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*original scientific paper*

# Integrated Approach to Mathematical Modelling of Atrazine Degradation in Different Reaction Systems**

*Marijan Bošnjak[1]*, Nikolina Udiković Kolić[2], Ines Petrić[2], Darin Cihlar[3] and Dubravka Hršak[2]*

[1]Croatian Academy of Engineering, HR-10000 Zagreb, Kačićeva 28, Croatia
[2]Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia
[3]Infoptim Ltd, HR-10000 Zagreb, Croatia

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**Summary**

Based on the known approaches and published mathematical models, as well as on theoretical consideration using our experimental data, the integrated approach to mathematical modelling of atrazine biodegradation processes has been employed and sophisticaed mathematical models for different reaction systems have been developed. The applicability of these models which take into account physical, chemical, biochemical and biological complexity of atrazine biodegradation was further analyzed in comparison with mathematical models describing simple consecutive reaction systems using first-order kinetics. Kinetics of atrazine degradation in liquid media and soil contaminated with atrazine at the temperatures of 10 and 30 °C was assessed and compared. Biodegradation experiments in liquid media were conducted at atrazine concentrations ranging from 0.14 to 25 mmol/L, while the experiments in soil were conducted at atrazine concentration of approx. 0.44 μmol/g. Computer simulations were applied to explain experimental results and test the adequacy of mathematical models. Detailed analysis of computer simulation data showed that the developed integrated mathematical models could be considered as the most convenient for describing kinetics of atrazine biotransformation in both liquid media and contaminated soil, although even simple mathematical models are suitable for explaining some experimental results, especially when evaluating the temperature effects on biodegradation efficacy of the applied mixed bacterial culture.

*Key words*: atrazine, biodegradation kinetics, mathematical modelling, computer simulation, mixed bacterial cultures

## Introduction

Application of pesticides demands detailed studies of kinetics of their degradation, which has resulted in a series of papers referring to this problem. As pointed out in the report of Soulas (*1*), Hill *et al*. (*2*) modelled the soil degradation of several substituted urea herbicides by applying simple first-order kinetics. Applicability of mathematical models based on fundamental chemical theory and on biocatalysis defined in accordance with Michaelis–Menten kinetics as well as on the theory of microbial processes was discussed. Discussion was extended to integrated models taking into account physicochemical processes with suggestions how to better describe pesticide degradation processes in soil. As a continuation, Soulas and Lagacherie (*3*) focused on the adaptation of the models taking into account microbial interac-

*Corresponding author; E-mail: marijan.bosnjak@hatz.hr

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tions with soil environments. Methodological and technical limitations were mentioned.

Atrazine is the most commonly used herbicide worldwide (*4*). However, in European countries, and therefore in Croatia, its use in agriculture has recently been restricted. Remarkable relevance to the study of atrazine biodegradation was given in Croatia, where for the last 10 years the studies have been oriented towards the discovery of mixed microbial cultures capable of degrading atrazine efficiently under different reaction conditions and towards describing kinetics relationships of degradation process. The experimental studies resulted in the acceptance of patent application and publication of research results (*5–9*). It is also worth mentioning that they contributed to the advances in this area worldwide.

Park *et al*. (*4*) applied three atrazine-degrading bacterial strains (*Pseudomonas* sp. strain ADP, *Agrobacterium radiobacter* strain J14a and *Ralstonia* sp. strain M91-3) in detailed studies of sorption equilibria in soil slurries, $CO_2$ production, as well as in the evaluation of distribution coefficients and desorption parameters. Mathematical model describing kinetics of particular events, consisting of 8 equations (three of them were expressed as differential equations), was applied to explain experimental results. Experimental results fitted quite well those theoretical. It was established that sorbed atrazine quantities for different sorbents were proportional to aqueous atrazine concentration. High importance was also given to atrazine and/or other pesticide sorption-desorption kinetics in publications of other authors (*10–14*).

Wenk *et al*. (*15*) studied rapid atrazine mineralization applying an atrazine-degrading *Pseudomonas* sp. strain. Detailed experimental investigation of biodegradation efficiency depending on the applied biomass quantity, soil moisture and atrazine adsorption-desorption rates was performed, but without expressing any mathematical model. However, their finding that the rate of atrazine removal from the contaminated soil was proportional to the water content of the soil and the amount of bacteria added to the soil is relevant for the development of reliable mathematical model convenient to explain atrazine biodegradation kinetics.

Kinetics of atrazine biodegradation in the water contaminated with this herbicide was studied by Goux *et al*. (*16*). The microbial community designated as COM1 originating from Belgian maize field was applied as biocatalyst. To describe biodegradation kinetics, two different mathematical models were used. The first was the conventional convection dispersion equation, whereas the other was the equation expressing the first order kinetics. In the experiments, media with relatively low initial atrazine concentration (20 mg/L) were used, and the applied microbial consortium COM1 showed to be efficient as biocatalyst.

The aim of this work is to demonstrate the advantages of integrated approach to mathematical modelling in explaining atrazine biodegradation kinetics, and to show how the developed mathematical models are suitable for describing atrazine biodegradation kinetics. To this purpose computer simulations have been applied.

## Materials and Methods

### *Postulation of a simple mathematical model*

At the very beginning of the study of atrazine biodegradation, we estimated that experimental results should be evaluated on the basis of recognised biodegradation kinetics relationships. The insight into the results of the first series of experiments suggested the application of mathematical model to the already obtained experimental data, describing degradation process kinetics with differential equations that express the reaction rates in accordance with the first order reaction kinetics and based on the reaction scheme:

$$A \xrightarrow{k_{AB}} B \xrightarrow{k_{BC}} C \xrightarrow{k_{CD}} D$$

**Scheme 1.** First order reaction kinetics

where A refers to atrazine, B to hydroxyatrazine, C to cyanuric acid, and D to the final degradation products $CO_2$ and $NH_4^+$.

Consequently, the mathematical model MAM1, defined by Eqs. 1–3, is obtained:

$$dc_A/dt = -k_{AB} \cdot c_A \qquad /1/$$

$$dc_B/dt = k_{AB} \cdot c_A - k_{BC} \cdot c_B \qquad /2/$$

$$dc_C/dt = k_{BC} \cdot c_B - k_{CD} \cdot c_C \qquad /3/$$

where $c_A$, $c_B$ and $c_D$ are the molar concentrations of A, B and C substances respectively, while $k_{AB}$, $k_{BC}$ and $k_{CD}$ are the corresponding reaction rate constants.

### *Development of integrated (structured) mathematical models*

Based on the literature data (*4,15,16*), relatively low initial concentrations of atrazine were applied in the first series of experiments. That is why there was no interest in developing more adequate mathematical model at the beginning of experiments. However, later during research the mixed microbial culture capable of degrading atrazine efficiently even in reaction media with much higher atrazine concentrations provoked an interest for developing a more adequate mathematical model taking into account both the role of microbial culture as a biocatalytic agent and the relevance of atrazine solubility in water. Prior to establishing the final mathematical model, the model represented by Eqs. 1–3 (MAM1) was transformed into the model describing the growth and biocatalytic action of microbial biomass during atrazine biodegradation. Therefore, if biomass was expressed in mass units, the following mathematical model (MAM2) was obtained:

$$d\gamma_x/dt = \mu_{xm} \cdot \gamma_x \cdot (1 - \gamma_x/\gamma_{xm}) \qquad /4/$$

$$dc_A/dt = -k_{ABX} \cdot \gamma_x \cdot c_A/(c_A + K_A) \qquad /5/$$

$$dc_B/dt = k_{ABX} \cdot \gamma_x \cdot c_A/(c_A + K_A) - k_{BCX} \cdot \gamma_x \cdot c_B/(c_B + K_B) \qquad /6/$$

$$dc_C/dt = k_{BCX} \cdot \gamma_x \cdot c_B/(c_B + K_B) - k_{CDX} \cdot \gamma_x \cdot c_C/(c_C + K_C) \qquad /7/$$

where $\mu_{xm}$ is maximal specific growth rate of microbial biomass in $h^{-1}$, $\gamma_x$ is microbial biomass concentration, $\gamma_{xm}$ is maximal microbial biomass concentration in g/L, and $K_A$, $K_B$ and $K_C$ are mean biocatalytic constants for reaction substances A, B and C, respectively. When biomass decay is observed, instead of logistic Eq. 4, the modified Eq. 8 is suggested:

$$d\gamma_x/dt=\mu_{xm}\cdot\gamma_x\cdot(1-\gamma_x/\gamma_{xm})\cdot c_A/(c_A+K_{ag})-k_d\cdot\gamma_x \qquad /8/$$

where $K_{ag}$ is biocatalytic constant of biomass growth with reference to substance A in mmol/L, while $k_d$ is specific biomass decay rate in $h^{-1}$.

If biomass was expressed as a number of microbial cells (CFU), then instead of Eq. 4, Eq. 9 should be applied:

$$dN_x/dt=\mu_{Nm}\cdot N_x\cdot(1-N_x/N_{xm}) \qquad /9/$$

where $N_x$ is the number of microbial cells in $L^{-1}$, $N_{xm}$ is maximal number of microbial cells in $L^{-1}$, $\mu_{Nm}$ is maximal specific rate of cell number increase in $h^{-1}$. In this case, a simultaneous transformation of rate constant values with respect to microbial cell units must be done.

In media with higher atrazine concentrations, it is only partly dissolved, while the other part is undissolved or adsorbed on soil or other carrier particles. In such cases, mathematical model MAM2, shown in Eqs. 4–7 or 9, should be transformed taking into account the changes of undissolved and total atrazine concentrations. Reaction system analysis led to the conclusion that the extended system of differential equations (MAM3) should be applied, *i.e.* in addition to Eqs. 4 or 9 and Eqs. 6 and 7, the following equations should be included:

$$dc_A/dt=-k_{ABX}\cdot\gamma_x\cdot c_A/(c_A+K_A)+k_{sol}\cdot c_{Ains}\cdot(c_{Am}-c_A) \qquad /10/$$

$$dc_{Ains}/dt=-k_{sol}\cdot c_{Ains}\cdot(c_{Am}-c_A) \qquad /11/$$

$$dc_{AT}/dt=dc_A/dt+dc_{Ains}/dt \qquad /12/$$

where $k_{sol}$ is the specific rate of undissolved atrazine dissolved in L/(mmol·h), $c_{Ains}$ is the concentration of undissolved atrazine in mmol/L, $c_{Am}$ is atrazine solubility in mmol/L and $c_{AT}$ is total atrazine concentration in mmol/L. The other conditions for the use of this model are the same as those in the model applicable for reaction media where atrazine is dissolved completely.

The range of applicability of any mathematical model is very important. Mathematical models shown in Eqs. 1–12 were originally developed to explain atrazine biodegradation kinetics in liquid media. However, literature data suggest that more relevance needs to be given to the kinetics of atrazine biodegradation in contaminated soil. Wenk *et al.* (*15*) established that the rate of atrazine removal from contaminated soil was proportional to the water content in the soil and depended on the amount of bacteria added to the soil. Therefore, hypothetically the developed mathematical model can be adapted for the explanation of kinetics of atrazine removal from contaminated soil. In order to verify its applicability, model parameters were converted with respect to the water content in the soil. This can be demonstrated with the following example:

If the soil containing 20 % of water is contaminated with atrazine 0.5 mmol/kg, inoculated with $10^7$ cells of a mixed microbial culture and then homogenised, the soil will act as liquid reaction medium containing (in aqueous phase) at the start: $10^7/0.2=5\cdot10^7$ of microbial cells/L, and atrazine 0.5/0.2=2.5 mmol/L. If atrazine solubility in the water is estimated to be 0.15 mmol/L, then the concentration of undissolved atrazine will be 2.35 mmol/L.

However, MAM3 cannot be considered as the final solution for describing atrazine biodegradation kinetics. It can be transformed giving its simpler modifications, or extended by taking into account other relevant physicochemical and biochemical biodegradation processes like those defined by Park *et al.* (*4*). Simpler model modifications can be recommended for use in order to support the advantages of more sophisticated mathematical models. One of the simpler modifications (MAM3a) can result from fixing the constant microbial biomass concentration and applying the first order kinetics (Eqs. 1–3), and the other (MAM3b) after fixing constant microbial biomass concentration. The extended mathematical model MAM4 can be defined by adding Eq. 13 and by modifying Eqs. 10 and 12:

$$dc_{Aim}/dt=-k_{dim}\cdot c_{Aim}+k_{im}\cdot c_A \qquad /13/$$

$$dc_A/dt=-k_{ABX}\cdot\gamma_x\cdot c_A/(c_A+K_A)+k_{sol}\cdot c_{Ans}\cdot(c_{Am}-c_A)+k_{dim}\cdot c_{Aim}-k_{im}\cdot c_A \qquad /14/$$

$$dc_{Ans}/dt=-k_{sol}\cdot c_{Ans}\cdot(c_{Am}-c_A) \qquad /15/$$

$$dc_{AT}/dt=dc_A/dt+dc_{Ans}/dt+dc_{Aim}/dt \qquad /16/$$

where $c_{Aim}$ refers to the sorbed (immobilized) atrazine concentration, and $c_{Ans}$ to the undissolved atrazine concentration, while $k_{im}$ and $k_{dim}$ indicate the rate constants with reference to dissolved atrazine ($c_A$) sorption and immobilised atrazine ($c_{Aim}$) desorption, respectively.

Prior to verifying whether the proposed mathematical models could explain the experimental data, it should be mentioned that microbial processes, especially those referring to mixed microbial cultures, are very complex and that they cannot be described perfectly with mathematical models because of insufficient information on all processes in every moment. However, mathematical models can be used to determine the differences in behaviour between particular microorganisms, as demonstrated recently (*17*) by comparing the behaviour of different *Streptomyces rimosus* strains. Simple systems of differential equations can usually be solved by known mathematical methods with analytical solutions. There is an analytical solution also for the system described by differential Eqs. 1–3. However, computer simulation can also be applied, and it appears as a method of choice even for very complex systems of differential equations. Therefore, it was also applied to verify the convenience of mathematical models in this work.

## *Computer simulation*

Due to the previous successful applications (*17–21*) of Scientist computer programme (Micromath, St. Louis, MO, USA), it was also applied in this work. Based on

the mathematical models developed in this work, adequate computer simulation kinetic models were prepared and applied. Fittings of computer simulation to experimental data were statistically validated applying the Jacobian matrix, installed as part of Scientist calculation programme.

### *Biodegradation experiments and experimental data*

The input parameters used for mathematical modelling are experimental data of our integrated studies on the mechanisms and kinetics of atrazine biodegradation using mixed and pure bacterial cultures originating from the soil exposed to long-term contamination with atrazine and other *s*-triazine compounds (*5–9*). Selected data of the biodegradation experiments in liquid media and soil were used to test the applicability of the developed mathematical models. The same, well characterized and very efficient atrazine-degrading mixed culture was used in all the experiments (*5*).

Biodegradation experiments in liquid media were performed at different initial atrazine concentrations (0.145–22 mmol/L) and two temperatures (10 and 30 °C) employing shake flask technique as described previously (*6*). It is important to note that in all experiments the culture media were composed of the same mineral salts – MS medium (*22*), and that in all cases except one (Fig. 1; computer simulation parameters with reference to all figures presented in Table 1) the MS medium was supplemented with sodium citrate (1 g/L), (MS-citrate medium). Quantitative measurements of atrazine and the formed intermediates (hydroxyatrazine and cyanuric acid) were performed by HPLC analysis as described previously (*6*).

The experiments in soil were performed in microcosms using soil that had not previously been exposed to atrazine. The procedure, including the preparation of atrazine-contaminated soil, was similar to that described elsewhere (*9*). The experiments were carried out under aseptic conditions at 10 and 30 °C using a mixed culture grown in liquid medium (MS-citrate medium containing 0.145 mmol/L of atrazine) (*6*). The culture biomass was centrifuged, resuspended in MS-citrate medium and added to the soil to the final water content of 20 %. Quantitative determination of atrazine and the formed intermediates was performed by using the same HPLC method as in liquid culture experiments after soil extraction with methanol.

## Results and Discussion

Series of computer simulations were performed based on MAM1 and it was established that this model can be applied to explain experimental results when media with relatively low atrazine concentrations were investigated. Selected computer simulations shown in Fig. 1 give evidence on the convenience of the applied mathematical model.

Because the role of microbial biomass cannot be ignored in the process of atrazine biodegradation, a series of computer simulations was performed using MAM2, shown in differential Eqs. 4–9. Data in Fig. 2 testify that MAM2 model is also convenient for explaining experimental data shown in Fig. 1.

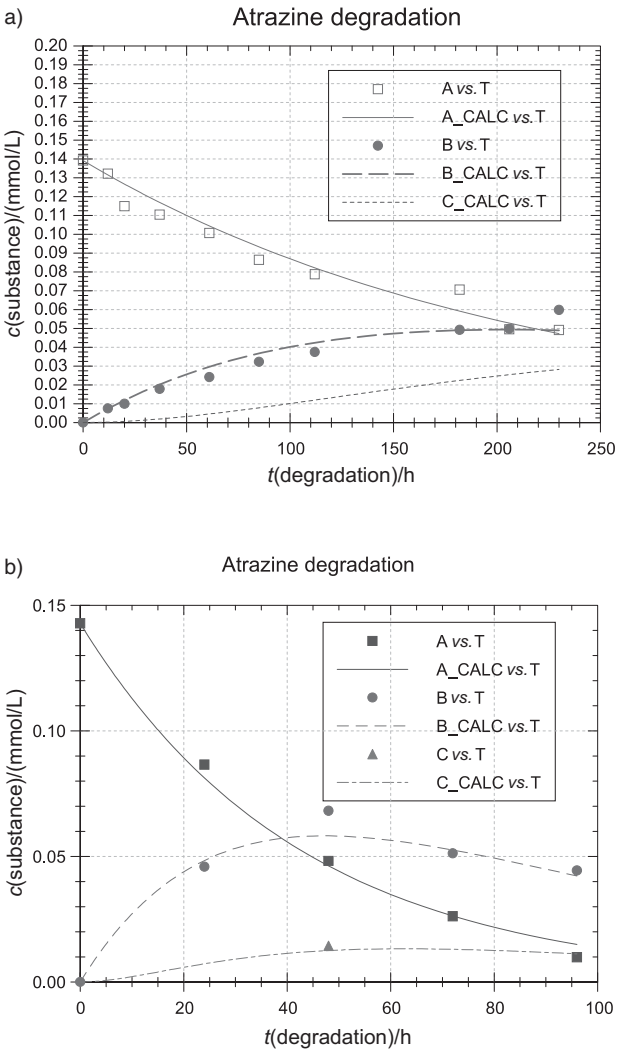



**Fig. 1.** Application of mathematical model MAM1 in describing atrazine biodegradation kinetics, and fitting computer simulation to experimental data by applying parameters shown in Table 1: a) experiment with MS culture medium, b) experiment with MS-citrate culture medium. Points: experimental data, curves: simulated data

With respect to the convenience of the application of mathematical model for larger range of reaction conditions, much more was expected when applying the extended model MAM3, represented by Eqs. 9–12. Computer simulation data mainly confirmed the expected, as demonstrated in Figs. 3–8, especially when considering atrazine biodegradation kinetics in soil aqueous phase (Figs. 7 and 8). However, it should be pointed out that the simplest mathematical model (MAM1) can also be applied to explain the same experimental data established for soil aqueous phase (Fig. 9). Although this finding could be surprising, there is no doubt that it represents useful information, since the simple MAM1 can also be recommended for use in evaluating the efficiency of atrazine removal from the contaminated soil, if taking into account the fact that differences in microbial biomass in MAM1 were neglected. Discrepancies be-

Table 1. Applied values of computer simulation parameters with reference to particular figures and applied mathematical models

| Fig. | Model | Parameter names and values | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MAM1 | $k_{AB}$ | $k_{BC}$ | $k_{CD}$ | | | | | | | | | | | | |
| 1a | | 0.0047 | 0.00505 | 0.005 | | | | | | | | | | | | |
| 1b | | 0.0235 | 0.019 | 0.08 | | | | | | | | | | | | |
| 2 | MAM2 | $k_{ABX}$ | $k_{BCX}$ | $k_{CDX}$ | $\mu_m$ | $\gamma_{xm}$ | $K_A$ | $K_B$ | $K_C$ | $k_{sol}$ | $c_{Am}$ | | | | | |
| | | 0.0225 | 0.0155 | 0.05 | 0.35 | 0.34 | 0.165 | 0.165 | 0.165 | | | | | | | |
| 3 | MAM3 | 1.75 | 1.75 | 1.25 | 0.15 | 0.272 | 0.14 | 0.14 | 0.14 | 0.90 | 0.1428 | | | | | |
| 4a | | 1.9625 | 4.5 | 4.5 | 0.15 | 0.278 | 0.14 | 0.14 | 0.14 | 0.90 | 0.1428 | | | | | |
| 4b | MAM3a | $k_{AB^*}$ | $k_{BC^*}$ | $k_{CD^*}$ | | $\gamma_x$ | | | | | | | | | | |
| | | 6.5 | 11.5 | 11.5 | | 0.28 | | | | 0.90 | 0.1428 | | | | | |
| 4c | MAM3b | $k_{ABX}$ | $k_{BCX}$ | $k_{CDX}$ | | | | | | | | | | | | |
| | | 1.55 | 3.75 | 3.75 | | 0.28 | 0.14 | 0.14 | 0.14 | 0.90 | 0.1428 | | | | | |
| 5a | MAM3 | $k_{ABN}$ | $k_{BCN}$ | $k_{CDN}$ | | $N_{xm}$ | | | | | | | | | | |
| | | $7.6\cdot10^{-13}$ | $8.0\cdot10^{-13}$ | $7.5\cdot10^{-13}$ | 0.222 | $1.62\cdot10^{12}$ | 0.15 | 0.15 | 0.15 | 1.5 | 0.1428 | | | | | |
| 5b | | $k_{ABX}$ | $k_{BCX}$ | $k_{CDX}$ | | $\gamma_{xm}$ | | | | | | $k_d$ | $K_{ag}$ | | | |
| | | 0.55 | 0.7 | 0.6 | 0.258 | 1.65 | 0.15 | 0.15 | 0.15 | 1.5 | 0.1428 | 0.0745 | 0.010 | | | |
| 6 | | $k_{ABN}$ | $k_{BCN}$ | $k_{CDN}$ | | $N_{xm}$ | | | | | | | | | | |
| | | $5.0\cdot10^{-13}$ | $1.2\cdot10^{-11}$ | $1.23\cdot10^{-13}$ | 0.045 | $9.8\cdot10^{10}$ | 0.30 | 0.30 | 0.50 | 1.5 | 0.1428 | | | | | |
| 7 | | $7.5\cdot10^{-13}$ | $2.5\cdot10^{-13}$ | $2.5\cdot10^{-13}$ | 0.23 | $5.0\cdot10^{13}$ | 0.14 | 0.14 | 0.14 | 1.0 | 0.1428 | | | | | |
| 8 | | $1.6\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | 0.0425 | $5.0\cdot10^{13}$ | 0.25 | 0.35 | 0.35 | 0.5 | 0.1428 | | | | | |
| 9a | MAM1 | $k_{AB}$ | $k_{BC}$ | $k_{CD}$ | | | | | | | | | | | | |
| | | 0.1 | 0.45 | 0.44 | | | | | | | | | | | | |
| 9b | | 0.0235 | 0.15 | 0.1 | | | | | | | | | | | | |
| 10a | MAM4 | $k_{ABN}$ | $k_{BCN}$ | $k_{CDN}$ | $\mu_m$ | $N_{xm}$ | $K_A$ | $K_B$ | $K_C$ | $k_{sol}$ | $c_{Am}$ | $N_{x0}$ | $c_{Aim0}$ | $k_{dim}$ | $k_{im}$ | $c_{Ans0}$ |
| | | $1.6\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | 0.0425 | $5.0\cdot10^{13}$ | 0.25 | 0.35 | 0.35 | 0.5 | 0.1428 | $1.8\cdot10^{11}$ | 0.20 | 0.01 | 0.1 | 1.374 |
| 10b | | $1.75\cdot10^{-13}$ | $1.4\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | 0.0425 | $5.0\cdot10^{13}$ | 0.25 | 0.35 | 0.35 | 0.5 | 0.1428 | $1.8\cdot10^{11}$ | 0.00 | 0.1 | 0.1 | 1.574 |
| 10c | | $1.6\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | $1.2\cdot10^{-13}$ | 0.0425 | $5.0\cdot10^{13}$ | 0.25 | 0.35 | 0.35 | 0.5 | 0.1428 | $1.8\cdot10^{9}$ | 1.00 | 0.01 | 0.1 | 0.574 |
| 10d | | $7.5\cdot10^{-13}$ | $2.5\cdot10^{-13}$ | $2.5\cdot10^{-13}$ | 0.23 | $5.0\cdot10^{13}$ | 0.14 | 0.14 | 0.14 | 1.0 | 0.1428 | $1.8\cdot10^{11}$ | 0.00 | 0.1 | 0.1 | 1.574 |

tween theoretical and experimental data suggest that their main cause appears to be neglecting the differences in microbial biomass quantities, which is especially evident when considering the process kinetics at lower temperature, where changes of microbial growth kinetics take place more slowly. In the process range of lower biomass amount, the experimental values referring to atrazine amount are higher than those theoretical (simulated), whereas in the range of larger biomass amount, theoretical values are higher than the corresponding experimental ones, if changes of atrazine quantities in the contaminated soil during atrazine biodegradation are compared. Concerning the applicability of simpler mathematical models, it was also established that they could be applied in describing the degradation process presented in Fig. 4. Computer simulation data (Figs. 4b and c) resulted from the transformation of MAM3 into its modifications MAM3a and MAM3b. As shown, computer simulation data are in agreement with experimental data. The statistically estimated values of model selection criteria for data in Figs. 3 and 4 support such an impression (Table 2). Since the data in Fig. 3 evidently support the application of MAM3, the same can be said for experimental data shown in Fig. 4.

More pronounced discrepancies between experimental and theoretical values can be observed when applying the extended mathematical model MAM3, as shown in Figs. 5 and 6. Probable reasons for the observed discrepancies could be the exhaustion of some nutrient during the later biodegradation phase (Fig. 6), or difference between experimental and theoretical microbial growth rates during the first process phase (Fig. 5).

On the other hand, good agreement between experimental and theoretical data in cases of atrazine removal from contaminated soil (Figs. 7 and 8, Table 2) suggests that the soil enriched with liquid nutrient medium and inoculated with larger amount of microbial culture showed to be more convenient for biodegradation than the corresponding liquid medium itself for the same processes in shake flasks when these were started with markedly smaller amounts of microbial culture.

Regarding the atrazine biodegradation kinetics in the experiments on contaminated soil (Figs. 8 to 10), it can be concluded that the obtained results confirmed the observations of Wenk *et al.* (*15*), giving an emphasis to the fact that the extended mathematical model MAM3 can be recommended for application in explaining atrazine biodegradation kinetics in contaminated soil. The

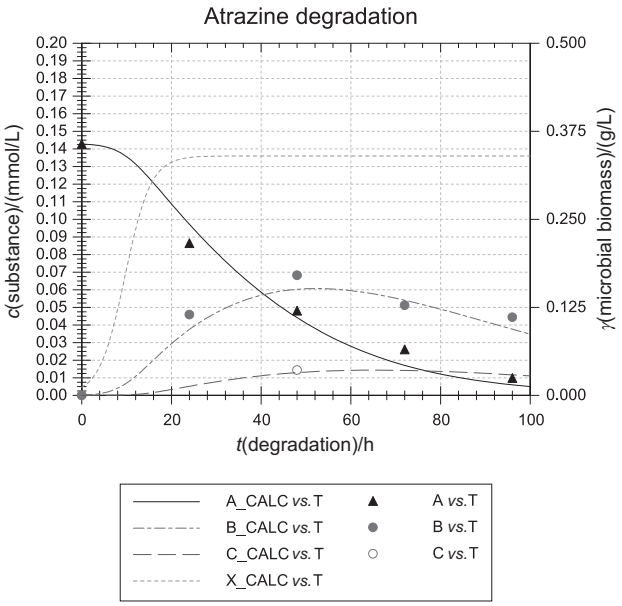



**Fig. 2.** Application of mathematical model MAM2 in describing atrazine biodegradation kinetics, and fitting computer simulation to experimental data as those presented in Fig. 1b. Parameter values are shown in Table 1. Points: experimental data, curves: simulated data

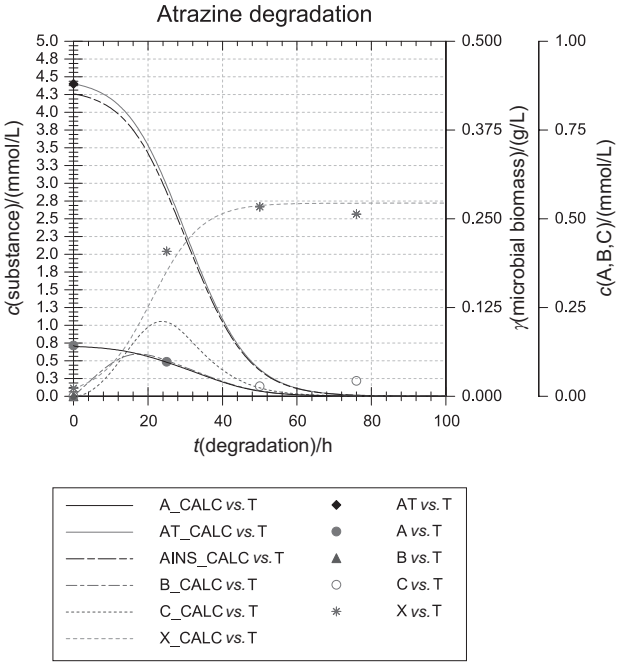



**Fig. 3.** Application of mathematical model MAM3 in describing atrazine biodegradation kinetics, and fitting computer simulation to experimental data by applying parameters shown in Table 1. Points: experimental data, curves: simulated data

obtained results are in agreement with findings of Park *et al.* (4), who found that atrazine quantities sorbed by different sorbents were proportional to aqueous atrazine concentrations. The fact that the majority of other pesticides actually being in use in agriculture are of lower solubility in water than atrazine implies that MAM3 could also be applicable in describing their biodegradation ki-

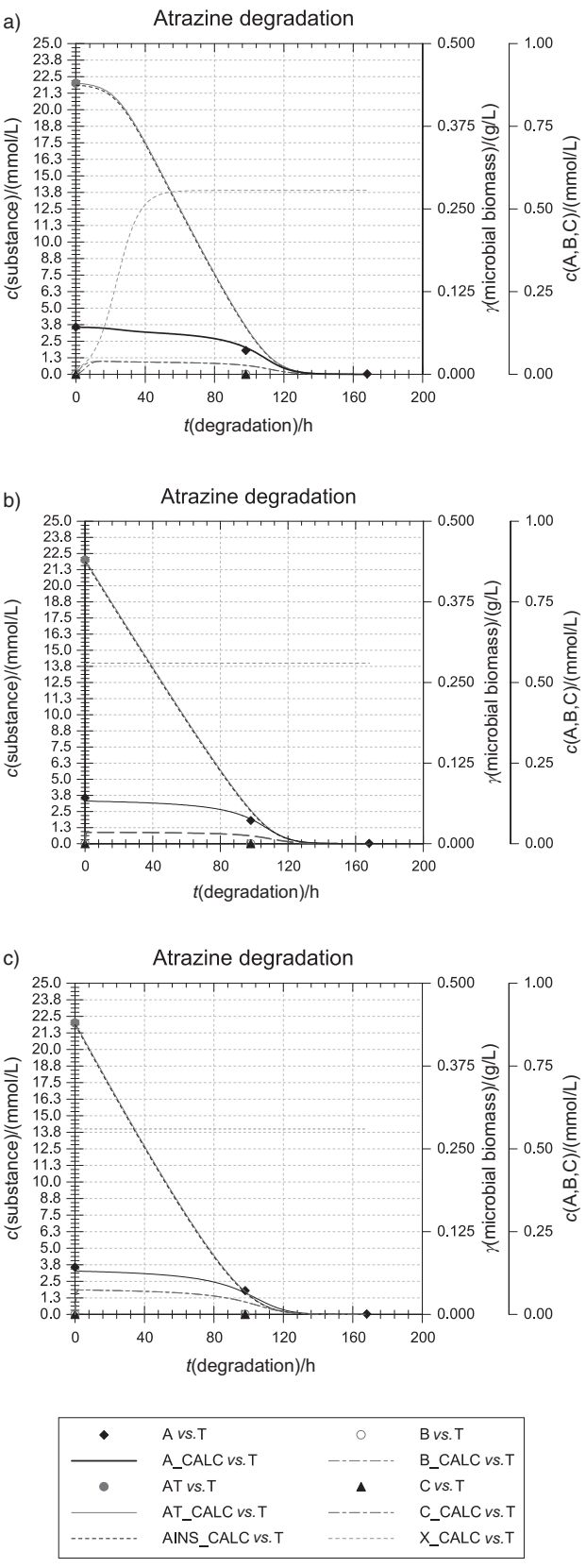
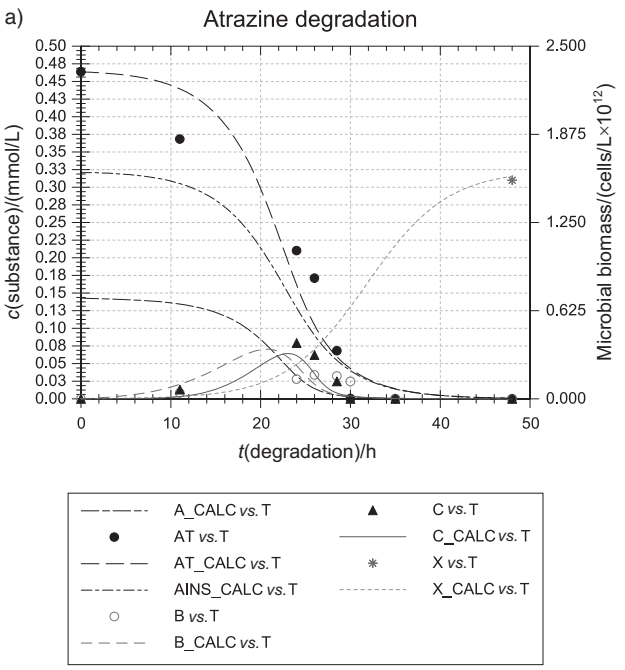



**Fig. 4.** Application of mathematical models MAM3 (a), MAM3a (b) and MAM3b (c) for description of atrazine biodegradation kinetics, and agreement of computer simulation with experimental data for simulation parameters indicated in Table 1. Points: experimental data, curves: simulated data



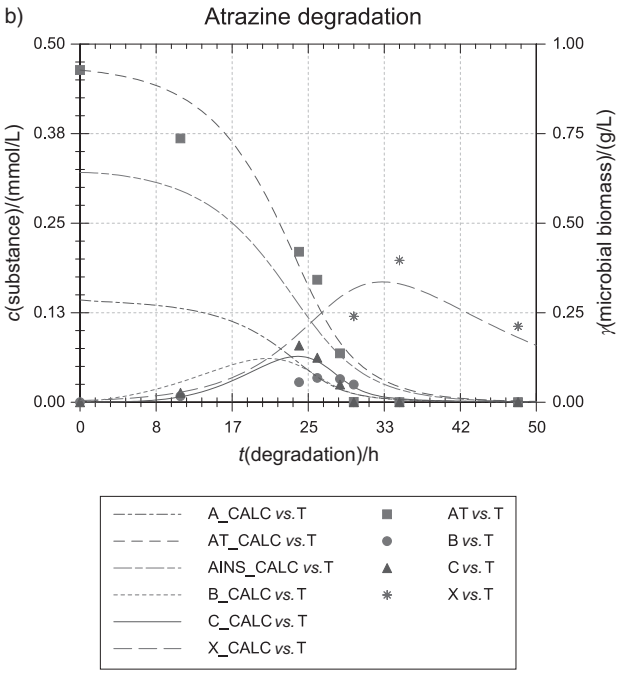




**Fig. 5.** Application of mathematical model MAM3 in describing atrazine biodegradation kinetics, and agreement of computer simulation with experimental data for simulation parameters (a,b) specified in Table 1. Points: experimental data; curves: simulated data, applied biodegradation temperature $T$/K=303

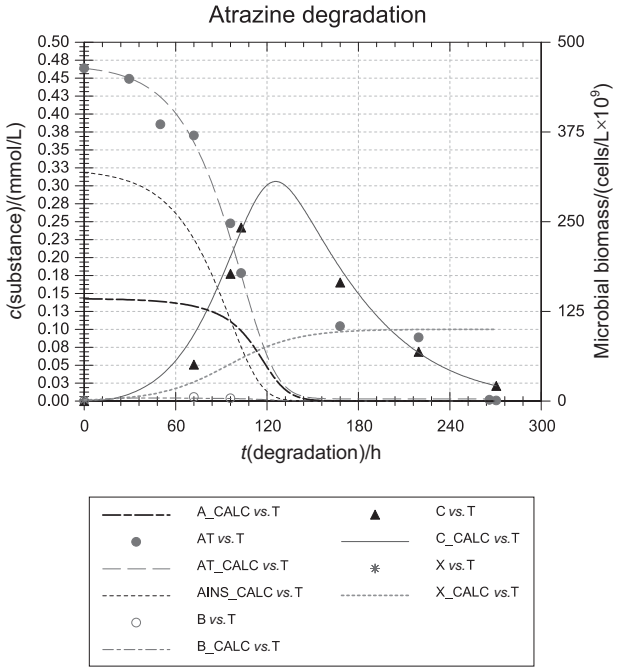



**Fig. 6.** Application of mathematical model MAM3 in describing atrazine biodegradation kinetics, and agreement of computer simulation with experimental data for simulation parameters specified in Table 1. Points: experimental data, curves: simulated data; applied biodegradation temperature $T$/K=283

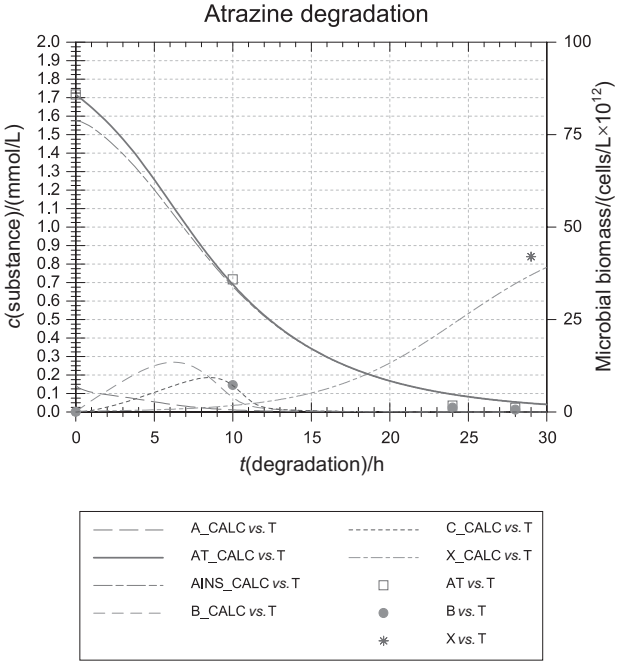



**Fig. 7.** Application of mathematical model MAM3 for description of atrazine removal from the wet soil contaminated with atrazine, and fitting computer simulation to experimental data for simulation parameters specified in Table 1. Points: experimental data, curves: simulated data; applied biodegradation temperature $T$/K=303

netics. One of the possibilities is the transformation of MAM3 by modifying Eqs. 10–12 and by adding the equation expressing the kinetics of atrazine sorption and desorption. The new model, MAM4, can also be tested for its applicability with reference to experimental data of this work. Chosen examples of computer simulations are shown in Fig. 10, which consists of four diagrams. Presented data explain well the relevance of sorption-desorption phenomena and the applied inoculum quantities for atrazine biodegradation efficiency. Data also suggest

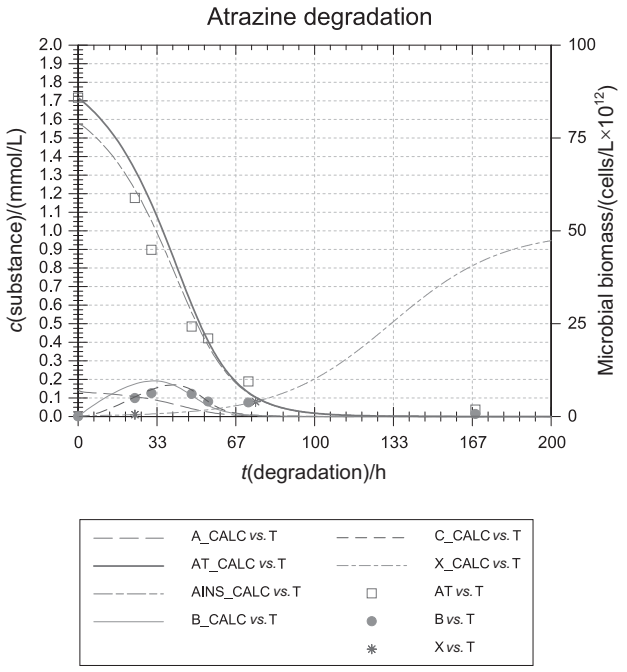



**Fig. 8.** Application of mathematical model MAM3 for description of atrazine removal from the soil contaminated with atrazine, and fitting computer simulation to experimental data for simulation parameters specified in Table 1. Points: experimental data, curves: simulated data; applied biodegradation temperature $T$/K=283

Table 2. Agreement of computer simulation with experimental data evaluated through the application of Jacobian matrix. Calculated values for data sets refer to the presented figures

| Data set referring to figure: | Correlation | Determination coefficient | Model selection criterion |
|---|---|---|---|
| 1a | 0.992911110 | 0.983743322 | 3.73829909 |
| 1b | 0.995879887 | 0.990581313 | 3.99839293 |
| 2 | 0.991665879 | 0.977348241 | 1.95418443 |
| 3 | 0.999926563 | 0.999831791 | 6.69030284 |
| 4a | 0.999998851 | 0.999996436 | 9.79457653 |
| 4b | 0.999997376 | 0.999993221 | 9.15166817 |
| 4c | 0.999999053 | 0.999997212 | 10.0402509 |
| 5a | 1.0000 | 0.999682868 | 7.17619325 |
| 5b | 0.976993968 | 0.953076535 | 2.09627444 |
| 6 | 1.0000 | 1.0000 | 44.8208048 |
| 7 | 0.999995944 | 0.984289663 | 1.95343639 |
| 8 | 0.989976731 | 0.966118367 | 1.81345363 |
| 9a | 0.995577867 | 0.988252516 | 3.44411622 |
| 9b | 0.990292771 | 0.978580451 | 3.27202271 |
| 10a | 0.974996018 | 0.898684957 | 0.432377519 |
| 10b | 0.991432169 | 0.97266063 | 1.74248338 |
| 10d | 0.999995944 | 0.984289663 | 1.55343639 |

that the decision whether to apply smaller or larger inoculums depends on whether the soil needs to be protected for selected agriculture or it needs to be efficiently detoxified. Also, soil properties (amounts and properties of sorbents in the soil reflect on $k_{dim}$ and $k_{im}$ values) could markedly influence kinetics of atrazine removal from the contaminated soil. This observation is in accordance with literature data (*4*,*10*–*14*).

The extended mathematical model MAM3 could also be improved by modifying Eqs. 4 and 9. One of the possibilities is the addition of the term which expresses kinetics of viable biomass decay (Eq. 8, Fig. 5). There are also other possibilities of improving the fitting of computer simulation to the experimental data. Models as those adapted for cluster analysis application are recommended (*23*). Also, more sophisticated approaches based on the structure of mixed microbial culture and the roles of individual members of the same culture can be suggested for consideration (*18*–*20*) prior to defining the improved models.

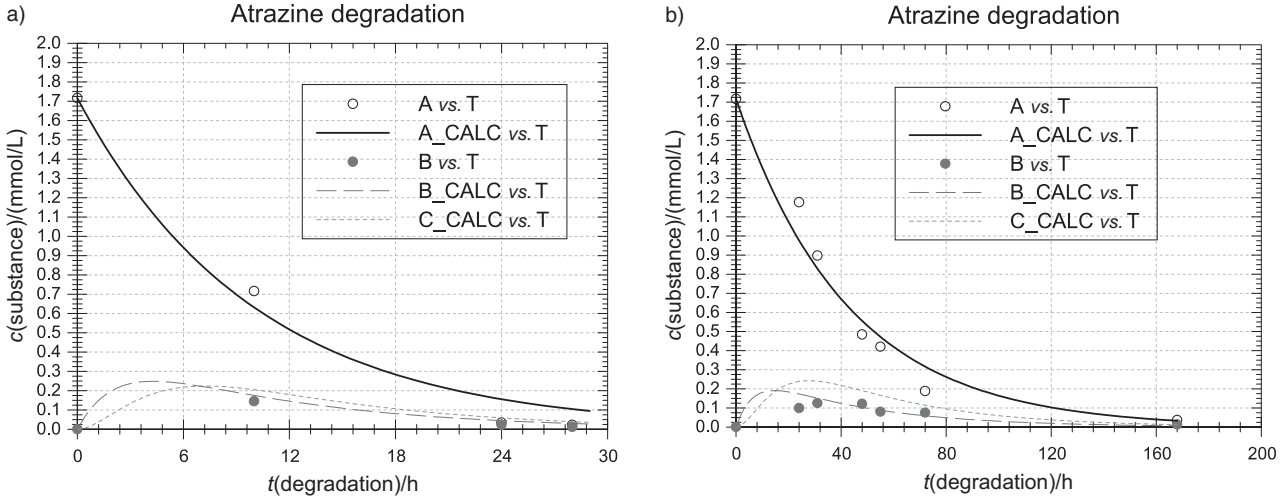



**Fig. 9.** Application of the simple mathematical model MAM1 for description of atrazine removal from the soil contaminated with atrazine, and fitting computer simulation to experimental data for applied simulation parameters specified in Table 1. Points: experimental data referring to Fig. 7 (a) and Fig. 8 (b), curves: simulated data

Based on the comparison of the data presented in Figs. 5–10, it can be concluded that biodegradation temperature had a strong influence on biodegradation kinetics. The results are in accordance with theoretical expectation. Comparing Figs. 5 and 6, it can be observed that kinetics of atrazine biodegradation was roughly 4 to 5 times faster at 30 than at 10 °C. Faster biodegradation rate was mainly a consequence of much faster biomass growth at 30 °C (about 5 times) than at 10 °C. Since in the later process phases the kinetics of particular events was not analogous, it can be concluded that the mixed microbial cultures differed in their physiology. Probable cause of such differences could be inadequate estimations of growth kinetics and/or culture differences with respect to the participation of particular microbial population members present in the mixed microbial cultures at later process phases. The differences between the two microbial cultures were much less expressed when they were applied as inoculums for contaminated soil (Figs. 7 and 8). Therefore, the mean rates of atrazine removal from contaminated soil differed, as expected, more than four times (Fig. 9). As it is already known (*24*), the application of different cultivation temperatures can lead to mixed microbial cultures with different properties, because the temperature affects differently particular members of a mixed microbial culture.

Special advantage of the present work is the use of mixed microbial culture capable of degrading atrazine even when it is present in the media with relatively high atrazine concentrations (*5*). Data shown in Figs. 3 and 4 testify that much higher biodegradation efficiency resulted in cases when higher atrazine quantities were present. Further series of experiments should be designed with an aim to give more data on particular processes (especially those referring to microbial biomass concentration and structure, oxygen availability and mass transfer) in order to confirm the applicability of mathematical models used in this work. Reliable predictions of the consequences of different atrazine applications in protecting different types of soil could be expected as desired benefit. Therefore, there is need for more detailed studies of atrazine biodegradation kinetics in contaminated soil. The experimental data of this work enable estimation of some parameters which could serve as a tool for useful predictions.

Based on the data shown in Figs. 5–9, activation energy for particular biodegradation can be calculated. Also, relationships relevant for the prediction of consequences of different applications referring to mixed microbial cultures and atrazine quantities can be found. The estimated values of activation energies referring to different events are presented in Table 3, which gives evidence that the highest values of activation energy refer to microbial biomass growth. When the sums of activation energies referring to degradations of atrazine, hydroxyatrazine and cyanuric acid are compared, differences can be observed. These are a consequence of the application of different mathematical models as a basis for activation energy calculations. This observation, together with the finding that the highest activation energy refers to the mixed microbial biomass growth, suggests that the biocatalytic activity of microbial biomass is the most decisive factor for atrazine biodegradation efficiency.

When the effects of temperature on atrazine biodegradation rates are compared, the half-life times could be considered as good criterion. Computer simulation data demonstrated in Fig. 9 appear to be convenient for half-life time calculations. The estimated values $(t_{1/2})_{30}$= 6.93 h and $(t_{1/2})_{10}$=29.50 h indicate that atrazine biodegradation at 30 °C was 4.26 times more efficient than that at 10 °C. Such a ratio between half-life times suggests that estimations of activation energies and half-life times refer to the suboptimal temperature range of biodegradation process conditions.

On the basis of previous discussion, the applied culture media and cultivation conditions can be validated. Cultivation media influenced biodegradation kinetics depending on carbon and nitrogen sources (Fig. 1), as expected on the basis of the already published data (*25*). Applied biodegradation conditions possibly differed with respect to oxygen availability and mass transfer rates (especially if comparing shaken liquid cultures with inoculated soil), and such differences reflected on the biodegradation kinetics. The applied values of computer

Table 3. Activation energies of atrazine biodegradation present in the contaminated soil. Calculation based on computer simulation data fitted well to the experimental data of atrazine biodegradation at 10 and 30 °C

| Reaction system | Temperature range | Activation energy/ ($10^3$ J/mol) | Referent kinetic parameter | Fig. |
|---|---|---|---|---|
| Soil samples contaminated with atrazine, and inoculated with mixed microbial culture | 283 to 303 K | 60.260 | specific growth rate, $\mu$ | 7 and 8 |
| | | 55.133 | atrazine (A) degr. rate const., $k_{AB}$ | |
| | | 26.193 | hydroxyatrazine (B) degr. rate const., $k_{BC}$ | |
| | | 26.193 | cyanuric acid (C) degr. rate const., $k_{CD}$ | |
| | | 51.675 | atrazine (A) degr. rate const., $k_{AB}$ | 9 |
| | | 39.206 | hydroxyatrazine (B) degr. rate const., $k_{BC}$ | |
| | | 52.874 | cyanuric acid (C) degr. rate const., $k_{CD}$ | |
| Microbial culture in liquid medium containing atrazine | 283 to 303 K | 56.957 | specific growth rate, $\mu$ | 5 and 6 |

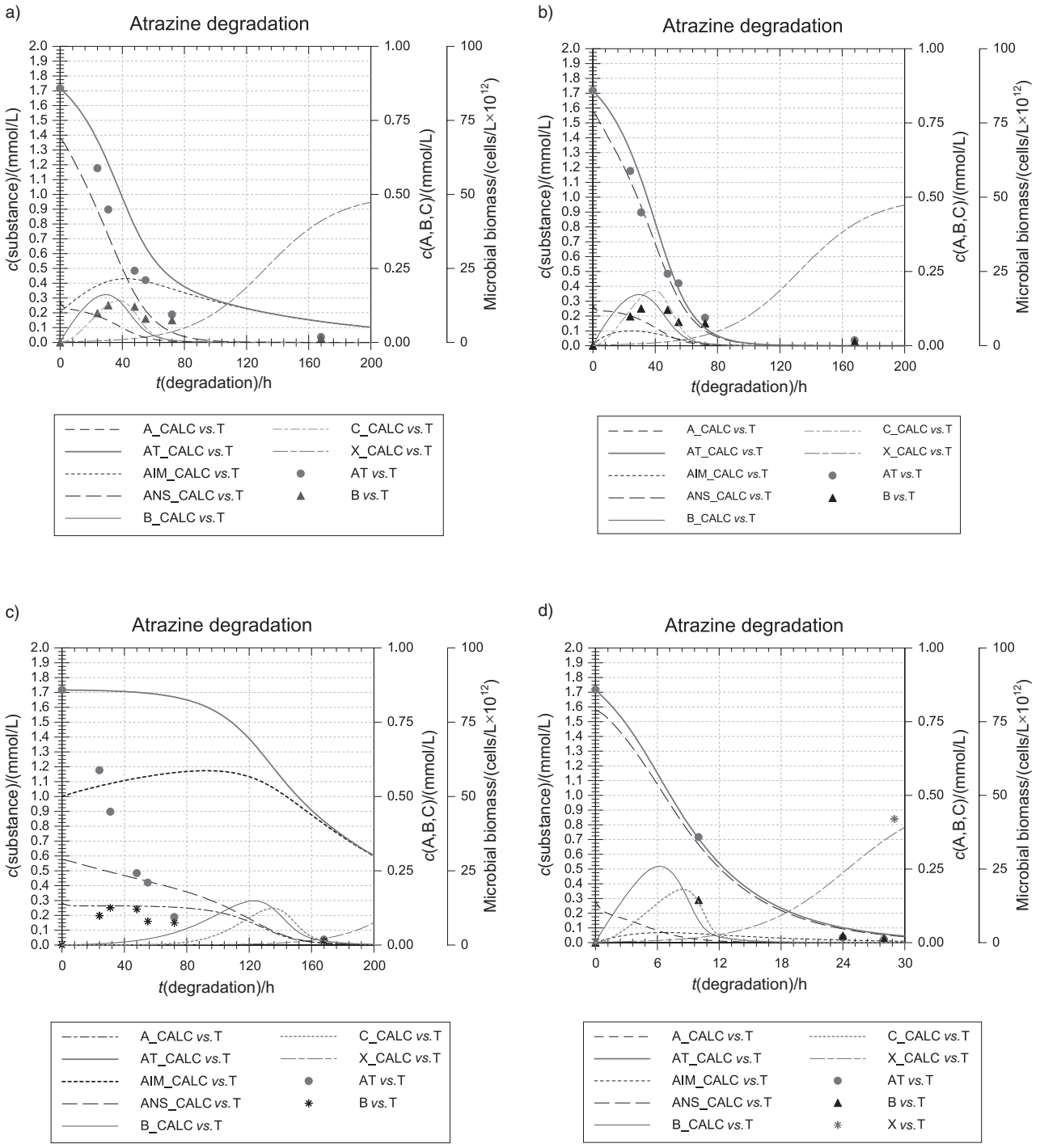



**Fig. 10.** Application of extended mathematical model MAM4 for description of atrazine removal from wet soil as a function of applied microbial culture inoculums quantity and soil properties referring to atrazine sorption-desorption phenomena. Fitting of computer simulation to experimental data for applied simulation parameters specified in Table 1. Points: experimental data referring to Fig. 8 (a,b,c) and Fig. 7 (d), curves: simulated data

simulation parameters resulted as a consequence of both the applied mathematical models and experimental conditions.

Finally, the results of this work and the applied approach in explaining them are in accordance with findings of Soulas (*1*) and Soulas and Lagacherie (*3*).

## Conclusion

General conclusion which can be drawn from the results of mathematical models presented in this work combined with the applied methods of evaluation of experimental and computer simulation data suggests

that the integrated approach to methematical modelling of biodegradation kinetics of atrazine and other pesticides can be recommended for application in further studies.

## Symbols

| | |
|---|---|
| A | atrazine |
| B | hydroxyatrazine |
| C | cyanuric acid |
| D | degradation products $CO_2$ and $NH_4^+$ |
| $c_A$, $c_B$, $c_C$/(mmol/L) | concentrations of reaction substances A, B and C |
| $c_{Aim}$/(mmol/L) | concentration of immobilised (sorbed) substance A (atrazine) |
| $c_{Aim0}$/(mmol/L) | initial $c_{Aim}$ |
| $c_{Am}$/(mmol/L) | substance A (atrazine) solubility |
| $c_{Ains}$/(mmol/L) | concentration of undissolved substance A (atrazine) |
| $c_{Ans}$/(mmol/L) | net concentration of undissolved substance A (atrazine) |
| $c_{Ans0}$/(mmol/L) | initial $c_{Ans}$ |
| $c_{AT}$/(mmol/L) | total substance A (atrazine) concentration |
| d | mathematical derivation operation |
| $E_a$/(J/mol) | activation energy |
| $k_{AB}$, $k_{BC}$, $k_{CD}$/$h^{-1}$ | reaction rate constants with reference to the conversion of A into B, B into C, and C into D |
| $k_{ABN}$, $k_{BCN}$, $k_{CDN}$/(mmol/h) | reaction rate constants with reference to concentration of microbial cells and conversions of A into B, B into C, and C into D |
| $k_{ABX}$, $k_{BCX}$, $k_{CDX}$/(mmol/(g·h)) | reaction rate constants with reference to concentration of microbial biomass and conversion of A into B, B into C, and C into D |
| $k_{AB*}$, $k_{BC*}$, $k_{CD*}$/(L/(g·h)) | reaction rate constants with reference to the conversions of A into B, B into C, and C into D catalysed with constant biomass concentration |
| $k_d$/$h^{-1}$ | specific biomass decay rate |
| $k_{dim}$/$h^{-1}$ | rate constant with reference to immobilised atrazine desorption |
| $k_{im}$/$h^{-1}$ | rate constant with reference to immobilised atrazine sorption |
| $k_{sol}$/(L/(mmol·h)) | specific rate of undissolved substance A (atrazine) dissolving |
| $K_A$, $K_B$, $K_C$/(mmol/L) | biocatalytic constants referring to substances A, B and C |
| $K_{ag}$/(mmol/L) | biocatalytic constant of biomass growth with reference to substance A |
| $N_x$/$L^{-1}$ | concentration of mixed microbial population cells |
| $N_{x0}$/$L^{-1}$ | initial $N_x$ |
| $N_{xm}$/$L^{-1}$ | theoretically maximal concentration of mixed microbial population cells |
| $T$/K | absolute temperature |

*Greek letters*

| | |
|---|---|
| $\gamma_x$ /(g/L) | microbial biomass concentration |
| $\gamma_{xm}$/(g/L) | theoretical maximum of microbial biomass concentration |
| $\mu_{Nm}$/$h^{-1}$ | maximal specific rate of microbial population cell number increase |
| $\mu_{xm}$/$h^{-1}$ | maximal specific rate of microbial biomass growth |

*Abbreviated computer simulation symbols*

A=atrazine, AIM=sorbed (immobilized) atrazine, AINS and ANS=undissolved atrazine, AT=total atrazine, B=hydroxyatrazine, C=cyanuric acid, X=microbial biomass

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## References

1. G. Soulas: Modeling of Biodegradation of Pesticides in the Soil. In: *Soil Ecotoxicology*, J. Tarradellas, G. Bitton, D. Rossel (Eds.), CRC Press, Boca Raton, FL, USA (1997) pp. 117–138.
2. G.D. Hill, J.W. McGahen, H.M. Baker, D.W. Finnerty, C.W. Bingeman, The fate of substituted urea herbicides in agricultural soils, *Agron. J. 47* (1955) 93–104.
3. G. Soulas, B. Lagacherie, Modelling of microbial degradation of pesticides in soils, *Biol. Fertil. Soils, 33* (2001) 551–557.
4. J.H. Park, Y. Feng, P. Ji, T.C. Voice, S.A. Boyd, Assessment of bioavailability of soil-sorbed atrazine, *Appl. Environ. Microbiol. 69* (2003) 3288–3298.
5. D. Hršak, M. Havriluk, Mixed bacterial culture for atrazine degradation. *US patent* 0026135 (2009).
6. N. Udiković Kolić, D. Hršak, A. Begonja Kolar, I. Petrić, S. Stipičević, G. Soulas, F. Martin-Laurent, Combined metabolic activity within an atrazine-mineralizing community enriched from agrochemical factory soil, *Int. Biodeter. Biodegr. 60* (2007) 299–307.
7. N. Udiković, D. Hršak, G. Mendaš, D. Filipčić, Enrichment and characterization of atrazine degrading bacterial communities, *Food Technol. Biotechnol. 41* (2003) 211–217.
8. N. Udiković Kolić, F. Martin-Laurent, M. Devers, I. Petrić, A. Begonja Kolar, D. Hršak, Genetic potential, diversity and activity of an atrazine-degrading community enriched from a herbicide factory effluent, *J. Appl. Microbiol. 105* (2008) 1334–1343.
9. N. Udiković-Kolić, D. Hršak, M. Devers, V. Klepac-Ceraj, I. Petrić, F. Martin-Laurent, Taxonomic and functional diversity of atrazine-degrading bacterial communities en-

riched from agrochemical factory soil, *J. Appl. Microbiol. 109* (2010) 355–367.

10. I.S. Fomsgaard, The influence of sorption on the degradation of pesticides and other chemicals in soil, *Environmental project No. 902*, Danish Environmental Protection Agency, Denmark (2004).
11. E. Barriuso, W. Koskinen, B. Sorenson, Modification of atrazine desorption during field incubation experiments, *Sci. Total Environ. 123/124* (1992) 333–344.
12. L. Guo, W.A. Jury, R.J. Wagenet, M. Flury, Dependence of pesticide degradation on sorption: Nonequilibrium model and application to soil reactors, *J. Contam. Hydrol. 43* (2000) 45–62.
13. L. Ma, H.M. Selim, Predicting atrazine adsorption-desorption in soils: A modified second order kinetic model, *Water Resour. Res. 30* (1994) 447–456.
14. R.D. Wauchope, R.S. Myers, Adsorption-desorption kinetics of atrazine and linuron in freshwater-sediment aqueous slurries, *J. Environ. Qual. 14* (1985) 132–136.
15. M. Wenk, T. Baumgartner, J. Dobovšek, T. Fuchs, J. Kucsera, J. Zopfi, G. Stucki, Rapid atrazine mineralisation in soil slurry and moist soil by inoculation of an atrazine-degrading *Pseudomonas* sp. strain, *Appl. Microbiol. Biotechnol. 49* (1998) 624–630.
16. S.J. Goux, M. Ibanez, M. Van Hoorick, P. Debongnie, S.N. Aghatos, L. Pussemier, Biodegradation of atrazine in sand sediments and in a sand-filter, *Appl. Microbiol. Biotechnol. 54* (2000) 589–596.
17. M. Bošnjak, A. Bago Joksović, J. Pigac, Ž. Bošnjak Cihlar, D. Hranueli, Applicability of mathematical models in defining the behaviour kinetics distinction among microbial strains, *Chem. Biochem. Eng. Q. 20* (2006) 375–388.
18. M. Bošnjak, S. Bogdan, Introduction to the theoretical fundamentals for increasing the probability of heterologous contacts between biological particles, *Food Technol. Biotechnol. 39* (2001) 183–196.
19. M. Bošnjak, S. Bogdan, Mathematical modelling of process relationships in the mixed microbial culture with probable new product formation, *Annual Croat. Acad. Eng. 2002* (2002) 15–21.
20. M. Bošnjak: *Introduction to the Kinetics of Microbial Processes*, Graphis, Zagreb, Croatia (2009) (in Croatian).
21. K. Mihaljević, M. Bošnjak, Defining of the kinetics of microbial oxidation process events with reference to L-sorbose formation in a large range of culture conditions, *Chem. Biochem. Eng. Q. 23* (2009) 239–250.
22. R.T. Mandelbaum, L.P. Wackett, D.L. Allan, Mineralization of the *s*-triazine ring of atrazine by stable bacterial mixed cultures, *Appl. Environ. Microbiol. 59* (1993) 1695–1701.
23. V. Topolovec, M. Bošnjak, Cluster analysis and simulation of microbial population differentiation during the process of antibiotic biosynthesis *Proceedings of the 6th International Symposium* 'Computers at the University', Dubrovnik, 1984, 627.1–627.9, SRCE, Zagreb, Croatia (1984) (in Croatian).
24. D. Hršak, M. Bošnjak, V. Johanides, Temperature effects on growth kinetics of linear alkyl-benzene sulphonate (LAS) degrading bacteria in continuous culture, *Proceedings of the World Surfactant Congress*, Munich, Germany (1984) pp. 249–261.
25. R. Abdelhafid, S. Houot, E. Barriuoso, Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils, *Soil Biol. Biochem. 32* (2000) 389–401.