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Received Jul 2; Accepted Nov 4. To view a copy of this license, visit http: Abstract Bioprocess limitations imposed by microbial cell-to-cell phenotypic diversity remain poorly understood. To address this, we investigated the origins of such culture diversity during lipid production and assessed the impact of the fermentation microenvironment. We measured the single-cell lipid production dynamics in a time-invariant microfluidic environment and discovered that production is not monotonic, but rather sporadic with time. To characterize this, we introduce bioprocessing noise and identify its epigenetic origins. We linked such intracellular production fluctuations with cell-to-cell productivity diversity in culture. This unmasked the phenotypic diversity amplification by the culture microenvironment, a critical parameter in strain engineering as well as metabolic disease treatment. The emerging paradigm of constructing target phenotypes for the production of chemical products and biofuels is attracting considerable interest and has met with significant success in recent years 1 , 2 , 3 , 4. Additionally, single cell analysis reveals a considerable productivity variance within a clonal population. Whereas few individual cells greatly outperform the median productivity, others lag in productivity and are even less efficient than non-engineered strains. This in essence limits our ability to register the true phenotype of a construct and, as such, it limits its bioprocessing reliability. Here we investigate the aforementioned limitations in bioprocesses at the single cell level with a specific focus on the de-novo lipid biogenesis of Yarrowia lipolytica, a promising candidate in the production of oleochemicals 5 , 6 , 7. First, we determined the phenotypic diversity in culture during batch growth of an over-producing and an under-producing strain. Subsequently, the lipid production dynamics of both strains were analyzed at the single cell level using microfluidics, primarily for two reasons. The first was to perform longitudinal investigations by tracking the lipid content at the single cell level over time. The second was to deterministically control the extracellular hydrodynamics, thus generating a quasi-time-invariance through ultra-fast nutrient supply and byproduct removal. Under such conditions, the inherent intracellular fluctuations of lipid abundance within an ideal extracellular environment were unmasked. We identified the epigenetic origins of such fluctuations and then compared them to the lipid content diversity between individuals in culture, similar to recent gene expression noise investigations 8 , 9 , 10 , 11 , Results Phenotypic Diversity in Culture: The Po1g and MTYL strains of Yarrowia lipolytica were investigated, as previously developed and extensively characterized 5. DGA1 is the final step of the triglyceride synthesis pathway thereby enabling MTYL with enhanced lipid production 5. As shown in Fig.

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Lumped compositions, molecular weight and reductance degree of cellular protein, monoclonal antibody, biomass and amino acid consumption excluding glutamine and alanine are found to be relatively constant for different hybridoma cell lines and may be used as regularities. The calculated rates of oxygen uptake and carbon dioxide evolution agree well with experimental values of several different cultures reported in the literature. This simple method gives the same results as calculated on the basis of a detailed metabolic reaction network. Selected References These references are in PubMed. This may not be the complete list of references from this article. Aeration in cell culture bioreactors. Determination of the respiration quotient in mammalian cell culture in bicarbonate buffered media. Metabolic flux analysis of hybridoma cells in different culture media using mass balances. The covalent structure of an entire gamma G immunoglobulin molecule. Application of mass and energy balance regularities in fermentation. On-line gas analysis in animal cell cultivation: Methods for oxygen uptake rate estimation and its application to controlled feeding of glutamine. On-line estimation of viable cells in a hybridoma culture at various DO levels using ATP balancing and redox potential measurement. Glucose and glutamine metabolism of a murine B-lymphocyte hybridoma grown in batch culture. Cell volume measurement as an estimation of mammalian cell biomass. CO2 in large-scale and high-density CHO cell perfusion culture. A kinetic analysis of hybridoma growth and metabolism in continuous suspension culture on serum-free medium. Cell retention â€" chemostat studies of hybridoma cells â€" analysis of hybridoma growth and metabolism in continuous suspension culture on serum-free medium. The effect of dissolved oxygen on the metabolic profile of a murine hybridoma grown in serum-free medium in continuous culture. Use of on-line gas analysis to monitor recombinant mammalian cell cultures. A combined cell-cycle and metabolic model for the growth of hybridoma cells in steady-state continuous culture. Network analysis of intermediary metabolism using linear optimization. I Development of mathematical formalism. Applications of improved stoichiometric model in medium design and fed-batch cultivation of animal cells in bioreactor. Material balance studies on animal cell metabolism using a stoichiometrically based reaction network. Continuous real-time monitoring of oxygen uptake rate OUR in animal cell bioreactors. Use of respiratory quotient as a control parameter for optimum oxygen supply and scale-up of 2,3-butanediol production under microaerobic conditions. Effect of CO2 absorption on the measurement of CO2 evolution rate in aerobic and anaerobic continuous cultures. A new balance equation of reducing equivalents for data consistency check and bioprocess calculation. High viable cell concentration fed-batch cultures of hybridoma cells through on-line nutrient feeding. Intracellular flux analysis applied to the effect of dissolved oxygen on hybridomas.

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Datar, R. V., T. Cartwright, and C. G. Rosen. "Process Economics of Animal Cell and Bacterial Fermentations - A Case Study Analysis of Tissue Plasminogen Activator." Bio/Technology 11, no. 3 ()