Characterization of *Desulfovibrio vulgaris* Grown in Extremophile Turbidostat Reactors

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We are studying the response of sulfate reducing bacteria *Desulfovibrio vulgaris*, an obligate anaerobe, to a range of environmental stressors (such as a salt, pH change or oxygen). We are using turbidostats to obtain a steady-state concentration, as controlled by the dilution rate, or flow rate through the reactor before applying the stress. *D. vulgaris* is grown in a defined medium in FairMenTec reactors (Bioengineering, Switzerland). The reactors are designed for growth of extremophiles, and are constructed of all non-metallic materials. The reactors are equipped with a temperature controlled water jacket, pH control, and a controllable agitator. The reactor is inoculated with 10% culture, and grown at 30°C until cell density has increased to the desired level. pH is maintained at 7.2. Sterile media is then pumped at a constant rate to the reactor, and effluent is continuously withdrawn from an overflow tube to maintain a constant reactor volume. The reactor is purged with nitrogen gas to maintain anaerobic conditions. The reactor is periodically sampled for cell density (by optical density at 600 nm, and AODC) and protein concentrations. A dilution rate of 0.25 1/h produces cells in log phase (low $1x10^8$ cells/ml), that are well-dispersed. At lower dilution rates, when cell concentrations are near $1x10^9$ cells/ml and are in stationary phase, the biomass clumps into large flocs. At low dilution rates, and a pH below 7, the biomass flocs were less dense. At this pH, the reduced sulfate is mostly H_2S, which is stripped from the reactor by the nitrogen purge gas. At a higher pH (7.2), the biomass flocs were denser and more dendritic – FeS with adhered biomass. Under log phase conditions, where the biomass was well-dispersed, we observed clusters of cells. The effect of the clusters could be seen in the shift in the correlation between optical density (absorbance at 600 nm) and cell density when the reactor stir-rate was increased from 125 RPM to 150 RPM. Biomass flocculation, and the characteristics of the floc, are dependent upon biomass age and environmental factors, important considerations for obligate anaerobe and extremophile bacteria.