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Chemically defined medium for *Desulfovibrio vulgaris* stress studies and biomass production

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A defined medium for optimal growth and maximum reproducibility of *Desulfovibrio vulgaris* was developed for biomass production for stress response studies. The medium was optimized by evaluating a variety of chemical components, including the removal of yeast extract, excess sulfate, and Fe, and redox conditions to optimize cell density and generation times, and to reduce lag times. Growth was monitored using direct cell counts, optical density, and protein concentration. The generation time for *D. vulgaris* in the original Baar's medium was 3 h, reaching a maximum density of 10^8 cells/ml and 0.4 $OD_{600\ nm}$. The newly developed medium, lactate-sulfate defined medium, version 4 (LS4D), supplies 0.06 M sodium lactate and 0.05 M sodium sulfate. Both ATCC-prepared Wolfe's vitamins and laboratory-prepared Thauer's vitamins were tried. It was determined that use of Wolfe's vitamins caused a >15 h increase in the lag phase, though no change in the generation time. Three differences were observed in the formulations: (1) Thauer's vitamins have 10x the concentration of Vitamin B12, (2) Thauer's 10X vitamin stock also includes 2 g choline chloride /l , and (3) Thauer's vitamins were prepared in our laboratory. Further LS4D medium includes tungsten, selenium, and copper as trace minerals. The generation time for *D. vulgaris* on LS4D was 5 h, with a maximum cell density of 10^9 cells/ml and a 0.9-1.0 $OD_{600\ nm}$. The reduction of $FeCl_2$ to 12.5mg/l (~60 uM) as a trace mineral reduced precipitates in the medium without affecting growth rates or generation times significantly. LS4D is well suited for the monitoring protocols, as well as the equipment and large scale processing needed for biomass production. Several reductant use protocols were tested, including titanium citrate, cysteine HCl, and sodium hyposulfite. It was found that more reproducible cultures and shorter lag time could be achieved by inoculating non-reduced medium (no reductant) with 10% actively growing mid-log phase culture. The mid-log phase culture had adequate reducing power in the fresh media to lower the redox sufficiently to allow for continued growth of the cells.