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KINETICS OF PHENOL AND BENZOATE BIODEGRADATION IN STATIC CULTIVATION SYSTEM BY *Burkholderia cepacia* G4.

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ABSTRACT

Kinetics of phenol and benzoate degradation by *Burkholderia cepacia* G4 in classical batch mode of static culture were investigated over a wide range of initial substrate concentrations applied as single substrates. Due to better adaptation to changing conditions, faster degradation of both phenol and benzoate was observed when higher concentrations were used. The results show decrease of the biomass yield coefficient, $Y_{X/S}$, from 0.83 to 0.31 g g^{-1} when the initial phenol concentration was increased from 0.54 to 0.73 g L^{-1}, supporting the well known inhibitory effect of phenol. On the other hand, linear increasing of the yield coefficient was observed with increasing benzoate concentration. During all experiments, maximum specific substrate consumption rates, r_{Smax}, were reached to 0.27 g $g^{-1}h^{-1}$ for phenol and to 0.40 g $g^{-1}h^{-1}$ when benzoate was the sole source of carbon in the bioreactor. In the present study, minor inhibitory effect of benzoate was observed during their investigated concentration range.

To explain their degradation modelling, experimental data of both phenol and benzoate biodegradation were fitted using various kinetic models. The results demonstrated that the Yano and Koga equation gave the best fit, compared with the other models. It means that biodegradation of phenol and benzoate can be described by the same kinetic model. Based on the kinetic data, all experiments were not sensitive to change in the saturation constant, K_S; therefore, K_S-value was fixed at 0.042 and 0.068 g L^{-1} for phenol and benzoate, respectively. After that, r_{Smax} and the inhibition constant, K_i, parameters were re-fitted in all experiments. In general, the kinetic parameters of both phenol and benzoate degradation were influenced by the initial substrate and bacterial cell mass concentrations, in addition to adaptation with changing in the culture conditions.

INTRODUCTION

Organic pollutants are widespread compounds produced in relatively high concentrations in many agricultural and industrial activities. Pesticides play an important role in plant protection, but contaminate our environment, and efficient treatment methods are necessary to reduce their concentration in wastewater to acceptable levels. Metabolites of these compounds are, maybe, more toxic than the parent compound (Amoros *et al*. 2000). Biological treatment, therefore, is the most effective technique, because it has the potential to degrade these contaminates with innocuous end products and minimum secondary waste generation (Goudar *et al*. 2000). Phenol and benzoate are among compounds that result from agricultural and industrial effluents causing environmental pollution. The widespread use of both compounds in various applications has led to extensive soil and wastewater toxicity. Phenol is a toxic and hazardous substance even at low

concentrations (Babish and Davis, 1984 and Li and Humphrey, 1989). Biodegradation of phenol, therefore, has long been the subject of numerous investigations using a wide variety of microorganisms (Cho *et al*. 2000 and Léonard *et al*. 1999). Benzoate is considerably less toxic compound released into the environment through routine disposal in waste treatment facilities (Hamzah and Al-Baharna, 1994). Biodegradation of benzoate was also investigated by Hickey and Focht (1990) and Van der Woude *et al*. (1995).

Burkholderia cepacia G4 (formerly: *Pseudomonas cepacia* G4) is one of the most effective bacterial strains that have the ability to use a wide range of the environmental pollutants. This strain is capable of mineralizing several aromatic compounds, like phenols, toluol, cresols and benzoic acid, with relatively high conversion rates (Nelson *et al*. 1987). The degraded strain has also used for phenol degradation as a sole source of carbon via unstable steady states in continous culture by Schrö̈der *et al*. (1997). Degradation of phenol and benzoate was also studied in a fluidized-bed reactor using the same strain (Hecht *et al*. 2000). Phenol and benzoate are typically broken down via the *meta*-cleavage pathway (Farell and Quilty, 1999), and catechol is the main intermediate produced in this way. Induction of *meta*- cleavage was stated by Mörsen and Rehm (1990) for phenol and by Harayama *et al*. (1987) for benzoate degradation. Accumulation of intermediates during degradation of phenol and benzoate was commonly observed by Allsop *et al*. (1993).

Information about the kinetics of phenol and benzoate biodegradation is necessary for optimal design and operation of biological treatment systems. Based on the growth limitation, Monod kinetics was the more suitable model to describe benzoate biodegradation (Ampe and Lindley, 1996). For phenol, their inhibitory nature is well known, and a variety of substrate inhibition models have been used to describe its kinetics. The well-known Haldane equation for enzymes has been used extensively to describe phenol degradation (D'Adamo *et al*. 1984 and Goudar *et al.* 2000). On the other hand, Schrö̈der *et al*. (1997), who compared the fitting of kinetic data for phenol degradation by *Burkholderia cepacia* G4, derived from unstable steady states, to various kinetic equations, found the best fitting for the equation of Yano and Koga, (1969).

The aim of the present work is to investigate degradation of phenol and benzoate individually by a pure culture of *Burkholderia cepacia* G4 under conditions as exist in the environment. Therefore, classical batch was the suitable cultivation technique chosen to guarantee reproducible and fitness initial biomass concentration. This work aims also to detect if degradation of phenol and benzoate via the same pathway (*meta*-clavage), can be described by the same kinetic model.

MATERIALS AND METHODS

1. Microorganism:

Burkholderia cepacia G4 (Nelson *et al*. 1986) was kindly obtained from K. N. Timmis (Division of Microbiology, GBF, Braunschweig, Germany). For

the biomass composition, an average value for *Pseudomonas* given by Roels (1983), was assumed to be $CH_{1.79}O_{0.50}N_{0.20}$. The ash content was considered as 7.5 %.

2. Chemicals:

Phenol (synthesis grade) was purchased from Merck (Darmstadt, Germany). All other chemicals used were of the highest purity commercially available.

3. Media:

The microorganism was cultivated in a mineral salt medium containing per liter deionized water: 100 mL buffer solution, 1.3 mL trace elements solution, 0.7 mL solution A and 4 mL solution B. The buffer solution consisted of (in grams per liter): $Na_2HPO_4 . 2H_2O$, 87.8; KH_2PO_4, 30.0; and $(NH_4)2SO_4$, 12.37. Trace elements solution (in grams per liter) contained: MgO, 10.75; $CaCO_3$, 2.0; $FeSO_4 . 7H_2O$, 4.5; $ZnSO_4 . 7H_2O$, 1.44; $MnSO_4 . 2H_2O$, 0.87; $CuSO_4 . 5H_2O$, 0.25; $CoSO_4 . 7H_2O$, 0.28; $H_3BO_3 . 7H_2O$, 0.06; and HCl (conc.), 51.3 mL. Solution A was 246.48 g L^{-1} $MgSO_4 . 7H_2O$, and solution B was 3.20 g L^{-1} $FeSO_4 . 7H_2O$, and 9.45 g L^{-1} ethylene diamine tetraacetic acid (EDTA). The medium was prepared by autoclaving water and buffer solutions. The trace elements solution and the solutions A and B were filter sterilized and added aseptically to the autoclaved part after cooling to prevent precipitation.

4. Analytical methods:

Ten-milliliter samples were taken from the reactor and centrifuged for 15 min at 15000 rpm (24652 g) at a temperature of 15ºC (Biofuge Stratos, Heraeus instruments, Kendro Hanau, Germany) in stainless steel tubes. The pellets were dried at 60ºC for 48 h in a vacuum (VT 6025, vacutherm, Kendro, Henau, Germany), and weighed after cooling for biomass estimation. The supernatants were filtered (Rezist 30/0.2 µm, Schleicher and Schüll, Dassel, Germany) and stored at -20 ºC for subsequent analysis. Phenol was quantified by using HPLC (Biotronik UV-Detector BT 3030) using a reversed-phase column (Nucleosil 120-3C_{18} (721721.46); front column (721606.40)). The mobile phase was methanol and water (6:4) and the flow was set at 1 mL $min.^{-1}$. Phenol was detected at 270 nm. For benzoate, HPLC (Jasco, UV-970) (intelligent UV/VIS Detector) with a reverse phase column (Chromasil 100-5C_8 (728043.40); front column (728057.40) Chromcart was used. The mobile phase gradient composed from $K_3PO_4.H_2O$, water and methanol and the flow was also set at 1 mL $min.^{-1}$. Benzoate was detected at 210 nm.

5. Cultivations:

As classical batch, cultivations of phenol and benzoate were individually carried out. Prior to the batch experiment, a studied substrate was added to the reactor through a sterile filter (Rezist 30/0.2 µm, Schleicher & Schüll, Dassel, Germany) by a syringe via a membrane seals part. After the added substrate was completely used, the next batch can be started out. All cultivations were carried out in a steam sterilizable 3.5 L stirred tank bioreactor (FZ 2000, Chemap AG, Volketswil, Switzerland). The working volume was 2.52 L. The reactor was equipped with a console for regulation of temperature, pH value, and agitation. The pH and the dissolved oxygen

content were measured by heat autoclavable calibrated electrodes (Ingold, Urdorf, Switzerland).

All cultivations were carried out at 25ºC. The pH was maintained at 7.0 by automatic addition of 1.0 mol L^{-1} sodium hydroxide solution and 0.5 mol L^{-1} sulfuric acid solutions. The pH values were checked routinely during cultivation by sampling. Aeration was done with compressed air at a flow at 162 L h^{-1} (STP) by using a mass flow controller (PR-3000, MKS, Germany). The inlet gas was sterilized with membrane filter and the stirrer speed was adjusted at 300 rpm. The exhaust gas analyzer, all probes, balances and pumps were connected with the bioprocess control computer type UBICON (Universal Bioprocess Control System) (ESD, Hanover, Germany) for data acquisition and control strategies of the reactor.

6. Kinetic models:

Based on a material balance for substrate in the batch cultivation, specific substrate consumption rate can be expressed as:

$$r_S = dC_S / (dt\, C_X) = \mu / Y_{X/S}$$

To describe the relationship between the maximum specific substrate consumption rate and concentration of non inhibitory substrate on a limitation growth, Monod (1949) proposed the following equation:

$$r_S = r_{S\,max}\ C_S / (K_S + C_S)$$

To describe the growth kinetics of the inhibitory substrates, different kinetic models, were used. Substrate inhibition is most often expressed by the Andrews equation (Andrews, 1968) (equal to the Haldane equation of enzyme kinetics):

$$r_S = r_{S\,max}\ C_S / (K_S + C_S + C_S^2 / K_i)$$

There are also several other equations describing substrate inhibition kinetics, some of them derived from Monod equation (Wayman and Tseng, 1976), some not (Tan *et al*. 1996). A very versatile equation for substrate inhibition was proposed by Yano and Koga (1969):

$$r_S = r_{S\,max}\, C_S / \left(K_S + C_S + C_S \sum_{J=1}^{p} (C_S / K_{i\,j})^{j}\right)$$

Here, the information of inactive enzyme-substrate complex with more than two substrate molecules is considered. The Andrews or Haldane equation is obtained by j = 1. The Yano and Koga equation was used by Schröder *et al*. (1997). Yano and Koga model for p = 2 is a four parameter equation. Due to the very low values, the inhibition constant (C_S^2/K_{i1}) was negligible compared with $C_S^3/K_{i\,2}^2$. The four-parameter equation can therefore be reduced to a three-parameter equation without reducing the quality of the fit [Yano and Koga 2] as follows:

$$r_S = r_{S\,max}\ C_S / (K_S + C_S + (C_S^3 / K_{i\,2}^{\,2}))$$

The inhibitory models containing four-parameters of Luong (1987),

$$r_S = r_{S\,max}\ C_S\, (1- C_S/C_S^*)^{\,n} / (K_S + C_S)$$

and containing five-parameters of Han and Levenspiel (1988) were also compared as follow:

$$r_S = r_{S\,max}\ C_S\, (1- C_S/C_S^*)^{\,n} / (C_S + K_S\, (1- C_S/C_S^*)^{\,m})$$

Where: C_S = substrate concentration (g L^{-1})
C_S^* = critical inhibitor concentration (g L^{-1})
K_S = half-saturation constant (g L^{-1})
K_i, $K_{i\,1}$, $K_{i\,2}^2$ = inhibition constants (g L^{-1}, g L^{-1}, g^2 L^{-2}).
$r_{S\,max}$ = maximum specific consumption rate (g g^{-1} h^{-1}).
μ = specific growth rate (h^{-1})
$Y_{X/S}$ = biomass yield coefficient (g g^{-1})

RESULTS AND DISCUSSION

1. Biodegradation of phenol and benzoate

All experiments were carried out as "classical batches" to guarantee fitness initial biomass concentrations, compared to adjusted batch cultivations. As sole source of carbon, degradation of phenol and benzoate via *Burkholderia cepacia* G4 was individually investigated. For each substrate, three batch experiments were carried out using different initial substrate concentrations. For phenol, initial substrate concentration, C_{S0}, starting biomass, C_{X0}, degrading time, as well as biomass yield coefficient are given in Table 1.

Table 1. Experimental data for phenol degradation by *B. cepacia* G4 in classical batch cultivations.

Experiments	C_{S0} g L^{-1}	C_{X0} g L^{-1}	Time h	$Y_{X/S}$ g g^{-1}
Phen-1	0.50	1.83	4.53	0.61
Phen-2	0.54	2.06	4.53	0.83
Phen-3	0.73	1.57	2.02	0.31

The first run (Phen-1) was started when 0.50 g L^{-1} phenol was added to the reactor. After 4.53 h, the substrate was completely consumed. The biomass increased from 1.83 g L^{-1} to 2.14 g L^{-1} during these time periods which correspond to a yield of 0.61 g g^{-1}. Due to further increase in the biomass concentration to 2.17 g L^{-1}, the yield reached 0.68 g g^{-1}, which is considerably lower when compared with the chemostat data recorded by Schröder *et al.* (1997). The second and the third runs (Phen-2 and Phen-3) were carried out with initial phenol concentrations of 0.54 and 0.73 g L^{-1} which was completely consumed after 4.53 and 2.02 h, respectively. The overall biomass yield reached 0.83 g g^{-1} in Phen-2, but decreased to 0.31 g g^{-1} in the third run. It means that, the lowest yield resulting in higher initial phenol concentration in Phen-3, indicating growth inhibition. This is in accordance with the observations of Babish and Davis (1984) and Li and Humphrey (1989), who stated that the inhibitory effect of phenol can be occurre even at low concentrations. In Fig. 1, the time courses for phenol biodegradation of the three batches are compared.

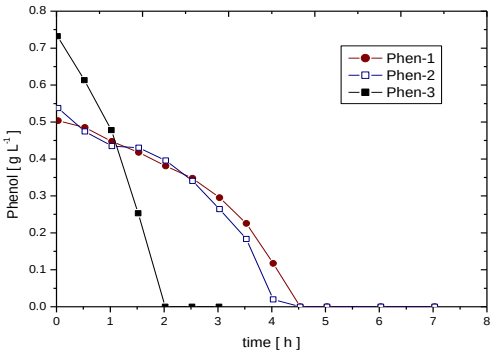

Fig. 1. Time courses for phenol biodegradation of the investigated batches.

It shows faster degradation of phenol in Phen-3 compared with the other runs, indicating better adaptation of the cell performance on the excess substrate concentration. So, the cells were adapted well to a higher consumption rate in this batch. There seems to be an influence of the initial phenol concentration. These results, therefore, are more in conjunction with the theory of Kovárová-Kovar and Egli (1998), who assumed that cell performance is influenced by adaptation and its training to the higher substrate concentration. This is also represented when specific phenol conversion rates of the three batches were plotted (not shown) as a function of phenol concentration. Maximum specific phenol consumption rate, r_{Smax}, was, then estimated as 10 g g^{-1} h^{-1} for both first and second runs, but reached to 0.27 g g^{-1} h^{-1} in the third batch.

A correlation between amount of phenol dose and its degradation rate was studied by Vojta *et al.* (2002). The authors observed that the decrease of the degradation rate resulted in increasing of phenol concentration in addition to other effects. It means that, 0.73 g L^{-1} phenol was not enough to inhibit its degradation rate. On the other hand, the experimental data are in accordance with Jayakumar and Lim (1989), who observed increasing in phenol degradation rate by *Methylomonas mucosa* in the inhibitory branch with increasing its concentration. Decreasing of the biomass yield observed in Phen-3 is perhaps due to other factors such as accumulation of intermediates in the reactor. This is, in agreement with the findings of Wang and Loh (1999), who suggested that accumulation of intermediates is one of the factors that inhibited the conversion rate. In the inhibitory branch, growth of *Methylomonas* strain L3 was influenced by high concentrations of accumulated toxic formaldehyde, which is reported to be inhibitory for cell production of L3 strain (DiBiaso *et al*. 1981).

For benzoate, three batch experiments were also carried out as described for phenol using different initial benzoate concentrations. Table 2 shows the data for the three experiments.

Table 2. Experimental data for benzoate degradation by *B. cepacia* G4 in classical batch cultivations.

Experiments	C_{S0} g L^{-1}	C_{X0} g L^{-1}	Time h	$Y_{X/S}$ g g^{-1}
Benz-1	0.73	0.48	4.92	0.16
Benz-2	0.98	0.77	4.70	0.24
Benz-3	1.30	1.64	3.03	0.37

To compare its degradation with phenol, 0.73 g L^{-1} benzoate, similar used in Phen-3, was used with the first batch (Benz-1). Due to its minor inhibitory effect, benzoate was completely converted after 4.92 h, considerably slower than phenol, resulting in a biomass yield of 0.16 g g^{-1}. So, the degrading strain needs longer time to prepare adaptive enzymes for mineralizing the new substrate. In the second run (Benz-2), 0.98 g L^{-1} benzoate is completely used after 4.70 h from starting, resulting in a biomass yield of 0.24 g g^{-1}. In Benz-3, the initial biomass is higher because the cells in the previous batches were better adapted. Here, 1.30 g L^{-1} benzoates are only consumed in 3.03 h, considerably faster than in the previous runs. The overall biomass yield reached 0.37 g g^{-1} in this run. Time courses of the three runs for benzoate biodegradation were compared in Fig. 2.

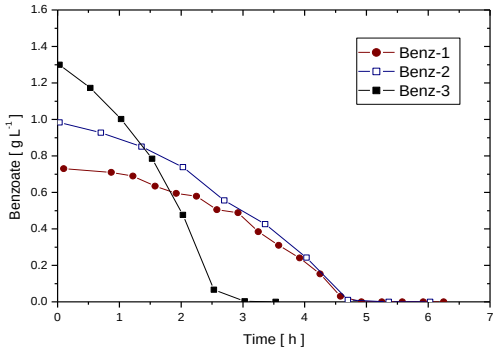

Fig. 2. Time courses for benzoate biodegradation of the investigated batches.

As for phenol, the batch performed out of the highest initial substrate concentration shows a deviation to the other runs, is considered faster conversion. So, the results of benzoate experiments, as for phenol, are more in conjunction with the theory of Kovárová-Kovar and Egli (1998). It is interesting, as for phenol, to mention that the use of a higher initial concentration of benzoate leads to higher maximum specific degradation rate. Based on the results plotted in Fig. 3, the cell mass yield coefficient ($Y_{X/S}$) was influenced by the initial substrate concentration.

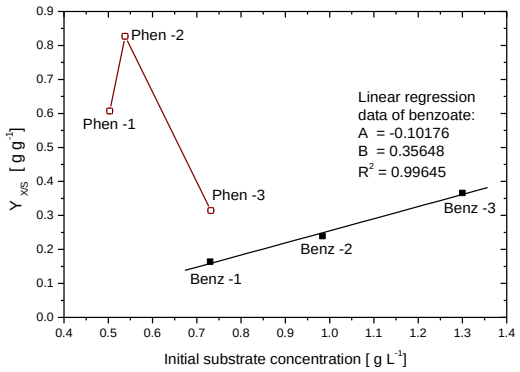

Fig. 3. Influence of the initial substrate concentration on the yield coefficient of all batch runs for phenol and benzoate biodegradation.

For phenol, the biomass yield increased when the initial phenol concentration reached to 0.54 g L^{-1} in Phen-2, after that, the yield sharply decreased, resulting in higher initial substrate concentration in the third batch. As can be seen, the biomass yield varied between 0.83 and 0.31 g g^{-1} when the initial phenol concentration ranged from 0.54 to 0.73 g L^{-1}. Wang and Loh (1999) found that the mass yield coefficient ranged between 0.94 and 0.43 g g^{-1} when the initial phenol concentration varied from 0.25 to 0.80 g L^{-1} under batch conditions. It indicates that the inhibitory influence of phenol was done within the experimental concentration range.

On the other hand, linear increase of the $Y_{X/S}$ values corresponding to the initial benzoate concentration was obtained, and an excellent regression degree of R^2 was found to be larger than 0.996. This, however, may reflect the overall non-, or at least, less inhibitory effect of benzoate concentration during the studied concentration range. The inhibitory effect of benzoate against *Pseudomonas putida* ATCC 49451 at higher initial concentration was also stated by Loh and Chua (2002). The inhibitory effect of benzoate was also observed on the growth of *E. coli* (Salmond *et al.* 1984).

2. Modeling in kinetics of phenol and benzoate biodegradation:

To establish a kinetic model valid for both phenol and benzoate degradation by *B. cepacia* G4 subjected to different substrate concentrations under classical batch conditions, the experimental data were fitted to different models. For this purpose, the nonlinear least squares fitting routine of MicroCal's ORIGIN® software package was used. All experiments were firstly fitted with investigated models, and then one batch of both substrates was selected for their specific consumption rate versus substrate concentration in Fig.4. The fitted functions for Yano and Koga, Haldane, Luong and Han & Levenspiel are plotted with the experimental data of phenol degradation in Fig. 4a. Due to very low regression degree, Luong's model was avoided for benzoate degradation. To examine wether benzoate degradation can be described by the substrate limitation kinetics, Monod model was compared with the other models and plotted in Fig. 4b. The low data scatter allows a clear differentiation between the fitted models.

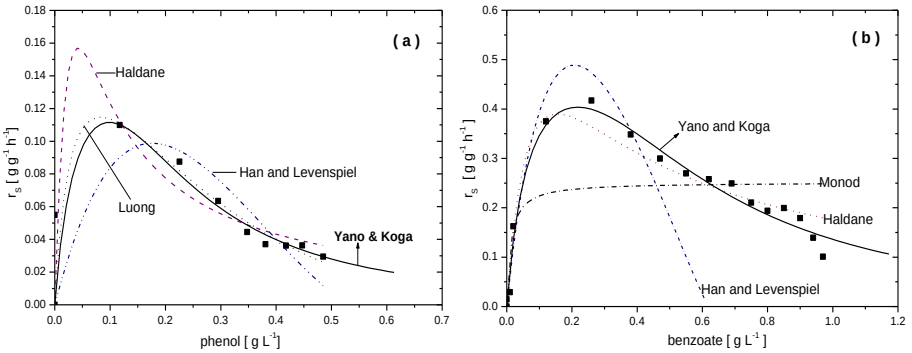

Fig. 4. Specific substrate consumption rate, r_S, versus substrate concentration with different kinetic models for phenol (a) and benzoate (b) batch cultivations.

The results show that, the Yano and Koga model, which is based on multiple inactive enzyme-substrate complexes, provides excellent data regression degrees for both phenol and benzoate degradation under classical batch conditions. This is in accordance with observations of Schröder *et al*. (1997), who compared the fitting of kinetic data for phenol degradation by *Burkholderia cepacia* G4 to various kinetic equations, found the best fitting for the equation of Yano and Koga (1969).

The extension to the four parameters of Luong (1987) model as well as to the five parameters of Han and Levenspiel (1988) did not result in a better fit. Additionally, poorest fit of all the tested equations was achieved for the Haldane model, which is most commonly used to describe substrate inhibition. This, however, is in contrary to the findings of Andrews (1968), D'Adamo *et al.* (1984) and Goudar *et al*. (2000), who well described phenol degradation by Haldane equation. Wang and Loh (1999) found that, the Haldane equation was not sufficient for modelling phenol degradation profile. For benzoate, poorst fit with very low regression was obtained when Monod equation was compared. Despite its inhibitory effect, the results are on contrary to Ampe and Lindley (1996), who described the kinetic parameters of *Alcaligenes eutrophus* 335 on low and high concentrations by using Monod equation.

For phenol, the lower bound of the fitting algorithm was set to 0.042; the estimated K_S-values are around this value. Due to their unsensitivity to a variation of K_S-value, K_S was fixed at 0.042 g L^{-1}. For phenol, specific substrate consumption rates, with the better model simulation of Yano and Koga after fixing of K_S-value, versus phenol concentration are re-plotted in Fig. 5. For benzoate, K_S-value was also fixed, but at 0.068 g L^{-1}. The resulting r_{Smax}-values and the fitting quality (R^2-values) for all phenol and benzoate cultivations are given in Table 3.

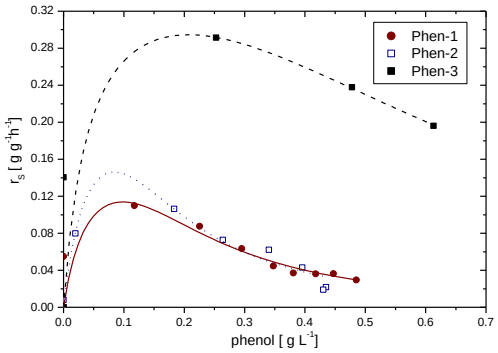

Fig. 5.Specific phenol consumption rates, r_S, (symbols) and their simulation by using Yano and Koga model after fixing of K_S-value (lines) versus phenol concentration for all batches.

Table 3. Fitting of all experimental data for phenol and benzoate biodegradation by Yano and Koga model after fixing of K_S-values.

Experiments	Individual simulation after fixed K_S		
	$r_{S\,max}$ g g^{-1}h^{-1}	K_i g L^{-1}	R^2
Phen-1	0.19	0.212	0.76
Phen-2	0.25	0.169	0.96
Phen-3	0.38	0.655	0.94
Benz-1	0.99	0.334	0.89
Benz-2	0.60	0.552	0.97
Benz-3	0.51	0.896	1.00

In contrast with phenol, these results reveal no dependency increasing of the modeled r_{Smax} with the increasing of initial benzoate concentration was obtained. Due to lower initial biomass (0.48 g L^{-1}), maximum specific benzoate consumption rate of Benz-1 was reached with higher value than those of the other batches. This, however, may reflect the overall effective role played by the initial biomass concentration and the initial substrate concentration on the r_{Smax} values. For all variables, good agreement between the kinetic model and experimental data was achieved.

It could be concluded that, conversion of phenol and benzoate by *B. cepacia* G4 can be described by Yano and Koga kinetics under classical batch conditions quite well. It seems evident that the performance of the cells is dependent of the substrate inhibition, adaptation and probably the initial biomass concentration. However, influence of cell adaptation was already stated by Sokoł (1987). This was shown by the enzyme regulation. Therefore, it is probable that the cells control their enzyme level, adjusting it to face the current conditions. This corresponds also with the theory of Kovárová-Kovar and Egli (1998), who state that the cells constantly adapt to the environmental conditions by varying their kinetic parameters.

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ديناميكية الهدم الحيوى للفينول والبنزوات فى نظام الأستزراع الثابت بواسطة بكتيريا *BURKHOLDERIA CEPACIA* G4

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درس ديناميكية الهدم الحيوى لكل من الفينول والبنزوات بواسطة السلالة البكتيرية *Burkhulderia cepacia* G4 فى نظام أستاتيكى متعاقب مستخدما مدى واسع من التركيزات أضيفت كمواد نمو فردية. نتيجة للتأقلم الجيد على الظروف المتغيرة, لوحظ هدم سريع للفينول والبنزوات عند إضافتهما بتركيزات عالية. ولقد أوضحت النتائج إنخفاض المعامل المحصولى للكتلة الحية $Y_{X/S}$ من 0.83 إلى 0.31 جم/ جم عندما أزداد تركيز الفينول المضاف من 0،54 الى 0،73 جم/ لترمؤيدا بذلك التأثير التثبيطى المعروف للفينول. على الجانب الأخر, لوحظ زيادة خطية فى المعامل المحصولى مع زيادة تركيز البنزوات. أثناء كل التجارب, فان المعدلات العظمى لهدم مادة النمو r_{Smax} وصلت الى 0،27 جم/(جم. ساعه) للفينول, والى 0،40 جم/(جم. ساعه) عندما كانت البنزوات هى المصدر الوحيد للكربون فى المفاعل الحيوى. هذا وقد دلت النتائج على تأثير تثبيطى محدود للبنزوات خلال مدى التركيز المدروس.

ولتوضيح نمطها الهدمى, فان النتائج التجريبية لهدم كل من الفينول والبنزوات حيويا قد تم ملائمتها رياضيا باستخدام نماذج ديناميكية متنوعة. وقد أوضحت النتائج أن معادلة Yano and Koga أعطت أفضل تطابق مقارنة بالنماذج الأخرى. هذا يعنى أن الهدم الحيوى للفينول والبنزوات يمكن أن يوصف بنفس النموذج الديناميكى. إستنادا الى هذه النتائج, فقد كانت حساسية كل التجارب للتغير فى قيمة ثابت التشبع K_S , تكاد تكون منعدمة, لذلك فقد تم تثبيت قيمة K_S عند 0،042 وعند 0،068 جم / لتر لكل من الفينول والبنزوات على التوالى. بعد ذلك, فان قيم r_{Smax} و K_i قد أعيد تكيفها مع كل التجارب. وبصفة إجمالية, فقد تأثرت المقاييس الديناميكية للهدم الحيوى لكل من الفينول والبنزوات بتركيز البداية لكل من مادة النمو وكتلة الخلايا البكتيرية, بالإضافة إلى التأقلم مع التغير فى الظروف المزرعية.

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