Recent Advances in Fermentation Technology (RAFT tm)

Saturday, October 28

7:30 AM - 8:30 AM Workshop Breakfast

Blue Heron AB, Lobby Level

7:30 AM - 8:30 AM Workshop Registration (no RAFTtm registration)

Great Egret, Lobby Level

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

Great Egret, Lobby Level

12:00 PM - 1:00 PM Workshop Lunch

Blue Heron AB, Lobby Level

4:00 PM - 7:00 PM Registration-Preregistrants only; onsite registration begins Sunday

Calusa Prefunction, Lobby Level

Sunday, October 29

9:00 AM - 2:00 PM Exhibits setup

Calusa Ballroom D-H, Lobby Level

9:00 AM - 5:00 PM Registration

Calusa Prefunction, Lobby Level

10:00 AM - 11:30 AM General Session: Welcome and Keynote "The dawn of sustainable bio-based industrial chemicals" Jason Crater, Genomatica

Calusa Ballroom ABC, Lobby Level

11:30 AM - 1:00 PM Lunch - All Registered Attendees

Calusa Terrace, Lobby Level

12:15 PM - 12:45 PM Exhibitor Showcases: 12:15 pm Sartorius-Stedim Biotech; 12:35 Aber Instruments

Calusa Ballroom ABC, Lobby Level

1:00 PM - 4:45 PM Session 1: Advances in microbial expression

Conveners: **Himanshu Dhamankar**, GreenLight Biosciences Inc., Medford, MA, USA and **Dr. Peter Becker**, Glycom, Hørsholm DK, Denmark

Calusa Ballroom ABC, Lobby Level

1:00 PM S6: Mechanistic understanding of the role of FkpA in improved soluble expression and folding of IgG4 half antibodies in *Escherichia coli*

E. Dong[*]*, Genentech, South San Francisco, CA, USA*

While producing recombinant antibody therapeutics in *E. coli* has its advantages over a mammalian expression system, it also comes with challenges associated with soluble expression and effective folding of these complex proteins. Developing a high titer *E. coli* process for producing IgG4-format half antibodies for bispecific antibody therapeutics required optimization of both molecular conditions, in the form of chain ratio modulation and chaperone overexpression, as well as process conditions, including temperature and agitation shifts prior to induction of recombinant expression. An overview of both optimization efforts will be presented, with a focus on the development of a chaperone overexpression strategy. To better understand the role of chaperone proteins in our process, we looked to the mammalian expression system to develop a hypothesis for how the *E. coli* chaperone FkpA could be acting as an analog to both the mammalian chaperone BiP and ppiase CypB involved in antibody folding in CHO cells.

1:25 PM Systematic genome engineering, HTP fermentation and machine learning as key drivers towards improved performance

C. Isaac[*]*, Zymergen, Emeryville, CA, USA*

Zymergen has developed a platform that uses automation and machine learning to design, engineer, and optimize microbes. We use this platform today for three distinct purposes: improve economics of partners' existing fermentation products, accelerate commercialization of partners' new fermentation products and

produce Zymergen's novel molecules to enable rapid materials science innovation. Our process involves an atheoretic search for improvements in the genome. Knowing which changes worked and identifying patterns to guide future changes is more important than gaining a mechanistic understanding regarding exactly why each change worked (or didn't). We rapidly generate and test in parallel, multiple hypotheses in an automated, high-throughput wet lab. Our computer systems allow rapid generation of many hypotheses and allow the use of machine learning to continually optimize our process and our input set of hypotheses. This high-throughput approach is fast, predictable, and reaches high performance.

1:50 PM S8: Enabling next generation genetic library screening by automated microbial fermentation

N. Janzen[*]*, J. Jarmer, M. Voigtmann, S. Abad and D. Reinisch, Boehringer-Ingelheim Regional Center Vienna GmbH & Co KG, Vienna, Austria; G. Striedner, University of Natural Resources and Life Sciences Vienna, Vienna, Austria*

In a competitive environment, selecting the right production strain, fermentation media and process parameters as soon as possible from the widest possible pool is crucial for successful bioprocess development.

Progressing developments in microbial expression systems exacerbate the already stressed timelines as a multitude of possible production strains arises with each new genetic element. As every strain still can be screened in various process conditions the number of experiments is multiplied. The development of one soluble target results in hundreds and thousands of feasible fermentations, exceeding the capabilities of the so far used 5L stirred-tank bioreactor systems.

Consequently to tap the benefits of expression system developments new process technologies are needed. To serve the need for scalable high-throughput early-stage microbial bioprocess development the multifermenter device is being established in the process science department of the Boehringer-Ingelheim Regional Center Vienna.

Combining up to 32 miniaturized stirred-tank bioreactors (2mag) with a laboratory robot (Tecan) enables automated millilitre scale microbial fed-batch fermentation and sampling. Full databank embedment ensures data integrity of process information. A complete automated process chain including recovery, downstream and analytics ensures timely follow-up processing.

This directly impacts timelines as the full potential of strain libraries in combination with media screening and early stage process development provides a novel, compact approach. And at the same time delivers comprehensive process knowledge at an early clinical trial phase. Strategies and first approaches to cope with costumer's expectations will be presented here.

2:15 PM S3: Applications of bacteriocins to control microbial strains in industrial process

R. Sodoyer[*]*, SYNGULON SA, Liege, Belgium*

Régis Sodoyer & Philippe Gabant. Syngulon SA Belgium

During the past decade, the domain of synthetic biology has drawn inspiration for engineers and microbiologists to design new industrially compliant microorganisms.

One of the major drivers was the insatiable need for large amounts of biotherapeutic molecules and also today an onward movement towards of production of bio-based chemical compounds. Molecular biology techniques have, in parallel, gained in sophistication and ease of use, leveraging the creativity associated to the definition of novel expression systems and more sophisticated genetic circuits. The industrial needs in terms of productivity, cost effectiveness and safety considerations became increasingly demanding. The consequence is the definition or strengthening of new and logical quality and safety constraints.

Most of these issues have been managed historically in the context of biopharmaceutic production constraints. However industrial microbiology for bioproduction of biobased products is different due the specific production context often associated with an open environment.

We propose alternatives technologies, based on the use of bacteriocins that can provide an unique and innovative genetic firewall to boost fermentation either in close or open environments. These natural effectors have recently been revisited for their potential applications and proven efficiency in bio-based production

https://syngulon.com/

US Patent 9,333,227

"Antibiotic-free selection in *E. coli*:…" Sodoyer R. SIMB Meeting (San Diego 2013)

Antibiotic-free selection in biotherapeutics: now and forever.. doi: 10.3390/pathogens4020157.

Antibiotic-free selection in E. coli: new considerations for optimal design and improved production. doi: 10.1186/1475-2859-9-65.

2:40 PM Break

3:05 PM S1: Strain engineering and process development for robust heterologous protein production using *Pichia pastoris*

S. Balatskaya, E. Colasante, C. Cox, M. Hoyt, R. Maples and S. Shankar, Impossible Foods, Redwood City, CA, USA*

In the last few decades, the methyltropic yeast *Pichia pastoris* has been used for production of several heterologous proteins of industrial relevance. Specifically, the methanol inducible promoter pAOX1 has the potential to lead to very high levels of protein expression. The most common technique for obtaining high expressing *P. pastoris* strains has been to screen for high copy number insertions of the heterologous genes. In this work, we describe novel strain engineering approaches that led to improved expression of an intracellular heme-bound protein, Leghemoglobin, from the pAOX1 promoter. These include manipulation of the endogenous heme biosynthetic pathway and overexpression of pAOX1 transcriptional activator, Mxr1. Furthermore, we describe a robust, fed-batch, high cell density fermentation process that can support production of Leghemoglobin at industrial scale.

3:30 PM S7: Innovative strategies and tools for reliable production of chemicals by engineered *Pichia pastoris* strains

A. Glieder and T. Vogl, Graz University of Technology, Graz, Austria; M. Geier, Austrian Centre of Industrial Biotechnology , ACIB GmbH, Graz, Austria*

Komagataella phaffii (Pichia pastoris) is one oft he most used microbial strains for protein expression. Recently more and more studies show the advantages of this host also for chemical production by metabolic engineering and synthetic biology. However mostly just proof of concept studies were performed in small lab scale experiments ignoring possible genetic instability of the engineered strains which makes them useless for long term cultivation and production processes in bioreactors. However, expressing biosynthetic pathways for example for ß-carotene production or derivatives thereof we have seen loss of parts of the expression cassettes due to the physiological stress caused by product accumulation. Most probably due to homologous recombination deletions of parts of the pathway led to non producing cells, overgrowing producers. Employing a new tool set of sequence diversified promoters and termnators and alternatively by polycistronic multigene expression using 2A linkers such negative effects could be avoided. Resulting in highly productive and stable engineered strains.

3:55 PM S2: Integration of process technology and synthetic biology development to build a carbon capturing gas fermentation platform

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

LanzaTech has developed a platform for commercial-scale production of fuels and chemicals from simple gas substrates: CO, CO_2 and H_2. These substrates exist in cheap, point sourced waste gas streams from industry, such as in steel making and ferroalloy processes. They can also be generated by gasification of waste biomass / municipal solid waste (syngas), or reformation of biogas.
Using classical strain selection, LanzaTech isolated a highly efficient platform strain of the acetogenic microbe, Clostridium autoethanogenum, which serves as a base industrial strain and model organism. In just a handful of years, the company has developed an advanced strain engineering platform based on this chassis. The comprehensive genetic toolbox includes bioinformatics tools and metabolic modelling, complemented by process reaction rate and mass transfer modelling, and validated by hundreds of continuous steady state fermentation runs. Production of over 40 different molecules has been demonstrated at lab scale, and the base process has been scaled-up all the way through pilot and demo scales, to commercial facilities currently under construction in China and Europe, totaling 100,000+ ton/year production capacity.

Multiple factors should be considered in directing and focusing research in strain development, including process integration with reactor design/arrangement, cell recycle/retention, product separation, and wastewater treatment technologies.

4:20 PM Poster Highlights

A. Mohagheghi, National Renewable Energy Laboratory, Golden, CO, USA*

Poster highlights - 4 Poster presenters

5:30 PM - 6:30 PM David Perlman Award Lecture "Stuart Stocks: half a career in biotechnology" Dr. Stuart Stocks, LEO Pharma A/S

Calusa Ballroom ABC, Lobby Level

6:30 PM - 8:30 PM Poster Session 1/Opening Reception

Calusa Ballroom ABC, Lobby Level

P1 Biofuel from lignocellulosic biomass: Identification of inhibitors affecting microbial fermentation performance

Y. Zhang, GLBRC, UW-Madison, Madison, WI, USA; D. Xie, J. Serate, E. Pohlmann, M. Young and R. Landick, University of Wisconsin-Madison, Madison, WI, USA; T.K. Sato, DOE Great Lakes Bioenergy Research Center, Madison, WI, USA*

To identify and overcome key barriers to sustainable conversion of lignocellulosic biomass to biofuels, we compared microbial fermentation performance in hydrolysates produced from various feedstocks, including corn stover, switchgrass, miscanthus, sorghum, and mixed prairie. These feedstocks were harvested from the same year, as well as some feedstocks from different years. We found that there are variations of chemical components and inhibitors in different feedstocks, as well as in the same feedstock harvested in different years. For example, compared to other feedstocks, highest levels of several lignocellulose-derived inhibitors (lignotoxins), were found in year 2014-corn stover hydrolysate, including coumaric acid, coumaroyl amide, ferulic acid, and feruloyl amide. Supplementation experiments indicate that high concentration of these lignotoxins hinders the microbial fermentation performance, especially for

xylose utilization. To further investigate the mechanism of lignotoxins affecting microbial fermentation, we generated synthetic hydrolysates (SynH) with or without lignotoxins, based on the chemical analysis of hydrolysate prepared from AFEX-pretreated corn stover (ACSH). Comparative multiomic fermentations with SynH were performed, and samples were collected for end product analysis, gene expression, and metabolomic and proteomic analyses. The multiomic data analysis will be presented, which will help us to identify bottlenecks for how lignotoxins affecting the conversion of biomass to biofuel.

P3 The production of the industrially relevant enzymes: 1. nitrile hydratase, 2. 1-hexene monooxygenase, and 3. amidase by the fermentation of *Rhodococcus rhodochrous DAP 96253*

K. Cannon, M. de la Croix, N. Amadasun, N. Wijewantha, G.E. Pierce and S.A. Crow Jr., Georgia State University, Atlanta, GA, USA; S. Belshazzar, University of Southern California, Los Angeles, CA, USA*

Rhodococcus rhodochrous is a ubiquitous, non-pathogenic soil-dwelling bacterium. *R. rhodochrous* strain DAP 96253 was originally isolated from soil in New Jersey and has shown active metabolism of nitriles, hydrocarbons, and amines. The wild-type organism has numerous potential applications such as: 1) The treatment of contaminated waste water by the cleavage of toxic nitriles and conversion into non-toxic acid salts 2) The delayed ripening of climacteric fruit by the interruption of plant signaling 3) The treatment of congenital disorders by the transamination of biological monomers. The 2-step pre-pilot scale fed-batch fermentation of this organism was conducted, using food grade materials to upregulate the expression of certain enzymes. The induced whole cell paste and purified enzymes have shown high activity at ambient temperature and 37°C.

P5 Dissolved oxygen-driven expression of recombinant protein during fermentation of *E. coli* Nissle

P. Reeder, N. Kotadia, M. Momin, C. Bergeron, E. Antipov and R. Schwartz, Synlogic, Cambridge, MA, USA*

The global anaerobic transcription regulator FNR is used to regulate protein expression cassettes under the control of the pFnrS promoter. Expression is controlled by the level of dissolved oxygen in the culture. Optimum control of dissolved oxygen enables concurrent biomass growth and protein expression. Fast, high cell density E. coli biomass increase is balanced against protein expression levels as a function of dissolved oxygen concentration in the culture. Data will be presented demonstrating the balance between biomass increase and protein expression based on dissolved oxygen for a live biotherapeutic product being developed at Synlogic to treat urea cycle disorders.

P7 Methanol-free protein production employing AOX1 promoter from *Komagataella phaffii* and activator plasmids

S. Ertl, bisy e. U., Hofstätten an der Raab, Austria; T. Vogl, J.E. Fischer, L. Sturmberger, C. Schmid and A. Glieder, Graz University of Technology, Graz, Austria*

The methylotrophic yeast *Komagataella phaffii* is a powerful host for large scale heterologous protein production. Like *Saccharomyces cerevisiae*, *K. phaffii* is particularly well-suited for fermentative growth due to its ability to grow to very high cell densities thus being able to produce recombinant proteins in the gram per litre range in secretory fashion. Typical expression strategies giving highest yields rely on the methanol inducible alcohol oxidase 1 promoter (P_{AOX1}). However, the use of toxic and flammable methanol may constitute a considerable safety risk in establishing industrial production processes. To make P_{AOX1} independent from methanol, we have designed activator plasmids that enable the conversion of already existing P_{AOX1} based production strains into methanol free systems. This is achieved by coexpressing transcription activators for P_{AOX1} under the control of a carbon source repressable promoter

from *K. phaffii* (e.g. P_{CAT1}) from a seperate plasmid. At low carbon source levels, the P_{AOX1} activator is expressed and acts on P_{AOX1} thus initiating target protein production without prior methanol induction.

P9 New *Pichia pastoris* platform strains for multi gene expression and antibiotic free selection

M. Gerstmann, bisy e.U., Hofstaetten/Raab, Austria and A. Glieder, Graz University of Technology, Graz, Austria*

Some applications on larger scale depend on available tools for the design and construction of production strains, which do not rely on antibiotic resistance markers. In addition the application of Zeocin as an antibiotic for strain construction is a significant cost factor in the lab. Auxotrophy markers on the other hand rely on the preparation of minimal media lacking individual amino acids which is more time consuming than the use of simple complex media. Recently we developed Pichia platform strains where the GUT1 gene (glycerol kinase 1) was knocked leading to strains which can not use glycerol as a carbon source. These strains grow like normal on glucose containing media and allow to use media with glycerol as a sole carbon source for selection of transformants. New vectors for transformation enable high transformation rates and in vivo recombination of vector and insert by Pichia transformation without prior cloning in vitro and also self cloning. A double deletion strain was generated by crossing with a HIS4 deletion strain to obtain a new platform strain which now allows to integrate at least two copies of the same or alternative genes for coexpression without the use of antibiotics for selection or later cultivation.

P11 Functional characterisation of methanol-free promoters for *Pichia pastoris*

D. Mächler, C. Braun, S. Weichart, V. Looser and K. Kovar, Zürich University of Applied Sciences ZHAW, Wädenswil, Switzerland; A.M. Hatzl and A. Glieder, Graz University of Technology, Graz, Austria*

The alcohol oxidase I promotor (AOX1) is frequently used to tightly control production of foreign proteins in *Pichia pastoris*. However, since induction of gene expression in *P. pastoris* under AOX1 requires methanol, which is highly inflammable and toxic, it is not desirable for industrial-scale manufacturing. Innovative replacements of methanol are being sought whilst still maintaining tight control of gene expression. Novel promoters (CAT and PDF) have been developed that can be switched on and off depending on whether glucose is available in limiting or excess concentrations in the culture supernatant. Using strains that secrete lipase B from *Candida antartica* (CalB), we compared these two promoters in a systematic manner to elucidate control at the transcript level. Strains with different promoters were compared under identical conditions in several fedbatch bioreactor cultures, with exponentially increasing feed rates of substrate (glucose) and, thus, different constant specific growth rates (μ) throughout the entire production phase. At μ of 0.075 h^{-1}, about six-fold higher maximum CalB-activities were achieved with a PDF-promotor strain, i.e. (30'926.4 ± 1'688.2) U L^{-1}, than with CAT. The $q_p(\mu)$-relationship (production kinetics) for strains with the CAT-promoter were bell-shaped with a maximum at 0.055 h^{-1}, whilst specific productivity (q_p) for the PDF-promoter strains increased linearly with μ. The PDF-promoter exhibits a similar product formation kinetics shape to the well-established GAP-promoter, but has an exceptionally high q_p maximum. Moreover, as confirmed by transcript analysis (RT-qPCR), both novel promoters can be controlled by excess of or limited glucose, which is not possible with GAP.

P13 Sheff-Coli CD Express: A chemically-defined, two component medium and feed system for the production of recombinant human serum albumin in *Escherichia coli* BL21 (DE3)

F. Inman, B. Wrage and J.F. Menton, Kerry, Beloit, WI, USA*

Sheff-Coli CD Express is a chemically-defined, two-component system consisting of a base medium and a complete feed supplement that targets high cell density and recombinant protein expression in

Escherichia coli BL21 (DE3). The base medium, Sheff-Coli CD, is an optimized proprietary blend of nutrients that will support growth rates up to 1.0 h^{-1} depending upon carbon source and cultivation parameters. Under semi-optimal batch conditions, *E. coli* BL21 (DE3) obtained a specific growth rate of 0.78 h^{-1} and reached a cellular density close to 40 OD_{600} (DCW ~ 32 g/L) within 8.5 hr using an initial carbohydrate concentration of 15 g/L. The feed component, Sheff-Coli CD Feed, is another proprietary blend of nutrients and carbon sources that is designed to support high productivity of recombinant proteins. When used with Sheff-Coli CD, Sheff-Coli CD Feed supported protein expression of recombinant human serum albumin (rHSA) when fed at a constant flow rate of 3.8 g/hr. IPTG induction of *E. coli* BL21 (DE3) resulted in a 6 hr period of increased productivity as verified by SDS-PAGE. After 24 hours post-induction, Sheff-Coli CD Feed give rise to rHSA concentrations of 700 mg per gram of dry cell weight. Sheff-Coli CD is compatible with many carbon sources; however, it's most attractive feature is its ability to be heat sterilized without effects on clarity, color and performance. Unlike many feeds that target increased biomass, Sheff-Coli CD Feed was explicitly tailored to control, maintain and balance the relationships between maintenance metabolism and production.

P15 CFD application as a tool for designing industrial fermenters

I. Rosinha Grundtvig, C. Bach, U. Krühne and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

Industrial fermenters are commonly characterized by non-uniform substrate, oxygen and microorganism concentration profiles due to poor mixing which subsequently results in low yield reaction systems. The impeller and the baffles are two critical components for promoting the mixing inside fermenters.
The main goal of this work is to present computational fluid dynamics (CFD) as a potential tool to model and improve the design and the performance of fermenters. This new design strategy includes a shape optimization routine which couples the computational fluid dynamics (CFD) code (Ansys CFX®) to Matlab®. Ansys CFX® will perform the discretization of the fermenter into finite elements and its fluid dynamics analysis. Matlab® implements the optimization routine by making changes to the geometry of the impeller or baffles. The performance of the system is then evaluated by a cost function which will measure the optimal dispersion of substrate, air and microorganism concentrations

This approach will give the opportunity to identify the optimal process conditions, and to collect detailed information regarding the flow characteristics which influence the mixing in the fermenters. Moreover, ultimately it should allow obtaining an improved reactor design and/or operating conditions before performing detailed experimental work.

P17 Simple shake flask cultivation of *Komagataella phaffii* with constant glycerol feed and online monitoring

J. Fischer and S. Schein, bisy e.U., Hofstätten/Raab, Austria; C. Schmid, A. Weninger and A. Glieder, Graz University of Technology, Graz, Austria*

Large scale methanol free protein production in yeast species like *Komagataella phaffii* is a very important application in the field of industrial biotechnology. Therefore, a good performing and characterized production strain for is an essential requirement. Small scale bioreactor cultivations up to five litres working are commonly used to evaluate a strain and its protein production behaviour prior to more expensive upscaling. Nevertheless, also small bioreactor cultivations in fed batch mode are laborious, time-consuming and rely on expensive equipment, especially if multiple strains need to be evaluated in parallel. To simplify initial cultivations of a new protein production strain we established a simple shake flask system for methanol free expression that simulates bioreactor conditions including a constant slow glycerol feed and online monitoring. Recombinant protein production is driven by the carbon source repressed promoters *PDC* from *K. phaffii*. Polymer discs, releasing a constant amount of glycerol assure a feed rate that keeps the promoters de-repressed and active while keeping biomass production low.

P19 Growth of lactic acid bacteria in traditional glass bioreactors versus single use bioreactors

M. Jensen, J. Kaya and J. Westman, Chr Hansen A/S, Copenhagen, Denmark*

Lactic acid bacteria (LAB) are a diverse group of microorganisms and hence their production also requires different bioreactor setups. Some species, such as oenococci, are slow growing, requiring long fermentation times that result in a greater susceptibility to contamination. Other species, such as bifidobacteria, are sensitive to oxygen, requiring anaerobic conditions for optimal growth, while yet others such as lactococci grow best while respiring.
In a process development laboratory, a fast turnover of bioreactors is a requirement for efficient upscaling of production processes. A way to enable this is through the use of disposable bioreactors made of plastic, rather than the traditional reusable, autoclavable, bioreactors made of glass. To investigate whether single use bioreactors could be used for different LAB, we compared fermentations in a DASbox® Mini Bioreactor System (Eppendorf AG) using single use BioBlu® (Eppendorf AG) and conventional bioreactors in parallel for four different species: *Streptococcus thermophilus*, *Oenococcus oeni*, *Bifidobacterium animalis* subsp. *lactis* and *Lactococcus lactis*, grown in both anaerobic and aerobic mode.

It was found that the single use bioreactors could indeed be used with indistinguishable results for most processes. However, a longer lag phase was observed for *Bifidobacterium* in the single use bioreactors. Also, the single use and re-usable bioreactors required different rotor motors that needed to be installed on the DASbox® limiting the flexibility.

In conclusion, single use fermenters have a great potential for screening of fermentation parameters for LAB. However, for strict anaerobes, care has to be taken to avoid inhibition by oxygen.

P21 Enhanced volatile fatty acid production by *Megasphaera elsdenii* via fed-batch, pertractive fermentation

R.S. Nelson, D.J. Peterson, E.M. Karp, G. Beckham and D. Salvachúa, National Renewable Energy Laboratory, Golden, CO, USA*

The “Carboxylate Platform” , the production of volatile fatty acids (VFAs, and specifically C2-C8) from biomass sugars via anaerobic fermentation either through mixed cultures or monoculture, has emerged as an attractive approach to generate high yields of promising biofuel and biochemical precursors. Acetic (AA) butyric (BA) and hexanoic (HA) acids, are produced by certain anaerobic microbes and can be catalytically upgraded to fuels and chemicals. The bacterium *Megasphaera elsdenii* represents a promising host for production of VFAs. However, due to the toxicity of these acids, *in situ* product removal is required to achieve high titers and productivities. Here, we examine multiple aspects of extractive separations to produce AA, BA and HA from glucose and lignocellulosic hydrolysate with *M. elsdenii*. After extractant composition and fermentation conditions were established, a fed-batch, pertractive fermentation was run for over 230 hours, producing over 57 g/L of purified VFAs from glucose. In addition, batch fermentations of lignocellulosic sugars were performed, without pH control, to produce and extract up to 17 g/L of AA, BA, and HA. These results indicate that *M. elsdenii* might be a potential host in the Carboxylate Platform.

P23 Production of an Escherichia coli culture with high bactericidal activity by fermentor

J.H. Chen, National Chung Hsing University, Taichung, Taiwan*

A bacterial toxin gene was cloned in to one of the two high-copy plasmids and transformed into Escherichia coli, each with $(His)_6$ tag at the C terminus of the recombinant toxin. One plasmid carries a T5 promoter and the promoter can be induced by IPTG, whereas the other plasmid carries an SOS promoter and the promoter can be induced by mytomycin C. The bacteria carrying the former plasmid had 4-fold lower bactericidal activity than those carrying the later plasmid, which was used for purification of the

recombinant toxin by Ni column-based affinity chromatography to near 95% homogeniety, as demonstrated by SDS-PAGE and Western hybridization. A standard curve of bactericidal activity vs. protein concentration was generated with r^2 equal to 0.9941. By varying the induction time, size of the culture flask, and volumn of the medium in the flask, the condition of 100 ml LB in 300 ml flask and induction time of 6 hrs was obtained, in which the best bacterial yied was OD_{600} to be 4.031 and best activity was 2^{16} per OD_{600}. On the other hand, using a 5 liter fermentor, the condition for best yields was as follwing. Glusose of 20 g/liter was added into the LB medium, the bacteria was first grown overnight, and then the culture was induced by 0.1 µg/mL mitomycin C for 2 hr followed by 0.2 µg/mL mitomycin C for 6 hr. The best bacterial yield was OD_{600} to be 6..25 and the best activity was 2^{15} per OD_{600}.

P25 Optimization of *Thermus thermophilus* growth in bioreactors for protein production

M. Edwards, J. Rust and D. Blum, University of Georgia, Athens, GA, USA*

Thermus thermophilus (Tth) is a gram-positive aerobic thermophile that has been documented to grow at temperatures up to 85C. Tth is a model organism used in systems biology experiments. A range of thermostable enzymes have been studied in Tth and are of great interest in the biotechnology field. Total cell protein has been produced from Tth HB8 for the purification of homologous proteins at the Bioexpression and Fermentation Facility (BFF), however, not under optimized conditions. The DasGip parallel bioreactor system (Eppendorf) was used to test a variety of conditions to increase the maximum OD600 reached during exponential growth phase. The timing and concentration of antifoam addition did not affect final ODs, but increased growth rate and decreased lag phase time by almost 80%. The addition of 6g/L glucose to the growth media increased the maximum OD600 during exponential growth phase from 2.97 ± 0.60 to 9.00 ± 0.56. Further increasing the glucose concentration had negative effects on the OD600. Batch-fed fermentations with glucose fed at a rate to maintain approximately 6g/L of glucose in the growth medium also negatively affected the growth of Tth HB8. Comparison of the original conditions used at the BFF with new optimized conditions decreased the harvest time by 45%, increased the cell weight at harvest by 1.8x and increased the total protein by 1.5x.

P27 ROQUETTE pea protein (NUTRALYS® S85F) and pea protein hydrolysate (NUTRALYS® H85): characterization of physical properties and fermentation performance evaluations

R. Cheng, M. Li, E. Lee, D. Wenzel, S. McCann and T. Feron, Roquette America Inc., Geneva, IL, USA; S. Maubourguet, A. Billa, R. Marmulla, L. Segueilha and B. Plancke, Roquette Freres, Lestrem, France*

As a sustainable, non-GMO vegetable resource with no major allergen concerns, pea protein is well situated to meet growing consumer demands. Due to wide-ranging acceptance and compatibility with increasing regulatory restrictions, pea protein is rapidly gaining acceptance for use in food, beverage, probiotic, nutraceutical, and fermentation applications.

ROQUETTE has developed pea protein (NUTRALYS® S85F), and a lower viscosity/reduced foaming pea protein hydrolysate (NUTRALYS® H85), specifically as nitrogen sources for fermentation applications. In this presentation, we will share physical properties for both NUTRALYS® S85F and NUTRALYS® H85, along with fermentation performance data generated from models using *B. subtilis; S. cerevisiae; E. coli; L. delbrueckii;* and *C. glutamicum* for cell growth and cell viability. The data obtained demonstrated significant viscosity reduction for the pea protein hydrolysate and demonstrated comparable performance and potential advantages when using ROQUETTE NUTRALYS® H85 as fermentation nitrogen source in comparison to current commercially available industrial soy flour and yeast extract.

Based on the data collected, and products characteristics that demonstrated compatibility with the most commercial benchmarks, indicating NUTRALYS® S85F & NUTRALYS® H85 are excellent nitrogen source options for a wide range of fermentation applications.

P29 Application of Electromicrobiology for In-situ Bioprocess Monitoring

P. Satjaritanun, B. Devivo, J. Przywara, S. Shimpalee and J. Weidner, University of South Carolina, Columbia, SC, USA; C. Turick, C. Milliken and C. Bagwell, Savannah River National Laboratory, Aiken, SC, USA; S. Greenway, Savannah River Consulting, Aiken, SC, USA*

Bioconversion of organic feedstocks to fuels and chemicals offers significant economic and environmental advantages. For bioconversion efficiency to be cost-effective, bioprocesses need to operate at near optimum conditions with sufficient chemical, biochemical and microbial monitoring. Bioprocess operational assessment is usually accomplished with periodic grab samples from bioreactors followed by laboratory analysis. This often presents a dilemma in determining how much data are enough for cost-effective and successful bioprocess operation. In-situ monitoring can be less expensive but is often problematic because of sensor fouling. Anaerobic bioconversion of waste organics to methane exemplifies the need for robust monitoring strategies in order to maintain balanced conditions and to avoid bioreactor under-performance or even failure. Over the last decade or so electrochemical techniques have been used to define extracellular electron transfer by microorganisms. This field of study, referred to as electromicrobiology, opens up new opportunities and techniques in bioprocess monitoring. For instance, voltammetric techniques provide precise information regarding extracellular electron transfer and electrochemical impedance spectroscopy offers a data rich platform for evaluation of microbial physiological status. In addition, voltammetric stripping is an established electrochemical technique used to clean electrodes in-situ. This creates the opportunity for in-situ, real time monitoring, thereby providing a cost-effective opportunity to monitor bioprocesses in real time with greater regularity. Application of electrochemical techniques for microbial analysis and bioprocess monitoring will be discussed in relation to bioprocess operation.

P31 Development of Temperature Shift Strategies for Chinese Hamster Ovary Cell Culture Based on Kinetic Modeling

J. Xu, BMS, Devens, MA, USA*

Development of Temperature Shift Strategies for Chinese Hamster Ovary Cell Culture Based on Kinetic Modeling

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Abstract

Temperature is a key parameter for Chinese hamster ovary (CHO) cell culture. Temperature shift (TS) strategies are widely used for improving therapeutic protein productivity and quality. Though a couple of studies have been published regarding the temperature's effect on CHO cell culture, there is no systematic method of TS strategy development available for wide application on different CHO cell lines. In this study, the temperature impact was systematically studied using two model CHO cell lines. Kinetic models were applied for rapid TS strategy development. The experiments were performed in short-duration (2 - 8 days) with two sets of seeding density of 0.6×10^6 and 10×10^6 cells/ml. Parameters derived from the resulting experimental data were subsequently used to compute the cell culture performance at extended periods (10 – 14 days) and various TS strategies via kinetic modeling. This study aims to develop an efficient method for systematic CHO cell bioprocess TS optimization while providing an understanding of temperature's effect on biomass accumulation, therapeutic protein productivity and quality.

P33 Applying high-throughput genome engineering to optimize microbes for industrial bioprocesses

R. Phan, P. Boccazzi, S. Lieder, D. Pascoe and C. Isaac, Zymergen, Emeryville, CA, USA*

Zymergen designs and engineers cells for enhanced traits that positively impact existing fermentation-based manufacturing. We believe innovation in biology will spur the next industrial revolution. Zymergen uses a systematic, comprehensive genome-perturbation approach to strain improvement. Radical Empiricism effectively identifies changes in off-pathway genes that would not be considered using hypothesis-driven, mechanism-focused engineering approaches. Zymergen's automation platform and analysis infrastructure enables this via a data driven "search" algorithm applicable to industrial fermentation processes at all stages of commercial development.

P35 Strategy for characterizing microbial physiology across scales in fermentation processes

G. Nadal Rey, A.E. Lantz and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark; S. Cornelissen, Novozymes A/S, Bagsvaerd, Denmark*

Heterogeneous conditions in large-scale fermentation processes can lead to suboptimal physiological performance that negatively affects process yields, productivity and product quality. In order to improve scale-up of fermentation processes and prevent the negative influence of heterogeneous fermentation environments on productivity, a thorough characterization of gradients in different scales should be performed from a physiological point of view.

In this work, a strategy to investigate the practical occurrence and the physiological impact of gradients on pilot- and production-scale fermentation processes will be presented, and advantages/disadvantages of different approaches will be discussed. In short, scale-down (SD) experiments will be firstly performed to study the effect of gradients and local occurrence of suboptimal conditions, based on information about gradient formation from larger scale. To that purpose, Computational Fluid Dynamics (CFD) simulations will be used to rationally design the SD experiments. The formation of metabolic markers will be investigated, with the aim of identifying a number of markers that can be used in later experiments as indicators for suboptimal conditions at different scales. Furthermore, based on the collected data, experimental protocols to rapidly characterize the effect of gradients on the cells will be elaborated. Subsequently, pilot- and production-scale fermentations will be performed to validate and exploit the results obtained.

Once the above-explained methodology has been executed and validated, improvements of the current scale-up approach can be defined. Unlike traditional scale-up strategies, the physiology of the microorganisms will be considered as a relevant variable for the development and optimization of fermentation processes.

P37 Accelerating the development of a scalable fermentation process with limited oxygen demand using definitive screening design

M. Kam, L.B. Wang, A. Tran, J. Lu and Y. Tang, BioMarin Pharmaceutical Inc., Novato, CA, USA*

Ensuring process scalability is a key step when developing a fermentation process to support early or late stage projects. Physical parameters such as power input per volume (P/V), mixing time (t), and oxygen transfer rate (OTR) are often major constraints during process scale up and can have significant impact on process performance. While scaling up a fermentation process from lab scale to clinical production scale, a decrease in process productivity was found to be associated with lower observed oxygen uptake rate (OUR) due to a lower OTR at such scale.

To overcome the scale up issue, a mathematical model was first used to estimate theoretical OUR of the culture based on the carbon source consumption rate during the fermentation process. Empirical observation revealed a constant correlation between theoretical and measured cellular OUR during cell mass accumulation phase. To develop a scalable process with less oxygen demand, a definitive screening design (DSD) experiment was performed to evaluate operational parameters with lab scale fermenters using the estimated OUR derived from theoretical OUR as an output.

Using DSD, a scalable fermentation process was developed, optimized, and confirmed with a peak OUR less than the maximal OTR at the target scale. Comparable or better process productivity was achieved without impacting product quality. The process was then successfully scaled up to a pilot scale fermenter

without issues. Using mathematically estimated OUR in a DSD experiment proved to be an efficient way to develop a scalable fermentation process to address issues with limited OTR.

P39 Redox potential control in anaerobic *Clostridium beijerinckii* fermentation using single-use bioreactors

Y. Yang and M. Sha, Eppendorf, Inc., Enfield, CT, USA*

Redox potential is an important physiochemical factor which measures the tendency of the medium to acquire electrons. In *Clostridium beijerinckii* fermentation, redox potential indicates the status of the $NAD(P)^+$ pool regeneration which directs the electron flow leading to solvent production including butanol. In this study, anaerobic *C. beijerinckii* (ATCC 6014) fermentation was conducted in the Eppendorf BioBLU® 3f Single-Use Vessel controlled by the BioFlo® 120 bioprocess control station. The parameters being monitored throughout the fermentation were redox potential and pH using the ISM® redox/pH sensors. The objectives of this study are (1) to investigate the effects of redox control on the growth and butanol production of *C. beijerinckii*; and (2) to validate the feasibility of using the BioFlo 120 and BioBLU 3f Single-Use Vessel for anaerobic fermentation applications. When *C. beijerinckii* was grown without redox control, a continuous and tremendous change of redox potential between -600 and 0 mV was observed in the broth. When fermentation ended at 124 h, OD_{600} was 0.8, glucose consumption was 33% and butanol production was limited. When redox potential was controlled at -500 mV by redox sensor guided addition of $Na_2S{\cdot}9H_2O$ solution, OD_{600} was 1.6, glucose consumption was 51%, and butanol production showed a 2-fold increase. In summary, with the combination of ISM Redox sensors and BioBLU Single-Use Vessels, the high variability of redox potential during *C. beijerinckii* fermentation can be actively controlled to increase biomass growth and solvent production.

P41 Fermentative production of butanol from sweet sorghum syrup in the presence of natural nutrients and inhibitor

K.T. Klasson and G. Eggleston, United States Department of Agriculture, ARS, New Orleans, LA, USA; N. Qureshi, USDA-ARS-NCAUR, Peoria, IL, USA; R. Powell, Delta BioRenewables LLC, Memphis, TN, USA; M. Heckemeyer, Heckemeyer Biorefinery, Sikeston, MO, USA*

Sweet sorghum syrups represent a renewable raw material that can be available year-round for production of biofuels and biochemicals. Sweet sorghum sugars have been used as sources for butanol production in the past but most often the studies focused on sweet sorghum juice and not on sweet sorghum syrups. Therefore, we investigated the feasibility of using the syrups as feedstock.
Initial studies showed that diluted sweet sorghum syrups, to 60 g/L of glucose equivalents, could not be used as a direct replacement of a synthetic growth medium for industrial butanol-producing strain *Clostridium beijerinckii*. Further studies revealed that supplemental nutrients (most notably, phosphate and ammonium) were required for successful fermentation. This was found true for two manufacturing sources of sweet sorghum syrups from commercial cultivars and hybrids. Typically, 15 g/L of total acetone, butanol, and ethanol (ABE) were produced with approximately half of that being butanol. Some minor statistical difference was noted between the production potential of the two sources of syrup. However, aconitic acid, which was present at similar levels in both syrups, was ruled out as a butanol fermentation inhibitor at the fermentation pH>4.5.

P43 Improved bioethanol production from rice husk using locally isolated stress tolerant yeasts

T.N. Nwobodo, University of Nigeria, Nsukka, Nigeria*

The aim of this research was to improve bioethanol production by isolating, screening and characterizing stress tolerant yeast strains capable of ethanol production from pretreated rice husk without the introduction of amylase. 310 thermotolerant yeasts were isolated from orchard soil, fermenting palm wine, sorghum spent grain, decaying wood and spoilt fruits after which four yeast strains- y11, y45, y146, y172

were selected as the best ethanol producers from pretreated rice husk. The selected yeast isolates- y11, y45, y146 were tentatively identified to be *Saccharomyces sp* while y172 was *Kluveromyces sp*. based on their morphological appearance and biochemical characteristics. Isolates were thermotolerant, acetic acid tolerant, ethanol tolerant and osmotolerant, with y172 showing highest tolerance to extreme osmotic condition (50% glucose and 40% xylose), elevated temperature (50^{o}C) and internal ethanol while y146 and y45 showed highest tolerance to acetic acid (0.8%) and external ethanol (15%) respectively. Stationary fermentation was carried out in Erlenmeyer flasks for bioethanol production from hydrolysed and unhydrolysed (control) rice husk. It was observed that ethanol produced by the yeasts from the hydrolysed rice husk was significantly higher than that of the unhydrolysed sample. Percentage ethanol production was estimated and y146 gave highest ethanol yield of 6.192% in 120h. This compares with 5.791%, 5.602% and 6.189% produced in 120h, 144h and 168h by y11, y45 and y172 respectively at pH 5.0, 30^{o}C and initial reducing sugar concentration of 9.653mg/ml. Hence, bioethanol production capacity of the yeast strains was ranked statistically as y146 > y172 > y11 > y45.

P45 Optimizing *E. coli* protein supernatant extraction

M. Yamada[*]*, Genentech, Inc, South San Francisco, CA, USA*

This work discusses the development of an *Escherichia coli* (*E. coli*) recombinant protein fermentation process in which high yields of recombinant protein were obtained in the supernatant. Our current approach for recombinant protein production in *E. coli* requires cell lysis using a homogenizer; however, the presence of recombinant protein in the supernatant enables protein recovery without this unit operation. Several factors including production host, glucose feed rate, and agitation rate were evaluated to understand the mechanism of action and further increase recombinant protein accumulation in the supernatant. This process development was performed at 10 L scale and recombinant protein titers up to 7 g/L were obtained in the supernatant.

P47 Multi-fold titer increase of an antibody fragment through host and process development for use as a bi-specific for oncology

M. Shimazu[*]*, Ambrx, Inc., La Jolla, CA, USA*

Ambrx technology platforms combine the power of conventional medicinal chemistry with cutting edge recombinant DNA-based protein biosynthesis. It allows us to incorporate non-native amino acids at selected sites in natural proteins as they are being made within the cells. This opens up new opportunities in the use of bi-and multi-specific antibodies and antibody fragments as therapeutics. Traditional fusion proteins generally have been restricted due to limitations in what the producing cell is able to synthesize. Ambrx's technology allow the introduction of non-natural peptides, small molecule drugs or other ligands into the bio-conjugate to create new specificities and activities.

We have engineered into Escherichia coli. new tRNA/tRNA synthetase pairs (orthogonal sets) that specifically recognize and incorporate non-natural amino acids at positions we define in the therapeutic proteins. These amino acids can be specifically chemically modified, allowing us to add functionality and empower the protein. This protein medicinal chemistry can be performed on any therapeutic protein or antibody using one of our proprietary bacterial (ReCODE) to create long acting therapeutic peptides and proteins and modified antibody and bi-specific fragments.

Here we present work describing a multi-fold increase in expression titers through host engineering and process development to produce antibody fragments (Fab). Starting with our proprietary ReCode expression system, we engineered components to facilitate the expression and assembly of our target Fab. Basal medium, feed composition, timing and duration were optimized in parallel to give a robust and scalable production platform.

P49 Use of ambr®250 mini-bioreactor to produce isotopically labeled antibody from *E.coli*

S.H. Grieco, Genentech, South San Franciso, CA, USA*

In the past it has been challenging to make sufficient quantities of isotopically labeled complex proteins for structural studies. Media components needed to achieve 100% isotope incorporation are expensive. The small scale shale flask culture can reduce the cost of the media, however, we have shown that shake flasks aren't predictive of fermentation results, and often have very low titers. The ambr®250 mini-bioreactor has ability to perform fermentation in much smaller working volume. Therefore, it allows us to perform fermentation that requires expensive material, such as isotopically labeled media. This study shows that we can successfully use ^{13}C-labeled media to express antibodies in the ambr®250. First, we tested if ambr®250 can produce hAb using the algae-based hydrolysate (Celtone). Media optimization to lower the osmo was required, but after the optimization of the media, the fermentation performance was successful and produced comparable amounts of hAb compared to our standard media. We then tested ^{13}C-labeled Celtone, which also showed comparable fermentation performance and final titer. We're currently testing ^{13}C-labeled glucose in addition to the ^{13}C-labeled Celtone to produce fully isotopically labeled hAb.

P51 Microbial evaluation of dry garri sold in three towns of ijebu – north, ogun state, nigeria

T. Akindele, Abraham Adesanya Polytechnic Ijebu-igbo, iIebu igbo, Nigeria and J.B. bilesanmi-Awoderu, Abraham adesanya polytechnic ijebu-igbo, ijebu igbo, Nigeria*

The sale and distribution of garri in local markets is associated with practices such as display of product in open buckets, bowls and mats at points of sale and the use of bare hands during handling and sales. These unhygienic practices may lead to the microbial contamination of garri. This study was carried out to evaluate the microbial quality of garri sold in Ijebu community. Six garri samples were randomly collected from six retail sellers in three towns of Ijebu-igbo, Ago-Iwoye and Oru Ijebu in Ijebu-North Local Government Area of Ogun State. Samples were serially diluted to 10^{-2} and inoculated by pour plate method onto Nutrient agar, MacConkey agar and Potato-Dextrose agar plates for Total aerobic plate count (TAPC), Coliform count (CC) and Fungal count (FC) respectively. The Coliform counts of garri ranged from 3.0 x 10^2 to 3.0 x 10^3CFU/ml while Fungal counts ranged from 3.0 x 10^3 to 4.0 x 10^3 CFU/ml. The pH ranged from 4.78 to 4.90. A total number of fourteen (14) bacterial isolates belonging to five genera were isolated. The occurrences were Escherichia coli (4) ,Staphylococcus aureus (3), Klebsiella pneumoniae (3), Bacillus spp.(2) and Pseudomonas aeruginosa (2). Nine (9) fungal organisms: Aspergillus flavus 1(11.11%), Aspergillus niger 2(22.22%), Penicillium sp. 2(22.22%), Fusarium sp. 1(11.11), Candida albican 2(22.22) and molds 1(11.11%) were also identified. Application of Good Manufacturing Practices (GMP) in garri handling post- processing is important.

P53 Comparative characterization of the oxidative and synergistic activities of *Tt*AA9E and *Ta*AA9A on cellulose and pretreated lignocellulose

I.J. Kim, Korea University Graduate School, Seoul, Korea, Republic of (South), J.H. Kim, Chungnam National University, Daejeon, Korea, Republic of (South), P. Harris, Novozymes, Davis, CA, USA and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Lytic polysaccharide monooxygenases (LPMOs) are a group of proteins capable of catalyzing the cleavage of glycosidic linkages of polysaccharides, such as cellulose, hemicellulose, and chitin, via the oxidative mechanism. Through an oxidative cleavage exerted by LPMOs, the hydrolytic performance of cellulases could be improved. Auxiliary activity family 9 (AA9) belongs to the fungal LPMO that targets crystalline cellulose as the substrate. Here, comparative characterization regarding oxidative and synergistic activities was carried out against cellulose and pretreated lignocellulose using *Tt*AA9E from *Thielavia terrestris* and *Ta*AA9A from *Thermoascus aurantiacus*, which belong to phylogenetically based Type 1 and 3, respectively. First, product analysis reveals a different oxidative regioselectivity of *Tt*AA9E and *Ta*AA9A. Specifically, *Tt*AA9E exclusively generated an aldonic acid forms of cellodextrins while *Ta*AA9A generated both aldonic acid and 4-ketoaldose forms of cellodextrins, which are evidences of C1 and C1/C4 oxidations, respectively. Next, when synergistic study was conducted, a higher activity for

*Tt*AA9E was exerted compared to *Ta*AA9A against pure cellulose. For the hydrolysis of pretreated substrates using rice straw, the synergistic activity of *Tt*AA9E was higher on acid-pretreated rice straw compared to *Ta*AA9A but it was lower on alkaline-pretreated rice straw, indicating different synergistic behaviors of the two AA9s depending on the pretreatment. Overall, different behaviors of *Tt*AA9E and *Ta*AA9A were shown in this study regarding oxidative and synergistic activities, which imply the functional variations and substrate specificities of AA9s, and further suggest the systematic customization of AA9s depending on substrate is required for obtaining an efficient hydrolytic process.

P55 Unraveling fermentation data – a Novozymes case study

A. Baum, L. Vermue, R. Moiseyenko and T.M. Jørgensen, Technical University of Denmark, Lyngby, Denmark; R. Devantier, Novozymes Pilot A/S, Bagsværd, Denmark*

Industrial fermentation processes are monitored using a variety of sensors. Typically, measurements are taken through-out the entire production process. Production may be carried out under supervision of different operators (operator variation), on different sites (global variation), in different buildings and/or in different tanks (local variation). However, up to now processes are mainly controlled according to traditional recipes and experience.

The massive amount of available process data combined with multivariate statistics enable new “Big Data” approaches to identify and extract significant patterns in the process control which may lead to considerable improvements with respect to process efficiency and yield maximization.

In our case study we propose a three-step approach to unravel the data. All analysis were performed using machine learning libraries available for Python and R.

1. Data integration of distinct and consistent data blocks (from multiple data bases);
2. Multiblock Partial Least Squares regression for prediction modeling of the product yield;
3. Comprehensive and easy-to-understand visualization of the results.

The data typically consist of various types of variables such as set points (input parameters), measured data (performance variables) and material input/output variables. We show that comprehensive multivariate analysis of such data leads to an improved understanding of the relationship between input parameters and performance giving potential for improved process control. The results are displayed using interactive visualization techniques which enables engineers to understand how to optimize process control on a single parameter level.

Impact of dynamic online fed-batch strategies on metabolism, productivity and n-glycosylation quality in CHO cell cultures

W. Miller and Z. Swilling, YSI Life Sciences, Yellow Springs, OH, USA*

In an effort to improve yields in order to meet the growing demands of therapeutics, the impact of process controls on glycosylation patterns can be employed as a means of ensuring increased efficacy and consistency. By utilizing an online fedbatch model based on maintaining levels of glutamine/glucose; impact on cellular metabolism, productivity, and N-glycosylation quality of the model recombinant protein, interferon gamma (IFN-y), can be quantified. Glutamine concentrations of 0.3mM provided a 10-fold increase in yield, and maintained an unequivocal macro- and microheterogeneity of IFN-y. It was also observed that low concentrations of glutamine and glucose (<0.1mM and <0.7mM respectively) led to decreased sialylation and increased presence of minor glycan species. In addition to nutrient limitation, N-glycosylation can be adversely affected by decreased cell viability and presence on inhibitory lactate and ammonia. Therefore it is imperative to measure both the culture viability as well as nutrient set points in order to optimize N-glycosylation quality.

6:30 PM - 8:30 PM Poster Session 1/Opening Reception/Exhibits open

Calusa Ballroom D-H, Lobby Level

Monday, October 30

7:00 AM - 8:00 AM Breakfast All Registered Attendees

Calusa Terrace, Lobby Level

7:00 AM - 5:00 PM Registration

Calusa Prefunction, Lobby Level

8:00 AM - 11:30 AM Session 2: Bench to manufacturing suite

Conveners: **Dale Brown**, Dow AgroSciences LLC, Indianapolis, IN, USA;**Farzaneh Rezei**, Novozymes, Durham, NC, USA and **Billy Allen**, Eli Lilly and Company, Indianapolis, IN, USA

Calusa Ballroom ABC, Lobby Level

8:00 AM S9: Estimation of oxygen transfer rates and gas hold-up in pilot-scale fermentors

B. Allen, Eli Lilly and Company, Indianapolis, IN, USA*

The topic of oxygen transfer has been the subject of dozens of publications over the last six decades. One would expect given the shear amount of information that has been developed that both fundamental principles governing oxygen transfer rates and practical design questions (e.g., optimum impeller configuration) would be fully elucidated by now. However, this is clearly not the case as one can find conflicting data on almost any topic in this field and almost no data to ascertain the accuracy of the correlations used to estimate oxygen transfer rates.

Data will be presented that quantify the ability to estimate gas transfer rates in pilot-scale fermentors based on correlations developed with a similarly sized, geometrically-scaled vessel and fermentation broth simulant. Correlations for k_La (oxygen transfer coefficient) and Φ (fractional gas hold) will be presented. The accuracy of these correlations were evaluated by collecting k_La and Φ data from pilot-scale fermentation processes over a two-year period. It will be shown that the correlations accurately predict oxygen transfer rates to within +/- 20 percent of the experimental values during the initial growth phase of the fermentations. During this period, the physical properties of the fermentation broth are accurately represented by the fluid used to develop the correlations. The difficulties in generalizing the results to include media composition effects, fermentor design differences and application to production-scale vessels will be briefly reviewed.

8:30 AM S10: How Genomatica and Novamont successfully started up a 30,000 ton/yr bio-based 1,4-butanediol plant

J. Crater and J. Lievense, Genomatica, Inc., San Diego, CA, USA*

The last decade has seen many difficult plant starts in industrial biotechnology with challenges in operational performance, product quality, and economics. During the 2nd half of 2016, Genomatica executed a successful scale-up and technology transfer of our bio-based 1,4-butanediol (GENO BDO™) process with our commercial and manufacturing partner, Novamont, at the world's first bio-based BDO plant in Bottrighe, Italy. This culminated in a June 2017 announcement that the Bottrighe plant has met all performance guarantees committed to by Genomatica. This represents the first example of large-scale fermentation production of an established bulk petrochemical. Underpinning this success was a comprehensive approach to process scale-up beginning with rigorous scale-down of the full-scale plant conditions. This presentation will focus on the fermentation part of the GENO BDO™ process, including the construction of a black box kinetic model of the engineered *Escherichia coli* BDO production strain that also accounts for the heterogeneous environment in the plant fermentors; application of that model to identify key scale parameters; evaluation of those parameters in laboratory fermentations; and use of laboratory data to anticipate and resolve scale related issues through a combination of strain and process solutions prior to tech transfer to the Bottrighe plant. The modeling approach and scale-down/scale-up methodologies are broadly applicable and are an indispensable component of Genomatica's integrated approach to developing robust, scalable strains and fermentation-based processes. They are equally effective for troubleshooting any unexpected performance deviations that may occur from time-to-time at the plant.

9:00 AM S11: Exploring industrial scale fermentation tank liberation through increased airflow and evaporation - a cost/benefit model.

J. Noel and M. Bredwell, Novozymes, NA, Franklinton, NC, USA; A. Jensen, Novozymes, DK, Kalundborg, Denmark*

Productivity of industrial scale fermentation tanks is an important factor for a growing biotech company like Novozymes. Investment in new fermentation vessels is expensive, making optimization of already installed capacity a cost effective way to avoid or delay capital expense. This study explores adjustment of the fermentation air handling system as a means of liberating capacity at the Novozymes production site in Franklinton, NC. Tank exhaust air is saturated with water making aeration a major avenue for mass removal during fed-batch fermentation. Increasing airflow rates would increase evaporation with the added benefit of increased oxygen transfer. Increasing airflow would require either higher differential pressure or lower pressure loss between the air source and tank entry point. This work explores the increased energy costs and potential capacity benefits of operating at higher air header pressures and by minimizing the pressure drop across the sterile filters.

9:30 AM Break

10:00 AM S12: Jet loop bioreactors as alternative to overcome mass transfer limitation in large scale fermentation

S. Schaepe, S. Weber, M.H. Kopf, S. Freyer and C. Dietzsch, BASF SE, Ludwigshafen, Germany*

Whenever the achievable space time yield is determined by the mass transfer performance of the reactor, energy efficiency plays an important role to meet the requirements regarding low investment- and operating costs. Based on theoretical calculations, compared to bubble column, airlift reactor and aerated stirred tank, the jet loop reactor owns the potential for an enhanced energetic efficiency at high mass transfer rates. Interestingly, its technical application in standard biotechnological production processes has not been realized, yet. Compared to a stirred tank reactor powered by Rushton turbines, maximum oxygen transfer rates about 200 % higher were achieved in a jet loop reactor at identical power input in a fed batch fermentation process. Moreover, a model based analysis of yield coefficients and growth kinetics showed that *E.coli* can be cultivated in jet loop reactors without significant differences in cellular metabolism. Based on an aerobic fermentation process, the assessment of energetic oxygen transfer efficiency [$kgO_2\ kW^{-1}\ h^{-1}$] for a jet loop reactor yielded an improvement by almost 100 % whereas the jet

loop reactor could be operated at mass transfer rates 67% higher compared to a stirred tank. Thus, an increase of 40% in maximum space time yield [kg m^{-3} h^{-1}] could be observed.

10:30 AM S13: Integrated process development for Inatreq™, a novel fungicide from a secondary metabolite

M. Mikola, D. Brown, C. Stowers, P. Speakman and K. Hill, Dow AgroSciences LLC, Indianapolis, IN, USA; D. Tyagi, Moderna Therapeutics, Cambridge, MA, USA*

The Bioprocess and Bioengineering Research and Development (BBRD) department at Dow AgroSciences has been engaged in the development of an industrial secondary metabolite fermentation process to produce UK-2A used in the manufacture of a novel agricultural fungicide, Inatreq™ active. The process is being developed through a highly collaborative and integrated use of engineering and biological disciplines. Strain improvement methods included classical random mutagenesis with high throughput selection for identification of improved strains, as well as a concerted synthetic biology rational design approach informed by transcriptomic, proteomic, and genomic data from the Dow AgroSciences Next Generation Sequencing center of expertise. New strains and fermentation process improvements are vetted in bioreactors at multiple scales and the performance of the downstream process is evaluated. Process performance results guide subsequent strain and process improvement targets resulting in a highly integrated development program. Final product design requirements and performance targets with a line of sight to final manufacturing process constraints have been incorporated into the development program from the start. This presentation will review how these facets contributed to a rapid acceleration in productivity gains resulting in a 75% improvement in titer over a one-year period, more than a 2 fold improvement in 4 years and the successful scale-up to final commercial scale.

11:00 AM S14: Large scale simulations of *Trichoderma reesei* fermentation using computational fluid dynamics: approach and early successes

C. Bach, U. Krühne and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark; M.O. Albaek, Novozymes A/S, Bagsværd, Denmark*

This work investigates the ability of commercial computational fluid dynamics (CFD) software to describe and predict the process gradients in industrial scale fermenters. The opportunity to calculate concentration gradients in full-scale fermenters is highly desirable since it can explain problems occurring at production scale, as well as anticipate potential problems with new products or processes.
The oxygen concentration was modelled and predicted for a 100 m^3 fermenter with an industrial *Trichoderma reesei* strain producing cellulase and compared with measurements. Oxygen concentrations in the fermenter were measured using optical electrodes mounted at different positions throughout the vessel. The mass transfer was modelled by a well-established Euler-Euler approach for two-phase flow in gas liquid systems at similar power input and gas flow rate. The decisions and assumptions involved in developing models for these systems will be discussed and evaluated in terms of complexity. In particular, the implementation of microorganism kinetics in CFD simulations, and how they affect the predicted gradients, will be highlighted. We would like to present this application of CFD at industrial scale to the fermentation community to receive feedback on our approach.

11:30 AM - 1:00 PM Lunch - All Registered Attendees

Calusa Terrace, Lobby Level

12:15 PM - 12:45 PM Exhibitor Showcases: 12:15 pm Moubio LLC; 12:25 pm BlueSens; 12:35 pm Keit Spectrometers

Calusa Ballroom ABC, Lobby Level

1:00 PM - 2:00 PM Session 2: Continued

Conveners: **Dale Brown**, Dow AgroSciences LLC, Indianapolis, IN, USA;**Farzaneh Rezei**, Novozymes, Durham, NC, USA and **Billy Allen**, Eli Lilly and Company, Indianapolis, IN, USA

Calusa Ballroom ABC, Lobby Level

1:00 PM S15: Upscaling of aerobic bioprocesses in bioreactors designed for anaerobic growth

J. Westman, M. Jensen and J. Kaya, Chr Hansen A/S, Copenhagen, Denmark*

Growth of lactic acid bacteria (LAB) is often thought of purely as an anaerobic fermentation process. It is also true that many LAB are inhibited by the presence of oxygen during growth and for certain species it is even fatal. However, it has also been found that certain LAB benefit from aerobic growth, resulting in a higher biomass yield, higher growth rates and improved long term survival after production. *Lactococcus lactis* is one example of these LAB. In order to respire, a heme source needs to be added to the growth medium. Externally supplied heme is necessary for synthesis of cytochrome bd oxidase, which is in turn required for a functional electron transport chain.

Upscaling of aerobic processes presents issues that are not observed in anaerobic processes, such as issues with foaming and insufficient oxygen supply. Foaming might make it impossible to fill the bioreactor to the same extent as can be done while running anaerobic fermentations, thereby lowering the yield of each fermentation. Insufficient aeration limits the respiration of the cells. This also leads to decreased yields of the process, since the cells will use homolactic fermentation rather than respiration for their growth. A too high aeration, on the other hand, can lead to a toxic environment as well as depletion of the heme source.

Here we present the upscaling of respiring *L. lactis*, from laboratory scale reactors up to production scale in >20 m^3 bioreactors, with emphasis on the above mentioned issues.

1:30 PM S16: Modelling commercial bubble column bio-reactors using computational fluid dynamics.

J. Kavanagh, D. McClure, G. Barton and D. Fletcher, The University of Sydney, Sydney, Australia*

Bubble column bio-reactors are widely used for the industrial-scale production of a range of products, which include organic acids (e.g. citric acid) and chemical intermediates (e.g. 1,3-propanediol). Due to their large size (more than 100 m^3) gradients exist in such reactors, which can be damaging to the overall process performance. For example, gradients in substrate concentration can lead to both overflow metabolism and starvation, both of which are detrimental in terms of maximising the yield of product. Ideally large-scale bio-reactors would be designed such that any gradients are minimised, however experimentally identifying the optimum design is a considerable challenge.

An alternative to costly and time-consuming large-scale experimentation is the use of Computational Fluid Dynamics (CFD) to quantify the effect of the reactor design on the process performance. In this presentation we will present validation results from both the pilot (120 L) and production (80,000 L) scales. We will also examine the inclusion of microbial kinetics in the model, using a range of industrially

relevant case studies including the production of baker's yeast, the production of recombinant proteins (using *Escherichia coli*) and the production of 1,3-propanediol.

2:00 PM Break

2:30 PM - 4:40 PM Session 3: Innovative fermentation processes designed to deliver tomorrow's products

Conveners: **Dorothea Reilly**, Genentech Inc., South San Francisco, CA, USA;**Keith Alsaker**, Evonik Corporation, Lafayette, IN, USA and **Kim Olofsson**, AAK, Malmo, Sweden

Calusa Ballroom ABC, Lobby Level

2:30 PM S17: Sartorius ambr 250 automated bioreactor system: accelerating fermentation process development and deployment in Ginkgo's foundry

E. Greenhagen and P. Iyer, Ginkgo Bioworks, Boston, MA, USA*

There is an emerging demand for sourcing plant-derived extracts (nutraceuticals, flavors, fragrances, sweeteners, etc.) from engineered microbes. While recent advances in synthetic biology and metabolic engineering provide feasible approaches to engineering such organisms, commercial success for developing these "cultured" ingredients presents specific challenges. Unlike commodities, where efforts can be focused on one particular molecule given the enormous market size, cultured ingredients require developing different organism lines and production processes in a rapid and low cost fashion. This requires a scalable solution for both bio-manufacturing of organisms and process development, which is provided by our state of the art foundry. I will describe how Ginkgo utilizes the Sartorius ambr 250 automated bioreactor system to accelerate fermentation process development, optimization, and successful deployment. In particular, I will describe the process development, optimization, and scale-up for a cultured ingredient produced in a multiphase yeast fermentation, showing identical process performance across the 250mL, 250L, and 50,000L scales.

2:50 PM S18: Advanced feed rate control and modelling of industrial fermentation processes

B. Cassells, Novozymes A/S, Bagsvaerd, Denmark*

Novozymes is the world's largest producer of industrial enzymes. With our products, we help our customers and partners to attain more sustainable production processes, e.g. by reducing their water, energy and raw material needs. For Novozymes to maintain a front-runner position, introduction of new products and processes is vital, while optimization of current processes is important to stay competitive. Novozymes' enzymes are often manufactured using large scale aerobic fermentation processes, using different expression organism throughout multiple sites all over the globe. This diversity can make process control, e.g. feed rate control, challenging, but also provides a good base to gain a deeper understanding of our process, across sites. We're trying to stay ahead of the game by continuously increasing our process knowledge and one way of doing this is by analysing process data and use it to develop and validate models of our different processes. These models then serve as a great platform, and starting point, for further development and testing/evaluation of new concepts, such as advanced feed rate controls. This type of work is often done through collaborations, e.g. with academia. This presentation covers a discussion about feed control challenges and opportunities when running diverse

processes at multiple locations across the world. Examples of different types of feed control concepts will be discussed with focus on the role of models during development.

3:10 PM S19: High gravity lignocellulose bioprocess development for ethanol and lactic acid production by multi-feed simultaneous saccharification and fermentation

C.J. Franzén, S. Fusco, D. Nickel, L. Olsson and R. Wang, Chalmers University of Technology, Gothenburg, Sweden; M. Aulitto, S. Bartolucci and P. Contursi, University of Naples "Federico II", Naples, Italy*

Second generation bioethanol production is becoming established in production plants across the world. The process can also be viewed as a model biorefinery concept for biotechnological conversion of recalcitrant lignocellulosic raw materials to chemicals and other products. We have developed a Multi-Feed SSCF process: a systematic, model-driven design of fed-batch simultaneous saccharification and co-fermentation of steam-pretreated lignocellulosic materials in standard stirred tank reactors. The design includes feeding of solid substrate, enzymes, and active, robust cell factories adapted to the present substrate. The concept has been applied not only to ethanol production with *S. cerevisiae*, but also to lactic acid production from wheat straw by the thermophilic, cellulolytic strain *Bacillus coagulans* MA-13, isolated from bean processing waste.

High Gravity operation, i.e. fermentation at high concentrations of water insoluble solids (WIS), pushes the process towards higher product concentrations and productivities, and improved energy and water economy. By using the multi-feed SSCF approach, the ethanol process was pushed towards final product concentrations above 60 g/L, at about 90% of the theoretical yields on consumed substrate, using 22% w/w accumulated WIS additions of acid- and steam explosion-pretreated wheat straw.

Bacillus coagulans MA-13 was found to secrete cellulolytic enzymes and ferment lignocellulose-derived sugars to lactic acid; thus, it may be a potential platform for consolidated bioprocessing of lactic acid. We investigated its performance in multi-feed SSF and found that pre-adaptation of cells to the liquid fraction of the steam-pretreated lignocellulosic material improves lactate productivity and reduces the SSF time from 33 to 12 hours.

3:30 PM Break

3:40 PM S20: Converting air into sugar: the design and in-vivo evolution of an *E. coli* strain capable using CO_2 as a source of biomass production

G. Jona, N. Antonovsky, S. Gleizer, E. Noor, Y. Zohar, E. Herz, U. Barenholz, L. Zelcbuch, S. Amram, A. Wides, D. Davidi, Y. Bar-On, T. Bareia, D.G. Wernick, I. Shani, S. Malitsky, A. Bar-Even and R. Milo, Weizmann Institute of Science, Rehovot, Israel; N. Tepper, Technion, Israel Institute of Technology, Haifa, Israel*

The development of microorganisms with improved features and enhanced capabilities has been the focus of many researchers for the past few decades. Among the aims and fields of study associated with the above one can find: the production of cellular biomass, the conversion of a substrate into products and metabolites, the production of biofuel, the expression of useful proteins, and more. While in many of these fields the implementation of such designs is efficient and does not have an apparent negative impact, there are many opposite cases where the introduction of a small change might result in an inefficient process, and in the more severe cases in the inability of the host to support growth. Two of the major technologies used to achieve the above are genetic manipulations and genome evolution; yet each of the two technologies by itself poses many limitations, which might be manifested, in the best scenario, in a functional strain, yet a non-optimized one. Here I show how we combine the two strategies to increase the probability to reach the target phenotype, and exemplify it using results from our recent study, where using genetic manipulations based on computational design, accompanied by direct *in-vivo* evolution using chemostats, we developed *E. coli* strains capable of producing sugars and biomass from

CO_2. I will further provide insight on the challenges we have encountered, as well as on our achievements, and future directions.

4:00 PM S21: A novel bacteriophage in an industrial fermentation process and CRISPR-based acquired resistance

M. Halter and J. Zahn, Dupont Tate & Lyle Bio Products, LLC, Loudon, TN, USA*

Bacterial-bacteriophage interactions are a well-studied and ecologically important aspect of microbiology. However, in the context of industrial-scale fermentation, the presence of bacteriophage is unwelcome, and can have major negative production and economic implications. Here, we have isolated, identified, and characterized a novel bacteriophage capable of lysing an *E. coli* MG1655 strain developed for the production of 1,3-propanediol in large-scale, fed-batch fermentation (DTL-phage). The bacteriophage genome was sequenced and annotated, identifying 67 potential open reading frames (ORF). The tail fiber ORF, the largest in the genome at 3426 bp, aligned closely (90%) to a phage previously identified by a similar fermentation facility in Germany (RTP-phage), but far enough away to confidently describe this as a novel phage. Upon identification of potentially important ORF's, a customized *Streptococcus thermophilus* CRISPR3 plasmid was developed, removing the ability of the CRISPR operon to acquire new spacers in order to avoid production plasmid degradation, and replacing all of the native *Streptococcus thermophilus* CRISPR3 spacers with spacers located within important ORF's identified within the genome, each of which was found to be located adjacent to a CRISPR PAM sequence (protospacer adjacent motif). The native *Streptococcus thermophilus* CRISPR3 operon, which was also assayed, was shown to decrease phage susceptibility by up to 96%, while the customized CRISPR3 operon, which contained 7 spacers tailored to the specific phage, and therefore did not require a spacer integration step, provided 100% resistance.

4:20 PM S22: NanoSpun's active biological fabric & biological membranes harnesses the power of biology for next-gen fermentation processes

D. Weinstein-Fischer and O. Bendror, NanoSpun Technologies, Yokneam, Israel*

NanoSpun Technologies (NanoSpun) is a leading biotech platform company which develops and manufactures disruptive biological-physical structures that harness, enable and improve biological-based fermentation processes, especially in challenging or inhibiting conditions. NanoSpuns technology enables the efficient encapsulation of any biological agents e.g. microorganisms, mammalian cells as well as enzymes. The encapsulation empowers the performance of the biological agents leading to an optimized cost-effective process. NanoSpun ground-breaking system empowers the biological process in all aspects; cell, fermenter, yield and downstream processes. The bio fabrics encapsulated with optimized biological agents and the unique Modular Biological Cassette Fermenter (MBCF) engineering properties allows high gas and mass transfer therefore reducing heat accumulation. The innovative engineering of the cassette bio-reactor and the inherent separation of the biological agents from the medium opens the opportunity for easy continuous process while maintaining the high cell biomass. Moreover, the inherent separation reduces the requirement for downstream processes as centrifuge or filtration. NanoSpun next-gen 'active biological fabrics' and 'biological cassettes' are the core of the company's MBCF units - revolutionary fermentation processes, which offers a game-changing solution for advanced, highly controlled, cost effective, and environmentally friendly fermentation or bio-conversion processes.

5:30 PM - 7:30 PM Poster Session 2/Reception/Exhibits open

Calusa Ballroom D-H, Lobby Level

5:30 PM - 7:30 PM Session: PS2: Poster Session 2

Calusa Ballroom ABC, Lobby Level

P2 DNA from cell lysis drives increased viscosity of *E. coli* fermentation broth

R.S. Kuczenski[*] and J. Baker, Genentech, South San Francisco, CA, USA; D. Reilly, Genentech Inc., South San Francisco, CA, USA

Increased broth viscosity can challenge bioprocessing by reducing mixing and the oxygen transfer rate as well as complicating downstream recovery operations. Here we characterize the rheology of *E. coli* fermentation broth. The data supports increased broth viscosity being driven by two mechanisms. First, the increased cell density over the growth phase is coincident with an initial rise in broth viscosity. Second, an increase in broth viscosity and shear thinning can be attributed to an increase in supernatant DNA. The rheological data is fit to a composite model that includes contributions from both cell density and supernatant DNA. This model captures the contributions of cell density and supernatant DNA to viscosity but is challenged to predict the end-of-run rheology when the cell density drops.

P6 Dynamics of phenotypical adaptation of yeast to main inhibitors found in lignocellulocidhydrolysate: a single cell analysis approach

P. Cabaneros[*], Technical University of Denmark, Lyngby, Denmark; C.T. Peng and N. Arneborg, University of Copenhagen, Copenhagen, Denmark; A.E. Lantz and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark

The inhibitors generated during the pretreatment of lignocellulocic biomass reduce the performance of yeast to produce 2G bioethanol. In this context, several studies have reported that phenotypical adaptation of the cells during their propagation step results in signicant improvements of the ethanol production.

In this study, single cell analysis was used to elucidate the mechanisms of inhibition and the dynamics of phenotypical adaptation of Saccharomyces cerevisiae to inhibitors commonly found in biomass hydrolysate (acetic acid, furfural and vanillin). Their effects were studied using a circumscribed central composite design. The phenotype of the yeast was assessed every 2 hours using multi-parametric ow cytometry based on three criteria: cell membrane integrity (relates to viability), membrane potential and cytosolic ROS concentration (both indicating metabolic stress). The results revealed that treatments with acetic acid (7.5g/L) and furfural (3.5g/L) entail a quick drop of the membrane potential, and an increase of the ROS concentration, indicating high metabolic stress. After 10 hours, the ratio of dead cells increased notoriously. Whilst cells treated with acetic acid never recovered from the shock, the cells treated with furfural recovered normal metabolic stress levels after 15 hours, indicating detoxication of furfural. The cells treated with vanillin (1.5g/L) also showed increased levels of metabolic stress, but were able to recover after 5 hours of fermentation.

This study shows the dynamics of phenotypical adaptation of yeast to inhibitors at a single cell level, and will be used to optimize the propagation of yeast to improve the production of 2G bioethanol.

P8 High cell density fed-batch fermentation in micro-scale bioreactors – feed rate optimisation and strain selection for the production of HMO in *Escherichia coli*

P. Becker[*] and N. Fierfort, Glycom, Hørsholm DK, Denmark; C. Bernal Martinez and C. Santos Fernandes, Applikon Biotechnology, Delft, Netherlands

Human Milk Oligosaccharides (HMO) are a diverse group of natural oligosaccharides which are present in high concentrations in human milk. They are the 3rd most abundant component of human milk and contribute to many of the health benefits associated with breastfeeding. With the introduction of fermentation-based processes for the production of HMOs, it has become feasible to develop improved infant formula and to look at other applications outside the infant-space. At the core of the fermentation process is an *E. coli* strain which has undergone extensive metabolic engineering to be able to synthesize HMOs. The fermentation strategy is based on a classical fed-batch culture with glucose as the only carbon and energy source and lactose as an acceptor for the production of activated sugars such as GDP-fucose.
This work focusses on the application of micro-scale bioreactors in the development of a fermentation process for the production of HMOs. Applikon's micro-Matrix platform, operating 24 microbioreactors at a working volume of 2-5 mL with individual control of pH, dO_2 and temperature, was evaluated against the use of benchtop fermenters and a conventional deep-well assay. A feeding strategy based on an automated pH-triggered feed start was applied as part of a high-cell density process using a defined medium. Four different feed regimes/flow rates were tested to establish conditions that give an overall productivity similar to that of a benchtop fermenter. The results demonstrate that the micro-Matrix is useful for the screening of different flow rates and evaluation of new production strain candidates.

P10 Mass transfer improvements in industrial fermentors

Z. Baumer, K. Alsaker, S. Woodard, D. Hester and T. Rau, Evonik Corporation, Lafayette, IN, USA*

The oxygen transfer properties of fermentation processes are critical for optimizing productivity. Enhanced mass transfer can facilitate other process improvements through media reformulation, higher cell densities, and improved regulation of oxygen-sensitive pathways. We analyzed k_La at production scale (60,000 L) for a viscous fermentation process utilizing multiple flights of Rushton and pitched blade impellers and examined the effects of altering (a) sparger design, (b) blade count and diameter, and (c) agitator replacement with low-shear A340 hydrofoils. The modification results varied from k_La reductions to significant, 20-35% improvements. The enhancement in oxygen transfer can be used as a path forward toward implementing other productivity improvement strategies at multiple scales.

P12 Production of agarobiose by acid hydrolysis of agarose from red macroalgae

D.H. Kim, S.H. Lee and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Macroalgae contain large amounts of carbohydrates. Therefore, they are considered as renewable resources of carbohydrates. Among various polysaccharides in macroalgae, agarose is the major component of red macroalgae. Oligosaccharides of agarose are being revealed to exhibit various physiological functions. To predominantly produce agarobiose among various agarooligosaccharides from agarose, we have developed an acid hydrolysis process using phosphoric acid in this study. To effectively produce agarobiose, prehydrolysis conditions were optimized. The optimal conditions were found to be the solids loading of 30.7% (w/v), the operating temperature of 110°C, the operating time length of 10 min, and the phosphoric acid concentration of 2% (w/v). At these optimal conditions, the agarobiose yield of 70.0% based on the input mass of agarose was obtained.

P14 Enhanced fatty acids production by *Saccharomyces cerevisiae* engineered by CRISPR-Cas9

D. Kim and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Saccharomyces cerevisiae is a Generally Recognized As a Safe (GRAS) strain. Therefore, *S. cerevisiae* is widely used as a fermenting starter for the food and beverage industries. To fully meet the industrial needs, it is sometimes necessary to genetically modify *S. cecerevisiae*. For genetically modified organisms (GMO), always legal and consumers' concerns are followed. To possibly avoid the GMO issue,

in this study, we exploited the CRISPR/Cas9 system that is the marker- and scar-free genome editing tool. Using the CRISPR/Cas9 system, we engineered *S. cerevisiae* to increase fatty acid production. More specifically, isocitrate dehydrogenase genes (*idh1* and *idh2*) in the TCA cycle was disrupted to accumulate citrate and then ATP-citrate lyase genes (*ylacl1* and *ylacl2*) were overexpressed to convert citrate into Acetyl-CoA that is the essential precursor of fatty acids biosynthesis. These engineering resulted in 37.1% increase of total fatty acids compared with that of WT strain. This marker- and scar-free engineered *S. cerevisiae* could be used as an alternative starter with high fatty acid production capability.

P16 Production of high titer 3,6-anhydro-L-galatose from agar by chemical liquefaction and enzymatic saccharification

K. Cho, D.H. Kim, N. PARK and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Red macroalage are regarded as renewable resources due to their high contents of carbohydrates. The main carbohydrate in red algae is agarose that is composed of 3,6-anhydro-L-galactose (AHG) and D-galactose. In particular, AHG is found to possess diverse biological properties such as anticancer and skin-whitening, moisturizing activities. To produce AHG from agar, the process combining chemical liquefaction and enzymatic sacchrification was developed by our group. In this study, to produce AHG from agar at high titers, we have optimized the three steps of AHG production process: chemical liquefaction of agar into agarooligosaccharides using Tris−HCl buffer; enzymatic hydrolysis of agarooligosacchrides into agarotriose and neoagarobiose using crude cell free extract of exo-β-agarase (Aga50D); and enzymatic hydrolysis of agarotriose and neoagarobiose into AHG and D-galactose using crude cell free-extract of both agarolytic β-galactosidase (ABG) and α-neoagarobiose hydrolase (NABH). The optimal chemical liquefaction conditions were 150°C and 40 min using 20 mM Tris−HCl buffer at a 20% (w/w) solids loading. The optimal loadings of the three crude enzymes, Aga50D, ABG, and NABH, were 60, 58, and 60 U/g agar, respectively. The final titer and yield of AHG achieved using the optimal conditions were 39.0 g/L and 28.2% (w/w), respectively. These results can be used as basic information for the industrial production of AHG from agar.

P18 Lacto-vinegar production by *Zymomonas mobilis*

P22 A three-zone predictive scale up method for system biology

D.G. Mou, Moubio Knowledge Co., Taipei, Taiwan*

Shake flask culture is known for limited surface aeration and atmospheric gas exchange. Ultimate enhancement of oxygen transfer in shaker culture relies on atmospheric oxygen enrichment or culture volume reduction or both. They made high throughput studies of micro-reactor cultures a success as soon as micro titer plates and their automation became available. However, problem remains in the low shear and low mixing surface aeration which cannot be resolved simply by a matching k_La – mass transfer coefficient k_L, multiplied by specific gas-liquid interfacial area, *a*. On the other hand, impeller dispersed line gas sparging in production scale stirred tank reactor (STR) is not easily scale down to or reproduced in mini and micro scale vessels like shake flask or micro titer plate without compromising their advantage in simplicity and economy. As success of combinatorial genomic DNA reconstruction, editing and subsequent high volume phenotype screen and validation spread in the new system and synthetic biology, predictive scale up conditions, like realistic impeller stirring, gas-liquid mixing and gas phase oxygen and carbon dioxide partial pressures, in mini or micro scale stirred reaction vessels have driven the high throughput fermentation device market ever since. This presentation will go over pros and cons of mini jar (in 10-100s mL) and micro well (in 100s µL) reactors in gas-liquid mixing design, and how and why a 3-zone mixing model and reactor vessel innovation (US Patent 8,162,295), made without engineering jargon, can benefit high throughput fermentation in phenotype validation and predictive scale up.

P24 Yeast extracts & peptones: optimal nutritional sources for culturing lactic acid bacteria

L. Jacob, Sensient Technologies, Hoffman Estates, IL, USA and D. Antibus, SENSIENT TECHNOLOGIES, Hoffman Estates, IL, USA*

The increasing demand for the growth of dairy & probiotic cultures has led to a strong interest in understanding microbial physiology and establishing optimal fermentation media for these lactic acid bacteria . This study highlights how yeast extracts serve as highly effective complex nitrogen sources and have strong influence on both cell biomass quantity and quality. They are abundant in peptides, amino acids and various growth factors that meet the high nutritional demands of fastidious bacteria. The supplementation of yeast extracts with peptones resulted in further increase in biomass production. M17 & de Man Rogosa Sharpe (MRS) culture media were each modified using animal-free and allergen-free yeast extracts and peptones to enhance & optimize the growth of various lactic acid bacteria.

P26 Use of bio-capacitance probes as an advanced process analytical tool at the manufacturing scale

J. Carvell, Aber Instruments Ltd, Aberystwyth, United Kingdom*

Real-time bioprocess monitoring is fundamental for maximizing yield, improving efficiency and process reproducibility, minimizing costs, and optimizing product quality. . The FDA's Process Analytical Technology initiative (PAT) encourages bioprocess workflows to operate under systems that provide timely, in-process results.The detection of biomass is one of the most requested parameters in industrial cell cultivation. The knowledge of the biomass progress during a fermentation process gives deeper process knowledge and control and helps to define harvest or infection points.
The radio frequency impedance (RFI) or bio-capacitance method for online *in-situ* detection of viable biomass has become well established in biopharmaceutical and the technology has an added appeal that it can now be applied to rocking motion and stirred disposable bioreactors.

Many of the modern cell culture processes are often operating at very high cell densities and in these cases the product titer and quality can be very sensitive to the amount of nutrient feed added. This paper will focus on how the industry is now using bio-capacitance probes for controlling nutrient addition. It will provide examples of how integral bio-capacitance is used in manufacturing processes up to 15,000L and will also discuss the merits of using uncorrected capacitance values rather than attempting to convert this into a figure that matched the offline VCD.

This paper will also focus on how several groups have used RFI scanning, from 100KHz to 20 MHz, to comparatively profile multiple bioreactor runs and elucidate fine details concerning cell viability and mechanism of cell death

P28 Dissolved carbon dioxide (dco2) as a critical process parameter in upstream bioprocessing

J. Lattari, Mettler Toledo, Billerica, MA, USA*

This presentation would be a general review on the importance of measuring CO2 in bioprocessing. DCO_2 is a critical parameter from a quality and performance perspective, impacting productivity, growth rates, and product quality.

DCO2 concentration directly impacts key metabolic pathways and intracellular and extracellular pH

Inline DCO2 measurement can be critical for process control and optimization, scale-up and scale-down models, and process understanding.

P30 Development of a new fast fermentation screening system for cell and process optimization

J. Rupprecht[*] and T. Adams, Sartorius Stedim Biotech, Goettingen, Germany; B. Zoro and A. Rees-Manley, Sartorius Stedim Biotech, Royston, United Kingdom

The development of biopharmaceuticals or biotechnological products derived from microbial fermentation is a financially risky endeavors and time consuming process, requiring technical upstream solutions which help to speed up this route and increase likelihood of success.
We have identified in particular the early steps of strain and process development offering best prospects to speed up the entire process significantly by using a reliable screening system.

Based on the well-proven ambr® principle we designed with ambr 15 fermentation an instrument perfectly matching the demand of early steps in the development of microbial fermentation products. The multi-fermentation unit allows, with a working volume just large enough to resemble larger scale processes, the screening for suitable clones, strains or growth conditions.
In two case studies with industrial partners using *E. coli* and *P. pastoris*, consistent and efficient control of fermentations across a variety culture conditions (e.g. feed, temperature, duration, pH) could be demonstrated.

A comparison of multiple replicates proved the reproducibility of ambr 15 fermentation and reliability of the system for screening processes.

In particular two procedures are of high relevance in microbial fermentations. High cell densities could be achieved for both strains in concentrations typical for bench-top scale systems. Furthermore the system allows with fed-batch cultivation one of the most commonly used procedures already at milli-scale, opening up the opportunity to scale-up such a process as shown for a 1L bench-top and 30L stainless steel system.

P32 Engineer *Aspergillus niger* by CRISPR/Cas9 for industrial bioreactor

L. Leynaud-Kieffer[*], Lawrence Berkeley National Laboratory, Emeryville, CA, USA

The conversion of the biomass into advanced biofuels faces many challenges, one of which is finding the right organism for the job. The filamentous fungus *Aspergillus niger* has been chosen as a biocatalyst for cellulose, hemicellulose, and lignin degradation because it can secrete numerous hydrolytic enzymes, such as lignin modifying enzymes (LMEs) and its genome sequence is available.
However, we currently lack efficient tools for editing and augmenting the *A. niger* genome. While genome editing techniques such as CRISPR/Cas9 editing function in *A. niger*, we are limited by the difficulty of making multiple mutations, restricted selection of markers, and inefficient, expensive and time-consuming methodologies for genome engineering.

Here I present progress towards developing a method for efficiently making multiple genomic mutations via Cas9/gRNAs without the use of selective markers. This technique utilizes several approaches; 1) pyrG positive and negative selection for transient plasmid maintenance, 2) a self-targeting plasmid for selection of Cas9 activity. Once complete, this strategy should remove the need for screening of colonies to identify mutants. Our objective is to first establish this method for genome engineering, and build a library of *Aspergillus niger* strains. Then we will design two type of bioreactor, a submerged fermentation and solid state fermentation. The objective is to define the best strains and conditions for the productivity of LMEs in bioreactors at a pilot scale for industries.

P34 Boosting bioprocess performance by using the right yeast extract

K.M. Bekers[*] and M.J. van der Werf, Ohly GmbH, Hamburg, Germany

Yeast extract is widely used in industry to boost cell growth and enhance productivity, titer and product yields in fermentation processes. Opposed to chemically defined media, it has the benefits of providing ready to use complete building blocks for cells, in addition to a range of cofactors, vitamins and growth factors. However, being such a complex mixture of nutrients, generally little is known on which components in the yeast extract are responsible for its beneficial results. Moreover, it is generally not known to biotechnologists that yeast extract can be highly different in composition. As a result, process optimization by adding yeast extract is generally only reviewed on a basic level.

To review the impact yeast extract can have on fermentation processes, a study was conducted in which the effect of three highly different yeast extract (combination)s on the performance of industrial relevant organisms was evaluated. In particular, (combinations of) Ohly® KAT (yeast extract high in free amino acids), Ohly® CTT-R (yeast extract containing relatively high nucleotide concentrations) and Ohly® PTU (yeast extract high in peptides) were tested in this study. They were reviewed with a 10-point experimental design to find an optimal blend.

The results show that there is clearly a potential for process optimization, if the right combination of yeast extracts is supplied. The experimental approach of testing ten blends as applied in this research shows to be a simple pragmatic approach to improve industrial production processes without the need for indepth knowledge about the exact composition of the yeast extract.

P36 Evaluation of industrial-grade commercial cellulases for the enzymatic hydrolysis of hydrothermally-pretreated empty fruit bunches

J.K. Kim, J. Yang and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Performance of cellulase in the enzymatic hydrolysis of lignocellulosic biomass largely depends on the characteristics of biomass feedstocks. Pretreatment methods are known to significantly affect the characteristics of lignocellulose. To obtain high sugar yields from pretreated lignocellulose, wise selection of effective cellulase for specifically pretreated biomass, which is based on the characteristics of cellulase and pretreated biomass, is important. In this study, we have evaluated industrial-grade commercial cellulases from major enzyme companies, such as Accellerase 1000, Accellerase 1500, and Spezyme CP from DuPont and Cellic CTec2 from Novozymes, for their hydrolysis efficiency with hydrothermally-pretreated empty fruit bunches (EFBs). Among the four cellulases tested, Cellic CTec2, which indicated the highest cellobiohydrolase, xylanase, and β-glucosidase activities, showed the highest glucose yield. The highest glucose yields of 91.3% and 84.7% (both based on the theoretical maximum glucose) were attained with 30 FPU of Cellic CTec2/g glucan with and without Cellic HTec2, respectively. These results would be valuable information for the selection of enzymes for the industrial-level enzymatic hydrolysis of hydrothermally-pretreated EFBs.

P38 Use of osmotic and temperature shock to improve viability of spray-dried microbial fungicides

S. Koganti and A. Schlesinger, AgBiome, Durham, NC, USA*

Despite high-quality agronomic practices, chemistries, and germplasm, growers continue to experience crop yield losses of approximately 30% due to pests and diseases. At AgBiome, we discover solutions to combat these problems based on a large and expanding core collection of fully-sequenced microbes from the plant-soil microbiome. It is well-known that production cost is a key factor in the commercial success of biological pesticides, particularly for use in agronomic crops such as soy and maize. Our lead biological product candidate, Howler™, is a wettable powder, and is efficacious against multiple fungal pathogens in field testing across multiple locations. Manufacturing process development has focused on low-cost drying options, including spray drying. The microbial active in Howler, *Pseudomonas chlororaphis* AFS009, is a novel Gram-negative isolate which has been formulated to be shelf-stable for 24 months and counting when blended with formulants and tray-dried. Spray drying is a much more attractive drying technology due to its lower cost and higher capacity; however, the use of high temperature and high shear can negatively impact the viability of biopesticides. The viability of this isolate in formulations which are spray-dried has been improved by 100 fold by the use of osmotic shock in the fermentation stage by the use of NaCl. Temperature shock in stationary phase produced improvements of 5-10 fold. Taken together these process changes represent a significant improvement in the ability to dry this product via lower-cost technologies and reduce the overall production cost of the biopesticide.

P40 *E. coli* culture and kLa comparison of single-use and steel tank fermenters

J. Brown, C. Brau and N. Jones, Thermo Fisher Scientific, Logan, UT, USA*

Recent advancement has led to the development of single-use reactors for use in microbial fermentation. The Thermo Fisher Scientific Hyperforma Single-Use Fermentor (SUF) is the first specifically designed fermentor to deliver equivalent performance to stainless steel SIP/CIP reactors for research and pilot scale microbial bioproduction at 30L and 300L liquid working volume. Due to the single-use film wall, sparge, and agitation implementation; there can be limitations when moving aggressive dense cultures from steel tank to single-use reactors. To characterize the limitations of available single-use bioreactors a rigorous comparison was undertaken in collaboration with multiple vendors. For kLa evaluation the same procedure, salt solution formula, and single-use dissolved oxygen probes were used to obtain comparable results. The various reactors were tested at optimal preset RPM and air flow rates without any oxygen supplementation allowed. Results were supplied to reactor vendors without disclosing the supplier names. Here we present the performance of Thermo Fisher Scientific Hyperforma Single-Use Fermentor (SUF) 30L and 300L in comparison to other unnamed vendor options. In addition we compare growth and protein production in the SUF to traditional stainless steel fermentors standard procedures, which show equivalent growth of *E.coli* to 200 OD_{600} with product yield of 8 g/L as were seen in SIP/CIP fermenters.

P42 The effect of design and scale on the mixing and mass transfer in U-loop bioreactors

L. Petersen, J. Villadsen, S.B. Jørgensen, A.E. Lantz and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark; I. Christensen, Unibio A/S, Odense M, Denmark*

A system capable of handling a large volumetric gas fraction while providing a high gas to liquid mass transfer is a necessity if the metanotrophic bacterium Methylococcus capsulatus is to be used in single cell protein (SCP) production.
Previous studies have proven that a U-loop fermenter, a novel vertical forced flow loop reactor where gas and liquid are driven through a series of static mixers in a U-shaped pipe, is quite capable of coping with these challenges in pilot scale. The critical question remains; what happens when the scale undergoes a more than 10 fold increase and the geometry is altered?

In this study we have investigated the mixing time and mass transfer capabilities of U-loop reactors of different geometries (high vs. diameter ratio) in pilot (0.15m^3) and semi-industrial scales (2.2m^3). A new expression for the mechanical power input into the system is also proposed, which indicates that an even more favorable relationship between power input and mass transfer rate (compared to previous literature) applies to U-loop fermenters.

P46 Imaging for monitoring downstream processing of fermentation broths

R. Moiseyenko, A. Baum and T.M. Jørgensen, Technical University of Denmark, Lyngby, Denmark; S. Glanville, Novozymes A/S, kalundborg, Denmark; C.N. Laursen, ParticleTech, Farum, Denmark; S.S. Mansouri, Technical University of Denmark, kongens lyngby, Denmark; K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

In relation to downstream processing of a fermentation broth coagulation/flocculation is a typical pre-treatment method for separating undesirable particles/impurities from the wanted product. In the coagulation process the negatively charged impurities are destabilized by adding of a clarifying agent thereby neutralizing the charges on the particles. Particles thus agglomerate. Larger agglomerates are formed in the flocculation process by adding a polymer, which forms bridges between the particles. The operation of coagulators, flocculators and clarifiers requires trained operators implying the human factor to play a major risk with regard to performance. Better process monitoring will provide the means for improved control giving higher yield, better quality, and minimize the consumption of water. In particular,

the optimal separation of biomass from a soluble enzyme phase is often dependent on an initial coagulation of the biomass and a final flocculation of the solids just prior to separation. We investigate flocculation processes at Novozymes facilities so that the response time and risk of error is minimized. We use oCelloScope [1], an automated microscope, for imaging samples from the flocculation process and subsequently we extract image features for qualitative and quantitative image characterization. The processing include image morphology, image segmentation and image quantification. The aim is to correlate image information to "quality" of the separation process. Here we report our initial finding.
[1] M.Fredborg et al. Journal of Clinical Microbiology Vol 51 Number 7 p. 2047–2053 (2013);
http://www.biosensesolutions.dk

P48 Bioprocess optimization achieved two-fold increase in terpene and ketone titers in two non-canonical hosts

M. Mirsiaghi[*]*, F. Masson, D. Tanjore, T. Pray and E. Sundstrom, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

The primary objective of this study was to develop industrially relevant processes with two non-canonical hosts, *Rhodosporidium toruloides* and *Streptomyces albus*, for conversion of cellulosic hydrolysate into renewable fuels. Collaborators at the Joint Bioenergy Institute (JBEI) engineered *R. toruloides* and *S. albus* for terpene and short-chain ketone production, respectively. At the Advanced Biofuels Process Development Unit (ABPDU), we scaled each process from shake flask to 2L fermentor and optimized production of target molecules by varying carbon sources, pH, dissolved oxygen concentration, and feeding regimes.
R. *toruloides* is capable of producing fatty acids along with terpenes, both of which can be used as biofuel, and is known to ferment glucose, xylose, and aromatic byproducts of lignin degradation. High gravity batch fermentation doubled terpene (bisabolene) titer as compared to fed-batch fermentation with corn stover hydrolysate. Two engineered *Streptomyces albus* strains were tested for production of a mixture of C5-C7 ketones. *Streptomyces* species generally require complex media for production of secondary metabolites; we were able to replace complex media components with cellulosic hydrolysates from corn stover, poplar, and bagasse. The optimized process eliminated several high-cost media components while doubling ketone titers as compared to the control medium. Alkali-pretreated corn stover hydrolysate led to the highest ketone titers reported to date (>500 mg/L in shake flasks); this feedstock was chosen for scale-up to 2L fed-batch fermentation. Full volatilization of C5 and C6 ketones was observed during fermentation process optimization at 2L-scale, highlighting the potential for low-cost recovery of gas-phase fuel molecules.

P50 Application of a model-based soft sensor to monitor lactic acid bacteria fermentations at pilot scale

R. Spann[*]*, A.E. Lantz, K.V. Gernaey and G. Sin, Technical University of Denmark, Kgs. Lyngby, Denmark; C. Roca, Chr. Hansen Holding A/S, Hoersholm, Denmark*

A model-based soft sensor was applied to monitor a 700 L *Streptococcus thermophilus* fermentation. The soft sensor was based on a data reconciliation module and a first principles mechanistic model. The data reconciliation module used a general process stoichiometry model to update some of the mechanistic model parameters with 5 minutes intervals using the very limited available on-line measurements, which were ammonia addition and pH. The updated parameters were used as input to the mechanistic model that combined biological (biomass growth and lactic acid production) and chemical (pH) mechanisms of the fermentation process. The model was then used to predict unmeasured, important process parameters, such as biomass, lactose, and lactic acid, and the measured pH. This process analytical technology (PAT) monitoring system was applied in MATLAB® (The MathWorks®, Natick, MA) to a historical data set of a 700 L fermentation where the on-line data was used as available on-line. A good prediction accuracy was obtained with an error less than 10 % of the biomass concentration. Uncertainty analysis was also performed using the Monte Carlo technique to quantify model prediction uncertainty when making predictions for the fermentation batches. The presented monitoring system is a promising

tool to improve the operation of LAB fermentations as it provides the operators with additional information of the process in real-time.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 643056. We are grateful to Chr. Hansen A/S for the experimental support.

P52 Lean process validation

J. Gunson, N. Krishnan and R. Hamilton, Genentech, Inc, South San Francisco, CA, USA*

A process validation (PV) effort was performed for an antibody fragment (Fab) produced in *Escherichia coli*. Using a Quality by Design (QbD) approach, the impact of eight production culture process parameters on eight potential critical quality attributes (pCQAs) was evaluated in a single PV study. The study consisted of a one statistical design of experiment (DOE), resolution V fractional factorial, in addition to several univariate assessments. No PV studies were performed for the inoculum culture steps in the fermentation process. The reduced number of studies along with the smaller number of process parameters and pCQAs evaluated in the single production culture study was the outcome of process parameter and pCQA risk ranking and filtering (RRF) exercises. These assessments leveraged existing process data and relevant stress model results to inform the lean PV effort.

P54 Raman spectroscopy for in situ, real-time fermentation monitoring

K. Esmonde-White, M. Cuellar, S. Gilliam and I. Lewis, Kaiser Optical Systems Inc., Ann Arbor, MI, USA; C. Uerpmann and B. Lenain, Kaiser Optical Systems SARL, Saint Priest, MI, France*

In situ Raman during bioprocesses enables simultaneous measurement of multiple cellular biochemistry and bioreactor parameters and allows for in-process monitoring and control. Raman is well-suited for many upstream and downstream bioprocess applications because of its ability to directly measure aqueous systems, strong history as a process analytical technology in small molecule pharmaceutical manufacturing, excellent model transferability and sampling versatility.

Raman spectroscopy can be used to measure gases, liquids, solids and turbid media. We describe technologic and ergonomic considerations in sampling probe design and how these factors affect implementation of *in situ* Raman spectroscopy for bioprocessing applications. Immersion probes are compatible with the bioreactor environment and sterilization protocols, provide simultaneous *in situ* measurements of multiple bioprocess parameters, and enable real-time process monitoring and control. Examples of immersion Raman probes will be shown in bioreactor and fermentation biogas production. New sampling systems compatible with single-use bioreactors (SUB) will be introduced and data comparing SUB-compatible optics with immersion probes an analytical standards model will be discussed. Through representative examples, we show Raman spectroscopy as a robust and reliable analytical technology for in situ fermentation application.

P56 The narrow balance of feeding charged nutrients

C. Sellers, Heliae Development, LLC, Gilbert, AZ, USA and E. Ganuza, Heliae Development LLC., Gilbert, AZ, USA*

A pH-auxostat is a self-titrated fed-batch system that provides a nutrient (i.e acetic acid) on demand to the culture. As the name implies, this system is designed to maintain the residual nutrient (auxo-) concentration constant (-stat). However, along with the acetate oxidation there are other biochemical processes would impact the medium alkalinity, resulting in the buildup of toxic acetate concentrations or conversely the displacement of all the acetate from the system. Our goal was to study the impact of the nitrogen source on the residual acetate concentration and ultimately design a process that could operate according to the auxostat principles. We used an oleaginous microalgae strain, *Aurantiochytrium* sp. HS399, that uses acetic acid as building block for lipid synthesis and energy. Real time monitoring of residual nutrients was achieved thanks to the Cedex Bio Analyzer (Roche Diagnostics (Schweiz,

Switzerland), which conveniently produced results in less than 20 min. When glutamate was used as a nitrogen source residual acetate built up to potentially toxic concentrations. In turn, when ammonium chloride was used as a nitrogen source residual acetate was displaced from the media resulting in the arrest of the pH-auxostat system. Finally, when ammonia was provided as ammonium acetate, residual acetate was balanced to minimize toxicity while avoiding the auxostat interruption. Feeding charged nutrients such as ammonia, acetic acid and managing their toxicities requires a deep understanding of the metabolic processes that go beyond the pH-auxostat control of one particular nutrient.
[CS1]Average test is 12-18 minutes

P58 Development of generic Raman models for process monitoring

B. Hadley and T. Webster, Lonza Biologics, Portsmouth, NH, USA*

Manufacturing safe and consistent bio-therapeutic products requires robust process monitoring and control of production bioreactors. This historically required sample removal, expensive and sometimes toxic reagents and skilled operators. The implementation of novel process analytical technologies for continuous monitoring has been proposed by regulatory agencies as a means of improving process control. Inline Raman spectroscopy coupled with multivariate software to develop predictive models for critical process parameters was used to create, generic, cell line-independent models to continuously monitor critical process parameters in bioreactors operating Lonza's platform processes.
Generic Raman models were developed by culturing two different GS-CHO cell lines using a platform process in 12 five-liter bioreactors. Raman spectra and offline measurements were collected twice daily during the course of cell culture in order to construct predictive models. Metabolites, VCC & TCC, and titer were measured offline using a Nova Bioprofile 400, Vi-Cell XR Analyzer, and Protein-A HPLC respectively. Projections on latent structure models for each parameter of interest were created in SIMCA v13.0.3 by regressing Raman spectra with their corresponding offline measurements.

Validation against a third independent GS-CHO cell line provided predictive error of the models. Raman was capable of monitoring changes in the concentration of glucose, lactate, ammonium, viable cell concentration, and total cell concentration with prediction errors of 0.44 g/L, 0.24 g/L, 0.028 g/L, 1.92 x 10^6 cells/mL, and 1.89 x 10^6 cells/mL respectively with Lonza's platform process. With online continuous monitoring, process improvements or realtime feedback control could be used for Lonza's platform processes in the future.

P60 Application of novel free-floating sensor device: Flow characteristics in stirred vessels

J. Bisgaard, J. K. Huusom, N. K. Poulsen and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark; O. Skyggebjerg and L. V. Petersen, Freesense ApS, Copenhagen N, Denmark; S. Cornelissen, Novozymes A/S, Bagsvaerd, Denmark*

In this initial study, a novel process analytical technology (PAT) tool for online monitoring of industrial bioreactors is presented. The technology is a free-floating sensor device which is robust, steam sterilizable and capable of measuring critical culture parameters. The sensors are designed for high sample rates and fast response times, while a patented positioning system gives information on the position at which the parameters are measured. Consequently, this novel sensor can also be used for analysis of flow characteristics and gradients in industrial scale bioreactors.
The free-floating sensors have been tested in a large-scale bioreactor (>100 m^3) filled with water, at different agitation speeds. Pulses of sodium hydroxide were added to compare the pH response of the free-floating sensor with the response of commercial pH-sensors fixed to the tank wall. Circulation times and spatial distributions were determined based on the collected pressure measurements. It could be seen from the distributions and axial velocities that mixing was limited in the top and bottom of the reactor. As expected, a noticeable decrease in the circulation time was found when increasing the impeller speed. Using free-floating sensors rather than fixed sensors can provide unexplored data which has not previously been available. This data can link measurements of culture parameters with positions and flow characteristics within the reactors. This can serve as an important tool in validation, control and

regulation of industrial processes, as well as a tool for process optimization where it should be possible to reduce development cycle time during process scale-up.

Biology's role in energy storage: A unique 2-step process to turn renewable electricity into renewable methane

K.W. Harrison[*] and N. Dowe, National Renewable Energy Laboratory, Golden, CO, USA

The cost of wind- and solar-generated electricity has decreased significantly over the past few decades and is now competitive with fossil-based generation. Low-cost and otherwise curtailed renewable electricity is beginning to open up new markets in the areas of gas fermentation for long-duration energy storage. The National Renewable Energy Laboratory (NREL) and Southern California Gas Company (SoCal Gas) are collaborating on a first-of-its-kind in the United States Power-to-Gas project to shift energy and enable even higher penetrations of renewable electricity generation on the utility grid. The two-step process first involves splitting water electrochemically via low-temperature water electrolysis to produce renewable hydrogen. Secondly, a biologically-based anaerobic methanation process converts the hydrogen and carbon dioxide in to methane, heat, and water. The methanogen, *Methanothermobacter thermautotrophicus,* is at the heart of the NREL/SoCal Gas collaboration and will be run in a new 700 Liter bioreactor at pressures up to 18 bar in Golden, Colorado. The team also includes Electrochaea GmbH, the company commercializing the technology, and Burns & McDonnell, the engineering firm that oversaw the design and fabrication of the system. NREL will characterize the system performance under steady-state and highly variable (i.e., wind and solar) power profiles to understand the microorganisms performance over the pressure, temperature and agitation range that the system will operate in. The NREL/SoCal Gas pilot project will be used to determine the commercial viability of this power-to-gas approach to energy storage and provide insights into megawatt-scale system designs.

Real-time optimization of fermentations and cell cultures profitability - from R&D up to biomanufacturing

J. Sirois[*], BioIntelligence Technologies inc, Sherbrooke, QC, Canada

Biomanufacturing and bioprocess development suffer from a lack of probes to perform a valuable monitoring of product biosynthesis and its optimization. Many samples have to be taken and analyzed with instruments such as HPLC to have an idea of the kinetics and yields during fermentations and cell cultures. This constrain induces delays and losses. The BioAnalyst is a new instrument collecting data from any source, being probes on a bioreactor, datafile from a HPLC or user entries from manual analysis, and performing real-time calculations to unveil hidden information such as biokinetics. The embedded algorithms push information on personal sets of dashboards for each user enabling them to accelerate decision making and implement real-time optimization. A layer of algorithms is dedicated to translate scientific information into financial information. This interconnectivity hub reduces time wasted on data capture, data management and data analysis, increases profit margin by reducing costs and losses, and increases throughput with no change on actual bioprocesses.

Tuesday, October 31

7:00 AM - 8:00 AM Breakfast All Registered Attendees

Calusa Terrace, Lobby Level

7:00 AM - 5:00 PM Registration

Calusa Prefunction, Lobby Level

8:00 AM - 11:30 AM Session 4: Advances in fermentation and cell culture

Conveners: **Zhibiao Fu**, GlaxoSmithKline Vaccines, Rockville, MD, USA and **Jim Xu**, BMS, Devens, MA, USA

Calusa Ballroom ABC, Lobby Level

8:00 AM S23: Small scale model qualification for a *Pichia pastoris* platform process employing Raman Spectroscopy-based monitoring as a PAT tool

J. Aon, GlaxoSmithKline, King of Prussia, PA, USA*

Pichia pastoris, a methylotrophic yeast, is an attractive expression system for the high level expression of recombinant proteins due to its prokaryotic growth characteristics to reach high cell densities, its efficient secretory system, and the many tools available for molecular manipulation. *P. pastoris* has one of the strongest and tightly regulated promoters, alcohol oxidase I (*AOX*1), and the ability to perform post-translational protein modifications available only in eukaryotic systems.

For the development of the platform, the two main objectives included: (*i*) a robust fermentation process; and (*ii*) a scalable platform for fermentations. The range explored in this study ranged from small (e.g., 0. 250-L vessels) to large (e.g., 150-L vessels) scales that would provide the needed flexibility to meet different business objectives, including throughput screening of multiple yeast strains or target proteins to fulfilling large-order customer requests of drug substance as well as validate some aspects of scalability of the process.

Results are based on a *P. pastoris* fermentation platform, which is a fed-batch, high-cell density culture process. The platform, tested on two different recombinant proteins, can be readily applied to future programs and early phase development work. Preliminary characterization of this platform was performed by measuring multiple bioprocess metabolites *in situ* by employing real-time Raman spectroscopy. From a process development perspective, monitoring the changes of key nutrients over time utilizing the *in situ* Raman spectroscopy, (*i*) increases process knowledge, and (*ii*) provides real-time data to evaluate the process “state of control” to make informed process decisions.

8:30 AM S24: Understanding Free Thiol Variability in a Recombinant Antibody Produced in *Escherichia coli*

K. Veeravalli, Genentech, Inc, South San Francisco, CA, USA*

Variability in the levels of free thiol (up to ~10-fold difference in % free thiol peaks) was observed in mAb1 drug substance batches manufactured using *E. coli* as the production host. Free thiol was one of the product quality attributes of particular interest during the technical development of mAb1. The process step and the enzyme responsible for generation of free thiols were identified. The root cause of the variability and some potential approaches to control this issue will be discussed.

9:00 AM S25: Lipid production using methane as the sole carbon soure in high cell density cultication

Q. Fei, Xi’an Jiaotong University, Xi’an, China*

Methanotrophs have been utilized for bioremediation and biocatalysis for decades due to the versatile enzyme, methane monooxygenase (MMO). This enzyme catalyzes the first step in methanotrophy, the controlled oxidation of methane to methanol and allows the methanotroph to utilize methane as both carbon and energy source. Methanotrophic bacteria have the ability to convert methane to all cellular components and thus have the potential to serve as a production platform for the conversion of methane into valuable products including biofuels via lipid biosynthetic pathways and catalytic hydrodeoxygenation upgrading. Because of the relatively low price of natural gas and increasing demands of liquid transportation fuels, attention has begun to turn to methantrophic bacteria for biofuel production. In this study, *Methylomicrobium buryatense* was investigated to achieve high lipid titer and productivity in high cell density cultivations (HCDC). The cell growth and lipid production from both strains were studied and compared in order to elucidate the influence of culture conditions on lipid production, gas uptake/evolution rate and glycogen accumulation in batch cultures of both strains. Finally, the fatty acid composition of membrane lipids was analyzed and characterized for diesel fuel production.

9:30 AM Break

10:00 AM S26: Application of Process Analytical Technologies (PAT) to improve process consistency for N-1 perfusion seed bioreactor for a recombinant IgG Chinese Hamster Ovary (CHO) process

T. Erlandson, J.C. Yee, S. Ray, S. Patel, M. Borys and Z. Li, Bristol Myers Squibb, Devens, MA, USA*

Recombinant CHO cells are the vehicle of choice for production of antibody therapeutics and other biologics. In GMP manufacturing, CHO cells are expanded in a series of shake flask, cell bags and seed bioreactors operated at batch mode prior to inoculation of production bioreactors. Recent efforts in process intensification has utilized perfusion seed bioreactors, to reduce the duration of seed expansion and enable the inoculation of production bioreactors at higher cell densities.
Biomass capacitance probes were installed in the perfusion bioreactor to monitor online cell densities. Based on a robust, linear correlation of biomass capacitance output to viable cell densities, the online capacitance data was utilized to dynamically change the media exchange rate to maintain a constant cell-specific perfusion rate (CSPR). The result confirms steady levels of glucose was achieved in a perfusion run based on a constant, low CSPR. The deployment of these online analytical technologies enabled a robust perfusion process while avoiding nutrient depletion in culture. This presentation will also highlight the impact of using perfusion seed culture compared to traditional batch seed culture, from the perspective of better improving cell health, productivity and reducing manufacturing cadence.

10:30 AM S27: Production of C4-C8 terminal alcohols through the '+1 pathway': using proteomics and metabolomics to focus strain development

C. Stowers, P. Sanghani, M. Devarapalli, S. Delaplane and R. Hill, Dow AgroSciences LLC, Indianapolis, IN, USA; D. Rosenfeld, Dow Chemical Company, Freeport, TX, USA*

C4 – C8 alcohols have a wide range of uses including fuels, lubricant additives, plasticizers, corrosion inhibitors and are also precursors to other important chemicals. Production of these alcohols through petrochemical means can be challenging since existing technologies, such as linear alpha oligomerization, are not selective. An important opportunity exists for the development of a bioprocess to selectively produce C4-C8 alcohols from renewable resources. The Dow Chemical Company has developed novel enzymes and micro-organisms for selective production of these alcohols using a '+1 pathway'. Initial studies revealed that pathway performance was highly sensitive to the design of the expression system. OMICs technologies were applied to interrogate the differences in performance between two different expression systems. A proteomic analysis revealed differential expression of pathway genes that was leveraged to guide strain development. Metabolomics and co-feeding studies revealed an enzymatic bottleneck which was alleviated by enzyme engineering.

11:00 AM S28: How can systems biotechnology add values to biopharmaceutical development and manufacturing?

S. Yoon, University of Massachusetts Lowell, Lowell, MA, USA*

The biopharmaceutical industry has grown rapidly over the last two decades. One mission gaining more and more urgency in biopharmaceutical today is to possess indepth knowledge and technology to make production faster and safer. In the recombinant protein area, mammalian cells are mainly used as the hosts for synthesizing and secreting products. Several parameters known to be important to bioprocess quality include cell line stability, productivity and protein quality. The mechanism how cells are responsive to culture environment and affected on its paths to the product amount and quality is elusive. Traditionally, optimization to bioprocesses is made based on imperial observation. Complementarily, systems biology emerges as an approach to gain an understanding of cellular mechanism by seizing 'omics at multiple levels including metabolome, transcriptome, proteome and fluxome. Systems biology enables to make the cellular behavior predictable by mechanistic models so bioprocess optimization and control can be carried out using *in-silico* simulation. In the presentation, discussed are a few case studies showing systems biology approach being applied to investigate problems related with productivity, product quality and cell line stability: 1) A CHO genome-scale model integrated with 'omics to predict cell growth, production and N-linked glycan profile; 2) Glycosylation control by medium supplementation guided by a mathematical model; 3) Understanding and controlling epigenetic changes in long-term continuous cell cultures for biotherapeutic antibody production.

11:30 AM - 1:00 PM Lunch - All Registered Attendees

Calusa Terrace, Lobby Level

12:15 PM - 12:45 PM Exhibitor Showcases: 12:15 pm Applikon

Calusa Ballroom ABC, Lobby Level

1:00 PM - 2:00 PM Session 4: Continued

Conveners: **Jim Xu**, BMS, Devens, MA, USA and **Zhibiao Fu**, GlaxoSmithKline Vaccines, Rockville, MD, USA

Calusa Ballroom ABC, Lobby Level

1:00 PM S29: Versatility in *A. niger*: By combining DNA modifications with cultivation restrictions one can direct high productivities of either acid or protein

K.M. Overkamp, A. Hossain, W. de Bonte and P.J. Punt, Dutch DNA Biotech BV, Utrecht, Netherlands*

The filamentous fungus *Aspergillus niger* is widely used in industry for its secretion capabilities. Interestingly enough, this fungus can produce two very different products, i.e. enzymes or organic acids. The differences between fermentative production of a protein or an acid starts with the genetic make-up of the specific fungal strain used. Furthermore, the applied fermentation conditions play a essential part in optimizing production of the acid or protein. For protein production, which is closely coupled to biomass production, the medium should contain all nutrients (no limitations) for optimal growth. The challenge is to maintain a sufficient supply of oxygen even when viscosity rises at higher biomass concentrations. For

organic acid production the opposite is true, most of the carbon in the medium should go to the product, not to biomass, in order to increase the yield of the acid. The challenge in this case is to find the right limiting factor(s) which keeps the biomass at a low concentration but sufficiently energized to quickly produce high titers of acid. In this presentation examples of both protein and acid production are given. Highlighted are the interdependency of DNA modifications and fermentation protocol changes. Usually several iterative rounds of optimization are needed to arrive at a high, industrially relevant productivity.

1:30 PM S30: Orbital shaken bioreactors: scale up fast track (upto 2500L)

T. Anderlei and T. Buergin, Kuhner AG, Birsfelden (Basel), Switzerland; D. Laidlaw, Kuhner Shaker Inc., San Carlos, CA, USA*

Since more than 60 years the shake flask is the standard shaken bioreactor in biotechnology. Regarding cell cultivation the shake flask has been used intensively since more than 10 years. The presentation gives an overview of the variety of shaken bioreactors from 10L to 2500L scale (ORBShake) focusing on the engineering parameters (eg mixing time, kLa, ...). Furthermore, application and scale up results of antibody and vaccine productions from reasearch institutes and industry using shaken bioreactors will be shown.
Since more than 63 years Kuhner AG, Switzerland is building shakers for the biotechnology field. Kuhner works strongly together with the EPFL, Switzerland, ExcellGene SA, Monthey and the Technical University of Aachen, Germany.

2:00 PM Break

2:30 PM - 4:30 PM Session 5: Industrial collaborations

Conveners: **Christopher McDowell**, Novozymes Biologicals Inc, Salem, VA, USA;**Tiffany D. Rau**, BioProcess Technology Consultants (BPTC), Lake Charles, LA, USA and **Frank Agbogbo**, Cytovance Biologics, Oklahoma City, OK, USA

Calusa Ballroom ABC, Lobby Level

2:30 PM S31: Industrial collaborations: Technical University of Denmark (DTU) and Novozymes

S. Cornelissen, Novozymes A/S, Bagsvaerd, Denmark and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

Novozymes is the world's largest producer of industrial enzymes. The company's products help customers to save energy, water and raw materials, to reduce waste and emissions, and to make everyday products more sustainable. Novozymes' enzymes are often manufactured using large scale aerobic fermentation processes. Scale-up, optimization and streamlining of these processes are important activities within the company and there's an ongoing effort to improve these by increasing process knowledge.
Making models and validating them using real process data and development and application of new control strategies are two valuable ways to increase process understanding. Modelling and control work is often done together with various external partners, i.e. other companies and universities. A major collaboration partner is the Technical University of Denmark (DTU). Over the years, DTU and Novozymes have built up an intensive partnership, which includes the establishment of a professorship in industrial fermentation technology held by Krist V. Gernaey since 2013. This talk will provide an overview of the partnership between DTU and Novozymes and will give a couple of examples that resulted from this collaboration, demonstrating the mutual benefits for both partners.

2:40 PM S32: Biotech innovation through external collaboration

C. Stowers and S. Webb, Dow AgroSciences LLC, Indianapolis, IN, USA*

Dow AgroSciences has a long history of leveraging external collaborations to catalyze innovation and accelerate product development. To date, Dow AgroSciences has engaged in hundreds of collaborations spanning both academic and industrial institutions under a variety of partnership models. These collaborations have proven to be an economical means for technology advancement in areas where the current technology is fragmented or is rapidly evolving. Furthermore, external collaborations provide a low-cost means to quickly evaluate alternative technologies. This presentation will summarize the strategic advantages of using external collaborations for technology development. The critical success factors for productive external collaborations will also be reviewed. Lastly, a few examples of how external collaborations have been used to accelerate bioprocess development will be shared.

2:50 PM S33: Lessons learned in the multi-partner collaboration to develop and commercialize a new Vitamin D2 production process

H. Meerman, P. Zacherl, L. Treiber and S. Levine, Nucelis LLC, San Diego, CA, USA; N. Fong, Nucelis, Inc., San Diego, CA, USA*

Nucelis is focused on delivering naturally sourced and sustainable solutions to the personal care, nutrition and flavor and fragrance markets. Nucelis' business model is to work with partners to apply our Rapid Trait Development System (RTDS™), a suite of proprietary, non-GMO precision gene editing technologies, coupled with extensive fermentation and downstream process development expertise to improve the economics of manufacture of microbial fermentation products. As an internal demonstration project Nucelis developed a novel process to produce and purify ergosterol (which is subsequently photo-chemically converted to Vitamin D2) and squalene in the oleaginous yeast *Yarrowia lipolitica*. Along the path to commercialization of these products we overcame multiple challenges in collaborating with several external organizations to scale-up our fermentation, downstream and photo-conversion technologies. We will discuss how we can apply the lessons learned to future projects.

3:00 PM S34: Industrial collaborations in contract research and manufacturing organizations

J. McCool and F. Agbogbo, Cytovance Biologics, Oklahoma City, OK, USA*

As more and more companies choose to utilize outsourcing partners for clinical and commercial supply of biological drugs, open innovation becomes an increasingly prevalent line item of a CDMOs operating budget for driving core value. In a recent industry survey about CDMO outsourcing, two key criteria for partner selection are "timeline" and "one stop shop". Platform processes and corporate alliances are vital tools for delivering these core value but their implementation can fall short without the appropriate up-front focus and effort. Open innovation is critical for driving the development and implementation of platforms in addition to enabling technologies, product and process analytics and QbD approaches. These are important factors in making CDMOs competitive and better able to cope with the inherent risks in the business (many molecules but few cGMP batches). In this presentation, some tangible approaches for creating and managing goals in the open innovation area that lead to value creation and critical solutions supporting contract partners will be discussed.

3:10 PM S35: Accelerating the bioeconomy: minimizing capital investment through industry-government partnerships

E. Sundstrom, M. Mirsiaghi, F. Masson, F. Tachea, J.P. Prahl, T. Pray and D. Tanjore, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; C.S. Chen, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

Shepherding novel fermentation processes from proof-of-concept to industrial-scale reality requires substantial investment in time, labor, and capital. The technical and market risk associated with these investments necessitates patience over a decade-long time horizon. A growing number of government-funded incubator programs seek to de-risk these early stage investments by providing seed funding, laboratory space, strain development capabilities, process analytics, process demonstration, and technical expertise to technology developers. In response to these resources, a new generation of lean biotechnology startup has evolved to leverage partnership opportunities, enabling small teams of inventors to rapidly achieve pilot or demonstration scale with non-dilutive funding and limited laboratory capabilities. At the Advanced Biofuels Process Development Unit (ABPDU) within Lawrence Berkeley National Laboratory, we have partnered with over 30 small and large companies, helping to accelerate process development and validate novel bioprocesses. As part of a national lab ecosystem that includes the Agile Biofoundry, the Cyclotron Road Program, and DOE SBIRs, Industrial Seedling grants and Small Business Vouchers, we can now provide a full suite of services including strain development, fermentation process development, downstream processing, analytical services, technoeconomic assessment, and expert technical advising. A number of small companies have leveraged the full extent of these capabilities; we will discuss four illustrative case studies (Kalion, Checkerspot, Visolis, and Perfect Day Foods), highlighting the impact of government partnerships on entrepreneurs, technologies, and the broader bioeconomy.

3:20 PM Round table Discussion

6:00 PM - 7:00 PM Reception - Sponsored by NOVOZYMES

Calusa Ballroom D-H, Lobby Level

7:00 PM - 8:30 PM Banquet

Calusa Ballroom ABC, Lobby Level

7:00 PM - 10:00 PM Exhibits dismantle

Calusa Ballroom D-H, Lobby Level

Wednesday, November 1

7:00 AM - 8:00 AM Breakfast All Registered Attendees

Calusa Terrace, Lobby Level

7:00 AM - 10:00 AM Exhibits dismantle

Calusa Ballroom D-H, Lobby Level

7:00 AM - 11:30 AM Registration

Calusa Prefunction, Lobby Level

8:00 AM - 11:30 AM Session 6: Process control, monitoring, PAT and new data analysis methods and tools

Conveners: **Firehiwot Tachea**, Lawrence Berkeley National Laboratory, Berkeley, CA, USA and **Christopher McDowell**, Novozymes Biologicals Inc, Salem, VA, USA

Calusa Ballroom ABC, Lobby Level

8:00 AM S36: Model-based control for fed-batch fermentation: Control for final tank fill

L. Mears, M.O. Albaek and B. Cassells, Novozymes A/S, Bagsværd, Denmark; S.M. Stocks, LEO Pharma A/S, Ballerup, Denmark; G. Sin and K.V. Gernaey, Technical University of Denmark, Kgs. Lyngby, Denmark*

A novel model-based control strategy is developed for filamentous fungal fed-batch fermentation processes. The system of interest is a pilot scale (550L) filamentous fungus process operating at Novozymes A/S. In such processes, it is desirable to maximise the total product achieved in a batch in a defined process time. It is therefore important to maximise both the product concentration, and also the total final mass in the fed-batch system.

In order to achieve this goal, a mechanistic model-based control strategy is developed to drive the process to reach a target mass at a specified time, whilst maintaining the dissolved oxygen concentration above a minimum constraint. A mechanistic model is used to predict the future mass trajectory and determine the error between the target mass and the trajectory in order to adjust the current feed rate accordingly. The model based strategy is implemented successfully in 550L fermenters at Novozymes A/S, with four different sets of process operating conditions.

8:30 AM S37: Real-time online monitoring of fermentations using an FTIR solid state sensor

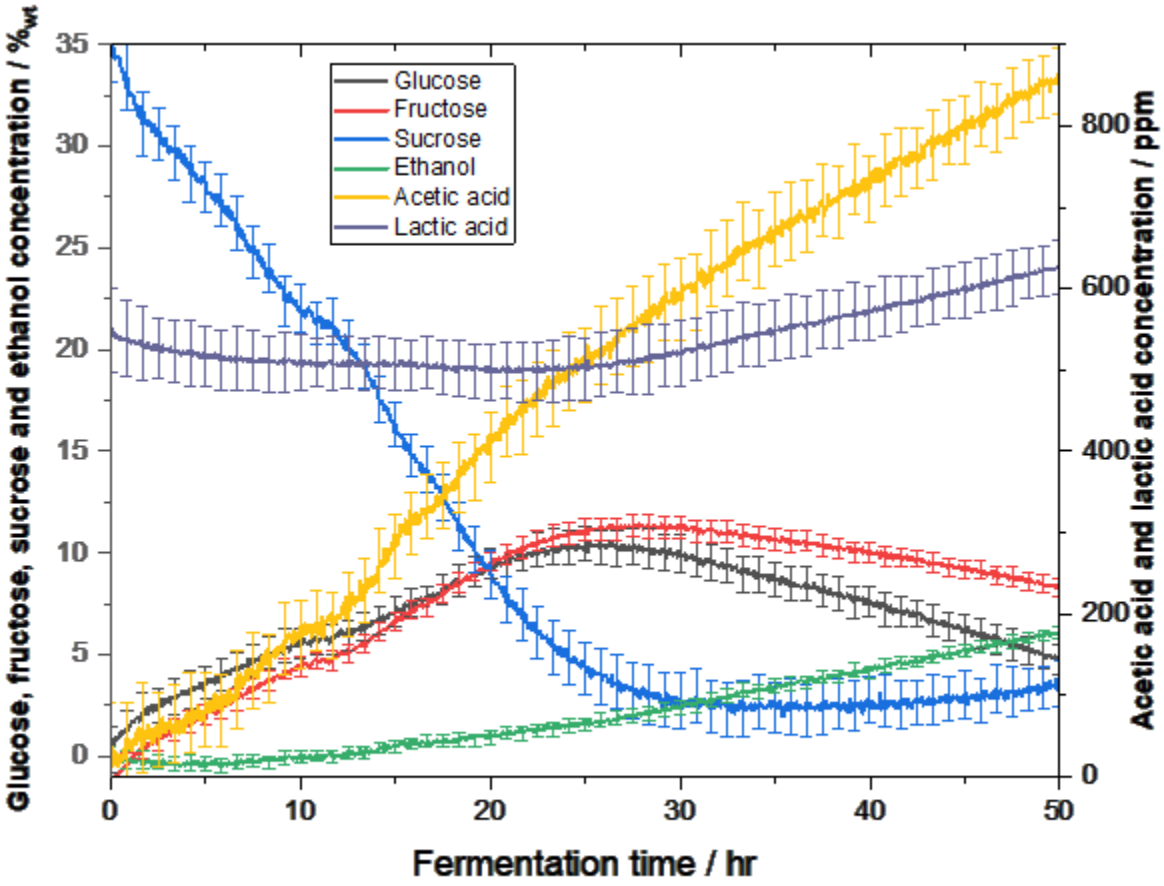
J. Speed, Keit Spectrometers, Oxford, United Kingdom*

The complex nature of all biochemical processes makes real time analysis of reaction progression very difficult, with scientists having to resort to off-line extractive techniques, such as HPLC. This can provide highly sensitive analysis of the process, but it is a slow and complicated method.

Vibrational spectroscopy is a common method of studying biochemical processes in the laboratory, but has not been widely taken up in industrial environments. Raman and NIR suffer from low sensitivity, and conventional FTIR is not a robust technique due to the inclusion of fragile fibre probes and moving parts. Here we present the use of a solid state FTIR sensor that is capable of monitoring a wide range of species present in fermentations simultaneously. This is due to the replacement of conventional optics (including moving mirrors), with a unique array of static optics, resulting in a robust and rugged sensor.

We present the results of ethanolic fermentations, monitoring the concentration of sugars (sucrose, glucose and fructose), ethanol, lactic acid, acetic acid and pyruvic acid. The sugars range in concentration from 0 – 30 %, whilst the acids are below 1000 ppm. The LOD for lactic acid was in the 10s of ppm range. We also present results in the quantification of up to 7 different sugars simultaneously in complex mixtures.

The use of simple static options also allows for calibration transfer from instrument to instrument, which is critical for industrial processes. Lastly, we show a comparison between the FTIR sensor and NIR based sensors.



8:50 AM S38: Inline process monitoring of industrial fermentation processes using Raman spectroscopy and multivariate data analysis

P. Ödman, K. Eisen and M. Brunsch, BASF SE, Ludwigshafen am Rhein, Germany*

Raman spectroscopy was evaluated as a tool for inline process monitoring of several types of industrial fermentation processes in lab and pilot scale. Multivariate data analysis was used to build prediction models for substrates and products based on the spectroscopic data. The chosen fermentation processes varied in complexity, ranging from simple processes with low aeration, low biomass concentrations and high product concentrations > 100 g/l to challenging processes like strongly aerobic high cell density cultivations with relatively low product concentrations. Both bacterial and fungal production hosts were used.

Univariate and multivariate data analysis methods were used to build calibration models for substrates and/or products (e.g. carbohydrates, amino acids and enzymes). In the simplest process, an area-under-the-curve (AUC) approach combined with univariate regression was sufficient to predict the product concentration. In all other cases, multivariate methods, i.e. partial least squares regression (PLSR, a linear regression method) and support vector regression (SVR, a non-linear regression method) were required. With increasing complexity (i.e. aeration and biomass concentration), only SVR could yield a satisfactory predictive performance.

In all cases, the spectroscopic signal quality was satisfactory and for most products, useful prediction models could be calibrated. Overall, Raman spectroscopy is a very promising method for inline bioprocess monitoring of industrial fermentation processes.

9:20 AM Coffee Break

9:40 AM S39: Data integration for high throughput fermentation: addressing the new bioprocess bottleneck with Antha

J. Rutley, S. Brown and C. Gershater, Synthace Ltd, London, United Kingdom; M. Gershater, S. Bryan, C. Alia, C. Bird and J. Arpino, Synthace Limited, London, United Kingdom*

To address the complexities inherent in stirred tank bioprocesses, sophisticated approaches such as optimal experimental design are highly beneficial. Many runs in stirred tanks are required for this approach, which have historically been exceptionally labour intensive to carry out. However, the development of automated, single use bioreactor arrays such as the Sartorius ambr®250 have been transformational in the number of fermentations that can be carried out in parallel. Dynamic sampling across multiple bioreactors can produce in excess of 800 samples per run, each of which can require multiple analytics on diverse equipment. The task of handling all these samples and integrating the data from the multitude of on- and off-line analytics then becomes the major bottleneck. We have found that manual collation and manipulation of data sets on this scale is not practical, with the generation and structuring of raw data alone often requiring 4-6 FTE weeks per fermentation run. Here, we present how we use Antha software to automate the process of data collation and organisation, integrating the auto generation of structured data tables and combined on-/off-line analytics plots for rapid preliminary analysis, while facilitating more sophisticated interrogation of fermentation data streams. Antha can interface with a multitude of lab equipment for liquid handling and analytics, recording the provenance of every sample such that each can be traced from its origin to the resulting highly structured data sets. This permits the dynamic analysis of highly complex, fermentation data sets across multidimensional experimental designs.

10:10 AM S40: No longer a black box: Ease Scale-Up through online measurement of oxygen, OUR, pH, CO2, T, rpm and biomass in traditional shake flask cultures

G. John, PreSens Precision Sensing GmbH, Regensburg, Germany and C. Ude, Leibniz University Hannover, Hannover, Germany*

Although novel parallel cultivation systems were developed in the last couple of years, shake flasks are still the workhorse of microbial cultivation. Typically, these vessels are still used as black boxes because no online measurement is integrated. The non-invasive measurement of oxygen and pH using chemical-optical sensors has already been commercially available for several years and online measurement of biomass has recently been introduced. Here, we present the new CO_2 sensor that was developed recently and integrated into a multi-parameter platform.

Applications are various: Although the CO_2 sensor is only a prototype it is possible to follow a diauxie of *E. coli* cultivations online, while small changes in the growth curve detected by the biomass sensor indicate the exact time of limitations which was shown for different organisms. Non-invasive oxygen measurements deliver the critical process parameter k_La – which is even the basis of online OUR determination..

However, our focus was not just to add more parameters but also to let them complement each other. Measuring several parameters - with all of them showing the same characteristics - enhances the measurement security. It was shown that combined oxygen and biomass measurement offers a solid conclusion about the metabolic status of the culture. In summary, multisensory monitoring enables to

adjust the conditions in shake flask cultivations to be far more comparable to stirred bioreactors. Therefore, scale-up with yield optimization can be performed more reproducibly.

10:30 AM S41: Real-time monitoring of a fermentation process: linking yeast morphology to insulin production by image analysis

K. Pontius[*] and A. Eliasson Lantz, Technical University of Denmark (DTU), Lyngby, Denmark

Fermentation production processes are often the most complex step within bio-manufacturing. Nevertheless, due to a highly challenging environment inside the bioreactor, industrial fermentation processes are presently rather limited regarding analytical tools for process control. There is a deficit in suitable monitoring devices that can cope with the complexity of the dynamic fermentation environment without compromising the integral success of the process.
Therefore, we want to take advantage of the recent advances in microscopy image analysis and evaluate its potential for on-/ at-line monitoring of yeast physiology. In yeast cultures, cell size (distribution) has been shown to be correlated with cell viability (dead/alive, osmotically stressed) and growth rate. Furthermore, the cell size was recently correlated to the accumulation of an internal product (fatty acids) in microalgae. Consequently, image analysis seems to be a promising tool for getting a snapshot of the physiological state of a yeast culture during a production process.

The lately developed oCelloScope instrument enables rapid imaging and image analysis of a growing yeast culture. By analyzing images over the cultivation time we investigate the distribution dynamics of single cells, budding cells and cell aggregates, aiming at correlations between morphological features and process performance. Ideally, we want to develop a real-time monitoring tool that may be used in industrial bioprocess setups. Within this approach, methodologies for automatic distinction between image objects (single cells, budding cells, cell aggregates) are developed and first time trends of the morphology dynamics of an insulin production process are discussed.