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SHORT COMMUNICATIONS

The Effect of Sulfur on the Growth of Hydrocarbon-Oxidizing Bacteria of Different Genera

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Our previous investigations showed that hydrocarbon-oxidizing bacteria belonging to different genera (*Pseudomonas, Arthrobacter,* and *Rhodococcus*) differ in their survival in a model association of sulfate-reducing and hydrocarbon-oxidizing bacteria, *Pseudomonas* and *Arthrobacter* species being the most tolerant of the products of sulfate reduction [1, 2]. It was suggested that the small number of rhodococci under conditions of sulfate reduction was due to the unfavorable effect of sulfur formed during the oxidation of hydrogen sulfide. This work was undertaken to verify this suggestion.

The hydrocarbon-oxidizing strains *Rhodococcus erythropolis* E-15, *Pseudomonas aeruginosa* P-20, and *Arthrobacter globiformis* 2F used in this study were obtained from the laboratory collection of microorganisms. The strains were grown on a shaker at 22°C in a Czapek medium with 1 vol % paraffin (C_{14}–C_{19} *n*-alkanes) as the sole source of carbon and energy. The medium was inoculated with bacterial cells washed out from Czapek agar slants. The effect of sulfur on the growth of hydrocarbon-oxidizing bacteria was studied using sulfur dissolved in liquid paraffin at concentrations of 0.2, 1, 2, and 4 mg/ml. The last concentration corresponded to the saturation concentration of sulfur in the paraffin at 22°C. The biomass yield was determined gravimetrically.

Experiments were performed in triplicate. In the experiments with *P. aeruginosa* strain P-20, the purity of M, S, and R dissociants was controlled by plating them onto nutrient broth–wort (1 : 1) agar [3].

Sulfur is known to dissolve well in organic solvents. Unlike arthrobacters and pseudomonads, rhodococci have a lipophilic cell wall, which allows the latter bacteria to take up hydrophobic substrates by means of simple diffusion [4]. Inasmuch as sulfur was introduced into the cultivation medium in the form of a solution in liquid paraffin [4], it could be anticipated that sulfur would penetrate through lipophilic cell walls together with *n*-alkanes. Consequently, rhodococci would show the least tolerance to sulfur.

The following experiments confirmed our anticipations. The addition of sulfur at a concentration as low as 0.2 mg/ml extended the lag-phase of *R. erythropolis* by a factor of 2 and reduced the biomass yield by more than three times as compared to the control (table). The effect of the higher sulfur concentrations on the growth parameters of *R. erythropolis* cells was still stronger. At a sulfur concentration of 4 mg/ml, bacterial growth began 5.5 days later than in the control, while the biomass yield comprised only 7.6% of the control biomass yield. At the same time, *A. globiformis* showed no response to the addition of sulfur to the growth medium. Even when added at the maximum concentration of 4 mg/ml, sulfur did not affect either the lag-phase duration or the biomass yield.

As for *P. aeruginosa*, this bacterium dissociates into M, S, and R variants (also called dissociants), which differ in their resistance to chemical and physical environmental factors [5]. These variants responded differently to sulfur too. For instance, sulfur did not affect the duration of the lag-phase of the M and S variants (these variants grow well on paraffin), whereas the biomass

The effect of sulfur on the growth of hydrocarbon-oxidizing bacteria in a Czapek medium with liquid paraffin

Strain		Sulfur concentration, mg/ml liquid paraffin	Lag phase duration, days	Dry biomass, g/100 ml
Rhodococcus erythropolis E-15		0	0.5	0.423
		0.2	1	0.125
		1	3	0.092
		2	4	0.049
		4	6	0.032
Arthrobacter globiformis 2F		0	0.5	0.465
		4	0.5	0.467
Pseudomonas aeruginosa P-20	M-variant	0	0.5	0.151
		4	0.5	0.059
	S-variant	0	0.5	0.129
		4	0.5	0.066
	R-variant	0	1	0.082
		4	1	0.079

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yield of these variants decreased twofold. In the case of the R variant, sulfur affected neither its lag-phase nor its biomass yield.

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