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-ketoacidipate 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:

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

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

**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, \( S_0 \) – Initial substrate concentration, g·l⁻¹, Y – Yield constant, g cell dry mass·g substrate⁻¹, μ – Specific growth rate, h⁻¹, \( \mu_{\text{max}} \) – 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-ketoacidipate pathway:

\[
\text{Phenol} \rightarrow \text{Catechol} \rightarrow \text{cis,cis-Muconate} \rightarrow \text{β-Keto-adipate} \rightarrow \text{Succinate}
\]

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: Na₂HPO₄·12H₂O – 9.0 g, KH₂PO₄ – 1.5 g, NH₄Cl – 1.0 g, MgSO₄·7H₂O – 0.2 g, CaCO₃ – 0.02 g, FeSO₄·7H₂O – 0.01 g, H₃BO₃ – 50 μg, CuSO₄·5H₂O – 10 μg, KJ – 10 μg, MnSO₄·4H₂O – 40 μg, ZnSO₄·7H₂O – 40 μg, Na₂MoO₄·2H₂O – 24 μg ad 11 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.

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. 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).

Fig. 3. Growth and phenol degradation at cultivation on a high phenol concentration. S₀ = 2.4 g·l⁻¹; — dry mass; —— living cells; —— phenol concentration.