPs very effectively remove benzene when influent concentrations are sufficiently high to pose environmental concern. This analysis also indicates that biodegradation serves as the principal mechanism for benzene removal. The primary role that biodegradation plays is further supported by Namkung & Rittman (1987) who estimated that approximately 94.8-95.3% of the benzene loss from two municipal WWTPs was due to biodegradation while only 2.4-2.6% were attributed to volatilization. These estimates are in reasonable agreement with SimpleTreat predictions if the biodegradation rate obtained from simulation studies is used for model calibration (Table 2).

Recommendation

Based on the information available, default WWTP fate calculations presented in the current benzene RAR appear to significantly underestimate the extent to which biodegradation limits benzene releases to the environment. While this approach may be appropriate for initial screening, further refinement is required if the contribution of WWTP emissions to the overall benzene emission inventory is to be more accurately characterized.

APA therefore recommends that the RAR is amended by the insertion of the following paragraph in Section 3.1.1.3.2, to reflect the effect that the acclimation of WWTP microorganisms has on benzene biodegradation rates :

"A significant body of data now exists (Namkung & Rittman, 1987; Cano & Wilcox, 1998; Temminck, 2001) to indicate that the aerobic biodegradation rates achieved in industrial WWTPs significantly exceed those derived from standard biodegradation tests. Where WWTP microorganisms have become acclimated to benzene (at influent concentrations >100 ug/l), the actual WWTP biodegradation rate will be between one to two orders higher than that predicted from OECD ready test protocols using activated sludge from domestic sewage treatment plants."

Benzene Concentrations in Industrial WWTPs

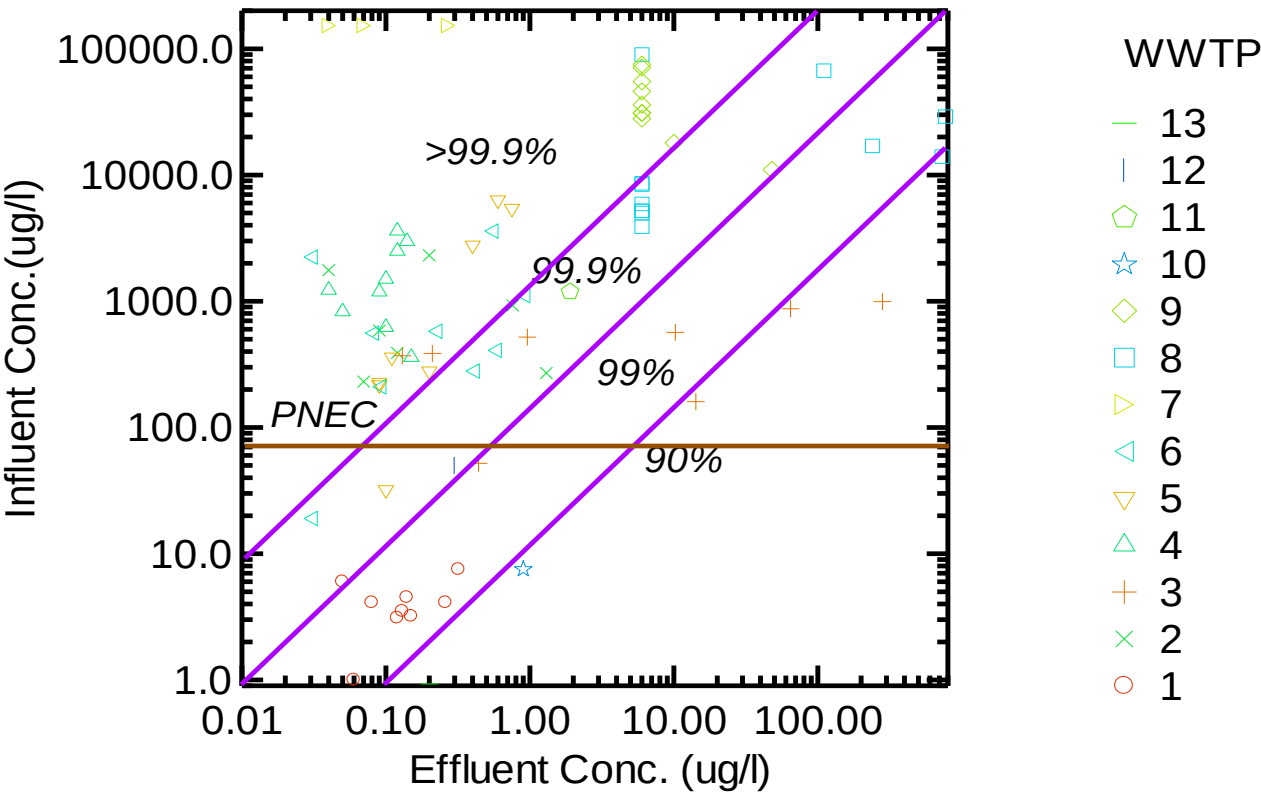


Figure 1.

Influent Benzene Concentration vs. WWTP Removal

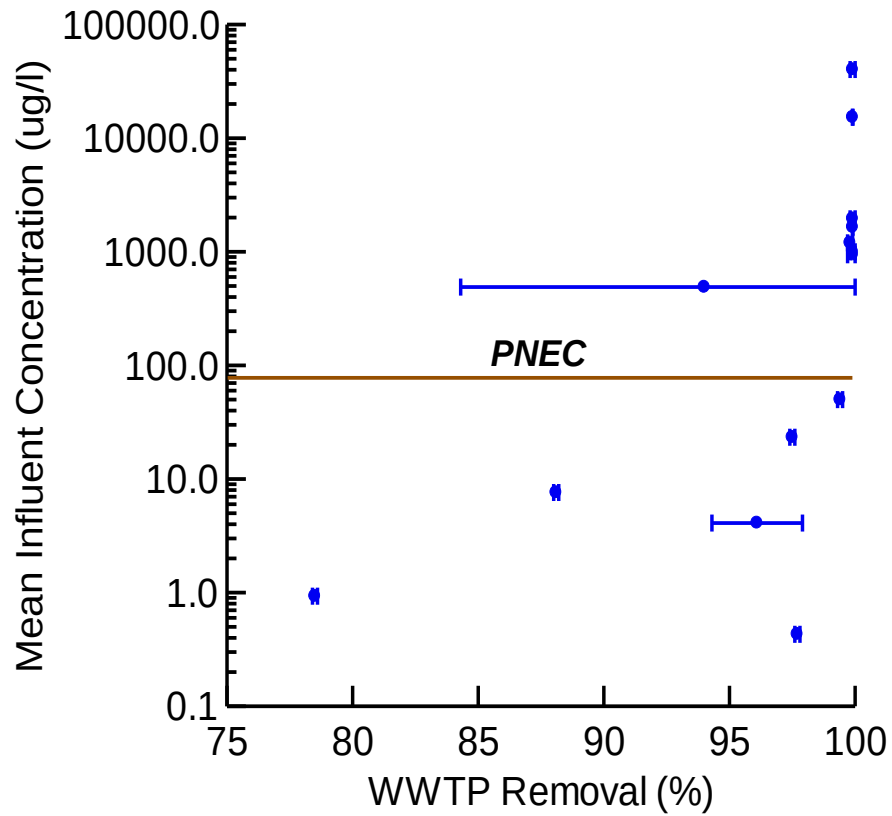


Figure 2.

Literature Cited

API (1981). Refinery wastewater priority pollutant study-sample analysis and evaluation data, American Petroleum Institute, publication 4346, Washington, DC., 101pp + Addendices.

Cano, M.L., M.E. Wilcox (1998). A direct comparison of activated sludge biodegradation kinetics determined by substrate removal (BOX experiment) and the extant kinetics respirometric (EKR) test. Proceedings of the Water Environment Federation 71st Annual Conference and Exposition, Orlando, Florida.

DGMK (1984). Analysis of refinery effluent components, DGMK German, Society for Petroleum Sciences and Cool Chemistry, project report no. 283, Hamburg, Germany, 95pp.

DGMK (1991). Assessment of wastewater in specific sidestreams and total effluent from petroleum and lute oil refineries, DGMK German Society for Petroleum Sciences and Cool Chemistry, project report no. 414, Hamburg, Germany, 112pp.

Kincannon, D.F., A. Weinert, R. Padorr, E.L. Stover (1982). Predicting treatability of multiple organic priority pollutant wastewaters from single-point treatability studies. Proceedings of the 37th Industrial Waste Conference, May-11-13, Perdue University, West Lafayette, Indiana.

Namkung, E., B.E. Rittmann (1987). Estimating volatile organic compound emissions from publicly owned treatment works J. Water Pollut. Control Fed. 59:670-678.

Naziruddin, M., C.P.L. Grady Jr., H.H. Tabak (1995). Determination of biodegradation kinetics of volatile organic compounds through the use of respirometry, water res. 67:151-158.

Struijs, J. (1996). Simple treat 3.0. A model to predict the distribution and elimination of chemicals by sewage treatment plants, National Institute of Public Health and Environment, report no. 719101025, Bilthoven, The Netherlands, 49pp.

Temmink, H. (2001). Reliability of models that predict the fate of organic trace pollutants in municipal activated sludge plants, PhD dissertation, Wageningen University, Wageningen, The Netherlands, 184 pp.

Weber, W.J., Jr., B.E. Jones (1984). Toxic Substance Removal in Activated Sludge and PAC Treatment Systems, Paper Presented at the 1984 Annual Conference of the Water Pollution Control Federation, New Orleans, LA, USA.

APPENDIX I

A DIRECT COMPARISON OF ACTIVATED SLUDGE BIODEGRADATION KINETICS DETERMINED BY SUBSTRATE REMOVAL (BOX EXPERIMENT) AND THE EXTANT KINETICS RESPIROMETRIC (EKR) TEST

M.L. Cano and M.E. Wilcox

Shell Development Company, P.O. Box 1380, Houston, TX 77251

ABSTRACT

We have conducted experiments which directly compared extant biodegradation kinetics determined with two different procedures: (1) the batch test with oxygen addition (BOX) and (2) the extant kinetics respirometric (EKR) test. The BOX tests use substrate removal to determine biodegradation kinetics, while the EKR tests use oxygen consumption. Biodegradation kinetics testing was performed for benzene with a refinery activated sludge that was (1) tested immediately and (2) acclimated to a higher level of benzene (~10-fold higher concentration) for ~3 weeks. Biodegradation kinetics were modeled with the Monod equation. This resulted in three types of biodegradation kinetic parameters: (1) the maximum substrate removal rate (Q_m), the half saturation constant (K_s), and a first order rate constant (K_d). The results of the comparison tests showed that the BOX and EKR tests provided equivalent biodegradation kinetic parameters. This was demonstrated with analysis of variance (ANOVA) statistical techniques. In addition, the data also indicated that when the feed concentration of benzene was increased, the benzene biodegradation rate increased in an amount proportional to the amount of feed COD contributed by the benzene (after acclimation had occurred). This supported the concept of "specialist" microorganisms which biodegrade specific compounds. Thus, BOX and EKR tests can both be used to effectively determine activated sludge biodegradation kinetics.

Keywords

activated sludge, benzene, biodegradation kinetics, BOX test, EKR test, Monod

INTRODUCTION

The removal of organic compounds from wastewater and the primary mechanism for their natural destruction is biodegradation. Data on biodegradation kinetics is essential for the design of biological treatment systems and for the process of establishing limits on the discharge of these compounds to the environment. Moreover, accurate kinetic models and reliable kinetic parameter estimates are necessary to predict effluent quality and emissions in wastewater treatment systems.

Many different types of batch experimental approaches have been used to determine biodegradation kinetic parameters. These have included respirometric approaches whereby oxygen consumption is used as a surrogate measure of biodegradation (Cech et al., 1984; Naziruddin et al., 1995; Dang et al., 1989; Ellis et al., 1996), infinite dilution approaches (Williamson and McCarty, 1975), and direct measurement approaches in which specific analytical techniques are used to quantify the biodegradation of a substrate (Alvarez-Cohen and McCarty, 1991; Rajagopalan et al., 1998; Cano et al., 1997). Additional techniques for measuring biodegradation rate constants are summarized by Pitter and Chudoba (1990).

Recently, at the Shell Westhollow Technology Center (WTC), we have been developing and implementing methods for determining biodegradation rate constants for specific compounds in activated sludge units. We have been working with tests that focus on specific substrate removal (Rajagopalan et al., 1998; Cano et al., 1997) as well as tests which measure oxygen consumption (Ellis et al., 1996). These two types of tests measure extant (currently existing) biodegradation kinetics. Extant tests provide parameters that are more representative of the conditions in the continuous biological reactor at the time they are performed. These kinetics may have a greater ability to predict effluent quality from continuous bioreactors because they are determined under conditions which do not alter the physiological state of the bacterial culture (Grady et al., 1996b). The conditions for determining extant biodegradation kinetic parameters are described in detail in Grady et al. (1996a) and Ellis et al. (1996). We have termed this experimental approach the extant kinetics respirometric (EKR) test.

The kinetics of biomass growth, substrate removal, and oxygen consumption are coupled through stoichiometry. If we use biomass growth as a basis, we can describe the rate of substrate removal and oxygen consumption due to biodegradation with the following equations using units of chemical oxygen demand (COD):

$$r_s = \frac{-r_x}{Y} \quad (1)$$

$$r_o = \frac{-r_x(1-Y)}{Y} \quad (2)$$

where r_s is the rate of substrate removal (mg substrate COD/L-hr), r_x is the rate of biomass growth (mg biomass COD/L-hr), r_o is the rate of oxygen consumption (mg of oxygen/L-hr), and Y is a biomass growth yield coefficient (mg biomass COD formed/mg substrate COD consumed). Biomass growth can be modeled using the Monod equation which has been widely applied to the growth of biomass on a noninhibitory substrate, at a concentration S (Grady and Lim, 1980):

$$r_x = \hat{\mu} \left(\frac{S}{K_s + S} \right) X \quad (3)$$

where $\hat{\mu}$ is the maximum specific growth rate (hr^{-1}), K_s is the half saturation coefficient (mg substrate COD/L), and X is the biomass concentration (mg biomass COD/L).

In order to describe the biodegradation of a specific compound, it is necessary to determine $\hat{\mu}$, K_s , and Y under a specific set of conditions. When $\hat{\mu}$ and Y are known, the kinetics can be used to model cell growth and substrate removal. Alternatively, the ratio $(\hat{\mu}/Y) = Q_m$ (a maximum substrate removal rate (mg substrate COD/g biomass COD-hr)) can be used to model substrate removal. Thus, kinetics determined by measuring r_s should be equal to kinetics determined by measuring r_o for growth inducing substrates.

To compare biodegradation kinetic parameters determined by substrate removal or by oxygen consumption measurements, experiments were conducted to measure benzene biodegradation kinetics for activated sludge that had been acclimated to two different levels of benzene. Herein, we summarize results of experimental work to directly compare biodegradation kinetics determined by substrate removal (BOX experiments; Rajagopalan et al., 1998; Cano et al., 1997) with those determined by measuring oxygen consumption (EKR test; Ellis et al., 1996).

EXPERIMENTAL

Activated sludge unit

The biomass used for the biodegradation kinetics experiments was obtained from a refinery activated sludge unit and placed in a bench scale continuous activated sludge unit at WTC. The bench scale unit consisted of a 7 liter (working volume) glass aeration basin and a funnel shaped 4 liter (working volume) glass clarifier. The reactor was started up by adding ~2 gallons of refinery activated sludge to the unit. When the biomass sample was received at WTC, it contained a mixed liquor suspended solids (MLSS) concentration of 2500 mg/L and mixed liquor volatile suspended solids (MLVSS) concentration of 1900 mg/L. The dissolved oxygen (DO) concentration was 3 mg/L and the pH was 7.5. The biomass was aerated immediately, but the feed was not started until a baseline set of biodegradation kinetic parameters were determined (see below). The time between sample collection and initial aeration at WTC was 1.5 hr. After ~3 hr, the feed was started to the unit. The unit was fed a synthetic wastewater feed composed of benzene (15 mg/L), toluene (7 mg/L), and methyl ethyl ketone (MEK, 176 mg/L) dissolved in unchlorinated WTC well water. These concentrations were substantially higher than what the refinery biomass was normally fed. Nutrients (N, P) were added to the reactor from a solution of NH_4Cl and NaH_2PO_4 in the ratio of 100:5:1 (feed COD:N:P). Table 1 shows the typical operational parameters for the activated sludge unit during this study.

Table 1: Operational parameters for activated sludge unit.

Parameter	Units	Value
Hydraulic residence time	hr	24
Feed flow	ml/min	5
Recycle flow	ml/min	100
Air flow	scfh	2.5
Dissolved Oxygen	mg/L	5-8
pH		7.5
MLSS	mg/L	2500
MLVSS	mg/L	1900
Sludge wasting	ml/day	148

This unit was only operated for a short period of time (~3-4 weeks) since its only purpose was to provide biomass to use for the comparison tests.

Biodegradation kinetics tests

Two types of biodegradation tests were performed: (1) BOX tests and (2) EKR tests. Details for these methods have been described elsewhere. The BOX test is described in Cano et al. (1997) and the EKR test is described in Ellis et al. (1996). Briefly, the BOX test is a batch experiment which uses an aerated draft tube reactor to measure the biodegradation of a specific compound via substrate removal. The BOX experiment considers the competition between stripping and biodegradation in the batch reactor and a mass balance approach is used to quantify biodegradation kinetic parameters which describe the substrate removal observed in the test. The EKR test is a batch experiment which uses a headspace-free reactor to measure biodegradation kinetics via oxygen consumption data. Oxygen consumption is related to substrate removal using a mass balance approach (Equations 1-3). Biodegradation kinetic parameters are determined for both types of experiments by non-linear least squares fitting of the batch reactor model equations to either the substrate removal or oxygen consumption data.

Benzene was the only compound tested in this study. The BOX experiments used MLVSS concentrations of 87-104 mg/L while the EKR experiments used MLVSS concentrations of 920-1940 mg/L. Reactor effluent was used to dilute the biomass when necessary. The

concentrations of benzene added for each batch biodegradation test were 0.5-1.0 mg/L. Two sets of biodegradation kinetic experiments were conducted. The first set was performed immediately after the biomass was brought to WTC. Four BOX and five EKR tests were performed. The second set of tests was conducted ~3 weeks later. During this time, the biomass had an opportunity to acclimate to the higher benzene concentration. This second set consisted of four BOX and four EKR tests.

Analytical measurements

Benzene analyses for the BOX experiments were conducted using a Photovac 10S-70 gas chromatograph (GC) to measure the off-gas concentrations. Henry's law was used to determine the liquid phase concentrations based on equilibrium assumptions (Rajagopalan et al., 1998; Cano et al., 1997). The Henry's law constant used for benzene (4.7×10^{-3} atm-m³/mol) was determined using the procedures described in Cano et al. (1997). Samples were drawn into the instrument using its internal sampling pump. The carrier gas was ultra-zero ambient monitoring air, and calibrations were performed using certified gas standards purchased from Scott Specialty Gases.

Benzene, toluene, and MEK analyses were performed by purge and trap gas chromatography. These samples were analyzed with a Varian 3600 gas chromatograph (GC) equipped with a flame ionization detector and a DB-624 column (30 m length x 0.53 mm ID x 3 micron film thickness). Prior to analysis, samples were concentrated with a Tekmar ALS2016 sample concentrator and a #9 trap containing TenaxTM, silica gel, and charcoal.

Dissolved oxygen measurements for the EKR test were made with a Yellow Springs Instrument (YSI) Company Model 5739 dissolved oxygen (DO) probe connected to a YSI Model 58 DO meter. The output from the DO meter was interfaced with a microcomputer using a data acquisition board and data acquisition software. Data acquisition was performed at a rate of 10 Hz.

RESULTS AND DISCUSSION

Activated sludge unit operation

The bench scale activated sludge unit was operated at WTC for ~3-4 weeks and served as the source of biomass for the biodegradation kinetic experiments. Typical operating parameters are shown in Table 1. The synthetic feed provided to the unit was intended to result in biomass acclimation to higher levels of benzene (~10-fold increase in benzene concentration).

Biodegradation kinetics for benzene

Benzene biodegradation kinetics experiments were conducted on two separate days: (1) immediately after collection of the biomass sample from the refinery and (2) after ~3 weeks of acclimation to the synthetic organic feed with elevated benzene levels. Two types of biodegradation kinetics experiments were performed: (1) BOX experiments and (2) EKR tests. Sample experimental results for BOX and EKR tests are shown in Figures 1 and 2, respectively. For the BOX experiments, biodegradation kinetics were determined by measuring benzene removal while for the EKR tests, they were determined by measuring changes in the oxygen uptake rate following an injection of benzene.

The measured biodegradation kinetics are summarized in Table 2. Data for biodegradation kinetics based on the EKR test were converted from oxygen equivalents (COD) to benzene concentrations by using the theoretical factor of 3.08 g COD/g benzene. For both types of experiments, the Monod biodegradation kinetics model fit the data well. Thus, the data are reported as a maximum substrate removal rate ($Q_m = \hat{\mu} / Y$) and a half saturation constant (K_s). In

addition, these two parameters were combined to calculate a first order biodegradation rate constant ($K_1 = Q_m/K_s$). Due to the low substrate concentrations in typical activated sludge units, this K_1 is likely to be the most important parameter to characterize biodegradation rates. For the BOX experiments of 7/10/96, it was not possible to distinguish the individual Q_m and K_s parameters due to the low initial substrate concentrations used for these tests. The data fitting routines used for analysis would only provide a correlated set of parameters and thus it is only possible to report K_1 (Grady et al., 1996a).

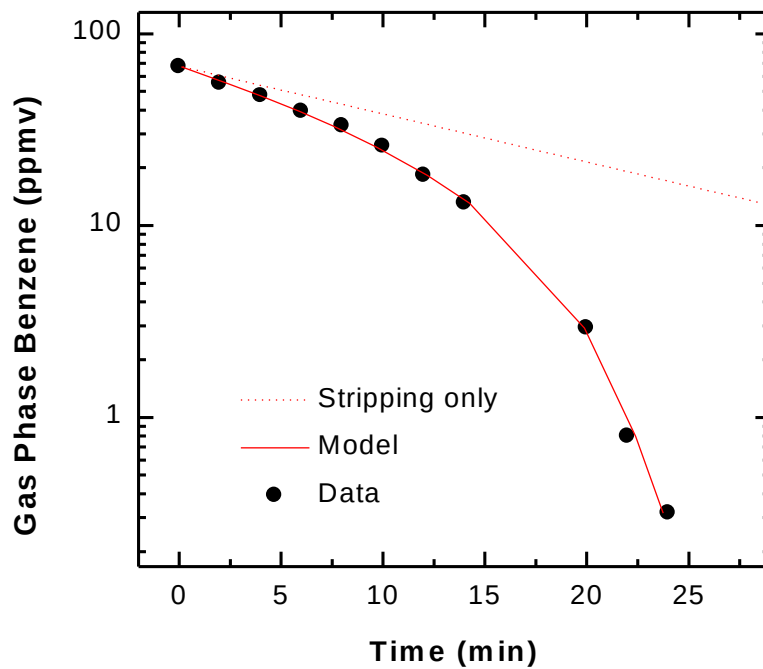


Figure 1: Example benzene biodegradation kinetics experiment with a BOX test (Experiment #1, 8/2/96).

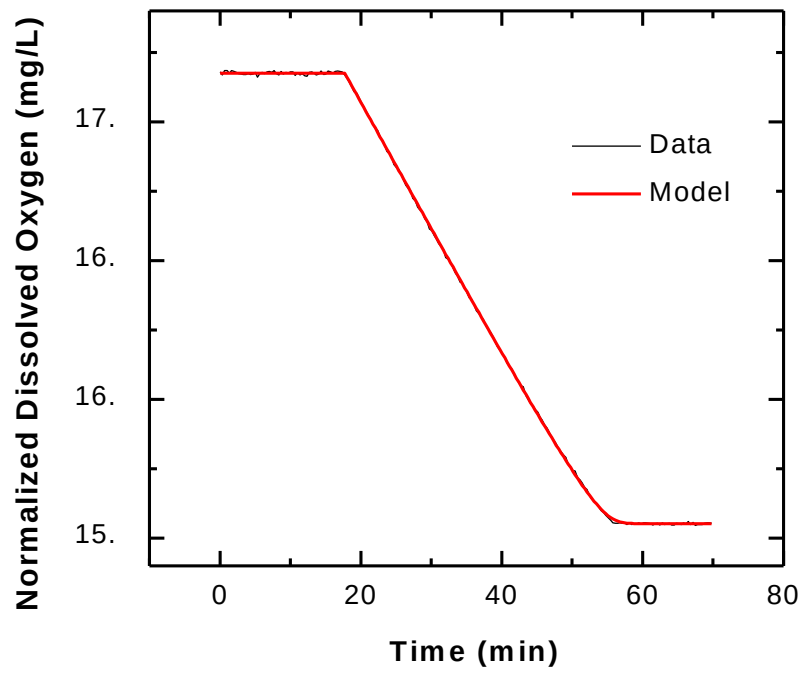


Figure 2: Example benzene biodegradation kinetics experiment with an EKR test (Experiment #2, 7/10/96).

Table 2: Benzene biodegradation kinetics based on BOX and EKR experiments.

Date	BOX Experiments			EKR Experiments		
	Q_m^a (mg/gVSS/hr)	K_s (mg/L)	K_1^b (L/gVSS/hr)	Q_m (mg/gVSS/hr)	K_s (mg/L)	K_1 (L/gVSS/hr)
7/10/96	- ^c	-	18	0.66	0.043	15
7/10/96	-	-	15	0.90	0.025	36
7/10/96	-	-	17	1.04	0.017	62
7/10/96	-	-	19	1.13	0.036	31
7/10/96	-	-		1.23	0.031	40
Average	-	-	17	0.99	0.030	37
Standard Deviation	-	-	2	0.22	0.010	17
8/2/96	18.20	0.042	438	11.40	0.022	519
8/2/96	16.11	0.027	603	12.92	0.025	518
8/2/96	18.61	0.039	483	10.88	0.021	517
8/2/96	18.57	0.031	604	12.53	0.024	523
Average	17.87	0.034	532	11.93	0.023	519
Standard Deviation	1.19	0.010	85	0.96	0.002	3

^a All data are reported using benzene mass as a basis (mg benzene).

^b $K_1 = Q_m/K_s$.

^c It was not possible to independently estimate Q_m and K_s due to the low initial benzene concentration used in these experiments.

The first order biodegradation rate constants for benzene were 17 ± 2 and 37 ± 17 L/gVSS/hr on 7/10/96 based on the BOX and EKR tests, respectively. After the ~3 weeks of acclimation to the higher benzene levels in the synthetic feed, these rate constants increased to 532 ± 85 and 519 ± 3 L/gVSS/hr. Analysis of variance (ANOVA) was used to compare the first order rate constants based on BOX and EKR tests. This analysis indicated that the first order rate constants determined from the two types of experiments were not statistically significantly different ($p > 0.05$). This suggests that the two approaches provided equivalent biodegradation kinetics. In addition, the benzene biodegradation kinetics observed in this study were consistent with previously measured values of K_1 (30-250 L/gVSS/hr) in other refinery activated sludge systems (Shell Development Company, unpublished).

Effects of benzene feed concentration

In this study, we have reported biodegradation kinetics based on total biomass (MLVSS) in the activated sludge reactor (units of Q_m are mg/gVSS/hr). This was done for convenience since this parameter is easy to measure. However, this convention assumes that all of the biomass participates in the biodegradation of each compound. This is probably not the case for xenobiotic compounds. There are likely to be "specialist" bacteria which degrade individual compounds. It has been proposed that the fraction of biomass capable of degrading a specific compound is proportional to the fraction of feed COD contributed by that compound (Grady et al., 1996b). If this is the case, then when the concentration of a compound increases in the feed to a bioreactor, the biodegradation kinetic parameters should increase proportionately if they are based on total MLVSS concentrations as we have done in this report. Previous work (Shell Development Company, unpublished) in our laboratories suggests that this proportionality approach is valid. The benzene biodegradation data in this report were also analyzed to see if they support this hypothesis. The results of this analysis are described below.

The benzene feed concentration was increased after the biomass was placed in the laboratory reactor. The benzene feed concentrations was increased ~10-fold while the contribution of benzene to the feed COD was increased by ~8-fold. Table 3 compares the increase in the first order biodegradation rate constant (K_1) based on the two types of kinetic experiments (BOX and EKR) with the increases in feed benzene concentration and the contribution of benzene to the feed COD.

Table 3: Comparison of feed benzene concentrations with biodegradation kinetics.

Parameter	Units	Value
<u>7/10/96</u>		
Benzene in feed ^a	mg/L	1.3 ± 0.4
COD in feed ^a	mg/L	330 ± 120
Fraction benzene COD in feed	-	0.012 ± 0.006
K_1 based on BOX expt.	L/gVSS/hr	17 ± 2
K_1 based on EKR expt.	L/gVSS/hr	37 ± 17
<u>8/2/96</u>		
Benzene in feed	mg/L	14
COD in feed	mg/L	439
Fraction benzene COD in feed	-	0.098
K_1 based on BOX expt.	L/gVSS/hr	532 ± 85
K_1 based on EKR expt.	L/gVSS/hr	519 ± 3
<u>Ratios of 8/2/96 to 7/10/96 ^b</u>		
Ratio fraction benzene COD	-	8 ± 4
Ratio BOX expt. K_1	-	31 ± 6
Ratio EKR expt. K_1	-	14 ± 6

^a Data were obtained from the refinery technical database. The values reported represent an average ± standard deviation of data collected by the refinery from 6/10/96 to 7/10/96.

^b Calculations were performed using standard approaches for error propagation (Taylor, 1982).

The data indicate that on average, the benzene K_1 increased by 14- to 31-fold. However, when we consider the uncertainty (see Table 3) in some of the measurements used for these calculations, the data show that the K_1 increase approximately parallels the increase in the fraction of feed benzene COD. Thus, the benzene data in this study also support the “specialist” concept. It may be possible to use COD ratios (compound specific COD/total feed COD) to scale biodegradation kinetic parameters for use in the modeling of similar systems receiving different concentrations of a specific compound. Caution should be used when applying this approach as it is known that biodegradation kinetic parameters are generally site and system specific.

CONCLUSIONS

We have described experiments directly comparing biodegradation kinetics determined using two different procedures: (1) the BOX test and (2) the EKR test. The BOX tests used substrate removal to determine biodegradation kinetics, while the EKR tests used oxygen consumption. Biodegradation kinetics testing was performed for benzene in a refinery activated sludge that was (1) tested immediately and (2) acclimated to a higher level of benzene (~10-fold higher concentration). The results of the comparison tests showed that the BOX and EKR test provided equivalent biodegradation kinetic parameters. This was demonstrated by using ANOVA statistical techniques. In addition, the data also indicated that when the feed concentration of benzene was increased, the benzene biodegradation rate increased in an amount proportional to the amount of feed COD contributed by the benzene (after acclimation had occurred). This

supported the concept of “specialist” microorganisms which biodegrade specific compounds. Thus, BOX and EKR tests can both be used to effectively determine activated sludge biodegradation kinetics.

REFERENCES

Alvarez-Cohen, L. and P.L. McCarty. 1991. A cometabolic biotransformation model for halogenated aliphatic compounds exhibiting product toxicity. *Environ. Sci. Tech.* 25: 1381-1387.

Cano, M.L., M.E. Wilcox, and R. van Compernelle. 1997. A direct comparison of EPA's Method 304B and batch tests for determining biodegradation rate constants of hazardous air pollutants. *Proceedings of the Water Environment Federation 70th Annual Conference and Exposition*, Chicago, IL, October 18-22, 1997.

Cech, J.S., J. Chudoba and P. Grau. 1984. Determination of kinetic constants of activated sludge microorganism. *Water Science Technology*, Vol. 17, Amsterdam, pp. 259-272.

Dang, J.S., D.M. Harvey, A. Jobbagy, and C.P.L. Grady Jr. 1989. Evaluation of biodegradation kinetics with respirometric data. *Research Journal WPCF* 61: 1711-1721.

Ellis, T.G., D.S. Barbeau, B.F. Smets, and C.P.L. Grady Jr. 1996. Respirometric technique for determination of extant kinetic parameters describing biodegradation. *Water Environment Research* 68: 917-926.

Grady, C.P.L., and H.C. Lim. 1980. *Biological Wastewater Treatment Theory and Applications*. Marcel Dekker, Inc., New York.

Grady, C.P.L., B.F. Smets, and D.S. Barbeau. 1996a. Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. *Water Res.* 30: 742-748.

Grady, C.P.L., Jr., B.S. Magbuana, R.L. Buddin, A.G. Rodieck, R.W. Sanders II, W.W. Sowers, J.C. Stanfill, B.F. Smets, and S. Brau. 1996b. Relative efficacy of extant and intrinsic kinetic parameter estimates for predicting the removal of synthetic organic chemicals by activated sludge. *Proceedings of the Water Environment Federation 69th Annual Conference and Exposition*, Dallas, TX, October 5-9, 1996.

Naziruddin, M., C.P.L. Grady, Jr., and H.H. Tabak. 1995. Determination of biodegradation kinetics of volatile organic compounds through the use of respirometry. *Water Environment Research* 67: 151-158.

Pitter, P. and J. Chudoba. 1990. *Biodegradability of Organic Substances in the Aquatic Environment*. CRC Press, Boca Raton, FL.

Rajagopalan, S., R. vanCompernelle, C.L. Meyer, M.L. Cano, and P.T. Sun. 1998. Comparison of methods for determining biodegradation kinetics of volatile organic compounds. *Water Environment Research* 70: 291-298.

Taylor, J.R. 1982. *An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements*. University Science Books, Mill Valley, CA.

Williamson, K.J., and P.L. McCarty. 1975. Rapid measurement of Monod half-velocity coefficients for bacterial kinetics. *Biotechnol. Bioeng.* 14: 915.