RAFT®14

Saturday, November 5

7:00 AM - 8:00 AM Workshop Breakfast (workshop attendees only)

7:00 AM - 8:00 AM Workshop Registration

8:00 AM - 9:00 AM Board Breakfast

8:00 AM - 4:00 PM Board Meeting

8:00 AM - 4:30 PM Workshop: Advanced Fermentation Concepts

Conveners: Tim Cooper, Danimer Scientific, Bainbridge, GA, USA

12:00 PM - 1:00 PM Board Lunch

12:00 PM - 1:00 PM Workshop Lunch (workshop attendees only)

Sunday, November 6

2:00 PM - 5:00 PM Exhibits & Poster Set up

5:00 PM - 6:00 PM RAFT Welcome & Perlman Awardee: Dr. Michael V. Arbige, DuPont's BioSciences Group, VP

Conveners: Mark Berge, AstraZeneca, Gaithersburg, MD, USA and **Kat Allikian**, South Pacific Sera

6:00 PM - 8:00 PM Opening Reception | Poster Session 1 | Exhibits Open

6:00 PM - 8:00 PM Poster Session 1

P1 GFP pipeline for model biopharmaceutical

E. Mahdinia, Stack Family Center for Biopharmaceutical Education & Training (CBET), Albany, NY, USA; A. Parry and R. Hobson, Department of Basic & Clinical Sciences, USA*

Green Fluorescent Protein (GFP) is a bioluminescence molecule natively produced by *Aequorea victoria* that is used extensively in scientific research. Currently, GFP is sustainably produced using recombinant DNA technology in *E. coil K12*. The purpose of this work was to construct an upstream and downstream pipeline for recombinant GFP as a model recombinant biopharmaceutical to serve The Stack Family Center for Biopharmaceutical Education & Training (CBET)'s education and training scopes. Here, recombinant GFP was produced in *E. coli* K-12 using the T7-promoter-driven pFluoroGreen plasmid. The bacteria were transformed using heat-shock transformation and selected transformants were cultured in Terrific Broth (TB). The transformants were fermented in a 3 L BioFlo 320 Fermenter with a 2.5 L working volume for 24 h, and GFP production was induced with 0.5 mM IPTG at t=6 h. Measurements of optical density at 600 nm and protein content were taken throughout the fermentation run to quantify biomass accumulation and GFP production, respectively. The maximum biomass concentration achieved was 1.6 g/L and the GFP concentration at the end of the fermentation run was 510 mg/L. The presence of GFP was confirmed throughout the production process via visualization under UV light. Downstream processing proceeded with centrifugation of the fermentation broth at 3900 rpm for 15 minutes, cell lysis via lysozyme addition and flash freezing at -80°C, vacuum aspiration with a 0.2 µm PES filter, and TFF with 50 kD and 100 kD filter cut-offs in 1X TE buffer. The crude GPF in 1X PBS was aseptically aspirated and then aseptically dispensed into 20 mL glass vials, which were subsequently finished, resulting in the completion of the pipeline. Ongoing work includes FPLC and UHPLC work-flow optimization for future analytical purposes.

P3 Production of high-quality plasmid in a novel chemically defined medium.

N. Sengupta, PhD, A. Jones, E. O'Hanlon, PhD, A. Gurunathan, S. Holdread and J. Brooks, PhD, Thermo Fisher Scientific, Hunt Valley, MD, USA*

With the advent of new treatment modalities, such as conjugated monoclonal antibodies (mAbs), DNA vaccines for cancer therapeutics, cell and gene therapy, and mRNA-based vaccines, there has been a renewed interest in the use of *Escherichia coli* (*E. coli*), an established workhorse for microbial-based recombinant bioproduction technology. The lack of capacity to produce GMP-grade plasmids has created a bottleneck in the biotechnology industry. This has triggered a new interest for advanced *E. coli* platforms using chemically-defined (CD) media to deliver consistency, when compared to complex broths, and to alleviate the risks associated with animal-origin components.

P5 AI Models applied in RQ control to optimize recombinant protein production in *Pichia pastoris*

X. Garcia-Ortega, A. Gasset, A. Sales, J.L. Montesinos and F. Valero, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain; J. Van Wijngaarden and T. Manzano, AIZON, Barcelona, Spain*

The yeast *Pichia pastoris* is currently considered the second preferred microbial host for recombinant protein production (RPP), specially since numerous products biomanufactured with this cell factory are FDA approved for therapeutics and food applications. Oxygen limitation has been shown to improve significantly RPP productivities and yields in bioprocesses regulated by GAP promoter. This environmental stress (hypoxia) can be monitored by the respiratory quotient (RQ), being then a transferable parameters between different fermentation systems. A step further is to design and to implement a control algorithm using RQ as a controlled variable to maintain the desired hypoxic conditions in the culture acting on agitation rate.

This strategy has been studied at three different stages: first, a manual-heuristic control system was tested, obtaining 2-fold higher productivities with respect to oxygen non-limiting conditions with a *Pichia* strain producing *Candida rugosa* lipase 1 (Crl1) as a model protein. Then, an automatic control algorithm has been implemented, considerably reducing labour and time costs while maintaining the 2-fold increase in productivity and also increasing precision and stability. Finally, as a more attractive and scalable strategy from an industrial perspective, an RQ-control algorithm based on artificial intelligence (AI) has been designed, implemented and tested. Continuous data feeding is conducted from the fermenter to an on-line server, where an AI model predicts on-line the proper agitation to maintain a constant RQ throughout the fermentation process.

As conclusion, it can be stated that an innovative physiological control has been implemented based on an AI approach allowing to achieve results comparable to those obtained with classical methods, increasing 2-fold yields and productivities compared to standard oxygen conditions (normoxia). This opens a window for this data-driven technologies to be implemented in bioprocesses based on microbial fermentation including both pharma and biotech industrial sectors.

P7 Bridging scales; from compartmental modelling to the transcriptomic analysis of industrially relevant oxygen concentrations

A. Kerssemakers, PhD student, G. Sin and S. Sudarsan, Danish Technical University, Kgs Lyngby, Denmark*

Process development of fermentative systems often comes with a wide range of challenges that should be overcome for successful commercialization. The significant changes in vessel size as compared to initial laboratory setups can lead to adverse effects with regard to e.g. mixing behavior and substrate availability [1]. Ideally, strains are developed to have a good tolerance towards these production conditions, creating the so-called *fermenterphiles* [2]. However, as large-scale fermentations are expensive to run and do not typically form part of early stage process development, scale-down experiments are needed. Often, researchers struggle to select the right conditions to be tested as industrial values are typically not shared publically.

Research from Nadal-Rey et al. has provided a compartmental understanding of a large-scale fed-batch fermentation (40 to 90 m^3) through computational fluid dynamics [3]. *Yarrowia lipolytica* specific kinetic parameters are then estimated based on continuous fermentation cultures. These parameters are inputted into microbial kinetic equations and combined with the compartmental understanding of the reactor vessel.

The simulated values provide an overview of expected O_2 concentrations and microbial kinetics throughout the fed-batch process. These values are used to create a framework of relevant conditions. Continuous cultures within this range, coupled with RNA-seq analysis, yields useful insights into *Y. lipolytica*'s behavior and regulation. A suboptimal or otherwise non-desired response provides a good starting point to make recommendations for further engineering efforts.

Acknowledgements

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References

[1] Lara, A.R., et al., Living with heterogeneities in bioreactors. Mol. Biotechnol. 2006 343 2006, 34, 355–381.

[2] Straathof, A.J.J., et al., Grand Research Challenges for Sustainable Industrial Biotechnology. Trends Biotechnol. 2019, 37, 1042–1050.

[3] Nadal-Rey, G., et al., Development of dynamic compartment models for industrial aerobic fed-batch fermentation processes. Chem. Eng. J. 2021, 420.

P9 From equations to data science: modeling fermentation process to its success

D. Weinstein-Fischer, VAYU Sense, Petach Tikva, Israel*

VAYU-Athena is a unique controller based on Artificial Intelligence (AI) tools for the optimization of bioprocesses. Our controller is free of prior process assumptions; it is entirely data-driven, enabling generic, fast, and precise solutions to enhance process performances. Equations are frequently used to model fermentation processes and strain behavior. But what guarantees that the chosen equations correctly describe and predict the process's pattern? Though it appears that this approach may have some success occasionally, clearly, it is highly limiting and would require a new set of equations, and assumptions, for each new process. We decided to change our mindset to look for a generic and scalable solution that would allow a controller's fast yet accurate development. We realized that the process dynamic could be best learned from its historical data—this database, combined with our unique modeling technique, is the heart for Athena's training. Once trained, Athena utilizes the online data to optimize the process outcomes in real-time. As Athena acts on process data alone, it will boost any multi-data process. Athena operates in a cloud environment which gives additional advantages to its users. The inimitable VAYU-Athena controller empowers companies performing fermentation-based processes to fully realize their production potential, bringing the fermentation industry to the next level of automation while maximizing yields and profitability.

P11 Engineering a microbial chassis for enhanced therapeutic protein expression

T. Telander, E. Nordwald, R. Todd, J. Johnson and A. Pilling, KBI Biopharma, Boulder, CO, USA*

Key words: E. coli strain engineering, minimal genome, microbial therapeutic protein expression, cell line development

Challenges in therapeutic protein manufacturing ultimately impact the cost of the drug product and the risk associated with developing it. Process and product related impurities can increase purification intensity and decrease the safety or efficacy of the drug product. All impurities as well as the quantity (titer) of product made starts from the host strain used for expression. Near wild-type host strains possess genomic elements, acquired over the course of evolution, that are either superfluous or even deleterious toward the goal of robust, clean, and high titer recombinant protein expression. The vast knowledge *E. coli's* genome allows for extensive tuning of the host to produce high titer product of interest with fewer process and product related impurities. We have generated an E. coli host strain with over 55 genomic edits (>900 genes) aimed at improving the quantity and quality of recombinant proteins expressed from this *E. coli* strain. This platform strain has a genotype associated with reduced translational and post-translational modifications (product-related impurities), improved stability, reduced excess and virulent genes (process-related impurities), improved phage resistance, and an improved metabolism. This platform strain with generically improved attributes can be leveraged into a library of strains for optimizing expression toward unique proteins of interest, including protein classes previously not viable for commercial expression in *E. coli*. To date, we have shown robust growth of the platform strain in fully chemically defined medium to high OD_{600}'s (> 100) and strong yet tunable recombinant protein expression capable of outperforming competing *E. coli* protein expression strains.

P13 Impact of feeding regime on cell growth & peptide production

C. Foune, A. Koyley, G. Campana-Paez, A. Lemons and J. Ladd, Vestaron Corporation, Kalamazoo, MI, USA*

Commercial fermentation of recombinant proteins requires productive biomass production. Fermentation conditions must be optimized to maximize protein production per cell. Oftentimes this is achieved through fed-batch fermentation. There are many different feed strategies that may be utilized including ramp, step, exponential growth and sensor-based. In this research, five different carbon feeding regimes were evaluated for biomass and recombinant peptide production using an industrial yeast strain excreting a recombinant protein. Batch fermentation was performed to determine baseline biomass and protein production. There was a 3.8- 5.2x increase in biomass production depending upon feeding strategy employed. Protein titers improved 5.2 – 8.8x over batch fermentation. This research highlights the importance of optimizing feeding strategy to maximize biomass productivity

P15 Challenges in downscaling fungal fermentations

K. Overkamp, Ginkgo Bioworks NL, Utrecht, Netherlands*

Fungal host strains such as *Aspergillus niger* are used to produce a wide variety of enzymes and proteins. Their capacity to produce and secrete proteins at high levels has made them the organism of choice for industrial protein production processes. However, one of the challenges of these fungal production strains is their inherently higher viscosity in comparison to bacterial or yeast strains. Nevertheless, the right combination of strain characteristics with a correct fermentation protocol can go a long way towards solving this challenge.

At Ginkgo, we have a low viscosity *A. niger* platform strain which, in combination with a tailored fermentation process at 5 Liter

scale, has produced very high native protein titers reaching up to 120 g/L. To guide further strain and process development for heterologous protein production, DoE setups to quickly find optimized fermentation settings will be required. However, the 5L scale is not suitable for high throughput work as it is cumbersome and labor intensive. Therefore, we looked for smaller volume systems to which we could downscale our process.

In this presentation I will compare different small-scale systems on their suitability for fungal fermentation parameter screening and outline the challenges faced during downscaling to our system-of-choice, the Ambr250. Some of the main challenges turned out to be the scalability of fungal viscosity behavior over the different cultivation volumes and the analysis methods to reliably determine this viscosity.

P17 Environmental ethanol feed tricarboxylate acid cycle in fermenting yeasts on glucose

T. Xiao[*] and J. Rabinowitz, M.D., Ph.D., Princeton University, Princeton, NJ, USA; A. Khan, Rockfeller University, New York City, NY, USA; C. Li, Ph.D., Fudan University, Shanghai, NJ, China

Glucose fermentation carries an important metabolic role in not only mammals but also yeasts. Both the alcoholic beverage and biofuel industries are based on glucose fermentation to ethanol by yeast. In this study, isotope tracing in combination with advanced analytical chemistry methods (high-resolution LC-MS and NMR) reveals that ethanol, the product of glucose fermentation, is a major source of acetyl units and NAD(P)H in fermenting yeasts. We find that, even as yeast are excreting ethanol, they are surprisingly also consuming excreted ethanol as a TCA cycle and redox fuel. Hence, even if historically viewed as a bottle neck of fermentation bio-platforms, overflow metabolism is a key turning point for systematic redox homeostasis to ensure feasibility of excessive biosynthesis.

P19 Overcoming aeration, OPTURA bio-reflectance sensors for real time total biomass monitoring in highly aerated process environments

A. Bhat[*], Aber Instruments Inc., Alexandria, VA, USA and L. Male, Aber Instruments Ltd., Aberystwyth, United Kingdom

One of the most critical process parameters in microbial processes is biomass, since it directly impacts critical quality attributes of the process. Typically, biomass in such processes is measured offline. The challenges of offline methods, such as infrequent sampling, contamination risk during sampling, intrinsic dilution errors, inter-operator errors, time & effort investment etc are well known. The ideal biomass measurement technique should be in-situ, real time, continuous and plug & play. In this paper, we present a real time and continuous optical method based on bio-reflectance for cell density measurement. This method has several advantages over other methods - including a wider linear range between 0.01 - >175 g/l DCW yeast and a patented bubble correction algorithm which successfully eradicates the effects of bubbles on biomass measurements up to 2 VVM. The bio-reflectance systems, presented as Aber's Optura range, also have reliable non-contact measurement options, which make the offering even more valuable. Typically, microbial process conditions present challenging conditions for optical sensors due to the high aeration rates used. The claims made above were validated when the Optura bio-reflectance sensor was successfully used to monitor the biomass growth during a bacteriophage amplification application using *E.coli* in an Airlift bioreactor, the most challenging environment for biomass monitoring technologies, producing real time biomass growth curves with high linearity to offline OD600 spectrophotometer measurements and CFU counts. Measuring biomass in such a challenging application enables process fingerprinting, real time troubleshooting, real time intervention and control of feeds.

P21 Human IgG Fc production through methanol-free *Pichia pastoris* fermentation

Y. Yang and M. Sha, Eppendorf, Inc., Enfield, CT, USA; J. Longsworth[*], Eppendorf Americas, Framingham, MA, USA; K. Madden, BioGrammatics, Inc., Carlsbad, CA, USA

Nowadays, therapeutic monoclonal antibodies (mAb) are predominantly produced with mammalian cell culture systems such as those using Chinese hamster ovary (CHO) cells. Efforts are underway to reduce the costs of this process to meet the increasing global demand in biopharmaceuticals; meanwhile cheaper and faster expression systems are being investigated as alternatives. The yeast *Pichia pastoris* has become a substantial workhorse for recombinant protein production. However, the N-linked glycosylation in *P. pastoris*, namely high mannose glycosylation, is significantly different from that in CHO or other mammalian cells including human cells. In this study, a SuperMan5 strain of *P. pastoris* was constructed using Pichia GlycoSwitch® technology to successfully produce a more mammalian like immunoglobulin fragment IgG Fc, which showcases the potential of *P. pastoris* as a next generation mAb production platform. Importantly, in this study a strong methanol-independent promoter P_{UPP} was applied which only requires glycerol feeding for protein production. Most *P. pastoris* promoters used for protein expression are derived from genes in the methanol metabolism pathway, creating safety concerns due to the flammable nature of methanol especially at large scale. Here, a fed-batch SuperMan5 *P. pastoris* fermentation was carried out in which methanol induction, as well as its affiliated safety risks, was eliminated. The 3 L bench scale bioprocess demonstrates the feasibility of using *P. pastoris* for IgG Fc production in a methanol independent environment, providing insight for future industrial mAb production seeking a safe and cost-effective approach to compete with mammalian cell culture.

P23 Microscale cultivation of *Trichoderma reesei* RutC30 enables strain phenotyping and bioprocess development in batch and fed-batch mode with higher throughput

K. Rohr, L. Gremm, B. Geinitz, W. Wiechert and M. Oldiges, Forschungszentrum Juelich, Juelich, Germany*
As our society transforms from fossil resources to renewable alternatives, processing of biomass remains a key challenge. Plant biomass conversion requires hydrolytic enzymes such as cellobiohydrolases and β-glucosidases; thus, cost-effective production of these enzymes is critical. Filamentous fungi are excellent enzyme producers due to their high secretion efficiency. However, due to morphological challenges, high-throughput, parallelized cultivation of filamentous fungi using micro-bioreactors is not widely established. One important producer of cellulolytic enzymes is *Trichoderma reesei* RutC30. In contrast to other industrially relevant filamentous fungi, RutC30 is used with a microfilamentous morphology, leading to culture broth with higher viscosity.

In this study, a micro-bioreactor workflow at milliliter-scale for time- and cost-efficient characterization of *T. reesei* strains is established. The applicability of batch and fed-batch mode with online measurement of scattered light (measure for biomass) and dissolved oxygen was examined. To this end, the cellulase hyperproducing strain RutC30 was cultivated. Cellobiohydrolase and β-glucosidase activity were measured by photometric assays on a liquid handling robotic platform.

A protocol for reproducible micro-bioreactor batch cultivation of RutC30 was successfully developed. Round Well Plates shaken at 1000 rpm and 35 % oxygen were found to work best, showing microfilamentous morphology and only minor wall growth. Furthermore, the applicability of microfluidic fed-batch with different feeding rates of 0.3-0.75 g/(l*h) lactose was shown. Cellobiohydrolase and β-glucosidase activities in fed-batch mode were increased 2.8-fold and 4.4-fold respectively compared to a batch cultivation under the same conditions. A morphological change from microfilaments only to a mixture with pellets was found during fed-batch.

To conclude, micro-bioreactors are a promising tool for parallelized cultivation workflows of *T. reesei*. The presented workflow can be applied for strain library characterization, providing advantages in terms of increased phenotypic information and reduced experimental resources. Moreover, bioprocess optimization under industrially relevant fed-batch conditions is shown to be feasible.

P25 Use of AI models to inform media combinations yields increased protein expression across platforms

K. Witherell, PhD, R. Caguiat, R. Olson, PhD, A. Sastry, PhD, S. Akella, MS, U. Sipetic, MS, K. Venkatamaranan, PhD, M. Gander, PhD, C. Kohnert, MS, A. Brown, PhD, B. Knight, C. Orona, W. Throndset, PhD, MBA and J. Plassmeier, PhD, Absci, Vancouver, WA, USA*
Recombinant protein technology is revolutionizing medicine, manufacturing, and agriculture, but efficient production and proper folding of many recombinant proteins is often challenging. Using Yellow fluorescent protein (YFP) expressing strains as proof of concept for more complex biologics expressed in our patented SoluPro™ *E. coli* strains and proprietary AI models, we rapidly optimize chemically defined media formulations and fermentation processes for robust protein production of YFP. Our genetic algorithm improved upon our chemically defined media using 18-24 unique media components and sterile deionized water. Results showed that over the course of five generations, a total of 11 media conditions increased total YFP signal by up to 20% compared to the control media. Results also showed adding additional media components increased YFP signal in the fifth generation after a local maximum was reached in the previous generations. Additional studies included using this genetic algorithm focused on improving semi defined media and fermentation conditions in SoluPro™ *E. coli* strains making a biologic. These studies show that recombinant protein signal increases with each generation. High protein signal was associated with low stress response, high ribosome production, amino acid starvation, and low metal. Based on results from these two studies, our genetic algorithm works to optimize either chemically or semi defined media to improve protein expression across platforms and and protein types.

P27 How spectroscopy is changing the face of modern fermentation

J. Speed, Keit Spectrometers, Oxford, United Kingdom*
One of the biggest limitations in modernising fermentation approaches is the need to truly understand what is happening within the fermenter. Whether the vessel is a million gallon tank as seen in the fuel ethanol space, or a bespoke plug flow continuous reactor the challenge is the same: if you cannot measure what is happening within the vessel you cannot improve it. Off-gas analysis, pH probes, temperature probes all give glimpses and hints but they only provide part of the information available. Off-line measurements such as titres or HPLC give more detailed pictures, but are a snapshot in time - with results available long after the same was originally taken.

Here we present the exciting work being done in a variety of fermentation process using static optics FTIR spectroscopy. We will predominantly use fuel ethanol as an example, because the industry is reasonably standardised (allowing comparisons from plant to plant), and the intellectual property surrounding it is more relaxed - enabling more interesting results to be shared. We will present how customers have overcome the following problems:

- ensuring representative sampling of the fermentation is achieved

- calibrating spectrometers for multiple fermenters efficiently and cost effectively

- deciding what to do with the data made available with spectroscopy

We'll briefly discuss the benefits and drawbacks of common spectroscopic techniques, and give an overview of where the uptake of spectroscopic monitoring and control of fermentation is headed.

P29 Enhancing growth and bioproduct output in engineered methanogens

S. Carr[*] and N. Buan, University of Nebraska Department of Biochemistry, Molecular Trait Evolution, LLC, Lincoln, NE, USA

Methanogens are obligately anaerobic archaea noteworthy for producing methane from C1 compounds and acetate. Their ability to convert low-energy, otherwise inaccessible carbon into methane is a result of their highly efficient central respiration, which accounts for approximately 99% of the chemistry in the cell. A result of this respiratory strategy is a high substrate:product conversion ratio which is industrially relevant for the production of biomethane, and may also be harnessed for the production of value-added commodities through strain engineering and synthetic biology. One area of interest are terpene compounds, as methanogen membranes are composed 5% by dry weight of isoprenoid lipids and flux through the isoprenoid biosynthetic pathways is naturally high in Archaea compared to Eukarya and Bacteria. To assess the metabolic plasticity of methanogens, our laboratory has engineered *Methanosarcina acetivorans* to produce the hemiterpene isoprene. We found that engineered methanogens directed up to 4% of total carbon substrate towards isoprene with increased overall biomass. Optimization of isoprene synthesis by archaea will require developing large-scale process conditions to capture methane and isoprene. While methanogens are routinely grown at large scale in municipal and agricultural anaerobic digesters for biogas, process conditions for scaling up pure cultures on defined culture medium have not yet been optimized. We are systematically evaluating bioreactor conditions including gas exchange and nutrient flow rates to improve methanogen growth in pure culture. To date we have increased final OD_{600} in batch 1.5L scale from 0.165 to 0.705 while trapping 5.58mg isoprene in a 1.5ml oil trap accounting for 0.16% of substrate carbon. Future experiments will assess growth in continuous culture and effects of process parameters on isoprene yield while optimizing isoprene capture.

P31 Bioprocess development for the production of polyphosphates with novel applications

P. Demling[*], J. Fees, A. Deitert and L.M. Blank, RWTH Aachen University, Aachen, Germany; P. Ehlert Jensen, A. Worberg and S. Sudarsan, Technical University of Denmark, Lyngby, Denmark

Phosphate is an essential resource required to meet the growing food demand. As the reserves in the form of phosphate rock are being depleted, a transformation to a circular phosphate economy is essential. The baker's yeast *Saccharomyces cerevisiae* was found to accumulate phosphate in the form of long-chain polymers, so-called polyphosphates. Polyphosphates with various chain length-specific properties are value-added materials with many applications. Whereas chemical synthesis is limited to a chain length of 40 subunits, polyphosphates in biological systems reach chain lengths of up to 1,000 subunits. The unique physicochemical properties of those long-chain polyphosphates and the modification of the counter-ion enable innovative applications such as antimicrobial coatings for surfaces. Further, if accumulated in *S. cerevisiae*, polyphosphate-enriched yeast extract can be produced as a novel food additive.

In this study, the technology for polyphosphate production in *S. cerevisiae* has been translated from small-scale shake flask setup to a tripartite high cell density fed-batch cultivation in a stirred-tank reactor. To evaluate viability for industrial-scale settings, the robustness of the process has been assessed by varying cultivation parameters and introducing perturbations. Further, metabolic engineering and a high-throughput platform for screening novel yeast strains for enhanced polyphosphate production will be presented. Ultimately, the combined strategies enable an efficient industrial-scale production process for polyphosphates with novel applications.

P33 Utilizing the AMBR250 for late-stage process development: defining a robust fermentation process for a polysaccharide vaccine antigen

A. Innes[*], Merck, West Point, PA, USA

As part of commercialization of vaccine bioprocesses, large process development data sets are required to define robust process parameters for the commercial process prior to qualification of the commercial facility. The required size of these data sets can linearly scale with multiple serotype vaccine antigen products, as was the case for a bacterial polysaccharide vaccine antigen produced by Merck & Co., Inc. Accordingly, it becomes necessary to pursue process scale down to achieve the data set required.

Historically for fermentation processes requiring stainless steel pressurized bioreactors, scale down of the fermentation process has presented a challenge, as many common high throughput reactor systems were not developed to support pressurized processes. In this case however, we present the successful scale down of a stainless-steel fermentation process to the Sartorius ambr® 250 small scale, single use, bioreactor platform through k_La characterization and computational fluid dynamics modeling,

The successful scale down to the ambr® 250 platform enabled rapid completion of a design of experiment (DOE) statistical approach to process parameter range definition. Specifically, the objective was to perform a 2 parameter DOE to define

temperature and glucose concentration at inactivation ranges for the production stage of the fermentation process for all serotypes of the product. Process parameter ranging studies on the inactivation step of the fermentation process were also performed. Through this work, robust fermentation and inactivation processes were defined with process parameter ranges defined to account for serotype specific sensitivities observed in the ambr® 250 process development studies.

P35 Smaller, faster, more robust: scaling down filamentous fungi in single-use vessels

K. Kneller, A. Dave and G. Venburg, Valent Biosciences, Libertyville, IL, USA*

Filamentous fungi produce a diverse collection of secondary metabolites such as enzymes, antibiotics, and isoprenoids. Potential applications for these fungal-based products are equally diverse, ranging from herbicides and insecticides to antimalarial and anticancer active ingredients. However, there are also major challenges associated with growing filamentous fungi by submerged fermentation in stirred-tank vessels. At high cell densities, the viscosity can drastically limit oxygen transfer and mixing of pH control agents and feeds. By implementing a dissolved oxygen feedback control for the substrate feed, these obstacles were mitigated.

Pilot-scale steam in place (SIP) vessels have the advantage of translating well to production-scale vessels. However, this comes at the cost of throughput. To optimize the secondary metabolite production of filamentous fungi, 250 mL single-use, plastic vessels were utilized to scale down a process first developed in 40 L SIP vessels. Due to the precise liquid transfers by an automated liquid handler, this miniature model proved to be more robust than its pilot-scale predecessor. The small footprint of this fermentation system enabled the cultivation of up to 12 vessels in parallel, which increased the number of process parameters that could be screened by 3-fold.

P37 Scalable ultrafiltration-diafiltration process of clarified plasmid DNA (pDNA) using T-Series cassettes with Omega™ membrane

A. Lorenzo, Pall BioPharm, Westborough, MA, USA and A. Armengol, Akon Bio, Sarasota, FL, USA*

Plasmid DNA (pDNA) is an important genetic engineering tool used to clone and amplify or express genes for a wide range of applications. Production of viral vectors and mRNA are dependent on pDNA. With the emergence of SARS-CoV-2, the development of new vaccines including mRNA/DNA vaccines sped up significantly. The manufacturing process for pDNA poses challenges for process development and for regulatory authorities tasked with assuring quality, efficacy, and safety of the final product. For each process, the design of a suitable purification and concentration strategy will depend on many variables including the pDNA interaction with filtration module and the nature of the pDNA. The development studies presented here confirm the Ultrafiltration-Diafiltration (UF-DF) process solutions after clarification of the pDNA. The main objectives of the UF-DF step are to concentrate and buffer exchange the pDNA prior to a chromatography step. One of the challenges for UF-DF step involves plasmid compaction promoted by use of high salt buffers. This compaction can result in pDNA passage through the UF membrane, resulting in low yields. Critical process parameters during these studies were predetermined to avoid plasmid damage due to shear stress. Membrane fouling was prevented, and an effective recovery method was performed, both to avoid yield loss. We have developed a process that confirmed retention of pDNA, increasing the pDNA yield post-clarification.

P39 Carbon-Negative Chemical Production Platform

A. Thompson, M. Martin and S. Brown, LanzaTech, Skokie, IL, USA; R. Giannone, R. Hettich and T. Tschaplinski, Oak Ridge National Laboratory, Oak Ridge, TN, USA; S. Ragsdale, University of Michigan Medical School, Ann Arbor, MI, USA*

LanzaTech™ (LT), with partners from the University of Michigan and Oak Ridge National Laboratory, will work to create transformative technology to enable direct conversion of CO_2 to ethanol – a building block for low carbon intensity fuels and chemicals – at 100% carbon conversion efficiency. The LanzaTech team are developing a gas fermentation process that leverages affordable, renewable hydrogen (H_2) as the reducing power to capture and fix carbon dioxide (CO_2) directly into valuable products. Inputs to the proposed process are carbon-free renewable energy, water, and CO_2. The availability of carbon-free renewable energy for H_2 production unlocks the opportunity to use H_2 to produce low carbon commodities such as fuels and chemicals from CO_2 at scale. The carbon-optimized conversion technology developed under this project is expected to be integrated with multiple CO_2 sources, such as corn grain ethanol refining and direct air capture (mid-long term). LanzaTech™, the global leader in microbial gas fermentation, is well positioned to rapidly bring this new technology to the market. Ultimately, the technology has the potential to improve the sustainability of fuels and chemicals and add value to the growing circular carbon economy.

P41 Production of melanin by fermentation of *Flavobacterium Kingsejongi*

P.C. Lee, Ajou University, Suwon, Korea, Republic of (South)*

Melanins are brown-black biopolymeric pigments and are widely used as bioactive materials and functional polymers in the biotechnology industry. Here, the high-level melanin production using a new melanogenic *Flavobacterium kingsejongi* strain is reported. Melanin biosynthesis of *F. kingsejongi* was investigated via genome analysis of the melanin biosynthesis pathway, and

structural analysis of purified melanin from *F. kingsejongi*. Analysis using FT-IR and NMR confirmed the chemical structure of melanin from *F. kingsejongi*. Next, the kinetics of growth and melanin production in *F. kingsejongi* was investigated by performing batch fermentation (5 L-bioreactor scale). Supplementation of tyrosine enhanced production and yield of melanin during fermentation of *F. kingsejongi.*

P43 Growth-uncoupled Fed-batch Strategies for Recombinant Protein Production in *Pichia pastoris*

N. Bernat Camps, M.A. Nieto and X. Garcia-Ortega, Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Barcelona, Spain; J. Fischer, K. Ebner and A. Glieder, bisy GmbH, Hofstaetten, Austria; F. Valero, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*

New expression tools are constantly being developed for the yeast *Pichia pastoris* (*Komagataella phaffii*), which is one of the preferred hosts for recombinant protein expression. A driving aim is to provide methanol-independent alternatives to the strong and methanol inducible promoter P_{AOX1}. The regulation and strength of the system can drastically change the optimal fermentation strategy, forcing to move away from standard processes. In this study an optimized fed-batch approach was developed for a new growth-uncoupled and methanol-free promoter, called P_{DH}. The industrially relevant lipase B from *Candida antarctica* (CalB) was chosen as model protein, while the strong and constitutive glycolytic promoter P_{GAP} was used as benchmark.

The growth-decoupled regulation of P_{DH} was confirmed in shake-flask cultivations, which were fed with glycerol FeedBeads® upon the initial glycerol was depleted. This slow-release feeding technology enables to achieve pseudo-starving conditions, which led to a 5.2-fold increase in the lipolytic activity of P_{DH} strain compared to P_{GAP} strain. Fed-batches at a low constant specific growth rate (μ) were performed in benchtop bioreactors with both promoters. The final CalB titer of P_{DH} strain outperformed the P_{GAP} strain by 1.4 times, suggesting room for improvement in the operational approach.

To optimize the P_{DH}-based fed-batch, the shake-flask pseudo-starving conditions were reproduced in benchtop bioreactor. Therefore, after increasing the final batch biomass concentration at a high constant μ, protein expression was induced by applying pseudo-starving conditions with a slow glycerol addition. Comparing to the benchmark, this approach successfully achieved 3.4-fold more specific productivity. To further increase titers, a new strategy was implemented combining several growth-induction cycles at different biomass concentrations, which enhanced the final titer but not the specific productivity. In summary, the single growth-induction fed-batch was the best approach for P_{DH}-based process, which could be transferred to other growth-uncoupled expression systems.

P45 Exhaust gas analysis in fermentation processes

S. Neumann, InProcess Instruments Gesellschaft für Prozessanalytik mbH, Bremen, Germany and D. Chen, InProcess Instruments for Analytic Process, North America, LLC, Irvine, CA, USA*

A benefit of mass spectrometry in online gas analysis is the ability to monitor gaseous compounds over a wide range of concentrations (ppb to %). This allows the control, improvement and automation of (bio)-chemical processes in research or industry. In contrast to gas specific sensors the mass spectrometer is able to measure all gaseous components in the fermenter exhaust lines.

The GAM 2000 Mass Spectrometer System that we are going to present is able to sequentially monitor up to eight fermenters. If more fermenters have to be monitored during operation the number of gas inlets can be increased up to 192 with optional gas stream selectors.

Due to the sophisticated nature of the pressure-controlled gas inlet system the actual size of the fermenters does not influence the measurement data. Therefore, the displayed solution is fully scalable in every aspect.

IPIs easy-to-use software package allows the user to specify gases, cycle durations and the fermenters to be measured. The data is being automatically analyzed and the gas concentrations are calculated and displayed within the IPI software. The data can also be transferred to third-party software via several different communication standards like MQTT, OPC UA and others.

In the field case that is displayed a GAM 2000 Mass Spectrometer System is connected to the exhaust lines of eight Sartorius Biostat A Advanced fermenters. The measurement data is being analyzed and the calculated concentration values are transferred into the Sartorius BioPat MFCS Software. The GAM 2000 provides concentration values of O_2 and CO_2 (other gaseous components can be added depending on the measurement task), which can be clearly displayed in a software interface together with other parameters from third-party suppliers (e.g. pH value). This enables to get an insight into the fermentation process for research purpose and to optimize the process.

P46 Impact of nutrient selection on fermentation and downstream processing of lactic acid bacteria

G. Venkat and S. Urbance, Sensient BioNutrients, Hoffman Estates, IL, USA*

Lactic acid bacteria (LAB) are key microbes in the probiotics and food industries. However, they can be challenging to work with. They are fastidious and require more complex nutrition sources when compared to other industrially relevant microbes. Nutrient requirements differ between species and strains. Yeast extracts are a standard ingredient in fermentation media for LAB. Yeast extracts provide a complex source of nutrition with protein in the form of amino acids and short and long peptides, vitamins, co-factors, and nucleic acid material. However, yeast extracts also vary in their nutritional composition. It is important to consider both upstream and downstream impacts when selecting a yeast extract because a fermentation yielding high cell counts may not equate to a stable, viable product after downstream processing, including lyophilization. We have investigated a variety of yeast extracts to determine their effects on both fermentation and downstream processing of *L. acidophilus, L. rhamnosus, L. plantarum, L. cremoris,* and *B. bifidum.* Increases of 27% to 59% were observed in end of fermentation cell counts and greater than 50% increase in yield was achieved.

P47 Ready Scalability for Ease of Manufacturing Development Using Pelican Expression Technology® (PET)

T. Bruck, D. Retallack and E. Orchard, Ligand Pharmaceuticals, San Diego, CA, USA*

Ready Scalability for Ease of Manufacturing Development Using Pelican Expression Technology® (PET)

The Pelican Expression Technology® (PET) platform (formerly Pfenex Expression Technology®) is a microbial expression system for developing protein therapeutics from concept-to-clinic. The platform is designed to enable rapid, and cost-effective production of proteins, antibodies, and peptides for use in human therapeutics, vaccines, and other applications and includes five commercially approved products. With over 20 years of experience developing *P. fluorescens* based processes, we have refined the method of scaling up fermentations successfully from HTP (High Throughput Screening) scale volumes to >10,000L seamlessly and rapidly. Pelican starts with an innovative approach to strain screening that leverages the unique properties of its proprietary bacterial strain, *Pseudomonas fluorescens,* such as general robustness of growth and expression over a wide range of conditions, tolerance of high concentrations of various carbon sources, and some of the advantages of being an obligate aerobe. Promising strains are down-selected from HTP screening to an optimal manufacturing strain at 2L fermentation scale. Each strain is evaluated under several induction conditions, such as induction temperature and pH, which generally have a significant impact on product quality and quantity. The best strain chosen is the one that shows the highest titer, quality, and robustness (best performance across a range of conditions). Additionally, special attention is paid to scalability in factors such as oxygen transfer rates, cell viability, and process simplicity. Indeed, this is achieved at Pelican by scaling the process to the 10-100L scale in-house, in anticipation of technical transfer to a CMO. In this poster we review several case studies demonstrating the effectiveness of the Pelican fermentation scale-up approach and show how initial HTP and smaller scale fermentation data were used to enable subsequent process development, tech transfer and cGMP manufacturing.

P49 Development of a multi-parameter electrochemical sensor for bioprocess monitoring based on a flow system

A. Hasanzadeh, B. Rezaei and M. Kilstrup, Technical University of Denmark, Lyngby, Denmark; P. Ramin and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

Bioprocesses require strict control of metabolites and nutrients to achieve optimal cell growth and maximize product yields. However, when comparing full-scale operation to lab-scale, the number of on-line sensors for monitoring of full-scale bioreactors is usually limited, consisting of traditional sensors (e.g. temperature, pH, and dissolved oxygen) [1]. Especially in a production environment, the detailed monitoring of cell performance is usually achieved by taking regular samples from the fermentation medium during the process, followed by off-line measurement of substrate, metabolite, and product levels in the samples. Thus, real-time monitoring of such critical process parameters is often lacking, and therefore, controller set points are often based upon experience rather than on-line data (e.g. golden batch approach). Some of these challenges can be alleviated by using biosensors for on-line monitoring which is also aligned with Process analytical technologies (PAT) [2].

In this work, we report on the design and development of a multi-parametric analytical flow system integrating three electrochemical sensors. The developed flow-system allows for simultaneous determination of ammonium, lactate, and glucose remotely in real-time. Amperometric sensors for the determination of glucose and lactate are based on a thin film of nanomaterials. A metal-organic framework (MOF) based amperometric sensor was developed for ammonium measurement. A fast detection was recorded for the sensors over wide concentration ranges for the mentioned analytes. The as-prepared sensors indicated highly reproducible responses and stable sensitivity. The successful application of the sensors set-up is demonstrated in different fermentation processes.

P51 Evaluation of alternative statistical approaches for fermentation media optimization

B. Forkus, J. Francis, D. Brown, E. Sherer and M. Chase, Corteva, Indianapolis, IN, USA*

When working with novel fermentation processes, a large effort is typically placed on optimizing the process conditions for desired outputs such as increased product titer, reduced viscosity, and minimization of unwanted side products. Commonly, fermentation experiments are developed using statistical design approaches, such as the response surface method. However, these traditional approaches often require upfront screening to reduce the number of independent variables to keep experiment

sizes manageable. For example, doing a two-factorial study on 12 fermentation parameters would yield >4000 individual experiments. Traditional methods are also inherently sequential, requiring success in previous iterations for further advancement. These limitations can cause higher order interactions to be missed and often require limited ranges on individual parameters which decreases the overall design space.

Applying global optimization approaches to fermentation process development may enable higher dimensions and broader experimental ranges to be explored, potentially leading to a more diverse set of solutions while dramatically reducing the amount of testing required. Bayesian optimization (BO) is a machine learning technique which can be applied to black-box functions for global optimization. It is particularly useful when attempting to explore design spaces with limited sampling capacity. In this work, a BO methodology was applied to the formulation of a complex media for the fermentation of a bacterium known to produce a promising metabolite of interest with crop protection properties. The BO algorithm was developed with metabolite titer as the optimization variable and was compared in parallel to a full-factorial design approach. Several media formulations were identified using both methodologies that outperformed the currently used control media. In each subsequent iteration of BO, a media formulation yielding higher titers of the metabolite of interest was identified, suggesting the algorithm was repeatedly moving towards better solutions.

Monday, November 7

8:30 AM - 11:30 AM Overcoming fermentation failure: Lessons learned Sponsored By: Kuhner Shaker, Inc.

Conveners: Tim Cooper, Danimer Scientific, Bainbridge, GA, USA and **Dr. Christopher Stowers**, DSM, Columbia, MD, USA

8:30 AM S1: Fits, mis-fits, and retrofits: Adventures in the biotech boneyard

M. Hoehne, Danimer Scientific, Winchester, KY, USA*

During the development of upstream processes from the lab through commercial scale, equipment selection is critical to the success or failure of the subsequent phase of scale-up. The ability to efficiently produce operating data and reliably produce fermentation broth are key deliverables at smaller scales. As process development progresses, these facilities must continuously adapt to more versatile roles and foster collaboration across an increasing number of sites and disciplines.

With these requirements in mind, how do you plan, design, and build a fermentation suite that has the capability to meet immediate objectives but the flexibility to adapt as the breadth of the research program evolves? This presentation explores the challenge of fitting the disparate and often mismatched equipment, facilities, and protocols into a cohesive unit that functions as the flywheel to advance a product through its lifecycle.

8:55 AM S2: Applying process knowledge to minimize scale-up challenges

T. Davies, R. Cobb, N. Hanspal and D. Feria-Gervasio, Corteva Agriscience, Indianapolis, IN, USA*

Corteva Agriscience manufactures a range of natural products and biologicals by fermentation. Product volume growth and cost reduction is supported by development and deployment of improved production strains and fermentation process conditions. R&D is undertaken in lab scale fermentations at volumes of 400 mL to 30 L and are validated at pilot scale (100 L). Production volumes can be orders of magnitude larger. Successful transfer of strains and processes to manufacturing requires knowledge of scale impacts. Research scale fermentation processes are intended to be an accurate scale-down of the production conditions. However, not all conditions can be perfectly represented. Differences between fermentation scales remain despite best efforts and continual improvement of models.

In this paper, the authors will present some of the techniques that Corteva has used to minimize differences between fermentation scales and to refine scale-down models. The methods employed include a multi-omics approach to analyzing microbial performance; an assessment of mixing and mass transfer through the application of computational fluid dynamics and fermentation studies; and the use of on-line process analytical technology for real-time process monitoring.

9:20 AM S3: Geno's lessons learned from 10+ years of fermentation scale-up

C. Mehrer, Ph.D., Genomatica, San Diego, CA, USA*

Over the past 10+ years of deploying fermentation processes across multiple sites and scales, Geno has learned many lessons on how to minimize risks when successfully scaling-up new fermentation processes. With the philosophy of "begin with the end in mind," sustainable materials leader, Geno has developed a rigorous approach to anticipate, prepare for, and learn to navigate areas with higher likelihood of failure. In this talk, we will discuss Geno's approach to characterizing process robustness, scaling-down fermentations prior to scaling-up, developing trust across the team beginning with technology transfer, and supporting initial scale-up efforts with laboratory staff. Geno developed this approach with to develop products like 1,4-butanediol

(BDO), butylene glycol (e.g, Brontide™), and has continued to refine and improve process deployment and scale-up with plant-based nylon and palm oil alternatives.

9:45 AM Coffee Break - Join the Placement Committee for Coffee and Conversations during all coffee breaks!

10:15 AM S4: “We do not learn from experience... we learn from reflecting upon experience”

W. Van Winden, DSM N.V., Delft, Netherlands*
The throughput of both automated strain construction and strain performance testing steadily increase, following rapid developments in genetic protocols as well as automation and robotization of molecular biology and small scale strain screening workflows. These developments increasingly move the challenge of fast and successful commercialization of new biotechnological processes and products towards first-time-right scaling up to commercial scale production sites.

The key risk factors for successful and fast scale up are:

(1) inability to operate the small scale fermentation protocol at large scale because of fundamental equipment limitations,

(2) inability to stably operate the intended protocol because of hardware, control software or human operation issues after start-up of the process,

(3) sensitivity of the developed microbial production strain (a.k.a. ‘lab champions’) to large scale conditions, even when applying and stably operating the intended protocol.

These three classes of potential causes of ‘fermentation failure’ are complementary, so success depends on effective mitigation of all three. This contribution will discuss examples of mitigation of each category, by applying a set of best practices that have evolved during introduction of many new fermentation processes to existing and new manufacturing plants.

10:40 AM S5: Scaling up lysozyme production: Challenges and solutions

M.O. Kaiser-Albæk, Novozymes, BAGSVAERD, Denmark*
Novozymes is the world leader in biological solutions, and we are in the progress of commercializing a microbial lysozyme produced by fermentation. *Trichoderma reesei* has been chosen as the production organism due to its ability to produce high levels of extracellular protein. During commercialization we have overcome a number of challenges related to large-scale production of the product – many of which are related to the (intrinsic and perhaps foreseeable) challenges of manufacturing a microbial lysozyme.

Because its crystals can easily be grown, hen egg white lysozyme of one of the most studied proteins. In our process development we have – during painful trial and error - learned a great deal about lysozyme solubility in the fermentation broth and how to deal with this. In this talk we will outline how we have found a viable way to control the solubility in the fermentation with a downstream recovery process.

The solutions to all challenges encountered have been created in collaboration between scientists from different backgrounds. Also based on experiences from this project, Novozymes has recently re-thought our way of working and now organize scientists involved in process- and product development in product teams with end-to-end responsibility of the process from fermentation through downstream processing to final formulation. Being part of empowered teams with shared goals increases motivation, collaboration, and productivity. Motivating examples of this will be discussed.

11:05 AM S6: Scaling up industrial bioprocesses: what could go wrong? (R)

J. Lievense, Lievense Bioengineering LLC, Ft Meyers Beach, FL, USA*
Scaling up industrial microbial processes for commercial production is a high-stakes endeavor, requiring time and investment often exceeding that for laboratory microbe and process development. Omissions, oversights, and errors can be costly to the program. Unfortunately, the author has seen and personally participated in several failures during his 40-year career with consequences spanning minor to serious to fatal. These are briefly summarized in the form of a personal project “scorecard” and associated ingredients for failure. The latter is also translated into a recipe for success. Two case studies are presented and discussed in more detail. One of these had a fatal outcome. The other very likely should have been fatal but – due to specific, preemptive actions – had a successful outcome. In closing, three guiding principles for successful project execution will be given: begin with the end in mind; be diligent in the details; prepare for the unexpected.

12:15 PM - 12:45 PM Exhibitor Monday Showcase: Aber Instruments, BlueSens and Biolog

1:00 PM - 4:00 PM Model driven strain & fermentation process development

Conveners: Dr. Mads Orla Kaiser-Albæk, Novozymes A/S, Bagsvaerd, Denmark and **Steve Van Dien**, Persephone Biosciences

1:00 PM S7: Guiding synthetic biology via Automated Recommendation Tool (ART)

T. Radivojevic, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

One of the most important challenges in bioengineering is effectively using -omics data to guide metabolic engineering towards higher production levels. Here, we present the Automated Recommendation Tool (ART), a tool that leverages machine learning and probabilistic modeling techniques to guide synthetic biology in a systematic fashion, without the need for a full mechanistic understanding of the biological system. ART provides a set of recommendations for the next engineering cycle, alongside probabilistic predictions of their outcomes. It can be used as a python library or through a web-based graphical frontend that does not require coding expertise.

1:25 PM S8: Bringing a scalable adaptive hybrid modelling framework closer to industrial use: application on a multi-scale fungal fermentation

T. Rydal and G. Nadal Rey, Novozymes A/S, Bagsvaerd, Denmark; J. Frandsen and P. Ramin, Technical University of Denmark, Kgs. Lyngby, Denmark*

Digitalization has paved the way for concepts such as digital shadows and digital twins for fermentation processes, opening the door for real-time process monitoring, control and optimization. With a digital shadow, real-time model adaptation to accommodate complex metabolic phenomena such as metabolic shifts of a process can be monitored. Despite the many benefits of digitalization, the potential has not been fully reached in the industry.

In this study, the development of a digital shadow for a very complex fungal fermentation process in terms of microbial physiology and fermentation operation on pilot-scale at Novozymes, and the challenges thereof are investigated. The process has historically been difficult to optimize and control due to lack of offline measurements and absence of biomass measurements.
Pilot-scale, lab-scale and production-scale fermentations were conducted for model development and validation.

With all available pilot-scale data, a data-driven soft-sensor was developed to estimate the main substrate concentration (glucose) with a normalized root mean squared error (N-RMSE) of 2 %. This robust data-driven soft-sensor was able to estimate accurately in lab-scale (volume < 20x pilot) with a N-RMSE of 7.8% and in production-scale (volume > 80x pilot) with a N-RMSE of 16.5%.

A hybrid soft-sensor was developed by combining the data-driven soft-sensor with a mechanistic model to estimate the glycerol and biomass concentrations on pilot-scale data with N-RMSEs of 11% and 21%, respectively.
A digital shadow modelling framework was developed by coupling a mechanistic model with the hybrid soft-sensor. The digital shadow modelling framework significantly improved the predictability compared with the predictive model.

The contribution of this study brings the application of digital shadows closer to industrial implementation. It demonstrates the high potential of using this type of modelling frameworks for scale-up and leads the way to a new generation of *in-silico* based process development.

1:50 PM S9: Modelling of gradients in industrial bioreactors to enhance fermentation process performance

G. Nadal Rey, B. Cassells and S. Cornelissen, Novozymes A/S, Bagsvaerd, Denmark; D. McClure, Brunel University London, Uxbridge, United Kingdom; J. Kavanagh and D. Fletcher, The University of Sydney, Camperdown, Australia; K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

Process economics and profitability have been shown by many authors to be affected by gradients in relevant bioreactor conditions (e.g., substrate concentration and pH) in large-scale fermentation processes, resulting in decreased yields. Thus, understanding, predicting and minimizing the occurrence and magnitude of gradients is of great importance from an industrial perspective. To this end, numerical modelling of large-scale bioreactors is key, as it can simulate the spatial-temporal conditions experienced by the cells in the fermentation environment.

To model industrial bioreactors, both hydrodynamic and microbial kinetics need to be considered. Therefore, a kinetic model describing the different metabolic regimes that the industrial workhorse *Saccharomyces cerevisiae* experiences (e.g., overflow and starvation) will be combined with three types of hydrodynamic models (Computational Fluid Dynamics models, compartment models and novel dynamic compartment models) for a 90 m^3 stirred tank bioreactor. These modelling techniques will allow the evaluation of the occurrence and magnitude of gradients in an industrial aerobic fed-batch fermentation process.

First, a comparison between the type, magnitude and occurrence of gradients will be provided, with focus on the local glucose

and dissolved oxygen concentrations, as well as the metabolic regimes that cells undergo in the different zones of the bioreactor (e.g., close to the feeding position). Yield differences between ideally and non-ideally mixed bioreactors will allow the quantification of the impact of gradients in industrial fermentation process performance. Finally, a comparison between the different hydrodynamic modelling strategies will be provided, with focus on their advantages, limitations and ease of implementation in industrial fermentation process development.

The modelling techniques developed and outlined in this contribution allow the characterization and minimization of gradients in industrial fermentation processes, helping to guarantee adequate process performance despite potential non-homogeneous cultivation conditions.

2:15 PM Coffee Break- Join the Placement Committee for Coffee and Conversations during all coffee breaks!

2:45 PM S10: Data driven tools to support process control and process development

K. Smith, BEng (Hons), PhD, MIET, C.Eng. and M. McEwan, BSc. PhD MIET, C.Eng., Applied Materials, Warrington, United Kingdom*

Data driven tools are becoming prevalent in the understanding of complex biological processes. Machine Learning (ML), in particular, is being put to great use. With the ability to process huge amounts of data and determine novel or unexpected patterns in data, machine learning tools can map and predict outcomes that accelerate product development.

In process development, there has been a journey from human guided trial and error through quality by design (notably in the Pharma space) and now machine learning is starting to play a role.

In this paper we present example Machine Learning approaches and show the beneficial combination of adaptive ML coupled with Advanced Process Control (APC). The combination of ML with APC addresses an important consideration, which is all too often overlooked. That is the effect of disturbance upon the process and how the process must be able to react to a disturbance and maintain product quality and yield.

3:10 PM S11: Explore or exploit? A model-based screening strategy for autonomous microbial phenotyping

L.M. Helleckes, T. Griesbach, C. Mueller, M. Osthege, B. Geinitz, E. von Lieres, W. Wiechert and M. Oldiges, Forschungszentrum Juelich, Juelich, Germany*

With modern tools of synthetic biology, many genetic variants are available for biotechnological processes. For example, strains with different signal peptides for secretion are constructed for protein production. However, *a priori* prediction of secretion performance is often not possible. Since it is not feasible to test all possible production strains in large-scale bioreactors, robust decisions are needed to derive a reduced subset in small-scale screening. Here, a typical exploration-exploitation trade-off is faced: On the one hand, new candidates need to be screened to explore strain libraries, on the other hand, the most promising candidates should be cultivated more often to evaluate them with higher statistical certainty.

In this work, we employ an automated microbioreactor platform to generate high-throughput (HT) data for microbial phenotyping. Concerning data analysis, we obtain suitable key performance indicators (e.g. titers or rates) for strain rankings from process data using a probabilistic process model with robust uncertainty quantification. For solving the exploration-exploitation problem, Thompson sampling is used as an experimental design strategy.

We applied our methods to screen two libraries of *Corynebacterium glutamicum,* each secreting a different heterologous polyethylene terephthalate hydrolase (PETase): Leaf-Branch Compost Cutinase (LCC) and Polyester Hydrolase (PE-H). In our study, we found that the rankings of 24 different Sec secretion signal peptides differ significantly between the two PETases, confirming that extensive screening is required. Due to the efficient optimization policy, only three batch runs (72 main cultures), were needed to make a statistically sound distinction. Within those rounds, Thompson sampling assigned 14 and 20 replicates to the best peptide for PE-H and LCC respectively. For comparison, in a standard triplicate design, the best performing strain would be screened only three times (3 replicates * 24 peptides = 72 cultures). Overall, this work paves the way for more efficient HT screening in early-stage bioprocess development.

6:00 PM - 8:00 PM Poster Session 2

P2 Development of a scalable single-use fermenter suitable for high demanding microbial cultivations

J. Rupprecht and M. Leupold, Sartorius Stedim Biotech, Goettingen, Germany; T. Ulrich, Sartorius North America Inc., Bohemia, NY, USA; L. Nascimento-Brooks, Sartorius Stedim UK Ltd, Royston, United Kingdom*

Microbial hosts offer advantages over cell cultures systems in terms of growth rate, protein production, media costs and process robustness, but until recently they have not been able to take advantage of the benefits of single use fermenters.

Large consistent, scalable, single-use fermenter solutions are needed to meet the obstacles of modern microbial process development and pilot manufacturing. Challenges such as mass transfer, heat transfer, mixing, foam formation, etc. are all amplified by the increased growth and production rates of microbial organisms, and often demonstrate the limitations of single-use fermenters in such applications.

We will deep dive into the technical challenges overcome in its development, such as foam detection, pH measurement, scalability, heat transfer, mixing, and mass transfer. Consistency was demonstrated between scales by measurement with standard guidelines for engineering characterization principles.

We will also discuss the benefits of microbial process development using consistent scalable single-use fermenter solutions, such as the Sartorius single-use Biostat STR® Microbial. We will present the capabilities of system, including comparison of characterization data with existing systems like the Sartorius Ambr® and an industrial case study highlighting the scalability to existing systems.

P4 Enhanced single-use fermentor for improved cooling and oxygen mass transfer

J. Brown, Thermo Fisher Scientific, Logan, UT, USA and C. Bergeron, Synlogic Inc., Cambridge, MA, USA*
Highly intensive microbial fermentation processes have necessitated design upgrades to the Thermo ScientificTM HyPerformaTM Single-Use Fermentor (S.U.F.) system, leading to the new Thermo ScientificTM HyPerformaTM Enhanced S.U.F. (eS.U.F.TM) system. Here, we display the key differentiators between these two systems; an increased jacket area with the ability to maintain a temperature setpoint at intensive run conditions and a redesigned impeller drive train that increases maximal dissolved oxygen (DO) delivery, minimizes foaming, and reduces process gas consumption. The suitability of the new system was ultimately tested by side-by-side culture comparisons between the HyPerforma S.U.F. and eS.U.F. systems, which demonstrated superior performance.

P6 *Pichia pastoris,* A PROMISING MICROBIAL CELL FACTORY FOR CONTINUOUS BIOMANUFACTURING

X. Garcia-Ortega, J.L. Montesinos-Seguí and F. Valero, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*
Such other industries did in the past, currently, the Industrial Biotechnology is involved in the transition from batch to continuous biomanufacturing in order to exploit the benefits of the continuous production. Among them, some are considered specially relevant such as; flexibility in operations, higher productivity and quality, decreased cost, smaller facilities as well as the integration and the simplification of the bioprocesses. However, the use of these cultivation mode in large scale biomanufacturing processes is still infancy and several drawbacks need to be addressed in the next future, as well as to the bioprocesses end-to-end full downstream integration.

The cell factory *Pichia pastoris*, is currently considered to be one of the most effective and versatile systems for the production of heterologous proteins and metabolites of interest. As a yeast, it combines the advantages of the microbial expression systems, such as fast and robust growth in defined media, and the ability to perform typical eukaryotic post-translational modifications. Furthermore, this host is able to target proteins extracellularly while secreting very low levels of native proteins. This combination of features allowed *Pichia* to become the second most preferred microbial host for recombinant protein production.

Currently, in the market are available different alternative expression systems that allows to regulate the expression of the target protein by the selection of the carbon source and/or the cultivations conditions. By the understanding of this regulation from the strain engineering point of view, is essential to develop efficient culture strategies from a bioprocess engineering approach. In this sense, continuous cultures based on alternative strategies have been implemented with *Pichia* producing different products of interest. For its application in large-scale production processes, is essential to develop stable and robust strains and bioprocesses which assure to maintain high production rates, product quality and bioprocess reproducibility over long operation times.

P8 Scale-up of anaerobic bacteria cultivation from 50 to 5,000 L in single-use bioreactors

J. Brown, Thermo Fisher Scientific, Logan, UT, USA, J. Heslep, independent consultant, Gainesville, FL, USA and A. Cowley, PhD MBA, Arranta Bio, Watertown, MA, USA*
The demand for anaerobically produced bacterial products has increased causing a need for rapid scale-up using single-use systems in contract manufactured operations. In this collaboration between Arranta Bio and Thermo Fisher Scientific, we show culture of a strict anaerobic bacteria strain in the worst-case surface to volume ratio 50 L Thermo ScientificTM HyPerformaTM Single Use Bioreactor (S.U.B.). Then with three similar oxygen sensitive processes we show scale-up in the 250 L HyPerforma S.U.B. and with one of those strain we further scale up in the 5,000 L Thermo ScientificTM DynaDriveTM S.U.B..

P10 Accurate, scalable microfermentation screening for microbial cell line development of therapeutic proteins

E. Nordwald, R. Todd, J. Johnson, T. Telander and A. Pilling, KBI Biopharma, Boulder, CO, USA*
The development of therapeutic proteins requires first selecting an appropriate cell line. In creating a cell line, both the microbial host strain and plasmid DNA sequence can have profound effects on the titer, purity profile, and overall manufacturability of a recombinant therapeutic protein. Choice of E. coli host strain can optimize titer, improve plasmid DNA stability, support disulfide formation, and/or minimize product-related impurities or degradation. Simultaneously, various plasmid elements including promoters, RBS, origins, tags, fusions, codon optimization strategies, and protein mutants can also affect titer, construct stability, purity, and overall manufacturability of the protein. Assessing these host strain-plasmid combinations should be done under representative fermentation conditions. Fermentation parameters may also need to be varied alongside the cell lines. We have implemented the Biolector microfermentation system to screen cell lines under relevant and varying fermentation conditions. This plate-based parallel microfermentation system can simultaneously screen cell lines while examining various fermentation parameters including media, feed-rates, pH profiles, and induction strategies. In one instance, we identified a cell line and fermentation strategy that improved upon the classical T7 (DE3)-system by more than ten-fold. After microfermenter screening, one or more strains may be further optimized in KBI's Ambr250 system. Although, integrating cell-line selection with fermentation development has streamlined subsequent development activities and even allowed direct scaling from the microfermenter to 10L stainless-steel or 30L single-use systems, in some cases. As a result of the integrated fermentation and cell line development, we are consistently exceeding 4 g/L titers after scale-up.

P12 Engineering a microbial platform for enhanced therapeutic protein expression

T. Telander, E. Nordwald, R. Todd, J. Johnson and A. Pilling, KBI Biopharma, Boulder, CO, USA*
Key words: E. coli strain engineering, minimal genome, microbial therapeutic protein expression, cell line development

Challenges in therapeutic protein manufacturing ultimately impact the cost of the drug product and the risk associated with developing it. Process and product related impurities can increase purification intensity and decrease the safety or efficacy of the drug product. All impurities as well as the quantity (titer) of product made starts from the host strain used for expression. Near wild-type host strains possess genomic elements, acquired over the course of evolution, that are either superfluous or even deleterious toward the goal of robust, clean, and high titer recombinant protein expression. The vast knowledge *E. coli's* genome allows for extensive tuning of the host to produce high titer product of interest with fewer process and product related impurities. We have generated an E. coli host strain with over 55 genomic edits (>900 genes) aimed at improving the quantity and quality of recombinant proteins expressed from this *E. coli* strain. This platform strain has a genotype associated with reduced translational and post-translational modifications (product-related impurities), improved stability, reduced excess and virulent genes (process-related impurities), improved phage resistance, and an improved metabolism. This platform strain with generically improved attributes can be leveraged into a library of strains for optimizing expression toward unique proteins of interest, including protein classes previously not viable for commercial expression in *E. coli*. To date, we have shown robust growth of the platform strain in fully chemically defined medium to high OD_{600}'s (> 100) and strong yet tunable recombinant protein expression capable of outperforming competing *E. coli* protein expression strains.

P14 Ambr250 as a screening tool for yeast-based vaccine process

S. Schmidt, Merck & Co., Inc., West Point, PA, USA*
The emphasis on high throughput and data-rich experiments in vaccines and biologics development is ever increasing, as patient needs and market demands compress filing timelines. The Ambr® 250 system is being leveraged as a high throughput scale for a multi-valent recombinant vaccine in a yeast expression system. Custom Ambr® 250 protocols were developed to scale down the 6000L commercial fed-batch fermentation to the 250mL small-scale model. Real time oxygen and carbon dioxide off-gas readings allow for advanced process control techniques, in which key metabolic indicators, such as oxygen uptake rate, are controlled at a setpoint. Automated sampling protocols allow for data-rich experiments with only light staffing. From proof-of-concept studies, online off-gas data largely falls within 3 standard deviations of an average 6000L commercial batch. At the Ambr® 250 scale, biomass levels are within the key process attribute limits for the commercialized vaccine. Leveraging the Ambr® 250 system results in a 4-fold increase in experimental throughput compared to the state-of-the-art 20L stainless steel model. As an example, a 5 factor, 3 level DOE could take upwards of half a year to complete in the 20Ls, while the AMBR system could complete this in a month and half with the same personnel, leading to significant time and cost savings. The results in early process development achieved thus far show the potential value of the Ambr® 250 system for this yeast-based process.

P16 Quality modeling with genomics: high-resolution metagenomics of closely related dairy bacteria using nanopore sequencing

D. Stefanova and L. Krych, University of Copenhagen, Copenhagen, Denmark; D. Lucena, Arla Foods, Aarhus N, Denmark*
Dairy starter cultures often consist of complex communities of closely related microbial strains. In such communities, subtle genetic differences can lead to distinct phenotypic features that define product traits such as flavor and quality. The genotypic characterization of bacterial communities holds therefore great promise in establishing a link between the genomes and the phenotypes that determine starter culture properties. Unfortunately, assembly of full genomes from closely related strains using

short-read technologies is not feasible, given the high level of genetic similarity among them. Instead, the use of a long-read DNA sequencing strategy offered by Oxford Nanopore Technologies (ONT) depicts an unprecedented opportunity for the recovery of high-quality full genomes from mixed communities. Moreover, closely related starter communities produce aroma compounds that are central for the quality and flavor profile of dairy products. This projects targets on the development of machine-learning based tools that combine detailed genomics with precise data on aroma formation during fermentation. We have recovered complete genomes (including plasmids) of bacterial strains from a mesophilic starter culture used in dairy production. Detailed metagenomic analyses disclosed unique strain-specific features of closely related bacteria, including phage-resistance mechanisms, mobile elements, and gene clusters related to flavor formation. With this data we seek not only to elucidate the biodiversity and composition of starter cultures, but also to reconstruct the metabolic pathways that lead to the production of flavor compounds of high relevance to the dairy industry.

P18 Advanced cell culture analysis of count ,viability and health by dynamic imaging using AI

T. Canty, PE, J.M.Canty Inc, Lockport, NY, USA*

P20 Real-time identification of problems in fermentations and quick adaptation of the conduct of operations, using inline fluorescence spectroscopy in combination with machine learning and artificial intelligence.

J. Sirois and S. Paquette-Pare, BioIntelligence Technologies Inc., Sherbrooke, QC, Canada; S. Cyr, F. Grondin and J. Sirois, University of Sherbrooke, Sherbrooke, QC, Canada*

This communication will address the most common failures encountered during industrial fermentation and will propose inline monitoring of metabolic activities using fluorescence metabolic indicators as a reliable and generic tool for early identification of problems and for adapting the conduct of operations. The authors will present the strengths and challenges of using fluorescence for inline monitoring and will explain how Machine Learning and Artificial Intelligence resolves these complex problems and enables real-time monitoring of fermentation and process deviations.

The communication will also share some real-life examples where the combination of these technologies has enabled a better understanding of the root cause of failures, and how these problems have been circumvented. The authors will conclude with a discussion on the limitations of the current metabolic activity technology and recommendations for future development.

P22 Optimization of a chemically defined medium for growth and antibiotic production from a new endophytic fungal isolate

S. Villas Boas and H. Fernandez-Lahore, Luxembourg Institute of Science and Technology, Luxembourg, Luxembourg*

Endophytic fungi represent the second largest reservoir of fungal diversity on Earth, second just to soil. Therefore, these fungi are a valuable bioresource for the discovery of new and structurally diverse biomolecules. We have isolated a new fungal species from an endophytic association with the New Zealand King fern. Culture crude extracts presented intense red pigmentation and showed broad antimicrobial activity against both fungi and bacteria, including various human and plant pathogens. The challenge, however, for exploring the bioactive metabolites produced by this new fungal isolate is our limited knowledge of its growth characteristics and requirements. The isolate grows well in complex rich media and in chemically defined minimal media, both under solid-state and liquid-state cultures. However, it only produces bioactive compounds under certain conditions, and the yield and diversity of secondary metabolites drastically change depending on the growth medium and condition used. We observed pigment production only when the fungus grows on complex media containing protein-rich substrates. Despite growing well on chemically defined minimal media, no pigment production nor antimicrobial activity was observed. Besides, antimicrobial production was observed only in extracts obtained from cultures grown on PDA, even though cultures grown on YPD presented a more intense pigment production. Therefore, we carried out a growth screening on minimal media supplemented with individual amino acids to determine which amino acids could induce pigment and/or antibiotic production. The fungus grew well in all 20 protein-derived amino acids with pigment production observed in all of them except in proline. Despite the intense pigment production, a clear indication of an active secondary metabolism, only five amino acids induced antibiotic production including proline medium. Therefore, antibiotic and pigment production by our endophytic fungal isolate seems to be highly influenced by the culture media composition, in particular by organic nitrogen sources.

P24 Development of optimized media for growth, sporulation, and metabolic output in *Bacillus* species for industrial fermentation.

C. Reedy and S. Veeravalli, Ph.D., Procelys, Cedar Rapids, IA, USA; S. Nelson, Ph.D., Procelys, Milwaukee, WI, USA; J. Holt, Ph.D., Envera, West Chester, PA, USA; J. Bencheikh and E. Oriel, Ph.D., Procelys, Maisons-Alfort, WI, France*

The use of Bacillus species as a biological tool is widespread across many industries including Environmental, Agricultural, Probiotics, and others. The growth of these microbes is not “one size fits all” as each strain requires a unique media formulation and growth conditions. Here we describe the process we use at Procelys to develop custom media formulations for Bacillus species of diverse industrial uses. We rely on a process which goes beyond cell growth and sporulation. We also look critically at cellular outputs and key process parameters which can be more indicative of commercial function. We will look more closely

at work on *Bacillus amyloliquefaciens*. In the end we were able to develop optimized media formulations for this strain by starting with the correct selection of yeast-based nutrient.

P26 Computational investigation of mixing behavior in a single vessel multi-compartment reactor

S. Dasgupta, Postdoctoral Researcher, Technical university of DEnmark, Lyngby, Denmark and S. Sudarsan, Technical University of Denmark, Lyngby, Denmark*

Small scale reactors (330 mL) are characterized by ideal mixing with mixing time in the order of seconds. On the contrary, large scale or industrial scale reactors (1000 L) exhibit significant gradients which results in higher mixing times in the order of minutes. The primary objective of this work is to obtain a computational model of a rational laboratory scale down reactor. Single vessel multi compartment reactors have widely been used by researchers in the past to scale down industrial reactors by introducing poor mixing. Contextually, this work is based on CFD modeling of a single vessel multi compartment reactor with 6 Rushton-turbine impellers. A 3-D computational geometry was setup in ANSYS Spaceclaim 2020 R1 for the reactor design. A The gas-liquid hydrodynamics of air and water as dispersed and continuous phases was simulated using a 2-phase Eulerian model. The liquid phase turbulence was resolved by the realizable k-epsilon and tracer diffusion was solved using the species transport equation in the commercially available CFD solver ANSYS Fluent 2020 R1. Transient simulations were carried out for a single phase (liquid) at different impeller rotational speeds until a steady state is attained based on liquid velocity. Subsequently, air is sparged from the inlets at the bottom of the reactor followed by introduction of a tracer from the top. It was observed that higher air sparging adversely affected mixing, thereby leading to gradients and increased mixing time, as observed in a large scale. This work will not only serve as a priori, but also provide a fundamental understanding of the fluid mixing in the novel reactor design and pave the way for future kinetics and mass transfer studies relevant to practical applications.

P28 High-throughput scale-down design to accelerate fermenterphile selection

J. Bafna-Rührer and S. Sudarsan, Technical University of Denmark, Lyngby, Denmark*

Successful scale-up of fermentation processes relies on the selection of fermenterphiles i.e., robust production strains that maintain optimal performance under industrial conditions. Screening and selection of strains with a fermenterphile phenotype require scale-down experiments in suitable scale-down bioreactors that mimic relevant industrial process conditions. Commonly used scale-down bioreactors often have a large footprint and therefore a low throughput, hence they can be used during the later stages of bioprocess development. However, including high-throughput scale-down systems at the early stages of bioprocess development would improve the chances of finding fermenterphiles that will perform best under full-scale process conditions. In this study, we present strategies to design high-throughput scale-down experiments using the Sartorius Ambr® 250 system. To demonstrate the strategy, we designed an intermittent feeding regime in cycles of 2 minutes to simulate the impact of glucose (substrate) gradients on *Escherichia coli*. Further, with the liquid handling solutions of Ambr® 250, metabolome and transcriptome analysis can be performed to elucidate the response of the tested strains under industrially relevant conditions. We show that the rational high-throughput scale-down design can be used efficiently to characterize fermenterphile candidate strains at the early stages of bioprocess development and thereby enable rapid bioprocess development.

P30 Towards Industry 4.0 in biomanufacturing: comprehensive vs. simple methods for HPLC modelling and simulation

C. Loureiro da Costa Lira Gargalo, S. Shiv, M. Muldbak, U. Krühne and K. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark; I. Udugama, University of Tokyo, Tokyo, Japan*

High performance liquid chromatography (HPLC) processes are a vital element of most downstream operations in the biomanufacturing industry. Although extensively used, for example, for the separation and purification of proteins, HPLC processes are still challenging to model to an adequate level of accuracy.

Therefore, this work endeavors to (i) review simple and complex modeling methods by which HPLC processes can be modeled, (ii) simulate and compare these models by replicating pilot-scale experiments, (iii) investigate the impact of process parameters on model fidelity, and finally (iv) identify the best strategies in the light of it being further used as digital models in a digital twin. Consequently, a comprehensive mass balance model and a simple plate model were developed. These models are compared by simulating a 3-component system under pilot-scale settings. The solutions obtained from each model were found to be in reasonably good agreement.

Furthermore, first thoughts on the possible economic ramifications of both model simplifications and increased complexity are also drawn and discussed under the digitalization context.

P32 Development of optimized media for growth, sporulation, and metabolic output in Bacillus species for industrial fermentation

S. Nelson, Ph.D., Procelys, Milwaukee, WI, USA; J. Holt, Ph.D., Envera, West Chester, PA, USA; J. Aldridge, Ph.D. and S. Veeravalli, Ph.D., Procelys, Cedar Rapids, IA, USA; J. Bencheikh, R. Rangel, Ph.D. and A. Sourabié, Ph.D., Procelys,*

Maisons-Alfort, WI, France
The use of Bacillus species as a biological tool is widespread across many industries including Environmental, Agricultural, Probiotics, and others. The growth of these microbes is not “one size fits all” as each strain requires a unique media formulation and growth conditions. Here we describe the process we use at Procelys to develop custom media formulations for Bacillus species of diverse industrial uses. We rely on a process which goes beyond cell growth and sporulation. We also look critically at cellular outputs and key process parameters which can be more indicative of commercial function. We will look more closely at work on three strains of Bacillus, *Bacillus thuringiensis*, *Bacillus coagulans*, and *Bacillus amyloliquefaciens*. In the end we were able to develop optimized media formulations for each strain by starting with the correct selection of yeast-based nutrient.

P34 Centrifuge-ready single-use BioProcess Containers enable closed-system handling

J. Brown and M. Wight, Thermo Fisher Scientific, Logan, UT, USA*
The CentriPAK centrifuge fills a key gap in bioproduction workflows by offering a competitive solution in harvest applications. Intensified upstream processes and the adoption of large-scale single-use bioreactors place increased stress on downstream unit operations. The CentriPAK system demonstrates a significant value-add in the single-use bioproduction workflow.

P36 Using small scale turbidostats to study the variation and evolution of identical unsupervised barcoded *S. cerevisiae* populations at high resolution

G. Jona, S. Rezenman, M. Knafo, I. Tsigalnitski, S. Barad, D. Levi, O. Dym, Z. Reich and R. Kapon, Weizmann Institute of Science, Rehovot, Israel*
Cellular lineage tracking provides the means to observe population makeup at the clonal level, allowing exploration of heterogeneity, evolutionary and developmental processes as well as the relative fitness individual clones.

Its use, however, is limited because existing methods are highly specific, expensive, labour-intensive, and, critically, do not facilitate the repetition of experiments.

To address these issues, we developed ***gUMI-BEAR*** (*genomic Unique Molecular Identifier Barcoded Enriched Associated Regions*), a modular, cost-effective method for tracking populations at high resolution.

Using parallel small scale turbidostat bioreactors, we first demonstrate how we apply the system to track, in high resolution, tens of thousands of *Saccharomyces cerevisiae* lineages growing together across multiple generations. This has revealed fitness differences, lineage-specific adaptations to environmental changes and subtle dynamic shifts. Then, we demonstrate how gUMI-BEAR can be used to perform parallel screening and optimization of virtually any number of gene variants, thus enabling unsupervised selection and identification of individual clones optimized for particular tasks. Finally, comparison between multiple, identical libraries allows us to reveal the interplay between stochastic and deterministic outcomes in these set of experiments.

P38 Continuous extractive fermentation at scale improves productivity and economics of de-novo 2-phenylethanol production

A. Brewster, K. Steinbusch, E. van den Berg and A. Oudshoorn, DAB.bio, Delft, Netherlands; M. Luttik and J.M. Daran, Delft University of Technology, Delft, Netherlands*
Novel biomolecules are rapidly being introduced to the scientific world through innovations in synthetic biology in the shift to a more sustainable bioeconomy. However, product toxicity and related microbial inhibition phenomena frequently hamper the maximum product level that can be achieved, limiting productivity and economic attractiveness.

In situ product recovery (ISPR) is a means to overcome this limitation by removing the product from the fermentation broth. Conventional two-phase fermentations apply an organic overlay to withdraw and concentrate the product in the overlay. Although an improvement, the capacity of a batch overlay is intrinsically limited and the achieved extension of the production phase is often insufficient. To overcome this limitation, DAB.bio has developed a reactor concept to continuously add and remove organic overlay, eliminating the limitation of conventional batch overlay fermentations and enabling indefinite extension of production processes. This FAST™ bioreactor platform (Fermentation Accelerated by Separation Technology) integrates conventional (fed)-batch fermentation with continuous extraction and in-situ product removal in one reactor without the use of membranes or mechanical aids and allows precise control of the product concentration in the aqueous phase. This robust design is easily scalable to industrial scale and can be seamlessly integrated with existing down-stream processing, dramatically improving the productivity of bioprocesses.

We present the case study on a fed-batch process for *de-novo* production of 2-phenylethanol with *Saccharomyces cerevisiae*. The performance of a conventional process with batch overlay was compared with continuous overlay with ISPR in a 500L FAST™ reactor. As expected, continuous extraction with FAST™ extended the production phase and dramatically increased the volumetric productivity of 2-phenylethanol production. By decreasing the cost of bioprocesses, continuous extraction and product removal with FAST™ has enormous potential to accelerate market entry of specialty chemicals.

P40 Improved understanding and monitoring of fermentations in plant-based protein production using Raman - spectroscopy - WITHDRAWN

K. Esmonde-White[*] and I. Lewis, PhD, Endress+Hauser, Ann Arbor, MI, USA; R. Wolthuis, PhD, Endress+Hauser, Naarden, IN, Netherlands

Fermentation processes in food production are important for spirits production, fermented foods, and plant-based proteins. Particular to plant-based proteins, fermentation provides the ability to acidify proteins, impart nutritional value, control flavor profiles, and improve emulsification. As food production expands into using sophisticated cell culture or fermentation manufacturing processes, it becomes increasingly more important to use process analytical technology (PAT) principles in order to understand composition, improve processes, and ultimately ensure a consistent product. Raman as a bioprocess PAT is well-suited for many upstream, downstream, formulation, and molecular structure applications because it can directly measure aqueous systems, has a strong history as a PAT in bioprocessing, and sampling versatility. We will present studies that demonstrate Raman spectroscopy as a tool for real-time, in-line feedback control of yeast or microbial fermentations. In these examples, Raman was used to simultaneously monitor sugars and alcohols around the clock without needing to extract a sample. A historical limitation of the Raman technique has been fluorescence interference. In this example we present a fluorescence mitigation strategy using a longer wavelength excitation, at 1000 nm, to reduce fluorescence interference and thus enable laboratory or process monitoring uses of Raman spectroscopy in fermentation and plant-based product measurements.

P42 Contamination and strain mutation resistant continuous fermentation at laboratory and pilot scale - WITHDRAWN

O. Wang[*], Pow Bio, Inc, Berkeley, CA, USA

While biomanufacturing may be one of the most technologically complex industries, the underlying production process, (fed)batch fermentation, has not changed in decades. Currently, most industrial-scale bioreactors are operated as (fed)batch reactors. Replacing the (fed)batch with a continuous fermentation system would drastically reduce Capex/Opex, increase volumetric productivity, and reduce production costs. Reduced production cost is essential for the economic viability of SynBio based products, as the production cost is a big impediment to commercialization. Here, we present a two-chamber, high-cell-density continuous fermentation process for the production of a high-value organic acid in *Escherichia coli* under mesophilic conditions (37 ˚C, pH 7). This process decouples the growth and production phases by establishing a fast-growing genetically stable culture that can be triggered into the production phase, thus enabling robust continuous fermentation and limiting host genetic drift. We operated this continuous process for 500 hours and produced 992g of total product from a 2-liter bioreactor. The productivity stayed >2g/L/hr for 101 hours during the continuous mode, with peak productivity of 2.18g/L/hr. The productivity (g/L/hr) observed in the continuous system represents a 5-fold increase compared to the fed-batch process of the same strain, which was optimized and scaled to 9000L scale. This 2L process was then optimized and scaled to 30L, which resulted in a titer of 67g/L, a 4.1x increase compared to the 2L continuous runs. A techno-economic analysis was conducted to model the benefit of continuous vs. fed-batch. For identical production capability (10 Kilo Tons per Annum), the model predicted a 78% reduction in facility capital investment ($40M vs $187M) and a 51% reduction in the cost of goods. The transition from the traditional fed-batch fermentation processes to the continuous fermentation process could be a key to solving the scale-up and production economy challenges faced by many synbio-based companies.

P44 A modelling tool for gas fermentation in hollow fibre membrane bioreactors

M. Perdigão Elisiário[*], L. Puiman and A. Straathof, Prof., Delft University of Technology, Delft, Netherlands; C. Picioreanu, Prof., King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia; H. Noorman, Prof., DSM, Delft, Netherlands

P48 Challenges and opportunities for the use of inductive logic programming in fermentation technologies

S. Caño de las Heras, C. Loureiro da Costa Lira Gargalo, K. Gernaey and U. Krühne, Technical University of Denmark, Kgs. Lyngby, Denmark; M. Law, ILASP Limited, London, United Kingdom; A.M. Russo, Imperial College of London, London, United Kingdom*

Three pillars support the digitalization of biomanufacturing processes: i) data; ii) models; and iii) the connections and services established among them. However, data from fermentations has proven to be what it is called “extreme data”. This data has characteristics that make the current technological implementation fail. Commonly, fermentations collect data with the following characteristics: sparse, missing, insufficient, or containing extreme variations. In addition, fermentations provide multi-source data, for example where variables are measured concentrations (g/L), pH, and impeller speed (rpm). These characteristics complicate implementation of some of the conventional machine learning (ML) methods to fermentation data. However, other types of ML, such as Inductive Logic Programming (ILP), can form an excellent option to use the available data to "intelligently” model and optimize the process. ILP is a logic-based form of machine learning that supports learning interpretable rules in the context of existing background knowledge, which decreases the amount of data needed to learn. Although ILP could tackle the issue of “extreme data” in the fermentation operation, this approach has not yet been sufficiently explored. Therefore, this raises the following question: (i) can ILP be effective when applied to fermentation?; (ii) how can this method be employed?; and, (iii) how can the digitalization and operation of fermentations benefit from it?

This work aims to answer those questions, and it presents opportunities and challenges that ILP faces when integrated into fermentation operations. Furthermore, to answer the last two questions, a case study is presented for the aerobic growth of *Corynebacterium glutamicum*, using a mechanistic model as background information. By applying the ILP method, it was observed that operational requirements can be inferred from a reduced number of examples and with low computation time. Hence, our case study demonstrates that ILP can be a beneficial strategy for digitalization of fermentation operations.

P50 *Saccharomyces cerevisiae* fed-batch fermentation scale-down case study: a practical approach to establish AMBR250 model from manufacturing-relevant process

Z. Liu, A. Innes and M. Woodling, Merck, West Point, PA, USA*

Process scale-down is a practical approach to identify optimal processes with increased throughput compared to experimentation at larger scale. In this study, we report a successful scale-down in the AMBR250 platform for a *Saccharomyces cerevisiae* fed-batch fermentation process to produce recombinant protein as drug substance material. A gas-transfer-guided scale-down approach was prioritized, assessing oxygen k_La in the current benchmark lab scale process and the AMBR250 system. Beyond gas-transfer approaches, the scale down model was further optimized to account for medium preparation sensitivity, bulk mixing and foam-out challenges not experienced at larger scales. Ultimately, the Ambr250 process demonstrates similar key metabolites and off-gas profiles as the larger scale operations and showed productivities within the manufacturing range.

This ‘scale-down model increases upstream process throughput to support tasks with wider design space, such as clone evaluation and screening as well as process parameter ranging. Current work focusing on investigating low pressure’s impact on process performance is ongoing, and further work is also planned to demonstrate the scale down models’ ability to predict commercial scale performance, enabling the ability of the scale down model data to be used to support commercial troubleshooting.

P52 *Towards a self-sufficient fermentation-based educational platform inspired by digital twins: a co-participatory design experience with conference participants as co-designers*

C. Loureiro da Costa Lira Gargalo, F. Caccavale, S. Caño de las Heras, K.V. Gernaey and U. Krühne, Technical University of Denmark, Kgs. Lyngby, Denmark*

Digitalization is the future of the bioprocess industries, which translates into embodying the concept of Digital Twins (DT). Therefore, engineering education must keep pace with the industry's developments. Furthermore, noteworthy is that the integration of online platforms in (bio)chemical engineering has progressively become part of the teaching/learning tools at all levels of education. Traditionally, such tools are designed by experts, meaning that the final users are not involved in co-designing the tool. However, if given the opportunity, we are convinced they provide valuable feedback regarding tool improvement when the virtual lab goals are clear.

In this work, we present the first stages of a DT-based education platform, BioVL, focusing primarily on teaching fermentation processes and modelling. Our vision is to turn BioVL into a self-sufficient fermentation-oriented educational platform valuable for all education levels, academics, and industrial partners. Considering this, we present a co-participatory design experience using the RAFT conference attendees as co-designers. This will give us valuable feedback from a group of professionals we cannot reach under normal circumstances. Qualitative and quantitative data will be collected, bringing several insights to ultimately help us boost the learning and engagement of the users. We aim to understand which features are more relevant for the learners, how to best embed them in the platform, and possibly come across other features that will make BioVL relevant at the academic and industrial levels. Some of the features that we demonstrate and respectively collect qualitative and quantitative data on are: (i) a

theory tab, (ii) a fully integrated database, (iii) teaching different modeling types (mechanistic, data-driven, and hybrid), (iv) chatbot, and, (v) virtual and augmented reality. Finally, we will also collect suggestions provided by the attendees in open conversation. After analysis, the features will be deployed into the software and re-tested for feasibility, usefulness, and user-friendliness.

6:00 PM - 8:00 PM Reception | Poster Session 2 | Exhibits Open Sponsored by: BioP2P Network

Tuesday, November 8

8:30 AM - 11:30 AM Natural product biosynthesis

Conveners: Nigel Mouncey, Joint Genome Institute, Berkeley, CA, USA and **Esha Khullar**, Cargill, USA

8:30 AM S12: Mining of fungal genomes for novel bioactive natural products

Y. Tang[*]*, University of California Los Angeles, Los Angeles, CA, USA and C. Harvey, Hexagon Bio, Menlo Park, CA, USA*
Fungal natural products are important sources of pharmaceuticals, including lovastatin, cyclosporine and echinocandin. Genome sequencing has revealed significantly untapped biosynthetic potential among fungi. Here, we present the efforts at UCLA, as well as in the company Hexagon Biosciences, to build platforms that combine genome sequencing, data science, and synthetic biology to enable targeted mining of fungal genomes for the discovery of new therapeutics for a variety of indications. Novel bioinformatic approaches for targeted mining of biosynthetic gene clusters from genomic data will be discussed along with a suite of synthetic biology tools available for the activation of the selected clusters.

8:55 AM S13: The Actinobacterial Strain Collection and Genome Database at NPDC

B. Shen[*]*, UF Scripps Biomedical Research, Jupiter, FL, USA*
The Actinobacterial Strain Collection and Genome Database at the Natural Products Discovery Center (NPDC) is a community resource, consisting of a physical resource (i.e., the actinobacterial strains) and a computational resource (i.e., the sequenced genomes) (https://npdc.rc.ufl.edu). It provides users an interactive interface to access at one site: (i) the Actinobacterial Strain Collection for a comprehensive analysis of the geographical, temporal, and taxonomic diversity of all the strains in the collection, (ii) the Actinobacterial Genome Database for the Microbial Minimal Draft genomes curated with each of the strains in the collection that are cross-searchable, (iii) the Enabling Bioinformatics and Computational Tools for the discovery and analysis of biosynthetic gene cluster (BGCs) and mapping of natural product biosynthetic landscape, and (iv) User Community for download of the curated genomes, request of strains, and support and improvement of accessibility and utility of the strain collection and genome database. The Actinobacterial Strain Collection and Genome Database at NPDC therefore strives to serve the broad scientific community by providing both the strains and their curated genomics information to launch new research initiatives and promote natural products training, research, and associated applications.

9:20 AM S14: New Tools For Targeted Cloning And Over Expression Of Biosynthetic Gene Clusters

D. Mead[*]*, Terra Bioworks, Middleton, WI, USA*
Over 130+ biosynthetic gene clusters (BGCs) ranging from 12 to 150 kb from over 100 diverse bacterial and fungal strains were successfully captured and cloned using CRISPR-based enzymes to precisely excise pathways of interest. To improve BGC heterologous expression, we developed a new Actinobacteria BGC expression vector (pDualP) and a companion vector for *Bacillus* expression that uniquely includes two inducible promoter elements that flank the cloning site. BGCs cloned and conjugally transferred to Actinobacteria or *Bacillus* heterologous hosts include ACT, RED, nystatin, erythromycin, vancomycin, difficidin, bacillusin A, and many dozens of novel clusters. We de-orphaned the stravidin BGC from *Streptomyces* sp. NRRL S-98 in two months using the same inducible approach. Second, we observed a substantial enhancement of the antimicrobial activity of heterologously-expressed, soil-derived metagenomic BGCs through induction with pDualP and demonstrate the ability to reconstitute complete BGCs from fragments of metagenomic libraries. Finally, we applied these directed BGC cloning tools to known and novel fungal BGCs for successful heterologous expression in *Aspergillus*, including imizoquin and pestalamide. These results indicate that sequenced BGCs can be cloned intact from complex (meta)genomes, and that direct cloning into a dual-inducible expression vector can greatly accelerate downstream small molecule expression and characterization.

9:45 AM Coffee Break:Coffee Break - Join the Placement Committee for Coffee and Conversations during all coffee breaks!

10:15 AM S15: Leveraging omics tools for studying biosynthesis to industrialize natural product discovery

K. Clevenger, PhD, Corteva Agriscience, Indianapolis, IN, USA*
Corteva Agriscience™, the largest pure-play agricultural biotech in the world, is leveraging advances in genomics and metabolomics to reinvigorate industrial Natural Products discovery. Over the last twenty years most major multinational corporations have abandoned Natural Products discovery due to a failure to identify novel bioactive scaffolds. Yet in that same time Corteva successfully advanced multiple Natural Product and Natural Product-inspired agrichemicals to market. The most recent examples of UK2A-based fungicides Inatreq™ and Adavelt™ demonstrate the enormous value proposition of Natural Products for industrial biotechnology at large. However, solutions to access novel scaffolds and avoid rediscovery are necessary for continued growth. To address this need, Corteva is deploying high-throughput integrated genomics and metabolomics to analyze biosynthesis and discover novel Natural Products to deliver sustainable agrichemicals and ensure the global food supply amid a changing climate and growing population.

10:40 AM S16: Biomanufacturing of Biorationals: A More Sustainable Alternative to Conventional Chemicals in Agriculture

K. Kneller, A. Dave, M. Bosserman and G. Venburg, Valent Biosciences, Libertyville, IL, USA; S. Park, Valent Biosciences, Osage, IA, USA*
Valent Biosciences has a rich history in the discovery, development, and commercialization of natural products that we call biorationals. Our portfolio of products begins with the launch of the plant growth regulator ProGibb® sixty years ago and has grown to encompass a full suite of plant growth regulators, biological insecticides, nematicides and fungicides and most recently biostimulants. This diverse portfolio of bioactives is cultivated via biomanufacturing of primarily wild type bacterial and fungal microorganisms. These organisms have been commercialized following iterative cycles of process development and improvement to ensure the strains are stable, robust, and capable of amplified biosynthesis of target actives with economic viability in the market. Going forward, greater emphasis will be directed towards mutagenized or engineered microorganisms.

11:05 AM S17: Sweet Sustainability: commercialization of fermentation-derived steviol glycoside sweeteners

H. Speirs, Cargill, Incorporated, Minneapolis, MN, USA*
The demand for nature-sourced high intensity sweeteners to reduce calories in food and beverage continuously increases as consumers avoid added sugars in their daily life. The glycoside rebaudioside M (Reb M) is a sweet-tasting, non-caloric compound that is found in very low abundance in the *Stevia rebaudiana* plant. Utilizing a fermentation process that includes an engineered yeast host allows for production from renewable sources, decreases water and land usage, and provides a better life cycle analysis score than commercial-scale production from Stevia leaves.

DSM and Cargill combined their expertise to form the joint venture Avansya. This JV commercialized fermentation-derived Reb M, starting up full-scale manufacture in Blair, NE USA in 2019. Industry partnerships allow for streamlined efforts to generate and screen improved strains, validate fermentation process parameters and inputs and capitalize on learning from established operations. Fermentation process optimization efforts are aided by high throughput automation and replication to understand key process metrics.

This presentation will describe the fermentation development and scaleup, as well as challenges and mitigation strategies for production of EverSweet™, a stevia sweetener.

12:15 PM - 12:45 PM Exhibitor Tuesday Showcase: Aber Instruments, Global BioIngredients, Inc & InProcess Instruments NAÂ

1:00 PM - 4:00 PM Alternative systems to animal cell culture

Conveners: Shushil Machhi, AstraZeneca and **Firehiwot Tachea**, Culture Biosciences, South San Francisco, CA, USA

1:00 PM S18: Rapid, High Yield “C1” Eukaryotic Gene Expression Platform to Rival CHO; Faster, Higher Yield, Lower Cost Way to Develop & Manufacture Biologics

M. Emalfarb, N. Valbuena and R. Tchelet, Dyadic International Inc., Jupiter, FL, USA*
Dyadic will present data and information regarding its C1 hyper productive protein production platform which is based on the thermophilic fungus *Thermothelomyces heterothallica*.

The C1 cells along with the genetic elements used to engineer these cells have been further improved to speed development, increase yields, and improve stability and purity of secreted recombinant protein antigens, monoclonal antibodies, bi/tri specific antibodies, FC fusion proteins as well as other classes and types of therapeutic proteins which can be manufactured using

standard stainless-steel or single use microbial bioreactors at flexible commercial scales.

Based on a number of animal studies in non-human primates, cattle, lambs, rabbits, hamsters, and mice, including a toxicology study for a SARS-CoV-2 C1 cGMP manufactured vaccine (DYAI-100) and a GRAS notification by the FDA that was granted to Dyadic on September 29, 2009, for the production of enzymes for food and feed applications, the data demonstrates that C1 is a safe production host for use in producing proteins for human clinical trials.

For Multiple Classes of Recombinant Proteins and Nanoparticle Vaccines – The C1 cell line has already been used to produce several recombinant protein-based vaccines. Stable cell lines can be developed in very short time at high yields, efficacy, and safety such as the RBD against SARS-CoV-2, HA and NA against influenza and other animal vaccines such as SBV, RVFV and a well-known avian virus. In addition, C1 cells can also express certain secreted VLP's that form the correct particle structures. C1 cells have also been used to express ferritin nanoparticle vaccines for COVID-19.

For Therapeutic Proteins – C1 cells have demonstrated major benefits over CHO cell lines in terms of high productivity (e.g., mAb productivity of 24 g/L in 7 days), low production cost, shorter time to develop stable cell lines and the ability to produce "difficult to express proteins" such as bi/tri specific antibodies, FC fusion and other therapeutic proteins stably and at high levels. Since the C1 filamentous fungi has Eukarya post-translational modification, the C1 produced proteins have certain inherent CHO like structure. In addition, C1 cells have been glycoengineered to produce N glycosylated proteins with human like glycoprotein structures such as G2/G1 and GO.

1:25 PM S19: Enabling Robust Cell-free Synthesis of Conjugate Vaccines for Decentralized Biomanufacturing

K. Warfel[*] and M. Jewett, Northwestern University, Evanston, IL, USA

E. coli cell-free expression (CFE) systems have been widely adopted in recent years for prototyping and biomanufacturing due to their ease-of-use and control over reaction conditions. By supplementing resources for transcription and translation to a soluble cell extract, cell-free systems can be used to produce proteins directly *in vitro* from DNA templates. Cell-free expression systems provide a modular, flexible, and scalable biomanufacturing platform without the viability constraints imposed by living cells. Additionally, CFE reactions can be easily lyophilized and reconstituted, making them a versatile platform for production of diagnostics and therapeutics at the point of care. However, increased robustness such as low reaction cost and high thermostability are necessary for further technology adoption and implementation as a decentralized biomanufacturing strategy. In this talk, I will highlight our recently developed platform for cell-free glycoconjugate vaccine production. Specifically, I will describe my efforts to improve glycoprotein yields, lower cost, and increase thermostability of the cell-free vaccine synthesis platform. Overall, this work contributes to the development of accessible biomanufacturing strategies.

1:50 PM S20: *Numaswitch^{TM} – the first viable alternative for chemical synthesis of peptides and pepteins*

M. Prescher[*], Numaferm GmbH, Duesseldorf, Germany

Pepteins (PEPTides and small protEINS) are 10 – 500 aa long, non-structured, complex, and highly active ingredients for pharma and non-pharma applications. So far, chemical synthesis supplies the demands, however, this approach comes with various limitations, such as insufficient purity levels (< 95%), target-related impurities and low titers, resulting in high manufacturing costs. Numaferm has established a new biotechnological platform approach, utilizing inclusion body fermentation in *E. coli*, meeting technical and commercial needs in peptein manufacturing.

Non-modified pepteins are usually difficult to express in *E. coli*. They tend to degrade, resulting in low expression titers. Making use of inclusion bodies in this case is familiar, however, until now, chaperones and chemical folding helpers were needed to make this precious material accessible. We have developed a technology that systematically harnesses inclusion body fermentation. Even better, Numaswitch^{TM} is able to produce challenging pepteins which need proper folding, e.g. containing up to six disulfide bridges.

At RAFT 2022, we will highlight the application of Numaswitch^{TM} for pepteins containing non-natural modifications, i.e. N-term. acetylation, C-term. amidation, side-chain acylation and other non-natural amino acids.
Numaswitch^{TM} is the first viable alternative to produce pepteins containing natural and various non-natural amino acids at scale, purity, cost, in time and in a sustainable way.

2:15 PM Coffee Break- Join the Placement Committee for Coffee and Conversations during all coffee breaks!

2:45 PM S21: Production of biologics in spirulina for low-cost, large-scale production

C. Behnke[*], B. Jester, J. Ferrara, H. Zhao, M. Heinnickel, M. Gewe, T. Adame and J. Roberts, Lumen Bioscience, Seattle, WA, USA

Spirulina (*Arthrospira platensis*) is a photosynthetic microbe farmed globally for use as a food and in the production of natural food coloring with annual production exceeding 10,000 tons/year and with very low costs of production, on the order of $10/kg dry matter. Spirulina as an organism is a specialist in expressing and accumulating soluble proteins in its cytoplasm, with wild type spirulina able to accumulate a single soluble protein (c-phycocyanin) to very high levels—approximately 10% of the cell dry weight. Further, spirulina has an excellent safety profile and has been evaluated by the United States Pharmacopeial Convention and assigned a Class A safety rating. Despite this low cost of production, ability to accumulate soluble proteins at high levels, and excellent safety profile, spirulina has not been useable as an expression host for biologic therapeutics due to challenges in genetically engineering the organism and in growth of the organism under suitable conditions for pharmaceutical manufacturing.

Lumen Bioscience has developed a robust, reliable technology for transforming spirulina and driving cytoplasmic accumulation of therapeutic proteins including single chain antibodies and enzymes, up to 15% of dry weight. In parallel, Lumen has developed a controlled manufacturing system to grow and process this photoautotrophic microbe under cGMP conditions. Because spirulina is already well accepted as a food material, the organism expressing and accumulating the therapeutic proteins can be used directly as an orally delivered drug, and with minimal processing can be utilized for other non-parenteral delivery modes. We will describe how transgenic spirulina strains are engineered, grown, and processed for use as therapeutics, and how the economics of cGMP spirulina production compare to those of traditionally produced biologic therapeutics.

3:10 PM S22: Corynex®: Suitable protein expression system for antibody mimetics using *Corynebacterium glutamicum*

H. Nagano, AJINOMOTO Co., Inc., Kawasaki, Japan*

Corynebacterium glutamicum discovered as a natural producer of glutamate is a gram-positive, non-sporulating, and non-pathogenic bacterium. For several decades, this strain has been heavily used in industrial production of amino acids, which are used in human food additive, animal feed additive, and pharmaceutical products. We have developed a unique recombinant protein secretion system by using *C. glutamicum* and protein expression service Corynex®.

This system has some advantages compared with other protein expression systems. First, *C. glutamicum* hardly secrete host cell proteins of itself into the culture supernatant. Therefore, the target protein can be secreted with high purity. Second advantage is about correct folding of the secreted protein. A protein which has complex structure such as including disulfide bonds, homo-dimer, and hetero-dimer can be secreted as active form. Third advantage is endotoxin-free. *C. glutamicum* is a gram-positive bacterium, which does not produce endotoxin natively. So, it is not needed to care about endotoxin clearance during manufacturing process.

To expand the versatility of this system, we developed the CspB-fusion method to increase the secretion titer, and identified the Tat-pathway, which is a novel protein secretion pathway that differs from general protein-secretion pathway well known as Sec-pathway. Furthermore, we have developed efficient breeding technologies, such as signal peptide library and host strain library, to improve the productivity.

Now we are applying this system to produce biopharmaceuticals. So far, several proteins produced by this system have already been tested in clinical trials in US and EU. Recently, there are focused on antibody mimetic proteins as well as conventional major antibody therapeutics in the biopharma field, so we have tried the strain development to produce these proteins. As a result, various kinds of proteins could be successfully secreted, so it was demonstrated that Corynex® is very suitable system to produce antibody mimetics and fragment antibodies.

3:35 PM : Nano-sized solution for a massive challenge: NanoAbs as a platform for COVID-19, asthma, and psoriasis therapies

D. Fischer, BiondVax Pharmaceuticals LTD, JERUSALEM, Israel*

Since 1985 approximately 100 monoclonal antibody (mAb) therapies have received regulatory marketing approval, and additional mAb therapies are under development. Available mAbs are directed against many antigens for treating immunologic diseases, reversal of drug effects, and cancer therapy. Though mAbs are known for their efficient, targeted treatment, their production process is convoluted, with long duration and high costs, which is reflected in high treatment prices. Moreover, proteins are sensitive to shear forces and heat, hence requiring a stringent cold chain. We at BiondVax Pharmaceutical Ltd (Israel), in collaboration with the Max Planck Institute for Multidisciplinary Sciences (MPG), and the University Medical Center Göttingen (UMG), harnessed the targeted treatment qualities of mAbs with an easy-to-produce and operated yeast-based expression system to manufacture alpaca-derived recombinant nano-sized VHH-antibodies (NanoAbs). NanoAbs' are human mAbs 'bio-betters' in-terms-of cost-effective production process, heat, and shear force stability, as well as their high target affinity (pM concentrations observed at *in-vitro* tests). VHH production employs a high-cell-density fermentation process using the well-established expression system of *Pichia pastoris (P.P)*. Their small size (15KDa) enables them to be secreted from the yeast, already folded to their active form. Constructed of a single low molecular weight heavy-chain, VHHs allowes a magnet-like binding to their target with high efficiency (vs. mAbs). Our lead therapies are inhaled NanoAbs against SARS-CoV2, administered directly to the target with high lung deposition efficiency.

Increased demand for improved COVID-19 therapies is anticipated as most experts believe that while Covid-19 will remain circulating for the foreseeable future, vaccine uptake will decline. The BiondVax-MPG-UMG collaboration's advantage lies in promptly reacting to emerging viral variants with high yield and cost-effective production. The highly efficient *P.P* expression system sets the foundation of NanoAbs platform products for additional illnesses such as asthma and psoriasis for which development has also been recently initiated.

4:15 PM - 5:15 PM Future faces of fermentation: round table

Conveners: Michelle Lewis[1];**Ehsan Mahdinia**[1];**Mark Berge**[2];**Kat Allikian**[3] and **James Dekloe**[4], (1)Stack Family Center for Biopharmaceutical Education & Training (CBET), Albany, NY, USA(2)AstraZeneca, Gaithersburg, MD, USA(3)South Pacific Sera(4)Director and Founder, Industrial Biotechnology Program, Solano Biotech, Fairfield, CA, USA

6:00 PM - 7:00 PM Banquet Reception | Exhibits Open

7:00 PM - 8:30 PM Banquet

8:30 PM - 9:30 PM Mixer

Wednesday, November 9

7:00 AM - 8:00 AM Breakfast - Registered attendees and exhibitors with meal plans

8:30 AM - 11:30 AM Alternative fermentation systems

Conveners: Jason Brown, Thermofisher, Logan, UT, USA and **Silas Villas-Boas**, Luxembourg Institute of Science and Technology, Belvaux, Luxembourg

8:30 AM S23: Microbial screening strategies for scalable expression of therapeutic proteins

E. Nordwald[*]*, R. Todd, J. Johnson, T. Telander and A. Pilling, KBI Biopharma, Boulder, CO, USA*

The development of therapeutic proteins requires first selecting an appropriate cell line. In creating a cell line, both the microbial host strain and plasmid DNA sequence can have profound effects on the titer, purity profile, and overall manufacturability of a recombinant therapeutic protein. Choice of *E. coli* host strain can optimize titer, improve plasmid DNA stability, support disulfide formation, and/or minimize product-related impurities or degradation. Simultaneously, various plasmid elements including promoters, RBS, origins, tags, fusions, codon optimization strategies, and protein mutants can also affect titer, construct stability, purity, and overall manufacturability of the protein. Combinatorial optimization of plasmids and host strains can create several logs worth of strains to assess. As the throughput of screening strains goes up, the representativeness and scalability tends to goes down. We have implemented the Biolector microfermentation system to screen cell lines under relevant and varying fermentation conditions. This plate-based parallel microfermentation system can simultaneously screen cell lines while examining various fermentation parameters including media, feed-rates, pH profiles, and induction strategies. Integrating cell-line selection with fermentation development has streamlined subsequent development activities and even allowed direct scaling from the microfermenter to 10L stainless-steel or 30L single-use systems, in some cases. As a result of the integrated fermentation and cell line development, we are consistently exceeding 4 g/L titers after scale-up. In one instance, we identified a cell line and fermentation strategy that improved upon the classical T7 (DE3)-system by more than ten-fold. Looking forward, we aim to increase screening ahead of the Biolector and develop a deeper library of host strains and plasmids to enable higher titers, better product quality, and microbial expression of more molecule types.

8:55 AM S24: Implementation of single use bioreactors in cultivating strict anaerobic bacteria

A. Cowley, PhD MBA[*]*, Arranta Bio, Watertown, MA, USA*

The Live Biotherapeutic Products (LBPs) sector is emerging rapidly into a new frontier for preventing, treating and curing diseased conditions. To manufacture these diverse set of products utilizing multiple strains of anaerobic organisms in the same production space and reactors, it is essential to use single-use systems from inoculation through final product encapsulation. This is because anaerobic strains are typically challenging to cultivate, have the potential to sporulate, can carry prophage genes, and make it particularly challenging to demonstrate the sterility of shared bioprocessing surfaces between cultures.

In collaboration with Thermo Scientific, Arrant Bio has been able to successfully adopt and implement Single-Use Bioreactors (SUBs), anaerobic organisms, and Single-Use Fermenters (SUFs), aerobic organisms, at our facility in Watertown MA. To date, we have successfully scaled and manufactured a large dataset of natural and genetically modified organisms at volumes up to 250L. More recently, we cultured a strict anaerobic bacterial strain in the 5,000L Thermo Scientific™ DynaDrive™ (SUB) system, demonstrating the ability of the system to maintain adequate anaerobicity to successfully cultivate these organisms at much larger scale.

Arranta Bio (Contract Development and Manufacturing Organization, CDMO) offers the innovation, knowledge and resources necessary to work together to develop and manufacture promising new microbiome therapies to meet the needs of patients around the world. Within this talk, we will discuss how implementing single-use systems has been vital to making our mission a reality.

9:20 AM S25: Scale-up by scaling down: simulating industrial-scale microbial performance on the bench

J. Bromley, LanzaTech, Skokie, IL, USA*

Bioprocess technologies enabling the conversion of abundant, low value, carbon-rich waste streams are important to accelerate the transition to a sustainable circular carbon economy. The scales required to biologically produce commodity fuels and chemicals are an order of magnitude above current bioreactor technologies, with industrial plants requiring thousands of cubic meters of total bioreaction volume. However, bioreactors of this size have long mixing times and substantial environmental gradients, affecting biological characteristics. With gas fermentation as an example technology, we present an industry perspective on the importance of bioprocess optimization and piloting, and report on the development of bench scale bioreactor systems designed to mimic the spatial-environmental heterogeneities of commercial scale bioreactors.

9:45 AM Coffee Break- Join the Placement Committee for Coffee and Conversations during all coffee breaks!

10:15 AM S26: A mesophilic biological methanation process to upgrade anaerobic digestion biogas

S. Lemaigre, X. Goux, M. Calusinska and J. Roussel, Luxembourg Institute of Science and Technology, Belval, Luxembourg*

Anaerobic digestion of organic wastes has become a key sustainable technology to replace natural gas by green gas alternative. However, the biogas produced is made up of CH_4, (50-65%) and CO_2 (35-50%). Currently, biomethane plants use physico-chemical technologies to upgrade the biogas to gas grid quality (CH_4> 95%). The proposed work aims to convert the CO_2 present in the biogas into CH_4 using hydrogenotrophic methanogens in biological methanation reactor under mesophilic conditions and low hydrogen pressure (1.5 bars).

The experiment was conducted over a 42 day period with two pairs of reactors (each pair contained 1 anaerobic digester coupled with 1 methanation reactor). One reactor pair (R2-R3) was supplied with hydrogen (H_2) through one of our novel membrane-based gas injection modules whilst the second pair, the control set (R0-R1), went without. The biogas from the anaerobic digester was pumped and circulated to the methanation reactor using a fishstone-type gas sparger placed in the bottom of the reactor.

Results show that the H_2 fed reactor (R3) efficiently converted the CO_2 to CH_4 and reached methane concentrations over 95%. In comparison, the average methane concentration in the control set (R0/R1) reached only 55%. Moreover, the total methane production by R2/R3 reactors was 1.7 - 2.3 times higher than in the control pair (R0/R1). The method of hydrogen injection in the reactor plays a crucial role in the rate of conversion. The use of the membrane-based module allowed the full conversion of the hydrogen thanks to a slow permeation. This resulted in lower than 0.2% residual hydrogen compared to 1.5% when the hydrogen was directly injected by gas sparging.

In conclusion, the experiment demonstrated that our robust biological methanation process can upgrade the biogas to biomethane (gas grid quality) while doubling the total methane production.

10:40 AM S27: Microbial production of methyl ketones in the novel bioreactor multiphase-loop reactor MLPR - integrated in situ extraction for simple product recovery

C. Grütering, T. Tiso, M. Neumann, C. Honecker, A. Jupke, S. Pischinger and L.M. Blank, RWTH Aachen University, Aachen, Germany*

Aliphatic, medium chain length methyl ketones (MKs) are naturally occurring, highly reduced platform chemicals. Favorable cetane numbers allow their usage as advanced biofuel blends for diesel engines. Microbial production of MKs was achieved in *Pseudomonas taiwanensis* VLB120. The product yield of 0.17 $g_{MK} \cdot g_{glucose}^{-1}$ is the highest reported yield using microorganisms. The bioprocess for MK production involves the addition of an organic solvent as a second liquid phase for *in situ* product extraction to simplify product purification. However, the formation of stable emulsions leads to product losses in conventional

stirred-tank bioreactors, hindering a truly efficient bioprocess.

In this context, this work combines two innovative approaches that enable cost-efficient and scalable MK production.

First, a reductive solvent screening was performed. The comprehensive procedure revealed a product congener, the C_{11} MK, to be a solvent candidate with superior properties with respect to decisive aspects such as product partition and biocompatibility.

The *in situ* extraction-based bioprocess was transferred from stirred-tank bioreactors to an innovative multiphase loop reactor (MPLR) where insufficient solvent recovery can be circumvented. Here, sparging ensures gas supply and power input in the inner compartment (riser). Countercurrent liquid-liquid product extraction takes place in the outer compartment (downcomer). In this new bioreactor setup, comparable yields to stirred-tank bioreactors can be realized without loss of the organic phase. Furthermore, by using the product congener as a solvent, the organic phase can be used, e.g., as an advanced biofuel blend without further costly purification steps such as distillation. The combination of these two innovations thus enabled the development of an efficient bioprocess for MK production. The produced MK mixture was successfully applied in combustion experiments, demonstrating its usability as a drop-in fuel for diesel engines.

11:05 AM S28: Performance of an enhanced single-use fermentor demonstrating improved cooling and oxygen mass transfer

M. Wight[*] and J. Brown, Thermo Fisher Scientific, Logan, UT, USA

The Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) has proven to a be a powerful process development and manufacturing platform for drug and enzyme production for biotechnology. HyPerforma™ single-use products reduce downtime and waste volume, as deep cleaning is not necessary, in contrast to stainless-steel vessels. As single-use technology continues to be accepted as a favorable option for fermentation, it is critical to continue product improvement to enable the achievement of higher cell mass or product titer in less space. As users of HyPerforma S.U.F.s continually push to increase production levels in their fermentors, we continue to increase our product capabilities to meet their needs. Here we share how we have further enhanced the HyPerforma S.U.F. to meet higher cooling requirements of exponentially growing, high oxygen–consuming cultures. We illustrate how the jacketed surface area was enlarged to increase cooling capacity of the support vessel. We discuss loading of Thermo Scientific™ BioProcess Containers (BPCs) to achieve optimal cooling performance. We show how we developed larger single-use parabolic turbine impellers with greater oxygen delivery capabilities and lower power consumption than equivalently scaled Rushton impellers on the original HyPerforma S.U.F. We demonstrate how these improvements deliver improved oxygen delivery with reduced oxygen consumption, comparable to the abilities of stainless-steel vessels.

11:30 AM - 12:00 PM 2023 RAFT 15 Planning Meeting

12:15 PM - 12:45 PM Exhibitor Wednesday Showcase: Keit Spectrometers, Getinge & Thermo Fisher ScientificÂ

1:00 PM - 4:00 PM Fermentation foods of today and tomorrow

Conveners: Helene Ver Eecke, Metropolitan State University of Denver, CO, USA and **David Welch**, Synthesis Capital

1:00 PM S29: Physical, Chemical, and Microbiological Properties of Date Fruit-Flavored Yogurt

A. Ayad, Ph. D.[*] and M. Ortiz de Erive, Ph. D., Postdoctoral Research Associate/ Center for Excellence in Post-Harvest Technologies (CEPHT), NC A&T State University, Kannapolis, NC, USA; D. Gad El-Rab, Ph. D., Research Scientist / National Research Center, Egypt, Cairo, NC, USA; G. Chen, Ph. D., Assistant Research Professor / Center for Excellence in Post-Harvest Technologies (CEPHT), NC A&T State University, Kannapolis, NC, USA; L. Williams, Ph. D., MBA, Professor/ Center for Excellence in Post-Harvest Technologies (CEPHT), NC A&T State University, Kannapolis, NC, USA

Drinkable flavors-yogurt and probiotics is currently in high demand. Yogurt is the most popular fermented milk products in the world with a numerous health benefits. Date fruits have a high nutritional value a good source of fiber and carbs. This study aim was to assess the microbiological, physicochemical, and rheological characteristics of the drinkable yogurt fortified with different concentrations (0.0, 0.4,0.8, 1.0, and 1.5 % (w/w)) of date fruit powder (DFP) for 15 days at 5-7°C. As a control sample, fresh milk was inoculated with (1.3 g/L, w/v %) starter cultures was used. Our results indicated that the pH values of the control and other DFP treatments (4.8, 4.86, 4.62, 4.55, and 4.58) were slightly decreased to (4.57, 4.66, 4.59, 4.54, and 4.56, respectively) during the storage time. Microbiological examination revealed that the initial culture counts (8.73±0.01, 8.70±0.02, 8.80±0.6, 8.83±0.6, and 8.34±0.01 Log CFU/mL) in fortified samples were significantly increased as DFP ratio increased and decreased (7.96±0.01,8.00±0.03, 7.31±0.01, 7.18±0.01, and 6.25±0.05 Log CFU/mL) as the storage period progressed compared to the control sample (without DFP). The rheological study displayed shear thinning behavior on the control and fortified drinkable yogurt samples. The samples fortified showed an increase in viscosity (96, 266, 346, and 141 Pa. s, respectively) compared to

the control 27 Pa. s. After 15 days of storage, the samples fortified with 0.4 and 1% of DFP showed the highest increase in viscosity 282 and 1162 Pa. s, respectively compared to the control 16.7 Pa.s. Our results showed that adding DFP significantly increased the redness parameter (a*) and decreased the lightness parameter (L*). The samples fortified with 1.5% of DFP showed the highest a* (1.31±0.16) and lowest L* (83.85±.0.09). Our results thus indicated that date fruit powder could enhance the functional, physicochemical, acidic, and survivability of the flavored drinkable yogurt.

1:25 PM S30: Evaluation of Torula yeast cultivation on sugar beet hydrolysis products to produce a high-protein product

K. Williams, PhD, J. Park, PhD and R. Ekmay, PhD, Arbiom Inc, Durham, NC, USA*

Microbial biomass represents an opportunity for sustainable global protein production. Torula yeast, in particular, can be produced from a variety of carbon sources including both C5 and C6 sugars; this allows it to leverage lignocellulosic substrate that are not fit for many applications. Sugar beet pulp (SBP) is a byproduct of beet sugar production and its potential as a substrate for single cell protein production has been previously evaluated (Athar et al., 2009). Herein, we report the production of a high-protein torula yeast cultivated on enzymatically hydrolyzed SBP.

Sugar beet pulp was hydrolyzed with a pectinase and cellulase, centrifuged, and the supernatant decanted. The composition of the resulting sugar-rich hydrolysate consisted of 6.5 g/L glucose, 3.0 g/L arabinose, 0.6 g/L galactose, 0.5 g/L mannose, 0.4 g/L xylose, and 0.3 g/L cellobiose. Torula yeast cultivation was evaluated in shake flasks using a minimal media with SBP hydrolysates as the only carbon source, as a blend with glucose, or with glucose as the only carbon source. Cultivations were normalized for total sugar content. The minimal media included ammonium sulfate as a nitrogen source and trace minerals and vitamins. A crude protein content of 54% was achieved under both conditions. This is a value that is significantly higher than has previously been reported in literature (44% CP by Athar et al., 2009). Yield was significantly greater with the blended stream compared with the single stream approach; however, yield was lower for both SBP cultivations compared with the glucose-only cultivation. The growth curve for the blended cultivation was distinctly biphasic, and likely indicated a transitory attempt by the organism to utilize arabinose and other carbon products. Results of these evaluations indicate that SBP may be utilized in the cultivation of high-protein torula yeast.

1:50 PM S31: Sustainable Production of two Essential Omega-3 Fatty Acids (EPA and DHA) using one Heterotrophic Algae Strain

D. Dong, R. Gladue and M. Johnson, DSM, Columbia, MD, USA*

DHA and EPA are two essential fatty acids that have significant health benefits to people from all ages, from brain and eye development for infant and children, to cardiovascular and brain health for adults. Consumers have been looking for non-fish, non-GMO and natural form of DHA and EPA for infant formula, fortified foods and dietary supplement. We will present how DSM collected the unique heterotrophic algae strain, improve the strain and the fermentation process for different food related applications. The scale-up challenges and how we overcame them will also be discussed.

2:15 PM Coffee Break- Join the Placement Committee for Coffee and Conversations during all coffee breaks!

2:45 PM S32: Mixed-culture metagenomics of the microbes making sour beer

R. Piraine and F. Leite, Universidade Federal de Pelotas, Pelotas, Brazil; M. Bochman, Indiana University, Bloomington, IN, USA*

Mixed microbial cultures create sour beers but many brewers do not know which microbes comprise their cultures. The objective of this work was to use deep sequencing to identify microorganisms in sour beers brewed by spontaneous and non-spontaneous methods. Twenty samples were received from brewers, which were processed for microbiome analysis by next generation sequencing. For bacteria, primers were used to amplify the V3-V4 region of the 16S rRNA gene; fungal DNA detection was performed using primers to amplify the entire internal transcribed spacer region. The sequencing results were then used for taxonomy assignment, sample composition, and diversity analyses, as well as nucleotide BLAST searching. We identified 60 genera and 140 species of bacteria, of which the most prevalent were *Lactobacillus acetotolerans*, *Pediococcus damnosus*, and *Ralstonia picketti/mannitolilytica*. In fungal identification, 19 genera and 26 species were found, among which the most common yeasts were *Brettanomyces bruxellensis* and *Saccharomyces cerevisiae*. In some cases, genetic material from more than 60 microorganisms was found in a single sample. In conclusion, we were able to determine the microbiomes of various mixed cultures used to produce beer, providing useful information to better understand the sour beer fermentation process and brewing techniques.

3:10 PM S32: Programing future food with Ginkgo

P. Manga, S. Srikrishnan, S. Srinivas, S. Jactel, S. Venkatraman, D. Burman, J. Ramchandani, Y. Li, K. Loving, P. Punt and P. Boyle, Ginkgo Bioworks, Boston, MA, USA*

To feed a rapidly growing population on a warming planet, society needs to develop innovative new technologies to grow and distribute food. The cattle industry, which includes both beef and dairy products, represents a $1 trillion global market opportunity and contributes to 9% of global greenhouse gas emissions. At Ginkgo, we are working towards a future where genetic engineering can help make foods of the future that are sustainable, healthier, delicious, and accessible to everyone. The most important thing to program this century isn't computers. It's DNA. Biology is the most advanced manufacturing technology on the planet, and we can program cells to make everything from food to materials to therapeutics. Among the various paths towards this end, we are building a cell programming platform to make biology easier to engineer with Pichia and Aspergillus driven protein production platforms. The Cell Development Kit (CDK) provides modular access to our extensive cell programming capabilities, expertise, and scale. With target protein or enzyme sequence as primary input, CDKs provide data on validated key metrics including process, rate, titer, and proteomics using the Ginkgo Design-Build-Test-Ferment and Learn cycle. Design: We generate multiple unique designs with high potential for expression. Build: We synthesize and assemble designs in a validated host
Test: We use high-throughput screening to evaluate strain performance at lab scale
Ferment: We validate the highest-performing strains in scalable fermentation mode.

The Cell Development Kit is the first step to de-risking future investments in protein production. CDK unlocks the potential of biology with a unique modular combination of our codebase, automation, software, computational capabilities, and experts in the field. Ginkgo's platform and collaborations with companies like Motif FoodWorks holds the potential to change the future of food by discovering and developing new meat, dairy, and plant-based proteins made by fermentation, not animal agriculture.

1:00 PM - 5:00 PM Exhibits Dismantle and Poster Removal