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**SHORT COMMUNICATIONS**

# The Relative Value of Redox Potential in *Rhodococcus rhodochrous* Cells as a Function of Their Growth Rate

**M. A. Baboshin[1] and L. A. Golovleva**

*Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

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Bacteria of the genus *Rhodococcus* are extensively studied due to their ability to transform and degrade various organic substances [1]. This work is a comparative investigation in vivo of redox potential in the *Rhodococcus rhodochrous* 172 cells growing on different substrates.

The *Rhodococcus rhodochrous* strain 172 used in this work was obtained from the collection of rhodococci at the Laboratory of Enzymatic Degradation of Organic Compounds of the Institute of Biochemistry and Physiology of Microorganisms. The basal mineral medium contained (g/l) $NH_4NO_3$, 1.0; $KH_2PO_4$, 1.0; $MgSO_4$, 0.2; and $CaCl_2$, 0.02. The medium was supplemented with two drops of a saturated $FeCl_3$ solution. The pH of the medium was approximately 7.5. Organic substrates (glucose, fructose, sucrose, sodium propionate, potassium lactate, or potassium succinate) were added to the medium at a concentration of 1 g/l in the form of sterile aqueous solutions. The ability of *R. rhodochrous* strain 172 to oxidize the naphthenoaromatic alcohol fluorenol (FOL) into the corresponding ketone fluorenone (FON) and to catalyze the reverse reaction was studied as follows: A flask with the incubation medium was inoculated with 1 ml of *R. rhodochrous* 172 culture grown in the mineral medium with glucose (2 g/l) to the stationary growth phase and incubated on a shaker (120 rpm) at 29°C. The cell suspension was sampled at regular intervals to measure optical density (OD) at 540 nm with a KFK colorimeter and a 0.3-cm cuvette. As soon as the OD of the culture reached 0.2, it was supplemented with either FOL or FON in an amount of 0.25 mg dissolved in 100 μl of methanol, and incubation was continued. After the measurements of the OD of the culture sample, bacterial cells were removed by centrifugation and the UV spectrum of the culture liquid was recorded with a Shimadzu UV 160 spectrophotometer against the reference solution (the culture liquid of *R. rhodochrous* 172 incubated without FOL or FON). The specific growth rate $\mu_m$ was calculated in the coordinates $\ln D_{540}(t)$. The concentrations of FOL and FON were determined by the Firodt method [2]. Measurements were performed at the wavelengths $\lambda_1 = 251$ nm and $\lambda_2 = 271$ nm. The molar extinction coefficients measured in the incubation medium were found to be [l/(mol cm)] $\varepsilon_{\lambda_1}^{FON} = 50000$, $\varepsilon_{\lambda_1}^{FOL} = 2000$, $\varepsilon_{\lambda_2}^{FON} = 10000$, $\varepsilon_{\lambda_2}^{FOL} = 21000$. The isosbestic point of the FOL–FON system was at $\lambda \approx 262$ nm. To avoid the effect of biomass on the reaction rate, the kinetic curves were analyzed with the use of the exposure variable $\tau = \int D_{540}\,dt$, where $D_{540}$ is the optical density of the culture measured at 540 nm (as a measure of the culture biomass) [3] rather than with the use of the time variable $t$. In the case of exponential growth, the exposure variable is defined by the formula $\tau = \frac{D_{540}^0}{\mu}(e^{\mu t} - 1)$, where $\tau$ has the dimensions OD min. Plots were constructed either in the $C(\tau)$ coordinates or in the coordinates of the reversible first-order reaction $[\ln C^\infty - \ln(C^\infty - C)]$ versus $\tau$ for the product and $[\ln(C^0 - C^\infty) - \ln(C - C^\infty)]$ versus $\tau$ for the substrate. The asymptotic concentrations $C_{FOL}^\infty$ and $C_{FON}^\infty$ (i.e., the concentrations that could be established at an infinitely long incubation time) were calculated as described by Schmid and Sapunov [3].

The culture showed exponential growth on all the organic substrates used. The processes of FOL oxidation and FON reduction followed the equation of the reversible first-order reaction. The ratio of the $C_{FOL}^\infty$ and $C_{FON}^\infty$ concentrations did not depend on the ratio of the initial FOL and FON concentrations, indicating the absence of inhibitory action on the reaction rate. In the controls without biomass, the UV spectrum remained unchanged. The cell-free culture liquid and the biomass killed by boiling in the mineral medium induced no changes in the UV spectrum. There was a distinct positive correlation between the degree of reduction of the FON/FOL system, $F = C_{FOL}^\infty / (C_{FOL}^\infty + C_{FON}^\infty)$, and the

[1] Corresponding author. E-mail: mbaboshin@rambler.ru

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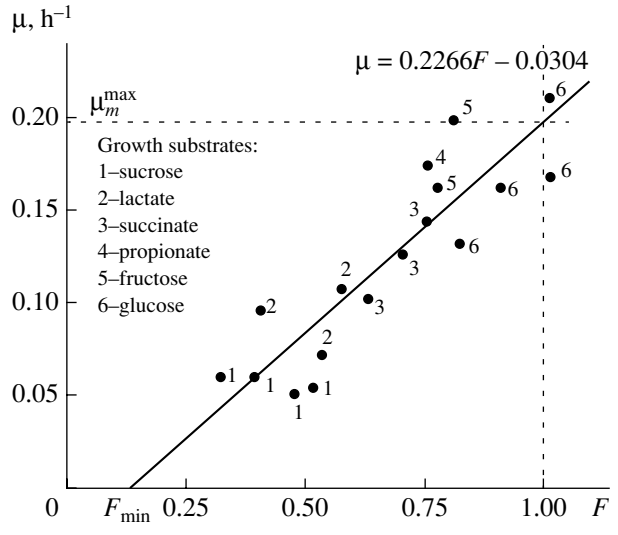


Correlation between the degree of reduction of the FON/FOL system and the specific growth rate of the *R. rhodochrous* 172 cells.

specific growth rate of the biomass, μ (figure). The observed phenomenon can be accounted for by one of two mechanisms: (1) the reversible reaction $X$ + FOL ⟷ FON + $XH_2$ or (2) the cyclic reaction FOL + $X$ ⟶ FON + $XH_2$, FON + $YH_2$ ⟶ FOL + $Y$. In the case of (1), the FON/FOL pair acts as an indicator of redox potential in the *R. rhodochrous* 172 cells, the ratio of the equilibrium concentrations of FOL and FON being solely determined by the ratio of the stationary concentrations of the reduced and oxidized forms of the redox pair: $C^{\infty}_{FOL} / C^{\infty}_{FON} = 1/K_{eq} C_{XH_2}/C_X$, where $K_{eq}$ is an equilibrium constant. If reaction (2) is valid, the concentration ratio $C^{\infty}_{FOL} / C^{\infty}_{FON}$ depends not only on the redox potential of the system but also on the ratio of the kinetic parameters of this reaction. Irrespective of the actual mechanism (either reversible or cyclic), the rhodococci may possess a system whose redox potential is determined by the growth rate (in the case of the reversible mechanism or in the presence of fluorenone in the medium, this is beyond doubt). The degree of reduction of the FON/FOL system cannot be greater than $F = 1$, which makes it possible to extrapolate the absolute maximum of the specific growth rate $\mu_m^{max}$ (the greatest value of $\mu_m$ on various growth substrates under given conditions), as shown in the figure. The accumulation of reducing equivalents during bacterial growth on high-efficiency substrates indicates that their utilization is saturated and that a further increase in the growth rate is impossible (figure). This situation corresponds to the saturation of the growth–limiting enzyme with reducing equivalents. Thus, we showed that fluorenol added to the growing *Rhodococcus rhodochrous* 172 culture undergoes a partial biocatalytic oxidation to fluorenone. Conversely, fluorenone added to the culture is partially reduced to fluorenol. Irrespective of the substance added (FOL or FON), their concentrations tend to the values $C^{\infty}_{FOL}$ and $C^{\infty}_{FON}$. This process can be described by the equation of the reversible first-order reaction. A positive correlation between the degree of reduction of the FOL/FON system, $F = C^{\infty}_{FOL} / (C^{\infty}_{FOL} + C^{\infty}_{FON})$, and the specific growth rate is revealed. The inference is made that the growth-limiting enzyme of *R. rhodochrous* 172 is saturated by reducing equivalents.

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