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KINETICS OF COMETABOLIC BIODEGRADATION OF 4-CHLOROPHENOL AND PHENOL BY *STENOTROPHOMONAS MALTOPHILIA* KB2

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Dedicated to Professor Andrzej Burghardt on the occasion of his 90th birthday

The cometabolic biodegradation of 4-Chlorophenol (4-CP) by the *Stenotrophomonas maltophilia* KB2 strain in the presence of phenol (P) was studied. In order to determine the kinetics of biodegradation of both substrates, present alone and in cometabolic systems, a series of tests was carried out in a batch reactor changing, in a wide range, the initial concentration of both substrates. The growth of the tested strain on phenol alone was described by Haldane kinetic model ($\mu_m = 0.9$ 1/h, $K_{sg} = 48.97$ g_g/m^3, $K_{Ig} = 256.12$ g_g/m^3, $Y_{xg} = 0.5715$). The rate of 4-CP transformation by resting cells of KB2 strain was also described by Haldane equation and the estimated parameters of the model were: $k_c = 0.229$ $g_c/g_x h$, $K_{sc} = 0.696$ g_c/m^3, $K_{Ic} = 43.82$ g_c/m^3. Cometabolic degradation of 4-CP in the presence of phenol was investigated for a wide range of initial 4-CP and phenol concentrations (22–66 g_c/m^3 and 67–280 g_g/m^3 respectively). The experimental database was exploited to verify the two kinetic models: *CIModel* taking only the competitive inhibition into consideration and a more universal *CNIModel* considering both competitive and non-competitive inhibition. *CNIModel* approximated experimental data better than *CIModel.*

Keywords: 4-Chlorophenol, phenol, cometabolism, kinetics

1. INTRODUCTION

Halogenated aromatic compounds, e.g. chlorophenols, constitute a major class of contaminants, which enter environment with industrial wastewaters and as a consequence of human agricultural activity. The negative impact of these compounds on the environment, human health, and quality of life is exhaustively discussed in literature and various physical, chemical and biological methods of wastewater and polluted soils treatment have been developed and applied (Busca et al., 2008; Danis et al.,1998; Jain at al., 2004; Krastanov et al. 2013).

Practice shows that the physicochemical methods are generally efficient but costly and might generate secondary pollutants such as substituted phenols or dioxins. Therefore, biological methods are preferred

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for being considered as more economical and environmentally friendly (Essam et al., 2010; Krastanov et al., 2013). Phenol and chlorophenols are not easily biodegradable because they are toxic to most microorganisms and in higher concentrations they can even inhibit the growth of microbial strains. After many years of research a number of fungi and bacterial species which show the ability to assimilate phenol and its derivatives have been identified, although in low concentrations in most cases. The microorganisms used are usually aerobes because they grow faster than anaerobes and usually achieve complete mineralization of toxic compounds rather than transformation, as in the case of anaerobic treatment (Goswami et al., 2002; Kim et al., 2002; Leontievsky et al., 2000; Sahinkaya and Dilek, 2005; Wang et al., 2000). Despite a significant amount of information gathered during the years, the problem is still topical and significant and stimulates research into the characterization of new and more effective microbial species (Aruthelvan et al., 2005; Farrell and Quilty 2002; Krastanov et al., 2013; Margesin et al., 2005; Shourian et al., 2009).

Chlorophenols are usually poorly degraded in conventional biological treatment systems because only very few microorganisms are able to use such compounds as the only source of carbon and energy (Field and Sierra-Alvarez, 2008); most often they are transformed in cometabolic processes (Alvarez-Cohen and Speitel, 2001).

Cometabolism is the biological transformation of a non-growth substrate by non-specific enzymes of bacteria. The synthesis of these enzymes in microbial cells can only be induced by a growth-substrate which provides energy for cell growth and maintenance of bacteria. Therefore, a growth substrate must be available at least periodically to grow new cells, provide an energy source and induce the production of the cometabolic enzymes (Criddle, 1993; Aktas, 2012).

The aerobic cometabolic transformation of chlorinated phenols involves oxygenases, molecular oxygen and a source of reducing equivalents, typically NAD(P)H. Since enzymes that catalyze cometabolic reactions have active sites that can react with a number of different substrates, the competition for an active site may occur when multiple substrates are simultaneously available resulting in an apparent decrease in enzyme affinity for each substrate (competitive inhibition). Competitive inhibition between a growth substrate and a cometabolic substrate has been observed for many oxygenase-utilizing microorganisms (Chang and Alvarez-Cohen, 1995; Saez and Rittmann, 1991).

4-Chlorophenol (4-CP) provides an excellent example of environmentally significant compound that is toxic, first of all, to the nervous system causing demyelination of nerve fibers and lowering the concentration of every neurotransmitter. It is used in industry as a semi-finished product in the synthesis of insecticides, herbicides, preservatives, antiseptics and disinfectants (Aktas, 2012). 4-Chlorophenol is a cometabolite that can be degraded by phenol-induced cells. The choice of phenol as a growth substrate seems to be an optimal solution since degradation pathways of both substrates are characterized by a great similarity of enzymes (Arora and Bae, 2014). The used microorganisms are usually aerobes, including *Pseudomonas* sp. (Aruthelvan et al., 2005; Farrell and Quilty 2002; Krastanov et al., 2013; Margesin et al., 2005; Shourian et al., 2009), *Acinetobacter* (Kim and Hao, 1999), *Rhodococcus* sp. (Monsalvo et al., 2009), *Candida tropicalis* (Jiang et al., 2007; Jiang et al., 2008; Liu et al., 2012), *Alcaligenes eutrophus* (Hill et al., 1996), *Comomonas testosterone* (Bae et al., 1996; Tobajas et al., 2012) and *Burkholderia tropicalis* (De Los Cobos-Vasconcelos et al., 2006).

The major objective of this study was to investigate the kinetics of 4-chlorophenol (4-CP) cometabolic transformation by the *Stenotrophomonas maltophilia* KB2 strain in the presence of phenol (P) as the growth substrate and to elaborate a kinetic model of the process. For validation of the kinetic model, our own experimental database was used; the minimum of loss function, defined as the sum of squared differences of the experimental and computed values of the concentrations of both substrates was used as the validation criterion.

Stenotrophomonas maltophilia KB2 used in this study is known to metabolize a broad range of aromatic compounds including phenol, benzoic acids, catechols, nitrophenols and others (Greń et al., 2010; Guzik et al., 2009; Wojcieszyńska et al., 2007); the efficiency of *Stenotrophomonas maltophilia* KB2 strain in cometabolic transformation of 4-CP in the presence of phenol as the growth substrate was tested in this study for a broad range of changes in concentrations of both substrates.

2. MODELING ASPECTS

Several kinetic models have been developed to simulate the behavior of cometabolic degradation (Alvarez and Speitel, 2001; Chen et al., 2008; Criddle, 1993; Hao et al., 2002; Sinha et al., 2011; Wang et al., 2015).

The enzyme inhibition caused by simultaneous degradation of growth and cometabolic substrates has generally been modeled by including competitive inhibition terms in the half-saturation constant of the equations describing the rate of degradation of the growth and cometabolic substrates respectively. If the Haldane substrate inhibition equation is used to model the inhibitory effect of phenol and 4-CP on their own biotransformation, the equations describing the specific rate of degradation of both substrates take the form:

$$q_g = -\frac{1}{X}\frac{dS_g}{dt} = \frac{k_g S_g}{K_{sg}\left(1+\frac{S_c}{K_{isc}}\right)+S_g+\frac{S_g^2}{K_{Ig}}} \tag{1}$$

$$q_c = -\frac{1}{X}\frac{dS_c}{dt} = \frac{k_c S_c}{K_{sc}\left(1+\frac{S_g}{K_{isg}}\right)+S_c+\frac{S_c^2}{K_{Ic}}} \tag{2}$$

where K_{isg} and K_{isc} are the competitive inhibition coefficients for K_{sc} and K_{sg}, respectively.

The specific rate of cell growth is typically expressed as a function of growth substrate consumption and cell decay as follows (Alvarez-Cohen and Speitel, 2001; Criddle, 1993; Jesus at al., 2016):

$$\mu = \frac{1}{X}\frac{dX}{dt} = Y_{xg}q_g - \frac{q_c}{T_c^{b*}} - b \tag{3}$$

where T_c^{b*} is true biomass transformation capacity defined as the mass of non-growth substrate that would be transformed by the unit mass of cells in the absence of endogenous decay. Thus q_c/T_c^{b*} is the specific decay rate attributed to cometabolism alone (Criddle, 1993).

Finally, Eqs. (1)–(3) provide a complete mathematical description of the utilization rates of the growth and non-growth substrates and growth rate of biomass throughout the growth and decay periods. The model was named *Competitive Inhibition Model (CIM)*.

A number of researchers have used modifications of the above expressions for competitive inhibition to successfully model the degradation of chlorinated solvents in the presence of growth substrate by a range of oxygenase expressing organisms (Alvarez-Cohen and Speitel, 2001; Chang and Criddle, 1997; Chen et al., 2008; Hao et al., 2002). In the absence of precisely estimated values of K_{isg} and K_{isc} some researchers used the approximation $K_{isg} = K_{sg}$ and $K_{isc} = K_{sc}$, but studies of Frascari et al. (2006; 2008) showed that this assumption is not correct (Jesus et al., 2016).

Ely et al. (1995) derived a conceptual model that incorporated both competitive and noncompetitive inhibition into cometabolic enzyme kinetics but, for the analysis experimental results, the model was simplified to the situations with competitive inhibition alone (Ely et al., 1995a; Ely et al., 1997).

Saez and Rittmann (1993), considering the Haldane kinetics for the biodegradation of the growth substrate, postulated that the presence of cometabolite affects the Haldane k_g and K_{sg} coefficients via interaction terms of the form:

$$\left[1+\frac{z_i^{n_i}}{K_i}\right], \qquad i=1,2 \tag{4}$$

where $z_i = S_c$ or S_c/S_g, K_i are inhibition constants and n_i are positive empirical exponents. Introducing these terms into the Haldane equation yields the governing equation for the phenol biodegradation rates in a dual system, which takes the form:

$$q_g = -\frac{1}{X}\frac{dS_g}{dt} = \frac{\dfrac{k_g}{1+\dfrac{S_c^{n_1}}{K_1}}}{K_{sg}\left(1+\dfrac{(S_c/S_g)^{n_2}}{K_2}\right)+S_g+\dfrac{S_g^2}{K_{Ig}}} \tag{5}$$

where K_1 andK_2 are noncompetitive and competitive inhibition coefficients, respectively.

Because the non-growth substrate is transformed via the electrons generated by the growth substrate and/or through electrons generated by biomass oxidation, the rate of cometabolite transformation should be proportional to the rate of growth-substrate oxidation and biomass decay. Therefore, the specific rate of cometabolite transformation can be described as:

$$q_c = -\frac{1}{X}\frac{dS_c}{dt} = T_c^g\left(-\frac{1}{X}\frac{dS_g}{dt}\right) - T_c^b b \tag{6}$$

whereas the rate of biomass growth is given by:

$$\mu = \frac{1}{X}\frac{dX}{dt} = Y_{xg}^s\left(-\frac{1}{X}\frac{dS_g}{dt}\right) - b \tag{7}$$

For short experiments (of the order of a few hours) endogenous decay can be neglected (Jesus et al., 2016; Saez and Rittmann, 1993) and Eqs. (6)–(7) take the form:

$$q_c = T_c^g\left(-\frac{1}{X}\frac{dS_g}{dt}\right) \tag{8}$$

$$\mu = Y_{xg}^s\left(-\frac{1}{X}\frac{dS_g}{dt}\right) \tag{9}$$

where Y_{xg}^s is biomass growth yield for dual-substrate tests.

Finally, the system of differential Eqs. (5), (8) and (9) constitutes the mathematical description of the process of cometabolic transformation of the substrates; the model was named *Competitive/Noncompetitive Inhibition Model (CNIM)*.

3. EXPERIMENTAL SECTION

3.1. Microorganism and culture conditions

The *Stenotrophomonas maltophilia* KB2 strain from the collection of Department of Biochemistry Faculty of Biology and Environmental Protection of the University of Silesia in Katowice, Poland (stored under number E-113197 in VTT Collection in Finland), was isolated from activated sludge of the sewage

treatment plant in Bytom-Miechowice in Poland as described previously (Guzik et al., 2009). The strain was stored on the agar slopes at 4 °C and transferred to the new ones every month.

The composition of mineral salts medium for bacteria culturing as well as for biodegradation experiments was as described in the study by Wojcieszyńska et al. (2011). In order to prepare microorganisms for the kinetics studies, *S. maltophilia* KB2 cells were transferred from the agar slopes to tubes containing 20 ml of sterile mineral salts medium supplemented with 150 μl of 0.4 M phenol. After a 24 hour incubation (130 rpm, 30 °C) the cell suspension was placed in a sterile Erlenmeyer flask, filled up to volume of 100 ml with mineral medium and 750 μl of 0.4 M phenol. The next day freshly proliferated cells were transferred to a 500 ml sterile Erlenmeyer flask, mineral salts medium was added to obtain the culture volume of 300 ml and 2.25 ml of 0.4 M phenol was introduced. Culture flasks were kept on an orbital shaker at 130 rpm at 30 °C for 72 hours, and every 24 hours a new dose of phenol was applied. After that the cells were harvested by centrifugation (4500× g, 15 min, 4 °C), washed with sterile mineral medium and after being suspended in 20 ml mineral medium were kept at 4 °C up to 7 days.

Kinetic tests were carried out in a periodic Biostat B fermenter (Sartorius, USA) with the working volume of 1.5 dm^3 (total volume 2.7 dm^3) equipped with temperature, pH and O_2 sensors. The tests were conducted at 30 °C, oxygenation was maintained at the level of 5 mg/dm^3 (by bubbling the suspension with air), pH 7, and the stirrer speed was 300 rpm. A constant value of pH was maintained by feeding a 10% solution of KOH or KH_2PO_4.

Bacterial cell density was determined spectrophotometrically (spectrophotometer HACH DR3900) at $\lambda =$ 550 nm. Before the kinetic studies the calibration curve was prepared which made it possible to convert optical density into grams of dry weight mass.

The changes of growth substrate (phenol) and cometabolite (4-chlorophenol) concentration in the liquid culture were determined by a Waters HPLC equipped with Waters 1525 gradient pump and two-wave detector UV-VIS Waters M2487. The separation was carried out on a reverse phase column (Spherisorb ODS 2, 5 μm, 150 × 4.6 mm). The mobile phase was the system methanol and 1% acetic acid (40:60 v/v) with the flow rate of 1 ml min^{-1}; detection was carried out at a wave length of 272 nm. Samples were taken from the bioreactor and subjected to centrifugation (15 000 rpm, 15 min, 4 °C), next filtration (2 μm pore diameter) and diluted with Millipore water before chromatographic analyses.

To determine the values of the parameters found in the proposed models, it is necessary to perform three series of experiments, including:

1) the study of the phenol biodegradation process by the tested strain; the obtained database will enable the determination of the following parameters: k_g, K_{sg}, K_{Ig}, Y_{xg}.
2) the study of a 4-CP transformation by phenol induced cells of the tested strain in the resting phase; the experimental database will make it possible to determine the values of: k_c, K_{sc}, K_{Ic}, T_c^b.
3) the study of a 4-CP cometabolic transformation in the presence of phenol; the experimental database will make it possible to estimate the parameters of the *CIModel* (K_{isg} and K_{isc}) and the *CNIModel* (K_1, K_2, n_1, n_2).

3.2. Phenol – alone tests

The studies on the phenol biodegradation kinetics by *Stenotrophomonas maltophilia* KB2 cells were conducted for the initial phenol concentration changing within the range of 25–500 g/m^3. During experiments, in particular time intervals, the changes in concentration of biomass and growth substrate were detected, and next the $\ln X = f(t)$ graph was prepared. In the phase of sustainable growth the graph of this function

is a straight line whose slop determines the value of the specific growth rate μ. The obtained experimental database $\mu = f(S_g^0)$, made the selection of the kinetic model and the estimation of its parameters possible.

The experiments have shown that for growth substrate concentrations exceeding 100 g/m^3 the negative effect of phenol on bacterial growth was observed. For the description of the process kinetics the Haldane model was applied:

$$\mu = \frac{\mu_m \cdot S_g}{K_{sg} + S_g + \dfrac{S_g^2}{K_{Ig}}} \tag{10}$$

and the values of the kinetic equation parameters were estimated to give: $\mu_m = 0.9\ \mathrm{h}^{-1}$, $K_{sg} = 48.97$ g/m^3, $K_{Ig} = 256.12$ g/m^3 (Nonlinear Regresion Analysis program – NLREG (Sherrod, 2010)).

The resulting kinetic equation with a mean percentage error not exceeding 5% ($R^2 = 0.95$) approximates the experimental data. The test results and the comparison shown in Fig. 1 confirmed a high activity of the tested strain, making it a promising candidate for industrial applications (short lag phase, high biodegradation activity, i.e. phenol in the concentration of 400 g/m^3 was degraded completely within 5.5 hours, the optimal growth rate achieved at relatively high concentrations of phenol). KB2 strain showed also great resistance to inhibition of their growth by phenol (a high value of the substrate inhibition constant, similar to the values obtained for *Pseudomonas putida* ATCC 49451 (Wang and Loh, 1999) and *Bacillus cereus* (Zhang et al., 2013).

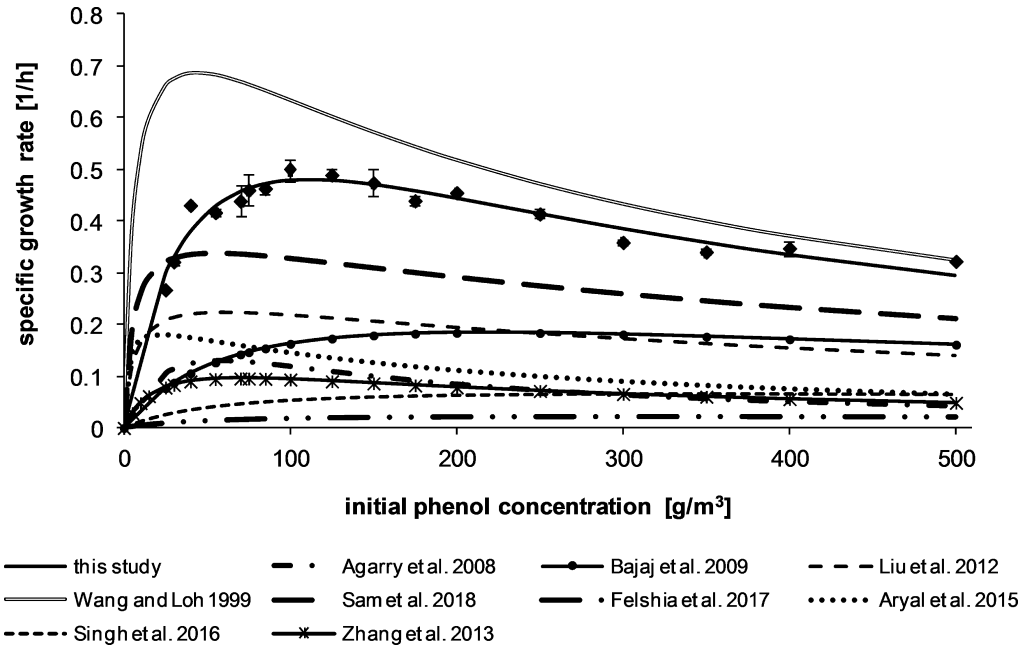

Fig. 1. The specific growth rate of various microorganisms in the presence of phenol as the sole carbon and energy source

The transformation of Eq. (10) describing the kinetics of microbial growth into the equation describing the rate of phenol biodegradation requires a knowledge of the biomass yield coefficient Y_{xg}, (k_g constant in Eq. (1) equals μ_m/Y_{xg}). For each culture the value of this parameter was determined by following the changes in the concentration of biomass and substrate over time (Fig. 2), and the slope of the straight line approximating experimental points determines the essential Y_{xg} value. The integral average of the experimentally determined Y_{xg} values was 0.5715.

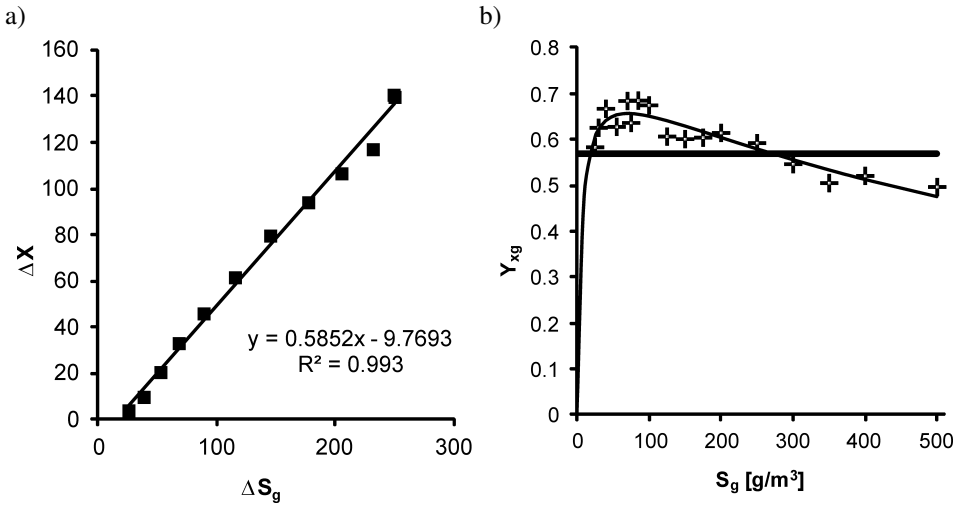

Fig. 2. Estimation of the biomass yield coefficient (a), $S_g^0 = 250$ g_g/m^3 and biomass yield coefficient as a function of initial phenol concentration (b) (the straight line corresponds to mean value of Y_{xg})

3.3. Resting cell experiments

Resting cell cultures were prepared after the growth in a batch reactor in mineral medium containing 282 g/m^3 of phenol. When cell growth approached 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 medium and centrifuged again. The concentrated cell pellet was mixed with a fresh medium to receive the solution with the required cell concentration (Elango et al., 2011).

The resting cell transformation experiments were performed at a constant initial cell concentration ($X^0 \sim 92$ g_x/m^3), for the initial 4-CP concentration changing in the range of 10–70 g_c/m^3. It was observed that the concentration of 4-CP was immediately reduced in the initial period ($\sim 2.5-5$ g_c/m^3) by substrate-assimilating resting cells. The same phenomenon was observed by Hao et al. (2002) for *Acinetobacter* sp. After the initial reduction, 4-CP concentration decreased linearly but with reduced rates at the higher initial concentration of 4-chlorophenol. It implies that 4-CP acts as a self-inhibitor. Therefore, the Haldane's model was applied to describe the 4-CP transformation rate by resting cells of *Stenotrophomonas maltophilia* KB2 strain and kinetic equation parameters were estimated using the least – square error method with NLREG program (Sherrod 2010) giving: $k_c = 0.229$ 1/h, $K_{sc} = 0.696$ g_c/m^3, $K_{Ic} = 43.82$ g_c/m^3 ($R^2 = 0.997$).

Finally, the equation describing the specific transformation rate of 4-CP by resting cells of KB2 strain takes the form:

$$q_c = -\frac{1}{X}\frac{dS_c}{dt} = \frac{0.229\,S_c}{0.696 + Sc + \dfrac{S_c^2}{43.82}} \tag{11}$$

The observed biomass transformation capacity T_c^b can be obtained by dividing $(-q_c)$ by (μ) (Criddle, 1993):

$$T_c^b = \frac{1}{\dfrac{\left(b - Y_{xg}^M q_g\right)}{q_c} + \dfrac{1}{T_c^{b*}}} \tag{12}$$

where T_c^{b*} is the true biomass transformation capacity. For resting cells ($q_g = 0$) Eq. (12) simplifies to:

$$T_c^b = \frac{1}{\dfrac{b}{q_c} + \dfrac{1}{T_c^{b*}}} \tag{13}$$

As can be seen from the above dependence, to calculate the observed value of this parameter it is necessary to know T_c^{b*}, which is determined by fitting the experimental profiles of changes in 4-CP and biomass concentration with those obtained as a result of solving the system of equations:

$$q_c = -\frac{1}{X}\frac{dS_c}{dt} = \frac{k_c S_c}{K_{sc} + S_c + \frac{S_c^2}{K_{Ig}}} \tag{14}$$

$$\mu = \frac{1}{X}\frac{dX}{dt} = -\frac{q_c}{T_c^{b*}} - b \tag{15}$$

Because the transformation time used in studies of biodegradation of the initial doses of 4-CP did not exceed several hours, therefore, according to the suggestion of Jesus et al. (2016), the value of $b = 0$ was assumed in the calculations; hence, $T_c^{b*} = T_c^b$. With this assumption, the value of the sought parameter can also be determined by preparing for each experimental point a graph of changes of 4-CP and biomass concentrations during the experiment. The slope of the straight line approximating the experimental data in $S_c = f(X)$ graph indicates the demanded T_c^b value.

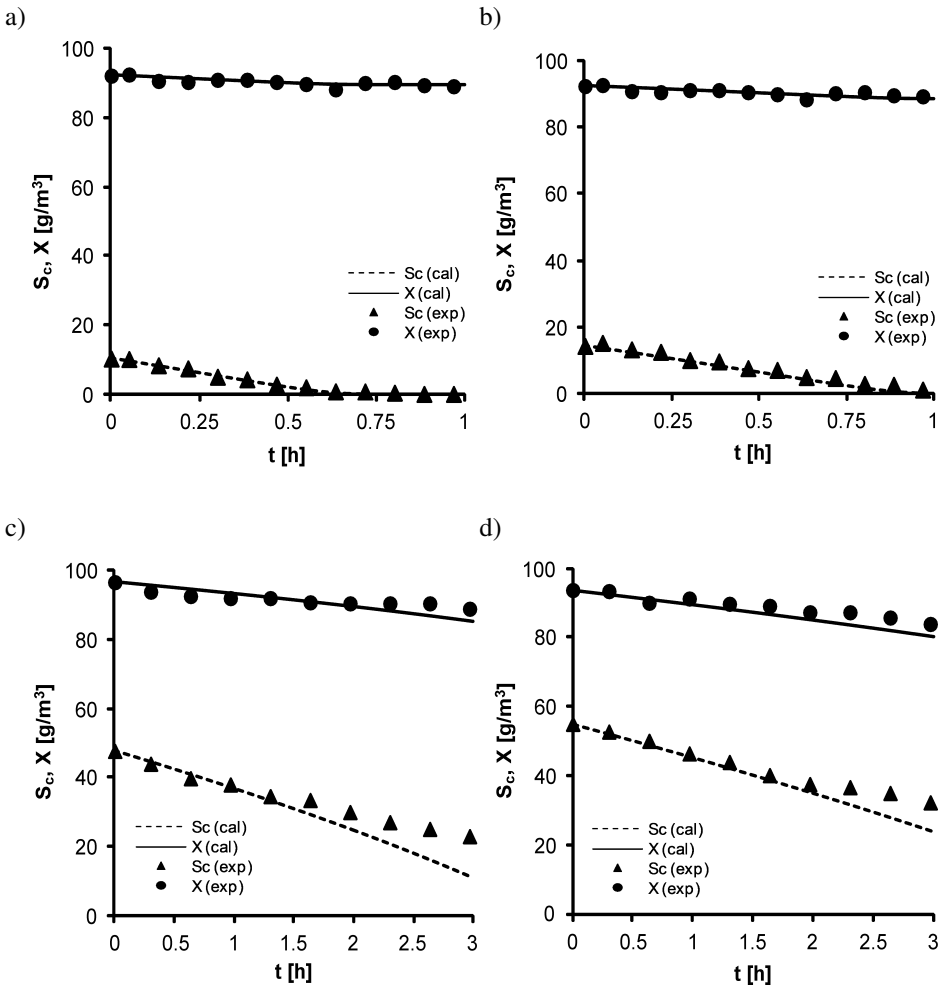

Fig. 3. Experimental and calculated profiles of changes in the biomass and 4-CP concentration. Resting cell experiments, $X^0 = 94$ g_x/m^3 a) $S_c^0 = 10.23$ g_c/m^3, $T_c^b = 3.78$ g_c/g_x; b) $S_c^0 = 14.42$ g_c/m^3, $T_c^b = 3.78$ g_c/g_x; c) $S_c^0 = 47.8$ g_c/m^3, $T_c^b = 3.21$ g_c/g_x; d) $S_c^0 = 55.05$ g_c/m^3, $T_c^b = 2.32$ g_c/g_x

In Fig. 3 the experimental data and the calculated profiles of changes in 4-chlorophenol and biomass concentration were compared for several selected sets of the initial data.

3.4. Cometabolic 4-CP transformation in the presence of phenol

To test whether the *Stenotrophomonas maltophilia* KB2 strain can use 4-chlorophenol as the only source of carbon and energy for cell growth, a series of tests was carried out, changing the initial concentration of 4-CP within the range of 10–50 g/m^3. There was neither a significant loss of substrate nor an increase in the amount of biomass. The experiments confirmed that the KB2 strain cannot use 4-chlorophenol as the growth substrate.

Two series of experiments were then carried out at the same initial biomass concentration equal to ~ 60 g$_x$/m^3:

- for the constant initial concentration of 4-CP equal to ~ 50 g$_c$/m^3, the initial phenol concentration was changed in the range of 88.6–280 g$_g$/m^3,
- for the constant initial concentration of phenol (two set of cultures: $S_g^0 = \sim 88$ g$_g$/m^3 and 68 g$_g$/m^3) the initial 4-CP concentrations were changed in the range of 22–66 g$_c$/m^3.

While analyzing the results of the first series of measurements it was found that for the whole studied range of changes in 4-CP/phenol mass ratio ($1 \div 6$), both substrates were degraded simultaneously, and the time of their degradation did not exceed 6 hours. These results confirmed a high activity of the tested KB2 strain in comparison with e.g. *Acinetobacter* sp. described by Hao et al. (2002).

A large initial dose of phenol is the first to be degraded, and the significant degradation of 4-chlorophenol begins when the phenol concentration decreases to ~ 150 g/m^3. At the time of complete depletion of phenol, the cell growth phase practically ceased. The profiles of concentration changes of a cometabolite in the cultures with increasing initial concentration of phenol presented in Fig. 4 indicate that both substrates compete for an active site of the enzyme, which results in a decrease in the 4-CP degradation rate.

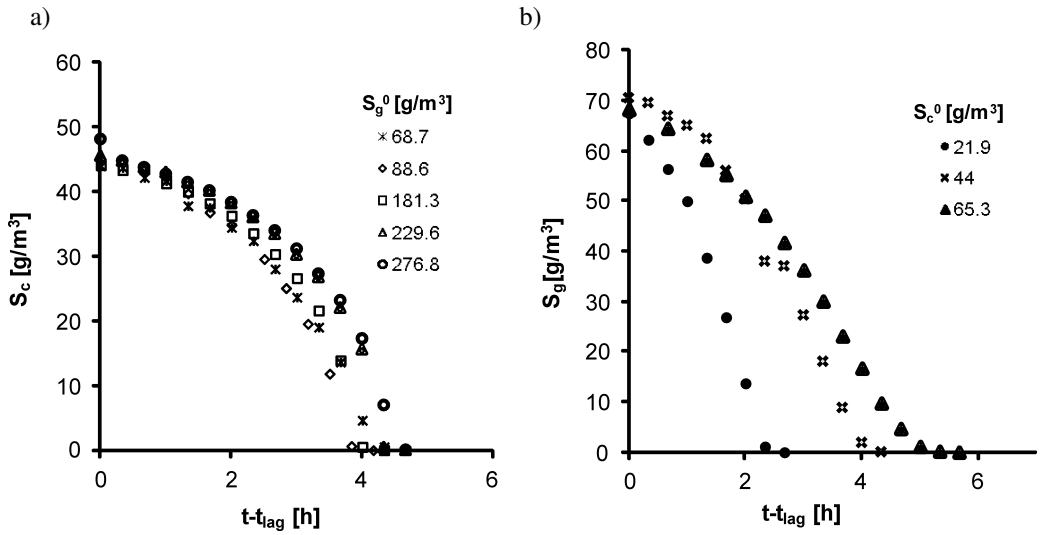

Fig. 4. Profiles of changes in 4-CP concentration with an increase in the initial concentration of phenol (a); $S_c^0 = 45$ g$_c$/m^3 and phenol concentration with an increase in the initial concentration of 4-CP (b), $S_g^0 = 68$ g$_g$/m^3

The results of the second series of measurements indicate that increase of the 4-CP initial concentration, when $S_g^0 = \text{const}$, significantly extended the decay of the entire phenol dose and limited the growth of biomass (not presented). A detailed analysis of the experiments carried out for various growth and non-growth substrates ratios was presented in the previous study (Szczyrba et al., 2016).

3.5. Observed growth substrate transformation yield, T_c^g

A graph of changes in the concentration of 4-CP and phenol in one single culture $(S_c = f(S_g))$ should be drawn for each experimental point to determine the value of T_c^g. The slope of the straight line approximating the experimental point determines the value of growth substrate transformation yield. The T_c^g value, determined in such a way, changes with the (S_g^0/S_c^0) ratio (Fig. 5).

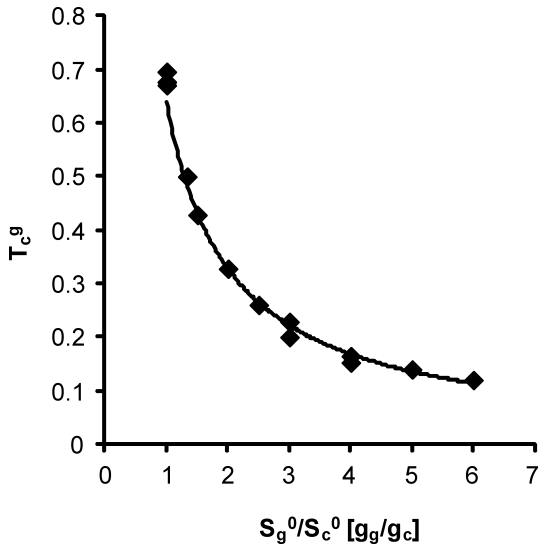

Fig. 5. The dependence of T_c^g on the initial ratio of growth substrate and cometabolite concentrations

4. RESULTS AND DISCUSSION

In the first series of calculations, the *CIModel* was tested. Therefore, for each set of experimental data the fitting K_{isg} and K_{isc} parameters were searched, for the application of which in the calculations ensures that the computed and experimentally obtained profiles of phenol, 4-CP and biomass concentrations were consistent. In the second series of calculations for the same set of experimental data, the *CNIModel* was tested to search for the optimal parameter values of: K_1, K_2, n_1, n_2.

In all calculations, the parameter values of: k_g, K_{sg} and K_{Ig} and k_c, K_{sc} and K_{Ic} were accepted as known and equalling the values determined in the biodegradation tests of phenol alone and the transformation of 4-CP by resting cells.

In order to estimate the changes in time of the phenol, 4-CP and biomass concentrations, the systems of ordinary differential equations (Eqs. (1)–(3)) for *CIModel* and (5, 8, 9) for *CNIModel*) with initial conditions were solved. The calculations were done using the MATLAB software. The standard MATLAB function ode45, which uses the 4th order of Runge-Kutta algorithm, was used to integrate the proper system of differential equations. The calculations were performed with a maximum relative error not exceeding 10^{-6}. To estimate the values of fitting parameters, the MATLAB fminsearch function from the MATLAB Optimization Toolbox was used. This function uses the simplex method to search for the loss function minimum. The loss function was defined as the sum of squared differences of the calculated and experimental values of phenol and 4-CP concentrations (the statistical weight factors were assumed equal to 1, 1 and 0 for phenol, 4-CP and biomass, respectively):

$$L = \sum_{i=1}^{n} \left(S_{g,\exp,i} - S_{g,calc,i}\right)^2 + \sum_{i=1}^{n} \left(S_{c,\exp,i} - S_{c,calc,i}\right)^2 \tag{16}$$

When testing the *CNIModel* it was found that by estimating the values of four fitting parameters very good adjustment of concentration profiles can be achieved but often with unrealistic values of these parameters. Therefore, following Wang et al. (2013), the estimation of n_1 and n_2 exponents was omitted, accepting the suggested by Saez and Rittmann (1993) values: $n_1 = 1$ and $n_2 = 2.5$. Finally, by testing the *CNIModel*, only the values of K_1 and K_2 coefficients were estimated. Figures 6a–6d show, as an example, the results of calculations for 4 sets of the initial phenol and 4-CP concentrations, while in Table 1 the values of the estimated parameters of both models together with the statistical parameters (correlation coefficients) of the selected model are presented.

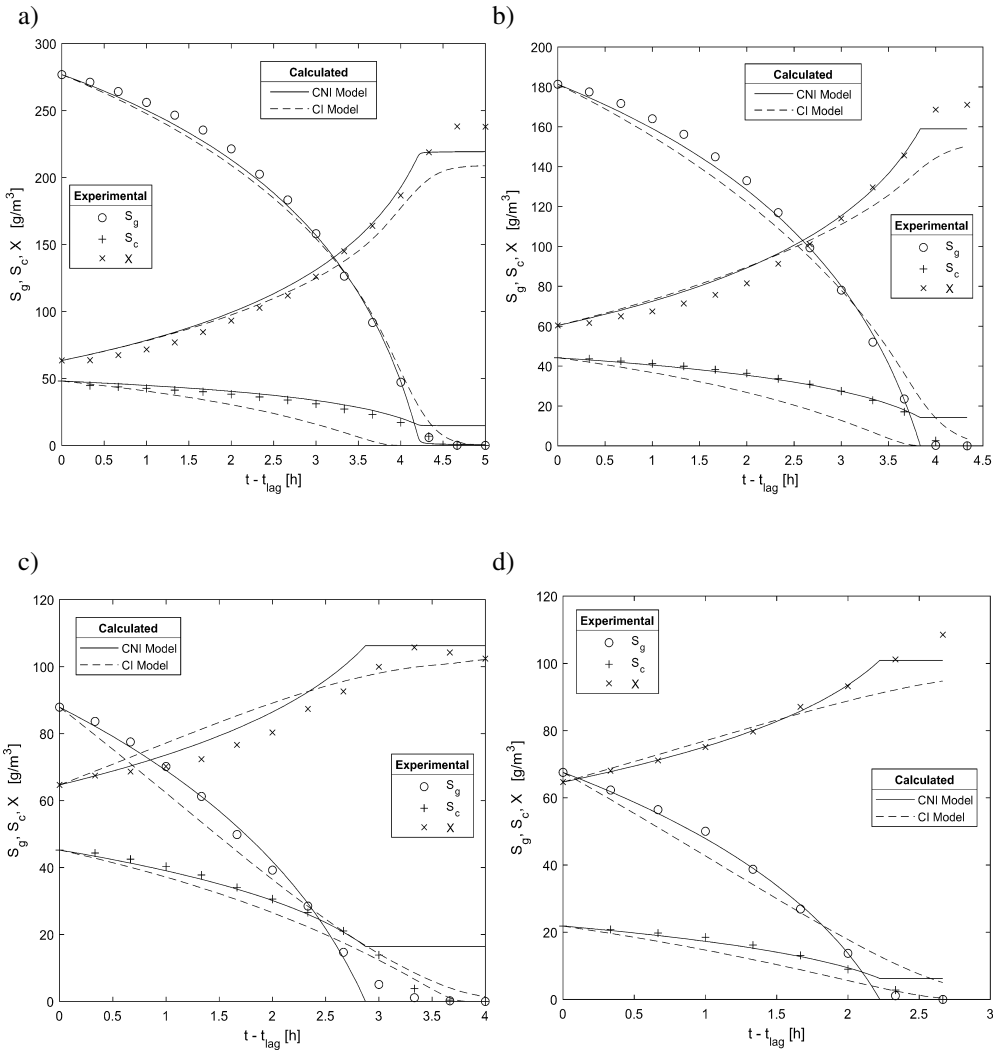

Fig. 6. Experimental and calculated profiles of 4-CP, phenol and biomass concentration: a) $S_g^0 = 276.8$ g$_g$/m^3, $S_c^0 = 48.4$ g$_c$/m^3, $t_{lag} = 1$ h; b) $S_g^0 = 181.8$ g$_g$/m^3, $S_c^0 = 44.2$ g$_c$/m^3, $t_{lag} = 1$ h; c) $S_g^0 = 88.6$ g$_g$/m^3, $S_c^0 = 43.95$ g$_c$/m^3, $t_{lag} = 0.667$ h; d) $S_g^0 = 67.6$ g$_g$/m^3, $S_c^0 = 21.9$ g$_c$/m^3, $t_{lag} = 0.667$ h

Analyzing the test results of both models it can be concluded that, in general, the *CNIModel* approximates experimental data better. Further, the values of the estimated K_1 and K_2 constants indicate that both changes in 4-CP and phenol concentrations modify mainly k_g value which proves that both phenol, 4-CP and their oxidation products compete for regulatory sites of monooxygenase in a non-competitive manner. Similar conclusions can be found in studies of Saez and Rittmann (1993) and Wang et al. (2013). The disadvantage of this model is that when phenol is depleted, it is not possible to continue calculating the changes in 4-CP and biomass concentration. Meanwhile, as experiments indicated, even when there is no

Table 1. Experimental data and predictions of the CI and CNI models for the process of cometabolic transformation of 4-CP on the presence of phenol

N	S_g^0/S_c^0	Y_{xg}^s	CI model		CNI model				
			K_{isg}	K_{isc}	$K1$	$K2$	R^2		
							P	4-CP	biomass
1	276.8/48.1	0.588	31.36	5.19	43.86	243.1	0.999	0.986	0.995
2	229.6/45.5	0.579	30.12	5.015	31.49	354.89	0.9994	0.977	0.995
3	181.3/44.2	0.5548	28.5	5.706	23.37	2234.87	0.9993	0.9755	0.995
4	129.7/44	0.4918	20.1	6.47	14.97	25518.96	0.9992	0.967	0.988
5	88.6/43.95	0.4675	23.5	8.22	12.41	143364.4	0.9984	0.958	0.988
6	88.8/65.8	0.366	21×10^{12}	8.34	10.34	30888.4	0.9974	0.966	0.99
7	91.86/22.1	0.6498	2.82	9.33	9.39	15.465	0.9997	0.957	0.99
8	68.8/65.3	0.249	∞	8.25	7.41	141404.45	0.996	0.972	0.967
9	67.6/43.95	0.4149	2.38	5.8	5.81	614567	0.995	0.976	0.981
10	67.56/21.9	0.5521	2.56	6.92	5.53	145861.4	0.9993	0.979	0.989

phenol in the culture medium, both the transformation of 4-CP by active cells and the growth of biomass on phenol oxidation products were observed. Therefore, as shown for example in Fig. 6, the concentration of 4-CP equal to 0 was not achieved because phenol was the first to be depleted.

Only in one case (point 7) a slight effect of competitive inhibition on the half-saturation constant was recorded. It seems advisable to carry out a detailed analysis of both the form of z_2 correction factor and n_2 exponent because, in the form proposed by Saez and Rittmann (1993), the correction term for the half-saturation constant was always much smaller than one.

5. CONCLUSIONS

The subject of the analysis presented in the study was the cometabolic biodegradation of 4-CP by *Stenotrophomonas maltophilia* KB2 strain in the presence of phenol as the only source of carbon and energy. The examined strain was isolated from the activated sludge of a sewage treatment plant. As demonstrated by the experiments, the KB2 strain effectively degraded the phenol alone (the time of total biodegradation of the ~ 500 g_g/m^3 dose did not exceed 6 hours) but was not able to use 4-CP as a growth substrate. In dual-substrate cultures, carried out for a wide range of changes in the initial ratio of substrate concentrations, both compounds were simultaneously degraded and the time of their total biodegradation did not exceed several hours. Thus, the experiments confirmed the high potential of *Stenotrophomonas maltophilia* KB2 strain as an efficient and versatile microorganism for the bioaugmentation of highly contaminated effluents and soil. A wide range of growth and non-growth substrate concentrations tested made it possible to create the experimental data base which was used to verify the two selected kinetic models: *Competitive Inhibition Model (CIM)* and more universal *Competitive/Noncompetitive Inhibition Model (CNIM)*. Satisfactory consistency of experimental and calculated data qualified CNIM as a practical tool for analyzing also other cometabolic systems. Nevertheless, additional verification of correction terms of the *CNIModel* was recommended.

SYMBOLS

b	decay coefficient, 1/h
CIM	competitive inhibition model
CNIM	competitive/noncompetitive inhibition model
g	acceleration due to gravity, m/s^2
k_k	Haldane model parameter, g_k/g_x h
K_{sk}	half-saturation coefficient, g_k/m^3
K_{Ik}	substrate self-inhibition constant, g_k/m^3
K_{isk}	competitive inhibition coefficient, g_k/m^3
K_1	inhibition constant associated with k_g (Eq. (5)), g_c/m^3
K_2	inhibition constant associated with K_{sg} (Eq. (5)), $(g_c/g_g)^{n2}$
L	loss function
n_1, n_2	positive empirical exponents (Eq. (5))
P	phenol
4-CP	4-Chlorophenol
S_k	substrate concentration, g_k/m^3
t	time, h
T_c^b	biomass transformation capacity, g_c/g_x
T_c^{b*}	true biomass transformation capacity, g_c/g_x
T_c^g	growth substrate transformation capacity, g_c/g_g
X	biomass concentration, g_x/m^3
Y_{xg}	observed biomass yield coefficient, g_x/g_g
Y_{xg}^S	biomass growth yield for dual-substrate tests, g_x/g_g

Greek symbols

q_k	specific substrate degradation rate, g_k/g_xh
μ	specific biomass growth rate, 1/h
μ_m	Haldane model coefficient, 1/h

Subscripts

av	average values
c	cometabolite (4-CP)
g	growth substrate (P)
k	denotes *k*th substrate ($k = g$ (phenol) or c (4-CP))
lag	refers to lag phase

Superscripts

0	initial value
M	maximum value

REFERENCES

Agarry S.E., Solomon B.O., 2008. Kinetics of batch microbial degradation of phenols by indigenous Pseudomonas fluorescence. *Int. J. Environ. Sci. Tech.*, 5, 223–232 DOI: 10.1007/BF03326016.

Aktas Ö., 2012. Effect of S_0/X_0 ratio and acclimation on respirometry of activated sludge in the cometabolic biodegradation of phenolic compounds. *Bioresour. Technol.*, 111, 98–104. DOI: 10.1016/j.biortech.2012.02.027.

Alvarez-Cohen L., Speitel Jr. G.E., 2001. Kinetics of aerobic cometabolism of chlorinated solvents. *Biodegradation*, 12, 105–126. DOI: 10.1023/A:1012075322466.

Arora P.K., Bae H., 2014. Bacterial degradation of chlorophenols and their derivatives. *Microb. Cell Fact.*, 13, 31. DOI: 10.1186/1475-2859-13-31.

Arutchelvan A., Kanakasabai V., Nagarajan S., Muralikrishnan V., 2005. Isolation and identification of novel high strength phenol degrading bacterial strains from phenol formaldehyde resin manufacturing industrial wastewater. *J. Hazard. Mater*., 27, 238–243. DOI: 10.1016/j.jhazmat.2005.04.043.

Aryal M., Liakopoulou-Kyriakides M., 2015. Phenol degradation in aqueous solutions by Pseudomonas sp. isolated from contaminated soil of mining industry. *J. Water Sustainability*, 5, 45–57. DOI: 10.11912/jws.2015.5.2.45-57.

Bae H.S., Lee J.M., Kim Y.B., Lee S.T., 1996. Biodegradation of the mixtures of 4-chlorophenol and phenol by *Comamonas testo*steroni CPW301. *Biodegradation*, 7, 463–469. DOI: 10.1007/BF00115293.

Bajaj M., Gallert C., Winter J., 2009. Phenol degradation kinetics of an aerobic mixed culture. *Biochem. Eng. J*., 46, 205–209. DOI: 10.1016/j.bej.2009.05.021.

Busca G., Berardinelli S., Resini C., Arrighi L., 2008. Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J. Hazard. Mater*., 160, 265–288. DOI: 10.1016/j.jhazmat.2008.03.045.

Criddle C.S., 1993. The kinetics of cometabolism. *Biotechnol. Bioeng*., 41, 1048–1056. DOI: 10.1002/bit.260411107.

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

Chen Y-M., Lin T-F., Huang C., Lin J-C., 2008. Cometabolic degradation kinetics of TCE and phenol by *Pseudomonas putida*. *Chemosphere*, 72, 1671–1680. DOI: 10.1016/j.chemosphere.2008.05.035.

Danis T.G., Albanis T.A., Petrakis D.E., Pomonis P.L., 1998. Removal of chlorinated phenols from aqueous solutions by adsorption on alumina pillared clays and mesoporous alumina phosphates. *Water Res*., 32, 295–302. DOI: 10.1016/S0043-1354(97)00206-6.

De Los Cobos-Vasconcelos D., Santoyo-Tepole F., Juarez-Ramirez C., Ruiz-Ordaz N., Galindez-Mayer C.J.J., 2006. Cometabolic degradation of chlorophenols by a strain of *Burkholderia* in fed-batch culture. *Enzyme Microb. Technol*., 40, 57–60. DOI: 10.1016/j.enzmictec.2005.10.038.

Elango V., Kurtz H.D., Freedman D.L., 2011. Aerobic cometabolism of trichloroethane and cis-dichloroethane with benzene and chlorinated benzenes as growth substrates. *Chemosphere*, 84, 247–253. DOI.10.1016/j.chemosphere.2011.04.007.

Ely R.L., Williamson K.J., Guenther R.B., Hyman M.R., Arp D.J., 1995. A cometabolic kinetics model incorporating enzyme inhibition, inactivation, and recovery. I. Model development, analysis, and testing. *Biotechnol. Bioeng*., 46, 218–231. DOI: 10.1002/bit.260460305.

Ely R.L., Kenneth J.W., Hyman M.R., Arp D.J.,1997. Cometabolism of chlorinated solvents by nitrifying bacteria: Kinetics, substrate interactions, toxicity effects, and bacterial response. *Biotechnol. Bioeng*., 54, 520–534. DOI: 10.1002/(SICI)1097-0290(19970620)54:6<520::AID-BIT3>3.0.CO;2-L.

Essam T., Amin M.A., El Tayeb O., Mattiasson B., Guieysse B., 2010. Kinetics and metabolic versatility of highly tolerant phenol degrading *Alcaligenes* strain TW1. *J. Hazard. Mater*., 173, 783–788. DOI: 10.1016/j.jhazmat.2009.09.006.

Farrell A., Quilty B., 2002. Substrate dependent autoaggregation of *Pseudomonas putida* CP1 during the degradation of mono-chlorophenols and phenol. *J. Indus. Microbiol. Biotechnol*., 28, 316–324. DOI: 10.1038/sj/jim/7000.

Felshia S.Ch., Karthick N.A., Thilagam R., Chandralekha A., Raghavarao K.S.M.S., Gnanamani A., 2017. Efficacy of free and encapsulated *Bacillus lichenformis* strain SL10 on degradation of phenol: A comparative study of degradation kinetics. *J. Environ. Manage.*, 197, 373–383. DOI: 10.1016/j.jenvman.2017.04.005.

Field J.A., Sierra-Alvarez R., 2008. Microbial degradation of chlorinated benzenes. *Biodegradation*, 19(4), 463–480. DOI: 10.1007/s10532-007-9155-1.

Frascari D., Pinelli D., Nocentini M., Baleani E., Cappelletti M., Fedi S., 2008. A kinetic study of chlorinated solvent cometabolic biodegradation by propane-grown *Rhodococcus* sp PB1. *Biochem. Eng. J*., 42, 139–147. DOI: 10.1016/j.bej.2008.06.011.

Frascari D., Zannoni A., Pinell D., Nocentini M., 2007. Chloroform aerobic cometabolism by butane-utilizing bacteria in bioaugmented and non-bioaugmented soil/groundwater microcosms. *Process Biochem.*, 42, 1218–1228. DOI: 10.1016/j.procbio.2007.05.018.

Goswami M., Shivaraman N., Singh R.P., 2005. Microbial metabolism of 2-chlorophenol, phenol and *p*-cresol by Rhodococcus erythropolis M1 in co-culture with *Pseudomnas fluorecens* P1. *Microbiol. Res.*, 160, 101–109 DOI: 10.1016/j.micres.2004.10.004.

Greń I., Guzik U., Wojcieszyńska D., Łabużek S., 2008. Molekularne podstawy rozkładu ksenobiotycznych związków aromatycznych. *Biotechnologia*, 2(81), 58–67 (in Polish).

Greń I., Wojcieszyńska D., Guzik U., Perkosz M., Hupert-Kocurek K., 2010. Enhanced biotransformation of mononitrophenols by Stenotrophomonas maltophilia KB2 in the presence of aromatic compounds of plant origin. *World J. Microbiol. Biotechnol.*, 26, 289–295. DOI.10.1007/s11274-009-0172-6.

Guzik, U., Greń, I., Wojcieszyńska, D., Łabużek, S., 2009. Isolation and characterization of a novel strain of Stenotrophomonas maltophilia possessing various dioxygenases for monocyclic hydrocarbon degradation. *Braz. J. Microbiol.*, 40, 285–291. DOI: 10.1590/S1517-838220090002000014.

Hao O.J., Kim M.H., Seagren E.A., Kim H., 2002. Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species. *Chemosphere*, 46, 797–807. DOI: 10.1016/S0045-6535(01)00182-5.

Hill G.A., Milne B.J., Nawrocki P.A., 1996. Cometabolic degradation of 4-chlorophenol by Alcaligenes eutrophus. *Appl. Microbiol. Biotechnol.*, 46, 163–168. DOI: 10.1007/s002530050799.

Horvath R.S., 1972. Microbial co-metabolism and the degradation of organic compounds in nature. *Bacteriol. Rev.*, 36(2), 146–155.

Jesus J., Frascari D., Pozdniakowa T., Danko A.S., 2016. Kinetics of aerobic cometabolic biodegradation of chlorinated and brominated aliphatic hydrocarbons: a review. *J. Hazard. Mater.*, 309, 17–52. DOI: 10.1016/j.jhazmat.2016.07.065.

Jain A.K., Gupta V.K., Jain S., 2004. Removal of chlorophenols using industrial wastes. *Environ. Sci. Technol.*, 38, 1195–1200. DOI: 10.1021/es034412u.

Jiang Y., Ren N., Cai X., Wu D., Qiao L. Lin S., 2008. Biodegradation of phenol and 4-chlorophenol by the mutant strain CTM 2. *Chin. J. Chem. Eng.*, 16, 796–800. DOI: 10.1016/S1004-9541(08)60158-5.

Jiang Y., Wen J., Lan L. Hu Z., 2007. Biodegradation of phenol and 4-chlorophenol by the yeast *Candida tropicalis*. *Biodegradation*, 18, 719–729. DOI: 10.1007/s10532-007-9100-3.

Kim J.H., Oh K.K., Lee S.T., Kim S.W., Hong S.I., 2002. Biodegradation of phenol and chlorophenols with defined mixed culture in shake-flasks and a packed bed reactor. *Process Biochem.*, 37, 1367–1373. DOI: 10.1016/S0032-9592(02)00007-9.

Kim M.H., Hao O.J., 1999. Cometabolic degradation of chlorophenols by *Acinetobacter* species. *Wat. Res.*, 33, 562–574. DOI: 10.1016/S0043-1354(98)00228-0.

Krastanov A., Alexieva Z., Yemendzhiev H., 2013. Microbial degradation of phenol and phenolic derivatives. *Eng. Life Sci.*, 13, 76–87. DOI: 10.1002/elsc.201100227.

Lee C.-Y., Lee Y.-P., 2007. Degradation of 4-chlorophenol by enriched mixed cultures utilizing phenol and glucose as added growth substrate. *World J. Microb. Biot.*, 23, 383–391. DOI: 10.1007/s11274-006-9235-0.

Leontievsky A.A., Mysoedova n.m., Baskunov B.P., Evans C.S., Golovleva L.A., 2000. Transformatin of 2,4,6-trichlorophenol by the white rot fungi *Panus tigrinus* and Coriolus versicolor. *Biodegradation*, 11, 331–340. DOI: 10.1023/A:1011154209569.

Liu J., Jia X., Wen J., Zhou Z., 2012. Substrate interactions and kinetics study of phenolic compounds biodegradation by *Pseudomonas* sp. cbp1-3. *Biochem. Eng. J.*, 67, 156–166. DOI: 10.1016/j.bej.2012.06.008.

Loh K.-Ch., Wu T., 2006. Cometabolic transformation of 2-chlorophenol and 4-chlorophenol in the presence of phenol by *Pseudomonas putida*. *Can. J. Chem. Eng.*, 84, 356–367. DOI: 10.1002/cjce.5450840312.

Margesin R., Fonteyne P.A., Redl B., 2005. Low-temperature biodegradation of high amounts of phenol by *Rhodococcus* sp. and *Basidiomycetous* yeasts. *Res. Microbiol.*, 156, 68–75. DOI: 10.1016/j.resmic.2004.08.002.

Monsalvo V.M., Mohedano A.F., Casas J.A., Rodríguez J.J., 2009. Cometabolic biodegradation of 4-chlorophenol by sequencing batch reactors at different temperatures. *Bioresource Technol.*, 100, 4572–4578. DOI: 10.1016/j.biortech.2009.04.044.

Saéz P.B., Rittmann B.E., 1993. Biodegradation kinetics of a mixture containing a primary substrate (phenol) and an inhibitory co-metabolite (4-chlorophenol). *Biodegradation*, 4, 3–21. DOI: 10.1007/BF00701451.

Sahinkaya E., Dilek F.B., 2005. Biodegradation of 4-chlorophenol by acclimated and unacclimated activated sludge – Evaluation of biokinetic coefficients. *Environ. Res*., 99, 243–252. DOI: 10.1016/j.envres.2004.11.005.

Sam S. P., Ng S.L., Rohana A., 2018. Kinetics of biodegradation of phenol and p-nitrophenol by acclimated activated sludge. *J. Phys. Sci*., 29(Supp. 1), 107–113. DOI: 10.21315/jps2018.29.s1.14.

Shourian M., Noghabi K.A., Zahiri H.S., Bagheri T., Karbalaei R., Mollaei M., Rad I., Ahadi S., Raheb J., Abbasi H., 2009. Efficient phenol degradation by a newly characterized *Pseudomonas* sp. SA01 isolated from pharmaceutical wastewaters. *Desalination*, 246, 577–594. DOI: 10.1016/j.desal.2008.07.015.

Singh S., Singh B.B., Chandra R., 2009. Biodegradation in batch culture by pure mixed strains of Paenibacillus sp. and Bacillus cereus. *Pol. J. Microbiol.*, 58, 319–325.

Sinha P.K., Sinha A., Das M., 2011. Microbial removal of phenol and *p*-chlorophenol from industrial waste water using *Rhodococcus* sp. RSP8 and its growth kinetic modeling. *J. Water Resource Prot*., 3, 634–642. DOI: 10.4236/jwarp.2011.38073.

Tobajas M., Monsalvo V.M., Mohedano A.F., Rodriguez J.J., 2012. Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by *Comamonas testosteroni*. *J. Environ. Manage*., 95, 116–121. DOI: 10.1016/j.jenvman.2010.09.030.

Wang J., Ma X., Liu S., Sun P., Fan P., Xia Ch., 2012. Biodegradation of phenol and 4-chlorophenol by *Candida tropicalis*. *W. Procedia Environ. Sci.*, 16, 299–303. DOI: 10.1016/j.proenv.2012.10.042.

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

Wang S.J., Loh K.C., 1999. Modeling the role of metabolic intermediates in kinetics of phenol biodegradation. *Enzyme Microb. Tech.*, 25, 177–184. DOI: 10.1016/S0141-0229(99)00060-5.

Wang S.J., Loh K.C., 2000. New cell growth pattern on mixed substrates and substrate utilization in cometabolic transformation of 4-chlorophenol. *Water Res*. 34, 3786–3794. DOI: 10.1016/S0043-1354(00)00144-5.

Wojcieszyńska D., Greń I., Łabużek S., Respondek M., 2007. Specyficzność substratowa oraz wrażliwość monooksygenazy fenolowej ze szczepu *Stenotrophomonas maltophilia* KB2 a jej potencjalne zastosowanie w bioremediacji środowiska. *Biotechnologia*, 2 (77), 181–191 (in Polish).

Wojcieszyńska D., Hupert-Kocurek K., Greń I., Guzik U., 2011. High activity catechol 2,3-dioxygenase from the cresols-degrading *Stenotrophomonas maltophilia* strain KB2. *International Biodeterioration and Biodegradation*, 65, 853–858. DOI: 10.1016/j.ibiod.2011.06.006.

Zhang Y., Lu D., Ju T., Wang L., Lin Sh., Zhao Y., Wang Ch., He H., Du Y. 2013. Biodegradation of phenol using *Bacillus cereus* WJ1 and evaluation of degradation efficiency based on a graphene modified electrode. *Int. J. Electrochem. Sci.*, 8, 504–519.

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