A simultaneous assessment metric for MAb quantity and glycan quality

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Received: 20 March 2016 / Accepted: 16 July 2016 / Published online: 9 August 2016
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Abstract As a critical quality attribute, glycosylation represents an important consideration when analyzing the success of a glycoprotein production process. Though critical, glycosylation is not the only measure of culture success; other factors, including culture size, maintenance, and productivity, are also critical. A new metric was developed to address both product quality, as measured through glycosylation, and product quantity, as measured through product concentration. A monoclonal antibody Chinese hamster ovary cell culture model system was used to assess this metric across various media formulations. In a model test system, the metric discriminated that some media supplements had a net positive impact on productivity and glycosylation, while others had a net negative impact on productivity and glycosylation.

Keywords Chinese hamster ovary cells (CHO) · Monoclonal antibodies · Productivity · Glycosylation · Critical quality attributes

Introduction

Monoclonal antibodies, or MAbs, make up a major portion of the rapidly growing therapeutic protein

market, capturing 7 % of the nearly \$600 billion sales in 2010 (Elvin et al. 2013). In tandem with drug discovery, process development activities are focused on improving product quality while decreasing the cost of goods sold. An important tool in product quality and quantity optimization is the manipulation of the cell culture environment, such as the temperature and culture medium in which the MAb is biosynthesized.

The success of a culture medium requires consideration of all aspects of culture performance, including cell growth, culture maintenance, productivity and product quality. With a focus strictly on one aspect of performance, other critical aspects of performance can suffer, even to the point of a net loss in overall performance; for example, some media supplements may promote productivity but have a negative effect on product quality (or vice versa). The goal of culture medium optimization is the high productivity of high quality MAbs; our proposed metric seeks to quantify this combined goal. To better understand this metric, we must first understand the various quality and productivity goals of cell culture system optimization.

Recombinant Chinese hamster ovary (CHO) cell lines are the dominant platform used to produce MAbs due to their genetic stability, highly characterised safety record, and capacity to produce MAbs of high fidelity (Hossler et al. 2009). Glycosylation, which has been identified as a Critical Quality Attribute (CQA), has become a research focus. Importantly, CHO cell lines possess the cellular

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machinery required to produce glycosylated MABs (Butler 2006; Hossler et al. 2009). However, many different glycoforms exist, each exhibiting different functional properties.

A number of different therapeutic effects have been linked to specific MAB glycan chain conformations. High levels of terminal galactose improve complement-dependent cytotoxicity (CDC), while a reduction of fucose levels increases antibody-dependent cell-mediated cytotoxicity (ADCC) (Jefferis 2009a, b; Mori et al. 2007). Both ADCC and CDC are immune system responses that mediate the death of antibody-bound cells, and so have substantial implications in the treatment of many forms of cancer. Increased levels of terminal sialylation have been identified as improving anti-inflammatory responses, though these results are controversial (Kaneko et al. 2006; Scallan et al. 2007). Additionally, terminal sialylation superseding terminal galactosylation potentially reduces the CDC response.

Many methods have been proposed to manipulate MAB glycan profiles, including targeted nutrient feed strategies, the manipulation of environmental conditions, and media supplementation (Gramer et al. 2011; Zhang et al. 2013).

The benefits of media supplements are not limited to the effects they have on glycosylation; they have been identified as improving CHO culture success in many ways. For example, growth factors such as insulin promote cellular glucose uptake. Plant or yeast hydrolysates contain undefined growth factors capable of stimulating cell growth and productivity, while simultaneously supplying additional nutrients (Babcock and Antosh 2012).

CHO culture success is currently measured and analyzed in many different ways, including cell growth, maintenance of cell viability, volumetric productivity, [P], specific productivity, q_p , and the glycan profile. Although MAB glycosylation has become a recent focus in the improvement of MAB quality, clarification as to the most ideal glycoforms, as well as a more comprehensive examination of other aspects of culture performance, particularly productivity, is required.

This study presents a metric that simultaneously addresses both product quantity and product quality, in order to facilitate assessment of the glycosylation CQA and to apply it in media optimization.

Materials and methods

Cell line

The model cell system used in this investigation is a recombinant CHO cell line producing the anti-epidermal growth factor receptor (EGFR) human-llama chimeric heavy chain MAB, designated EG2. This antibody consists of an active llama Fab domain fused to a human IgG1 Fc region (Bell et al. 2010). It was developed from the CHO-DXB11 parental cell line, a variation of the dihydrofolate reductase deficient (*dhfr*−) CHO cell line. Stable transfection was achieved using polycation polyethylenimine (PEI), then chosen through puromycin selection. The theoretical protein output of this cell line is 100 mg/L (Agrawal et al. 2012). The cell line was provided by Dr. Durocher (University of Montreal, NRC) as part of the MABNet research network (NSERC 2010–2016).

Cell culture growth medium

Ham's F12K basal medium (Sigma N-3520) was used in all experiments, enriched with additional glucose and amino acids (Table 1), and supplemented with 28.8 $\mu\text{mol/L}$ sodium hypoxanthine, 180 $\mu\text{mol/L}$ thymidine (HT, Invitrogen, Burlington, ON, Canada), and 0.9 g/L pluronic F68 (Sigma P5556, Oakville, ON, Canada). BIOGRO CHO (BIOGRO Technologies, Winnipeg, MB, Canada), supplied by Dr. Butler (University of Manitoba) through the MABNet research network (2010–2016), was employed as a positive control.

Fractional factorial design of experiment

Four supplements were selected to illustrate the effectiveness of the metric developed in this paper. In particular, the experiment demonstrates the potential trade-offs between productivity and glycosylation. A fractional factorial design of experiment (DoE) was employed to screen four different media supplements added to the enriched basal medium for their effect on cell growth, productivity and glycosylation. Four supplements that have been shown to have varying positive effects on a CHO cell culture, including the improvement of cell growth, productivity and glycosylation, were selected:

- the SITE liquid media supplement (Sigma S4920), typically used to enhance cell growth,

Table 1 Glucose and amino acid concentrations in supplemented Ham's F12K basal medium

Metabolite	Concentration (mmol/L)
Glucose	24.000
L-Alanine	0.580
L-Arginine	2.100
L-Asparagine	0.480
L-Aspartic Acid	1.260
L-Cysteine	0.100
L-Glutamic Acid	0.570
L-Glutamine	4.000
L-Glycine	0.700
L-Histidine	0.650
L-Isoleucine	1.220
L-Leucine	2.000
L-Lysine	1.500
L-Methionine	0.422
L-Phenylalanine	0.810
L-Proline	0.320
L-Serine	0.700
L-Threonine	1.250
L-Tryptophan	0.090
L-Tyrosine	0.750
L-Valine	1.350

- a uridine, manganese and galactose cocktail (UMG, Sigma U6381, 529680, G5388), developed to promote galactosylation (Gramer et al. 2011),
- the Lipid Mixture 1 (LM1) cocktail (Sigma L0288), which has been shown to promote both cell growth and productivity (Parampalli et al. 2007), and
- the soy hydrolysate HYPEPTM 1510 (Sheffield Bio-Science), which has been shown to promote cell growth (Murayyan 2013).

A fractional factorial DoE was selected because it is capable of resolving main effects of factors, while also being capable of estimating two way interactions between factors. This is accomplished through the sparsity-of-effects principle (Murayyan 2013) (Parampalli et al. 2007).

The fractional factorial experiment consisted of eight experimental treatments, comprising high and low levels of each of the four supplements (Table 2). The DoE, summarized in Table 3, was augmented with two centre points, a negative control (the enriched basal Ham's F12K) and a positive control of BIOGRO CHO.

Analytical techniques

Viability cell concentration

Total cell concentration and cell viability were determined using the trypan blue exclusion method (Schrek 1936), performed with a 1:1 dilution of cell

Table 3 Fractional factorial DoE

Mixture#	HYPEP TM 1510	LM1	SITE	UMG
1	Low	Low	Low	Low
2	High	Low	Low	High
3	Low	High	Low	High
4	High	High	Low	Low
5	Low	Low	High	High
6	High	Low	High	Low
7	Low	High	High	Low
8	High	High	High	High
9	MID	MID	MID	MID
10	MID	MID	MID	MID
BIOGRO CHO	N/A	N/A	N/A	N/A
Ham's F12K	N/A	N/A	N/A	N/A

Table 2 The high and low levels of four supplements tested through a DoE

	High level	Low level
Soy hydrolysate (HYPEP TM 1510)	5 g/L	1 g/L
SITE liquid media supplement	10 mL/L	2 mL/L
Lipid mixture 1 (LM1)	7 mL/L	1 mL/L
UMG (Uridine, Manganese, Galactose)	8 mM uridine 0.016 mM manganese 40 mM galactose	2 mM uridine 0.004 mM manganese 10 mM galactose

culture media with trypan blue dye (0.2 %), and counted on a haemocytometer. Cell viability was expressed as a percentage of living (non-stained) cells versus the total number of cells.

IgG concentration analysis

EG2 concentrations were determined through the use of a protein A column. 100 µl of centrifuged and filtered culture supernatant samples were transferred to low volume HPLC inserts and injected into a POROS A20 analytical column (Applied Biosystems cat# 2-1001-00). A Waters UV detector (Waters e2695 and 2489) was set to 280 nm. The EG2 concentrations were calculated using a calibration curve from the response to EG2 protein at varying known concentrations.

Glycan analysis

Samples from the culture at the maximum protein concentration were analyzed for their glycan profiles. Between 10 and 20 mL of medium was harvested from the culture depending on the estimated protein concentration.

The collected samples were concentrated to approximately 400 mg/L, using a 15 mL 10,000 nominal molecular weight limit (NMWL) centrifuge filter (Amicon Ultra-15, UFC901024) operating at 500 g for 10 min. 15 mL of PBS (Sigma P4417) was then added to the concentrates, and the concentration step repeated, exchanging the medium for PBS. The samples were rinsed a second time, before being concentrated to a final volume of 1 mL and frozen at -20°C .

Prior to glycan analysis, the samples were kept on ice overnight. Glycan structures were removed from the protein with Peptide-*N*-Glycosidase (PNGase F), tagged with 2-aminobenzamide (2-AB) and analysed using HILIC analysis as described in the literature (Liu et al. 2014). Elution times were compared to a dextran ladder standard to generate glucose unit (GU) values, and a tentative assignment of structure was based on the glycan database Glycobase (www.NIBRT.ie). Glycan structural assignments were confirmed using exoglycosidase digests (Royle et al. 2007). The glycan analysis was performed by Dr. M. Spearman (University of Manitoba) as part of the MAbNet research network (NSERC 2010–2016).

Calculations

Viability index

The viability index (VI) is a parameter that describes cell population size, endurance, and, importantly, the associated biological capacity for production (e.g., biosynthesis of protein product), and is therefore useful in assessing culture and media performance (Dutton et al. 1998). VI is calculated as the numerical integral of the log mean viable cell density at time steps ($t_{j+1} - t_j$) over the period i to f :

$$VI = \sum_{j=i}^f \frac{(X_{V_{j+1}} - X_{V_j})}{\ln(X_{V_{j+1}}/X_{V_j})} (t_{j+1} - t_j).$$

Results and discussion

MAB glycan profile

Currently, there are no metrics to simultaneously assess the quality and quantity of MAb production. That said, various metrics do exist that express only product quantity including specific productivity, volumetric productivity, and final protein concentration. Similarly, various metrics do exist that assess the chemical reactions that occur during the glycosylation process.

One method frequently employed to assess the progression of glycosylation is a series of independent glycosylation indices (Aghamohseni et al. 2014; Ohadi et al. 2013; Liu et al. 2014; Majid et al. 2007). Two common forms that measure the galactosylation and sialylation processes include the galactosylation index (GI) and sialylation index (SI):

$$GI = \frac{G2F + 0.5 \times G1F}{G2F + G1F + G0F}, \quad (1)$$

$$SI = \frac{G2S2 + 0.5 \times G2S1}{G2S2 + G2S1 + G2}, \quad (2)$$

Each of these indices focus on the percentage of glycans within a specific glycan species: the fucosylated galactosylated form (G2) in (1) and the fucosylated disialylated form (G2S2) in (2). Each individual glycosylation index is calculated as a value between

zero and one, where a higher value indicates a high percentage of the completed biantennary diglycosylated form (G2F/S2), and a low percentage of the monoglycosylated (G1F/S1) and aglycosylated (G0F/S0) forms.

Importantly, each of these metrics is normalized to their respective glycosylated form through the denominator, and therefore describes the success of their respective glycosylation process within a species. These metrics cannot, however, be easily combined (via, for example, addition) to obtain a measure of the overall glycan quality of the product, especially where multiple glycosylation patterns may be desirable, since they focus on the glycosylation process, not the glycosylated product. The process of normalizing the indices to a subjective subset of the glycoforms has the potential effect of misrepresenting the overall glycan profile of the proteins produced. For example, consider a case where a high degree of galactose is desired. If 5 % of the glycans are in the G2F form (the most desired form), 1 % are in the G1F form, 0 % are in the G0F form, and 94 % are in the G2S2 form, the resulting galactosylation index would be 0.92, indicating a nearly complete galactosylation process. However, only 5 % of the overall glycans were of the most highly desired form.

Here, we present a method to quantify glycosylation that is product focused, rather than process focused. This method measures the overall quality of the various glycan patterns without normalizing to a subjective glycan subset. The proposed metrics are hereby termed galactosylation and sialylation quality indices, QI_{Gal} , and QI_{NeuAc} , respectively:

$$QI_{Gal} = \frac{G2 + 0.5 \times G1}{100}, \quad (3)$$

$$QI_{NeuAc} = \frac{G2S2 + 0.5 \times G2S1}{100}. \quad (4)$$

QI_{Gal} and QI_{NeuAc} , much like GI and SI , apply a factor of 0.5 to the mono-glycosylation forms (G1 and G2S1) as a stoichiometric representation of both potential mono-glycosylated antennae. The difference between the two sets of metrics is that GI and SI are normalized to their respective glycan species while QI_{Gal} and QI_{NeuAc} are not normalized to a sub species.

As with the previous metrics, each of these indices is individually bounded by one. A key difference between these metrics and the previous ones is that

these metrics are normalized to all glycans in a batch, so the sum of these indices is also bounded by one, and are therefore a measure of the glycan quality of the entire batch.

Consider, again, the example described above (5 % G2F, 1 % G1F, 0 % G0F, 94 % G2S2). Though the calculated GI may be 0.92, the calculated QI_{Gal} of this culture is 0.055, reflecting the relative absence of the most desired glycan structure.

A simple sum of our new indices is possible, but would not reflect the fact that different glycan structures have different functional properties and therefore may not be equally desirable. To reflect this variation in desirability, we employ a weighted sum, which we label the total Quality Index, QI :

$$QI = \frac{\alpha \times QI_{Gal} + \gamma \times QI_{NeuAc}}{\max\{\alpha, \gamma\}}, \quad \alpha, \gamma > 0, \quad (5)$$

where for example, supposing that galactose levels are 10 times more desirable than sialic acid levels, we would set $\gamma = 1$ and $\alpha = 10$.

At this point, we have proposed a metric, QI , which is an objectively normalized quality metric that can assess the glycan quality of the entire batch. We now wish to expand QI into a new metric. In order to do so we must select a measure of quantity. Specific productivity, a measure of the per-cell, per-hour productivity of a culture (Dutton et al. 1998), is a useful tool for analyzing the cellular efficiency of a cell culture system, but the primary metric of interest to industry is the volumetric productivity, $[P]$, measured at the end of the culture (final titer). With this measure of quantity made the focus, we can expand QI into another metric, Titer Quality, TQ , to simultaneously assess both the protein quantity and protein glycan quality of an entire batch:

$$TQ = QI \times [P]. \quad (6)$$

In this equation, QI is a unitless factor between 0 and 1 that represents the fraction of glycans that exhibit the most ideal glycan structure, while $[P]$ is the protein concentration at the same point of analysis. Therefore, the product of these two values, TQ , is a measure of the protein concentration with the most ideal glycan structure. TQ can equal $[P]$ in the event that the QI of the batch measures 1 (indicating that all of the glycans in the batch are in the most ideal form) or TQ can be 0 in the event that the QI of the batch measures 0 (indicating that none of the glycans were in

Fig. 1 There is an inverse relationship between the final protein titer [P] (grey bars) and the glycan quality *QI* (black bars) (left). Titer Quality, as a measure of both product quality and product quantity, is useful when evaluating net culture performance (right). BIOGRO CHO outperformed all other treatments in

terms of *TQ*, while M6 exhibited a *TQ* approaching that of BIOGRO CHO. This differs from a focus strictly on productivity, where the final protein concentration in BIOGRO CHO is nearly double that of the next best formulation (mixture #6)

an ideal form). As *QI* varies from 0 to 1, *TQ* varies from 0 to [P].

With this new metric that addresses both glycan quality and protein quantity, culture success from both perspectives can be assessed simultaneously. For example, though the final protein concentration achieved by a batch may be 100 mg/L, if only 50 % of the glycans are of the most desired form (with a calculated *QI* of 0.5), the *equivalent* concentration of ideal protein is 50 mg/L. *This metric, therefore, allows trade-offs between productivity and glycan quality to be addressed.*

This is a critical benefit of *TQ*, as direct or inverse relationships between glycan quality and productivity are possible. In the event that a direct relationship exists, the merits of a supplement or media formulation are clear. However, in the event that an inverse relationship exists, net gains must be assessed in order to address optimization.

It is critical to recognize that the Quality Index and, by extension, Titer Quality are highly modular metrics in the sense that they can be manipulated to reflect a number of different quality requirements. In the above examples, Quality Index was defined through a modification of *GI* and *SI*. This does not, however, indicate that those metrics (and therefore Quality Index) are accurate reflections of glycan quality for a particular treatment goal.

One way to generalize the total Quality Index to allow for the assessment of any combination of glycans is by eliminating the two underlying glycan indices (3) and (4), and instead using a weighted sum (α_i) of the percentage of specific glycans (X_i):

$$QI = \sum_{i=1}^n \frac{\alpha_i \cdot X_i}{\max_{i=1 \dots n} \{\alpha_i\}}, \quad \sum_{i=1}^n X_i \in [0, 1], \quad \alpha_i > 0 \quad (7)$$

This general form of *QI* (7) is a powerful tool, as it is not limited to any glycan species or sub species. Rather, any desired glycan, diglycosylated, monoglycosylated, or otherwise, can be measured in a single metric. For example, a pharmacological study may find that the G2, S2 and the bisecting *N*-acetylglucosamine (bisGlcNAc) forms are the most desired glycoforms for a specific therapeutic, where bisGlcNAc is found to be 10× more desirable than S2, and G2 is 2× more desirable than S2. The *QI* for this specific therapeutic, using the general form of *QI* shown in (7) would be:

$$QI = \frac{2 \times G2 + S2 + 10 \times \text{bisGlcNAc}}{10} \quad (8)$$

It is also important to recognize that the method by which *QI* is calculated allows glycans that are highly correlated to be measured using a single metric. Measurements for glycan concentrations are based on

Fig. 2 Chromatograms for the glycan analysis of M3, M6 and BIOGRO CHO. Percentage of each glycan in a sample is measured as the relative percentage of the area under each peak. Identification of the species is conducted using a dextran ladder

percentages of glycans in an entire batch (as measured through a sample of culture broth), so in any event where a percent increase of one type of glycan relies on the percent decrease of another type of glycan (as is the case with sialylation superseding galactosylation), QI and TQ will remain representative of the overall glycan profile as they are not used to measure progression or glycan conformation within a species,

but represent the glycan concentration of an entire batch.

Future research is needed to evaluate the suitability of monoglycosylated glycans and alternative glycans for particular MABs and their indications, and hence assign specific weighting factors. In the absence of a specific desired glycan conformation, QI_{Gal} , and QI_{NeuAc} and their associated Titer Quality metric is

Table 4 Raw data of the glycan analysis. Structures with less than 1 % of the total area are excluded

Media formulation #3 (M3)				Media formulation #6 (M6)				BIOGRO CHO				Structure	
Retention time	Area	% area	GU units	Retention time	Area	% area	GU units	Retention time	Area	% area	GU units		
26.684	59,484	2.38	4.38	26.683	566,491	3.34	4.38	26.667	1,278,420	4.18	4.38	M3	
31.501	11,303	0.45	5.41	29.323	100,562	0.59	4.92	29.314	498,043	1.63	4.92	A1	
33.227	65,005	2.61	5.83	31.496	595,084	3.5	5.41	31.436	1,666,517	5.45	5.40	A2 or F(6)A1	
34.452	94,063	3.77	6.15	33.222	1,704,914	10.04	5.83	33.203	4,561,768	14.91	5.83	F(6)A2G0	
36.035	405,458	16.25	6.58	34.437	1,406,712	8.28	6.15	34.416	5,665,498	18.52	6.14	A2G1	
36.446	85,066	3.41	6.70	36.035	2,612,303	15.38	6.58	36.016	2,608,616	8.53	6.58	F(6)A2G1	
37.646	101,351	4.06	7.06	36.445	809,844	4.77	6.70	36.428	1,037,373	3.39	6.70	F(6)A2G1 isomer	
38.937	1,192,005	47.77	7.46	37.639	325,499	1.92	7.06	37.515	704,938	2.3	7.02	A2G2	
40.284	181,939	7.29	7.91	38.937	6,141,714	36.17	7.46	38.917	6,607,910	21.6	7.46	F(6)A2G2 or A3G2	
40.5	36,154	1.45	7.99	40.282	1,133,037	6.67	7.91	40.261	1,854,431	6.06	7.91	F(6)A2G2S1	
41.621	54,496	2.18	8.39	40.517	158,672	0.93	7.99	40.483	302,673	0.99	7.98	F(6)A2G2S1 isomer	
42.664	57,328	2.3	8.78	41.582	405,870	2.39	8.37	41.566	590,766	1.93	8.37	F(6)A2G2S2	
				42.658	157,087	0.93	8.78	42.633	513,325	1.68	8.77	A4G3	

assigned an equal weight of desirability [i.e., (5) and (6) with $\alpha = \gamma = 1$].

Culture performance using QI and TQ

To demonstrate the utility of the Quality Index and Titer Quality metrics, a fractional factorial study was conducted, as described in the materials and methods section.

If we focus on productivity as the only measure of success, BIOGRO CHO performs well, reaching a final protein concentration of nearly 40 mg/L (Fig. 1). Whether alone or in a mixture in the fractional factorial DoE, two of the supplements increased productivity (SITE and HYPEP 1510TM), while two of the supplements decreased productivity (UMG and LM1) (Fig. 1). Conversely, if we focus on glycan quality as the only measure of culture success, UMG and LM1 were found to have a positive impact (Fig. 1). This was expected for the UMG cocktail, since this cocktail was designed to achieve this goal (Gramer et al. 2011) (Fig. 2; Table 4).

These results imply a trade-off between improving glycan quality and MAb quantity through media supplementation. While HYPEPTM 1510 and SITE improve productivity and reduce glycan quality, LM1 and UMG have the opposite effects. This trade-off must be quantified in order to assess optimization, a purpose for which *TQ* can be used.

A focus strictly on productivity suggests that BIOGRO CHO outperforms mixture #6 (Fig. 1)—approaching double the protein concentration (Table 5); however, the success of BIOGRO CHO is tempered by a relatively low *QI*, resulting in a *TQ* approximately equal to mixture #6 (Fig. 1; Table 6). The higher *QI* for mixture #3 and mixture #6, regardless of lower productivity, improves their relative success compared to BIOGRO CHO when focusing on *both* protein quantity and glycan quality. This demonstrates the utility of *TQ* in assessing the performance of a culture with regards to both product quality and quantity, providing an assessment of culture performance from a net perspective.

As another measure of culture success, cell population expansion and maintenance were assessed using the viability index. *VI* is the total viable cell hours per unit volume from start of culture to time of measurement, and can be likened to the business concept of the “person hour”: in biopharmaceutical culturing terms,

Table 5 Mixture formulations, cell growth, and protein concentration response variables

Mixture#	HYPEP™ 1510	LM1	SITE	UMG	Max cell density (1E6 cells/mL)	Viability Index (1E8 cell * h/mL)	Final MAb concentration (mg/L)
3	Low	High	Low	High	1.69	2.18	12.81
6	High	Low	High	Low	3.58	6.80	23.90
BIOGRO CHO	N/A	N/A	N/A	N/A	3.72	3.63	39.05

Table 6 Calculated QI and TQ of each species

Mixture#	Galactose		Sialic acid		Total	
	QI	TQ	QI	TQ	QI	TQ
3	0.576	7.381	0.066	0.839	0.642	8.22
6	0.426	11.051	0.062	1.479	0.524	12.53
BIOGRO CHO	0.276	10.763	0.055	2.130	0.330	12.89

Fig. 3 M6, containing high levels of HYPEP™1510 and SITE but low levels of UMG and LM1, exhibited a nearly twofold increase in the maintenance of population viability over the positive control BIOGRO CHO

the “cell hour” is simply a measure of the cumulative presence of viable cells to perform “work” in terms of biologically mediated functions (e.g., making the biopharmaceuticals of interest). It can be measured at any point in the culture period including both growth (lag and exponential) and non-growth (stationary and decline) associated phases, and can be measured for any culture mode (e.g., batch or continuous). Essentially, *VI* expresses how many viable cell hours were available through the duration of the culture or culture phase, and can be used to accurately assess how much (useful) work was performed per

viable cell hour during the relevant time span. BIOGRO CHO is characterized by a rapid rise in *VI* before reaching an early final *VI*. By comparison, mixture #6 exhibited an extended stationary and decline phase resulting in a nearly twofold increase in maintenance of population viability over BIOGRO CHO (Fig. 3).

An added benefit of *TQ* being a measure of protein concentration is that it can be used in any equation that uses protein concentration as a factor. For example, volumetric *TQ* could be calculated, indicating not only the volumetric productivity throughout the course of a

cell culture, but the volumetric productivity *and* quality throughout the course of a culture. Furthermore, specific TQ (q_{TQ}) can be calculated using the concept of VI , as described by Dutton (1998), describing the per-cell, per-hour productivity *and* glycan quality of a particular cell culture system:

$$q_{TQ} = \Delta QI \times [P]/VI. \quad (9)$$

Overall, the inverse relationship observed between productivity and product quality demonstrates the need for metrics that are capable of quantifying net benefits. With a narrow focus on productivity or cell growth, HYPEP™ 1510 and SITE could be falsely identified as the superior supplements. Similarly, with a narrow focus on glycosylation, UMG and LM1 could be falsely identified as the superior supplements. It is not until net benefits are considered, an analysis made possible through TQ , that a truly beneficial supplement mixture can be identified.

It is also critical to note that while high levels of terminal galactose have been identified as improving CDC, this may not be the most desired attribute of the EG2 MAb. The EG2 MAb is an anti-cancer MAb (targeting EGF receptors overexpressed in various forms of cancer), and may therefore benefit from increased CDC activity; however, other glycoforms, such as the afucosylated form, may be even more beneficial, in which case a QI generated with a focus on afucosylated glycoforms using (7) may be the main response variable of interest.

Specific research must be conducted in order to identify the most desired glycan pattern for a specific indication of each specific MAb. The selection of glycans and appropriate weight factors for QI requires careful consideration, as it is relative and subjective to the particular case, i.e., the optimal glycan profile for the specific pharmacological target. The modification of QI for a particular application requires the identification of all relevant glycans and their respective weight factors. Once identified, the general form of QI can be applied, enabling simultaneous optimization of *both* productivity and product quality, as defined by TQ ,

Conclusion

Quality Index (QI) and the associated metric Titer Quality (TQ) are both useful tools when assessing culture performance. In the event that a trade-off is

required between the two performance attributes of glycan quality and product quantity, Titer Quality can be used to assess the net benefit. The simultaneous assessment of these two critical quality attributes, facilitated by the use of the Titer Quality metric, allows a convergence to optimal productivity. The complementary nature of supplements shown to improve either productivity or glycan quality illuminates the use of the metric. The general forms of Quality Index allows for customization to a specific application. Titer Quality, which can be measured on a volumetric or cell specific basis, is a powerful new entry for the culture optimization tool box.

Acknowledgments We would like to thank Dr. Spearman at the University of Manitoba for conducting the glycan analysis, Dr. Butler at the University of Manitoba for supplying BIOGRO CHO, and Dr. Durocher at the University of Montreal for supplying the cell line, as well as MAbNet and NSERC for providing the funding for this research.

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