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^{13}C metabolic flux analysis identifies limitations to increasing specific productivity in fed-batch and perfusion

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ABSTRACT

Industrial cell culture requires substantial energy to generate protein. The protein generated is not only the product of interest (IgG in this case), but also the protein associated with biomass. Here, ^{13}C-Metabolic Flux Analysis (^{13}C-MFA) was utilized to compare the stationary phase of a fed-batch process to a perfusion process producing the same product by the same clone. The fed-batch process achieved significantly higher specific productivity, approximately 60% greater than the perfusion process. In spite of this, a general lack of difference was observed when globally comparing glycolysis, pentose phosphate pathway, and TCA cycle fluxes. In contrast, gross growth rate was significantly different, approximately 80% greater in the perfusion process. This difference was concealed by a significantly greater death rate in the perfusion process, such that net growth rates were both similar and near-zero. When considering gross growth rate and IgG specific productivity, total protein specific productivity (Biomass + IgG) differed little, offering rationale for the observed central carbon pathway similarities. Significant differences were identified in anaplerotic branched-chain amino acid (BCAA) contributions by ^{13}C-MFA. The perfusion process exhibited markedly higher (up to three times) BCAA catabolism, an observation often associated with increased death.

1. Introduction

A current focus in the biologics industry is to maximize upstream productivity. This allows for a potential reduction in cost of goods and manufacturing footprint (Xu et al., 2016). Cost of goods and manufacturing footprint are directly influenced by the selection of fed-batch or continuous operation. Among several factors, market size, existing infrastructure, product stability, and historical experience all dictate the selection of fed-batch vs. perfusion for a given process (Konstantinov et al., 2006). Given the inherent advantage of platform options, we sought to understand how each mode of operation affects cell culture process performance at a systems level.

To examine cell culture at a systems level, ^{13}C Metabolic Flux Analysis (^{13}C-MFA) was utilized to examine the impact of reactor mode of operation upon the cell. Gene library, mRNA expression, protein expression, and post-translational modifications (PTMs) all influence metabolic fluxes. Flux measurements offer direct pathway quantitation, and provide a holistic description of exhibited cellular phenotypes. In contrast, protein expression does not always correspond with pathway activity (Burgess et al., 2007). Fluxes eventually saturate, regardless of enzyme expression levels. mRNA expression does not consistently correspond with pathway activity (Chubukov et al., 2013). Enzymatic activity of protein does not necessarily correlate with observed flux in Chinese Hamster Ovary (CHO) cells (Wahrheit et al., 2014).

In addition to providing direct pathway quantitation, MFA enables a straightforward comparison between fed-batch and perfusion processes because it is based on specific rates. Comparison of perfusion to fed-batch time-course profiles (e.g. glucose concentration or titer) is otherwise challenging. Glucose is continuously consumed by culture, continuously added in the feed, and continuously removed in the effluent (varying with dilution rate) of a perfusion process. Likewise, IgG is continuously produced by the culture, but it too is being continuously removed in effluent. Glucose is continuously consumed and product continuously produced in fed-batch, but since there is no continuous feed or effluent, time-course profiles do not lend themselves to a fair comparison. MFA views cell specific consumption or production rates, allowing a fair comparison of cellular metabolism. In addition to MFA, Flux balance analysis (FBA) also allows cellular metabolism to be compared. However, it requires the usage of biological objective functions to constrain the solution space (Feist and Palsson, 2010). To avoid

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Nomenclature

μ_{gross}	Gross growth rate (day^{-1})
μ_{net}	Net growth rate (day^{-1})
^{13}C-MFA	^{13}C Metabolic Flux Analysis
3PG	3-Phosphoglycerate
AcCoA	Acetyl-CoA
ACL	ATP Citrate Lyase
aKG	α-Ketoglutarate
Ala.e	Alanine.extracellular
Ala	Alanine (intracellular)
ALT	Alanine transaminase
Arg.e	Arginine.extracellular
Asn.e	Asparagine.extracellular
Asp.e	Aspartate.extracellular
ATF	Alternating tangential flow
ATP	Adenosine-5′-triphosphate
BCA	Bicinchoninic acid
BCAA	Branched-chain amino acid
BCAT	Branched-chain amino acid aminotransferase
BCKDC	Branched-chain α-ketoacid dehydrogenase
C	Concentration (mM)
CD	Chemically-defined
Cit	Citrate
D	Dilution rate (day^{-1})
DHAP	Dihydroxyacetone phosphate
DO	Dissolved oxygen
DW	Dry weight (pg)
E4P	Erythrose-4-phosphate
F6P	Fructose 6-phosphate
Fum	Fumarate
G6P	Glucose-6-phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
G6PDH	Glucose-6-phosphate dehydrogenase
Galactose	Galactose.extracellular
GAP	Glyceraldehyde-3-phosphate
GC-MS	Gas chromatography mass spectroscopy
Glc.e	Glucose.extracellular
Gln.e	Glutamine.extracellular
Glu.e	Glutamate.extracellular
Gly.e	Glycine.extracellular
GS-CHO	Glutamine synthetase-Chinese Hamster ovary
HCP	Host cell protein
His.e	Histidine.extracellular
HPLC	High performance liquid chromatography
IDH	Isocitrate dehydrogenase
Ile.e	Isoleucine.extracellular
k_d	Death rate (day^{-1})
Lac.e	Lactate.extracellular
LDH	Lactate dehydrogenase
Leu.e	Leucine.extracellular
Lys.e	Lysine.extracellular
Mal	Malate
Met.e	Methionine extracellular
MSX	Methionine sulfoximine
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide phosphate
OAA	Oxaloacetate
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PEP	Phosphoenolpyruvate
PGI	Phosphoglucose isomerase
Phe.e	Phenylalanine.extracellular
PPP	Pentose phosphate pathway
Pro.e	Proline.extracellular
Pyr	Pyruvate
q	Flux (C or N pmol/cell/day)
R5P	Ribose-5-phosphate
RCF	Relative Centrifugation Force
RIPA	Radioimmunoprecipitation
ROS	Reactive Oxygen species
Ru5P	Ribulose-5-phosphate
S7P	Sedoheptulose-7-phosphate
Ser.e	Serine.extracellular
Suc	Succinate
t	Time (day)
TCA Cycle	Tri-Carboxylic Acid Cycle
Thr.e	Threonine extracellular
Trp.e	Tryptophan.extracellular
Tyr.e	Tyrosine.extracellular
Val.e	Valine.extracellular
X	Viable cell density (cell/mL)
X5P	Xylulose-5-phosphate
Y_{LDH}	LDH content per cell (μU activity/cell)

user-defined objective functions, MFA was selected over FBA.

The central carbon pathways of glycolysis, the pentose phosphate pathway, and the TCA cycle are all considered through MFA in this work. Catabolic contributions from branched-chain amino acids (BCAA), and their relationship with death pathways, are also examined. The relationship between total anabolic protein loads and IgG production are evaluated, specifically as they relate to metabolic phenotype. Since a multitude of phases make up a fed-batch, we examined the stationary-like phase (negligible net growth) of predicted peak volumetric IgG productivity and matched this condition in the perfusion process (optimized for volumetric productivity). To our knowledge, this is the first ^{13}C-MFA study to directly compare perfusion and fed-batch modes of operation at any phase.

2. Materials and methods

2.1. Cell line

The same Glutamine Synthetase (GS) CHO clone expressing an IgG1 (pI ≈ 8.15) was cultured in both the perfusion and fed-batch processes. The clone was transfected by electroporation, and selected by methionine sulfoximine (MSX) amongst approximately 1000 clones.

2.2. Bioreactor operation

The same 3 L glass bioreactors (Sartorius Stedim, Gottingen, Germany) were used for all experiments, with geometry and operation similar to previous work (Xu and Chen, 2016). Two biological replicates were performed for both processes. Via base addition and CO_2 sparging, pH was maintained at 6.75–7.05 for the course of the experiments. Temperature was controlled at 36.5 °C and dissolved oxygen (DO) maintained at 30% of air saturation with pure O_2.

In the perfusion process, a 0.2 μm polyether sulfone (PES) hollow fiber membrane (Part# F2:RF02PES, Repligen Corporation, Waltham, MA) was utilized in conjunction with an ATF-2 (alternating tangential flow) unit (Repligen Corporation, Waltham, MA). The exchange rate was regulated at 1 vessel volume daily (VVD), controlled by external pumps (ChemTec, model 108, SciLog, Inc, Middleton, WI) and scales (Explorer Pro, model EP32001, Ohaus Corporation, Pine Brook, NJ). A working volume of 1.3 L was maintained throughout the 30-day run. ^{13}C tracers were continuously introduced to the culture from day 26–29.