Treatment of Hazardous Substances in Wastewater Treatment Plants

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*For many types of hazardous wastes, particularly those contained in high volume,
low concentration wastewaters, biological treatment in existing facilities,
perhaps in selected publicly owned treatment works (POTWs), may be a better alternative.
The motivation for using existing biological facilities to treat hazardous wastes
is very high since there is insufficient hazardous waste treatment capacity.
This problem will become exacerbated in the future unless the current
hazardous waste facility problems are resolved.*

INTRODUCTION

Since the passage of the PL 92-500, most municipalities in the United States have constructed some type of secondary wastewater treatment facilities. The vast majority of these use the activated sludge process to remove the organic soluble and colloidal pollutants. In this process, biodegradable material is oxidized to carbon dioxide, water, or converted to biomass. Non-biodegradable pollutants may pass through the process, but are more often removed from the liquid phase, either by adsorption onto biological flocs that are subsequently removed in secondary clarifiers or volatilization to the atmosphere during aeration.

These publicly owned treatment works (POTWs) were designed to treat municipal wastewaters comprised primarily of biodegradable materials from domestic operations, such as human wastes, kitchen wastes, and washing byproducts. Consequently, the process has been optimized for treating these easily biodegraded materials. Commercial and industrial discharges almost always have separate treatment systems. These treatment systems often discharge their effluents into POTWs, and for this reason they are actually functioning as pretreatment systems.

Municipalities like Los Angeles have many industries discharging into their treatment system. Consequently, the wastewaters are composed not only of municipal wastes but also of industrial components. The industrial contribution results from imperfect pretreatment systems, fugitive emissions, or illegal discharges. Table 1 shows

TABLE 1. SOURCES OF TOXIC COMPOUNDS.

Compound	Anthropogenic Sources	Natural Sources
Cresols	Auto Exhaust Roadway Runoff Asphalt Runoff Petroleum Distillates Oils, Lubricants	Coal Petroleum Wood Constituent Natural Runoff
Xylenols	Roadway Runoff Asphalt Runoff Fuels, Solvents Plastics Pesticides Catalytic Cracking	Coal
Isophorone	Solvent for Resins Lacquers and Finishes Pesticides Organic Chemical Mfg.	
PAHs*	Air Pollution	Plant and Animal Pigments
	Processing of Fossil Fuel	Crude Oil
	Combustion of Fossil Fuel	Grass and Forest
	Roadway Runoff	Fires
	Catalytic Cracking	

* Polycyclicaromatic hydrocarbons

nificant, "semi-hazardous" waste. Wastes that can be biodegraded or adsorbed to sludge, and those that cannot be conveniently isolated from municipal waste or urban runoff, are the best candidates for treatment in POTWs.

To evaluate the suitability of conventional plants to treat these wastes, a survey of industrially significant candidate compounds was made and several were selected for experimental investigation. A two year experimental investigation was conducted using 12 liter complete mixing biological reactors, with integral clarifiers. This paper reports our findings for a significant fraction of these compounds, representing alicyclic and aromatic compounds, using isophorone, several isomers of xylenol, and all three cresol isomers. Biodegradation rates and a model to predict volatilization are presented.

potential sources for the compounds being investigated in this project.

In the current environment of increasing control of hazardous wastes, municipal plants are being evaluated for a larger treatment roll. Few economical and reliable hazardous waste treatment alternatives exist, and those that do are reserved for the most hazardous and problematic wastes. It is proposed that municipal systems be re-evaluated as a treatment technology for commercially sig-

BIODEGRADATION

It is generally believed that before aromatic compounds can undergo ring-fission, the benzene nucleus must contain at least two hydroxyl groups. This usually results in the formation of a catechol, protocatechutate, or gentisate [1]. Chapman and co-workers [2–4] found that the degradation of 2,4 xylenol was initiated by the oxidation of the methyl substituent para to the hydroxyl group and that p-cresol and 3,4 xylenol were attacked in a similar manner [5]. The further degradation of 4-hydroxy-3-methylbenzoic acid, from 2,4 xylenol, involves oxidation of the methyl substituent to a carboxyl group forming 4-hydroxy-isophthalic acid followed by an oxidative decarboxylation of the newly formed carboxyl to give protocatechuic acid. Protocatechuic acid is then metabolized to b-ketoadipic acid by protocatechuic acid-3,4-dioxygenase. Figure 1 shows the overall pathway. In 2,4 xylenol both methyl groups undergo oxidation in succession

β-Ketoadipic acid   Protocatechuic acid
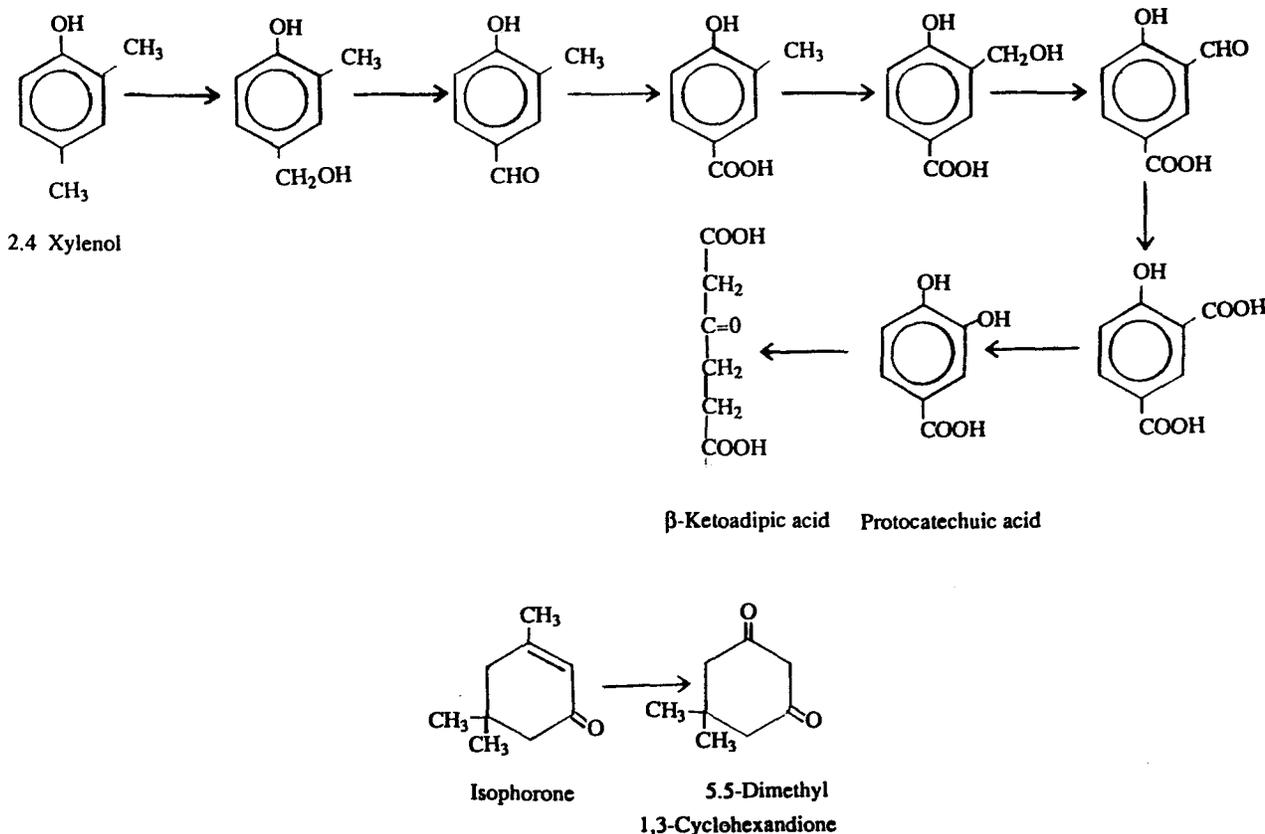
Isophorone   5.5-Dimethyl
1,3-Cyclohexandione

Figure 1. Biodegradation pathways for 2,4 xylenol (after Chapman (1971)) and isophorone (speculated).

with the original ortho methyl group being replaced by hydroxyl to produce protocatechuic acid. For the xylenols utilizing the pathways involving catechol and gentisate, one or both of the methyl groups remain intact until after ring cleavage. Hooper [3] showed that a species of Pseudomonas initiated the degradation of cresols and xylenols by oxidizing a methyl group placed meta to the hydroxyl group, and added a second hydroxyl group para to the first. As a result m-cresol, 2,5 xylenol, and 3,5 xylenol were oxidized to gentisic acid, 4-methylgentisic acid, and 3-methylgentisic acid, respectively. Dagley and Patel [5] found that *Pseudomonas putida* was able to utilize 2,3 xylenol and 3,4 xylenol, as well as phenol and o-, m-, p-cresol. They suggested that the catabolism of these compounds proceeded via direct ring hydroxylation and meta ring cleavage of the resulting catechols.

The pathways of the alicyclic hydrocarbons are not as well researched. Alicyclic hydrocarbons are resistant to microbial attack and microorganisms capable of utilizing them as sole carbon sources are not easily isolated. The difficulty in isolating single microorganism may arise because degradation occurs primarily under conditions of co-oxidative metabolism, and in commensal situations. Trudgill [6] achieved the best results when using mixed cultures to study the degradation of alicyclic compounds. From our results, using mass spectrophotometry, isophorone undergoes oxidation resulting in the number 3 methyl group being replaced by an oxygen. The resulting intermediate is 5,5-dimethyl-1,3-cyclohexandione. Our results show that ultimately isophorone is completely degraded.

The results presented indicate that many of these reactions are mediated by enzymes of broad specificity, inducible by structurally related compounds. Exposure of the bacteria to a single compound can induce the production of enzymes that can partially or completely metabolize a whole class of compounds. In activated sludge there is the potential for the induction of many degradation pathways. Enriching activated sludge in microorganisms that utilize a particular pathway can enhance the degradation other aromatic or alicyclic hydrocarbons that may also be degraded by that pathway.

EXPERIMENTAL METHODS
Reactor Description

Three identical continuous flow activated sludge reactors were operated in parallel and used as a source of microorganisms for experimentation. Initially, one received 2,4 xylenol, one received isophorone, and the third served as the control. All three reactors received a glucose solution, which served as the primary substrate. This feed stock, reactor geometry, and operating conditions have been described by Ng, et al. [7].

The concentrated glucose feed was prepared and automatically diluted before being pumped into the reactors. This concentrated feed was diluted 404 times resulting in influent glucose concentration of 250 mg/L. The feed solutions were refrigerated at 5°C.

The toxic compounds were pumped from separate reservoirs. The concentration of toxic compound in the reactor influent was gradually increased over time to allow acclimation of the activated sludge cells. The 2,4 xylenol solution in water was prepared at a concentration such, that when combined with the glucose feed, the maximum concentration, after acclimation was 110 mg/L. Isophorone was similarly prepared with a maximum concentraton of 118 mg/L.

The activated sludge used to initially seed the reactors was obtained from the City of Los Angeles's Hyperion

TABLE 2. ACTIVATED SLUDGE OPERATING PARAMETERS (MEAN VALUES).

MCRT	13.8 days
HRT	13.8 hrs
MLVSS	20 g/L
Glucose, as BOD_5	250 mg/L
pH	7.0
DO	>3.0 mg/L
Q	24 L/day
F/M	0.2

Treatment facility. To increase the probability of acclimation by maintaining a diverse cell population, cells from other treatment plants in the Los Angeles area were added periodically until acclimation was achieved. The operating parameters maintained for all reactors during the period of experimentation are shown in Table 2.

Analytical Methods

Isophorone was concentrated using liquid/liquid extractions [8]. Three successive extractions using 5 ml of dichloromethane to 25 ml sample were made. Excess water remaining in the dichloromethane fraction was removed after extraction and removal using anhydrous sodium sulfate. The solvent was then filtered and volume was reduced using roto-evaporation.

Xylenols, cresols, and trimethylphenol were extracted using bonded phase silica sorbents. A CH (cyclohexyl) bonded phase sorbent (Bond Elute from Analytichem International, Harbor City, CA) was used. Sample pH was adjusted to 1–2, and 5% wt/vol of NaCl was added to enhance recovery. The procedure used to complete the extraction is the same as that previously described by Chladek and Marano [9].

Compounds adsorbed onto the surface of the suspended solids in the activated sludge were extracted using a modified method from Warner [10]. One hundred ml aliquots were removed from each reactor and centrifuged. After removing the supernatant 1 ml of distilled water was added to the centrifugation vial to facilitate cell removal. Using a syringe, 5 ml of cells were removed and placed in a screw top test tube. The cells were then extracted with three successive portions of dichloromethane. Each extraction involved shaking for one minute followed by a three minute centrifugation. The dichloromethane layer was removed from the bottom of the test tube using a long needle syringe. The extract was dried and concentrated as before.

The final analysis of the samples was done using GC or GC/MS. Fused silica Supelcowax 10 column was used for both isophorone and 2,4 xylenol. For isophorone the initial temperature was 90°C, increased at 8°C/min until 270°C then held at 270°C for two minutes. For xylenols, cresols, and trimethylphenol the initial temperature was 70°C, which was then increased at 8°C/min until the final temperature of 240°C was reached. The injector temperator was 250°C, the FID detector temperature was 280°C for both compounds.

Batch Assay Procedure

Batch assays to determine biodegradation rates of isophorone, xylenol, trimethylphenol, and cresol were performed with samples of activated sludge in 150 ml bottles. For each assay, 100 ml aliquots were collected from the experimental and control reactors, and placed in separate bottles. Each bottle was then centrifuged and the supernatant discarded. One hundred ml of an aqueous solution containing appropriate amounts of the compound

being tested and feed solution were added to each bottle. This mixture was then incubated and aerated for the appropriate time interval. At the end of the incubation period the reaction was stopped by adding one drop (.1 ml) of a 1 + 1 H_2SO_4 solution in H_2O. The final volume was measured and in some cases adjusted to the original volume. The sample was then refrigerated until the cells could be separated from the supernatant by centrifugation. The cells or supernatant were then extracted as previously described.

For the batch assays in which 2,4 xylenol, 2,3 xylenol, and o-cresol were tested for degradability alone and combined in a mixture, 1 and 2 liter containers of activated sludge were used. For each compound tested separately, two 1 liter beakers were incubated with the appropriate amount of toxic compound and feed solution, one beaker containing acclimated activated sludge and one containing unacclimated sludge. For the mixture, 2 liter flasks were used in place of 1 liter beakers. At periodic intervals, 25 ml of sample were removed from the beaker and flasks, acidified and immediately extracted.

VOLATILIZATION

Volatilization or stripping of organics from activated sludge plants has been investigated by Roberts, et al. [11], and Nanking and Rittmann [12]. These investigators and others have estimated the volatilization rates of trace organics using oxygen as a tracer. In general this appears to be a valid approach, and the two-film model used to estimate oxygen transfer can also be used to estimate volatilization losses.

The following analysis is presented to show that volatilization rates for xylenols and isophorone are negligible for high rates of biological destruction. In the case of both compounds, adsorption to sludge particles does not occur, and has been confirmed by experimental measurements. The material balance equations for a single volatile species C, which decays at a first order rate, become

$$\frac{dC_g}{dt} = \frac{Q_g(C_{go} - C_g)}{V_g} - K_L a_C (C^*_{g\infty} - C_l) \frac{V_l}{V_g} \qquad (1)$$

$$\frac{dC_l}{dt} = Q_l \frac{(C_o - C_l)}{V_l} + K_L a_C (C^*_{g\infty} - C_l) - KC_l \qquad (2)$$

where

Q_g, Q_l = gas and liquid volumetric flow rates, respectively

C_g, C_l = gas and liquid volumetric concentration of species C

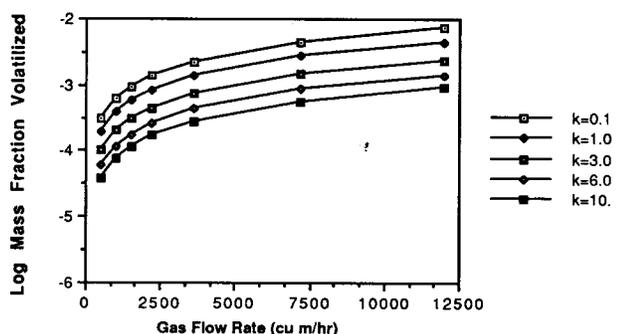C_{go}, C_o = inlet gas and liquid phase concentrations of species C

K = first-order decay rate.

The equilibrium concentration $C^*_{g\infty}$ is calculated as

$$C^*_{g\alpha} = C_g \frac{RT}{H_e} e_d \qquad (3)$$

where

H_e = Henry's Law constant $\left(\frac{atm\ m^3}{mol}\right)$

R = Universal gas constant, $8.206 \times 10^{-5} \left(\frac{atm\ m^3}{mol\ °K}\right)$

T = absolute temperature, °K

e_d = effective saturation depth, dimensionless, which accounts for the effect of hydrostatic pressure in the aeration system.

The decay parameter, K, is shown as a first-order reaction rate coefficient. In many situations it will be first-order, and in other situations it may be zero-order. Most biological modeling investigators use a saturation function to describe the decay rate. Such functions can be zero-order at high substrate concentrations and first-order at low concentrations. The rate is usually a function of active biomass concentration, often measured as volatile suspended solids concentration, or MLVSS. The reactors were modeled as first-order for this volatilization analysis, and K is considered a function of MLVSS.

The volumetric mass transfer coefficient, $K_L a_C$, is estimated from the oxygen mass transfer coefficient, $K_L a_{02}$. The oxygen mass transfer coefficient is generally known or can be estimated for particular conditions [13]. Roberts, et al. [11] have suggested that $K_L a$ be modeled as

$$K_L a_C = \Psi\ K_L a_{02} \qquad (4)$$

In clean water tests they have tabulated ranges of Ψ between 0.5 and 0.7 for a variety of 2 or 3 carbon chlorinated organic solutes. Considering this as a worst case for larger molecules, which should have lower diffusivity coefficients, is conservative and provides an upper bound for stripping estimates. The maximum transfer rate is also further reduced by contaminants in the wastewater, and is generally correlated by an empirical alpha factor, ranging from 0.2 to 0.8.

Figures 2A and 2B show the results of using the model described by Equations (1) to (4) for a typical domestic wastewater treatment plant. Figure 2A shows the losses for complete mixing reactor and Figure 2B shows the losses for a plug flow system simulated by four CSTRs in series. The aeration rate is tapered 2 to 1. The volume and size parameters were selected for a 1,000 m³/hr (6.5 MGD) treatment plant with 6-hour hydraulic retention time. The gas is also modeled as a complete mixing reac-
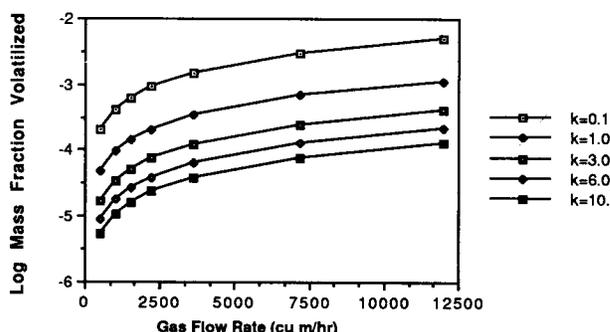
Figure 2a. Volatilization losses of 2,4 xylenol in a CSTR for varying gas flow rates.

Figure 2b. Volatilization losses for 2,4 xylenol in a dispersed flow reactor (4 tanks-in-series) with tapered aeration rates.

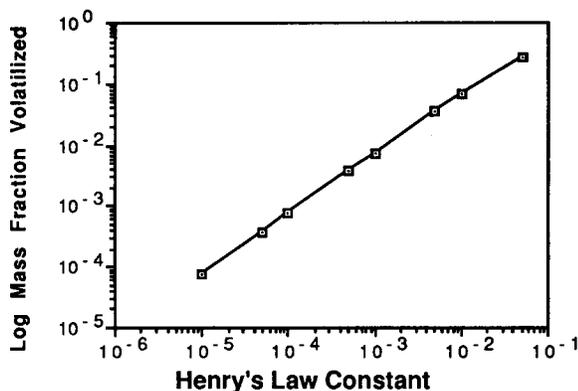Figure 3. Volatilization losses for varying Henry's Law constant.

Figure 4. Time series destruction data for xylenol and isophorone, illustrating zero order rates.

tion, and a hold up gas volume of 140 m^3 is typical for a subsurface aeration system in a 4.5 m (15 ft) deep tank. A typical value of $K_L a_{02}$ would be 5 hr^{-1} for a fine bubble diffused aeration system transferring approximately 7% oxygen. Gas flow rates for these conditions should be approximately 500 to 1500 m^3/hr. This represents typical operation for a modern activated sludge process system found in a municipal treatment plant. The possible range of all anticipated gas flow rates for all conceivable aeration systems is approximately 500 m^3/hr for the highest efficiency subsurface aeration system, to 12,000 m^3/hr for the lowest efficiency spiral roll system.

For the lowest K the treatment efficiency is only about 40%. At this rate approximately 0.006 mass fraction is lost by volatilization with the lowest efficiency aeration system. The typical aeration system using 1500 m^3/hr loses less than 1 × 10^{-3} mass fraction. For greater degrees of biological treatment the losses are much less, as low as 10^{-5} mass fraction. The plug flow reactor loses much more at lower biological treatment efficiencies, due to the higher per unit volume gas flow rate in the early stages. Figure 3 shows the results for a CSTR for varying Henry's Law coefficients. Volatilization losses become significant when the Henry's Law constant becomes 10^{-2} to 10^{-3}. For the compounds used in this study we expect no measurable volatilization losses. The rate of volatilization is affected by gas flow rate. Therefore high efficiency submerged aeration systems such as fine bubble diffusers should be used to minimize volatilization rates.

Biodegradation Results

Table 3 shows the results of our reactor studies for xylenols, cresols, and isophorone. Degradation rates were cal-

culated from time series data, as shown in Figure 4. Both first-order and zero-order kinetic coefficients were calculated. Zero-order kinetics describe the experimental results better than first-order results, which should be expected in this high concentration range.

Figure 5 shows the results of an experiment designed to show that the reaction rate, while zero-order with respect to substrate concentration, is first-order with respect to MLVSS concentration. The data shown in Figure 5 were collected in batch assays. Cells were diluted with distilled water to obtain the desired concentration. Figure 5 shows the ratio concentration measured at one hour into the batch assay divided by the initial concentration. At 2.1 g/L MLVSS concentration approximately 64% of the 2,4 xylenol was destroyed after one hour. At 1.05 g/L approximately 28% was destroyed. The results indicate that the zero-order reaction rate constants tabulated in Table 3 are approximately first-order with respect to MLVSS concentration.

The continuous experiments were conducted for several months for each compound. After acclimation no detectable (<5.0 μg/L) effluent concentrations of the experimental compound were found. No stable intermediates or byproducts were observed. An intermediate was found in the isophorone batch assays, and was tentatively identified as 5,5 dimethyl-1-3-cyclohexadine. The extremely low effluent concentrations indicate that complete destruction of the compounds is possible. No experiments were performed to determine Monod half-saturation coefficients. However, they must be quite low, as indicated by the less than detectable effluent concentrations.
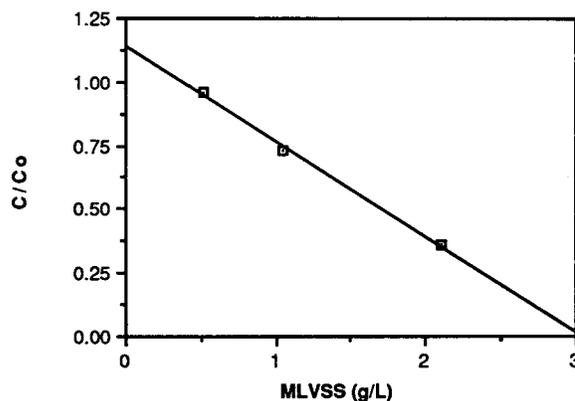
TABLE 3. TYPICAL RESULTS FROM A CULTURE ACCLIMATED TO 2,4 XYLENOL

Compound	Minimum Degradation Rate (Co-C)/ (MLVSS *ΔT)	MLVSS (gm/L)
2,4 xylenol	15.37	2.160
2,3 xylenol	3.04	2.240
2,5 xylenol	2.87	1.582
2,6 xylenol	0.94	1.909
3,4 xylenol	4.62	1.909
3,5 xylenol	0.00	3.102
2,4,6 trimethylphenol	2.18	1.909
o-cresol	1.49	2.518
p-cresol	1.46	2.518
m-cresol	1.47	2.518

Figure 5. Xylenol concentration versus MLVSS concentration after one hour exposure.
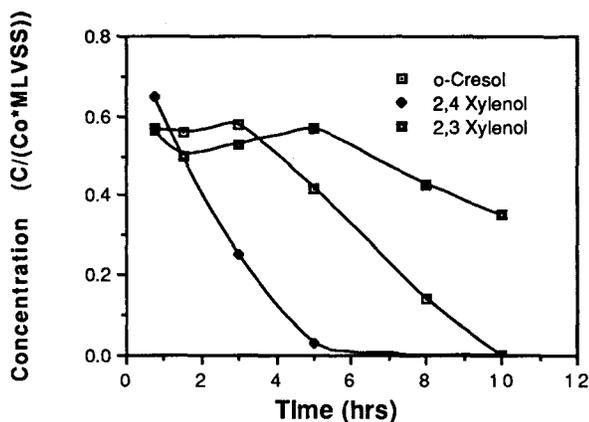
Figure 6. o-Cresol, 2,4 xylenol, and 2,3 xylenol versus time.

Return Activated Sludge

Figure 7. Activated sludge process with enrichment reactor (E).

Figure 6 shows the results of a mixed substrate experiment using 2,4 and 2,3 xylenol and o-cresol. The culture was previously acclimated to 2,4 xylenol, which disappears first. After the disappearance of 2,4 xylenol the 2,3 xylenol and o-cresol begin to degrade.

The compounds used in the study do not adsorb onto the microbial solids. If this were to happen then their ultimate fate would depend upon sludge disposal technique. In some cases, adsorption might be desirable and in other cases it would not.

CONCLUSIONS

Previous work by Chapman [4] has shown that the degradation of 2,4 xylenol undergoes hydroxylation at the 2 and 4 position to give protocatechutate, which is then metabolized by ortho fission of the benzene nucleus. The enzymes of the first part of this reaction have been shown to have broad specificity, oxidizing 3,4 xylenol, and p-cresol. The enzymes catalyzing the remaining degradation steps in the conversion of p-hydroxybenzoic acid to b-ketoadepate possess a high degree of specificity.

Our results indicate that the mechanisms that occur in the degradation of 2,4 xylenol in pure culture can also be found in our activated sludge process enriched for 2,4 xylenol degradation. The same culture will also oxidize the other isomers of xylenol that have methyl groups in the 2 or 4 position, all isomers of cresol, and 2,4,6 trimethylphenol. All compounds that appear to degrade well with these cultures have a methyl group in the 2 or 4 position. Of these, o-cresol degrades the slowest. 3,5 xylenol did not biodegrade in any batch assay experiments, even those that lasted 14 hours.

There were no intermediates of degradation identified by gas chromatography when the test compound had reached nondetectable levels. Consequently, although the initial oxidation of the methyl group of the phenols most likely was oxidized by the enzymes provided by the cells that degrade 2,4 xylenol, the completed oxidation of the compound must involve other microorganisms. Since their numbers are much less, due to lack of enrichment, the compounds that utilize alternate pathways are degraded at a much slower rate.

Enrichment of activated sludge that contains microorganisms that utilize cometabolism and/or commensalism, as well as produce enzymes of broad specificity could be an efficient and cost effective way to degrade toxic waste. The concept here could be expanded, such that the activated sludge could be acclimated to all three pathways involving the intermediates gentistic, protocatechuic acid, and catechol by feeding it 2,4 xylenol, 2,5 xylenol
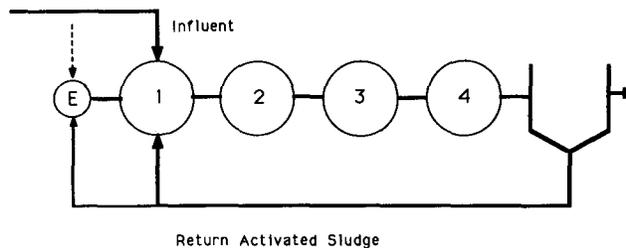
and 2,3 xylenol, respectively. The resulting enriched activated sludge could then degrade, with expediency, a wide range of phenolic compounds. Figure 7 shows an enrichment factor concept whereby a small reactor treating a selected substrate, as described above, could continuously seed a large activated sludge process.

Our modeling results show that volatilization of xylenol and compounds with similar Henry's Law constants ($\sim 10^{-5}$) is insignificant in the presence of high rates of biodegradation. Typically, mass fraction of less than 10^{-4} to 10^{-5} percent will occur with effective biodegradation. Without degradation losses increase to slightly less than 0.01 mass fraction.

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