

Expression of EGFP Reporter Protein with a Recombinant Vaccinia Virus - Comparison of Microcarrier and Cell Suspension Based Bioreactor Systems

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ABSTRACT

A recombinant vaccinia virus expression system was studied as an alternative method to produce recombinant proteins in a scalable bioreactor system. A recombinant vaccinia virus containing the gene for the reporter protein, enhanced green fluorescent protein (EGFP), was used to study the system parameters that affect final protein expression. Evaluations of a microcarrier based method for adherent cell growth and infection in a bioreactor were performed. Controllable parameters such as temperature and dissolved oxygen level during production were tested to observe the effect on the level of protein production. Additionally, a cell suspension system was compared to the microcarrier method to determine if the levels of cell growth, viral infection, and ultimately protein expression could be maintained with a simpler production scheme.

BACKGROUND

• Objective - Develop a protein production process with recombinant vaccinia virus

• Vaccinia Virus

- Transcription occurs in cytoplasm of infected cell
- Wide host range
- VOTE[®] inducible expression system for high, T7 promoter controlled expression
- Utilize previously constructed recombinant virus containing the gene for the reporter protein, enhanced green fluorescent protein (EGFP), to examine expression characteristics

• High Cell Density

- HeLa and HeLa S3 cells – human cervical adenocarcinoma
- Compare attachment dependent growth on microcarriers to suspension growth

• Develop bioreactor strategies for growth

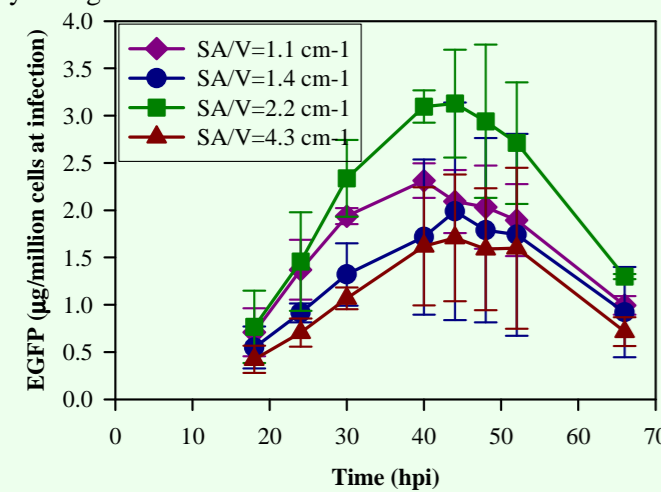
• Study infection processes for these two types of systems

PARAMETER OPTIMIZATION

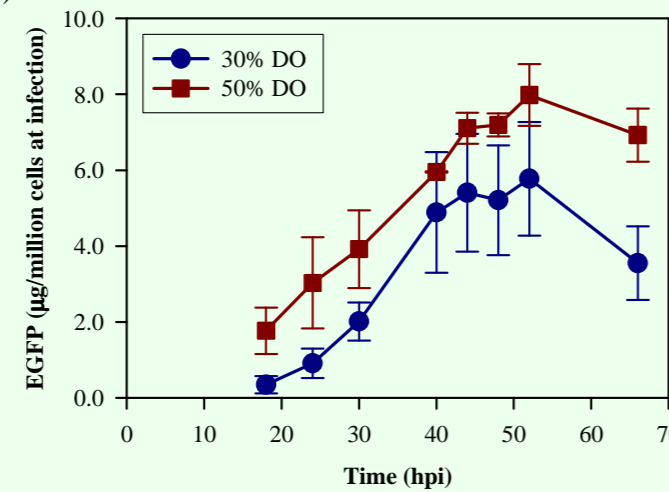
Production with the Microcarrier System

DO Levels in Spinner Flasks

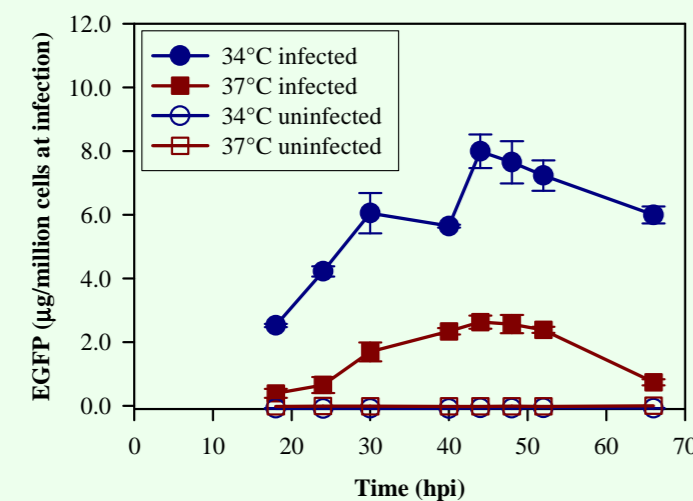
(by changes in surface area to volume ratio of media in flasks)



DO Levels in Bioreactor



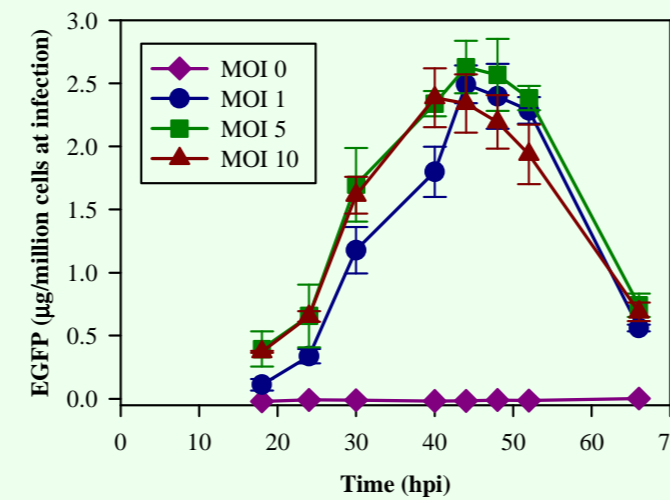
Temperature in Spinner Flasks



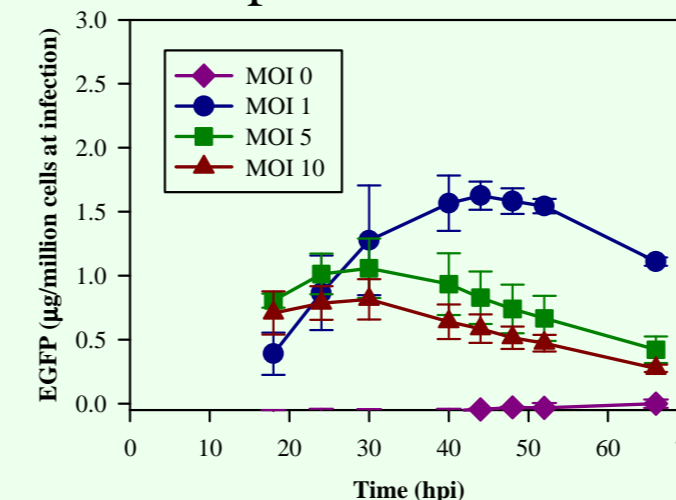
COMPARISON OF MICROCARRIER AND SUSPENSION SYSTEMS

Effect of MOI on Expression in Spinner Flasks

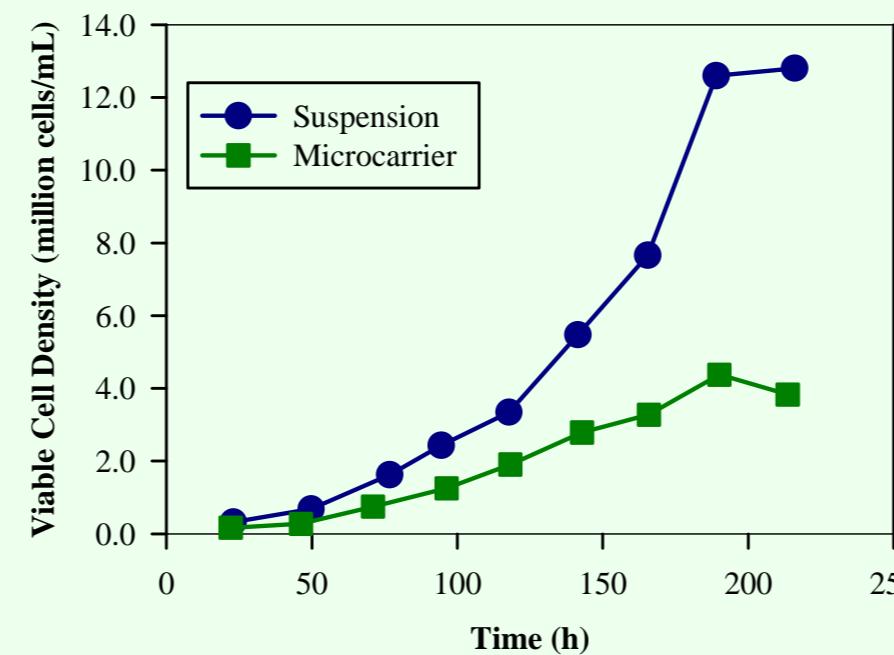
Microcarrier Culture



Suspension Culture

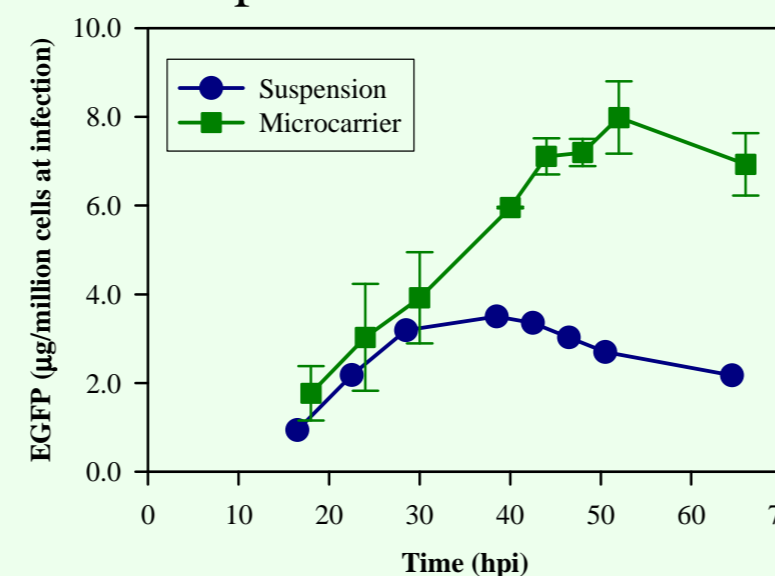


Growth Comparison in Bioreactors

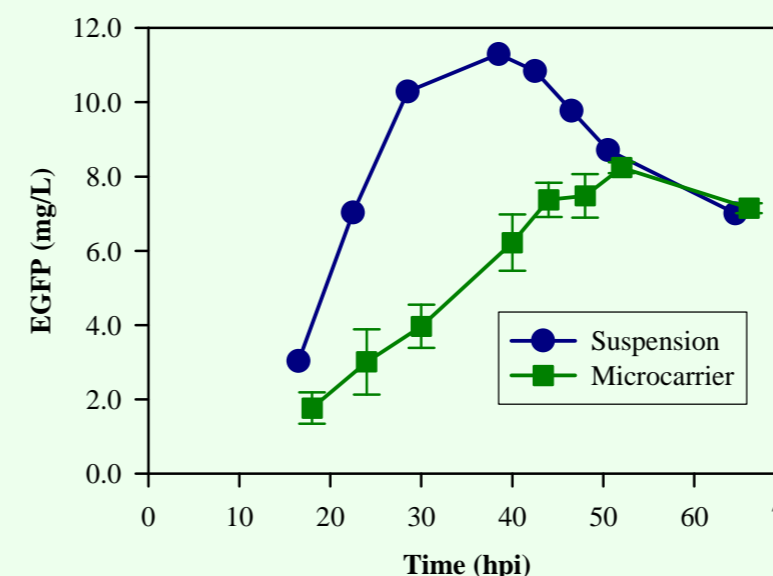


Protein Production Comparison in Bioreactors

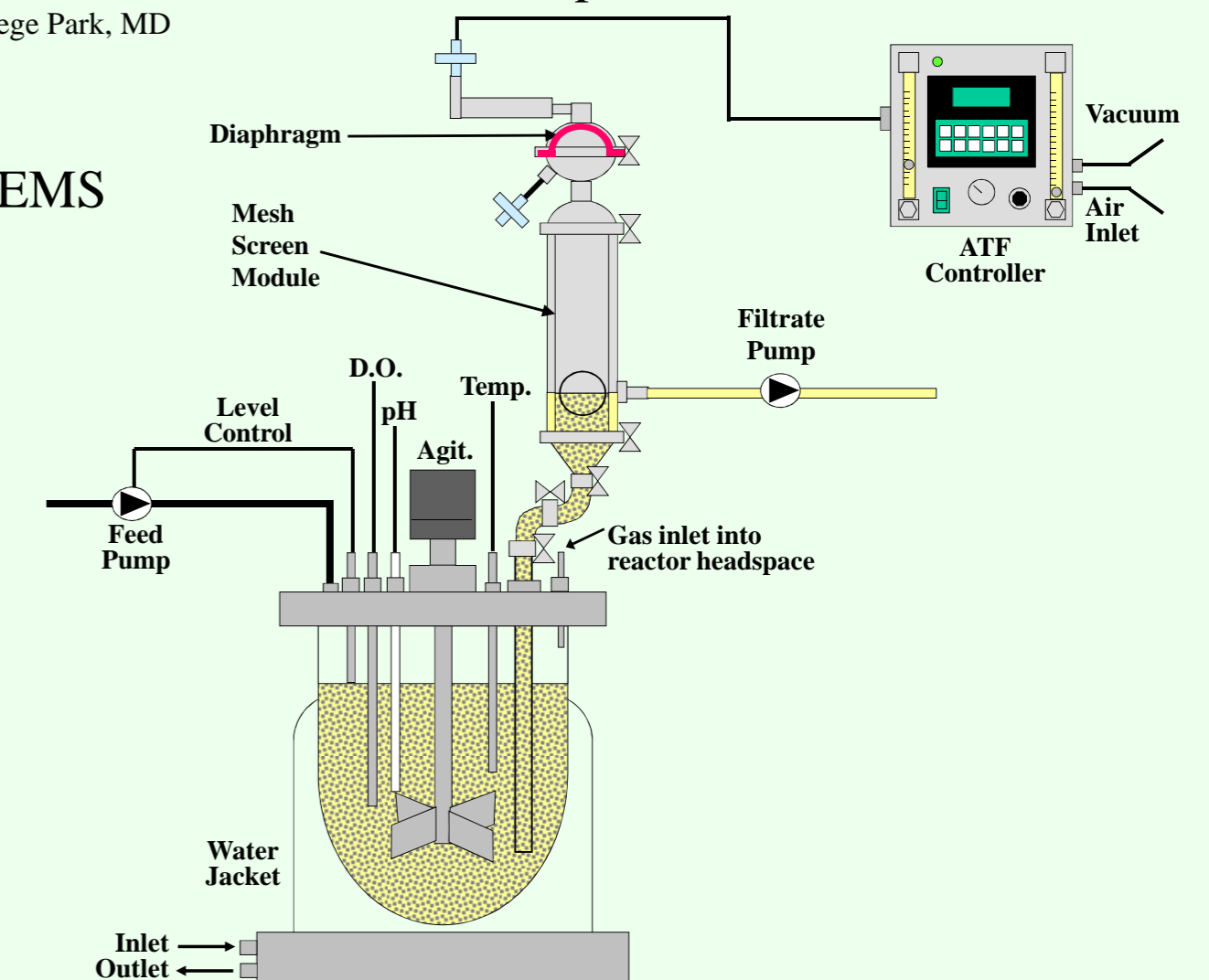
Specific Production



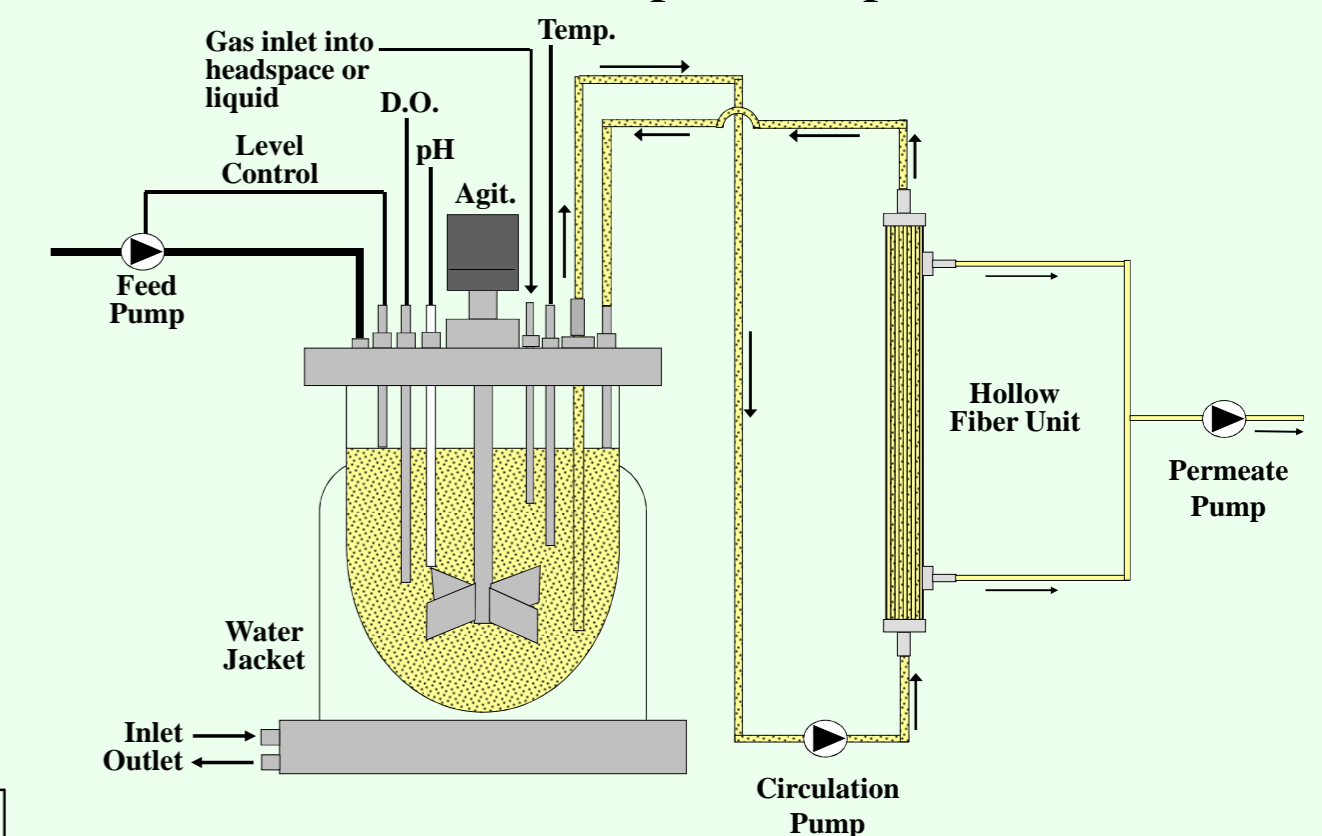
Overall Production



Bioreactor Setup for Microcarrier Culture



Bioreactor Setup for Suspension Culture



CONCLUSIONS

- Higher dissolved oxygen and lower temperature during production increase expression levels
- Both microcarrier and suspension systems can produce proteins
 - MOI above 1 appears to be more detrimental to suspension production than microcarrier production
 - Microcarrier system has a higher specific production
 - Suspension system has a higher overall production due to a higher cell density