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Enhanced Production and Expression of Recombinant DNA Ligase by Using Modified Growth Media in Bioreactor

Abstract

The ligase enzyme is also called synthetase due to its synthetic activity as conservation of chemical energy and provides a couple between energy demanding synthetic process. DNA ligase is one of the top-ranking enzymes in DNA diagnostics because of its ability to form bond between two DNA molecules. It also plays a very crucial role for the detection of oncogenic diseases. The ligTK gene was isolated from bacterium *Pyrococcus abyssi*. The plasmid pET22b (+) was used as expression vector for ligTK gene. The *Escherichia coli* (BL21(RIPL codon plus)) was used as host strain for the expression of DNA ligase enzyme. The fermentation parameters were optimized during shake flask fermentation and volumetric yield was scaled up by batch fermentation. Enhanced production of recombinant protein (2.115g h⁻¹) with higher biomass concentration (29.78g L⁻¹) and expression level (30 %) was efficaciously achieved in 8 hours of batch fermentation with modified M9NG medium auto-induced with 10 mM lactose fermented at 37°C, pH: ±7.0 agitated at 1000 rpm dissolve oxygen concentration 30 %. Optimization of media formulation facilitated in improving favorable conditions for better cell growth. The specific growth rate attained was 0.424 h⁻¹ and observed to be gradually reduced in periodic manner whereas substrate consumption associated with the highest production was 0.026 g h⁻¹. This study concluded that the influence of media formulation, inducer supplementation and fermentation conditions on the kinetics of recombinant DNA Ligase crux for the production and expression enhancement of cell density or enzymes in shortest fermentation time frame.

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