## ENRICHMENT OF BACTERIA WITH GLYCEROL AND SULFATE FROM ANAEROBIC BIOREACTOR SLUDGES

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## Abstract:

Biodiesel is a biofuel that is produced by the transesterification of vegetable oils or animal fats with ethanol or methanol, resulting in the formation of crude glycerol as a main by-product. Crude glycerol is a cheap and attractive substrate for biotechnological application, such as growth of sulfate-reducing bacteria to produce sulfide formetal precipitation. In this work, sulfate reduction with glycerol was studied at neutral pH using sludge samples from bioreactors as inoculum. Bacteria were isolated from the serial dilutions of primary enrichments using a basal medium that contained glycerol as substrate and sulfate as electron acceptor. Sulfate reduction activity of cultures was monitored by measuring sulfidecolorimetrically. Bacterial growth was assessed by measuring the optical densities. Organic acids and alcohols were analysed by highpressure liquid chromatography. Sulfate-reducing (SRB) and fermentative bacteria were co-enriched. A 16S rRNA gene based clone library revealed that Desulfomicrobium (99%similarity), Spirochaeta (97%similarity) and Proteiniphilum (95% similarity) were dominant in the cultures enriched. Glycerol conversion was coupled to sulfate reduction, but the substrate was incompletely oxidized to acetate in the enrichments. Three strains belonging to Desulfomicrobium (99% similarity), Clostridium (99% similarity) and Proteiniphilum (95% similarity) genera were isolated, but the first two isolates were not able to use glycerol. Likely, in the enrichments glycerol was used by fermentative bacteria that formed products that could be utilized by sulfate-reducing bacteria. We suppose that there is a syntrophic relationship among glycerol-degrading fermentative bacteria and SRB. Our data indicate that glycerol promotes growth of sulfate-reducing communities, which might be used to precipitate and recover heavy metals.

Key words: glycerol, sulfate-reducing bacteria, fermentative bacteria

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