

Abstract of Thesis:

The proposed thesis work is aimed at optimization and intensification of a biochemical process for producing hydrogen from biodiesel-derived crude glycerol. The micro-organism used for in this study is *Clostridium pasteurianum*, which has a higher potential for biohydrogen production. This study aims in enriching H₂ producing *Clostridium pasteurianum* by optimizing the physio-chemical parameters for maximal H₂ production by using response surface methodology. This is followed by kinetic and thermodynamic analysis of hydrogen production for both pure and crude glycerol as substrate for fermentation. Similarly statistical optimization of the media components was also considered as one of the approach to intensify H₂ production. Further to maximize valuable metabolite production, extensive analysis and understanding of metabolic pathways is required so as to redirect the cellular metabolic pathways primarily towards its production. An *in silico* metabolic flux model has been formulated for this analysis that determines the complete intracellular fluxes of all metabolites from experimentally measured fluxes. This methodology was used for comparative analysis of mechanical shaking and ultrasound-assisted fermentation for H₂ production. The flux analysis results were further corroborated by targeting the genes involved in glycerol-hydrogen pathway of *C. pasteurianum* which involved overexpression of Fe-only hydrogenase encoded by *hydA* and the enzymes involved in glycerol metabolism encoded by *dhaD* and *dhaK*. The hydrogen production efficiency was compared between the wild type and recombinant strain of *C. pasteurianum* using crude glycerol as the substrate .