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Research review paper

Growing *E. coli* to high cell density—A historical perspective on method development

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Abstract

E. coli is the major bacterial platform for expressing simple heterologous proteins. Growing *E. coli* to high densities has been the subject of numerous studies since the early 1970s, exploring the limits of bacterial culture density in order to achieve maximum productivity. Research strategies were focused on improving the cultivation techniques, manipulating the bacteria's physiology or both. As a result, batch, fed batch and dialysis fermentation techniques had been developed. These growth strategies, together with optimization of media composition and the application of molecular biology methods, made it possible to grow *E. coli* to cell densities of up to 190 g/l (dry weight), while avoiding media precipitation and preventing acetate accumulation. Additional research on the effects of heterologous protein biosynthesis on signal transduction, proteolysis and post transcription events in *E. coli* may improve its productivity.
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Keywords: *E. coli*; Growth strategies; Acetate excretion; High density

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1. Introduction

Growing *E. coli* to high density is currently the method of choice for the production of recombinant proteins, mainly because of the high volumetric productivity associated with this method. Exploring the growth limits of microorganisms in general and *E. coli* in particular, engaged industrial microbiologists many years before it was possible to convert *E. coli* to a "production machine" for heterologous proteins. Today, techniques to obtain the highest possible density of productive *E. coli* in submerged cultures are well developed.

Current commercial products obtained from *E. coli* cultures include mainly recombinant proteins from prokaryotic and eukaryotic sources which are considered to be low-volume–high-value products (Riesenberg et al., 1990). In addition, recent advancements in metabolic engineering made it possible to use *E. coli* as a platform to produce high-volume–low-value products. Products such as poly-hydroxy-butyrate, succinic acid, octanoic acid, aromatic compounds, ethanol, acetone and styrene oxide are a few examples of the latter (Lee, 1996). For high-volume–low-values products, high cell density and high volumetric yield are essential conditions for economical feasibility. For low-volume–high-value products high cell density significantly reduces capital investment and operation costs of the GMP production facilities. This reduction in cost is achieved due to reduction in size of the fermentation equipment, upstream utilities such as purified water, purified steam, clean air supply and clean room environments. The size of downstream process units such as centrifuges, micro and ultra filtration devices, and purification apparatus is also being reduced. Hence, obtaining high-density cultures and improving the volumetric productivity is a major objective of any *E. coli*-based process.

Early studies on high cell density growth of aerobic gram-negative bacteria, including *E. coli* were performed either to investigate the limits of bacterial growth in liquid cultures (Hestrin et al., 1943; Gorelick et al., 1951; Vinet and Fredette, 1951; Tyrrell et al., 1957; Gerhardt and Gallup, 1963) or to obtain large quantities of exponentially grown *E. coli* needed for biochemical studies (Bauer and Shiloach, 1974; Shiloach and Bauer,