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Kinetics of Phenol Oxidation by *Candida tropicalis*: Effects of Oxygen Supply Rate and Nutrients on Phenol Inhibition

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ABSTRACT. The kinetics of phenol degradation was estimated in a fed-batch reactor system. Effects of oxygen and nutrient excess or limitation as well as the presence of several essential ions on the phenol- and oxygen-specific uptake rates achieved simultaneously in a bioreactor were shown. *Candida tropicalis* was grown on phenol as the only carbon and energy source. Applying the best fit of polynomial function, the maximum specific uptake rates of phenol and oxygen, the critical concentrations of phenol, the half-saturation constants and inhibition constants were determined. Linear relationship between specific phenol uptake rate and the exogenous respiration rate was found regardless of the kind and presence of essential nutrients. At oxygen limitation both the phenol uptake rate and the cell affinity to phenol decreased more strongly compared with those under nutrient limitation. Oxygen in excess resulted in a significant increase of cell tolerance toward phenol. The presence of essential nutrients increased the specific phenol degradation rate and led to complete phenol oxidation.

Phenol, like other xenobiotics, belongs to widespread pollutants that have still to be removed from many contaminated sites. Its inhibitory concentration is not constant but is linked closely to environmental conditions. For this reason many studies have focused on biodegradation of phenol and phenolic compounds with respect to degradation pathways (Dagley 1985; Katayama-Hyraiama *et al.* 1991*a*), the energy-yielding conversion (Müller and Babel 1994), the kind of degraders – bacteria (Hill and Robinson 1975; Bechard *et al.* 1990; Heipieper *et al.* 1991; Li *et al.* 1989; Koturi *et al.*1991; Folsom *et al.*1990; Okaygun *et al.* 1992; Müller and Babel 1996; Straube *et al.* 1990; Hensel and Straube 1990; Collins and Daugulis 1997; Marek *et al.* 2001; Muñoz *et al.* 2001), fungi (Mörtberg and Neujahr 1987; Spanning and Neujahr 1987, 1990; Anselmo *et al.* 1989; Stephenson 1990; Katayama-Hyraiama *et al.* 1991*a,b*; Zilly *et al.* 2002) and mixed cultures (Fan *et al.* 1987; Watson-Craik and Senior 1989; Hobson and Millis 1990; Mörsen and Rehm 1990; Martius *et al.* 1996; Páca and Martius 1996; Branyik *et al.* 2000; Weigner *et al.* 2001). Relatively less information on yeast cultures grown in media with phenolics can be found (Shimizu *et al.* 1973*a,b*; Yang and Humphrey 1975; Krug *et al.* 1985; Mörtberg and Neujahr 1987; Spanning and Neujahr 1987, 1990; Stephenson 1990; Katayama-Hyraiama *et al.* 1991*a,b*; Collins and Daugulis 1997; Chang *et al.* 1998; Ruiz-Ordaz *et al.* 1998).

For description of aerobic phenol degradation some authors (Hensel and Straube 1990; Straube *et al.* 1990; Müller *et al.* 1995; Ruiz-Ordaz *et al.* 1998; Sanchez *et al.* 1998) used mathematical models describing the growth of microorganisms. Limbert and Betts (1995) and Stephenson (1990) evaluated the biodegradation kinetics using respirometry alone. Anselmo *et al.* (1989) and Mason (1994) evaluated both the phenol and oxygen uptake rates. Except for Mason (1994), others performed respiration measurements using Warburg (Anselmo *et al.* 1989; Limbert and Betts 1995) or Gilson respirometers (Stephenson 1990) where artificial conditions with subsequent gradual transition to oxygen limitation can occur. For this reason we used here the dynamic method for a simultaneous measurement of the specific phenol and oxygen uptake directly in the bioreactor under conditions of excess oxygen.

Previously we focused on phenol degradation and oxygen consumption by a mixed microbial population with respect to the oxygen supply rate, to the presence or absence of nutrients (Páca and Martius 1996) and, using the actual phenolic wastewater from coking, to the effects of dissolved oxygen concentration and temperature (Martius *et al.* 1996).

Here we carry out a kinetic study with *C. tropicalis*. We aim to elucidate the effect of oxygen supply rate and the presence of essential nutrients on the kinetics of phenol removal; we also show relationships

between the specific rate of phenol degradation and exogenous respiration rate under conditions with and without a partial inhibition by phenol.

MATERIALS AND METHODS

Microorganism. The strain of *Candida tropicalis* was isolated from an undefined mixed culture originally obtained from oil-polluted sandy aquifer. The culture was maintained on an agar slope with glucose and phenol as carbon and energy sources with mineral salts at 4 °C; however, the strain was able to use phenol as the only carbon and energy source.

Media and culture conditions and kinetic measurement. The growth medium (YNB) contained (in g/L): Yeast Nitrogen Base (YNB, *Difco*) 1.7, $(NH_4)_2SO_4$ 5, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, NaCl 0.1; 1 mL per L medium of amino acid solution (g/L; L-methionine 4, L-tryptophan 4, L-histidine 2). Phenol and oxygen uptake measurements were done in YNB, tap water with (in g/L) $(NH_4)_2SO_4$ 0.3, with KH_2PO_4 0.2, with NaH_2PO_4 0.2 and tap water only.

The cells were obtained from a fed-batch oxistat-culture system. Phenol, as the sole carbon and energy source, was added according to the adjusted level of dissolved oxygen concentration to avoid the effect of substrate inhibition. The culture parameters were 28 °C, pH 5.2 and the volumetric oxygen transfer coefficient ($K_L a$) of 340 h^{-1}. The cultivations were performed in an LF 2 bioreactor (*Development Workshop, Academy of Sciences of the Czech Republic*, Prague) with a working volume of 2 L. After separation, the cells were washed three times with tap water.

Phenol degradation and oxygen consumption were examined either in the same bioreactor and under the same conditions or in Erlenmeyer flasks (working volume 0.1 L) on a shaker ($K_L a$ = 12 h^{-1}). The starting biomass concentration was 3 g/L. Each biodegradation experiment was repeated four times.

Analytical methods. Dry cell mass was determined gravimetrically. The samples were centrifuged, washed, and dried for 1 h at 70 °C and for 2 h at 105 °C.

Phenol was determined by HPLC equipped with UV detector–Spectro monitor 3200 set at 270 nm and column Nucleosil 12-5 C18, 250 × 4 mm (*Watrex*, Czechia) under isocratic conditions (eluent mixture methanol–water–H_3PO_4, 50 : 49 : 1). The signal was evaluated by Chromatography Station for Windows version 1.7 (CSW).

Oxygen uptake was determined in the bioreactor using a Pt-Ag–AgCl electrode coupled with Oxytest analyzer (*Developmental Workshop, Academy of Sciences of the Czech Republic*, Prague).

Using the dynamic method (Páca and Grégr 1977) the volumetric oxygen transfer coefficients ($K_L a$) and the endogenous and whole respiration rates were calculated. From these values the exogenous respiration rate was calculated and plotted (Figs 1, 3–5).

RESULTS AND DISCUSSION

Degradation kinetic models. Two models were tested for their ability to describe substrate inhibition regarding the specific phenol removal rate q_S and exogenous respiration rate q_{O_2}. The first model used the Haldane equations given by

$$q_S = q_{s(max)}\, S/(K_S + S + S^2/K_i) \qquad (1)$$

or

$$q_{O_2} = q_{O_2(max)}\, S/(K'_S + S + S^2/K'_i) \qquad (2)$$

where S (mg/L) is the phenol concentration, $q_{S(max)}$ (mmol/g dry mass per h) the maximum specific phenol removal rate, $q_{O_2(max)}$ (mmol/g dry mass per h) the maximum exogenous respiration rate, K_S and K'_S (mg/L) are the half-saturation constants, and K_i and K'_i (mg/L) are inhibition constants. The kinetic constants of the Haldane model were found both by a method of the random number regression and by linear analysis after transformation to a second-order polynomial.

In the second model, experimental data were approximated by n-order polynomials to estimate the kinetic constants. The linear regression analysis of least squares with Gauss method for the solution of linear equations set was used for the best fit.

Fig. 1 shows both fitting methods. The Haldane model did not fit the experimental data as well as the polynomial function in both ranges of the phenol limitation and the phenol inhibition. Also Hobson and

Millis (1990) and Mörsen and Rehm (1990) found a poor fit of the Haldane equations. We evaluated therefore all kinetic parameters using the fitting by the *n*-order polynomial curve.

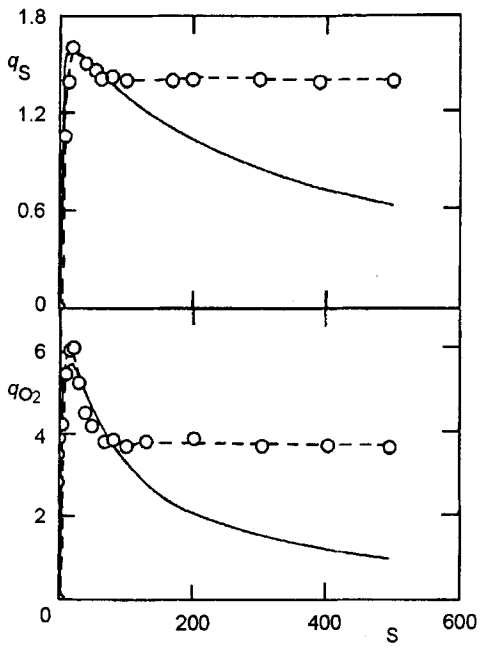

Fig. 1. Specific phenol removal rate (q_S *above*) and specific oxygen consumption rate (q_{O2}; *below*) (both in mmol/g dry mass per h) in tap water at K_La = 340 h^{-1} (oxygen in excess); S – phenol, mg/L; *circles* – experimental data, *full lines* – Haldane equations, *dashed lines* – polynomial function.

Effect of oxygen supply rate on phenol degradation. The plot of specific uptake rates of phenol *vs.* phenol concentrations in the growth medium at two different oxygen supply rates shows (Fig. 2 *left*) that under oxygen limitation in shaking flasks (K_La = 12 h^{-1}) typical inhibition kinetics occurred.

At oxygen in excess (K_La = 340 h^{-1}), significantly higher rates of phenol degradation were achieved. Under these conditions the maximum specific phenol uptake rate was more than twice higher than under oxygen limitation. The kinetic parameters $q_{S(max)}$, S_{crit} (critical phenol concentration; mg/L), K_S and K_i calculated are shown in Table I. The excess of oxygen caused both a significant increase in affinity of the yeast cells to phenol and a decrease of cell sensitivity to phenol inhibition (comparing K_S and K_i). A similar positive effect of oxygen excess on the cell sensitivity to phenol was observed in our previous study with a mixed culture (Páca and Martius 1996) and by Anselmo *et al.* (1989).

Effect of nutrients on phenol degradation. After cultivation in the bioreactor under oxygen excess (K_La = 340 h^{-1}; Fig. 2 *right*) the kinetic parameters were calculated (Table I). Addition of ammonia ions did not affect the q_S values. However, the presence of phosphate, K^+, Na^+ and all nutrients resulted in an increase of the specific phenol degradation rate. The presence of nutrients did not affect the affinity of the cells to phenol or the K_S value.

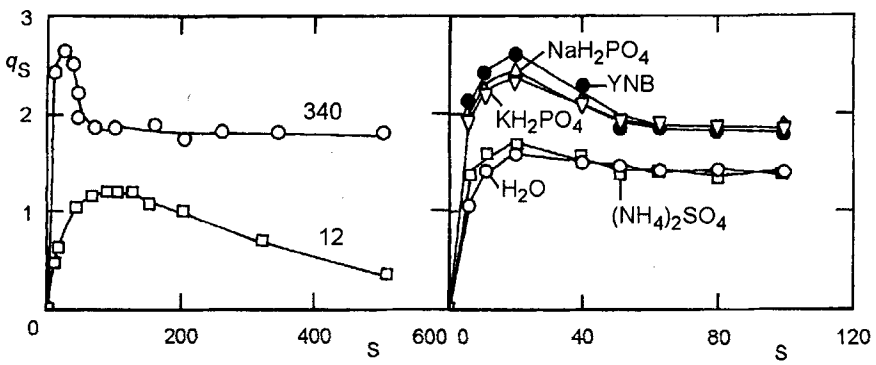

Fig. 2. Effect of oxygen supply rate (*left*; K_La = 12 and 340 h^{-1}) and nutrients (*right*; K_La = 340 h^{-1}; in the YNB medium, tap water with and without inorganic salts) on the specific rate of phenol degradation; average values from four experiments.

Effect of nutrients on oxygen consumption. From the dynamic method (Páca and Grégr 1977) the endogenous respiration ($q_{O2(e)}$, mmol/g dry mass per h) was evaluated. The $q_{O2(e)}$ value in the YNB medium and in tap water with addition of salts was 0.6 mmol/g dry mass per h and in tap water 0.8 mmol/g dry mass per h, respectively. The exogenous respiration rates at different phenol concentrations are shown in Fig. 3. It is necessary to stress that the q_{O_2} values describe the exogenous respiration rate, *i.e.* the quantity of oxygen consumed for phenol oxidation alone. The calculated kinetic parameters are shown in Table II. Comparing Figs 2 and 3 the presence of every nutrient can be seen to increase the maximum respiration rate at critical phenol concentration in the range of 5–62 % while the maximum specific phenol uptake rate increased in the range of 26–86 %. The presence of nutrients also increased the affinity of cells for phenol (K'_S values). On the other hand, the effect of phenol inhibition was much more pronounced with respect to the oxygen uptake

rate. Nevertheless, also here the significant protective effect of oxygen excess resulted in stable q_{O_2} up to the phenol concentration of 500 mg/L, and the inhibition constant (K'_i) therefore could not be determined. The presence of nutrients did not affect the critical phenol concentration (Table II).

Table I. Kinetic parameters[a] of phenol uptake by *C. tropicalis* at oxygen and nutrient limitation

Nutrient[b]	K_La h^{-1}	$q_{s(max)}$ mmol/g per h	S_{crit} mg/L	K_s mg/L	K_i mg/L
Without limitation					
YNB	340	2.60 ± 0.05	28.0 ± 1.0	4.6 ± 0.9	>500
Oxygen limitation					
YNB	12	1.20 ± 0.06	90.5 ± 2.5	14.9 ± 0.8	376 ± 15
Nutrient limitation[c]					
None[d]	340	1.60 ± 0.05	21.3 ± 1.0	4.5 ± 0.8	>500
$(NH_4)_2SO_4$	340	1.73 ± 0.06	21.1 ± 1.1	3.7 ± 0.8	–
NaH_2PO_4	340	2.33 ± 0.05	19.8 ± 2.0	3.1 ± 0.8	–
KH_2PO_4	340	2.41 ± 0.07	18.6 ± 1.5	3.3 ± 0.9	–

[a]K_La – oxygen supply rate, $q_{S(max)}$ – the maximum specific phenol removal rate, K_s – half-saturation constant, K_i – inhibition constant, S_{crit} – critical phenol concentration.
[b]YNB-complex medium or tap water with inorganic salt.
[c]Oxygen in excess.
[d]Tap water.

Under oxygen limitation the rate of phenol degradation was less than one-half as under oxygen excess (cf. Fig. 2) and the cell affinity for phenol also significantly decreased. This fact consequently postpones the critical concentration value at which the phenol inhibition starts to occur (Fig. 2, Table I). Oxygen and nutrient limitations during phenol degradation result in a lower specific phenol degradation rate and a more significant effect of phenol inhibition (K_i). The nutrient limitation did not influence the cell affinity for phenol and the critical phenol concentration.

The highest specific phenol degradation rate together with the high cell affinity for phenol, were achieved with excess of both oxygen and nutrients in the medium. In addition, the protective effect of an excess oxygen concentration on the phenol inhibition was found to be more pronounced in *C. tropicalis* (Páca and Martius 1996; Fig. 2).

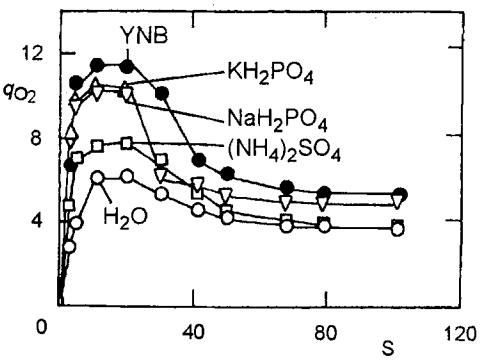

Fig. 3. Effect of nutrients on the specific oxygen consumption rate (exogenous respiration rate only) at K_La = 340 h^{-1}; average values from four experiments.

Table II. Effect of nutrients on kinetic parameters of oxygen uptake at excess oxygen (K_La = 340 h^{-1})

Nutrient	$q_{O2(max)}$ mmol/g per h	S'_{crit} mg/L	K'_S mg/L
None	6.2 ± 1.0	15.5 ± 0.8	3.3 ± 0.7
$(NH_4)_2SO_4$	7.9 ± 0.8	17.5 ± 0.9	1.6 ± 0.5
NaH_2PO_4	11.0 ± 0.8	15.6 ± 0.8	1.4 ± 0.5
KH_2PO_4	11.4 ± 0.7	15.6 ± 0.8	1.4 ± 0.5
YNB	11.7 ± 0.7	16.5 ± 0.8	1.6 ± 0.5

[a]$q_{O2(max)}$ – maximum exogenous respiration rate, K'_S – half-saturation constant, for oxygen, S'_{crit} – critical phenol concentration for oxygen.

For *C. tropicalis* growing on phenol Ruiz-Ordaz *et al.* (1998) found K_S = 3.7 mg/L and K_i = 484 mg/L, Shimidzu *et al.* (1973*a*) then 110 and 1200 mg/L. Yang and Humphrey (1975) reported these parameters for *Trichosporon cutaneum* to be K_S = 1.66 mg/L, K_i = 380 mg/L and for *Pseudomonas putida* K_S = 99.4 mg/L, K_i = 106 mg/L. On the other hand, Sanchez *et al.* (1998) found for *P. putida* K_S = 4.64 mg/L and K_i = 398 mg/L, and Seker *et al.* (1997) found for *P. putida* the relevant parameters to be 75.9 and 99.4 mg/L. In *Pseudomonas* sp., K_S and K_i were found to be 75.9 and 105.9 mg/L, respectively (Limbert and Betts 1995). Hobson and Millis (1990) determined in mixed culture K_S = 40 mg/L and K_i = 374 mg/L. The broad variation of the kinetic values may be explained by different culture conditions, in many cases even not quite precisely defined. Our kinetic parameters (obtained under oxygen or nutrient limited and unlimited conditions as well as in the presence of different ions in the medium; Table I) correspond with the above data.

The value of $q_{S(max)}$ = 39.3 mmol/g per h found by Hobson and Millis (1990) was relatively high due to the use of mixed culture and the two-stage chemostat system. Using the formula $q_{S(max)} = \mu_{max}/Y_{X/S}$ (where μ_{max} is the maximum specific growth rate and $Y_{X/S}$ is the biomass yield from phenol) and neglecting the maintenance energy we could calculate the $q_{S(max)}$ to be 11 mmol/g per h according to Seker *et al.* (1997), 7.8 (Tang and Fan 1987), 6.1 (Worden and Donaldson 1987), and 2.5 (Sanchez *et al.* 1998). Our $q_{S(max)}$ values (Table I) were 1.6–2.6 mmol/g per h.

To describe the effects of the individual nutrients and the phenol inhibition we evaluated the correlations between the metabolic quotients (Fig. 4). This correlation was carried out for the phenol degradation without any substrate inhibition (at S_{crit}) and for two increasing partial phenol inhibitions (at S = 40 and 100 mg/L). Despite the presence or absence of nutrients the ratio of q_{O_2}/q_S remained constant. When no phenol inhibition (at S_{crit} and $q_{O_2(max)}/q_{S(max)}$) was observed the oxygen consumption was very close to that expected for complete phenol oxidation. Comparing our value of $Y_{O_2/S}$ (equal to $q_{O_2(max)}/q_{S(max)}$ = 4.48 mmol O_2 per mmol phenol) with those described by Seker *et al.* (1997) – 4.53 mmol O_2 per mmol of phenol (steady state of continuous culture), Arvin *et al.* (1991) – 7.35 (mixed culture), Livingston and Chase (1989) – 4.12, Tang and Fan (1987) – 4.12 and Worden and Donaldson (1987) – 4.11, much better agreement was seen than with the recalculated q_S values.

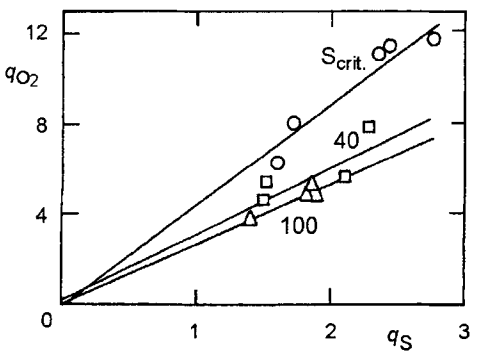

Fig. 4. Effects of nutrients and phenol inhibition at excess oxygen on the correlations of metabolic quotients; S_{crit}, q_{O_2}/q_S = 4.48 mol/mol; S = 40 mg/L, q_{O_2}/q_S = 2.97 mol/mol; S = 100 mg/L, q_{O_2}/q_S = 2.69 mol/mol.

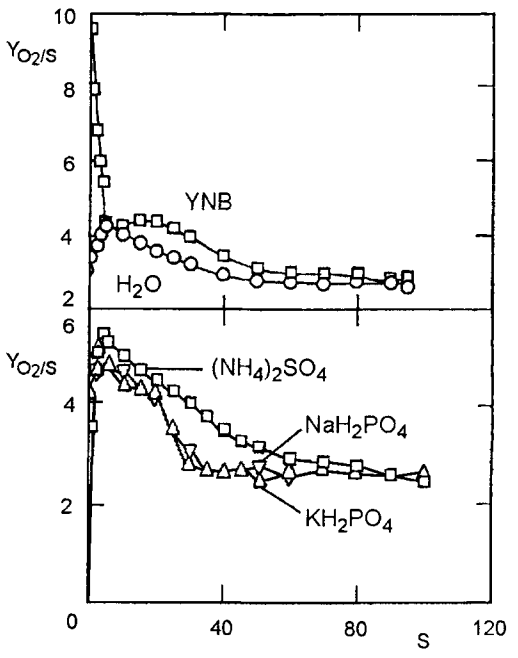

Fig. 5. Effect of nutrients and phenol inhibition at excess oxygen on the ratio of metabolic quotients; *above* – with and without nutrient limitation, *below* – with inorganic salts.

For a more accurate description of the inhibitory effect of phenol and its physiological interpretation we compared q_{O_2}/q_S with the initial phenol concentrations in the different media (Fig. 5). In the growth YNB medium and in the presence of each nutrient the highest values of q_{O_2}/q_S were achieved below S_{crit} (Figs 2 and 3). The exogenous respiration values were closely related to those that we expected for complete phenol oxidation. Similar behavior (but only in the case of a growth medium and an immobilized biofilm reactor) was also observed by Arvin *et al.* (1991). Fig. 5 demonstrate that the oxygen excess significantly decreases the sensitivity of cells to phenol inhibition and a decrease in respiration acti-

vity at increased phenol concentration; this indicates that phenol primarily suppresses the cell respiratory enzymes.

Comparing our kinetic parameters of the exogenous respiration (Table III) with those reported by Anselnmo *et al.* (1989) ($q_{O_2(max)} = 7$ mmol/g per h, $K_S = 85$ mg/L) our *C. tropicalis* showed the same $q_{O_2(max)}$ values but a much higher affinity for phenol.

We can summarize that q_S was significantly affected by the oxygen supply rate; under oxygen limitation q_S showed typical inhibitory kinetics; excess of oxygen resulted not only in higher q_S values but increased the cell resistance to phenol; Haldane kinetics could not be recommended for fitting of experimental data (the application of a polynomial function gave most realistic kinetic parameters); the lack of nutrients caused a lesser decrease of $q_{S(max)}$ than did oxygen limitation; the presence of essential nutrients increased the cell affinity for phenol and the exogenous respiration rate; the kind and presence of essential nutrients influenced the value of phenol concentration at which substrate inhibition began; phenol inhibition affected primarily the respiratory enzymes, resulting in incomplete phenol oxidation.

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REFERENCES

ANSELMO A.M., CABRAL J.M.S., NOVAIS J.M.: The adsorption of *Fusarium flocciferum* spores on cellite particles and their use in the degradation of phenol. *Appl.Microbiol.Biotechnol.* **31**, 200–203 (1989).

ARVIN E., JENSEN B.K., GUNDERSEN A.T.: Biodegradation kinetics of phenols in a aerobic biofilm at low concentrations. *Water Sci. Technol.* **23**, 1375–1384 (1991).

BECHARD G., BISAILLON J.G., BEAUDET R.: Degradation of phenols by a bacterial consortium under methanogenic conditions. *Can.J. Microbiol.* **36**, 573–578 (1990).

BRANYIK T., KUNCOVÁ G., PÁCA J.: The use of silica gel prepared by sol–gel method and polyurethane foam as microbial carriers in the continuous degradation of phenol. *Appl.Microbiol.Biotechnol.* **54**, 168–172 (2000).

CHANG Y.H., LI C.T., CHANG M.C., SHIEH W.K.: Phenol degradation by *Candida tropicalis* and its fusant. *Biotechnol.Bioeng.* **60**, 391–395 (1998).

COLLINS L.D., DAUGULIS A.J.: Characterization and optimization of a two-phase partitioning bioreactor for the biodegradation of phenol. *Appl.Microbiol.Biotechnol.* **48**, 18–22 (1997).

DAGLEY S.: Microbial metabolism of aromatic compounds, pp. 483–505 in M. Moo-Young (Ed.): *Comprehensive Biotechnology, Vol. 1.* Pergamon Press, Oxford (UK) 1985.

FAN L.S., FUJIE K., LONG T.R., TANG W.T.: Characteristics of draft tube gas–liquid–solid fluidized-bed bioreactor with immobilized living cells for phenol degradation. *Biotechnol.Bioeng.* **30**, 498–504 (1987).

FOLSOM B.R., CHAPMAN P.J., PRITCHARD P.H.: Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates. *Appl.Environ.Microbiol.* **56**, 1279–1285 (1990).

HEIPIEPER H.J., KEWELOH H., REHM H.J.: Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. *Appl.Environ.Microbiol.* **57**, 1213–1217 (1991).

HENSEL J., STRAUBE G.: Kinetic studies of phenol degradation by *Rhodococcus* sp. P1 – II. Continuous cultivation. *Antonie van Leeuwenhoek* **57**, 33–36 (1990).

HILL G.A., ROBINSON C.W.: Substrate inhibition kinetics: phenol degradation by *Pseudomonas putida*. *Biotechnol.Bioeng.* **17**, 1599–1615 (1975).

HOBSON M.J., MILLIS N.F.: Chemostat studies of a mixed culture growing on phenolics. *Res.J.Water Pollut.Control Fed.* **62**, 684–691 (1990).

KATAYAMA-HYRAIAMA K., TOBITA S., HIRAYAMA K.: Metabolic pathway of phenol in *Rhodotorula rubra*. *J.Gen.Appl.Microbiol.* **37**, 379–388 (1991*a*).

KATAYAMA-HIRAYAMA K., TOBITA S., HIRAYAMA K.: Degradation of phenol by yeast *Rhodotorula*. *J.Gen.Appl.Microbiol.* **37**, 147–156 (1991*b*).

KOTURI G., ROBINSON C.W., INNIS W.E.: Phenol degradation by a psychrotrophic strain of *Pseudomonas putida*. *Appl.Microbiol. Biotechnol.* **34**, 539–543 (1991).

KRUG M., ZIEGLER H., STRAUBE G.: Degradation of phenolics compounds by the yeast *Candida tropicalis* HP 15 – I. Physiology of growth and substrate utilization. *J.Basic Microbiol.* **25**, 103–110 (1985).

LI J., HUMPHREY A.: Kinetic and fluorometric behaviour of a phenol fermentation, pp. 190–206 in T.K. Ghose (Ed.): *Bioprocess Engineering. The First Generation.* Ellis Horwood, Chichester (UK) 1989.

LIMBERT E.S.B., BETTS W.B.: Kinetics of bio-oxidation of medium comparising phenol and mixture of organic contaminants. *Appl. Microbiol.Biotechnol.* **43**, 165–170 (1995).

LIVINGSTON A.G., CHASE H.A.: Modeling phenol degradation in a fluidized-bed reactor. *Am.Inst.Chem.Engin.J.* **35**, 1980–1992 (1989).

MAREK J., PÁCA J., HALECKÝ M., KOUTSKÝ B., SOBOTKA M., KESHAVARZ T.: Effect of pH and loading manner on the start-up period of peat biofilter degrading xylene and toluene mixture. *Folia Microbiol.* **46**, 205–209 (2001).

MARTIUS G.G.S., STOTTMEISTER U., JECHOREK M., PÁCA J.: Inhibition concentration of phenolic substances under different cultivation conditions – part II: impact of dissolved oxygen concentration and temperature on degradation kinetics. *Acta Hydrochim.Hydrobiol.* **24**, 168–175 (1996).

MASON J.R.: The induction and repression of benzene and catechol oxidizing capacity of *Pseudomonas putida* ML2 studied in perturbed chemostat culture. *Arch.Microbiol.* **162**, 57–62 (1994).

MÖRSEN A., REHM H.J.: Degradation of phenol by a defined mixed culture immobilized by adsorption on activated carbon and sintered glass. *Appl.Microbiol.Biotechnol.* **33**, 206–121 (1990).

MÖRTBERG M., NEUJAHR H.Y.: *In situ* and *in vitro* kinetics of phenol hydroxylase. *Biochem.Biophys.Res.Commun.* **146**, 41–46 (1987).

MÜLLER R.H., BABEL W.: Phenol and its derivatives as heterotrophic substrates for microbial growth – an energetic comparison. *Appl. Microbiol.Biotechnol.* **42**, 446–451 (1994).

MÜLLER R.H., BABEL W.: Growth rate-dependent expression of phenol-assimilation pathways in *Alcaligenes eutrophus* JMP 134 – the influence of formate as an auxiliary energy source on phenol conversion characteristics. *Appl.Microbiol.Biotechnol.* **46**, 156–162 (1996).

MÜLLER R.H., BLEY T., BABEL W.: Transient state cultivation as a means for determining maximum growth rates of microorganism in inhibition kinetics. *J.Microbiol.Meth.* **22**, 209–219 (1995).

MUÑOZ J.A., PÉREZ-ESTEBAN B., ESTEBAN M., DE LA ESCALERA S., GÓMEZ M.A., MARTÍNEZ-TOLEDO M.V., GONZÁLEZ-LÓPEZ J.: Growth of moderately halophilic bacteria isolated from sea water using phenol as the sole carbon source. *Folia Microbiol.* **46**, 297–302 (2001).

OKAYGUN M.S., GREEN L.A., AKGERMAN A.: Effects of consecutive pulsing of an inhibitory substrate on biodegradation kinetics. *Environ.Sci.Technol.* **26**, 1746–1752 (1992).

PÁCA J., GRÉGR V.: Method for the determination of oxygen transfer coefficients (K_La) with the correction for the actual cultivation conditions. *J.Appl.Chem.Biotechnol.* **27**, 155–164 (1977).

PÁCA J., MARTIUS G.G.S.: Inhibition concentration of phenolic substances under different cultivation conditions – part I: phenol oxidation by mixed microbial population in a model system. *Acta Hydrochim.Hydrobiol.* **24**, 127–131 (1996).

RUIZ-ORDAZ N., HERNANDEZ-MANZANO E., RUIZ-LAGUNEZ J.C., CRISTIANI-URBINA E., GALINDEZ-MAYER J.: Growth kinetic model that describes the inhibitory and lytic effect of phenol on *Candida tropicalis* yeast. *Biotechnol.Progr.* **14**, 966–969 (1998).

SANCHEZ J.L.G., KAMP B., ONYSKO K.A., BUDMAN H., ROBINSON C.W.: Double inhibition model for degradation of phenol by *Pseudomonas putida* Q5. *Biotechnol.Bioeng.* **60**, 560–568 (1998).

SEKER S., BEYENAL H., SALIH B., TANYOLAÇ A.: Multi-substrate growth kinetic of *Pseudomonas putida* for phenol removal. *Appl. Microbiol.Biotechnol.* **47**, 610–614 (1997).

SHIMIZU T., AKITAYA K., FUKUCHI M., NEI N., ICHIKAWA K.: Basic decomposition parameters of phenol by *Candida tropicalis*. *J.Ferment.Technol.* **51**, 803–808 (1973*a*).

SHIMIZU T., UNO T., DAN Y., NEI N., ICHIKAWA K.: Continuous treatment of waste water containing phenol by *Candida tropicalis*. *J.Ferment.Technol.* **51**, 809–812 (1973*b*).

SPANNING A., NEUJAHR H.Y.: Growth and enzyme synthesis during continuous culture of *Trichosporon cutaneum* on phenol. *Biotechnol.Bioeng.* **24**, 464–468 (1987).

SPANNING A., NEUJAHR H.Y.: The effect of glucose on enzyme activities and phenol utilization in *Trichosporon cutaneum* grown in continuous culture. *J.Gen.Microbiol.* **136**, 1491–1495 (1990).

STEPHENSON T.: Substrate inhibition of phenol oxidation by a strain of *Candida tropicalis*. *Biotechnol.Lett.* **12**, 843–846 (1990).

STRAUBE G., HENSEL J., NIEDAN C., STRAUBE E.: Kinetic studies of phenol degradation by *Rhodococcus* sp. P1 – I. Batch cultivation. *Antonie van Leeuwenhoek* **57**, 29–32 (1990).

TANG W.T. FAN L.S.: Steady state phenol degradation in a draft tube, gas–liquid–solid fluidized bed-biofilm reactor. *Am.Inst.Chem. Engin.J.* **33**, 239–247 (1987).

WATSON-CRAIK I.A., SENIOR E.: Treatment of phenolic waste waters by co-disposal with refuse. *Water Res.* **23**, 1293–1303 (1989).

WEIGNER P., PÁCA J., LOSKOT P., KOUTSKÝ B., SOBOTKA M.: The start-up period of styrene degrading biofilters. *Folia Microbiol.* **46**, 211–216 (2001).

WORDEN R.M., DONALDSON T.L.: Dynamics of a biological fixed film for phenol degradation in a fluidized bed reactor. *Biotechnol. Bioeng.* **30**, 398–405 (1987).

YANG R.D., HUMPHREY A.E.: Dynamic and steady-state studies of phenol biodegradation in pure and mixed cultures. *Biotechnol. Bioeng.* **17**, 1211–1235 (1975).

ZILLY A., SOUZA C.G.M., BARBOSA-TESSMANN I.P., PERALTA R.M.: Decolorization of industrial dyes by a Brazilian strain of *Pleurotus pulmonarius* producing laccase as the sole phenol-oxidizing enzyme. *Folia Microbiol.* **47**, 273–277 (2002).