INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	SAUMYA AHLAWAT
Roll Number	:	126106021
Programme of Study	:	Ph.D.

Thesis Title: Development of a bioprocess for enhanced butanol production: Integrating laboratory experiments with *in-silico* predictions

Name of Thesis Supervisor(s)	:	PROF. DEBASISH DAS
Thesis Submitted to the Department/ Center	:	BIOSCIENCES AND BIOENGINEERING
Date of completion of Thesis Viva-Voce Exam	:	27. 09. 2019
Key words for description of Thesis Work	:	Biofuels; Butanol; Bioprocess development; Systems biology; *Clostridium* sp.

SHORT ABSTRACT

Increasing global population and energy intensive lifestyle has resulted in increased global energy demand. Butanol, produced by clostridial fermentation, is a promising transportation alternative as a drop-in fuel due to its physiochemical properties. The present study aims to address pressing bottlenecks and develop a suitable bioprocess for butanol production using *Clostridium* sp. Suitable strain for fermentation, *C. acetobutylicum* MTCC 11274, was selected from procured cultures based on a maximum butanol production capability of 11.5 g L^{-1}. The maximum butanol titer obtained under screening experiments restricted by butanol toxicity. Hence, for media optimization, maximization of biomass productivity and maximization of butanol productivity were selected as objective functions. Based on batch experiments conducted using the media obtained for maximization of biomass productivity and maximization of butanol productivity, a two-stage fed-batch fermentation strategy was developed. The process involved sequential use of biomass productivity media followed by butanol productivity media, where butanol induction served as the transition point from one stage to the second stage. The strategy resulted in a 77 % improvement in the butanol productivity (0.55 g L^{-1} h^{-1}) when compared to batch fermentation using the optimized medium for maximization of butanol productivity. Further, studies on limitation and starvation of nutrients in stage one yielded magnesium limitation as the most suitable factor and addition of 5 g L^{-1} calcium carbonate in stage two was found to be the best among supplementation experiments. Based on these results, a combinatorial approach was designed which improved butanol titer and butanol productivity to 16.5 g L^{-1} and 0.59 g L^{-1} h^{-1}, respectively, one of the highest values reported in literature. Further improvement in butanol titer was obtained by integrating the combinatorial strategy with *in-situ* product recovery. Intermittent gas stripping served the dual purpose of alleviation of butanol toxicity and product recovery. To reduce the cost of nutrients used, glucose and peptone were replaced with a rice straw hydrolyzate and instant dry yeast, respectively. The final process resulted in a 54.3 g L^{-1} butanol titer, 0.58 g L^{-1} h^{-1} butanol productivity, 40% replacement of glucose with rice straw hydrolyzate, and 100% replacement of peptone with instant dry yeast. Further reduction in substrate cost would be possible upon utilization of a highly concentrated sugar hydrolyzate. Hence, the strategy demonstrated potential for sustainable butanol production. Flux balance analysis was performed to understand the metabolic flexibility exhibited by the organism in response to nutritional modulation during batch and fed-batch fermentations. Early induction of solventogenesis during magnesium limited two-stage fed-batch fermentation was attributed as the secondary effect of improved growth. In order to fulfill the ATP demand of improved growth, elevated carbon flux was diverted toward acid synthesis pathways, which serve as ancillary source of energy generation after glycolysis. Pathways related to biomass and butanol synthesis remained concomitantly active during acidogenesis and solventogenesis under combinatorial usage of magnesium limitation and calcium supplementation in two-stage fed-batch fermentation. This can be attributed to the presence of calcium in second stage, which is reported to aid in upregulation of heat shock proteins, DNA synthesis, transcription, repair, and carbohydrate catabolic enzymes, and stabilize membrane proteins. Thus, calcium alleviated the negative effects of butanol and stalled the downregulation of solventogenic reactions.