European Biotechnology

Autumn 2014

Biomanufacturing

SPECIAL

Assuring film quality

LEACHABLES Plastics additives or their degradants rarely leach from single-use bags into the process fluid. However, because leachables can seriously affect drug potency, affect analytical assay results or impact cell growth, drug manufacturers are keeping a close eye on the issue.

Flexible cell-culture bags are made of multilayer plastic films. In rare cases, these have been shown to release leachables that can diffuse into the medium.

Over the last decade, single-use cell culture bags and bioreactors have become established tools in R&D, as well as in the production of preclinical and clinical batches of biopharmaceuticals and vaccines. But rare reports of diminished cell growth due to substances leaching out of plastic biocontainers or bioreactor film materials have raised concerns in the industry. According to the latest annual survey released by science market intelligence company BioPlan Associates, the number of biomanufacturing experts concerned about leachables and extractables (L&Es) has doubled since 2009. "20% of responders cite L&Es as their single most important reason out of 23 reasons for not increasing use of disposables," stressed Bioplan Managing Partner Eric Langer. The issue was drawn even more into the limelight by a highly-publicised article in the PDA Journal of Pharma Science & Technology last year (doi: 10.5731/pdajpst.2013.00905). In it, Amgen researcher Matthew Hammond identified high concentrations of a degradation product [Tris (2,4-di-tertbutylphenyl] phosphate (bDtBPP)) from the photostabiliser Irgafos 168 (BASF) as the reason for growth inhibition of sensitive cell cultures exposed to a specific biofilm.

> "Consistency of raw materials is a must."

"Growth inhibition caused by leachables does not occur very often," says Wieland Wolf, the CEO of Berlin-based CMO Probiogen. "However, as media composition and biopharmaceutical compounds vary individually for every single process, the interaction of films, process fluid, and cells have to be considered when cell growth lags behind what has been achieved with other cultivation devices that are, for example, made of glass or stainless steel." Choosing other antioxidants, however, is not really an acceptable solution to the problem, as Irgafos is already used widely in several different industries to prevent photo-oxidation in thermoplasts. Researchers also know a great deal about the additive's characteristics.

Reduce Irgafos concentration

"A new additive always means new risks," comments Stefan Schlack, Senior Vice President Marketing at Sartorius Stedim Biotech. However, controlling the Irgafos concentration, the extrusion process of the film and the manufacturing process of the bags – including the gamma irradiation – mitigates the risk of any growth inconsistencies, he says. According to Schlack, researchers at both his company and partner Südpack, which is a major player in film-extrusion and medical-grade plastics in Europe, identified important parameters that affect the cytotoxicity of polyethylene film materials. They include:

- concentration of Irgafos (Sartorius Stedim Biotech used a standardised cell-growth assay that showed dose dependency to establish resin and film specifications)
- heat transfer during extrusion (analyses identified resin melting temperature, film-cooling temperature and quantity/hour as critical parameters affecting Irgafos oxidation).

Last summer, Schlack's company launched a range of single-use bags based on a new film dubbed S80, with minimised concentration of Irgafos and no bDtBPP detected in water extracts. According to the company, they provide lot-to-lot consistent growth performance and extractable and leachable profiles. "With

Pictures: RimBio

a standardised assay, we were able to demonstrate that the new film does not inhibit cell growth directly after gamma -irradiation, nor during accelerated aging or extended media extraction studies," says Schlack. The first products to be manufactured from S80 are Flexsafe RM bioreactor bags that have a volume of up to 200 litres. The three-layer film is composed of an inner and outer sheet of LLDPE (linear low density polyethylene) with EVOH (ethylene vinyl alcohol) in-between, which acts as a gas barrier. According to Schlack, Sartorius plans to shift production of its other single-use bioreactor, the Biostat STR, to S80 by the end of the year. Subsequently, 2D and 3D bags for upstream, downstream and fill and finish applications will be offered.

Urgent need for validated assays

"Consistency of raw materials is a must," confirms Hélène Pora, Vice President of Single-Use Technologies at Pall Life Sciences, a company that is marketing two films. Pall's TK8 and Pall's Allegro film have also been tested negatively for growth inhibition, both internally and in round robin tests, but she believes even that isn't enough. Validated assays able to predict cytotoxicity for a specific process under true-to-life conditions are still missing. "At the end of the day, it's the combination of one cell line in a given, defined medium that can give you a defined answer," says Pora. "At the moment, no test is good enough to claim to be the gold standard."

Pora also warns that it's important not to underrate but also not to overhype the leachables problem: "The Amgen case was one study and related to one compound, but I don't think that's enough data yet for the industry to know if there is any compound that could create problems," she says. "The challenge is to have a test that gives you a good indication but – as reports of leachables are not sufficient – isn't so sensitive that all films appear to behave poorly when in the real world. That's not the case." The Pall VP says her company has looked for Irgafos degradation compound in its products, but didn't find it. According to experts, as long as growth media with high protein content were commonly used, growth inhibition due to leachables could not be observed. "Adoption of chemically defined media, however, appears to make production cell lines more sensitive," says Pora.

Those suspicions appeared to be confirmed by recent round robin tests conducted by the DECHEMA working group "Single-Use Technology", which is headed by Dieter Eibl from the Zurich University of Applied Sciences. It recommends using a model cell line (CHO XM 111-10) and a standard medium (CHO Master HP-1 medium) for the basic validation of cytotoxicity. Out of eleven multilayer films provided by suppliers, the researchers identified three that cause growth inhibition when compared with culture in glass bottles.

A film for every application

While most suppliers use multilayer films to produce rocking-motion and stirred-tank single-use bioreactors (SUBs), storage, mixing, shipping, as well as freeze & thaw bags, Eppendorf offers rigid wall SUBs. Made from mainly polystyrene and some polycarbonate, which can contain the Irgafos component tDtBPP, granulates for BioBLU single use vessel production are Irgafos-free, confirmed Karl Rix, Vice President Sales & Support Bioprocess, Europe, in mid-September. The company also said it was not able to find any signs of reduced cell growth when performing cell-growth studies like the DECHEMA assay.

In the absence of an appropriate generic cytotoxicity assay that could replace the US Pharmacopeia 87 assay, suppliers should provide extensive and traceable information on product characteristics, changes in film composition and manufacture, as well as expanded process validation services. Only that will promote the further adoption of single-use technology in more critical applications, such as vaccine and biologics production. ■

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Growing potential: mAb production with Fibra-Cel

BIOMANUFACTURING The market for monoclonal antibodies continues to grow steadily, but there are still bottlenecks in the development of new products, including long development times mostly due to R&D. Advanced bioprocess equipment that meets the specific demands of mAb-producing hybridoma can accelerate design and production, and reduce overall development costs along the way.

› Claudia M. Huether-Franken, Eppendorf AG, Bioprocess Center, Juelich, Germany; Ray Rose, Stacey Willard and Ma Sha, Eppendorf Inc., Enfield, USA

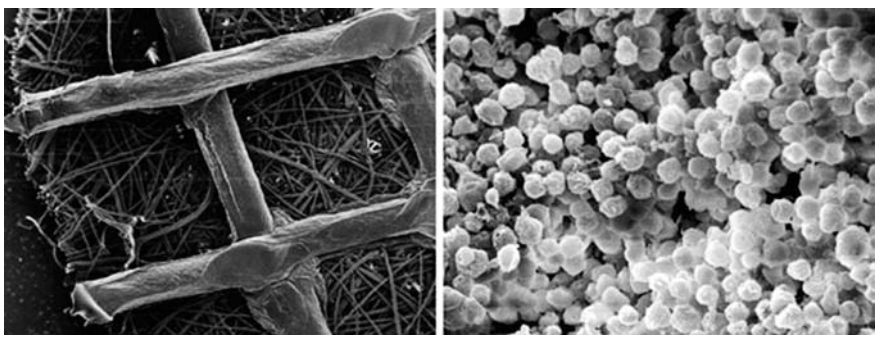


Fig. 1: Scanning electron micrograph of Fibra-Cel disks (left); mouse-mouse hybridoma DA4.4 immobilized on Fibra-Cel disks during production at 1 × 10^8 cells/cm^3 of packed-bed volume (right).

Recent years have seen a continuous rise in the market for monoclonal antibody (mAb)-based therapeutics, diagnostics and imaging modalities. In particular, mouse mAbs produced in hybridoma cells are experiencing a resurgence in growth because of the increasing demand for immunodiagnostic tests, which identify circulating tumor cells, stem cells and pathogens. For example, the diagnostic used to differentiate between leukemia subtypes employs hybridoma-generated mAbs to detect B and T cell subsets. Another imaging technique employed in the diagnosis of prostate cancer hinges on a mAb specific for a human prostate cancer cell surface marker which was created using hybridoma technology. In addition, common pregnancy tests detect the condition with the help of mAbs specific to the β-chain of the pregnancy hormone hCG. This growing market segment is predicted to reach US$19.83bn by 2015, and is expected to continue to expand throughout the next decade.

The effective hybridoma

The most common method of producing mAbs for diagnostics and imaging in the biopharmaceutical industries is hybridoma technology. Hybridomas are hybrid cell lines generated from fusing a B cell producing an epitope-specific antibody with a myeloma cell carrying the ability to grow in cell culture and lacking antibody chain synthesis.

Stirred-tank bioreactors are often used in the large-scale production of hybridoma-derived diagnostic mAbs. Besides the culture volume, the advantage of bioreactors compared to conventional cell culture methods is the automated and precise control of all important culture conditions and process parameters.

Proven technology with scale-up potential

The Fibra-Cel® technology has been established as an excellent method for the growth of suspension and anchorage-dependent cell lines. The three dimensional structure of the Fibra-Cel disk provides an excellent solid-support matrix for the entrapment or attachment of animal cells, allowing constant perfusion of nutrients in a low-shear environment. It is used predominantly in perfusion processes for the production of secreted products such as recombinant proteins and viruses. Since the 1980s, scientists around the globe have been using Fibra-Cel to grow a wide range of mammalian and insect cell lines. Recently it was also shown that hybridoma cells such as DADA4.4, 123A, 127A, GAMMA, 67-9-B can be successfully cultivated on Fibra-Cel disks at high cell densities (see Fig. 1). By im-

Pictures: Eppendorf/DASGIP

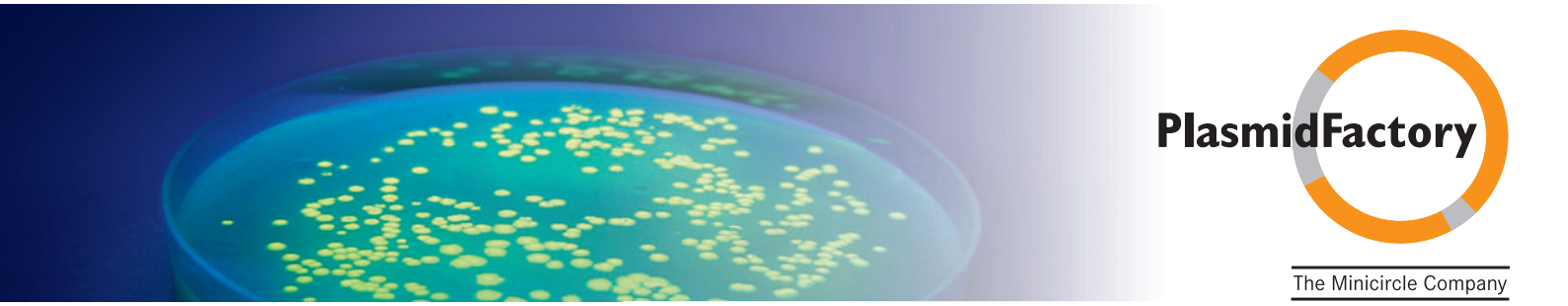
proving cell densities, the mAb titers in production processes can be massively increased.

Originally used in autoclavable CelliGen® cell culture bioreactors (Eppendorf), Fibra-Cel technology has now been successfully adapted to sterilizable-in-place systems as large as 150 liters, allowing for seamless scale-up to commercial production. With the BioBLU® packed-bed, single-use vessels (Eppendorf), Fibra-Cel technology is also available for those who prefer the advantages of disposable systems.

Increasing productivity fivefold

Scientists at the Eppendorf Research & Development Lab in Enfield, CT, USA have been evaluating DA4.4 hybridoma cell cultures on Fibra-Cel disks. To demonstrate that the proprietary packed-bed basket impeller is capable of robust, reproducible high-density hybridoma culture under perfusion conditions, two independent trials were conducted using the suspension-adapted DA4.4 hybridoma cell line in a CelliGen 310 bioreactor (Fig. 2). This packed-bed impeller creates a low differential pressure at the base of the impeller tube, which circulates the medium throughout the system. The medium receives gases through a sparger located at the bottom of the inner tube, protecting the cells from exposure to the gas liquid interface. This results in low turbulence and low shear stress on the culture.

In preparing the inoculum, DA4.4 hybridoma cells were grown in 1 L shake flasks at 37 °C with 5% O_2 and agitation set at 95 rpm. The culture medium was prepared using Gibco® Hybridoma-SFM complete DPM powder supplemented with 5% Hyclone® Fetal Bovine Serum and 1% Gibco liquid Pen/Strep. For bioreactor cultivation, the 1.75 L vessel working volume was inoculated with a target total of 4.1 x 10^8 cells. Actual viable cell numbers were 3.5 x 10^8 cells (2.2 x 10^5 cells/mL) for the first run and 4.8 x 10^8 cells (3 x 10^5 cells/mL) for the second run. For both runs, hybridoma cells were cultured in CelliGen 310 bioreactors for nine consecutive days, using the basket impeller system packed with 75 g of Fibra-Cel disks. Perfusion was initiated for each bioreactor on day 3 and continued through day 9. Initially, the main objective was to increase the perfusion rate to maintain a glucose concentration at or above 1 g/L. For the second bioreactor experiment, the perfusion rate was adjusted to match the first bioreactor rate in order to make the two runs as similar as possible. Daily off-line measurements of glucose concentration were performed from both bioreactors, and the glucose consumption



Cultivation Setpoints	
Agitation	80 rpm
Temperature	37 °C
pH	7.15
DO	50 %
Gas supply	CO_2 for pH control
Gas flow conditions	0.4 SLPM
Vessel	1 L glass water jacketed
Fibra-Cel	75 g

Day	Perfusion volume (L)
3	0.73
4	1.81
5	4.25
6	5.50
7	4.25
8	4.75
9	5.00

Packed-bed basket impeller including Fibra-Cel disks

CelliGen 310 with 1L vessel

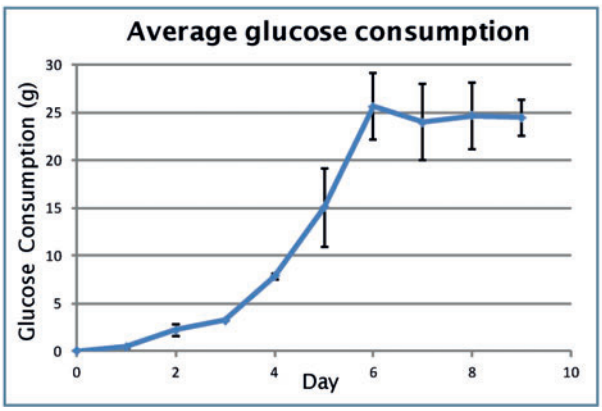


Fig. 2: Experimental setup and results; cultivation parameters, perfusion volumes and equipment used (left and upper right). Average glucose consumption of both runs as an indicator of cell productivity (lower right). Error bars indicate standard error of the mean.

was calculated for each time point and plotted as an average of the two independent runs.

The performed experiments have demonstrated that the implementation of packed-bed Fibra-Cel growth conditions in addition to perfusion production methods greatly increase yields of hybridoma cells, which are inherently sensitive to waste buildup. Fig. 2 shows the rate of glucose consumption across both trials. Comparable consumption was observed, indicating reproducible growth performance of hybridoma cells in this environment. One conclusion is that the use of Fibra-Cel in the basket impeller system on the CelliGen 310 is an excellent method for high-density hybridoma culture. In batch runs with common pitched blade impellers, hybridoma cells usually peak at approximately 5 g/day of glucose consumption (data not shown). The packed-bed basket impeller system presents significantly higher productivity, with glucose consumption peaking at an average 25 g/day. In addition, if growth conditions are maintained by continued fresh media perfusion and glucose concentration is never allowed to fall below 1 g/L, hybridoma can be continuously cultured in the basket many days after the nine-day window observed in this study. This further increases productivity and decreases overall antibody production costs. No optimisation of growth conditions were attempted for either bioreactor run.

Conclusions

In summary, Fibra-Cel provides benefits in research laboratories as well as for commercial production of mAbs. Because higher yields can be achieved, smaller bioreactors can be used to substantially reduce initial capital expenditure, as well as to reduce the utilities required for operation (such as electricity, water, and steam if required). In addition, because the cells remain entrapped, the packed bed eliminates the need for cell filtration to separate cells from the end product, thus simplifying harvesting. Finally, product recovery and downstream processing can be more easily controlled because users can determine the volume of harvest material that is to be processed at any given time.

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Taking mAb purification to the next level

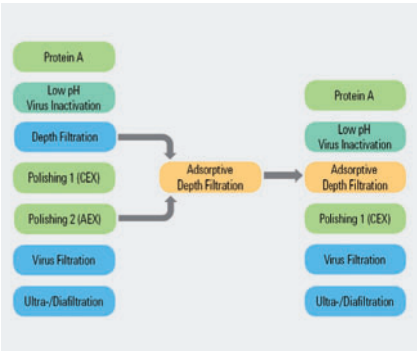
BIOMANUFACTURING From a processing perspective, mAbs are usually manufactured using a typical platform process that consists of three purification steps based on chromatography – and increasingly relies on membrane adsorption. German contract manufacturer Rentschler has now come up with a simplified two-step programme that is more economical, yet maintains quality and safety standards throughout the purification process.

› Dr. Dethardt Müller, Rentschler Biotechnologie GmbH, Laupheim, Germany

Most generic purification platforms for monoclonal antibodies and Fc fusion proteins include a Protein A affinity chromatography step and two polishing steps that employ cation and anion exchange chromatography, or membrane adsorption. The primary objective of these polishing steps is to remove process-related impurities, in particular residual host cell proteins (HCP) and DNA, as well as product-related impurities (aggregates, fragments). Moreover, each polishing step decisively contributes to process safety in direct relation to its virus removal capabilities.

Adsorptive depth filtration

While depth filtration is a widely used unit operation specifically aimed at removing precipitated particles that occur upon acidic virus inactivation, a new generation of adsorptive depth filters is also able simultaneously to remove process-related impurities. These single-use hybrid clarifiers contain a Q-functional anion exchange (AEX) hydrogel media that is integrated with a fine-particle bioburden reduction membrane. Rentschler has now set up a shortened purification process for mAbs that capitalises on this hybrid function, allowing users to skip the AEX-based polishing step (see above). The new two-step purification platform has been shown to increase overall yields of the target drug substance, while at the same time maintaining high product quality, process safety and robustness.



Novel two-step downstream process for mAb purification when applying adsorptive depth filtration.

Removing viral contaminants and process-related impurities

Rentschler has tested different adsorptive depth filters for their ability to remove process-related impurities and viral contaminants, including the functionalised non-woven Emphaze™ AEX Hybrid Purifier (3M, USA). All virus clearance studies in the test employed enveloped X-MuLV (Xenotropic Murine Leukemia Virus) and small non-enveloped MVM (Minute Virus of Mice). Both model viruses are negatively charged at neutral or basic pH, and both putatively bind to the positively charged surface of a filter membrane.

The study demonstrated significant virus removal by anionic adsorption, with samples reaching log reduction values (LRV) of 2.0-5.0 for X-MuLV and 2.5-7.0 for MVM – comparable to values achieved by classic AEX-based polishing steps. The viral clearance potential of the Emphaze™ AEX Hybrid Purifier by electrostatic retention could moreover be viewed as an orthogonal method for the currently adopted virus filtration step, which is based on size exclusion. Adding adsorptive depth filtration to the shortened two-step antibody purification process (Protein A, CEX) removed process-related impurities like HCP and DNA as effectively as the classic three-step process (Protein A, CEX, AEX), while overall product yields rose.

The process simplification has allowed Rentschler to be more economical in its mAb purification platform, while improving overall process safety and yield. ■

Picture: Rentschler Biotechnologie

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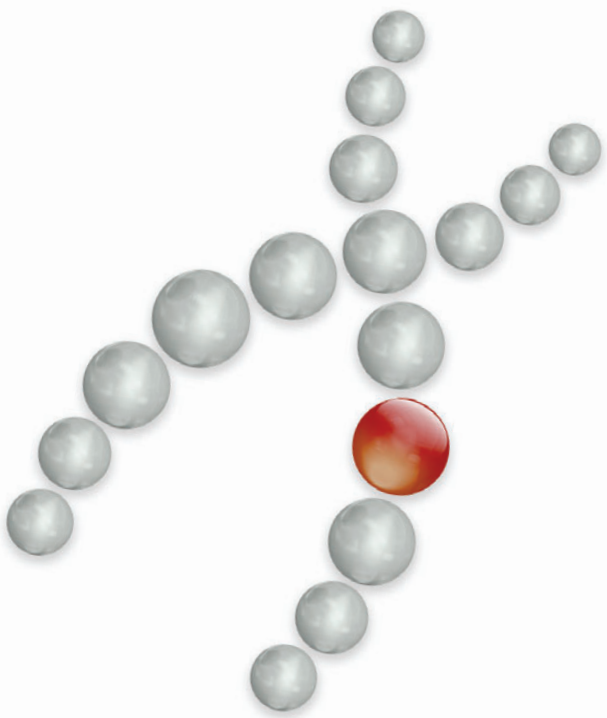

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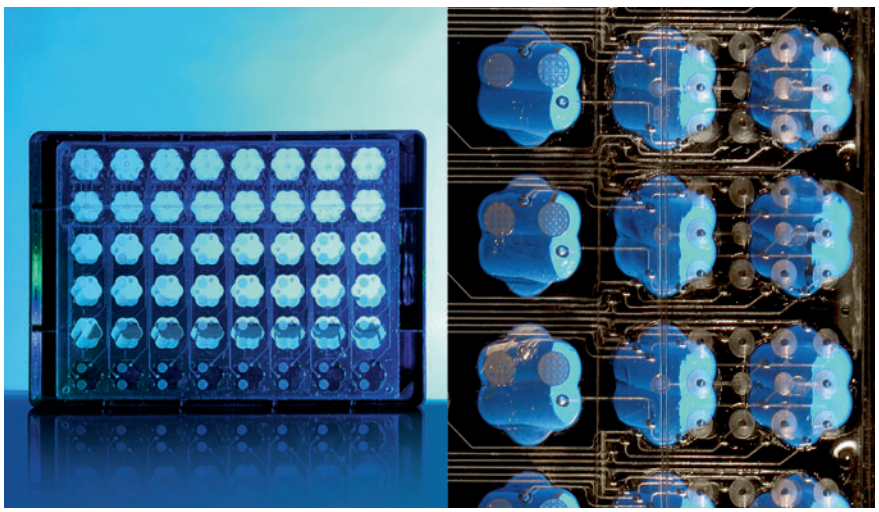
BIOPROCESS DEVELOPMENT Developing efficient bioprocesses as quickly as possible while at the same time reducing costs is a tall order. To achieve high yields and product titers, you have to conduct intensive screening – a process that involves a vast number of experiments relating to strains, media composition and process conditions. High-throughput fermentation at the microliter scale provides profound understanding and optimised bioprocess parameters for scale-up.

› Till Olfers, Sebastian Hofzumanns, Frank Kensy, m2p-labs GmbH, Baesweiler, Germany

Already established on the market for a few years, as well as in both academia and industry, the BioLector® system offers highly parallel fermentations in a 48-well microtiter plate with a standard SBS footprint. The system's FlowerPlate® technology provides full online measurements of biomass, DO and pH-value, as well as up to three additional fluorescences for each individual well in 800-1500 µl working volumes. Due to its special shape, the FlowerPlate can reach oxygen transfer rates (OTR) of up to 120 mmol/L/h – comparable to bench-top stirred-tank reactors. The disposable plates are delivered pre-calibrated, sterile and ready-to-use.

Proof of concept

In a recent study, this versatile tool was used to rapidly determine the metabolic effects of a variety of oxygen transfer conditions in the cultivation of *C. glutamicum* [Käß et al., Bioprocess Biosyst Eng 2014, 37:1151-1162]. Oxygen supply is crucial in industrial application of microbial systems. With the oxygen transfer screening approach, the investigating researchers studied metabolic effects within a broad range of supply conditions in a fraction of the time that is normally required for common protocols. The study identified and examined systematic effects on growth, productivity and side-product formation. Monitoring respiration activity additionally provided relevant information for scale-up and transfer to other reactor systems. It has been shown that oxygen supply – like established screening procedures for strains or media components – is yet another suitable development parameter during the earliest stages of bioprocess optimisation. The use of high-mixing, high-gas transfer microtiter cultivation devices like the BioLector provides a suitable alternative to stirred tank or shake-flask cultivations in bioprocess development. It reduces the number of required cultivations for reaching optimised operating conditions in aerobic bioprocesses, and thus boosts the efficiency of many developments in current industrial biotechnology.



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The BioLector® Pro

system offers individually and fully-controlled reactor wells with liquid pH-regulation and fed-batch possibilities. A chip replaces the usual plate bottom, allowing investigators to pump nanoliter-amounts of liquid independently into individual wells without losing the online monitoring capabilities of the basic system. While the first two well-rows act as reservoirs for the desired feeding solutions, the remaining 32 wells work as distinct bioreactors. The task for each reservoir row can be chosen independently, enabling the system to deliver two different feeding solutions: one feed and one pH-value up-/down-regulation, or full pH-control for each well column. Constant, linear or exponential feeding can also be set, while a PI-controller derivate ensures proper pH-control. The microfluidic technology utilises pressurised air to actuate membrane valves at the bottom of the microfluidic chip. The liquids are pumped through the chip via microchannels directly into the wells. The complete plate, a disposable item, remains a closed system. The system's proprietary software provides a user-friendly interface that allows experiments to be defined and supervised. The progress of the 32 parallel cultivations can be controlled in realtime, and displayed promptly. Additionally, calibration sets of biomass concentration and optodes for DO and pH-value can be imported. With a few clicks, you can access different analysis functions that allow the evaluation and comparison of measurement data and derivatives from one or more experiment runs of 32 parallel fermentations.

A solid bioprocessing investment

With the help of microbioreactors, a researcher can set up and analyse a wide variety of experiments and gain a profound understanding of the studied bioprocess within a short time frame. Microfluidic technology brings full bioprocess control and precise dosing to the micro-scale, allowing parameters to be optimised at an early stage. Down the line, only a few confirmation runs are necessary at the lab-scale for further up-scaling. BioLector Pro technology is paving the way for much faster R&D and the application of increasingly advanced industrial bioprocesses.

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The inner (mini)circle

INTERNATIONAL CONFERENCE Some of Europe's leading scientists met up in Bielefeld (Germany) to exchange information about new approaches in minicircle and DNA vector technology. As regulatory requirements for transfection grow stricter, bullseye targeting has become even more significant.

› Erika Sahrhage, PlasmidFactory GmbH & Co. KG, Bielefeld, Germany

The range of current projects and results presented at the 3rd Minicircle & DNA Vector Conference in Bielefeld highlighted how much DNA vector technology has evolved since the first two forerunner conferences took place back in 2007 and 2008. Over 60 scientists from many nations took part. "We wanted specifically to invite young scientists to present their research and give them the chance to learn more about the latest developments in Minicircle technology and its applications in DNA vaccination and gene therapy," said PlasmidFactory CEO Dr Martin Schleef, who was also the conference's scientific organiser.

Platform for gene therapy and vaccination

Minicircles (MCs) are circular non-viral DNA elements that can be generated by intramolecular (cis-) recombination from a parental plasmid (PP). The difference between MC and standard plasmid vectors for gene therapy or nucleic acid vaccination is that minicircles don't contain a bacterial origin of replication (for amplification of plasmids in bacteria), antibiotic resistance markers (ABR) or other selection systems to keep high amounts of the plasmid within the producer cell.

Since regulatory authorities require developers to avoid ABR and unnecessary (or CpG-containing) sequence elements in plasmids for pharmaceutical use, their removal is a major goal in non-viral vector development, as well as in the area of support for the plasmid-based production of viral vectors (e. g. AAV, LV).

"Minicircles are a promising tool in meeting demand for increased efficacy, as well as in terms of regulatory requirements involving future clinical applications," Schleef pointed out at the conference. Europe's leading service provider in the field, PlasmidFactory also owns the most relevant patents and IP, according to Schleef.

The first day of the conference, which focused on MC technology and applications, featured presentations on the history of plasmid size reduction (Dr. D. Scherman, Université René Descartes, Paris, FR) and progress in the production of custom-made minicircle DNA (Dr. M. Schmeer PlasmidFactory, Bielefeld, DE).

The second day of the meeting was dedicated to quality assurance (Dr. H.G. Eckert, Gempex GmbH, Mannheim, DE) and the current patent situation (Patent Attorney Dr. Martin Grund, GRUND IPG, Munich, DE). Additionally, the conference looked in-depth at quality standard requirements in gene therapy. Dr. A. Constanzo, (EDQM, Strasbourg, FR) explained the importance of the Gene Therapy Working Group of the General European OMCL Network in the quality control of gene therapy products.

Supported by PlasmidFactory, the Young Investigator Session for the first time also played an important part in the programme by giving young scientists the chance to present their recent work in the field of novel, non-viral DNA vectors.

Participants in the 3rd Minicircle & DNA Vector Conference at the conference venue in Bielefeld.

Picture: Plasmid Factory

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Broad P. pastoris expression platform

CONTRACT DEVELOPMENT The recombinant protein production market is rising steadily every year. In many cases, the complexity of a target protein determines the choice of a manufacturer's expression system.

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Several classes of expression systems – ranging from bacterial hosts to mammalian cells – are in routine use today. In the search for the perfect platform, yeast is an excellent choice. *Pichia pastoris,* for example, combines the advantages of prokaryotes (fast growth to high cell densities on inexpensive and chemically-defined media, easy genetic manipulation) with eukaryotic features that include the subcellular protein processing machinery needed for post-translational modification and secretion. The *P. pastoris* expression system is recognised as an established and highly-competitive host for the fast and economic production of recombinant proteins. A wide range of diverse *Pichia*-based products is already on the market, with many more in development.

VTU Technology has developed a *P. pastoris* expression platform that is both broad and comprehensive; one that stands out due to the diversity of its expression tools and ingenious expression strategies. VTU´s key technology – its synthetic AOX1 promoter libraries – has unprecedented genetic diversity, with superior regulatory properties for fine-tuning gene expression. It works by selecting the perfect match from among a huge range of different combinations of promoters and a target gene.

VTU´s exclusive, first-generation library of synthetic methanol-inducible PAOX1 promoter variants is at the core of the company´s cutting-edge in-house *P. pastoris* toolbox. It provides a foundation that enables high-level protein production, and the secretion of often more than 20g/L of target protein. This library was complemented with groundbreaking and unique methanol-free second-generation PAOX1 promoter variants, which facilitate strong expression, even when glycerol or glucose are the only available carbon sources. It clearly outperforms conventional promoter systems.

The versatility and effectiveness of VTU´s *P. pastoris* system is further underlined by a set of proprietary expression-enhancing helper factors, several platform strains with different genetic backgrounds, elaborated cloning and transformation protocols, a high-throughput microscale screening and cultivation regime, and effective fermentation protocols for maximisation of product yield and overall process performance. ■

Picture: VTU Technology

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