XIII Congreso Nacional de Biotecnología y Bioingeniería
SIPAL VII Simposio Internacional de Producción de Alcoholes y Levaduras

BIOREACTOR SYSTEM DEVELOPMENT FOR LOW-COST BIOPRODUCTION: BIOFUELS, BIOPHARMACEUTICALS & PLANT PROPAGATION.

Wayne R. Curtis, 226B Fenske Laboratory, Dept. Chemical Engineering, Penn State University, University Park, PA 16802; Fax: 814-865-7846 WRC2@psu.edu

Keywords: Algae biofuel, Protein production, Ethanol fermentation

Introduction: The development of commercial bioreactors predominantly for pharmaceutical production has biased reactor development towards autoclaves and steam sterilizable materials. This presentation will overview several bioproduction processes we are developing which include components of low-cost bioreactor design and operational principles. Transient expression of proteins in plant tissue culture and plant propagation can be implemented in simple plastic bioreactors. The ability of purple non-sulfur bacteria to grow under anaerobic photoheterotrophic conditions which are not permissive to growth of contaminants permits membrane protein expression in reactors with only simple surface sterilization. Biofuels represent the greatest challenge to low-cost, high-volume production.

Methods: The low cost plastic-lined bioreactor system is patented (1), with several implementations described therein. Our most recent publication on transient expression describes the delivery of heterologous genes into plant tissue cultures of *Nicotiana glutinosa* using a co-cultured *Agrobacterium* auxotroph (2). The strains of *Rhodobacter sphaeroides* used for heterologous membrane protein expression are described in detail in work at Argonne National Lab (3). Biomass fermentation is being developed for *Clostridium phytofermentans* (4). Bioreactor designs for plant propagation have been implemented with loblolly pine and cacao; while most remain to be published, design principles are available (5). Algae culture is being conducted with *Botryoccocus braunii* using a stoichiometrically balanced media as described in a recent patent application (6) which also describes photobioreactors.

Results and discussion: Plant cell and tissue culture can be grown in simple reactors where offline measurements combined with structured models of growth allow for operating conditions to be set without feedback control based on probes. The ability to grow plant cells in a simple plastic reactor and introduce genes transiently opens the opportunity to rapidly produce proteins in 3-5 days without waiting for transformation. This technology provides the flexibility to produce vaccine epitopes in response to a sudden demand such as an outbreak of a flu variant. Similarly, *Rhodobacter* growth on organic acids allows simple control of nutrient conditions by fed-batch addition of succinate with excess nitrogen. We have achieved heterologous cytochrome membrane protein expression to 2-6% of the dry weight! Bioreactor cell concentrations approaching 9 grams dry weight per liter are achieved under photoheterotrophic conditions (where quorum sensing stops growth). Similar bioreactor technology with nutrient mass-balanced growth of *Botryococcus* has resulted in several month-long steady-state cell concentrations greater than 25 gDW/L and oil productivities of 15 mg/L/photo-hr (which is one of the highest oil productivities ever reported).

Conclusions: Low capital investment bioreactors with low operating costs impose different constraints on bioprocess design and operation. This is particularly true for bioprocesses with operation time scales of weeks or months. The ability to accommodate slower process kinetics greatly reduces demand for process control which permits additional cost reduction by allowing off-line measurements and feed-forward process control. Maturing biotechnological products for non-pharmaceutical applications and products that can be validated based on improved analytics will reduce reliance on process validation. These changes will facilitate a paradigm shift towards reduced constraints for maintaining asepsis and greater flexibility in the implementation of biological processing.

Acknowledgements: Collaborators include Susan Leshine (U.Mass, Amherst), Deborah Hanson & Philip Laible (Argonne National Lab) and Joe Chappell (U. Kentucky). Part of this work was supported by Monsanto and Weyerhaeuser as well as support by the National Science Foundation under grants no. BCS-0003926, BIR-941304, and CBET-0828648. Any opinions, findings, and conclusions or recommendations expressed in this material are thoseof the authors and do not necessarily reflect the views of the National Science Foundation.

References:

1. Curtis, WR. (2004). Growing cells in a reservoir formed of a flexible sterile plastic liner, U.S. Patent # 6,709,862.
2. O'Neill KM, Larsen JS, Curtis WR (2008). Scale-up of *Agrobacterium*-mediated transient protein expression in bioreactor-grown *Nicotiana glutinosa* plant cell suspension culture. *Biotechnol Prog* 24(2):372-376.
3. Laible PD, Mielke DL, Hanson DK. (2008). Foreign gene expression in photosynthetic bacteria. In: *The Purple Phototrophic Bacteria*. Hunter, CN, Daldal, F, Thurnauer, MC, Beatty, JT, (Eds.) Springer-Verlag, New York, Chapt 43.
4. Warnick TA, Methe BA, Leschine SB. 2002. *Clostridium phytofermentans* sp. nov., a cellulolytic mesophile from forest soil. *Int J Syst Evol Microbiol* 52: 1155-1160.
5. Curtis, WR. (2005). Appl of bioreactor design principles to plant micropropagation. *Plant Cell Tissue Organ Cult* 81:255-264.
6. Curtis, WR. Trickle-film bioreactor for growth of photo-heterotrophic anaerobes for membrane protein production and growth of photosynthetic algae. (U.S. Patent Application # 12/387140).