
ANAEROBIC BIODEGRADATION OF TOLUENE IN A PLUG-FLOW REACTOR

S. Ghosh, T. Liu, and K. Fukushi, Department of Civil and Environmental Engineering, The University of Utah, Salt Lake City, UT, 84112, Phone: 801-581-5152, FAX: 801-581-6937

ABSTRACT This paper presents the application of a continuous-flow, mesophilic (35°C), plug-flow digester to cometabolic degradation of toluene by an anaerobic microbial consortium in the presence of conventional pollutants serving as the major carbon and energy sources. In contrast to conventional practice, digester contents were not mixed. Reactor effluents were recirculated to the influent end at a rate of 20 vol. percent of the influent flow rate. The anaerobic digester was charged with 50 mg/l of toluene. The total COD (chemical oxygen demand) concentration of the mixed, non-hazardous carbon sources was 11,000 mg/l. Experimental results showed that anaerobic digestion occurring in a plug-flow reactor can simultaneously stabilize and detoxify conventional and hazardous wastes with the production of a methane-rich (up to 80 mol% methane) fuel gas. The observed gas yield of 0.36 ± 0.01 SCM/kg VS added was higher than that of conventional, complete-mix digesters under similar operating conditions of hydraulic retention time (HRT) and organic loading rate (OLR). Toluene did not inhibit anaerobic biogasification of non-hazardous substrates. A toluene degradation efficiency of 95% and a total COD reduction of 96% were observed at an HRT of 13 days and an OLR of 0.81 kgCOD/m³-day. Digester effluents contained about 2 mg/l of toluene under these operating conditions. Toluene released to the reactor head gas was about 1.4% of the toluene introduced into the anaerobic reactor. The small concentration of toluene in the gas-phase would not be a problem if the digester gas were subjected to high-temperature combustion. A simple model based on the Monod growth-kinetic equation was developed to describe cometabolic toluene-degradation profiles in the plug-flow digester. The kinetic parameters of half-velocity constant (K_s) and the maximum toluene utilization rate per unit culture volume (C) were estimated by nonlinear regression analysis of steady-state toluene concentration profiles in the reactor liquid phase. Values of K_s and C were 12.2 mg/l and 6.2 mg/l-day, respectively. This research showed that a simple, plug-flow anaerobic digester gasified solid and liquid wastes with simultaneous destruction of toluene at efficiencies higher than 95%. There was no evidence of inhibition of the anaerobic digestion process by 50 mg/l of toluene. The reactor produced up to a 80-mol% methane-content product gas.

KEYWORDS: anaerobic, biodegradation, kinetics, plug-flow, toluene

INTRODUCTION

Most communities south of the U.S.-Mexico border region do not have any organized waste management systems. Waste collection is infrequent or non-existent. Residential wastes are often mixed with hazardous industrial effluents. Uncontrolled decomposition of these unstable and toxic materials has led to serious public health hazards, soil pollution, and deterioration of water quality. Similar conditions of

environmental pollution are encountered in other parts of the world.

Toluene is one of the frequently discharged hazardous substances in the U.S.-Mexico border area. It is often mixed with non-hazardous, conventional organic pollutants of domestic origin. Several treatment methods including chemical oxidation and combustion, activated carbon adsorption, and biological stabilization may be used for the conversion of toluene to a non-toxic substance. Biological treatment is favored

because of the capability of microorganisms to degrade low concentrations of toluene in large volumes of aqueous wastes economically. Toluene can be degraded under aerobic as well as anaerobic conditions. Aerobic biological treatment has the disadvantage that toluene is released into the atmosphere due to agitation and aeration of the waste waters creating an air pollution problem. Anaerobic treatment is preferred to aerobic treatment because it does not require the introduction of compressed air and the associated energy input. Also, most contaminated sites polluted with mixed wastes develop anaerobic conditions after an initial period of aerobic biological activity and depletion of oxygen in void spaces and pore water. For these reasons, anaerobic biodegradation of toluene in a simple novel anaerobic digester was investigated in this project.

Biodegradation of toluene under anaerobic conditions

Several investigators studied biodegradation of toluene and other toxic pollutants in low concentrations in waste water treatment plants [1]. As expected, exposure of waste water organisms to toluene results in the selection of resistant organisms that can produce appropriate enzymes to degrade this toxic substance. Anaerobic degradation of toluene in aquifers, sediments, and waste waters was shown to be effected by cultures of sulfate-reducing, denitrifying, iron-reducing, and mixed methanogenic and acidogenic cultures [2-9].

Most studies on anaerobic biodegradation of toluene were conducted under batch conditions with pure cultures of bacteria fed with toluene as the sole source of carbon and energy [10-14]. However, in many cases, anaerobic degradation of toluene

occurs in the presence of mixtures of organic compounds. Toluene in low concentrations is metabolized with the simultaneous utilization of *primary substrate(s)* serving as the source(s) of carbon and energy required for growth. Xenobiotic compounds, such as toluene, act as *secondary substrates* that do not contribute to the anabolic process leading to cell growth. This type of metabolism is called *cometabolism*. Cometabolism was defined by Alexander as a process in which a xenobiotic compound is mineralized or converted to non-hazardous metabolic products; it does not provide energy or nutrients for microbial growth [15].

Research needs

To simulate the real-world conditions such as those prevalent near the U.S.-Mexico border area, it was necessary to work with a multi-substrate medium in which toluene was present as a minor source of organic carbon. Consideration of the socio-economic nature of the communities in this area pointed to the need for a simple anaerobic process that did not require skilled operation. A continuous-flow simple reactor design that minimized capital cost and the input of purchased external energy needed to be developed and tested for possible application to mitigate the pollution problem on the Mexican side of the international border.

An innovative plug-flow reactor consisting of three zigzagging channels in series with continuously sloping floors was developed for this research. The efficiency of such a reactor for stabilization and gasification of non-hazardous wastes, as well as for destruction of toluene, benzene, xylene, and other hazardous substances needed to be determined. In this paper, the cometabolic destruction of toluene will be discussed.

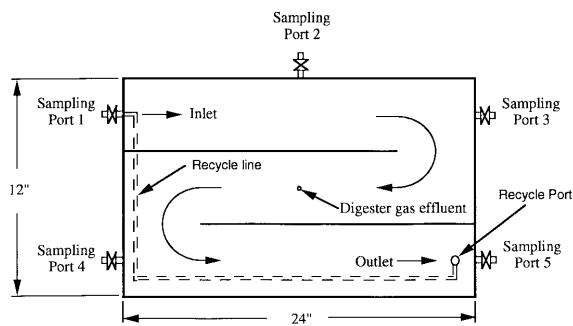


FIGURE 1. SECTIONAL VIEW OF THE CONTINUOUS ANAEROBIC PLUG-FLOW REACTOR.

Research objectives

The objectives of this research were: (1) to develop and test the performance of a simple plug-flow, anaerobic digester in stabilizing and gasifying conventional solid and liquid wastes; (2) to investigate toluene degradation by cometabolism in the presence of conventional wastes serving as the major sources of carbon and energy; (3) to develop and apply a mathematical model to describe toluene degradation in a plug-flow reactor; and (4) to determine kinetic parameters of the model.

MATERIALS AND METHODS

A synthetic feed slurry qualitatively simulating the mixed waste characteristics of the U.S.-Mexico border area was prepared for the anaerobic biodegradation study. A custom-made plug-flow reactor with a sludge recycle system was designed, fabricated, and operated with the synthetic feed. The reactor was simple in design and expected to be inexpensive to construct in the field. The performance of the reactor was evaluated by monitoring the effluent for concentrations of toluene, total substrate COD, and several other parameters.

Feed composition

A synthetic feed slurry mixed with a hazardous compound, toluene, was used for this research. The feed slurry was prepared from commercial dog food, yeast extract, and other inorganic nutrients (1 g/l NH_4Cl , 1 g/l KH_2PO_4 , 1 g/l $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.22 g/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.75 g/l FeCl_3 in the reactor feed stream). The dog food contained protein, fat, and fiber to simulate mixed residential solid and liquid wastes; yeast extract was included to provide soluble organics. Inorganic nutrients were added to provide buffer capacity to the feed and to support the needed inorganic nutrients.

Apparatus

A 41-liter capacity (32 liters of culture volume with 9 liters of head space) bench-scale plug-flow anaerobic reactor was used for this research. A cross-sectional plan view of the plug-flow reactor is shown in Figure 1. The rectangular, box-shaped reactor was fabricated from aluminum panels welded at the seams. Inside surfaces of the reactor were coated with Teflon to resist corrosive actions of organic acids and toluene. A Teflon-coated aluminum lid with a Viton rubber gasket was used to seal the reactor from the atmosphere. Electrical heating pads were installed on the exterior of the vertical walls. Operation of the heating pads was controlled by temperature thermister probes and controllers to maintain a constant mesophilic temperature of $35 \pm 1^\circ\text{C}$. Ball valves were installed on the reactor walls, the bottom floor, and the top cover for feeding, sampling, and withdrawing effluents. Locations of sampling ports are shown in Figure 1. Sampling ports on the vertical walls were installed 2.54 cm below the culture level. One gas collection port was installed on the reactor cover plate for gas venting, collection, and sampling. Settled sludge withdrawn through a bottom port

near the outlet was recycled to the inlet end with a peristaltic pump to seed fresh feed with acclimated anaerobic organisms. The recycle flow rate was set at 20% of the feed flow rate.

The digester gas production rate was measured by passing the gas through a three-way Teflon solenoid valve and collecting it over a salt saturated, acidic salt solution contained in a tri-tube manometric gas collector. As gas is collected over the acid solution in the first tube, the liquid level is gradually depressed until it touches the end—an electrode inserted in the tube—and signals the opening of the exhaust port of the solenoid valve. Upon complete venting of the collected gas, the liquid level in the tube returns to the top level set by another electrode. The solenoid gas vent is closed, and collection of digester gas in the first leg of the tritube starts again, completing a cycle. An electrically connected counter registers a “gas count” each time a gas-collection-venting cycle is completed. Gas production is calculated by knowing the volume between the tips of the two level probes and the gas count.

The reactor feed slurry was stored in a 15-liter cylindrical polyvinylidene fluoride (PVDF) reservoir installed inside a refrigerator kept at $5 \pm 2^\circ\text{C}$. The surface of the feed slurry in the reservoir was covered with a floating Teflon-coated cover to minimize evaporation of toluene. The feed was mixed with a motor-driven propeller mixer at a rotating speed of about 100 revolutions per minute to avoid settling of solid materials. The feed was delivered to the reactor with a timer-operated, laboratory-scale progressive cavity pump. All the materials that came in contact with the toluene-containing feed stream, reactor culture, or product gas were glass, Teflon, Viton, or PVDF.

Reactor start-up and operation

The anaerobic plug-flow reactor was started with 3 liters of feed slurry and 29 liters of anaerobic inoculum collected from digesters in the North Salt Lake City municipal waste water treatment plant. Reactor operation was started in a semicontinuous feeding mode at a hydraulic retention time (HRT) of about 50 days. The reactor HRT was carefully and gradually reduced in several steps to a final HRT of about 13 days during a period of about four months.

Digester operation was continued at an HRT of 13 days until steady-state conditions were reached as indicated by the daily gas production rate, gas composition, effluent COD and volatile fatty acids (VFA) concentrations, pH, ORP, and other parameters. In a subsequent period of operation, toluene was added to the feed first to produce a concentration of 5 mg/l in the feed. The feed toluene concentration was then gradually increased in nine steps to obtain a final influent concentration of 50 mg/l. Steady state data were collected for about two months of operation with toluene.

Analytical methods

For the purpose of monitoring reactor performance and characterizing the reactor environment, samples were withdrawn from five sampling ports installed to monitor parametric profiles along the flow path in the plug-flow reactor. Liquid, solid, and gas samples were analyzed immediately after sampling or were preserved as outlined in the APHA Standard Methods [16]. Culture pH and oxidation reduction potential (ORP), reactor gas production rate and gas composition, and concentrations of total and soluble chemical oxygen demand (COD), organic nitrogen, total and bicarbonate alkalinities, toluene, individual VFAs, total solids (TS), and volatile solids (VS) were

measured. Volatile fatty acids (VFAs) concentrations were measured with a Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector (FID) and a Chromosorb 101-packed column. The column was kept at 180°C, and nitrogen was used as the carrier gas.

Toluene concentrations in liquid and gas phases were determined by using a Perkin-Elmer Series 8000 gas chromatograph equipped with an FID and a DB-624 capillary column (J & W Scientific, Inc.). A guard column was installed between the injection valve and the column to avoid column contamination from deposits of nonvolatile materials. A direct injection method was used for toluene analysis. Temperature programming was done according to the recommendations of the column supplier. Helium was used as the carrier gas. Other chemical analyses were performed as per APHA Standard Methods [16].

KINETIC MODEL DEVELOPMENT

Grady suggested that the Monod growth kinetic equation was appropriate to describe the biodegradation kinetics for most xenobiotic compounds if the prevailing concentration did not inhibit microbial growth [17]. Edwards and Galic showed that toluene can be inhibitory to methanogenic consortiums at concentrations above 165 mg/l [4]. Since a toluene concentration much lower than 165 mg/l was used in this research, it was assumed that the Monod equation can be used to develop a model for cometabolic degradation of this xenobiotic compound. Because the toluene COD was about 1% of the total feed COD of 11,000 mg/l, it was assumed that this xenobiotic compound did not support anaerobic cell growth and that organisms growing at the

expense of the primary carbon sources cometabolized toluene.

The rate of toluene degradation in the plug-flow reactor was expressed as follows:

$$-\frac{dS}{dt} = \frac{1}{Y} \left(\frac{dX}{dt} \right) = \frac{1}{Y} \mu X, \quad (1)$$

where S is the toluene concentration at a distance L from the inlet (mg/l), L is the distance from the inlet of the plug-flow reactor (cm) = v.t, v is the average longitudinal flow velocity in the reactor, t is the retention time (days), Y is the growth yield coefficient (mass of cells formed per unit mass of substrate consumed, mg/mg), X is the concentration of toluene-degrading microorganisms (mg/l), and μ is the specific growth rate of toluene-metabolizing microorganisms (day^{-1}).

In solving Equation 1, it was assumed that the concentration of toluene-degrading organisms was approximately constant in the reactor under steady-state conditions. This assumption is supported by the observation of Lawrence and McCarty, who reported that concentration of microorganisms in the effluent of a plug-flow reactor with recycle is generally not increased significantly over that in the reactor influent [18].

Using the Monod model for μ ,

$$\mu = \frac{\mu_m S}{K_s + S} \quad (2)$$

and

$$dS = - \left(\frac{\mu_m X}{Y} \right) \frac{S}{K_s + S} dt, \quad (3)$$

where μ_m is the maximum specific growth rate (day^{-1}) and K_s is the half-velocity constant (mg/l).

Let

$$C = \frac{\mu_m X}{Y} \quad (4)$$

and

$$dt = \frac{dV}{Q} = \frac{A}{Q} dL, \quad (5)$$

where C is the maximum rate of toluene utilization (mg/l-day), V is the culture volume (cm^3), Q is the feed flow rate (cm^3/day), and A is the cross-sectional area perpendicular to the direction of flow (cm^2).

Substituting Equations 4 and 5 into Equation 3,

$$dS = -C \frac{S}{K_s + S} \left(\frac{A}{Q}\right) dL. \quad (6)$$

The solution to Equation 6 is

$$K_s \ln\left(\frac{S}{S_0}\right) + (S - S_0) = -C \left(\frac{A}{Q}\right) L, \quad (7)$$

where S_0 is the toluene concentration in the reactor (mg/l).

TABLE 1. CHEMICAL CHARACTERISTICS OF SYNTHETIC DIGESTER FEED SLURRY (WITHOUT ADDING TOLUENE).

Parameters	Feed
pH	6.2
ORP, mV	-
Total organic acid, mg/l	148
Total alkalinity, mg CaCO_3/l	3,554
Bicarbonate alk, mg CaCO_3/l	3,449
Total COD, mg/l	11,043±40
Soluble COD, mg/l	1,790
$\text{NH}_3\text{-N}$, mg-N/l	278
Org-N, mg-N/l	117
Crude protein, mg/l	733
Total solids, g/l	6.67±0.08
Volatile solids, g/l	3.77±0.05

RESULTS AND DISCUSSION

Feed fermentability

The chemical composition of the synthetic digester feed slurry is presented in Table 1. The feed slurry had a total COD concentration of about 11,000 mg/l and a soluble COD concentration of about 1,800 mg/l. Thus, about 84 wt% of the total COD was due to organic solids. The TS and VS analyses indicated that about 57% of the synthetic feed was organic in nature. Available information on the nature of mixed solid and liquid wastes found on the Mexican side of the international border indicated that a COD concentration of approximately 10,000 mg/l, a solids content of about 0.7 wt%, and a VS concentration of about 4 g/l represented the general characteristics of mixed residential wastes south of the border. A high fixed solids (FS) content of 43% was adopted because inclusion of ash, grits, and dirt in the waste streams in unsewered communities tend to increase the FS content above the levels of 30-40 wt% observed with wastes in the United States.

A total COD concentration of 11,000 mg/l represents a feed that is more dilute than that used to operate conventional, completely mixed (or "high-rate") anaerobic digesters in the United States. It is known that the VS reduction efficiency in completely mixed digesters decrease as the influent organic concentration is decreased. A plug-flow digester, on the other hand, is expected to afford a higher stabilization efficiency with lower feed VS concentration than a completely mixed anaerobic digester. In addition to simplicity in design, this was also a reason why a plug-flow reactor was selected for this research. As discussed in the following section, VS reduction exhibited by the plug-flow digester was considerably higher than the 30 to 50 percent VS

reductions commonly observed with high-rate digesters.

Alkalinity and nitrogen analyses reported in Table 1 indicated that the simulated feed had a reasonable buffer capacity and would not require the addition of external alkali to maintain balanced acidogenic and methanogenic fermentations, as needed, to achieve a neutral culture pH. Overall, it was felt that the synthetic feed represented a reasonable simulation of the mixed solid and liquid wastes generated south of the U.S.-Mexico border. Finally, it may be mentioned that a simulated digester feed was needed since it was not feasible to procure mixed Mexican wastes for prolonged periods of operation of a continuous-flow system.

Plug-flow reactor performance without toluene

The plug-flow reactor was operated for about six months to obtain steady state data at an HRT of about 13 days and a loading

rate of 0.29 kgVS/m³-day. A 13-day HRT was selected because most digesters charged with residential wastes are operated at HRTs between 13 and 40 days. The attainment of steady-state was indicated by values of daily gas production rate (GPR), gas composition, COD and toluene reduction efficiencies, and culture pH and ORP to be generally within 5 to 10% of their average values.

Steady-state performance of the plug-flow digester with the simulated feed excluding any toluene is presented in Tables 2 and 3. A culture pH of 6.8 and an effluent ORP of -335 mV with respect to the calomel electrode indicated that the anaerobic reactor was operating under acceptable methanogenic conditions [19, 20]. A total effluent VFA concentration of less than 100 mg/l indicated that balanced digestion was achieved and that there was no evidence of acid accumulation. The plug-flow reactor showed good performance in terms of total COD and crude protein reductions of 96 and 71%, respectively (Table 2). This digester

TABLE 2. CHEMICAL CHARACTERISTICS OF PLUG-FLOW DIGESTER FEED AND EFFLUENT, AND OBSERVED REMOVAL EFFICIENCIES UNDER STEADY-STATE CONDITIONS AT AN HRT OF ABOUT 13 DAYS AND A LOADING RATE OF 0.81 kgCOD/m³-DAY.

Parameters	Feed	Effluent	Removal efficiency (%)
pH	6.2	6.8 ± 0.1	-
ORP, mV	-	-335 ± 7	-
Total organic acid, mg/l	148	93 ± 24	37
Total alkalinity, mg CaCO ₃ /l	3,554	1106 ± 110	-
Bicarbonate alk, mg CaCO ₃ /l	3,449	989 ± 72	-
Total COD, mg/l	11,043 ± 40	394 ± 26	96
Soluble COD, mg/l	1,790	317 ± 14	82
NH ₃ -N, mg-N/l	278	384 ± 20	-
Org-N, mg-N/l	117	34 ± 2	-
Crude protein, mg/l	733	213 ± 11	71
Total solids, g/l	6.67 ± 0.08	3.42 ± 0.2	49
Volatile solids, g/l	3.79 ± 0.05	0.81 ± 0.04	77 ^a

^aThis efficiency was calculated from the Van Kleeck formula, $[(VS_i - VS_o)/(VS_i - (VS_i)(VS_o))]$, where VS_i and VS_o are decimal fractions VS in the influent and effluent solids.

also exhibited an excellent VS reduction efficiency of 77% compared to typical values for conventional anaerobic digesters of 30% to 50% achieved at HRTs higher than 13 days. Methane content in the digester gas ranged between 72% and 80%, compared with 50-60% for conventional anaerobic digesters (Table 3). The plug-flow digester consistently produced a high methane-content product gas that can be used by the community. The system gas production rate of 0.14 standard volume/culture volume-day was lower than that of high-rate digesters because the reactor loading rate was also lower than that of conventional systems. Consideration of data reported in Tables 2 and 3 indicated that the plug-flow digester exhibited excellent performance in terms of product gas quality and conversion efficiencies.

Plug-flow reactor performance with toluene

After completing the steady-state run without any toluene in the digester feed,

TABLE 3. STEADY-STATE GAS PRODUCTION RATE AND GAS-QUALITY DATA OBTAINED FROM THE OPERATION OF THE ANAEROBIC PLUG-FLOW DIGESTER AT AN HRT OF 13 DAYS AND AN ORGANIC LOADING RATE OF 0.81 kgCOD/m³-DAY WITHOUT ANY TOLUENE INCLUDED IN THE DIGESTER FEED.

Parameters	Values
Organic loading rate, kgVS/m ³ -day	0.29
Organic loading rate, kgCOD/m ³ -day	0.81
Gas production rate, std l/l-day,	0.14 ± 0.01
Total gas yield, SCM ^a /kgCOD added	0.16 ± 0.02
SCM ^a /kgVS added	0.46 ± 0.05
Methane yield, SCM ^a /kgCOD added	0.13 ± 0.01
SCM ^a /kgVS added	0.36 ± 0.01
Methane content, %	76.3 ± 4.5

^aSCM = standard cubic meter (1 atm and 0°C).

digester operation was continued under the same operating conditions of HRT, loading rate, temperature, etc., as before, but with toluene included in the continuous feed stream. The purpose of this run was to investigate if 50 mg/l toluene in the feed would cause inhibition of the plug-flow digestion process.

As discussed before, toluene concentration in the feed was increased in several steps. There was no evidence of any toluene inhibition during the stepwise increase in toluene concentration. Comparison of digester performance data collected without and with toluene present in the feed showed that toluene did not inhibit gasification of the non-hazardous organic feed (Tables 4 and 5). As indicated by Edwards and Galic, a toluene concentration of 50 mg/l was not expected to cause inhibition of the digestion process [11].

Evaluation of toluene degradation kinetics

Several toluene-concentration profiles were developed from analyses of liquid samples withdrawn from the different ports shown in Figure 1. Five different toluene profiles are plotted in Figure 2. A toluene profile based on the average toluene concentration for each port is shown in Figure 3. Figures 2 and 3 show that the concentration of toluene decreased along the distance from the inlet, as expected in a plug-flow reactor. The concentration of toluene at the reactor inlet was about 41.5 mg/l, which was lower than the feed concentration of 50 mg/l because of dilution of the feed by the recycled digester effluent.

Undegraded toluene in the reactor was expected to partition between the liquid and the gas phase. It was anticipated, therefore, that some toluene would escape with the reactor gas. Analysis of the digester gas showed that the concentration of toluene in the gas phase was about 0.71 mg/l. This meant that about 3.11 mg/day of toluene was released from the liquid phase. The amount of toluene released to the gas phase was approximately 1.4% of the toluene added to the reactor. The gas-phase toluene concentration was lower than that predicted from the partition coefficient probably because toluene was sorbed by the heterogeneous solid particles and removed from the liquid phase. It was expected that with continuous feeding of toluene, the

processes of biodegradation, adsorption, and back-dissolution would come to equilibrium. Under this condition, liquid-phase toluene profiles in the reactor would reflect the steady-state situation. Information presented in Figures 2 and 3 indicated that for all practical purposes there were no great differences between the five toluene profiles.

Table 6 presents a toluene mass balance for the plug-flow reactor; it shows a 95% bioconversion of the feed toluene at an HRT of 13 days and an organic loading rate of 0.81 kgCOD/m³-day. The ultimate fate of the added toluene is not known since it was difficult to identify and quantify small amounts of toluene breakdown products in a heterogeneous system. However, there was

TABLE 4. COMPARISON OF PLUG-FLOW DIGESTER PERFORMANCE AT AN HRT OF ABOUT 13 DAYS AND AN ORGANIC LOADING RATE OF 0.81 kgCOD/m³-DAY WITHOUT AND WITH TOLUENE INCLUDED IN THE DIGESTER FEED.

Parameters	Without toluene	With toluene
Effluent total COD, mg/l	394 ± 26	422 ± 3
Effluent soluble COD, mg/l	317 ± 14	417 ± 84
Effluent crude protein, mg/l	213 ± 11	202 ± 10
Effluent volatile solids, mg/l	810 ± 40	840 ± 30
Gas production rate, std l/l-day,	0.14 ± 0.01	0.13 ± 0.01
Total gas yield, SCM ^a /kgCOD added	0.16 ± 0.02	0.15 ± 0.02
SCM ^a /kgVS added	0.46 ± 0.05	0.45 ± 0.04
Methane yield, SCM ^a /kgCOD added	0.13 ± 0.01	0.13 ± 0.01
SCM ^a /kgVS added	0.36 ± 0.01	0.36 ± 0.01
Methane content, %	76.3 ± 4.5	74.5 ± 5.0

^aThis efficiency was calculated from the Van Kleeck formula, $[\text{VS}_i - \text{VS}_o]/[\text{VS}_i - (\text{VS}_i)(\text{VS}_o)]$, where VS_i and VS_o are decimal fractions VS in the influent and effluent solids.

TABLE 5. COD, PROTEIN, AND VS CONVERSION EFFICIENCIES WITH AND WITHOUT TOLUENE IN THE FEED.

Parameters	Without toluene (%)	With toluene (%)
Total COD	96	96
Soluble COD	82	77
Crude protein	71	72
Volatile solids	77	75

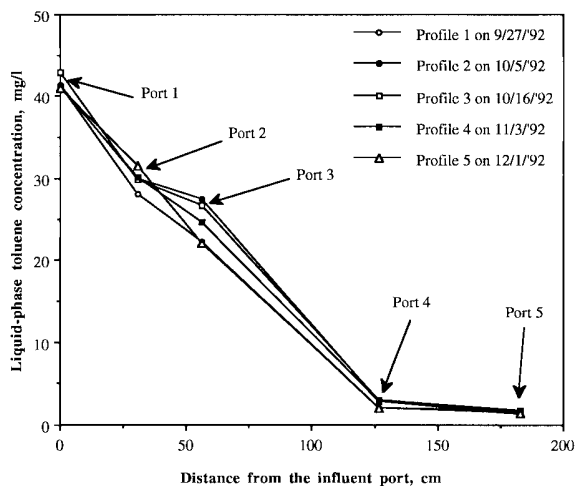


FIGURE 2. TOLUENE CONCENTRATION PROFILES IN THE MESOPHILIC PLUG-FLOW DIGESTER OPERATED AT AN HRT OF 13 DAYS AND AN ORGANIC LOADING RATE OF 0.81 kgCOD/m³-DAY.

evidence that toluene was bioconverted, but it cannot be definitely concluded that it was *mineralized*. Another evidence that toluene was biodegraded was provided by the fact that the observed toluene profile could be satisfactorily described by a bioconversion model (Equation 7 presented previously under Model Development).

Kinetic parameters for toluene degradation by the anaerobic microbial consortium in the plug-flow reactor were determined by analyzing the toluene degradation profiles in terms of the kinetic model—Equation 7—presented in a previous section. The half-velocity constant (K_s) and the maximum rate of toluene utilization (C) were estimated by a nonlinear regression technique applied to the “average” steady-state toluene concentration profile shown in Figure 3. Values of K_s and C were 12.2 mg/l and 6.2 mg/l culture-day, respectively. That these estimates of K_s and C were satisfactory was indicated by an R^2 value of 0.92. A predicted toluene concentration profile was plotted on

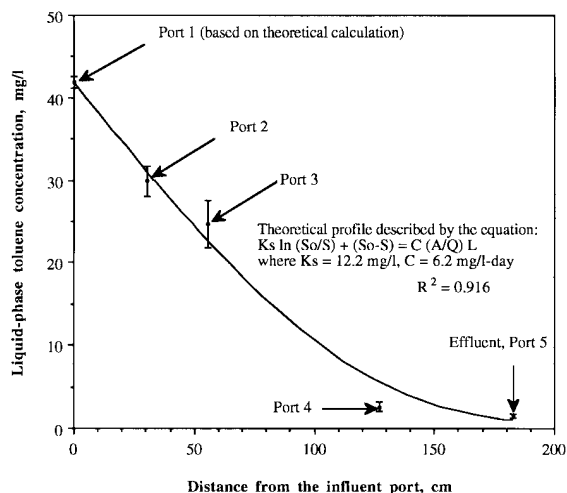


FIGURE 3. THE AVERAGE TOLUENE CONCENTRATION PROFILE IN THE MESOPHILIC PLUG-FLOW DIGESTER OPERATED AT AN HRT OF 13 DAYS AND AN ORGANIC LOADING RATE OF 0.81 kgCOD/m³-DAY. THE ERROR BAR WAS PLOTTED TO PRESENT THE STANDARD DEVIATION OF THE MEASURED VALUES OF TOLUENE. THE CURVE IN THE FIGURE WAS CALCULATED BASED ON THE KINETIC MODEL WITH KINETIC PARAMETERS OF THE HALF VELOCITY CONSTANT (K_s) AND THE MAXIMUM RATE OF TOLUENE UTILIZATION (C).

Figure 3 by using values of these kinetic parameters. It can be concluded from this plot that the cometabolic toluene-bioconversion model developed for the plug-flow anaerobic digester described the observations satisfactorily.

Maximum toluene utilization rates observed in anaerobic environments including methanogenic, sulfate-reducing, and denitrifying fermentations were calculated from literature data and reported in Table 7. All data were taken from small batch reactors charged with toluene concentrations ranging from 6.5 mg/l to 100 mg/l. However, most tests utilized a toluene concentration of less than 55 mg/l. The maximum rates were observed under methanogenic conditions.

The highest rate of 6.1 mg/l-day was obtained at a batch reactor detention time of 20 days. The maximum toluene utilization rate of 6.2 mg/l-day exhibited by the plug-flow anaerobic digester operated at an HRT of 13 days compared very well with the literature data.

SUMMARY AND CONCLUSIONS

This project was undertaken to develop a simple waste-to-energy bioconversion system for the benefit of urban areas such as those south of the U.S.-Mexico border where there is no organized collection of solid, liquid, and hazardous wastes. Data collected from operation of a small, bench-scale pilot system showed that conventional liquid and

solid wastes could be efficiently converted to a high methane-content (70-mole percent methane) fuel gas with simultaneous destruction of hazardous chemicals such as toluene. The continuous plug-flow reactor used in this research effected 96% COD stabilization, 77% VS reduction, and 95% toluene degradation by cometabolism. A kinetic model developed for cometabolic biodegradation described the reactor toluene concentration profile satisfactorily. It could predict toluene removal efficiency by cometabolic degradation under methanogenic fermentation conditions. The kinetic parameters of the half-velocity constant (K_s) and the maximum rate of toluene utilization (C) were estimated at

TABLE 6. TOLUENE MASS BALANCE IN THE MESOPHILIC PLUG-FLOW REACTOR OPERATED AT AN HRT OF 13 DAYS AND AN ORGANIC LOADING RATE OF 0.81 kgCOD/m³-DAY.

Toluene concentration	Influent (mg/ day)	Effluent (mg/ day)	Percent of the feed (%)
Feed	120.0		100.0
Gas phase		1.7	1.4
Liquid phase		4.3	3.6
Bioconversion		114.0	95.0

TABLE 7. TOLUENE BIODEGRADATION RATES DETERMINED FROM LITERATURE DATA UNDER DIFFERENT ANAEROBIC ENVIRONMENTS.

Ref. No.	Authors	Operating mode	Cometabolism	Toluene concentration (mg/l)	Environment	Degradation periods (days)	Temp. (°C)	Maximum ^a removal rate (mg/l-day)
3	Beller, <i>et al.</i> (1992)	batch	No	14.0	sulfate-reducing	14	35	1.0
4	Edwards and Galic (1994)	batch	No	6.5	methanogenic	5	35	3.5
			No	50.0	methanogenic	13	35	5.2
			No	100.0	methanogenic	20	35	6.1
9	Seyfried, <i>et al.</i> (1994)	batch	No	55.2	denitrifying	30	25	2.8
10	Beller, <i>et al.</i> (1992)	batch	No	64.4	sulfate-reducing	23	35	3.2
11	Edwards, <i>et al.</i> (1992)	batch	No	8 to 12	sulfate-reducing	38	20	1.0
12	Evans, <i>et al.</i> (1991)	batch	No	9.2	denitrifying	30	30	0.65

^aBased on the authors' calculation.

12.2 mg/l and 6.2 mg/l culture-day, respectively. This kinetic model enabled quantitative estimation of toluene degradation in the presence of organic pollutants serving as the major carbon and energy source.

ACKNOWLEDGMENTS

Research described in this paper was funded in part by the U.S. EPA under assistance agreement W-11 to the University of Utah through the Southwest Center for Environmental Research and Policy. It has not been subjected to the Agency's peer and administrative review and may not necessarily reflect the views of the Agency. No official endorsement should be inferred. The authors acknowledge the assistance provided by Ms. Athena Chan, an undergraduate student assistant, in performing sample analyses, and Mr. David Wiley, an undergraduate student assistant, in helping to assemble the anaerobic reactor system.

REFERENCES

1. M.S. Stenstrom, L. Cardinal, and J. Libra, Treatment of hazardous substrates in wastewater treatment plants, *Environ. Prog.*, 8:2 (1989) 107-112.
2. U. Altenschmidt and G. Fuchs, Anaerobic degradation of toluene in denitrifying *Pseudomonas* sp.: Indication for toluene methylhydroxylation and benzoyl-CoA as central aromatic intermediate, *Arch. Microbiol.*, 156 (1991) 152-158.
3. H.R. Beller, D. Grbic-Galic, and M. Reinhard, Microbial degradation of toluene under sulfate-reducing conditions and influence of iron on the process, *Appl. Environ. Microbiol.*, 58 (1992) 786-793.
4. E.A. Edwards and D. Grbic-Galic, Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium, *Appl. Environ. Microbiol.*, 60 (1994) 313-322.
5. S. Ghosh and M.L. Sun, Anaerobic biodegradation of benzene under acidogenic fermentation condition, In: L.E. Erickson, S.C. Grant, and J.P. McDonald (Eds.), *Proceedings of the Conference on Hazardous Waste Research, Engineering Extension*, Kansas State University, 1992, pp. 208-218.
6. S.R. Hutchins, G.W. Sewell, D.A. Kovacs, and G.A. Smith, Biodegradation of aromatic hydrocarbon by aquifer microorganisms under denitrifying conditions, *Environ. Sci. Technol.*, 25 (1991) 68-76.
7. D.R. Lovley and D.J. Lonergan, Anaerobic oxidation of toluene, phenol, and *p*-cresol by the dissimilatory iron-reducing organism GS-15, *Appl. Environ. Microbiol.*, 56 (1990) 1858-1864.
8. R. Rabus, R. Nordhaus, W. Ludwig, and F. Widdel, Complete oxidation of toluene under strictly anoxic conditions by a new sulfate-reducing bacterium, *Appl. Environ. Microbiol.*, 59 (1993) 1444-1451.
9. B. Seyfried, G. Glod, R. Schocher, A. Tschech, and F. Zeyer, Initial reaction in the anaerobic oxidation of toluene and *m*-xylene by denitrifying bacteria, *Appl. Environ. Microbiol.*, 60 (1994) 4047-4052.
10. H.R. Beller, M. Reinhard, and D. Grbic-Galic, Metabolic by-products of anaerobic toluene degradation by

- sulfate-reducing enrichment cultures, *Appl. Environ. Microbiol.*, 58 (1992) 3192-3195.
11. E.A. Edwards, L.E. Wills, M. Reinhard, and D. Grbic-Galic, Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions, *Appl. Environ. Microbiol.*, 58 (1992) 794-800.
 12. P.J. Evans, D.T. Mang, K.S. Kim, and L.Y. Young, Anaerobic degradation of toluene by a denitrifying bacterium, *Appl. Environ. Microbiol.*, 57 (1991a) 1139-1145.
 13. D. Grbic-Galic and T.M. Vogel, Transformation of toluene and benzene by mixed methanogenic cultures, *Appl. Environ. Microbiol.*, 53 (1987) 254-260.
 14. R.J. Schocher, B. Seyfried, F. Vazquez, and J. Zeyer, Anaerobic degradation of toluene by pure cultures of denitrifying bacteria, *Arch. Microbiol.*, 157 (1991) 7-12.
 15. M. Alexander, Biodegradation of organic chemicals, *Environ. Sci. Technol.*, 18 (1985) 106-111.
 16. American Public Health Association, American Water Works Association, Water Environment Federation, In: A.E. Greenberg, L.S. Clesceri, and A.D. Eaton (Eds.), *Standard Methods for Examination of Water and Wastewater*, 18th edition, APHA, 1992.
 17. C.P.L. Grady, Biodegradation of toxic organics: Status and potential, *J. Environ. Eng.*, 116 (1990) 805-828.
 18. A.W. Lawrence and P.L. McCarty, Unified basis for biological treatment design and operation, *J. Sanit. Engng Div., Am. Soc. Civ. Engrs.*, 96 (1970) 757-778.
 19. Metcalf and Eddy, *Wastewater Engineering: Treatment, Disposal, and Reuse*. 3rd ed., McGraw-Hill, Inc., New York.
 20. A.J.B. Zehnder and W. Stumm, Geochemistry and biogeochemistry of anaerobic habitats. In: A.J.B. Zehnder (Ed.), *Biology of Anaerobic Microorganisms*, Wiley-Liss, New York, 1988.