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Biotechnologie

REPORT OF THE TEMPORARY WORKING GROUP

“Single-use Technology in Biopharmaceutical Production”

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1. Introduction to single-use technology

In the biopharmaceutical industry, the term “single-use” (also commonly known as “disposable”) refers to product that is intended for a one-time use. Generally such objects are made from a plastic (polyamide {PA}, polycarbonate {PC}, polyethylene {PE}, polyethersulfone {PESU}, polypropylene {PP}, polytetrafluorethylene {PTFE}, polyvinyl chloride {PVC}, cellulose acetate {CA}, ethylene vinyl acetate {EVA}, see also Point 3.1) and are disposed of after use. Accordingly, single-use technology (SUT) is to be understood as a technology based on single-use systems (SUS).

The foundation for SUT was set in 1953 by the company Fenwal Laboratories (today Fenwal Blood Techniques, Illinois) with the first plastic blood bag [Codner and Chat 2005]. In the 1960’s, the market saw the advent of plastic bottles, flasks, Petri dishes and 96-well plates, which increasingly replaced their glass counterparts for routine tasks in cell culture laboratories (passaging, cell expansions, screening). Another key milestone in SUT was set by Knazek and his team in the early 1970’s [Knazek et al. 1972]. They developed the first hollow fibre bioreactor and demonstrated that mammalian cells could be cultured at high cell densities under *in vivo* like conditions, where hollow fibre membranes could be used in a disposable cartridge for a continuous culture processing in perfusion mode. This formed the basis for the popular *in vitro* diagnostic and therapeutic mg-scale production of antibodies in the 1980’s. Similarly, in the mid 1970’s Nunc and Bioferon (now Rentschler) began producing polystyrene Cell Factories [Schwander and Rasmusen 2005]. These systems were primarily used for cultivation of adherent mammalian cells; in the 1990’s they replaced the roller bottles used for cell expansion and simple biopharmaceutical products in GMP vaccine production (including vaccines against polio, rotavirus and hepatitis A).

Over the past decade, the number and variety of SUS available on the market in biopharmaceutical development and production processes has continued to increase steadily. In 2009, a 35% growth rate was reached, largely from products for Upstream Processing (USP) [Langer 2009]. In early 2000, Hyclone (today a part of the ThermoFisher Group) introduced the first disposable bag for storage and transport of buffers and media. In addition, dialysis membrane reactors such as CeLLine [Trebak et al. 1999], the MiniPerm System [Falkenberg 1998] and wave-mixed bag bioreactors [Singh 1999] made their way into modern biotechnology research labs. Undisputedly, the driving force for the ensuing rapid further development of SUT was the Wave Bioreactor 20 (the first wave-mixed bag bioreactor) and above all its successful application despite initial scepticism against the new mixing principle and its subsequent scale-up (up to 500 L of culture volume).

Today, users can choose from a large selection of SUS products from a whole range of different suppliers. The products can be categorized into systems for everyday lab use, simple peripheral systems and systems for basic operations and process platforms, as shown in Figure 1 [Eibl et al. 2011a]. Most SUS products are used in processes where protein-based biotherapeutics made from

mammalian cells are the target product. With the availability of single-use connectors, sampling and transfer systems, mixers and bioprocessing containers and other single-use bioreactors as well as sensors and single-use pumps, today complete single-use USP for culture volumes up to 2 m^3 is possible (see also Point 3.4). Although the wave-mixed bioreactors (Wave Bioreactor by GE Healthcare and Biostat CultiBag RM by Sartorius Stedim Biotech) have dominated inoculum production and can no longer be done without, new protein-based biotherapeutics are preferred for the stirred

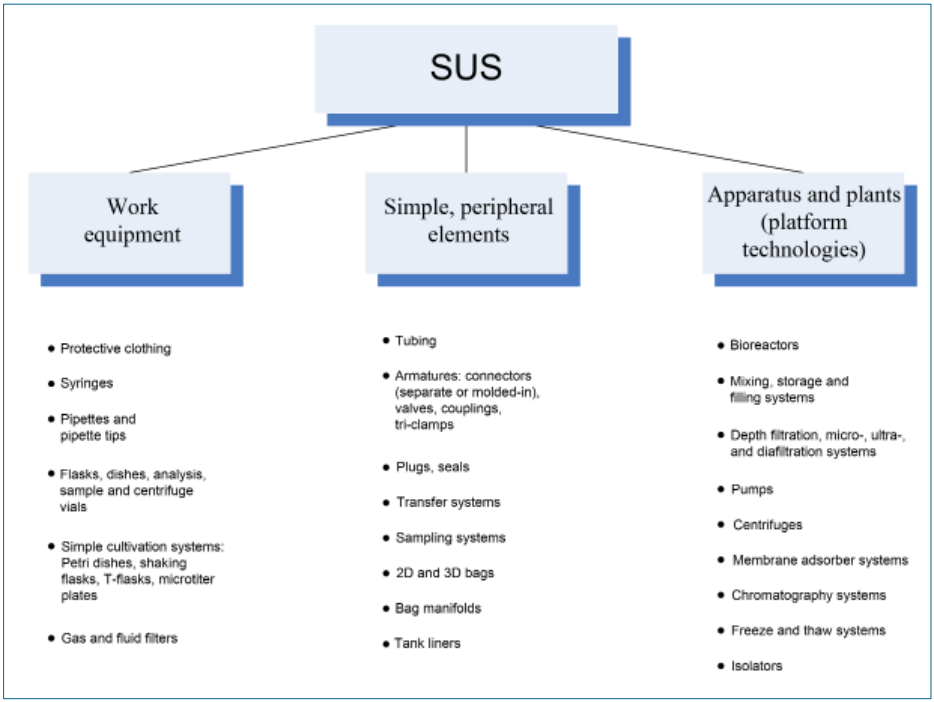

Figure 1: Categories of the SUS currently available on the market [modified after Eibl et al. 2011a]

single-use bioreactors (SUBs). These latter can be equipped with standard or disposable sensors [Lindner et al. 2011] (see also Point 3.6) and are available as benchtop-scale with rigid plastic tanks (e.g. Mobius CellReady, UniVessel SU, CelliGEN BLU) or as flexible bag systems for culture volumes of 4.5 L (S.U.B., Biostat CultiBag STR, XDR Bioreactor) with heat sleeves or temperature-controlled steel containers that hold the bag and maintain its shape [Eibl and Eibl 2009b].

Although the increasing use of SUT in USP also led to the development of SUS for Downstream Processing (DSP) (centrifuges, freeze-thaw systems, filling systems, tangential and depth filtration systems, chromatography columns, membrane adsorbers), SUS is not as significant a factor in

DSP as it is in USP (see also Section 3.5). Instead, DSP and especially chromatography, which is restricted to 20 L by pre-packaged and sanitized columns (“Plug and Play”) are currently the limiting factors [Blackwell 2010]. However, the limitations of SUS also include the limits with respect to pressure, flow rates, centrifugal forces, temperature and O_2- and CO_2 stripping rates. Other restrictions include potential leachables and extractables [Jenke 2007], size limits, higher costs for consumables, lack of standardization or categorization, supply security, and the current lack of sensor technologies in automation. Finally, the successful implementation of SUT also depends on changes and new approaches in system design, employee training, quality assurance and production processes (see also Section 3.3), all of which start already in the planning stage [Sinclair 2009].

Nonetheless, the SUS and SUT available on the market, when used and handled correctly, provide a means for smaller, cheaper, greener, safer and faster development and production [Ott 2011]. This fact also undoubtedly explains why these systems can no longer be done without in all the main process steps in small to medium scale production of biopharmaceuticals and biosimilars, particularly in USP. This pertains to the rapid development and time to market for new biotherapeutics such as antibodies and veterinary and human vaccines. The majority of biotherapeutics producers (particularly contract manufacturers) deploy SUS wherever possible. In German-speaking countries, this includes Baxter Austria, Boehringer Ingelheim, Merckle-Biotec, Hoffmann-La Roche Germany and Switzerland, Merck Serono Germany and Switzerland, Novartis Switzerland and Austria, Rentschler and Werthenstein BioPharma.

Hybrid production systems still dominate in these companies. Single-use and traditional systems using glass or stainless steel are combined. However, there are plans for the first production facilities that are fully based on SUS. In these cases, there is a distinction between closed production types of facilities (those in which the SUS is pre-set and tied to the sequence of individual steps in the process and there is the potential for free draining or hydrostatic pressure for the transport of source materials, intermediate and end products from one process step to the next) and production facilities with operations divided into stations (where source materials, intermediate and end products are transported in mobile containers from one step to the next) [Peuker and Eibl 2011].

The global developers and manufacturers of SUS listed in Table 1 are also included in this trend with their products. The largest portfolio is currently held by GE Healthcare, Merck Millipore and Sartorius Stedim Biotech. Among German-speaking countries, Sartorius Stedim Biotech is the leader in the field of Single-Use with their research and development and production centres in Göttingen (Germany) and Tagelswangen (Switzerland).

Table 1: Overview of selected developers and manufacturers of SUS (worldwide, alphabetic order)

Company	Website	Single-use products
Aber Instruments	www.aber-instruments.co.uk	Sensors
AC Engineering	www.acengineering.co.il	Pumps
Adolf Kühner AG	www.kuhner.com	Bioreactors, incubation shakers for single-use bioreactors
Advanced Scientifics	www.advancedscientifics.com	Bags, containers, connectors, filters, filling systems, sampling systems, tube welders
AmProtein	www.amprotein.com	Bioreactors
Applikon Biotechnology	www.applikon-bio.com	Bioreactors
ATMI Life Sciences	www.atmi.com	Bags, mixers, bioreactors
Bayer Technology Services	www.bayertechnology.com	Mixers, bioreactors
Bosch Pharma	www.boschpackaging.com	Dosing and filling systems
C-Cit	www.c-cit.ch	Sensors
CeLLution Biotech	www.celltainer.com	Bioreactors
Charter Medical	www.chartermedical.com	Bags
Cole-Parmer	www.coleparmer.de	Sensors
Corning Life Sciences	www.corning.com	Plates, flasks, dishes, disposables for routine work in cell culture labs
Eppendorf	www.eppendorf.com	Bioreactors, disposables for routine work in cell culture labs
ExcellGene	www.excellgene.com	Disposables for routine work in cell culture labs, bioreactors
Finesse Solutions	www.finesse.com	Sensors, systems control, bioreactors
Fogale Biotech	www.fogalebiotech.com	Sensors
Fluorometrix	www.fluorometrix.com	Sensors
GE Healthcare	www.gehealthcare.com	Bags, containers, connectors, filters, tubing, sampling systems, mixers, bioreactors, filling systems, chromatography columns, platform solutions for USP and DSP, tube welders, re-sealers
Gemue GmbH	www.gemue.de	Membrane valves

Company	Website	Single-use products
Gore	www.gore.com	Sampling systems, tubing, valves, freeze- and freezedry dishes
Hamilton Medical	www.hamilton-medical.com	Sensors
HyNetics	www.hynetics.com	Mixers
ICM Technologies	www.icmtechnologies.com	Bags, containers
Infors AG	www.infors-ht.com	Incubation shakers for single-use bioreactors
JM Separations	www.jmseparations.com	Bags, tubing, manifolds, mixers, filters, platform solutions for USP and DSP
Jobst Technologies	www.jobst-technologies.com	Sensors
Levitronix	www.levitronix.com	Pumps
Meissner	www.meissner.com	Bags, bioreactors, containers, filters
Merck Millipore	www.merckgroup.com	Bags, containers, connectors, filters, tubing, sampling systems, mixers, bioreactors, incubation shakers for single-use bioreactors, filling systems, platform solutions for USP and DSP
mp2-labs	www.mp2-labs.com	Bioreactors
Nalgene Labware	www.nalgenelabware.com	Bags, plates, flasks, filters
Nestlé	www.nestle.com	Bioreactors
Nunc Brand	www.nuncbrand.com	Plates, flasks, dishes, disposables for routine work in cell culture labs
Ocean Optics	www.oceanopticsensors.com	Sensors
Pall	www.pall.com	Mixers, bags, bioreactors, connectors, filter, filling systems, platform solutions for USP and DSP
PBS Biotech	www.pbsbiotech.com	Bioreactors
PendoTECH	www.pendotech.com	Sensors
PreSens	www.presens.de	Sensors
Qattroflow	www.quattroflow.com	Pumps
Saint-Gobain	www.medical.saint-cobain.com	Connectors, tubing

Company	Website	Single-use products
Sartorius Stedim Biotech	www.sartorius-stedim.com	Bags, containers, connectors, filters, tubing, sampling systems, mixers, bioreactors, filling systems, membrane adsorber, freeze and thaw systems, platform solutions for USP and DSP, tube welders, re-sealers
Sebra	www.sebra.com	Tube welders
Schulte Bagtainer	www.schulte-bagtainer.de	Containers
SciLog	www.scilog.com	Sensors
Terumo	www.terumo-europe.com	Bags, tube welders, re-sealers
Thermo Scientific	www.thermo.com	Bags, containers, mixers, bioreactors
TPP	www.tpp.ch	Plates, flasks, dishes, disposables for routine work in cell culture labs
TRACE Analytics GmbH	www.trace.de	Sensors, sampling systems
Xcellerex *(now part of GE Healthcare)*	www.xcellerex.com	Mixers, bioreactors, platform solutions for USP
3M Purification	www.solutions.3m.com	Filters

Things are in the state of flux in the business sector of developers and manufacturers of SUS. Occasionally new players arrive on the scene. However, it is generally believed that there will be a consolidation among the many producers within the next few years. Those companies with a wide range of products and who can guarantee versatile support for the user throughout the whole process are at an advantage.

Since the beginning of 2000, working groups from academic institutions in Germany have been involved in research activities associated with SUT (Leibnitz University in Hannover, Technical University of Magdeburg) and Switzerland (École Politechnique Féderalé, Eidgenössische Technische Hochschule Zürich [Swiss Federal Institute of Technology in Zurich], Zurich University of Applied Sciences). This research is primarily focussed on bioreactor and sensor developments, as well as process development and the associated applications for animal and human cells.

Although the Bio-Process Systems Alliance (BPSA, www.bpsalliance.org) has supported and guided the development and application of SUT as well as training in English-speaking countries for six years, in German-speaking countries no such coordination is taking place despite the activities within industry and academia. With this in mind, in spring of 2010 the temporary working group (TWG) “Single-use technology in biopharmaceutical production” was formed from the “Bioprocess technology” working committee of DECHEMA.

2. TWG "Single-use technology in biopharmaceutical production"

At present the TWG consists of 64 specialists from 37 companies and 12 academic institutes in Germany, Belgium, Switzerland and Austria. Following an analysis of the imminent challenges and future perspective of SUT, the TWG members were organized into the following seven working groups (WGs):

- WG on Materials and their Properties, Qualification and Validation
- WG on Component Harmonisation and Logistics
- WG on Project Planning
- WG on Bioprocess Technology USP
- WG on Bioprocess Technology DSP
- WG on Bioprocess Technology Sensors
- WG on New Application Areas for Single-use Systems

The prime goals of the "Single-use technology in biopharmaceutical production" TWG are the promotion of SUT and the compilation of relevant activities in the areas of research, production and application in German-speaking countries. In addition, the standardisation of SUS and SUT takes high priority. The status paper produced by the working groups should call attention to the current possibilities and limits of SUS in biopharmaceutical production (from the point of view of the user and the manufacturer) and in addition should specify the key needs for action in this area among German-speaking countries.

3. Contributions of the working groups

3.1. WG on Materials and their Properties, Qualification and Validation

Despite widespread use and acceptance of SUS in biopharmaceutical production processes, users are currently faced with nine challenges, listed in Box 1. These challenges originate primarily from materials and the associated qualification and validation of SUS and the processes arising thereby. Hence, pharmacopoeias describe test for polymers (EP: 3.1.x, USP <87>, USP <88>, USP <381> or USP <661>), but they present these screening tests. However, not all polymers from which SUS can be made have been entered into the pharmacopoeias.

CURRENT CHALLENGES IN THE INTRODUCTION OF SINGLE-USE SYSTEMS

1. There are no defined, pharma-grade polymers.
2. The qualification and validation data of all the producers are variously compiled and informative. Often not all additives are specified.
3. There are no regulatory requirements for SUS for the overall biotechnology process, only for individual SUS at best (e.g. for filters) and for the end product. Consequently, the requirements for inspections by authorities are not defined for the individual production steps.
4. For a complete review, a universal approach for all SUS is required. In addition to bags and filters, this also includes tubing, tube connectors, membranes, etc.
5. Within the individual clinical phases, the same SUT should be used whenever possible in order to highlight its suitability.
6. In general, there have been few risk analyses for SUS
7. Development is proceeding at a rapid pace for base materials (plastics) of SUS and its processing.
8. The requirements for SUS can vary widely, depending on the length of time of contact with the SUS, the importance of individual extractable substances for each process or surface-active substances such as Tween can influence an extraction.
9. There are no evaluated analytical methods with acceptance criteria for leachables and extractables.

Box 1: Typical user-defined problems in the introduction SUS

The suppliers of SUS provide the user with a clean, fully assembled, ready-to-function and sterile SUS and this presupposes a high safety level in the production process of SUS. The suppliers must indicate this production safety in their qualification and validation brochures. The most important aspects in the implementation of SUS in this context are the supplier's qualifications, the SUS qualification, the sterility and the testing of extractables/leachables.

The manufacturers of SUS basically supply the user with information regarding the material qualification and the suitability of SUS for the respective use (see Table 2). The regulatory requirements for the material qualification of SUS are set by EU GMP Guide Part II, 21 CFR 211.65(a) and ICH

(Q7A). It is necessary here to ensure that the interactions (extractables/leachables, adsorption, by-products or decomposition products) between the product and the SUS will have no effect on the quality of the active agent/pharmaceutical to be produced. These specifications, however, do not apply to the overall biotechnological process, but to the end product. In addition to these legal requirements, compliance with the guidelines provided in pharmacopoeias must also be observed. For the analysis and evaluation of extractable substances, depending on the intended use, the publications summarized in Table 3 may be consulted.

Ultimately, the pharmaceuticals manufacturer assumes more of the responsibility with the decision to introduce SUS. That is to say, the pharmaceuticals manufacturer as a user of SUS must demonstrate that the selected materials will result in the target product of a defined quality under the specified process conditions (process solution, temperature, time, pressure, etc.), and this will be documented in the process validation [Merseburger 2011]. Which additional studies and validations will be necessary is something the user should be able to determine by means of a risk-based approach using the SUS manufacturers' qualification/validation documentation.

Table 2: Overview of manufacturer-provided information on SUS

Information on	Specification	Valid documents	Object of qualification
Material suitability	QA system of supplier	ISO 9001 et seq. or alternative system based on EC 2023/2006	Raw material qualification
	Data on substances extractable from the raw material	EP chapter. 3.1.x and USP <87>, <88>, <661> and <381>; EU 2002/72	
	Certificates: free from TSE/BSE	EMA/410/01, Rev. 2 - October 2003 (under consideration of EC 1774 / 2002; Annex VI Chapter III)	
	Compliance with REACH	ECHA/PR/08/38-REV, including DEHP	
	Information on bisphenol A	Public health authority of Canada: bisphenol A is orbidden for critical applications	
	Allergenic substances		

Information on	Specification	Valid documents	Object of qualification
Chemical tests	Water tests according to the monographs for sterile water	USP and EP	Product qualification
	Extractables analysis	Manufacturer-specific	
	Chemical compatibility	Manufacturer-specific, on the basis of ASTM D 543-06	
Physical tests	Temperature, through flow, gas transmission, physical stability	Manufacturer-specific	
Function tests	Stability after evaporation and gamma irradiation	Manufacturer-specific	
	Accuracy applica-tion area: pressure, through flow		
	Test of joint strenght	HIMA document; ASTM F-838-05	
	Bacterial deposition tests, storage stability	Manufacturer-specific	
Hygiene	Bioburden	ISO 11737	
	Particle release	USP <788>, E.P. 2.9.19	
	Endotoxins	USP<85> and E.P. 2.6.14	
Package	Primary package (for medical devices)	ISO-11607-1	
Storage stability	Real storage tests	Manufacturer-specific	
	Tests under accelera-ted conditions	Manufacturer-specific, on the basis of ASTM F 1980-02	

Remark: The abbreviations used in this table are given in the list of abbreviations (point 6).

Table 3: Publications for research on and evaluation of extractables

Document	Title
EMA	Guideline on Plastic Intermediate Packaging Materials
FDA	Guidance on Container Closure Systems for Packaging Human Drugs and Biologics
Guidance for Industry	Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
ICH Guideline Q8	Pharmaceutical Development
ICH Guideline Q6A	Test Procedures and Acceptance Criteria for New Drug Substances and Drug Products
PQRI	Safety Tresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products
PQRI (enacted in 2011)	Thresholds and Best Practices for Parenteral and Ophthalmic Drug Products
BPSA	Recommendations for Testing and Evaluation of Extractables from Single-use Process Equipment
PDA (enacted in 2011)	Technical Report on Single Use Systems

Remark: The abbreviations used in this table are given in the list of abbreviations (point 6).

3.2. WG on Component Harmonisation and Logistics

Highly diverse manufacturing processes for synthetic products attempt to meet the rapidly increasing demand for SUS in all areas of the biopharmaceutical production method. While simple components can quite easily be manufactured with automated procedures (e.g., injection moulding, extrusion, welding), the manufacture of more complex systems such as peripheral single-use elements involves a highly manual process [Vanhamel and Masy, 2011]. For that reason, the exponential growth in capacity presents an increased statistical risk for the occurrence of manufacturing errors which must be reduced.

Manufacturers of SUS address this issue with regulatory provisions (e.g., PDA, ISO9001) for the qualification of the production processes. Methods for quality assurance must also be established to prevent damage to sub-components and to ensure that transitions and connections within the system design are linked in such a way as to prevent leakage and non-sterility within the components during use. Currently, this is being safeguarded by the implementation of tensile and compression testing at the respective transition points (e.g., between tube clips and the tube connecting piece) and their mechanical fastening is defined by means of fixing devices such as cable ties, BarbLock ® or alternatively, clamps. In this manner, a qualification matrix is created which corresponds to a statistical test mask and substantiates a system's conformity. To date it is not possible, however, to verify the correct procedure for manufacturing a complete system design whereby correlated measurement techniques are applied to complex end products which simultaneously provide proof of integrity in instances of external influence factors.

In the meantime, the market shows an increasing need for these kinds of test procedures which can be employed by manufacturers as well as by users. Because such procedures are not currently available, the user's application of SUS in critical production stages is often limited. The risk of the system's failure to perform (leakage and the associated contamination of the pharmaceutical product) is accompanied by the risk of financial losses. A test procedure can be of use here to provide evidence for the user that there were no instances of damage to the system's integrity before, during or after its use. In particular, test procedures to be used before the actual use are of interest, as they provide prior assurance that no defective components are being used. By the same token, these procedures should not compromise the compatibility of the components nor are the efforts and expenses to be influenced so as to match the actual reason for using the SUS.

In view of the fact that such tests are not currently available, great significance is attached to the correct storage as well as to the transport locking of the finished and most often gamma-irradiated end products. For example, objects with sharp edges should be packed in such a way that no ad-

jacent connected components (e.g., bags) can be damaged during the transport and storage. Even the most minor damage to the surface may endanger the system's stability during later use and thus present a hazard for the user in terms of both safety and cost-efficiency.

It must therefore be taken into consideration that packaging materials must meet the requirements of pharmaceutical production facilities and cleanrooms. Thus foams or papier-mâché are not very suitable, because particles may be released in the handling of these materials and this is to be avoided in cleanrooms or (material) air locks. The chemical compatibility of the working materials should also be tested, since often surface disinfection is used during the introduction of the packaged SUS. Next to the selection of the material, the design of the packaging should be convenient and easy to manage.

The multitude of working steps required in order to customise process-specific SUS results in more laborious documentation and certification processes. At the same time, within the framework of their quality assurance, end users of these systems have a significant need for documentation related to the monitoring and tracking of the components as well as the procedures involving the manufacturing, the delivery and the warehousing/storage. Modern solutions for dealing with this tracking by means of a single system such as so-called Radio Frequency Identification (RFID) chips have not yet been generally accepted. The advantage of these RFID chips is the fact that they can be directly linked to the system and all the product-relevant data can be stored in them. It must be possible, though, that not only the manufacturer, but also third parties (e.g., sterilization services, forwarding agents, warehouse clerks etc.) have access to the chip and can read it and write on it, in the best case scenario without removing the outer packaging. Uniform standards should be established for the handling of these data, so that the supplier and the user can use the respective technology to discuss the products to be exchanged without a problem, while data protection and data security provisions are to be respected. Ultimately this also ensures that official requirements for the testing and documenting of a pharmaceutical production system are being met. This course of action also follows the original idea behind SUS, i.e. to reduce unprofitable steps of the procedure. A high potential for this rests with the documentation management as well as, where applicable, in the clarification of variations where it is often requisite that previous product related processes are reviewed and updated.

3.3. WG Project Planning

To date, numerous projects for the installation of SUS in the field of pharmaceutical cell cultures have been and are being set up in German-speaking countries. Thus, in Germany in the last 12 months, SUS contracts worth multi-millions have been implemented by the corporations Hoffmann-La Roche and Rentschler alone. By implication, there are an adequate number of experienced project planning companies and suppliers present in German-speaking countries. In spite of all this, the use of SUS in biopharmaceutical companies is not yet as widespread in Germany as it is in the USA. Instead, the extent of the use of SUS is limited to a handful of internationally active pharmaceutical companies. Because of the limited number of users, training customers is generally part of the project planning, since many customers are using SUS for the first time. Pre-existing challenges in the field of project planning primarily include facility layout, proper handling and waste management.

Facility Layout

The growing demand for the quality of biopharmaceutical products is also reflected in the design of production facilities. In addition to conditions imposed by companies, the process is largely driven by regulatory conditions and must focus on the potential risk of a contamination of the active agent. This includes first and foremost the technological measures to ensure that the production process takes place in completely sealed off areas. If that is not possible, the general conditions should be designed in such a way as to minimise or exclude the risk of contamination. The multiproduct character of many production facilities often means that the facilities are designed for the most unfavourable case of application, i.e. that the process sequences for the USP and DSP areas are often conducted in ISO 8 or 7 according to cleanroom classification. This results in high requirements for the quality of the cleanroom design as well as the related ventilation and climate control technology. Next to the necessary investment expenses, the operating expenses in particular represent a considerable portion of these demands.

Based on the increased use of SUS, which by their nature are combined in such a way that expanded, separate process areas are created, the risk assessments as carried out in conventional production facilities should be re-evaluated. Because of the closed manner of operating, conventional cleanroom requirements should be challenged. The use of local laminar flow units in a pharmaceutically controlled environment (CNC) or the processing in enclosed areas in controlled, but not classified environments are approaches under discussion in relation to the use of SUS. In this area, there is a need for scientifically verified work to examine new conceptional approaches for cleanroom requirements in the use of SUS. The personnel and material flow concepts under special consideration of the SUT present a further object of study regarding the facility's design. Frequently

the media storage/media supply areas are kept in a separate location from the basic technological operations. The transfer past cleanroom boundaries requires technical solutions for tube connectors, some of which are already available on the market, but where a considerable potential for development is evident, especially with regard to multiple tube connections.

Handling

The use of SUS is most often associated with numerous manually performed steps. Generally speaking, the automation level of the systems is lower than that of comparable conventional systems. This fact leads to the task of providing training for these manual steps and of monitoring and documenting them. Consequently most users need a reasonably priced coding system for single-use components, for example, to facilitate the integration of the components into Manufacturing Execution Systems (MES) by scanning them. There is also a need for “intelligent” single-use components that contain the information regarding batch data, assembly and technical data integrated in the single-use components.

Often an overall system for carrying out one process step is composed of individual components. In some areas of application (e.g., the use of dangerous substances or organisms with a hazard potential) the integrity of the overall system should be tested before the system is used. At present, neither a safe method nor the equipment necessary for on-site testing is available, since classic helium and hydrogen leak tests are hardly applicable in the case of plastics due to the permeability of the materials for these gases.

Waste Management Concepts

The successful implementation of SUT in a biopharmaceutical manufacturing process includes a proper waste management system. Since with SUS it is often a question of composite materials, the separation of the materials presents a challenge. Aside from various synthetics, sometimes metals are also used as components.

Since already nowadays all the process steps for selected applications are carried out using SUS, the volume of waste is correspondingly large. All the bags, tubing, filters, etc. used in the process are likely to be contaminated with organisms and/or environmentally hazardous chemicals and must be handled with that in mind prior to their disposal. Thermal or chemical procedures may be used. The synthetic material is usually disposed of by incineration or sometimes even by landfill [Baier, 2011].

At present, systems that can compact or reduce to smaller pieces the large amounts of waste are available on the market only to a limited extent. Especially when it comes to deactivation/decontamination, the market offers next to no solution. There is also no option available to separate the composites on-site and, if possible, recycle or reuse them. This means that elaborate and cost-intensive logistics are required which would compromise the advantages of using SUT in the process application. A great need exists here which must be met with innovative solutions. As previously mentioned in Point 1, the concepts for the material flow of SUT must be taken into account already in the planning stages of implementation projects with SUS. Since similar problems have arisen in other sectors of the industry, for example in the food industry, and solutions have been found, an adaption to the biotechnology sector should be possible.

3.4. WG on Bioprocess Technology USP

In general, USP includes the manufacturing, storage and preparation of culture media, production of inocula and seed inocula as well as the basic fermentation. Depending on the philosophy of scale, the separation of biomass following fermentation can also be a part of the USP. The technical unit operations listed in Box 2 are used in putting these processes into action. As described in Point 1, there are many different suppliers of SUS for the technical implementation of the unit operations. They differ on the basis of size, the mode of action and mixing, as well as instrumentation and are characterized by a defined fluid dynamic. In addition, the lowest shearing during mixing is stipulated with simultaneous homogenous energy distribution in various applications, primarily in cell culture. To implement continuously repeating tasks (mixing, storing and transfer, inoculum production and fermentation as well as biomass separation), the centralization of technical unit operations into process platforms has also proved successful. Process platforms are technically-implemented, well-defined procedures of operations or process steps. They have already been created for various sizes and numbers and sequences of the process steps in the production of media, fermentation and in biomass separation.

TECHNICAL UNIT OPERATIONS IN UPSTREAM PROCESSING

- Storage and transfer of solids
- Storage and transfer of liquids
- Dissolving of solids and liquids
- Filtration of mixtures of substances
- Homogenisation of fluids
- Suspension of solids
- Dispersal of droplets and gas bubbles
- Processes for transfer of materials to obtain phase interfaces
- Heat transfer processes

Box 2: Overview of the typical technical unit operations in USP in biopharmaceutical production

For sampling, distribution, storage and transfer, 2- and 3-dimensional plastic bags (2D-, 3D-bags) and so-called tank liners are used as individual systems or as manifolds (multiple distribution systems) in sizes from 50 ml to 3000 L. Depending on the intended use or customer-specific needs, the bags are equipped with appropriate ports, connectors, tubing, filters and sensors. Solids can be processed using special powder bags with appropriately-sized apertures. For secure transport, bag handling systems such as bowls, tubs, racks and transport containers are convenient. Larger bag systems are generally placed and fixed into containers or trays. Such systems are available that are either stackable or foldable and made from either plastic or stainless steel [Riesen and Eibl 2011].

Single-use Mixers (SUM) [Werner et al. 2011] and SUB [Eibl et al. 2011b] are used for dissolving, mixing and cultivation. At present, SUM are available up to 5000 L and SUB up to 2000 L in bag format as well as rigid cultivation vessels in benchtop scale up to 14 L. The range of shapes and sizes of the cultivation vessels, the power inputs, instrumentation, mixing and stirring elements as well as shaft sealings and their mounting variants depend on the numerous variations of SUM and SUB available on the market. A systematisation of these systems can be performed, which, like their conventional counterparts, is based on the type of power input (i.e. whether mechanical or pneumatic (Figure 2) or hybrid (a combination of mechanical and pneumatic, not shown)). Single-use filter systems are used in USP for media and gas filtration, biomass retention and biomass separation (compare Table 4).

Table 4: Single-use filter systems

Single-use filter system	Technical data (min. and max. per capsule resp. single-use entity)
Filter cartridge	0.02 to 3.3 m^2
Depth filter	0.0025 to 2 m^2
Microfiltration system (deposition rate in µm)	0.06 to 3.5 m^2 (0,65 µm)
Ultrafiltration system (MWCO in kDa)	0.001 to 3.5 m^2 (10 to 30 kDa)
Spin filter (pore size in µm)	0.031 m^2 to 0.851 m^2 (10 µm)

Remark: The filter area can be extended by parallelisation.

In addition, there are single-use centrifuges (max. 120 L min^{-1}, max. volumes 3000 L) and for delivering the media there are single-use peristaltic pumps up to 4000 L min^{-1}, 4-fold piston diaphragm pumps up to 4000 L min^{-1} and single-use magnetic levitation centrifugal pumps up to 8400 L min^{-1}.

Technical limitations on the use of SUT in USP arise from the material itself used for construction (plastics). The limitations from these are set based on stability, area of use, scale and handling. At present the size limitations for the user are between 1000 L and 2000 L bag volumes and 30 inch filter cartridges, although producers offer larger bag systems (up to 5000 L). System capacities beyond these magnitudes can currently be accomplished by the user by means of parallelisation. According to the most recent surveys conducted by Aspen Brook Consulting, this is sufficient for over 80 % of users.

All technical issues associated with SUS which involve power input greater than 100 W m^{-3} and which are performed at pressure and temperature gradients are also problematic. This in particular

includes viscous material systems, suspensions with high solid content and media that need heat treatment.

A major shortcoming of the SUS for USP available on the market is similarly the lack of compatibility and comparability. Although there are various studies on the characterization of SUM and SUB and comparisons with conventional systems, generally the results of these cannot be generalized and are difficult to carry over. This is the result of the study conditions and different methods of determining the characteristic technical size scales. The shortcomings include: (1) a unified list of methods for determining mixing and retention times, k_La values, power inputs, shearing loads and

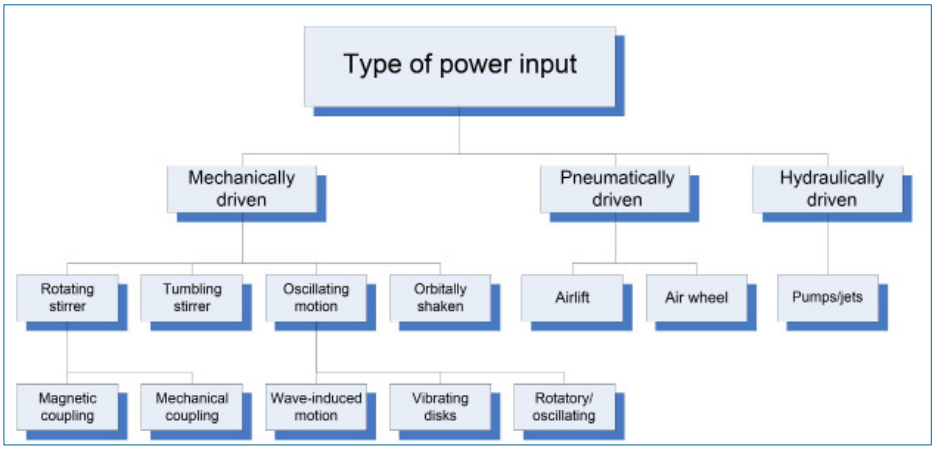

Figure 2: Categorization of SUM and SUB

flow profiles, which are secured by suitable interlaboratory tests, (2) key figure models for determining material and heat transfers and (3) methods or criteria for characterisation of the suspension materials in SUS.

Closed scale-up transfer chains should be derived for SUS use recommendations and suggestions for a scale-up transfer that is the most geometrically similar. In this case, in addition to the conventional technical investigations, Computational Fluid Dynamics (CFD) simulations should be performed, standard processes (mixing, cultivation and filtration processes) should be defined and the results should be used as evaluation criteria. However, there is also urgent need for action for transfer mechanisms of SUS that are not geometrically comparable. Because SUS are not available for all scale-up transfers in USP, or are available only for application specific systems or in specific sizes and geometries, at present, scale-up transfer in SUS that are geometrically comparable is only possible in certain cases and for special applications. There are no scientifically-based criteria or methods for transfers in SUS that are not geometrically comparable (e.g. wave-mixed systems

and stirred systems or shaken systems and stirred systems). Presently, transfers take place empirically, are time-consuming and often suboptimal. Generally, standardized sampling and probe ports on the SUS would also be beneficial.

3.5. WG on Bioprocess Technology DSP

The technical unit operations used in DSP for the production of biopharmaceutical products include classic filtration procedures and chromatographic steps, as well as innovative technologies such as functional filtration/adsorption methods and "mixed-mode" techniques. The term "mixed-mode" refers to the multiple retention mechanisms that are the basis for the interactions between sample and sorbent. In contrast, in biopharmaceutical production, the filling process for the formulated end product is generally a classic fluid transfer with or without a final lyophilisation (freeze drying). Box 3 lists the standard unit operations used in filtration and chromatography processes. The appropriate methods for isolation and purification of the product are selected from these unit operations and are merged into a downstream sequence. The sequence and quality of the methods used varies depending on the characteristics and requirements for the quality of the product being purified.

DOWNSTREAM PROCESSING

Filtration

- Dead-end filtration
  - *Steril filtration*
- Tangential (crossflow) filtration
  - *Ultrafiltration (UF)*
  - *Diafiltration (DF)*
- Functional filtration
  - *Depth filter*
  - *Membrane adsorber (affinity, ion exchanger)*

Chromatography

- Affinity chromatography
- Cation exchange chromatography
- Anion exchange chromatography
- Hydrophobic interaction chromatography
- Mixed-mode chromatography

Box 3: Standard process operations in DSP

Figure 3 illustrates an example of a generic sequence for purification of a monoclonal antibody.

As with USP, there are key advantages to using SUT versus conventional, re-usable systems in DSP: (1) lower investment costs, (2) reduced development and implementation times, (3) reduced qualification and maintenance expenses and (4) increased flexibility [Laukel et al. 2011]. However, compared to the rapid development of SUS in USP and its potentially complete application, the situation in DSP has been different. Disposable mixers up to 1000 L and disposable versions of classic microfiltration (0.1/0.2 µm) and depth filtration systems have already become mainstream. The latter have even allowed cell separations in high cell density culture processes (fed batch) with animal cells up to 1000 L scale [Dudziak, 2010]. Alternatively, single-use centrifuges such as the UniFuge (Carr Centritech) are available for cell separation.

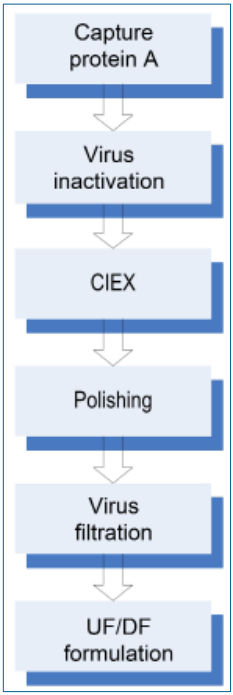

Figure 3: Typical purification of a monoclonal antibody

As before, ultrafiltration is still a bottleneck. Disposable systems for tangential filtration are available from various manufacturers only up to 3.5 m^2 [Laukel et al., 2011], which is why in 1000 L scale the UF/DF step must be carried out in several stages. The first tandem systems can be implemented for up to 7 m^2 [pers. comm., T. Peuker, Sartorius Stedim Biotech 2011]. To date, there are no standard disposable systems for virus filtration. Various manufacturers do offer customer-specific solutions, such as FlexAct or the Mobius FlexReady solution for virus filtration.

The situation is quite different for the chromatographic systems. The advantages of flexibility and reduced time and expenses gained through pre-packaged, ready-to-use columns are offset by the costs for the chromatographic gels that are used. At present, disposable chromatographic systems with column volumes up to 20 L are available. This requires yields in 1000 L scale to be purified in several cycles. Although under these conditions the columns can be more economical, at the same time the standing time and hence the entire procedure time is lengthened by the multiple cycles. In chromatography, the further development of SUS is highly dependent on the market trend in the price of chromatography medium. Disposable systems in chromatography are currently not an attractive solution for processes with frequent harvests and purifications in columns with longer lifetimes. New developments are underway for improving performance while reducing costs associated with SUT. These include the use of “mixed mode” sorbents as well as sequential chromatography sorbents that allow significant reduction in the chromatography medium required through selectivity in protein capture and by more efficient use.

However, the rather slow development of SUS in chromatography has led to the development of alternative purification techniques. Functional filtration with membrane adsorbers combine the advantages of disposable filtration and functional surfaces, especially with ion exchange and affinity properties [Wagner and Müller, 2011]. Although the dynamic binding capabilities are significantly lower than in chromatography columns, in Figure 3, which illustrates the typical purification of a monoclonal antibody, significantly higher flows in the adsorber can take effect. Membrane adsorbers are designed from the outset as SUS.

As in USP, in DSP the technical characterisation of the SUS and its standardization are inadequate. Cost-effective standard systems are needed for product reprocessing and filling.

3.6. WG on Bioprocess Technology Sensors

Until now, SUS has not made as much avail of the full range of functions with respect to process monitoring and automation technology as their traditional counterparts. They are equipped with both *in situ* and *ex situ* sensors [Glindkamp et al. 2009]. *In situ* sensors, which are in contact with the culture medium, must be able to be sterilized. *Ex situ* sensors, which allow non-invasive monitoring, such as optical sensors that measure via a transparent window or classical sensors that measure in a sample flow outside of the sterile barrier, do have this requirement. Table 5 provides an overview of the sensors currently available, including their measuring principle and commercial availability and the degree to which they can be integrated.

Table 5: Currently available sensor systems

Group	Sensor system	Availability	Integration in single-use systems
Pressure	piezo	++	yes
	physical	++	-
	mechanical	+	yes
Temperature	electrical	++	yes
Flow	optical	++	yes
Conductivity	impedance	++	yes
pO_2	optical	+++	yes
	electrochemical	+	behind sterile filter
CO_2	optical	+++	behind sterile filter
	electrochemical	+	behind sterile filter
pH	optical	+++	yes
	electrochemical	++	yes
Glucose	amperometric	++	behind sterile filter
Glucose, lactate, glutamine	amperometric	++	behind sterile filter
Glucose, lactate, glutamate	amperometric	+	in situ
Methanol	optical	+	behind sterile filter
Ethanol	optical	+	behind sterile filter
Biomass	impedance	++	yes

+++ several suppliers, ++ few suppliers, + limited availability, - no information available

Various Single-Use Analytics (SUA) for measurable process parameters are still in the research and development stages or are not ready for market. In part SUA are too specific and too expensive for cost-effective use in SUS and only partially meet the key requirements for the USP Class VI and Leachables/Extractables certification, such as sufficient range of measurement and precision level. For the measurement of standard process parameters such as pressure, temperature and some-

times also pH and pO_2, there are several systems that do meet these requirements. Measurability at this scale can be possible for SUS. Further improvements are desirable in certain areas (e.g. increase in the measurement range). In contrast, there are limitations for measurements of other process parameters such as flow, conductivity and pCO_2. Only very few systems are available. The same applies to the measurement of cell counts and concentrations of substrates, respective to metabolites including glucose, lactate, glutamine and glutamate.

In general, all *ex situ* measuring analyses can be used in SUS. In addition to analysis of exhaust for measuring quantities of substances such as ethanol, carbon dioxide, methane, methanol and oxygen, sample streams (cell-free or samples with cells) can be taken for transfer to the appropriate measuring device or connection to optical measuring systems can be made. The entire range of devices are theoretically possible for this, including those for conventional systems. The only restrictions is the connection with a sterile sampling unit or the coupling to the SUS.

The simplest method of cell-free sampling is via a sterile filter with connected tubing. However, even in smaller systems, a disadvantage is the relatively high sample volumes taken and the time delay of the measurement. In such cases, an advantage is found with miniaturized sensors, which are coupled to a suitable microfluidic and are close to the SUS and which analyze continuously with minimal time delay and low medium requirements. These problems do not apply to filtration probes that are immersed directly in the container. However, these systems were developed for conventional equipment and are not yet available in single-use versions. For sampling with cells, there is a range of SUS available. However, in general only few samples can be taken and the sampling mechanism can rarely be automated.

Optical measurement systems (IR and fluorescence spectra) are generally also suitable for measuring cell counts and concentrations of substrates and metabolites. They are still not commercially useful in SUS, since the necessary optical windows in the system are not present, among other reasons.

As a result of differing measuring principles, the lack of standard ports in *in situ* sensors and the lack of standardized mechanical interfaces in order to reproducibly position *ex situ* sensors without contacting the medium at the periphery of SUS, a simple connection of sensors to SUS is difficult. Because of this problem, an exchange or transfer of the make is virtually impossible. The development and integration of a standard port would allow the manufacturer to integrate a connection point in the bag, which would permit the incorporation of probes with pre-determined design in SUS with and without contact with the medium, positioned in a manner that is reproducible and mechanically stable. This integration of a standard port can be verified by the manufacturer. If the size and geometry as well as the material of the port are pre-set, the probe manufacturer can adjust accordingly so

that a quick and secure implementation can be made via this port. By way of example, the US patent number 8,008,065 describes a port that meets the requirements described above [Selker et al. 2011]. An urgent objective is also the creation of a single interface of sensor and the whole system, in order to provide the user with all measurement data as well as sensor-specific data, such as the most recent calibration, error messages, etc.

Finally, the requirements for sensors and analysis systems for single-use application are presented in Box 4; they are based on the national and international roadmaps and strategy recommendation [NAMUR and VDI/VDE-GMA 2009, AMA 2010, Biotechnology 2020plus 2011]. Core activities include the development and evaluation of the most comprehensive, sensor-based process intelligence for individual applications.

DEFINITIONS AND NEEDS FOR DEVELOPMENT

- Identification of relevant measurement parameters and sites
- Definition of process limits
- Selection of appropriate, scalable measurement principles (e.g. sensor, input feed)
- Single-use sampling systems and connection to diagnostics
- Increase in sensor sensitivity and selectivity in single-use sensors
- Calibration of sensors for *in situ* applications
- Optimizing sterilising capability through radiation (resistance)
- Standardization of electrical ports per sensor principle

Box 4: Definitions and needs for development

3.7. New application areas for single-use systems

It is believed that the SUT for the production of protein-based therapeutics will not continue to grow at the same rate it has. As described earlier by other working groups, it is necessary to expedite the standardization efforts for SUS, to overcome the limitations and to further develop them. This is especially the case for the field of sensors with respect to process-relevant measurement parameters as well as the scale of instruments and devices for depth-, ultra- and diafiltration, chromatography and filling. If there is success in these areas, complete single-use production systems and hence the " Single-Use Factory in a Box" will be that much closer to a reality.

Biotherapeutics of the newest generation will be important in shaping the further development of SUT. These latter are produced with autologous (produced naturally in the body) or allogenic (produced extraneous to the body) human stem cells or T-cells and therefore are also known as cell therapeutics. These cell therapeutics are the most important product segment in personal medicine and include the products urging on the market since the beginning the 1990's for regenerative medicine (skin, cartilage, bone) [Buckler 2011] as well as the first personal vaccine, Sipuleucel-T from Dendreon Corporation (www.dendreon.com), which was released by the FDA in April 2010 for the treatment of prostate cancer. More than 200 cell therapeutic agents are in the clinical trial stage, including those for transplant medicine, cancer and AIDS treatment [Shaw 2011]. Because cell therapy is still in its infancy compared to established manufacturing of protein therapeutics, innovative equipment and new technology are urgently required in order for it to reach commercial success [Burger 2010]. SUS are conditioned by product demands and usage to a "must".

SUS for USP, DSP and filling in the production of cell therapeutics

The manufacturing process for cell therapeutics is characterized by significantly smaller culture volumes (currently 1 to 30 L) [Rios 2011] in USP. For some stem cell types such as mesenchymal stem cells, for preservation of their biological function adherence must also be ensured, whereby the perfusion and microcarrier technologies regain importance. One key issue in the use of stem cells is the preparation of a sufficient quantity of cells. Stem cells can lose their ability to differentiate (multipotency) during proliferation. The solution to this critical problem is inevitable for clinical application of cell-based therapies and involves the production of suitable cultivation systems. For this too, the development of new reactor concepts based on substrates for the preparation of a sufficiently large surface area (e.g. fixed or rotating bed) as well as their configuration as SUS is required. There are also differences in product harvest (enzymatic) and the subsequent processing steps (centrifugation, cell selection, growth, filling, cryoconservation). The product processing of cell-based therapeutics is focused on the isolation of bioactive cells which the patient receives either directly via infusion or after previous cryoconservation.

However, still lacking with SUB are GMP-compliant platform solutions that allow efficient expansion and/or differentiation of the final cell products. There is also potential for development in enzymatic product harvest and the subsequent processing steps. The centrifuge tubes and equipment used until now in approved processes are derived from the processing of blood [Brandenberger et al. 2011]. These are not suitable for the DSP of greater quantities of cell culture broth. Solutions are also imperative in the filling process and its automation, as well as in large-scale cryoconservation.

Further, new applications for SUB appear likely that will focus on (1) the manufacture of microbial niche products, (2) production processes with algae and (3) cell suspensions of plant origin, root cultures and mesenchymal tissue based products for the pharmaceutical, food and cosmetic fields. Thus, as a consequence of the low energy requirements and the substantial avoidance of purification and sterilization process in SUB, for example in food technology, the opportunity arises for decentralization of production processes. The first concept studies for the use of SUS for a more effective processing of milk directly in the pasture have already been carried out (pers. comm., D. Eisenkrätzer, Hoffmann-La Roche 2011).

Manufacture of microbial niche products with SUB

Until now, the significance of SUT for microbial products was mainly in screening applications at ml-scale. Thus, 96-well microplates, deep-well plates and Falcon tubes were used for high-throughput screening of strains, constructs and mutation libraries. In particular, the high costs for SUS associated with cultivation containers stood in the way of applications at larger scales. As a result, SUT only became established in the field of microbial fermentation where security issues offer a distinct advantage (e.g. in the production of pathogens for inactive vaccines) or where small or medium quantities of high-priced products are produced.

One limitation of SUB is posed – as shown by [Mikola et al. 2007] for a wave-mixed bioreactor in yeast cultivation – by the limited oxygen transfer and the achievement of high cell densities in microbial processes. However, disposable wave-mixed bioreactors that were originally designed for cultivation of animal cells have been successfully used for facultative anaerobics (*Escherichia coli*, *Schizosaccharomyces pombe*, *Erynia neoaphidis*) and their products (immunomodulators, chiral building blocks, biological insecticides) [Eibl et al. 2009a]. The most recent studies on recombinant protein production with *Escherichia coli* have shown that high cell densities and maximum optical densities (OD_{600}) between 67 (dry weight of 25.5 g L^{-1}) and 141 (dry weight of 47 g L^{-1}) are possible using optimized feeding strategies, just as they are with stirred bag systems [Glazyrina et al., 2010, pers. comm., G. Greller, Sartorius Stedim Biotech 2011]. On the other hand, today's user can resort to SUB that are specially designed for the cultivation of microorganisms such as the CELL-tainer (a wave-mixed bag bioreactor) or the microbial version of the stirred SUB from Xcellerex, in which ODs

of 370 (dry biomass 125 g/l) can be reached [Galliher et al. 2011]. However, compared to animal cell culture, microbial applications with SUB today still play a secondary role. Since a large number of parallel cultivations can be performed quickly and with low expenditure with SUB, they are likewise favourable for the cultivation of slowly growing microorganisms (e.g. streptomycetes for the production of antibiotics and other secondary agents) and the production of plasmids for transient gene expression in cell cultures. In the latter case, several mg L^{-1} of plasmid are required and the number of constructs as well as the required product quantity is generally quite high. It is likely that the importance of SUS in microbial applications will continue to grow along with the availability of suitable ready-to-use media that support the simple handling of SUB as well as with the development of additional microbial SUB versions [Glaser 2011].

Production processes with algae and SUB

The established SUB are directly applicable to the cultivation of heterotrophic algae for the production of secondary materials and fatty acids. In a feasibility study at the Technical University in Berlin, it was shown in a heterotrophic dinoflagellate as an example, that disposable systems are advantageous for the development of high cell density processes. In this example, there was a successful scale-up of 24 deep-well plates via ultra yield flasks, as well as cultivation in CELL-tainers and SBX-200. The establishment of disposable systems has brought a variety of advantages with respect to rapid process development [pers. comm., P. Neubauer, TU Berlin 2011]. An important aspect in this regard is the dependency of marine microorganisms on high salt concentrations, especially chloride ions. Standard steel reactors corrode under these conditions and the specialized coatings are expensive and are expensive to use in stirred systems.

The most recent development in the field of light diodes has brought new perspectives to SUB and phototrophic cultures. Sartorius Stedim Biotech and Applikon have developed the third generation of wave-mixed SUBs that can be equipped with LED lighting. The cultivation of microalgae and microalgal cell cultures was already possible in the second generation wave-mixed bioreactors at the Anhalt University of Applied Sciences. Also worth mentioning are the low-cost single-use foil bioreactors such as the Novagreen bioreactor (www.novagreen-microalgae.com) and the Vertigro algae bioreactor (www.valcent.net) that can be used for microalgae (chlorella, spirulina, nannochloropsis, scenedesmus, chlamydomonas).

SUB for the production of plant cell and tissue culture based products for use in pharmaceuticals, food products and cosmetics

Plants and their cell (suspension) and tissue culture (root cultures such as hairy roots, filamentous and meristematic tissue and embryonic cultures) are potent production organisms for niche

products in the fields of pharmaceuticals, foods and cosmetics. This primarily involves secondary metabolites and glycoproteins. Since the mid 1990's, SUB has already been used for these product expressions [Eibl et al. 2011b]. Since then, users have resorted to wave-mixed [Eibl et al. 2006, Werner et al. 2010] and stirred systems [Eibl et al. 2011b], pneumatic powered bubble columns [Curtis 2004, Ziv 2005, Shaaltiel et al. 2007, Terrier et al. 2007], reactors with vertically-oscillating disks [Reichert 2011], bed bioreactors [Sivakumar et al. 2010] and hybrid systems [Ducos et al. 2009] up to a maximum of 400 L of culture volume (compare Table 6). Plant cell and tissue cultures are predicted to have increasing significance especially in the fields of food products and cosmetics. At present, there is a need for bags customized especially for the requirements of cultures, which can also ensure the desired growth and homogenous production in non-Newtonian culture broths or tissue while maintaining their integrity. Since in general there are no GMP-requirements for the latter processes, this raises the issue of more favourable bags for such applications.

Table 6: SUB successfully used for plant cell and tissue culture

Reactor categorie	Reactor name	Culture volume [L]	Culture type	Product
Wave-induced motion SUB	Wave/BioWave/ BIOSTAT CultiBag RM	1-10	Suspension culture	Pharma/cosmetics: biomass, secondary metabolites, antibodies
			Embryo culture	Food: secondary metabolites
		10-100	Filamentous tissue	Pharma: recombinant proteins
		0.3-5	Hairy roots	Pharma: secondary metabolites, antibodies
	AppliFlex	5-10	Suspension culture	
	Wave and Under-tow Bioreactor	10-100		Food: biomass, secondary metabolites
Stirred SUB	Single-Use-Biore-actor	25		Pharma: antibodies
Single-use reactor with vertically os-cillating perforated disc	Saltus Vibromix Bioreactor (ehe-mals bio-t-bag),	100		Cosmetics: secondary metabolites
Single-use bubble column	LifeReactor	1.5-5	Meristematic tissue, embryo culture	Food: biomass for plant breeding
			Hairy roots	Cosmetics: secondary metabolites
	Plastic-lined Bio-reactor	100	Suspension culture, hairy roots	Pharma: biomass, secondary metabolites
	Slugg Bubble Bioreactor	10-70	Suspension culture	
	Protalix Reactor*	400		Pharma: recombinant proteins
Single-use hybrid reactor**	Box-in-Bag Reactor	5	Embryo culture	Food: biomass for plant breeding

*bases on LifeReactor
**Combination of a static and a dynamic system with mechanical power input
Remark: Further information about these bioreactors is provided by [Eibl et al. 20 09] and [Eibl et al. 2011b und 2011c].

4. Training and development for the advancement and use of single-use systems

To date, SUS and SUT have not had the same attention of the education sector that has already been achieved in practice. Thus, only a few institutions (e.g. Bielefeld University of Applied Sciences, Biberach University of Applied Sciences, Zurich University of Applied Sciences) have introduced SUS in the context of cell culture technology training at the Bachelor level. This is likely due to the fact that, as a result of the high costs, practical training in cell culture at the mL to benchtop scale is concentrated at only a few institutions in Germany (Leibnitz University at Hannover, Bielefeld University, Hamburg Technical Institute at Harburg, Aachen University of Applied Sciences, Biberach University of Applied Sciences, Bielefeld University of Applied Sciences, Gießen Friedberg University of Applied Sciences), Switzerland (Zurich University of Applied Sciences) and Austria (Vienna University for Natural Resources and Life Sciences, Krems University of Applied Sciences). In addition, work with SUS is made even more costly as a benchmark for training in biotechnology as a result of the increased costs for the consumables. This prevails over the existing pressure to cut costs so that the use of SUT is only possible with the technical or material support from the manufacturers and suppliers of SUS.

In the context of the Master's program "Master Life Sciences CH", the Zurich University of Applied Sciences offers a whole one-week course "SUT". In addition to lectures on theory, participants are involved in a project with the proper selection of SUS and the drafting of a pilot facility for antibody production based on plant, insect and mammalian cells. Currently the Zurich University of Applied Sciences is planning a summer school on the subject of "Mammalian cell-based upstream processing with standard and single-use bioreactors" in collaboration with other members of the Swiss Biotechnet (www.biotechnet.ch) and HAN BioCentre (Nijmegen) as well as the Arnheim and Nijmegen University of Applied Sciences (The Netherlands).

Similarly in conjunction with the Swiss Biotechnet, for 6 years the Zurich University of Applied Sciences has successfully offered advanced education courses that are generalized or customized to customer needs on the use of SUS in cell culture technology. For these, the most commonly available SUS from various suppliers are used. Similar further education courses have only been available until now in the training centres of Sartorius Stedim Biotech in Göttingen and GE Healthcare in Munich, where the training with internal products was at the forefront. The need for further education courses on the use of SUB in association with culture technology has also been acknowledged by the European Society for Animal Cell Culture Technology (ESACT, www.esact.com) and has been integrated into their course "Animal Cell technology" as an important focal point. Similarly, the company Sourcin (www.sourcin.com) has a new proposal it wants to develop for a multi-media package for training with SUT.

However, further training in the field of SUT can take place at international conferences, which are organized on a regular basis especially to address these issues by the BPSA, the ISPE (International Society for Pharmacoepidemiology, www.ispe.org), Informa Life Sciences (www.ibclifesciences.com), Pharma IQ (www.pharma-iq.com) and the Zurich University of Applied Sciences as well as the Swiss Biotechnet. Among German-speaking countries, Concept Heidelberg (www.concept-heidelberg.de) annually organizes a Single-Use conference for users. In general today, oral presentations and posters on issues surrounding SUT are featured at all conferences where the development and production of biotherapeutic products are the theme.

5. Conclusions for the future activities of the TWG

In biopharmaceutical production with animal cells, SUT has gained great national and international significance. The equipment available meets the needs for production-level scale, with the exception of sensor technology and chromatography, although it remains in its infancy. The greatest weakness, which affects all process levels and the SUS needed for them, is the lack of standardization and comparability among systems as well as with traditional systems. To date, there have been no design and use recommendations for SUS or single-use facilities nor risk analyses for SUS. Moreover, there are no tests to assess the integrity of SUS prior to implementation, nor are there regulatory requirements for SUS for the complete biotechnology process, nor evaluated analytical methods with acceptance criteria for leachables and extractables. Nevertheless, an application of SUT beyond the biopharmaceutical field (food, cosmetics) and animal cell culture (microorganisms, plant cell and tissue cultures, algae) is clearly forthcoming. However, the use of SUT for the production of low-cost products permits cost-effective SUS.

The TWG "Single-use Technology in Biopharmaceutical Production", with its seven working groups, will therefore focus its future activities on the following priorities:

- the preparation of a recommendation for categorization of bags (2D, 3D) of different quality depending on use and differing requirements (Lead: WG on Materials and their Properties, Qualification and Validation)
- the establishment of a method catalogue on biotechnical process characterization for SUB and SUM, the definition of scale-up criteria for geometrically similar and dissimilar SUB, the planning and implementation of interlaboratory tests for data acquisition and evaluation (Lead: WG on Bioprocess Technology USP)
- the drafting and verification of a catalogue with methods for the technical characterization of SUS for DSP (Lead: WG on Bioprocess Technology DSP)
- the development of ideas for intensification of DSP (dynamic binding capacities and crossflow performance) when using SUT with the goal of minimizing the work volume and the production costs (Lead: WG on Bioprocess Technology DSP)
- the evaluation of available, sometimes patent-protected ports or the development of a new standard port for *in situ* sensors and standardised, mechanical interfaces for *ex situ* sensors for SUS (Lead: WG on Bioprocess Technology Sensors)
- the preparation of an on-site test for evaluating the integrity of SUS (Lead: WG on Component Harmonisation and Logistics)
- the preparation of layout recommendations for single-use production facilities considering the closed processing and personnel and material flow concepts (Lead: WG on Project Planning)
- the categorization of appropriate disposable bioreactors for microorganisms, plant cell and tis-

sue cultures, algae and stem cells based on technical parameters and cultivation results as well as the development of more favourable technologies based on SUS for the cosmetic and food industries (Lead: WG on New Application Areas for Single-use Systems)

In this context, public support for both the basic and practical issues would be desirable. As the next item of action, the TWG plans a special issue with the Chemie Ingenieur Technik (CIT) [Chemical Engineering and Technology] for 2013, titled “Single-use technology in biotechnological processes”, with reports from the working groups and current technical contributions.

6. Abbreviations

ASTM	American Society for Testing and Material
BSE	bovine spongiform encephalopathy
BPSA	BioProcess Systems Alliance
CA	cellulose acetate
CFR	Code of Federal Regulation
CIEX	high-resolution cation exchange chromatography
DF	diafiltration
DSP	downstream processing
ECHA	European Chemicals Agency
EMA	European Medicines Agency
EP	European Pharmacopoeia
ESACT	European Society for Animal Cell Technology
EVA	ethylene vinyl acetate
FDA	Food and Drug Administration
Glc	glucose
GMP	Good Manufacturing Practice
HIMA	Health Industry Manufacturing Association
ICH	International Conference on Harmonization
IR	infrared
ISO	International Organization for Standardization
ISPE	International Society for Pharmacoepidemiology
Lac	lactate
LED	light emitting diode
MES	Manufacturing Execution System
OD	optical density
OPC	protocol standard for industrial communication
PA	polyamide
PC	polycarbonate or personal computer
PDA	Parenteral Drug Association
PE	polyethylene
PESU	polyethersulfone
PP	polypropylene
PQRI	Product Quality Research Institute
PTFE	polytetrafluorethylene
PVC	polyvinyl chloride
pCO_2	partial pressure of carbon dioxide
pO_2	partial pressure of dioxygen
QA	quality assurance
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RFID	radio frequency identification
RM	rocking motion
STR	stirred
SUA	single-use analytics
SUB	single-use bioreactor/s
SUM	single-use mixer/s
SUS	single-use system/s
SUT	single-use technology/technologies
TSE	transmiscible spongiform encephalopathy
TWG	Temporary Working Group
UF	ultrafiltration
USP	United States Pharmacopoeia or upstream processing
WG	Working Group
2D-	two-dimensional
3D-	three-dimensional

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