

**Title:** A redox mediated UME biosensor using immobilized chromobacterium violaceum strain R1 for rapid biochemical oxygen demand measurement

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**Abstract:** An effective ferricyanide-mediated microbial biochemical oxygen demand (BOD) biosensor was constructed and used for BOD determination in a water system. This BOD sensor uses ultramicroelectrode (UME) technology in which the tip of the sensor consists of a two-electrode system (10- $\mu$ m Pt working electrode and Pt counter electrode). Because of their small size, UMEs have relatively large diffusion layers and small overall currents enabling rapid achievement of useful steady-state conditions with very high scan rates. Living *Chromobacterium violaceum* R1 cells (isolated from pineapple industry wastewater) were immobilized on the surface of the UME working electrode using a calcium alginate gel and further enclosure by a layer of polyamide membrane. Glucose-glutamic acid (GGA) solution was used as the standard solution. The amperometric measurement was optimized at +450 mV operating potential and 30 mM ferricyanide in a 0.1 M phosphate buffer (pH 7.0) at 26 °C. The sensor exhibited a linear response ranging from 20 to 225 mg O<sub>2</sub> L<sup>-1</sup> BOD<sub>5</sub> for standard GGA solution and 25 to 230 mg O<sub>2</sub> L<sup>-1</sup> BOD<sub>5</sub> for OECD synthetic wastewater with a response time of 30 min. Repeatability and reproducibility of the biosensor were within the limits set by the APHA; i.e., less than 15.4%. The rapid BOD estimation of the biosensor is applicable for measuring samples with a high content of fast and easily assimilated compounds. When used to estimate the BOD of various wastewaters, the developed biosensor provided values comparable to those obtained using the conventional BOD<sub>5</sub> method.