Total Mineralization of Aqueous Atrazine in the Presence of Ammonium Nitrate Using Ozone and *Klebsiella terragena* (Strain DRS-I): Mechanistic Considerations for Pilot Scale Disposal

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s-Triazines have been found to be somewhat recalcitrant to enhanced biomineralization (preoxidation prior to microbial degradation). Atrazine ozonation studies provided evidence for a hydroxy radical process in which kinetics are sterically rather than electronically controlled. These results enabled the optimization of the ozonation procedures and the isolation of an organism better adapted for pesticide waste streams. *Klebsiella terragena* (strain DRS-I) was shown to effectively utilize chlorodiamino-*s*-triazine (CAAT), the final oxidation product of atrazine, as a nitrogen source in the presence of NH_4NO_3, a fertilizer often found in pesticide waste. Evidence for the metabolic pathway is provided, which was shown to proceed via aminochlorohydroxy-*s*-triazine (COAT) followed by dechlorination and total mineralization. Formulated atrazine was successfully mineralized in a pilot scale system (*ca.* 200 L) with and without NH_4NO_3 present. Ozonation efficiencies were nearly identical in both cases; degradation of CAAT and COAT was readily achieved after *ca.* 1 day of incubation.

Keywords: *Atrazine; remediation; oxidation; biomineralization*

INTRODUCTION

Development of remediation strategies for pesticide wastes, application equipment rinsates, and contaminated soil and water has received considerable attention over the past decade (Krueger and Seiber, 1984; Bourke *et al.*, 1992). In the past, rinsates and other pesticide wastes were typically deposited on nearby soil to evaporate and presumably degrade; however, this method has proven to be inadequate as evidenced by the contamination of applicator sites and nearby wells (Aharonson 1987; Parsons and Witt, 1988). In some regions of the country, applicators have initiated programs to minimize waste by collecting and reusing rinsates in subsequent applications. Waste elimination is not always possible, however, particularly in areas where multiple crops are grown and/or a variety of pesticides are used.

Laboratory studies have shown that ozonation of pesticides enhances the rate of microbial mineralization, *i.e.*, breakdown to CO_2, H_2O, NH_3^-, or NO_3^-, and other inorganic salts (Kearney *et al.*, 1988; Somich *et al.*, 1988; Hapeman-Somich, 1991). Preliminary on-site evaluation of ozonation and subsequent biodegradation by indigenous soil microflora has demonstrated the potential usefulness of this binary process (Somich *et al.*, 1990; Hapeman-Somich *et al.*, 1991), although ozonation efficiencies were less than desirable. Results further indicated that the *s*-triazinyl herbicides, such as atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], one of the most widely used herbicides, were somewhat recalcitrant to ozonation (and their ozonation products to biomineralization) as compared to other pesticides. A pseudomonad (PSA, strain A) that could utilize *s*-triazines as sole nitrogen sources was found to be more effective in mineralizing atrazine ozonation products than indigenous soil microorganisms in laboratory studies; however, degradation was completely inhibited in field experiments (Somich *et al.*, 1990). Pesticide rinsate typically consist of an aqueous mixture of formulating agents, emulsifiers, surfactants, fertilizers, and pesticides at concentrations of 10−1000 ppm. High ammonia concentrations (*ca.* 1%) were found *a priori* to be responsible for the inhibition of microbial degradation (Leeson *et al.*, 1993).

Thus, successful optimization and scaleup of this binary process require extensive knowledge of the ozonation chemistry, isolation and characterization of tolerant organisms, and development of a more efficient ozonation chamber. Atrazine was used as a test compound because it was not readily oxidized or biomineralized as compared to the phenoxy and chloroacetanilidyl herbicides commonly found in pesticide waste.

METHODS AND MATERIALS

Standards and Analytical Methods. For convenience, the nomenclature system used by Cook (1987) is used here: A, amino; C, chloro; E, ethylamino; I, isopropylamino; O, hydroxy; and T, *s*-triazine ring. Several ozonation products contained an acetamide group so, in keeping with this nomenclature, D has been added to denote this moiety (Table 1). Atrazine (CIET) [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], simazine (CEET) (2-chloro-4,6-bis(ethylamino)-*s*-triazine), propazine (CIIT) [2-chloro-4,6-bis(isopropylamino)-*s*-triazine], 6-amino-2-chloro-4-(isopropylamino)-*s*-triazine (CIAT), and 6-amino-2-chloro-4-(ethylamino)-*s*-triazine (CEAT) were gifts from Ciba-Geigy (Greensboro, NC). Chlorodiamino-*s*-triazine (CAAT) was purchased from Aldrich (Milwaukee, WI). 4-Acetamido-2-chloro-6-(isopropylamino)-*s*-triazine (CDIT), 4-acetamido-2-chloro-6-(ethylamino)-*s*-triazine (CDET), 2-chloro-4,6-diacetamido-*s*-triazine (CDDT), and 6-amino-4-acetamido-2-chloro-*s*-triazine (CDAT) were synthesized as described elsewhere (Hapeman-Somich *et al.*, 1992). Aminochlorohydroxy-*s*-triazine (COAT) was acquired from melamine-grown *Pseudomonas* strain A which converts CAAT to COAT with no further metabolism (Grossenbacher *et al.*, 1984). Resting cells were incubated with 1 mM CAAT at 28 °C, and the sample was used as a chromatographic standard with no further isolation except centrifugation (6000*g*, 10 min) to remove microorganisms. COAT was identified in unknown samples by comparison of the retention time and UV spectra

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Table 1. 2-Chloro-*s*-triazines and Their Ozonation Products

compound	CIET product	CEET product	CIIT product
CIET atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine]			
CIIT propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine]			
CEET simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine]			
CAAT chlorodiamino-*s*-triazine	X	X	X
CDAT 6-amino-4-acetamido-2-chloro-*s*-triazine	X	X	X
CDDT 2-chloro-4,6-diacetamido-*s*-triazine	X	X	X
CDET 4-acetamido-2-chloro-6-(ethylamino)-*s*-triazine	X	X	
CDIT 4-acetamido-2-chloro-6-(isopropylamino)-*s*-triazine	X		X
CEAT 6-amino-2-chloro-4-(ethylamino)-*s*-triazine	X	X	
CIAT 6-amino-2-chloro-4-(isopropylamino)-*s*-triazine	X		X
COAT aminochlorohydroxy-*s*-triazine			

obtained from a Waters (Milford, MA) Model 990 photodiode array detector on the HPLC system described below with the standard sample.

Ozonation samples were analyzed directly (*i.e.*, no extraction procedures were necessary) by HPLC employing two Waters Model 510 HPLC pumps equipped with a Waters Model 490 UV detector (210, 225, and 235 nm monitored), an NEC APC-IV controller, and Maxima 820 software. Separations were achieved using a 15–50% acetonitrile/phosphoric acid buffer (pH 2) 10 min gradient (Waters Curve 10) at a flow rate of 0.4 mL/min on a standard Beckman C_{18} (ODS, 5 μm) end-capped 4.6 mm × 25 cm steel jacketed column or using a 0–50% acetonitrile/phosphoric acid buffer (pH 2) gradient (Waters Curve 8) in 5 min at a flow rate of 2 mL/min on a Waters NOVAPAK 4 μm C_{18}, 8 mm × 10 cm radial compression module column. Concentrations of CDDT, CDAT, and CAAT in hydrolysis samples were determined directly using a NOVAPAK column and a 20–30% acetonitrile/phosphoric acid buffer (pH 2) linear gradient (3 min); 225 and 210 nm were monitored. CAAT and COAT concentrations in biological studies were determined at 210 nm and using a Waters Resolve C_{18} column; separations were achieved using an ion pair solvent system (pH 2.0) consisting of 2.863 g of heptanesulfonic acid, 13.7 mL of diethylamine, and 18.05 mL of 85% H_3PO_4 in 1 L of water. All *s*-triazine concentrations, except COAT, were determined using response factors or concentration standard curves.

Ozone concentrations in solution were determined using the indigo method described by Bader and Hoigné (1981). Chloride ion concentrations were determined using an Orion chloride electrode. Ammonia and nitrate concentrations were determined colorimetrically using a flow injection system (Lachat, Milwaukee, WI).

Ozonation Procedure. Laboratory ozonation experiments were carried out in a previously described standard photoreactor retrofitted with a bottom-feed sintered glass frit (Somich *et al.*, 1988). Ozone was generated using a PCI Ozone generator Model GL-1B (PCI Ozone Corp., West Caldwell, NJ) with oxygen feed and delivered to the reactor at 1 L/min with an ozone concentration between 0.2 and 0.4% as determined using a PCI Ozone monitor Model HC. Initial concentrations of starting material ranged from 15 to 150 μmol in ultrapure water (Modulab, Type I HPLC, Continental Water System Corp., San Antonio, TX) at circumneutral pH.

Isolation of Microorganism. An organism that would preferentially use organic nitrogen for growth was enriched from sewage sludge using 0.1% Tru-Sweet 42 (high fructose corn syrup, American Fructose Corp., Decatur, AL) as the carbon source and CAAT as the sole nitrogen source. The sludge was amended with 20 mM phosphate buffer (pH 7.0) and a trace metals solution (Shelton and Somich, 1988). Growth was monitored turbidimetrically. After three transfers, enrichment cultures were serially diluted and spread plated onto agar plates containing the same medium. The most dominant colony type was restreaked to ensure purity and then tested in liquid culture to verify its ability to utilize CAAT as a sole nitrogen source. The isolate was identified as *Klebsiella terragena* and was designated strain DRS-I (D. R. Durham, W. R. Grace and Co., Columbia, MD, personal communication, 1992).

Growth of DRS-I on CAAT. Nitrogen-free basal salts medium (NFB) described by Karns *et al.* (1986) made chloride free by replacing the chloride salts with sulfate salts (Karns *et al.*, 1983) was amended with glycerol (40 mM final concentration) and melamine or CAAT (1 mM final concentration). Medium was filter sterilized and placed into sterile Erlenmeyer or Fernbach flasks and incubated after inoculation on a gyrorotatory shaker at 28 °C. Starter cultures (50 mL) were inoculated with colonies of DRS-I from NFB–glycerol–CAAT agar plates and incubated for 48–96 h until adequate turbidity was achieved. The entire starter culture was added to 1 L of fresh medium, and samples were taken at various time points. The turbidity of the samples was read in a Klett-Summerson colorimeter with a no. 66 filter. The sample was centrifuged (6000*g*, 10 min), and supernatant was saved for chloride and triazine analyses. Starter cultures for pilot reactor studies consisted of six 1 L cultures (6×10^8 cfu/mL) of NFB medium amended with glycerol (40 mM) and CAAT (1 mM), inoculated with 50 mL of starter culture each, and grown for 96 h.

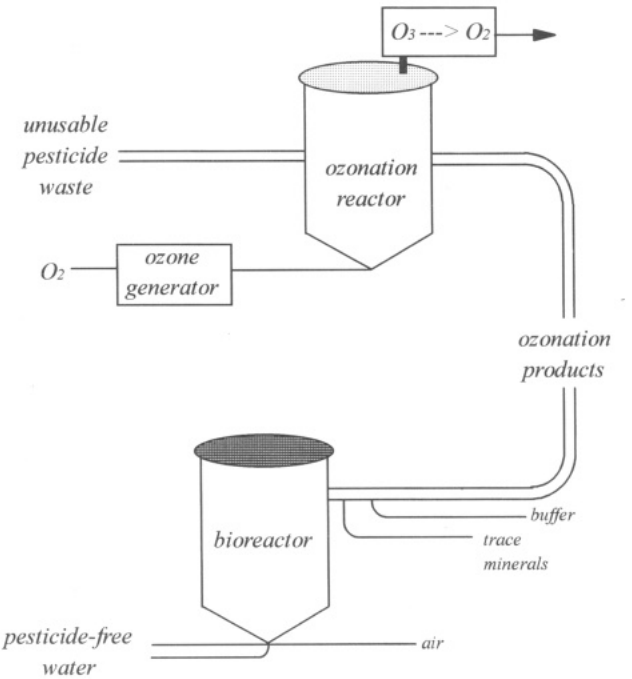
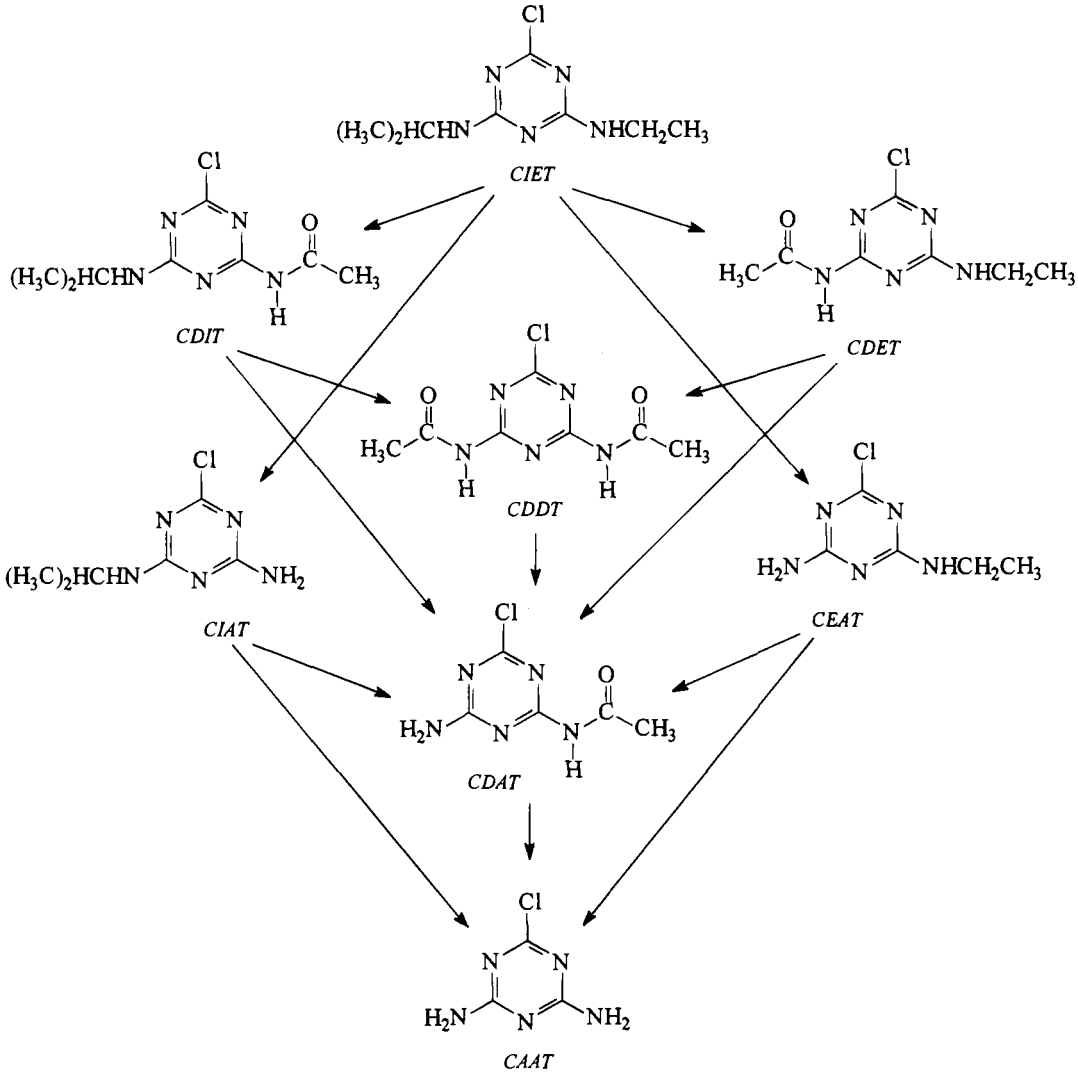


Figure 1. Pilot scale unit.

Pilot Scale Experiments (Figure 1). The ozonation chamber consisted of a 55 gal (208 L) cylindrical [56 cm (diameter) × 92 cm (height)], cone-bottomed Nalgene (cross-linked polyethylene) tank (106 cm total height) fitted with a Nalgene 6 cm wide outer lip (total diameter 68 cm), a Nalgene lid, a 5 mm thick Viton seal, and aluminum brackets above and below bolted every *ca.* 5 cm through all five layers. The contents of the tank were removed through a side outlet (28 cm from the top) using a recirculating electric jet pump (Marathon, Wausau, WI) that forced the stream through a venturi injector and returned it via a bottom inlet at a rate of 55 gal/min (208 L/min). All plumbing parts were made of PVC. Ozone was generated as above and introduced into the circulating stream through the venturi injector at a rate of 5 L/min (an additional 1 L/min was sent to the ozone monitor) at a concentration of *ca.* 3% O_3/O_2 (w/w).

Scheme 1. Degradation Pathway for Atrazine Ozonation



The ozonation chamber was filled with *ca.* 55 gal (208 L) of distilled water, and 22.85 g of formulated atrazine (*ca.* 0.4 mM), Aatrex Nine-O (85.5% atrazine, 4.5% simazine, and propazine), was added. Six hundred grams of NH_4NO_3 (36 mM) was also added in the nitrogen-containing experiment. Chamber off-gases were monitored for ozone concentration by splitting the stream, with 1 L/min routed to the ozone monitor and 4 L/min to the ozone kill unit and then to vent. Ozone consumed was calculated by subtracting the off-gas concentration from the average concentration of the stream introduced into the chamber. Concentrations of ozone, nitrate, ammonia, atrazine, and degradation products in solution were determined as above from samples obtained via a small sample port mounted on the side of the vessel *ca.* 80 cm from the top. Typical ozone concentrations in solution were 5.0–7.3 μM.

After ozonation, the solution containing CDDT, CDAT, and CAAT was transferred to a second 55 gal Nalgene cone-bottomed cylindrical vessel equipped with continuous aeration and side sample port *ca.* 75 cm from the top. The pH was adjusted to *ca.* 10 with KOH to facilitate hydrolysis of the amide moieties. The pH was readjusted to 7.0 with H_3PO_4 when the concentrations of CDAT and CDDT were below the limit of detection (*ca.* 50 ppb). The solution was further amended with trace elements (1.0 g of $MgCO_3$, 1.5 g of $MnSO_4$, 1.0 g of $CaCO_3$, 0.16 g of $FeCl_3$) and 136 g of KH_2PO_4. If NH_4NO_3 was added, then sufficient phosphate was presumed to be present after neutralization [*ca.* 250 mL of H_3PO_4 (85%) was required]; thus, no KH_2PO_4 was added. The solution was inoculated with six 1 L large starter cultures of DRS-I (*ca.* 100 Klett units). Samples were removed periodically via side sample port, and 1 mL aliquots were centrifuged (6000*g*, 2 min). The supernatant was removed and the pellet resuspended in 1 mL of 0.1 N NaOH. Both samples were frozen until analysis.

RESULTS AND DISCUSSION

Ozonation Chemistry. The degradation pathway of atrazine ozonation has been described previously and is shown in Scheme 1 (Hapeman-Somich *et al.*, 1992). Similar reactions, *i.e.*, dealkylation and amidization followed by deacylation, were also observed for propazine and simazine (Table 1) (Hapeman, 1994). Competitive kinetic experiments using propazine (CIIT) versus simazine (CEET) and CIAT versus CEAT demonstrated that the reaction with the ethyl moiety was favored 5:1 over the reaction with the isopropyl group (Hapeman, 1994). Examination of the early stages of atrazine degradation also showed a 5:1 reactivity preference of ethyl versus isopropyl (Figure 2).

Further examination of the reaction profile for atrazine (Figure 2) revealed that dealkylation was preferred over oxidation (amidization). The concentration ratio of CEAT to CDET, which reflects the dealkylation and oxidation of the isopropyl group, respectively, was *ca.* 10:1. The initial concentration ratio of CIAT to CDIT, the products formed from the dealkylation and oxidation of the ethyl moiety, respectively, was *ca.* 2:1. As the

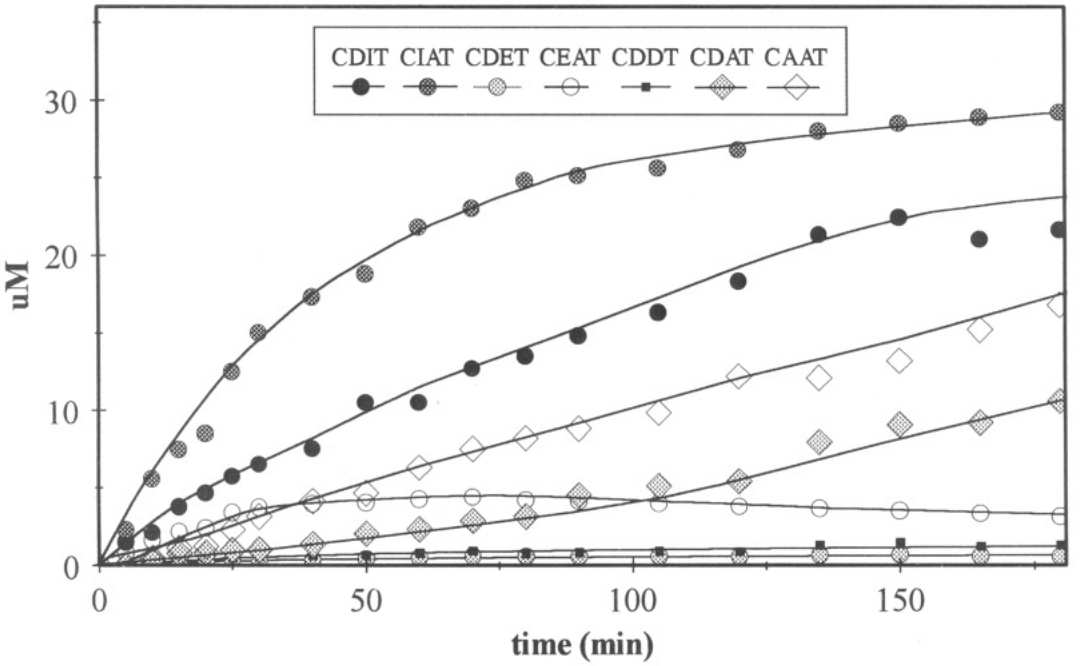


Figure 2. Reaction profile of atrazine ozonation (laboratory scale).

reaction continues, this ratio becomes smaller, suggesting that the degradation of CDIT is slower than that of CIAT.

Mechanisms of amine ozonation in organic solvents have been described and generally involve direct attack by ozone. However, these mechanisms cannot explain the formation of the acetyl moiety from the ethyl group observed in this study; *i.e.*, the formation of CDET from atrazine and simazine and the formation of CDAT from CEAT are precluded (Bailey *et al.*, 1978; Bailey, 1982). In addition, experiments conducted with diisopropylethylamine showed that ozone attack preferentially occurred at the tertiary hydrogen of the isopropyl (Bailey *et al.*, 1978). In this study, however, reaction with the ethyl moiety was overwhelmingly preferred over reaction with the isopropyl. A more plausible explanation for the aqueous ozonation of 2-chloro-*N*-(alkylamino)-*s*-triazines, therefore, involves hydroxy radical oxidation rather than direct reaction with ozone.

If hydroxy radical attack occurred at the primary methyl, then the isopropyl group, with two methyls, would be expected to react at a rate twice that of the ethyl moiety. However, the hydroxy radical prefers more electron rich sites, which in this case is the hydrogen on the carbon α to the amine nitrogen, which provides additional electron density. Furthermore, the bond energy of the C−H bond of the isopropyl methyl group (104 kcal/mol) is greater than that of the α-carbon−hydrogen bond of the ethyl moiety, H−CH(CH_3) (98 kcal/mol), and the α-carbon−hydrogen bond of the isopropyl group, H−C(CH_3)$_2$ (95 kcal/mol) (Golden and Benson, 1969). Thus, if the reaction is thermodynamically controlled, hydroxy radical attack would be expected to occur preferentially at the more weakly bonded hydrogen on the α-carbon of the isopropyl group over that on the ethyl group. (The resultant tertiary carbon radical would also be more stable than the secondary carbon radical.) Yet, if the reaction is actually sterically controlled, then the more hindered isopropyl hydrogen would be less reactive. The results conclusively showed that reaction was preferred 5:1 in favor of the ethyl substituent, suggesting, therefore, that attack occurs at the electron rich α-carbon and that the reaction is sterically controlled.

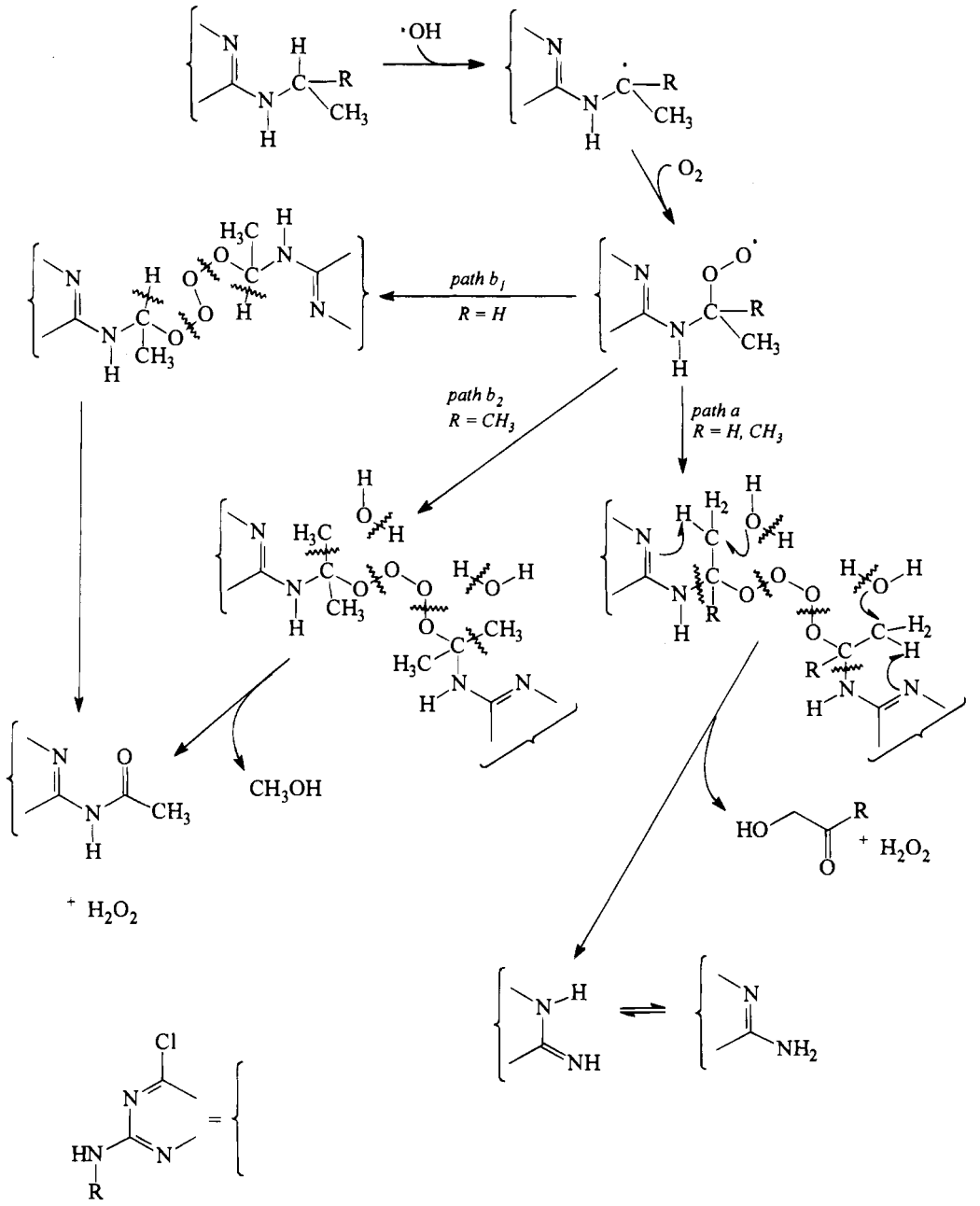
The mechanism proposed for the ozonation of *N*-alkylated *s*-triazines involves removal of the α-hydrogen by hydroxy radical and trapping of the resultant radical with O_2 to form peroxy species (Scheme 2). Russell-type rearrangements (Howard, 1983) with some solvent (H_2O) assistance would give rise to either the acylated or dealkylated nitrogen. The above results further indicate that path a is significantly less favored as compared to path b_2 for the isopropyl, while paths a and b_1 are more equal for the ethyl group; however, dealkylation is still favored.

Additional evidence for a hydroxy radical mechanism has been demonstrated using ozone consumption data (G. R. Peyton, Illinois State Water Survey, Champaign-Urbana, IL, personal communication, 1994). Thus, attempts to raise the pH to facilitate ozonation using carbonate (Somich *et al.*, 1990) would, in fact, be expected to decrease the reaction rate for these compounds because carbonate is an effective hydroxy radical scavenger (Glaze, 1987) and would decrease the rate of atrazine degradation. Decreasing the concentration of hydroxy radical may not be a factor, however, for other species that can also react directly with ozone such as the acetanilides (Hapeman-Somich, 1991). Increasing the pH for ozonation with carbonate, which could readily be removed later as CO_2 and would therefore not interfere with the subsequent microbial degradation, was abandoned.

Conversion of CDAT and CDDT to CAAT. In addition to CAAT, the final products of atrazine ozonation included CDDT and CDAT. Deacylation of these compounds during ozonation is very slow and expensive (energy and oxygen are needed to generate ozone) relative to simple base hydrolysis. In laboratory scale reactions, the pH was simply increased to 10 with the addition of KOH and hydrolysis was complete within 2 h (data not shown).

Microbial Mineralization of CAAT with *K. terragena*. The *s*-triazine ring of CAAT is at its maximum oxidation state (*i.e.*, has no hydrogens); consequently,

Scheme 2. Proposed Mechanism for *s*-Triazine Ozonation



CAAT cannot be used as a carbon or energy source. Thus, the mineralization of CAAT requires microorganisms that can effectively utilize CAAT as a nitrogen source even in the presence of inorganic nitrogen sources, such as ammonium nitrate (a typical fertilizer found in pesticide waste). Such an organism was isolated from sewage sludge and identified as a bacterium (*K. terragena*, strain DRS-I) (Leeson *et al.*, 1993).

In laboratory studies in which DRS-I was grown in NFB with glycerol as a carbon source and CAAT as the sole nitrogen source, CAAT decreased to below detectable limits within 8 h with concomitant increase in turbidity of the culture and release of chloride ion (Figure 3). Release of chloride ion lagged behind CAAT disappearance somewhat, and a transient unknown peak was observed in the HPLC chromatogram. This peak was subsequently identified as COAT on the basis of identical retention time and spectrum with material made using PSA. COAT accumulated to a maximum at 7 h and then rapidly disappeared. Quantitation of COAT was not possible as no standard is available, nor can it be readily isolated. Concentrations of COAT were estimated by calculating the difference between the moles of CAAT consumed and the amount of chloride released. A plot of the HPLC peak area versus this calculated concentration had a correlation coefficient of 0.989 (data not shown). A small amount of COAT was present in the culture initially from the inoculum which was grown on CAAT. This initial concentration is assumed to be equal to the additional amount of chloride released and confirmed the validity of this approach.

Although DRS-I can ultimately mineralize CAAT, a portion of the triazine moiety can remain in solution as COAT even though CAAT is completely consumed. Confirmation of total triazine destruction, therefore, requires that COAT degradation be monitored in addition to CAAT. The fact that no other products were observed to accumulate indicates that the metabolism of COAT is the rate-limiting step in triazine metabolism. Complete mineralization of the *s*-triazine ring has

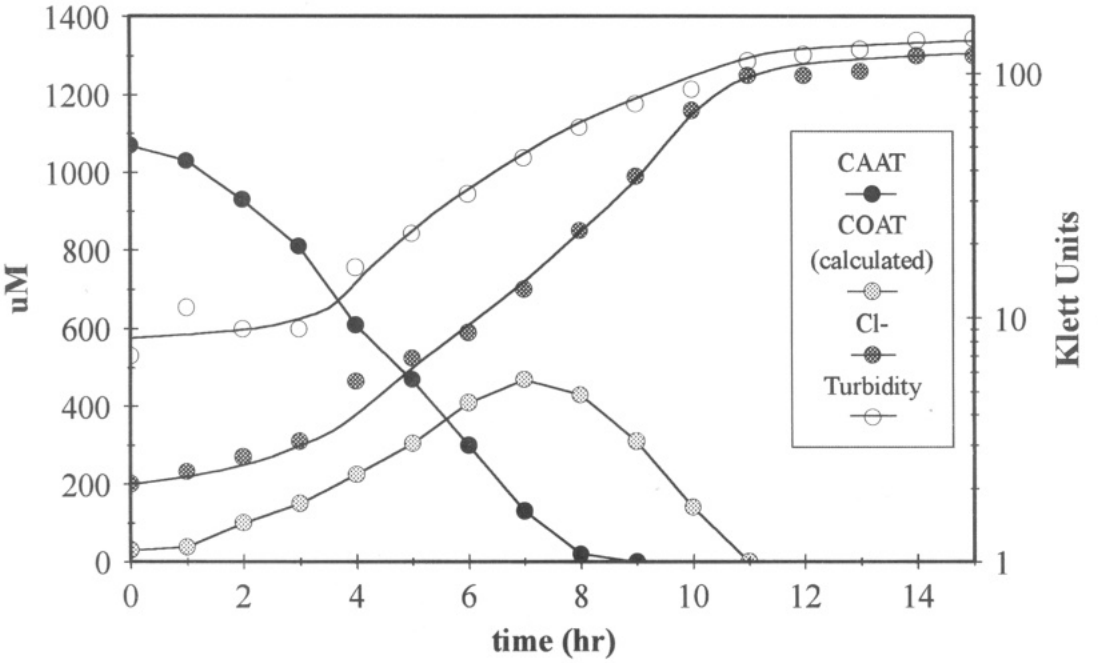


Figure 3. Biodegradation profile of CAAT.

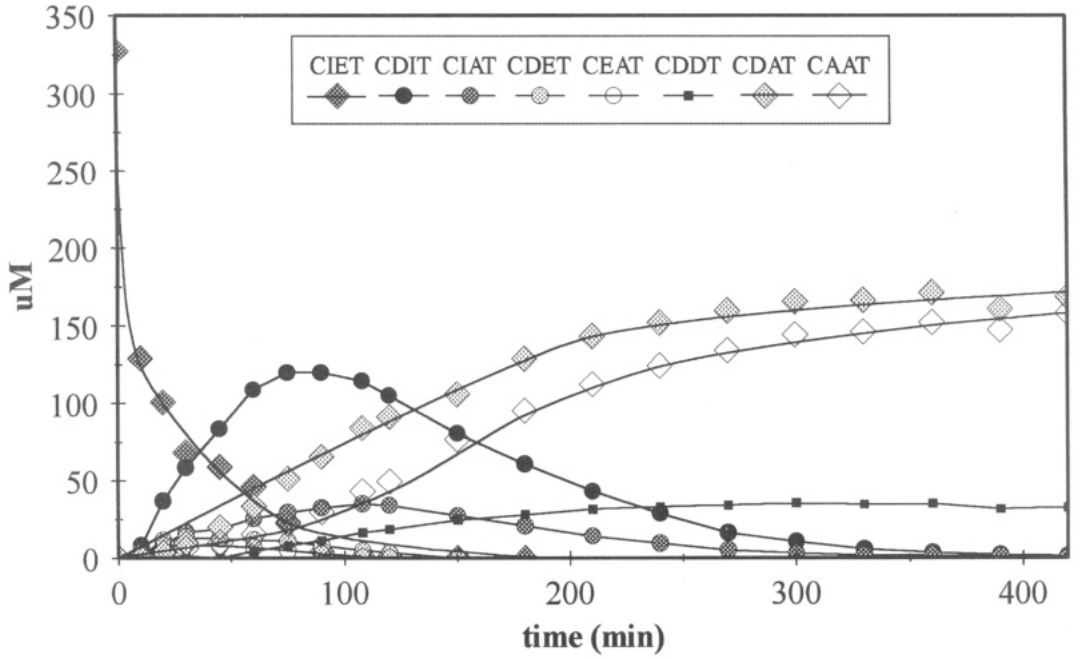


Figure 4. Reaction profile of atrazine ozonation (pilot scale).

previously been demonstrated using CAAT-*U-ring-*^{14}C, where 77% of the initial activity was recovered as carbon dioxide (Leeson *et al.*, 1993).

Results of Pilot Scale—No NH_4NO_3. A 208 L capacity disposal system was built using readily available PVC piping and valves and Nalgene products. A venturi injector and recirculating pump were used to enhance mass transfer of ozone into the solution. Ozonation of formulated atrazine, Aatrex, afforded CDDT, CDAT, and CAAT as final products via the same intermediates observed in the laboratory (Figure 4). Interestingly, the initial ratio of CIAT to CDIT (*ca.* 1:3) was opposite to what was observed in the laboratory, indicating that oxidation of the ethyl group was preferred to dealkylation. The ratio of CEAT to CDET was nearly 1:1, as compared to 10:1 ratio in laboratory experiments, suggesting that no preference exists between dealkylation and oxidation for the isopropyl moiety of atrazine in the formulated material. A possible explanation for this phenomenon is that the formulation, which consists mostly of clay minerals, stabilizes the intermediate(s) involved in amidization more than those involved in dealkylation for both the isopropyl and ethyl substituents. The ozonation efficiency is defined as the number of moles of atrazine per the number of moles of ozone required to accomplish total degradation to CAAT and was calculated by the difference in the measured concentrations of ozone in the input and off-reactor gas streams. In the pilot scale reactor without NH_4NO_3, the efficiency was determined to be 0.0022 mol of atrazine/mol of ozone.

The ozonated material was transferred to a second chamber, and the pH was adjusted to 10 with the addition of KOH. Facile hydrolysis of CDDT and CDAT

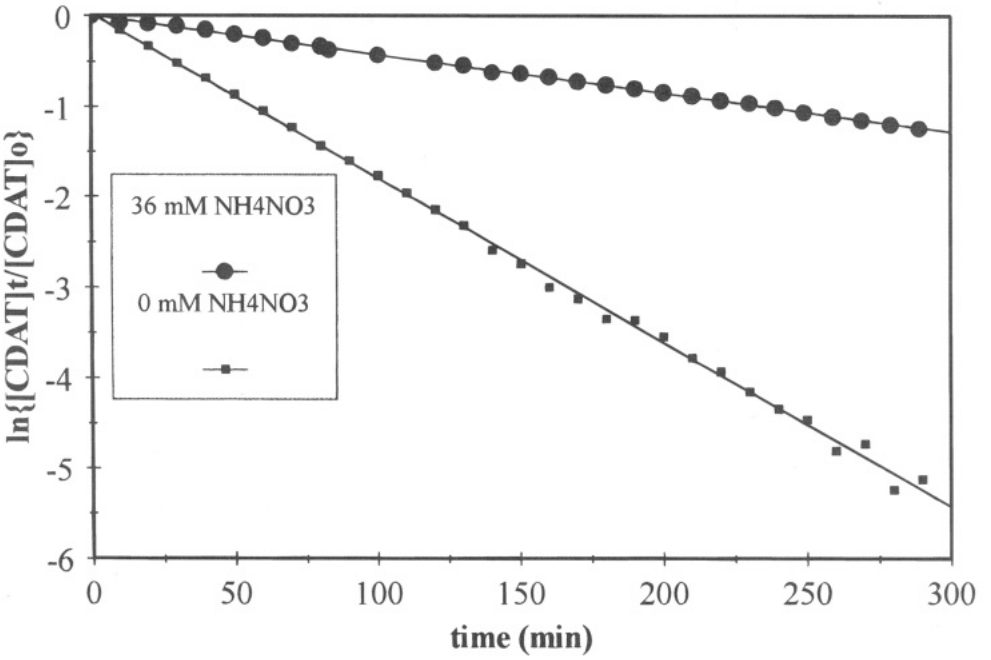


Figure 5. Base hydrolysis of CDAT to CAAT with and without NH_4NO_3, $k_{H_2O} = -0.0182$ ($r^2 = 0.996$); $k_{H_2O(NH_4NO_3)} = -0.00428$ ($r^2 = 0.999$).

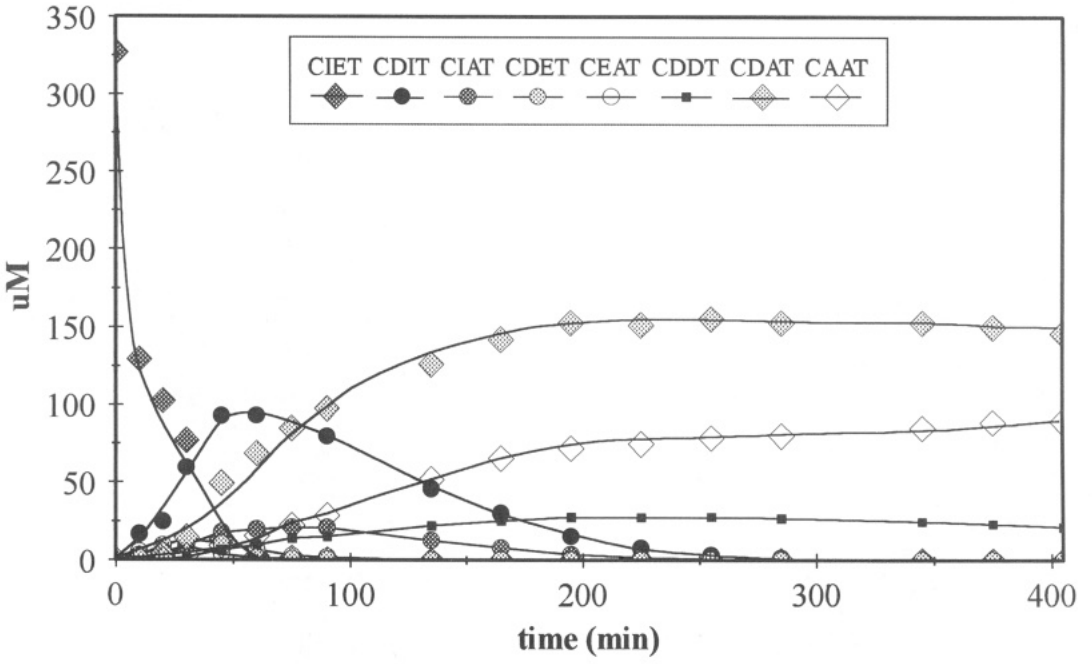


Figure 6. Reaction profile of atrazine ozonation in the presence of NH_4NO_3 (pilot scale).

to CAAT was achieved, and a first-order rate constant of -0.0182 min^{-1} was determined for CDAT (Figure 5). The solution was subsequently neutralized with the addition of phosphoric acid and amended with mineral salts and high fructose corn syrup. As expected, CAAT was readily degraded by DRS-I. After 4 h, 75% of the CAAT was degraded with the concomitant appearance of COAT and an increase in biomass from 3 to 34 Klett units. No COAT or CAAT was detected after 20 h of incubation (data not shown).

Results of Pilot Scale with NH_4NO_3. Ozonation of Aatrex in the presence of ammonium nitrate proceeded in a fashion similar to that observed without the addition of nitrogen. Again, the final products were CDDT, CDAT, and CAAT, although there appears to be a slight decrease in the concentration of CAAT (Figure 6). This is probably an anomaly due to the large amount of nitrate present in the reaction, the tail of which coelutes with CAAT in the HPLC chromatogram. Oxidation is also preferred over dealkylation for the ethyl moiety (CIAT:CDIT *ca.* 1:10); the two pathways appear to be nearly equal for the isopropyl group. Ozonation efficiency was determined to be 0.0025 mol of atrazine/mol of ozone. Hydrolysis of CDAT and CDDT to CAAT was an order of magnitude slower in the presence of NH_4NO_3; $k_{H_2O} = -0.00428$ min^{-1} for CDAT (Figure 5).

Biomineralization of CAAT by DRS-I occurred even in the presence of the large molar excess of nitrogen (Figure 7). CAAT was degraded to undetectable levels after 10 h of incubation while the concentration of COAT increased. COAT was subsequently slowly degraded over the next 20 h. Biomass in the culture increased from 2×10^7 colony-forming units (CFU) mL^{-1} at the

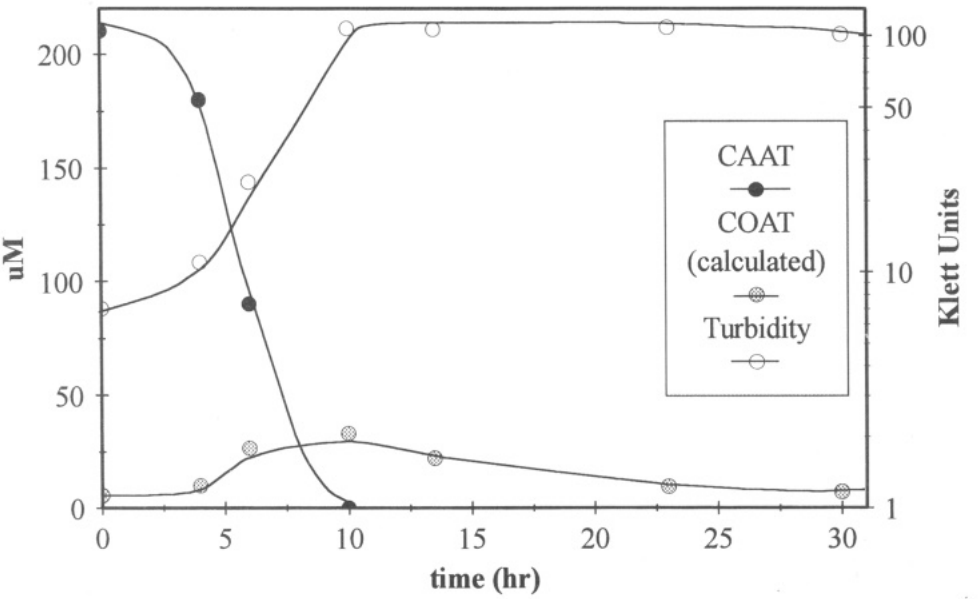


Figure 7. Biodegradation profile of CAAT in the presence of NH_4NO_3 (pilot scale).

time of inoculation to 3×10^9 CFU mL^{-1} at 30 h. Again, COAT concentrations were determined on the basis of the previously calculated standard curve of moles of CAAT consumed and chloride released versus peak area (absorption units × minutes).

CONCLUSION

Optimization of the binary disposal process (ozonation and biomineralization) requires the identification of ozonation and microbial products as well as elucidation of the respective degradation pathways. In the present study, the ozonation mechanism of atrazine was fully described and the rate-limiting step of CAAT metabolism identified.

Ozonation efficiencies (total moles of pesticide per mole of ozone consumed) from previous pilot scale studies, using different ozonation vessels/configurations, were determined to be 0.0067 (Somich *et al.*, 1990) and 0.0058 (Hapeman-Somich *et al.*, 1991). These values reflect the ozonation efficiencies for only the parent materials, since only disappearance of parent material was monitored. In the current study, the ozonation efficiencies (0.0022 and 0.0025 mol of atrazine/mol of ozone) were calculated on the basis of the degradation of atrazine as well as the degradation of all intermediate products. Recalculation of the ozonation efficiencies based only on atrazine degradation, consistent with previous studies, results in efficiencies of 0.0088 and 0.0108 without and with NH_4NO_3, respectively. In addition, previous studies were conducted with pesticide mixtures, some of which were more reactive with ozone; the *s*-triazines represented about one-third of the total pesticide concentration. These data indicate that the current pilot scale unit is more effective than the previous systems.

Results from this study also demonstrate that *K. terragena* (strain DRS-I) is capable of metabolizing the *s*-triazine ring in the presence of ammonia and nitrate under pilot scale conditions. This organism is somewhat unusual because of its ability to degrade CAAT even in the presence of ammonia; organic N catabolic pathways in most bacteria are typically highly regulated and repressed in the presence of ammonia (Magasanik, 1982). Thus, *K. terragena* (strain DRS-I) is ideally suited for use in disposal systems involving the destruction of triazine herbicides in environments with high nitrogen concentrations, such as pesticide applicator wastes.

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