438n Modeling and Optimization of DNA Plasmid Production from E. Coli Fermentation

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Interest in DNA plasmid production stems from the fact that approximately 25% of the ongoing clinical trials for gene therapy products use DNA plasmids to carry the the corrective gene. DNA plasmids are also finding application in vaccine development. Most DNA plasmids currently in clinical trials are produced from fermentation of E. coli bacteria strains with a recombinant DNA plasimd that contains the corrective gene for gene therapy products or the appropriate gene for vaccine development. These plasmids also typically contain a gene for antibiotic resistance so that the presence of antibiotic in the media acts as a selective pressure on the E. coli bacteria to encourage the production and retention of the plasmid during fermentation. In this work, we are interested in optimizing plasmid productivity in the fermentation operation.

Although there have been a number of detailed, distributed parameter, metabolic pathway-based models describing the production of plasmids in E. coli bacteria, these models are not particularly useful for productivity optimization. The most significant drawback is the complexity of the model and the inability to identify numerical values for all of the rate expression constants in the model from typical ferementation operating data. We present a reduced-order model for the bacterial system that predicts cell mass and plasmid productivity where the parameters are identifiable from operating data. We then use this model to explore non-conventional fermentation operating conditions to maximize plasmid productivity. Although continuous fermentation is very efficient at increasing the production of cell mass, DNA plasmid production is not directly related to cell mass production. At high cell mass production rates, the production of DNA plasmids actually decreases due to a shift in the growth-related production of DNA plasmid in E. coli. Our studies indicate that semi-continuous operation produces both a high productivity of cell mass and a high DNA plasmid concentration at appropriate antibiotic concentrations in the media. These results are illustrated using the E. coli strain Life Technologies DH5a which produces the DNA plasmid pUC18. Experimental plasmid productivity is compared and an optimal operating policy based on the reduced-order model is presented.