Process Analytical Technologies for Small-scale Cell Culture: ARACUS Amino Acid Analyzer

By

Bishouy Sharoubim

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Thesis

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Date 4/15/2024 Signature: DocuSigned by: Mathiowitz, Edith E063C09A6AB6428...

Dr. Edith Mathiowitz, Advisor

Date 4/15/2024 Signature: DocuSigned by: 03C08343FDFE45F...

Dr. Jacquelyn Schell, Reader

Date 4/15/2024 Signature: DocuSigned by: 610D0644D5AC4E0...

Dr. Diana Horrigan, Reader

Approved by the Graduate Council

Dr. Thomas A Lewis, Dean of the Graduate School

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Chapter 1: Introduction

Biotechnology companies have challenges in their production processes due to the necessity to innovate to gain a competitive edge and meet the demands of the modern world regarding the safety and environmental impact of their processes and products (REBEL Cell Culture Analyzer | 908 Devices, 2023). Creating a strong and reliable control strategy for cell culture processes to keep process performance and finished product quality within strictly defined parameters is one of these technological challenges. In this section, I'll be addressing the importance of Amino Acids to cell culture, the ARACUS technology which can measure Amino Acid levels, benefits of implementing the ARACUS at AMGEN, a comparison between ARACUS and the standard method for Amino Acid quantification: HPLC, competitors to the ARACUS technology, and the role cell culture process development can play in implementing new technologies such as ARACUS.

Importance of Amino Acids to cell cultures

Amino acids are chemical compounds that possess an amino (−NH2) group in addition to a carboxyl (−COOH) or acidic group. Amino acids are the necessary components of cell culture medium because they are the building blocks of proteins. Certain amino acids, such as L-glutamine, L-tyrosine, and L-cystine, have low solubility and stability, which places restrictions on the formulation of media, the stability of storage, the intensification of processes, and eventually, total productivity (High-quality Ingredients for Cell Culture Applications, 2023). The metabolic flux and the rates of amino acid consumption are influenced by the genetic makeup of cells, their gene expression profiles, the phases of the cell cycle, and the environment in which

they are found (Salazar A et al, 2016). The determination of the ideal amino acid concentrations is especially critical for fed batch and perfusion cultures. These techniques allow for the external supply of nutrients throughout the culture phase, which has the potential to change the equilibrium of metabolic pathways.

ARACUS Technology

With continuous flow ion exchange liquid chromatography, which was first developed in 1958 at The Rockefeller Institute for Medical Research (New York) by Spackman, Moore, and Stein, amino acid analyzers can provide both qualitative and quantitative information about the amino acid composition of a protein or peptide hydrolysate. Chromatographic separation of amino acids is performed by ion-exchange chromatography, followed by post-column derivatization with ninhydrin (Stein & Moore) and photometric determination using LED.

ARACUS utilizes the ninhydrin reaction, which is a common bioanalytical tool for methods involving the analysis of amino acids. "Ruhemann's purple" is the result of ninhydrin's reaction with the α-amino group of primary amino acids. For every main amino acid, the identical chromophore is generated. A chromophore is a molecule which absorbs light at a particular wavelength and emits color as a result. Chromophores are commonly referred to as colored molecules for this reason (Ref, 17). The quantity and composition of the amino groups under investigation determine how intense the color becomes. The ideal pH for the entire reaction is 5.5.. (Ref# 16: ARACUS Amino Acid Analyzer using ninhydrin reaction). Figure 2 shows a flow chart of the ARACUS setup and how the samples run.

Figure 1: ARACUS Amino Acid Analyzer (Ref 16)
file:///C:/Users/bsharoub/Desktop/misc/Thesis/ARACUS-2014.pdf

The chart shows the structure of the setup: Figure 2

- The pump allows the **simultaneous operation of two fluidic lines** (eluent side and reactive side), which guarantees a constant mixing.
- The ARACUS has **two photometers**, 440 nm and 570 nm.
- The system is equipped with an **autosampler with 100 µL loop.**

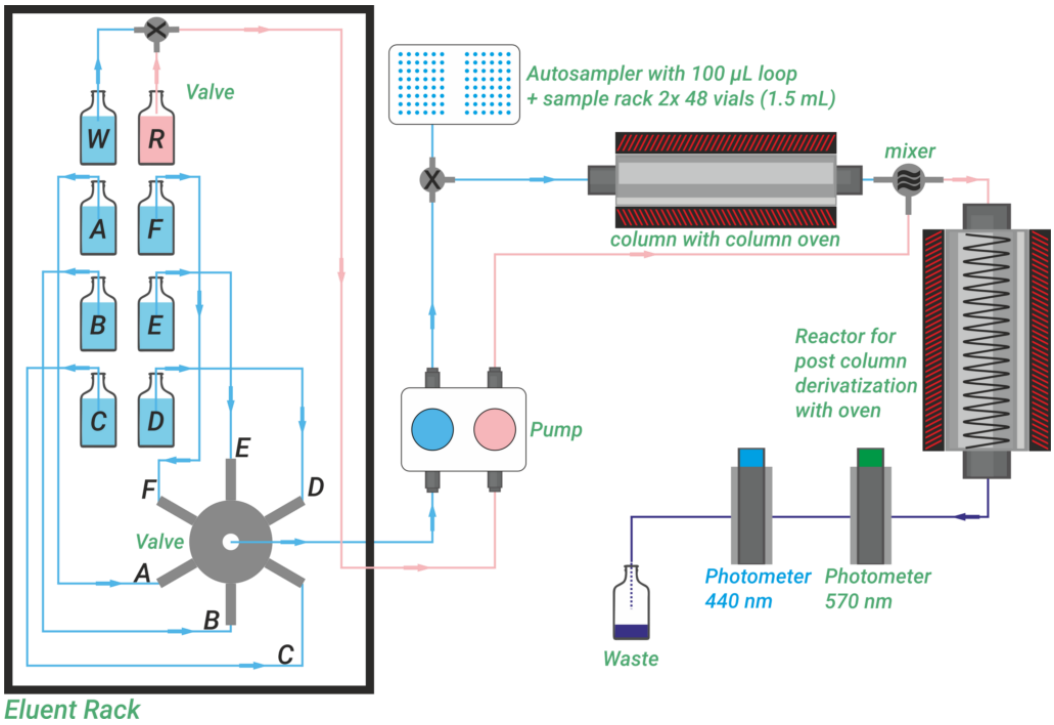

Figure 2: ARACUS Flow Chart (Ref 16)
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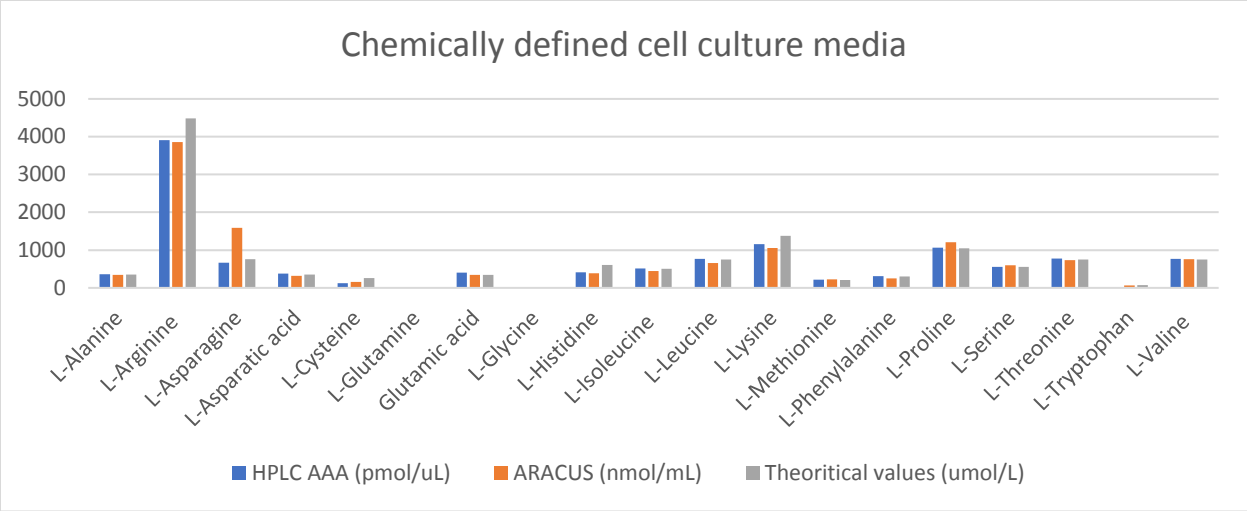
Comparison between ARACUS and HPLC

ARACUS has showed its comparability against HPLC (the current standard for quantifying amino acids) by running the same sample on both instruments and the results are shown below in Figure 3. The sample contained a chemically defined media. The data below showed most amino acids in the sample had a difference within 15% between ARACUS and HPLC while only two had a difference within 25% which shows the robustness of the ARACUS against HPLC. Two amino acids were not detected by either method.



	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Val
Within 15%	Y	Y		Y		N/A	Y	N/A	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y
Within 25%					Y	N/A		N/A						Y					

Figure 3: The graph and table show the difference in performance between the HPLC and ARACUS and the theoretical values. HPLC and ARACUS data are very similar which the ARACUS is capable of providing the same information without the tedious buffer preparation HPLC requires.

Benefits of the ARACUS to AMGEN

The team at Amgen Rhode Island Process Development Cell Culture assessed and bought an ARACUS analyzer in 2021. Without the laborious standard preparation of an HPLC, it takes only one hour for it to run 42 amino acids analysis in each sample. To minimize human error, it uses a ready-to-use kit which takes about 15 minutes of labor time to prepare each sample, integrate, and analyze results. It is also possible to download ARACUS data directly to Spotfire and Holistic Lab Electronic Notebook (HLEE) for ease of data analysis.

The ARACUS was used for development of a high mass process to determine amino acid consumption and requirements to add desired amino acids while the experiment was in

progress, within an hour of sampling. Prior to the ARACUS, amino acid samples needed to be collected and shipped to Amgen Thousand Oaks, California to run on their HPLC. This often takes approximately 2 weeks and does not allow Rhode Island scientists to make changes to an in-progress experiment.

The determination of the ideal amino acid concentrations is especially crucial for fed batch and perfusion cultures. These techniques allow for the external supply of nutrients throughout the culture phase, which has the potential to change the equilibrium of metabolic pathways. The function of glycine and l-serine in the tetrahydrofolate (THF) cycle helps to explain this. Through the THF cycle, these two amino acids are implicated in the metabolism of nucleic acid precursors (Amelio et al. 2014; Locasale 2013). However, adding glycine as a supplement causes the body to produce more l-serine when there is a shortage of it. Metabolites are pulled out of the THF cycle by this. According to Labuschagne et al. (2014) and Duarte et al. (2014), a delayed THF cycle inhibits cell proliferation. (Fukuda et al., 2001)

The ARACUS tool may also be used to gain additional information during investigations. The team was able to analyze the media from a slow growing culture in manufacturing using the ARACUS to help rule out the media as the potential root cause.

In summary, the ARACUS is simple to use, inexpensive to maintain and the vendor has been great to work with. (4.Salazar, Andrew & Keusgen, Michael & Hagen, Jörg. (2016)).

Introducing competitors to the ARACUS Technologies

As ARACUS showed its capability in quantifying the different amino acids in media samples, REBEL also has showed itself as an amino acid analyzer. The REBEL is a brand-new, specialized cell culture medium analyzer made for quick, simple, and effective nutrition and amino acid analysis. The REBEL gives process development scientists instant access to vital data online by fusing 908 Devices' well-shown capillary electrophoresis and high-pressure mass spectrometry technology with an award-winning design (Elliott et al., 2020). ARACUS was chosen over the REBEL because it was cheaper and easier to maintain than the REBEL. Also ARACUS customer service provided better warranty coverage and a loaner on site to test out the samples in AMGEN and we can have a hands on experience with the instrument.

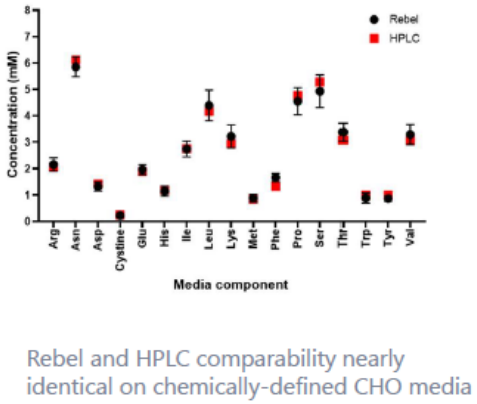


Figure 4; REBEL AAA and its comparability against HPLC (Ref 6). The left figure shows the comparability between REBEL and HPLC.
https://908devices.com/products/rebel/

Cell Culture Process Development's Role

One of the most essential components in the effectiveness of in vitro cell culture is the composition of the culture medium. To fulfill the material and energy requirements for full cell synthesis, cell metabolism, and other biochemical reactions, the cell culture medium must include an adequate amount of nutrients. The use of automated microbioreactor systems has increased dramatically in process development and cell culture labs (Salazar A et al, 2016). Analyzing spent cell culture media is frequently a daily routine when working on the development of biotherapeutic processes, especially when optimizing bioreactor settings and media composition. Based on the nutritional requirements and consumption patterns of the cells for each experimental condition, as well as the state of stress, the components of the monitored media can be identified by such technologies to improve the cell productivity. The purpose of this work is to use small-scale experiments to demonstrate possible applications for the ARACUS within process development.

Aims

Aim 1: Use the ARACUS during development of a new High Mass Processes by changing nutrient feed media timing and nutrient feed media strategy for better performance and higher productivity. We hypothesize that by using the ARACUS, we'll be able to;

- Maintain sufficient nutrient feed media volumes administered to the cells during production step.
- Increase cell productivity of specific protein.
- Analyze and troubleshoot amino acid consumption.

Aim 2: Have a baseline of all Amino Acid run on the ARACUS for all media used in our experiment to support in future investigations and used for batch verification. We hypothesisze that having the baseline of amino acids for each media will be helpful to;

- Compare differences in media compositions and the effect over the course of a multi-day process.
- Maintain consistency in preparation of culture media.

Chapter 2: Methods

Cell culture media and cell lines

The cell culture media and cell lines were proprietary to Amgen. Most of the cells used in the experiments were CHO (Chinese hamster ovary) cells. The rules and procedures of Amgen Process Development were utilized by all processes.

Bioreactor assembling and setup

Mix of two- and three-liter bioreactors were used during the experiments. The glass bioreactors are Chem Glass Brand (Chemglass Life Sciences LLC, Vineland, NJ) with metal headplates as seen in Figure 5. Each bioreactor was assembled using the parts in Figure 6. Each headplate consists of weldable c-flex and silicon tubing, sparge lines, a condenser, vent filters, a stirrer, a pH probe and a dO2 probe. Prior autoclaving the bioreactor, pH and DO probes are calibrated per station then the bioreactor is washed and screwed together to be autoclaved. Once sterilized, the bioreactors are set up, dO2 probes are spanned and the process media is added to the bioreactor prior to inoculation.

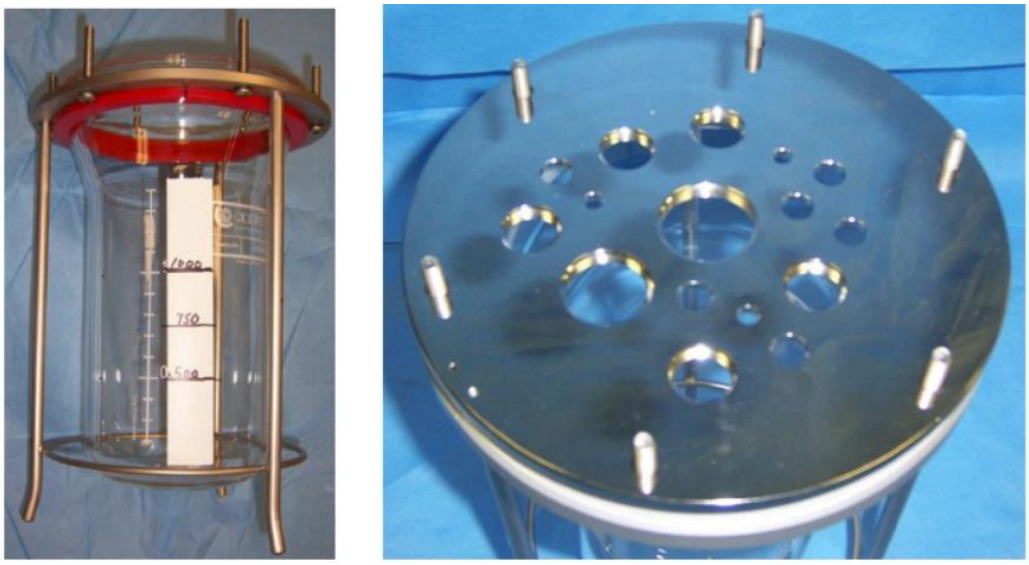


Figure 5; 2L Glass Bioreactor and Stainless-steel Head Plate

Figure 6; Bioreactor head plate parts

Inoculation and sampling bioreactors

Bioreactors are inoculated using a culture source. The inoculum volume (V_{Inoc},) is calculated using the following equation:

$$V_{Inoc} = \frac{VCD_{Target} \times V_{Brx}}{VCD_{Inoc}}$$

VCD_{Target} = Target VCD of bioreactor, cells/mL

V_{Brx} = Desired bioreactor working volume, mL

VCD_{Inoc} = Final viable cell density of the inoculum source, cells/mL

Equation 1; the calculation of the inoculum volume

Bioreactor sampling is done daily through a nonsterile syringe and a leur lock fastened to the bioreactor. To make sure that any hold-up in the sample line is eliminated and that the sample tested is a homogenous solution, a sample flush is taken out of the bioreactor prior to sampling.

Different instruments are used during the sampling the process, Table 1. The pH and carbon dioxide and oxygen concentrations in the culture are measured using a Siemens Medical Solutions, Malvern, PA RapidLab 1240 Blood Gas Analyzer. The determination of protein and metabolite concentrations is done using a Cedex BioHT (Roche Diagnostics Corp., Indianapolis, IN). The following metabolites are examined: glucose, ammonia, potassium, sodium, glutamine, glutamate, and lactate. Viable cell density (VCD), total cell density, cell aggregation, average cell diameter, and viability are measured using a Cedex HiRes (Roche Diagnostics Corp.,

Indianapolis, IN). The samples need to be spun down with a benchtop centrifuge before the sample is analyzed for osmolality, which is done with an Osmo Tech Pro (Advanced Instruments, Norwood, MA). For analytical purposes, offline sampling devices are assumed to have a 95% precision.

Days/tasks	1	2	3	4	5	6	7	8	9	10	11	12
Blood Gas Analyzer	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
	CO2	CO2	CO2	CO2	CO2	CO2	CO2	CO2	CO2	CO2	CO2	CO2
	O2	O2	O2	O2	O2	O2	O2	O2	O2	O2	O2	O2
Cedex Hires	VCD	VCD	VCD	VCD	VCD	VCD	VCD	VCD	VCD	VCD	VCD	VCD
	Viability	Viability	Viability	Viability	Viability	Viability	Viability	Viability	Viability	Viability	Viability	Viability
Osmometer	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity	Osmolarity
BioHT	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose
	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate
	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine	Glutamine
	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia	Ammonia
	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium	Potassium
	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium	Sodium

Table 1: Bioreactor Sampling Daily tasks. All the bioreactors were sampled daily on Blood Gas Analyzer (for pH, CO2, and O2), Cedex Hires (for VCD and viability), Osmometer (for osmolarity), and BioHT (for all metabolites like glucose, lactate, glutamate, glutamine, ammonia, potassium, and sodium). The process duration was 12 days in the production step.

Feeding the Bioreactors

The feeding process is a critical step for the productivity and the viability of the cells. The process begins by batching the raw materials to make the feed medias. The feeds are combination of three different batches, nutrient, cystine/tyrosine, and 50% glucose. Bioreactors are fed post daily sampling based on the scheduled feed days. Nutrient and cystine/tyrosine feed schedules varied by experiment and will be detailed in Chapter 3 as part of the experimental

design. However, glucose is fed daily as needed based on the glucose results from the Roche BioHT. Figure 7, shows the glucose concentration for experiment 1 and 2 throughout the days of experiment. Based on the concentrations, Equation 2 for glucose calculator is used to feed the bioreactors with the right amount of glucose to reach glucose target on a daily basis.

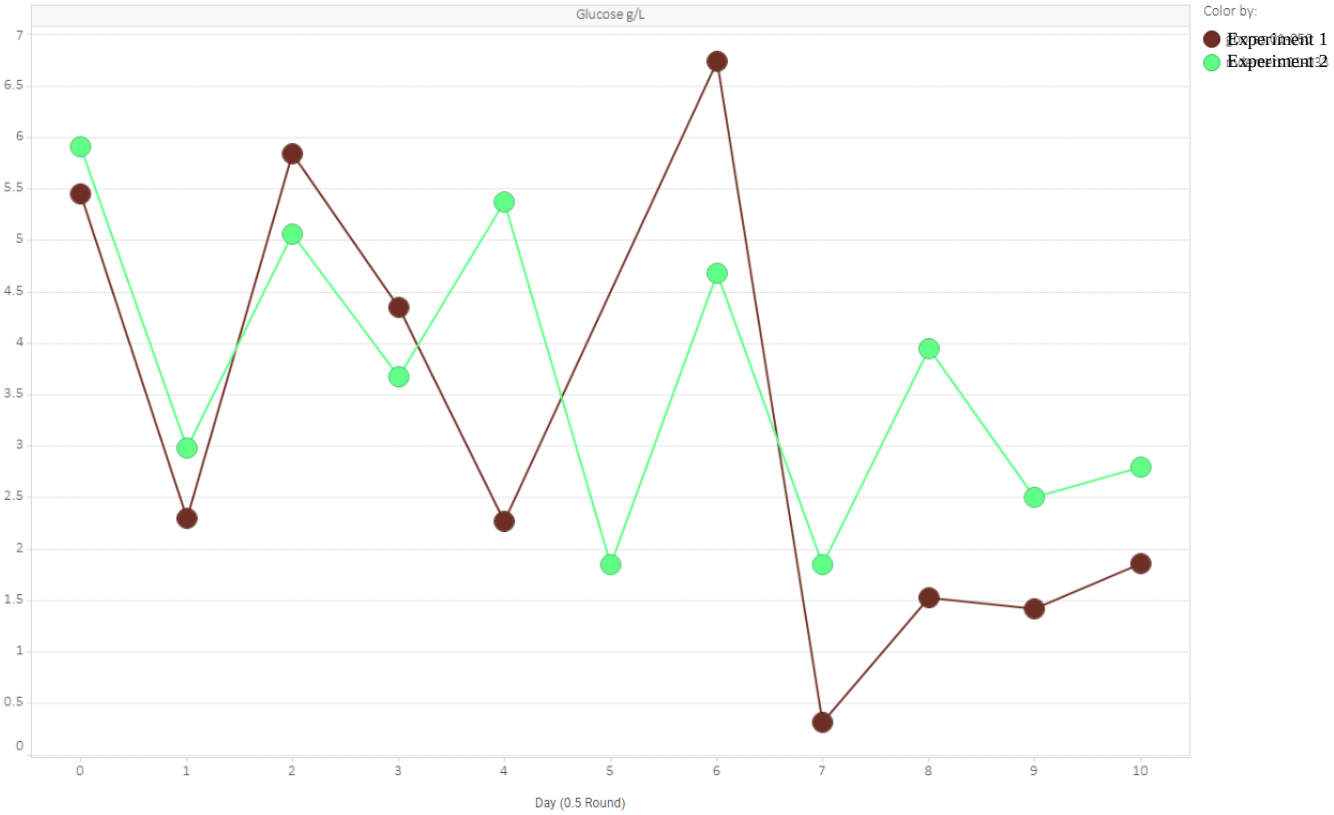


Figure 7: Glucose Concentrations between experiment 1 and 2. Based on glucose concentration and glucose calculator, bioreactors were fed with 50% glucose to reach the glucose target.

50% (w/v) glucose feed calculation for fed-batch bioreactor (bolus feed):

$$\text{Glucose addition (kg)} = \frac{C_T \times \left(\frac{V + V_F}{1.00\,\frac{\text{kg}}{\text{L}}}\right) - \left(\frac{V}{1.00\,\frac{\text{kg}}{\text{L}}} \times C_G\right)}{500\,\frac{\text{g}}{\text{L}}} \times 1.19\,\frac{\text{kg}}{\text{L}}$$

Where:
C_T = target glucose concentration (g/L) – defined in PTD
C_G = measured glucose concentration (g/L)
V = bioreactor level prior to feeds (kg)
V_F = total amount of feeds for that day besides glucose, for example, cystine tyrosine and nutrient feed (kg)
V_F = (Nutrient feed amount) + (Cystine tyrosine feed amount), determined using the following

Equation 2: Glucose calculator formula

Harvesting the Bioreactors

The harvesting process happens on final day of the process. The process starts after final day sampling and feeds are not necessary on that day. Either partial samples are taken from the bioreactors or the full volume of the bioreactors to be centrifuged. After centrifuging the protein quality samples, each sample's supernatant is collected to be used in the protein quality tests. Using an HPLC cation exchange chromatography method, and HPLC size exclusion chromatography method, each protein quality sample is examined for charged variations, and aggregates, and glycosylation as needed.

ARACUS setup and analysis

To initialize a run, a log in is required by the software. Checking the fluid level of the eluents and reagents and expiration dates in the system prior every run is recommended to avoid any failures in the runs. The system is calibrated monthly per the vendor recommendation. Once

calibrated, the calibration data is integrated in the system and the peaks are identified according to a chart given by the vendor. Now, the instrument is ready to run actual samples. The samples are prepared by diluting the centrifuged samples at 1:10 (1 mL of supernatant with 9 mL of DPBS). Samples are placed in the refrigerated autosampler to run. After samples are done running, an integration is required to identify the peaks within each sample by utilizing the calibration identified earlier. Then the data is exported to our electronic notebook and can be graphed easily once saved for easier analysis.

Chapter 3: Results

A set of experiments were used to understand the differences in amino acid data to reach our goal for both aims. The goal of this work is to understand the importance of feeding the right amounts of nutrients and amino acids and its effects on the productivity of the cells.

Aim 1:

Use the ARACUS during development of a new High Mass Processes by changing feed timing and feed strategy for better performance (VCD and Viability) and higher productivity (Titer). We hypothesize that by using the ARACUS, we'll be able to;

- Maintain sufficient nutrient feed volumes administered to the cells during production step.
- Increase cell productivity of specific protein.
- Analyze and troubleshoot amino acid consumption.

Two experiments were utilized to assess the feeding strategy and the importance of increasing the amino acids to the cell productivity. The first experiment used different feed days with lower feed volumes. The nutrient feed days were days 1 (5% the working volumes), 3 (7%), 6 (9%), 8 (9%), and 9 (7%). The cystine/tyrosine feed days were days 3 (5.5% of the nutrient feed volumes), 6 (4%), and 8 (4%). The second experiment changed the feeding days and volumes because of the low performance in first experiment. The ARACUS data collected during the first experiment provided valuable information on the amino acid consumption to design the second experiment for better performance. The nutrient feed days were days 1 (7% the working volumes), 3 (10%), 5 (12%), 7 (12%), and 9 (5%). The cystine/tyrosine feed days were days 3

(4% of the nutrient feed volumes), 5 (4%), 7 (4%) and 9 (4%). Reference **Error! Reference source not found.**

Table 2: Feed days for experiment 1 and 2. All bioreactors were fed based on this table. Experiment 1 had a different feeding strategy than experiment 2. Experiment 2 feed total volume increased by 20% more than experiment 1.

Days	1	2	3	4	5	6	7	8	9	10	11	12
Exp 1 Nutrient, Cys/Tyr	5%, 0%	None	7%, 5.5%	None	None	9%, 4%	None	9%, 4%	7%, 0%	None	None	None
Exp 2 Nutrient, Cys/Tyr	7%, 0%	None	10%, 4%	None	12%, 4%	None	12%, 4%	None	5%, 4%	None	None	None

Figure 8 and Figure 9 show the difference in cell growth based on viable cell counts and cell viability respectively between both feeding strategies. The brown trend represents the Experiment 1 feeding strategy while the green trend represents the Experiment 2 feeding strategy. The difference in feeding between each strategy is extremely different as mentioned above.

Viable Cell Density analysis (VCD)

In Figure 8, experiment 1 started off with a higher cell density than experiment 2 for the first four days due to seeding the reactor much higher on inoculation day which kept the data continuously higher until day 5. Due to the low feeding volumes and higher seeding density, experiment 1 cell density started dropping significantly after day 5. After investigating the data using the ARACUS daily sample (Figure 11, Figure 12,

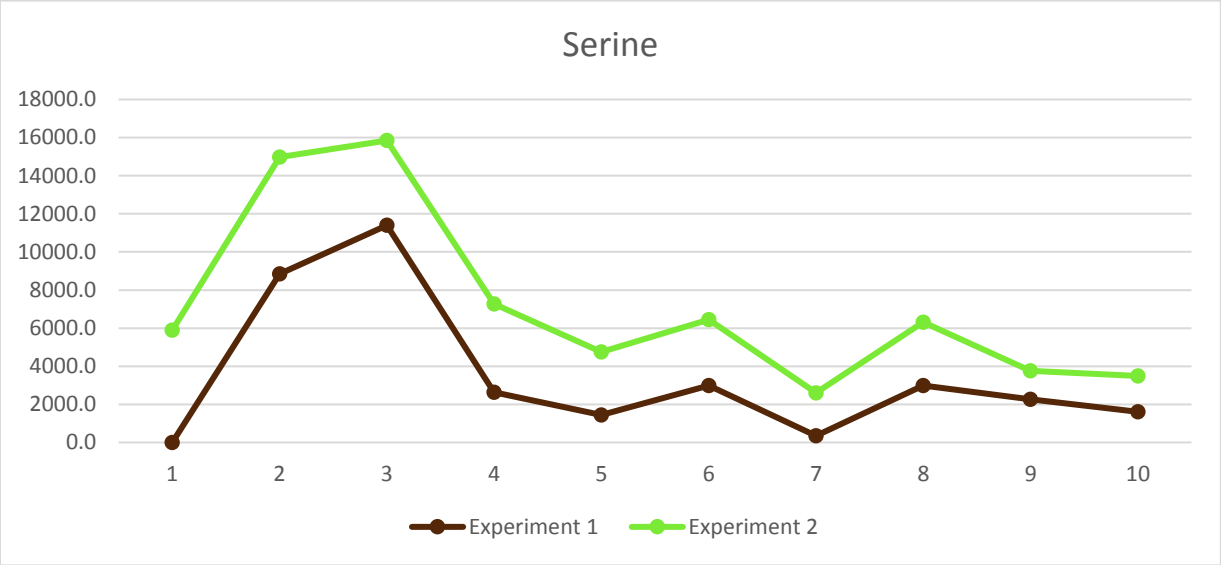


Figure 13, and
Figure 14), the amino acids were found to be depleted quicker which led to lower growth (viable cell count). As a result, titer production for experiment 2 was higher than experiment 1 by 15.8% as noted in Figure 10.

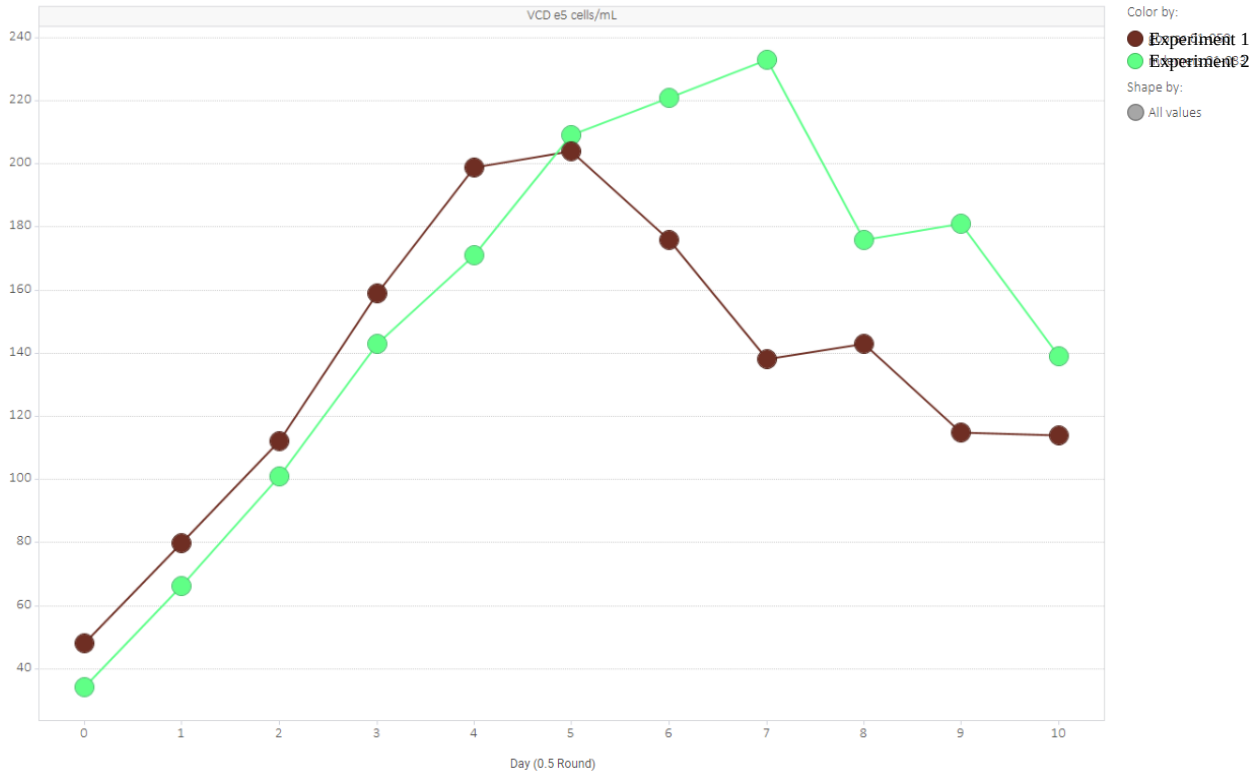


Figure 8; Viable cell density (VCD) between experiment 1 and 2. The brown trend represents the old feeding strategy. The green trend represents the new feeding strategy. VCD trended higher for experiment 2 than experiment 1 due to the different feeding strategies. The trends showed the difference between both feeding strategies. The peak VCD (day 7) increased by about 100 e^5 cells for experiment 2.

Cell viability analysis

Cell viability is the measurement of the quantity of live cells, and it's typically measured by percentage (Heney, 2021). Figure 9 shows the effect in cell viability for Experiment 1 compared to Experiment 2 after day 5. The viability dropped significantly on days 6 through 8 because of the lower feed, which correlates to the viable cell data in Figure 8.

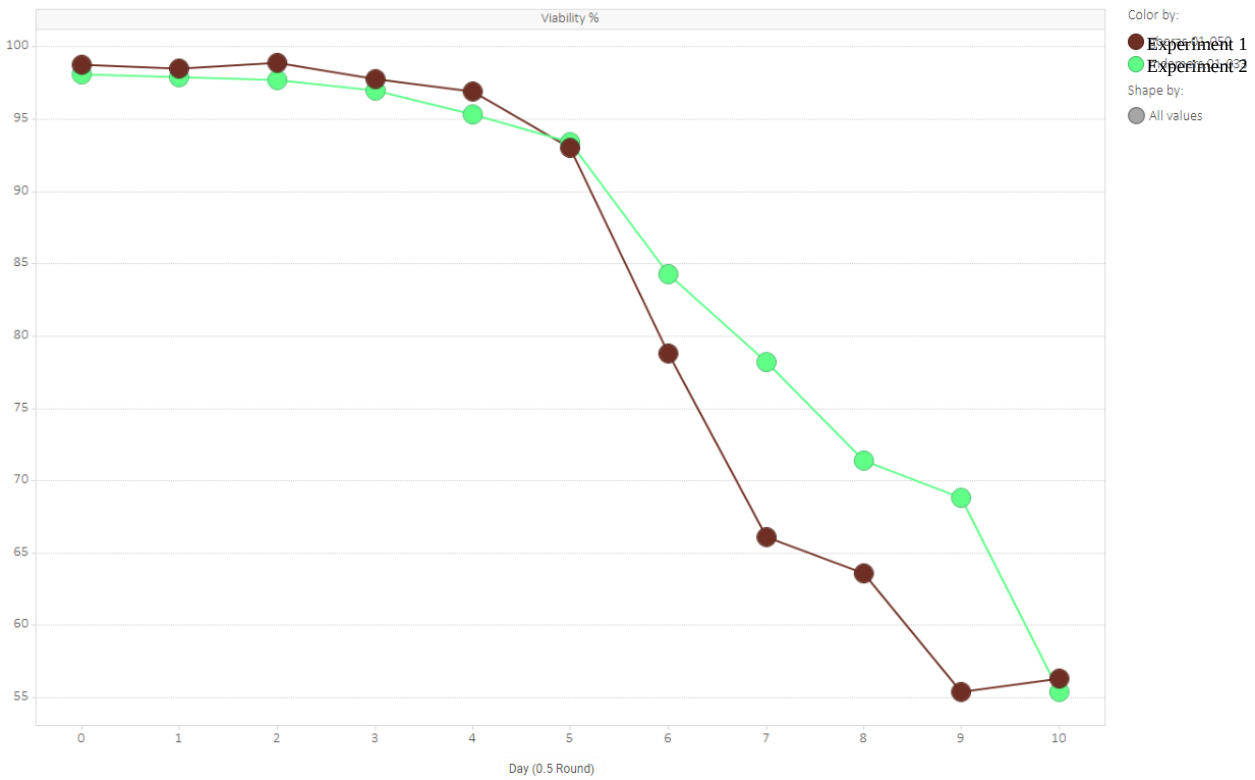


Figure 9: Cell Viability count between experiment 1 and 2. The brown trend represents the old feeding strategy. The green trend represents the new feeding strategy. Viability trended higher for experiment 2 after day 5 than experiment 1 due to the different feeding strategies and feed volumes. Experiment 2 viability maintained higher than experiment 1 for the rest of the experiment.

Cell Productivity analysis (protein titer)

A titer is a way of describing a concentration, based on successively performing a binary (positive or negative) test on increasingly diluted samples. A popular test for figuring out how

much of a certain protein is in a sample is protein titer monitoring. It can be used, for instance, to monitor the production process for a protein-based biopharmaceutical or to find antibodies. Monitoring of protein titers might be automated or carried out manually but we decided to use Roche BioHT to measure the IGG values (protein titer) (Alhuthali S, Kotidis P, Kontoravdi C, 2021). Figure 10 shows the cumulative titer production for experiment 2 is higher than experiment 1 by 15.8% because of the additional feed volumes and timing. Experiment 1 final day titer was 3.5 g/L while experiment 2 final day titer was 4.1 g/L.

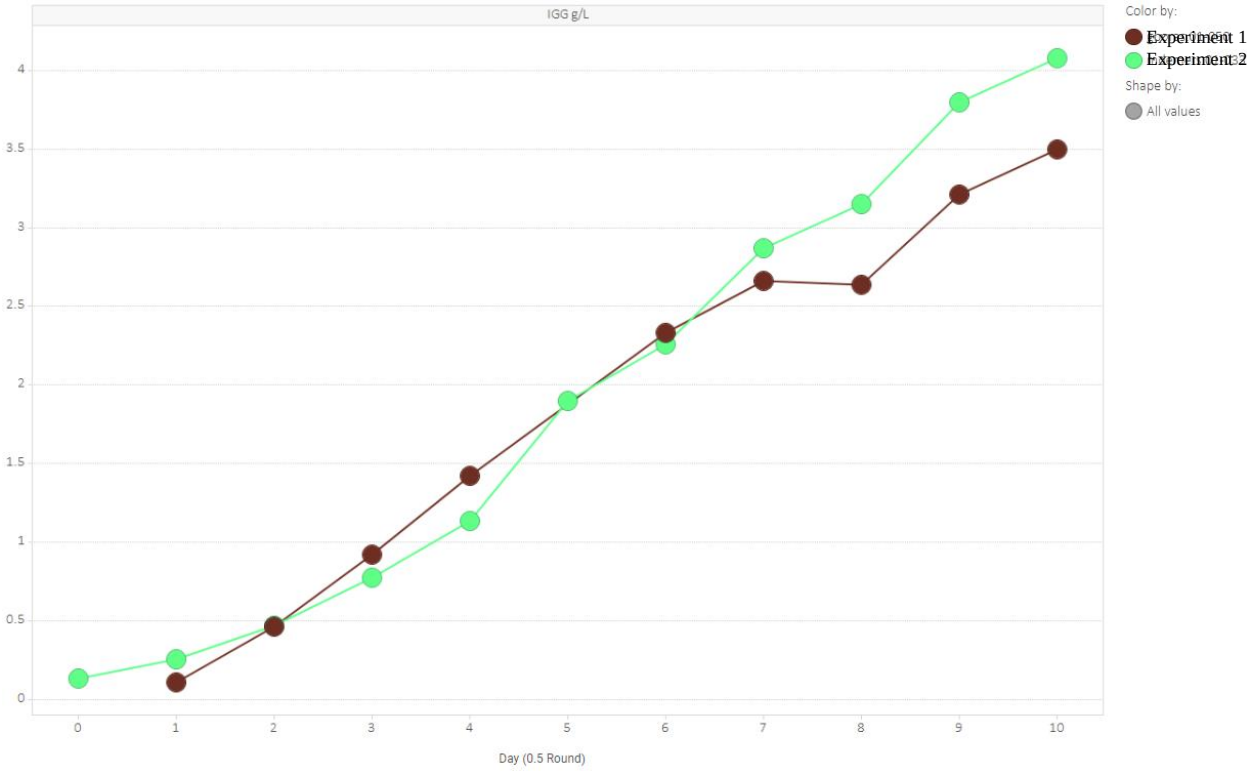


Figure 10: IGG represents the titer (protein production) between experiment 1 and 2. The brown trend represents the old feeding strategy. The green trend represents the new feeding strategy. Protein concentration increased by about 16% in experiment 2 than experiment 1 due to the change in feeding strategy as a result of amino acid data.

Amino Acid analysis.

When designing the new high mass process experiments, feeding the cells the right amount of nutrients was challenging. Overfeeding and underfeeding may, in fact, result in lower cell

density (indicated by VCD) resulting in lower cell productivity in protein titer (L-Tyrosine in Cell Culture, n.d.). By running the daily samples on the ARACUS from both experiments, the data showed the amino acids deficiencies over the days. Based on the results from experiment 1, experiment 2 increased the nutrients feed volumes to supply the cells with the sufficient volume of amino acids and to increase cell productivity and growth. Further highlighting this point, Figure 11, Figure 12, Figure 13, and Figure 14 below show the difference between experiment 1 (low feed volume) and experiment 2 (high feed volume).

Cystine analysis

Cystine is one critical amino acid that is frequently utilized in cell culture conditions and is crucial for the synthesis of proteins. Figure 11 has shown that experiment 1 had lower feeds and

almost no feeds on days 6 and 7 which are the peak days in growth and requires the most nutrient. Experiment 2 fixed the issue by feeding the cells more on the essential days and maintain a higher feed volume throughout the experiment. However, Day 6 in experiment 2 has almost reached 0 and more experiments are still in the works to maintain a higher cystine concentrations on day 6. An optimized cysteine supply during fed-batch cultivation supports the protein production capacity of recombinant CHO cell lines.

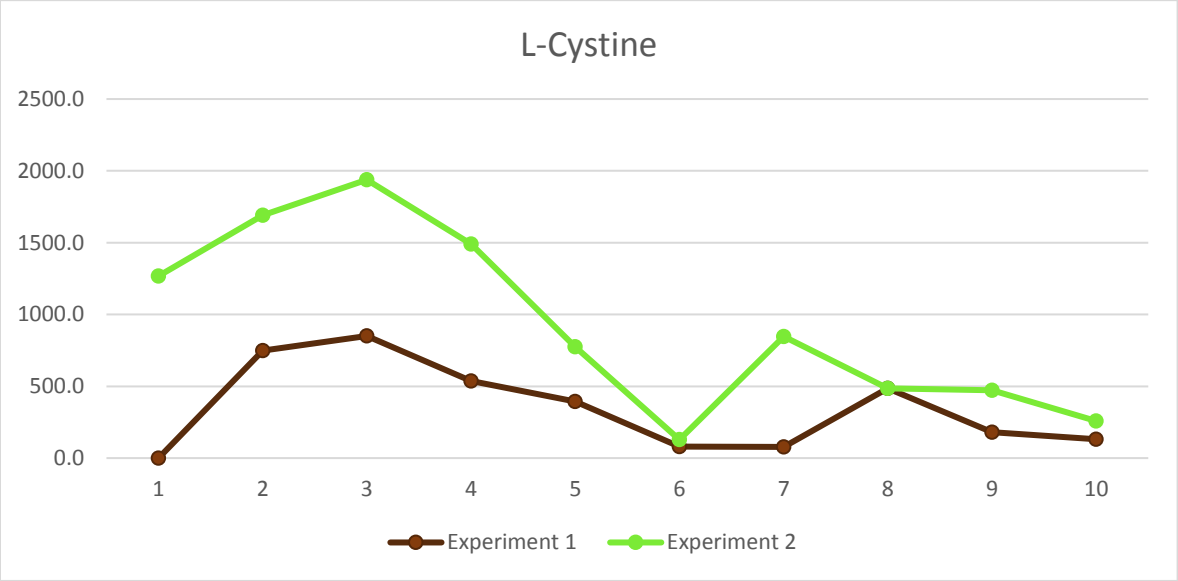


Figure 11; L-Cystine graph trend between experiment 1 and experiment 2. Cystine concentrations on the Y-axis are measured in umol/L. Note; experiment 1 had no data for day 1. The trend show the cystine was depleted by day 6 and 7 in experiment 1 while it maintained slightly higher for day 7 in experiment 2 due to the nutrient feed addition on day 7. Experiment still in progress for day 6 to adjust the feed volumes and times.

Tyrosine analysis

L-tyrosine is a non-essential amino acid; however, it can still be given to the cell culture media to promote the cultures' growth and viability. The cells can retain more energy and stay clear of the

hazardous byproducts created during biosynthesis when non-essential amino acids are introduced to the medium. Additional L-tyrosine supplementation is still required for certain cell lines (Amelio et al., 2014). Figure 12 has shown experiment 1 tyrosine to be depleting starting day 5 and completely depleted by day 7 due to low feed volumes and feed strategy, feed rates are referenced in page xxiv. Experiment 2 fixed the issue by feeding the cells more nutrients on the essential days and maintain a higher feed volume throughout the experiment.

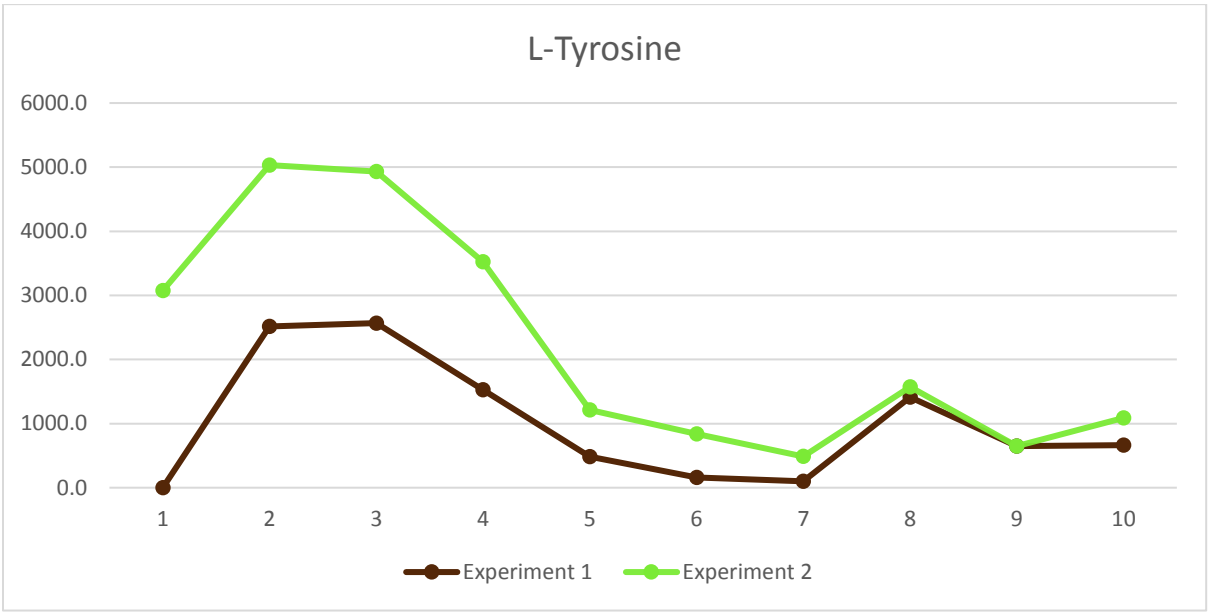


Figure 12; L-Tyrosine graph trend between experiment 1 and experiment 2. Tyrosine concentrations on the Y-axis are measured in umol/L Note; experiment 1 had no data for day 1. The trend shows the tyrosine was depleted by day 6 and 7 in experiment 1 while it maintained higher in experiment 2 to the nutrient feed addition on days 5 and 7.

L-Serine and Glycine analysis

Serine and glycine are essential amino acids. Together, they are the building blocks of proteins, nucleic acids, and lipids required for cell growth (de Koning TJ et al, 2003). Through the tetrahydrofolate (THF) cycle, these two amino acids play a role in the metabolism of nucleic acid precursors. However, supplementing glycine causes L-serine synthesis when L-serine levels are low. As a result, cell growth is inhibited because of a delayed THF cycle (Sveistyte et al., 2022). Figure 13 and Figure 14, serine was completely depleted on day 7 while glycine was depleted on days 5 and 7 completely in experiment 1 which caused lower cell growth and productivity. Experiment 2 had more sufficient feeding strategy and more nutrient volumes. As a result, experiment 2 had higher growth and cell growth Figure 8.

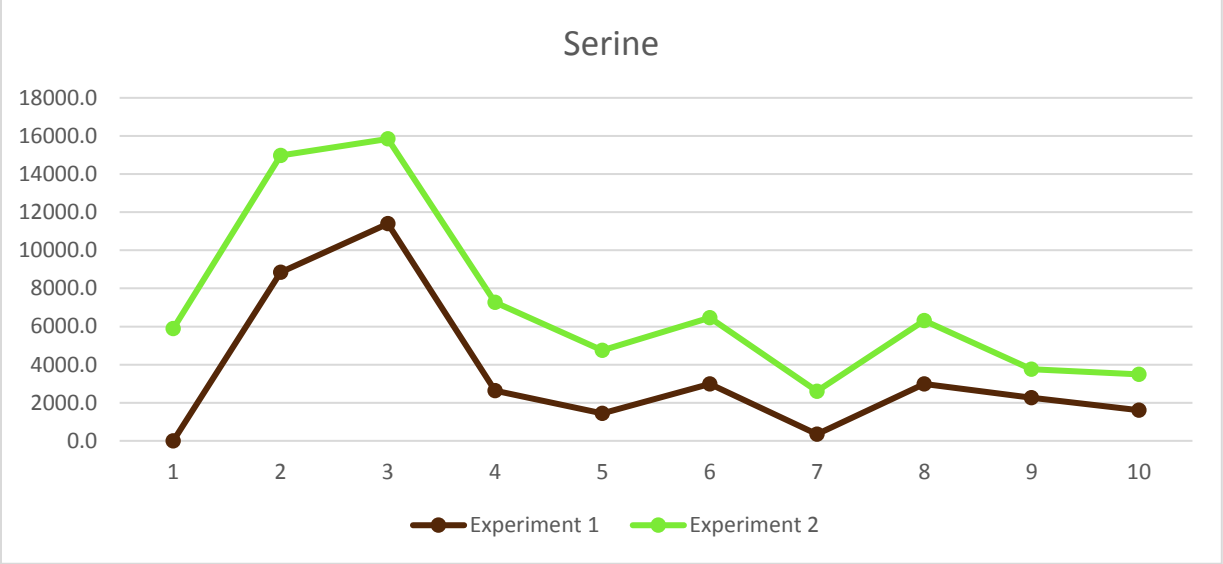


Figure 13; Serine graph trend between experiment 1 and experiment 2. Serine concentrations on the Y-axis are measured in umol/L. Note; experiment 1 had no data for day 1. The trend shows the glycine was depleted on day 7 in experiment 1 while it maintained higher in experiment 2 due to the nutrient feed addition on days 5 and 7.

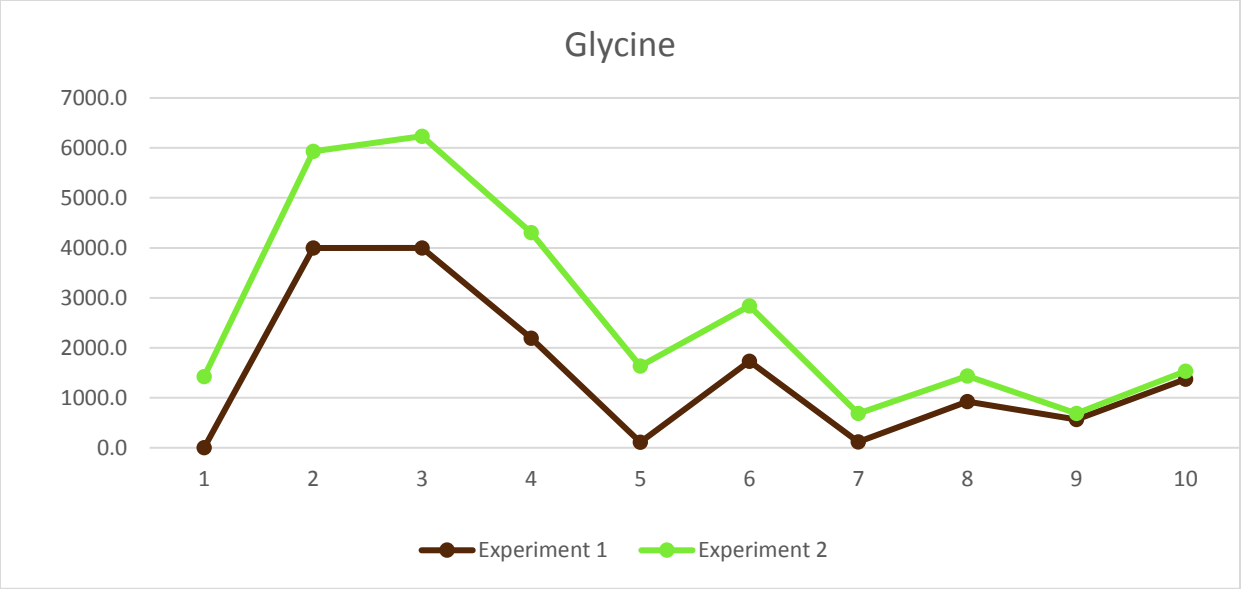


Figure 14; Glycine graph trend between experiment 1 and experiment 2. Glycine concentrations on the Y-axis are measured in umol/L. Note; experiment 1 had no data for day 1. The trend shows the glycine was depleted by days 5 and 7 in experiment 1 while it maintained higher in experiment 2 due to the nutrient feed addition on days 5 and 7.

Aim 2:

Have a baseline of all Amino Acid run on the ARACUS for all media used in our experiment to support in future investigations and used for batch verification. We hypothesize that by utilizing the ARACUS, we'll be able to.

- Increase the check points for media batching.
- Ensure the right materials were added during batching.
- Decrease the number of batches made by mistake due to error of addition of raw materials.

Different media samples have been collected from different media batches to be run on the ARACUS for amino acid analysis. The batched media tested were from the same chemically defined media powders. The goal was to see if the ARACUS data can be used as an additional check point on top of pH and DO. Currently only pH and Osmo are the only 2 check points for media batching which may not be enough to support troubleshooting if the right materials were added during batching. The data collected will be graphed with error bar and new media batches will be tested against historical data as an additional check.

Currently, this strategy has been tested on few programs where the media batch made was questionable and ARACUS confirmed it. The historical amino acid data showed the new media batch was fit for use. As a result, the run was continued as planned without re-batch nor rescheduling.

Figure 15 shows the average amino acid concentrations collected from 3 different batches of the same media. This establishes the baseline and anticipated variability for each amino acid. New media batches of the same media will be tested against this historical average and new medias

will be required to be within the error bars to pass for reproducibility. The goal is to catch an improperly batched media from being used in cell culture, adding another level of verification along with pH and DO.

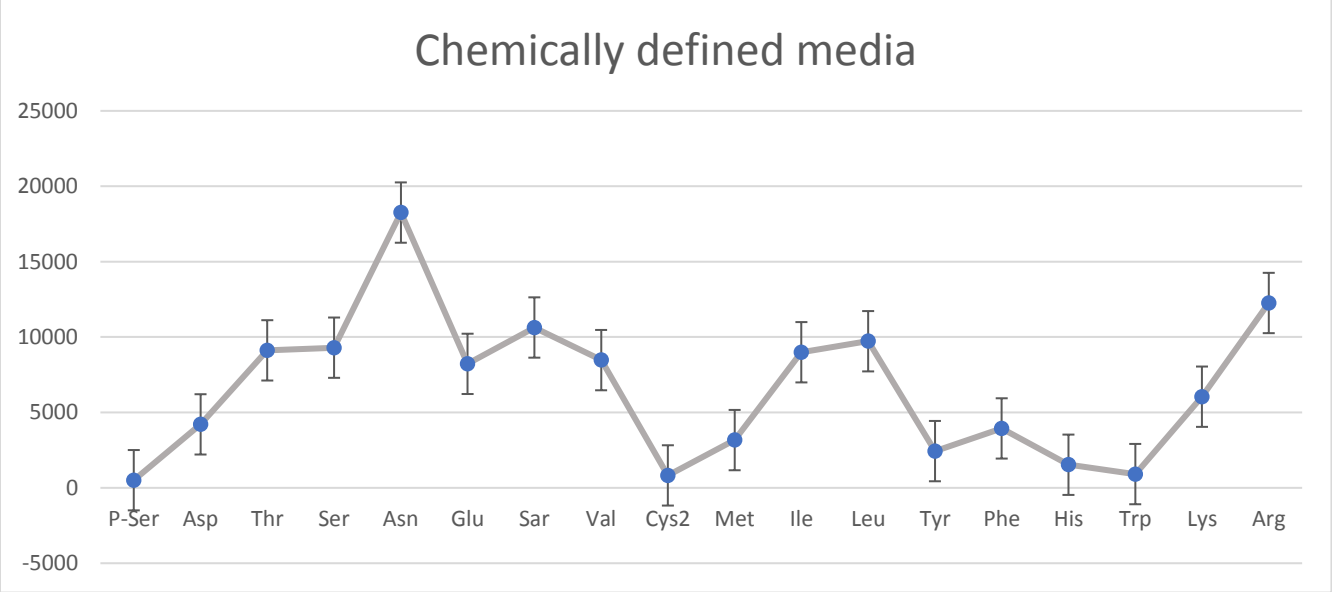


Figure 15; Amino acid concentrations baseline and expected variability for chemically defined media with error bars to identify if the new medias are within range. The idea is to run all new media batches of the same media along historical trends to identify if the new batch was done correctly. If any of the amino acids in the new media outside error bars, it means the new batch isn't safe to use.

Chapter 4: Discussion and conclusion

The purpose of this work was to assess the use of the ARACUS amino acid analyzer in the pharmaceutical process development area and its benefits during the cell culture development.

This work was processed over a year and conducted over multiple experiments. In conclusion, ARACUS has showed its capability during process development, and the aims mentioned above were achieved. It provided a clear understanding of the cell utilization of the nutrient feeds and the specific days where the cells require more nutrients.

During experiment planning, ARACUS played a role in decreasing the time spent on experimental design by only utilizing the ARACUS data and adjusting the feed amounts and times required. With previous technologies, it used to take longer time and more experiments to know the source of low productivity and low growth. To summarize the conclusion, ARACUS analyzer can speed up research, reduce manufacturing costs, and increase efficiency, all of which could lead to lower pharmaceutical drug pricing and greater profits for the corporations that develop them.

In addition to process design, ARACUS was able to decrease the number of media batches made daily by eliminating the uncertainty of batching the wrong the medias or adding the wrong amount of a specific raw material. This work was done by collecting multiple data of the same media from different batches and comparing it to new medias made for consistency.

The application of ARACUS technology in biotech process development has demonstrated that it adds value by facilitating efficient real-time process monitoring and glucose control, as well as upstream cell culture processing and process development operations. Technological advancement could lead to a significant increase in production efficiency and process development.

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