Vol.53, n. 2: pp. 481-486, March-April 2010
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Catechol Biodegradation kinetics Using *Candida parapsilopsis*

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ABSTRACT

This study evaluated the biodegradation of catechol by a yeast strain of Candida parapsilopsis in standard medium in Erlenmeyer flasks. Results shown that the highest concentration of catechol caused the longer lag period, demonstrating that acclimatized cultures could completely degrade an initial catechol concentration of 910 mg/L within 48 h. Haldane's model validated the experimental data adequately for growth kinetics over the studied catechol concentration ranges of 36 to 910 mg/L. The constants obtained for this model were $\mu_{max} = 0.246\ h^{-1}$, $K_s = 16.95$ *mg/L and* $K_i = 604.85$ *mg/L.*

Key words: *Candida parapsilopsis*; Biodegradation; Catechol; Growth kinetics; Equation of Haldane; Substrate inhibition

INTRODUCTION

Wastewater from coal conversion processes, petroleum refineries, manufacture of pharmaceuticals, fertilizer and dyes contain phenolics compounds, which represent a serious ecological problem due to their widespread use, toxicity and occurrence throughout the environment (Godjevargova et al., 2000; Marek et al., 2001; Jindrová et al., 2002). Biological treatment process is generally used to degrade these substances and have usually been carried out through aerobic process. However, phenol has high inhibitory and antibacterial activity influence on cells. It acts as a membrane-actived agent, which increases the permeability of the cytoplasmac membrane and causes a leakage of cytoplasmic material. Little information about the use of yeast cultures grown on phenolic compounds is found in the literature (Páca et al., 2002; Ruiz-Ordaz et al., 1998; Komarkova et al., 2003), especially the catechol that is considered more toxic than phenol Kumar et al.,(2005).

There are some phelolytic microorganisms that show different behaviour in terms of their ability to degrade these compounds. The biodegradation of phenol and its derivatives by microbial cultures has been the focus of research for a long time (Yang and Humphrey, 1975; Kumar et al., 2005; Yan et al., 2005; Gerrard et al., 2006). The knowledge of growth kinetics is essential to understand microorganism capabilities for

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degradation and treatment operation processes. There are several studies related to phenolics compounds degradation as summarized in Table 1. The main aim of this work was to select a strain that degraded catechol under high concentrations in order to generate kinetics experimental data describing the growth of this culture on catechol in batch experiments.

Table 1 - Kinetic constants for phenol or catechol biodegradation (the parameters were obtained from the Haldane's model).

Culture	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	Reference
Candida parapsilopsis [1]	0.246	16.95	604	This work
Candida parapsilopsis [2]	0.174	11.2	298	Rigo and Alegre (2004)
Thichosporon cutaneum [2]	0.464	1.66	380	Yang and Humphrey (1975)
Pseudomonas putida [2]	0.534	< 1	470	Monteiro *et. al.* (2000)
Pseudomonas putida [2]	0.569	18.5	99.4	Hill and Robinson (1975)
Fusarium flocciferum [2]	—	85	345	Beyenal *et. al.* (1997)
Candida tropicales [2]	0.48	11.7	207.9	Yan *et. al.* (2005)
Pseudomonas putida [1]	0.326	29.9	99.9	Kumar *et. al.* (2005)

[1]Catechol biodegradation. [2]Phenol biodegradation.

MATERIALS AND METHODS

Microorganisms

Samples from an industrial chemistry effluent contaminated with phenolic compounds were collected. The microorganisms in the samples were isolated by procedure described by Rigo and Alegre (2004). Each species of microorganism was tested as a pure culture for the ability to grow on phenol (50 to 1000 mg/L). The standard medium (SM; Table 2) was prepared in two solutions, A and B, which were steam-sterilized separately. After cooling to room temperature, the solutions were mixed aseptically. The final SM was prepared by adding catechol (modified according to Yang and Humphrey, 1975).

Table 2 - Standard medium**.**

Component		g/liter
Solution A	**Solution B**	
KH_2PO_4		1.6
KI		0.001
$(NH_4)_2SO_4$		3.0
	$CaCl_2 \cdot 2\ H_2O$	0.3
	$MgSO_4 \cdot 7\ H_2O$	0.5
Trace elements [a]		1 mL
Vitamins [b]		16 mL
Catechol		Variable

[a] Trace elements solution (in g/L): H_3BO_3 0.1; $ZnSO_4 \cdot 7H_2O$ 0.04; $(NH_4)Mo_7O_{24} \cdot 4H_2O$ 0.02; $MnSO_4 \cdot 7H_2O$ 0.04; $CuSO_4$ $5H_2O$ 0.045; $FeSO_4 \cdot 7H_2O$ 0.025.
[b] Vitamin solution (in mg/L): Ca-pantothenate 200; thiamine-HCl 40; pyridoxin-HCl 40.

The selected species were inoculated in SM containing 50 mg/L of catechol in 250 mL conical flasks with 50 ml of medium, and incubated at 30 °C and 2.5 Hz. When growth was detected, samples were inoculated into new flasks with SM containing 150 mg/L of catechol. This procedure was repeated increasing the catechol concentration to 450 mg/L and finally to 900 mg/L. Controls were made by following the same procedure without any addition of inoculum.

In order to estimate the specific growth rate (μ) in batch culture, a traditional method was used, which the exponential growth phase appeared as a straight line on a semi-logarithm plot of the cell concentration against time; the value of μ was readily calculated from the slope of the line (Doran, 2000). The assays were carried out by preparing 1-liter conical flasks containing 300 mL of standard medium with catechol concentration of 36 – 910 mg/L. Thirty milliliter of the inoculum

prepared with standard medium containing 150 mg/L catechol was added to the flasks and the absorbance and catechol concentration was measured during the fermentation course. For all the assays, the initial pH value was 7.0 and incubation at 30 °C under agitation.

A number of substrate-inhibition models have been proposed for use with inhibitory substrates (Sokol and Howell, 1981; Yang and Humphrey, 1975). The simplest and most commonly used model, which is the Haldane's model, is given as:

$$\mu = \frac{\mu_{max}}{\left(\frac{K_s}{S} + 1 + \frac{S}{K_i}\right)}$$

Where K_i is an inhibition constant, equivalent to the highest substrate concentration at which the specific grown rate (μ) is equal to a half part of the maximum specific growth rate (μ_{max}) in the absence of inhibition.

Catechol assay

Catechol was measured by a colorimetric method based on its rapid condensation with 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), followed by oxidation with tripotassium hexacyanoferrate under alkaline conditions to give a red product. The culture sample was filtered through a 1.2-μm filter (Whatman), and the filtrate was used to determine the catechol concentration APHA, (1989).

Microorganisms growth determination were done by measuring of the optical density at 600 nm using a Hach DR/4000 V spectrophotometer.

RESULTS AND DISCUSSION

The 22 microorganisms isolated were capable to grow on phenol as sole carbon and energy sources, in prior work (Rigo and Alegre, 2004). In this work, microorganisms were also cultured in pure culture with catechol as the only carbon source in SM medium. There was only one species capable to grow with catechol concentration up to 900 mg/L: a mycelial yeast identified as *Candida parapsilopsis*, based on its morphology, physiological and biochemical characteristics. This species was also capable of degrading phenol in high concentration (Rigo and Alegre, 2004). Although *C. tropicalis* has been referred as a phenol degrader (Ruiz-Ordaz et al., 2001; Yan et al., 2005), there was no report on *C. parapsilopsis* to have catechol degrading activity. Figure 1 shows the micrography of *C. parapsilopsis* growing in a catechol concentration of 1.0 g/L. The other species of bacteria could possibly grow in SM at low concentration of catechol, 50 mg/L (data not shown).

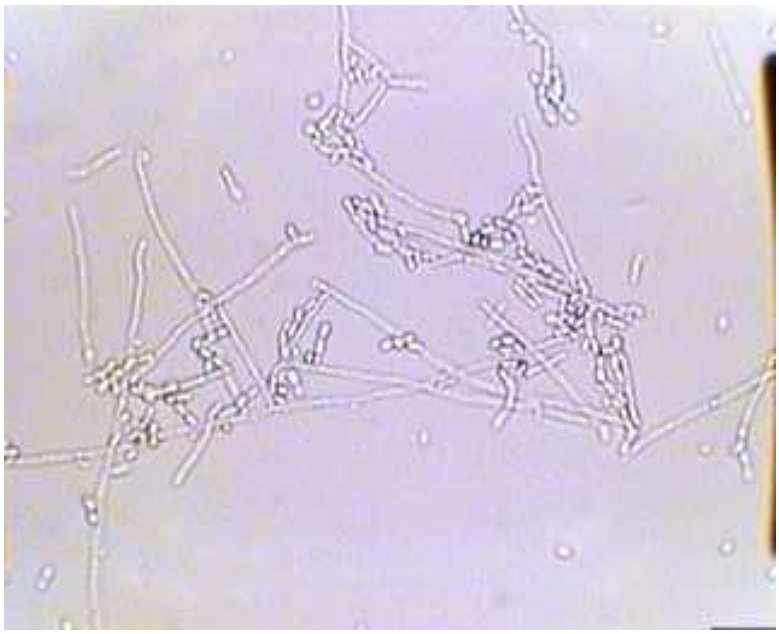

Figure 1 - *Candida parapsilopsis* (400 x).

Batch cultures of *C. parapsilopsis* were carried out in a standard medium containing initial catechol concentrations ranging from 36 to 910 mg/L. For each flask with a certain initial catechol concentration, the specific growth rate of biomass was calculated. Figure 2 shows typical curves for *C. parapsilopsis* where density optical was plotted against time, for some experiments using different initial catechol concentration. The maximum growth rate was 0.19 h^{-1} at an initial catechol concentration of 72 mg/L. That means that there was a small lag phase. As the initial catechol concentration increased to 213 and 308 mg/L the lag phase raised to 5 and 7 h, respectively.

Figure 3 shows a typical growth curve of *C. parapsilopsis* with the effective degradation of catechol. The equation of Haldane was adopted for assessing the dynamic behavior of *C. parapsilopsis* grown on catechol since the high catechol concentration caused the inhibition on the cell grown. Then, this equation was used to fit the experimental data of a specific growth rate of biomass for each batch culture with initial different catechol concentration. By using nonlinear least squares-regression analysis of the software Statistic, the value of the chi square coefficient was a small value (0.00004), indicating that the regression curve and the experimental data had an excellent agreement. Kinetic parameters for *C. parapsilopsis* grown on catechol were μ_{max} = 0.246 h^{-1}, K_s = 16.95 mg/L and K_i = 604.85 mg/L.

The comparison between the calculated and experimental specific growth rates of biomass at different initial catechol concentration is shown in Figure 4. As expected, the maximum specific grown rate occurred at low catechol concentration, and with further increase of initial catechol concentration, lower values of the specific grown rates were obtained, due to intense substrate inhibition at high catechol concentration. In addition to the ability of catechol degradation and high-level resistance to this compound, the best yeast strain chosen had low nutritional requirements. It could grow and degrade catechol in a medium containing 30 mg/L glucose and 3 mg/L of casein. These characteristics suggested its possible application for the removal of phenolic substances from wastewaters containing other carbons compounds beside phenolic compounds as suspended cells or in an immobilized form.

Values of the kinetic constants obtained in this work confirmed the same magnitude found in literature data. The value of the maximum specific growth rate was very low (Table 1). However, the medium used in this work had also a very low vitamin content.

The newly isolated *C. parapsilopsis* capable of using catechol as the only carbon and energy sources could be important in bioremediation and wastewater treatments, nevertheless the kinetic parameters were less suitable for practical use.

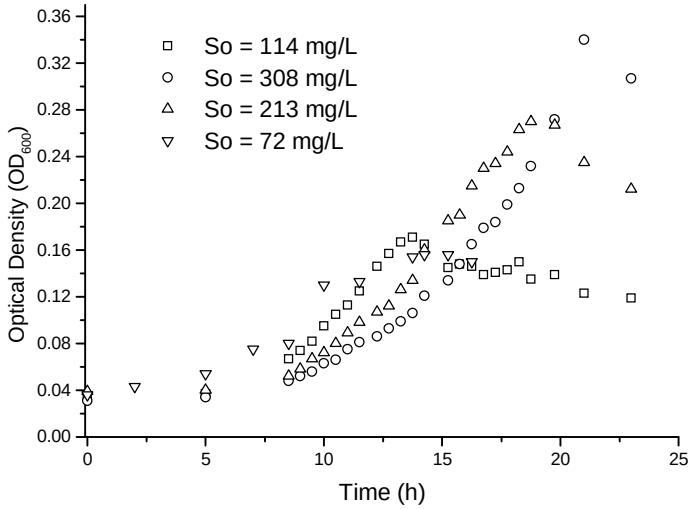

Figure 2 - Optical density at 600 nm as a function of time of catechol degradation by *C. parapsilopsis*, under various initial catechol concentrations (So).

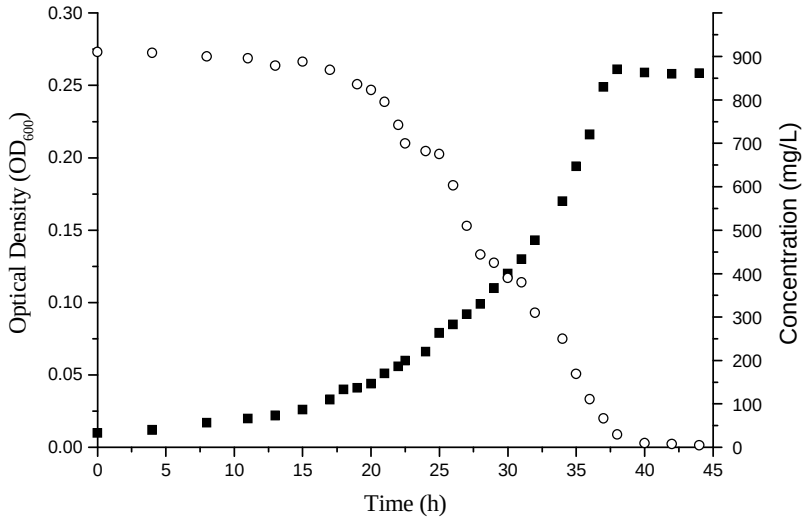

Figure 3 - Catechol degradation by *C. parapsilopsis* in standard medium into the Erlenmeyer flask (So = 910 mg/L), (○) represented catechol concentration, (■) represented optical density.

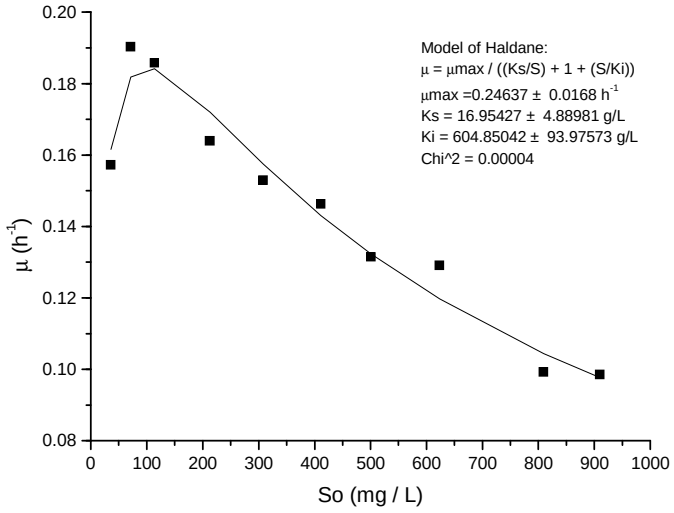

Figure 4 - Specific growth rate (μ) as a function of the initial catechol concentration (So). (■)Experimental data.

ACKNOWLEDGEMENTS

We are grateful to Universidade Estadual do Centro – Oeste (UNICENTRO), Universidade Estadual de Campinas (UNICAMP) and Fundação Tropical de Pesquisas e Tecnologia André Tosello for identification of *Candida parapsilopsis*.

RESUMO

Neste trabalho foi estudada a biodegradação de catecol em frascos de Erlenmeyers em água residuária sintética pela levedura *Candida parapsilopsis*. As respostas dos ensaios cinéticos mostraram que altas concentrações de catecol

ocasionaram uma fase lag longa para a levedura. Portanto, a aclimatização da cultura de levedura empregada para biodegradação de catecol é de fundamental importância, sendo possível reduzir toda a concentração inicial de catecol da água residuária sintética de 910 mg/L em 48 horas. Os dados experimentais da cinética de biodegradação do catecol foram ajustados pelo modelo de Haldane adequadamente, sobre a faixa de concentração de catecol investigada de 36 a 910 mg/L. Os parâmetros cinéticos obtidos do modelo de Haldane foram: μ_{max} = 0,246 h^{-1}, K_s = 16,95 mg/L e K_i = 604,85 mg/L.

ABREVIATIONS

K_i = inhibition constant for catechol (mg/L)
K_s = half-saturation constant for catechol (mg/L)
S = catechol concentration (mg/L)
S_o = initial catechol concentration (mg/L)
μ = specific growth rate of biomass (h^{-1})
μ_{max} = maximum specific growth rate of biomass (h^{-1})

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Received: August 24, 2007;
Revised: May 27, 2008;
Accepted: July 28, 2009.