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SCIENTIFIC PAPER

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MODELING OF AROMATIC COMPOUND DEGRADATION BY *Pseudomonas putida* ATCC 21812

Aniline degradation by Pseudomonas putida intact cells in a stirred batch reactor was studied. Since such kinetic data are of significant importance in view of the strain used in large-scale bioreactors, the biodegradation reaction was considered thoroughly. A mathematical model of the process is proposed. The model consists of differential equations describing the time-course of biomass growth and simultaneous aniline utilization, as well as the initial instantaneous substrate adsorption by the cell.

The kinetic parameters were determined by using an adapted numerical procedure. In this procedure, the yield coefficient is considered as a variable. The model proposed is adequate to describe the experimental results delivered both from the study and from the literature. The model was compared to several reference models from the literature concerning biodegradation reactions. Bearing in mind that no other model from the literature describes the initial phase of substrate degradation, this specific part of the model proposed is significant for control of the exponential phase of fermentation.

Key words: Aniline, Pseudomonas Putida, Model.

Aromatic containing compounds such as phenol and aniline occur widely in a variety of process streams. These species can have serious consequences when released in the environment, due to possible health effects for many organisms including humans. Before being discharged environmentally, aniline and phenol containing industrial effluents require proper previous treatment.

Conventional methods such as solvent extraction, activated carbon adsorption, and chemical oxidation often suffer from serious drawbacks including high costs and the formation of hazardous by-products. On the other hand, recently there has been a pronounced interest in phenol biodegradation [1,2]. Wastewaters containing aniline in the range of 5–2000 mg/l are also considered suitable for treatment by biological processes [3].

Aniline degradation by *Pseudomonas putida* ATCC 21812 intact cells in a stirred batch reactor was studied in the present study. Since such kinetic data are of significant importance in view of the strain used in large-scale bioreactors e.g. to determine the specific operation time and the reactor design, the biodegradation reaction was considered thoroughly. A mathematical model of the process is proposed. The model consists of differential equations describing the time-course of biomass (X) growth and simultaneous aniline utilization (S). The model was compared to several reference models from the literature concerning

biodegradation reactions. The aim of this study was to describe more precisely the initial phase of substrate degradation, which is significant for control of the exponential phase and to propose an integrated model. The model consists of differential equations describing the time-course of biomass growth and simultaneous aniline utilization. The model was compared to several reference models from the literature concerning biodegradation reactions.

MATERIALS AND METHOD

The experiments were carried out in a 1 liter stirred tank (working volume of 0.5 liters) with a propeller mixer. The temperature was maintained at 30°C. The reactor was equipped with an oxygen probe to ensure that the limiting substrate was aniline but not oxygen. The reactor was charged with sterile nutrient containing aniline, and aseptic conditions were observed throughout all the experiments. The salt composition of the medium contained: KH₂PO₄ 0.42 g/l, K₂HPO₄ 0.36 g/l, (NH₄)₂SO₄ 0.34g/l, Na₂SO₄ 0.50 g/l and CaCl₂·2H₂O 0.10 g/l.

At equal time intervals, the culture broth was sampled for aniline and the bacterial cell concentration of *Pseudomonas putida* ATCC 21812 was measured as dry weight biomass per liter. A sample (volume 5 ml) was dried during 48 hours and weighed with high precision.

Aniline was mainly determined by using a diazo coupling reaction. A 1 ml sample was incubated with 2 ml of 1N HCl and 0.4% NaNO₂ for 3 min at room temperature. Excess NaNO₂ was decomposed with 1 ml of 2% ammonium sulfamate. After 2 min, 1 ml of 0.6% N-naphthylethylenediamine dihydrochloride in 95% (v/v) ethanol was added with mixing. The absorbance was measured at 560 nm at 15 to 180 min later. The respective aniline concentrations were read in the range

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of zero to 20 μM . Aniline was also determined by measuring the absorbance at 230 and 280 nm where aniline has absorption peaks at neutral pH.

MATHEMATICAL MODEL

Let us recall the different mass balance equations:

$$\text{Biomass } \frac{dX}{dt} = \mu X \quad (1)$$

$$\text{Substrate } \frac{dS}{dt} = -\frac{\mu X}{Y} \quad (2)$$

Kinetic model from the literature

In the literature, some models exist to represent the specific growth rate. We tested some of these to determine their efficiencies. In Table 1 we show that any model correctly represents the experimental points. The used models have different specifications, for example the Monod model does not take into account the

Table 1. Maximum deviation error for different models found in the literature for microbial growth

Model name	μ	F value	Maximum deviation in biomass (%)	Maximum deviation in substrate (%)
Haldane	$\frac{\mu_m S}{K_S + S + S^2/K_I}$	721031	38	18
Monod	$\frac{\mu_m S}{K_S + S}$	152923	18	93
Teissier	$\mu_m S \left(1 - \exp\left(-\frac{S}{K_S}\right) \right)$	882935	32	98
Moser	$\frac{\mu_m S''}{K_S + S''}$	101948	9	23
Contois	$\frac{\mu_m S}{K_S B + S}$	437500	29	97

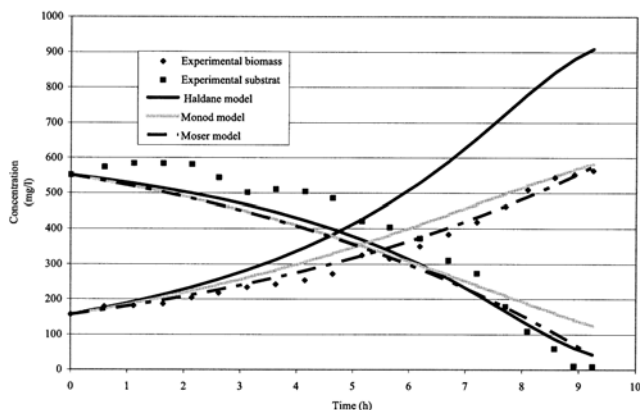


Figure 1. Representation of biomass and aniline profiles by literature models

substrate inhibitor effect, whereas the Haldane model takes it into account [4–6].

In Figure 1, the experimental points are realized at the operating conditions described in the paragraph "Materials and methods". Moreover, in this figure, we represent the typical model used for biodegradation, namely, the Haldane, Monod and Moser models. In fact, the Haldane model does not represent the experimental biomass evolution in particular if the biomass concentration is high. Whereas, the Monod model correctly represents the biomass evolution but it has a big difference between the substrate experimental and modeling points, in particular at the end of biodegradation. Moreover, this deviation exists with weak substrate concentration so, the quadratic deviation is low whereas the maximum deviation is too high. The Moser model represents at the same time biomass and substrate evolution. However, any model represents the start of the evolution of substrate biodegradation. Knowledge of this part, of the process is very interesting to control the exponential phase.

New kinetic models

In this part, the following assumptions were made: in the first period, the cell adsorbs a lot of substrate, and in the second period, the cell has a lot of substrate in the membrane, so the inverse phenomenon takes place. But, it is not instantaneous, it depends on the time.

With the objective to more precisely describe the initial phase of substrate elimination, two new models were tested:

$$\begin{cases} \frac{dX}{dt} = \mu X \\ \frac{dS}{dt} = -\frac{\mu X}{Y} + k(X - X_m) \end{cases} \quad (3)$$

and

$$\begin{cases} \frac{dX}{dt} = \mu X \\ \frac{dS}{dt} = -\frac{\mu X}{Y} + (X - X_m)^{n_d} \end{cases} \quad (4)$$

Equation (3) represents biodegradation by the so called desorption model 1 and Equation (4) by the so called desorption model 2. It is important to remark that the yield coefficient is a model parameter. It is not possible to determine it in any other way because aniline degradation corresponds to desorption and to biomass growth.

RESULTS AND DISCUSSION

In order to describe substrate biodegradation, it is necessary to evaluate the relationship between the specific growth rate, μ , and the substrate concentration S .

Two typical models are used, i.e. the Haldane equation

$$\mu = \frac{\mu_m S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \tag{5}$$

and the Monod equation:

$$\mu = \frac{\mu_m S}{K_s + S} \tag{6}$$

For solving this problem, the first assumption is to take the yield coefficient as a constant. The integration of equations (1) and (2) in both cases, i.e. the Haldane and Monod models, gives the following solutions:

$$X = X_0 \exp(\mu t) \tag{7}$$

$$t = -\frac{1}{\frac{\mu_m X_0}{K_s Y} + S_0 \frac{\mu_m}{K_s}} \ln\left(\frac{S}{S_0}\right) + \left(\frac{1}{\frac{\mu_m X_0}{K_s Y} + S_0 \frac{\mu_m}{K_s}} + \frac{1}{\mu_m} + \frac{B_0}{\mu_m K_i} + \frac{S_0}{\mu_m K_i}\right) \cdot \ln\left(1 + \frac{S_0 - S}{\frac{B_0}{Y}}\right) + \frac{S - S_0}{\mu_m K_i} \tag{8}$$

$$t = -\frac{1}{\frac{\mu_m X_0}{K_s Y} + S_0 \frac{\mu_m}{K_s}} \ln\left(\frac{S}{S_0}\right) + \left(\frac{1}{\frac{\mu_m X_0}{K_s Y} + S_0 \frac{\mu_m}{K_s}} + \frac{1}{\mu_m}\right) \ln\left(1 + \frac{S_0 - S}{\frac{B_0}{Y}}\right) \tag{9}$$

Consequently, for the two solutions, i.e. with the Haldane and Monod models (equations 8 and 9), it is impossible to have the function $S=f(t)$. Since biomass evolution is a function of the substrate concentration and time, we used a different method, i.e. we modified, and extended the method, as follows:

In the first step: the value of the yield coefficient using the biomass and substrate evolution in the exponential phase was determined.

In the second step: the values of the specific constants (μ_m , K_s , and K_i) of this stage were parameterized, as to minimize the quadratic difference between the experimental and modeling points for the substrate concentration. Referring to the literature, some authors stopped at this stage, whereas few authors continued the study. They realized the modeling biomass evolution as a reflection of the substrate curve [2,7 and 8]. The symmetric axis is based on the yield coefficient. Thus, the assumption realized was:

$$Y = \frac{\Delta S}{\Delta X} = \frac{S_t - S_0}{X_0 - X_t} \tag{10}$$

But, in reality, the yield coefficient is equal to. So, it is impossible to deduce the biomass curve with a symmetric point. The biomass curve and the substrate curve are different. According to this method, some

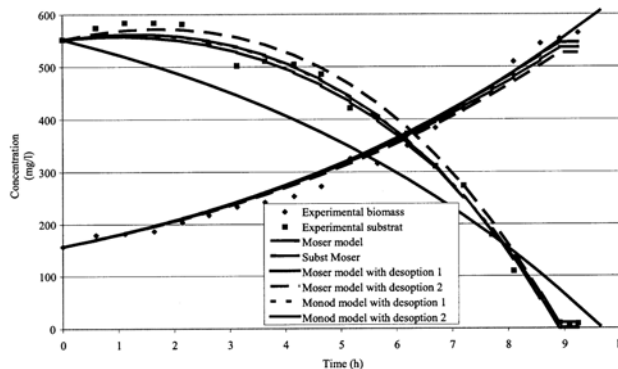


Figure 2. Representation of biomass and aniline profiles by the modified models

disadvantages exist. In particular, the biomass experimental curve was not used.

In this paper we present an adapted procedure based on the simultaneous use of the two evolutions (substrate and biomass). The goal was to solve the differential system of equations simultaneously using a numeric differential solver.

The parameterization method is based on two levels for the solution. The first level is a master program and the second level is a slave program. The slave program represents the kinetics, so it is based on the Runge-Kutta Method. The master program is an optimization tool, in this case, Quadratic Programming was used. The F criterion on the optimization tool is as follows:

$$F = \min (\Sigma(S_{exp} - S_{mod})^2 + \Sigma(X_{exp} - X_{mod})^2) \tag{11}$$

The yield coefficient is considered as a parameter in this procedure.

The model in Figure 2 represents the experimental points for the biomass and substrate concentrations.

The aniline biodegradation experimental evolution could be described according to three phases. In the first phase, the aniline concentration increases and returns to the initial point. In the second phase, a high diminution is observed. The last phase is non-evolution of the biomass and the substrate concentration.

Moreover, it is important to notice that when the aniline concentration varies that the concentration of

Table 2. Maximum deviation error for the proposed models of microbial growth

Model name	Desorption model	F value	Maximum deviation in biomass (%)	Maximum deviation in substrate (%)
Moser	1	12351	5.9	5.6
Moser	2	18751	7.6	9.8
Monod	1	10933	9.1	6.5
Monod	2	10727	8.9	6.7

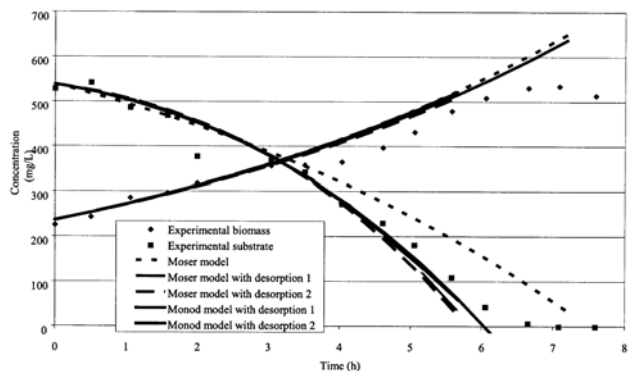


Figure 3. Validation of the proposed models for lower aniline concentrations

Table 3. Parameter values obtained by Quadratic Programming optimization (QP) with the Moser model

Desorption model	μ_m (h ⁻¹)	n	K _S (mg/L)	Y	X _m (mg/L)	Remarks
1	0.138	9	2.04	0.395	677.7	k=0.126
2	0.136	8.8	2.04	0.344	558.1	n _d =0.741

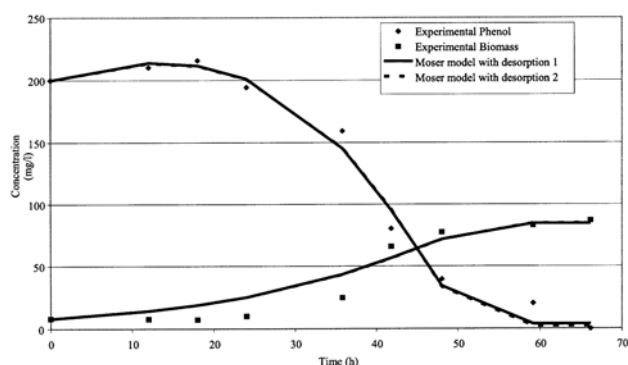


Figure 4. Representation of Kotturi's data (1991) by the proposed models

biomass does not increase. One obtains a modelled curve which resembles the biomass curve and the evolution of aniline decomposition simultaneously. This was confirmed with the results obtained in Table 2. Moreover, one can conclude that the model which creates the minimum of error is the model of Moser combined with desorption model 1.

In order to validate the good adequacy of our models, a series of validation runs were realized (Figure 3). Initially, we used the experimental points of aniline decomposition to a lower initial concentration. Then, we found the experimental points of phenol degradation by *Pseudomonas Putida* in the literature. It is important to specify that the stocks used were not the same ones.

The various parameters obtained by minimizing the standard deviation between the experimental points and model points for a given time and the two evolutions for the biomass and aniline are described in Table 3.

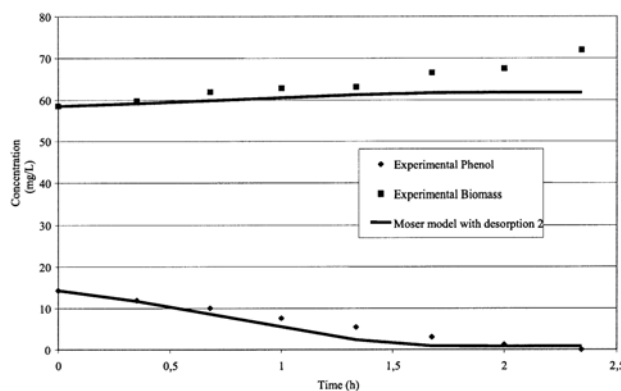


Figure 5. Validation of parameter models using the data of Kotturi et al. (1991)

Table 4. Parameter values obtained by QP optimization with the Moser model and Kotturi experimental points

Desorption model	μ_m (h ⁻¹)	n	K _S (mg/L)	Y	X _m (mg/L)	Remarks
1	5.2 10 ⁻²	0.97	8.5	0.18	983.58	k=4,1 10 ⁻³
2	5.1 10 ⁻²	1.15	8.5	0.17	980.60	n _d =0.21

Thus in Figure 4 we again took the same method described previously and the parameters of the various models were adjusted to the experimental points. The experimental points were those of Kotturi et al (1991) [9]. To validate this example, we used only the Moser model with the two models of desorption. Table 4 contains the indexed values of the various parameters. The results observed are very correct. Moreover, using the parameters obtained, we checked that the model obtained corresponded to the experimental points for a weaker concentration. Figure 5 illustrates the good adequacy between the experimental points and the values of the model.

To have even more certainty of our method, points of (Chung and Al, 2003) [1] were modelled according to the same approach as previously. We chose to represent only one figure. Thus, in Figure 6, the modelled curves and the experimental points can be visualized. One notes the same adequacy between the modelled curves and the experimental points.

CONCLUSIONS

The kinetics of aniline degradation by *Pseudomonas putida* ATCC 21812 intact cells in a stirred batch reactor were investigated.

A mathematical model of the process of aniline elimination by *Pseudomonas putida* ATCC 21812 is proposed. The model consists of differential equations describing the time-course of biomass growth and simultaneous aniline utilization, as well as the aniline initial adsorption by the cells.

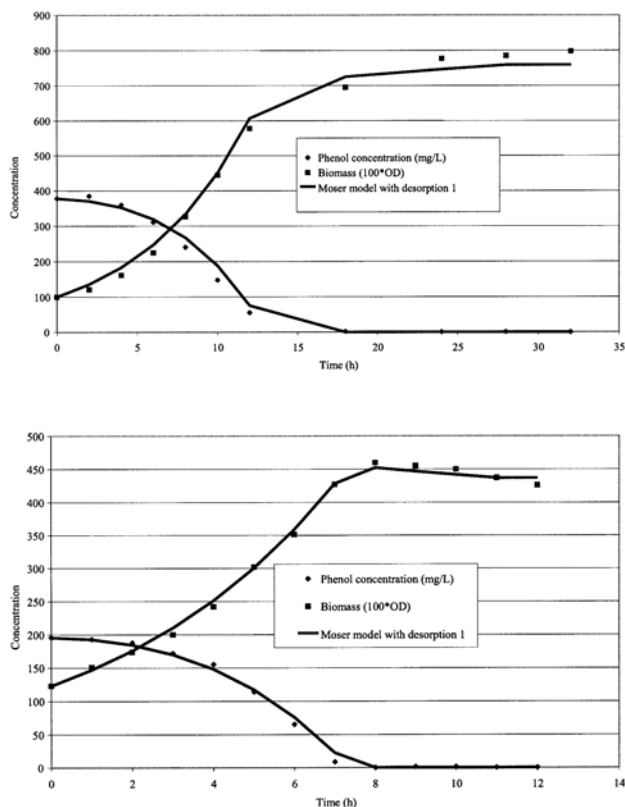


Figure 6. Validation of data of Chung et al., 2003 using model 1

The two modifications proposed are adequate to describe the experimental results from both this study and from the literature.

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NOMENCLATURE

X – biomass concentration (mg/l or OD)

X_0 – initial biomass concentration (mg/l or OD)
 S – substrate concentration (mg/l)
 S_0 – initial substrate concentration (mg/l)
 μ – specific growth rate (h^{-1})
 μ_m – maximum specific growth rate (h^{-1})
 K_S – Haldane's growth kinetics inhibition coefficient (mg/l or OD)
 K_I – half saturation coefficient (mg/l or OD)
 X_m – maximum biomass concentration (mg/l or OD)
 k – kinetic desorption parameter (h^{-1})
 n_d – exponential factor in desorption model
 Y – yield coefficient
 t – time (h)

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IZVOD

BIOHEMIJSKA RAZGRADNJA AROMATSKIH JEDINJENJA KORIŠĆENJEM *Pseudomonas putida* ATCC 21812

(Naučni rad)

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U radu se analizira razgradnja anilina pomoću mikroorganizma *Pseudomonas putida* realizovana u šaržnom reaktoru budući da je poznavanje kinetike ove reakcije od značaja za projektovanje industrijskih bioreaktora. Razvijen je matematički model koji omogućava izračunavanje povećanja biomase i iskorišćenje supstrata (anilina) odnosno uticaj anilina na efikasnost delovanja mikroorganizma. Razvijeni model omogućio je definisanje kinetičkih parametara i prinosa biomase kao vremenski promenljivog parametra. Model je omogućio dobro opisivanje rezultata eksperimentalnih ispitivanja sprovedenih u ovom radu ali i onih koji su objavljeni u literaturi.

Ključne reči: Anilin, *Pseudomonas putida*, Model, Biohemijski proces.