VH Berlin

Invitation

The International 31st VH Yeast Conference provides you with lectures and presentations on current topics in the fields of markets and quality, applied yeast research and process innovations in yeast production.

The focus is on "yeast as a cell factory and its metabolic ambiguities"'. You are invited to join the interdisciplinary dialogue with experts and partners from applied science and practical experience. VH members are called to invite their partner companies to enable reduced fees for attendants.

We look forward to welcoming you in Leuven, Belgium.

A. Chagnon — President of VH

M. Quantz — General manager of VH

Yeast As A Cell Factory And Its Metabolic Ambiguities

Monday, April 16th 2018

08:30 a.m. Registration

09:00 a.m. General Assembly of the VH members (on special invitation)

Markets

10:00 a.m. Conference opening and welcome

Pres. A. Chagnon

Lallemand Inc. (CAN)

10:30 a.m. Studying the yeasts of yesterday to generate the industrial yeasts of tomorrow

K. J. Verstrepen

Systems Biology Laboratory, VIB Center for Microbiology
Catholic University Leuven, (KU Leuven) (BEL)
Laboratory of Genetics and Genomics, KU Leuven (BEL)
Leuven Institute for Beer Research (LIBR), Leuven (BEL)

The common brewer's yeast *Saccharomyces cerevisiae* is used in a broad range of industrial applications, from the production of beer, wine and bread to biofuels and pharmaceuticals. Interestingly, there are hundreds of different industrial yeast strains, but their origins and specific characteristics are largely unknown. We combined large-scale robotized phenotyping with genome sequencing to track the genealogy and evolution of today's industrial yeasts. Using this knowledge allowed us to set up large-scale breeding and directed evolution programs to generate superior variants that increase production efficiency and expand the range of yeast-derived products and aroma's, allowing more efficient fermentation, improved stress tolerance and production of novel products.

Moreover, sequencing the genomes of several yeasts isolated from various industrial niches revealed the presence of several complex interspecific hybrids that combine characteristics from the parental species. This prompted us to create novel crosses between different yeast species, allowing to create novel hybrids with industrial relevance.

11:00 a.m. CHASSY: Model-based construction and optimisation of versatile chassis yeast strains for Production of Valuable Lipid and Aromatic Compounds

John Morrissey[1], Jean-Marc Daran[2], Jack Pronk[2], Verena Siewers[3], Jens Nielsen[3], Tristan Rossignol[4], Jean-Marc Nicaud[4], Mislav Oreb[5], Eckhard Boles[5], Harald Heider[6], Florian David[7], Anastasia Krivoruchko[7], Georg Schirrmacher[8], Henning Markmann[8]

[1] University College Cork, Ireland; [2] Technical University Delft, The Netherlands; [3] Chalmers Technical University, Sweden; [4] INRA, France; [5] Goethe University Frankfurt, Germany; [6] Evolva AG, Switzerland; [7] Biopetrolia AB, Sweden; [8] Clarian Products GmbH, Germany.

CHASSY is an EU-funded H2020-funded collaboration between 5 academic partners and 5 companies from across Europe that aims to build platform yeasts for production of diverse high-value products in the oleochemical and aromatic families.

These classes of molecules have applications in the cosmetic, chemical, nutraceutical and pharmaceutical sectors. Their production in yeast hosts also contributes to sustainability in two ways: (1) replacing petrochemicals/palm oil as a source of chemicals contributes to development of the European bio-based economy; (2) using heterologous hosts for the production of plant-originating metabolites (aromatics) avoids the need to harvest plants from fragile ecosystems to extract these compounds.

To date, *Saccharomyces cerevisiae* has been the preferred species for these type of applications and we have extensive knowledge of its physiology and many

relevant synthetic biology tools. Even in this species, however, it has been challenging to move from proof-of-principle experiments to industrial application.

CHASSY will address this gap in *S. cerevisiae* and will also broaden our knowledge of the central metabolism and compatibility of molecular tools for two other yeast species, *Kluyveromyces marxianus* and *Yarrowia lipolytica*.

The aim is to build robust, efficient, genetically accessible chassis strains of each of the three species that can be used in the production of high value compounds.

The scientific goals will be achieved by integrating the knowledge gained from systems biology with the engineering tools of synthetic biology, leading to redesign of metabolic pathways in the different yeast species. These redesigned strains, or "chassis", will have optimised levels of core precursors and thus the capacity to act as flexible hosts for biosynthesis of a wide variety of commercially-valuable metabolites. The focus in the initial phase of the project has been on developing the synthetic biology tools to engineer these yeasts and on improving genome-scale models.

The presentation will describe the context for the project, highlight some of its early successes, and explain the general strategies being implemented to achieve the project goals.

11:30 a.m. AIF/IGFproject: Upcycling of cropped brewer's yeast production of functional cell wall fragments (YCW)

M.Eigenfeld

Technical University Munich (GER)

Yeast is a major by-product of the brewing industry. Most of it is used as food additive for monogatric and only a small part is used as dietry supplement or yeast extract production. Due to the complex mixture of different molecular groups such as proteins and various polysaccharides, yeast cell walls meet the requirements as encapsulation material for use in the food industry. Because of their antioxidant properties, bioactive substances such as omega-3 fatty acids, carotenoids, anthocyanins or phytosterols are currently in the focus of food industries. By use of carbohydrate-based hydrocolloids like alginate or gummi arabicum these bioactive substances have been successfully encapsulated. This process improved their bioavailability significantly. The upcycling of brewer's waste yeast to a high-quality encapsulation material for food and non-food industry has a great potential for increasing the value of breweries.

To use the brewer's waste yeast, a method for removing the bitter substances from the yeast cell must first be developed. This is necessary because waste yeast is in contact with different by-products (e.g., hop bittering, residual ethanol) from wort production and alcoholic fermentation. Therefore,the bitterness must be reduced by a cleaning process below a sensory level to have no influence on later

food products. In previous methods, this has been done in the context of the production of yeast extract or yeast protein recovery. These approaches purify the yeast cells at high temperatures (45 °C – 50 ° C) and alkaline pH-value, which both stress the yeast cell wall and the environment, and are not energetically economical. These methods do not go easy on the structure and affect the functionality of the yeast cell walls. The challenge is to develop a gentle purification of the yeast cells, which should also be efficient in terms of environmental impact and the cost of energy. Subsequently, the purified yeast cells should be broken up and the resulting fragments examined for their suitability for encapsulation.

12:00 a.m. Short poster presentations

Poster 1: Production of muconic acid and protocatechuate from lignocellulose feedstock

T. Nicolaï, M.R. Foulquié Moreno, J. m. Thevelein

University of Leuven, Laboratory of Molecular Cell Biology, VIB
Center for Microbiology, GlobalYeast NV, Leuven-Heverlee, (BEL)

Development of sustainable processes to produce bio-based compounds is urgently required because of environmental problems caused by utilization of depleting fossil resources. Converting saccharomyces cerevisiae into microbial cell factories by engineering new metabolic pathways facilitates the establishment of a renewable bio-based industry. Muconic acid serves as an interesting platform chemical for the production of several bio-polymers, such as polyurethane, nylon and polyethylene terephthalate. In the past, saccharomyces cerevisiae has been tailored for the production of muconic acid by expression of a heterologous pathway consisting of three different genes. as such, dihydroshikimate (dhs), an intermediate of the shikimate pathway, is converted into muconic acid.

We are applying a similar strategy using an industrial pentose-utilizing s. cerevisiae strain and achieved initial maximum titers of 15 mg/l muconic acid with the intermediate protocatechuate (pca) accumulating. however, as expected, the majority of the glycolytic flux was still directed towards ethanol, which confirms the need of disrupting pyruvate decarboxylase activity by obtaining the triple knock-

out mutant for pdc1, pdc5 and pdc6. the pdc negative strain cannot grow with glucose as sole carbon source. It requires a partial deletion in mth1 to ensure production of cytosolic acetyl-coa and, therefore, to restore growth on glucose. Initial results with this modified strain show higher amounts of muconic acid with lower carbon loss towards ethanol production. Moreover, our results indicate that high titers of pca production is achievable, which could be an interesting alternative end product with industrial applications.

Poster 2: Current research for the production of Kombucha starter cultures

M. Schmacht, S. Malchow, H. Woest, M. Hageböck, M. Senz

Research and Teaching Institute for Brewing in Berlin (VLB), (GER)

There is a wide range of traditional fermented food and beverages that are produced by a multipart interaction of mixed culture of bacteria and yeast. Thereby the microbial metabolites often contribute to the preservation, flavor development and characteristic texture of the product. One such product is Kombucha, an acidic and healthy beverage that is the result of a well-matched co-culture fermentation process. Traditionally, Kombucha is produced by inoculation of sweetened tea with a certain amount of mostly unknown symbiotic culture of acid producing bacteria and yeast (SCOBY) that are associated with a characteristic cellulose matrix, the so called mother.

For industrial production, an adequate process control, including the exact knowledge of used microorganisms as well as reproducibility, is crucial. Therefore the usage of known starter cultures is prior art for many producers. While the provision of fresh cultures is established, the production of adequate dried starters is still challenging due to poor stability of the cultures during preparation. This contribution shows current investigations and developments at the VLB Berlin for the production and application of different Kombucha starter culture preparations. Thereby, the up- and downstream processing as well as the final application is taken into consideration.

Poster 3: Origins, diversity and evolution of industrial Saccharomyces interspecific hybrids

E. B. Gallone

Vlaams Instituut voor Biotechnologie (VIB) (BEL)

Interspecific hybridization is now recognized as a common phenomenon across the tree of life. The *Saccharomyces* species complex includes a group of closely related species able to cross and form hybrids that contain genomic contributions from two or multiple *Saccharomyces* species. Hybridization is thought to be a fast route to the development of new phenotypes by bridging together divergent genetic combinations in one genome and by inducing profound changes in the genomic structure. Despite a rapidly increasing body of work revealing how frequent *Saccharomyces* hybrids are, we still know relatively little about their origin and diversity.

We isolated a collection of more than >200 hundred yeasts from different environments (beer/brewery, wine, bread, bioethanol, sake, cider etc.) and by combining whole-genome-sequencing and high-throughput phenotyping we identified and characterised around 60 *Saccharomyces* interspecific hybrids with different combinations of Saccharomyces parental species. We show that the formation of these hybrids happened within the industrial environment and is niche-specific: beer and wine hybrids have different origins. We also describe how hybridization induced severe aneuploidies, chromosomal rearrangements and chimerisation between the sub-genomes, often unique to each hybrid combination and environment. Together these results shed a light on the diversity and evolution of Saccharomyces hybrids. on the "gas retention capacity" in doughs.

A variety of flours as well as yeasts with different raising power were kneaded in a measuring kneader and industrial kneaders, producing different dough elasticities and dough inflation behaviour.

It turns out that RPP measurements may be also useful to determine the dough elasticity as part of flour dough rheology measurements.

Poster 4: Monitoring the changes on the physiology of Saccharomyces cerevisiae during aerobic detoxification of common inhibitors found in biomass hydrolysate: a single cell analysis approach (MPFC)

Cabaneros Lopez P.

PROSYS, Technical University of Denmark

The inhibitors generated during the pretreatment of lignocellulocic biomass reduce the performance of yeast to produce 2G bioethanol. In this context, several studies reported that adaptation of the cell culture during the propagation step results in significant improvements in the ethanol production [1].

In this study, multi-parametric flow cytometry (MPFC) was used to study the changes in the physiology of *Saccharomyces cerevisiae* during fermentation containing different combinations of common inhibitors found in biomass hydrolysate (vanillin, furfural and acetic acid). Measurements of the cytosolic ROS concentration, membrane potential and membrane permeability were used to assess the physiological state of the cells during the adaptation process. The results showed that the presence of inhibitors results in long lag-phases characterized by high concentration of ROS compounds and low membrane potentials (corresponding to detoxification processes). Only cases with combinations of inhibitors resulted in premature loss of cell viability. MPFC is then applied to determine the effects of the aerobic capacity and of growth phase on the capacity of *S. cerevisiae* to adapt to hydrolysate. Seed cultures are propagated in high and low aerobic conditions and inoculated, at middle exponential and stationary phases, in a synthetic media simulating hydrolysate. The physiology of yeast is periodically followed with MPFC for a period of 3 – 6 days.

MPFC was first used to understand the adaptation of yeast to biomass hydrolysate, and then applied to assess the effect of aerobic capacity and growth phase on the capacity of adaptation of yeast.

Poster 5 : „Rapid Detection of Living Cell Count in Granulated Dry Yeast Using NIR-Spectroscopy

S. Liebold, O. Schewtschenko, F. Meuche

fzmb GmbH – Forschungszentrum für Medizintechnik und Biotechnologie

L. Friedrich,

Glatt Ingenieurtechnik GmbH

The detection of living cell count is one of the most time-consuming and costly methods for analyzing yeast. The NIR-spectroscopy could be a rapid non-destructive method for analyzing this parameter. This is a feasibility study in using NIR-spectroscopy to determine the CFU/g (colony-forming units per gram) in granulated yeast.

A total of 80 samples of granulated yeast, produced in a vacuum fluidized bed dryer, were measured. Diffuse reflectance spectra using an FT-NIR-spectrometer were measured in a spectral range of 10,000 – 3,800cm^{-1} [950 – 2,650 nm]. The living cell count was analyzed for each sample using classic microbiological methods. The range of values was between 2.9 x 10^{4} and 2.5 x 10^{10} CFU/g.

NIR spectra and lab results were used for calibration development. Several preprocessing steps were applied to the spectra in order to remove influences of particle size and packing factor. A PLS-algorithm was used for regression and a cross validation was carried out. A chemometric model with an RMScross = 1.23x10^{9} CFU/g, an R^2 = 96.1 as well as an RPD = 5.07 could be developed. This study demonstrates, that NIR-spectroscopy could be an useful tool to detect the living cell count in granulated yeast in a very fast and simple way.

Poster 6 : „"Engineering the yeast CB1 for xylose utilization to produce fuels and chemicals"

M. Matthijs
Cargill Europe (BEL)
t.b.d

12:30 p.m. Lunch break

Analytics/ Quality

02:00 p.m. Online cell density meausrement improves process insight in yeast fermentation

M.Frank

Hamilton (CHE)

Process reproducibility is the key parameter to maintain stable yields. Missing the optimal time for inoculation of the fermentation or the perfect induction time has significant effect on product quantity and quality. Therefore real-time measurements of the cell density and implementing control strategies based on this information is of great importance. Currently the most common methods for online control of biological processes are pH and dissolved oxygen measurements but they provide only limited information. The measurement of the cell density is still done by time consuming offline measurements so critical events to define control strategies might be missed.The benefit of using online monitoring of the cell density could be proven in different applications and will be in the focus of the talk.

02:30 p.m. In situ microscopy for the real-time monitoring of budding yeast cultivations

A. Marbà-Ardébol, P. Neubauer, S. Junne, Technical University Berlin

(GER),

J. Emmerich

SOPAT GmbH (GER)

Morphology of cells is altered by environmental stress, as it appears e.g. in large-scale production [1]. Among techniques, which are able to capture morphologic features of cells promptly, automated imaging technologies are promising, as they provide further information beyond the size about cellular structures, shape or cell aggregation. In situ photo-optical microscopy (ISM) and holographic digital microscopy (DHM) were used in this study to measure single-cell morphological dynamics of single budding yeast Saccharomyces cerevisiae in suspension.

Multi-compartment scale-down reactors were applied for studying the influence of gradients, as they appear in large scale, on the morphological heterogeneity of these yeast cultures [2]. Namely oxygen oscillating conditions, as they occur in aerated large-scale nutrient-limited fed-batch cultivations. The exposure time to oxygen limitation was critical, which was measured by an increment of cell heterogeneity by means of DHM.

Maturation of the yeast became trackable at the single-cell level when ISM was applied. The detection was achieved by coupling the microscopy with an automated image recognition based on neural networks. According to the relation of budding and total cells (budding index), a distinction was feasible between growth stages of a batch cultivation. The single-cell size distribution got narrower during the growth phase, so the population homogeneity increased. Under glucose starvation, the percentage of non-budding cells remain constant due to a reduced bud formation. It was demonstrated that morphologic population heterogeneity was accessible, as well as useful to predict growth and metabolic activity.

The possibility to monitor changes in cell morphology directly on line enables to gain new parameters for monitoring and control, in process development as well as in production scale.

03:00 p.m. Yeast based strategies to reduce FODMAP levels in whole wheat bread

N.Struyf, J. Verspreeta, K. J. Verstrepen, C. M. Courtina (BEL)

Laboratory of Food Chemistry and Biochemistry, and Leuven Food Science and Nutrition Research Centre (LfoRCe)
KU Leuven (BEL)
VIB Laboratory for Systems Biology & CMPG Laboratory for Genetics and Genomics (BEL)

The presence of FODMAPS (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols) in the diet is said to cause negative health effects for people suffering from irritable bowel syndrome (IBS). As wheat fructans are a major source of FODMAPs in the Western diet, wheat breads with reduced fructan levels can offer beneficial health effects for IBS patients. Several studies already showed that 50 to 80% of the fructans present in wheat whole meal are degraded during bread making by yeast invertase. Despite this degradation, wheat and rye breads generally contain FODMAP levels that are above the cut-off value for inducing symptoms in IBS patients.

Therefore, different strategies were developed to enhance fructan degradation during bread making using yeast-based strategies. In a first set-up, the extent of fructan degradation by different Saccharomyces cerevisiae strains during wheat whole meal dough fermentation was studied. The results revealed that fructan degradation is yeast dependent and linked with the industrial use of the yeast strains. Additionally, it was shown that the extent of fructan degradation is dependent on fermentation time and yeast dosage. In a second set-up, the fructan degrading capacity of other yeast species was analyzed. Inulinase-secreting Kluyveromyces marxianus strains degraded the fructans in wheat whole meal for more than 90% during fermentation, resulting in fructan levels of <0.2% dm in the final bread. As K. marxianus strains generally cannot ferment maltose, the main sugar in lean dough, alternative sugar sources have to be included in the bread making recipe to ensure sufficient production of CO2.

Another option is to use mixed cultures of S. cerevisiae and K. marxianus, which was shown to be an efficient way to ensure optimal production of CO2 and enhancement of fructan degradation. Different volatile aroma compounds were produced in significantly different levels when K. marxianus was used as starter culture compared with the conventional S. cerevisiae bakery strain. These differences were, however, not detected when sensory analysis of the crumb was performed, probably because of evaporation of aroma compounds during the baking phase of bread making. To conclude, this study revealed that fructan degradation can be enhanced by either using alternative yeast strains/species or by altering process parameters during bread making. Breads that are low in fructans, and hence in FODMAPS, can be beneficial for people suffering from IBS.

03:30 p.m. Coffee Break

Applied Research

04:00 p.m. Computational modelling of protein constraints in yeast cell

factories

E. Kerkhoven
Chalmers (SWE)

Yeasts such as Saccharomyces cerevisiae can function as microbial cell factories for the synthesis of a wide range of products. However, genome editing is often required to e.g. engineer a novel biosynthetic pathway or increase productivity. The identification of targets for genome editing has benefited from the use of genome-scale in silico models of metabolism. These models (or GEMs) aim to describe the complete metabolic network that is present in the cell, and GEMs can be used for a variety of different analysis, including the prediction of internal metabolic fluxes and as scaffold for multi-omics data analysis.

GEMs have indeed successfully predicted genome editing targets for increased productivity, however, one of the shortcomings of classic GEMs is that they do not consider that a cell has a limited protein capacity that can be allocated to perform metabolic tasks, while each protein further limited by their catalytic efficiency. In our research group, we have developed a novel modelling toolbox called GECKO, for Genome-scale model to account for Enzyme Constraints, using Kinetics and Omics. Application of this approach to S. cerevisiae resulted in an enzyme constrained model, ecYeast7, that is now able to accurately predict e.g. the Crabtree effect, growth rates on various carbon sources, and more realistic production envelopes. Inclusion of omics data can subsequently be used to identify potential targets for genome editing.

04:30 p.m.. End of Monday lecture sessions

05:30 p.m. Alternative 1: Tour: history of Begijnhof and KU Leuven,
Short beer tasting stop, starting point: reception Faculty Club

05:15 p.m. Alternative 2: AB InBev Brewery visit with beer tasting,
starting point: bus in front of Begijnhof Hotel

07:30 p.m. Conference dinner at restaurant “de klimop leuven”
and convivial evening (until 11: 00 p.m.)

10:30 p.m. Bus returns to hotels (second bus shuttle 11:00 p.m.)

Tuesday, April 17th 2018

Applied Research

9:00 a.m. Engineering efficient yeast-based cell factories

Petri-Jaan Lahtvee
Institute of Technology, University of Tartu, (Est)

Yeast is extensively used in various biotechnological processes with the global market reaching yearly into billions of dollars. However, to make bioprocesses more cost-efficient and more generally applied by industries, challenges to create a well-performing cell factory with increased production of precursor molecules and robustness towards stress factors must be addressed. We have focused on various aspects to make cells energetically more efficient and have dedicated to create synthetic switches to control these changes metabolically. Protein synthesis has shown to be the most energy consuming process in proliferating cells and, therefore, understanding what controls protein abundances and what are the most efficient ways to regulate it represent critical questions in biology and biotechnology.

I am going to present two case-studies, wherein the first one, we have quantified all the transcript and protein abundances in the cell under various environmental stress conditions and applied machine learning algorithms to understand which parameters control the protein abundances and resulting metabolic fluxes the most.

In the second case-study, we have focused on the protein turnover measurements and quantified protein turnover under a number of environmental conditions to understand what causes the differences in turnover rates, responsible for more than 15% increase in cellular efficiency.

The present dataset provides more than 250,000 quantitative data points on yeast metabolism and, therefore, represents a significant expansion of the current resources for future systems biology studies to improve the efficiency of yeast-based cell factories.

09:30 a.m. Superior industrial yeast strains for efficient conversion of Lignocellulosic biomass into bioethanol

J.M. Thevelein, M.R. Foulquié-Moreno, M. Demeke, S. Swinnen, A. Goovaerts, E. Belo, M. Stojiljkovic, Q. Deparis, P. Vandecruys, A. Claes, T. Nicolaï, S. Varghese, G. Vanmarcke

University of Leuven, Laboratory of Molecular Cell Biology
VIB Center for Microbiology
GlobalYeast NV, Leuven-Heverlee, (BEL)

The development of a new industry capable of producing biofuels and higher-value chemicals from waste streams and energy crops is essential for the transition from the unsustainable petroleum-based economy to the sustainable bio-based economy, but it is facing many challenges. The pretreatment and enzymatic hydrolysis of lignocellulosic biomass releases fermentable sugars with a quite satisfactory yield but also produces very high levels of fermentation inhibitors. The fermentable sugars include up to 35% xylose.

We have engineered bacterial xylose isomerases into a very robust industrial yeast strain used for first-generation bioethanol production with starch-derived fermentable sugars. The xylose fermentation rate was further enhanced continuously and very strongly by mutagenesis, evolutionary adaptation, genome shuffling and rational genetic engineering. The latter has been performed using superior alleles identified by polygenic analysis of complex yeast traits important for fermentation performance in lignocellulosic hydrolysates. These traits include tolerance to inhibitors like acetic acid, furfural and hydroxymethyl furfural, xylose fermentation capacity, ethanol tolerance, thermotolerance, etc.

Screening of our strain collection identified strains with superior characteristics for these traits and QTL mapping by pooled-segregant whole-genome sequence analysis followed by reciprocal hemizygosity analysis were performed to identify the responsible causative alleles. These superior alleles are then used for site-specific genetic engineering of the second-generation bioethanol strain to further improve the traits-of-interest, with minimal risk of negatively affecting other traits important for industrial application.

This combination of diverse methodologies has resulted in very robust industrial yeast strains able to ferment all glucose and xylose in concentrated, undetoxified lignocellulose hydrolysates within 48 h, routinely reaching ethanol titers higher than 5%. Hence, these strains are ready for successful application in the second-generation bioethanol industry. Future improvements of the second-generation bioethanol process might be obtained by optimizing the yeast for simultaneous saccharification and fermentation, and by expressing secreted lignocellulolytic enzymes in the yeast to reduce the high enzyme cost in the enzymatic hydrolysis process.

10:00 a.m. The synergy of rational design, natural genetic variation and evolutionary engineering in a high throughput biological foundry for industrial yeast strain improvement

D. Thompson, S. Srikrishnan, S. Agarwala, J.Wang, B. Renda, T. Curran, P. Rahda, N. Reppas, N. Maheshri

Ginkgo Bioworks (USA)

Ginkgo Bioworks is the organism company. We design custom microbes (including yeast strains) for customers across multiple markets. In biological engineering, living organisms are the factories that build new products. Designing the best organisms requires a different sort of factory, one where we combine state of the art methods in genetics, synthetic and systems biology with the best tools in automation and software to do biological engineering at scale. Bioworks1 was the world's first organism foundry, where engineers are prototyping thousands of biological designs. We have since built Bioworks 2 and 3 that greatly increases our capacity to breed, phenotype and genotype microbes in high throughput at increasingly lower costs.

We will describe the suite of transcriptomic, proteomic, and metabolomic pipelines developed in our foundries, and how they can be employed to characterize the natural genetic variation in diverse strains to select those with improved performance and yield in industrial fermentations. In some cases, we go further and identify the genetic determinants underlying the desired traits using the principles of quantitative trait mapping.

Finally, we will discuss the power of generating further diversity from natural genetic variation by leveraging a high-throughput breeding pipeline and/or directed evolution approaches, in context of a few examples. This enables us to rapidly generate improved yeast strains for a variety of applications in industries based on fermentation, including distilling, brewing, wine making, and baking.

10:30 a.m. Synthetic Transcription Amplifier System for Orthogonal Control of Gene Expression in Saccharomyces cerevisiae

M.Penttilä & D. Mojzita, A. Rantasalo, J. Kuivanen, J. Jäntti

Technical Research Center of Finland (FIN)

Sustainable production of chemicals, materials and pharmaceuticals is increasingly performed by genetically engineered cell factories. In order to obtain highest possible overall efficiencies and controllable production, it is important to have tools that enable orthogonal expression of the target genes, i.e. expression that is not impaired by host regulation and physiology or external culture conditions.

We have developed a synthetic expression system (SES) that consists of synthetic transcription factors and promoters, which enables tuneable expression at a range of expression levels. Furthermore, SES system works in a vast range of fungal hosts in addition to *S.cerevisiae*. The system is also extended to include synthetic repressors and promoter elements for repression of target genes.

A combination of the induction and repression elements to a bi-stable genetic circuit system was also constructed, which enables switching between expression and repression of alternative sets of pathway genes. Examples are given how the synthetic

expression system can be used for pathway engineering.

11:00 a.m. Coffee break

Process

11:30 a.m. E-bio Project „Yeast Scent“ results: IMS / model predictive control (MPC) fermentations

S. WEGERHOFF AND S. ENGELL, Technical University Dortmund (GER)

Process Dynamics and Operation groups, Technical University Dortmund

The yeast Saccharomyces cerevisiae is usually produced in a fed-batch reactor which is fed with molasses and oxygen as substrates. During the production process S. cerevisiae can switch to undesired ethanol production which is caused by the Crabtree effect or by an insufficient supply of oxygen which is especially a problem at the end of the production phase. The production of ethanol causes not only a reduced yield of biomass, with respect to the sugar fed to the reactor, also if the concentration of ethanol is greater than a tolerable range the batch produced cannot be sold.

During the industrial process usually only the concentration of ethanol is measured and the concentration of ethanol during the process is controlled by a PI or a PID controller which tries to follow a desired feed curve. In the case when ethanol is produced the controller decreases the feed rate such that the ethanol concentration declines until the concentration is shifted back to a tolerable range. This type of control strategy has the disadvantage that it feeds too less molasses to the reactor such that the growth potential of the cells is not exhausted which leads to an increased production time. Also it cannot react on changing environmental conditions or different substrates or yeast strains.

In this work a novel control strategy is presented which uses a mathematical model to control the process. The model-based control is extended by a state estimator which uses the model to estimate the unmeasured concentrations and to determine the current process phase. With the information of the current process phase and of all concentrations the model-based control is able to determine the optimal operating point at which the growth is at its maximum while no ethanol is produced. Also when the environmental conditions change or when different substrates or yeast strains are used the model-based control can still follow the optimal operation point that also under impending conditions the process can be finished.

The model-based control was tested in several fermentations in lab scale at the VH Berlin and it can be shown that the model based control can reduce the production time more than 20% compared with the classical control scheme which is applied in industry.

12:00 a.m. Looking at baker´s yeast fermentation through new glasses: The neglected potential of vinasse for biotechnological applications

J. LISIČAR, M. SEDAGHATI, S. BARBE

Technische Hochschule Köln, Faculty of Applied Natural Sciences (GER)

Baker’s yeast producers mostly concentrate their attention to the quality and quantity of biomass obtained after the fermentation. Looking at this process through another glasses (e.g. as a biochemist, an animal physiologist or a thermal engineer) leads to a different focus. In the present contribution, we demonstrate how this process can be regarded as an industrial approach for the production of invertase, one of the most significant technical enzymes used in food industry. Beet molasses is the main raw material used in the production of baker´s yeast and contains appreciable quantities of betaine, an important nutrition supplement used for animal feeding.

We confirmed that betaine is not being metabolized by S. cerevisiae during the fermentation, remains intact in the vinasse and can be easily recovered. The current demand for these two key molecules combined with the huge volumes of fermentation broth being processed during the industrial production of baker’s yeast, strongly increases the neglected potential of vinasse. Furthermore, large amounts of low grade fermentation heat have to be continuously removed during this large scale aerobic bioprocess. State-of-the-art heat pumps combined with advanced PCM based energy storage constructions would allow for supplying a surrounding city with district heat.

12:30 a.m. Centrifugal separation as key technology in the fermentation industry

A. PIEK , GEA Westfalia (GER)

Since the beginning of the last century centrifuges are used for separating biomass from fermentation broth.

Different kind of centrifuges such as clarifying separators, nozzle separators and decanter centrifuges are the key technology for molasses treatment, which is used as raw material for the culture media, for separation and washing of the yeast after the fermentation process as well as for separation of yeast extract from the cell walls.

Modern centrifuges are working fully automated and continuous, are completely CIP

capable and meet the hygienic standards of today. Their control system can be integrated in a superordinated process control system and their technical design can be adapted to the specific product.

The capacity of centrifuges has been continuously increased during the last decades while energy consumption was reduced by introduction of innovative drive systems.

01:00 p.m. Feedback and final remarks

01:15 p.m. Lunch/ End of conference