DOI: 10.2429/proc.2017.11(2)044 2017;11(2)

Elżbieta SZCZYRBA[1], Agnieszka GĄSZCZAK[1], Grażyna BARTELMUS[1]
and Daniel JANECKI[2]

MODELLING OF THE COMETABOLIC DECOMPOSITION OF 4-CHLOROPHENOL AND PHENOL BY THE *Stenotrophomonas maltophilia* KB2 STRAIN

MODELOWANIE KOMETABOLICZNEGO ROZKŁADU 4-CHLOROFENOLU I FENOLU PRZEZ SZCZEP *Stenotrophomonas maltophilia* KB2

Abstract: The present study investigated the degradation kinetics of growth (phenol) and non-growth (4-chlorophenol, 4-CP) substrates, present alone and in cometabolic system. Batch experiments were performed using very active *Stenotrophomonas maltophilia* KB2 strain. The methods of determining the model parameters describing the kinetics of changes in biomass and both substrates concentrations in a cometabolic system were presented.

Keywords: cometabolism, kinetics, phenol, 4-chlorophenol, self-inhibition, competitive inhibition

Introduction

Phenol and chlorophenols are one of the main groups of environmental pollutants because their toxicity seriously affects living organisms, even in very small concentrations. An example of such a compound is 4-chlorophenol (4-CP) which is toxic, first of all, to the nervous system causing demyelinization of nerve fibres and lowering the concentration of every neurotransmitter. 4-CP is used in industry as a semi-finished product in the synthesis of insecticides, herbicides, preservatives, antiseptics and disinfectants. Information on microbiological decomposition of 4-CP can be found in literature, however, only very few microorganisms are able to use 4-CP as the only source of carbon and energy; most often they are transformed in cometabolic processes.

Aerobic cometabolism is a process by which a non-growth substrate is oxidized by an oxygenase enzyme which has been synthetized by a microorganism and designed for the uptake of a growth substrate. The non-growth substrate does not support microbial growth, yielding no carbon or energy benefit to the cells. The rate of this cometabolic reaction may be limited by several factors, with enzyme inhibition, depletion of reductants and product toxicity considered the most relevant [1]. The subject of research in the presented study was cometabolic decomposition of 4-CP by the *Stenotrophomonas maltophilia* KB2 strain in the presence of phenol as a growth substrate. The choice of phenol as a growth substrate seems to be an optimal solution since degradation pathways of both substances are characterized by a great similarity of enzymes [2]. The aim of the study is to present the way of determining parameters describing kinetics of biomass growth and decomposition of phenol and 4-CP in a cometabolic system.

[1] Institute of Chemical Engineering, Polish Academy of Sciences, ul. Bałtycka 5, 44-100 Gliwice, Poland, email: gaszczak@iich.gliwice.pl
[2] Department of Process Engineering, University of Opole, ul. R. Dmowskiego 7-9, 45-365 Opole, Poland
Contribution was presented during ECOpole'17 Conference, Polanica Zdroj, 4-7.10.2017

Materials and methods

Stenotrophomonas maltophilia KB2 strain, coming from the collection of the Department of Biochemistry, the Faculty of Biology and Environmental Protection of The Silesian University in Katowice, Poland, was originally isolated from activated sludge coming from waste water treatment plant in Bytom-Miechowice. While researching the phenol biodegradation process [3] the strain showed a high activity and resistance to high phenol concentrations which inhibit its growth. Thus, checking its activity in 4-CP cometabolic transformation in the presence of phenol was the next stage of the research. The strain is stored on agar slopes at the temperature of 4°C.

The experiments were carried out in Biostat B bioreactor (Sartorius, USA) with working volume of 2.7 dm^3 (the culture volume - 1.5 dm^3). The apparatus was equipped with a temperature sensor and pH and pO_2 electrodes. The research was performed at optimal environmental conditions (pH 7, 30°C) and stirrer rotations 300 rpm. The air was supplied by external compressor and the concentration of dissolved oxygen was maintained at the level of 5-7 $g \cdot m^{-3}$. The composition of culture mineral medium was presented previously [3].

Microorganism concentration was determined by means of spectrophotometric method (HACH-Lange, Germany; λ = 550 nm). The changes of growth (phenol) and non-growth (4-CP) substrates concentrations in liquid were determined by HPLC method using Waters chromatograph equipped with UV/VIS detector and silica-based, reversed-phase column (Spherisorb ODS 2, 5 µm, 150 × 4.6 mm). Mixture of methanol and 1% acetic acid in water (40:60 v:v) was used as the mobile phase. The efluent flow rate was 0.06 $dm^3 \cdot h^{-1}$. Detection was performed at the wave length of λ = 272 nm. Before analysis samples drawn from biostat were centrifuged, filtered (pores diameter - 0.2 µm) and diluted with water at the ratio of 1 : 1. A detailed description of experiments was presented in earlier studies [3].

Description of the model

The following equations provide a complete mathematical description of the specific growth rate and specific utilization rate of the growth, q_g, and non-growth, q_c, substrates throughout the growth and decay periods [4]:

- growth substrate:

$$q_g = -\frac{1}{X}\frac{dS_g}{dt} = \frac{k_g S_g}{K_{Sg} + S_g + \frac{S_g^2}{K_{Ig}}} \tag{1}$$

- non-growth substrate:

$$q_c = -\frac{1}{X}\frac{dS_c}{dt} = \left(T_c^g q_g + k_c\right)\frac{S_c}{K_{Sc} + S_c + \frac{S_c^2}{K_{Ic}}} \tag{2}$$

- biomass:

$$\mu = Y^m q_g - b - \frac{q_c}{(T_c^b)^*} \tag{3}$$

where: q_i - a specific utilization rate [$g_i \cdot g_x^{-1} \cdot h^{-1}$], μ - specific growth [h^{-1}], rate S_i - substrate concentration [$g_i \cdot m^{-3}$], X - biomass concentration [$g \cdot m^{-3}$], k_i - maximum specific rate of utilization of substrate [$g_i \cdot g_x^{-1} \cdot h^{-1}$], K_S - half-saturation constant [$g \cdot m^{-3}$], K_I - inhibition constant [$g \cdot m^{-3}$], T_c^g - growth substrate transformation yield [$g_c \cdot g_g^{-1}$], T_c^b - biomass transformation capacity [$g_c \cdot g_x^{-1}$], Y^m - maximum growth yield [$g_x \cdot g_g^{-1}$], b - decay coefficient [h^{-1}].

Equation (2) points out that the maximum rate of non-growth substrate utilization depends upon two factors: the utilization rate of the growth substrate, q_g, and the maximum rate at which cells can transform non-growth substrate in the absence of a growth substrate, k_c.

The growth substrate transformation yield, T_c^g, equals the mass of non-growth substrate transformed per unit mass of growth substrate consumed during growth. Higher rate of degradation in the presence of growth substrate is attributed to elevated levels of catabolic enzyme activity when the growth substrate is present.

The endogenous term, b, accounts for loss of energy reserves in the absence of a non-growth substrate. This parameter represents the rate of loss of cometabolic activity in a suspension of resting cells that has not previously been exposed to a non-growth substrate [5].

Two important stoichiometric parameters are: the observed transformation capacity $(T_c^b)_{obs}$ and the observed transformation yield $(T_c^g)_{obs}$. The former is obtained by dividing - q_c by μ:

$$(T_c^b)_{obs} = \frac{1}{\frac{(b - Y^m q_g)}{q_c} + \frac{1}{(T_c^b)^*}} \tag{4}$$

where $(T_c^b)^*$ is true biomass transformation capacity. For resting cells (q_g = 0) Eq. (4) simplifies to:

$$(T_c^b)_{obs} = \frac{1}{\frac{b}{q_c} + \frac{1}{(T_c^b)^*}} \tag{5}$$

The observed transformation yield $(T_c^g)_{obs}$ is obtained by dividing q_c and q_g (Eqs. 1 and 2).

When there is competitive inhibition between the growth and non-growth substrates, $(K_{sg})_{obs}$ and $(K_{sc})_{obs}$ replace K_{sg} and K_{sc}, respectively, in Eqs. (1) and (2), where:

$$\left(K_{Sg}\right)_{obs} = K_{Sg} \frac{1}{\left(\frac{S_c}{K_{cI}}\right)} \tag{6}$$

$$(K_{Sc})_{obs} = K_{Sc} \frac{1}{\left(\frac{S_g}{K_{gI}}\right)} \tag{7}$$

It is necessary to carry out a few series of experiments both on active microorganisms and resting cells to determine constants occurring in the above equations.

Results and discussion

Phenol - alone tests

The experiments with phenol as the only source of carbon and energy for the tested microorganisms were conducted in a batch bioreactor for different initial concentrations of phenol (25-500 $g_{growth\ substrate} \cdot m^{-3}$($g_g \cdot m^{-3}$)). During the experiments, at regular intervals, the concentrations of biomass and growth substrate were determined. The experiments showed that, at concentration exceeding ~100 $g_g \cdot m^{-3}$, phenol began to act as an inhibitor of bacterial activity. Therefore, the Haldane's equation was selected for assessing the dynamic behavior of *Stenotrophomonas maltophilia* KB2 strain grown on phenol:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S_g}{K_{Sg} + S_g + \frac{S_g^2}{K_{Ig}}} \tag{8}$$

Based on the experimental data obtained in the tests of phenol biodegradation alone, the values of the kinetic equation parameters were estimated: μ_m = 0.9 h^{-1}; K_{sg} = 48.97 $g_g \cdot m^{-3}$; and K_{Ig} = 256.12 $g_g \cdot m^{-3}$. The developed kinetic equation with the mean percentage error not exceeding 5% approximates the experimental data (Fig. 1).

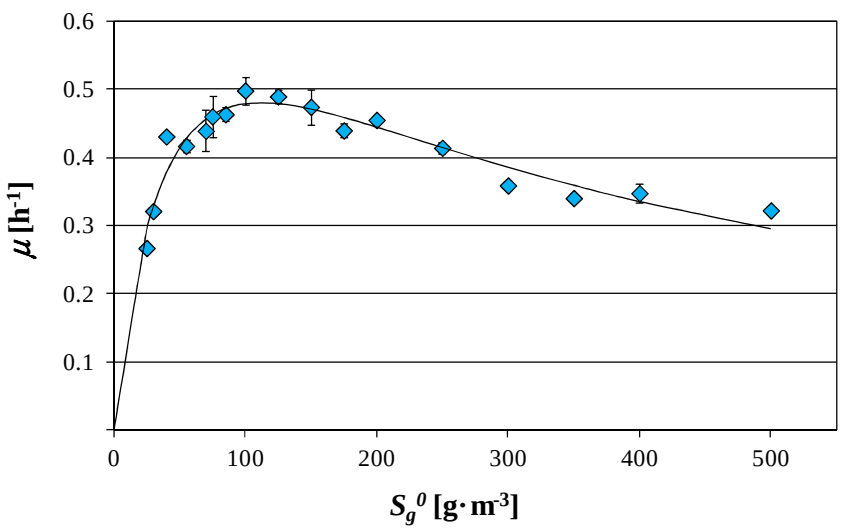

Fig. 1. Effect of initial phenol concentration on specific growth rate of *Stenotrophomonas maltophilia* KB2 strain

The conducted experiments made it possible to determine also the value of the biomass yield coefficient; the mean integral value of Y_{obs} determined in the range of changes of the initial phenol concentration in the solution of 25-500 $g_g \cdot m^{-3}$ was 0.65. Therefore, the specific rate of phenol biodegradation can be expressed as:

$$q_g = \frac{\mu}{Y_{obs}} = \frac{\frac{\mu_m}{Y_{obs}} S_g}{K_{Sg} + S_g + \frac{S_g^2}{K_{Ig}}} \tag{9}$$

where $\frac{\mu_m}{Y_{obs}} = k_g$ [$g_{growth\ substrate} \cdot g_{biomass}^{-1} \cdot h^{-1} 1 (g_g \cdot g_x^{-1} \cdot h^{-1})$].

Under high concentration of growth substrate and in the absence of non-growth substrate

$$Y^m = Y_{obs} \left(\frac{\mu + b}{\mu} \right) \tag{10}$$

Resting cell experiments

Resting cell cultures were prepared after growth in a batch reactor on a basal medium containing phenol. When cell growth neared the end of the exponential phase, the reactor was stopped and the known volumes of suspended culture were transferred to the tubes, centrifuged, washed with a mineral solution and recentrifuged. The concentrated cell pellet was mixed with a fresh mineral salt solution to receive the solution at demanded cell concentration [6].

Endogeneous decay coefficient

The endogeneous decay kinetics can be expressed as first-order reaction vs. biomass concentration, in the absence of growth and non-growth substrates, as follows:

$$\mu_d = \frac{1}{X} \frac{dX}{dt} = (-b) \tag{11}$$

This equation is commonly employed to estimate decay constant, *b*, by monitoring the biomass change using resting cell experiments. The semilog plot of cell concentration vs time yields a line with the slope of (–*b*). For the tested microorganisms the value of decay coefficient of 0.051 h^{-1} was obtained.

The rate of biodegradation 4-CP

Resting cell transformation experiments were performed in two series:

- at constant initial cell concentration (X^0 ~ 92 $g_x \cdot m^{-3}$), with varying initial 4-CP concentration (Fig. 2),
- at constant initial 4-CP concentration ($S_c^{\ 0}$ = 50 $g_{nongrowth\ substrate} \cdot m^{-3} (g_c \cdot m^{-3})$), with varying initial biomass concentration (Fig. 3).

It was observed that the concentration of 4-CP was immediately reduced in the initial period (~2.5-5 $g_c \cdot m^{-3}$) by substrate-assimilating resting cells. After the initial reduction, the 4-CP concentration decreased linearly but with reduced rates at a higher initial 4-CP concentration. It implies that 4-CP acts as an inhibitor. Therefore, the Haldane's model was applied to describe the 4-CP transformation rate by resting cells of *Stenotrophomonas maltophilia* KB2 strain. Because the transformation 4-CP is not coupled with cell growth, the kinetic equation simplifies to the form:

$$r_c = -\frac{dS_c}{dt} = \frac{k_c X^0 S_c}{K_{Sc} + S_c + \frac{S_c^2}{K_{Ic}}} \tag{12}$$

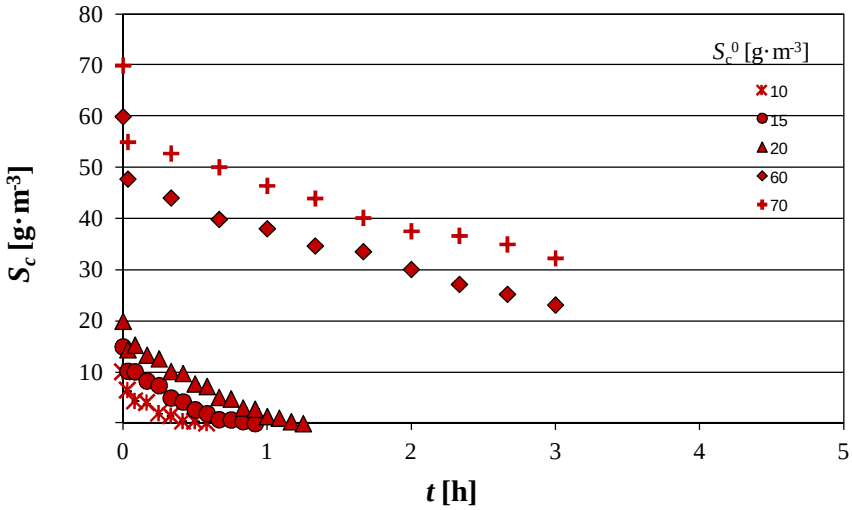

Fig. 2. Effect of initial 4-CP concentrations on transformation of 4-CP by resting cells

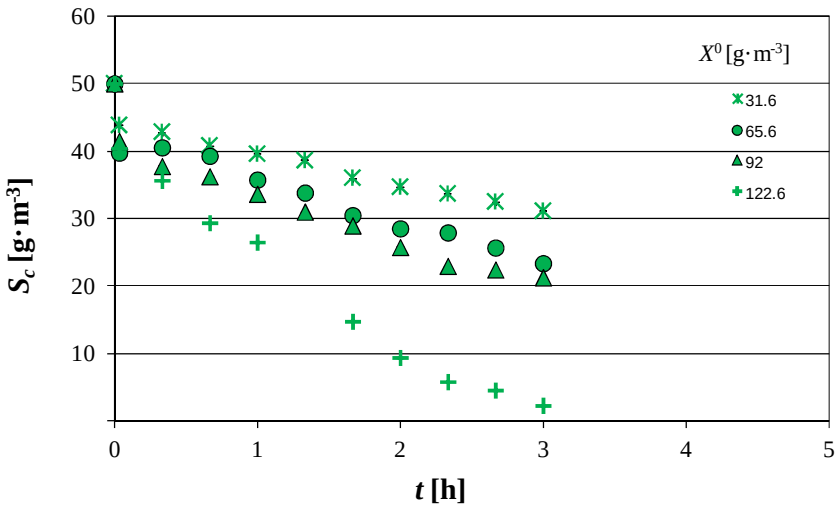

Fig. 3. Effect of initial cell concentrations on transformation of 4-CP by resting cells

In order to estimate the k_c value, the initial 4-CP transformation rates were plotted against the initial cell concentration (Fig. 4). By extrapolating the linear line, the k_c value of 0.158 $g_c \cdot g_x^{-1} \cdot h^{-1}$ was determined [6].

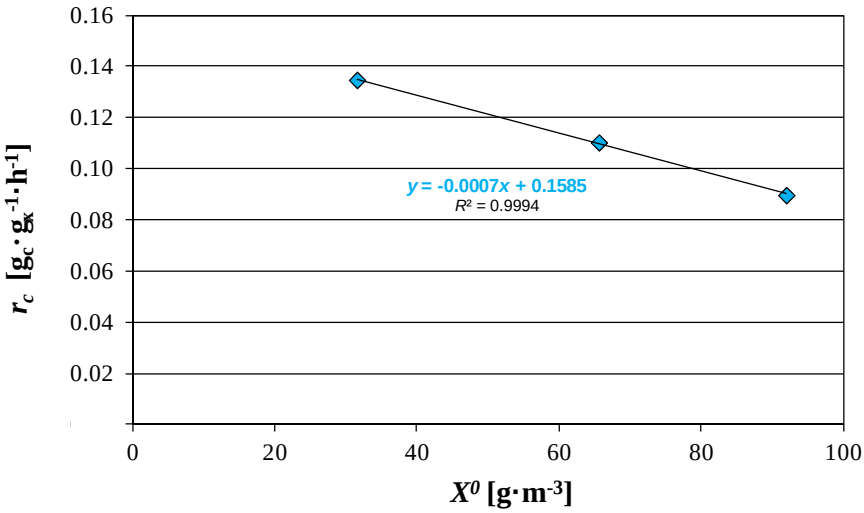

Fig. 4. Determination of k_c value from the specific initial 4-CP transformation rate

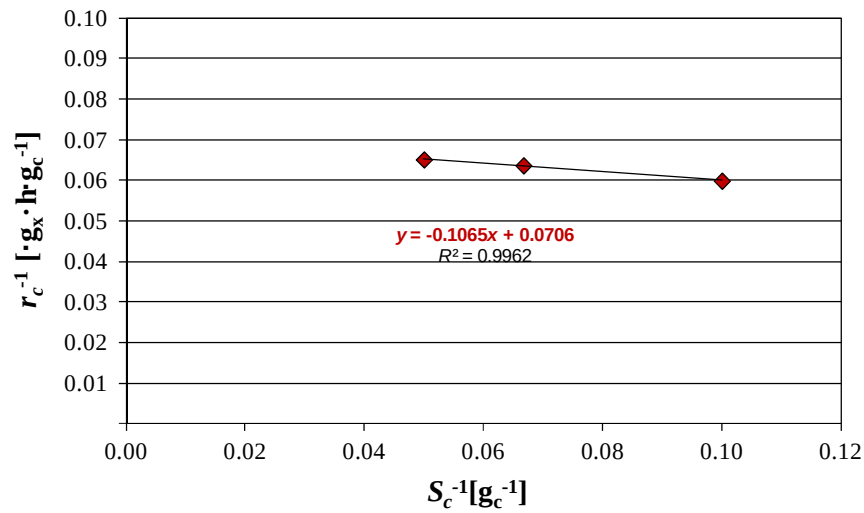

Fig. 5. The way of determination of k_c and K_{Sc} values

The influence of inhibition on the process can be omitted for small values of 4-CP initial concentration. In which case the equation (12) abridges and can be presented in the form of:

$$\frac{1}{r_c} = \frac{1}{k_c X^0} + \frac{K_{Sc}}{k_c X^0 S_c} \tag{13}$$

Plotting the inverse of the initial 4-CP transformation rate ($1/r_c$) vs inverse of the initial 4-CP concentration (Fig. 5) a straight line is obtained whose slope is $K_{Sc}/k_c\ X^0$, and the *y*-axis intercept is $1/k_c\ X^0$. In this way the values of k_c = 0.154 $g_c \cdot g_x^{-1} \cdot h^{-1}$ and K_{Sc} = 0.15085 $g_c \cdot m^{-3}$ can be calculated.

Next, for big values of S_c^0, the equation (12) can be abridged to the form of:

$$\frac{1}{r_c} = \frac{1}{k_c X^0} + \frac{S_c}{k_c X^0 K_{Ic}} \tag{14}$$

The slope of a straight line in the chart $1/r_c$ vs initial 4-CP concentration makes it possible to estimate the inhibition constant value K_{Ic} = 114.72 $g_c \cdot m^{-3}$ (not presented).

Cometabolic 4-CP transformation in the presence of phenol

Two series of studies were performed. In the first series cultures were grown at the initial concentration of 4-CP = 50 $g_c \cdot m^{-3}$, however, the initial phenol concentration was changed in successive cultures within the range of 50-300 $g_g \cdot m^{-3}$ (Ph/4-CP = 1;1.5;2;2.5;3;4;5;6). In the other series, for the constant initial phenol concentration (75 $g_g \cdot m^{-3}$), the initial 4-CP concentration was changed within the range of 25-100 $g_c \cdot m^{-3}$. For the whole range of changes of Ph/4-CP, both substrates were simultaneously degraded and the transformation time did not exceed 6 hours. It shows a great activity of the tested strain. Comparing phenol concentration changes profiles in a mono-substrate culture and in the presence of various doses of 4-PC it was stated that (Fig. 6) degradation time of the same initial phenol doses lengthens together with cometabolite concentration increase. This fact confirms the competitive inhibition of phenol biodegradation rate by 4-CP. A detailed analysis of experiments carried out for various growth and non-growth substrates ratios was presented in the previous study [7].

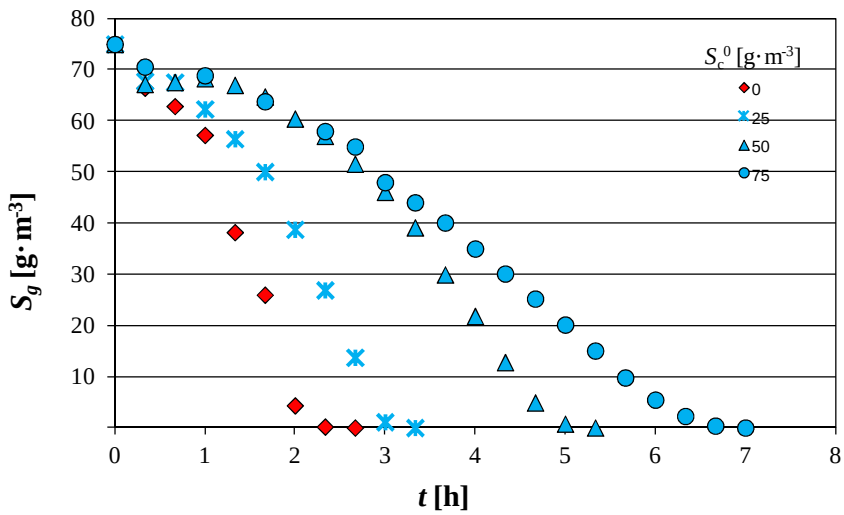

Fig. 6. Effect of initial 4-CP concentration on biodegradation of phenol (S_g = 75 $g \cdot m^{-3}$)

Observed growth substrate transformation yield $(T_c^g)_{obs}$

A graph of 4-CP and phenol changes during one single culture should be drawn for each experimental point to determine the value of $(T_c^g)_{obs}$ (Fig. 7).

The initial biodegradation rate is a straight line whose slope determines the $(T_c^g)_{obs}$ value.

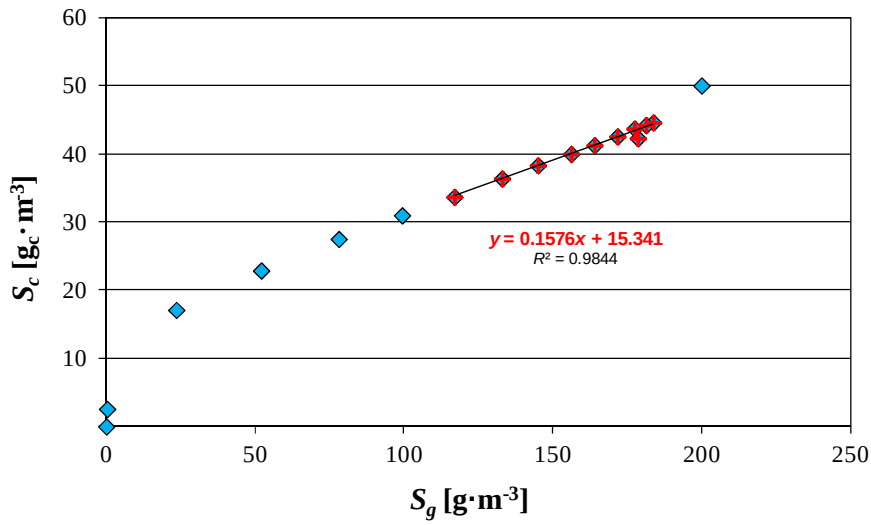

Fig. 7. Plot of function S_c vs S_g (S_c^0 = 50 $g_c \cdot m^{-3}$; S_g^0 = 200 $g_g \cdot m^{-3}$)

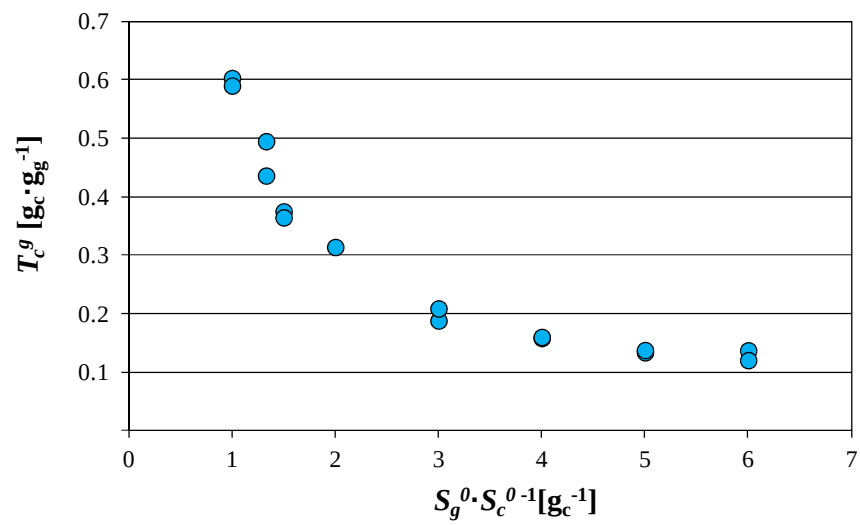

Fig. 8. Effect of the S_g/S_c ratio on $(T_c^g)_{obs}$ values

The $(T_c^g)_{obs}$ value, determined in such a way, changes with the S_g/S_c ratio; for high values of the ratio S_g/S_c (≥ 3) $(T_c^g)_{obs} = 0.1326\ g_c \cdot g_g^{-1}$ (Fig. 8).

Observed biomass transformation capacity $(T_c^b)_{obs}$

Eqation (5) indicates that $(T_c^b)_{obs}$ is a function S_c and time. Criddle [4] suggested that $(T_c^b)_{obs}$ can be obtained by simplifying Eq. (2) to $q_c = k_c$ for $S_c >> K_{Sc}$, $S_g = 0$ and in the absence of non-growth substrate inhibition. However, in the presented experiments, the high concentrations of 4-CP lead to the inhibition. Therefore, $(T_c^b)_{obs}$ cannot be obtained from simplified form of Eq. (2). The method of determining $(T_c^b)_{obs}$ and $(T_c^b)^*$ values will be presented in the next paper.

Conclusions

The kinetics of cometabolic 4-PC biodegradation by *Stenotrophomonas maltophilia* KB2 strain, in the presence of phenol as a growth substrate, was analyzed in the presented study.

It is necessary to determine the values of a lot of model parameters to solve the mathematical model equation of the process occurring in a cometabolic system. It requires carrying out a lot of series of experiments using both metabolically active microorganism and resting cells. Moreover, the research should be carried out at changing concentrations of both substrates present in the solution as a mixture and alone.

Only then, having a set of such parameters, by fitting the experimentally determined and calculated from the model (Eq. (1)-(4)) concentration profiles of biomass as well as growth and non-growth substrates, it can be assessed whether the suggested model describes the kinetics of the process well.

References

[1] Jesus J, Frascari D, Pozdniakowa T, Danko AS. Kinetics of aerobic cometabolic biodegradation of chlorinated and brominated aliphatic hydrocarbons: A review. J Hazard Mater. 2016;309:37-52. DOI: 10.1016/j.jhazmat.2016.01.065.

[2] Arora PK, Bae H. Bacterial degradation of chlorophenols and their derivatives. Microb Cell Fact. 2014;13:1-31; http://www.microbialcellfactories.com/content/13/1/31.

[3] Szczyrba E, Szczotka A, Bartelmus G, Gąszczak A, Greń I, Kolarczyk A. Kometaboliczna biodegradacja 4-chlorofenolu przez szczep Stenotrophomonas maltophilia KB2. Prace Naukowe IICh PAN. 2016;20:137-154; www.iich.gliwice.pl.

[4] Criddle CS. The kinetics of cometabolism. Biotechnol Bioeng. 1993;41:1048-1056. DOI: 10.1002/bit.260411107.

[5] Chang WK, Criddle CS. Experimental evaluation of a model for cometabolism: prediction of simultaneous degradation of trichloroethylene and methane by a methanotrophic mixed culture. Biotechnol Bioeng. 1997;56:492-501. DOI:10.1002/(SICI)1097-0290(19971205)56:5<492::AID-BIT3>3.0.CO;2-D.

[6] Hao OJ, Kim MH, Seagren EA, Kim H. Kinetics of phenol and chlorophenol utilization by Acinetobacter species. Chemosphere. 2002;46:797-807. DOI: 10.1016/S0045-6535(01)00182-5.

[7] Szczyrba E, Szczotka A, Bartelmus G. Modelling of aerobic biodegradation of phenol by Stenotrophomonas maltophilia KB2 strain. Proc ECOpole. 2016;10:533-543. DOI: 10.2429/proc.2016.10(1)057.

[8] Chen YM, Lin TF, Huang C, Lin JC. Cometabolic degradation kinetics of TCE and phenol by Pseudomonas putida. Chemosphere. 2008;72:1671-1680. DOI: 10.1016/j.chemosphere.2008.05.035.

[9] Wang Q, Li Y, Li J, Wang Y, Wang C, Wang P. Experimental and kinetic study on the cometabolic biodegradation of phenol and 4-chlorophenol by psychrotrophic Pseudomonas putida LY1. Environ Sci Pollut Res. 2015;22:565-573. DOI: 10.1007/s11356-014-3374-x.

MODELOWANIE KOMETABOLICZNEGO ROZKŁADU 4-CHLOROFENOLU I FENOLU PRZEZ SZCZEP *Stenotrophomonas maltophilia* KB2

[1] Instytut Inżynierii Chemicznej, Polska Akademia Nauk, Gliwice
[2] Katedra Inżynierii Procesowej, Uniwersytet Opolski, Opole

Abstrakt: Zaprezentowano model rozkładu 4-chlorofenolu przez szczep *Stenotrophomonas maltophilia* KB2 w obecności fenolu jako substratu wzrostowego. Opracowanie tego modelu wymagało wykonania czterech serii badań: biodegradacji różnych dawek czystego fenolu, biodegradacji różnych dawek czystego 4-CP, biodegradacji 4-CP w obecności fenolu przy różnym stosunku stężeń obu substratów oraz biodegradacji 4-CP przez komórki w fazie spoczynku, indukowane wstępnie fenolem. Szczegółowo omówiono sposoby wyznaczania poszczególnych parametrów równań opisujących szybkość degradacji substratów wzrostowego i niewzrostowego oraz przyrostu biomasy. W oparciu o stworzoną bazę danych obliczono m.in. stałe półnasycenia K_{Sg} i K_{Sc}, stałe inhibicji K_{Ig} i K_{Ic}, współczynnik zamierania endogennego b oraz wydajność transformacyjną substratu wzrostowego w stosunku do kometabolitu T_c^g.

Słowa kluczowe: kometabolizm, kinetyka, fenol, 4-chlorofenol, inhibicja