Enzymatic Approach to Waste Minimization in a Cattle Dipping Operation: Economic Analysis

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Cattle dipping liquid containing the pesticide coumaphos must be discarded when potasan, a dechlorination product of coumaphos, accumulates. An enzymatic strategy is examined in which the enzyme parathion hydrolase is used to selectively hydrolyze potasan relative to coumaphos, therefore permitting the extended use of the cattle dipping liquid and reducing the overall generation of waste from the cattle dipping operation. A large-scale experiment was performed to confirm that the observed rate of potasan hydrolysis is an order of magnitude larger than the rate of coumaphos hydrolysis. A mathematical model was adapted to permit an economic comparison of the enzymatic waste minimization approach to the alternative of dumping the potasan-containing dipping liquid and recharging the dip vat with fresh coumaphos. This analysis demonstrates that because the amount of enzyme required is small and the cost of the coumaphos pesticide is large, it is almost always economical to extend the lifetime of the dip vat by the addition of the enzyme.

Keywords: *Parathion hydrolase; organophosphate phosphotriesterase; coumaphos; potasan; waste minimization*

INTRODUCTION

Increased public concerns and regulatory mandates for the environment have changed the way wastes are managed. These concerns have stimulated the development of technologies to effectively treat effluents and remediate previously contaminated sites. Further, the escalating disposal costs and uncertain legal liabilites have provided significant economic incentives for the development of technologies to minimize the generation of waste. In this work, an enzymatic approach for waste minimization has been studied, and to assess the utility of this approach, we conducted a large-scale test and compared the economics of this enzymatic approach with current alternatives.

The problem we are investigating involves the use of the organophosphate pesticide coumaphos, which is used to control the population of the ticks *Boophilus microplus* and *Boophilus annulatus*, which transmit the disease cattle fever (Graham and Hourrigan, 1977). Although the population has been controlled in the United States, the ticks are endemic in Mexico. Thus, to prevent the spread of cattle fever, cattle in Mexico and at the Texas−Mexico border are dipped in vats containing an aqueous suspension of the pesticide coumaphos. A vat can be used for several months (and even years) provided a toxic byproduct of coumaphos does not accumulate. As illustrated in Figure 1, the potasan byproduct is produced from the anaerobic dechlorination of coumaphos; this byproduct is toxic to cattle (Shelton and Karns, 1988). In the United States, when the potasan level exceeds 300 mg/L, the dip vat must be taken out of service and the liquid disposed in a holding pond. In previous studies, it was observed

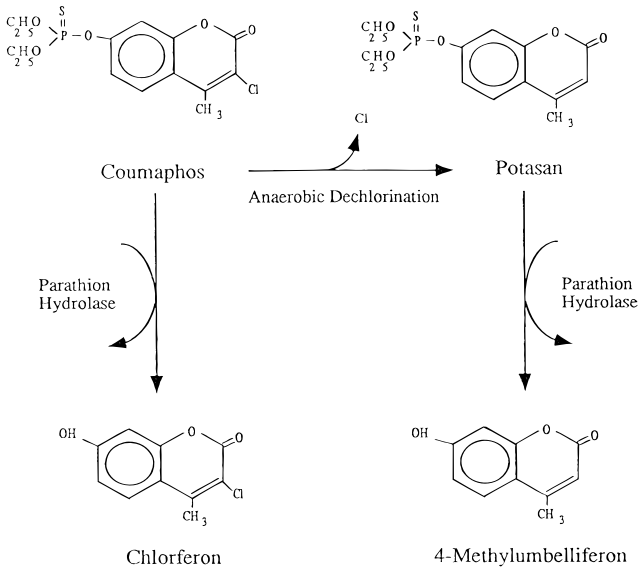

Figure 1. Schematic for waste minimization approach.

that the enzyme organophosphate phosphotriesterase, or parathion hydrolase, was capable of degrading both coumaphos (Kearney et al., 1986) and potasan and that under dip vat conditions the rate of potasan hydrolysis was considerably greater (Coppella et al., 1990). Later studies demonstrated that the difference in the hydrolysis rates was not the result of differences in the intrinsic kinetics of the enzyme toward coumaphos and potasan. Rather, the overall hydrolysis rates appear to be limited by how fast the coumaphos and potasan can be dissolved into the liquid (both potasan and coumaphos have low water solubilities), and because potasan has an 8-fold higher solubility than coumaphos, it is preferentially hydrolyzed in dip vat liquids (Smith et al., 1992).

The observation that potasan is hydrolyzed more readily than coumaphos suggested the potential for using this enzyme for waste minimization. Specifically,

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0021-8561/96/1444-0351$12.00/0

rather than disposing of coumaphos-containing vats when potasan accumulates, the enzyme could be added to selectively hydrolyze the toxic potasan byproduct. If the enzyme was inactivated after potasan was completely hydrolyzed, then further coumaphos hydrolysis could be prevented and the vat could be put back in service. Thus, this enzymatic approach would reduce the total amount of waste generated from the dipping operation. To inactivate the enzyme after potasan hydrolysis, a range of options could be considered. We believed the easiest approach for enzyme inactivation was to exploit the limited stability of the enzyme, which has a reported half-life on the order of 8−40 h (Munnecke, 1980; Coppella et al., 1990; Rowland et al., 1991). Thus, if just enough enzyme were added to completely hydrolyze the potasan and the enzyme was completely inactivated at the time that potasan hydrolysis was complete, then the loss of coumaphos would be minimized.

To determine the optimal amount of enzyme that must be added to ensure complete hydrolysis of potasan while minimizing the hydrolysis of coumaphos, a mathematical model must be developed which reasonably describes the operation. This model must account not only for the rates of hydrolysis of potasan and coumaphos and the inactivation of the enzyme but also for the rate at which potasan and coumaphos are dissolved from solid particles into the liquid. Description of the mass transfer in this system is particularly difficult given that the dip vat is not well mixed and considerable amounts of solids settle to the bottom of the vat. The first goal of this work was to adapt a model previously developed from laboratory-scale experiments (Smith et al., 1992) and to test this model under larger-scale, field conditions.

It is often cited that waste minimization programs are economically attractive not only because they reduce disposal and potential liability costs but also because raw material costs are reduced when the raw materials are used more efficiently (i.e. over a longer lifetime). The second goal of our study was to exploit the mathematical model to compare the economics between the use of enzymes that selectively hydrolyze potasan and permit reuse of the dip vat liquid and the disposal of potasan-containing dip vat liquid and recharging the vat with fresh coumaphos.

MATERIALS AND METHODS

The parathion hydrolase enzyme was obtained by fermentation as previously discussed. For small-scale studies, the enzyme was obtained from *Streptomyces lividans* strain 66 containing the plasmid pIJ702 (Steiert et al., 1989). Since this bacteria secretes the enzyme, recovery required only cell removal (Coppella et al., 1990). For large-scale studies, enzyme was obtained from *Escherichia coli* strain DH5α containing plasmid pJK33 (Mulbry and Karns, 1989a). In this case, the cells were lysed by French press and enzyme was separated from cells and cell debris by centrifugation (Mulbry and Karns, 1989b).

Analysis of the dip vat liquid for potasan, coumaphos, 4-methylumbelliferon, and chlorferon was performed by HPLC as previously described (Shelton and Karns, 1988; Coppella et al., 1990). The error bars in Figure 3 represent 95% confidence limits.

MATHEMATICAL MODEL

For this work, we developed a system of five differential equations, which are listed in Table 1. Equations 1 and 2 describe the total disappearance of coumaphos and potasan as a function of the active enzyme concentration (E) and the amount of dissolved substrate (C_{cd} and C_{pd}). As suggested by these equations, the model assumes that the rate of hydrolysis is linear with respect to the dissolved substrate concentration. Although saturation kinetics have often been reported for hydrolysis by parathion hydrolases, the solubilities of potasan (17 μM) and coumaphos (2 μM) reported by Smith et al. (1992) are below the half-saturation constants, which are typically reported to be 25−600 μM (Munnecke and Hsieh, 1976; Brown, 1980; Mulbry and Karns, 1989b; Dumas et al., 1989; Rowland et al., 1991). Also, the model assumes that only dissolved (and not solid phase) substrates can be hydrolyzed by the enzyme. The rate constants for enzymatic hydrolysis, k_c and k_p, were previously determined to be identical for both substrates in our system (Smith et al., 1992). Since both substrates have the same rate constant and are both exposed to the same enzyme concentrations, then eqs 1 and 2 suggest that the only difference in the rates of coumaphos and potasan degradation is due to differences in their dissolved concentrations.

Table 1. Model Equations and Parameters

$$dC_{ct}/dt = -k_c E C_{cd} \quad (1)$$

$$dC_{pt}/dt = -k_p E C_{pd} \quad (2)$$

$$dC_{cd}/dt = -k_c E C_{cd} + KA_c(C_c^* - C_{cd}) \quad (3)$$

$$dC_{pd}/dt = -k_p E C_{pd} + KA_p(C_p^* - C_{pd}) \quad (4)$$

$$dE/dt = -k_d E \quad (5)$$

k_c = rate constant for degradation of coumaphos	= 8600 L/mg·h
k_p = rate constant for degradation of potasan	= 8600 L/mg·h
E = concentration of active enzyme in the system	= mg/L
C_{cd} = concentration of dissolved coumaphos	= mg/L
C_{ct} = total concentration (dissolved and undissolved) of coumaphos	= mg/L
C_{pd} = concentration of dissolved potasan	= mg/L
C_{pt} = total concentration (dissolved and undissolved) of potosan	= mg/L
KA_c = mass transfer coefficient for coumaphos	= 44.7/h
KA_p = mass transfer coefficient for potasan	= 73.9/h
C_c^* = coumaphos equilibrium concentration	= 0.8 mg/L
C_p^* = potasan equilibrium concentration	= 5.6 mg/L
k_d = rate constant for enzyme inactivation	= 0.08/h
t = time	= h

The major difference between the model described here and that described previously (Smith et al., 1992) is that the rate constants k_c and k_p are normalized in terms of milligrams of pure enzyme. This change was made to compare results from different experiments and to assist in estimating the cost of this operation. To normalize the rate constants, we used an average observation that 2000 units (1 unit is equal to the amount of enzyme required to catalyze the hydrolysis of 1 μmol of parathion per minute) corresponds to 1 mg of pure parathion hydrolase (Mulbry and Karns, 1989b; Dumas et al., 1989; Rowland et al., 1991).

Equations 3 and 4 are material balances for coumaphos and potasan with respect to the liquid phase. These equations show that the dissolved concentrations change with time depending on how fast the substrates are disappearing from solution due to hydrolysis and how fast the solid phase substrates are dissolving into the liquid. As was stated above, the rate of disappearance by hydrolysis is described by eqs 1 and 2.

The rate at which solid substrates are dissolving into solution is determined by the mass transfer expression on the right-hand side of eqs 3 and 4. The mass transfer coefficients (K) depend on the level of agitation in the liquid, while the interfacial areas (A) depend on the number and shapes of the solid particles containing

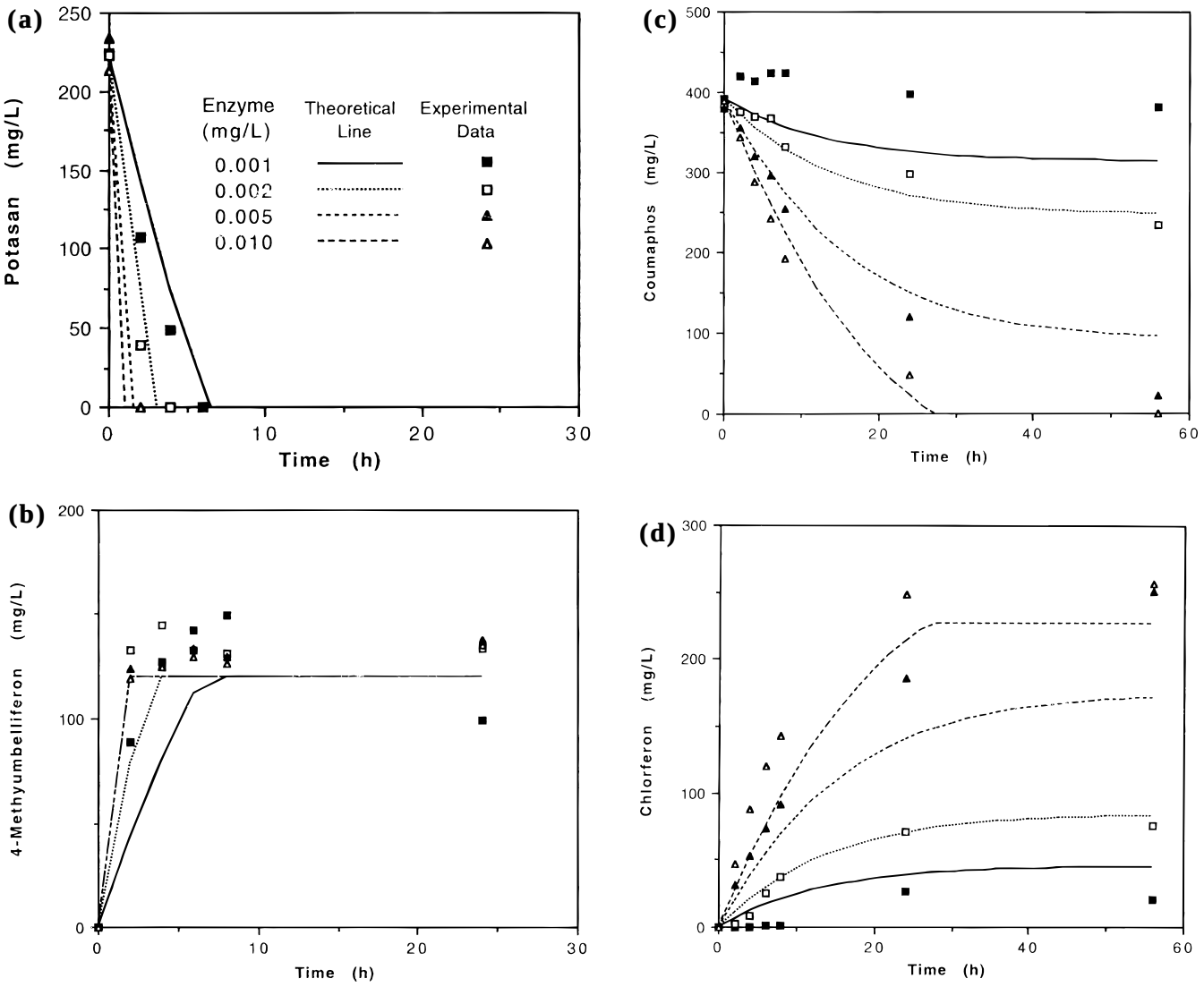

Figure 2. Enzymatic hydrolysis of potasan (a) and coumaphos (c) and the formation of the corresponding products 4-methylumbelliferon (b) and chlorferon (d) under small-scale, well-mixed laboratory conditions. Data were obtained from the addition of various amounts of enzyme to 5 mL samples of dip vat liquid. Curves represent model predictions using the parameters in Table 1. Data are from Smith et al. (1992).

coumaphos and potasan. Since agitation and particle size and shape are poorly defined in dip vat systems, we have lumped *K* and *A* together, assumed them to be constant throughout treatment, and fitted the values to agree with experimental data. The assumption that *KA* remains constant is clearly invalid in the limit of complete hydrolysis since when no particles of solid substrate are present, then the rate of mass transfer should go to zero ("*A*" would go to zero). Nevertheless, given the uncertainties in the mixing and mass transfer of the vats, the assumption of constant *KA* provides a reasonably simple starting point for modeling. From small-scale, well-mixed experiments the *KA* values for coumaphos and potasan were fitted to be 44.7 and 73.9 h^{-1}, respectively (Smith et al., 1992).

In addition to the *KA* terms, the rates at which the substrates dissolve depend on the difference ($C^* - C_d$), where C_d is the concentration of the dissolved substrate (either coumaphos or potasan) and C^* is an equilibrium concentration which we have assumed to be equal to the solubility of the substrates. The assumption that C^* is equal to the solubility is valid in clean systems involving pure substrate crystals, but it is likely to be less valid for complex systems (i.e. for dip vats) in which the substrates can be adsorbed onto various particles (e.g. dirt and cattle waste).

The next factor in our model is the inactivation of the enzyme. As indicated in eq 5 this inactivation is assumed to follow first-order kinetics with respect to the concentration of the active enzyme (*E*), and the inactivation rate constant, k_d, was assumed to be 0.08 h^{-1}. This inactivation rate constant was suggested previously for small-scale studies employing actual dip vat wastes (Smith et al., 1992). This value is similar to others reported for inactivation rate constants for parathion hydrolase (Munnecke, 1980; Rowland et al., 1991; Coppella et al., 1990).

RESULTS

Model Verification. The model was tested using previously reported data in which 5 mL samples of dip vat liquid were incubated with various amounts of enzyme extract. These solutions were incubated with agitation, and the experimentally measured concentrations are shown in Figure 2. As expected, increased levels of enzyme resulted in increased rates of loss of the substrate potasan and formation of its hydrolysis

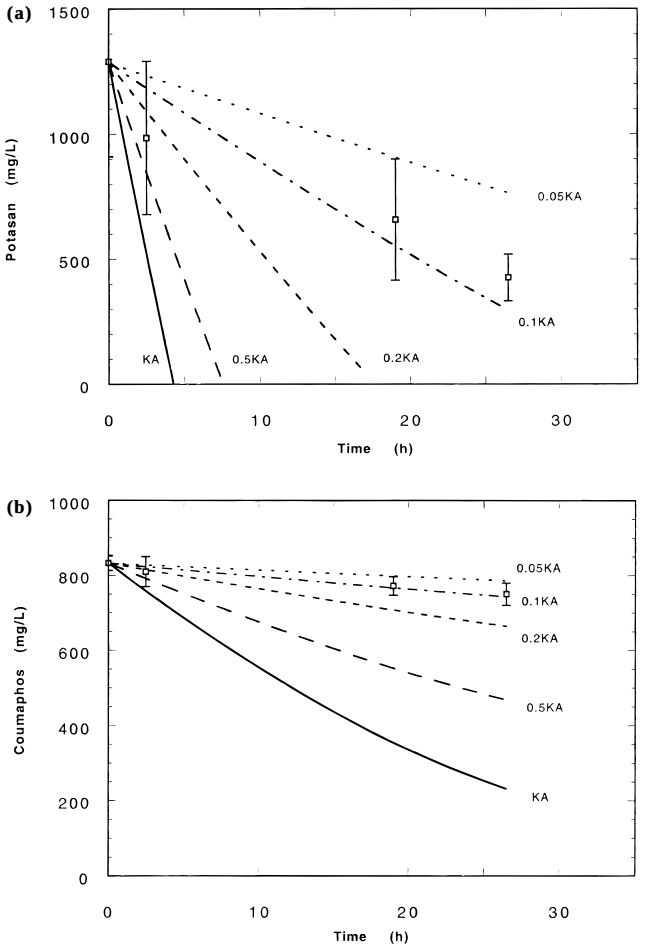

Figure 3. Enzymatic hydrolysis of potasan (a) and coumaphos (b) in a large-scale study. Enzyme (430 000 units) was added to dip vat liquid (2100 gal) and incubated without agitation. Points represent experimentally measured data, while the curves represent model predictions. The model parameters were the same as in Table 1 except that the mass transfer terms *KA* were varied to determine the sensitivity of the model to mass transfer and to fit the experimental observation. Error bars on the experimental data represent 95% confidence limits.

product, 4-methylumbelliferon. For all enzyme levels tested, potasan hydrolysis was complete within 7 h. For coumaphos, the hydrolysis rate was observed to increase with increasing enzyme levels. However, at the lower enzyme levels, coumaphos hydrolysis was incomplete and, according to our model, continued coumaphos hydrolysis did not occur because of the loss of enzyme activity. The model predictions are shown as the smooth curves in Figure 2. Given the complexities of the system, the simple model does a reasonably good job of predicting the enzymatic hydrolysis of coumaphos and potasan.

To test this enzymatic treatment approach under large-scale conditions, 2100 gal of liquid was pumped from a potasan-containing dip vat into a tank. Potasan was allowed to accumulate in this tank to a level of 1200 ± 100 mg/L. At that time, a crude extract which contained a total of 430 000 units of parathion hydrolase was mixed into the dip vat liquid. Assuming a specific activity of 2000 units/mg, this addition corresponds to 0.027 mg of enzyme added/L of liquid (it should be noted that the level of enzyme was chosen simply because of its availability). After the addition of enzyme, the suspension remained unmixed except when the tank was sampled for analysis. As can be seen by Figure 3, the potasan level in the tank was observed to decrease

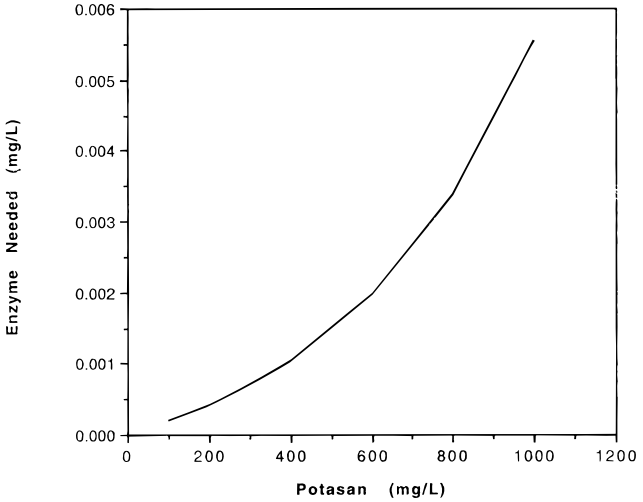

Figure 4. Simulation of the amount of enzyme that must be added to degrade a given amount of potasan. Simulations were performed using the parameters in Table 1 except that KA_p was reduced to 10% of the value observed in the small-scale study (i.e. to 7.39 h^{-1}). Simulations were conducted to 56 h when 99% of the initial enzyme activity was predicted to be lost.

from 1200 ± 100 to 400 ± 90 mg/L over the 26 h period of the test. During this period, the coumaphos level was observed to decrease by only 60 mg/L from 790 ± 20 to 730 ± 30 mg/L. Also evident in Figure 3 are the large experimental error bars which result because of the difficulty in obtaining representative samples from the poorly mixed suspension in the tank.

To apply the mathematical model to this system, more information would be required on the mass transfer characteristics of the dip vat. Specifically, to accurately predict hydrolysis rates, the effect of poor mixing on mass transfer (i.e. on the *KA* terms) would need to be understood. The bottom lines in Figure 3 are simulations performed in which the mass transfer terms (*KA*) were assumed to be identical to those from the small-scale, well-mixed experiments of Figure 2. As can be seen, when these *KA*'s are used, there is poor agreement between the model's predictions and the observed behavior. When simulations were conducted with various *KA* values, Figure 3 shows that the predicted concentration profiles are very sensitive to *KA*. When *KA* was set at 10% (*0.1KA*) of the values observed in the small-scale studies, Figure 3 shows reasonably close agreement between model predictions and measured concentrations. Thus, Figure 3 suggests that the poor mixing in the tank results in a 10-fold reduction in the mass transfer rate compared to the well-mixed laboratory study of Figure 2.

Economic Analysis. Using the model, we estimated the relative economics for this enzymatic waste minimization approach. In practice, this enzymatic approach would be used when routine analysis indicated that potasan levels approached or exceeded the action level of 300 mg/L. When these potasan levels were observed, sufficient enzyme would be added to degrade the potasan while minimizing the degradation of coumaphos. Thus, the costs for this approach are the cost of the enzyme and the cost required to replace the coumaphos that had been hydrolyzed by the enzyme.

To assess the cost of the enzyme, we performed the simulation shown in Figure 4. In this simulation, the amount of enzyme that would be just sufficient to degrade a given level of potasan was calculated. For

Table 2. Economic Comparison for Enzyme-Based Approach Using Mathematical Model

initial potasan level[a] (mg/L)	enzyme required[b] (mg/L)	coumaphos degraded[c] (mg/L)	cost of enzyme[d] ($)	cost for coumaphos replacement[e] ($)	total cost for treatment[f] ($)	cost to recharge vat[d] ($)
300	0.0007	37 (45)	0.18 (1.83)	43 (52)	54	1920
800	0.0034	87 (126)	0.89 (8.93)	102 (147)	156	1920

[a] If observed during routine analysis. [b] Required to completely hydrolyze observed potasan assuming 0.1 KA_p. [c] Assuming 0.1KA_c or 0.2KA_c for values in parentheses. [d] Assuming 3500 gal dip vat and enzyme cost of $20/g or $200/g for values in parentheses. [e] Assuming coumaphos cost of 8.8¢/g and 0.1KA_c or 0.2KA_c for values in parentheses. [f] Assuming enzyme cost of $200/g and 0.2$KA_c$. [g] Cost to add 22 kg of coumaphos to a fresh vat.

this simulation, we used the mass transfer values (0.1KA_p) from Figure 3 which had been observed to best describe the large-scale data and we assumed treatment was essentially complete at 56 h when 99% of the enzyme activity had been lost. As can be seen from Figure 4, if the dip vat was measured to contain 300 mg/L of potasan, then 0.0007 mg/L of enzyme is predicted to be required to completely degrade the potasan. For a 3500 gallon vat, this corresponds to 9 mg of enzyme. The important conclusion from this calculation is that a very small amount of enzyme is required to completely hydrolyze potasan.

Because the enzyme parathion hydrolase does not appear to be commercially available yet, we used two independent approaches to estimate its cost. First, we assumed the cost of parathion hydrolase would be comparable to the $20/g cost of other inexpensive and commercially available enzymes such as proteases. We believe the assumption that parathion hydrolase would be inexpensive to produce is justified because (i) it has been expressed and in some cases secreted in a variety of recombinant systems (Serdar and Gibson, 1985; Steiert et al., 1989; Serdar et al., 1989; Mulbry and Karns, 1989a; Dumas et al., 1989; Rowland et al., 1991) and (ii) since enzyme purity is unimportant for this application, the costs for enzyme recovery and purification would be minimal. A second approach for estimating the cost of parathion hydrolase involves the correlation suggested by C. L. Cooney (Massachusetts Institute of Technology, personal communication, 1989), who observed that the revenues from many fermentation products can be related to the productivity of the fermentation with an average value of 15¢/(L·day). For the fermentation production of parathion hydrolase, it was observed that 40 units/mL could be produced within a 4 day period (Payne et al., 1990; DelaCruz et al., 1992). Since the specific activity of this enzyme was estimated in that work to be approximately 1000 units/mg (and not the 2000 units/mg value used in the model), the productivity of the fermentation can be estimated to be 0.01 g of enzyme/(L·day). Using the 15¢/(L·day) correlation, the sale price of the parathion hydrolase enzyme is estimated to be $15/g. Thus, for an initial economic evaluation, we estimated the cost of parathion hydrolase to be $20/g. As shown in Table 2, if a typical 3500 gal vat contained 300 mg/L potasan, then the 0.0007 mg/L of enzyme required to completely hydrolyze this byproduct would amount to a total of 19¢. A 3500 gal vat containing 800 mg/L potasan would require 90¢ to completely hydrolyze the potasan. As can be seen from Table 2, the cost of the enzyme is rather small, and even an order of magnitude increase in enzyme cost would not substantially affect the economics of this enzymatic treatment approach.

The second cost for the proposed enzymatic treatment strategy would be the cost to replace the coumaphos that had been degraded by the addition of the enzyme. To estimate this cost, we performed a simulation in which enzyme was added to vats containing the operational level of 1650 mg/L coumaphos. The enzyme added would correspond to the amount needed to completely degrade the potasan, and thus Figure 5 shows the potasan concentration on the abscissa. The ordinate in Figure 5 is the concentration of coumaphos that would be degraded by the enzyme which had been added to completely degrade the potasan. As can be seen from Figure 5, two simulations were performed, the first at 0.1KA_c, which had been observed to fit the data from the large-scale test, and a second, more conservative, estimate at 0.2KA_c. For both cases, Figure 5 shows relatively small amounts of coumaphos are hydrolyzed during the treatment. For instance, for a vat in which 300 mg/L of potasan was to be degraded, Figure 5 indicates that less than 50 mg/L coumaphos would be degraded. To estimate the cost to replace the coumaphos, we used an estimate of 8.8¢/g of coumaphos. As can be seen from Table 2, if 300 mg/L of potasan were to be degraded, then $52 worth of coumaphos would be hydrolyzed and would therefore need to be replaced. For the hydrolysis of 800 mg/L of potasan, Table 2 indicates that $147 worth of coumaphos would be lost by hydrolysis.

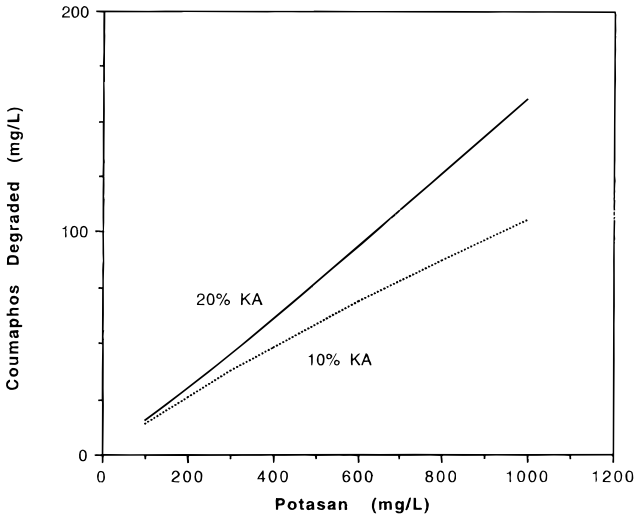

Figure 5. Simulation of the amount of coumaphos lost during the enzymatic hydrolysis of a given amount of potasan. Simulations were performed by determining the amount of enzyme required to degrade a given level of potasan and then determining how much coumaphos would be degraded by that level of enzyme. The parameters in Table 1 were used for these simulations except that KA_p was reduced to 10% of the value observed in the small-scale study (i.e. to 7.39 h^{-1}), while KA_c was either 10% or 20% of the values observed in small scale studies (i.e. either 4.47 or 8.94 h^{-1}). Simulations were conducted to 56 h when 99% of the initial enzyme activity was predicted to be lost.

To compare the costs for this waste minimization operation, we considered the alternative treatment of discarding the vat when potasan levels exceed 300 mg/L. A true estimate of the cost for this option would include the costs for draining and recharging the vats as well as the disposal costs for the waste dip vat liquid.

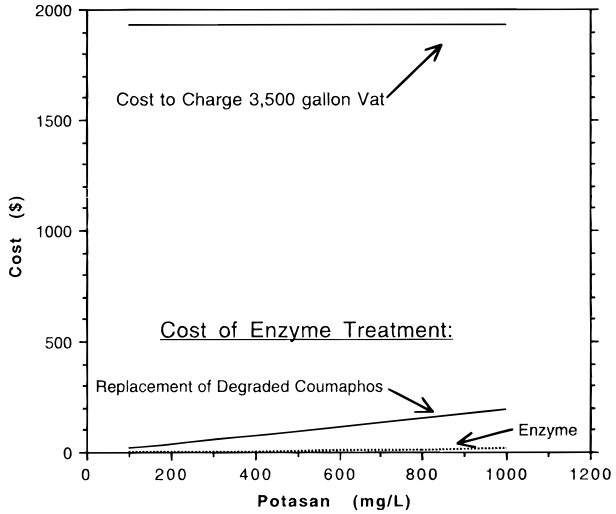

Figure 6. Economic comparison for using the enzyme to selectively hydrolyze potasan versus dumping the contents and recharging the vat with fresh coumaphos. Simulations were performed using the parameters in Table 1 except that KA_p and KA_c were 10% and 20% of the values observed in small-scale studies (i.e. 7.39 and 8.94 h^{-1}, respectively). Simulations were conducted for 56 h, and the costs for the enzyme and coumaphos were considered to be $200/g and 8.8¢/g, respectively. Vats were considered to be filled to 3500 gal with 1650 mg/L coumaphos.

Since waste vat liquid is currently stored in holding ponds, we have neglected the treatment cost and simply estimated the cost for the disposal option as the raw material cost (i.e. coumaphos cost) for recharging the vats. On the basis of the 8.8¢/g estimate for the price of coumaphos, the cost to add 22 kg of coumaphos to a 3500 gal vat to yield 1650 mg/L coumaphos would be $1920. As can be seen from comparison with Table 2, recharging the vat with coumaphos is considerably more expensive than adding enzyme to selectively degrade the potasan.

Figure 6 provides a general conclusion for this study. The bottom curve in Figure 6 shows how the cost of the enzyme increases with increased levels of potasan which must be treated. To be conservative, we estimated that the cost of enzyme would correspond to $200/g—an order of magnitude higher than our best estimate. Nevertheless, Figure 6 shows that the estimated cost of the enzyme is very low. The second curve from the bottom in Figure 6 shows the cost for replacing the coumaphos that was degraded by the enzyme. Again, to be conservative, we assumed that the mass transfer for potasan was reduced to 10% of the value observed in small-scale, well-mixed studies (i.e. simulations were performed with $0.1KA_p$), while that for coumaphos was only reduced to 20% (i.e. simulations were performed with $0.2KA_c$). As can be seen, higher potasan levels which require greater enzyme additions also increase the coumaphos replacement cost because of the increased hydrolysis of coumaphos. The upper horizontal line in Figure 6 shows the cost for replacing the dip vat and adding fresh coumaphos. As can be seen, for almost every reasonable level of potasan detected in the vat, it would be economically favorable to add the enzyme parathion hydrolase to selectively hydrolyze the potasan and continue using the vat. It should be noted, however, that one situation not considered in this economic analysis is that if a vat were to have both a high concentration of potasan and a low concentration of coumaphos, then it may not be worthwhile to use the enzyme (the amount of coumaphos remaining in the vat may not be sufficiently valuable to warrant a waste minimization strategy). However, the coumaphos level would have to be very low (below about 400 mg/L) before it would be economically more favorable to dispose of the liquid and recharge the vat with fresh coumaphos.

DISCUSSION

To assess the economics for adding parathion hydrolase to potasan-containing dip vats, it was necessary to develop a quantitative framework to permit calculation of the amount of enzyme required for treatment. The model adapted for this work accounts for the mass transfer, enzyme kinetics, and enzyme inactivation. The most serious limitation of the model concerns the mass transfer, which is difficult to describe for poorly mixed systems containing insoluble substrates. The importance of mass transfer is illustrated by the sensitivity of model predictions to the mass transfer term *KA* (i.e. Figure 3). Thus, to confidently determine what level of enzyme would be required to ensure complete hydrolysis of potasan while minimizing coumaphos degradation, more information would be required on the mass transfer characteristics of the actual dip vat system. Fortunately, for economic analysis, since the level of enzyme required for treatment is small, the enzyme cost should not be the dominant cost for this operation. Thus, the uncertainties in the mass transfer component of the model do not prevent the model from being a useful tool for estimating the economics of this enzymatic waste minimization option.

The technical and economic success of the enzymatic approach hinges on the observation that in both small- and large-scale studies potasan is hydrolyzed an order of magnitude more rapidly than coumaphos. The ability to quantitatively describe this selective hydrolysis (albeit with modifications to the mass transfer terms in the model) allows a reasonable prediction of the minimum loss of coumaphos which must be tolerated to completely hydrolyze potasan in dip vat systems (Figure 5). The cost to replace the coumaphos that is hydrolyzed represents the dominant cost for the enzymatic treatment approach.

As summarized in Figure 6, the addition of enzyme to selectively hydrolyze the potasan is economically attractive for two reasons. First, the selective enzymatic hydrolysis of potasan is inexpensive because the required amount of enzyme is small, the loss of coumaphos is minimal, and there are no capital equipment costs. Second, the alternative of disposing potasan-containing liquids and recharging the vats is costly because the coumaphos pesticide is expensive. It should be noted that for the disposal alternative we have not included a cost for treating the waste dip vat liquid because this liquid is currently being "treated" in a holding pond. If more extensive treatment becomes necessary, then the enzymatic approach would become even more economically attractive.

There are three caveats to this work. First, if a vat contains a low concentration of coumaphos, then the value of the coumaphos may be insufficient to warrant a waste minimization approach. In our simulations, we only considered the case in which coumaphos was present at 1650 mg/L—the level which is maintained during dipping operations. Second, our simulations have been limited to cases in which potasan levels are below 1000 mg/L. Although higher potasan levels have been encountered, such levels are unusual. Finally, our cost estimates are based on the addition of an optimal

amount of enzyme (i.e. the minimum amount of enzyme to completely hydrolyze all of the potasan). If suboptimal levels of enzyme were added, then potasan hydrolysis would be incomplete. If excessive enzyme were added, then active enzyme would be present after potasan had been completely hydrolyzed and there would be increased losses of coumaphos. Since coumaphos replacement is the dominant cost, the use of excessive enzyme would reduce the cost savings of this enzymatic waste minimization approach.

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Received for review June 20, 1995. Accepted October 11, 1995.[⊗] This work was partially supported by the National Science Foundation (BES-9315449).

JF950375H

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1995.