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Kinetic studies of phenol degradation by *Rhodococcus sp.* P1 I. Batch cultivation

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Abstract

Rhodococcus sp. P1 utilizes phenol as the sole carbon and energy source via the β-ketoadipate pathway. In batch cultivation, concentrations up to 2.8 g · l^{-1} phenol were degraded. The highest values for the specific growth rate of 0.32 h^{-1} were obtained at concentrations near 0.25 g · l^{-1}. At higher concentrations, substrate inhibition was observed, characterized by increases in lag phase and decreasing growth rates. A mathematical expression was proposed to fit the kinetic pattern of phenol inhibition on the specific growth rate μ:

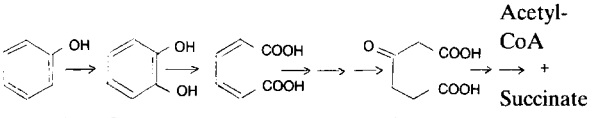
$$\mu = \mu_{max} \frac{S}{S + K_S} \exp\left(-\left(\frac{S}{K_I}\right)^K\right)$$

with $\mu_{max} = 0.33\,h^{-1}$, $K_S = 0.00032$ g · l^{-1}, $K_I = 1.264$ g · l^{-1}, $K = 1.44$.

Nomenclature: K – Exponent of the inhibition function, K_S – Monod saturation constant, g · l^{-1}, K_I – Inhibition constant, g · l^{-1}, S – Substrate concentration in culture broth, g · l^{-1}, S_o – Initial substrate concentration, g · l^{-1}, Y – Yield constant, g cell dry mass · g substrate^{-1}, μ – Specific growth rate, h^{-1}, μ_{max} – Maximum growth rate, h^{-1}

Introduction

Phenols belong to the category of toxic chemical pollutants of industrial origin. However, some microorganisms are able to degrade these compounds. We isolated a strain of *Rhodococcus* (Hensel 1980), which can be induced to metabolize phenol as a sole carbon and energy source via the β-ketoadipate pathway:



Phenol Catechol cis,cis-Muconate β-Keto-adipate

In the present work, we study the kinetic behavior of this organism in batch cultures and discuss substrate inhibition. In another paper (Hensel & Straube 1989) some data of continuous cultivation experiments will be presented.

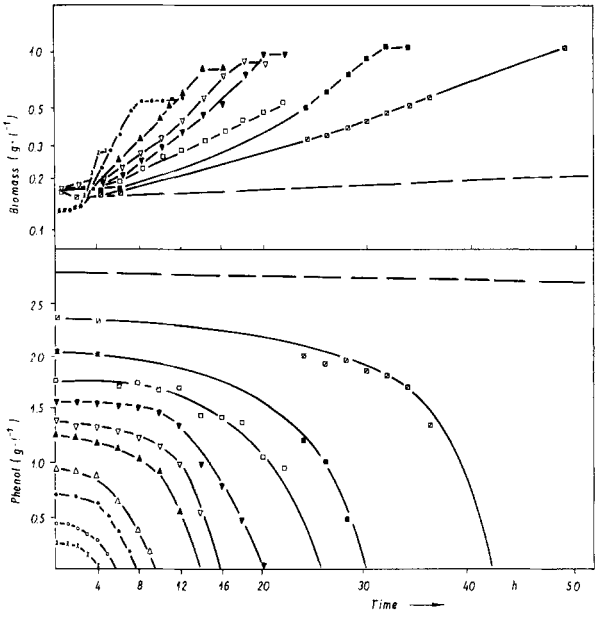


Fig. 1. Growth and phenol degradation by induced cells on various phenol concentrations. Broken line: 2.8 g · l^{-1} phenol were exhausted completely after 270 hours.

Materials and methods

Organism

The bacterial strain *Rhodococcus sp.* P1 was isolated from the Saale river near Halle (Hensel 1980).

Cultivation

The strain was grown on a rotary shaker at 30° C in a mineral salt medium containing: $Na_2HPO_4 \cdot 12H_2O$ – 9.0 g, KH_2PO_4 – 1.5 g, NH_4Cl – 1.0 g, $MgSO_4 \cdot 7H_2O$ – 0.2 g, $CaCO_3$ – 0.02 g, $FeSO_4 \cdot 7H_2O$ – 0.01 g, H_3BO_3 – 50 μg, $CuSO_4 \cdot 5H_2O$ – 10 μg, KJ – 10 μg, $MnSO_4 \cdot 4H_2O$ – 40 μg, $ZnSO_4 \cdot 7H_2O$ – 40 μg, $Na_2MoO_4 \cdot 2H_2O$ – 24 μg ad 1 l distilled water. Phenol was sterilized by filtration, and added after sterilization of the medium.

The inoculum was prepared in a standard manner: 48-hours-old agar slant cultures were inoculated into the mineral salt medium with 0.5 g · l^{-1} phenol. 0.5, 0.5 and 1.0 g · l^{-1} phenol was added after 7, 21 and 28 h, respectively. After 40 h the cells were harvested and washed twice and resuspended with carbon-free medium.

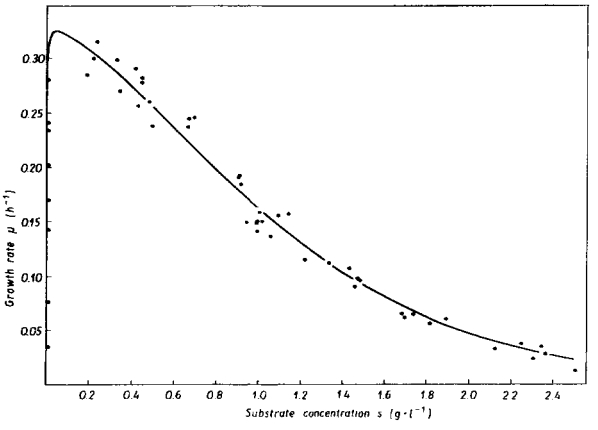


Fig. 2. Dependence of the specific growth rate μ on the phenol concentration S. – computer fitted model; ● experimental data (below S = 0.1 from continuous cultivations).

Cell concentration

Growth was followed by measuring the optical density at 578 nm. The number of surviving cells was determined by plating on a complex medium.

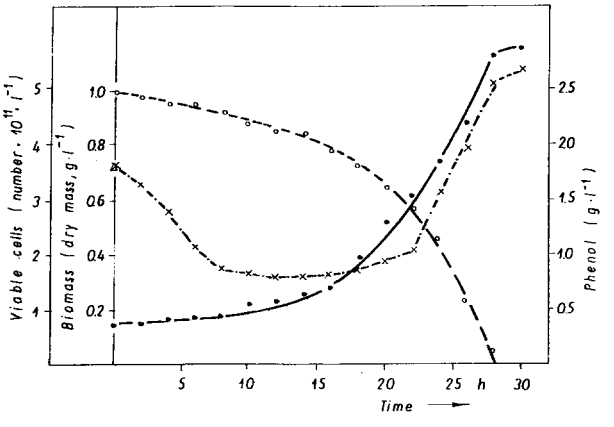


Fig. 3. Growth and phenol degradation at cultivation on a high phenol concentration. S_o = 2.4 g · l^{-1}; —●— dry mass; –·–×–·– living cells; – –○– – phenol concentration.

Phenol concentration

Phenol was assayed by the method of Lacoste et al. (1959), using 4-aminoantipyrine as colour indicator.

Specific growth rate

The specific growth rates were determined with the help of a cubic spline interpolation to reduce data scatter (Späth 1973).

Results

In batch cultivation, *Rhodococcus sp.* P1 was able to grow on phenol concentrations up to $2.8\,g \cdot l^{-1}$ (Fig. 1). But, at higher concentrations above $0.25\,g \cdot l^{-1}$, substrate inhibition was observed, characterized by increasing lag phases at cultivation of non-induced cells and by decreasing growth rates (Fig. 2). The highest values for the specific growth rate of about $0.32\,h^{-1}$ were obtained at phenol concentrations near $0.25\,g \cdot l^{-1}$. Experimental data showed that the often-used Haldane-model describing substrate inhibition is not useful here. The following equation, which gives a much faster decrease than the Haldane-model, would appear to best fit the data up to substrate concentration of approximately $2.5\,g \cdot l^{-1}$:

$$\mu = \mu_{max} \frac{S}{S + K_S} \exp\left(-\left(\frac{S}{K_I}\right)^K\right)$$

with $\mu_{max} = 0.33\,h^{-1}$, $K_S = 0.00032\,g \cdot l^{-1}$, $K_I = 1.264\,g \cdot l^{-1}$, $K = 1.44$.

The yield coefficient (Y_{phenol}) decreases with increasing phenol concentrations as summarized in Table 1.

At very high phenol concentrations (above $2.0\,g \cdot l^{-1}$) a decrease in the number of living cells was observed within the first hours after inoculation. An example is shown in Fig. 3. Incubation of cells adapted to phenol in medium with a phenol concentration ($5\,g \cdot l^{-1}$) high enough to prevent growth resulted in almost complete death within 48 hours (Table 2). The longer the cells had been exposed to this high phenol concentration, the more time was needed to resume growth if cells were transferred to media with growth-permitting phenol concentrations (not shown).

Table 1. Yield coefficient (Y_{phenol}) in dependence on the initial phenol concentrations (S_o).

S_o ($g \cdot l^{-1}$)	Y (g dry mass · g phenol^{-1})
0.25	0.64
0.50	0.62
0.75	0.60
1.00	0.60
1.20	0.56
1.30	0.55
1.50	0.54
2.30	0.40
2.50	0.38

Discussion

Rhodococcus sp. P1 degrades phenol in batch cultivation in concentrations up to $2.8\,g \cdot l^{-1}$. This is a very high concentration and only some yeasts have been found to exhibit a similar activity (Shivaraman et al. 1978; Krug et al. 1985). These yeasts split the aromatic ring of catechol, like *Rhodococcus,* by the ortho-cleavage (β-ketoadipate pathway).

With increasing phenol concentration the growth lag times increase drastically. This was also described for other systems, e.g. in *Pseudomonas putida* grown on phenol, lag phases of about 10

Table 2. Number of surviving cells after incubation with a high phenol concentration of $5.0\,g \cdot l^{-1}$.

Incubation time (h)	Living cells (%)
0	100 ($\hat{=}$ $3.3 \cdot 10^{11}$ cells $\cdot\, l^{-1}$)
0.5	97
3	66
5	37
10	20
24	8
48	1

hours at $S_o = 0.2\,g \cdot l^{-1}$ and of 7 days at $S_o = 0.7\,g \cdot l^{-1}$ were measured (Hill & Robinson 1975). In that case, the extent of the increase in lag time was dependent on the inhibition constant and the inoculum size. Compared with the cited data from *Pseudomonas* the lag times of *Rhodococcus* were very short.

For description of the relation between specific growth rates and substrate concentration, most authors used the classical Haldane-model of substrate inhibition (Jones et al 1973; Hill & Robinson 1975; Yang & Humphrey 1975; Brilkov et al. 1980). But, in some cases, this model did not fit the experiments for very high phenol concentrations. There was a greater inhibitory effect at high substrate concentrations than was indicated by this model (Yang & Hymphrey 1975; Brilkov et al. 1980). We obtained a similar result with *Rhodococcus sp.* P1. Therefore we proposed an other model, developed by computer fitting of the experimental data. At concentrations above $2.5\,g \cdot l^{-1}$ a threshold substrate concentration above which growth is completely inhibited can be expected, as discussed also by Luong (1987). An exact experimental determination of this value is very difficult and would be possible only with phenolstat cultivations.

At very high phenol concentrations a part of the cells was damaged. We observed the same phenomenon in studies with the phenol utilising yeast *Candida tropicalis* HP 15 (Krug et al. 1985). Therefore, negative values for the growth rate μ have to be assumed at very high substrate concentrations. Besides the decrease in metabolic activity, the death of a part of the cells seems to contribute further to substrate inhibition of growth.

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