J Ind Microbiol Biotechnol (2008) 35:1297–1301
DOI 10.1007/s10295-008-0410-1

ORIGINAL PAPER

# Phenol and cresol mixture degradation by the yeast *Trichosporon cutaneum*

**Z. Alexieva · M. Gerginova · J. Manasiev · P. Zlateva · N. Shivarova · A. Krastanov**

Received: 20 March 2008 / Accepted: 29 July 2008 / Published online: 20 August 2008


**Abstract** Most industrial wastes contain different organic mixtures, making important the investigation on the microbial destruction of composite substrates. The capability of microbes to remove harmful chemicals from polluted environments strongly depends on the presence of other carbon and energy substrates. The effect of mixtures of phenol- and methyl-substituted phenols (*o*-, *m*-, *p*-cresol) on the growth behaviour and degradation capacity of *Trichosporon cutaneum* strain was investigated. The cell-free supernatants were analysed by HPLC. It was established that the presence of *o*-, *m*- and *p*- cresol has not prevented complete phenol assimilation but had significant delaying effect on the phenol degradation dynamics. The mutual influence of phenol and *p*-cresol was investigated. We developed the kinetic model on the basis of Haldane kinetics, which used model parameters from single-substrate experiments to predict the outcome of the two-substrate mixture experiment. The interaction coefficients indicating the degree to which phenol affects the biodegradation of *p*-cresol and vice versa were estimated. Quantitative estimation of interaction parameters is essential to facilitate the application of single or mixed cultures to the bio-treatment of hazardous compounds.

Z. Alexieva (✉) · M. Gerginova · J. Manasiev · N. Shivarova
Institute of Microbiology, Bulgarian Academy of Sciences,
Acad.G.Bontchev str., bl.26, 1113 Sofia, Bulgaria
e-mail: zlatkama@microbio.bas.bg

P. Zlateva
Institute of Control and System Research,
Bulgarian Academy of Sciences,
Acad.G.Bontchev str., bl.2, 1113 Sofia, Bulgaria

A. Krastanov
Department of Biotechnology, University of Food Technologies,
Maritza blvd. 26, 4002 Plovdiv, Bulgaria

**Keywords** Cresol · Degradation · Kinetic models · Phenol · *Trichosporon cutaneum*

## Introduction

The capability of microbes to remove harmful chemicals from polluted environments strongly depends on the presence of other compounds. Most industrial wastes contain different organic mixtures, making important the investigation on the microbial destruction of composite substrates. The removal or degradation of one or all components can be delayed and/or ceased depending on the composition of the studied mixture. Wastewaters from petroleum refineries, coal mining and variety of industrial chemical syntheses contain many aromatics as phenol, cresols, nitrophenols, etc. [17]. The metabolism of aromatic compounds, particularly phenol and its derivatives, has been intensively studied in prokaryotic microorganisms [11, 14]. Some members of yeast genera *Candida, Rodotorula, Trichosporon* and others that can metabolize phenolic compounds as a sole carbon and energy source are described in the literature [7, 17]. The first step in aerobic metabolism is phenol hydroxylation to catechol by phenol hydroxylase. Catechol, the product of the reaction catalysed by phenol hydroxylase, is a central intermediate in the degradation pathways of various aromatic compounds. It is metabolized by different strains via either the *ortho*- or the *meta*-fission pathway [15, 18]. Previously, we have isolated and described a strain of *Trichosporon cutaneum* which could grow aerobically and assimilate 1 g $l^{-1}$ phenol for a period of 18–20 h [2, 22].

The effect of other compounds in a mixture of homologous carbon and energy substrates on the biodegradation of a chemical can be positive as in the case of

co-metabolism [19, 21] or induction of required enzymes [13]. More commonly, negative interactions are reported [4, 5, 8]. Enhanced degradation of benzene and *p*-xylene in the mixture with toluene has been observed with a *Pseudomonas* strain [3]. Systems that exhibit co-metabolic behaviour with chlorinated aromatics have been reported by many authors [10, 16].

Quantitative estimation of interaction parameters is essential to facilitate the application of single or mixed cultures to the bio-treatment of hazardous compounds. The most models reported in the literature have been developed to describe only one substrate biodegradation. Recently, a few mathematical models of mixed homologous substrate consumption and microbial growth have been proposed. The experimental results with *Pseudomonas putida* and *Burkholderia* sp. strains growing individually and together on benzene, toluene, phenol and their mixtures are presented and mathematical models created to describe these results are compared [19]. They demonstrate that the simple models do not accurately predict the outcome of these biodegradation experiments, and describe the development of a new model for substrate mixtures, the sum kinetics with interaction parameter (SKIP) model. Recently, the biodegradation behaviour of *Candida tropicalis* in dual-substrate system has been described by kinetic equations adapted to fit the investigated process [23].

Simultaneous metabolism of phenol and cresol is reported by Hutchinson and Robinson [12], who have studied the degradation kinetics of both phenol and cresol by *P. putida* in mixture where the concentrations are kept well below the inhibitory level of the toxic substrate. The patterns of multiple substrate utilization and substrate interactions in the biodegradation of paired substrates (phenol/*p*-cresol, phenol/*o*-cresol) by *Arthrobacter* sp. MTCC1553 are quantified and categorized [13].

In this study, the effect of mixtures of phenol- and methyl-substituted phenols (*o*-, *m*-, *p*-cresol) on the growth behaviour and degradation capacity of *Trichosporon cutaneum* strain are investigated. The kinetic model describing the mutual influence of phenol and *p*-cresol is developed.

## Materials and methods

### Microorganisms and growth conditions

All data for biodegradation kinetics modelling were obtained from batch cultivation of the strain *T. cutaneum* R57 (National Bank of Industrial Microorganisms and Cell Cultures, N2414/1994). The cultivation was carried out on the carbon-free medium for yeast containing 6.7 g/l Yeast Nitrogen Base (YNB w/o AA, Fluka AG, Bucks, Switzerland) and appropriate concentrations of cresol isomers and phenol were added. All experiments were done at pH 5.5, and at ambient temperature (28–30 °C) on a New Brunswick rotary shaker (200 rev/min).

### Analytical methods

Cell density was monitored spectrophotometrically (UV–Vis Ultraspec 1000, LKB Vienna, Austria) by measuring the optical density at $\lambda = 610$ nm. The cell-free supernatants were analysed by HPLC performed on a reversed phase C18 column (Lichrosorb RP18, Perkin Elmer, Waltham, MA, USA) with methanol–water (50:50) liquid phase using an UV detector at 220 nm.

All chemicals were of the highest purity grade available (Fluka AG, Bucks, Switzerland; Merck, Whitehouse Station, NJ, USA).

## Results and discussion

### Degradation of phenol/cresol mixtures

Microorganisms capable of degrading one aromatic compound are often able to degrade other similar compounds. The capacity of *T. cutaneum* R57 strain to utilize up to 1 g/l phenol was already established [22]. Our previous investigations showed that *T. cutaneum* R57 could grow in a complete yeast extract/peptone media (YEP) comprising some hydroxy- and nitro-phenols [1]. The experiments involving the use of the investigated strain to degrade phenol, *o*-, *m*-, and *p*-cresol demonstrated a significant difference in the degradation capacity of the strain in relation to the investigated cresol isomers. No degradation of *o*-cresol was observed. The degradation of 0.1 g/l *m*-cresol was almost completely accomplished (85%). The degradation of 0.1 or 0.2 g/l *p*-cresol was completed out in 24 h [24].

The ability of *T. cutaneum* R57 to degrade the binary mixtures of phenol and its methylated derivatives was investigated in the present study. In the mixture of phenol and *o*- or *m*-cresol, we observed complete phenol and *m*-cresol degradation but no degradation of *o*-cresol was detected. The influence of substrate mixtures on *T. cutaneum* R57 growth was traced out. All experiments were performed in triplicate. The results are illustrated in Fig. 1. The data obtained showed no significant effect of all cresol isomers studied on the culture growth.

### The influence of cresol isomers concentrations on phenol degradation by *T. cutaneum* R57

The analysis of the effect of different cresol isomers' concentrations in the studied mixtures showed the

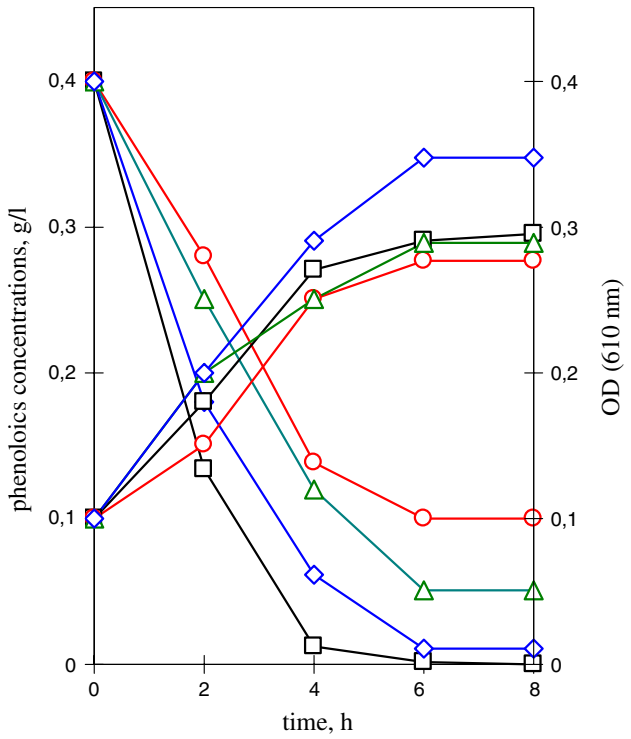


**Fig. 1** Growth and degradation curves of *T. cutaneum* R57 cultivated in a medium comprising dual mixtures of 0.3 g/l phenol and 0.1 g/l *o-*, *m-*, and *p*-cresols, one by one: *o*-cresol (*circle*); *m*-cresol (*triangle*); *p*-cresol (*diamond*); and 0.4 g/l phenol as a single carbon source (*square*)

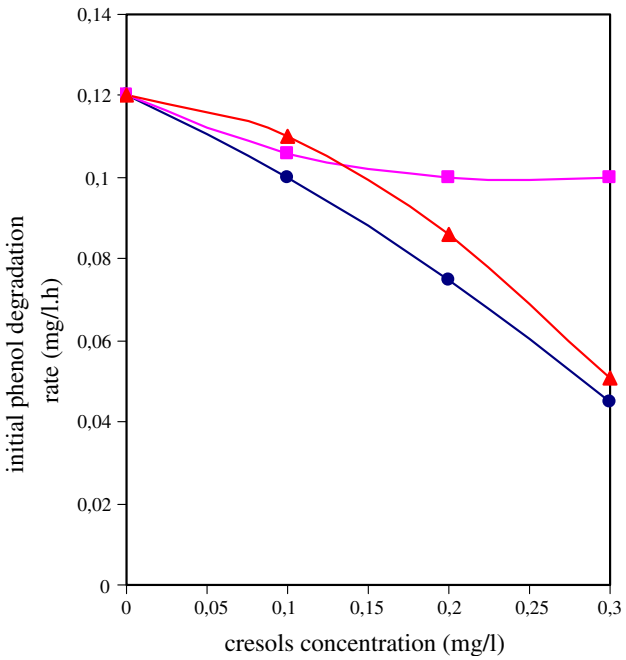


**Fig. 2** Effect of *o*-cresol (*filled circle*), *m*-cresol (*filled square*) and *p*-cresol (*filled triangle*) on the phenol degradation rate in 60 min

noticeable influence of higher *o*- and *p*-cresol concentrations on the initial phenol degradation rates. The assumed initial period of degradation was 60 min. The presence of *m*-cresol exerted a lighter effect on phenol degradation in this period of time (Fig. 2).

It is evident that *o*-, *m*-, and *p*-cresol have an equal inhibitory effect on phenol degradation. These data were in accordance with reported data about *ortho*-pathway of phenol and cresol degradation in *T. cutaneum* which was a precondition for the similar interactions between each of cresol isomers and phenol [15, 17]. The same type of experiments for a biodegradation of aromatic compounds mixtures has been carried out with different prokaryotes [9, 13, 20]. Recently, Yan et al. [23] reported results obtained by cultivating of a yeast strain of *C. tropicalis* in phenol/*m*-cresol mixture. They observed an intensive inhibition of the phenol degradation caused by *m*-cresol presence.

## Substrate interactions model for biodegradation of phenol/*p*-cresol mixture by *T. cutaneum* R57

Since *p*-cresol was the best degradable investigated compound, we used *p*-cresol/phenol mixture in the following experiments. *T. cutaneum* strain R57 consumed *p*-cresol and phenol simultaneously during most of the cultivation, but phenol degradation had begun before *p*-cresol degradation, and phenol was depleted first. The results of biodegradation experiments with mixtures of *p*-cresol (0.2 g/l) and phenol (0.3 g/l) are shown in Fig. 3.

A common model for cell growth on homologous substrate mixtures is a no-interaction sum kinetics model, in which the specific growth rate is a sum of the specific growth rates for each of the substrates. Models incorporating competitive interactions have also been used but none has given satisfactory results [18]. To create a kinetic model which was accounting for inhibitory interactions between phenol and *p*-cresol in mixture we used the idea for "sum kinetics with interaction parameters" (SKIP) model [19]. In SKIP models, the effect of one substrate presence $S_1$ ($S_2$) on some other substrate degradation $S_2$ ($S_1$) is given by $S_1 I_{1/2}$ ($S_2 I_{2/1}$) terms. The values of interaction coefficients $I_{1/2}$ ($I_{2/1}$) express the degree of inhibition exerted by substrate $S_1$ ($S_2$). The larger values of interaction coefficient correspond to stronger inhibition. In contrast to modified Monod model [19], we used a Haldane equation:

$$\mu(S_{\mathrm{ph}}) = \frac{\mu_{\max(\mathrm{ph})} S_{\mathrm{ph}}}{k_{\mathrm{s(ph)}} + S_{\mathrm{ph}} + S_{\mathrm{ph}}^2 / k_{\mathrm{i(ph)}}} \quad (1)$$

$$\mu(S_{\mathrm{cr}}) = \frac{\mu_{\max(\mathrm{cr})} S_{\mathrm{cr}}}{k_{\mathrm{s(cr)}} + S_{\mathrm{cr}} + S_{\mathrm{cr}}^2 / k_{\mathrm{i(cr)}}} \quad (2)$$

where $S_{\mathrm{ph}}$ was the phenol concentrations, $S_{\mathrm{cr}}$ was the cresol concentrations, $k_{\mathrm{s(ph)}}$ was the saturation constant of the

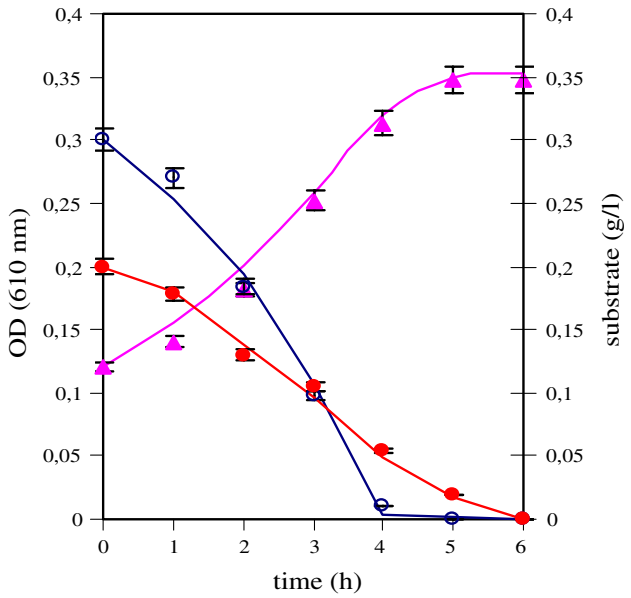


**Fig. 3** Experimental data and model output for biodegradation of a phenol/*p*-cresol mixture by *T. cutaneum* R57. *Symbols* indicate biomass (*filled triangle*), phenol (*open circle*) and *p*-cresol concentrations (*filled circle*)

phenol, $k_{s(cr)}$ was the saturation constant of the cresol, $\mu_{max(ph)}$ was the maximum specific growth rate for phenol, $\mu_{max(cr)}$ was the maximum specific growth rate for cresol, $k_{i(ph)}$ was the inhibition constant of the phenol, and $k_{i(cr)}$ was the inhibition constant of the cresol.

The growth kinetic model describing biodegradation of binary mixture of phenol and *p*-cresol can be presented as follows:

$$\frac{dX(t)}{dt} = \mu(S_{ph}, S_{cr})X(t) \quad (3)$$

$$\frac{dS_{ph}(t)}{dt} = -k_{1(ph)}\mu(S_{ph}, S_{cr})X(t) \quad (4)$$

$$\frac{dS_{cr}(t)}{dt} = -k_{2(cr)}\mu(S_{ph}, S_{cr})X(t) \quad (5)$$

where $X$ was the biomass concentration; $k_{1(ph)}$ and $k_{2(cr)}$ were the metabolic coefficients; $\mu(S_{ph}, S_{cr})$ was the specific growth rate for mixed substrates which was given in the following equation [21]:

$$\mu(S_{ph}, S_{cr}) = \frac{\mu_{max(ph)}S_{ph}}{k_{s(ph)} + S_{ph} + S_{ph}^2/k_{i(ph)} + I_{cr/ph}S_{cr}} + \frac{\mu_{max(cr)}S_{cr}}{k_{s(cr)} + S_{cr} + S_{cr}^2/k_{i(cr)} + I_{ph/cr}S_{ph}} \quad (6)$$

In this study, the parameter identification problem was reduced to estimation of values of the interaction parameters ($I_{ph/cr}$ and $I_{cr/ph}$) and the metabolic coefficients ($k_{1(ph)}$ and $k_{2(cr)}$). The interaction coefficients ($I_{cr/ph}$, $I_{ph/cr}$) indicated the degree to which *p*-cresol affected the phenol biodegradation and vice versa.

The optimization method for direct search was used. It is well known that the nonlinear optimization procedure is strongly sensitive to the initial values and the variation intervals of the model parameters. For this reason, the search for the values of the kinetic constants was constrained within boundaries predetermined on the basis of the process knowledge and experimental data. The SKIP model prediction was compared with experimental data. It was found that the designed model described the trend of experimental data satisfactorily. Computer simulations and experimental data are shown in Fig. 3.

The values of metabolic and interaction coefficients are given in Table 1. According our experimental data *p*-cresol and phenol degraded simultaneously but it was obvious that cells were unable to utilize the growth substrates in a way to produce as much as the sum of established biomasses ($X$) in single-substrate experiments. Such data have been reported by others [5, 6].

The obtained kinetic parameters in single-substrate experiments for phenol and cresol to be more specific $k_i$ and $k_s$ presumed the easier phenol degradation when compared with cresol. The higher value of $k_{s(cr)}$ demonstrated the lower rate of cresol utilization than that established for phenol. Correspondingly $k_{i(ph)}$ had higher value than $k_{i(cr)}$ showing the stronger toxic effect of cresol on the *T. cutaneum* R57 strain development [24]. This conclusion was in accordance with interaction coefficients values received. The high value of interaction coefficient $I_{cr/ph} = 10$ in contrast with $I_{ph/cr} = 1$ demonstrated as well the stronger influence of the *p*-cresol on phenol degradation.

## Conclusions

The results obtained demonstrated that the presence of *o*-, *m*- and *p*-cresol have not prevented complete phenol assimilation in the mixture but had significant delaying

**Table 1** Parameters for SKIP model of biodegradation of phenol/*p*-cresol mixture by *T. cutaneum* R 57

| Parameters | Growth substrates | | |
|---|---|---|---|
| | *p*-Cresol | Phenol | Phenol/*p*-cresol |
| $S_0$ (g/l) | 0.20 | 0.30 | 0.30/0.20 |
| $k_{1(ph)}$ | – | 1.25 | 1.30 |
| $k_{2(cr)}$ | 0.71 | – | 0.75 |
| $I_{ph,cr}$ | NA | NA | 1 |
| $I_{cr,ph}$ | NA | NA | 10 |

effect on the phenol degradation dynamics in *T. cutaneum* R57 cells.

The SKIP model was developed on the basis of model parameters from single-substrate experiments. It could be used to predict the outcome of the two-substrate mixture experiment. The investigations on specificity of interaction between different compounds are meaningful for invention of effective remediation technologies for industrial wastes where the mixed substrates are common occurrence.

**Acknowledgments** The support by National Counsel of Science Research of the Bulgarian Ministry of Education and Science under project K 1205/02 is gratefully acknowledged.

## References

1. Aleksieva Z, Ivanova D, Godjevargova T, Atanasov B (2002) Degradation of some phenol derivatives by *Trichosporon cutaneum* R57. Proc Biochem 37:1215–1219
2. Alexievaa Z, Gerginova M, Zlateva P, Peneva N (2004) Comparison of growth kinetics and phenol metabolizing enzymes of *Trichosporon cutaneum* R57 and mutants with modified degradation abilities. Enzyme Microb Technol 3–4:242–247
3. Alvarez J, Vogel T (1991) Substrate interactions of benzene, toluene, and *p*-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl Environ Microbiol 57:2981–2985
4. Bielefeldt A, Stensel H (1999) Modeling competitive inhibition effects during biodegradation of BTEX mixtures. Water Res 33:707–714
5. Chang M-K, Voice C, Criddle C (1993) Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene and *p*-xylene by two. Biotechnol Bioeng 41:1057–1065
6. Dapaah S, Hill G (1992) Biodegradation of chlorophenol mixtures by *Pseudomonas putida*. Biotechnol Bioeng 40:1353–1358
7. Georgieva N, Yotova L, Betcheva R, Valchev I, Chadjiska C (2007) Biotransformation of lignin in linen with *Trichosporon cutaneum* R57. J Cellulose Chem Technol 2:25–27
8. Haigler B, Pettigrew C, Spain J (1992) Biodegradation of mixtures of substituted benzenes by *Pseudomonas* sp. strain JS150. Appl Environ Microb 58:2237–2244
9. Heinaru E, Viggor S, Vedler E, Truu J, Merima M, Heinaru A (2001) Reversible accumulation of *p*-hydroxybenzoate and catechol determines the sequential decomposition of phenolic compounds in mixed substrate cultivation in pseudomonads. FEMS Microbiol Ecol 37:79–89
10. Hill G, Milne B, Nawrocki P (1996) Cometabolic degradation of 4-chlorophenol by *Alcaligenes eutrophus*. Appl Microbiol Biotechnol 46:163–168
11. Hinteregger C, Leitner R, Loidl M, Ferschl A, Streichsbier F (1992) Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. Appl Microbiol Biotechnol 37:252–259
12. Hutchinson D, Robinson C (1988) Kinetics of the simultaneous batch degradation of *p*-cresol and phenol by *Pseudomonas putida*. Appl Microbiol Biotechnol 29:599–604
13. Kar S, Swaminathan T, Baradarajan A (1997) Biodegradation of phenol and cresol isomer mixtures by *Arthrobacter*. World J Microbiol Biotechnol 13:659–663
14. Muller R, Babel W (1996) Growth rate-dependent expression of phenol-assimilation pathway in *Alcaligenes eutrophus* JMP 134-the influence of formate as an auxiliary energy source on phenol conversion characteristics. Appl Microbiol Biotechnol 46:156–162
15. Neujahr H, Varga J (1970) Degradation of phenols by intact cells and cell-free preparations of *Trichosporon cutaneum*. Eur J Biochem 13:37–44
16. Oh Y-S, Shareefdeen Z, Baltzis B, Bartha R (1994) Interactions between benzene, toluene and *p*-xylene during their biodegradation. Biotechnol Bioeng 44:533–538
17. Powlowski B, Dagley S (1985) $\beta$-Ketoadipate pathway in *Trichosporon cutaneum* modified for methyl-substituted metabolites. J Bacteriol 16:1126–1135
18. Reardon K, Mosteller D, Rogers J (2000) Biodegradation kinetics of benzene, toluene and phenol as single and mixed substrates for *Pseudomonas putida*. Biotechnol Bioeng 69:385–400
19. Reardon K, Mosteller D, Rogers J, Teau N, Kim K-H (2002) Biodegradation kinetics of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures. Environ Health Perspect 110:1005–1011
20. Rogers J, Reardon K (2000) Modelling substrate interactions during the biodegradation of mixtures of toluene and phenol by *Burkholderia* species JS150. Biotechnol Bioeng 70:428–435
21. Schmidt S, Alexander M (1985) Effects of dissolved organic carbon and second substrates on the biodegradation of organic compounds at low concentration. Appl Environ Microbiol 49:822–827
22. Shivarova N, Zlateva P, Atanasov B, Christov A, Peneva N, Gerginova M, Alexieva Z (1999) Phenol utilization by filamentous yeast *Trichosporon cutaneum*. Bioproc Eng 20:325–328
23. Yan J, Jianping W, Jing B, Daoquan W, Zongding H (2006) Phenol biodegradation by the yeast *Candida tropicalis* in the presence of *m*-cresol. Biochem Eng J 29:227–234
24. Zlateva P, Gerginova M, Manasiev Y, Atanasov B, Peneva N, Dimova N, Alexieva Z (2005) Kinetic parameters determination of the phenolic derivatives assimilation by *Trichosporon cutaneum* R57. Biotechnol Biotechnol Equip 19:93–97