INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Sustainable production of biofuels from *Clostridium sporogenes* NCIM 2918: Process strategies and system biology approach

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SHORT ABSTRACT

Global warming coupled with depleting fossil fuel resources has warranted nations to produce fuels which are renewable and carbon neutral. Liquid biofuel (ethanol and butanol) production using *Clostridium* spp. has gained immense attention in recent times. Present study reports a non-acetone producing *Clostridium* strain as a potential producer of liquid biofuels. Biochemical characterization, phylogenetic analysis and 16S rRNA gene sequence analysis identified the strain as *Clostridium sporogenes* NCIM 2918. Alcohol production was positively regulated by sorbitol and instant dry yeast as carbon and nitrogen sources respectively. Media optimization resulted in maximum butanol and ethanol titer (g L^{-1}) of 12.1 and 7.9 respectively. Depending on the combination of carbon sources, the organism was found to manipulate its metabolism towards synthesis of either ethanol or butanol, thereby affecting the total alcohol titer. The first metabolic network for *C. sporogenes* was constructed and Flux Balance Analysis (FBA) was performed to elucidate the reason behind substrate dependent modulation observed in alcohol biosynthesis using *C. sporogenes* NCIM 2918. The elevated ATP demand due to improved growth was satisfied by an upregulated carbon flux towards butyric acid synthesis during glucose and glucose-glycerol fermentations. Possible repression of pyruvate carboxylase resulting in lower carbon flux towards TCA cycle through anaplerotic reaction may be responsible for reduced growth in glycerol fermentation. The ammonium acetate mediated induction of acetic acid utilization, during acidogenesis, led to excess acetyl-CoA generation and its subsequent metabolism to butyric acid or ethanol. Amongst various dual substrate combinations, glucose-glycerol mixture in the ratio of 60:40 resulted in maximum butanol and ethanol titer (g L^{-1}) of 11.9 and 12.1 respectively with total alcohol productivity of 0.59 g L^{-1} h^{-1}. Finally a cost effective process was demonstrated by culturing the organism on cheaper raw materials sugar hydrolysate from lignocellulosic biomass and crude glycerol, a waste from biodiesel industry. Batch fermentation resulted in a total alcohol titer and productivity of 23.5 g L^{-1} and 0.52 g L^{-1} h^{-1} respectively. An improvement in total alcohol titer (26.4 g L^{-1}) and productivity (0.69 g L^{-1} h^{-1}) was observed when batch fermentation was coupled with intermittent gas stripping of the alcohols. To extend the cultivation period, the mode of fermentation was changed from batch to fed-batch. Fed-batch fermentation with intermittent feeding of sugar hydrolysate and crude glycerol coupled with an *in situ* product recovery via gas stripping resulted in further improved total alcohol titer of 44.4 g L^{-1} (butanol 21.5 g L^{-1} and ethanol 22.9 g L^{-1}) with a productivity and yield of 0.62 g L^{-1} h^{-1} and 0.37 alcohol per gram of sugar (glucose + glycerol) respectively.