INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

The present study has been designed and executed with a keen interest on eliminating the major bottlenecks of butanol fermentation, in order to render the developed bioprocess strategies economically feasible and commercially realizable. *Clostridium acetobutylicum* ATCC 824 has been chosen as the platform for butanol biosynthesis. Initially, the strain has been characterized on a wide variety of carbon and nitrogen sources, assessing its maximum butanol potential. Glucose and peptone were screened as suitable carbon and nitrogen source which resulted in a maximum butanol titer of 11.6 g/Land 11.68 g/L respectively. After, the initial screening, statistical media optimization was carried out to optimize the initial concentration of glucose, peptone and trace metal composition with the objective function of maximization of butanol titer. Optimization of media components resulted in a butanol titer of 11.76 g/L , which was an insignificant improvement of 1.3%. Further, with the objective of enhancing butanol production and elucidating the roles of metal ions, the strain was cultivated under individual starvation of magnesium, manganese, iron and sodium. To that end, magnesium starvation positively influenced butanol production with significant increase in butanol titer (13.72 g/L) and earlier onset of solvent formation (6 h). Furthermore, zinc supplementation at 10 mg L-1 in the optimized media proved beneficial for butanol production. Therefore, a novel medium engineering strategy was developed coupling zinc supplementation in magnesium starved optimized medium which resulted in an enhanced butanol titer of 19.18 g L-1 with a maximum productivity of 0.63 g L-1 h -1 . The novel medium strategy resulted in an improvement of 61.5% and 110% with respect to butanol titer and productivity. With the aim of elucidating the metabolic regulations behind inflected phenotypic response of the organism under the influence of metal ions were captured via obtaining temporal expression profile of the key metabolic enzymes in glycolytic, ethanol, butanol and acetone formation pathways. The elevation in butanol biosynthesis was associated with raised glucose utilization, reduced ethanol production and early induction of solventogenesis. Change in these phenotypic traits of the organism may be attributed to multi-level modulation in central carbon metabolism e.g., upregulation of glycolytic pathway; upregulation in thiolase activity; key intermediate enzyme for biosynthesis of acids and solvent; upregulation in the activity of butyrylaldehyde dehydrogenase & butanol dehydrogenase, the enzymes responsible for butanol biosynthesis and downregulation in alcohol dehydrogenase, redirecting carbon flux from ethanol to butanol. In order to enhance process feasibility, a fed-batch was demonstrated with intermittent feeding of glucose and zinc which resulted in 24% enhancement in butanol productivity. However, it was inferred that the high butanol titer was detrimental for cellular metabolism and the fermentation terminated at an early stage. Thus, in order to alleviate butanol toxicity the fed-batch was coupled with in-situ product recovery through optimized gas stripping parameters which resulted in a cumulative titer of 54.2 g L-1 with average productivity of 0.66 g L-1 h -1 . However, the strategy was demonstrated using expensive laboratory grade peptone and glucose as nitrogen and carbon source respectively. Hence, present study also demonstrates a novel two-stage sequential bioprocess for production of biobutanol using low cost substrates, corn steep liquor and industrial grade maize starch. Improved butanol titer using low cost substrates was achieved via combinatorial approach of: (i) optimization of initial concentration of corn steep liquor and starch; (ii) hydrolysis of starch into fermentable sugar using industrial grade amylase and (iii) coupling attributes of butanol upregulation via magnesium starvation and zinc supplementation. The strategy resulted in a butanol titer of 16.54 g L-1 with a yield of 0.28 g g-1 . The produced butanol was further distilled (90% purity) and the qualitative and engine performance was analyzed for its potent application as an alternate transportation fuel. Physicochemical properties of butanol-diesel blends e.g. kinematic viscosity, absolute viscosity, density, flash point, fire point, cloud point and pour point corroborate well with pure diesel. Engine performance analysis revealed enhanced brake thermal efficiency with negligible change in key engine performance parameters under butanol–diesel blends as compared to diesel, depicting suitability of butanol as potent alternate to petroleum fuel. An economic analysis of the developed bioprocess strategies was also performed which revealed that the bioprocess strategy using low cost substrates resulted in a price of 0.8 USD for every litre of butanol, offering economic feasibility for possible commercial realization which was a significant reduction of 99% with respect to the media using peptone and glucose as nitrogen and carbon source. Hence, the current study successfully demonstrates suitable and sustainable bioprocess strategies using low cost substrates which aids towards commercial realization of butanol biosynthesis