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Comparison of different models of substrate inhibition in aerobic batch biodegradation of malathion

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Abstract: In this study, the biodegradation of malathion by a culture of acclimated indigenous activated sludge was investigated under aerobic conditions. Specific substrate consumption rates (r_s) of the culture under different initial malathion concentrations of from 5 mg/L to 140 mg/L were calculated. The results showed the potential for using local activated sludge for malathion biodegradation. However, malathion exhibited inhibition of substrate degradation rate at 140 mg/L. Various substrate inhibition models were compared by fitting them to the experimental data using Statistica 7.0 software. Experimentally it was observed that the kinetic biodegradation of malathion was best described by both the Andrews and Yano and Koga models, which gave high coefficients of determination (0.97 and 0.98, respectively). On the other hand, the degradation ability of the activated sludge was found to be weak when the pesticide was used as the sole source of sulfur or phosphorus.

Key words: Activated sludge, inhibition, kinetic, malathion, models, phosphorus, sulfur

1. Introduction

Malathion S-[1,2-di(ethoxycarbonyl)ethyl] dimethyl phosphorothiolothionate] (CAS No. 121-75-5; $C_{10}H_{19}O_6PS_2$) (Figure 1) is one of the most widely used organophosphate insecticides throughout the world. It is commonly used to control mosquitos and a variety of insects that attack fruits, vegetables, landscaping plants, and shrubs (WHO, 2004). Removal of this pesticide can be attained by physicochemical and biological processes. The latter (also called bioremediation) can be defined as any process that uses bacteria, fungi, green plants, or their enzymes to return an environment altered by contaminants to its original condition (Vaishnav and Demain, 2009). The popularity of bioremediation is increasing because it often consumes less energy and fewer resources and thus is less expensive and more sustainable than physicochemical treatment approaches (Becker and Seagren, 2010).

Several studies have examined the degradation of malathion by microbes (Xie et al., 2009; Goda et al., 2010; Singh et al., 2012) and most of these studies were carried out using pure cultures. Little information is available concerning degradation of malathion by activated sludge culture (Barik et al., 1984; Kanazawa, 1987).

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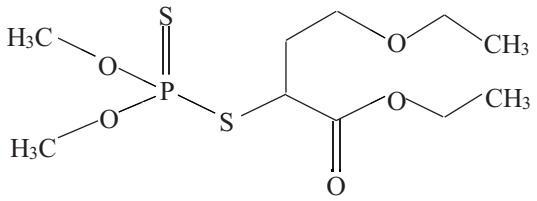

Figure 1. Structure of malathion.

Evaluation of substrate inhibition becomes an important consideration in the treatment of toxic compounds in engineered systems such as activated sludge processes (Hao et al., 2002), and mathematical modeling can be helpful for understanding the behavior of biological processes and predicting the component concentrations in the system (Tziotzios et al., 2008). The kinetics of biodegradation can be normally described by a cell growth model (μ_x) or a substrate utilization model (r_s). Several mathematical models have been used to quantify the inhibitory effect of toxic substrates on their own transformation or to express growth kinetics of microorganisms inhibited by substrate. Most of these equations have been adapted from models of the substrate inhibition of enzymatic reactions and involve a common substrate inhibition term (Ki) (Tziotzios et al., 2008).

To the best of our knowledge, there are no works evaluating different kinetic models of the inhibitory effect of malathion on its own degradation. The objective of this work was to determine statistically the best kinetic model for malathion degradation inhibition using a local activated sludge.

In most studies of xenobiotic degradation in general, and malathion degradation in particular, the compounds under consideration have been supplied to microorganisms exclusively as sources of carbon. Their utilization as a source of phosphorus and sulfur has been studied less well until now. Therefore, the ability of local activated sludge to use malathion as a source of phosphorus and sulfur nutrition was evaluated.

2. Experimental

2.1. Microorganisms and cultivation media

A mixed activated sludge culture, obtained from the aeration basin of a wastewater treatment plant in Ain Benian, Algiers, was used in this study. The mineral salt medium (MSM) used during the experiments had the following composition per liter of deionized water: 0.1 g K_2HPO_4, 0.2 g $MgSO_4$, 0.001 g $FeSO_4$, 1.0 g $(NH_4)_2SO_4$, 1.0 g NaCl, and 0.0033 g $NaMoO_4$ (Barik et al., 1984). Technical malathion (95 %) with molecular weight of 330 g/mol was obtained from Alphyte Co., Algeria. For the experiments in which malathion was used as the sole phosphorus or the sole sulfur source, the composition of the mineral medium, prepared in deionized water, was as follows, in g/L units: KH_2PO_4 0.038, $MgSO_4$ 0.05, $CaCl_2$ 0.05, urea 0.2 (Kargi and Konya, 2007). KH_2PO_4 and $MgSO_4$ were substituted with equimolar concentrations of KCl and $MgCl_2$, respectively.

2.2. Acclimatization procedure

The microorganisms used for malathion degradation were required to be acclimatized to the malathion environment because malathion removal was not observed with an unacclimated activated sludge culture. To initiate the acclimatization, an aerobic sludge of about 7 L was incubated in an Erlenmeyer flask (capacity: 9 L) at room temperature and fed with the synthetic wastewater (mineral salt medium + glucose + malathion) for the appropriate growth of microorganisms. The concentration of glucose was decreased gradually to 0, and the concentration of malathion was increased in small stepwise increments for a period of 1 month to allow the culture to acclimatize.

2.3. Malathion biodegradation

2.3.1. Model equations used for malathion biodegradation

In the present study, 8 models are considered, which are expressed by substrate degradation rate and are described below.

The Andrews equation (Edwards, 1970), based on specific growth rate, is one of the most commonly used models due to its mathematical simplicity and wide acceptance for describing the growth inhibition kinetics of microorganisms. The Andrews inhibitory growth kinetic equation is as follows:

$$\mu_x = \mu_{\max} \times S/(K_s + S + (S^2/K_i)), \tag{1}$$

where K_s, K_i, μ_x, $\mu_{x\max}$, and S are the half saturation constant (mg/L) (substrate-affinity constant), inhibition constant (mg/L), specific growth rate (h^{-1}) (positive constant), maximum specific growth rate (h^{-1}), and substrate concentration (mg/L), respectively. The corresponding form of this equation for substrate utilization is:

$$r_s = r_{s\ \max} \times S/(K_s + S + (S^2/K_i)), \tag{2}$$

where r_s and $r_{s\max}$ are the specific substrate consumption rate (h^{-1}) and maximum specific substrate consumption rate (h^{-1}), respectively.

Webb (Edwards, 1970) proposed a modified form of the Andrews model, derived his model from enzyme kinetics, and integrated an allosteric effect with β (dimensionless) as the reaction rate, as given by Eq. (3).

$$r_s = r_{s\ \max} \times S((1 + (\beta \times S/K_i))/(Ks + S + (S^2/K_i)) \tag{3}$$

Teisser (Edwards, 1970) proposed another model to predict substrate inhibition at higher substrate concentrations, as given by Eq. (4).

$$r_s = r_{s\max}(\exp(-S/K_i) - \exp(-S/K_s)) \tag{4}$$

Yano and Koga (1969) proposed a model based on a theoretical study on the dynamic behavior of single-vessel continuous fermentation subject to growth inhibition at high concentrations of rate-limiting substrates, e.g., the gluconic acid fermentation from glucose. The model form is given in the following equation:

$$r_s = r_{s\ \max} \times (S/(K_s + S + S^2/K_i + (S^3/K_i \times K))), \tag{5}$$

where K (mg/L) is the positive constant.

The model developed by Aiba et al. (1968), as in Eq. (6), is an empirical correlation. Nevertheless, simulated data with substrate inhibition agree well with empirical data from laboratory experiments.

$$r_s = (r_{s\ \max} \times S \times \exp(-S/K_i))/(K_s + S)) \tag{6}$$

Luong (1987) proposed the application of substrate inhibition to microorganism growth, describing butanol inhibition on yeast growth with the following kinetic model (7):

$$r_s = (r_{s\ \max} \times S/(Ks + S))(1 - (S/S_m))^n, \tag{7}$$

where S_m is the substrate concentration above which net growth ceases (mg/L) and n (dimensionless) is an empirical constant.

Han and Levenspiel (1988) proposed a generalized nonlinear model (Eq. (8)) to describe growth kinetics of a culture even at inhibitory levels of the substrate and covering a wide of product inhibition situations:

$$r_s = r_{s\,\max} \times S \times (1 - (S/S_m))^n / (S + K_s \times (1 - (S/S_m))^m), \quad (8)$$

where n and m (dimensionless) are empirical constants.

Tseng and Wayman (1975) proposed a substrate inhibition model (Eq. (9)) to describe the effect of alcohol concentrations on the growth rate of 2 *Candida* species and 1 *Saccharomyces* in batch tests:

$$r_s = (r_{s\,\max} \times (S/K_s + S)) - K_i \times (S - S_m). \quad (9)$$

All these models consider that substrates act as inhibitors at higher concentrations and behave as activators at lower levels. Knowledge of such kinetic models could be necessary for improvements in process control and malathion removal efficiency.

2.3.2. Effect of initial malathion concentration on its own biodegradation

All biodegradation experiments were performed in 500-mL Erlenmeyer flasks containing 250 mL of MSM as described above, containing malathion at concentrations ranging from 5 mg/L to 140 mg/L and inoculated with 250 mL of activated sludge culture containing approximately 4000 mg volatile suspended solids (VSS)/L biomass. The final biomass concentration was 2000 mg VSS/L.

2.3.3. Biodegradation of malathion present as the sole phosphorus and sulfur sources

Experiments were conducted in 1000-mL Erlenmeyer flasks containing 125 mL of the mineral medium, described above, and inoculated with 150 mL of activated sludge culture (3850 mg VSS/L), previously washed to remove any phosphorus present on the cell surfaces. The final biomass concentration was 1925 mg VSS/L. Pesticide concentration in the culture flasks was 60 mg/L. The control experiment was conducted using malathion as the sole source of carbon under the same conditions.

In all experiments, the flasks were immediately covered with aluminum foil to protect the solution from light and were supplied with oxygen by fine bubble air diffuser. The pH was maintained at 6.0. Malathion in the open environment undergoes chemical hydrolysis. In fact, the rate of this hydrolysis depends heavily on the pH of the environmental medium. Malathion is quite stable under pH 6 and susceptibility to hydrolysis increases with increasing alkalinity. All cultures were incubated at room temperature (25 °C, max. deviation ±1 °C) with a speed of 200 rpm using a magnetic stirrer.

2.4. Analytical procedures

2.4.1. Estimation of malathion concentration

The malathion residuals during biodegradation were determined using the colorimetric method proposed by Naidu et al. (1990). A UVmini-1240 UV-Vis spectrophotometer was used (Shimadzu, Japan). The relative standard deviation (RSD) was 5%.

2.4.2. Estimation of biomass concentration

Biomass concentrations were determined by measuring mixed liquor volatile suspended solids (MLVSS) as follows: mixed liquor suspended solids (MLSS) were obtained by drying the residue on filter paper (Millipore,

0.45 μm) for 2 h at 105 °C and weighed. MLVSS analyses were carried out by igniting the MLSS analysis residue for 1 h at 550 °C. The MLVSS amount was calculated as the weight difference in MLSS before and after the combustion step. The RSD was less than 2%.

2.4.3. Kinetic study

For the growth kinetic studies, specific growth rate (μ_x) values were calculated using the exponential growth phase data according to the following formula:

$$\ln(X/X_0) = \mu_x \times t, \tag{10}$$

where X_0 and X indicate the initial biomass and the biomass at time t, respectively.

Furthermore, the specific substrate consumption rate r_s (h^{-1}) was calculated according to the following equation:

$$r_s = -dS/(dt \times X), \tag{11}$$

where X and S are the biomass and substrate concentrations in mg/L at time t (h).

2.4.4. Software used

In the present study, for all substrate concentration values, all 8 substrate consumption kinetic models were fitted to the experimental data. The model equations were solved using the nonlinear regression method. Statistica 7.0 software (StatSoft Inc.) was used to analyze the data. This software utilizes the nonlinear least squares model estimation (Levenberg–Marquardt method) for minimizing the sum of squares of residuals.

All experimental measurements were carried out in duplicate and average values were used.

3. Results and discussion

3.1. Modeling the biodegradation kinetics of malathion

The experimental data and model predictions for the specific malathion consumption rate under different initial malathion concentrations are shown in Figure 2. It could be seen from the fitting of experimental data of malathion degradation that variation in specific consumption rate with respect to initial malathion concentration was observed. The concentrations of malathion between 5 mg/L and 80 mg/L did not show any inhibitory effect on the substrate biodegradation as indicated by the increasing of the specific malathion consumption rate value. A maximum specific degradation rate of 0.046×10^{-2} h^{-1} was observed at 80 mg/L and 120 mg/L concentrations of malathion. However, at a malathion concentration of 140 mg/L, the specific consumption rate dropped clearly, indicating substrate inhibition of the biodegradation at this concentration.

On the other hand, among all the models tested, which are normally used to represent substrate degradation kinetics even at inhibitory levels of the substrate, the Andrews, Yano and Koga, Luong, Han and Levenspiel, and Webb models were found to fit the data well. However, the Teissier and Aiba et al. models do not fit the experimental data at all.

The P-value and determination coefficient (R^2) statistic criteria were applied to choose the best kinetic model among those described above. Considering $\mathrm{P}-$values of less than 0.05, it can be said that the independent variable can be used to predict the dependent variable. Larger R^2 values (close to 1) indicate that the equation is a good description of the relation between the independent and dependent variables. As shown in Table 1, among the several models fitted, not only the Andrews model but also the Yano and Koga model proved

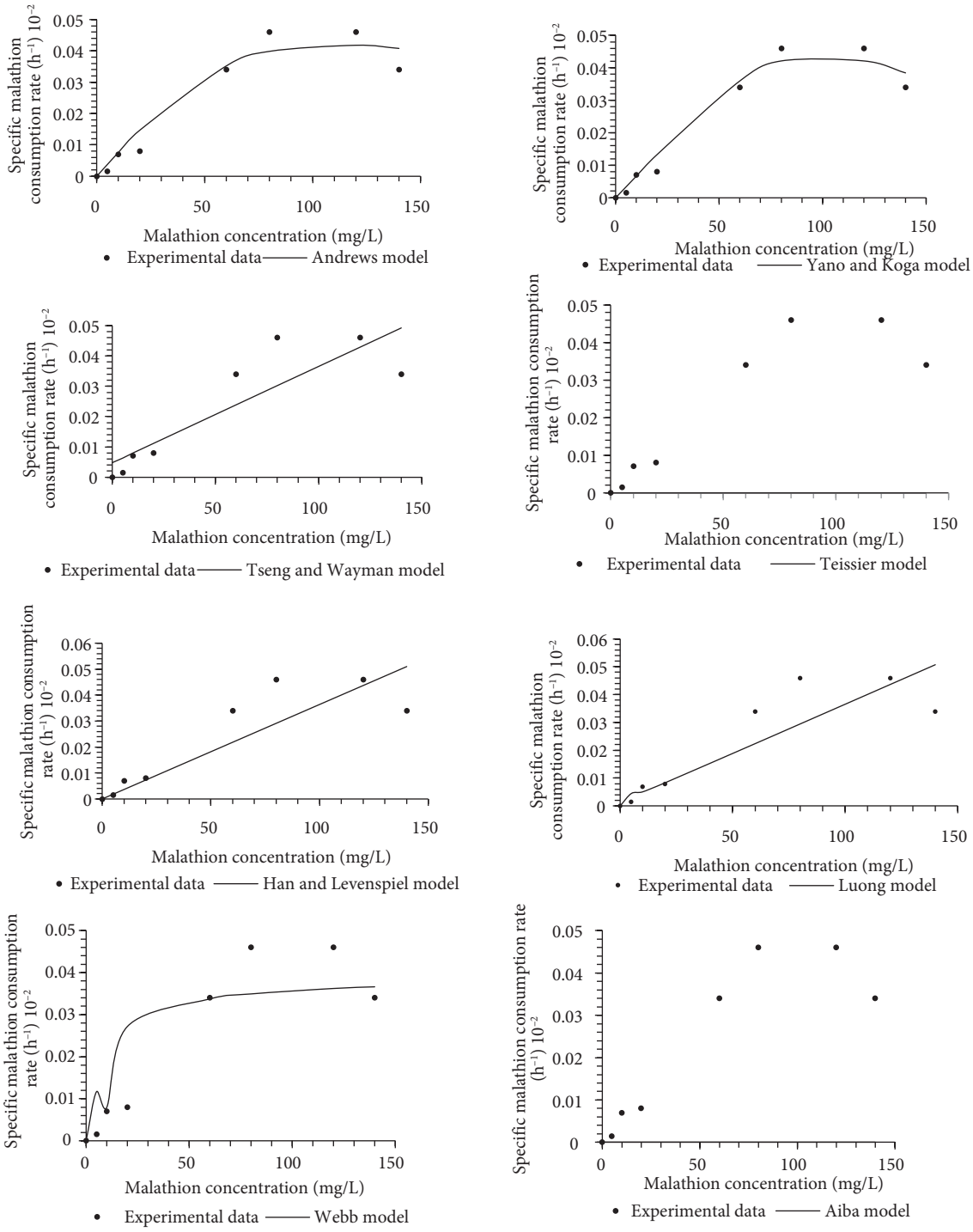

Figure 2. Experimental and predicted specific substrate consumption rates at different malathion concentrations due to different models.

to be better fits as determined by their R^2 results between the experimental and model predicted values of specific degradation rates. These results show that there were no large differences between the fittings of the Andrews and Yano and Koga models. Both are thus good kinetic models to describe the inhibition of malathion

degradation in batch mode using activated sludge. However, the Andrews model had a smaller P-value as compared with the Yano and Koga model. This result could lead to the conclusion that the Andrews model is more sensitive than the Yano and Koga model.

Table 1. Determination coefficients and P-values for various kinetic models employed for the fitting of experimental data of malathion degradation.

Model	R^2	P-value*
Andrews (Edwards 1970)	0.97	0.00016
Yano and Koga (1969)	0.98	0.00048
Han and Levenspiel (1988)	0.89	0.0079
Luong (1987)	0.89	0.0072
Tseng and Wayman (1975)	0.9	0.027
Webb (Edwards 1970)	0.89	0.03
Teissier (Edwards 1970)	0.00	1
Aiba et al. (1968)	0.00	1

*: $P < 0.05$ was considered to be significant.

Based on the same criteria, it can be said that the Teissier and Aiba et al. models are not statistically valid to describe the inhibition of malathion degradation. This could be explained by the fact that these models have generally been used to describe substrate inhibition on the growth of a pure microbial culture.

The biokinetic parameters of the models of inhibition used are also listed in Table 2. It was observed that the biokinetic constants values ($r_{s\ max}$, K_s, K_i) of malathion degradation of the culture obtained from these models differ substantially from one another. This diffiference in the models' constants values is probably due to the fact that the models tested for the present experiment differ in their origin of development. Each has been used successfully for certain organisms growing under certain growth conditions. On the other hand, the magnitude of K_s values inherent to the Andrews and Yano and Koga models indicate that the biomass has a slight affinity to malathion.

Table 2. Estimated values of parameters for various kinetic models employed for the fitting of experimental data of malathion degradation.

Model	Estimated value of parameters							
	$r_{s\ max}$ (h^{-1}) 10^{-2}	K_s (mg/L)	K_i (mg/L)	K (mg/L)	S_m (mg/L)	n	m	β
Andrews (Edwards 1970)	2.96	2318	2.718	-	-	-	-	-
Yano and Koga (1969)	58.7	87806	6.25	3.6	-	-	-	-
Han and Levenspiel (1988)	0.1	0.1	-	-	0.11	1	1	-
Luong (1987)	0.018	2.89	-	-	0.13	1	-	-
Tseng and Wayman (1975)	0.067	0.16	1.42	-	0.0033	-	-	-
Webb (Edwards 1970)	1.2	3326	0.78	-	-	-	-	0.05
Teissier (Edwards 1970)	/*	/	/	-	-	-	-	-
Aiba et al. (1968)	/	/	/	-	-	-	-	-

*: Undetermined values.

The inhibition constant K_i is the substrate concentration at which bacterial growth or substrate degradation reduced to 50% of the maximum specific growth rate or maximum specific degradation rate of the substrate due to substrate inhibition. The magnitude of this parameter indicates the inhibition tendency. It also indicates the degree of toxicity of the substrate towards the microorganisms. A lower K_i value indicates that microorganisms have a higher sensitivity to substrate inhibition. The K_i values inherent to the Andrews and Yano and Koga models are shown in Table 2. The low values of K_i indicate that the inhibition effect of malathion can be observed in a low concentration range, indicating high substrate inhibition.

It is to be mentioned that while attempting to compare the results obtained in this study, no other relevant studies with malathion carried out by other authors were found in the literature (no data for $r_{s\ \max}$, K_s, and K_i values). However, there are some articles comparing different kinetic models to express the kinetic of substrate inhibition. Carrera et al. (2004) found that the Aiba equation was the best model to describe ammonium inhibition of the nitritation process in a suspended biomass system and an immobilized biomass system, whereas the Haldane equation (similar to the Andrews equation) was the best model to describe nitratation inhibition by nitrite in both systems. Agarry et al. (2008) investigated the kinetics of phenol degradation using an indigenous binary mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* and showed that the Yano and Koga equation is the best model to describe the phenol biodegradation. On the other hand, the substrate inhibition observed during the biodegradation of phenol by a mixed microbial culture was explained by Saravanan et al. (2008) using the Haldane and Han–Levenspiel substrate inhibition models. Between the 2 models, the Han–Levenspiel model gave a better fit for the experimental data. However, more recently, the Haldane model was best fitted for phenol degradation by mixed microbial culture (Dey and Mukherjee, 2010) and for the aerobic biodegradation of hydroquinone and catechol by an activated sludge acclimated to consume p-nitrophenol (Pramparo et al., 2012).

3.2. Biodegradation of malathion present as the sole phosphorus and sulfur source

Malathion was tested as a source of phosphorus and sulfur by supplementing the basal salt medium with 60 mg/L malathion. Growth kinetics of activated sludge were studied and the nonbiodegraded residual malathion, expressed in percentage, was determined. The initial biomass concentration was 1925 mg VSS/L. It is to be mentioned that the maximum percentage degradation obtained for the control was 77% after 67 h of incubation. The degrading ability of local activated sludge in liquid culture with malathion as the sole phosphorus source was observed to be very slight (Figure 3). Indeed, the maximum malathion percentage degradation obtained was only 15% after 71 h of incubation. Moreover, the results showed that almost no cell growth was observed ($\mu \approx 0\ h^{-1}$), suggesting that malathion-tolerant microorganisms have slower growth ability. These results lead us to suggest that the microorganisms tested in this work do not carry the necessary combination of microbial metabolism and exoenzyme activity to release phosphorus from the insecticide. This is probably due to the fact that the phosphorus limitation in the medium seems to prevent the synthesis of enzymes involved in providing both phosphorus and carbon to the cell from the pesticide supplied as a sole source of phosphorus and carbon for growth. Similar results were reported by Subramanian et al. (1994), who established that malathion was used by *Aulosira fertilissima* ARM 68 as the sole source of phosphorus in the absence of inorganic phosphate in the medium. Under these conditions, the use of malathion in phosphorus nutrition may be connected to the role of extracellular phosphatases (acid and alkaline).

Figure 4 shows the time profile of malathion degradation and the growth of bacteria measured as MLVSS when malathion was added as the sulfur source. It is evident from this figure that no cell growth was observed,

as indicated by the specific growth rate value (μ), which approaches 0 h^{-1}. Moreover, the experimental results obtained showed that only 25% of the malathion had disappeared after 71 h, indicating that the mixed culture was not capable of utilizing malathion very efficiently. Kertesz et al. (1994) explained possible underlying reasons for this phenomenon. They suggested that the conditions under which environmental isolates were enriched were crucial in selecting for strains not only with the desired degradative enzyme systems but also with specific regulation mechanisms for the degradation pathways.

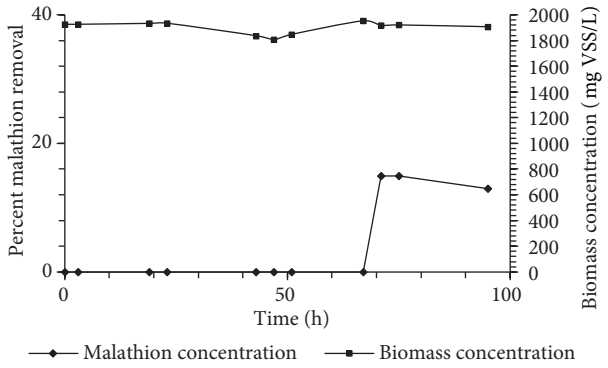

Figure 3. Malathion degradation and growth kinetics of activated sludge with malathion as sole phosphorus source (initial malathion concentration and initial cell concentration were 60 mg/L and 1925 mg VSS/L, respectively).

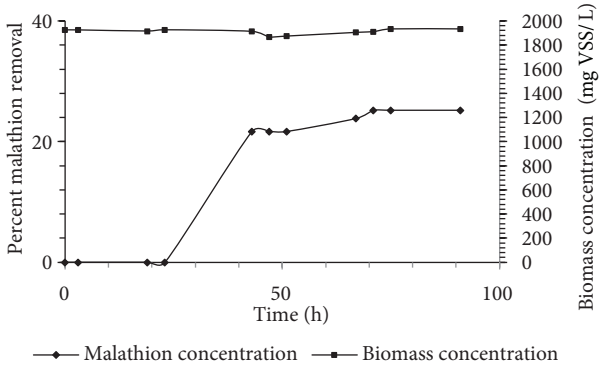

Figure 4. Malathion degradation and cell growth kinetics with malathion as sole sulfur source (initial malathion concentration and initial cell concentration were 60 mg/L and 1925 mg VSS/L, respectively).

4. Conclusion

The capability of acclimated activated sludge for malathion biodegradation was studied using a batch culture under aerobic conditions. In the range of malathion concentrations used in the study, specific degradation rate was observed to follow substrate inhibition kinetic. Indeed, it was observed that malathion concentrations below 140 mg/L showed no inhibitory effect. However, at 140 mg/L of malathion, a distinct substrate inhibition effect was found. The substrate inhibition due to malathion was evaluated by comparing various substrate inhibition models. Of these models, the Andrews and the Yano and Koga models gave a better fit to the experimental data and hence may be proposed to describe the malathion degradation behavior of local acclimated activated sludge. These results would lead to greater insights about the predictive understanding of success required for malathion treatment. On the other hand, the activated sludge used in the present study was found to be unable to use the pesticide as either the sole sulfur source or as the sole source of phosphorus. Based on the results, the acclimated activated sludge used in the present work is a potential culture that can be used for malathion biodegradation in real-life wastewater treatment. However, the cell inhibition mechanisms involving this pesticide should be studied more in detail.

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