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Sustainability analysis for a multi-product biotech API production facility: A holistic comparison between single-use technology and a fully automated traditional stainless steel build facility

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Declaration

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Zusammenfassung/Abstract

Die globale Erderwärmung hat das Potential Fauna und Flora, sowie das Leben von Millionen von Menschen in den kommenden Dekaden maßgeblich zu verändern. Anthropogene Treibhausgas Emissionen gelten als eine der Hauptursachen der globalen Erderwärmung und entstehen unter anderem bei der Herstellung und Benutzung eines jeden Produkts. Der CO_2-Fussabdruck eines Produkts erlaubt die direkten und indirekten Treibhausgas Emissionen während dem Lebenszyklus eines Produktes zu quantifizieren.

Wie auch die Herstellung von Lebensmitteln, Fahrzeugen, etc., resultiert die Produktion von therapeutischen Produkten (z.B. monoklonale Antikörper) unumgänglich in der Freisetzung von Treibhausgasen. In dieser Thesis werden zwei verschiedene Bauweisen für Produktionsanlagen zur Herstellung monoklonaler Antikörper bezüglich ihres CO_2-Fussabdrucks untersucht. Die klassische Stahl-Bauweise (Akronym: SST) mit ihren fest installierten Stahlrohren und Stahltanks wird einer Bauweise unter Einsatz von «single-use Technologie» (Akronym: SUT; Entsorgung von Prozesskomponenten nach einmaliger Benutzung) gegenübergestellt.

Der CO_2 Fussabdruck einer Produktionsanlage für monoklonalen Antikörper hängt von verschiedenen Variablen ab, was einen pauschalisierten Vergleich kategorisch ausschliesst. In dieser Arbeit werden exemplarisch Fallbeispiele gegenübergestellt. Die drei erarbeiteten Fallbeispiele betrachten unterschiedliche Standorte (Basel, Boston, Shanghai) mit ihren regionsspezifischen Einflussparametern wie wetterabhängiger Energieverbrauch für die Klimaanlage oder unterschiedlich nachhaltiger Strom. Die Auslegung der Anlage fußt in erster Linie auf der Auswahl der volumetrischen Dimensionierung der Gesamtbioreaktorkapazität. Die Berechnungen basieren auf einem in EXCEL/VBA entwickelten Programm. Untersucht werden der Gesamtwasserverbrauch, pendelnde Arbeiter, Heizung, Lüftung, Klimatechnik technologiespezifische Kategorien wie Clean-in-place (CIP) und Sterilisation-in-place (SIP) für SST Anlagen sowie die Herstellung und anschliessende thermische Verwertung von SUT typische Plastikbeutel und Filtergehäuse aus Kunststoff. Das entwickelte Modell vergleicht SST Anlagen mit einem Gesamtreaktorvolumen von wahlweise 2000 L

(2k) oder 18000 Litern (18k) mit einer bereits vorab ausgelegten SUT Anlage mit einem Gesamtreaktorvolumen von 2000 L.

Die Berechnungen für die verschiedenen Standorte (Basel, Boston, Shanghai) zeigen, dass der regionale Strom Mix erheblich zur Gesamtmenge an ausgestossenem CO_2 beiträgt. Für Anlagen gleicher Bioreaktorkapazität (2000 L) ergeben sich für alle drei Standorte ein geringerer CO_2 Ausstoss für die klassische Stahlbauweise. Mit steigender Gesamtreaktorkapazität (*scale-up*) der SST Anlage lässt sich eine weitere Reduktion des CO_2-Ausstoß pro produzierter Menge an monoklonalen Antikörpern (t_{CO2}/t_{mAb}) feststellen. Die maximale Bioreaktorgrösse (aktuell 2000 L; Stand 2019) schliesst ein *scale-up* für SUT Anlagen aus. Dies hat zur Folge, dass die absolut produzierte Menge an monoklonalen Antikörpern bei SUT Produktionsanlagen nur durch ein *numbering-up* (d.h. parallele Produktionsanlagen) gesteigert werden kann. Da der CO_2- Ausstoss pro SUT Anlage konstant ist, ist keine CO_2-Einsparung wie beim *scale-up* Ansatz möglich. Die CO_2 Emissionen pro Tonne monoklonaler Antikörper beträgt über einen Zeitraum von fünf Jahren und unter Berücksichtigung der getroffenen Annahmen:

Basel:	SUT 2k: 1463 t_{CO2}/t_{mAb} SST 2k: 479 t_{CO2}/t_{mAb} SST 18k: 152 t_{CO2}/t_{mAb}	Boston:	SUT 2k: 6428 t_{CO2}/t_{mAb} SST 2k: 2757 t_{CO2}/t_{mAb} SST 18k: 1509 t_{CO2}/t_{mAb}	Shanghai:	SUT 2k: 11170 t_{CO2}/t_{mAb} SST 2k: 4988 t_{CO2}/t_{mAb} SST 18k: 2828 t_{CO2}/t_{mAb}

Wird die emittierte Menge CO_2 auf die produzierte Menge monoklonaler Antikörper bezogen, ergeben sich folgende Vergleiche:

- Eine 2k SUT Anlage emittiert im Durchschnitt das 2-fache an CO_2, wenn sie mit einer 2k SST Anlage verglichen wird.
- Eine 2k SUT Anlage stösst im Vergleich mit einer 18k SST Anlage durchschnittlich das 4-fache an CO_2 aus.

Das entwickelte Programm mit dem zugrundeliegenden Modell bietet über die in dieser Thesis präsentierten Fallbeispiele hinaus, die Möglichkeit eine große Anzahl an Szenarien miteinander zu vergleichen. Die grundlegenden Einflüsse auf die CO2 Bilanz einer Produktionsanlage für monoklonalen Antikörper wurden untersucht und ausgewertet. Das aktuelle Modell unterliegt gewissen Einschränkungen und Annahmen die in zukünftigen Weiterentwicklungen verbessert werden können.

Abstract

Global warming has the potential to significantly change biota as well as the lives of millions of people in the coming decades. Anthropogenic emissions of greenhouse gasses originate from the production and use of every product and are counted among one of the main causes of global warming. The carbon footprint of a product allows quantification of direct and indirect greenhouse gas emissions during the life cycle of a product.

The production of therapeutic products (e.g. monoclonal Antibodies) results inevitably in the release of greenhouse gases, as does the production of groceries, automobiles and so on. In this thesis, two different designs for production facilities for monoclonal antibodies are compared regarding their carbon footprint. The conventional hard-piped stainless steel build facility (acronym: SST) with stainless steel tanks is compared to a design that relies on single-use technology (acronym: SUT; Disposed of used product-contacting material after one use).

The carbon footprint of a production facility for monoclonal antibodies depends on different variables and therefore excludes generalized comparisons. This work compares exemplary case studies with distinct locations (Basel, Boston, and Shanghai) and their region specific influence parameters like weather dependant energy consumption of the air conditioning system or varying carbon intensive electricity. The facility design is primarily driven by the volumetric dimensioning of the total bioreactor capacity. Calculations are performed with an EXCEL/VBA tool. Reviewed parameters include total water consumption, commuting employees, HVAC (heating, ventilation and air conditioning) and technology specific categories like clean-in-place (CIP) or sterilization-in-place (SIP) for SST facilities, as well as production and incineration of single-use plastic bags or filter housing for SUT facilities. The developed model allows a comparison of SST facilities with a total bioreactor capacity of optionally 2000 L (2k) or 18000 L (18k) with a pre-dimensioned 2000 L single-use facility.

The calculations for the different locations (Basel, Boston, and Shanghai) show that the regional electricity mix has a substantial impact on the emitted carbon dioxide. The conventional stainless steel facilities emit less carbon dioxide than the SUT facilities for

the three locations evaluated. In the case of a SST facility, scaling up reduces the carbon emissions per ton of produced monoclonal antibodies (t_{CO2}/t_{mAb}). The maximum single-use bioreactor capacity (current state 2019: 2000 L) for the SUT facilities excludes the possibility for scale-up. As a result, the absolute amount of produced monoclonal antibodies can only be increased by a numbering-up approach (viz. parallel production lines). Since CO_2 emissions per SUT line is constant, savings in carbon emissions are not possible. The CO_2 emissions per ton of monoclonal Antibodies over the course of five years come to:

	SUT 2k: 1463 t_{CO2}/t_{mAb}		SUT 2k: 6428 t_{CO2}/t_{mAb}		SUT 2k: 11170 t_{CO2}/t_{mAb}
Basel:	SST 2k: 479 t_{CO2}/t_{mAb}	Boston:	SST 2k: 2757 t_{CO2}/t_{mAb}	Shanghai:	SST 2k: 4988 t_{CO2}/t_{mAb}
	SST 18k: 152 t_{CO2}/t_{mAb}		SST 18k: 1509 t_{CO2}/t_{mAb}		SST 18k: 2828 t_{CO2}/t_{mAb}

When the emitted amount of CO_2 is related to the amount of produced monoclonal antibodies, the resulting comparison shows the following:

- On average, a 2k SUT facility emits the 2-fold amount of CO_2 when compared to a 2k SST facility.
- On average, a 2k SUT facility emits the 4-fold amount of CO_2 when compared to a 18k SST facility.

The developed EXCEL/VBA tool with its base model allows for research of various scenarios beyond the presented case studies. The fundamental influences on the carbon footprint of production facilities for monoclonal antibodies has been reviewed and evaluated. The current model is subject to certain limitations und assumptions that leave room for improved by further development.

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Abbrevations

18k	18000 L bioreactor volume
2k	2000 L bioreactor volume
AEX	Anion exchange chromatography
barg	Bar gauge
BDS	Bulk drug substance
CAPEX	Capital expenditure
CFP	Carbon footprint of a product
CFP-PCR	Carbon footprint of a product - product category rules
cGMP	Current good manufacturing practice
CH_4	Methane
CHO	Chinese hamster ovary cells
CIP	Cleaning-in-place
CO_2e	Carbon dioxide equivalent
dLUC	Direct land use change
DSP	Downstream process
EDI	Electro-deionisation
EF	Emission factor
EIO	Environmental Input-Output
EMA	European Medicine Agency
EUPH	European Pharmacopeia
GHG	Greenhouse gas
GLSP	Good large scale practice
GMO	Genetically modified organisms
GMP	Good manufacturing practice
GTP	Global temperature change potential
GWP	Global warming potential
H_2O	Dihydrogen Monoxide
H_3PO_4	Phosphoric acid
HCl	Hydrogen chloride
HIC	Hydrophobic Interaction Chromatography
IEX	Ion excahnge chromatography
iLUC	Indirect land use change
IPCC	Intergovernmental panel on climate change
IR	Infrared
LCA	Life cycle assessment
LCI	Life cycle inventory analysis
LCIA	Life cycle impact assessment
LU	Land use
LUC	Land use change

mAb	Monoclonal antibody
MCB	Master cell bank
MSW	Municipal solid waste
N_2O	Nitrous oxide
NaOH	Sodium hydroxide
NO_x	Nitrogen oxides
O_2	Oxygen
PA	Process analysis
PCR	product category rules
pkm	Passenger-kilometers
PM_{10}	Coarse particulate matter, PM10
PW	Purified water
RO	Reverse osmosis
SIP	Sterilizing-in-place
SO_2	Sulfur dioxide
SPC	supplementary protection certificate
SPC	Supplementary protection certificate
SST	Stainless steel
SUT	Single-use technology
UF	Ultrafiltration
UF/DF	Ultrafiltration/diafiltration
USP	Upstream process
WCB	Working cell bank

Nomenclature

Disclaimer:

The terms and definitions for the estimation of the carbon footprint originate from the copyrighted document “Greenhouse gases — Carbon footprint of products - Requirements and guidelines for quantification” (ISO 14067:2018; ÖNORM, EN ISO 14067,Edition: 2019-03-15; [1]), published by the European Committee for Standardization.

1 Definition of the carbon footprint of a product

Carbon footprint of a product (CFP): sum of GHG missions and GHG removals in a product system, expressed as CO_2 equivalents and based on a life cycle assessment using the single impact category of climate change

Partial carbon footprint of a product (partial CFP): sum of GHG emissions and GHG removals of one or more selected process(es) in a product system expressed as CO_2 equivalents and based on the selected stages or processes within the life cycle

Carbon footprint of a product systematic approach (CFP systematic approach): set of procedures to facilitate the quantification of the CFP for two or more products of the same organization

Carbon footprint of a product study (CFP study): all activities that are necessary to quantify and report a CFP or a partial CFP

Carbon footprint of a product study report (CFP study report): report that documents the CFP study, presents the CFP or partial CFP, and shows the decisions taken within the study

Quantification of the carbon footprint of a product (quantification of the CFP): activities that result in the determination of a CFP or a partial CFP

Carbon offsetting: mechanism for compensating for all or a part of the CFP or the partial CFP through the prevention of the release of, reduction in, or removal of an amount of GHG emissions in a process outside the product system under study

Note to entry: carbon offsetting is not allowed in the quantification of a *CFP* and communication of carbon offsetting is outside of the scope of this document.

Product category: group of products that can fulfil equivalent functions product category rules (PCR): set of specific rules, requirements and guidelines for developing Type III environmental declarations and footprint communications for one or more product categories

Carbon footprint of a product – product category rules (CFP–PCR): set of specific rules, requirements and guidelines for CFP or partial CFP quantification and communication for one or more product categories

Carbon footprint of a product performance tracking (CFP performance tracking): comparing the CFP or the partial CFP of one specific product of the same organization over time

2 Greenhouse gases (GHGs)

Greenhouse gas (GHG): gaseous constituent of the atmosphere, both natural and anthropogenic, that absorbs and emits radiation at specific wavelengths within the spectrum of infrared radiation emitted by the Earth's surface, the atmosphere and clouds

Carbon dioxide equivalent (CO_2 equivalent; CO_2e): unit for comparing the radiative forcing of a GHG to that of carbon dioxide

Global temperature change potential (GTP): index measuring the change in global mean surface temperature at a chosen point in time in response to a GHG emission pulse, relative to the change in temperature attributed to carbon dioxide

Global warming potential (GWP): index, based on radiative properties of GHGs, measuring the radiative forcing following a pulse emission of a unit mass of a given GHG

in the present-day atmosphere integrated over a chosen time horizon, relative to that of carbon dioxide

Greenhouse gas emission (GHG emission): release of a GHG into the atmosphere

Greenhouse gas removal (GHG removal): withdrawal of a GHG from the atmosphere

Greenhouse gas emission factor (GHG emission factor): coefficient relating activity data with the GHG emission

3 Products, product systems and processes

Product: goods or service

Product system: collection of unit processes with elementary flows and product flows, performing one or more defined functions and which models the life cycle of a product

Co-product: any of two or more products coming from the same unit process or product system

System boundary: boundary based on a set of criteria representing which unit processes are a part of the system under study

Process: set of interrelated or interacting activities that transforms inputs into outputs

Unit process: smallest element considered in the life cycle inventory analysis for which input and output data are quantified

Functional unit: quantified performance of a product system for use as a reference unit

Declared unit: quantity of a product for use as a reference unit in the quantification of a partial CFP

Reference flow: measure of the inputs to or outputs from processes in a given product system required to fulfil the function expressed by the functional unit

Elementary flow: material or energy entering the system being studied that has been drawn from the environment without previous human transformation, or material or energy leaving the system being studied that is released into the environment without subsequent human transformation

Service life: period of time during which a product in use meets or exceeds the performance requirements

4 Life cycle assessment

Cut-off criteria: specification of the amount of material or energy flow or the level of significance of GHG emissions associated with unit processes or the product system to be excluded from a CFP study

Life cycle: consecutive and interlinked stages related to a product from raw material acquisition or generation from natural resources to end-of-life treatment

Life cycle assessment (LCA): compilation and evaluation of the inputs, outputs and the potential environmental impacts of a product system throughout its life cycle

Life cycle inventory analysis (LCI): phase of life cycle assessment involving the compilation and quantification of inputs and outputs for a product throughout its life cycle

Life cycle impact assessment (LCIA): phase of life cycle assessment aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts for a product system throughout the life cycle of the product

Life cycle interpretation: phase of life cycle assessment in which the findings of either the life cycle inventory analysis or the life cycle impact assessment, or both, are evaluated in relation to the defined goal and scope in order to reach conclusions and recommendations

Sensitivity analysis: systematic procedures for estimating the effects of the choices made regarding methods and data on the outcome of a CFP study

Impact category: class representing environmental issues of concern to which life cycle inventory analysis results may be assigned

Waste: substances or objects that the holder intends or is required to dispose of

Critical review: activity intended to ensure consistency between the CFP study and the principles and requirements of this document

Area of concern: aspect of the natural environment, human health or resources of interest to society (e.g. water, climate change, biodiversity)

5 Organizations

Organization: person or group of people that has its own functions with responsibilities, authorities and relationships to achieve its objectives

Supply chain: those involved, through upstream and downstream linkages, in processes and activities relating to the provision of products to the user

6 Data and data quality

Primary data: quantified value of a process or an activity obtained from a direct measurement or a calculation based on direct measurements

Site-specific data: primary data obtained within the product system

Secondary data: data, which do not fulfil the requirements for primary data

Uncertainty: parameter associated with the result of quantification that characterizes the dispersion of the values that could be reasonably attributed to the quantified amount

7 Biogenic material and land use

Biomass: material of biological origin, excluding material embedded in geological formations and material transformed to fossilized material

Biogenic carbon: carbon derived from biomass

Fossil carbon: carbon that is contained in fossilized material

Land use (LU): human use or management of land within the relevant boundary

Direct land use change (dLUC): change in the human use of land within the relevant boundary

Indirect land use change (iLUC): change in the use of land which is a consequence of direct land use change, but which occurs outside the relevant boundary

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1 Introduction

In the last decades, global warming has led to widespread shrinking of the cryosphere. Mass loss from the Antarctic ice sheet over the period 2007–2016 tripled relative to 1997–2006, resulting in potential sea level rise of several meters within a few centuries. [2]

An increasing proportion of the world´s population is living in mega cities located in coastal regions [3]. Therefore, the numerous negative effects of anthropogenic greenhouse gas emission such as extreme weather patterns or rising sea levels represent an enormous threat to people that live in those areas.

In recent years, the focus of the sustainability community did not include the (bio-) pharmaceutical sector in terms of its contribution to reduction of the global carbon footprint [4]. The urgency to meet the greenhouse gas reduction set in the United Nations Framework Convention Agreement and Unit in the year 2016 calls for a method to quantify the carbon footprint of (bio-) pharmaceutical products [5]. This should provide a tool to identify key processes that are responsible for greenhouse gas emissions during the manufacturing of products for the healthcare sector.

This Master´s thesis aims to analyse the difference in carbon footprints of two different facility types for the production of monoclonal antibodies. By using the ISO 14067 [1] framework, case studies compare single-use technology and conventional stainless steel based facilities. The examination of both facility types is based on the development of a model (EXCEL/VBA tool) that allows the calculation of carbon emissions by implementation of different system inputs.

A carbon footprint assessment of the product is conducted by a hybrid approach combining bottom-up analysis including Process Analysis (PA) and top-down analysis based on Environmental input-output (EOI) with respective system boundaries. The process trains for single-use technology and conventional stainless steel design are reviewed to identify differences in the production process as well as the impact on the respective carbon footprint.

2 State of Art

Recombinant DNA technology spawned a new era in the treatment of disease and the role of biologics and their manufacturing process in the mid-1970s. In the early 1980s, large scale manufacturing became an urgent need and biologics manufacturers turned to the plasma purification, dairy, food/beer/winemaking and antibiotics industry to copy the manufacturing systems that are traditionally dominated by stainless steel technology. In the 1990s, first generation blockbuster mAbs (US trade names: Orthoclone OKT3®, ReoPro®, Rituxan®, Zenapax®, Synagis®, Remicade®, Herceptin®, Enbrel®, Simulect® [6, S. 30]) manufactured in large stainless steel facilities, dominated the market. These large facilities had the capacity for the rapidly growing demand for 1st generation blockbuster drugs in western markets. Large changes began with the patent expiration of the first generation blockbuster drugs that were followed by "generic biologics", so called biosimilars. Global competition demanded higher efficiency, lower cost and faster agility. Second generation drugs would be more potent resulting in lower doses and improved cell line genomics resulted in boosted product titers. In the early- to mid-nineties, mammalian cell-based production processes had product titers of below 1 g/L. Over the course of the 2000s, product titers reached 2 to 4 g/L and further increased to 5 g/L or more for more recent processes with highly optimized cell cultures [7, S. 87]. The increase in production titers for monoclonal antibody production facilities resulted in a possible scale down in bioreactor volume and made the large-scale implementation of single-use equipment possible [8, S. 352]. The increased productivity further developed the trend towards smaller markets, reducing the manufacturing scale on average. [7, S. 557-558]

The rise of biosimilars with their generally lower prices increased the pressure on the industry and demanded a re-examination of the entire basis of drug production costs. Research & development and successful market introduction became more risky, challenging and expensive, as more complex diseases were attacked. This lead to large pharma consolidations, mergers and acquisitions to share and therefore reduce the risk. Smaller facility scales are on the rise due to improved cell line productivity, purification yield and lower doses that are more potent. Slow and large build single product stainless

steel manufacturing technology began to be re-examined from a financial risk and return perspective due to increased chance of becoming obsolete before completion or being too slow to respond to market demand [9, S. 50, 10].

As a result of price pressure, manufacturers of biopharmaceutical products started to look for new technologies that could transform cost and speed without reducing product quality, by examination of different risk and cost factors [11–13]. As an additional way to save costs, manufacturing agility or flexibility for multi-product operation was analysed to maximize facility utilization and efficiency [11, 14, 15]. Single-use technology offers many of the desired attributes but did not originate in sector of biopharmaceutical manufacturing.

By the 1960s, glass syringes (which were re-usable after sterilisation) were being replaced by disposable plastic syringes (polystyrene) and single-use needles. The idea of avoiding cross contamination by immediate disposal after use became reality. [16, S. 4]

From the 1960s until early 1970s, single-use technology (SUT) gained a foothold in form of cultivation devices like petri dishes, T-flasks and roller bottles [8, S. 45]. In the 1990s, the first single-use items became applicable in form of cartridge filters, t-flasks, cell factories, sample bags as well as media and buffer storage bags. The first “disruptive” SUT innovation came to the industry in 1996, with development of the first disposable Wave Bioreactor™. The system consisted of a pre-sterilized bag as cell culture chamber and a rocking platform with integrated heating as a bag holder [16, S. 7]. The development of single-use wave bioreactors of a volume up to 300 L is an approach to scalable SUT devices.

The goal of the first SUT devices was to support operation of stainless steel facilities. Main drivers were patient safety, ease and simplicity of use, lower capital expenditure, speed of installation, fast turnaround as well as disposability that makes CIP/SIP systems (that are needed for traditional stainless steel facilities) redundant [11, 15]. The reduction of risk of cross contamination results in reduced administrative effort due to extensive validation and quality systems that are required by regulatory agencies. The chance for cross contamination between batches is reduced since single-use items arrive pre-sterilized and are discarded after use either for incrimination or for disposal at a landfill. This is especially important for manufacturers that consider multi-product

operations to increase facility utilization and therefore lower cost of goods and operational expenditure. By the 2010s, single-use technology evolved from solely “support systems” to “production systems”. In the last decade, single-use technology became more widespread with increasing industry pressure to reduce capital/operational cost, batch turnover time as well as the implementation of more flexible multi-product manufacturing processes. [7, S. 557-559]

Monoclonal antibodies represent a growing share of all biopharmaceuticals produced worldwide and are mainly used in treatment of autoimmune diseases and cancer [17]. In 1979, Georges Köhler and César Milstein discovered that antibodies can be produced by lymphocite fusion. Monoclonal antibody-mediated therapy started with mouse monoclonal antibodies, moved to mouse-human chimaeras, humanized monoclonal antibodies and later completely human antibody. Nowadays monoclonal antibody production systems include gram-negative and gram-positive bacteria, insect cell lines, yeasts and filamentous fungi, mammalian cells as well as transgenic plants and animals [18]. Diagnostic antibodies are produced with the help of yeast and bacteria as production systems. For treatment in humans Chinese ovary hamster (CHO) cell lines are the main choice for commercial bioprocesses due to their ability to produce the glycosylation patterns that are required to achieve therapeutic efficacy [19, S. 13]. The major challenge is to synthesize mAbs with human glycosylation since inappropriate glycosylation can result in reduced activity, limited half-life in circulation and unwanted immunogenicity [20]. Today a large proportion (60-70%) of all recombinant protein pharmaceuticals are produced using mammalian cell lines [21].

Monoclonal antibody therapy covers the fields of cancer, infectious disease, transplantation, allergy, asthma and some autoimmune disease. Their major therapeutic advantage is their high specificity, namely their high affinity with which they bind to targets and the limited side effects associated with their use.

In-vivo methods are able to produce antibodies in high concentrations and animal models allow post-translational modifications as glycosylation. The major disadvantage is the use of animals and its associated bio-ethical concerns. In-vitro production offers the advantage of large-scale production in short time with easy antibody purification. Currently, there are robust and scalable bioreactor processes that achieve high cell

densities and product yields with titers of the order of 10 g/L for monoclonal antibodies in fed-batch culture [22, S. 12]. Cell lines for monoclonal antibody production were originally cultured in media containing animal serum. Because of concerns about potential contamination (e.g. viruses, bacteria, fungi, bovine spongiform encephalopathy (BSE)), batch-to-batch variation in serum performance in culture media, the cost of serum, cell lines have been adapted to grow in culture media that are free from serum or any other animal-derived components [22, S. 13]. Stable and scalable platform processes allow manufacturers of biopharmaceutical products to enter the market more rapidly, with patent protection as the backbone of their products.

Patent protection only lasts 20 years with an additional 5 years for special cases. This leaves approximately 10 to 15 years after the clinical phases have been completed and the product is launched and marketed. In this time-frame profit generation has to account for initial investment as well as financing for future research which can easily account for 1-2 billion in capital investment. [22, S. 324]

Blockbuster drugs that were developed in the 1990s are expiring and emerging biosimilars further increase the pressure on producers of monoclonal antibodies, due to ever-increasing economic pressure to reduce or sustain healthcare expenses [17]. This creates a demand for highly flexible production facilities with low initial investment and without the risk of cross contamination due to batch changes. With the use of disposable bioprocessing growing at a rate of 30% per year [23, S. 5] there is legitimate concern regarding environmental impact and sustainability.

Life Cycle Assessment

Life Cycle Assessments (LCA="compilation and evaluation of the inputs, outputs and the potential environmental impacts of a product system throughout its life cycle" [1]) started to develop in the early 1970s with the goal to derive an international standard for ecological analysis. The international standards on life cycle assessment (LCA) ISO 14040 and ISO 14044 got released in October 2006 and are the foundation for the development of ISO 14067:2018. This ISO specifies principles, requirements, and guidelines for the quantification and reporting of the carbon footprint of a product (CFP), in a manner consistent with International Standards on life cycle assessment (ISO 14040 and 14044). The carbon footprint of a product (CFP) only addresses a single

impact category: climate change [1]. The CDP is defined by clear and consistent methodology and looks at a product from a “cradle-to-grave” perspective. It measures the amount of greenhouse gas (GHG) emitted to the environment by a certain process or a product

With the rise of single-use technology from a “support technology” to a “production technology”, the aspect of sustainability needs detailed investigation to determine the carbon footprint of conventional stainless steel technology and single-use technology.

3 Theoretical Basis

This chapter aims to provide the necessary knowledge in a compact manner for different subject areas, needed for the understanding of this thesis. This overview does not claim comprehensiveness regarding the different subject areas but offers the most important information available from the time range (April to September 2019) writing this thesis.

3.1 Greenhouse gas balance

The total amount of greenhouse gas emitted by a process (e.g. the production of a product) can be determined with a material flow balance. This procedure can be described as a "carbon footprint". What exactly is a "carbon footprint" and what aspects are included during the calculation? Take, for example, a pack of pasta. Should the greenhouse gases that are involved in production of the plastic packaging be included, or the cardboard box the pasta is packed in during transport on a pallet? What about the ingredients of the pasta itself and the energy needed to boil the pasta to make it eatable?

«Carbon Footprint» is a term that was used over decades in countless studies in the field of energy and ecological economics but scientific literature is surprisingly void of a clear definition [24, S. 2]. There is still some confusion what "carbon footprint" actually means and measures and what unit is to be used. The common baseline is, that the "carbon footprint" stands for a certain amount of gaseous emissions that are relevant to climate change and are associated with human consumption or production activities. To this date, there is no clear definition and a lack of consensus in regards to carbon footprint quantification or measurement [24, S. 2]. There is a wide spectrum of "carbon footprints", ranging from direct CO_2 emissions to full life-cycle greenhouse gas emissions without clear units of measurements. In most cases, a "carbon footprint" represents a generic synonym for emissions of carbon dioxide or greenhouse gases expressed in CO_2 or CO_2 equivalents (CO_2e) as seen as in Figure 1.

Source	Definition
BP (2007)	"The carbon footprint is the amount of carbon dioxide emitted due to your daily activities – from washing a load of laundry to driving a carload of kids to school."
British Sky Broadcasting (Sky) (Patel 2006)	The carbon footprint was calculated by "measuring the CO_2 equivalent emissions from its premises, company-owned vehicles, business travel and waste to landfill." (Patel 2006)
Carbon Trust (2007)	"… a methodology to estimate the total emission of greenhouse gases (GHG) in carbon equivalents from a product across its life cycle from the production of raw material used in its manufacture, to disposal of the finished product (excluding in-use emissions). "… a technique for identifying and measuring the individual greenhouse gas emissions from each activity within a supply chain process step and the framework for attributing these to each output product (we [The Carbon Trust] will refer to this as the product's 'carbon footprint')." (CarbonTrust 2007, p.4)
Energetics (2007)	"… the full extent of direct and indirect CO_2 emissions caused by your business activities."
ETAP (2007)	"…the 'Carbon Footprint' is a measure of the impact human activities have on the environment in terms of the amount of greenhouse gases produced, measured in tonnes of carbon dioxide."
Global Footprint Network (2007)	"The demand on biocapacity required to sequester (through photosynthesis) the carbon dioxide (CO_2) emissions from fossil fuel combustion." (GFN 2007; see also text)
Grub & Ellis (2007)	"A carbon footprint is a measure of the amount of carbon dioxide emitted through the combustion of fossil fuels. In the case of a business organization, it is the amount of CO_2 emitted either directly or indirectly as a result of its everyday operations. It also might reflect the fossil energy represented in a product or commodity reaching market."
Paliamentary Office of Science and Technology (POST 2006)	"A 'carbon footprint' is the total amount of CO_2 and other greenhouse gases, emitted over the full life cycle of a process or product. It is expressed as grams of CO_2 equivalent per kilowatt hour of generation (gCO_2eq/kWh), which accounts for the different global warming effects of other greenhouse gases."

Figure 1 Various definitions of «carbon footprint» according to research literature from Scopus and ScienceDirect [24, S. 3].
BP=British Petroleum; ETAP=Environmental Technologies Action Plan

There are several different questions to be answered:

- Should a carbon footprint include just carbon dioxide emissions or other greenhouse gas emissions (e.g. methane) as well?
- Should molecules be included that do not have carbon in their structure (e.g. N_2O)?
- Should the measure include all sources of emissions, even those that do not stem from fossil fuels (e.g. carbon dioxide emissions from soils)?
- Are indirect emissions from upstream production processes included or are just direct (on-site) emissions of the product, process or person relevant?
- Where should the boundaries be set and how can these impacts be quantified (e.g. are workers commuting to the production facility factored in?)?

The terminology "footprint" implies an area-based use of units (e.g. m^2, km^2, ha etc.) where the total amount of carbon is physically measured in mass units like kilograms or tonnes. A conversion into land area requires a variety of assumptions and increases the uncertainties and errors associated with a particular footprint estimate [25, S. 192]. With no clear definition by academia, consultancies, businesses, NGOs and governments provide their own definitions and various methodologies with their pro's and con's [24, S. 4].

There are several methodological issues related to greenhouse gas records and measurements that have to be considered:

a) **Greenhouse gases (GHGs):** The choice of the GHGs depends on the nature, requirement and the guidelines of the sector/activities and is specific for each individual case. In case of wastewater, the most important gas emitted is CO_2, as a result of bacterial metabolism and along with it are traces of CH_4 and N_2O. [26, S. 7]

b) **System boundaries:** The system boundary defines the target region and considers all activities within the set boundary. The two different boundary types are the organizational boundaries and the operational boundaries. The boundary of the organization on the economic and business grounds and its associated activities form the organizational boundary. The direct (on-site) and indirect (embodied emissions- consumption of purchased power) emissions are within the operational boundaries. Indirect emissions include the consumption of purchased energy, acquired electricity, heating, cooling and so on. Such

emissions are an outcome of activities within the organizational boundaries but are emitted from sources that are controlled or owned by some other organization (e.g. central electricity supplier). Other possible indirect emission sources are:

- GHG emissions from the supply chain as upstream/downstream transport of materials, fuels, chemicals and upstream production
- The reuse of biosolids including land application or other methods that are outside the organizational boundaries
- The landfilling of biosolids
- Emissions from services contracted with outside vendors (e.g. wastewater treatment by municipalities)
- The emissions that stem from employee commuting and business travel

All indirect emissions are difficult to quantify and require additional guidelines for facilitating inventories. [26, S. 8] The scope of the system boundary has a great impact on the overall greenhouse gas balance due the possibility to include or exclude certain GHG sources. Figure 2 shows an example where the overall GHG emissions depend on where the system boundaries are drawn.

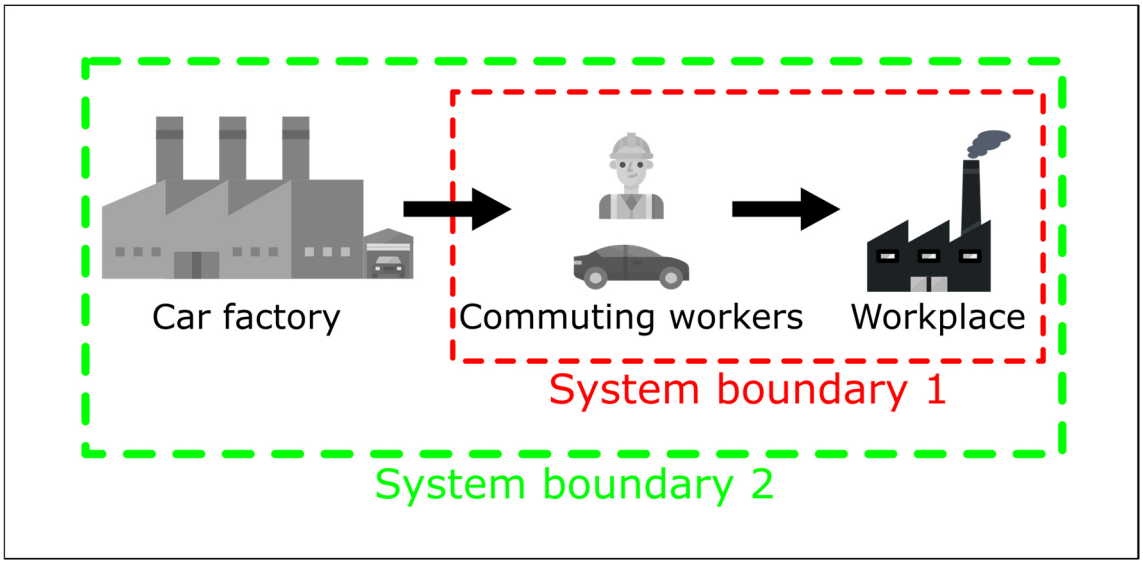


Figure 2 The definition of system boundaries according to the scope. System boundary 1 includes the greenhouse gas emissions from workers commuting to the workplace by car. System boundary 2 includes all activities within system boundary 1 but extents the scope to include the production process of the car in the factory with the associated greenhouse gas emissions.

c) **Compilation of GHG emission data:** If emissions are not quantified directly by on-site real time measurements, they have to be quantified by estimations based on emission factors and various empirical models. Emission factors or the model based data generation are the most used means for acquiring data and are calculated from specific emission factors using the data on fuel consumption, efficiency of the process, energy and other activities resulting in emissions. Emission factors for individual industrial processes, various sectors and land-use are available from different sources (e.g. European Environment Agency). Relevant emission factors are listed in section *3.1.3 Emission factors (EF) for greenhouse gas inventories*.

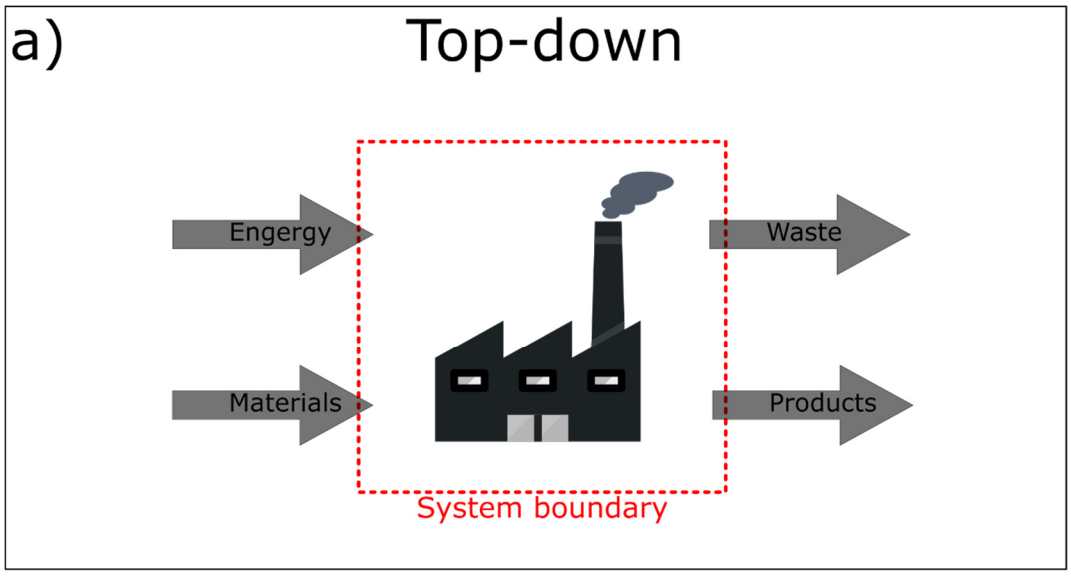
   Direct measurement of fugitive GHG emissions is possible via infra-red (IR) sensors, quantitative IR spectroscopy, quantitative gas chromatography, optical or biochemical sensors. Primary data collection techniques with these direct methods are the most precise and accurate but the cost for data acquisition by experiments and analyses is high. An alternative are secondary databases that are economic (e.g. World Resource Institute/ World Business Council for Sustainable Development) and involve the usage of approximated values instead of the values that stem from primary data [26, S. 8]

d) **Calculation of the carbon footprint:** All GHG quantities/flux are converted in CO_2-equivalents by impact factors. The undertaking to calculate the carbon footprint can be approached methodologically from two different directions (see Figure 3) [24, S. 5, 26, S. 9]:

   - Bottom-up approach based on Process Analysis (PA)
   - Top-down approach based on Environmental Input-Output (EIO) analysis

Bottom-up models start from detailed understanding of the fundamental elements and processes of the system, and then generate aggregate system behaviour by simulating the relations between the individual entities of the system. A Process analysis, for instance, begins with mass and energy balances of the final production process and then works backwards to determine the energy and material needs of each contributing input.

On the other hand, top-down models begin with an overall description of aggregate performance of the system and then proceed to subdivide the system to understand its functioning. An input-output method uses data on bought energy and materials by a particular production facility or industrial sector. This data coupled with information on physical production, yields average values for energy-/material demand for the produced products. [27, S. 18]



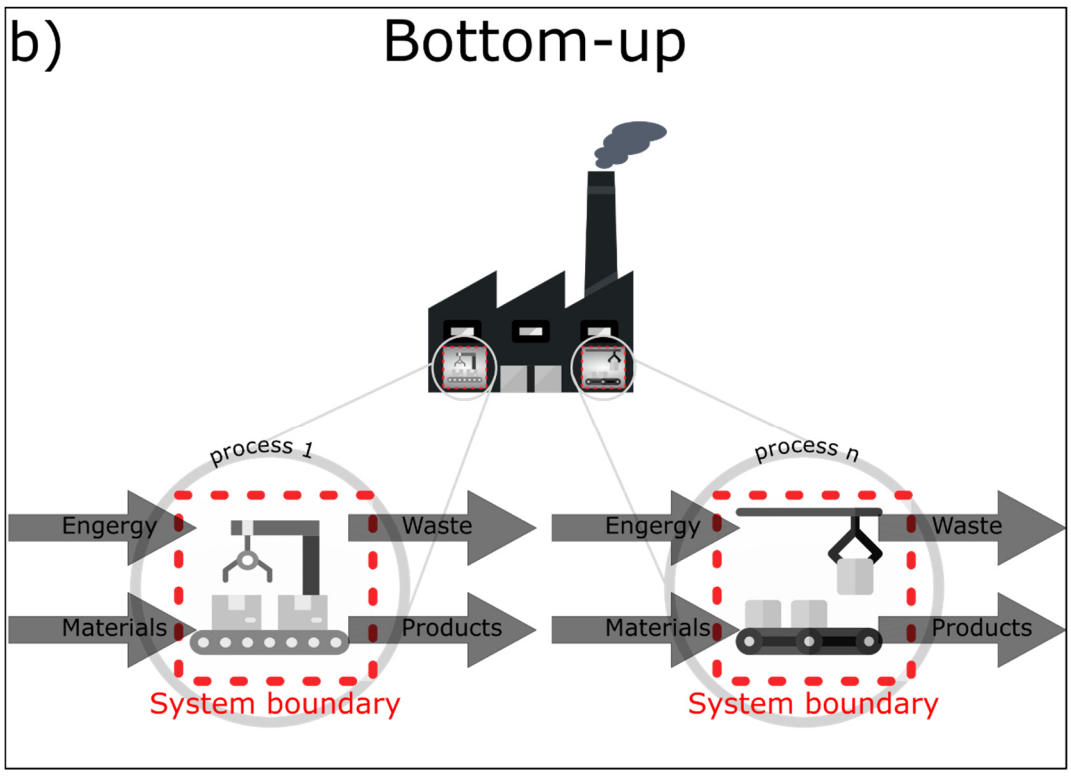


Figure 3 Differences in top-down and bottom-up approach.
a) The top-down approach has one system boundary that takes a look at total input-/output-flux (e.g. energy, materials, waste, products, etc.).
b) The bottom-up approach assigns a system boundary to each individual process (process 1 to n) with their input- and output flux. To receive the total flux according to the top-down approach, the individual process flux (process 1 to n) can be sumed up.

Process Analysis is a bottom-up method developed to understand the environmental impacts of individual products from cradle to grave [24, S. 5]. PA-LCAs suffer from system boundary problems because of the omission of resource requirements or pollutant release of upstream stages of the production process [28, S. 1]. When PA-LCAs are used to calculate carbon footprint estimates, a strong emphasis should be on the identification of appropriate system boundaries to minimise truncation errors. Further problems occur for PA-LCAs when carbon footprints for larger entities such as governments, households or particular industrial sectors have to be established. When extrapolating information contained in life-cycle databases results will get increasingly patchy. This is the result of the assumption that a subset of individual products are representative for a larger product grouping and the use of information of different databases.

EIO analysis is a top-down method and provides a picture of all economic activities at the sector level. The application of this approach to microsystems such as products or processes is limited since homogeneity of prices, outputs and carbon emissions at a sector level is assumed [26, S. 8].

The best way for a comprehensive and robust analysis is a hybrid approach that combines the PA and the EOI method.

e) **Carbon footprint assessment of a product:** The carbon footprint of a product (CFP) can be derived either by the bottom-up method based on Process Analysis (PA) or by the top-down method based on the Environmental Input-Output (EIO) considering the full-life cycle impacts through an Life Cycle Assessment (LCA). The bottom-up method involves a Process Analysis that considers the environmental impact of individual products from cradle to grave. For this approach, the appropriate identification of system boundaries is crucial. The hybrid approach that involves the combination of bottom-up Process Analysis and top-down Environmental-Input-Output allows one to preserve the detail and

accuracy of bottom-up approaches in lower-order stages, whereas higher-order requirements are covered by the input-output part of the model. [26, S. 9]

f) **Carbon footprint standards for products:** What costs more, a kilogram of cherries or a kilogram of beef? This question is easy to answer in terms of price in Euros or Dollars but what about an answer in grams of carbon dioxide? Calculating the cost of a product in mass of CO_2 is known as carbon footprint. The carbon footprint of a product covers the measuring, managing and communicating GHG emissions related to the product's goods and services. The goal is to constitute the first step towards a more comprehensive environmental assessment. There are three commonly used carbon footprint standards:

- ISO 14067
- PAS 2050
- GHG Protocol

The British Standards Institution developed PAS 2050 and came into effect in October 2008. The WRI/WBCSD developed the GHG Protocol, which launched in October 2011. The European Committee for Standardisation published ISO 14067 in September 2018 and it aims to provide clarity and consistency for quantifying, monitoring, reporting and validating or verifying GHG emissions. [26, S. 9]

3.1.1 Carbon footprint of a product (CFP)

Every product that is manufactured has a life-cycle that is relevant for climate (see Figure 4 for a basic schematic). A car in the garage, the pizza in the freezer, every clothing article, a smartphone, a drug product or a vacation trip – every product causes the emissions of greenhouse gases during manufacturing, transport, storage, use and disposal. The CFP is a measure for greenhouse gas emissions in the life-cycle of a certain product. It is not an easy undertaking to calculate the carbon footprint of a product since many products follow a long and complicated path before they reach the final consumer. Many products consist of a wide range of crude materials. For some products, the primary source of greenhouse gas emissions is the production process itself, while other products have the focus on the use or disposal (e.g. short-lived packaging). The carbon footprint of an electricity consumer (e.g. refrigerators), has its focus on operation rather

than manufacturing and disposal. For this reason there has to be a clear definition how and how long a product is used before calculating the carbon footprint of a product. [29, S. 4]

The carbon footprint of a product has its focus on the determination and evaluation of the relevance for climate. Other aspects such as eutrophication, land use, energy- and resource consumption or toxicity for water and soil are often not considered. For a CFP the accuracy and reproducibility are afflicted by variances. This is the result of varying quality or origin of used data as well as definitions of certain assumptions regarding the different phases in the life-cycle of a product. [29, S. 25]

The direct comparison between different products might not make sense as variations in methodology have a significant impact. A comparison between products of the same category with identical applied methodologies is favourable.

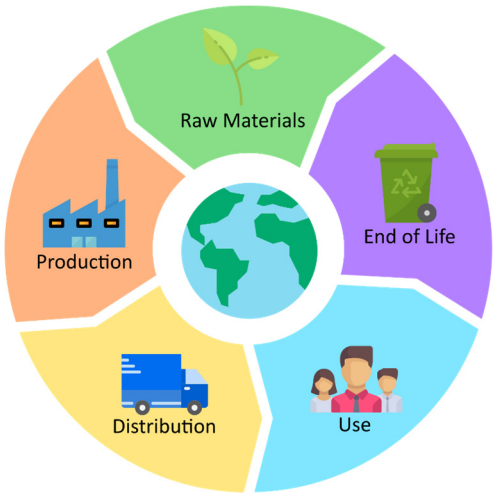


Figure 4 The life cycle of a product with different phases. At the beginning, the raw materials are obtained to supply production. The product is then distributed and utilized by the user. The last step is the end of life stage where some of the products materials might be recycled to support the raw materials stage. Adapted from [30, S. 2]

Disclaimer:

For the making of this thesis, ISO 14067 “Greenhouse gases – Carbon footprint of products – Requirements and guidelines for quantification” is used and provides the definitions for the glossary. The document specifies principles, requirements and guidelines for the quantification and reporting of the carbon footprint of a product

(CFP), in a manner consistent with international standards on life cycle assessment (LCA) (ISO 14040 and ISO 14044). The used version of ISO 14067 was published at the 15th of March 2019 by the European Committee for Standardization.

3.1.2 CO_2-equivalents (CO_2e)

Carbon dioxide equivalents (CO_2e) are a measuring unit for the unification of the global warming potential of different greenhouse gases. Carbon dioxide equivalents describe the impact of a chemical compound on the greenhouse effect, namely the average warming potential of the atmosphere over a certain period (usually 100 years). The CO_2-equivalent specifies how much a certain mass of a greenhouse gas adds to global warming compared to the same mass of carbon dioxide. The greenhouse effect of the different gases is estimated by the global warming potential (GWP; see nomenclature) that depends on radiative forcing and the timeframe of consideration (usually taken as 100 years). The GWP base factor for carbon dioxide (CO_2) is 1 for a 100 year period, 28 for methane (CH_4) and 265 for nitrous oxide (N_2O) [31, S. 87]. This means, over a period of 100 years, one ton of methane will have the equivalent warming effect as 28 tons of carbon dioxide [26, S. 6]. It is important to note that values for the different greenhouse gases vary according to different data sources (e.g. 100-year GWP by the International Energy Agency CO_2: 1, CH_4: 25, N_2O: 298 [32, S. 32]).

3.1.3 Emission factors (EF) for greenhouse gas inventories

The quantification of emissions is done directly via on-site real-time measurements or through estimations based on the emission factors and various empirical models. Emission factors or model-based data generation are the most used means for acquiring data. The specific emission factors are calculated by using data on fuel consumption, efficiency of the process, energy usage and other activities that result in emissions. [26, S. 8]

With emission factors it is possible to determine the amount of greenhouse gases that are emitted for processes like burning of gasoline/plastic or the production of a defined mass of steel. Estimations of emission factors are highly dependent on the used system boundaries and the used data (e.g. for electricity generation). This makes comparison between different emission factors very challenging. As Kanako Tanaka pointed out in her 2008 article: ”Energy consumption and energy intensity are often estimated based on different definitions of an industry’s boundaries, making comparison at best difficult, at worse invalid.” [33, S. 2891]

The required emission factors for the balancing of a production facility either with conventional stainless steel design or single-use technologies are listed in the following tables.

Table 1 Emission factors (EF) for production of stainless steel according to different sources

Name	EF	Unit	Source	Comment
Stainless steel (Europe)[1]	2.90	t_{CO2}/t_{SSt}	International Stainless Steel Forum [34, S. 9]	Produced with scrap steel
Stainless steel (Europe)[1]	4.2	t_{CO2}/t_{SSt}	International Stainless Steel Forum [34, S. 9]	Produced solely from raw materials
Stainless steel (Germany)[2]	1.708	t_{CO2}/t_{SSt}	Article in "Resources, Conservation and Recycling" [35, S. 127]	-
Stainless steel (China)[2]	2.148	t_{CO2}/t_{SSt}	Article in "Resources, Conservation and Recycling" [35, S. 127]	-
Stainless steel (USA)[2]	1.736	t_{CO2}/t_{SSt}	Article in "Resources, Conservation and Recycling" [35, S. 127]	-

Table 2 Emission factors (EF) for electricity generation in different countries/regions

Name	EF	Unit	Source	Comment
Electricity (Switzerland)	9	g_{CO2}/kWh	"Umweltbilanz Strommix Schweiz 2014", federal office for environment - Bundesamtes für Umwelt (BAFU) [36, S. 5]	Renewable electricity mix
Electricity (Switzerland)	23.6	g_{CO2}/kWh	"Umweltbilanz Strommix Schweiz 2014", federal office for environment - Bundesamtes für Umwelt (BAFU) [36, S. 5]	Average electricity mix
Electricity (Germany)	523	g_{CO2}/kWh	[37, S. 8]	Average electricity mix
Electricity (USA)	428	g_{CO2}/kWh	[38]	Average electricity mix
Electricity (Asia)	845	g_{CO2}/kWh	[39]	Average of Asia

Table 3 Emission factors (EF) for different fuel types

Name	EF	Unit	Source	Comment
Gasoline	3.15	$t_{CO2}/t_{gasoline}$	"Factsheet" of the "greenhouse gas-inventory" (Switzerland) [40, S. 2]	-
Diesel	3.15	t_{CO2}/t_{diesel}	"Factsheet" of the "greenhouse gas-inventory" (Switzerland) [40, S. 2]	-
Natural gas	2.67	$t_{CO2}/t_{natural\ gas}$	"Factsheet" of the "greenhouse gas-inventory" (Switzerland) [40, S. 2]	-

Table 4 Emission factors (EF) for plastic production

Process	EF	Unit	Source	Comment
Primary plastic production	3.12	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastics
Primary plastic production	2.58	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastic (film)
Primary plastic production	3.19	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastic (rigid)

Table 5 Emission factors (EF) for cargo transport on rail and roads

Process	EF	Unit	Source	Comment
Cargo transport rail	15.6	$\frac{g_{CO2}}{t_{cargo} \cdot km}$	European Environment Agency[42]	Cargo in Europe
Cargo transport road	139.8	$\frac{g_{CO2}}{t_{cargo} \cdot km}$	European Environment Agency[42]	Cargo in Europe

Table 6 Emission factors (EF) for incineration of plastic waste

Name	EF	Unit	Source	Comment
Combustion of plastics	0.02138*	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastic (with energy recovery)
Combustion of plastics	0.02138*	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastic film (with energy recovery)
Combustion of plastics	0.02138*	$kg_{CO2e}/kg_{Plastic}$	UK Government GHG Conversion Factors for Company Reporting [41]	Average plastic rigid (with energy recovery)

*"These factors cannot be used to determine the relative lifecycle merit of different waste management options. This is because the benefits of energy recovery and recycling are attributed to the user of the recycled materials, not the producer of the waste, in line with GHG Protocol Guidelines.

Table 7 Emission factors (EF) for allocation of clean, and the treatment of contaminated water

Name	EF	Unit	Source	Comment
Potable water supply	0.344	kg_{CO2e}/m^3	UK Government GHG Conversion Factors for Company Reporting [41]	-
Water treatment	0.708	kg_{CO2e}/m^3	UK Government GHG Conversion Factors for Company Reporting [41]	-

Table 8 Emission factors (EF) for steam generation

Name	EF	Unit	Source	Comment
Heat and steam generation	0.18746	kg_{CO2e}/kWh	UK Government GHG Conversion Factors for Company Reporting [41]	Onsite heat and steam

3.2 Production of monoclonal antibodies (mAb)

Since the first monoclonal antibody was produced in 1975 and the first monoclonal antibody was licensed in 1986, the pharmaceutical industry's demand for mAbs increased exponentially [43]. Caesar Milstein and Georges Köhler developed the method to fuse murine B cells from mice that were injected with an antigen to produce an antibody agent, with immortal murine myeloma cells. The result were cell lines (hybridomas) derived from single hybridoma cell (clone) that were capable of producing the respective antibody [6, Preface]. Available cell lines include Chinese hamster ovaries cells as well as human cell lines such as PERC6 [22, S. 5]. Monoclonal antibodies have been isolated against a wide range of targets introducing them to a broad range therapies such as different types of cancer, *multiple sclerosis* and immunological disorders such as *rheumatid arthritis* and *psoriasis* [44]. Monoclonal antibody therapeutics serve a large patient population and often involve chronic therapy with high doses. This requires a production process that is able to produce large quantities of pharmaceutical-grade mAbs.

The production of monoclonal antibodies (see Figure 5) requires the use of mammalian cells because they contain the cellular machinery to express proteins that contain multiple disulfide bonds, glycans and other modifications in an efficient manner. Continuous process development involves the optimization of cell lines, cultivation conditions, media and the implementation of new technologies in upstream and downstream processing, that increased product titers from 0.1 g/L to more than 10 g/L in fed-batch processes [22, S. 11-13].

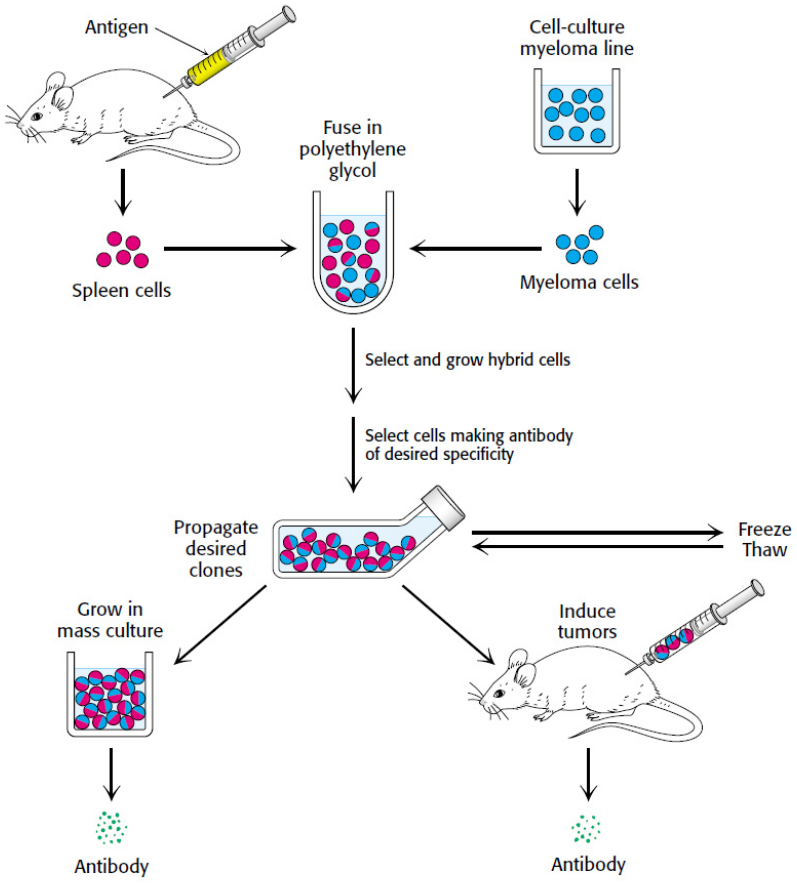


Figure 5 Preparation of monoclonal antibodies. A mouse is injected with the antigen. The goal is to produce antibodies that specifically target this antigen. Antibody producing spleen B-cells and immortal myeloma cells are fused in polyethylene glycol to from hybridoma cells that are able to continuously form antibodies against an antigen. The hybrid cells are the grown in selective medium to screen for the ones that produce antibodies of desired specificity. [45, S. 101]
Antibodies can now be produced “in-vitro” as represented by the left pathway or “in-vivo” as represented by the right pathway.

The current state of technology in mammalian cell platforms involves manufacturing in fermenters up to 25 m^3 in batch, fed-batch modes or perfusion mode in small scale, followed by a sequence of filtration, chromatographic separation and concentration steps to deliver product batch sizes of 50 to 100 kg. Due to their generic structure, platform purification process can be implemented for mAb purification, as seen as in Figure 6 and Figure 7.

To use a cell line over many manufacturing cycles, a two-tiered cell banking system consisting of a master cell bank (MCB) and a working cell bank (WCB) is used. The master cell bank is established from a single clone and must be characterized and tested extensively for contaminants such as bacteria, fungi, and mycoplasmas as well as sterility

and PCR testing for viruses. Cells from the MCB are expanded from the WCB, which is characterized for cell viability prior to the usage in the manufacturing process. [46]

This typically involves clarification by multistep filtration or centrifugation, followed by protein A capture chromatography and orthogonal downstream chromatography steps to separate the product from host cell proteins and product related impurities [44, S. 257].

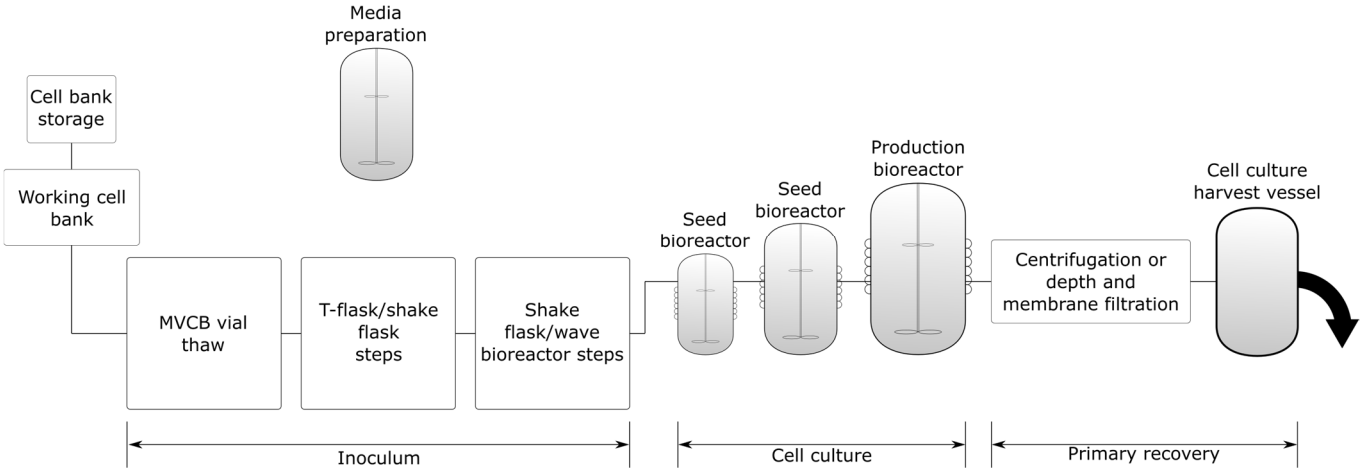


Figure 6 Typical mAb production upstream process. Vial thaw and expansion of cells via a series of inoculum steps is followed by an expansion in a series of seed bioreactors. In the production bioreactor, the monoclonal antibodies are expressed into the medium. Primary recovery involves centrifugation and a series of filtration steps to harvest the cell culture broth from cells and cell debris. (Adapted from [44, S. 256])

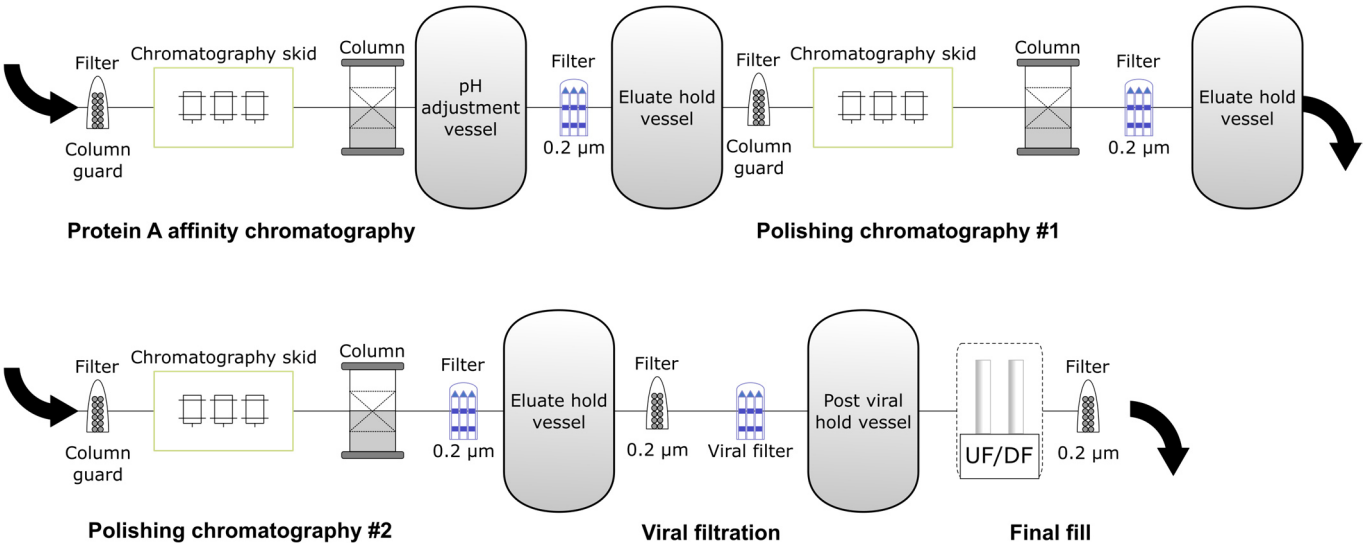


Figure 7 Typical mAb production downstream process. The upstream process is followed by protein A affinity chromatography and involves two subsequent chromatographic polishing steps to remove impurities. Viral clearance is ensured with two orthogonal steps: low pH viral inactivation after protein A affinity chromatography and viral filtration. The last step is ultrafiltration/diafiltration (UF/DF) to formulate and concentrate the product.(Adapted from [44, S. 257])

Conventional fed-batch bioreactors require a volume of up to 25000 L with a typical operation time of 5-18 days. Perfusion-based processes are able to reduce the reactor volume to 1000 L and last for production cycles up to 3 month.

For multi-product mAb production facilities, there is a distinct difference in cleaning effort between conventional stainless steel construction method and the single-use equipment approach. In the past decades production facilities for monoclonal antibodies relied on the use of in somewhat inflexible, hard-piped equipment. The growing trend of more flexible single-use technology (SUT) throughout the entire manufacturing process demands for a direct comparison between SUT and the conventional stainless steel manufacturing processes.

3.3 Conventional stainless steel technology vs single-use technology

The term “Single-Use” (often referred to as “disposables”) in the biopharmaceutical industry, is defined as an item that is dedicated to be used only once. Generally, the item is made from plastics (polyamide, polycarbonate, polyethylene, polypropylene etc.) and is disposed after its use. Accordingly, Single-Use-Technology (SUT) is a technology based on Single-Use-Systems (SUS). [47]

In the early 1980s, large-scale manufacturing became an urgent need as the demand for human forms of the legacy biologics such as insulin, Factor VIII or growth hormones as well as therapeutic enzymes, hormones and cytokines began to increase. Biologics manufacturers turned to manufacturing systems from the plasma purification, dairy, food and beer/winemaking and antibiotics industries, which are traditionally dominated by stainless steel manufacturing technology. At that time, low cell line expression levels ($\ll$ 1 g/L) and growing market demand drove manufacturing scales to 10000 L and up to 25000 L for mAb bulk production [48, S. 2]. This resulted in large single product “six-pack” facilities (e.g. 6 x 10000 L) that require complex stainless steel installations.

The increasing market demand and the launch of the first high-dose monoclonal antibody blockbuster drugs led to manufacturing scales of 20000 – 25000 L. These large facilities were hugely successful in meeting the rapidly increasing demand in western markets for 1st generation blockbuster biologics and will continue to produce originator products according to the respective market demand as well as new blockbusters for the steadily growing United States and EU markets.

The contract manufacturing industry experienced a growth spurt in order to share the high cost risk of production facilities. To accommodate multiple clients, the contract manufacturing industry had to design multiproduct facilities. The resulting necessities are validated cleaning operations to eliminate the risk of cross contamination between different products. For different drug manufacturing campaigns extensive validation and quality systems are required to reduce potential cross contamination and to comply with regulatory agencies.

Production facilities at this scale demand industrial grade mechanical, architectural and process engineering design and construction with a need for large clean utility facilities with kilometres of welded and borescoped stainless steel piping.

Those steel installations demand steam in place (SIP) and clean in place (CIP) systems, which can require hundreds of validated SIP/CIP circuits with sophisticated automation to monitor and control all unit operations and support systems. This complexity resulted in high capital cost and long timelines for erecting such a facility.

Patent expiration of the 1st generation blockbuster drugs (starting around 2015), emerging biosimilars as well as global competition will demand higher efficiency, lower cost and higher agility. Average manufacturing facility scale requirements will decrease as 2nd generation drugs require lower dosage. [7, S. 557]

The requirement of some nations like Russia or China for «in country, for country» manufacturing challenges drug manufacturing companies to build small footprint facilities in these countries to serve the local markets [49, S. 38][50]. Large increase in mammalian cell expression levels and product expression levels, improved downstream purification yields as well as the domination of monoclonal antibodies whose manufacturing process could be platformed and optimized for many different antibody drugs collectively pushed the trend towards smaller manufacturing scale in general. [7, S. 558]

In the beginning, single-use technology was limited to the early inoculum stages of the cell expansion process incorporating the use of shake flasks and T-flasks. Single-use systems for large-scale got introduced in 1996 in form of WAVE bioreactors with up to 100 L in volume [16, S. 7, 51].

From the 2000s, the variety and number of available SUS steadily increased in biopharmaceutical development- and production processes. Currently, hybrid production facilities that combine single-use and traditional systems made out of glass and stainless steel still dominate [47].

Biopharmaceutical facilities rely on several single-use unit operations as an integral part of their hard-piped setups (filters, etc.) but real innovation approaching fully disposable processing occurred during the launch of single-use bioreactors. Almost all processing

steps in biologics production facilities can be realized with disposables up to a plastic bag volume of 2000 – 3000 L. Single-use unit operations include:

- mixing/holding/distributing culture media and buffers
- cell seed expansion and product fermentation,
- cell removal by depth filtration or centrifugation
- disposable chromatography systems and columns
- ultrafiltration/diafiltration/virus filtration

Across the pharmaceutical industry suppliers, end users and regulators compare the implementation of single-use technology against conventional hard-piped stainless steel technology. Some of the risks and benefits for the application of single-use technology are listed in Figure 8.

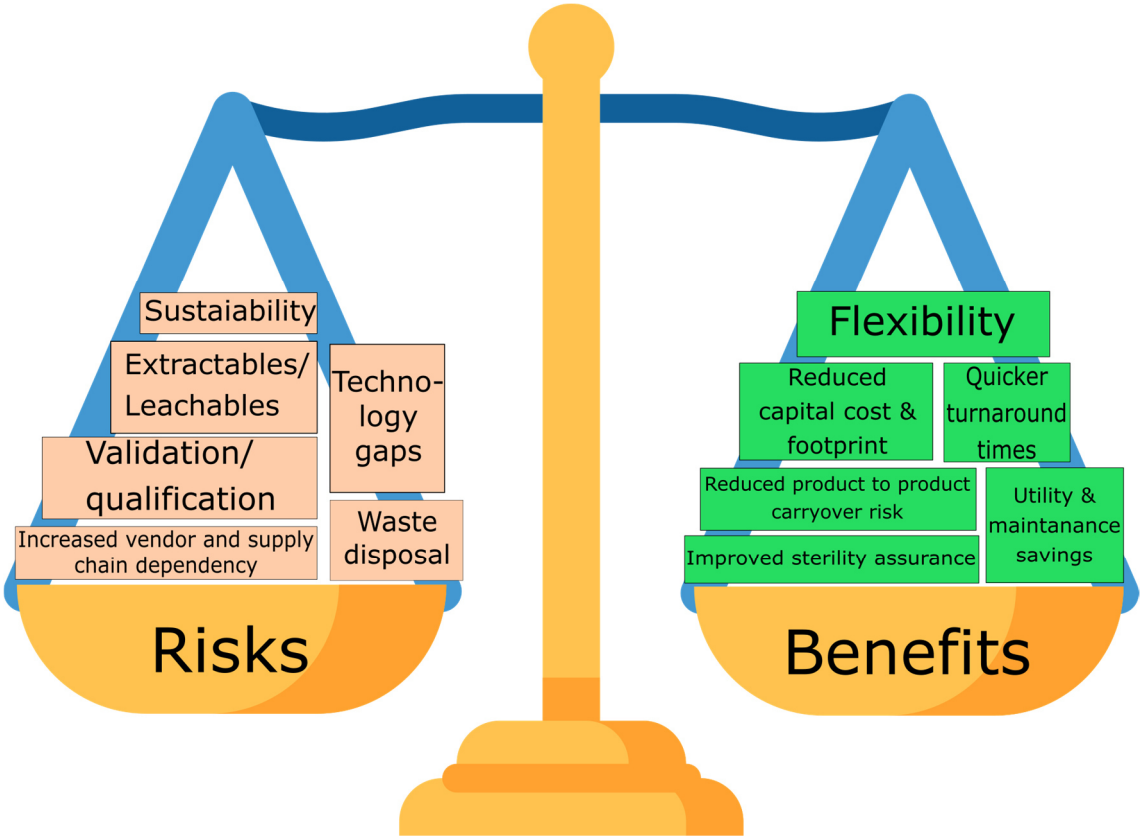


Figure 8 Risk vs Benefits of single-use technology. Potential benefits and risks associated with implementation of single-use technology in comparison with conventional stainless steel technology. [52, S. 355]

Risks associated with SUT involve the following aspects:

- Increased vendor and supply chain dependency: so far, no industry wide standard for single-use items was established. The result is a dependency on one certain supplier to guarantee compatibility (e.g. connectors) as well as quality (e.g. extractables/leachables; structural integrity). If the bag or single-use bioreactor ever cannot be delivered, production could come to a stop. This increased reliance increased the demand for support, supply-chain contingencies and documentation. [13]

- Waste disposal: the main component of single-use items are heterogeneous plastics. As a result, mono-material recycling is not possible and incineration is the method of choice regarding disposal. Landfilling is accepted in the USA but prohibited in Switzerland. The *Bundesamt für Umwelt BAFU* in Switzerland categorizes landfills with increasing potential for harming the environment by the assigned letters A to E in the according document «*Verordnung über die Vermeidung und die Entsorgung von Abfällen (Abfallverordnung, VVEA)»*. The BAFU is the authority that aims to protect the environment by sustainable usage of natural resources. Single-use items made from plastic are suitable for energy recovery and therefore landfilling is not allowed. [53]

- Validation/qualification: the supplier of the single-use items becomes the product due to close proximity to the patient and often process experts. Quality of single-use items in terms of extractables/leachables

- Technology gaps: the production of monoclonal antibodies on an industrial scale require different consecutive unit operations. Some unit operations (e.g. chromatography following 2000 L fermenters with high titers) are limited in their scale and need further development. At the moment there is no supplier that is able to provide all available technologies [8, S. 9].

- Extractables/leachables: this is a serious challenge for disposable systems since these substances can cause health issues for patients. The source can be all disposable equipment that is used during the manufacturing process (storage containers, sensors, filters, final drug containers, tubing, etc.). Many of these items are sealed with elastomers or glues that are a possible source of contamination. [8, S. 169].

- Sustainability: as the category “waste disposal”, this topic is an unsolved problem with single-use items. The overall impact of single-use items that are mainly composed of heterogeneous plastics in terms of recyclability has to be evaluated in comparison to stainless steel that has a very good recyclability [34].

Benefits associated with SUT involve the following aspects:

- Improved sterility assurance: single-use items arrive pre-sterilized and are usually gamma irradiated [8, S. 275]. Since SUT items are generally disposed after use, there are no issues with sterility assurance.

- Utility and maintenance savings: SUT eliminates the need for SIP and CIP systems that require large validated circuits with sophisticated automation.

- Reduced product-to-product carry over risk: the disposal of single-use items after their use inherently reduces the risk of cross contamination.
- Reduced capital cost & footprint: single-use systems require less instrumentation and fever utilities. Sterilization and cleaning processes are eliminated, while installation and support systems are reduced. The smaller footprint is a result of decreased demand for piping, valves, instrumentation and related space for maintenance purposes. [15, S. 2]
- Quicker turnaround times: lower up-front investment cost, which themselves lower variable costs, generally tip the scales in favour of SUT systems when it comes to turnaround times, in comparison with conventional stainless steel facilities. [15, S. 2]
- Flexibility: SUT enables a modular facility design with improved flexibility [54]. The lack of hard-piped equipment allows adaptions regarding the layout and the portability allows multi-product manufacturing processes.

3.3.1 Single-use systems (SUS) in production processes

What makes up a „typical“ single-use system? Single-use systems consist of fluid path components. The common systems are made up of bag chambers, connectors, tubing, and filter capsules. More complex unit operations such as cross-flow filtration or cell cultures will include other functional components such as agitation systems, aeration and single-use sensors. [55, S. 358]

The demand scenario is changing for many biopharmaceutical drugs including mAbs. The landscape now includes standard hard-piped units, hard-piped/disposables hybrids as well as completely SUT-based production facilities. Nowadays almost all steps for biologics production can be done in disposables up to a bag volume of around 2000 to 3000 litres. The following segments describe the current status of single-use technologies for all process unit operations during a standard mAb production procedure. [22, S. 181]

<u>Single-use bioreactors (SUBs)</u>

Disposable stirred bag bioreactors entered the market in 2006. A specially designed disposable bag (see Figure 9 & Figure 10) is inserted into a permanent stainless steel support structure. [16, S. 11]

SUBs can be used to produce mammalian cells of up to 2000 L in bag volume. Bioreactors are available in a rocking motion (see Figure 11) and as stirred-tank bioreactors (STBs; see Figure 12). Additionally, there are vibratory mixers commercially available to be used with single-use systems [56]. STBs are available from 10 to 3000 L, while rocker systems range from 300 mL to 50 L. To ensure desired quality, the used bags are tested for extractables/ leachables as well as robustness of the bag film under various conditions. Rocker-style bioreactors work well for seed train operations and initial inoculation. The bag sits on a platform/tray that can be heated and a back-and-forth movement of the platform achieves agitation (non-invasive). Most rocker bags come with integrated pH and dissolved oxygen sensors. STBs bags come with integrated filters and sensors (pH, dissolved oxygen, temperature). The agitator is connected to the motor after the bag is installed in the support structure that also contains a jacket for heating/cooling. [22, S. 182-184]

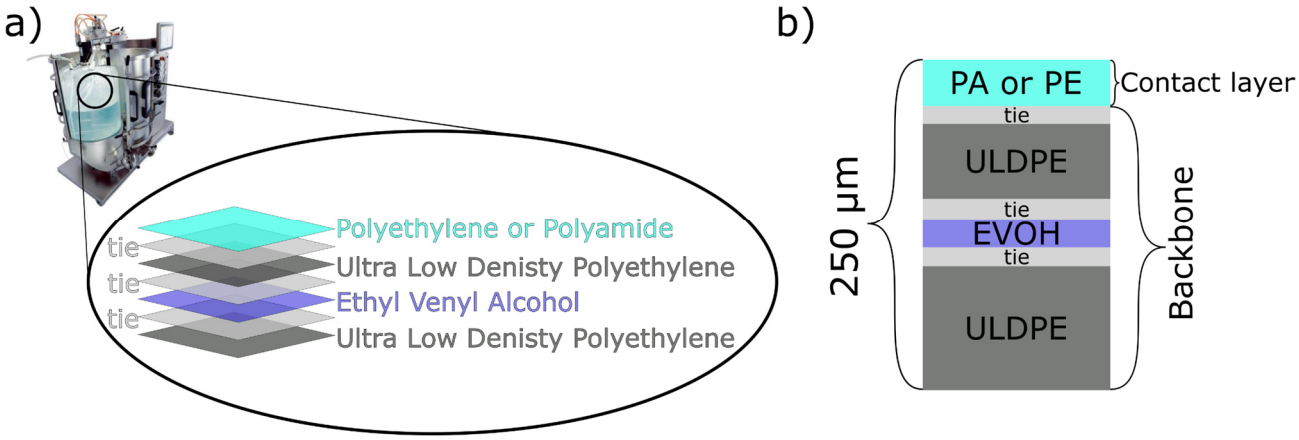


Figure 9 a) General single-use multi-layer film bioreactor bag composition.
b) Schematic bag layer thickness and dedicated materials. The tie layers are necessary to bind adjacent layers. Illustrations adapted from: [52, S. 354]

Bag film layer	Typical film layer components	Purpose of layer
Contact layer	► Ultra Low Density Polyethylene (ULDPE) ► Low Density Polyethylene (LDPE) ► Polyethylene (PE) ► Ethyl Vinyl Acetate (EVA) ► Polyvinyl Chloride (PVC) ► Polyamide (PA)	► Acts as the fluid contact layer ► Provides a clean, inert, and highly chemical-resistant contact layer
Inner layer	► Ultra Low Density Polyethylene (ULDPE) ► Low Density Polyethylene (LDPE) ► Polyethylene (PE) ► Mono Anhydride Polyethylene	► Provides durability and strength ► Reduces gas transmission through the film
Gas barrier layer	► Ethyl Vinyl Alcohol (EVOH)	► Acts as the main gas barrier ► Minimizes transmission of gases such as CO2 and O2 through the
Outer layer	► Low Density Polyethylene (LDPE) ► Nylon ► Polyester Elastomer	► Acts as light, strong, and clear protective outer layer ► Provides robustness ► Contributes to the reduction of gas transmission through the film

Figure 10 Multiple polymer layers provide a range of different functions: biological compatibility tensile properties, puncture resistance, wide operating temperature, transportability, optical transparency, pH stability, minimal extractables/leachables, low product adsorption and low degradation. Illustration adapted from: [52, S. 354]

Figure 11 Single-use WAVE bioreactor by GE Healthcare. [58]

Figure 12 STB by Sartorius Stedim Biotech. [58]

Single-use harvesting

Currently there are three equipment options:

- Single-use acoustic chamber (e.g. Cadence™ system by Pall)
- single-use centrifuge followed by single-use depth filter
- direct harvesting through single-use depth filters

The latter is more prevalent because single-use large-scale centrifuges are not commercially available at the moment. [22, S. 184-185]

The acoustic separator allows a continuous removal of CHO cells and cell debris [57]. Cell debris from cell culture fluid can be separated via centrifugation. Alternatively, the supernatant is further processed through depth filters where solid cells and cell debris are discarded. The filtered pool from depth filtration is further processed through sterile filtration to obtain the harvested cell culture fluid.

Single-use centrifuges

SUT centrifuges (Figure 13) contain disposable conical shaped chambers or single-use separation modules and tubing sets co clarify cell culture fluid in a fully closed disposable format. Peristaltic pumps are typically used for feed inlet and the fluid path is controlled via pinch valves. Tubing manifolds can be sterilely welded to upstream and downstream unit operations or come supplied with any number of sterile connectors. Key considerations to assess single-use centrifuges involve processing time, particle size, turbidity, depth filter area and sterile filter area required post-centrifugation. [22, S. 185-186]

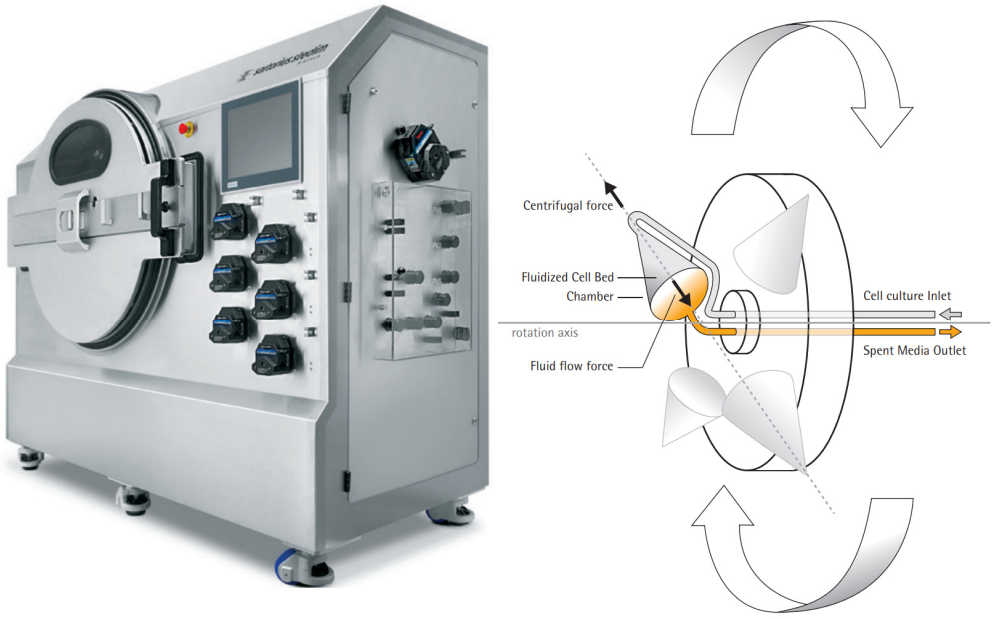


Figure 13 Single-use centrifuge by Sartorius Stedim Biotech with conically shaped disposable chambers. [58]

Depth filters

Typically, depth filters are composed of layers of cellulosic fibres and diatomaceous earth held together with a resin binder (see Figure 14). The resin binder imparts a positive charge to the media to filter smaller negatively charged molecular components (DNA & RNA). Monoclonal antibodies are not retained because of their small size and weak charge. Depth filters require flushing with water or other liquid buffers to wash away inherent, loose organic material prior to pumping cell culture fluid across the filter. Filters can be blown down with pressurized air or nitrogen to push out liquid to reduce holdup volume. After depth filtration, filter cartridges are removed from their holders and disposed in appropriate manner. Disposable depth filters are available in different media grades within self-contained modules to meet application needs. Despite modular units, a holder is still required to force each capsule together to tighten the filter assembly to form a watertight seal. Depth filters can be used to harvest directly from a single-use bioreactor (up to 2000 L). The number of modules required to process 2000 L is high and analysis regarding finance, waste and footprint is necessary. [22, S. 186-187]

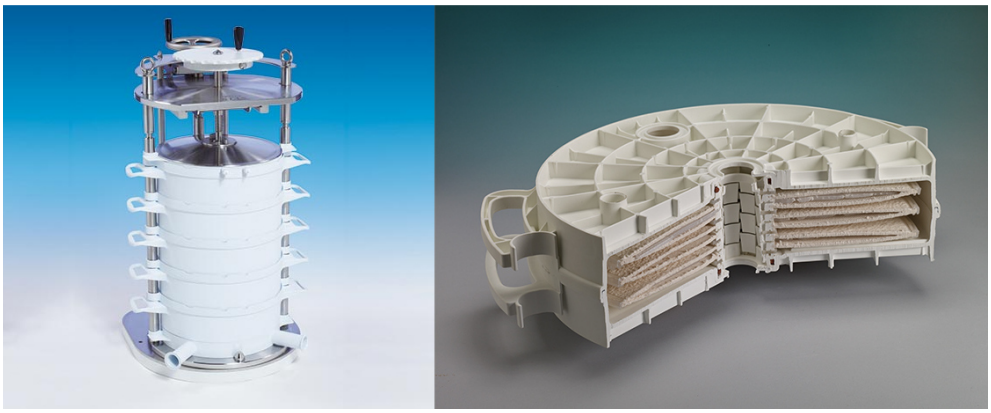


Figure 14 Left: disposable depth filter system by Pall [59]. Right: Single-use depth filter capsules by ErtelAlsop [60].

The combination of centrifugation coupled with depth filtration as primary recovery steps are intended to remove most particulates from cell broth to ease the burden of the subsequent purification steps [61, S. 1]. A depth filter is employed most frequently after the centrifugation step because there is a practical lower limit to the particle size that can be removed by centrifugation [61, S. 2]. Depth filters are usually followed by 0.2 µm absolute filters, to further polish the product and to prevent clogging of following process equipment.

Single-use chromatography

Disposable chromatography systems support up to 2000 L of bioreactor harvest depending on titers, column loading capacity and flow rates. Relevant factors are protein load onto the column and the maximum number of cycles on a column, affecting processing time and occupancy of the equipment. Flow rates of up to 5000 L/h are possible. Disposable chromatography systems consist of flexible flow kits supported by a network of pinch valves, single-use pump heads or peristaltic pumps, single use sensors and can be used with traditional columns or single-use pre-packed columns. Flow kits include single-use tubing, connectors, single-use sensors (pressure, UV, pH, conductivity, temperature, flow). Single-use flow kits usually come gamma pre-sterilized and pre-calibrated. The hardware itself is used to hold and operate the single-use components and is not itself single-use, since only process contact materials are designed to be disposable (see Figure 15).

Closed operation is possible by modifying the flow equipment path with aseptic connectors. Buffer bags can be connected to the inlet, and tubing manifolds can be connected to the product and waste lines, via aseptic connectors. Closed processing is critical if processing (e.g. chromatography via single-use columns) takes place in lower classification rooms or upstream and downstream processing operations are located in one room.

The commercially available single-use flow and pH sensors are limited in accuracy and range, where flow sensor errors can be as high as 10%. A traditional pH sensor can be used and disposed after use to avoid cleaning validation since commercially available disposable pH sensors are limited in pH range. [62, S. 188-189]

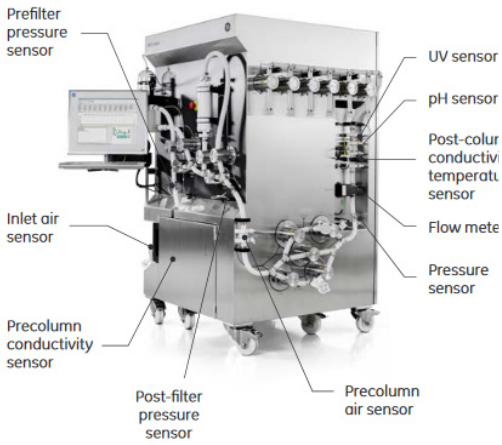


Figure 15 Single-use chromatography platform by GE Healthcare. [63]

Figure 16 Single-use tangential flow unit by Pall [61]

Single-use tangential flow filtration (Ultrafiltration/Diafiltration)

Tangential flow filtration is a method for further retentate concentration and buffer change, commonly applied in monoclonal antibody downstream processing. A typical single-use tangential flow unit is presented in Figure 16. It is widely used to concentrate antibodies (ultrafiltration) and also for buffer exchange (diafiltration). Single-use tangential flow filtration systems are compromised of pinch valves, recycle tanks, pumps (feed and buffer), sensor transmitters, tubing manifolds (feed, retentate, filtrate). Similar to other single-use systems, hardware is used as a support for various

disposables. Manifolds have integrated single-use pressure sensors, flow sensors, conductivity sensors, temperature sensors and temperature control valves on the retentate line. Manifolds and pre-calibrated sensors come gamma sterilized. Tangential flow filtration cassettes can be used in single-use or reusable format.

Single-use mixers

Disposable mixers range from 10 to 3000 L and can be used for upstream and downstream applications, buffer make-up, media make-up, pool holds, pool adjustments, et cetera. Mixers are either top-mounted or bottom-mounted agitator systems, where the bag comes with a pre-installed agitator. Mixers are equipped with a jacket for temperature control. Most of the bottom-mounted agitators are magnetically coupled impellers. Similar to other single-use equipment, mixers consist of a support structure to accommodate dedicated bags. Rocking systems can also be used for small scale mixing tasks. [62, S. 191-192]

Figure 17 Single use mixers by Merck Millipore, Sartorius Stedim and Thermo Scientific. [62, S. 192]

Single-use bulk freeze systems

Single-use bags for bulk-freeze storage and shipping applications are an area of increased focus. Bulk drug substance (BDS) containers for transportation and storage of active pharmaceutical ingredients is well established for clinical operations. Bulk freezing, transfer and storage are important steps to ensure that the final product is safely handled, stored and delivered (either to fill-finish sites or eventually patients). The vast majority of manufacturing for bulk-freeze applications is still dominated by steel tank systems with its associated disadvantages. Systems made from stainless steel must be maintained in a clean state pre- and post-use and require costly and labour intensive cleaning. The average large biotech company employs labour for many hours to maintain a steel tank's integrity. Additionally shipping validation is required to ensure that the steel tanks are in a state of microbial control at all times. For these reasons, disposable bulk freeze-thaw systems (e.g. systems by Zeta, Meissner, or systems by SUSupport as seen as in Figure 18) are developing towards single-use applications. [62, S. 192-194]

Figure 18 Single-use freeze-thaw system by SUSupport. [53]

3.3.2 Waste treatment

An inherent feature of any SUT is the larger quantity of (usually solid) waste compared to multi-use counterparts. The most common disposable items for the development and manufacture of biopharmaceuticals are listed in Figure 19. The solid waste stream generated by SUT (bags, transfer systems, reactors and downstream equipment) represents the major fraction of the plastic waste stream. Gaseous waste streams are more a function of the production process and less a function of the technology (Single-use vs SST). For conventional stainless steel facilities, liquid waste streams in form of CIP wastewater represents a significant exception. [8, S. 174]

For the application of SUT, buffer and media preparation is a significant source of liquid waste. The needed volume is generally increased by 15-20% to provide a surplus as a measure to prevent production halt. The leftover volume that has not been used during production, has to be routed to wastewater treatment. This results in an increased wastewater volume (mainly WFI) as well as a loss of buffer salts during the discharge.

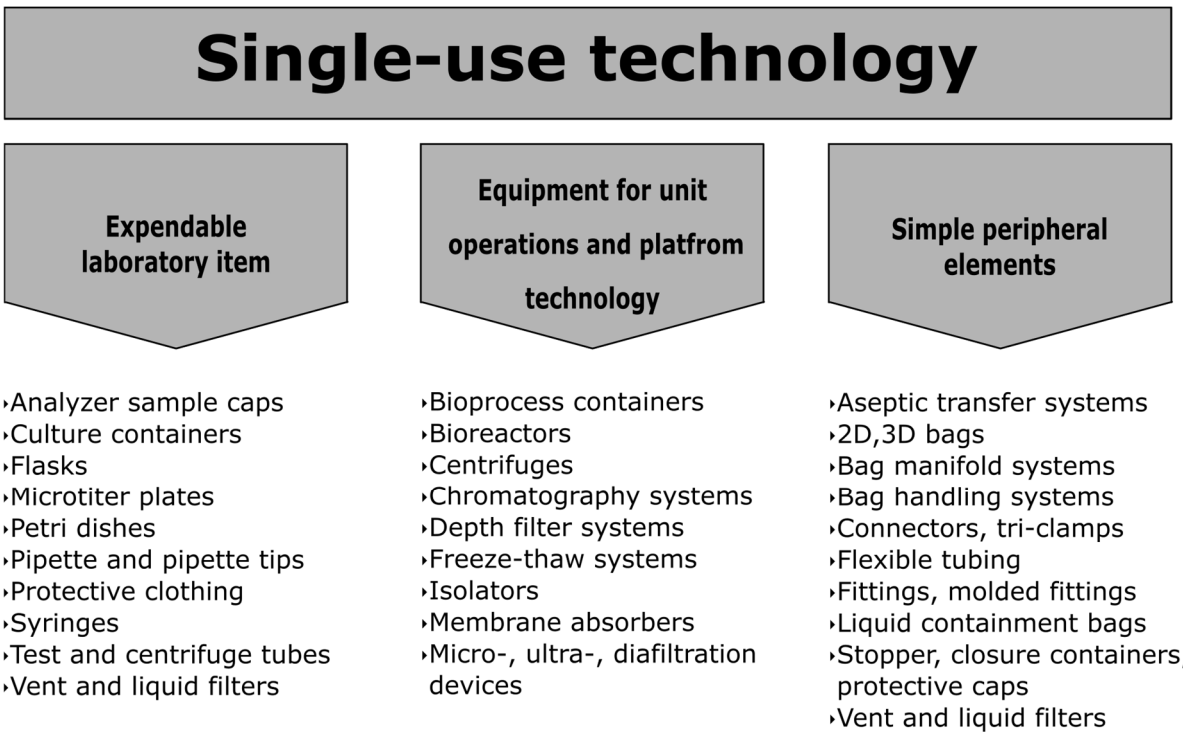


Figure 19 Primary categories for single-use technology equipment. Adapted from [8, S. 8]

Recycling of plastics often requires a relatively high material homogeneity but mixed wastes such as polymer bags and combined manifolds are unsuitable for most material recycling processes [64]. Recycling of SUSs combining components or layers of different plastics (polyethylene, polystyrene, polypropylene) require separation to produce homogenous fractions. Currently most SUS wastes are unsuitable for material recycling and direct or indirect reuse is simply impractical.

Solid waste

Disposable systems consist of a wide variety of seizes, structures, and quality. Sizes range from centimetres (e.g. connectors) to meters (e.g. tubing) or grams (e.g. bags) to kilograms (e.g. disposable bioreactors). Materials can be soft (silicone tubing, bags) and resistant to grinding or stiff (filter capsules, rigid bioreactors vessels, centrifuge cartridges) and hard to break. Solid waste can be categorized in respect to collection, further treatment or disposal as seen as in Figure 20.

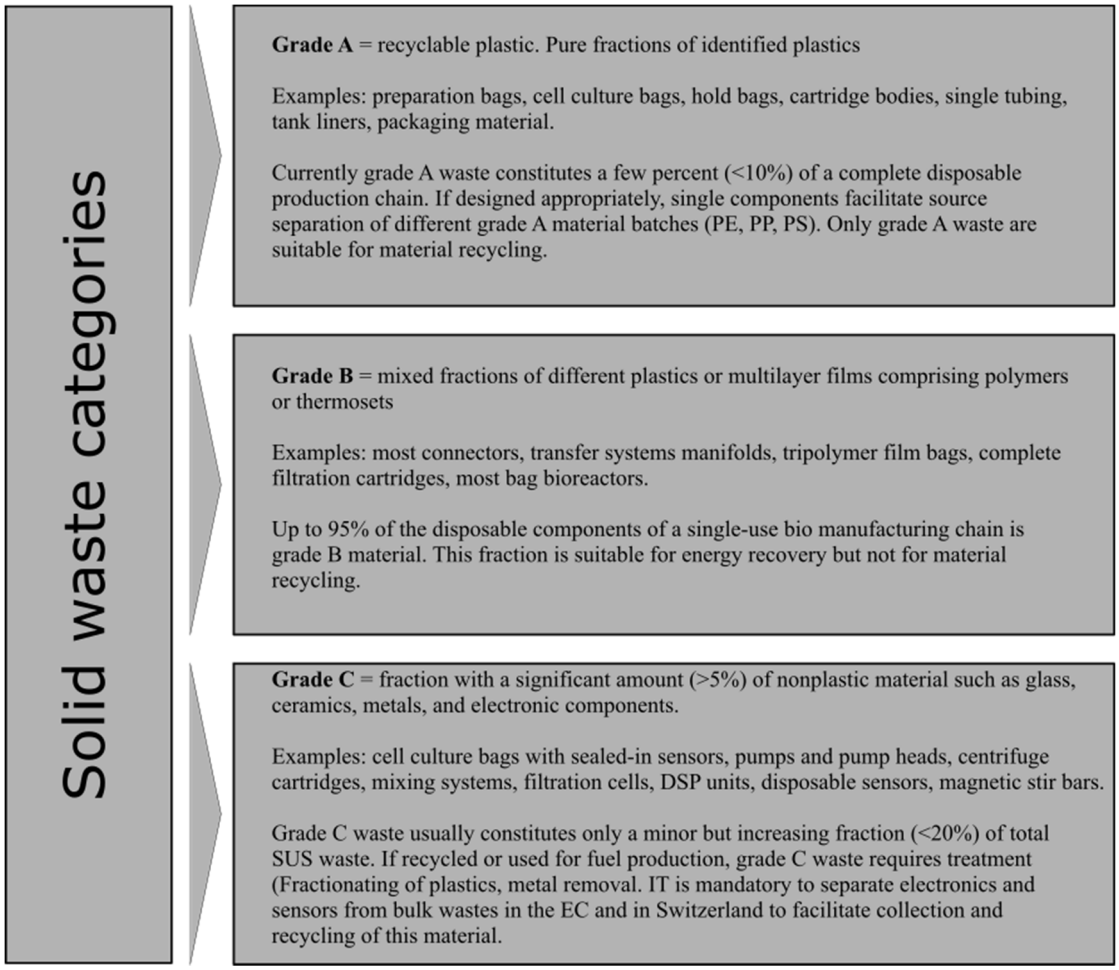


Figure 20 Solid waste categories of single-use systems. [8, S. 175]

The exact quantity of solid waste generated from SUSs is specific for each individual process. [8, S. 175]

Leveen and colleagues reported a total of 880 kg of solid waste per batch for a 3 x 2000 L scale commercial mAbs production process [65].

The continuing growth of single-use productions steps in biopharmaceutical manufacture increases the demand for design concepts for the reduction and prevention of solid waste. Several approaches can minimize the volume of solid waste generated:

- Thinner polymeric films: bag and liner products with thinner but stronger walls can reduce the volume of this waste category.
- Dual components: a combination of units made of multi-use disposable parts (nonsterile, without product contact) and single-use parts (sterile- with product contact). This approach offers significant waste reduction potential (e.g.

bioreactor liners, filter cartridges, holders with pre-sterilized disposable inserts, centrifuge units with disposable rotor-stator inserts.

- Reuse: even though the term “single-use technology” implies that an item is used only once, single-use items that do not have direct product contact can be utilized multiple times. One example is the refill of buffer bags that can lead to a waste stream reduction.
- Source separation: SUSs should be designed with recyclability in mind. This does not refer to multiple use but rather the potential for further valorisation as raw material or as energy carrier. This involves easy separation of electronic components, controls and sensors. Directive 2012/19/EU on waste electrical and electronic equipment makes the separate collection or even reuse of electronic components mandatory in the European Union [66].
- Bioplastics: the implementation of bioplastics for the production of SUSs will not have a significant impact on the amount of solid waste in the near future. Their lack of chemical and biological stability as well as incompatibility with certain pharmaceutical standards represent further obstacles. Bioplastics have a tendency to increase the amount of solid waste due to their typically lower mechanical strength and hence thicker walls. Nevertheless, the use of renewable resource-based materials and bioplastics would offer significant potential to reduce the overall carbon footprint of disposables.

During manufacture, several single-use items come in contact with microorganisms (particularly genetically modified organisms). Regulatory bodies require an inactivation of microorganisms prior to waste treatment off site. Onsite treatment greatly reduces material transport, treatment, and disposal expenses in comparison with hazardous haul-away services [67, S. 2]. Method of choice is the heat inactivation with an according autoclave cycle (121°C for 15-30 minutes [7, S. 963]). The single-use items are placed manually in an autoclave and to get routed for further waste treatment (e.g. incineration) after the inactivation of all microorganisms. Larger single-use items such as 2000 L stirred bioreactor bags are problematic to handle due to their dimensions and weight. Items that occur in larger numbers (e.g. filter pods) are also problematic since the autoclaving process is volume limited and equipment occupation can become a bottleneck. The volume capacity of commercially available autoclaves is limited to the range of 3.9 – 5.4 m^3 resulting in a time consuming and labour intensive process to sterilize all contaminated single-use items of a production campaign.

One possible solution is an on-site sterilization system consisting of shredding and a following steam-sterilization process (see Figure 21). This results in waste volumes reduction of more than 80% and treated material is rendered as common municipal

waste that is appropriate for recycling. Automation reduces employee handling of contaminated single-use material.

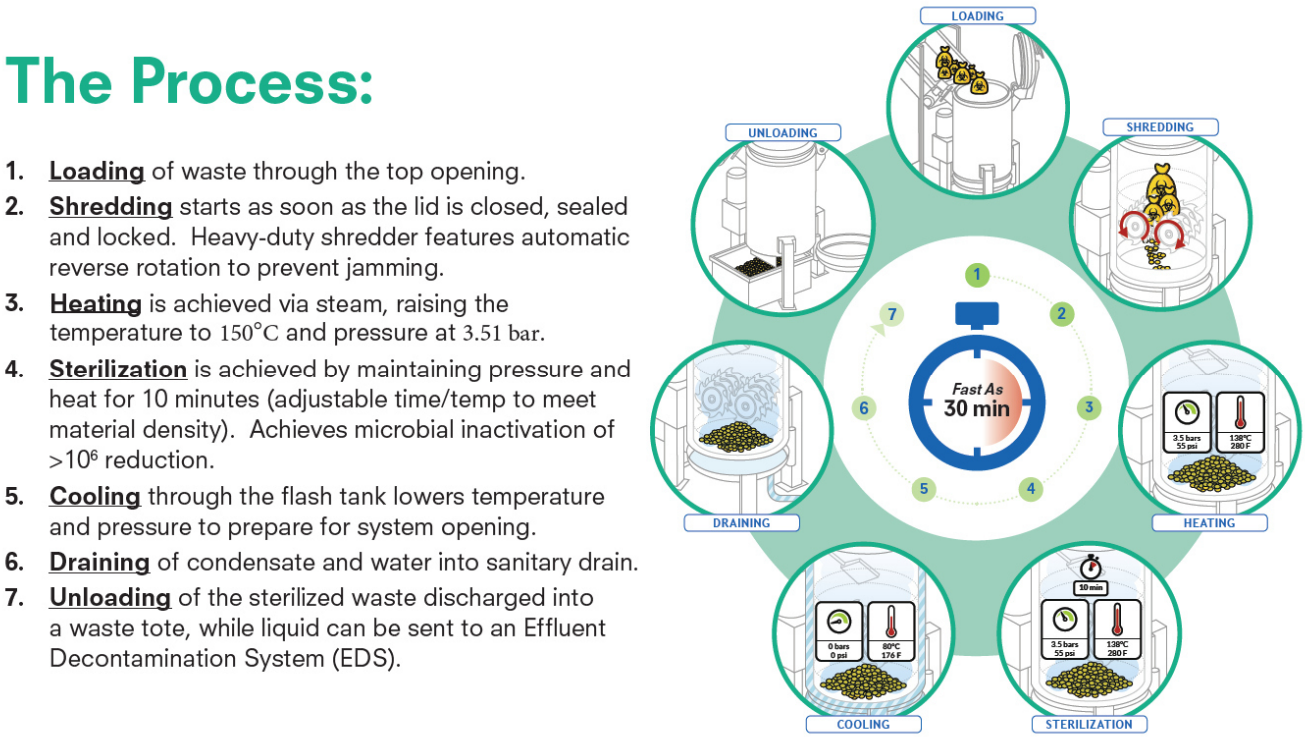


Figure 21 Process procedure for on-site shredding and steam sterilization of contaminated single-use materials. Adapted from [67, S. 3]

Important process data and available models for sterilization cycles are listed in Figure 22. The heterogeneity of material use in single-use items can cause problems in the shredding process. The magnetic stir bars that are used with single-use bioreactor bags can get stuck in the shredder shaft, causing a stop of the sterilization process or even damaging shredder components. The shredder rolls are manufactured from hardened steel that is ferromagnetic and therefore attracts fractions of the magnetic stir bars.

Data	X-300	X-700	X-1000	X-2000
Volume Capacity	350 L	700 L	1100 L	2500 L
Avg. Process capacity *see waste density below	35-52 kg/cycle	70-90 kg/cycle	110-165 kg/cycle	250-375 kg/cycle
Avg. Daily capacity 3 shifts (24 hrs/day)	2088 kg	4176 kg	5667 kg	10000 kg
Avg. Cycle time	30 min	30 min	35 min	45 min
Dimensions (L x W x H)	220 x 260 x 330 cm	490 x 460 x 520 cm	510 x 460 x 460 cm	510 x 460 x 460 cm
Electricity/Cycle	1.7 kWh	3.5 kWh	4 kWh	9 kWh
Water	25 L	30 L	35 L	50 L
Steam	15 kg	18 kg	20 kg	40 kg
Max Steam Flow	170 kg/h	230 kg/h	370 kg/h	500 kg/h

* Assumes Avg. Density of Regulated Medical Waste of 100-150 kg/M3
All Models Use 8 bar of Steam Pressure, and 6 bar of Compressed Air

Figure 22 Different models for on-site shredding and sterilization systems by PRIbio. Utility and energy consumption per cycle are listed as well as volume capacity per cycle. Adapted from [67, S. 4]

Liquid waste

Sources of liquid waste are CIP wastewater (e.g. contamination with Triton X-100), salt containing buffers from down stream processing, filtered spent broth, as well as contaminated streams from indirect cleaning. Condensates from water or steam heating or waste cooling water are not contaminated by raw materials or products and are therefore usually not a source of bio contamination. Indirect cleaning and CIP wastewater must be treated as water contaminated with raw materials, by-products, biocatalysts, products, caustic agents (e.g. NaOH) or acidic agents (e.g. H_3PO_4). These streams are generally characterized by high conductivity (salts) and medium to high eco-toxicity. If genetically modified organisms (GMOs) or bioactive substances have been used, liquid off streams from biopharmaceutical manufacturing processes may be classified as hazardous wastewater and require appropriate treatment (irrespective if

they originate from conventional SST or from SUS). Buffer usage in down stream processing represent the main source of waste water from biopharmaceutical manufacturing systems. Single-use systems with their disposable approach will inherently generate much less, if any waste water due to the lack of CIP (and SIP). SUSs contain only 2-5% of CIP agents (caustics, acids) compared with SST systems. [8, S. 174-175]

Since biopharmaceutical products are biological in nature, there might be special local city and state regulations that guide the manner of waste treatment required by the facility. Broad guidance exist with the cGMP (40 CFR Part 261, 40 CFR Part 264) outlining the minimum standards required. For the biotech industry the main concern of waste treatment is the recombinant host used at the start of the process during cell manipulation and cell culture. These organisms pose a health threat if they are released into the environment without prior treatment. Risks assessments are used to identify to what extend these substances are hazardous to health and environment. Bases on this information, waste is classified into a biological safety class that determines the waste treatment procedure. Most culture-based therapeutic processes (e.g. CHO cell lines) will have a biosafety level of good large scale practice (GLSP), that do require inactivation prior to disposal. Some local municipalities or state authorities may require inactivation prior to disposal [68, S. 601]. The protection of intellectual property also favours the thermal inactivation of single-use items prior to disposal or incineration. This step will eliminate concerns about theft of the high performance cell line and the associated investment in form of research and development.

Off-gas

The three sources of gaseous emissions from biopharmaceutical production lines are:

- Air streams from bioreactors: all aerobic and most anaerobic bioprocesses produce waste air streams from the bioreactors. This includes conventional SST as well as SUSs.
- Off-gas from post sterilization: this might be specific for SUSs if components are poststerilized in-house prior to disposal. Off-gas from autoclave processes is characterized by a discontinuous flow pattern, high humidity, and high biodegradability of its components.
- Secondary odour production: Disposable components which are contaminated with raw material, by-products, biocatalysts or final product, are often stored in-

house prior to their final treatment. Extended storage can lead to the production of secondary odour even with prior presterialization (130°C, 60 min).

The unit operations, storage, preparations and the use of SUSs or components do not represent a source of off-gas as such but the “cradle-to-grave” approach has to be applied to cover all aspects of SUSs application. [8, S. 175-176]

Energy recovery from SUS waste

The latest environmental regulations in Switzerland published by the Office for the Environment (BAFU), prohibit landfilling of plastic waste (*Verordnung über die Vermeidung und die Entsorgung von Abfällen 814.600*). For this reason, aspects for landfilling of plastic waste are not further investigated and covered in detail. A possible treatment option is state of the art incineration including thermal recycling with three different routes:

- In-house incineration: state-of-the-art dual combustion chambers are approved by the Environment Protection Agency (EPA) or certified by the Confromité Européenne (CE) and meet international emission standards. The units provide almost 100% waste volume reduction but require an external energy source (diesel, oil, liquefied natural gas). Operation is batch wise at temperatures between 1000 and 1300°C. Throughput can be as low as 150 kg of solid waste per day or 100 L per batch. About 3-5% of the initial mass of the waste has to be landfilled periodically as ash. Benefits are the significant reduction of transport and disposal cost

- Combined municipal solid waste (MSW) incineration: municipal incinerations accept a wide range of waste types like hospital waste and combined plastic waste. At temperatures between 800 to 1000°C there is complete destruction of biocontamination as well as chemical contamination making them suitable for solid waste from SUSs even despite their chemical or biohazard classification. Toxic components with extremely high temperature stability require industrial incinerators that are specifically designed for hazardous material. 3-5% of impure plastic waste will remain as ash or fly ash after incineration [64]. The disposal at municipal incineration facilities can attract high transportation and high tonnage cost of €120 to €250 (approximately $135 to $281) per ton [64].

The latest off-gas treatment technologies ensure compliance with stringent environmental standards for particulate matter (PM_{10}), nitrous oxide (NO_x), sulphur dioxide (SO_2) or hydrochloric acid (HCl). The high caloric value of dry plastic waste processed in modern MSW incineration plants (40000 kJ/kg; irrespectively of polymer type [69, S. 22]), results in an energy recovery of roughly 10000 kJ (35%) of electricity and 18000 kJ heat (65%) per 1 kg of plastic.

- Industrial incineration: The same benefits as in the MSW incineration facility apply, namely minimization of waste volume, and destruction of hazardous components and biomaterial. Industrial incineration of plastic waste might be beneficial from an energy perspective, since the kilns often require material of high caloric value.

Waste treatment for single-use items remains a challenging topic that requires further development. The inherent inhomogeneity of single use-items in terms of material use (e.g. different plastic types or magnetic stir bars for stirred bioreactor bags) requires adapted solutions for waste processing. Since landfilling is not an option in Switzerland and waste disposal sites are a burden for future generations, incineration remains the current solution for the disposal of single-use waste. State of the art incineration facilities are able to achieve a significant reduction in waste volume and eliminated many concerns regarding emissions (e.g. PM_{10}, NO_x, SO_2, HCl) from past generation incineration facilities. The emission of carbon dioxide is an indispensable characteristic of incineration facilities that further increases the carbon footprint of products that are produced with the use of disposables.

3.3.3 Technical and clean process utility systems

Biopharmaceutical production requires utilities (water, electricity, compressed air), while the sources of these utilities are typically located outside the clean room. [7, S. 966]

Production processes which have direct impact on product quality typically require clean utilities that include surfaces with direct product contact. For this reason, clean utilities need to be as pure, if not more so, than the product being produced to ensure that no new contamination is introduced into the production process. For biopharmaceutical production facilities following utilities are generally needed [7, S. 966]:

- Water for injection (WFI) and purified water (PW)
- Clean compressed air
- Clean process gases (e.g. O_2, N_2, CO_2, etc.)
- Clean steam

Clean utilities

WFI and PW are for makeup of buffers and cell culture media, both of which come into direct contact with the product, as well as for cleaning in place (CIP)/sterilizeing in place (SIP) operations to clean/sterilize product contacting surfaces. WFI and PW are also needed for HVAC (e.g. humidification) and aeration of SUBs. Clean compressed air is utilized for blow down of transfer pipes and drying of product contacting surfaces after cleaning and sterilizing as well as vacuum evacuation after SIP. Additionally, clean compressed air can be used in pneumatic valves within transfer pipe networks and unit operations. Clean process gasses are needed extensively within cell culture processes. [7, S. 966]

Technical (“black”) utilities

Technical utilities (or referred to as “black” utilities) are supporting the process operation but do not have direct contact with the product. Technical utilities are either used as inputs for clean utility generation (e.g. potable water for WFI/PW generation) or utilized in support of the manufacturing process (e.g. chilled water/steam for jacketed vessels). For biopharmaceutical production facilities following technical utilities are generally needed [7, S. 966]:

- Plant steam: room heating purposes
- Potable water: Generation of higher grades of water or domestic systems (e.g. bathrooms, kitchens, canteens)
- Fire water: safety reservoir for fire service or sprinkler systems
- Cooling water/glycol : for cooling applications via heat exchangers or jackets
- Hot water/technical steam: non-product heating applications
- Natural or liquefied gas: for firing gas boilers
- Electrical power
- Waste water collection/rain water/sanitary waste water

Water

Water for biopharmaceutical manufacturing has to be appropriate for the desired process step. Different qualities of water are specified regionally, namely the United States Pharmacopeia ,WHO, or the European Pharmacopeia (EUPH). The quality is based on conductivity, pH, total organic carbon (TOC) and endotoxin content. Standard potable (drinking water) contains contaminants (microorganisms, dissolved organic and particulate matter etc.) that could either react with the desired product (a protein in case of mAb production) directly or that it would have an adverse effect upon patient health if present in the final product [70, S. 131]. Purified water is used for all CIP rinse cycle except the final rinse as issued in the Guideline on the quality of water for pharmaceutical use, published by the European Medicines Agency (EMA) [71].

A basic schema for the generation of WFI and purified water is shown in Figure 23.

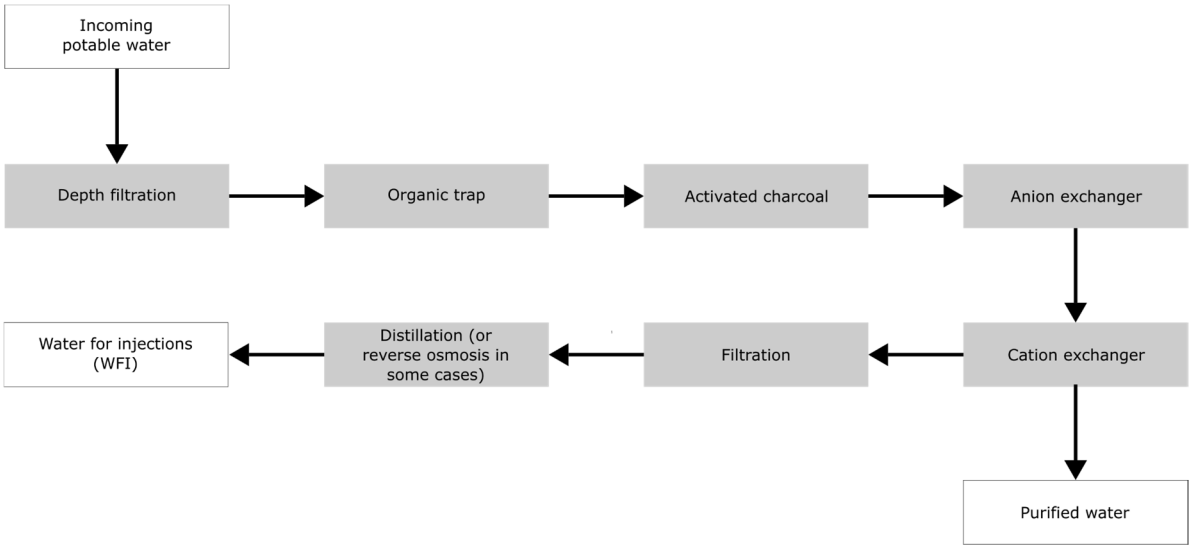


Figure 23 Overview of a generalized procedure by which purified water and WFI are generated (Adapted from [70, S. 134])

The highest grade of water is typically water for injections (WFI). WFI is generally utilized for all product-contacting solutions such as buffers, cell culture media, as well as CIP operations and room cleaning. WFI is typically generated in continuous water treatment systems (see Figure 24 for a basic schematic). Water treatment starts with potable water from municipal water supply. Good manufacturing practice (GMP) requirements by all international authorities demand the use of drinkable water of at least WHO-quality as raw water for the generation of pharmaceutical water. At first, the potable water is filtered to remove any particulates. A softener bed is used to remove calcium and magnesium (cations) form the water to minimize scale deposits in the plant's utility systems, water purification filters ,reverse osmosis, and distillation units. The softened water is passed through an activated carbon filter to remove oxidizing substances (e.g. chlorine and its compounds) and low molecular-weight organic material. The water is finally purified with reverse osmosis and/or distillation. A reverse osmosis filter system with an electro-deionisation (EDI) step is the most common way to meet requirements for conductivity, TOC, pH and bioburden. For the generation of WFI and clean steam, three different approaches are generally applied [7, S. 966-967]:

i. Distillation via vapour compression: vapour compression-distillation is a method where the process fluid is boiled on one side of a heat transfer surface and the compressed vapour generated, is directed to the other side of the heat transfer

surface where it is condensed (giving up its latent heat to the boiling liquid). Heating is achieved via steam or electricity. Compression is usually accomplished via steam jet ejectors or mechanical compression. With this method, it is possible to generate hot and cold WFI.

ii. Distillation via multi-effect: multi-effect distillation used distillation columns that perform both an evaporation and condensing process. Treated water us evaporated by technical steam and subsequently condensed in a series of distillation columns and heat exchangers for energy recovery. The number of columns has to be sufficient to obtain WFI without need for external cooling. WFI is produced at a minimum of 80°C.

iii. Reverse osmosis (RO) and Ultra filtration (UF): this method can be used, if it is allowed by the ruling pharmacopeia. While the US and Europe allow this method, China only allows WFI generation via distillation. Ultrafiltration or Reverse osmosis follow the same process water generation process prior to an additional RO or UF step for pyrogen removal.

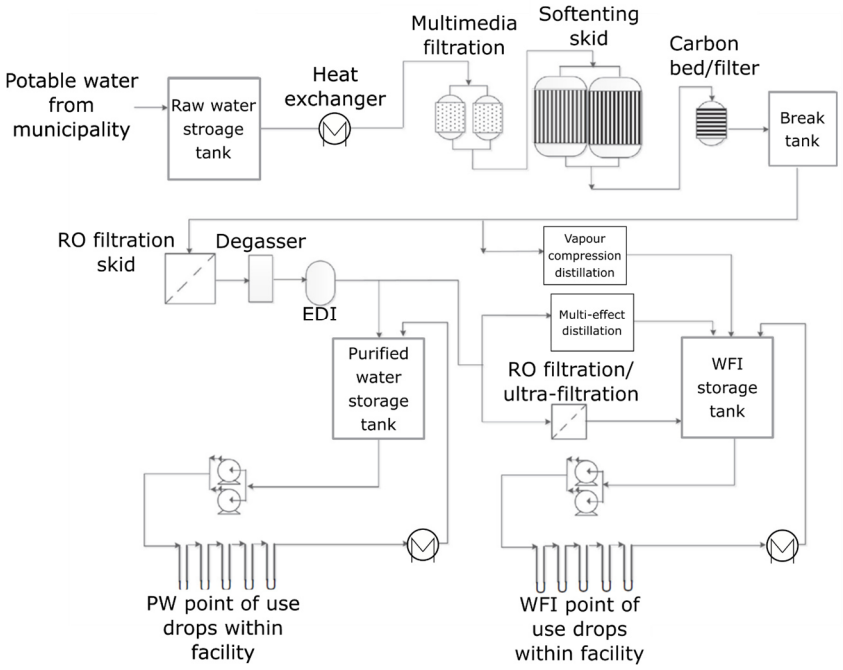


Figure 24 Schematic of water purification system for generation of water for injection (WFI) and purified water (PW). EDI=electro-deionisation; RO=reverse osmosis. Adapted from [7, S. 967]

The material choice for WFI/PW loop construction is of great importance to minimize the risk of microbial growth. High-grade stainless steel (316) or PTFE should be used with polished surfaces (roughness parameter Ra<0.5 µm) to eliminate ridges or crevasses that could stagnate water. As a result, WFI/PW storage tanks and distribution loops are very costly and contributes a significant portion to overall CAPEX of the facility.

Depending on the method used, WFI is generated and distributed hot (>80°C), chilled (20-25°C) or at ambient temperature. PW loops are generally cold loops (20°C). To ensure PW/WFI stays within specifications, microbial growth is minimized by keeping the water flowing at all times via continuous recirculation in the main loop through the distribution tank. Two separate cases have to be considered regarding the needed sanitization activities:

- Hot loop including storage: self-sanitizing, so there a no sanitization activities required
- Cold loop including storage, sanitization via ozone or heat sterilization (121°C)

UV systems are an option to disinfect, de-chlorinate and to break down the ozone (if used) into O_2 + H_2O. Sanitization via the application of heat is achieved by rising the temperature of the PW/WFI loop to 80-85°C for a defined period. From this standpoint, “hot” WFI loops have the additional advantage of being continuously self-sanitizing. [7, S. 968]

Steam

There are typically two types of steam used within the biopharmaceutical production facility [7, S. 968-969]:

- Plant or black steam (at 8-12 barg):this type of steam produced from a boiler: in most cases technical steam is produced by conventional fire-tube steam boilers. Such boilers are almost always operated with injected additives in the feed water to protect the boiler and steam distribution piping from scale and corrosion. The system designer has to determine what additives are used and verify that they are acceptable for application.

- Clean steam (at 3 barg): This type of steam is not produced by a boiler. Clean steam is generated from treated water that is free of volatile additives (e.g.

hydrazines or amines) and is used for thermal disinfection or sterilization processes. The main purpose of clean steam is humidification. Additionally, clean steam is used to sterilize products and more typically, equipment. To sterilize equipment or piping, clean steam is injected to create a sterile environment. Sometimes clean steam is used within HVAC operations for clean room humidification. There is no pharmacopeia standard for clean steam. Conservatively, manufacturers tend to produce clean steam with a quality whereby the condensate produced meets WFI requirements for conductivity, TOC, and endotoxins. Microbial limits are usually excluded since it is acknowledged that viable microorganisms cannot survive in steam systems. Clean steam is produced by boilers that are heated by plant steam.

Distribution systems for clean steam need to be inert to the aggressive nature of clean steam. Commonly used is corrosion-resistant 316 L grade steel. Surface roughness is generally not a point of concern due to the self-sanitizing nature of clean steam. Piping has to be designed to be able to handle thermal expansion and to drain condensate. [7, S. 968-969]

3.3.4 Cleaning in place (CIP)/sterilizing in place (SIP) process overview

Clean-in-place does not have it's origins in the biopharmaceutical industry but rather started in the 1960s with the necessity to clean pipelines of dairy farms. In the subsequent 15 years, CIP systems got adopted by beverage, brewery, winery, and food processing industry. CIP got widely adopted in the biopharmaceutical industry during the last three decades. Cleaning in the biopharmaceutical industry is recognized as a very important procedure to prevent cross contamination. Effective cleaning is the most important step prior to SIP, since sanitization/sterilization requires contact between steam and microorganisms. [72, S. 1]

Cleaning and sterilization are similar procedures but have different requirements for the outcome (free of residues and germ reduction, respectively). The extent of outcome is dependent on four factors that are known as Sinner's diagram (see Figure 25).

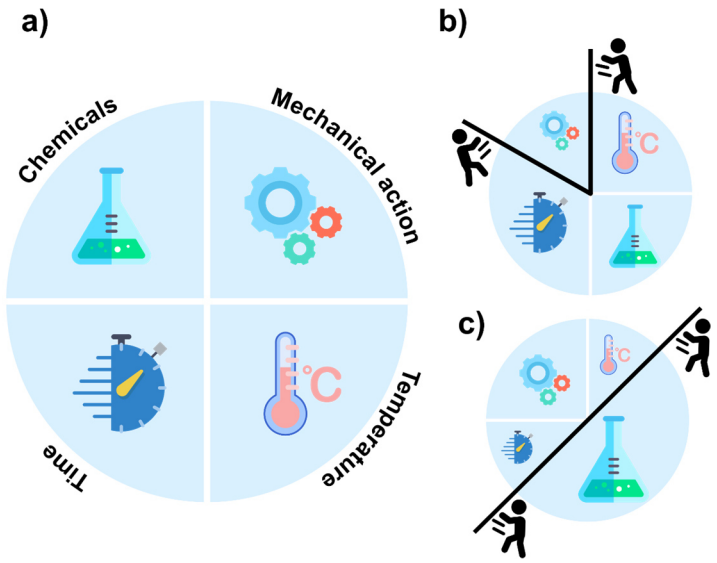


Figure 25 Sinner's diagram.
a) Sinner's diagram with the four parameters: chemicals, mechanical action, temperature, time.
b) Example of Sinner's diagram, where the reduction of mechanical action requires an increase in time.
c) Example of Sinner's diagram where the increased use of chemicals reduces the required time and temperature

The four factors (chemicals, mechanical action temperature and time) are interconnected but are variable in extent:

- Chemicals: this parameters involves the choice of detergents and its concentration of cleaning agents and their pH value
- Mechanical action: this parameter refers to the type of mechanical movement that is used for cleaning (e.g. water jet speed). Examples are spray ball design and turbulent flow in pipes
- Temperature: the temperature influences the cleaning process
- Time: this parameters describes the duration of other parameters (e.g. how long a cleaning agent can work into residues, how long a certain temperature is held, how often a cycle is repeated, how long a mechanical cleaning action is performed)

The four parameters have to be balanced against each other to achieve the desired results and always add up to the same grand total. [73, S. 64-65]

There is no universally applicable CIP system that suits all different scenarios in a pharmaceutical production facility. Different tank sizes, changing products and changes in operational scale due to changing market demand require specialized CIP systems that are individual for every production facility.

Goal of any CIP system is to pass the cleaning validation. The validation of a cleaning process is based on defining “clean” and developing and validating analytical methods to ensure proper sensitivity, accuracy, and reproducibility. For a multiproduct facility, there are two basic concepts for cleaning validation:

1.) Prove that the cleaning process is effective for each product

2.) Prove that the cleaning process is effective for the least soluble (or worst-case) product.

Generally, there are two methodologies to access cleaning: surface sampling and rinse solution sampling [74, S. 342]. After a CIP system is dimensioned and installed, the four parameters (chemicals, mechanical action, temperature, time) of the Sinner’s diagram can be used to adjust the CIP system to pass the cleaning validation. CIP systems can be designed in various ways and include the following approaches: boil out systems (fill/flood), total loss, single-use recirculation, re-use (recovery), multi-channel, fixed and

mobile systems. As an example, Figure 26 shows the basic setup of a total loss system and a re-use system with recovered water tank.

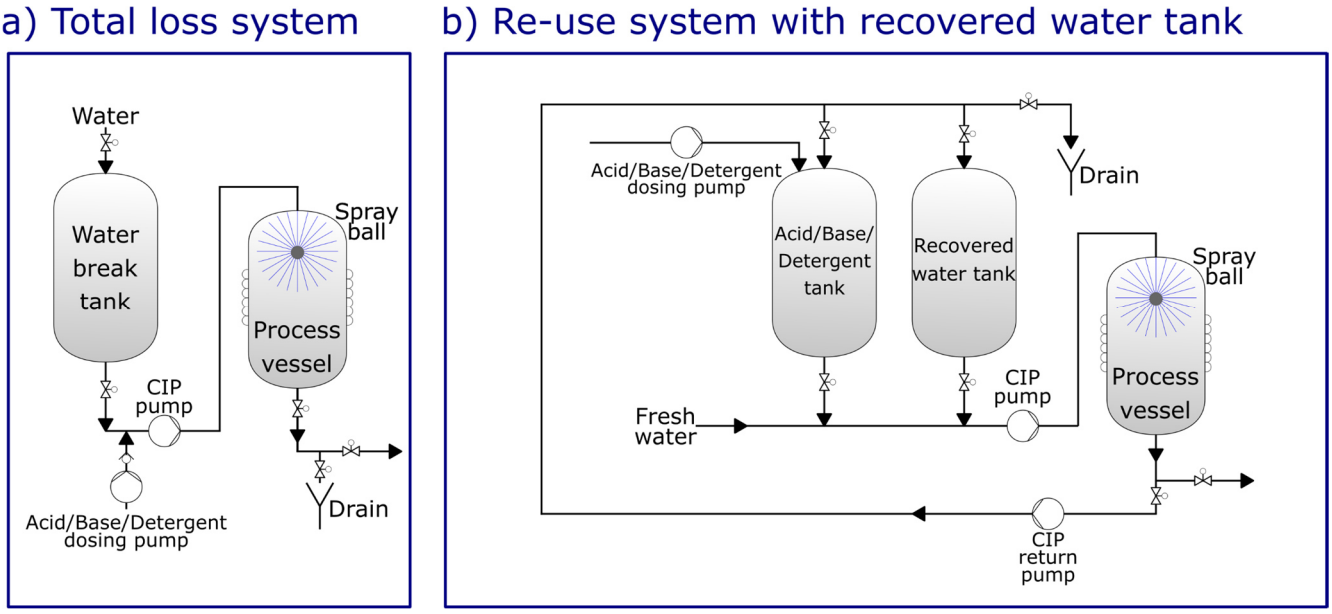


Figure 26 Exemplary basic scheme of a CIP system.
a) The total loss system does not recover the used water.
b) Re-use systems recover the used water to reduce overall water usage
Adapted from the presentation "Design for CIP" by Nicholas Jeffrey and Elliot Sutton (suncombe Ltd's)

The effectiveness of mechanical/chemical cleaning depends on the following factors: time, temperature, concentration and physical action. Physical action depends on proper design and engineering: the selection and application of the correct sprays, supply and return pumps and the sizing of CIP-supply/CIP-return and product piping to achieve the required flow velocity for cleaning. There is no definite "best way" to handle any particular cleaning program, as the first objective is "do what is necessary to get the equipment clean". Afterwards, further adjustments regarding limitations in temperature, time, or cleaning chemical cost are possible. Four decades of experience have demonstrated that fat-, protein-, and carbohydrate-based soils encountered in pharmaceutical and biotechnological processes can be removed by one or a combination of several of the following treatments:

1. Pre-rinse (water)
2. Alkali clean (2% caustic)
3. Inter-rinse (water)
4. Acid clean (1% phosphoric acid)
5. Final rinse (water)

The final rinse can be collected to be used as the first prewash rinse for the next CIP cycle.

This basic CIP cycle is tailored to the cleaning task of the dedicated process with suiting cleaning agents, temperatures and times. Typical CIP cycles of different sources can be found in the appendix on page267. The water/energy demand of CIP cycles is dependant on the number of spray balls, spray ball type, their flowrate as well as the duration and water temperature of each cycle step. The water quality to be used for CIP operations is regulated in the “Guideline on the quality of water for pharmaceutical use”, published by the European Medicines Agency and depends on the product type:

Cleaning/Rinsing of Equipment, Containers, Closures	**Product type**	**Minimum Acceptable quality of water**
Initial rinse	Intermediates and API	Potable Water
Final rinse	API	Use same quality of water as used in the API manufacture
Initial rinse including CIP* of equipment, containers and closures, if applicable.	Pharmaceutical products – non sterile	Potable Water
Final rinse including CIP* of equipment, containers and closures, if applicable.	Pharmaceutical products – non sterile	Purified Water or use same quality of water as used in manufacture of medicinal product, if higher quality than Purified Water
Initial rinse** including CIP* of equipment, containers and closures, if applicable.	Sterile products	Purified Water
Final rinse***including CIP* of equipment, containers and closures, if applicable.	Sterile non-parenteral products	Purified Water or use same quality of water as used in manufacture of medicinal product, if higher quality than Purified Water
Final rinse***including CIP* of equipment, containers and closures, if applicable.	Sterile parenteral products	WFI ****

* CIP = Clean In Place

** Some containers, e.g. plastic containers for eyedrops may not need an initial rinse, indeed this may be counter-productive since particulates counts could be increased as a result. In some cases e.g. blow-fill-seal processes rinsing cannot be applied.

*** If equipment is dried after rinsing with 70% alcohol, the alcohol should be diluted in water of the same quality as the water used for the final rinse.

**** Where a subsequent depyrogenisation step is employed the use of Highly Purified Water may be acceptable subject to suitable justification and validation data.

Figure 27 Water used for cleaning/rinsing. The minimum acceptable water quality depends on the product type. [71, S. 5]

Steam in place (SIP)

To prevent cross-contamination between batches and products it is important to ensure sterility within areas exposed to biologically active systems. In general, biologically active systems are exposed to equipment during cell cultivation and harvest. CIP operations or chemical sanitization may not be sufficient to prepare equipment for the next batch and to ensure complete sterility. As an additional challenge, equipment is often too large to fit in an autoclave. For this reasons, sanitization via chemical treatment or clean steam is undertaken. For clean steam sterilization, product contacting surfaces are heated up to a temperature of 121°C for 15-30 minutes. [7, S. 963]

To sterilize a vessel, clean steam is pumped directly into the vessel during the “heat-up phase”, depending on the sizing of the vessel. When the desired temperature is reached, it is maintained for up to 30 minutes, before steam is stopped from entering the vessel. This is followed by a “cool-down phase”. Heating and cooling maintenance of a vessel is facilitated by the jacket around the vessel, which can be supplied with technical (or “plant”) steam/hot water as well as chilled water. As with CIP operations, all transfer lines exposed to biologically active material should be sterilized in place. Case vent filters are also sterilized in place together with the unit operation. SIP processes inherently introduce liquid condensate into the system during the cycle. Equipment has to be designed for easy drainability to prevent stagnation of contaminated liquid. The largest amount of condensate is generated at the start of the SIP process because of the high temperature difference between the hot steam and the cold equipment. Steam traps are part of the inherent design of an automated SIP process. Steam traps automatically shut when steam exits the drain and vent valves and indicates that air and condensate have been removed from the system. Steam traps limit the steam flow and allow the system to reach and maintain the desired sterilization temperature. They will open intermittently to evacuate condensate and allow replacement with fresh saturated steam. The entire system has to be designed to be pressurizable with sterile air (or other sterile gases) during the cool-down phase of the SIP cycle to avoid creating a vacuum in the system that would draw in potential contamination sources or damage the equipment. [7, S. 963]

Lastly, with manufacturers reliant on stainless steel vessels, there is always a risk that minute quantities of product will be left behind to contaminate the next batch, regardless of how carefully a vessel is cleaned. The safe limits for residual product carryover become more and more challenging, as drugs become more potent. With the application of single-use technology the potential for cross-contamination is eliminated.[13]

3.3.5 Heating, ventilation and air conditioning (HVAC) demands

The basic requirement of the pharmaceutical industry regarding clean room systems is the manufacturing of drugs, where the possibility of contamination with unwanted substances or germs can safely be excluded. [75, S. 191].The goal of the heating, ventilation and air conditioning (HVAC) systems is to meet specific criteria for particle/microorganism contamination and ultimately guarantee patient safety.

For pharmaceutical cleanrooms air cleanliness is either based on EU GMP guidance (alphabetic notations) or ISO 14644 (numerical notations). [76, S. 189-190]

Table 9 Air change rates according to *GMP-Berater*. [77]

Cleanroom class	Air change rate n for design/concept [n/h]	Air change rate n for operation [n/h]
E,F, CNC	5-12	5-8
D (ISO 9)	10-15	8
C (ISO 8)	12-20	8-15
B (ISO 7)	20-40	15-30

To determine the carbon footprint of HVAC systems two different sources of energy consumption have to be quantified:

- Adjustment of air temperature, humidity and pressure to meet target air properties (human comfort zone; T=20°c, ϕ=0.6)
- Generation of the necessary volumetric air flux by fans to meet the cleanroom call specific air change rate

The area and ceiling height of a clean room determine the volume. Together with the cleanroom class, specific air change rate the volumetric flux of air is set. The volumetric air flux requirement for each room determines the fan dimensioning with according

electricity consumption. The volumetric air flux together with properties (temperature, humidity, pressure) from the air that is entering the HVAC system, dictate the energy consumption for air conditioning. The larger the volumetric flux that has to be adjusted to meet desired air properties, the larger the total energy consumption. Basic HVAC system types include:

- Once-thru: air is conditioned, enters the clean room and is discarded (see left hand side of Figure 28)
- Recirculated: air is conditioned, enters the space and a portion is reconditioned while another is discarded (see left hand side of Figure 28)

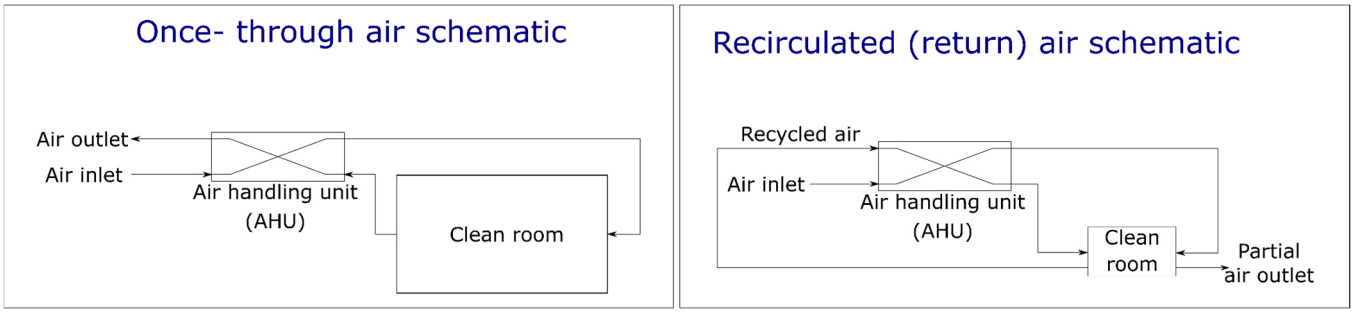


Figure 28 Schematic of a once-thru and a recirculated HVAC system. Adapted from [78]

The recirculation system is more efficient since the recycling of used air reduces the demand of fresh air form the outside. Central HVAC systems have combined devices in an air handling unit which contain supply air fan, humidifier, reheat coil, cooling coil, preheat coil and filter (Figure 29). For increased energy efficiency the preheat coil can be supported by a heat exchanger to heat/cool air that enters from the outside with the air that exits the clean room.

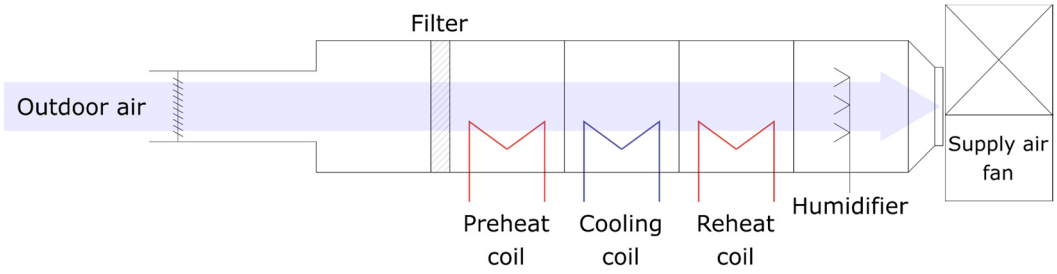


Figure 29 Arrangement for central HVAC systems. Adapted from: [79]

The footprint of clean rooms determines the dimensioning of the HVAC system with according energy demand for operation. This is particularly important for the comparison of conventional stainless steel facilities with the increasing number of hybrid or single-use facilities. The area that has to be supplied by the HVAC system is directly proportional to the amount of emitted greenhouse gases of the HVAC system. Several sources (Leveen et al. [65],Cochet et al. [10]) assign smaller area demand to single-use facilities.

3.4 Economical impact factors of biopharma plants

Plant operating times contributes to the overall carbon footprint. Some carbon emissions only occur once during the factory lifetime (e.g. emissions that stem from transport of steel tanks to the facility), making the total time of operation relevant to understand carbon emissions over a period of time

The continuing growth of single-use technology is a result of many different factors such as lower CAPEX, shorter time to market and lower space demand. The decrepit need of cleaning validation and the therefore resulting lack of WFI production. Another major aspect is the changing market because of patent coverage, the upcoming of biosimilars, the shift to target smaller patient groups and the “in country – for country” approach of countries like Russia or China. All of this affects the economics and therefore the overall production facility lifetime.

The biotechnology industry is continuing its growth with therapeutic monoclonal antibodies (mAbs) as an emerging sector. The global market evaluation is reaching US$ 130-200 billion [19] or US$727.1 [80] by the year 2025. Currently the world`s production is predominated by the United States of America (56 %) and Europe (36 %), with Asia currently manufacturing only 6 %. By 2021 the Asia-Pacific region is expected to be the third largest in sales and growth of mAbs following North America and Europe [19].

Patent expiry and the resulting rise in so-called generics resulted in a loss of 80-90 % in sales for the small-molecules drugs market in the first year off patent. This trend will likely continue in the biopharmaceutical industry as several best-selling drugs as well as many antibodies are coming off patent within the next years. According to an Allied Market Research report, biosimilars reached a market value of US$ 1.3 billion in 2013 and is forecasted to reach 35 billion by 2020 [81]. Patents expire on seven major biologics before 2020 and the European Medicine Agency (EMA) has approved so-called biosimilars or bio-betters of several products. [82, S. 3-4]

To fully understand all aspects that affect the typical biopharmaceutical product the drug life cycle has to be examined. The pharmaceutical market is based on the same

principles as other markets but some special features apply that shape the certain phases in the life-cycle curve (see Figure 30) of the drug.

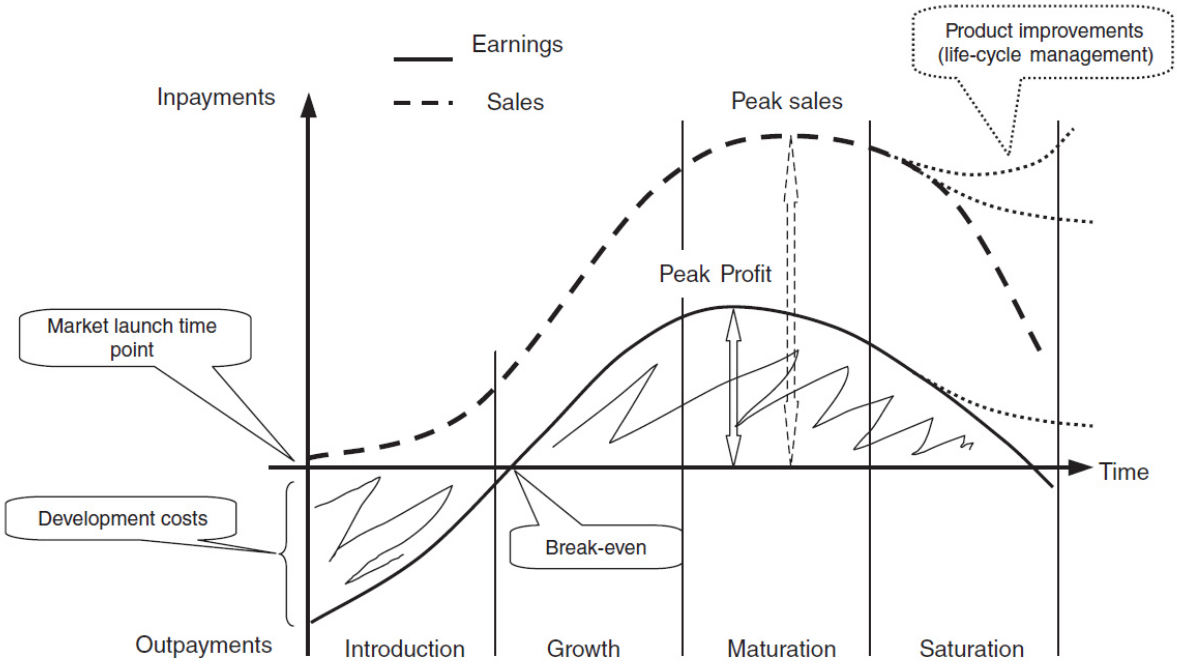


Figure 10.1 Life-cycle curve of a drug. Dashed line = sales; solid line = earnings (sales – efforts); and hatched area = cumulative profit.

Figure 30 Life-cycle curve of a drug. The solid black line is the cumulated balance between in- and out payments while the dashed grey line symbolizes the sales development. For sales, the slight gradient during the introduction phase is followed by a steep slope in the growth phase. Peak profits occur in the maturation phase where the product is well established and serves a large market. In the saturation phase the market is already saturated and competitive products let the sales volume decrease.[83, S. 324]

The start of the saturation phase is determined by the patent protection of the product. For patents there are generally two categories: process patterns and product patents. While process patterns do not protect the produced substance but only its manufacture or application, product patents protect the object itself. After the patent expires, generic drugs usually enter the market due to limited research and development effort and with a less expensive price points compared to the original preparations. This scheme might not be universally true for biosimilars since this is currently subject to intensive discussion whether complex biological generics can be brought to market without clinical trials.

During the saturation phase, the profit curve can be prolonged by life-cycle management activities that keep the product attractive. Typical life cycle management measures increase the comfort of physicians of patients, for example [83, S. 327]:

- Simpler route of administration
- Longer administration intervals
- Improved storage conditions
- Other dosages that justify price adjustment

The usual patent lasts for 20 years with a supplementary protection certificate (SPC) extending the period to a maximum of 25 years for products that require particularly intensive efforts for research and bear a high-cost risk. Normally the patent application is filed in the early research phase, leaving only 10-15 remaining years to obtain a return of the initial investment. Figure 31 provides a graphical overview of these timeframes.

Time to market is of key importance in drug development since positive payments that compensate for the spending on development only starts with market launch. A short time to market also offers a potential competitive advantage if the own product is available bevor the product of potential competitors. [83, S. 326]

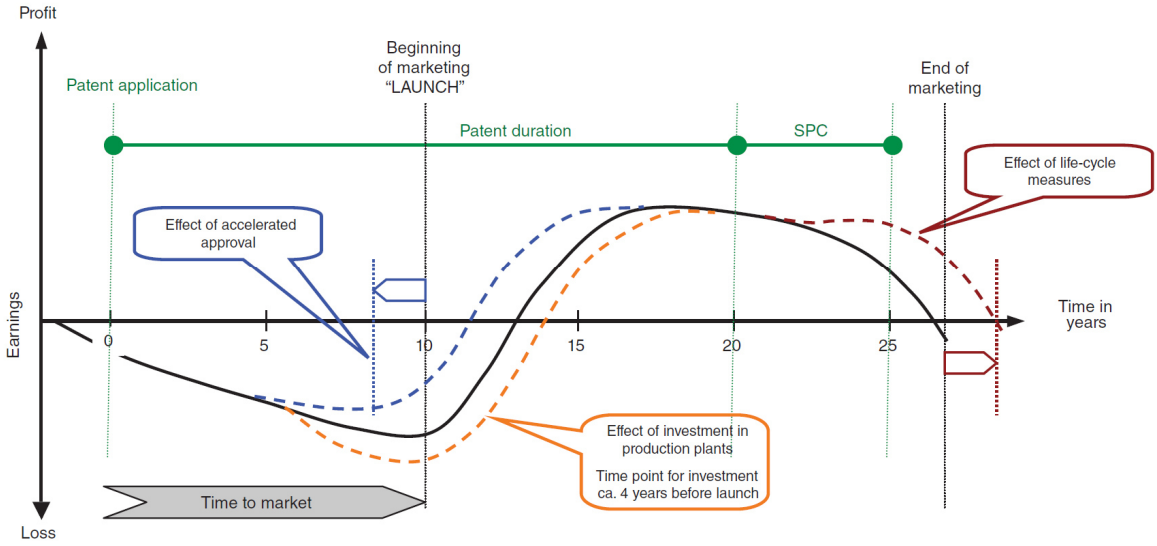


Figure 31 Profit/loss situation of a drug during development and commercialisation. Time to market is crucial to ensure a competitive advantage. The patent runtime of 20 years already starts to decrease from the point of patent application. From the point that the product is available on the market ("Launch"), there are an average of 10 or 15 years in case of a supplementary protection certificate (SPC) left.[81, S. 326]

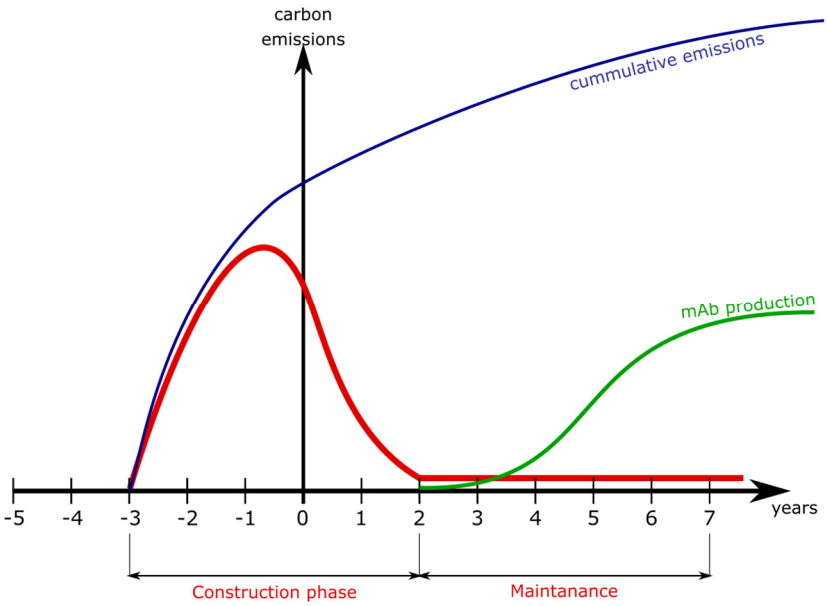


Figure 32 Exemplary time dependent carbon emissions. The red graph shows the emissions due to the construction of the facility and the maintenance activities that are necessary to allow continuous operation. The green curve shows the carbon emission because of beginning mAb production. The blue curve shows the cumulative carbon emissions.

One-time carbon emissions like facility construction or manufacturing of steel tanks and their transport to the construction site have a large impact if a factory only operates for a short duration. The importance of emissions from production activities and maintenance increase with advancing facility lifetime.

The adoption of single-use technology can be linked to economic aspects as a result of the current market situation combined with increased competition as well as many inherent advantages of SUT. Together all factors further increase the usage of disposable items and therefore shape the carbon footprint of products that are produced with the help of single-use technology.

4 Process & Assumptions

The goal of this thesis is to compare a conventional SST build facility to a facility that is based on SUT in regards to the carbon footprint. A well-founded comparison is based on a system of rules that allows unbiased comparison between both facility types. A multitude of parameters influences carbon emissions and a holistic approach is needed to cover all aspects that influence the total carbon footprint. Available information have to be supplemented by neutral assumptions when there is a lack of data. Assumptions are necessary to obtain a more detailed overview without sacrificing objectivity.

The method of choice sets variables for both facility types to observe the systems behaviour in respect to the resulting carbon footprint of the different building blocks of a mAb production plant. Both technologies have weaknesses and strengths that have to be highlighted by input variable selection. One way is to benchmark each facility type by keeping the total processing time for one batch constant. This ensures that both facilities meet the degradation time restrictions for the processing of a defined quantity of monoclonal antibodies by different unit operations.

Standardization for numerical results allows the direct comparison between facilities of various scales. The two major components that make up the total carbon footprint are water consumption in m^3 and energy consumption in kWh. Emission factors (e.g. t_{CO2}/kWh; t_{CO2}/m^3) are used, to obtain the output in carbon equivalents. To allow immediate comparison, carbon emissions are standardized to the mass of produced antibodies (t_{CO2e}/t_{mAbs}).

Numerical results are based on the following input variables and can be found in the appendix on page 168 as copies of the input mask of the developed EXCEL/VBA tool. Since the dimensioning of the facility is based on the production bioreactor size, following passages refer to scale in the sense of total bioreactor capacity with according dimensioning of all required unit operations. A scale of 2 m^3 refers to a total bioreactor capacity with accordingly dimensioned process, for instance.

Table 10 System input parameters

SST		SUT	
Process variables			
Volume of bioreactor [L]			
Product titer [g/L]			
Factory runtime [years]			
WFI generation			
Efficiency factor [%]			
T_{start} [°C]			
T_{end}[°C]			
Emission factors			
Electricity [kgCO2/kWh]			
SST production [t_{CO2}/t_{SST}]			
HVAC [kg_{CO2}/kWh]			
Commuting			
Number of workers			
Commuting distance (roundtrip) [km]			
CIP process			
CIP Model			
SIP process			
Insulation layer thickness [mm]			
Steel tank variables		**Bag support structure transport (SUM;SUB)**	
Transport distance [km]		Distance (one way)	
Transport method		Transport method	
Working volume increase	[%]		
Round up to	[L]		

4.1 Process overview

There are several ways to design or operate a GMP-compliant mAb production facility. [68, S. 553]. The production process of monoclonal antibodies on an industrial scale can acceptably prepared using a variety of technologies and at a range of different scales. Depending on process scale, investment and (development-) time limitations, the selection of unit operations and their arrangement in the process train is to a certain degree variable. Core elements like protein A affinity chromatography have their specific place in the process train but several unit operations and their layout of design are free to a certain degree. Examples are the number of chromatography polishing steps, chromatography media selection (e.g. HIC, AEX, IEX) or the choice if centrifugation or depth filtration is used prior to protein A affinity chromatography. A basic scheme is presented in *3.2 Production of monoclonal antibodies (mAb)* on page 24.

The process design with according unit operations that is used in this work is presented in the appendix for the SUT (*10.19 Downstream process flow chart for the SUT facility*) and SST (*10.20 Downstream process flow chart for the SST facility*) process.

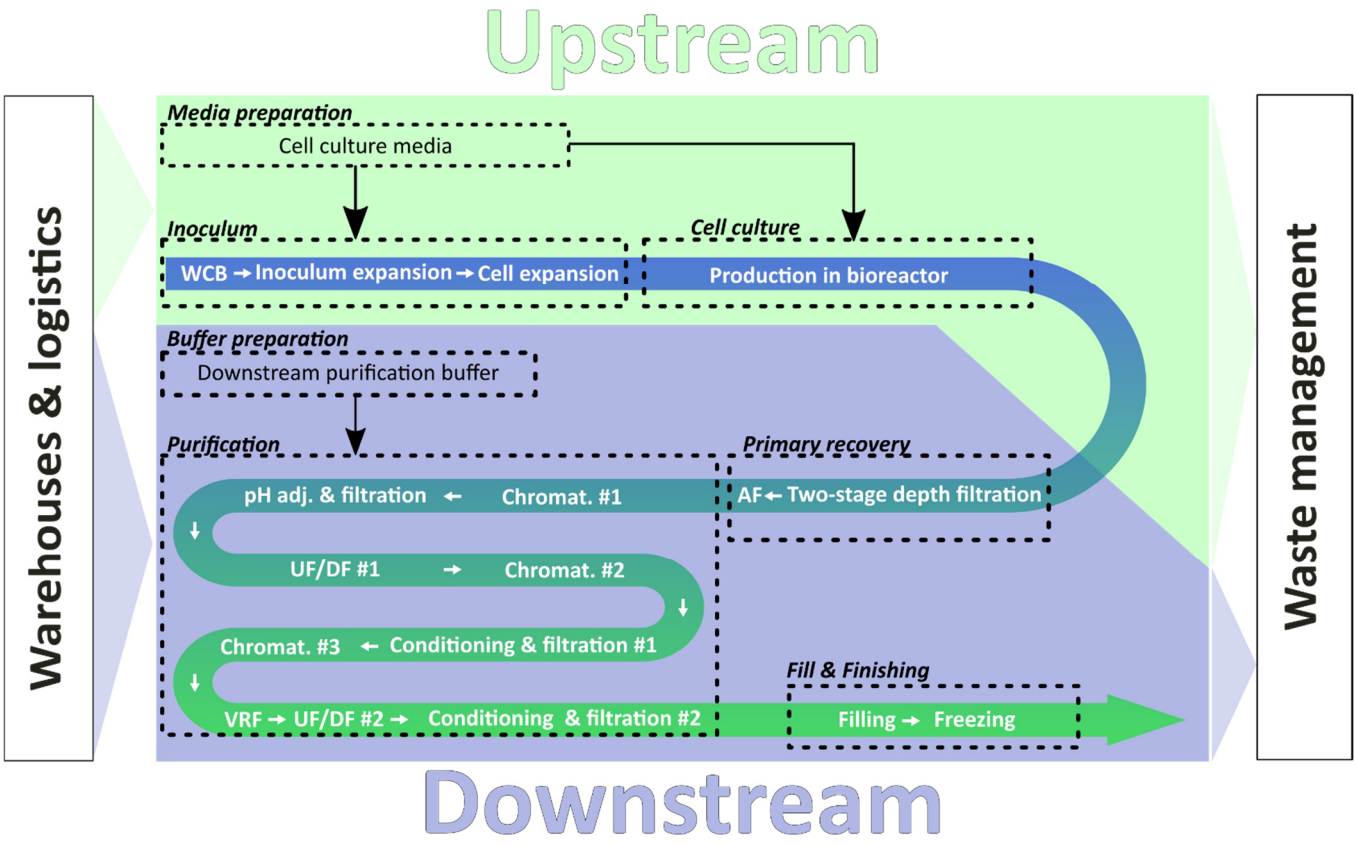


Figure 33 Consecutive production process for monoclonal antibodies

Warehouses & logistics supply upstream (mint green) and downstream (purple) operations. The inoculum stage starts with a vial thaw form the working cell bank (WCB) and includes early cell expansion. The cell culture stage involves production in a dedicated bioreactor. Primary recovery involves a two-stage depth filtration, followed by an absolute filtration (AF) step to remove cells and cell debris. Purification starts with protein A affinity chromatography(Chromat. #1), followed by a virus reduction step by pH shift (pH adj. & filtration). Ultrafiltration/diafiltration is used to further concentrate the retentate and to perform a buffer change. The first polishing chromatography step (Chromat #2) is ion exchange chromatography (IEX). Conditioning and filtration #1 is followed by Chromatography #3 (anion exchange chromatography). The second virus removal step (VRF) is performed by single-use filters. UF/DF #2 is performed achieve further concentration prior to the final conditioning & filtration #2 step. Fill & Finishing involves the filling of the monoclonal antibody prior to freezing for shipping or long-term storage.

The more detailed flow diagrams for the SST and SUT process are attached on page 273 and page 272 respectively.

4.2 Building blocks

For this thesis, the production process for monoclonal antibodies is divided into five building blocks with their according sub building blocks (Figure 34).

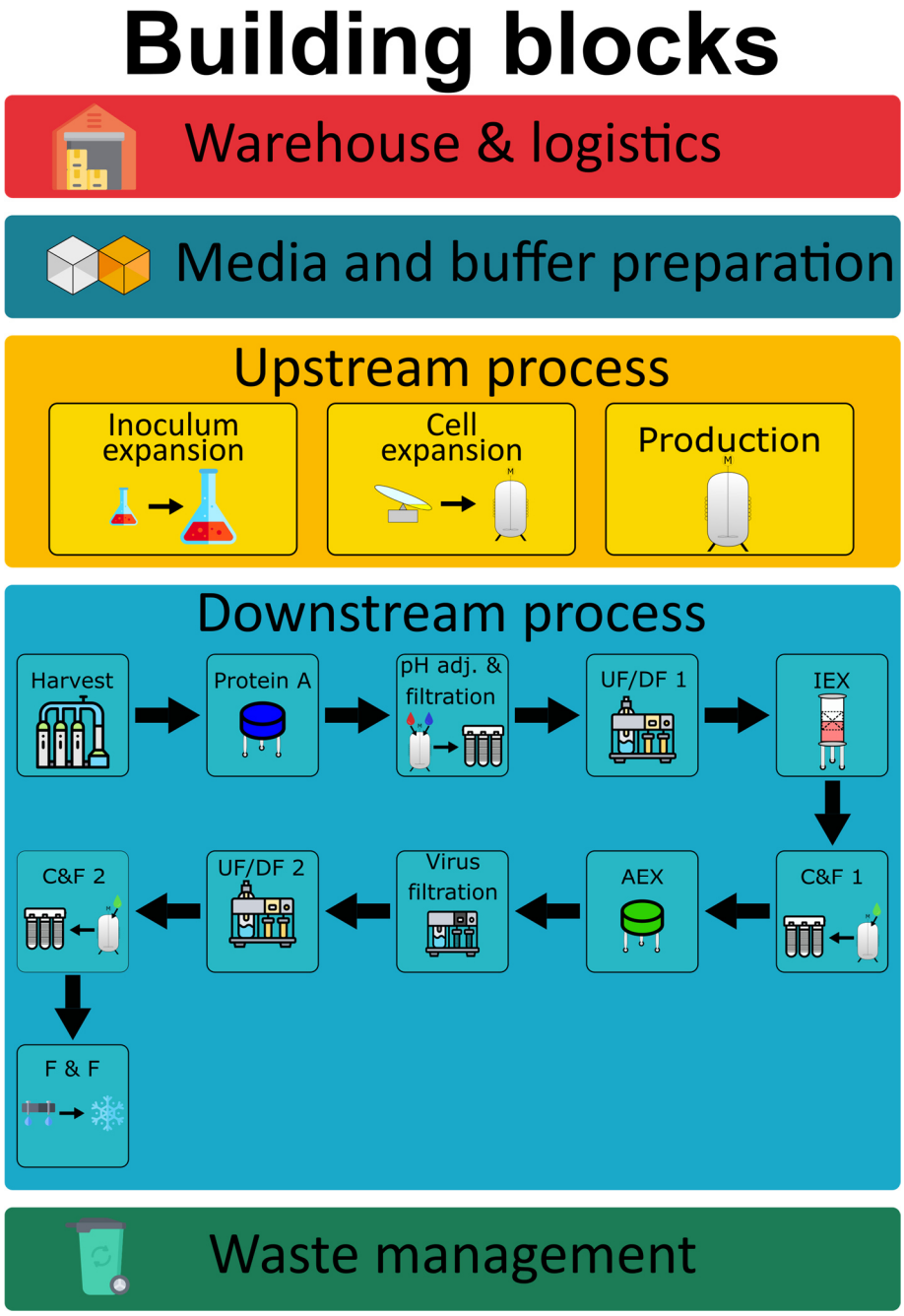


Figure 34 Arrangement of the five building blocks of monoclonal antibody production for this Master's thesis. Each building block serves a specific purpose and directly influences all other building blocks either directly or indirectly. There are distinct differences for single-use facilities and conventional stainless steel facilities, resulting in individual waste streams.

Each building block has to fulfil certain requirements and is linked with all other building blocks. The five building blocks (in no particular order) are:

- Warehouse & logistics: this building block manages the supply of all necessary charge materials. For the single-use facility, this building block has to handle the entire buffer demand that is produced externally is delivered by road via trucks or rail via trains.
- Media and Buffer preparation: this building block handles the buffer supply. For the single-use facility, all necessary buffers are produced externally and shipped to the site. The conventional stainless steel facility relies on an on-site production of all buffers.
- Upstream process: this building block covers all processing steps before the production in the large scale bioreactor can begin.
    - The sub block “Inoculum expansion” starts with a vial thaw form the working cell bank and covers early cell expansions steps.
    - Cell expansion is necessary before entering the bioreactor production stage and is commonly performed in 1:2 – 1:10 inoculation ratios for cell cultures. [84, S. 25]
    - Production: this building block manages the production of mAbs in bioreactors. For the conventional stainless steel facilities, the bioreactors are made from type 316 stainless steel, while the single-use facility uses SUBs.
- Downstream process: The downstream process is separated in eleven consecutive processing steps:

    - Harvest: this building block includes the harvest and its main purpose is to separate cells as well as other larger impurities to prepare the monoclonal antibodies for further downstream processing. This can be achieved by centrifugation or depth filtration as well as micro filtration.
    - Protein A: this building block is a capture step via protein A affinity chromatography.
    - pH adjustment and filtration. This building block is part of the orthogonal approach for virus filtration and aims to further purify the monoclonal antibodies via filtration
    - Ultrafiltration/diafiltration 1: this building block aims to perform a buffer chance
    - Ion exchange chromatography: this building block involves an ion exchange chromatography capture step
    - Conditioning and filtration 1: this building block involves buffer addition as well as filtration for further polishing of the product
    - Anion exchange chromatography: this building block involves an anion exchange chromatography capture step
    - Virus filtration: this building block is an essential step for the manufacturing of biopharmaceuticals and is crucial for patient safety. Viruses are removed via a dead end filtration step.
    - Ultrafiltration/diafiltration 2 this building block involves a buffer chance as well as a volume reduction before entering the next building block
    - Conditioning and filtration 2: this building block involves buffer addition as well as filtration for further polishing of the product

    - Fill and finish: this building block covers the filling, packaging and storage of the bulk drug substance
- Waste management: this building block manages the resulting waste streams of all other building blocks. Single-use facilities produce large quantities of heterogeneous plastic waste that has to be decontaminated via autoclaves before incineration. Conventional stainless steel facilities produce large wastewater as a result of the necessary CIP/SIP operations.

4.2.1 Upstream process

The upstream process is scaled according to the production bioreactor volume.

Inoculum expansion

The upstream process starts with thaw from WCB. 100 mL inoculum form the WCB and 0.3 L medium A are filled in to each Erlenmeyer flask. Incubation takes place at 37°C for 290 h. 100 mL from each Erlenmeyer flask is transferred into a shaking flask. 0.3 L medium A is added and the flasks are incubated for 290 h at 37°C. At this point the SST and the SUT process separate. The SUT inoculum expansion is a N-3 step process, meaning there are three cell expansion steps prior to entering the production bioreactor. The SST process train is a N-4 (four steps prior to entering the production bioreactor) step process that is carried out in stirred bioreactors to allow an incremental scale up (2000 L, 6000 L, 12000 L, 18000 L bioreactor volume). Total processing time is 484 h. A process schematic is presented in Figure 35.

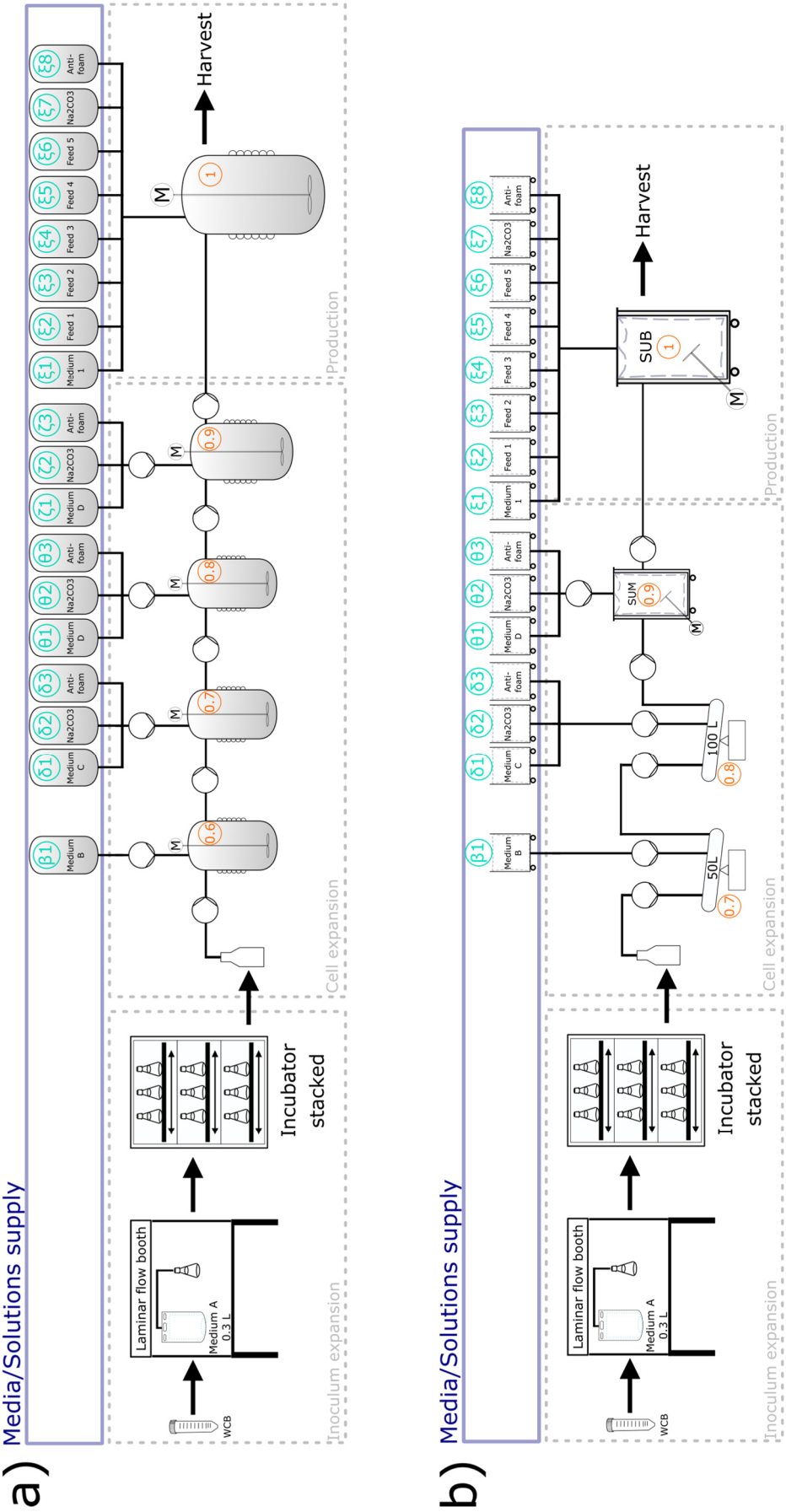


Figure 35 Upstream process for a) SST and b) SUT

Cell expansion

- SUT: the single use process train starts with inoculum expansion in a 50 L WAVE bioreactor. This step takes 96 h at 37°C and requires 21.4 L of medium B. 25 L are transferred to a 100 L WAVE bioreactor where 69.9 L medium C, 5 L Na_2CO_3 and 0.1 L anti-foam are added. Processing takes 96 h at 37°C. The last step before entering the production bioreactor is a 500 L single use bioreactor. 100 L form the WAVE bioreactor are filled to a volume of 500 L with 374.5 L medium, 5 L Na2CO3 and 0.1 L anti-foam. The total processing time is 294 h.
- SST: the SST process consists of four stirred SST bioreactors. The SST process uses the same media and buffer as well as Na_2CO_3 and anti-foam. Temperature conditions are the same and remain at 37°C for all steps during cell expansion. Due to the fact, that there are four stages before entering the production bioreactor an additional 96 h is needed. The total processing time for cell expansion is 394 h.

Calculations regarding the dimensioning of the inoculum and cell expansion are attached in the appendix:

- *10.1.1 SUT seed train calculations on* page 169
- *10.1.3 SST seed train calculations* on page 174

Production

The SUT/SST facility both used the same buffers. The limitation for buffer storage in single-use bags (1000 L) does require to supply production with multiple bags to meet the desired volume. The production takes 288 h at 37°C, followed by the harvest building block, which marks the beginning of the downstream processing.

4.2.2 Downstream processing

Downstream processing consists of elven generic but representative processing steps. So called “platform processes” allow for a multi-product production due to the similar properties of different monoclonal antibodies. The presented quantities are exclusive to the SUT process. The calculations that deliver the required numbers for the SST facility are presented in the appendix 10.2 Downstream process – preliminary calculations on page 179.

- Depth filtration

  The process consists of a three-stage filtration process. The first two steps use the same filters while the last step is a 0.2 µm absolute filtration. Total processing time for this building block is 8 h including an estimated preparation time of 2 h and a dismantling time of 2 h.

  - First stage depth filtration consists of 6 filter racks that hold 6 filters each, resulting in a total number of 36 filters that can process 500 L/h.
  - Second stage depth filtration consists of 3 filter racks that hold 6 filters each, resulting in a total number of 21 filters that can process 500 L/h.
  - 0.2 um absolute filtration requires 4 filters that can process 500 L/h.
  - The filtrate is pooled and cooled down from 37-15°C. in a 3000 L SUM. The process takes a total of 8 h with 2 h of estimated preparation time and 2 h of estimated dismantling time.

- Conditioning and filtration 1 (Virus reduction – Option 1)

  - For virus inactivation, 23.3 mL/L of detergent are added to a 2000 L pool vessel bag. The process takes 6 h total with an estimated 1 h preparation time and 1 h dismantling time at 15°C.
  - The two stage filtration step included a 0.8/0.45 um and a 0.45/0.2 um absolute filtration. Each stage requires one filter and with a flow rate of 500 L/h the total processing time including a 2 h preparation and a 2 h dismantling time comes to 8.1 h total.

- Protein A affinity chromatography

  The protein A chromatography system is not a single-use item due to immense cost of the column. The system has the following specifications:

  - Column volume (CV): 20.2 L
  - Column cross-sectional area: 10212 cm^2
  - Number of cycles: 16
  - Process steps:

- Rinse with 6 CV buffer A at 400 cm/h →121 L buffer A total
- Sanitization with 3 CV buffer B at 400 cm/h →61 L buffer B total
- Equilibration with 5 CV buffer B at 400 cm/h →1616 buffer B total
- Load with product at 400 cm/h → 2053 L total
- Elution with 6 CV buffer D at 400 cm/h → 1939 L buffer D total
- Post elution wash with 2 CV buffer E at 400 cm/h → 646 L buffer E total
- Sanitization with 3 CV buffer B at 400 cm/h →979 L buffer B total
- Regeneration with 5 CV buffer A at 400 cm/h → 1616 L buffer A
- Regeneration with 0.03 CV buffer F at 400 cm/h → 9.7 L buffer F total
- Rinse with 0.03 CV WFI for storage at 400 cm/h → 0.6 L WFI total
- Rinse with 3 CV Buffer G for storage at 400 cm/h → 61 L buffer G total

The total processing time is 36.86 h with an estimated 4 h of preparation time and 4 h of dismantling time.

- pH adjustment and filtration (Virus reduction – Option 2)

  This process step involves pH adjustment in a SUM and is followed by a two stage filtration step.

  - pH adjustment is done by adding >100 L buffer H. The process takes 5 h total with an estimated 2 h preparation and a 2 h dismantling time.
  - The two stage filtration step included a 0.8/0.45 µm and a 0.45/0.2 µm absolute filtration. Each stage requires one filter and with a flow rate of 500 L/h the total processing time including a 2 h preparation and a 2 h dismantling time comes to 5.9 h total.

- Ultrafiltration/diafiltration 1

  This step is performing a buffer exchange. The UF/DF skid has the following specifications:

  - Total membrane area 10 m^2
  - Initial volume: 940 L
  - Concentrated volume 277 L
  - The process involves the following steps:

    - Rinse with 30 L/m2 WFI → 300 L WFI total
    - Sanitization with 10 L/m2 0.5 M NaOH → 100 L NaOH total
    - Rinse with 63 L/m2 WFI -> 630 L WFI total
    - Equilibration with 50 L/m2 of Buffer J → 500 L buffer J total

- Concentrate product to 40 g/L→ 940 L product get concentrated to 277 L
- Diafiltration with one DV Buffer J → 1660 L buffer J total
- Recovery with one DV of Buffer J → 277 L buffer J total
- Flush to 35 g/L with buffer J → 24 L buffer J total

The total processing time is 13.8 h with an estimated 2 h of preparation and a 2 h dismantling time

- Filtration is a one-step absolute filtration with a 0.8/0.45 um filter. With one filter capsule the process takes 7 h total with an estimated 2 h of preparation and 2 h of dismantling time.

- <u>IEX chromatography</u>

  The single use IEX chromatography system has the following specifications:

  - Column volume: 1.2 L
  - Number of cycles: 18
  - Process steps:
    - Rinse with 20 CV buffer J → 24 L buffer J total
    - Sanitization with 20 CV buffer K→ 24 L buffer K total
    - Rinse with 20 CV buffer L → 24 L buffer L total
    - Equilibration with 10 CV buffer L → 216 L buffer J total
    - Wash with 20 CV buffer J →432 buffer J total
    - Load product → 298 L of product
    - Post load wash with 20 CV buffer J -> 432 L buffer J total
    - Elution with 20 CV buffer M → 432 L buffer N total
    - Regeneration with 20 CV buffer N -> 432 L buffer N total

  The overall processing time is 16.5 h with an estimated 4 h of preparation and 4 h dismantling time.

- <u>Conditioning and filtration 1</u>

  - For virus filtration, 149 L of buffer O are added to a 650 L pool vessel bag. The process takes 6 h total with an estimated 4 h preparation time and 0 h dismantling time at 21-25°C.
  - The filtration step includes a 0.8/0.45 µm absolute filtration with one filter capsule at a flow rate of 312 L per hour. Total processing time is 6.5 h with an estimated preparation time of 2 h and dismantling time of 2 h.

- <u>AEX chromatography</u>

  The AEX chromatography system has the following specifications:

- Column volume: 32 L
- Number of cycles: 16
- The processing steps are:
  - Rinse with 6 CV buffer P → 192 buffer P total
  - Sanitization with 3 CV buffer N → 96 L buffer N total
  - Equilibration with 3 CV buffer Q → 1536 buffer Q total
  - Load product → 528 L product
  - Post load wash with 3 CV buffer Q → 1536 L buffer Q total
  - Elution with 6 CV buffer R → 3072 L buffer R total
  - Regeneration with 3 CV WFI → 1024 L WFI total
  - Clean with 3 CV buffer N → 1536 buffer N total
  - Regenerate with 5 CV buffer P → 2560 buffer P total
  - Regenerate with 0.03 CV buffer S -> 15.4 L buffer S total

The total processing time is 25.4 h with an estimated 4 h preparation and 4 h dismantling time.

- Virus filtration

  Virus filtration involves two different virus filters that both operate at 300 L/h at a temperature range of 18-28°C. The processing steps are:

  - Filter wash with 152 L buffer R
  - Load 1229 L of product
  - Post use flush with 32 l buffer R

The total processing time is 8.7 h with an estimated 2 h of preparation and 2 h dismantling time.

- Ultrafiltration/diafiltration 2

  The UF/DF building block includes ultrafiltration/diafiltration followed by absolute filtration.

  - Initial process volume: 1261 L
  - Concentrated process volume (DV): 391 L
  - Processing steps:
    - Rinse with 30 L/m2 WFI → 300 L WFI total
    - Sanitize with 10 L/m2 0.5 M NaOH → 100 L NaOH total
    - Rinse with 63 L/m2 WFI → 630 L total
    - Equilibration with 50 L/m2 buffer R → 500 L buffer R total
    - Diafiltration with 10 DV buffer T → 3914 L buffer T total
    - Flush to 20 g/L with buffer T → 98 L buffer T total

The total processing time is 15 h with an estimated 2 h preparation and 2 h dismantling time

- One 0.8/0.45 µm filter is required to process the volume at 100 L/h. The total processing time is 8.6 h with an estimated 2 h preparation and a 2 dismantling time.

- Fill and finish

  The last building of downstream processing includes a formulation step followed by absolute filtration and a two stage bulk filtration before finally entering the filling station.

  - Formulation takes place in a 650 L pool vessel at 21-25°C. 411 mL buffer U is assed. The total processing time is 6 h with an estimated 2 h of preparation and 0 h dismantling time.
  - One 0.8/0.45 µm filter capsule is required for filtration at 200 L/h. The total processing time is 6.3 h with an estimated preparation time of 2 h and a dismantling time of 2h.
  - Bulk filtration is performed with three 0.5/0.2 µm and five 0.22 µm filter at a flow rate of 100 L/h. The total processing time is 8.5 h with an estimated 2 h preparation and 2 h dismantling time
  - A filling station fills 4 L aliquots at a rate of 100 L/h. The total processing time is 6.5 h with an estimated preparation time of 1 h and a dismantling time of 1 h

The entire downstream process takes 220.56 h or 9.19 days for one batch.

4.2.3 Media and buffer preparation

The production of monoclonal antibodies required a broad range of different buffers as well as acid/base for pH adjustment. Table 11 lists media, buffers, acid and base that are involved in upstream and downstream processing. The identifiers can be found in the process flow scheme in the appendix on page 272 and 273.

Table 11 List of Buffer/media/chemicals/WFI that are necessary for production

Building block/ sub building block		**Labelling**	**Identifier SUT**	**Identifier SST**
Upstream processing	Inoculum expansion	Medium A	(bag in laminar flow booth)	(bag in laminar flow booth)
	Cell expansion	Medium B	β1	β1
		Medium C	σ1	σ1
		Medium D	θ1	θ1,ζ1
		Na_2CO_3	σ2, θ2	σ2, θ2, ζ2
		Anti-foam	σ3, θ3	σ3, θ3, ζ3
	Production	Medium 1	ε1	ε1
		Feed 1	ε2	ε2
		Feed 2	ε3	ε3
		Feed 3	ε4	ε4
		Feed 4	ε5	ε5
		Feed 5	ε 6	ε 6
		Na_2CO_3	ε7	ε7
		Anti-foam	ε8	ε8
Downstream processing	Harvest	Detergent 1	w1	w1
		WFI (SST from loop)	t1	t1
		WFI (SST from loop)	t2	t2
	Protein A	Buffer A	a1	a1
		Buffer B	a2	a2
		Buffer C	a3	a3
		Buffer D	a4	a4
		Buffer E	a5	a5
		Buffer F	q1	q1
		Buffer G	a6	a6
		WFI (SST from loop)	p1	p1
		WFI (SST from loop)	c1	c1
	pH adj. & filtration	Acid	b1	b1
		Base	b2	b2
	UF/DF 1	0.5 M NaOH	d1	d1
		Buffer J	f1	f1
	IEX	Buffer J	g1	g1

		Buffer K	g2	g2
		Buffer L	g3	g3
		Buffer M	g4	g4
		Buffer N	g5	g5
		WFI (SST from loop)	r1	r1
	C&F 1	Buffer O	h1	h1
	AEX	Buffer S	i1	i1
		Buffer P	j1	j1
		Buffer N	j2	j2
		Buffer Q	j3	j3
		Buffer R	h4	h4
		WFI (SST from loop)	s1	s1
	Virus filtration	Buffer R	k1	k1
	UF/DF 2	0.5 M NaOH	l1	l1
		Buffer R	l2	l2
		Buffer T	m1	m1
		WFI (SST from loop)	n1	n1
	C&F 2	Buffer U	o1	o1
	F&F			

The difference between the SUT and SST facility is the location where the necessary buffer/media are produced. For the SST facility buffer/media are produced on site in steel tanks. For the SUT facility, the buffers/media are produced off site and transported to the facility. The transport distance as well as the method of transport (roads via trucks, rail via train) can be selected in the developed ECXEL/VBA tool.

4.2.4 Warehouses and logistics

Single-use facilities have different warehousing requirements than conventional stainless steel build facilities. SUT facilities require enough warehouse space to prevent production losses due to buffer/media shortages. As buffer/media is produced externally, its quality has to be tested on arrival at the production facility. Buffer/media storage bags as well as empty SUBs and SUMs are stored in high rack warehouses and are transported via forklift. Conventional stainless steel facilities rely on onsite production of buffer and are therefore less prone to production losses due to buffer/media scarcity. SST facilities require warehousing area for the storage of filters and spare equipment. The overall labour demand to handle warehousing for a SUT facility is estimated to be higher when compared to a similarly sized SST facility.

4.2.5 Waste management

This building block covers the treatment and disposal of solid waste. Waste categories include filter waste as well as single-use processing bags, SUMs and SUBs. Equipment that has direct contact with product will be autoclaved prior to thermal recycling in SUT facilities. Conventional stainless steel build facilities require a waste handling area with dedicated autoclave to decontaminate filters from depth filtration.

As the number of product contacting equipment per batch is known, an according autoclave with appropriate footprint can be selected to handle the decontamination.

Waste management areas that can store used equipment as well as autoclaves for decontamination have to be considered as they contribute to carbon emissions by HVAC operation.

4.3 Utilities

Production facilities for monoclonal antibodies require different utilities in order to supply different processing steps. Utilities can come in different qualities and their supply is related to carbon emissions. The required utilities for facility operation are:

- Electrical power
- Water for injection
- Water purified by reverse osmosis (RO water)

- Cooling water
- Steam

Electrical power

Electricity is the primary energy source of the SST and SUT facility. Many unit operations that require heating could also be designed to run by gas combustion (e.g. for WFI production via multiple effect distillation or vapour compression). The presented SUT and SST facility rely on electricity as their only source of energy.

For the developed model, electricity is used in various processing steps. This includes the supply of the required energy to heat water for WFI generation to produce buffer/media to supply upstream- and downstream operations. Heating, ventilation and air conditioning (HVAC) require electricity to adjust the temperature and humidity and pressure of air that is entering the facility from the outside. HVAC supply fans consume large quantities of electrical power to provide the necessary air flux that is determined by the cleanroom class. In contrast to other electricity consumers that consume energy periodically, fans draw electricity for 365 days per year running 24 hours a day and 7 days per week. SUT facility require electricity for steam autoclavation of single-use items such as buffer/media storage bags, single-use mixers, single-use bioreactors or depth filtration cassettes. SST facilities require energy for the generation of purified water by reverse osmosis as well as for heating water for CIP and SIP processes. Both facilities require electricity for heating and cooling of process fluids like media or product solution.

Water

Water is the essential for the production of monoclonal antibodies since all buffers and media are produced from WFI. Water is required to clean and sterilize steel tanks via CIP and SIP operations and to supply heating and cooling of process equipment via steam or cooling water. HVAC operations consume water to humidify air. There are five types of water present in the SUT and SST facilities:

1. Water for injections: WFI is used for production of all media/buffer as well as the final rinse during CIP procedure

2. Reverse osmosis water: RO water is used for all rinses prior to the final rinse during CIP operations as well as humidification in case that air is drier than the selected value for HVAC operation. RO water is also used for all SIP procedures.
3. Cooling water: during harvest, the volume leaving the depth filtration skid is pooled and cooled down. The pooling takes place in jacketed vessels with the help of cooling water
4. Heating water: jacketed tanks require warm water to supply the required 37°C for upstream processing.
5. Process waste water treatment by thermal inactivation: this category of water was not included in the scope of this work and is proportional for the SST and SUT process. To calculate the carbon footprint to this water type, the toxicity of different wastewater streams has to be evaluated.

Steam

Steam is generated via electrical steam boilers. The SST and the SUT facility require steam in different areas:

1. SIP procedure: steam has two functions during the SIP procedure as it is used to heat the tank to the desired temperature, fill the tank with steam to contact all surfaces.
2. Autoclaves: the sterilization process requires saturated steam at 132°C (2.5-3 barg) for 21 minutes to achieve effective sterilization.
3. Heating of water: warm water supply is needed to heat up tanks during upstream processing. For this work, electrical water heating is assumed.

Gases

The production of monoclonal antibodies requires different process gases.

1. CO_2: carbon dioxide is necessary during the cell expansion and production phase as it is necessary for hybridoma cell growth. For this work, the consumption of carbon dioxide for cell growth was not counted as a carbon sink and is excluded from the system boundaries.
2. N_2: nitrogen is used to blow out process fluid that would otherwise remain in the depth filters. The emission of nitrogen is not included in the system boundaries of this work.
3. O_2: oxygen is used as an overlay during pooling and cooling after harvest. All carbon emissions associated with oxygen are not included in the system boundaries of this work.
4. Process air: process air is required during the cell expansion and production phase as cells require oxygen for growth and gassing with oxygen is not economical. All carbon emissions associated with process air are not included in the system boundaries of this work.

Solid waste

The waste leaving the SUT facility includes single-use bags (buffer/medium storage bags, SUMs, SUBs) as well as different types of filters. The waste of the SST facility also includes different filter types. Both facilities produce large amounts of wastewater that has to be treated according to its toxicity. This work does not include wastewater treatment in its system boundaries.

The SUT facilities require on site or off site incineration of single-use equipment according to Swiss law. The legislation in other countries may allow landfilling of single-use waste. For this work it is assumed that all SUT facilities incinerate their waste on site. State of the art exhaust treatment is assumed resulting only in carbon instead of additional PM_{10}-, NO_x-, SO_2- and HCl emissions.

4.4 Key Assumptions

Data is the basis for the determination of the carbon footprint of a product. Incomplete data has to be supplemented by assumptions to allows for a more complete assessment. This chapter lists the assumptions for the SUT and SST facilities. The SST facility is based on a SUT process that is already available from data provided by the Chemgineering group. Data from the SUT process serves as a base to dimension the SST process accordingly.

Bioreactor volume

The SUT process has limited scale up potential due to the restriction in SUB capacity which is 2000 L at the moment. To increase production capacity a numbering up approach is necessary. The SST facility allows an incremental scale-up which begins by selecting the desired bioreactor volume (2000, 6000, 12000, 18000 L).

Product titer

The product titer of grams monoclonal antibodies per litre of bioreactor volume is set to 6 g/L for the SUT process. Scenarios of titer change is currently only available for the stainless steel facility. To ensure comparability the product tier remains constant for both facility types.

WFI generation

Both facility types require WFI for buffer production and various unit operations, so naturally the energy consumption of WFI generation has a significant impact on the carbon footprint. WFI is generated with electricity. The calculations for WFI generation are explained in detail in the appendix on page 215.

Emission factors

Electricity emission factors are available for the following locations: Switzerland, Germany, USA, Asia, as average grid mix values. These electricity emission factors are also used for HVAC calculations, but are relabelled to Basel (Switzerland), Boston (USA) and Shanghai (China) to represent the three currently available locations

that include HVAC dimensioning based on local weather trends for the year of 2018. The emission factors for stainless steel production are available for Germany, USA, China, the European Union (including scrap steel) and the European Union (steel from raw material only).

Emission factors for the transport of buffer, steel tanks and bag support structures are available for transport on roads via trucks or on rails via train. All used emission factors are listed in the section *3.1.3 Emission factors (EF) for greenhouse gas inventories*.

Commuting

Commuting is based on the estimated number of workers that commute to the production facility via car. The distance can be set in km for the round trip and incorporates the gasoline consumption of an average car. Detailed calculations regarding commuting can be found in the appendix on page 254. The SST facility requires 150 employees due to automatisation, the SUT facility requires 180 employees due to the lack of automatisation and increased labour requirement for buffer/media handling at a scale of 2000 L.

CIP process

This is only relevant for the SST facility, since SUT facilities do not require the cleaning of tanks due to the disposable nature of single-use equipment. For the SST facility two models are available for selection with detailed explanation in the appendix on page 228, including selected cycles with according temperatures and times.

Bag support structures transport

The transport distance as well as the method of transport (road, train) can be set to account for one-time carbon emissions that accrue during the transport of the support structures for the SUBs and SUMs. The conversion to carbon equivalents is implemented via transport emission factors presented in the section on page 262.

SIP process

SIP is only relevant for SST facilities since SUT equipment is disposable. SST tanks undergo the SIP procedure to ensure sterility. Input parameter is the insulation layer thickness of the tanks that directly influences the required energy demand. Detailed

information regarding the dimensioning of the SIP process are attached in the appendix on page 221.

Buffer transport

Buffer transport is an SUT facility exclusive since SST facilities rely on on-site production of the required buffer. The options include the distance of buffer transport in km and the transport method (rail, road). The conversion to carbon equivalents is implemented via transport emission factors presented on page 262. The calculations only include a one-way trip of the buffer/media to the facility, without accounting for the transport of empty totes back to the buffer production facility.

Steel tank transport

The core of SST facilities are the various tanks that are needed for plant operation. The large mass of steel that is used to manufacture the steel tanks has to be accounted for by transport distance as well as transport method (road via trucks, rail via trains). The conversion to carbon equivalents is implemented via transport emission factors presented in the section on page 262.

4.4.1 System boundaries

This work and the developed EXCEL/VBA tool includes the following aspects of monoclonal antibody production:

CIP

- CO_2 from RO water production
- CO_2 from WFI production
- Energy demand for secondary (for CIP) heating of water
- Total CO_2 output of CIP procedure

SIP

- WFI demand for SIP
- Energy demand to heat WFI to SIP temperature
- Total CO_2 output of SIP operations

SST tanks

- CO_2 from steel production
- CO_2 from transport of steel tanks to facility

HVAC

- Energy demand of HVAC air heating/cooling
- Energy demand of HVAC fan operation
- Energy demand of clean steam for humidification
- Total energy demand of HVAC operations

Single-use bags

- Incineration without energy recovery

General

- CO_2 from commuting workers
- Total buffer consumption
- CO_2 from filter cartridge waste
- CO_2 from steel production
- CO_2 from plastic film production
- Total CO_2 from bag use
- CO_2 of buffer transportation via trucks
- CO_2 of buffer/media transport via trucks
- Water demand for autoclave operation
- Energy demand for autoclave operations
- CO_2 from heating of production tank medium
- CO_2 from cooling after harvest

System boundaries can be extended almost infinitely resulting in a model with increased accuracy but unmanageable complexity. Some likely aspects that are not included in the system boundaries of the presented models are:

- The unclear system boundaries of the used emission factors
- The emission factors for single-use equipment that correctly displays the heterogeneous material mix in single use material
- The energy saving aspects of HVAC systems that recycle air instead of using the "once-through" method
- The energy saving aspects of the numbering up approach in SUT facilities (shared space e.g. warehouses)
- The CO_2 emission due to the radiation procedure of single-use equipment
- The CO_2 emissions as a result of developing a transportation system (production of trains, cars, railroads, etc.)

- The differences in energy consumption due to different pump dimensioning
- A more exact facility layout with dedicated room planning. The estimated facility size is based on dimensions of different unit operations including safety additions
- The limited timeframe of available weather data that reduces the accuracy of the carbon footprint due to HVAC operations
- The facility runtime is based on a 365 days,24/7 scheme that does not account for maintenance time or realistic work schedules
- HVAC demand for a buffer/media production area for the SST facility
- Dimensioning for a dedicated building for incineration of single-use waste

4.4.2 Facility size

Calculations regarding the facility size are based on a model that differs between circular objects (e.g. steel tanks) or rectangular objects (single-use bags). This method allows for a dynamic scaling for different production demands but does not deliver the accuracy of a carefully developed layout. The presented method approximated the required space by adding additional maintenance space to known dimensions of equipment and tanks. A detailed explanation of the calculations is attached in the appendix on page 246.

A March 2010 article by Niels Guldager shows a 25% reduction in space for SUT facilities with their SST build counterparts. [15]

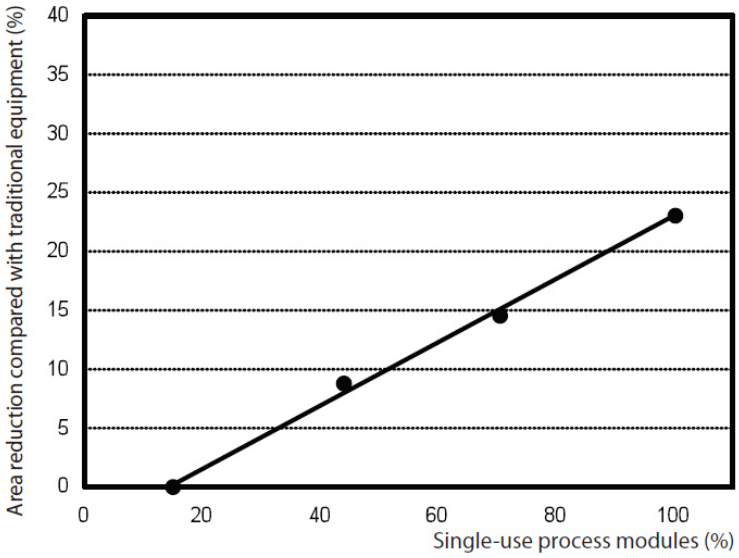


Figure 36 Area reduction as a result of single-use equipment. [15]

4.4.3 Labor

Labor requirement is an important factor due to the reoccurring distance that commuters have to cover. The lack of existing data that allows a direct calculation based on facility size (bioreactor volume) eliminates the usage of accurate numbers. The developed method only allows to estimate the number of workers for each facility. Various references in literature point to a reduced labor requirement of single-use facilities but do not account for the increased demand in labor due do unavailable process automation or the increased warehousing effort. The April 2013 article from

Howard L. Levine et al. suggest a higher FTE requirement for stainless steel build facilities which translates in a higher labor demand. [54, S. 43]

Table 4: Full-time employee (FTE) staffing estimates for a typical MAb facility

Function	Single-Use Facility	Stainless Steel Facility
Drug substance manufacturing	29 FTEs	34 FTEs
Drug product manufacturing	8 FTEs	8 FTEs
QA/QC	37 FTEs	42 FTEs
Engineering/maintenance	11 FTEs	13 FTEs
Purchasing/administration/other	17 FTEs	20 FTEs
Total Staff	**102 FTEs**	**117 FTEs**

Figure 37 Estimated labor requirements for a typical mAb facility (2000 L production scale bioreactor). [54, S. 43]

A March 2018 paper from Dr. Tina Lütke-Eversloh and Peter Rogge lists a higher cost-of-goods regarding labor, suggesting that single-use facilities require a larger amount of labor. [85]

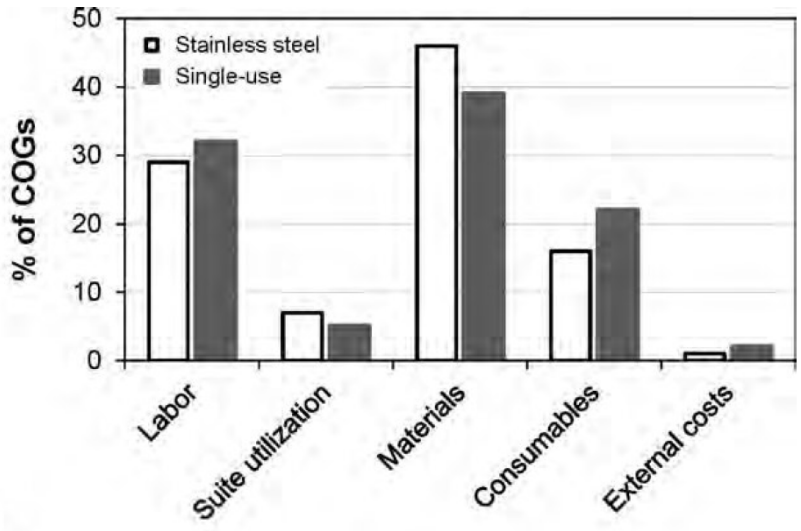


Figure 38 Cost-of-goods analysis for comparison biopharmaceutical manufacturing using stainless steel and single-use equipment. [85]

In the March 2018 article “The Expanding Landscape of Commercial Single-Use Bioreactors” by Feliza Mirasol, a facility with approximately 4500 L reactor volume created 150 jobs. A similar facility in Worcestor with 4500 L bioreactor capacity will provide positions for approximately 150 employees. [86, S. 20-21]

For a hard-piped (2 x 15000 L) vs single-use (10 x 2000 L) facility the labor is estimated by Elan Corp for a Bioprocessing Meeting in 2009. According to the presented table, single-use facilities only require 41% of the SST build facility staff. [87, S. 198]

Parameter	Hard-piped	Single-use
Titer (g/L)	3	3
Mfg capacity (tons/year)	1	1.2
Capital cost (millions of €)	350	145
Capital cost (€/kg)	100	35
Gas supply (%)	100	12
Electricity supply (%)	100	37
Water supply (%)	100	8
Manufascturing area (%)	100	17
Staff (%)	100	41

Facility assumptions: hard-piped with 2 × 15,000-L fermenters, single-use with 10 × 2,000-L fermenters

Figure 39 Assumptions on labor requirements for SUT and SST based facilities. [87, S. 198]

For this work, assumptions are based on an estimated 20% increase in labor demand for SUT facilities. Considerations include the following list:

- Increased warehousing supply chain for single use items
- Increased site logistics including buffer handling and quality control
- Increased waste handling of single-use bags and filter housing from depth filtration

Indications for labor demand in correlation to total bioreactor capacity can be found in the previously mentioned references, to roughly estimate the number of workers required. Planners of monoclonal antibody production plants as well as operators of already operating plants can used the EXCEL/VBA tool to investigate the impact on commuting workers on their carbon footprint.

4.4.4 Process parameters

The input process parameters include the bioreactor volume in litres, the product titer in g/L and factory runtime in years. Resulting process parameters are the amount of produced mAbs in t, the overall time in hours for one batch and the according number of batches per year as well as the factory footprint in square meters.

Input parameter: bioreactor capacity

The production bioreactor capacity for single-use facilities is limited to 2000 L, while SST build facilities can be scaled dynamically. The EXCEL/VBA tool offers 2000, 6000, 12000 and 18000 L bioreactor capacity for the SST based facility.

Input parameter: product titer

The SUT facility has a predetermined titer of 6 g/L that the entire process train is accustomed to. For the SST build facility the product titer can be set dynamically while the resulting changes in processing are also calculated dynamically by the developed EXCEL/VBA tool. With modern cell lines approaching the 10 g/L mark, a product titer of 6 g/L is reasonable and allows for a direct comparison between the SUT and SST facility.

Input parameter: factory runtime

The factory runtime is based on a 365 days per year operation and can be set in years for the SUT/SST facility. When the bioreactor volume and the product titer are set, the total processing time is calculated. This is the base for the calculation of the number of batches per year. The factory runtime is an important factor for the overall carbon footprint since some carbon emission have a one-time character. The delivery of steel tanks or bag support structures to the facility are examples for once in a factory lifetime carbon emissions. The overall impact of these emissions decrease the longer the facility produces product to meet market demands.

Output parameter: number of batches per year

The number of batches per year is the starting point for various calculations. Everything entering and exiting the production facility is determined by the number of batches that can be run per year.

Output parameter: facility size

The facility size is based on a model that respects the dimensions of tanks, single-use bags, single-use mixers, as well as equipment that is necessary for production such as chromatography columns, filtration skids, laminar flow benches or incubators.

4.4.5 Depth filtration

The SST and SUT facility use the same type of depth filtration filter cassettes for downstream calculations. For the calculation of the carbon footprint it is assumed, that the SUT facility emit CO_2 due to the production of the polypropylene filter housing and incineration after use. The cellulose filter media is not respected. On the other hand, the SST facility uses filtration skids made from stainless steel. The skids include filter cartridge holders which exclude the necessity for polypropylene filter housing. The CIP and SIP procedure of the stainless steel depth filtration skid as well as its production is not included in the carbon footprint. This simplification needs to be addressed by approaching the CIP and SIP procedure of the stainless steel depth filtration skid with a reduced complexity, stationary model. The implementation of a centrifugal clarification step for large scale operations can provide a viable solution.

4.4.6 Tank insulation layer for SIP operations

The energy demand of the SIP procedure is dependent on the thickness of the insulation layer and the insulation layer material that is used. For this work, data from existing tank dimensioning war implemented, suggesting a 175 mm rock wool insulation layer.

4.4.6 Transport of steel tanks/bag support structures

The mass of transported steel tanks determines its carbon footprint via emission factor conversion. The tanks do not include any processing equipment like agitators, sensors or other ancillaries and therefore underestimate the carbon footprint by transport. Suppliers of bag support structures state the exact weight including all ancillaries, making the calculations of transport carbon emissions more accurate for the single-use equipment.

5 Methodology

To determine the carbon footprint of a product, it is of key importance to list all aspects that have been respected against aspects that have not been covert to allow an unbiased judgement. Basis of this process is transparency. The three core elements of this work are system boundaries, used data for calculations and the impact of emission factors.

System boundaries

System boundaries are prone to exploitation since they allow two extremes: completely ignoring aspects by placing system boundaries in a fashion that favours exclusion or elevated focus that highlights certain aspects in an unbalanced manner.

Data

To perform all necessary calculations to determine the carbon footprint of monoclonal antibodies, data with sufficient quality have to be gathered. This work is based on existing data for a single-use technology based production facility for monoclonal antibodies using 2000 L SUBs. Not all data were disclosed to not infringe on intellectual property.

Emission factors

Emission factors are the link between mass balance and carbon emissions. The purpose is to convert energy usage (kWh) or mass flux (e.g. m^3_{water}/h) into an absolute mass of carbon (e.g. t_{CO2}) or carbon equivalents (e.g. t_{CO2e}). The selection of emission factors allows for manipulation as emission factors themselves are based on system boundaries. The lack of data and time constraints did not allow to calculate all emission factors for this work. This introduces an uncertainty since the obtained emission factors from external sources has to be trusted if they are used within the work. Emission factors are published by official sources like government agencies or are presented in scientific literature such as papers. The presented methodology and the statement regarding system boundaries has to be studied extensively. All used emission factors for this work come with a reference to investigate methology and used system boundaries (see *3.1.3 Emission factors (EF) for greenhouse gas inventories*).

5.1 Definition of system boundaries

Besides cell culture, operations that take place in a monoclonal antibody production facility do not emit CO_2 directly with the exception of bag incineration if this step is carried out on site. To cover the key aspects of carbon emissions, system boundaries have to include emissions that occur outside the production plant. Energy consumption for factory operation as well as transportation of staff and materials to the production site. Activities that consume electricity are the heating/cooling of water, CIP/SIP operations, HVAC and RO water generation derive carbon emissions form their according electricity emission factors (Table 2). Transport of steel tanks and bag support structures as well as commuting of staff involves the burning of fossil fuels and is included in the system boundaries of this work. Steel production is included in the system boundaries. Figure 40 offers an overview of included and excluded aspects within the system boundaries for this thesis.

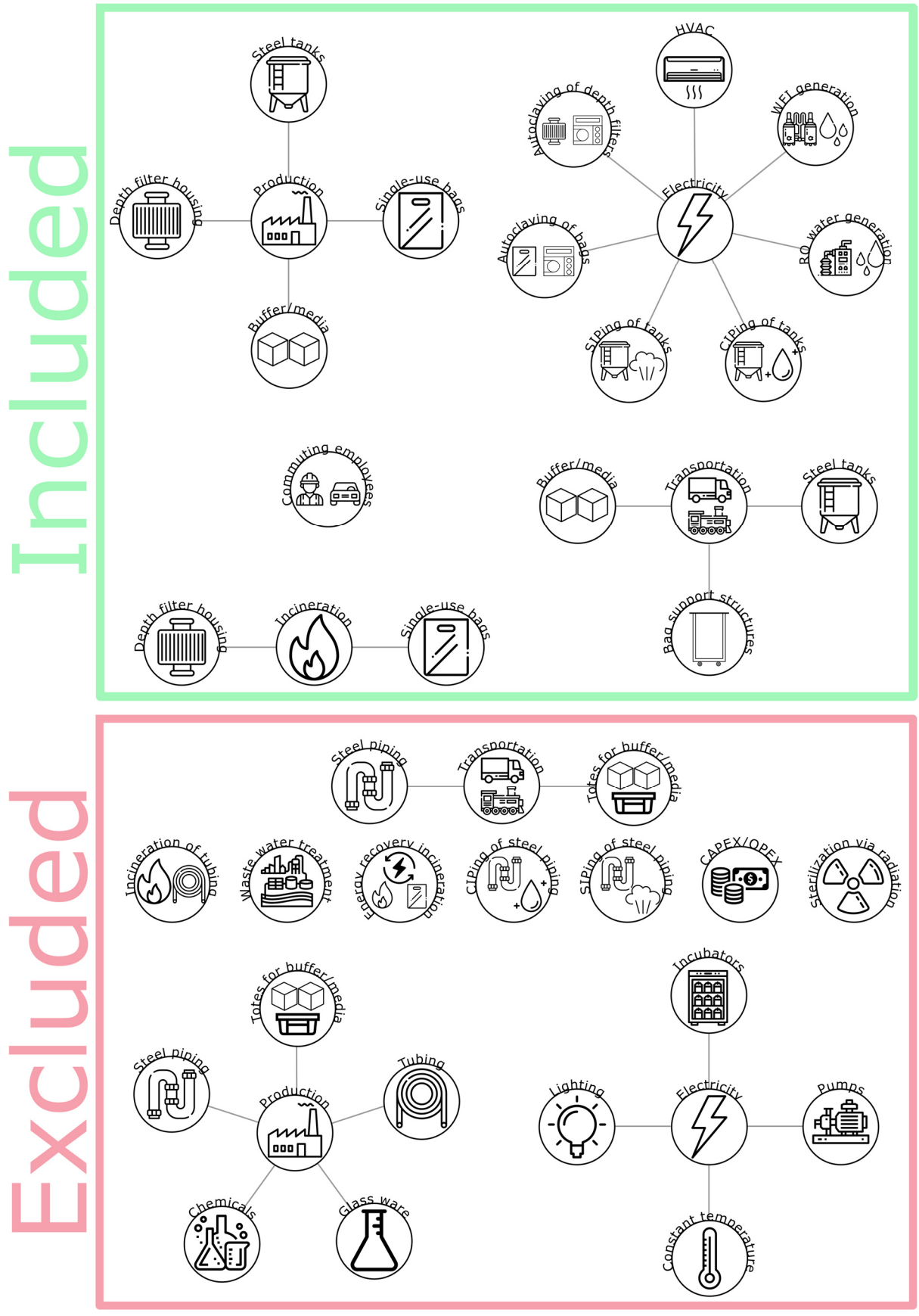


Figure 40 Visual representation of the aspects that are included and excluded from the system boundaries in this thesis.

Table 12 Aspects that ware included in the system boundaries of this work

Category	Sub-category	Comment	Reference
Production	Steel tanks	Emission factors for the conversion of steel mass to carbon emissions are available for different countries	external
	Single-use bags	Emissions for extrusion of plastic film (buffer/media storage, SUBs, SUMs) are calculated with according emission factors	external
	Buffer/media	The production of buffer/media from WFI is included	internal
	Depth filter housing	The production of the plastic depth filter housing is included	internal
Electricity	HVAC	Power consumptions for air conditioning as well as fan operation is included	external
	WFI generation	Generation of WFI using the hot method (e.g. distillation)	external
	RO water generation	Electrical energy required to generate RO water by forcing the feed water through filter membranes by pumps	external
	CIPing of steel tanks	Tank sizing determines the power consumption for CIP	external
	SIPing of steel tanks	Tank sizing determines the power consumption for SIP	external
	Autoclaving of bags	Bag dimensions are used to determine the carbon footprint of the autoclavation process	external
	Autoclaving of depth filters	Filter dimensions are used to determine the carbon footprint of the autoclavation process	external

Transportation	Steel tanks	Emission factors for transport via train/rail are avialable	external
	Bag support structures	Bag support structures are made from stainless steel and hold the single-use bags in place	external
	Buffer/media	The SUT facility obtains buffer/media from a external production facility	external
Incineration	Single-use bags	Due to inherent bag inhomogeneity, an emission factor for incineration of a 100% polyethylene film layer was calculated	internal
	Depth filter housing	An emission factor for incineration of a 100% polypropylene filter housing was claculated	internal
Commuting employees	Commuting employees	Emission factors for different commuter types (e.g. train, car, public transport) are available	External/internal

Table 13 lists all aspects that are not included in the system boundaries of this work. The table includes self-evident considerations but does not reserve the right to completeness.

Table 13 Aspects that are not included in the system boundaries of this work

Category	Sub-category	Comment
Transportation	Steel piping	No steel piping is included in this thesis. Therefore no transportation of steel tubes to the SST facility is required
	Totes for buffer/media	SUT facilities require plastic totes as a support structure for single-use bags to store buffer/media
Incineration of tubing	-	While the SST facility required piping, the SUT facility requires flexible tubing. Tubing is not included in the system boundaries of this study
Waste water treatment	-	Waste water treatment is not included in this thesis
Energy recovery from incineration	-	Energy recovery from incineration of single-use plastic waste is not included in this thesis. The incineration required the use of natural gas.
CIPing of steel piping	-	-
SIPing of steel piping	-	-
CAPEX/OPEX	-	Considerations that involve capital expenditure (CAPEX) or operational expenditure (OPEX) are not included in this thesis
Sterilisation via radiation	-	Single-use equipment is gamma sterilized before usage. The carbon emissions by this process are not included in this thesis
Production	Totes for buffer/media	The carbon emissions from production of plastic totes for buffer/media transport/storage are not included in this thesis
	Tubing	The carbon emissions from flexible tubing for the SUT facility are not included in this thesis
	Glass ware	The carbon emissions from the production of glass ware (Erlenmayer - or shaking flasks) is not included in this thesis
	Chemicals	The carbon emissions from the production/disposal of

		chemicals (e.g. Na_2CO_3 or anti-foam)
	Steel piping	The carbon emissions from production of steel piping for the SST facility is not included in this thesis
Electricity	Incubators	Emissions from incubator power consumption are not included in this thesis
	Pumps	Emissions from pump operation are not included in this thesis
	Constant temperature	The assumption of adiabatic tanks removes this consideration from the system boundaries. Therefore only the cooling after the harvest and the heating of media for production is respected.
	Lighting	Power consumption by lighting is not included in this thesis

6 Calculations

The large amount of different input parameters of the developed EXCEL/VBA tool allow an extensive analysis with according time and effort. The intention of this chapter is to demonstrate the possible calculations of an exemplary comparison between a conventional stainless steel build facility and a single-use facility. As it is not possible to demonstrate all possible scenarios, case studies are presented to explain different system inputs. The case studies are:

- Case study 1 - Basel
- Case study 2 – Boston
- Case study 3 – Shanghai

The four topics shown are partial results covering:

- Water consumption parameters
- CIP+SIP in comparison with bag use
- HVAC parameters
- Commuting employees

Calculations require different input variables that have to be set prior to performing the calculations automatically with the EXCEL/VBA tool. The performed calculations are supported by according preliminary calculation subchapters (10 Appendix) in the appendix.

6.1 System input variables

At first, the system input has to be defined before calculations can be executed.

General

For this case study, the facilities are both located in Basel (Switzerland) and aim to produce monoclonal antibodies over the course of 5 years. For the SST facility, the production bioreactor has a capacity of 2000 L or 18000 L, while the SUT facility has a production bioreactor capacity of 2000 L. The facility footprints are 850 m^2 for the 2000 L SUT facility, 720 m^2 for the 2000 L SST and 1547 m^2 for the SST facilities.

WFI generation

Both facilities require WFI and the efficiency of WFI generation is set to 90% with a starting temperature of 20°C and an end temperature of 105°C (vapour compression method [88]). Calculations are based on the method presented in *10.3 WFI and RO water generation – preliminary calculations*.

Emission factors

The electricity for both facilities is the average grid value for Switzerland (0.0236 kg_{CO2}/kWh). The emission factor for stainless steel production is Germany (1.708 t_{CO2}/t_{SST}). Emission factors are listed in *3.1.3 Emission factors (EF) for greenhouse gas inventories*.

HVAC

The location for HVAC dimensioning is set to perform weather data specific calculations that depend on average daily temperature, average daily relative humidity and average daily pressure. The average daily temperature for Basel, Switzerland in the year 2018 is 13.41°C. The average daily relative humidity is 67.10%. Calculations are based on the method presented in *10.7 HVAC – preliminary calculations*.

Commuting

The SST facility requires 150 employees to commute 50 km per day for 230 days per year by car, while the SUT facility employs 180 workers (20% increase compared with the SST facility) that commute 50 km per day for 230 days per year by car. Calculations are based on the method presented in *10.11 Commuting – preliminary calculations*.

CIP

CIP is only relevant for the SST facility and calculations are based on “Model 2” that is described in *10.4 SIP - preliminary calculations*.

SIP

SIP is only relevant for the SST facility and calculations require the input of the insulation layer thickness, which is set to 1 mm. Calculations are based on the method presented in *10.4 SIP - preliminary calculations.*

Steel tank transport

Steel tanks are transported form the tank production plant to the SST facility via trucks for a distance of 600 km. Calculations are based on the method presented in *10.14 Cargo transport emission – preliminary calculations*.

Buffer transport

Buffers are transported via trucks for a distance of 600 km. Calculations are based on the method presented in *10.14 Cargo transport emission – preliminary calculations*.

6.2 System output

The first step is to calculate the time to process one batch since this allows to calculate the number of possible batches in a defined period of time. The necessary time for one batch starts with the selection of a production tank volume and the product titer. These inputs determine the mass of monoclonal antibodies that has to be processed in one batch. The number of seed train steps as well as dimensioning of all downstream unit operations are governed by the total mass of mAbs. Unit operations like filtration ultrafiltration/infiltration, chromatography steps or cooling procedures are the same for the SST and SUT facility. To achieve the same time per batch as the SUT facility independent of scale, unit operation have to be scaled in a manner that the same processing time as its SUT counterpart is guaranteed. For chromatography columns this can be done by adjusting the column diameter and therefore the volume of the column. For ultrafiltration/diafiltration the number of filter cassettes and therefore the provided filter area is adjusted accordingly. Filtration steps the number of filters is adjusted to achieve the desired processing time. When the time per batch is calculated, the number of possible batches (without taking downtimes due to problems or maintenance into consideration) per year can be determined. With the desired number of operational years of the facility all time dependant parameters can be calculated. The system output is split for the SST facility and the SUT as seen as in *10.22 Exemplary input (case study one)* since some carbon emissions are exclusive for each facility type.

The considerations that go into the output parameter calculation are highlighted in the following section:

CO_2 from commuting workers

The values for the SST facility stem from the different number of workers. The commuting distance and transportation method is the same for both facility types. Calculations are based on the method presented in *10.11 Commuting – preliminary calculations*.

Buffer preparation

The buffer consumption of the SUT facility is already known and serves as a base for the calculations regarding the SST facility. Buffer is consumed by upstream as well as

downstream operations. Calculations start with the volume of the production bioreactor and the product titer. Those two input parameters determine the mass of monoclonal antibodies that is produced and therefore has to be handled by the unit operations of the downstream process. The bioreactor volume serves as starting point for the dimensioning of the upstream process. The number of necessary inoculation steps is proportional to buffer/media consumption. The calculations to obtain the buffer amounts of each unit operation is described in *10.1 Upstream process* – preliminary calculations and in *10.2 Downstream process – preliminary calculations*

When the total amount of needed buffer is known, the carbon emissions can be calculated by the emission factor that was derived in *10.3 WFI and RO water generation – preliminary calculations.*

CIP: CO_2 from RO water production

CIP cycles use RO water for all rinsing steps except the final rinse that uses WFI. The amount of needed RO water depends on tank size, the flow rate of the selected spray ball model and the duration of each CIP cycle step. When the total amount of RO water is determined, the energy demand in kWh for generation of the RO water can be calculated by dimensioning the pump that forces the feed water through the reverse osmosis filters. Generation of RO water is generally dependant on feed water solutes concentration. RO water energy consumption based on tap water solutes concentrations in Basel is presented in *10.3 WFI and RO water generation – preliminary calculations*.

Energy demand for secondary (for CIP) heating of water

The energy demand depends on the water that is used for the CIP rinse. Energy consumption of RO water is derived from pump dimensioning for RO water generation and the energy that is needed to heat the water to the desired rinse temperature. The WFI that is used for the final rinse consumes energy during its production via the hot method (*10.3 WFI and RO water generation – preliminary calculations*) and consumes energy if it is heated to the desired rinse temperature.

The amount of water per rinse depends on CIP cycle dimensioning. This involves the spray ball selection according to tank size, amount of soil and its composition as well as

CIP cycle times. The calculations for CIP water and energy demand are presented in *10.5 CIP –preliminary calculations*.

Pipes and transfer lines are omitted from these calculations.

Total CO_2 output of CIP procedure

The energy demand of RO water generation, WFI generation and the energy demand for heating the water to the desired rinse temperature is summed up to receive the total output in CO_2 per batch. This number can then be multiplied by the number of batches to receive the carbon emissions of the desired time period.

WFI demand for SIP

SIP is a SST facility exclusive. SIP for a SUT facility is not required since all material come gamma-sterilized and is disposed after use. To determine the amount of water needed for the SIP procedure, the tank dimensions (width, height, wall thickness) and the insulation layer thickness have to be known. The method to determine tank dimensions including the wall thickness according to the *AD 2000 Merkblätter* of a steel tank is presented in *10.6 Steel tank dimensioning – preliminary calculations*. From the tank dimensions the weight of the steel shell as well as lid and bottom can be calculated. The SIP process time as well as starting and end temperature are used to calculate the loss of heat due to the tank's surface, the energy required to heat the mass of steel of the tank to desired temperature, the mass flux of steam required to fill the tank and provide the required energy. The calculations regarding SIP process dimensioning are covered in *10.4 SIP - preliminary calculations*. The determined amount of steam is conform with the required amount of WFI.

Energy demand to heat water to SIP temperature

When the needed mass of water for the SIP procedure is known, the energy demand can be calculated by using the specific heat capacity of water as well as the starting and end temperature of the water. The calculations are presented in *10.4 SIP - preliminary calculations*.

Total CO_2 output of SIP operations

The energy associated with the amount of water needed for SIP operations as well as the energy demand to generate steam for SIP operations are summed up to receive the total carbon emission per tank. This is done for every tank that requires SIP and by multiplying the energy demand of all tanks per batch with the number of batches the overall carbon emissions are determined.

CO_2 from steel production

Steel production emissions concern SST and SUT facilities as both require the use of steel for certain process components. To determine the carbon emission from steel production the mass of used steel has to be determined. For machinery, this data can be obtained from datasheets or personal communication. For steel tanks the tank specifications are calculated from the tank volume by obtaining the optimized tank diameter and tank height for minimal material usage (*10.15 Tank diameter/height – preliminary* calculations). With the help of the *AD 2000 Merkblätter* the wall thickness can be determined. At this point the mass of the shell, lid and bottom part of a tank can be determined by calculating the volume and multiplying it with the density of steel. The calculations regarding this concern is presented in *10.6 Steel tank dimensioning – preliminary calculations*. With known steel masses and the help of emission factors for steel production, the total carbon emissions for steel production can be determined.

Transport of steel tanks to facility

Only the SST facility used stainless steel tanks that have to be transported from the production site to the SST facility. Transport is carried out either on roads via trucks or on rails via trains. The emission factors for road or rail transport require a mass and distance specification. The calculations regarding this method are presented in *10.14 Cargo transport emission – preliminary calculations*.

CO_2 from filter cartridge waste

The use of filter cartridges is crucial for SST and SUT based facilities. When the number of necessary filter cartridges is known, the mass can be multiplied with an emission

factor to receive the carbon emissions during production, transport or incineration. The dimensions of the filter cartridges are used to determine how many autoclave cycles are necessary to decontaminate filters that had product contact. The number auf autoclave cycles is directly proportional to the amount of used water/energy. A method to determine the number of autoclave cycles for single-use bags with a certain volume is presented in *10.10 Autoclaving of single-use equipment* – Preliminary calculations. The method to determine the number of necessary autoclave cycles for filters works accordingly.

For this work, only the filters from the harvest depth filtration are included. The total weight per filter cartridge is known and with an estimated 30% of polypropylene waste from filter housing the carbon footprint from autoclaving filters as well as incineration is determined.

CO_2 from steel production (bag support structures)

The principle to determine the carbon footprint of steel production for bag support structures of SUT facilities is basically the same as for steel tanks. Supplier data only lists the total weight of the bag support structure including other components such as driver units, pumps, control units and computers. For this reason it is not possible to precisely determine the amount of used steel. Estimation can be a large source of error and for this reason the carbon emission for the production of bag holding apparatus is not included in this work.

CO_2 from bag incineration

To calculate the amount of carbon emitted by the incineration of single-use bags (buffer storage bags, SUMs, SUBs), the number of bags and their according mass is determined. Data on the number and types of single-use bags for upstream and downstream operations are known and documented. Suppliers generally do not list the specific weights for their various models in the dedicated datasheets. Some suppliers kindly provided data on the weight of their products. Due to the inhomogeneous nature of bag films, it is not possible to calculate the carbon emissions associated with each individual bag layer or other components such as agitators, tubing and filters. With the assumption that the entire bag is made from polyethylene the carbon emissions for the incineration

of single-use bags can be approximated. In this work energy, recovery from incineration as well as needed energy demand (e.g. burning of plastic waste with natural gas) is not considered. Data or incineration and the resulting carbon emissions are listed in *10.13 Emission factor of PE/PP – preliminary calculations*.

CO_2 from plastic film production

To calculate the carbon emissions of plastic film production by extrusion the mass of bags has to be known. With data on weight provided by bag manufacturers, the carbon emissions can be calculated with the use of emission factors for plastic film extrusion. Due to unknown single-use bag composition the calculated values are only approximations to the exact carbon emissions of a multilayer multicomponent bag film. Calculations are presented in *10.12 Single-use bag production and incineration*.

CO_2 of buffer/media transportation

The mass of single-use buffer/media storage bags is multiplied with the transport emission factor for road/rail. Calculations are based on the method presented in *10.14 Cargo transport emission – preliminary calculations*

Total CO_2 from bag use

The total CO_2 emissions by bag usage in a SUT facility is the sum of bag production, bag transport, decontamination and bag incineration with according emission factors.

CO_2 from transport of buffers

When the amount of buffer is calculated as described in 10.1 Upstream process – preliminary calculations *and* 10.2 Downstream process – preliminary calculations, the carbon emissions from transport of buffers from the buffer production facility to the SUT facility is calculated. The available emission factors allow calculation for transport via roads/rails for a defined cargo mass for a defined distance. Calculations are based on the method presented in *10.14 Cargo transport emission – preliminary calculations*.

Electricity demand of autoclave operation

The energy demand for autoclave operation is calculated from data provided by the autoclave manufacturer. The dimensions of the autoclave chamber and the filling

degree limit the number of items that can be placed in the autoclave for one cycle. The minimum number of cycles is determined by placing the maximum allowed amount of items in terms of volume in the autoclave. The number of resulting cycles is multiplied with the energy consumption per cycle to receive the total energy consumption for sterilizing all items of one production batch. This value is multiplied with an according emission factor for electricity as well as total number of batches to receive the total amount of CO_2 emissions. Calculations are based on the method presented in *10.10 Autoclaving of single-use equipment* – Preliminary calculations.

CO_2 of autoclave steel production

The mass of steel that is used to manufacture the autoclave is multiplied with the emission factor for steel production to obtain the total carbon emissions. Calculations are based on the method presented in *10.6 Steel tank dimensioning – preliminary calculations*.

CO_2 from heating of production bioreactor medium

By setting the volume of the production bioreactor, the energy demand for heating the medium form a starting to an end temperature in a certain time can be calculated. The obtained value on kWh is then multiplied with the electricity emission factor to receive the total carbon emission for one batch. This value can then be multiplied with the number of batches to receive the overall carbon emissions. The method to calculate the heating energy demand is presented in *10.9 Tank heating/cooling – preliminary calculations*.

CO_2 from cooling after harvest

SST as well as SUT facility rely on cooling during the harvest .The entire volume of the production bioreactor is pooled during harvest (after depth filtration) and cooled down. The energy demand is calculated via the starting and end temperature the mass of the volume to be cooled down, the specific heat capacity and the duration of the cooling process. The method to calculate the energy demand for the cooling process is presented in 10.9 Tank heating/cooling – preliminary calculations.

CO_2 by energy demand of HVAC air heating/cooling/dehumidification/humidification

HVAC calculations are relevant for SUT and SST facilities. To calculate the energy demand for heating/cooling clean rooms several aspects have to be considered. The room volume has to be determined and multiplied by its height to receive the room volume. The cleanroom class with its according air change rate is selected to determine the volumetric flow. Weather data (average daily temperature, average daily relative humidity and average daily pressure) is used to calculate the saturation vapour pressure via Antoine's equation. The partial pressure of steam is calculated to calculate the absolute humidity. The specific enthalpy of each day and the specific enthalpy of the desired clean room conditions (temperature, relative humidity) are calculated. The difference of those two values represents the energy demand in kJ/kg_{air}. As a result a delta for each specific enthalpy for each day of the year is calculated. The volumetric air flux is converted to mass flux via air density and multiplied with the delta of specific enthalpy for each day of the year. The sum of all days delivers the energy demand for heating/cooling/dehumidification/rehumidifcation per year. The total energy demand per year is multiplied with the electricity emission factor to obtain the total carbon emissions per year.

CO_2 by energy requirement of HVAC fan operation

A room of defined volume with an according clean room class and its specific air change rate deliver the volumetric flux of air. Fan manufacturers and suppliers offer data sheets for their fan models including the maximum air flux in m^3/h and energy consumption in kW. After selecting the right model for the room either by suiting air change rate for the room or best efficiency ($m^3/h/kW$), the number of needed fans with according energy demand is set. The number of fans is multiplied with the energy consumption and the yearly operational hours resulting in an energy demand in kWh/year. By using an electricity emission factor the yearly carbon emissions are calculated. The described method is presented in *10.7 HVAC – preliminary calculations*.

CO_2 by energy demand of WFI for humidification

To determine the carbon footprint of the WFI that is used when the desired specific humidity falls below target, the necessary amount of WFI is determined. The cleanroom

air flux is calculated by multiplying the room's volume with the room class specific air change rate. The absolute humidity of the air is calculated from weather data (average daily temperature, average daily relative humidity and average daily pressure). A desired clean room temperature, pressure as well as desired relative humidity is set to calculate the absolute humidity of the desired set point. The difference in daily absolute humidity and set point humidity is determined in g_{water}/kg_{air}. The volumetric flux is converted into a mass flux by air density. Mass flux is then multiplied with the difference in absolute humidity to determine how much water has to be added. This process is done for each day of the year to receive the amount of RO water that has to be added to the air stream of the clean room per year. The described method is presented in *10.7 HVAC – preliminary calculations*.

CO_2 by total energy demand of HVAC operations

Energy demand for heating/cooling/dehumidification/humidification, fan operation and RO water generation for humidification are summed up to receive the total carbon emissions of HVAC operations. This work assumes 24/7, 365 days per year HVAC operation without the industry wide common practice of reduced mode (approximately 25% energy savings) during employee absence.

Mass of produced product

The mass of produced product after 5 years of production is 1.86 t for the SST facility and 1.77 t for the SUT facility. The value is derived from the process volume and the concentration of the fill and finish building block The mass of produced mAbs per time is important as this is value is used as a base for normalization that allows direct comparison between facilities of different scale (e.g. a 18000 L bioreactor capacity SST facility with a 2000 L bioreactor capacity SUT facility). Normalized values on CO_2 $[\frac{t_{CO2}}{t_{mAbs}}]$ or on water $[\frac{t_{water}}{t_{mAbs}}]$ allow interpretation regardless of scale.

The mass of produced mAbs is also important, as it has to suit market demand. A scale-up approach for the SST and a numbering up approach for the SUT-facility allow market adaption.

7 Results

The results are based on facilities located in Basel, Boston and Shanghai. Presented asepects are water consumption, CIP and SIP in comparison with single-use bag production and incineration, HVAC as well as the influence of commuting employees.

7.3 Case study 1: results for the facility in Basel

This case study shows the impact of changing the electricity emission factor from the Swiss average (23.6 g_{CO2}/kWh) to the German average (523 g_{CO2}/kWh). Areas of interest are impact on water consumption parameters, CIP and SIP operations in comparison with bag incineration, HVAC parameters and commuting employees. Calculations concern SST facilities at a bioreactor capacity scale of 2000 L (labelled as 2k SST), 18000 L (labelled as 18k SST) and an SUT facility at 2000 L (labelled as 2k SUT). The 2k SST facility has a footprint of 720 m^2, the 2k SUT facility a footprint of 850 m^2 and the 18k SUT facility a footprint of 1547 m^2, influencing HVAC operations. The 2k SST facility produces 1.86 t_{mAb} within 5 years. The 2k SUT facility produces 1.77 t_{mAb} within 5 years. The 18k STT facility produces 16.76 t_{mAb} within 5 years. The slight difference between the 2k SST and the 2k SUT facility stems from the upstream and downstream design of the SST facility, that relies on rounded numbers for different unit operations: like number of filters (e.g. 9.7 filters) or chromatography column diameter (e.g. 43.7 cm). This results in a more optimized process and an improved overall yield. The 2k SST facility produces 0.00938 t (9.38 kg) of mAbs, while the SUT facility produces 0.00894 t (8.94 kg) of mAbs per batch. With a total batch count of 198 over the course of 5 years, the 2k SST facility produces more mAbs than the 2k SUT facility.

7.3.1 Water consumption

Water consumption stems from different sources in regards of SST and SUT facilities and is linked to carbon emissions due to generation of high quality water that is necessary for biopharmaceutical production. Different water qualities like WFI, RO water or cooling water are linked to different carbon emissions. The SUT facility does not require CIP and SIP operations.

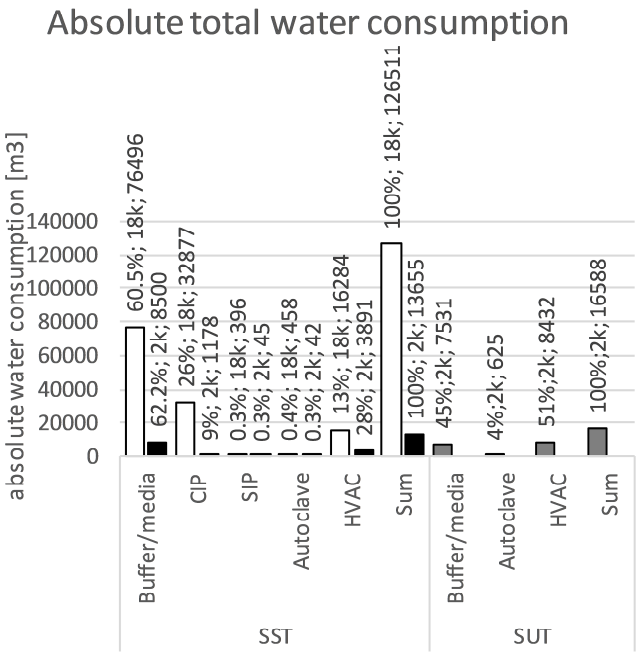


Figure 41 Absolute total water consumption of a 2k SST, 18k SST and a 2k SUT facility.

Figure 41 shows the absolute total water consumption for three different facilities. The black bars represent the 2k SST facility, the white bars the 18k SST and the grey bars the SUT facility. Direct comparison is only possible at a scale of 2000 L and the 18k facility only shows the influence of scale. All facilities consume the most of their water for buffer and media.

2k SST vs 2k SUT

The distribution for the 2k SST facility is 8500 m^3 (or 62.2%) for buffer/media, 3891 m^3 (28%) for HVAC, 1178 m^3 (9%) for CIP, 45 m^3 for SIP (0.3%) and 42 m^3 (0.3%) for autoclave operation, resulting in a total consumption of 13655 m^3. The 2k SUT facility consumes 7531 m^3 (45%) for buffer/media, 8432 m^3 (51%) for HVAC and 625 m^3 (4%) for autoclave operation, resulting in a total water consumption of 16588 m^3. The absolute water consumption of the SST facility is greater than the absolute water consumption of the SUT facility.

Influence of scale

The 18k SST facility consumes more water than the 2k SST facility. The distribution for the 18k SST facility is 76496 m^3 (or 60.5%) for buffer/media, 32877 m^3 (26%) for CIP,

16284 m^3 (13%) for HVAC, 396 m^3 for SIP (0.3%) and 458 m^3 (0.4%) for autoclave operation, resulting in a total consumption of 126511 m^3.
The scale up to 18 m^3 results in extended CIP operations since all tanks in the facility increase in size according to the increased bioreactor capacity.

Normalized total water consumption

Water consumption is normalized on the total mass of produced mAbs. While the total water consumption increases with scale so does the total produced of monoclonal antibodies.

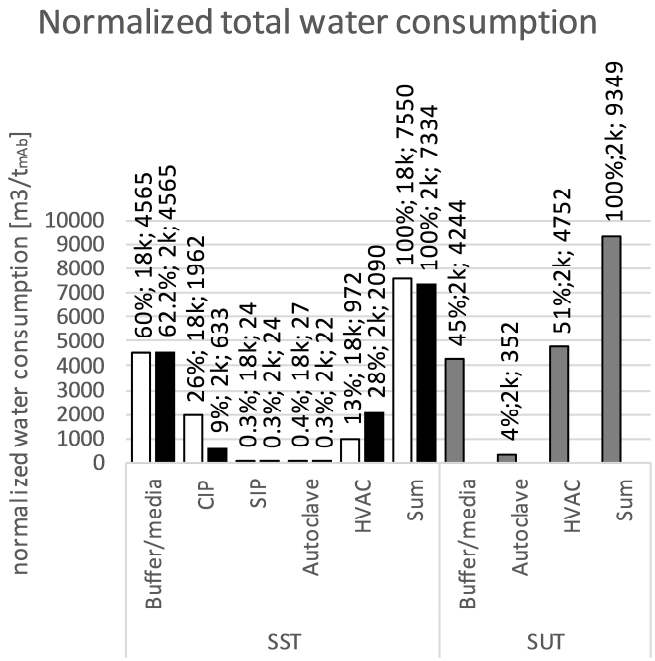


Figure 42 Normalized total water consumption of a 2k SST (black bars), 18k SST (white bars) and a 2k SUT (grey bars) facility. Black and grey bars allow a direct comparison, while the black bars emphasize the influence of possible scale up of SST facilities.

The carbon emissions (m^3_{water}) are divided by the total mass of produced mAbs (t_{mAb}) to allow a direct comparison between the facilities.

2k SST vs 2k SUT

The 2k SST facility consumes 4565 m^3 (62.2%) of water for buffer/media per produced ton of mAbs., 2090 m^3/t_{mAb} (28%) for HVAC, 633 m^3/t_{mAb} (9%) for CIP, 24 m^3/t_{mAb} (0.3%)

SIP and 22 m^3/t_{mAb} (0.3%) for autoclave operation. The 2k SST facility consumes a total of 7334 m^3 of water for every ton of mAbs that is produced. The distribution for the 2k SUT facility is 4244 m^3/t_{mAb} (45%) for buffer/media, 4752 m^3/t_{mAb} (51%), for HVAC and 352 m^3/t_{mAb} (4%) for autoclave operation. The 2k SUT facility consumes 9349 m^3 of water per ton of produced mAbs.

Influence of scale on the SST facility

The 18k SST facility consumes 4565 m^3/t_{mAb} (60%) for buffer/media, 1962 m^3/t_{mAb} (26%) for CIP, 972 m^3/t_{mAb} (13%) for HVAC, 27 m^3/t_{mAb} (0.4%) for autoclave operation and 24 m^3/t_{mAb} (0.3%) for SIP. The 18k SST facility consumes 7550 m^3 of water per ton of produced mAbs.

The comparison between the 2k SST and the 18K SST facility shows that with increasing scale water consumption grows faster than the mass of produced mAbs:

$$SST_{2k} = 7334[\frac{m^3{}_{water}}{t_{mAb}}]$$

$$SST_{18k} = 7550[\frac{m^3{}_{water}}{t_{mAb}}]$$

In terms of water preservation the 2k SST facility outperforms the 18k SUT facility

The SUT facility consumes more water than its 2k SST counterpart and the 18k SST facility, for every ton of mAbs that is produced:

$$SUT_{2k} = 9349\left[\frac{m^3{}_{water}}{t_{mAb}}\right] > SST_{18k} > SST_{2k}$$

The high water consumption of the 2k SUT facility stems from HVAC operations. The 2k SUT facility has a 850 m^2 footprint (2k SST: 720 m^2) with higher ceilings, resulting in a larger room volume and therefore required air flux. Additionally, there is not the option to place all the buffer/media bags in a separate room as it is done with the buffer/media tanks in the SST facility. Furthermore, the clean room class of the buffer/media stainless steel tanks is CNC (9 air changes per hour), while the clean room class for the buffer/media bags has to be C (20 air changes per hour) since the buffer/media is placed in the room with the rest of the equipment (SUMs, SUBs, filtration skids, chromatography columns, etc.).

7.3.2 CIP+SIP in comparison with bag usage

Two technology exclusive factors are calculated to investigate the carbon emissions of the CIP and SIP process of SST facilities in comparison with the carbon emissions from bag (buffer bags, SUMs, SUBs) production and incineration after use. Two different scales for the SST factory (2 m^3; 18 m^3) are compared to a 2 m^3 SUT facility. The factory is operating for 5 years with a product titer of 6 g/L.

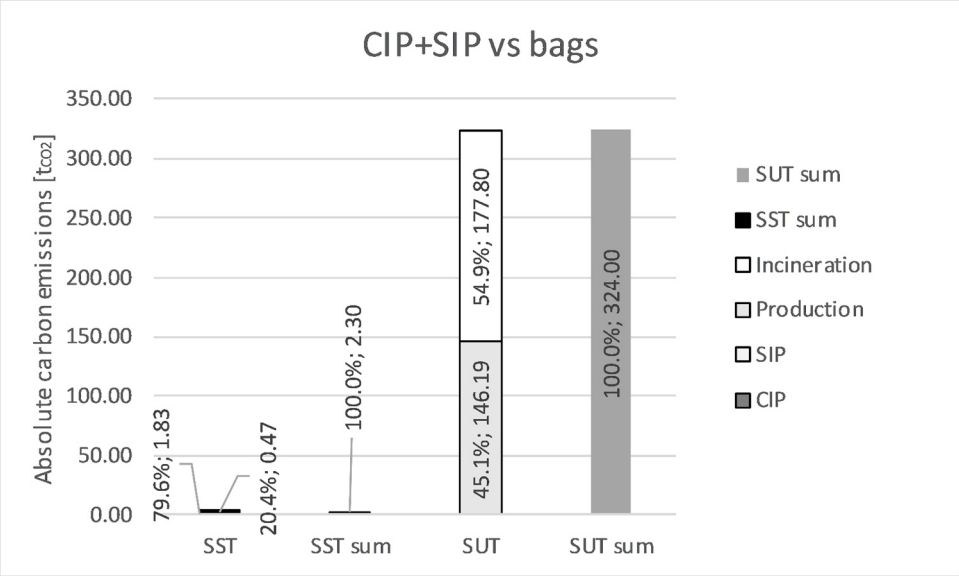


Figure 43 Comparison of 2k SST CIP and SIP procedure with the production and incineration of bags of a 2k SUT facility.

Figure 43 shows the total carbon emissions over the course of 5 years for a 2k SST (CIP and SIP) and a 2k SUT (production and incineration) facility. CIP emits 1.83 t_{CO2} (79.6%), while SIP emits (0.47 tCO2), resulting in total carbon emissions of 2.30 t_{CO2} over the course of 5 years. The production of bags results in 146.19 t_{CO2} (45.1%), while the incineration results in 177.80 t_{CO2} (54.9%), resulting in total carbon emissions of 324 t_{CO2}.

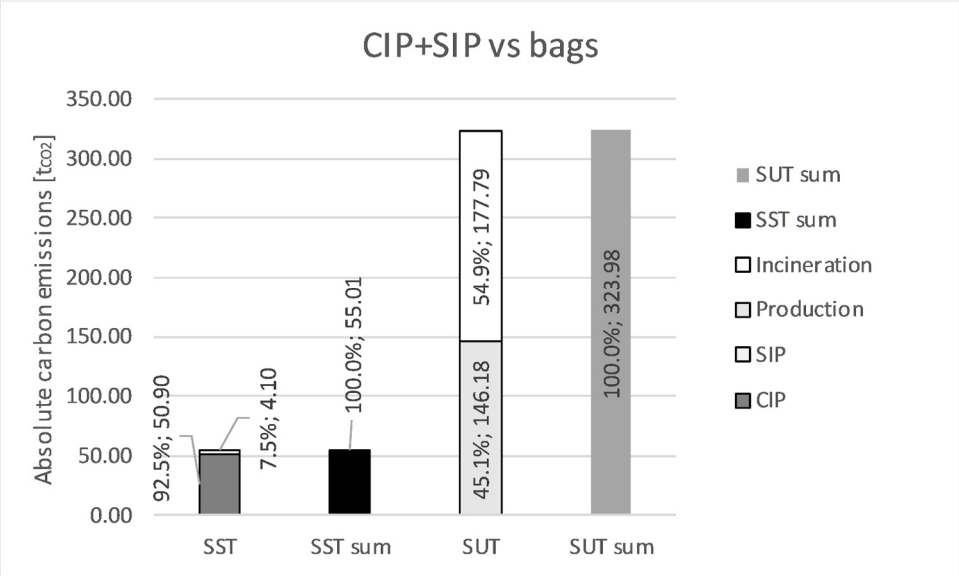


Figure 44 Comparison of 18k SST CIP and SIP procedure with the production and incineration of bags of a 2k SUT facility, running with average Swiss electricity.

Figure 44 shows the total carbon emissions over the course of 5 years for a 18k SST (CIP and SIP) and a 2k SUT (production and incineration) facility. CIP emits 50.90 t_{CO2} (92.5%), while SIP emits (4.10 t_{CO2}), resulting in total carbon emissions of 55.01 t_{CO2} over the course of 5 years. A comparison with Figure 43 shows that the 18k SST facility emits more carbon dioxide. This is a result of the larger tanks with larger diameters. The larger the tank the higher the flow rate of the spray balls. This results in an overall larger amount of water that is required clean the tanks sufficiently.

Normalized values

Normalization on the mass of produced mAbs allows a direct comparison between the facilities. The 18k SST facility emits 3 t_{CO2} per ton of produced mAbs. The 2k SUT facility emits 183 $_{tCO2}$ per ton of produced mAbs, while the 2k SST facility emits 1 t_{CO2} per ton of produced mAbs.

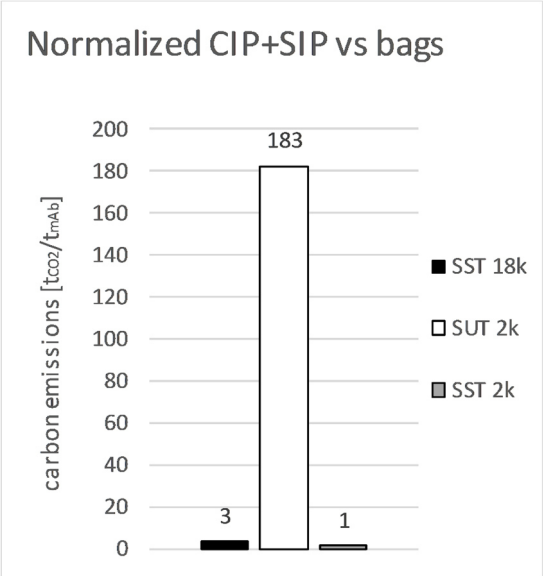


Figure 45 Normalized values for a 2k SST, 18k SST and a 2k SUT facility. The bars of the SST facilities include the carbon emissions of CIP and SIP procedure. The bars of the SUT facility include the emissions of bag production and bag incineration.

Figure 45 shows the increase in carbon emissions per produced ton of mAbs due to the increase in scale for the SST facility from 2k to 18k. This increase in a result of growing water and energy demand due to CIP and SIP operations.

Switching to German electricity

The emission factor for electricity is switched from the average Switzerland grind mix to the average German electricity grid mix. All other input variables are not changed.

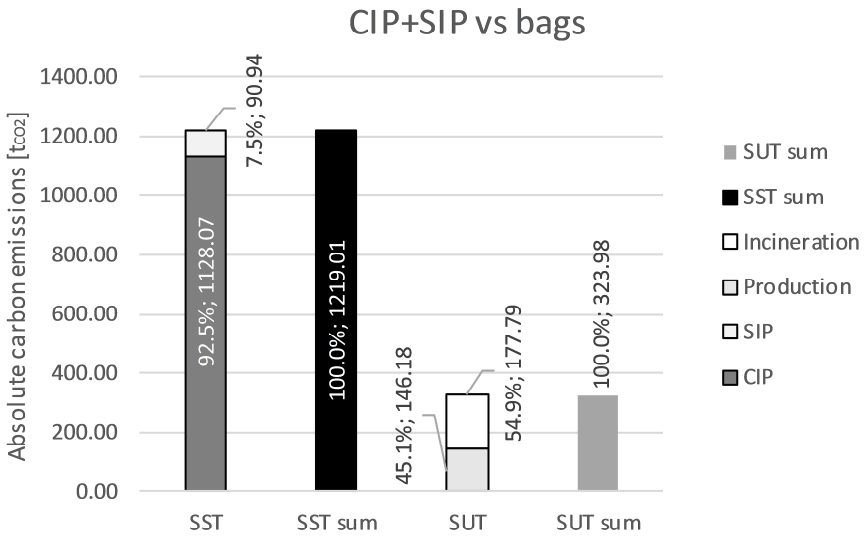


Figure 46 Comparison of 18k SST CIP and SIP procedure with the production and incineration of bags of a 2k SUT facility running with the average German electricity.

When Figure 46 is compared with Figure 44, the values for the SUT facility stay the same. The SST facility now emits 1128.07 t_{CO2} for CIP and 90.94 t_{CO2} for SIP operations. The generation of WFI and RO water for the CIP and SIP process consumes large amounts of electricity. When switching from the “clean” Swiss grind mix to the more carbon intensive German grid mix, the total carbon emissions are therefore amplified.

Influence of German electricity on normalized carbon emissions

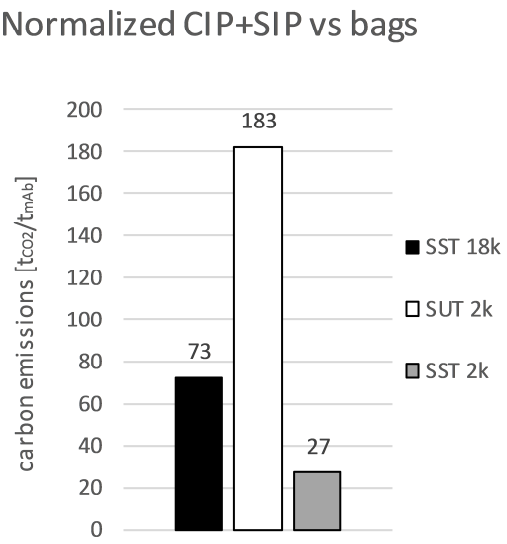


Figure 47 Normalized values of carbon emissions for a 2k SST, 18k SST and a 2k SUT facility. The bars of the SST facilities include the carbon emissions of CIP and SIP procedure. The bars of the SUT facility include the emissions of bag production and bag incineration. The factories operate with the average German electricity grid mix.

Figure 47 Shows the increase in normalized carbon emissions from 3 t_{CO2}/t_{mAb} to 73 t_{CO2}/t_{mAb} for the 18k SST facility and 1 t_{CO2}/t_{mAb} to 27 t_{CO2}/t_{mAb}, when compared to Figure 45. The normalized carbon emissions of bag production and incineration for a 2k SUT facility are higher than the normalized carbon emissions of a 2k or 18k SST facility, running on the average German electricity grid mix.

7.3.3 HVAC

HVAC operations require electrical energy for heating/cooling/dehumidification/humidification as well as fan operation. WFI is consumed when air that enters the HVAC unit has not the desired properties (temperature; relative humidity). HVAC energy and water consumption calculations are based on volumetric (or mass) flux. Clean room area and its according ceiling height (and therefore room volume) together with cleanroom class specific air change rate are the base of all HVAC calculations.

Calculations involve two different scales (2 m^3 and 18 m^3) and two different electricity sources (average Swiss electricity and average German electricity). The area of the SST facility is 528 m^2 and the area of the SUT facility is 604 m^2, both with a ceiling height of 3.5 m.

The area is calculated according to *10.8 Space requirement – preliminary calculations*. Area demand of all upstream and downstream operations are categorized. The categories include processing tanks/(processing bags, Media/buffer and Equipment (see Table 14). Ancillary room demand like water handling areas (with autoclave), WFI generation and column preparation are not categorized and receive their own clean room class. Ancillary rooms are based on estimated area demands and are listed in Table 14:

Table 14 Area requirement for upstream and downstream operations for the SST and SUT facilities

	SST Area [m^2]			SUT Area [m^2]		
	Processing tanks	Media/buffer	Equipment	Processing bags	Media/buffer	Equipment
Inoculum expansion	0.00	0.00	13.22	0.00	0.00	13.22
Cell expansion	31.76	2.40	0.00	16.77	8.14	0.00
Production	27.95	37.10	0.00	17.47	18.39	0.00
Harvest	28.24	3.51	633.53	8.05	29.80	7.45
Protein A	0.00	69.29	0.91	0.00	84.18	7.45
pH adj. & filtration	28.34	9.36	0.00	19.87	6.71	0.00
UF/DF 1	12.36	19.67	110.26	5.41	24.55	12.25
IEX	0.00	32.96	4.90	0.00	35.84	4.40
C&F 1	14.31	5.17	0.00	11.30	3.58	0.00
AEX	0.00	66.22	5.11	0.00	74.83	0.00
Virus filtration	17.33	5.60	0.00	12.11	4.78	0.00
UF/DF 2	24.35	32.64	110.26	12.11	35.04	0.00
C&F 2	11.21	3.33	0.00	10.81	0.00	0.00
F&F	0.00	0.00	150.22	0.00	0.00	16.00

Ceiling height are:

Table 15 Ceiling heights for the three categories (processing tanks/bags, buffer/media nad equipment)

Ceiling height [m]			
SST		SUT	
Processing tanks	4.05	Processing bags	4.555
Media/buffer	5.0	Media/buffer	4.555
Equipment	4.05	Equipment	4.555

The largest item in the SUT facility is a 3.55 m single-use bioreactor. The total ceiling height of 4.555 m adds one meter for HVAC maintenance purpose. All three categories have the same ceiling height for the SUT facility, since processing bags, media/buffer and equipment need to be in the same room for certain processing steps. For the SST facility buffer/media are stored in a separated room with a ceiling height of 5 m (highest items + 1 m maintenance. Processing tanks and equipment are in the same room and

respect the highest item with an addition of 1 m for maintenance purposes. The ceiling height for the SST facility are listed together with the area requirement in Table 16

Table 16 Area requirement and ceiling height for ancillary rooms.

Room	SST area [m²]	Ceiling [m]	SUT area [m²]	Ceiling [m]
Warehouse	60	3.5	250.00	5
Waste/ Autoclaving area	40	3.5	60	3.5
	5.28		5.28	
WFI generation	30	6.31	0	0
Column preparation	50	3.5	50	3.5

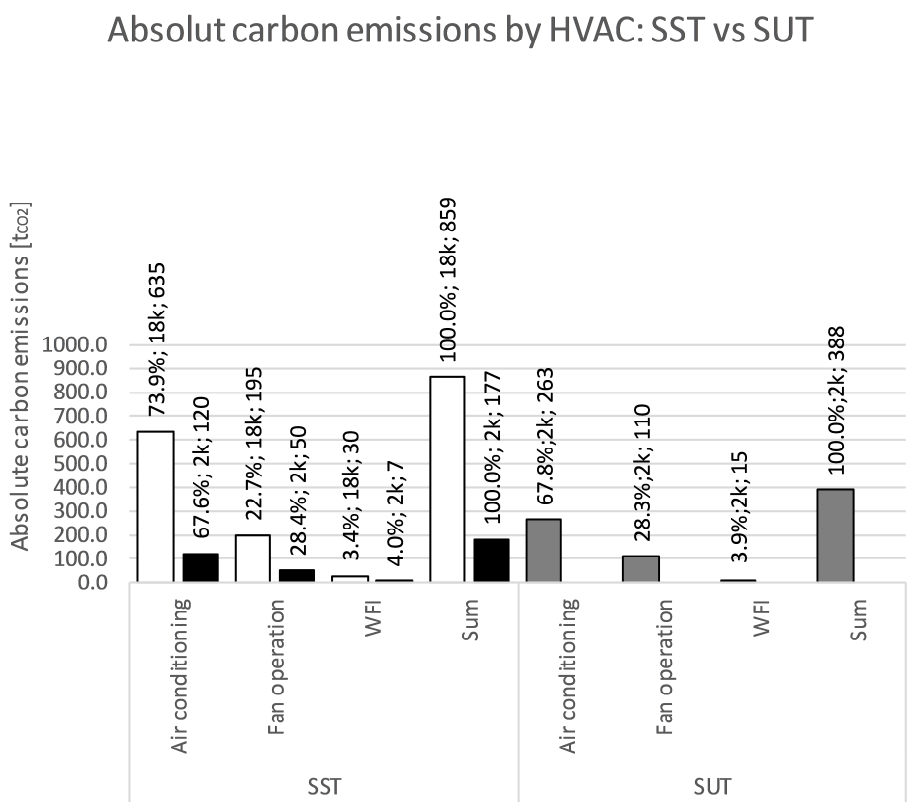


Figure 48 Absolute carbon emissions by HVAC operations for a 2k SST (black), 2k SUT (grey) and a 18k SST /white) facility.

2k SST vs 2k SUT

Figure 48 shows the absolute carbon emissions due to HVAC operations. For the 2k SST facility, the distributions are: 120 t_{CO2} (67.6%) for air conditioning, 50 t_{CO2} (28.4%) for fan operation, 7 t_{CO2} (3.4%) for WFI generation, resulting in 177 t_{CO2} over the course of 5 years. The 2k SUT facility emits 263 t_{CO2} (67.8%) for air conditioning, 110 t_{CO2} (28.3%) for fan operation and 15 t_{CO2} (3.9%) for WFI generation, resulting in total carbon emissions of 388 t over the course of 5 years. For both facility the conditioning of air (adjustment of temperature and humidity) is the most carbon intensive category, followed by fan operation and the generation of WFI for humidification.

Influence of scale

Figure 48 shows, that the 18k SST facility emits 635 t_{CO2} (73.9%) for air conditioning, 195 t_{CO2} (22.7%) for fan operation, 30 t_{CO2} (3.4%), resulting in total emissions of 388 t_{CO2}. The facility emits the most carbon dioxide due to air conditioning, followed by fan operation and generation of WFI.

Normalized values

The normalization of the carbon emissions on the mass of produced mAbs allows for a direct comparison of the three different facilities.

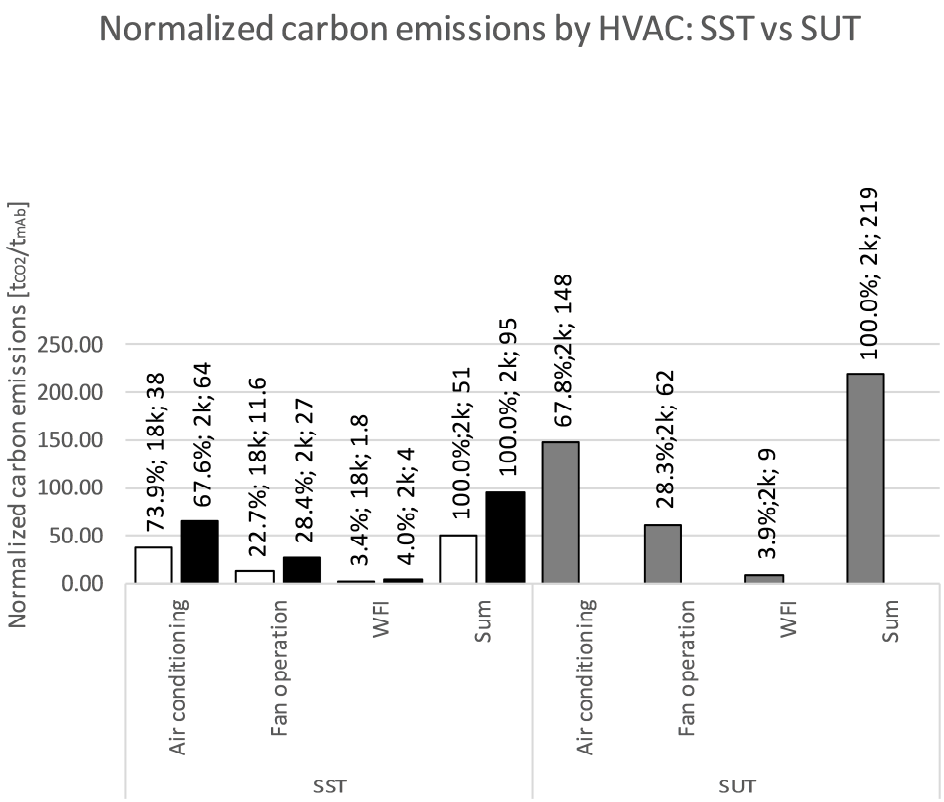


Figure 49 Normalized total carbon emissions due to HVAC operations. The white bars represent the 18k SST facility, the black bars the 2k SST facility and the grey bars the 2k SUT facility.

Figure 49 shows the normalized values (t_{CO2}/t_{mAb}). The 2k SST facility emits 64 t_{CO2} per ton of produced mAbs (67.6%) for air conditioning, 27 t_{CO2}/t_{mAb} for fan operation (28.4%) and 4 t_{CO2}/t_{mAb} (4.0%) for WFI generation, resulting in total emissions of 95 t_{CO2}/t_{mAb}. The 2k SUT facility emits 148 t_{CO2} per ton of produced mAbs (67.8%) for air conditioning, 62 t_{CO2}/t_{mAb} for fan operation (28.3%) and 9 t_{CO2}/t_{mAb} (3.9%) for WFI generation, resulting in total emissions of 219 t_{CO2}/t_{mAb}. Both facilities allocate most carbon emissions to the air conditioning category, followed by fan operation and WFI generation. The 2k SUT facility emits more carbon dioxide per ton of produced mAbs when compared to its 2k SST counterpart (56 t_{CO2}/t_{mAb} vs 54 t_{CO2}/t_{mAb}). The increase in scale to 18k for the SST facility reduces the carbon emissions for air conditioning to 38 t_{CO2}/t_{mAb} (73.9%), 11.6 t_{CO2}/t_{mAb} (22.7%) for fan operation and 0.1.8 t_{CO2}/t_{mAb} (3.4%) for WFI generation. The total carbon emissions for the production of one ton of mAbs is therefore 51 t_{CO2}. A nine times fold increase in total bioreactor capacity (2k to 18k) results in a 46.2% reduction in carbon emissions per ton of produced mAbs:

$$SST_{2k} = 54 \triangleq 100\%$$

$$SST_{2k} = \frac{100[\%]}{95\left[\frac{t_{CO2}}{t_{mAbs}}\right]} \cdot 51\left[\frac{t_{CO2}}{t_{mAbs}}\right] = 53.\overline{\overline{68}}[\%] \approx 53.8[\%]$$

$$100[\%] - 7.4[\%] = 46.2[\%]$$

Switching to the average German electricity grid mix

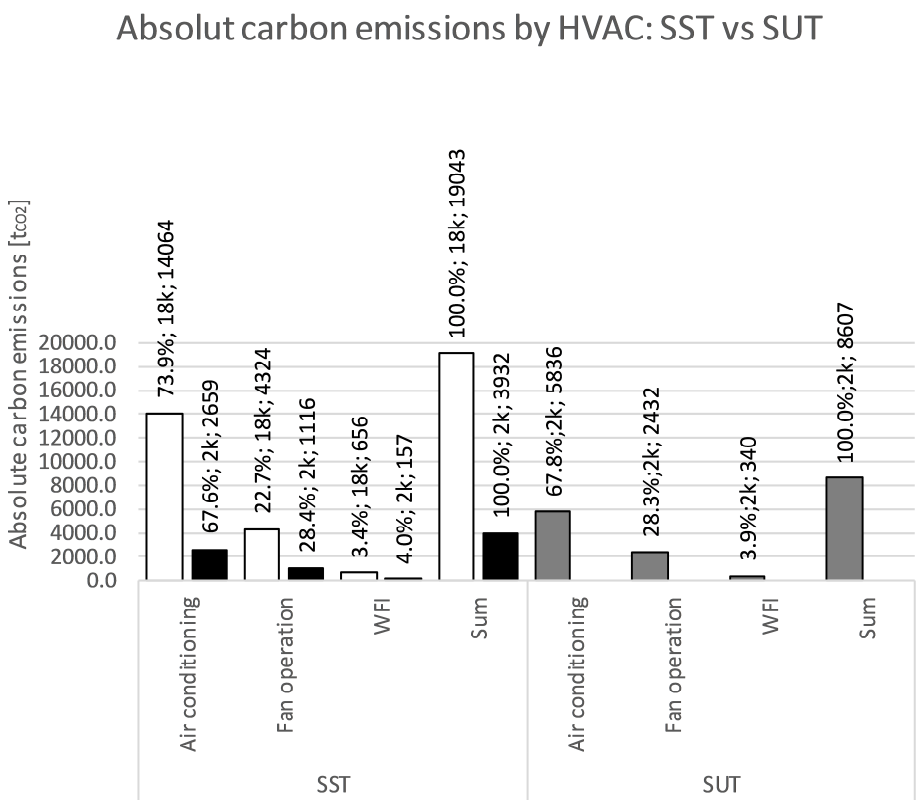


Figure 50 Absolute carbon emissions by HVAC operations for a 2k SST (black), 2k SUT (grey) and a 18k SST (white) facility. The factories operate with the average German electricity grid mix.

Figure 50 shows the increased carbon emissions from changing the Swiss average electricity mix to the German average electricity mix when compared to Figure 48. The total carbon emissions increased from 859 t_{CO2} to 19043 t_{CO2} for the 18k SST facility, from 177 t_{CO2} to 3932 t_{CO2} for the 2k SST facility and from 388 t_{CO2} to 8607 t_{CO2} for the 2k SUT facility.

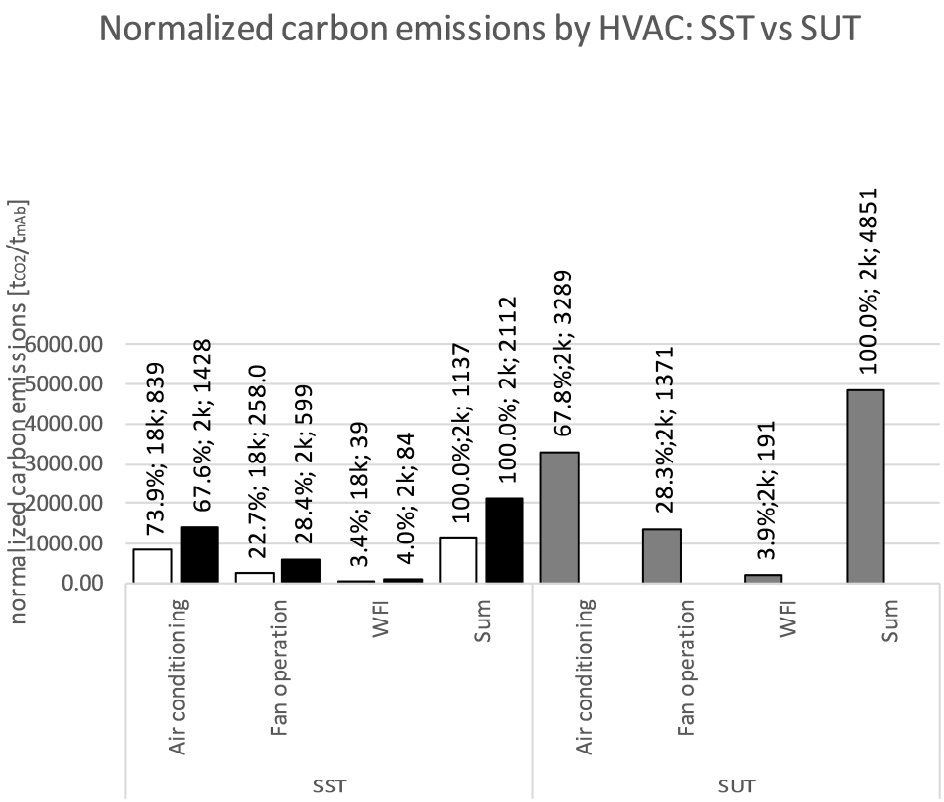


Figure 51 Normalized total carbon emissions due to HVAC operations. The white bars represent the 18k SST facility, the black bars the 2k SST facility and the grey bars the 2k SUT facility. The facilities run on the average German electricity grind mix.

Figure 51 shows the influence on the normalized carbon emissions of HVAC operation when the Swiss electricity (see Figure 49) is replaced by German electricity. The total carbon emissions per ton of produced mAbs for the 2k SST facility increase from 95 t_{CO2}/t_{mAb} to 2112 t_{CO2}/t_{mAb}, from 219 t_{CO2}/t_{mAb} to 4851 t_{CO2}/t_{mAb} for the 2k SUT and from 51 t_{CO2}/t_{mAb} to 1137 t_{CO2}/t_{mAb} for the 18k SST facility.

The production of the same mass of monoclonal antibodies is 22.2 times more carbon intensive when the production runs with the average German electricity grid mix, when compared to the exact same facilities running on the average Swiss grid mix:

$$SST_{2k,DE} = \frac{2112[\frac{t_{CO2}}{t_{mAbs}}]}{95[\frac{t_{CO2}}{t_{mAbs}}]} = 22.2\overline{3} \approx 22.2$$

$$SUT_{2k,DE} = \frac{4851[\frac{t_{CO2}}{t_{mAbs}}]}{219[\frac{t_{CO2}}{t_{mAbs}}]} = 210.\overline{15} \approx 22.2$$

$$SST_{18k,DE} = \frac{1137[\frac{t_{CO2}}{t_{mAbs}}]}{51[\frac{t_{CO2}}{t_{mAbs}}]} = 22.\overline{29} \approx 22.3$$

$$average = \frac{22.2 + 22.2 + 22.3}{3} = 22.\bar{3} \approx 22.3[\%]$$

The German grind mix is 22.2 times more carbon intensive than the Swiss grind mix:

$$EF_{CH\ vs\ DE} = \frac{523[\frac{g_{CO2}}{kWh}]}{23.6\left[\frac{g_{CO2}}{kWh}\right]} = 22.2\bar{3} \approx 22.2$$

7.3.4 Commuting employees

The influence of commuting workers is assessed by comparison with different carbon emission categories for SST and SUT facilities. Investigation concern the influence of the emission factor of the Swiss and the German average grid mix as well as different scales for the SST facility (2 m^3, 18 m^3) in comparison with 2 m^3 scale for the SUT facility. For the SST facility the selected categories are:

- Total CO_2 from commuting workers (10.11 Commuting – preliminary calculations see for details on calculations)
- Total media/buffer consumption (see *10.1 Upstream process – preliminary calculations* and *10.2 Downstream process – preliminary calculations* for details on calculations)
- Total CO_2 output of CIP operations (see 10.5 CIP –preliminary calculations for details on calculations
- Total CO_2 output of SIP operations (see *10.4 SIP - preliminary calculations* for details on calculations)
- Total CO_2 from steel production (see *10.6 Steel tank dimensioning – preliminary calculations* and *10.15 Tank diameter/height – preliminary calculations* for details on calculations)
- CO_2 from heating of production bioreactor medium (see *10.9 Tank heating/cooling – preliminary calculations* for details on calculations)
- CO_2 from cooling after harvest (see *10.9 Tank heating/cooling – preliminary calculations* for details on calculations)
- Total HVAC CO_2 emissions (see *10.7 HVAC – preliminary calculations* for details on calculations)

For the SUT facility the selected categories are:

- Total CO_2 from commuting workers (10.11 Commuting – preliminary calculations see for details on calculations)
- Total buffer/media consumption (see *10.1 Upstream process – preliminary calculations* and *10.2 Downstream process – preliminary calculations* for details on calculations)
- CO_2 from bag incineration (see 10.12 Single-use bag production and incineration for details on calculations)
- CO_2 from bag production (see 10.12 Single-use bag production and incineration for details on calculations)
- CO_2 from buffer transport via trucks (see 10.14 Cargo transport emission – preliminary calculations for details on calculations)
- Electricity demand of autoclave operations (see 10.10 Autoclaving of single-use equipment – Preliminary calculations for details on calculations)
- CO_2 from heating of production bioreactor medium (see 10.9 Tank heating/cooling – preliminary calculations for details on calculations)
- CO_2 from cooling after harvest (see 10.9 Tank heating/cooling – preliminary calculations)

- Total HVAC CO_2 emissions (see 10.7 HVAC – preliminary calculations for details on calculations)

The carbon emissions of all categories are calculated according to the chapters listed above. The emissions for all categories are summed up. Each individual category is divided by the sum and multiplied by one hundred to receive the percentage distribution. Facilities operate on the average Swiss electricity grind mix.

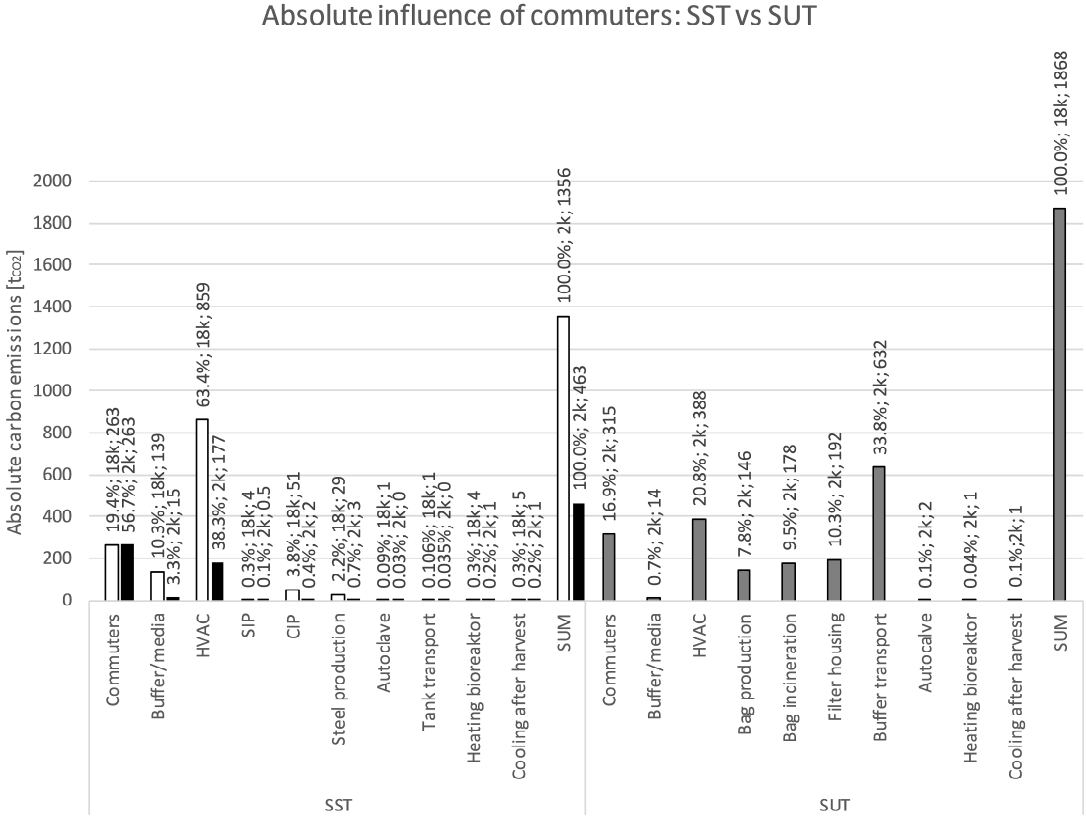


Figure 52 Absolute carbon emission of different categories for SST and SUT facilities including commuting workers.

Figure 52 shows that commuters account for 56.7% of the total carbon emissions for a 2k SST facility, followed by HVAC with 38.3%, followed by buffer/media preparation (3.3%). All other categories fall below the 1% mark. For the 2k SUT facility buffer transport accounts for 33.8% of the total carbon emissions, followed by HVAC (20.8%), followed by commuters (16.9%), followed by the production and incineration of depth filtration filter housing (10.3%), followed by bag incineration (9.5%), followed by bag production (2.8%). Each of the remaining categories account for less than 1% of the total carbon emissions. For a 18k SST facility the commuters account for 19.4% of the total

carbon emissions, while HVAC accounts for 63.4% of the total carbon emissions and buffer/media preparation accounts for 10.3% of the total carbon emissions. CIP accounts for 3.8%, steel production for 2.2%. The remaining categories are all below the 1% mark.

Normalized values

The normalization of carbon emissions on the mass of mAbs allows for direct comparison between the facilities.

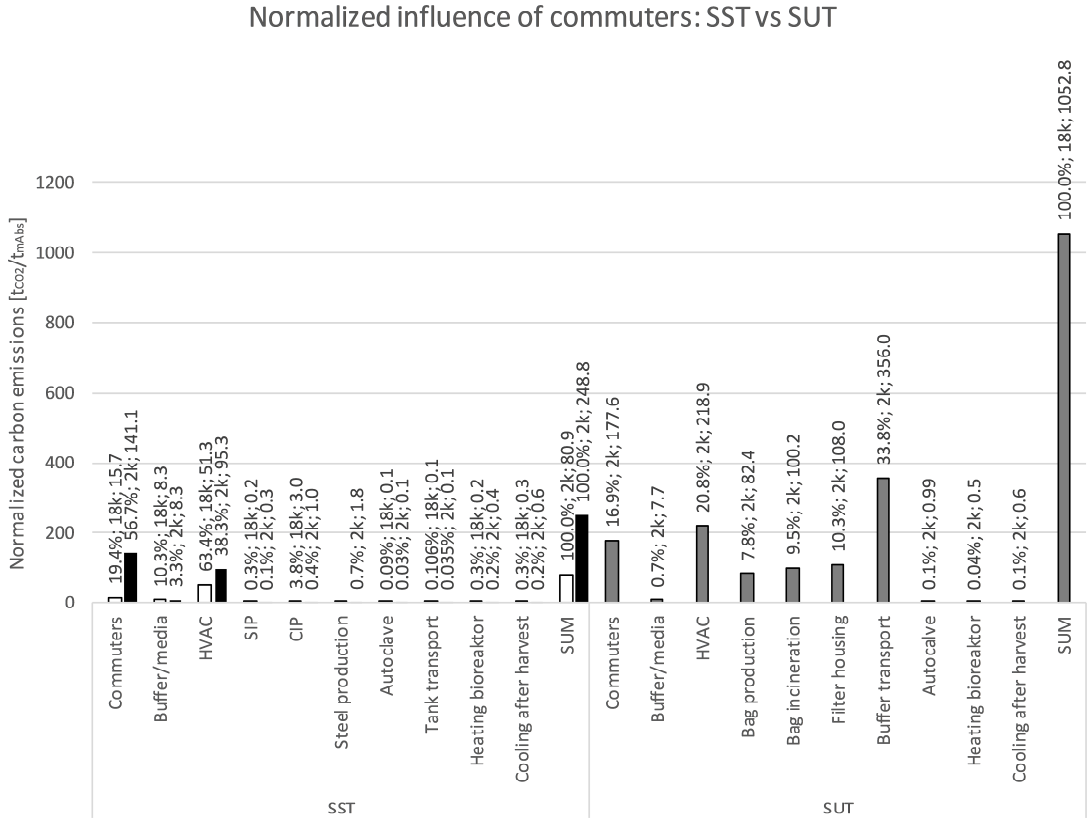


Figure 53 Normalized carbon emissions for commuting workers in comparison with technology specific categories. Black bars represent the 2k SST facility, grey bars represent the 2k SUT facility and white bars represent the 18k SUT facility.

Figure 53 shows the normalized carbon emissions for a 2k SST, 2k SUT and an 18k SST facility. Commuters emit 141.1 t_{CO2} for every ton of mAbs that is produced for the 2k SST facility and 15.7 t_{CO2} for the 18k SST facility, while the commuters of the 2k SUT facility emit 177.6 t_{CO2} per ton of mAbs that is produced (16.9%). For the 2k SUT facility, HVAC emits 218.9 tons of carbon dioxide for every ton of mAbs (20.8%), buffer transport emits 356.0 tons of CO_2 for every ton of mAbs (33.8%), the production and incineration of filter

housing emits 108.0 t_{CO2}/t_{mAb} (10.3%), bag incineration emits 100.2 t_{CO2}/t_{mAb} (9.5%) and bag production emits 82.4 t_{CO2}/t_{mAb} (7.8%). All other categories fall below the 1% mark.

Influence of German electricity

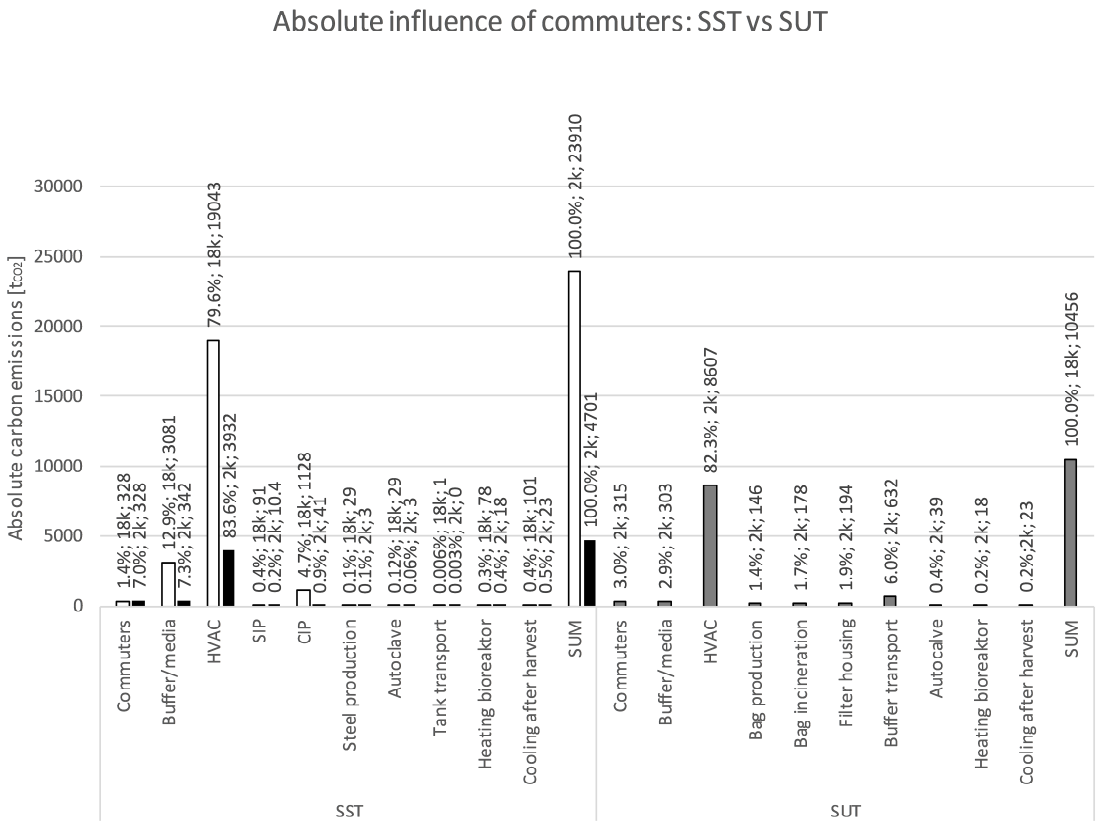


Figure 54 Absolute carbon emissions of commuters in comparison with other carbon emission categories for 2k SST (black), 18k SST (white) and 2k SUT (grey) facilities running on the average German grind mix.

Figure 54 shows the influence of commuting workers in comparison with other impact categories. For the 2k SST facility commuters account for 328 t_{CO2} (7.0%), HVAC accounts for 3932 t_{CO2} (83.6%), buffer/media accounts for 342 t_{CO2} (7.3%), resulting in a total of 4701 t_{CO2} .All other categories fall below the 1% mark. The 2k SUT facility emits 315 t_{CO2} (3.0%) for commuters, 303 (2.9%) for buffer/media, 8607 t_{CO2} (82.3%), 146 t_{CO2} (1.4%) for bag production, 178 t_{CO2} (1.7%) for bag incineration, 194 (1.9%) for filter housing production/incineration and 632 (6.0%) for buffer transport, resulting in a total of 10456 t_{CO2}. All other categories fall below the 1% mark. For the 18k SST facility the distribution is 328 t_{CO2} (1.4%) commuters, 3081 t_{CO2} (12.9%) buffer/media, 19043 t_{CO2}

(79.6%) for HVAC, 1128 t_{CO2} (4.7%) for CIP, resulting in a total of 23910 t_{CO2}. All other categories fall below the 1% mark. With increasing scale, the influence of commuters is reduced from 7.0% to 1.4% as other categories (mainly HVAC) gain in significance.

Normalized values

Normalized values allow for direct comparison between different facilities.

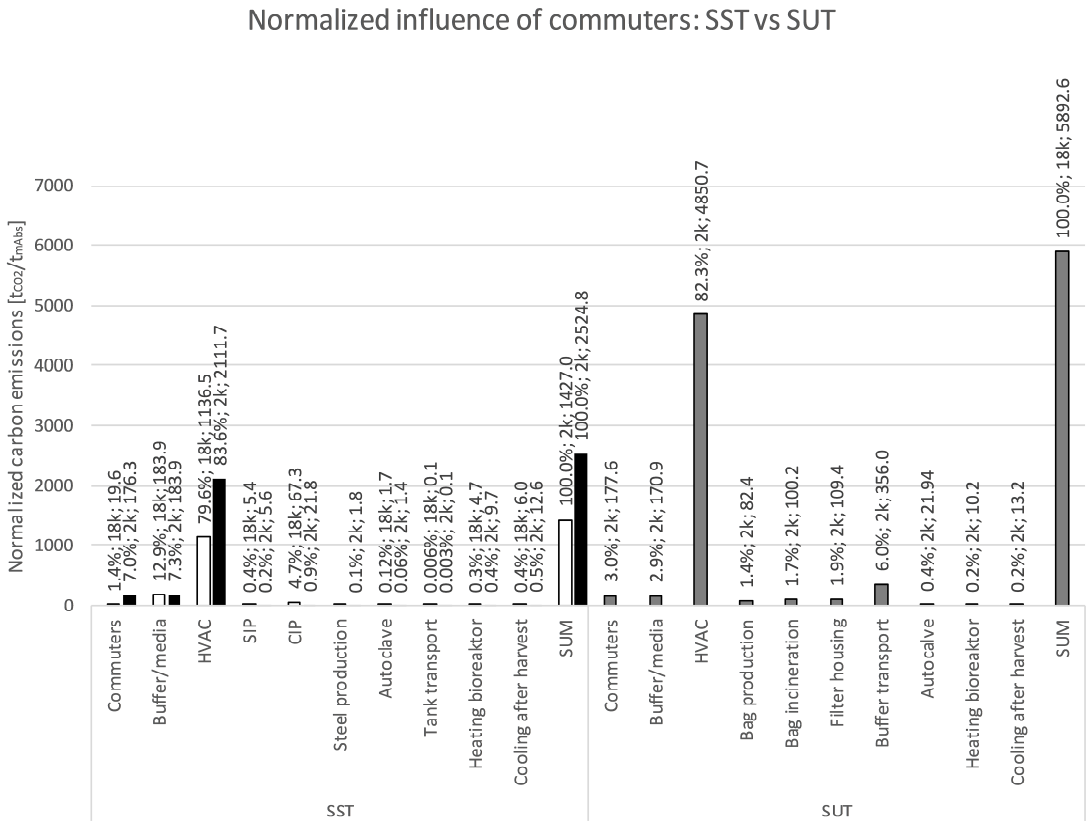


Figure 55 Normalized values for carbon emissions of commuters and other categories for 2k SST (black), 2k SUT (grey) and 18k SST (white) facilities.

Figure 55 shows the normalized values for a 2k SUT, 2k SST and an 18k SST facility. The commuters of the 2k SST facility emit 176.3 t_{CO2} for every ton of mAbs that is produced. Buffer/ media emits 183.9 t_{CO2}/t_{mAb} while HVAC emits 2111.7 t_{CO2}/t_{mAb}. All other categories fall below the 1% mark. The 2k SUT facility emits 177.6 t_{CO2} per ton of produced mAbs for commuters. Buffer/media emits 170.9 t_{CO2}/t_{mAb}, HVAC emits 4850.7 t_{CO2}/t_{mAb}, bag production emits 82.4 t_{CO2}/t_{mAb}, bag incineration emits 100.2 t_{CO2}/t_{mAb}, the production/incineration of filter housing emits 109.4 t_{CO2}/t_{mAb} and buffer transport emits356.0 t_{CO2}/t_{mAb}. All other categories fall below the 1% mark. The commuters of the 18k SST facility emits 19.6 t_{CO2} for every ton of produced mAbs.

Buffer/media emits 183.9 t_{CO2}/t_{mAb}, HVAC emits 1136.5 t_{CO2}/t_{mAb} and CIP emits 67.3 t_{CO2}/t_{mAb}. All other categories fall below the 1% mark.

The total carbon emissions for every ton of produced mAbs is: 2524.8 t_{CO2}/t_{mAb} for the 2K SST facility, 5892.6 t_{CO2}/t_{mAb} for the 2k SUT facility and 1427.0 t_{CO2}/t_{mAb} for the 18k SST facility. The direct comparison between SST and SUT is not meaningful since different categories influence the total carbon emissions. The graph aims to highlight the categories that have the largest contribution to carbon emissions.

Swiss vs German electricity

The comparison between Figure 53 and Figure 55 shows that on average the facilities running on German electricity emits more carbon dioxide than its Swiss counterparts do:

Electricity	**2k SST [t_{CO2}/t_{mAb}]**	**2k SUT [t_{CO2}/t_{mAb}]**	**18k SST [t_{CO2}/t_{mAb}]**
Switzerland	248.8	1052.8	80.9
Germany	2524.8	5892.6	1427.0

These numbers show that the local average electricity grind mix affects the carbon emissions per ton of produced product since electricity is used for almost all processing steps as well as ancillary systems like HVAC or WFI generation. HVAC optimization is crucial, as its operation is especially carbon intensive:

Electricity	**2k SST [t_{CO2}/t_{mAb}]**	**2k SUT [t_{CO2}/t_{mAb}]**	**18k SST [t_{CO2}/t_{mAb}]**
Switzerland	95.3	23.2	51.3
Germany	2111.7	624	1427.0

7.4 Case study 2: results for comparison between Basel, Boston and Shanghai

This case study aims to highlight aspects that are relevant for a SST/SUT facility based in Basel (Switzerland), Boston (USA) or Shanghai (China). Different weather data influence HVAC dimensioning, while average electricity efficiency factors influences the conversion of energy usage to carbon emissions. The setup for this case study is the same as in *7 Results*

The results are based on facilities located in Basel, Boston and Shanghai. Presented asepects are water consumption, CIP and SIP in comparison with single-use bag production and incineration, HVAC as well as the influence of commuting employees.

7.3 Case study 1: results for the facility in Basel but HVAC dimensioning is based on weather data for Basel, Boston and Shanghai from the year 2018. Instead of using the Swiss commuters model that was described in *10.11 Commuting – preliminary calculations*, it is assumed that all employees commute to the facilities via car. Emission factors for steel production are Germany for Basel, USA for Boston and China for Shanghai.

Average weather conditions for the year 2018:

- Basel:
  - average temperature: 13.41°C
  - average relative humidity: 67.10%
- Boston
  - average temperature: 11.57°C
  - average relative humidity: 67.00%
- Shanghai
  - average temperature: 17.26°C
  - average relative humidity: 78.93%

Normalized values are used for direct comparison. The current limitation in bioreactor volume for the SUT facility to 2000 L only allows for a numbering up instead of a scale up approach. While the numbering up approach is able to increase total mass of mAbs that is produced, the normalized carbon emissions will remain constant.

7.4.1 Water consumption

The normalized water consumption for each location is used to allow a direct comparison between the facilities of the same scale.

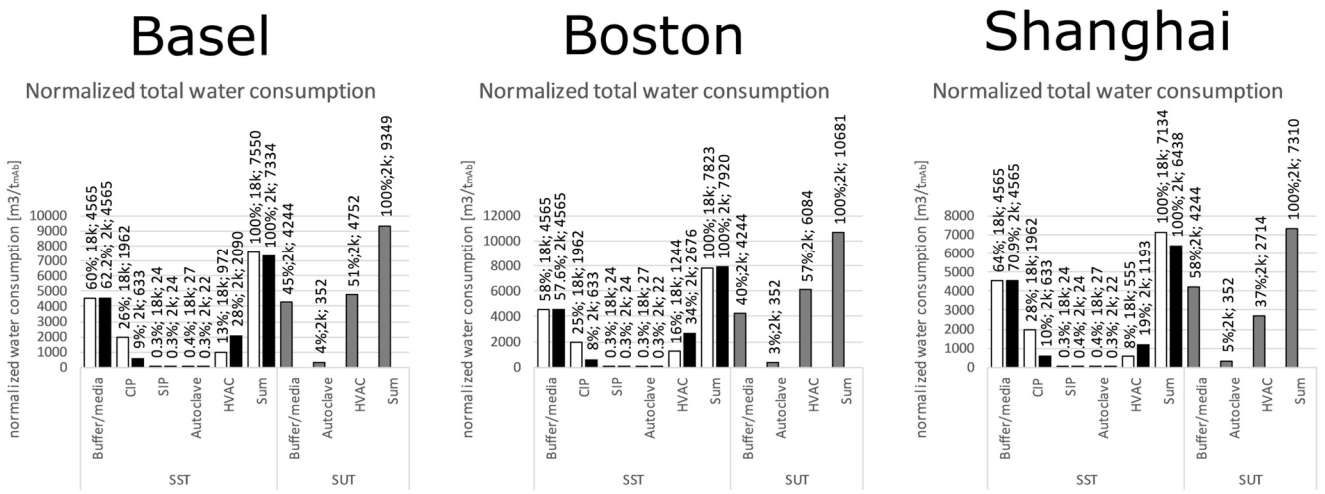


Figure 56 Normalized water consumption for a 2k SST (black), 2k SUT (grey) and a 18k SST (white) facility in Basel, Boston and Shanghai.

All categories except HVAC are location independent. The carbon emissions per ton of produced mAbs for the 2k SST facility is 2090 m^3_{water}/t_{mAb} (Basel), 2676 m^3_{water}/t_{mAb} (Boston) and 1193 m^3_{water}/t_{mAb} (Shanghai). As a result of yearly weather conditions the facility in Shanghai consumes the least amount of water, followed by Basel and Boston. Shanghai is the closest to the desired clean room conditions of 20°C and 60% relative humidity. With an average yearly relative humidity of 78.93%, the addition of WFI for humidification is reduced but excess water has to be removed to not exceed the desired 60% relative humidity.

7.4.2 CIP+SIP in comparison with bag usage

The production and incineration of bags is location independent, whereas CIP and SIP are influenced by the location specific electricity emission factor. The generation of RO water for CIP and SIP and WFI for CIP require electrical energy. The heating of water to the desired temperature or the generation of steam for SIP results in additional electricity consumption.

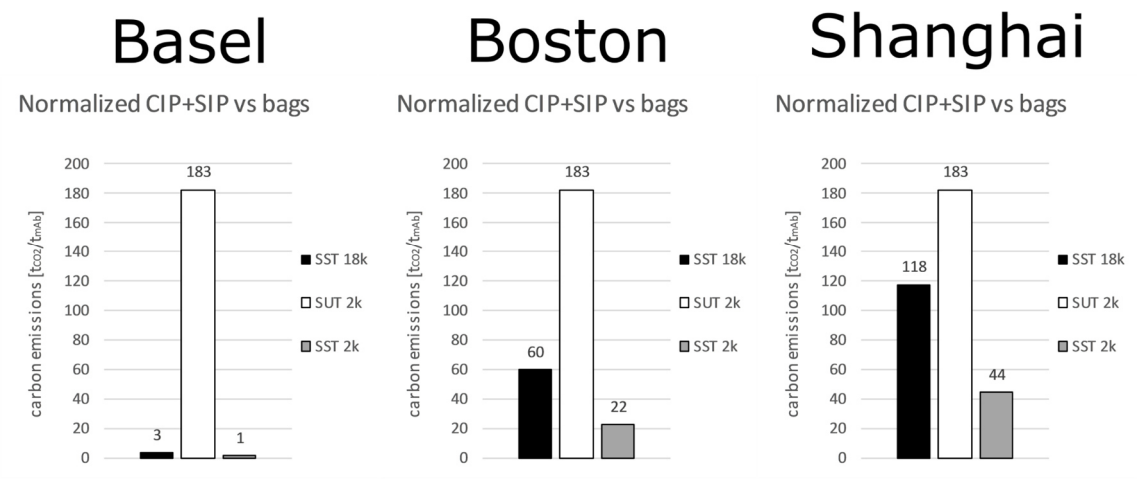


Figure 57 Comparison of the carbon emissions of CIP and SIP procedure of SST facilities with bag production and incineration in SUT facilities. 18k SST in black, 2k SUT in grey and 2k SUT in white.

Figure 57 shows the normalized carbon emissions (t_{CO2}/t_{mAb}) for the three different locations for the three different facilities. The white bar that represents the 2k SUT facility is location independent and is therefore constant. The 2k SST facility in Basel emits 1 t_{CO2}/t_{mAb}, the facility in Boston 22 t_{CO2}/t_{mAb} and the facility in Shanghai 44 t_{CO2}/t_{mAb}. The182k SST facility in Basel emits 3 t_{CO2}/t_{mAb}, the facility in Boston 60 t_{CO2}/t_{mAb} and the facility in Shanghai 118 t_{CO2}/t_{mAb}. This increase is a result of increased water demand due to scale up from 2k to 18k in combination with increasingly carbon intensive electricity emission factors (Basel: 23.6 g_{CO2}/kWh, Boston 428 g_{CO2}/kWh, Shanghai 845 g_{CO2}/kWh. The normalized carbon emissions for CIP are based on the assumption that every tank has to be CIPed after each batch. In reality it is common practice to CIP tanks after several campaigns. This results in reduction in water usage and energy consumption.

7.4.3 HVAC

The comparison of commuting employees with other categories (e.g. HVAC) in *7.3.4 Commuting employees* showed the large impact of HVAC ton the normalized carbon emissions of a facility. HVAC consumes electrical energy for air conditioning (adjustment of temperature, humidity and pressure) and for fan operation to achieve the desired air change rates according to the clean room class.

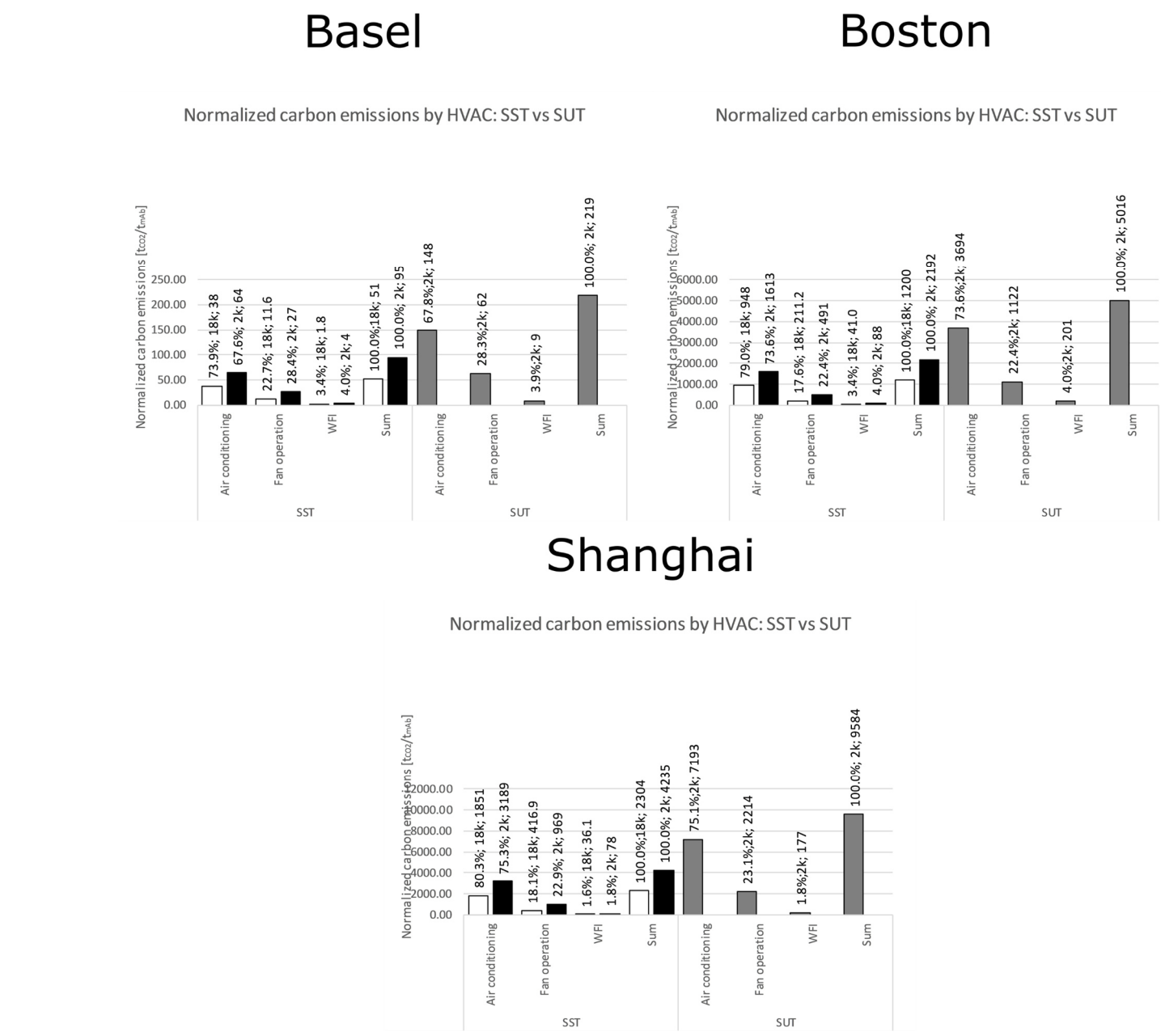


Figure 58 Normalized carbon emissions for different locations (Basel, Boston, Shanghai) and facilities. 2k SST in black, 2k SUT in grey and 18k SST in white.

Figure 58 shows a comparison of the HVAC carbon emissions for Basel, Boston and Shanghai. The 2k SST facility emits 64 t_{CO2}/t_{mAb} in Basel, 1613 t_{CO2}/t_{mAb} in Boston and 3189 t_{CO2}/t_{mAb} in Shanghai for air conditioning. Fan operation emits 27 t_{CO2}/t_{mAb} in Basel, 491 t_{CO2}/t_{mAb} in Boston and 969 t_{CO2}/t_{mAb} in Shanghai. WFI allocation emits 4 t_{CO2}/t_{mAb} in Basel, 88 t_{CO2}/t_{mAb} in Boston and 78 t_{CO2}/t_{mAb} in Shanghai. The total HVAC emissions for the 2k SST facility are 95 t_{CO2}/t_{mAb} in Basel, 2192 t_{CO2}/t_{mAb} in Boston and 4235 t_{CO2}/t_{mAb} in Shanghai.

The same 2k SST facility in Boston emits the 23-fold in carbon for HVAC operations compared to the facility in Basel:

$$\frac{2192[\frac{t_{CO2}}{t_{mAb}}]}{95[\frac{t_{CO2}}{t_{mAb}}]} = 23.0\overline{7} \approx 23$$

The 2k SST facility in Shanghai emits the 45-fold in carbon HVAC operations compared to the facility in Basel:

$$\frac{4235[\frac{t_{CO2}}{t_{mAb}}]}{95[\frac{t_{CO2}}{t_{mAb}}]} = 44.\overline{58} \approx 45$$

The 2k SST facility in Boston emits the 2-fold in carbon HVAC operations compared to the facility in Basel:

$$\frac{4235[\frac{t_{CO2}}{t_{mAb}}]}{2192[\frac{t_{CO2}}{t_{mAb}}]} = 1.93 \approx 2$$

The 2k SUT facility emits 148 t_{CO2}/t_{mAb} in Basel, 364 t_{CO2}/t_{mAb} in Boston and 7193 t_{CO2}/t_{mAb} in Shanghai for air conditioning. Fan operation emits 62 t_{CO2}/t_{mAb} in Basel, 1122 t_{CO2}/t_{mAb} in Boston and 2214 t_{CO2}/t_{mAb} in Shanghai. WFI allocation emits 9 t_{CO2}/t_{mAb} in Basel, 201 t_{CO2}/t_{mAb} in Boston and 177 t_{CO2}/t_{mAb} in Shanghai. The total HVAC emissions for the 2k SUT facility are 219 t_{CO2}/t_{mAb} in Basel, 5016 t_{CO2}/t_{mAb} in Boston and 9584 t_{CO2}/t_{mAb} in Shanghai.

The same 2k SUT facility in Boston emits the 23-fold in carbon for HVAC operations compared to the facility in Basel:

$$\frac{5016[\frac{t_{CO2}}{t_{mAb}}]}{219[\frac{t_{CO2}}{t_{mAb}}]} = 22.\overline{90} \approx 23$$

The 2k SUT facility in Shanghai emits the 44-fold in carbon HVAC operations compared to the facility in Basel:

$$\frac{9584[\frac{t_{CO2}}{t_{mAb}}]}{219[\frac{t_{CO2}}{t_{mAb}}]} = 43.\overline{76} \approx 44$$

The 2U SST facility in Shanghai emits the 2-fold in carbon HVAC operations compared to the facility in Boston:

$$\frac{9584[\frac{t_{CO2}}{t_{mAb}}]}{5016[\frac{t_{CO2}}{t_{mAb}}]} = 1.\overline{91} \approx 2$$

The 18k SST facility emits 38 t_{CO2}/t_{mAb} in Basel, 948 t_{CO2}/t_{mAb} in Boston and 1851 t_{CO2}/t_{mAb} in Shanghai, for air conditioning. Fan operation emits 11.6 t_{CO2}/t_{mAb} in Basel, 211.2 t_{CO2}/t_{mAb} in Boston and 416.9 t_{CO2}/t_{mAb} in Shanghai. WFI allocation emits 1.8 t_{CO2}/t_{mAb} in Basel, 41.0 t_{CO2}/t_{mAb} in Boston and 36.1 t_{CO2}/t_{mAb} in Shanghai. The total HVAC emissions for the 18k SST facility are 51 t_{CO2}/t_{mAb} in Basel, 1200 t_{CO2}/t_{mAb} in Boston and 2304 t_{CO2}/t_{mAb} in Shanghai.

The same 2k SUT facility in Boston emits the 23-fold in carbon for HVAC operations compared to the facility in Basel:

$$\frac{1200[\frac{t_{CO2}}{t_{mAb}}]}{51[\frac{t_{CO2}}{t_{mAb}}]} = 22.\overline{90} \approx 23$$

The 2k SUT facility in Shanghai emits the 45-fold in carbon HVAC operations compared to the facility in Basel:

$$\frac{2304[\frac{t_{CO2}}{t_{mAb}}]}{51[\frac{t_{CO2}}{t_{mAb}}]} = 45.\overline{18} \approx 45$$

The 2k SST facility in Shanghai emits the 2-fold in carbon HVAC operations compared to the facility in Boston:

$$\frac{2304[\frac{t_{CO2}}{t_{mAb}}]}{1200[\frac{t_{CO2}}{t_{mAb}}]} = 1.92 \approx 2$$

Geographical location and its associated weather together with the local average electricity grind emission factor influence the HVAC systems carbon footprint. For all three facility types the ranking from least to most carbon intensive is Basel, Boston, Shanghai.

7.4.4 Commuting employees

The influence of commuters on the total normalized carbon emission is location dependant. The scale of influence is highlighted for three different locations (Basel, Boston, and Shanghai) as well as three different facilities (2k SST, 2k SUT, 18k SST). Figure 59, Figure 60 and Figure 61 show the influence of commuters in relation to other categories. One hundred twenty employees commute 30 km (roundtrip) by car to the SST facility, while 144 employees commute 30 km (roundtrip) by car to the SUT facility. See *10.11 Commuting – preliminary calculations* for more details on the calculations

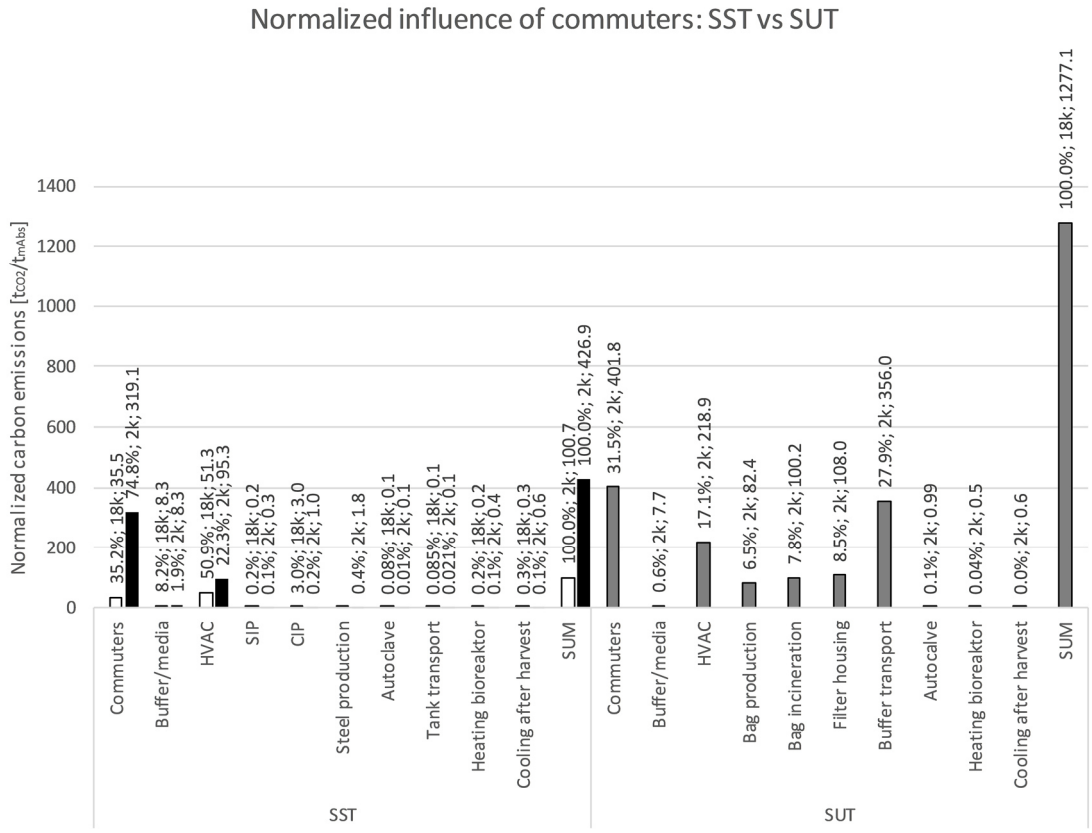


Figure 59 Influence of commuters on overall carbon emissions for the facilities in Basel. 2k SST (black), 2k SUT (grey) and 18k SST (white). 120 employees commute to work by car for the SST facility. 144 employees commute to work by car for the SUT facility.

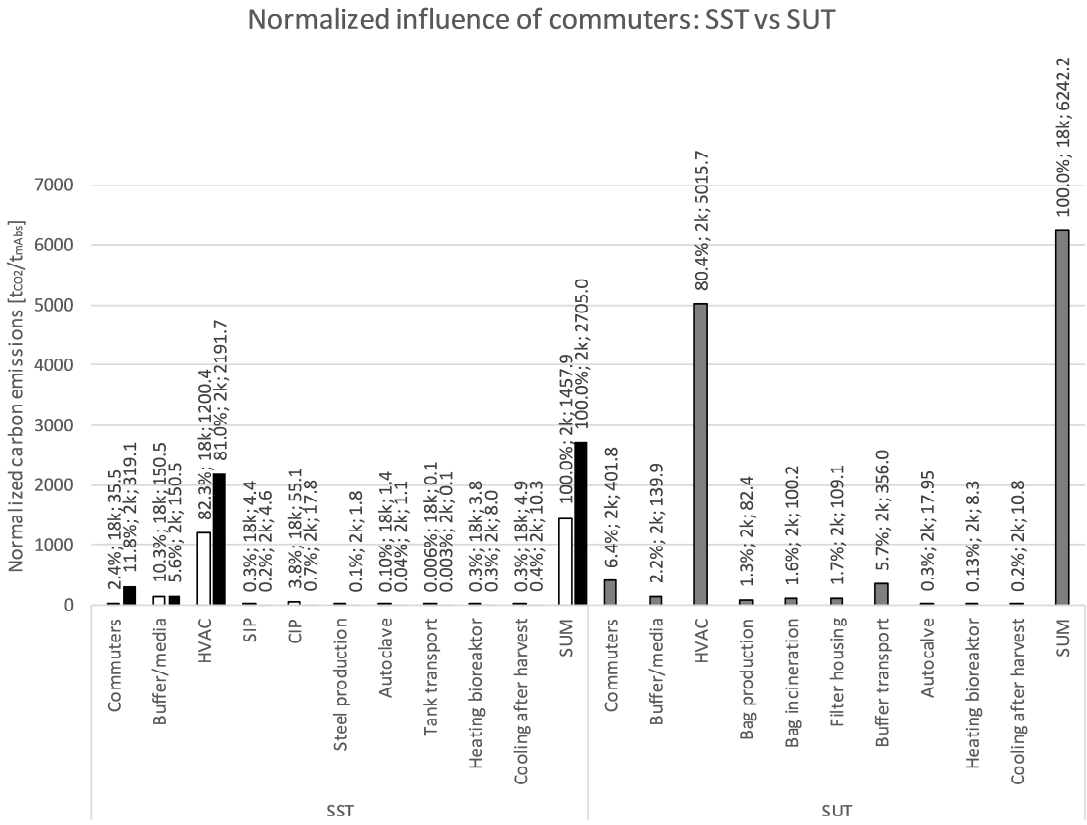


Figure 60 Influence of commuters on overall carbon emissions for the facilities in Boston. 2k SST (black), 2k SUT (grey) and 18k SST (white). 120 employees commute to work by car for the SST facility. 144 employees commute to work by car for the SUT facility.

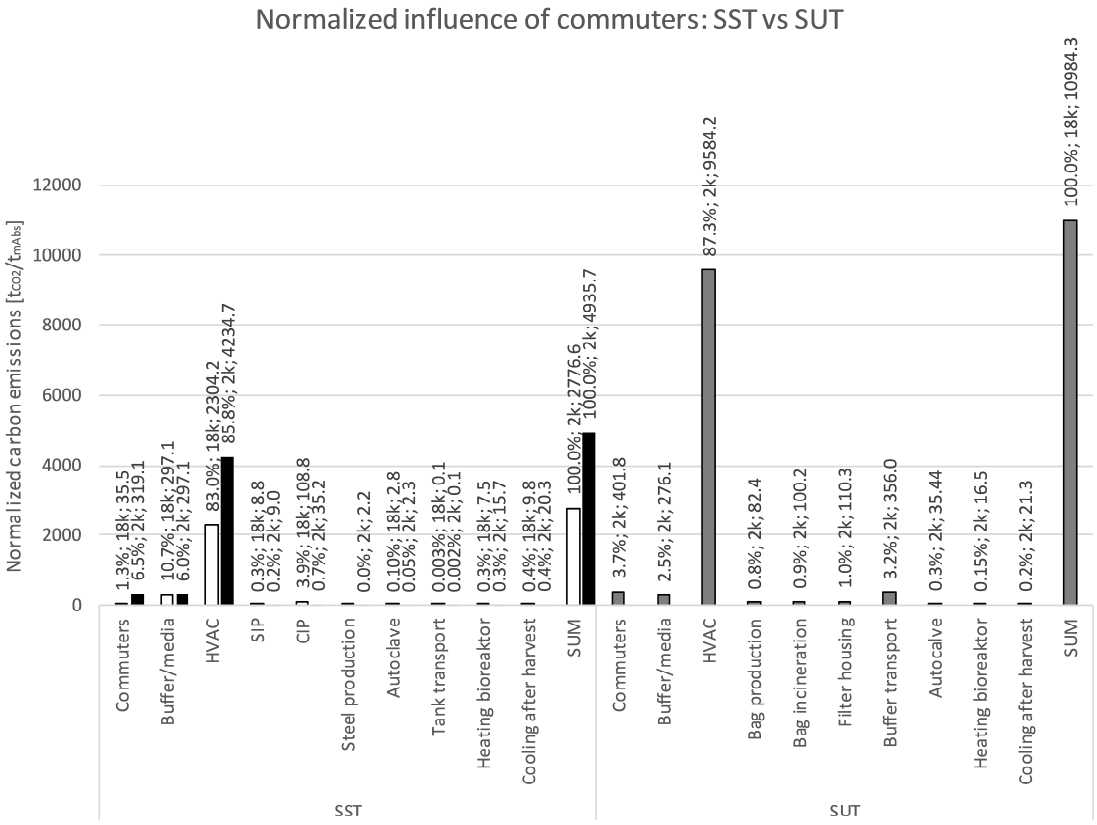


Figure 61 Influence of commuters on overall carbon emissions for the facilities in Shanghai. 2k SST (black), 2k SUT (grey) and 18k SST (white). 120 employees commute to work by car for the SST facility. 144 employees commute to work by car for the SUT facility.

Figure 59, Figure 60 and Figure 61 show that commuters have a larger impact on the total normalized carbon emissions for Basel, when compared to Boston and Shanghai.

	Basel	**Boston**	**Shanghai**
2k SST	74.8%	11.8%	6.5%
2k SUT	31.5%	6.4%	3.7%
18k SST	35.2%	2.4%	1.3%

In Shanghai the commuters account for less of the total normalized carbon emissions when compared with Basel and Boston. This is true for all three facilities (2k SST, 2k SUT and 18k SST). The combination of geographical location and local electricity emission factor influence the importance of commuter behaviour. In regions with low electricity emission factors (e.g. Basel) the commuters share in total carbon emissions is larger when compared to regions with more carbon intensive electricity emission factors (e.g. Boston or Shanghai).

7.4.5 Absolute/normalized carbon emissions- system output

The system output of all categories listed in *10.23 Exemplary output data (case study one) (SST: mint green/SUT: light blue)* is summed up and presented in two graphs, showing the absolute carbon emissions and the normalized carbon emissions for Basel, Boston and Shanghai. The graphs present the values for a 2k SST, 2k SUT and a 18k SST facility.

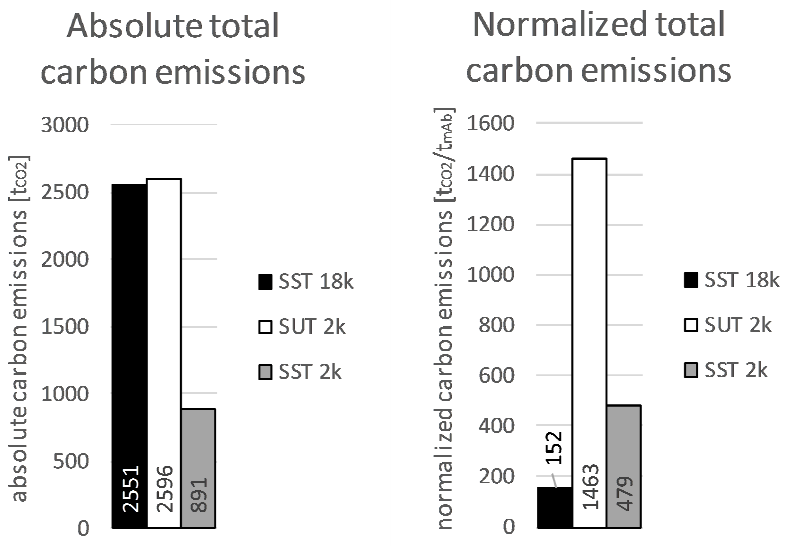


Figure 62Sum of absolute and normalized carbon emissions for a 2k SST (grey), 2k SUT (white) and a 18k SST (black) based in Basel.

The absolute total carbon emission for the facility in Basel over the course of 5 years is 2551 t_{CO2} for a 18k SST facility, 2596 t_{CO2} for a 2k SUT facility and 891 t_{CO2} for a 2k SST facility. Normalized total carbon emissions for the 18k SST facility are 152 t_{CO2}/t_{mAb}, 1463 for the 2k SUT facility and 478 for the 2k SST facility.

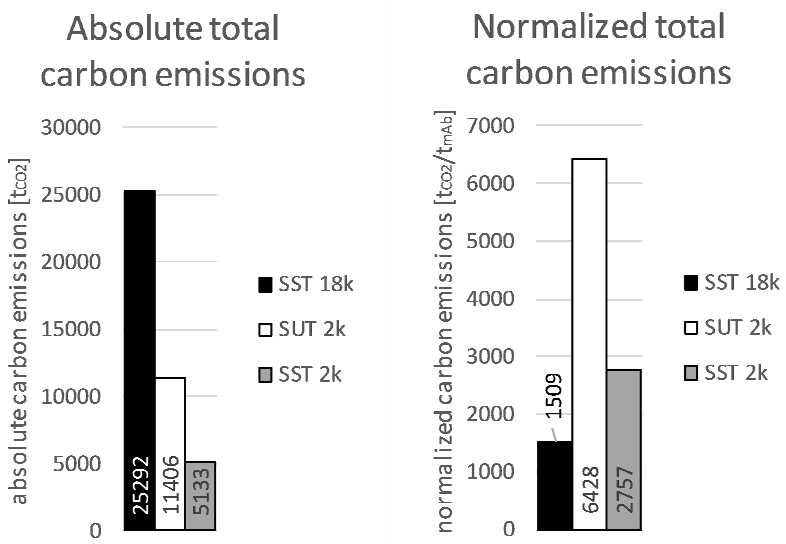


Figure 63 Sum of absolute and normalized carbon emissions for a 2k SST (grey), 2k SUT (white) and a 18k SST (black) based in Boston.

The absolute total carbon emission for the facility in Boston over the course of 5 years are 25292 t_{CO2} for a 18k SST facility, 11406 t_{CO2} for a 2k SUT facility and 5133 t_{CO2} for a 2k SST facility. Normalized total carbon emissions for the 18k SST facility are 1059 t_{CO2}/t_{mAb}, 6428 for the 2k SUT facility and 2757 for the 2k SST facility.

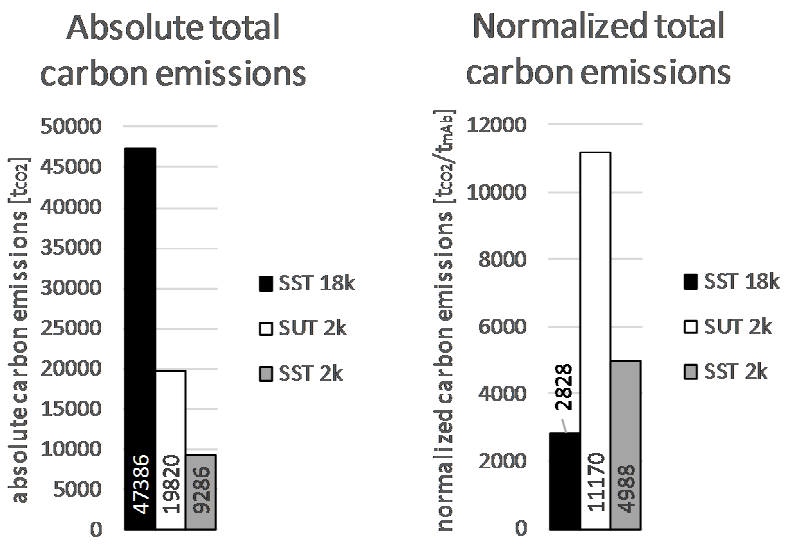


Figure 64 Sum of absolute and normalized carbon emissions for a 2k SST (grey), 2k SUT (white) and a 18k SST (black) based in Shanghai.

The absolute total carbon emission for the facility in Shanghai over the course of 5 years are 47286 t_{CO2} for a 18k SST facility, 19820 t_{CO2} for a 2k SUT facility and 9286 t_{CO2} for a 2k SST facility. Normalized total carbon emissions for the 18k SST facility are 2828 t_{CO2}/t_{mAb}, 11170 for the 2k SUT facility and 4988 for the 2k SST facility.

A comparison between the three locations and the three facility types (2k SUT, 2k SST, 18k SST) shows the following results:

<u>2k SUT</u>

The 2k SUT facility in Boston emits the 4-fold in carbon emissions when compared to the same facility in Basel:

$$\frac{6428\left[\frac{t_{CO2}}{t_{mAb}}\right]}{1463\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 4.\overline{39} \approx 4$$

The 2k SUT facility in Shanghai emits the 8-fold in carbon emissions to produce the same amount of mAbs when compared to the same facility in Basel:

$$\frac{11170\left[\frac{t_{CO2}}{t_{mAb}}\right]}{1463\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 7.\overline{63} \approx 8$$

2k SST

The 2k SST facility in Shanghai emits the 6-fold in carbon emissions to produce the same amount of mAbs when compared to the same facility in Basel:

$$\frac{2757\left[\frac{t_{CO2}}{t_{mAb}}\right]}{479\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 5.76 \approx 6$$

The 2k SST facility in Shanghai emits the 6-fold in carbon emissions to produce the same amount of mAbs when compared to the same facility in Basel:

$$\frac{2757\left[\frac{t_{CO2}}{t_{mAb}}\right]}{479\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 5.76 \approx 6$$

18k SST

The 18k SST facility in Shanghai emits the 10-fold in carbon emissions to produce the same amount of mAbs when compared to the same facility in Basel:

$$\frac{1509\left[\frac{t_{CO2}}{t_{mAb}}\right]}{152\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 9.93 \approx 10$$

The 18k SST facility in Shanghai emits the 19-fold in carbon emissions to produce the same amount of mAbs when compared to the same facility in Basel:

$$\frac{2828\left[\frac{t_{CO2}}{t_{mAb}}\right]}{152\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 18.6\overline{1} \approx 19$$

2k SUT vs 2k SST

On average, a 2k SUT shows a 2-fold increase in CO_2 emissions for the same amount of produced mAbs when compared to its 2k SST counterpart:

$$\frac{\sum 2k\ SUT}{\sum 2k\ SST} = \frac{2k\ SUT_{Basel} + 2k\ SUT_{Boston} + 2k\ SUT_{Shanghai}}{2k\ SST_{Basel} + 2k\ SST_{Boston} + 2k\ SST_{Shanghai}}$$
$$= \frac{1463\left[\frac{t_{CO2}}{t_{mAb}}\right] + 6428\left[\frac{t_{CO2}}{t_{mAb}}\right] + 11170\left[\frac{t_{CO2}}{t_{mAb}}\right]}{479\left[\frac{t_{CO2}}{t_{mAb}}\right] + 2757\left[\frac{t_{CO2}}{t_{mAb}}\right] + 4988\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 2.32 \approx 2$$

2k SUT vs 18k SST

On average, a 2k SUT shows a 2-fold increase in CO_2 emissions for the same amount of produced mAbs when compared to a 18k SST facility:

$$\frac{\sum 2k\ SUT}{\sum 18k\ SST} = \frac{2k\ SUT_{Basel} + 2k\ SUT_{Boston} + 2k\ SUT_{Shanghai}}{2k\ SST_{Basel} + 2k\ SST_{Boston} + 2k\ SST_{Shanghai}}$$
$$= \frac{1463\left[\frac{t_{CO2}}{t_{mAb}}\right] + 6428\left[\frac{t_{CO2}}{t_{mAb}}\right] + 11170\left[\frac{t_{CO2}}{t_{mAb}}\right]}{152\left[\frac{t_{CO2}}{t_{mAb}}\right] + 1509\left[\frac{t_{CO2}}{t_{mAb}}\right] + 2828\left[\frac{t_{CO2}}{t_{mAb}}\right]} = 4.25 \approx 4$$

8 Discussion

The calculations from chapter six are compiled and interpreted. Comparison between the absolute carbon emissions of the SST facilities (2k and 18k) show an increase according to the scale up of the bioreactor capacity. All upstream- and downstream operations are accustomed to the bioreactor capacity. As scale increases, the water requirement for CIP and SIP increases accordingly to the growth in tank diameter and tank mass. Increased water consumption involves increased energy consumption for WFI, RO water and steam generation. Facility footprint grows from 720 m^2 (2k SST) to 1547 m^2 (18k SST) and results in rinsing WFI demand for humidification and energy demand in form of electricity for fan operation.

For the 2k SUT facility the footprint remains at 850 m^2. Absolute carbon emissions are directly linked to the local electricity emission factor and local weather. The 2k SUT facilities' absolute carbon emissions increase the more carbon intensive electricity is used. The 2k SUT facility has a larger footprint when compared to the 2k SST facility. This is the result of increased warehouse are for buffer storage to prevent production losses. A key role in HVAC energy consumption is the clean room class specific air change rate. While all buffer and media tanks can be located in a separate area (CNC for majority of the tanks), all buffer and media bags have to be allocated to the clean room class where unit operations take place. In terms of carbon emissions from commuting employees the lacking automation as well as the increased demand in warehousing labour and QC staff for buffer/media control further increases the carbon footprint of the SUT facility.

Absolute carbon emissions do not take into account the increase in produced product. When absolute values are compared with normalized values, the increased water consumption or carbon emissions are justified by the growing mass of produced mAbs. Normalized values for the 18k SST facility show a better carbon footprint when compared with the 2k SST facility. For all three locations that are in this work, the normalized carbon footprint of the SUT facilities always remains higher than the one of the SST facilities.

The growing water and energy demand for CIP as a result of increasing total bioreactor capacity is a possible bottleneck for SST facilities with bioreactors beyond 18000 L capacity. The CIP process uses RO water for 4 out of five rinse cycles, contributing the majority of water usage. The simplified model to estimate the carbon footprint of RO water generation is based on tap water from Basel and does not account for additional pressure drop besides the Ro filter cartridge. The energy demand of RO water generation is directly linked to feed water quality. The energy demand of the pump correlates with the solutes in the feed water, that is location dependant. The carbon footprint of the RO filter media itself is also not included.

The model to calculate energy demand and carbon emissions of the HVAC system do not assign individual rooms for upstream and downstream operations but rather categorize in processing tanks/bags, media/buffer/ equipment. This leaves room for improvement to design SST and SUT facilities with accordingly structured room layout.

The measurement of carbon dioxide and carbon dioxide equivalents was not respected in this work due to a lack of available data (e.g. emission factors) and the fact that several emission factors were calculated while only respecting carbon dioxide emissions and ignoring all other possible greenhouse gas emissions as well as the occurrence of other environmentally hazardous side products. One example is the incineration of single-use plastic bags, where full oxidation of polyethylene was assumed while in reality there are several additional emissions that possess global warming potential. Future release of data, published as carbon dioxide equivalents, would resolve this issue.

The SST facility design includes all necessary tanks for buffer/media storage but does not include a dedicated buffer manufacturing area, that requires additional tanks and dedicated HVAC. The design of the SUT facility does not include a building for incineration of single-use waste nor the demand in natural gas that is required for the incineration process.

The following list summarized the findings:

- The 2000 L SUT facility emits more carbon dioxide than the 2000 L SST facility.
- Scale up of the SST facility from 2000 L to 18000 L reduces the carbon emissions per ton of produced mAbs
- Scale-up of the SST facility from 2000 L to 18000 L increases water consumptions per ton of produced mAbs
- Carbon intensity of the local average electricity grid mix has a large impact on overall carbon emissions

9 Conclusion and Outlook

The carbon footprint of monoclonal antibodies (mAbs) is calculated for conventional stainless steel (SST) build facilities, as well as facilities that incorporate single-use technology (SUT), in respect to various aspects. The impact of factory location specific parameters like weather and average electricity grid mix, the influence of run time, as well as scale dependant factors like tank size and overall factory size are respected. The developed model allows incremental scales of 2000 L, 6000 L and 18000 L bioreactor capacity for the stainless steel facility. The model for a 2000 L single-use technology (SUT) facility is based on available dimensioning data and only allows a predefined product titer of 6 g/L. For direct comparison this limits the product titer selection for the SST facility to 6 g/L even though the SST facility is dynamically scalable if no comparison is contemplated. The developed EXCEL/VBA tool allows to set up specific scenarios that incorporate technology specific impact factors. For both technologies, the factory run time can be adjusted to investigate the impact on the total carbon footprint. Electricity emission factors for the average Swiss, German, USA and Asian grid mix can be set. The location specific emission factor for stainless steel production can be set to Germany, China, USA and the EU. The number of workers and the commuting distance via car can be adjusted. SST facility specific impact factors involve two different models for the CIP process, as well as adjustable insulation layer thickness for the SIP process. The transport of steel tanks to the production facility can be adjusted by transport method (rail, road) and distance. SUT facility specific impact factors include the transport method (rail, road) and distance for the delivery of single-use bag support structures as well as buffer/media. For both facility types impact factors for WFI generation can be adjusted by an efficiency factor as well as starting and end temperature for the WFI generation process.

To determine the carbon footprint of monoclonal antibodies either produced in a SST facility or SUT facility involves the definition of clear system boundaries to ensure traceable results. System boundaries are set and explained to the best of knowledge and judgement. The determination of the carbon footprint required the usage of emission factors to convert energy and mass flux into carbon emissions. Emission factors

themselves are impacted by their system boundaries and depend on the scope of their publisher if they are obtained from external references. The quality and availability of emission factors varies greatly depending on area of interest, making them susceptible to impact the process of calculating the carbon footprint in an inadvertent nontransparent manner. Emission factors often are limited to carbon emissions instead of carbon equivalents ($CO2_e$) that also include the global warming potential of other greenhouse gases. Carbon footprinting is a relatively new field of interest, that does not concern many fields of daily live and industry sectors yet. Under this directive the emission factors for many items is not available. The sheer complexity of many items such as specific material composition and the lack of exact data on weight leave a gap that has to be filled in the future to receive a more accurate balance.

Inevitably, the lack of emission factors or data in general, results in justifiable assumptions. Production facilities for mAbs are impacted by a large amount of variables that generalization for the two different facility types are not possible. A better approach is to view balanced case studies where the number of variables becomes manageable. The question whether SST or SUT facilities are more sustainable can not be answered universally. Any economic considerations (CAPEX/OPEX) were excluded in this study but play a vital in decision making, regarding the implementation of single-use technology or conventional stainless steel technology.

There still remain unresolved issues with the determination of the carbon footprint for monoclonal antibody production. In summary future development steps are:

- Emission factors: identification of missing emission factors either by provision through reliable sources or internal determination. Introduction of natural gas as an alternative energy source to electricity.
- Accurate data on the weight of single-use bags like single-use bioreactors, single-use mixers and 2D/3D single-use storage bags for buffers/media. Additional distinction between the weight of the bag chamber as well as included packaging, filters and tubes.
- Scalability: implementation of a numbering-up approach for the SUT facility as well as incorporation of availability of bioreactor capacities greater than 2000 L. Full dynamic scalability for the SUT facility, which includes the possibility to set varying product tiers

- EXCEL/VBA tool: automatisation of the EXCEL/VBA tool to harness the full potential data analytics. Extension of the EXCEL/VBA tool by a weather data library to offer an extended location selection
- Transportation: implementation of an alternative commuting system via train for commuting employees. Option to select a maritime route for steel tank shipping.
- HVAC model: implementation of a more sophisticated HVAC model that includes pressure drop and explicit dimensioning of fans with according energy consumption as well as more granular clean room zoning

The determination of the carbon footprint of a product will gain in significance in the future, as global warming becomes an increasing thread with more extreme weather conditions affecting billions of people worldwide. The presented work approaches this topic in an unprejudiced way and serves as a base for future improvements to determine the carbon footprint of stainless steel and single-use facilities even more accurately.

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10 Appendix

10.1 Upstream process – preliminary calculations

The dimensioning of the inoculum expansion and cell expansion steps depends on production scale, namely the total production reactor volume. The inoculum expansion steps are commonly labelled as $N - distance\ from\ production\ bioreactor$. As an example N-1 is the inoculum expansion step prior to entering the production bioreactor N. Single-use technology has a limited volume capacity with limited scale-up potential compared to SST technology. In general there is a trade-off between time and space. The more inoculum expansion steps the longer the time bevor production can start. When lesser inoculum expansion steps are chosen the amount of incubators (to house the Erlenmeyer flasks for inoculum expansion from the WCB) and therefore the space demand. The following calculations are exemplary but aim to provide the necessary knowledge to scale the seed train to any given total production bioreactor volume.

It is recommended to see *10.19 Downstream process flow chart for the SUT facility* on page 272 and :*10.20 Downstream process flow chart for the SST facility* on page 273, to be able to link the calculations to tank numbering.

10.1.1 SUT seed train calculations

The exemplary seed train calculations are based on a 2000 L production bioreactor capacity. A basic scheme to follow the presented calculations is shown in Figure 65:

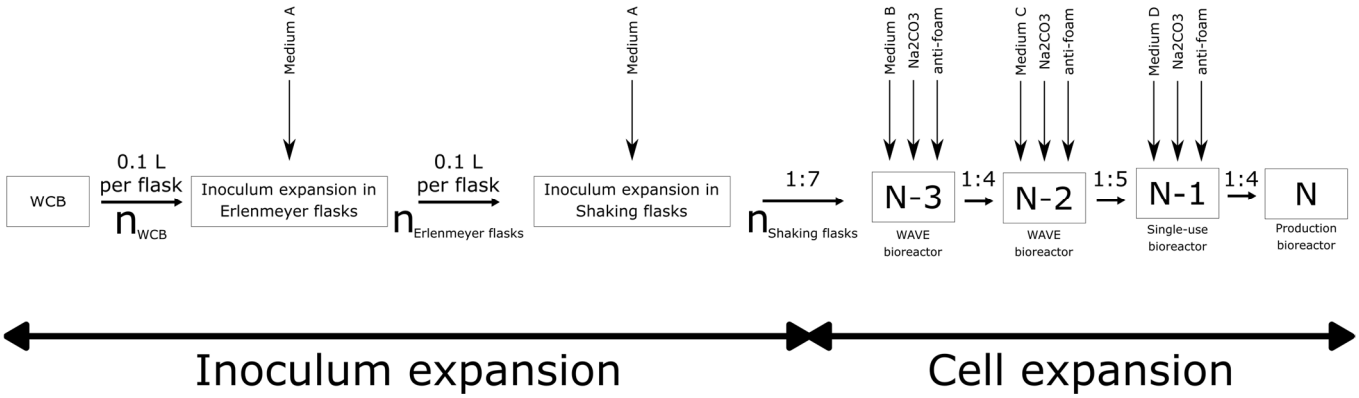


Figure 65 Seed train calculations scheme

The calculation starts by setting the production bioreactor volume N to a defined volume. From there all necessary seed train volumes are calculated. As calculation start from the production bioreactor volume, cell expansion is calculated first.

Cell expansion - volumes

The production bioreactor has a volume of 2000 L:

$$V_N = 2000[L]$$

The N-1 single use bioreactor has a volume of 500 L:

$$V_{N-1} = V_N \cdot \frac{1}{4} = 2000[L] \cdot \frac{1}{4} = 500[L]$$

The N-2 WAVE bioreactor has a volume of 100 L:

$$V_{N-2} = V_{N-1} \cdot \frac{1}{5} = 500[L] \cdot \frac{1}{5} = 100[L]$$

The N-3 WAVE bioreactor has a volume of 25 L:

$$V_{N-3} = V_{N-2} \cdot \frac{1}{4} = 100[L] \cdot \frac{1}{4} = 25[L]$$

A total volume of 3.6 L is needed to inoculate the N-3 WAVE bioreactor:

$$V_{N-3,inoc.} = V_{N-3} \cdot \frac{1}{7} = 25[L] \cdot \frac{1}{7} \approx 3.7[L]$$

With known seed train reactor volumes, the volumes of needed media and chemicals can be calculated.

N-1 additives

The required volume of medium D for the N-1 stage is 374.5 L:

$$V_{Medium\ D,N-1} = V_{N-1} \cdot \frac{374.5[L]}{500[L]} = 500[L] \cdot \frac{374.5[L]}{500[L]} = 374.5[L]$$

The require volume of Na2CO3 for the N-1 stage is 25 L:

$$V_{Na2CO3,N-1} = V_{N-1} \cdot \frac{25[L]}{500[L]} = 500[L] \cdot \frac{25[L]}{500[L]} = 25[L]$$

The require volume of anti-foam for the N-1 stage is 25 L:

$$V_{Na2CO3,N-1} = V_{N-1} \cdot \frac{0.5[L]}{500[L]} = 500[L] \cdot \frac{0.5[L]}{500[L]} = 0.5[L]$$

N-2 additives

The required volume of medium C for the N-2 stage is 374.5 L:

$$V_{Medium\ C,N-2} = V_{N-2} \cdot \frac{69.9[L]}{100[L]} = 100[L] \cdot \frac{69.9[L]}{100[L]} = 69.9[L]$$

The require volume of Na2CO3 for the N-1 stage is 25 L:

$$V_{Na2CO3,N-2} = V_{N-2} \cdot \frac{5[L]}{100[L]} = 100[L] \cdot \frac{5[L]}{100[L]} = 5[L]$$

The require volume of anti-foam for the N-1 stage is 0.1 L:

$$V_{Na2CO3,N-2} = V_{N-2} \cdot \frac{0.1[L]}{100[L]} = 100[L] \cdot \frac{0.1[L]}{100[L]} = 0.1[L]$$

N-3 additives

The required volume of medium B for the N-3 stage is 21.4 L:

$$V_{Medium\ B,N-3} = V_{N-3} \cdot \frac{21.4[L]}{25[L]} = 25[L] \cdot \frac{21.4[L]}{25[L]} = 21.4[L]$$

Required number of shaking flasks

One shaking flask has a total volume of 0.4 L (0.1 L from the Erlenmeyer flask plus 0.3 L of medium A). For this reason, 9 shaking flasks are needed:

$$\mathrm{n}_{\text{shaking flasks}} = \frac{V_{N-3,inoc.}}{V_{shaking\ flask}} = \frac{3.6[L]}{0.4[L]} = 9$$

Required number of Erlenmeyer flasks

Since 0.1 L are transferred from each shaking flask to each Erlenmeyer flask, amount of Erlenmeyer flasks is the same as the number of shaking flasks:

$$n_{shaking\ flasks} = n_{Erlenemeyer\ flasks} = 9$$

Volume of medium A

Every shaking or Erlenmeyer flask is filled with 0.3 L of medium A. For the preparation of 9 shaking flasks, 2.7 L of medium A is needed:

$$V_{Shaking\ flasks,medium\ A} = n_{shaking\ flasks} \cdot 0.3[L] = 9 \cdot 0.3[L] = 2.7[L]$$

Volume/number of WCB probes

Each Erlenmeyer flask requires 0.1 L from the WCB. For 9 Erlenmeyer flasks, 0.9 L of WCB inoculum is needed:

$$V_{WCB} = n_{Erlenmeyer\ flasks} \cdot 0.1[L] = 0.9[L]$$

With 0.1 L per Erlenmeyer flask, the number of necessary WCB probes is 9:

$$n_{WCB} = \frac{V_{WCB}}{0.1[L]} = \frac{0.9[L]}{0.1[L]} = 9$$

Seed train processing time

It takes 288 h to prepare the Erlenmeyer flaks at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 290 h.

It takes 192 h to prepare the shaking flaks at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 194 h.

It takes 96 h for the N-3 stage cell expansion at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

It takes 96 h for the N-2 stage cell expansion at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

It takes 96 h for the N-1 stage cell expansion at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

The total time for inoculum and cell expansion is 778 h (or $\approx 32.5\ d$):

$$t_{total} = 290[h] + 194[h] + 98[h] + 98[h] + 98[h] = 778[h]$$

10.1.2 SUT production bioreactor calculations

The calculations are based on a 2000 L production bioreactor. The necessary amount of Medium 1 is 572.4 L:

$$V_{Medium\,1} = V_N \cdot \frac{572.4[L]}{2000[L]} = 2000[L] \cdot \frac{572.4[L]}{2000[L]} = 572.4[L]$$

The necessary amount of Feed 1 is 408 L:

$$V_{Feed\,1} = V_N \cdot \frac{408[L]}{2000[L]} = 2000[L] \cdot \frac{408[L]}{2000[L]} = 408[L]$$

The necessary amount of Feed 2 is 326 L:

$$V_{Feed\,2} = V_N \cdot \frac{326[L]}{2000[L]} = 2000[L] \cdot \frac{326[L]}{2000[L]} = 326[L]$$

The necessary amount of Feed 3 is 16.5 L:

$$V_{Feed\,3} = V_N \cdot \frac{16.5[L]}{2000[L]} = 2000[L] \cdot \frac{16.5[L]}{2000[L]} = 16.5[L]$$

The necessary amount of Feed 4 is 33 L:

$$V_{Feed\,4} = V_N \cdot \frac{33[L]}{2000[L]} = 2000[L] \cdot \frac{33[L]}{2000[L]} = 33[L]$$

The necessary amount of Feed 5 is 44 L:

$$V_{Feed\,4} = V_N \cdot \frac{44[L]}{2000[L]} = 2000[L] \cdot \frac{44[L]}{2000[L]} = 44[L]$$

The necessary amount of Na2CO3 is 82 L:

$$V_{Na2CO3} = V_N \cdot \frac{82[L]}{2000[L]} = 2000[L] \cdot \frac{82[L]}{2000[L]} = 82[L]$$

The necessary amount of anti-foam is 1.6 L:

$$V_{anti-foam} = V_N \cdot \frac{1.6[L]}{2000[L]} = 2000[L] \cdot \frac{1.6[L]}{2000[L]} = 1.6[L]$$

10.1.3 SST seed train calculations

The exemplary seed train calculations are based on a 18000 L production bio reactor capacity. A basic scheme to follow the presented calculations is shown in

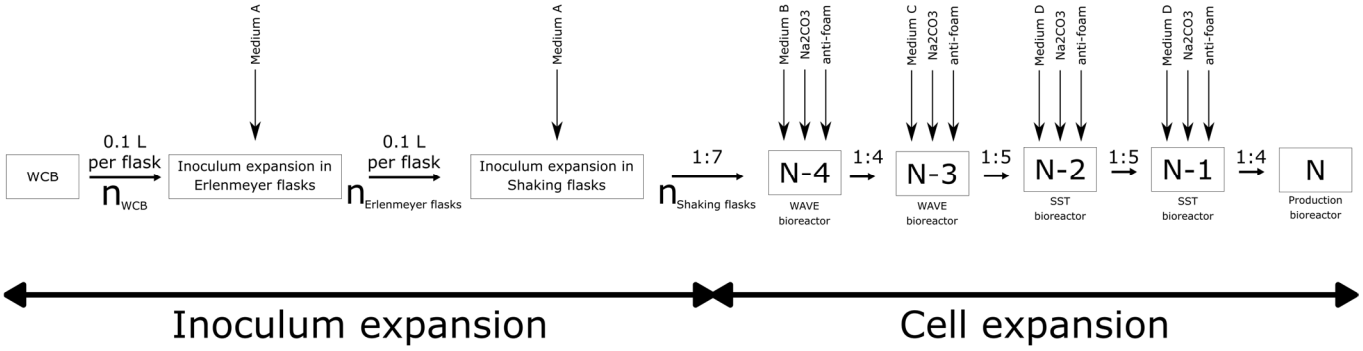


Figure 66 SST seed train schematic

The calculation starts by setting the production bioreactor volume N to a defined volume. From there all necessary seed train volumes are calculated. As calculation start from the production bioreactor volume, cell expansion is calculated first.

Cell expansion - volumes

The production bioreactor has a volume of 18000 L:

$$V_N = 18000[L]$$

The N-1 bioreactor has a volume of 4500 L:

$$V_{N-1} = V_N \cdot \frac{1}{4} = 180000[L] \cdot \frac{1}{4} = 4500[L]$$

The N-2 bioreactor has a volume of 900 L:

$$V_{N-2} = V_{N-1} \cdot \frac{1}{5} = 4500[L] \cdot \frac{1}{5} = 900[L]$$

The N-3 bioreactor has a volume of 180 L:

$$V_{N-3} = V_{N-2} \cdot \frac{1}{4} = 900[L] \cdot \frac{1}{5} = 180[L]$$

The N-4 bioreactor has a volume of 45 L:

$$V_{N-4} = V_{N-3} \cdot \frac{1}{4} = 180[L] \cdot \frac{1}{4} = 45[L]$$

A total volume of 3.6 L is needed to inoculate the N-4 bioreactor:

$$V_{N-4,inoc.} = V_{N-4} \cdot \frac{1}{7} = 45[L] \cdot \frac{1}{7} \approx 6.43[L]$$

With known seed train reactor volumes, the volumes of needed media and chemicals can be calculated.

N-1 additives

The required volume of medium D for the N-1 stage is 3370.5 L:

$$V_{Medium\ D,N-1} = V_{N-1} \cdot \frac{374.5[L]}{500[L]} = 4500[L] \cdot \frac{374.5[L]}{500[L]} = 3370.5[L]$$

The require volume of Na2CO3 for the N-1 stage is 225 L:

$$V_{Na2CO3,N-1} = V_{N-1} \cdot \frac{25[L]}{500[L]} = 4500[L] \cdot \frac{25[L]}{500[L]} = 225[L]$$

The require volume of anti-foam for the N-1 stage is 4.5 L:

$$V_{Na2CO3,N-1} = V_{N-1} \cdot \frac{0.5[L]}{500[L]} = 4500[L] \cdot \frac{0.5[L]}{500[L]} = 4.5[L]$$

N-2 additives

The required volume of medium D for the N-2 stage is 674.1 L:

$$V_{Medium\ D,N-2} = V_{N-2} \cdot \frac{374.5[L]}{500[L]} = 900[L] \cdot \frac{374.5[L]}{500[L]} = 674.1[L]$$

The require volume of Na2CO3 for the N-2 stage is 45 L:

$$V_{Na2CO3,N-2} = V_{N-2} \cdot \frac{25[L]}{500[L]} = 900[L] \cdot \frac{25[L]}{500[L]} = 45[L]$$

The require volume of anti-foam for the N-2 stage is 0.9 L:

$$V_{Na2CO3,N-2} = V_{N-2} \cdot \frac{0.5[L]}{500[L]} = 900[L] \cdot \frac{0.5[L]}{500[L]} = 0.9[L]$$

N-3 additives

The required volume of medium C for the N-3 stage is 125.82 L:

$$V_{Medium\ C,N-3} = V_{N-3} \cdot \frac{69.9[L]}{100[L]} = 180[L] \cdot \frac{69.9[L]}{100[L]} = 125.82[L]$$

The require volume of Na2CO3 for the N-1 stage is 9 L:

$$V_{Na2CO3,N-3} = V_{N-3} \cdot \frac{5[L]}{100[L]} = 180[L] \cdot \frac{5[L]}{100[L]} = 9[L]$$

The require volume of anti-foam for the N-1 stage is 0.18 L:

$$V_{Na2CO3,N-3} = V_{N-3} \cdot \frac{0.1[L]}{100[L]} = 180[L] \cdot \frac{0.1[L]}{100[L]} = 0.18[L]$$

N-4 additives

The required volume of medium B for the N-4 stage is 38.52 L:

$$V_{Medium\ B,N-4} = V_{N-4} \cdot \frac{21.4[L]}{25[L]} = 45[L] \cdot \frac{21.4[L]}{25[L]} = 38.52[L]$$

Required number of shaking flasks

One shaking flask has a total volume of 0.4 L (0.1 L from the Erlenmeyer flask plus 0.3 L of medium A). For this reason, 16 shaking flasks are needed:

$$\mathrm{n_{shaking\ flasks}} = \frac{V_{N-4,inoc.}}{V_{shaking\ flask}} = \frac{9.43[L]}{0.4[L]} \approx 16$$

Required number of Erlenmeyer flasks

Since 0.1 L are transferred from each shaking flask to each Erlenmeyer flask, amount of Erlenmeyer flasks is the same as the number of shaking flasks:

$$n_{shaking\ flasks} = n_{Erlenemeyer\ flasks} = 16$$

Volume of medium A

Every shaking or Erlenmeyer flask is filled with 0.3 L of medium A. For the preparation of 16 shaking flasks, 4.8 L of medium A is needed:

$$V_{Shaking\ flasks,medium\ A} = n_{shaking\ flasks} \cdot 0.3[L] = 16 \cdot 0.3[L] = 4.8[L]$$

Volume/number of WCB probes

Each Erlenmeyer flask requires 0.1 L from the WCB. For 16 Erlenmeyer flasks, 0.9 L of WCB inoculum is needed:

$$V_{WCB} = n_{Erlenmeyer\ flasks} \cdot 0.1[L] = 1.6[L]$$

With 0.1 L per Erlenmeyer flask, the number of necessary WCB probes is 9:

$$n_{WCB} = \frac{V_{WCB}}{0.1[L]} = \frac{1.6[L]}{0.1[L]} = 16$$

Seed train processing time

It takes 288 h to prepare the Erlenmeyer flaks at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 290 h.

It takes 192 h to prepare the shaking flaks at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 194 h.

It takes 96 h for the N-4 stage cell expansion at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

It takes 96 h for the N-3 stage cell expansion at 37°C. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

It takes 96 h for the N-2 stage cell expansion at 37°C.. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

It takes 96 h for the N-1 stage cell expansion at 37°C.. With an estimated preparation time of 2 h and 0 h dismantling time, the total time is 98 h.

The total time for inoculum and cell expansion is 876 h (or $\approx 36.5\ d$):

$$t_{total} = 290[h] + 194[h] + 98[h] + 98[h] + 98[h] + 98[h] = 876[h]$$

10.1.4 SST production bioreactor calculations

The calculations are based on a 18000 L production bioreactor. The necessary amount of Medium 1 is 5151.6 L:

$$V_{Medium\,1} = V_N \cdot \frac{572.4[L]}{2000[L]} = 18000[L] \cdot \frac{572.4[L]}{2000[L]} = 5151.6[L]$$

The necessary amount of Feed 1 is 3672 L:

$$V_{Feed\,1} = V_N \cdot \frac{408[L]}{2000[L]} = 18000[L] \cdot \frac{408[L]}{2000[L]} = 3672[L]$$

The necessary amount of Feed 2 is 2934 L:

$$V_{Feed\,2} = V_N \cdot \frac{326[L]}{2000[L]} = 18000[L] \cdot \frac{326[L]}{2000[L]} = 2934[L]$$

The necessary amount of Feed 3 is 148.5 L:

$$V_{Feed\,3} = V_N \cdot \frac{16.5[L]}{2000[L]} = 18000[L] \cdot \frac{16.5[L]}{2000[L]} = 148.5[L]$$

The necessary amount of Feed 4 is 297 L:

$$V_{Feed\,4} = V_N \cdot \frac{33[L]}{2000[L]} = 18000[L] \cdot \frac{33[L]}{2000[L]} = 297[L]$$

The necessary amount of Feed 5 is 396 L:

$$V_{Feed\,4} = V_N \cdot \frac{44[L]}{2000[L]} = 18000[L] \cdot \frac{44[L]}{2000[L]} = 396[L]$$

The necessary amount of Na2CO3 is 738 L:

$$V_{Na2CO3} = V_N \cdot \frac{82[L]}{2000[L]} = 18000[L] \cdot \frac{82[L]}{2000[L]} = 738[L]$$

The necessary amount of anti-foam is 14.4 L:

$$V_{anti-foam} = V_N \cdot \frac{1.6[L]}{2000[L]} = 18000[L] \cdot \frac{1.6[L]}{2000[L]} = 14.4[L]$$

10.2 Downstream process – preliminary calculations

Calculations are based on a previously dimensioned SUT facility with a bioreactor capacity of 2000 L. The dimensioning of the SST facility uses this single-use facility as a basis for reference. The developed EXCEL/VBA tool allows incremental scaling for the SST facility (2 m^3, 6 m^3,12 m^3, 18 m^3 bioreactor volume) while the SUT facility remains at a scale of 2 m3 due to limitations in maximum bioreactor capacity inherent to single-use technology.

Goal of the downstream process calculations is to determine all process tank and buffer volumes. Buffer is produced from WFI that consumes electrical energy during its making, resulting in a respective carbon footprint.

The optimized tank diameter and tank height are derived from tank volumes (*10.15 Tank diameter/height – preliminary calculations*). These tank dimensions are the base for CIP cycle design, space requirements as well as calculations for the tank wall thickness according to the *AD-2000 Merkblätter*. The CIP cycle design determines the water and energy consumption with dedicated CO_2 emissions. Space demand is crucial for HVAC design and operation, which have a large impact on energy demand and hence CO_2 emissions. Tank wall thickness is important since the steel production and the transport of the steel tanks to the production facility emits CO_2 accordingly.

The model requires user input based on operation scale, tither and factory runtime. Unit operations are scaled as shown in the following examples. The calculations for the downstream process are based on available data form a V_B=2000 L; c_{titer}=6 g/L SUT process.

It is recommended to see the process flow chart (*10.20 Downstream process flow chart for the SST facility*) in the appendix on page 273, to be able to link the calculations to tank numbering.

The calculation for the SST downstream processing are based on the following exemplary parameters:

Variable	Symbol	Unit	Exemplary value
Bioreactor tank volume	V_B	m^3	18000
Product titer	C_{titer}	$g \cdot L^{-1}$	6

Disclaimer: presented values stem from EXCEL/VBA calculations but are rounded manually. Deviations in decimal places might apply.

10.2.1 Harvest

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Volume [L]
Volume of tank 2	mAb solution	V_2	?
Volume of tank 3	mAb solution	V_3	?
Volume of tank w1	Detergent	V_{w1}	?
Processing time	-	$t_{final,Harvest}$	?
Volume after harvest	-	V_3	?
Concentration after harvest	-	c_3	?

The determination of tank sizes is based on buffer demand calculations. Minimum tank sizes are calculated from buffer requirement. The therefore received geometrical volume is later increased by 15-20% (selectable in the EXCEL/VBA tool) to receive the working volume.

The indices stand for the tank label in the process flow chart in the appendix on page 273.

Since there is no loss during filtration, the volume of tank 2 is 18000 L with a constant concentration of 6 g/L.:

$$V_2 = 18000[L]$$

$$c_2 = 6.00[\frac{g}{L}]$$

The harvest building block includes a two stage depth filtration process as well as an absolute friltration.

<u>First stage depth filtration</u>

Filter specifications: filter area=1.6 m^2, flow rate=$13.\overline{8}[\frac{L}{h \cdot filter}]$, filter loading = 35 L/m^2

$$Required\ filter\ area = \frac{18000[L]}{35[\frac{L}{m^2}]} = 514.29[m^2]$$

$$Number\ of\ needed\ filter\ capsules = \frac{514.29[m^2]}{1.6[m^2]} \approx 322$$

$$flowrate\ per\ filter = \frac{500[\frac{L}{h}]}{36} = 13.\overline{8}[\frac{\mathrm{L}}{\mathrm{h}\cdot\mathrm{filter}}]$$

$$Flowrate = 322 \cdot \frac{500\left[\frac{L}{h}\right]}{36\ filter} = 4472.22[\frac{L}{h}]$$

$$Processing\ time = \frac{18000[\mathrm{L}]}{4472.22[\frac{L}{h}]} = 4.02[h]$$

$$WFI\ to\ flush\ first\ stage\ filters = 54\frac{[L]}{[m^2]} \cdot 514.29[m^2] = 27771.66[L]$$

Second stage depth filtration

Filter specifications: filter area=1.6 m²; flow rate=$23.\overline{8}\frac{L}{h\cdot filter}$; filter loading = 60 L/m²

$$Required\ filter\ area = \frac{18000.00[L]}{60[\frac{L}{m^2}]} = 300.00[m^2]$$

$$Number\ of\ needed\ filter\ capsules = \frac{300.00[m^2]}{1.6[m^2]} \approx 188$$

$$Flowrate\ per\ filter = \frac{500[\frac{L}{h}]}{21} = 23.\overline{8}[\frac{\mathrm{L}}{\mathrm{h}\cdot\mathrm{filter}}]$$

$$Flowrate = 191 \cdot\ 23.\overline{8}[\frac{\mathrm{L}}{\mathrm{h}\cdot\mathrm{filter}}] = 4476.19[\frac{L}{h}]$$

$$Processing\ time = \frac{18288.00[L]}{4547.62[\frac{L}{h}]} = 4.02[h]$$

$$WFI\ to\ flush\ second\ stage\ filter = 54\frac{[L]}{[m^2]} \cdot 300.00[m^2] = 16200.00[L]$$

0.2 µm absolute filtration (2x20" capsules)

Filter specifications: filter area=1.8 m²; flow rate= 500 L/h; filter loading = 300 L/m²; pore size=0.2 µm

$$Set\ filtration\ time = 4[h]$$

$$Flow\ rate\ per\ filter = \frac{500[\frac{L}{h}]}{4} = 125[\frac{L}{h}]$$

$$Number\ of\ needed\ filter\ capsules = \frac{18000.00[L]}{4[h]\cdot 125[\frac{L}{h}]} \approx 36$$

$$Filter\ area = 36 \cdot 1.8 = 64.80[m^2]$$

The overall filtration time is 4.02 h and with an estimated 2 h preparation and 2 h dismantling time, the total processing time is 8.02 h.

After the three stage depth filtration, the filtrate is pooled and cooled down. Cooling time is independent from process volume and is held constant at 4 h to eliminate the risk of product degradation.

The calculations steps that are necessary to calculate the energy demand for heating/cooling is presented in *10.9 Tank heating/cooling – preliminary calculations*.

The energy consumption of cooling an 18 m^3 mAb solution from 37°C to 15°C within 4 h is 986.27 kWh:

$$E_{cooling} = 243.97[kW] \cdot 4[h] = 975.89[kWh]$$

The energy consumption of cooling an 2 m^3 tank from 37°C to 15°C within 4 h is 225.55 [kWh].

With 2 h preparation and 2 h dismantling time, the total processing time for this step is 8 h.

This step is followed by virus inactivation, where 26.3 mL of detergent are added per litre of mAbs solution.

$$V_{w1} = 18000[L] \cdot 0.0263 \left[\frac{L}{L}\right] = 473.4[L]$$

$$V_3 = 18000[L] + 473.4[L] = 18473.4[L]$$

The process takes 4 h and with 1 h preparation and 1 h dismantling time, the total processing time for this step is 6 h.

The last step is a 0.45 µm /0.2 µm absolute filtration *(2 x 30"capsules)* (Pore size=0.8/0.45 µm; membrane area = 1.8 m^2; Filter loading=1000 L/m^2; Flow rate per filter= 500 L/h).

$$Nuber\ of\ needed\ filters = \frac{18473.4[L]}{4.1[h] \cdot 500[\frac{L}{h} \cdot filter]} \approx 9$$

$$Filter\ area = \frac{18473.4[L]}{1000[\frac{L}{m^2}]} = 18.50[m^2]$$
$$Flow\ rate = 9 \cdot 500\left[\frac{L}{h}\right] = 4500.00[\frac{L}{h}]$$
$$Processing\ time = \frac{18473.4[L]}{4500[\frac{L}{h}]} = 4.1[h]$$

Second stage absolute filter (20") (Pore size=0.45/0.2 µm; membrane area = 1.8 m^2; Filter loading=1000 L/m^2).

$$Nuber\ of\ needed\ filters = \frac{18473.4[L]}{4.1[h] \cdot 500[\frac{L}{h} \cdot filter]} \approx 9$$

$$Filter\ area = \frac{18473.4[L]}{1000[\frac{L}{m^2}]} = 18.50[m^2]$$
$$Flow\ rate = 9 \cdot 500\left[\frac{L}{h}\right] = 4500.00[\frac{L}{h}]$$
$$Processing\ time = \frac{18473.4[L]}{4500[\frac{L}{h}]} = 4.1[h]$$

With an additional 2 h preparation and 2 h dismantling time, the total processing time comes to 8.1 h. This results in a final processing time of 30.12 h for the harvest:

$$t_{final,Harvest} = t_{depth\ filtration} + t_{pooling} + t_{virus\ inactivation} + t_{absolute\ filtration}$$
$$t_{final,Harvest} = 8.02[h] + 8.00[h] + 6.00[h] + 8.1[h] = 30.12[h] \cdot \frac{1[d]}{24[h]} = 1.26[d]$$

Leaving the harvest building block are 18473.4 L with a concentration of 5.85 g/L:

$$c_3 = \frac{c_1 \cdot V_1}{V_3} = \frac{6[\frac{g}{L}] \cdot 18000[L]}{18473.4[L]} \approx 5.85[\frac{g}{L}]$$

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank 2	mAb solution	V_2	18000[L]
Volume of tank 3	mAb solution	V_3	18473.4[L]
Volume of tank w1	Detergent	V_{w1}	$473.4[L]$
Processing time	-	$t_{final,Harvest}$	1.26 [d]
Volume after harvest	-	V_3	18473.4[L]
Concentration after harvest	-	c_3	5.85[g/L]

10.2.2 Protein A affinity chromatography

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank a1	Buffer A	V_{a1}	?
Volume of tank a2	Buffer B	V_{a1}	?
Volume of tank a3	Buffer C	V_{a1}	?
Volume of tank a4	Buffer D	V_{a1}	?
Volume of tank a5	Buffer E	V_{a1}	?
Volume of tank a6	Buffer G	V_{a1}	?
Volume of tank q1	Buffer F	V_{q1}	?
WFI loop supply c1	WFI	$V_{WFI\ loop,c1}$	
WFI loop supply p1	WFI	$V_{WFI\ loop,p1}$	
Volume after protein A affinity chromatography	-	V_4	?
Concentration after protein A affinity chromatography	-	c_4	?
Total processing time	-	$t_{total,Protein\ A}$	?

The column diameter of the protein A column is adjusted to suit the required processing time of 29 h depending on process scale. For the V_1=18000[L] the diameter is set to 1.12 m.

The protein A affinity chromatography column properties are:

Diameter:108 cm=1.08 m
Bed height:20 cm=0.2 m
Dynamic binding capacity:37 g/L

$$Area = \frac{\pi \cdot (108[cm])^2}{4} = 9085.84[cm^2] = 0.91[m^2]$$

$$\text{Column } volume\ (CV) = Area \cdot 0.2[m] = 0.91[m^2] \cdot 0.2[m] \cdot \frac{1000[L]}{1m^3} = 181.72[L]$$

$$Binding\ capacity = 181.72[L] \cdot 37\left[\frac{g}{L}\right] = 6723.52[g]$$

$$Liters\ that\ can\ be\ processed\ in\ one\ cycle = 6723.52[g] \cdot \frac{1}{5.85[\frac{g}{L}]} = 1150.06[L]$$

$$Number\ of\ cycles = \frac{18473.4[L]}{1150.06[\mathrm{L}]} = \approx 16$$

Table 17 Protein A affinity chromatography cycles. CV= column volume.

Media	Operation	Cycles	CV	Volume[L]	Linear flow rate [cm/h]	Volumetric flow rate [L/min]	Time [h]
A	Rinse 1	1	6	1090.30	400	60.57	0.30
B	Sanitization 1	1	3	545.15	400	60.57	0.15
A	Equilibration	16	5	908.58	400	60.57	4.02
Sample load	Load	16		1150.06	400	60.57	5.08
A	Wash 1	16	4	726.87	400	60.57	3.21
C	Wash2	16	4	726.87	400	60.57	3.21
D	Elution	16	6	1090.30	400	60.57	4.82
E	Post-elution wash	16	2	363.43	400	60.57	1.61
F	Sanitization 2	16	3	545.15	400	60.57	2.41
A	Regeneration 1	16	5	908.58	400	60.57	4.02
F	Regeneration 2	16	0.03	5.45	400	60.57	0.02
WFI	Rinse 2	1	0.03	5.45	400	60.57	0.00
G	Storage	1	3	545.15	400	60.57	0.15
Sum							29.00

Example for Rinse 1:

$$Volume = Column\ volume\ (CV) \cdot Volume_{Column} = 6 \cdot 181.72[L] = 1090.30[L]$$

$$Volumetric\ flow\ rate = \frac{400\frac{cm}{h}}{60} \cdot 9085.84[cm^2] = 60572.2\bar{6}\frac{mL}{min} \cdot \frac{1[L]}{1000[mL]} = 60.57[\frac{L}{min}]$$

$$t_{total,Protein\ A} = Volume[L] \cdot \frac{1}{Volumetric\ flow\ rate\left[\frac{L}{min}\right]} \cdot \frac{1[h]}{60[min]} \cdot CV$$

$$t_{toal,Protein\ A} = 1090.30[L] \cdot \frac{1}{60.57\left[\frac{L}{min}\right]} \cdot \frac{1[h]}{60[min]} \cdot 1 \approx 0.3[h]$$

Sanitization 1, Equilibration, Wash 1, Wash 2, Post-elution wash, Sanitization 2, Regeneration 1, Regeneration 2, Rinse 2 and Storage are calculated according to the example for Rinse 1

The total duration to process 18473.4 L with a concentration of 5.8462 g/L is 2.6929 days including an estimated 4 h preparation and a 4 h dismantling time:

$$t_{total,Protein\ A} = \frac{29[h] + 4[h] + 4[h]}{\frac{24[h]}{1[d]}} \approx 1.54[d]$$

The elution volume is 7583.35 L:

$$V_4 = Volume[L] \cdot Cycles \cdot (\frac{Elution\ fraction}{100})$$

$$V_4 = 1090.30[L] \cdot 16 \cdot (\frac{43.3}{100}) = 7553.60\ [\mathrm{L}]$$

To calculate the concentration of product after the protein A affinity chromatography one has to calculate the starting mass of mAbs first:

$$m_{mAbs,start} = 18473.4[\mathrm{L}] \cdot 5.85\left[\frac{g}{L}\right] = 108069.39[g]$$

If there is no loss of product the concentration after protein A affinity chromatography is:

$$c_4 = \frac{108069.39[g]}{7583.35\ [L]} = 14.61[\frac{g}{L}]$$

Leaving the protein A affinity chromatography are 7583.35 L with a concentration of 14.61 g/L.

Tank size and buffer amount

There is no limitation in tank size for a SST build facility and buffers of the same type can be pooled (e.g. buffer A in tank a1). The minimum amount of buffer A and the minimum tank size for a1 are 41794.78 L :

$$V_{a1} = \left(n_{Cycles,Rinse\ 1} \cdot Volume_{Rinse\ 1}\right) + \left(n_{Cycles,Equilibration} \cdot Volume_{Equilibration}\right) + \left(n_{Cycles,Wash\ 1} \cdot Volume_{Wash\ 1}\right) + \left(n_{Cycles,Regeneration\ 1} \cdot Volume_{Regeneration\ 1}\right)$$

$$V_{a1} = (1 \cdot 1090.30[L]) + (16 \cdot 908.58[L]) + (16 \cdot 726.87[L]) + (16 \cdot 908.58[L]) = 41794.78\ [L]$$

The minimum amount of buffer B and the minimum tank size for a2 are 545.15 L:

$$V_{a2} = \left(n_{Cycles,Sanitization\ 1} \cdot Volume_{Sanitization\ 1}\right) = 1 \cdot 545.15[L] = 545.15[L]$$

The minimum amount of buffer C and the minimum tank size for a3 are 11629.92 L:

$$V_{a3} = \left(n_{Cycles,Wash\ 2} \cdot Volume_{Wash\ 2}\right) = 16 \cdot 726.87[L] = 11629.86[L]$$

The minimum amount of buffer D and the minimum tank size for a4 are 17444.79 L:

$$V_{a4} = \left(n_{Cycles,Elution} \cdot Volume_{Elution}\right) = 16 \cdot 1090.30[L] = 17444.79[L]$$

The minimum amount of buffer E and the minimum tank size for a5 are 5814.93 L:

$$V_{a5} = \left(n_{Cycles,Post-elution\ wash} \cdot Volume_{Post-elution\ wash}\right) = 16 \cdot 363.43[L] = 5814.93[L]$$

The minimum amount of buffer G and the minimum tank size for a6 are 545.15 L:

$$V_{a6} = \left(n_{Cycles,Storage} \cdot Volume_{Storage}\right) = 1 \cdot 545.15[L] = 545.15[L]$$

The minimum amount of buffer F and the minimum tank size for q1 are 87.20 L:

$$V_{\mathrm{q1}} = \left(n_{\mathrm{Cycles,Regeneration\ 2}} \cdot Volume_{\mathrm{Regeneration\ 2}} + n_{Cycles,Sanitization\ 2} \cdot Volume_{\ Sanitization\ 2}\right) = 16 \cdot 5.45[L] + 16 \cdot 545.15[\mathrm{L}] = 8809.62[L]$$

The amount of WFI that has to be provided by the WFI loop is scaled from the 2k SUT process:

$$V_{WFI\ loop,c1} = 18473.4[L] \cdot \frac{1000[L]}{1016[L]} = 18182.48[L]$$

The amount of WFI that has to be provided by the WFI loop is scaled from the 2k SUT process:

$$V_{WFI\ loop,p1} = 18473.4[L] \cdot \frac{0.6[L]}{1016[L]} = 10.91[L]$$

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank a1	Buffer A	V_{a1}	41794.82
Volume of tank a2	Buffer B	V_{a1}	545.15
Volume of tank a3	Buffer C	V_{a1}	11629.86
Volume of tank a4	Buffer D	V_{a1}	17444.79
Volume of tank a5	Buffer E	V_{a1}	5814.93
Volume of tank a6	Buffer G	V_{a1}	545.15
Volume of tank q1	Buffer F	V_{q1}	8809.62
WFI loop supply c1	WFI	$V_{WFI\ loop,c1}$	18182.48
WFI loop supply p1	WFI	$V_{WFI\ loop,p1}$	10.91
Volume after protein A affinity chromatography	-	V_4	7553.60
Concentration after protein A affinity chromatography	-	c_4	14.61
Total processing time	-	$t_{total,Protein\ A}$	1.54[d]

10.2.3 pH adjustment and filtration

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 4	mAb solution	V_4	?
Volume of tank 5	mAb solution	V_5	?
Volume of tank 6	mAb solution	V_6	?
Volume of tank b1	Acid	V_{b1}	?
Volume of tank b2	Base	V_{b2}	?
Volume after pH adjustment and filtration	-	V_5	?
Concentration after pH adjustment and filtration	-	c_6	?
Total processing time	-	$t_{total,pH\ adj.\&filt.}$	?

Since 100 L acid/base are needed for the 2000 L reference SUT process, 1003.0805 L are needed for the 18000 L SST process:

$$V_{b1/b2} = 7553.60[L] \cdot \frac{100[L]}{840[L]} = 899.\overline{24}[L]$$

This results in a volume of 9428.9569 L after the pH adjustment:

$$Volume\ after\ pH\ adjustment = V_5 = V_6 = 7553.60[L] + 899.\overline{24}[L] = 8452.84[L]$$

To calculate the product concentration after pH adjustment and filtration the mass of mAbs before pH adjustment and filtration has to be calculated:

$$m_{mAbs} = V_{after\ Protein\ A} \cdot c_{after\ protein\ A} = 7553.60[\mathrm{L}] \cdot 14.61\left[\frac{g}{L}\right] = 110065.90[g]$$

With no loss of mass the concentration after pH adjustment and filtration is:

$$c_6 = \frac{110065.90[g]}{8452.84[L]} \approx 13.11[\frac{g}{L}]$$

The pH adjustment takes 5 h with an estimated preparation time of 1 h and an estimated dismantling time of 1 h.

$$t_{pH\ adj.} = 5[h]$$

pH adjustment is followed by a two stage absolute filtration.

Pre-filtration filter capsule (*30")* (Pore size=0.8/0.45 µm; membrane area=1.2 m^2; filter capacity=1000 L/m^2; Flow rate per filter 500 L/h)

$$Number\ of\ needed\ filter\ capsules = \frac{8452.84[L]}{1.9[h] \cdot 500[\frac{L}{h \cdot filter}]} \approx 9$$

$$Flow\ rate = 9 \cdot 500\left[\frac{L}{h}\right] = 4500[\frac{L}{h}]$$

$$Processing\ time = \frac{8452.84[L]}{4500[\frac{L}{h}]} \approx 1.9[h]$$

Main filtration filter capsule (30") (Pore size=0.45/0.2 µm; membrane area=1.2 m^2; filter capacity=1000 L/m^2)

$$Number\ of\ needed\ filter\ capsules = \frac{8452.84[L]}{1.9[h] \cdot 500[\frac{L}{h \cdot filter}]} \approx 9$$

$$Flow\ rate = 9 \cdot 500\left[\frac{L}{h}\right] = 4500[\frac{L}{h}]$$

$$Processing\ time = \frac{8452.84[L]}{4500[\frac{L}{h}]} \approx 1.9[h]$$

With an estimated preparation time of 2 h and a dismantling time of 2 h the processing time for the two stage absolute filtration is 5.9[h].

$$t_{filtration} = 1.9[h] + 2[h] + 2[h] = 5.9[h]$$

The total processing time of the pH adjustment and filtration building block is 10.9 h or 0.45 days:

$$t_{total,pH\ adj.\&filtration} = t_{pH\ adj.} + \mathrm{t}_{filtration} = 5[\mathrm{h}] + 5.9[\mathrm{h}] \cdot \frac{1[d]}{24[h]} = 0.45[d]$$

The volume of tank 6 is conform with the elution volume of the protein A affinity chromatography (page 187):

$V_4 = Elution\ volume$ =7553.60 [L]

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank 4	mAb solution	V_4	7553.60[L]
Volume of tank 5	mAb solution	V_5	8452.84[L]
Volume of tank 6	mAb solution	V_6	8452.84[L]
Volume of tank b1	Acid	V_{b1}	$899.\overline{24}$[L]
Volume of tank b2	Base	V_{b2}	$899.\overline{24}$[L]
Volume after pH adjustment and filtration	-	V_5	8452.84[L]
Concentration after pH adjustment and filtration	-	c_6	13.11[g/L]
Total processing time	-	$t_{total,pH\ adj.\&filt.}$	0.45[d]

10.2.4 Ultrafiltration/diafiltration 1

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 7 (9)	mAb solution	V_7	?
Volume of tank 8 (10)	mAb solution	V_8	?
Volume of tank d1	0.5 m NaOh	V_{d1}	?
WFI loop supply f1	WFI	$V_{WFI\ loop.e1}$	?
Volume of tank f1	Buffer J	V_{f1}	?
Volume after UF/DF1	-	V_8	?
Concentration after UF/DF 1	-	c_8	?
Total processing time	-	$t_{final,UF/DF1}$	?

The UF/DF 1 filter cassette (Filter area=2.5 m^2; number of filter cassettes: 36; Transmembrane pressure=1bar; Recirculation flow rate=360 [L/m^2•h]; Permeate flow rate=43 [L/m^2•h; Feed flow rate=100 L/h)

$$Total\ area = 36 \cdot 2.5[m^2] = 90[m^2]$$

$$Membrane\ load = \frac{V_{in} \cdot c_{in}}{Total\ area} = \frac{8452.84[L] \cdot 13.11[\frac{g}{L}]}{90[m^2]} \approx 1250.41[\frac{g}{m^2}]$$

$$Initial\ volume = 8452.84[L]$$

$$Concentrated\ volume = 8452.84[L] \cdot \frac{277[L]}{940[L]} = 2490.89[L] = V_7$$

$$Recirculation\ flow\ rate = 360[\frac{L}{m^2 \cdot h}] \cdot 90[m^2] = 32400[\frac{L}{h}]$$

$$Permeate\ flow\ rate = 43[\frac{L}{m^2 \cdot h}] \cdot 90[m^2] = 3870[\frac{L}{h}]$$

Table 18 Parameters form UF/DF 1. The UF/DF 1 process is performed till a concentration of 40 g/L is reached. The flush reduces this concentration from 40 to 35 g/L.

Medium	SUT Volume [L]	SST Volume [L]	Operation	Value	Unit	Time[h]
WFI (from loop)	300	2697.71	Rinse 1	-	L/m^2	0.71
0.5 M NaOH	100	899.24	Sanitization	-	L/m^2	0.24
WFI (from loop)	630	5665.20	Rinse 2	-	L/m^2	1.49
Buffer J	500	4496.19	Equilibration	-	L/m^2	1.18
Product	940	8452.83	Concentrate to	40	g/L	1.56
Buffer J	1660	14927.34	DF with 6 DV	-		3.92
Buffer J	277	2490.89	Recovery with 1 DV	-		0.65
Buffer J	24	215.82	Flush to	35	g/L	0.06
Sum						9.80

The SST volumes are calculated form the proportion of numbers from the SUT volumes, e.g.:

$$V_{SST,Rinse\ 1} = 8452.84[L] \cdot \frac{300[L]}{940[L]} = 2697.71[L]$$

The times for Rinse 1, Sanitization, Rinse 2, Equilibration, DF with 6 DV, Recovery with 1 DV and Flush are calculated the following way, e.g. Rinse 1:

$$t_{Rinse\ 1} = \frac{V_{SST,Rinse\ 1}}{Permeate\ flow\ rate} = \frac{2697.71[L]}{3870[\frac{L}{h}]} = 0.71[h]$$

The time to process the product is calculated the following way:

$$t_{Product} = \frac{V_{SST,Product}}{Permeate\ flow\ rate} = \frac{8452.84[L] - 2490.89[L]}{3870[\frac{L}{h}]} = 1.56[h]$$

The volume after UF/DF 1 is 3019.2723 L:

$$V_{afterUF/DF\ 1} = Concentrated\ volume + V_{Flush} = 2490.89[L] + 215.82[L]$$
$$= 2706.71[L] = V_8$$

The volume for tank d1 (V_{d1}) (0.5 M NaOH) is 899.24 L as seen as in Table 18

The volume that has to be supplied by the WFI loop ($V_{WFI\ loop.e1}$) (WFI) is 14927.34 L, as seen as in Table 18:

$$V_{WFI\ loop,e1} = 2697.71[L] + 5665.20[L] = 8362.91$$

The volume for tank f1 (V_{f1}) is 24688.8116 L as seen as in Table 18:

$$V_{f1} = 4496.19[\mathrm{L}] + 14927.34[\mathrm{L}] + 2490.89[\mathrm{L}] + 215.82[\mathrm{L}] = 22130.24[\mathrm{L}]$$

With an additional preparation time of 2 h and a dismantling time of 2 h, this results in a processing time of 13.8 h or 0.58 days:

$$t_{UF/DF\ 1} = 9.8[\mathrm{h}] + 2[\mathrm{h}] + 2[\mathrm{h}] = 13.8[\mathrm{h}]$$

The UF/DF procedure is followed by filtration (Filter area=1.2 m^2; filter loading=300 L/m^2; flow rate per filter=100 L/h) with a set time of 3 h:

$$Required\ number\ of\ filter = \frac{2706.71[\mathrm{L}]}{3[\mathrm{h}] \cdot 100[\frac{\mathrm{L}}{\mathrm{h} \cdot \mathrm{filter}}]} \approx 9$$

$$Flow\ rate = 9 \cdot 100\left[\frac{L}{h}\right] = 900[\frac{L}{h}]$$

$$Volume\ after\ filtration = 2706.71[L] - \left(2706.71[L] \cdot \frac{3[L]}{301[L]}\right) = 2679.02[L]$$

With an additional preparation time of 2 h and a dismantling time of 2 h, this results in a processing time of 7 h:

$$t_{filtration} = 3[\mathrm{h}] + 2[\mathrm{h}] + 2[\mathrm{h}] = 7[\mathrm{h}]$$

The final processing time is 20.8 h:

$$t_{final,UF/DF1} = 13.8[\mathrm{h}] + 7[\mathrm{h}] = 20.8[\mathrm{h}]$$

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank 7	mAb solution	V_7	2490.89[L]
Volume of tank 8	mAb solution	V_8	2706.71
Volume of tank d1	0.5 m NaOh	V_{d1}	899.24[L]
WFI loop supply f1	WFI	$V_{WFI\ loop.e1}$	14927.34[L]
Volume of tank f1	Buffer J	V_{f1}	22130.24[L]
Volume after UF/DF1	-	V_8	2706.71[L]
Concentration after UF/DF 1	-	c_8	35[g/L]
Total processing time	-	$t_{final,UF/DF1}$	20.80[h]

10.2.5 Ion exchange chromatography

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank g1	Buffer J	V_{g1}	
Volume of tank g2	Buffer K	V_{g2}	
Volume of tank g3	Buffer L	V_{g3}	
Volume of tank g4	Buffer M	V_{g4}	
Volume of tank g5	Buffer N	V_{g5}	
WFI supply loop r1	-	$V_{WFI\ loop,r1}$	
Volume after IEX	-	V_{g4}	
Concentration after IEX	-	c_4	
Total processing time		$t_{final,IEX}$	

The ion exchange chromatography column properties are:

Diameter: 130 cm=1.30 m
Height: 0.8 cm=0.008 m
Dynamic binding capacity=500 g/L

$$Area = \frac{\pi \cdot (130[cm])^2}{4} = 13228.26[cm^2] = 1.32[m^2]$$

$$Volume = Area \cdot 0.008[m] = 1.32[m^2] \cdot 0.008[m] \cdot \frac{1000[L]}{1[m^3]} = 10.56[L]$$

$$Maximum\ binding\ capacity = 10.56[L] \cdot 500\left[\frac{g}{L}\right] = 5280.00[g]$$

$$Liters\ that\ can\ be\ processed\ in\ one\ cycle = 5280.00[g] \cdot \frac{1}{35\left[\frac{g}{L}\right]} = 150.\overline{86}[L]$$

$$Number\ of\ cycles = \frac{2706.71[L]}{151.18[\mathrm{L}]} = 17.73 \approx 18$$

Table 19 Ion exchange chromatography cycles. CV= column volume.

Buffer	Purpose	Volume SUT [L]	CV	Volume SST [L]	Linear flow rate [L/h]	Volumetric flow rate [L/h]	n_{Cycles}	Time (one cycle) [h]	Time (total) [h]
J	Rinse 1	24	20	211.65	0.24	3174.78	1.00	0.07	0.07
K	Sanitization	24	20	211.65	0.24	3174.78	1.00	0.07	0.07
L	Rinse 2	24	20	211.65	0.24	3174.78	1.00	0.07	0.07
L	Equilibration	216	10	105.83	0.24	3174.78	17.73	0.03	0.59
J	Wash 1	432	20	211.65	0.144	1904.87	17.73	0.11	1.97
Product	Load	298	13.57	151.18	0.144	1904.87	17.73	0.08	1.41
J	Wash 2	432	20	211.65	0.144	1904.87	17.73	0.11	1.97
M	Elution	432	20	211.65	0.24	3174.78	17.73	0.07	1.18
N	Cleaning flush	432	20	211.65	0.24	3174.78	17.73	0.07	1.18
Σ									8.50

Example for “Rinse 1”:

$$Volume = CV \cdot Volume_{Column} = 20 * 10.56[L] = 211.65[L]$$

$$Time = Volume[L] \cdot \frac{n_{Cycles}}{Volumetric\ flow\ rate\left[\frac{L}{h}\right]} = 211.65[L] \cdot \frac{1}{3174.78\left[\frac{L}{h}\right]} = 0.07[h]$$

The total duration to process 2706.71L with a concentration of 35 g/L is 16.50 h including an estimated 4 h preparation and a 4 h dismantling time:

$$t_{total,IEX} = \frac{8.5[h] + 4[h] + 4[h]}{\frac{24[h]}{1[d]}} = 16.50[h]$$

The elution volume is 3915.68 L:

$$Elution\ volume = Volume[L] \cdot Cycles \cdot (\frac{Elution\ fraction}{100})$$

$$Elution\ volume = 211.65[L] \cdot 17.65 \cdot \left(\frac{100}{100}\right) = 3751.62[L]$$

To calculate the concentration of product after the IEX chromatography one has to calculate the starting mass of mAbs first:

$$m_{mAbs,start} = 2706.71[\mathrm{L}] \cdot 35\left[\frac{g}{L}\right] = 94734.85[g]$$

If there is no loss of product the concentration after IEX chromatography is:

$$c_4 = \frac{94734.85[g]}{3751.62[L]} = 25.00[\frac{g}{L}]$$

Leaving the IEX chromatography are 94734.85 L with a concentration of 25.00 g/L.

Tank size and buffer amount

There is no limitation in tank size for a SST build facility and buffers of the same type can be pooled (e.g. buffer J of Rinse 1,Wash 1, Wash 2; see Table 19). The minimum amount of buffer J and the minimum tank size for g1 are 9195.132 L :

$$V_{g1} = \left(n_{Cycles,Rinse\ 1} \cdot Volume_{SST,Rinse\ 1}\right) + \left(n_{Cycles,Wash\ 1} \cdot Volume_{SST,Wash\ 1}\right) + \left(n_{Cycles,Wash\ 2} \cdot Volume_{SST,Wash\ 2}\right)$$

$$V_{g1} = (1 \cdot 211.65[L]) + (17.73 \cdot 211.65[L]) + (17.73 \cdot 211.65[L]) = 7716.76\ [L]$$

The minimum amount of buffer K and the minimum tank size for g2 are 384.8 L:

$$V_{g2} = \left(n_{Cycles,Sanitization} \cdot Volume_{SST,Sanitization}\right) = 1 \cdot 211.65[L] = 211.65[L]$$

The minimum amount of buffer L and the minimum tank size for g3 are 2088.02 L:

$$V_{g3} = \left(n_{Cycles,Rinse\ 2} \cdot Volume_{SST,Rinse\ 2}\right) + \left(n_{Cycles,Equilibration} \cdot Volume_{SST,Equilibration}\right) = 1 \cdot 211.65[L] + 17.73 \cdot 105.83[L] = 2088.02[L]$$

The minimum amount of buffer M and the minimum tank size for g4 are 3752.55 L:

$$V_{g4} = \left(n_{Cycles,Elution} \cdot Volume_{SST,Elution}\right) = 17.73 \cdot 211.65[L] = 3752.55[L]$$

The minimum amount of buffer N and the minimum tank size for g5 are 4405.1855 L:

$$V_{g5} = \left(n_{Cycles,Cleaning\ flush} \cdot Volume_{SST,Cleaning\ flush}\right) = 17.73 \cdot 211.65[L] = 3752.55[L]$$

The amount of WFI from the loop $V_{WFI\ loop,r1}$ are scaled from the 2k SUT process:

$$V_{WFI\ loop,r1} = 2706.71[L] \cdot \frac{1000[L]}{294[L]} = 9206.46[L]$$

The following list shows all calculated variables:

Description	Content	Labelling	Value
Volume of tank g1	Buffer J	V_{g1}	7716.76[L]
Volume of tank g2	Buffer K	V_{g2}	211.65[L]
Volume of tank g3	Buffer L	V_{g3}	2088.02[L]
Volume of tank g4	Buffer M	V_{g4}	3752.55[L]
Volume of tank g5	Buffer N	V_{g5}	3752.55[L]
WFI supply loop r1	-	$V_{WFI\ loop,r1}$	9206.46[L]
Volume after IEX	-	V_{g4}	3752.55[L]
Concentration after IEX	-	c_4	25.00 [g/L]
Total processing time		$t_{final,IEX}$	16.50[h]

10.2.6 Conditioning and filtration 1

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 9	mAb solution	V_9	?
Volume of tank 10	mAb solution	V_{10}	?
Volume of tank h1	Buffer O	V_{h1}	?
Volume after C&F 1	-	-	?
Concentration after C&F 1	-	-5	?
Total processing time	-	-,	?

The amount of buffer O for conditioning is scaled from the 2k SUT process:

$$V_{h1} = 3752.55[L] \cdot \frac{148[L]}{384[L]} = 1445.94[L]$$

The minimum tank volume for tank 9 is the Volume of the eluate of the IEX chromatography step:

$$V_9 = 3751.62[L]$$

The volume of tank 10 can be calculated by adding the volume after filtration to the amount of buffer O:

$$V_{10} = 3751.62[L] + 1445.94[L] = 5197.56[L]$$

The loss due to filtration can be scaled from the 2 m^3 SUT process:

$$V_{after\ filtration} = 5197.56[L] \cdot \frac{523[L]}{533[L]} = 5510.73[L]$$

With a 4 h preparation time and a 0 h disassembling time the total conditioning time is 6[h]:

$$t_{conditiononing} = 2[h] + 4[h] + 0[h] = 6[h]$$

Filtration with cartridges (30") (Pore size=0.8/0.45 µm; membrane area=1.8 m^2; filter capacity=300 L/m^2; flow rate per filter=213 L/h).

$$Required\ filter\ capsules = \frac{5510.73[L]}{2.5[h] \cdot 213[\frac{L}{h \cdot filter}]} = 10.35 \approx 10$$

$$Flow\ rate = 9.76 \cdot 213 \left[\frac{L}{h \cdot filter}\right] = 2078.88[L/h]$$

With 2 h preparation and 2 h dismantling time the total time for filtration is 6.5 h:

$$t_{filtration\ total} = 2.5[h] + 2[h] + 2[h] = 6.5[h]$$

The concentration after the conditioning and filtration procedure is:

$$c_{post\ C\&F1} = c_{10} = c_{pre\ C\&F1} \cdot \frac{V_{pre\ C\&F1}}{V_{post\ C\&F1}} = 25.00\ [\frac{g}{L}] \cdot \frac{3751.62[L]}{5510.73[L]} = 18.05[\frac{g}{L}]$$

The overall processing time of the conditioning and filtration 1 processing step is 2.2299 d:

$$t_{total,C\&F1} = 6[h] + 6.5[h] = 12.5[h]$$

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank 9(11)	mAb solution	V_9	3751.62[L]
Volume of tank 10 (12)	mAb solution	V_{10}	5197.56[L]
Volume of tank h1	Buffer O	V_{h1}	1445.94[L]
Volume after C&F 1	-	V_{10}	5510.73 [L]
Concentration after C&F 1	-	-c_{10}	18.05[g/L]
Total processing time	-	$t_{total,C\&F1}$	12.5[h]

10.2.7 Anion exchange chromatography

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank i1	Buffer S	V_{i1}	?
Water from loop (s1)	$V_{WFI\ loop,s1}$	V_{s1}	?
Volume of tank j1	Buffer P	V_{j1}	?
Volume of tank j2	Buffer N	V_{j2}	?
Volume of tank j3	Buffer Q	V_{j3}	?
Volume of tank j4	Buffer R	V_{j4}	?
Volume after AEX	-	$c_{11,12}$	?
Concentration after AEX	mAb solution	$V_{11,12}$	?
Total processing time	-	$t_{total,AEX}$	?

Column properties for anion exchange chromatography are:

Diameter: 140 cm=1.40 m
Bed height: 20 cm=0.2 m
Dynamic binding capacity=20 g/L

$$Area = \frac{\pi \cdot (140[cm])^2}{4} = 15463.14[cm^2] = 1.55[m^2]$$

$$Volume = \ Area \cdot 0.2[m] = 1.55[m^2] \cdot 0.2[m] \cdot \frac{1000[L]}{1m^3} = 309.26[L]$$

$$Binding\ capacity = \ 309.26[L] \cdot 20\left[\frac{g}{L}\right] = 6185.26[g]$$

$$Liters\ that\ can\ be\ processed\ in\ one\ cycle = 5724.30[g] \cdot \frac{1}{18.05\left[\frac{g}{L}\right]} = 342.77[L]$$

$$Number\ of\ cycles\ = \frac{5510.73[L]}{342.77[\mathrm{L}]} = 16.08 \approx 16$$

Buffer	Purpose	SST Volume [L]	CV	Linear flow rate [cm/h]	Volumetric flow rate [L/h]	n_{Cycles}	Time (one cycle) [h]	Time (total) [h]
P	Rinse 1	1855.58	6	300	4638.94	1.00	0.40	0.40
N	Sanitization	927.79	3	300	4638.94	1.00	0.20	0.20
Q	Eqilibration	927.79	3	300	4638.94	16.08	0.20	3.22
Product	Load	342.77	1.11	300	4638.94	16.08	0.07	1.19
Q	Wash 1	927.79	3	300	4638.94	16.08	0.20	3.22
R	Elution	1855.58	6	300	4638.94	16.08	0.40	6.43

WFI	Regeneration	618.53	2	300	4638.94	16.08	0.13	2.14
N	Cleaning flush	927.79	3	300	4638.94	16.08	0.20	3.22
P	Regeneration 1	1546.31	5	300	4638.94	16.08	0.33	5.36
S	Regeneration 2	9.28	0.03	300	4638.94	16.08	0.002	0.03
Sum								25.4

Example for Rinse 1:

$$Volume = CV \cdot Volume_{Column} = 6 * 309.26[L] = 1855.58[L]$$

$$Volumetric\ flow\ rate = 300[\frac{cm}{h}] \cdot 15463.14[cm^2] = 4638942\,\frac{mL}{h} \cdot \frac{1[L]}{1000[mL]} = 4638.94[\frac{L}{min}]$$

$$Time = Volume[L] \cdot \frac{n_{Cycles}}{Volumetric\ flow\ rate\ \left[\frac{L}{h}\right]} = 1855.58[L] \cdot \frac{1}{4638.94\ \left[\frac{L}{h}\right]} = 0.40[h]$$

The total processing time for 5510.73 L with a concentration of 18.05 g/L is 33.40 h (1.39 d), including an estimated 4 h preparation and a 4 h dismantling time:

$$t_{total,IEX} = \frac{25.40[h] + 4[h] + 4[h]}{\frac{24[h]}{1[d]}} = 1.39[d]$$

The elution volume is 13689.9863 L:

$$Elution\ volume = Volume[L] \cdot Cycles \cdot (\frac{Elution\ fraction}{100})$$

$$Elution\ volume = 1855.58[L] \cdot 16.08 \cdot \left(\frac{40}{100}\right) = 11934.95[\mathrm{L}] = V_{11+12}$$

To calculate the concentration of product after AEX chromatography one has to calculate the starting mass of mAbs first:

$$m_{mAbs,start\ AEX} = 5510.73\ [\mathrm{L}] \cdot 18.08\left[\frac{g}{L}\right] = 99441.70[g]$$

If there is no loss of product the concentration after AEX chromatography is:

$$c_{post\ IEX} = c_{11,12} = \frac{99441.70[g]}{11934.95[L]} = 8.33[\frac{g}{L}]$$

Leaving the AEX chromatography are 11934.95 L with a concentration of 8.33 g/L.

Tank size and buffer amount

There is no limitation in tank size for a SST build facility and buffers of the same type can be pooled (e.g. buffer N from "Sanitization" or "Cleaning flush" in tank j2). The minimum amount of buffer N and the minimum tank size for j2 are 15844.04 L:

$$V_{j2} = \left(n_{Cycles,Sanitization} \cdot Volume_{SST;Sanitization}\right) + \left(n_{Cycles,Cleaning\ flush} \cdot Volume_{SST,cleaning\ flush}\right)$$

$$V_{j2} = (1 \cdot 927.79[L]) + (16.08 \cdot 927.79[L]) = 15844.04\ [L]$$

The minimum amount of buffer S and the minimum tank size for i1 are 149.16 L:

$$V_{i1} = \left(n_{Cycles,Regeneration\ 1} \cdot Volume_{SST,Regeneration\ 1}\right)$$

$$V_{i1} = 16.08 \cdot 9.28[L] = 149.16[L]$$

The minimum amount of buffer P and the minimum tank size for j1 are 26716.00 L:

$$V_{j1} = \left(n_{Cycles,Rinse\ 1} \cdot Volume_{SST,Rinse\ 1}\right) + \left(n_{Cycles,Regeneration\ 1} \cdot Volume_{SST,Regeneration\ 1}\right) = 1 \cdot 1855.58[L] + 16.08 \cdot 1546.31 = 26716.00[L]$$

The minimum amount of buffer Q and the minimum tank size for j3 are 29832.51 L:

$$V_{j3} = \left(n_{Cycles,Wash\ 1} \cdot Volume_{SST,Wash\ 1}\right) + \left(n_{Cycles,Equilibration} \cdot Volume_{SST,Equilibration}\right) = 16.08 \cdot 927.79[L] + 16.08 \cdot 927.79[L] = 29832.51[L]$$

The minimum amount of buffer R and the minimum tank size for j4 are 29832.51 L:

$$V_{j4} = \left(n_{Cycles,Elution} \cdot Volume_{SST,Elution}\right) = 16.08 \cdot 1855.58[L] = 29832.51[L]$$

The amount of WFI from the loop and therefore tank volume s1 are scaled from the 2k SUT process:

$$V_{WFI\ loop,s1} = 5510.73[L] \cdot \frac{1000[L]}{523[L]} = 10536.76[L]$$

The following list shows all variables that have been calculated:

Description	Content	Labelling	Value
Volume of tank i1	Buffer S	V_{i1}	149.16[L]
Water from loop (s1)	$V_{WFI\ loop,s1}$	V_{s1}	10536.76[L]
Volume of tank j1	Buffer P	V_{j1}	26716.00[L]
Volume of tank j2	Buffer N	V_{j2}	15844.04[L]
Volume of tank j3	Buffer Q	V_{j3}	29832.51[L]
Volume of tank j4	Buffer R	V_{j4}	29832.51[L]
Volume after AEX		V_{11+12}	11934.95[L]
Concentration after AEX		$c_{11,12}$	8.33[g/L]
Total processing time		$t_{total,AEX}$	33.40[h]

10.2.8 Virus filtration

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 11 (13)	mAb solution	V_{11}	?
Volume of tank 12 (14)	mAb solution	V_{12}	?
Volume of tank k1	Buffer R	V_{k1}	?
Volume after VF		V_{13+14}	?
Concentration after VF		$c_{13,14}$	?
Total processing time		V_{j4}	?

Virus clearance is assured by the use of specialized two stage virus filtration filters (Pore size=0.8/0.45 µm; membrane area=0.6 m^2; filter capacity=500 $L/m^2 \cdot h$; selected filter area=0.60 m^2)

The two stage filters are flushed with 33 L/m^2 buffer R, and then the mAb solution is filtrated. Afterwards, the filters are flushed with 7 L/m^2 to leave no product behind.

The process time is set to 4.7 h resulting in the selection of $9.73 (\approx 10)$ filtration capsules:

Second stage (Pore size=0.45/0.2 µm; membrane area=4 m^2; filter capacity=75 $L/m^2 * h$; selected filter area=4 m^2; selected quantity of filter capsules=1)

Washing of the filter is performed with 33 L/m^2, resulting in a buffer R demand:

$$V_{k1,filter\ wash} = 33\left[\frac{L}{m^2}\right] \cdot 8.73 \cdot (0.6[m^2] + 4[m^2]) = 1324.68[L]$$

Cleaning after the filtration is performed with 7 L/m^2, resulting in a buffer R demand:

$$V_{k1,cleaning\ flush} = 7\left[\frac{L}{m^2}\right] \cdot 8.73 \cdot (0.6[m^2] + 4[m^2]) = 280.99[L]$$

$$V_{k1,} = V_{k1,filter\ wash} + V_{k1,cleaning\ flush} = 1324.68[L] + 280.99[L] = 1605.68[L]$$

$$Flow\ rate = 75[\frac{L}{m^2 \cdot h}] \cdot 8.73 \cdot 4[m^2] = 300[\frac{L}{h}]$$

Table 20 Parameters of virus filtration

Buffer	Operation	Flow rate [L/h]	SST Volume [L]
R	Filter wash	2919	1477.75
Product	Load	2919	11934.95
R	Cleaning flush	2919	313.46

$$Flow\ rate, stage\ 1 = 500[\frac{L}{m^2 \cdot h}] \cdot 9.73 \cdot 0.6[m^2] = 2919[\frac{L}{h}]$$

$$Flow\ rate, stage\ 2 = 75[\frac{L}{m^2 \cdot h}] \cdot 9.73 \cdot 4[m^2] = 2919[\frac{L}{h}]$$

$$t_{filtration} = \frac{1477.75[L] + 11934.95[L] + 313.46[L]}{2919[\frac{L}{h}]} = 4.70[h]$$

With an estimated preparation time of 2 h and a dismantling time of 2 h the total duration is 8.70 h:

$$t_{total,VF} = 4.70[h] + 2[h] + 2[h] = 8.70[h]$$

The volume after virus filtration increases due to flushing activities:

$$V_{after\ virus\ filtration} = 11934.95[L] + 313.46[L] = 12248.41[L] = V_{11+12}$$

The tank volume of tank 11 and 12 is 7023.2192 L, since the volume that leaves the virus filtration is split in two:

$$V_{11/12} = \frac{12248.41[L]}{2} = 6124.21[L]$$

The product mass before virus filtration was:

$$m_{before\ virus\ filtration} = 11934.95\ [L] \cdot 8.33\left[\frac{g}{L}\right] = 99418.13[g]$$

With no loss of mass during virus filtration the concentration after virus filtration is:

$$c_{after\ virus\ filtration} = \frac{99418.13[g]}{12248.41[L]} = 8.12\left[\frac{g}{L}\right] = c_{11,12}$$

The following list shows all calculated variables:

Description	Content	Labelling	Value
Volume of tank 11 (13)	mAb solution	V_{11}	6124.21[L]
Volume of tank 12 (14)	mAb solution	V_{12}	6124.21[L]
Volume of tank k1	Buffer R	V_{k1}	1605.68[L]
Volume after VF		V_{11+12}	12248.41[L]
Concentration after VF		$c_{11,12}$	8.12[g/L]
Total processing time		$t_{total,VF}$	8.70[h]

10.2.9 Ultrafiltration/diafiltration 2

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 13 (15)	mAb solution	V_{13}	?
Volume of tank 14 (16)	mAb solution	V_{14}	?
Volume of tank 15 (17)	mAb solution	V_{15}	?
Volume of tank l1	0.5 M NaOH	V_{l1}	?
Volume of tank l2	Buffer R	V_{l2}	?
Volume of tank m1	Buffer T	V_{m1}	?
WFI from loop (n1)	WFI	V_{n1}	?
Volume after UF/DF2		V_{16}	?
Concentration after UF/DF 2		C_{16}	?
Total processing time		$t_{total,UF/DF2}$	?

Ultrafiltration/diafiltration requires several filter cassettes (Filter area=2.5 m^2; Transmembrane pressure=1bar; Recirculation flow rate=360 [L/m^2*h]; Permeate flow rate=63 [L/m^2*h; Feed flow rate=100 L/h).

With set filtration time of 11 h, this building block requires 38.86 (≈ 39) filter cassettes.

$$Total\ area = 38.86 \cdot 2.5[m^2] = 97.15[m^2]$$

$$Initial\ volume = 12248.41[L]$$

$$Concentrated\ volume = 12248.41[L] \cdot \frac{391[L]}{1261[L]} = 3797.88[L] = V_{15}$$

$$Recirculation\ flow\ rate = 360[\frac{L}{m^2 \cdot h}] \cdot 97.15[m^2] = 34974[\frac{L}{h}]$$

$$Permeate\ flow\ rate = 63[\frac{L}{m^2 \cdot h}] \cdot 97.15[m^2] = 6120.45[\frac{L}{h}]$$

Table 21 Parameters form UF/DF 2. The UF/DF 2 process is performed till a concentration of 25 g/L is reached. The flush reduces the concentration from 25 to 20 g/L.

Medium	**SUT Volume [L]**	**SST Volume [L]**	**Operation**	**Value**	**Time[h]**
WFI	300	2913.98	Rinse 1	-	0.48
0.5 M NaOH	100	971.33	Sanitization	-	0.16
WFI	630	6119.35	Rinse 2	-	1.00
Buffer R	500	4856.63	Equilibration	-	0.79
Product	1261	12248.41	Concentrate to	25	1.38
Buffer T	3914	38017.66	DF with 6 DV	-	6.21
Buffer T	98	951.90	Flush to	20	0.98
					11.00

The SST volumes are calculated form the proportion of numbers from the SUT volumes, e.g.:

$$V_{SST,Rinse\ 1} = 12248.41[L] \cdot \frac{300[L]}{1261[L]} = 2913.98[L]$$

The times for Rinse 1, Sanitization, Rinse 2, Equilibration, DF with 10 DV and Flush are calculated the following way, e.g. Rinse 1:

$$t_{Rinse\ 1} = \frac{V_{SST,Rinse\ 1}}{Permeate\ flow\ rate} = \frac{2913.98[L]}{6120.45[\frac{L}{h}]} = 0.48[h]$$

The time to process the product is calculated the following way

$$t_{Product} = \frac{V_{SST,Product}}{Permeate\ flow\ rate} = \frac{12248.41[L] - 3797.88[L]}{6120.45[\frac{L}{h}]} = 1.38[h]$$

The sum of all operation steps is 11.00 h.

With an additional preparation time of 2 h and a dismantling time of 2 h, this results in a processing time of 15 h:

$$t_{final,UF/DF2} = 11.00[\text{h}] + 2[\text{h}] + 2[\text{h}] = 15.00[\text{h}]$$

The volume after UF/DF is 4749.78 L:

$$V_{afterUF/DF} = Concentrated\ volume + V_{Flush} = 3797.88[L] + 951.90[L]$$
$$= 4749.78[L] = V_{16}$$

The volume of tanks 13 and 14 are the volume that enters UF/DF 2 divided by two:

$$V_{13/14} = \frac{V_{after\ virus\ filtration}}{2} = \frac{12248.41[\text{L}]}{2} = 6124.21[\text{L}]$$

The volume for tank l1 (Vl1) (0.5 M NaOH) is 971.33 L as seen as in Table 21

$$V_{l1} = 12248.41[L] \cdot \frac{100[L]}{1261[L]} = 971.33[L]$$

The volume of the tank l2 (V_{l2}) is 4856.63L as seen as in Table 21

$$V_{l2} = 12248.41[L] \cdot \frac{500[L]}{1261[L]} = 4856.63[L]$$

The volume of the tank m1 (V_{m1}) is 44689.1749 L as seen as in Table 21

$$V_{m1} = V_{SST,DF\ with\ 6DV} + V_{SST,Flush} = 38017.66[L] + 951.90[L] = 38969.56[L]$$

The volume of the tank n1 (V_{n1}) is L as seen as in Table 21

$$V_{n1} = V_{SST,Rinse\ 1} + V_{SST,Rinse\ 2} = 2913.98[L] + 6119.35[L] = 9033.33[L]$$

The ultrafiltration/diafiltration step is followed by absolute filtration (Filter area=1.2 m^2; filter loading=300 L/m^2; flow rate per filter=100 L/h).

With a set filtration time of 4.6 h the number of required filters is 10.

$$number\ of\ filters = \frac{4749.78\ [\text{L}]}{4.6[h] \cdot 100[\frac{l}{h \cdot filter}]} = 10.33 \approx 10$$

$$Flow\ rate = 10.33 \cdot 100\left[\frac{L}{h}\right] = 1033.00[\frac{L}{h}]$$

$$Volume\ after\ filtration = 4749.78\ [L] \cdot \frac{460}{465} = 4698.71[L] = V_{16}$$

With an additional preparation time of 2 h and a dismantling time of 2 h, this results in a processing time of 8.60 :

$$t_{filtration} = 4.60[\mathrm{h}] + 2[\mathrm{h}] + 2[\mathrm{h}] = 8.60[\mathrm{h}]$$

The total processing time is 23.60 [h]:

$$t_{final,UF/DF2} = 15.00[\mathrm{h}] + 8.60[\mathrm{h}] = 23.60[\mathrm{h}]$$

The following list shows all calculated variables:

Description	Content	Labelling	Value
Volume of tank 13	mAb solution	V_{13}	6124.21[L]
Volume of tank 14	mAb solution	V_{14}	6124.21[L
Volume of tank 15	mAb solution	V_{15}	4749.78[L]
Volume of tank l1	0.5 M NaOH	V_{l1}	971.33[L]
Volume of tank l2	Buffer R	V_{l2}	4856.63[L]
Volume of tank m1	Buffer T	V_{m1}	38969.56[[L]
WFI from loop (n1)	WFI	V_{n1}	9033.33[L]
Volume after UF/DF2		V_{16}	4698.71 [L]
Concentration after UF/DF 2		c_{16}	20[g/L]
Total processing time		$t_{total,UF/DF2}$	23.60[h]

10.2.10 Conditioning and filtration 2

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Volume of tank 16	mAb solution	V_{16}	?
Volume of tank 17	mAb solution	V_{17}	?
Volume of tank o1	mAb solution	V_{o1}	?
Volume after C&F2	-	V_{l2}	?
Concentration after C&F2	-	C_{17}	?
Total processing time	-	$t_{total,C\&F2}$	?

The amount of buffer U for conditioning is scaled from the 2k SUT process:

$$V_{o1} = 4698.71[\mathrm{L}] \cdot \frac{0.411[L]}{460[L]} = 4.20[L]$$

The minimum tank volume for tank 16 is the volume leaving the UF/DF 2 step:

$$V_{16} = 4698.71[L] + 4.20[L] = 4702.91[L]$$

The minimum volume of tank 17 is the same as the minimum volume of tank 16:

$$V_{17} = V_{16} = 4697.71[L] + 4.20[L] = 4701.91[L]$$

The time for conditioning is 4 h. With a 2 h preparation time and a 0 h disassembling time the total conditioning time is 6 h:

$$t_{conditioning} = 4[h] + 2[h] + 0[h] = 6[h]$$

Conditioning is followed by an absolute filtration (Pore size=0.8/0.45 µm; membrane area=1.2 m^2; filter capacity=300 L/m^2; flow rate per filter=200 L/h):

With set filtration time of 2.3 h, the number of filter comes to 9.04 (≈9):

$$number\ of\ filters = \frac{4702.91[L]}{2.3[h] \cdot 200[\frac{L}{h \cdot filter}]} = 10.22 \approx 10$$

The volume after filtration is 4656.95 L:

$$V_{17,out} = 4702.91[L] \cdot \frac{456}{460.5} = 4656.95[L]$$

With a filtration time of 2.3 h, a preparation time of 2 h and a dismantling time of 2 h, the total time for filtration is 6.30 h:

$$t_{filtration} = 2.30[h] + 2[h] + 2[h] = 6.30[h]$$

The concentration after the conditioning and filtration procedure is:

$$c_{post\ C\&F2} = 20[\frac{g}{L}] \cdot \frac{V_{pre\ C\&F2}}{V_{post\ C\&F2}} = 20.00\ [\frac{g}{L}] \cdot \frac{4656.95[L]}{4698.71[L]} = 19.80[\frac{g}{L}]$$

The mass of mAbs before C&F2 is 93974.2 g:

$$m_{pre\ C\&F2} = 20\ [\frac{g}{L}] \cdot 4698.71[L] = 93974.2[g]$$

The concentration drops to 19.98 g/L, when 4.20 L of buffer U are added:

$$c_{after\ conditioning} = \frac{93974.2[g]}{4698.71[L] + 4.20[L]} = 19.98\left[\frac{g}{L}\right] = c_{final}$$

Filtration results in a loss of volume but does not impact the concentration.

The total processing time of the conditioning and filtration 2 processing step is 12.30[h]:

$$t_{total,C\&F2} = 6.30[h] + 6[h] = 12.30[h]$$

The following list shows all calculated variables:

Description	Content	Labelling	Value
Volume of tank 16	mAb solution	V_{16}	4701.91
Volume of tank 17	mAb solution	V_{17}	4701.91 [L]
Volume of tank o1	mAb solution	V_{o1}	4.20
Volume after C&F2	-	$V_{17,out}$	4656.95 [L]
Concentration after C&F2	-	c_{final}	19.98[g/L]
Total processing time	-	$t_{total,C\&F2}$	12.30[h]

10.2.11 Fill and finish

The following list shows all unknown variables that have to be calculated:

Description	Content	Labelling	Value
Total processing time	-	$t_{total,C\&F2}$	?
Number of filling stations		$n_{filling\ stations}$	?
Number of nalgene bottles		$n_{nalgenes}$	?

Fill and finish includes a two-stage filtration process with a set processing time of 4.5h. Filter one (Pore size=0.5/0.2 µm; membrane area=0.16 m^2; filter capacity=1000 L/m^2; flow rate per filter =33.33 L/h):

$$Number\ of\ needed\ filter\ capsules = \frac{4656.95\ [L]}{4.5[h] \cdot 33.33[\frac{L}{h \cdot filter}]} = 31.05$$

$$\approx 31$$

$$flow\ rate\ of\ filter\ 1 = 31.05 \cdot 33.33[\frac{L}{h}] = 1034.90[h]$$

Filter two (Pore size=0.22 µm; membrane area=0.1 m^2; filter capacity=1000 L/m^2; flow rate=100 L/h):

$$Number\ of\ needed\ filter\ capsules = \frac{4656.95\ [L]}{4.5[h] \cdot 20.00[\frac{L}{h \cdot filter}]} = 51.74$$

$$\approx 31$$

$$flow\ rate\ of\ filter\ 2 = \ 51.74 \cdot 20.00[\frac{L}{h}] = 1034.90[L/h]$$

The volume after filtration is 4565.03 L:

$$V_{final} = 4656.95[\mathrm{L}] \cdot \frac{447}{456} = 4565.03[L]$$

The flow rate of one filling station is 100 L/h. To achieve the set filling time of 4.5 h, 10.14 (≈10) filling stations are needed:

$$n_{filling\ stations} = \frac{4565.03[L]}{4.5[h] \cdot 100[\frac{L}{h \cdot station}]} = 10.14$$

The total time for filling comes to 6.5 h, including a 1 h preparation and a 1 h dismantling time:

$$t_{filling} = 4.5[h] + 1[h] + 1[h] = 6.50[h]$$

Total processing time of the fill and finish building block is 15 h:

$$t_{total,F\&F} = t_{filtration} + t_{filling} = 8.50[h] + 6.50[h] = 15.00[h]$$

4565.03 L with a concentration of 19.98 g/L can be filled into storage bottles/bags with a volume of 5 L. This results in 862 bottles/bags total that can be freezed:

$$n_{bottles/bags} = \frac{4565.03[L]}{5[L]} = 913.01\ \approx 913$$

The following list shows all calculated variables:

Description	Content	Labelling	Value
Total processing time	-	$t_{total,F\&F}$	15[h]
Number of filling stations		$n_{filling\ stations}$	10.14
Number of nalgene bottles		$n_{nalgenes}$	913

10.3 WFI and RO water generation – preliminary calculations

Water for injections (WFI)

The energy demand for WFI generation by distillation is based on the following formula:

$$Q = m_{water} \cdot c_{p,water} \cdot \Delta T \cdot (2 - efficiency\ factor)$$

The energy demand to heat 1 kg (or 1 L) of water from 20°C to 105°C by also accounting for an efficiency factor of 0.9 is 0.1296 kWh:

$$Q_{1L,WFI} = 1[kg] \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (105 - 20)[K] \cdot (2 - 0.9) = 392.70[kJ]$$

$$Q_{1L,WFI} = 428.4[kJ] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 0.11[kWh]$$

As a result, 0.11 kWh are necessary to heat 1 kg (or 1 L) of water to the desired temperature of 105°C.

With the use of emission factors for electricity (see Table 2), the CO_2 output per kg of water generated can be calculated. For this example, an average emission factor for electricity generation that includes different countries and regions is used:

$$EF_{electricity,average} = \frac{EF_{Switzerland}+EF_{Germany}+EF_{USA}+EF_{Asia}}{4} = \frac{23.6\left[\frac{g_{CO2}}{kWh}\right]+523\left[\frac{g_{CO2}}{kWh}\right]+428\left[\frac{g_{CO2}}{kWh}\right]+845\left[\frac{g_{CO2}}{kWh}\right]}{4} = 454.9[\frac{g_{CO2}}{kWh}]$$

For the generation of 1 L of water for CIP operations 58.96 gCO2 are emitted:

$$\frac{0.11[kWh]}{1kg_{WFI}} \cdot 454.9\left[\frac{g_{CO2}}{kWh}\right] = 50.04[\frac{g_{CO2}}{kg_{WFI}}]$$

A common method to save energy is to preheat the incoming cold water with the hot water exiting the WFI distillation system. The water exiting the WFI distillation system has a temperature105°C (ϑ_1') and shall be cooled down to 80°C (ϑ_1'') for storage in the hot WFI loop. To calculate the temperature of the incoming feed water ϑ_2'' after passing through a heat exchanger, the basic heat exchanger design equation can be applied:

$$\dot{Q} = \dot{W}_1 \cdot (\vartheta_1' - \vartheta_1'') = \dot{W}_2(\vartheta_2'' - \vartheta_2')$$

- $\dot{Q}$ is the heat flux in [W]
- $\dot{W}_1$ is the thermal capacity flow of the hot WFI exiting the system in [W/K]
- $\dot{W}_2$ is the thermal capacity flow of the cold WFI entering the system in [W/K]
- ϑ_1' is the temperature of the hot WFI entering the heat exchanger (105°C)
- ϑ_1'' is the temperature of the hot WFI exiting the heat exchanger (80°C)
- ϑ_2' is the temperature of the cold feed water entering the heat exchanger (20°C)
- ϑ_2'' is the unknown temperature that the feed water has after being preheated by the heat exchanger

In a stationary state, the same mass of feed water is entering the WFI generation system as ready to use WFI is leaving the generation system.

$$\dot{m}_{in} = \dot{m}_{out}$$

For this reason, the thermal capacity flows $\dot{W}_1$ and $\dot{W}_2$ are the same:

$$\dot{W}_1 = \frac{\dot{m}_{in}}{c_{p,water}} = \dot{W}_2 = \frac{\dot{m}_{out}}{c_{p,water}}$$

Therefore, the basic heat exchanger equation can be rearranged to the temperature of the preheated feed stream:

$$\dot{Q} = \dot{W}_1 \cdot (\vartheta_1' - \vartheta_1'')$$

$$\dot{Q} = \dot{W}_2 (\vartheta_2'' - \vartheta_2')$$

$$(\vartheta_1' - \vartheta_1'') = (\vartheta_2'' - \vartheta_2')$$

$$\vartheta_2'' = \vartheta_1' - \vartheta_1'' + \vartheta_2'$$

$$\vartheta_2'' = 105°C - 80°C + 20°C = 45°C$$

The energy demand to heat 1 kg (or 1 L) of water from 45°C to 105°C by also accounting for an efficiency factor of 0.9 is 0.1296 kWh:

$$Q_{1L,water} = 1[kg] \cdot 4.2 \left[\frac{kJ}{kg \cdot K}\right] \cdot (105 - 45)[K] \cdot (2 - 0.9) = 277.20[kJ]$$

$$Q_{1L,water} = 277.20[kJ] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 0.08[kWh]$$

As a result, 0.07 kWh are necessary to heat 1 kg (or 1 L) of water to the desired temperature of 105°C. To generate one ton of WFI, 80 kWh are required:

$$E_{WFI} = 0.08\left[\frac{kWh}{kg_{WFI}}\right] \cdot \frac{1000[kg_{WFI}]}{1[t_{WFI}]} = 80\left[\frac{kWh}{t_{WFI}}\right]$$

This value is higher than the WFI generation systems by Meco (*10.17 Exemplary WFI generation systems*) that produce 1500 L of WFI per hour. The four systems by Meco consume between $6.1\bar{6}$ and 17.06 kWh per ton of WFI:

System 1 – WFI with multiple effect distillation:

$$E_{WFI,system1} = \frac{9.25[kW]}{1.5[\frac{t_{WFI}}{h}]} = 6.1\bar{6}[\frac{kWh}{t_{WFI}}]$$

System 2 – WFI with RO/EDI & Ultrafiltration

$$E_{WFI,system1} = \frac{9.5[kW]}{1.5[\frac{t_{WFI}}{h}]} = 6.\bar{3}[\frac{kWh}{t_{WFI}}]$$

System 3 – WFI with carbon filtration, water softening and vapour compression

$$E_{WFI,system1} = \frac{25.6[kW]}{1.5[\frac{t_{WFI}}{h}]} = 17.0\bar{6}[\frac{kWh}{t_{WFI}}]$$

System 4 – WFI with ultrafiltration and vapour compression:

$$E_{WFI,system1} = \frac{25.6[kW]}{1.5[\frac{t_{WFI}}{h}]} = 17.0\bar{6}[\frac{kWh}{t_{WFI}}]$$

With the use of emission factors for electricity (see Table 2), the CO_2 output per kg of water generated can be calculated. For this example, an average emission factor for electricity generation that includes different countries and regions is used:

$$EF_{electricity,average} = \frac{EF_{Switzerland}+EF_{Germany}+EF_{USA}+EF_{Asia}}{4} = \frac{23.6\left[\frac{g_{CO2}}{kWh}\right]+523\left[\frac{g_{CO2}}{kWh}\right]+428\left[\frac{g_{CO2}}{kWh}\right]+845\left[\frac{g_{CO2}}{kWh}\right]}{4} = 454.9[\frac{g_{CO2}}{kWh}]$$

For the generation of 1 L of water for CIP operations, 58.96 g_{CO2} are emitted:

$$\frac{0.07[kWh]}{1kg_{water}} \cdot 454.9 \left[\frac{g_{CO2}}{kWh}\right] = 31.84[\frac{g_{CO2}}{kg_{water}}]$$

This is a reduction of 27.12 g when compared to a method without energy recovery via a heat exchanger:

$$CO_2 emission\ without\ heat\ exchanger = 58.96[\frac{g_{CO2}}{kg_{water}}]$$

$$CO_2 emission\ with\ heat\ exchanger = 31.84[\frac{g_{CO2}}{kg_{water}}]$$

$$CO_2 emission\ reduction = 58.96 \left[\frac{g_{CO2}}{kg_{water}}\right] - 31.84 \left[\frac{g_{CO2}}{kg_{water}}\right] = 27.12 \left[\frac{g_{CO2}}{kg_{water}}\right]$$

This is a reduction of 46%:

$$100\% - \frac{100\%}{58.96 \left[\frac{g_{CO2}}{kg_{water}}\right]} \cdot 31.84 \left[\frac{g_{CO2}}{kg_{water}}\right] = 100\% - 54\% = 46\%$$

Reverse Osmosis (RO)

With the *van't Hoff* equation $\pi = c \cdot R \cdot T$ [89, S. 119] the necessary pressure for reverse osmosis operation can be calculated.

c is the concentration in $\frac{mol}{L}$, R is the ideal gas constant in $\frac{L \cdot bar}{mol \cdot K}$ and T is the temperature in K.

As an example, tap water analysis data(Industrielle Werke Basel) [90] from Basel is used:

Table 22 Tap water analysis data from Basel(IWB Industrielle Werke Basel) [90]

Substanz	Substance	Chemical formula	PubCHem CID	c [mg/L]	M [g/mol]		c [g/L]	c [mol/L]
Hydrogencarbonat	Bicabonate	HCO3	769	177	61.017		0.177	0.0029
Calcium	Calcium	Ca++	271	58	40.08		0.058	0.0014
Sulfat	Sulfate	SO4-2	1117	32	96.06		0.032	0.0003
Chlorid	Chloride	Cl-	312	16.5	35.45		0.0165	0.0005
Natrium	Sodium	Na	5360545	12.5	22.989769		0.0125	0.0005
Magnesium	Magnesium	Mg	5462224	7.9	24.305		0.0079	0.0003
Nitrat	Nitrate	NO3-	943	7.1	62.005		0.0071	0.0001
Kieselsäure	Silica	SiO2	24261	5.3	60.084		0.0053	0.0001
Kalium	Potassium	K	5462222	1.9	39.098		0.0019	0
Fluorit	Fluorite	CaF2	24617	0.11	78.07		0.00011	0
Aluminium	Aluminum	Al	5359268	0.005	26.981538		0.000005	0
Sum								0.0063

With a concentration of $0.0063[\frac{mol}{L}]$, an ideal gas constant of $0.0831433[\frac{L \cdot bar}{mol \cdot K}]$ and a temperature of 273.15 [K], the osmotic pressure is 0.1231 bar:

$$\pi = 0.0063[\frac{mol}{L}] \cdot 0.0831433 \left[\frac{L \cdot bar}{mol \cdot K}\right] \cdot 293.15[K] = 0.1528[bar]$$

Since 1 m water column is equal to 0.098 bar, 0.123 bar equates to 1.26 $m_{water\ column}$:

$$H_{RO} = \pi \cdot \frac{1[m_{water\ column}]}{0.098[bar]} = 0.1528[bar] \cdot \frac{1[m_{water\ column}]}{0.098[bar]} \approx 1.56[m_{water\ column}]$$

As an example, 88.94 m^3 need to generated over the course of 6 h. This results in a necessary production capacity of $\frac{88.94[m^3]}{6[h]} = 14.82[\frac{m^3}{h}]$, or $14.82\left[\frac{m^3}{h}\right] \cdot \frac{1}{3600[s]} = 0.00411\bar{6}[\frac{m^3}{s}]$.

With the assumption, that all other pressure drops in a reverse osmosis system can be neglected, the total energy consumption of the system due to pump operations is 63.61 W:

$$P_{pump} = \frac{\rho_{water} \cdot g \cdot flow\ rate \cdot H_{RO}}{\eta_{pump}}$$

$$P_{pump} = \frac{1000[\frac{kg}{m^3}] \cdot 9.81[\frac{m}{s^2}] \cdot 0.00411\bar{6}[\frac{m^3}{s}] \cdot 1.56[m_{water\ column}]}{0.8} = 78.75[W]$$

Since the pump has to operate for 6 h to generate the desired volume, the energy consumption comes to 0.38166 kWh:

$$Energy\ consumption_{RO} = 78.75[W] \cdot \frac{1[kW]}{1000[W]} \cdot 6[h] = 0.4725[kWh]$$

With the use of emission factors for electricity (see Table 2), the CO_2 output per kg of water generated can be calculated. For this example, an average emission factor for electricity generation that includes different countries and regions is used:

$$EF_{electricity,average} = \frac{EF_{Switzerland} + EF_{Germany} + EF_{USA} + EF_{Asia}}{4} = \frac{23.6\left[\frac{g_{CO2}}{kWh}\right] + 523\left[\frac{g_{CO2}}{kWh}\right] + 428\left[\frac{g_{CO2}}{kWh}\right] + 845\left[\frac{g_{CO2}}{kWh}\right]}{4} = 454.9[\frac{g_{CO2}}{kWh}]$$

For the generation of 88.94 [m^3] of water for CIP operations 58.96 g_{CO2} are emitted:

$$0.4725[kWh] \cdot 454.9\left[\frac{g_{CO2}}{kWh}\right] = 214.94[g_{Co2}]$$

This results in an emission of $1.95 \cdot 10^{-3}[\frac{g_{CO2}}{kg_{RO\ water}}]$

$$\frac{214.94[g_{CO2}]}{88.94[m^3]} \cdot \frac{1[m^3]}{1000[kg_{RO\ water}]} = 2.42 \cdot 10^{-3}[\frac{g_{CO2}}{kg_{RO\ water}}]$$

10.4 SIP - preliminary calculations

The objective of the following calculations is the determination of the tank dimensions form the tank volume. The height and diameter of the tank deliver a certain space requirement that is necessary for HVAC calculations. Furthermore, the tank wall thickness is determined to obtain the mass of the steel tanks. The mass of the steel tanks is relevant for steel production CO_2 emissions as well as emissions that are caused by transportation of the tanks from the production site to the monoclonal antibody production facility.

Tanks generally have torispherical heads and the shape is challenging for surface heat loss calculations. A simplified model is used to calculate the energy and steam demand of the SIP procedure. The tank model consists of an insulated shell with an insulated top and bottom in form of flat disks (see Figure 67).

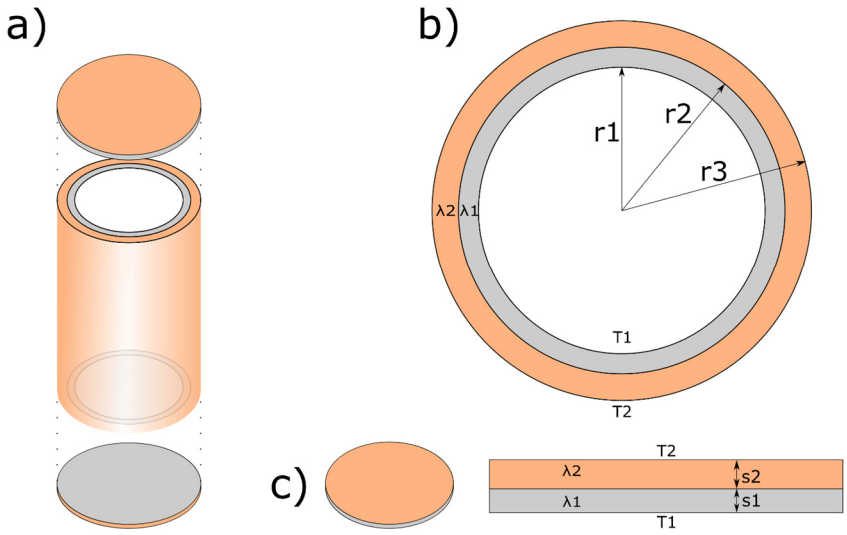


Figure 67 Simplified model to calculate the energy and steam demand of the SIP procedure. Steel: grey; insulation: peach
r1, r2 and r3 are the radii of the steel and insulating layers. T1 and T2 are the temperatures at the according locations. λ1 and λ2 are is the thermal conductivity of the steel and insulation layer. s1 and s2 are the layer thickness of the steel and insulation layer
a) Tank model: shell with lid and bottom
b) Cross section showing the steel layer (grey) and insulating layer (peach)
c) Cross section of the lid and bottom

The following variables are needed for calculating the energy demand of the SIP process:

Table 23 Input variables for the SIP procedure calculations with exemplary values

Variable	Symbol	Unit	Exemplary value
Steel density	ρ	$\frac{\mathrm{kg}}{\mathrm{m}^3}$	7950
Tank diameter	dT	m	2.84
Tank wall thickness	s1	m	0.00436
Tank height	hT	m	2.84
Insulation layer thickness	s2	m	0.08
T_{inside}	T1	°C	121
$T_{outside}$	T2	°C	20
Thermal conductivity steel	λ1	$\frac{\mathrm{W}}{\mathrm{m} \cdot \mathrm{K}}$	45
Thermal conductivity insulation	λ2	$\frac{\mathrm{W}}{\mathrm{m} \cdot \mathrm{K}}$	0.035
Specific heat capacity of steel	$cp_{,steel}$	$\frac{\mathrm{kJ}}{\mathrm{kg} \cdot \mathrm{K}}$	0.5
Duration of the SIP process	t_{SIP}	s	1800
Enthalpy of evaporation	h_V	$\frac{\mathrm{kJ}}{\mathrm{kg}}$	2147
Steam density at 2.5 barg	$\rho_{steam@2.5barg}$	$\frac{kg}{m^3}$	1.9084

Table 24 Variables that are calculated from the input parameters

Variable	Symbol	Unit	Exemplary value
Inner radius	r1	m	$\frac{d_T}{2}$ =1.42
Outer radius of steel layer; inner radius of insulation layer	r2	m	$r1 + s1$ =1.42436
Outer radius of insulation layer	r3	m	$r2 + s2 = 1.50436$

The energy and steam demand of the SIP procedure consists of the following elements:

I. Calculation of shell, lid, bottom volume and mass

The volume of the shell is calculated according to formula 1:

$$V_{shell} = \frac{(d_T+2\cdot s_1)^2}{4} \cdot h_t - \frac{{d_T}^2}{4} \cdot h_t \quad 1$$

The volume of the lid/bottom is calculated with formula 2:

$$V_{lid/bottom} = \frac{\pi*(d_T+2\cdot s_1)^2}{4} \cdot s_1 \quad 2$$

The mass of the shell is calculated via formula 3, while the mass of the lid is calculated with formula 4:

$$m_{shell} = \rho_{shell} \cdot V_{shell} \quad 3$$

$$m_{lid/bottom} = \rho_{steel} \cdot V_{lid/bottom} \quad 4$$

II. Loss of heat due to tank surface

The area of the shell is calculated with formula 5:

$$Area_{shell} = 2 \cdot \pi \cdot r_1 \cdot h_T \quad 5$$

The area of the lid and bottom disks are calculated with formula 6:

$$Area_{lid/bottom} = \frac{{d_T}^2 \cdot \pi}{4} \quad 6$$

The loss of energy due to the tank shell surface is calculated with formula 7 or with formula 8 for the lid/ bottom part of the tank. The loss of energy due to the surface of the lid and the bottom is calculated with formula 8:

$$Q_{surface,shell} = \frac{T_1 - T_3}{\left(\frac{1}{\lambda_1}\cdot\frac{ln(r1)-ln(r2)}{2\cdot\pi * h_T} + \frac{1}{\lambda_2}\cdot\frac{ln(r3)-ln(r2)}{2\cdot\pi\cdot h_T}\right)} \quad 7$$

$$Q_{surface,lid/bottom} = \frac{(T_1 - T_3)\cdot Area_{lid/bottom}}{\frac{s_1}{\lambda_1}+\frac{s_2}{\lambda_2}} \quad 8$$

The loss of energy due to the surface of the lid and the bottom is calculated with formula 9:

$$Q_{surface,lid+bottom} = 2\cdot\frac{(T_1 - T_3)\cdot Area_{lid/bottom}}{\frac{s_1}{\lambda_1}+\frac{s_2}{\lambda_2}} \quad 9$$

III. Energy required to heat the tank up to 121 °C

The energy required to heat up the shell to 121° C is calculated with formula 10, while the energy required to heat up the lid/bottom to 121°C is calculated with formula 11:

$$Q_{shell,121°C} = m_{shell} \cdot c_p \cdot \Delta T \quad 10$$

$$Q_{lid/bottom,121°C} = m_{lid/bottom} \cdot c_p \cdot \Delta T \quad 11$$

IV. Mass flux of steam that is required to fill the tank

To calculate the mass flux of steam that is required to fill the tank with steam, the volume of the tank has to be calculated according to formula 12:

$$V_{tank} = \frac{{d_T}^2\cdot\pi}{4} \cdot h_T \quad 12$$

The mass of steam required to fill the tank can be calculated according to formula 13:

$$m_{steam} = \varrho_{steam@2.5barg} \cdot V_{tank} \quad 13$$

The mass flux of steam required to fill the tank can then be calculated according to formula 14:

$$\dot{m}_{steam} = \frac{m_{steam}}{t_{SIP}} \quad 14$$

V. Energy that is required to fill the tank with steam

The energy required to fill the tank with steam can be calculated according to formula 15:

$$Q_{steam} = \frac{V_{tank} \cdot \rho_{steam@2.5barg} \cdot h_{V,steam}}{t_{SIP}} \quad 15$$

VI. Mass of steam required to compensate loss of heat, fill the tank with steam and heat the tank up to 121°C

The total mass stream fort he SIP procedure can be calculated with formula 16:

$$\dot{m}_{steam,total} = \frac{Q_{surface,shell} + Q_{surface,lid+bottom} + Q_{shell,121°C} + Q_{lid/bottom,121°C}}{h_{v,steam}} + \dot{m}_{steam} \quad 16$$

VII. The total energy demand of the SIP procedure

The total energy demand of the SIP procedure can be calculated with formula 17:

$$Q_{total} = Q_{surface,shell} + Q_{surface,lid+bottom} + Q_{shell,121°C} + 2 \cdot Q_{lid/bottom,121°C} + Q_{steam} \quad 17$$

An exemplary calculation using the values from Table 23 and Table 24 can be found below:

$$V_{shell} = \frac{\pi \cdot (2.84 + 2 \cdot 0.00436)[m]^2}{4} \cdot 2.84[m] - \frac{\pi \cdot 2.84[m]^2}{4} \cdot 2.84[m] = 0.1106[m^2] \quad 1$$

$$m_{shell} = 7950[\frac{kg}{m^3}] \cdot 0.1106[m^2] = 879.27[kg] \quad 3$$

$$V_{lid/bottom} = \frac{\pi * (2.84[m] + 2 * 0.00436[m])^2}{4} \cdot 0.00436[m] = 0.0278[m^3] \quad 2$$

$$m_{lid/bottom} = 7950[\frac{kg}{m^3}] \cdot 0.0278[m^2] = 221.01[kg] \quad 4$$

$$Area_{shell} = 2 \cdot \pi \cdot 2.84[m] \cdot 2.84[m] = 25.34\, m^2 \quad 5$$

$$Area_{lid/bottom} = \frac{2.84\,[m]^2 \cdot \pi}{4} = 6.33 m^2 \quad 6$$

$$Q_{surface,shell} = \frac{(273.15 + 121 - 273.15 + 20)\,[K]}{(\frac{1}{45\frac{[W]}{[m]*[K]}} \cdot \frac{ln(1.42\,[m]) - ln(1.42436\,[m])}{2 \cdot \pi * 2.84\,[m]} + \frac{1}{0.035\frac{[W]}{[m]*[K]}} \cdot \frac{ln(1.50436\,[m]) - ln(1.42436\,[m])}{2 \cdot \pi \cdot 2.84\,[m]})} \quad 7$$

$$= 1154.3\,[\mathrm{W}] = 1.1543[\mathrm{kW}]$$

$$Q_{surface,lid/bottom} = \frac{(121 - 20)[K] \cdot 6.33 m^2}{\frac{0.00436\,[m]}{45\frac{[W]}{[m]*[K]}} + \frac{0.08}{0.035\frac{[W]}{[m]*[K]}}} = 279.6950\,[W] \quad 8$$

$$Q_{surface,lid+bottom} = 2 \cdot Q_{surface,\frac{lid}{bottom}} = 2 \cdot 279.6950\,[W] = 559.39\,[W] = 0.55939[kW] \quad 9$$

$$Q_{shell,121°C} = 879.27[kg] \cdot 0.5\left[\frac{kJ}{kg \cdot K}\right] \cdot (121 - 20)[K] = 44403.135[kJ] \quad 10$$

$$Q_{shell,121°C} = \frac{4440.135[kJ]}{t_{SIP}} = \frac{44403.135[kJ]}{1800[s]} = 24.6684[kW]$$

$$Q_{lid/bottom,121°C} = 221.01[kg] \cdot 0.5\left[\frac{kJ}{kg \cdot K}\right] \cdot (121 - 20)[K] = 11161.005[kJ] \quad 11$$

$$Q_{lid/bottom,121°C} = \frac{11161.005[kJ]}{t_{SIP}} = \frac{11161.005[kJ]}{1800[s]} = 6.2006[kW]$$

$$V_{tank} = \frac{2.84[m]^2 \cdot \pi}{4} \cdot 2.84[m] = 17.9906[m^3] \quad 12$$

$$m_{steam} = 1.9084 \left[\frac{kg}{m^3}\right] * 17.9906[m^3] = 34.3333[kg] \quad 13$$

$$\dot{m}_{steam} = \frac{34.3333\ [kg]}{1800[s]} = 0.01907[\ \frac{kg}{s}] \quad 14$$

$$Q_{steam} = \frac{17.9906[m^3] \cdot 1.9084\left[\frac{kg}{m^3}\right] \cdot 2147[\frac{kJ}{kg}]}{1800[s]} = 40.9520[kW] \quad 15$$

$$\dot{m}_{steam,total} = \frac{1.1543[kW] + 0.55939[kW] + 24.6684[kW] + 2 \cdot 6.2006[kW] + 40.9520[kW]}{2147[\frac{kJ}{kg}]} + 0.01907\left[\frac{kg}{s}\right] = 0.0562\ [\frac{kg}{s}] \quad 16$$

$$\dot{m}_{steam,total,1800s} = 0.0562\ \left[\frac{kg}{s}\right] \cdot 1800[s] = 101.16[kg]$$

$$Q_{total} = 1.1543[kW] + 0.55939[kW] + 24.6684[kW] + 2 \cdot 6.2006[kW] + 40.9520 = 79.7353[kW] \quad 17$$

$$Q_{total,1800s} = 79.7353[kW] \cdot 1800[s] \cdot \frac{1[h]}{3600[s]} = 39.8677[kWh]$$

The SIP procedure for an approximately 18 m^3 tank with a wall thickness of 4.36 mm and an insulation layer of 80 mm consumes 39.8677 kWh of energy and 101.16 kg of steam.

10.5 CIP –preliminary calculations

The energy and water demand of CIP operations are based on two distinct models. The first model is based on empirical values (personal communication Chemgineering personnel). The number of spray balls is set to at least two per tanks to prevent spray shadows due to installations that are commonly present in tanks. The second model is based on empirical values but is extended with data for spray balls from Lechler GmbH [91, 92].

Model 1

Table 25 Typical CIP cycle times and spray ball selection (provided by Chemgineering personnel). RO= reverse osmosis water, WFI = water for injection

	Time [min]						
	First rinse [RO]	Caustic rinse [RO]	Second rinse [RO]	Acid rinse [RO]	Final rinse [WFI]	Number of spray heads	Flow rate [m^3/h] per head
Tank volume [m^3]							
120	6	3	4	3	5	3	20
10-15	1.5	0.75	1	0.75	1.25	2	15
3-5	0.75	0.375	0.5	0.375	0.625	2	7.5
0.5-1	0.25	0.125	0.167	0.125	0.208	2	2.5

Table 25 shows, that for every rinse except the final rinse, RO water is used. RO water is generated by reverse osmosis which is naturally a cold method. For CIP cycles, RO water has to be heated from room temperature to desired CIP cycle temperature (30-80°C). The final rise is done with WFI which comes from the WFI loop at a temperature of 80°C (see *10.3 WFI and RO water generation – preliminary calculations*). As a result no additional energy is required for the final CIP rinse with WFI.

The exemplary calculation refers to a tank with a volume of 18000 L.

For a 18 m^3 tank the number of necessary spray heads is two, according to Table 25.

The flow rate per head is $20\,\frac{[m^3]}{[h]}$ or $\frac{2}{3}\frac{[m^3]}{[min]}$ for two spray balls:

$$\dot{V}_{2\ spray\ balls} = 40[\frac{m^3}{h}] \cdot \frac{[h]}{60[min]} = \frac{2}{3}[\frac{m^3}{min}]$$

The times for a 18 m^3 tank are:

- First rinse: 6 min
- Caustic rinse: 3 min
- Second rinse: 4 min
- Acid rinse: 3 min
- Third rinse: 5 min

This results in a water consumption of 4 m^3 for the first rinse:

$$V_{first\ rinse} = \frac{2}{3}[\frac{m^3}{min}] \cdot 6[min] = 4[m^3]$$

2 m^3 for the caustic rinse:

$$V_{caustic\ rinse} = \frac{2}{3}\left[\frac{m^3}{min}\right] \cdot 3[min] = 2[m^3]$$

$2.\overline{6}$ m^3 for the second rinse:

$$V_{Second\ rinse} = \frac{2}{3}\left[\frac{m^3}{min}\right] \cdot 4[min] = 2.\overline{6}[m^3]$$

2 m^3 for the acid rinse:

$$V_{acid\ rinse} = \frac{2}{3}\left[\frac{m^3}{min}\right] \cdot 3[min] = 2[m^3]$$

$3.\overline{3}$ m^3 for the third rinse:

$$V_{final\ rinse} = \frac{2}{3}\left[\frac{m^3}{min}\right] \cdot 5[min] = 3.\overline{3}[m^3]$$

The total water consumption is 7 m^3:

$$V_{total} = V_{First\ rinse} + V_{Caustic\ rinse} + V_{Second\ rinse} + V_{Acid\ rinse} + V_{Final\ rinse}$$
$$= 4 + 2 + 2.\overline{6} + 2 + 3.\overline{3} = 14[m^3]$$

To calculate the energy demand a start and end temperature in combination with an efficiency factor has to be set for each CIP cycle step:

- First rinse: T_{start}=20°C, T_{end}=30°C, efficiency factor=0.8
- Caustic rinse: T_{start}=20°C, T_{end}=80°C, efficiency factor=0.8
- Second rinse: T_{start}=20°C, T_{end}=30°C, efficiency factor=0.8
- Acid rinse: T_{start}=20°C, T_{end}=70°C, efficiency factor=0.8
- Final rinse: T_{start}=80°C, T_{end}=80°C, efficiency factor=0.8

The energy demand is calculated with the following formula, including the specific heat capacity of water $c_{p,water} = 4.2[\frac{kJ}{kg \cdot K}]$

The energy demand for the first rinse is 100800 kJ:

$$Q_{first\ rinse} = 4[m^3] \cdot \frac{1000[kg]}{1[m^3]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (30 - 20)[K] \cdot (2 - 0.8)$$
$$= 201600[kJ]$$

The energy demand for the caustic rinse is 302400 kJ:

$$Q_{caustic\ rinse} = 2[m^3] \cdot \frac{1000[kg]}{1[m^3]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (80 - 20)[K] \cdot (2 - 0.8)$$
$$= 604800[kJ]$$

The energy demand for the second rinse is 67032 kJ:

$$Q_{second\ rinse} = 2.\bar{6}[m^3] \cdot \frac{1000[kg]}{1[m^3]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (30 - 20)[K] \cdot (2 - 0.8)$$
$$= 134400[kJ]$$

The energy demand for the acid rinse is 252000 kJ:

$$Q_{acid\ rinse} = 2[m^3] \cdot \frac{1000[kg]}{1[m^3]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (70 - 20)[K] \cdot (2 - 0.8)$$
$$= 504000[kJ]$$

The energy demand for the third rinse is 0 kJ:

$$Q_{final\,rinse} = 3.\overline{3}[m^3] \cdot \frac{1000[kg]}{1[m^3]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (80 - 80)[K] \cdot (2 - 0.8) = 0[kJ]$$

The total energy demand is 856800 kJ:

$$\begin{aligned} Q_{total} &= Q_{first\,rinse} + Q_{caustric\,rinse} + Q_{second\,rinse} + Q_{acid\,rinse} + Q_{thrid\,rinse} \\ &= 201600[kJ] + 604800[kJ] + 134400[kJ] + 504000[kJ] + 0[kJ] \\ &= 1444800[kJ] \end{aligned}$$

This can be converted into kWh:

$$Q_{total} = 1444800[kJ] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 401.\overline{3}[kWh]$$

Model 2

The exemplary calculation is based on a tank with a volume of 18000 L.

To apply this model the optimized (see Appendix page 265 for details) tank height is determined:

$$h_{tank,optimized} = \sqrt[3]{\frac{4 \cdot V_c}{\pi}} = \sqrt[3]{\frac{4 \cdot 18[m^2]}{\pi}} = 2.84[m]$$

The optimized tank diameter is calculated as following:

$$d_{tank} = 2 \cdot r_{tank} = \sqrt[3]{\frac{4 \cdot V_c}{\pi}} = \sqrt[3]{\frac{4 \cdot 18[m^2]}{\pi}} = 2.84[m]$$

Depending on the tank diameter, the spray ball type is selected. The two available types are a static spray ball (see Figure 72 in the appendix on page268) and a rotating spray ball (see Figure 73in the appendix on page 268).

Table 26 Spray ball flow rate @ ~2.8bar

Flow rate [US_{gal}/ min]	Type	Article number	Max. tank diameter [m]	Spray angle	Sprayball Model

4	Static	591.M11.17.00	2	360°	Static spray balls Series 591
15	Static	591.X11.17.00	2.2	360°	Static spray balls Series 591
22	Rotating	569.139.1Y	2.1	360°	Rotating cleaning nozzle "Whirly" Series 569
45	Rotating	569.279.1Y	3	360°	Rotating cleaning nozzle "Whirly" Series 569
80	static	591.B31.17.00	5.2	360°	Static spray balls Series 591

For the 18 m^3 tank with a diameter of 2.84 m, the rotating spray ball with the article number 569.279.1Y with a flow rate of 45 US_{gal}/min is selected.

45 US_{gal}/min is the same as 170.325 L/min:

$$45\left[\frac{US_{gal}}{min}\right]\cdot\frac{3.785[L]}{1[US_{gal}]}=170.325[\frac{L}{min}]$$

For two spray balls, the flow rate is 340.65 [L/min]:

$$V_{2\ spray\ balls}=2\cdot 170.325\left[\frac{L}{min}\right]=340.65\left[\frac{L}{min}\right]$$

The times for a 18 m^3 tank are (see Table 25):

- First rinse: 6 min
- Caustic rinse: 3 min
- Second rinse: 4 min
- Acid rinse: 3 min
- Third rinse: 5 min

This results in a water consumption of 2043.9 L for the first rinse:

$$V_{First\ rinse}=340.65\left[\frac{L}{min}\right]\cdot 6[min]=2043.9[L]$$

1021.95 L for the caustic rinse:

$$V_{Caustic\ rinse}=340.65\left[\frac{L}{min}\right]\cdot 3[min]=1021.95[L]$$

1362.6 L for the second rinse:

$$V_{Second\ rinse} = 340.65\ \left[\frac{L}{min}\right] \cdot 4[min] = 1362.6[L]$$

1021.95 L for the acid rinse:

$$V_{Acid\ rinse} = 340.65\ \left[\frac{L}{min}\right] \cdot 3[min] = 1021.95[L]$$

1703.25 L for the final rinse:

$$V_{Third\ rinse} = 340.65\ \left[\frac{L}{min}\right] \cdot 5[min] = 1703.25[L]$$

The total water consumption is 3576.795 L or 3.58 m^3:

$$\begin{aligned} V_{total} &= V_{First\ rinse} + V_{Caustic\ rinse} + V_{Second\ rinse} + V_{Acid\ rinse} + V_{Third\ rinse} \\ &= 2043.9[L] + 1021.95[L] + 1362.6[L] + 1362.6[L] + 1703.25[L] \\ &= 7494.30[L] \end{aligned}$$

$$V_{total} = 7494.30[L] \cdot \frac{1[m^3]}{1000[L]} = 7.4934[m^3] \approx 7.50[m^3]$$

To calculate the energy demand a start and end temperature in combination with an efficiency factor has to be set for each CIP cycle step:

- First rinse: T_{start}=20°C, T_{end}=30°C, efficiency factor=0.8
- Caustic rinse: T_{start}=20°C, T_{end}=80°C, efficiency factor=0.8
- Second rinse: T_{start}=20°C, T_{end}=30°C, efficiency factor=0.8
- Acid rinse: T_{start}=20°C, T_{end}=70°C, efficiency factor=0.8
- Final rinse: T_{start}=80°C, T_{end}=80°C, efficiency factor=0.8

The energy demand is calculated with the following formula, including the specific heat capacity of water $c_{p,water} = 4.2[\frac{kJ}{kg \cdot K}]$

The energy demand for the first rinse is 51506.28 kJ:

$$Q_{first\ rinse} = 2043.9[L] \cdot \frac{1[kg]}{1[L]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (30 - 20)[K] \cdot (2 - 0.8)$$
$$= 103012.56[kJ]$$

The energy demand for the caustic rinse is 154518.84 kJ:

$$Q_{caustic\ rinse} = 1021.95[L] \cdot \frac{1[kg]}{1[L]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (80 - 20)[K] \cdot (2 - 0.8)$$
$$= 309037.68[kJ]$$

The energy demand for the second rinse is 34337.52 kJ:

$$Q_{second\ rinse} = 1362.6[L] \cdot \frac{1[kg]}{1[L]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (30 - 20)[K] \cdot (2 - 0.8)$$
$$= 68675.04[kJ]$$

The energy demand for the acid rinse is 128765.7 kJ:

$$Q_{acid\ rinse} = 1021.95[L] \cdot \frac{1[kg]}{1[L]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (70 - 20)[K] \cdot (2 - 0.8)$$
$$= 257531.4[kJ]$$

The energy demand for the third rinse is 42921.9 kJ:

$$Q_{final\ rinse} = 1703.25[L] \cdot \frac{1[kg]}{1[L]} \cdot 4.2\left[\frac{kJ}{kg \cdot K}\right] \cdot (80 - 80)[K] \cdot (2 - 0.8) = 0[kJ]$$

The total energy demand is 369128.34 kJ:

$$Q_{total} = Q_{first\ rinse} + Q_{caustic\ rinse} + Q_{second\ rinse} + Q_{acid\ rinse} + Q_{final\ rinse}$$
$$= 103012.56[kJ] + 309037.68[kJ] + 68675.04[kJ] + 257531.4[kJ]$$
$$+ 0[kJ] = 738256.68[kJ]$$

This can be converted into kWh:

$$Q_{total} = 738256.68[kJ] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 205.07[kWh]$$

10.6 Steel tank dimensioning – preliminary calculations

The goal is to determine the weight of the steel tanks. The mass of steel that is used to produce steel tanks can be converted to CO_2 emissions via an emission factor. The mass

of steel is also relevant to calculate the amount of carbon dioxide that is emitted during the transport of the steel tanks to the production facility.

The selected steel for tank manufacturing is an austenitic stainless steel:

- Steel type: X5CrNiMo17-12-2 (1.4401)
- Elongation at break (A[%]) ≥ 40 [93, S. 95]
- Density: $\rho_{316} = 8000[\frac{kg}{m^3}]$ [94, S. 1]
- Yield strength ("Dehngrenze"): $R_{p1,0/\vartheta@20°C} \geq 260[\frac{N}{mm^2}]$ [93, S. 95]

Calculation of the necessary tank wall thickness is done according to the *AD-2000 Merkblätter* as this is a commonly used guideline in the European Union.

The following stability requirement has to be met by pressure vessels:

$$\sigma_{present} \leq \sigma_{acceptable}$$

$$\sigma_{acceptable} = \frac{K}{S}$$

$\sigma_{acceptable}$ for $\geq 35\%$: $\sigma_{acceptable} = max\left\{\frac{R_{p1,0/\vartheta}}{1.5}; min\left[\frac{R_{p1,0/\vartheta}}{1.2}\right]\right.$

For 20°C: $R_{p1,0/\vartheta} \geq 260[\frac{N}{mm^2}]$

$$\sigma_{acceptable,max} = \frac{R_{p1,0/\vartheta}}{1.5} = \frac{260[\frac{N}{mm^2}]}{1.5} = 173.\overline{3}\left[\frac{N}{mm^2}\right] \cdot \frac{1000^2[mm^2]}{[m^2]}$$
$$= 173.33 \cdot 10^6[\frac{N}{m^2}]$$

$\sigma_{acceptable,max}$ is selected since the resulting wall thickness is greater that the wall thickness resulting from $\sigma_{acceptable,min}$.

$$\sigma_{acceptable,min} = \frac{R_{p1,0/\vartheta}}{1.2} = \frac{260[\frac{N}{mm^2}]}{1.2} = 216.\overline{6}\left[\frac{N}{mm^2}\right] = 216.\overline{6} \cdot \frac{1000^2[mm^2]}{1[m^2]}$$
$$= 216.67 \cdot 10^6[\frac{N}{m^2}]$$

$$s_V = \frac{d_o \cdot p_i}{2 \cdot \frac{K}{S} \cdot v_N + p_i}$$

With $d_o = d_i + 2 \cdot s_V$ the formula can be rearranged to:

$$s_V = \frac{d_i \cdot p_{max}}{2 \cdot \sigma_{acceptable} \cdot v_N - p_{max}}$$

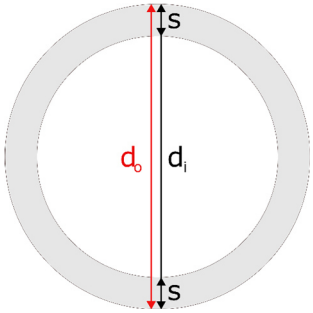


Figure 68 Tank cross section

Nowadays the welding seam factor ("Schweißnahtfaktor") v_n is set to 1.0 due to the high standard of welding technology [93, S. 93].

The minimum holding capacity of a tank is converted to working volume by a volume addition of 15-20%. A tank that has to hold 15000 L results in a tank with 18000 L working volume when 20% volume are added:

$$15000[L] \cdot 1.2 = 18000[L]$$

For this example the tank has a volume of 18 m^3, a height of 2.84 m and an inner diameter of 2.84 m (see page 231 for calculations where this values are derived). With a height of 2.84 m the maximum pressure during operation inside the tank is the atmospheric pressure plus the hydrostatic pressure:

$$p_i = p_{atmospheric} + p_{hydrostatic}$$

$$p_{hydrostatic} = \rho \cdot g \cdot h_{tank} = 1000 \left[\frac{kg}{m^3}\right] * 9.81 \left[\frac{m}{s^2}\right] \cdot 2.84[m] = 27860 \left[\frac{\frac{kg}{m \cdot s^2}}{m^2}\right]$$

$$= 27860[\frac{N}{m^2}]$$

$$p_i = 1.013 \cdot 10^5 \left[\frac{N}{m^2}\right] + 27860 \left[\frac{N}{m^2}\right] = 129160[\frac{N}{m^2}]$$

The SIP procedure is carried out at 2.5 barg, exceeding the regular pressure pi during operation:

$$p_{SIP} = 2.5[barg] \cdot 10^5 = 250000\ [\frac{N}{m^2}]$$

$$p_{SIP} > p_i \rightarrow p_{SIP} = p_{max}$$

$$s_V = \frac{2.84[m] \cdot 250000[\frac{N}{m^2}]}{2 \cdot 173.33 \cdot 10^6 \left[\frac{N}{m^2}\right] \cdot 1.0 - 250000[\frac{N}{m^2}]} = 2.05 \cdot 10^{-3}[m] \cdot \frac{1000[mm]}{1[m]}$$
$$= 2.05[mm]$$

From the minimum wall thickness s_V the order wall thickness ("Bestellwanddicke") can be calculated:

$$s = s_V + C_1 + C_2$$

For austenitic steel like 316 C_1 and C_2 are zero [93, S. 89]. The wall thickness is therefore:

$$s = 2.05[mm] + 0[mm] + 0[mm] = 1.06[\text{mm}] = 2.05 \cdot 10^{-3}[m]$$

The outer diameter d_a is therefore 2.8411 m:

$$d_o = d_i + 2 \cdot s = 2.84[m] + 2 \cdot 2.05 \cdot 10^{-3}[m] = 2.8441[m]$$

The shell volume is $13.4131 \cdot 10^{-3}[m^3]$:

$$V_{shell} = h_{tank} \cdot \frac{\pi}{4} \cdot ({d_o}^2 - {d_i}^2)$$

$$V_{shell} = 2.84 \cdot \frac{\pi}{4} \cdot ((2.8441[m])^2 - (2.84[m])^2) = 51.9821 \cdot 10^{-3}[m^3]$$

With the density of 8000 kg/m^3 the mass of the shell comes to 107.44 kg:

$$m_{shell} = V_{shell} \cdot \rho_{steel} = 51.9821 \cdot 10^{-3}[m^3] \cdot 8000 \left[\frac{kg}{m^3}\right] = 415.8568[kg]$$

With the assumption that the wall thickness s also applies to the lid and bottom of the tank, the volume of the lid can be calculated:

$$V_{lid/bottom} = \frac{d_o^2 \cdot \pi}{4} \cdot s = \frac{(2.8441[m])^2 \cdot \pi}{4} \cdot 1.06 \cdot 10^{-3} = 13.0237 \cdot 10^{-3}[m^3]$$

With the density of 8000 kg/m^3 the mass of the lid/bottom comes to 47.7648 kg:

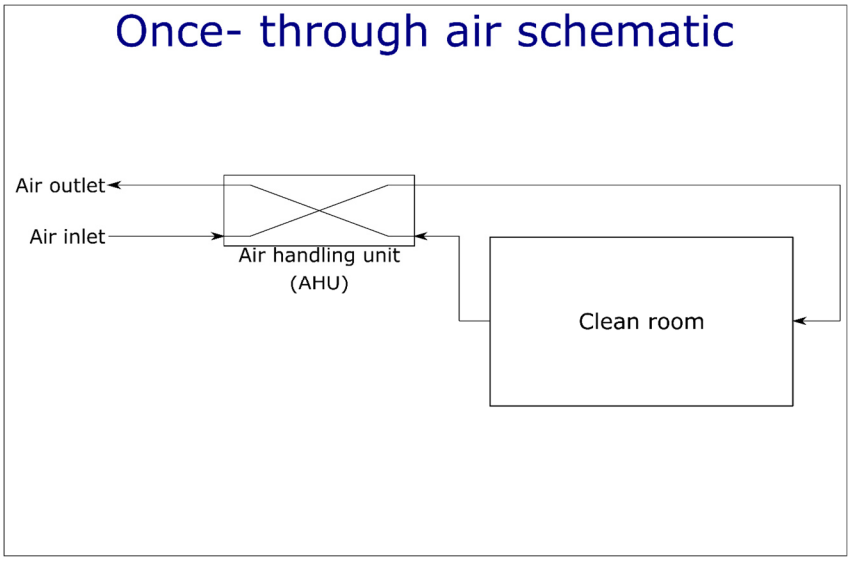
$$m_{\frac{lid}{bottom}} = V_{\frac{lid}{bottom}} \cdot \rho_{steel} = 13.0237 \cdot 10^{-3}[m^3] \cdot 8000 \left[\frac{kg}{m^3}\right] = 104.1896[kg]$$

The total mass of the tank is 624.2360 kg:

$$m_{tank,total} = m_{shell} + 2 \cdot m_{\frac{lid}{bottom}} = 415.8568[kg] + 2 \cdot 104.1896[kg]$$
$$= 624.2360[kg]$$

10.7 HVAC – preliminary calculations

The calculations to determine the energy demand of HVAC operations include heating/cooling, dehumidification/humidification and fan power requirement. Calculations are based on a once-through HVAC design with energy recovery via a heat exchanger as shown in the figure below:



The calculations are split in the following steps:

1. Obtain weather data for location of interest (Temperature T[°C], relative humidity φ[%] and absolute pressure $p_{abs}[Pa]$)
2. Determine clean room volume [m^3]
3. Determine the volumetric flow [m^3/h] and the mass flux [kg/day] of air
4. Determine the saturation vapor pressure in [Pa] via Antoine's equation
5. Determine the partial pressure of steam $p_{partial,steam}[Pa]$
6. Determine the absolute humidity $x[\frac{kg_{water}}{kg_{dry\ air}}]$ and the humidity at saturation state (φ=1) $x_s[\frac{kg_{water}}{kg_{dry\ air}}]$
7. Determine the specific enthalpy $\Delta h_{1+x}[\frac{kJ}{kg}]$
8. Set a desired temperature and relative humidity for the clean room
9. Calculate the difference in specific enthalpy $\Delta h[\frac{kJ}{kg}]$
10. Calculate the energy demand for heating/cooling/dehumidification/humidification $E_{HVAC}\ in[kWh]$
11. Calculate the necessary fan power consumption E_{fan} in kWh
12. Calculate the total energy demand E_{total} in kWh per day
13. Calculate the carbon emissions in kg per day

1.) Weather data

For this example, weather data from two days in February 2018 (Basel, Switzerland) is used as one day has a positive average (>0°C) temperature and one days has negative average temperature (<0°C). The data are obtained from meteoblue.com:

Table 27 Exemplary weather data

Date	$\boldsymbol{T_{average}}[°\boldsymbol{C}]$	$\boldsymbol{\varphi}_{\boldsymbol{average}}[\%]$	$\boldsymbol{p_{abs}}[\boldsymbol{hPa}]$
24.02.2018	0.3	62.92	1021.3
25.02.2018	-3.52	54.17	1022.93

2.) Room volume

HVAC operations of different rooms depend on clean room classification, as the air change rate is the driving factor of energy consumption. The clean room classes with according air change rates according to the *GMP Berater* are:

Table 28 CLeanroom classes accroding to the *GMP Berater*

Clean room class	**Maximum air changes per hour**
CNC	9
D	10
C	20
B	30

The volume of the clean room is derived by multiplying the area by ceiling height. For this example a 10 x 10 x 3 (L x W x H) is used:

$$V_{cleanroom} = 29.86[m^2] \cdot 3.5[m] = 104.52[m^3]$$

3.) Volumetric flow and mass flux

The volume flux according to the exemplary clean room class C is 104.52 m^3/h:

$$\dot{V}_{air} = 104{,}52[m^3] \cdot 15[\frac{1}{h}] = 1567.8[\frac{m^3}{h}]$$

With an average density of 1.2923 kg/m^3 at 20°C the mass flux is 48625.63 kg/d:

$$m_{air} = 1567{,}8\left[\frac{m^3}{h}\right] \cdot 1.2923\left[\frac{kg}{m^3}\right] \cdot 24\left[\frac{h}{d}\right] = 48625.63[\frac{kg}{d}]$$

4.) Saturation vapor pressure

Antoine equation is used to calculate the saturation vapor pressure of water at a given temperature:

$$p_{sat.} = 10^{A-\frac{B}{T+C-273.15}}$$

The Antoine parameters (T in [K], $p_{sat.}$ in [bar]) are:

A=5.11564

B=1687.537

C=230.17

For the 24th of February 2019 with an average daily temperature of 0.3°C, the saturation vapor pressure according to Antoine's equation is:

$$p_{sat.} = 10^{5.11564-\frac{1687.537}{(0.3+273.15+230{,}17-273.15}} = 6.2156 \cdot 10^{-3}[bar]$$

$$p_{sat.} = 6.22 \cdot 10^{-3}[bar] \cdot \frac{10^5[Pa]}{[bar]} = 621.5632[Pa]$$

For the 25th of February 2019 with an average daily temperature of -3.52°C, the saturation vapor pressure according to Antoine's equation is:

$$p_{sat.} = 10^{5.11564-\frac{1687.537}{(-3.52+273.15+230{,}17-273.15}} = 4.6781 \cdot 10^{-3}[bar]$$

$$p_{sat.} = 6.22 \cdot 10^{-3}[bar] \cdot \frac{10^5[Pa]}{[bar]} = 467.8173[Pa]$$

5.) Partial pressure of steam

The formula for relative humidity $\varphi = \frac{\boldsymbol{partial\ pressure\ of\ steam}}{\boldsymbol{saturation\ vapor\ pressure}} = \frac{\boldsymbol{p_{part.,steam}}}{\boldsymbol{p_{sat.}}}$ can be rearranged to calculate the partial pressure of steam:

$$p_{part,steam} = \varphi \cdot p_{sat.}$$

For the 24th of February 2019 with an average daily relative humidity of 62.92%, the partial vapor pressure is 391.09 Pa:

$$p_{part,steam} = 0{,}6292 \cdot 621.5632 = 391.09[Pa]$$

For the 25th of February 2019 with an average daily relative humidity of 54.17%, the partial vapor pressure is 253.42 Pa:

$$p_{part,steam} = 0{,}5417 \cdot 467.8173 = 253.42[Pa]$$

6.) Absolute humidity and absolute humidity at saturation

For positive temperatures, only the absolute humidity x is relevant. For negative temperatures the absolute relative humidity x and the absolute relative humidity at saturation x_S has to be calculated.

$$x = \frac{m_{water}}{m_{air}} = \frac{R_{air}}{R_{steam}} \cdot \frac{\varphi \cdot p_{sat.}}{p_{abs.} - \varphi \cdot p_{sat.}}$$

$$x_s(\varphi = 1) = \frac{m_{water}}{m_{air}} = \frac{R_{air}}{R_{steam}} \cdot \frac{1 \cdot p_{sat.}}{p_{abs.} - 1 \cdot p_{sat.}}$$

For the 24th of February 2019 with an average daily relative humidity of 62.92%, the absolute humidity is $2.41 \left[\frac{g_{water}}{kg_{air}}\right]$:

$$x = \frac{0.2871[\frac{kJ}{kg \cdot K}]}{0.46153[\frac{kJ}{kg \cdot K}]} \cdot \frac{0.6292 \cdot 621.5632[Pa]}{1021.3 \cdot 100[Pa] - 0.6292 \cdot 621.5632[Pa]}$$

$$= 2.41 \cdot 10^{-3}[\frac{kg_{water}}{kg_{aor}}]$$

$$x = 2.41 \cdot 10^{-3} \left[\frac{kg_{water}}{kg_{air}}\right] \cdot \frac{1000[g]}{1[kg]} = 2.41 \left[\frac{g_{water}}{kg_{air}}\right]$$

For the 25th of February 2019 with an average daily relative humidity of 54.17%, the absolute humidity is $1.55 \left[\frac{g_{water}}{kg_{air}}\right]$:

$$x = \frac{0.2871[\frac{kJ}{kg \cdot K}]}{0.46153[\frac{kJ}{kg \cdot K}]} \cdot \frac{0.5417 \cdot 467.8173[Pa]}{1022.93 \cdot 100[Pa] - 0.5417 \cdot 467.8173[Pa]}$$

$$= 1.55 \cdot 10^{-3}[\frac{kg_{water}}{kg_{aor}}]$$

$$x = 1.55 \cdot 10^{-3} \left[\frac{kg_{water}}{kg_{air}}\right] \cdot \frac{1000[g]}{1[kg]} = 1.55 \left[\frac{g_{water}}{kg_{air}}\right]$$

The absolute humidity at saturation state x_s at the 25th of February is $2.86 \left[\frac{g_{water}}{kg_{air}}\right]$:

$$x_s = \frac{0.2871[\frac{kJ}{kg \cdot K}]}{0.46153[\frac{kJ}{kg \cdot K}]} \cdot \frac{1 \cdot 467.8173[Pa]}{1022.93 \cdot 100[Pa] - 1 \cdot 467.8173[Pa]}$$

$$= 2.86 \cdot 10^{-3}[\frac{kg_{water}}{kg_{aor}}]$$

$$x_s = 1.55 \cdot 10^{-3}\left[\frac{kg_{water}}{kg_{air}}\right] \cdot \frac{1000[g]}{1[kg]} = 2.86\left[\frac{g_{water}}{kg_{air}}\right]$$

7.) Specific entropy

The enthalpy in kJ/kg to heat/cool or humidify/dehumidify air depends on the parameters temperature, humidity and pressure. Two separate calculation approaches have to be selected for positive (i) and negative (ii) temperatures. Calculations for temperatures below 0°C include the change in saturation enthalpy as a result of ice formation.

i. $h_{1+x(\geq 0°C)} = c_{p,air} \cdot T + x \cdot (\Delta h_V + c_{p,steam} \cdot T)$

ii. $h_{1+x(\leq °C)} = c_{p,air} \cdot T + x_s \cdot \left(\Delta h_V + c_{p,steam} \cdot T\right) + (x - x_s) \cdot (c_{p,ice} \cdot T - \Delta h_{melt})$

$$c_{p,air}: specific\ heat\ capacity\ of\ air = 1.004\left[\frac{kJ}{kg \cdot K}\right]$$

$$c_{p,steam}: specific\ heat\ capacity\ of\ steam = 1.86\left[\frac{kJ}{kg \cdot K}\right]$$

$$c_{p,ice}: specific\ heat\ capacity\ of\ ice = 2.04\left[\frac{kJ}{kg \cdot K}\right]$$

$$T: temperature\ in\ [°C]$$

$$x: absolute\ humidity\ \left[\frac{kg_{water}}{kg_{dry,air}}\right]$$

$$x_s: absolute\ humidity\ at\ saturation\ state\ \left[\frac{kg_{water}}{kg_{dry,air}}\right]$$

$$\Delta h_V: enthalpy\ of\ evaporation = 2501\left[\frac{kJ}{kg}\right]$$

$$\Delta h_{melt}: latent\ enthalpy\ of\ fusion = 333.5\left[\frac{kJ}{kg}\right]$$

With formula i applied on the 24th of February 2018, the specific enthalpy is 6.33 [kJ/kg]:

$$h_{1+x(\geq 0°C)} = 1.004\left[\frac{kJ}{kg \cdot K}\right] \cdot 0.3°C + 2.41 \cdot 10^{-3}\left[\frac{kg_{water}}{kg_{aor}}\right] \cdot \left(2501\left[\frac{kJ}{kg}\right] + 1.86\left[\frac{kJ}{kg \cdot K}\right] \cdot 0.3°C\right) = 6.33[\frac{kJ}{kg}]$$

With formula ii applied on the 25th of February 2018, the specific enthalpy is 4.09 [kJ/kg]:

$$h_{1+x(\leq°C)} = 1.004\left[\frac{kJ}{kg\cdot K}\right]\cdot -3.52°C + 2.86\cdot 10^{-3}\left[\frac{kg_{water}}{kg_{aor}}\right] \cdot\left(2501\left[\frac{kJ}{kg}\right] + 1.86\left[\frac{kJ}{kg\cdot K}\right]\cdot -3.52°C\right) + \left(1.55\cdot 10^{-3}\left[\frac{kg_{water}}{kg_{aor}}\right] - 2.86\cdot 10^{-3}\left[\frac{kg_{water}}{kg_{aor}}\right]\right) \cdot\left(2.04\left[\frac{kJ}{kg\cdot K}\right]\cdot -3.52°C - 333.5\left[\frac{kJ}{kg}\right]\right) = 4.09[\frac{kJ}{kg}]$$

8.) Desired clean room temperature/humidity/pressure

With a set temperature of 20°C and a relative humidity of 60%, the saturation vapour pressure is 2344.6552 Pa:

$$p_{sat.} = 10^{5.11564-\frac{1687.537}{(20+273.15+230{,}17-273.15}} \approx 23.45\cdot 10^{-3}[bar]$$

$$p_{sat.} = 23.45\cdot 10^{-3}[bar]\cdot\frac{10^5[Pa]}{[bar]} = 2344.6552[Pa]$$

The absolute humidity comes to 8.75 $[\left[\frac{g_{water}}{kg_{air}}\right]]$ with a slight over pressure of 100 Pa:

$$x = \frac{0.2871[\frac{kJ}{kg\cdot K}]}{0.46153[\frac{kJ}{kg\cdot K}]}\cdot\frac{0.6\cdot 2344.6552[Pa]}{(100[Pa] + 1.013\cdot 10^5[Pa] - 0.6\cdot 2344.6552[Pa]} = 8.75\cdot 10^{-3}[\frac{kg_{water}}{kg_{aor}}]$$

$$x = 8.75\cdot 10^{-3}\left[\frac{kg_{water}}{kg_{air}}\right]\cdot\frac{1000[g]}{1[kg]} = 8.75\left[\frac{g_{water}}{kg_{air}}\right]$$

With formula i applied, the specific enthalpy is 42.29 [kJ/kg]:

$$h_{1+x(\geq 0°C)} = 1.004\left[\frac{kJ}{kg\cdot K}\right]\cdot 20°C + 8.75\cdot 10^{-3}\left[\frac{kg_{water}}{kg_{aor}}\right] \cdot\left(2501\left[\frac{kJ}{kg}\right] + 1.86\left[\frac{kJ}{kg\cdot K}\right]\cdot 20°C\right) = 42.29[\frac{kJ}{kg}]$$

9.) Difference in specific enthalpy Δh[kJ/kg]

The difference in specific enthalpy for the 24th of February 2018 is 35.98 [kJ/kg]:

$$\Delta h_{(T>0°C)} = \left|6.33\left[\frac{kJ}{kg}\right] - 42.29[\frac{kJ}{kg}]\right| = 35.98[\frac{kJ}{kg}]$$

The difference in specific enthalpy for the 25th of February 2018 is 38.19 [kJ/kg]

$$\Delta h_{T<0°C} = \left|4.09\left[\frac{kJ}{kg}\right] - 42.29[\frac{kJ}{kg}]\right| = 38.19[\frac{kJ}{kg}]$$

10.) Energy demand for heating/cooling/humidification/dehumidification E [kWh]

The energy demand to adjust the temperature and humidity to the desired clean room parameters (20°C; 60% relative humidity) is calculated on a daily basis. For the 24th of February the energy demand is 515.84 [kWh/d]

$$E_{HVAC} = 35.98\left[\frac{kJ}{kg}\right] \cdot 48625.63\left[\frac{kg}{d}\right] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 485.99[\frac{kWh}{d}]$$

For the 25th of February the energy demand is 485.99 [kWh/d]:

$$E_{HVAC} = 38.19\left[\frac{kJ}{kg}\right] \cdot 48625.63\left[\frac{kg}{d}\right] \cdot \frac{1[kWh]}{3.6 \cdot 10^3[kJ]} = 515.84[\frac{kWh}{d}]$$

11.) Fan power consumption E_{fan}

The fan power consumption is based on data from *Industrie-Ventilatoren Dassler*. For a volume flux of $\dot{V}_{air} = 1567.8[\frac{m^3}{h}]$, model No. 2 (see page 274 for fan data) is selected due to matching volume flux.

Model no. 2 consumes 0.09 kW and is able to provide an air flux of 1550 m^3/h. The daily energy consumption is therefore 2.16 kWh:

$$E_{fan} = 0.09[kW] * 24h = 2.16\ [kWh/d]$$

12.) Total energy consumption

The total energy consumption of the 24th February 2018 is 488.15 kWh:

$$E_{total} = 485.99\left[\frac{kWh}{d}\right] + 2.16\ \left[\frac{kWh}{d}\right] = 488.15\left[\frac{kWh}{d}\right]$$

The total energy consumption of the 24th February 2018 is 518.00 kWh:

$$E_{total} = 515.84[\frac{kWh}{d}] + 2.16\ \left[\frac{kWh}{d}\right] = 518.00\left[\frac{kWh}{d}\right]$$

Explanatory note: It is possible to gain more accurate data on energy consumption by reducing the timeframe from days to hours or even minutes/seconds.

13.) Carbon emissions per day

With an emission factor of 23.6 g_{CO2}/kWh, the total carbon emissions for the 24th of February is 11.5 kg:

$$CO2_{per\ day} = 488.15\left[\frac{\text{kWh}}{\text{d}}\right] \cdot 23.6\left[\frac{g_{CO2}}{kWh}\right] = 11520.34[g_{CO2}]$$

or $11520.34[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \approx 11.5[kg]$

With an emission factor of 23.6 g_{CO2}/kWh, the total carbon emissions for the 24th of February is 12.2 kg

$$CO2_{per\ day} = 518.00\left[\frac{\text{kWh}}{\text{d}}\right] \cdot 23.6\left[\frac{g_{CO2}}{kWh}\right] = 12224.8[g_{CO2}]$$

or $12224.8[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \approx 12.2[kg]$

10.8 Space requirement – preliminary calculations

Processing equipment and tanks are allocated to be in the rectangular or in the circular shape category to calculate the required space demand in m^2.

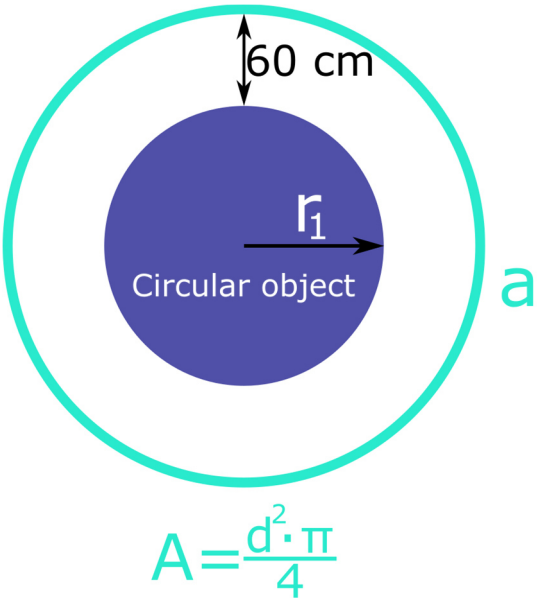


Figure 69 Area of circular objects

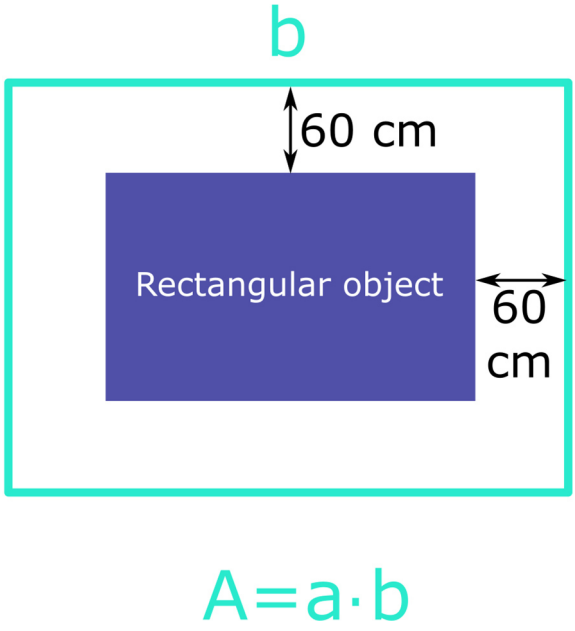


Figure 70Area of rectangular objects

The marine blue area marks the minimum space that the piece of equipment, reactor or tanks requires. For circular objects the radius is extended by 60 cm to allow easy access during operation and maintenance. For rectangular objects, side length is extended by 60 cm to allow easy access during operation and maintenance.

The turquoise line marks the total occupied area. The formula to calculate the turquoise area for circular objects is:

$$A_{circle} = \frac{(d_{object}[m] + 1.2[m])^2 \cdot \pi}{4}$$

The turquoise are for rectangular objects is:

$$A_{rectangular} = (a_{object} + 2 \cdot 0.6[m]) + (b_{object} + 2 \cdot 0.6[m])$$

Example for a circular object:

A 18 m3 production bioreactor tank with a 2.84 m diameter requires an area of 12.82 m²:

$$A_{circle} = \frac{(2.84[m[+1.2)^2 \cdot \pi}{4} = 12.82[m]^2$$

A single use chromatography skid with a length of 2.059 m and a width of 1.087 m requires an area of 7.45 m²:

$$A_{rectangular} = (2.059[m] + 2 \cdot 0.6[m]) \cdot (1.087 + 2 \cdot 0.6[m]) = 7.45[m^2]$$

10.9 Tank heating/cooling – preliminary calculations

The energy demand for cooling operations can be calculated with the following formula:

$$Q = k \cdot A \cdot \Delta T = k \cdot A \cdot (T_{high} - T_{low})$$

The area that is available for heat transfer is calculated from the tank diameter and tank height. For this calculation heat transfer is assumed to take place on the tanks shell as well as the tanks bottom plate (see Figure 71). Simplifications for calculations include the reduction of the torispherical bottom to a disk shape plate (see c) in Figure 67).

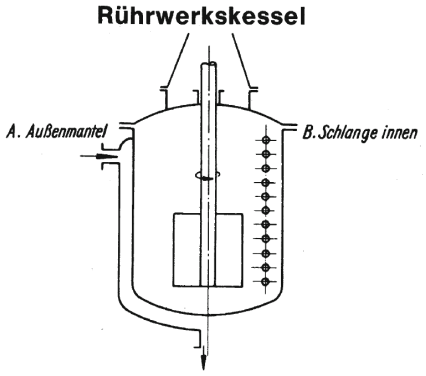


Figure 71Schematic to show the heat transfer area for a jacketed reactor [95, S. 88]

The tank diameter and height for a 18 m³ tank are 2.84 m (see page 231 for calculation). The area available for heat exchange is therefore 31.6735 m2:

$$A = A_{shell} + A_{bottom} = d_{tank} \cdot \pi \cdot h_{tank} + \frac{d_{tank}^2 \cdot \pi}{4} \cdot d_{tank}$$

$$= 2.84[m] \cdot \pi * 2.84[m] + \frac{2.84[m] \cdot \pi}{4} \cdot 2.84[m] = 31.6735[m^2]$$

For jacketed stirred reactors the k value can be assumed to be 350 $\frac{W}{m^2 \cdot K}$ according to the *VDI Wärmeatlas* [95, S. 88].

The calculation is based on the assumption that the reactor has to be cooled down from 37°C to 15°C:

$$Q = 350\left[\frac{W}{m^2 \cdot K}\right] \cdot 31.6735[m^2] \cdot (37 - 15)[K] = 241575.95[W]$$

$$Q = 241575.95[W] \cdot \frac{1[kW]}{1000[W]} = 214.5760[kW]$$

The cooling process takes 4 h resulting in an energy consumption of 966.304 kWh:

$$E_{cooling} = 214.5760[kW] \cdot 4[h] = 966.304[kWh]$$

To heat the same tank form 20°C to 37°C in 4 h it takes 188457.325 kWh:

$$Q = 350\left[\frac{W}{m^2 \cdot K}\right] \cdot 31.6735[m^2] \cdot (37 - 20)[K] = 188457.325[W]$$

$$Q = 188457.325[W] \cdot \frac{1[kW]}{1000[W]} = 188.4573[kW]$$

The heating process takes 4 h resulting in an energy consumption of 966.304 kWh:

$$E_{heating} = 188.4573[kW] \cdot 4[h] = 753.8292[kWh]$$

10.10 Autoclaving of single-use equipment – Preliminary calculations

Zirbus technology GmbH kindly provided data from their HST 12x10x15 autoclave model. The chamber measurements are 1.25 x 1.0 x 1.57 m (LxWxH). Autoclave programmes are individual and depend on items and filling degree. A general program has the following features:

Table 29 Average autoclave cycle for solids

Cooling water demand	**180 L**
Soft water demand	30-40 L
Pressurized air	800 L
Energy consumption	25-30 kWh
Cycle time	40-45 min
Temperature	134 °C
Sterilization time	7 min
Drying time	5 min

Total volume of the autoclave chamber is 1.9625 m3:

$$V_{autoclave} = 1.25\ m \cdot 1.0\ m\ \cdot 1.57\ m = 1.9625\ m^3$$

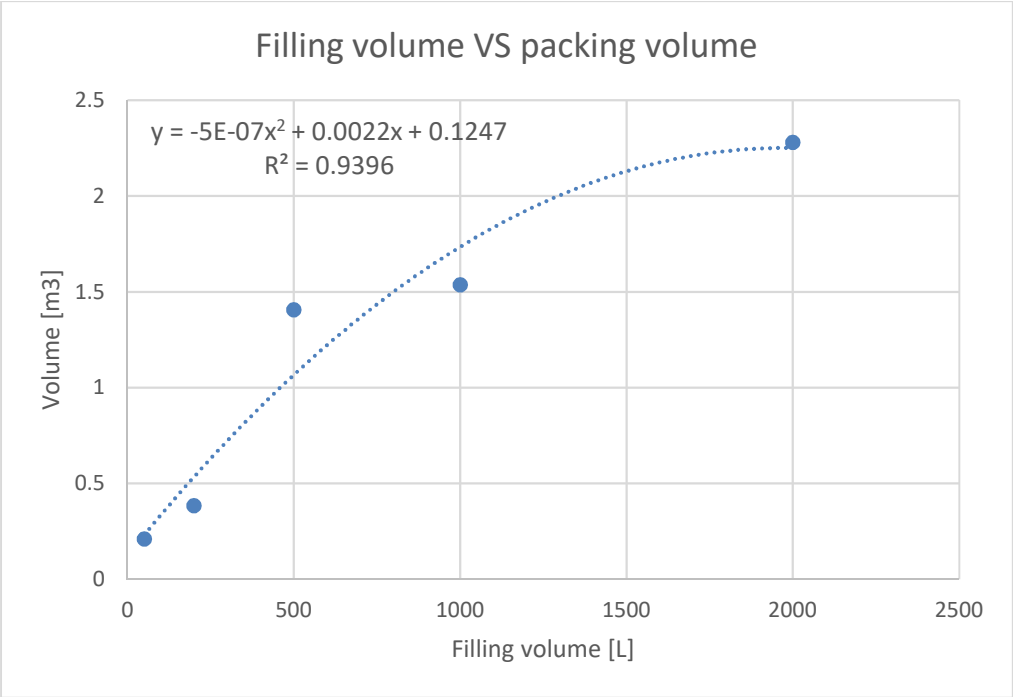
With a maximum load capacity of 80% the maximum loading volume of the autoclave is 1.57 m3:

$$V_{autoclave,80\%} = 0.8 \cdot 1.9625 m^3 = 1.57 m^3$$

With data provided by Sartorius, the volume per bag is calculated.

STR	**L [m]**	**B [m]**	**H [m]**	**Bags/pallete**	**Total Volume [m³]**	**Volume per bag [m³]**
50	1.2	0.8	1.315	6	1.2624	0.2104
200	1.2	0.8	1.6	4	1.536	0.384
500	1.2	0.8	1.465	1	1.4064	1.4064
1000	1.2	0.8	1.6	1	1.536	1.536
2000	1.2	1	1.9	1	2.28	2.28

This data was used to plot the volume demand of bags in m3 vs the filling volume in L, to determine the room requirement of unlisted bags. The second degree polynomial with a correlation coefficient of 0.9396 is used to determine volume demand of bags.



As an example, the space demand of a 1500 L pool vessel is 2.2997 m³:

$$V_{bag\ in\ m3} = -5 \cdot 10^{-7} V_{bag\ in\ L}^2 + 0.0022 \cdot V_{bag\ in\ L} + 0.1247$$

$$V_{bag\ in\ m3} = -5 \cdot 10^{-7} 1500L^2 + 0.0022 \cdot 1500 + 0.1247 = 2.2997m^3$$

When the bags can be compressed by at least 60% the volume of a 1500 L pool vessel bag is reduced to 1.38 m³:

$$V_{bag,compressed} = 0.6 * 2.2997 = 1.38m^3$$

The list below shows all bags that have product contact and have to be autoclaved for one batch.

Bag No.	**Description**	**SUT volume [L]**	**Volume [m3]**
1	SUB 2000 L	2000	1.51
2	Pool Vessel 3000 L	3000	1.33
3	SUM 2000 L	2000	1.51
4	SUM 1000 L	1000	1.09
5	SUM 1000L	1000	1.09
6	Break Bag 1000 L	1000	1.09
7	SUM 650 L	650	0.81
8	SUM 450 L	450	0.61
9	SUM 650 L	650	0.81
10	Break Bag 1000 L	1000	1.09
11	SUM 650 L	650	0.81
12	Break Bag 1000 L	1000	1.09
13	SUM 650 L	650	0.81
14	SUM 650 L	650	0.81
15	SUM 650 L	650	0.81
16	SUM 400 L	400	0.55
17	SUM 400 L	400	0.55

The cycle plan for the autoclave is:

Autocalve cycle	Bag No.	Volume
1	1	1.51482
2	2	1.33482
3	3	1.51482
4	4	1.09482
5	5	1.09482
6	6	1.09482
7	7	0.80607
8	8	0.60807
9	9&10	1.90089
10	11&18	0.88089
11	12	1.09482
12	13&19	0.88089
13	14	0.80607
14	15	0.80607
15	16	0.55482
16	17	0.55482

For a total of 16 cycles the Cooling water consumption is 2880 L

$$V_{cooling} = 180L \cdot 16 = 2880L$$

The soft water consumption is 480 L:

$$V_{soft\ water} = 30L \cdot 16 = 480\ L$$

The total energy demand is 400 kWh:

$$E_{batch} = 25kWh \cdot 16 = 400kWh$$

The total time is 720 min:

$$t_{total} = 45min \cdot 16 = 720min$$

All received values for water consumption, energy consumption and total time can be multiplied by the number of batches per year to receive yearly results.

Autoclaving of filters from depth filtration

The provided calculations for autoclaving of the filter capsules from depth filtration stem for the 2 m3 SUT process.

For the processing of one batch, 57 single-use depth filtration capsules are needed. One capsule has a diameter of 45.2 cm, a height of 20.3 cm and weights 10.7 kg. Every capsules has a volume of 0.0326 m3 (32.60 L) and is incompressible due to its ridged polypropylene shell.

$$V_{capsule} = \frac{(0.452[m])^2 \cdot \pi}{4} \cdot 0.203[m] = 0.0326[m^3]$$

$$V_{capsule} = 0.0326[m^3] \cdot \frac{1000[L]}{1[m^3]} = 32.60[L]$$

As previously mentioned, the autoclave chamber has a volume of 1.57 m^3 , fitting 48 filter capsules:

$$n_{filter\ capsules} = \frac{1.57[m^3]}{0.0326[m^3]} = 48.\overline{16} \approx 48$$

The remaining nine filter capsules can be autoclaved with together with bags in autoclave cycle 12, 13, 14 since there is empty space left in the named cycles. The total weight of all filter capsules is 609.9 kg:

$$m_{filter\ capsules} = 57 \cdot 10.7g] = 609.9[kg]$$

10.11 Commuting – preliminary calculations

To calculate the yearly carbon emissions of commuting by car, the total amount of gasoline consumption is determined. For 150 employees that commute to work on a 5 days per week basis (=230 days per year) with an average round trio distance of 50 km the total distance of all employees combined is 1725000 km:

$$D_{1\ year} = n_{employees} \cdot n_{working\ days} \cdot D_{round\ trip} = 150 \cdot 230 \cdot 50[km]$$
$$= 1725000\ [\frac{km}{year}]$$

With an emission factor of 2.392 $kg_{CO2}/L_{gasoline}$ the total carbon emissions come to 247.572 t_{CO2} per year:

$$m_{carbon,1year} = 103500[L] \cdot 2.392\left[\frac{kg_{CO2}}{L_{gasoline}}\right] = 247572[\frac{kg_{CO2}}{year}]$$

$$m_{carbon,1year} = 247572\left[\frac{kg}{year}\right] \cdot \frac{1[t]}{1000[kg]} = 247.572[\frac{t_{CO2}}{year}]$$

The average carbon emission per worker per year is 1.65 t_{CO2}:

$$m_{average} = \frac{247.572[\frac{t_{CO2}}{year}]}{150} \approx 1.65[\frac{t_{CO2}}{year}]$$

Swiss commuters

The *Bundesamt für Statistik* (*Schweizerische Eidgenossenschaft*) puplished data for commuters in the year 2017 [96]:

Average distance (roundtrip)	30 km
Way of transportation	
Car	52%
Train	17%
Public transport	14%
Motorbikes	2%
Bicycle	7%
By foot	9%

To estimate the carbon emissions of 150 employees in Switzerland that commute to work for 230 days per year, the commuters are split according to the percentage distribution, whereas the commuters that commute via car were split in 50% brackets for gasoline and diesel:

Way of transport	**Number of workers**	**Yearly distance (roundtrip) [km]**	**EF [g_{CO2e}/pkm]**	**Emissions [t_{CO2e}/year]**
Commute by car	78			
Car (gasoline)	39	269100	130.23	35.045
Car (diesel)	39	269100	106.01	28.527
Train	26	179400	0.05	0.009
ÖV	21	144900	0.12	0.017
Motorbike (Gasoline)	3	20700	99.48	2.059
Bike/by foot	22	151800	0	0.000
SUM		1035000		65.66

Exemplary calculation for “Car (gasoline)”:

$$distance\ per\ year = 39 \cdot 230[d] \cdot 30[km] = 26910[pkm]$$

$$carbon\ emissions\ per\ year = 23910[pkm] \cdot 130.23\left[\frac{g_{CO2e}}{pkm}\right] = 35.045[t_{CO2e}]$$

The average carbon emissions per km is:

$$\frac{65.66[t] \cdot 1000[\frac{kg}{t}] \cdot 1000[\frac{g}{kg}]}{1035000[pkm]} = 63.3[\frac{g_{CO2e}}{pkm}]$$

10.12 Single-use bag production and incineration

The life cycle of single-use bags, namely buffer/media storage bags, SUMs and SUBs start with the production of the film itself. The mass of single-use bags is then multiplied by the emission factor for incineration of polyethylene to receive the carbon emissions. The first step is to gather all necessary mass data for various single-use bags. Manufacturers and suppliers do not offer data on weight. Through personal communication three major manufacturers of single-use bags provided mass data. With the data on mass being incomplete regarding certain bag types and volumes, the data interpolation/extrapolation is applied.

The three manufacturers provided the following data:

Table 30 Manufacturer 1: 2D single-use bags. The mass includes the bag chamber without any filters or tubing

Volume [L]	Mass [kg]
0.5	0.0269
2	0.0578
5	0.115
10	0.185
20	0.2506

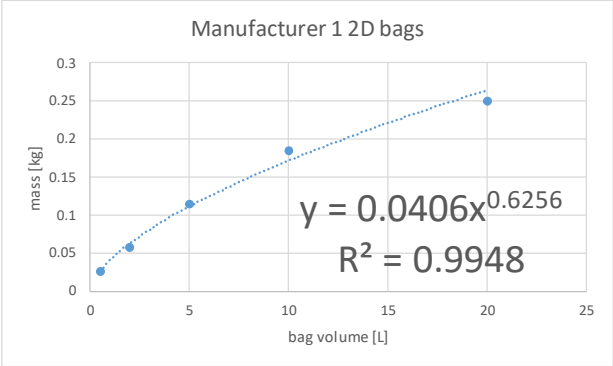


Table 31 Manufacturer 1: 3D single-use bags. The mass includes the bag chambers without any filters or tubing

Volume [L]	Mass [kg]
50	0.0269
100	0.0578
500	0.115

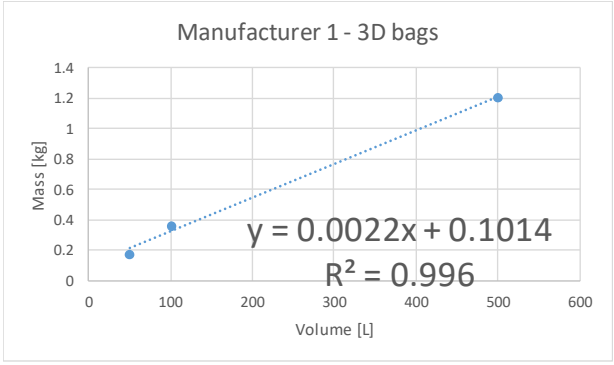


Table 32 Manufacturer 1: SUB bags. Provided data include weight of boxed product and product without packaging.

Volume [L]	Boxed product [kg]	Weight without packaging [kg]
200	9	6.5
1000	30	14
2000	34	17

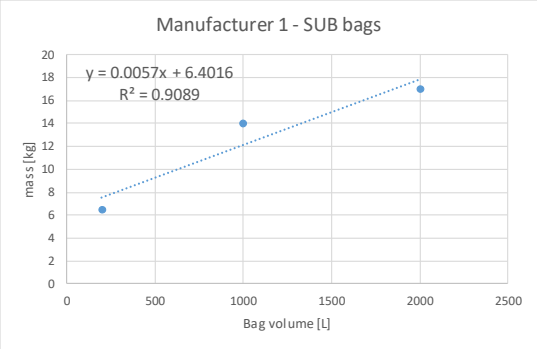
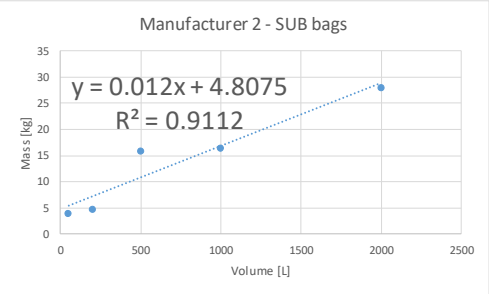


Table 33 Manufacturer 2: SUB bags. The mass includes packaging.

Volume [L]	Bag+packaging [kg]
50	14
200	17
500	30
1000	33
2000	56



Manufacturer 3 provided the following data on their single-use 3D bags:

Table 34 Manufacturer 3: 3D single-use bags. It is unclear weather the weight is packaging weight, the weight of the bags including filters and tubing or just the single-use bags themselves. The provided data also appears to be unrealistic when compared to data provided by other manufacturers.

Volume [L]	Mass [kg]
500	120.2
1000	163.3
1500	224.5
2000	281.2

The list that has to be filled to determine the weight of single-use bags that is used per batch, the following list has to be completed:

Type	Volume	Weight [kg]
2D bag	1	?
	10	?
	20	?
	25	?
	50	?
3D bag	100	?
	200	?
	250	?
	500	?
	650	?
	1000	?
	1500	?
WAVE	25	?
	50	?
	100	?
SUB	200	?
	500	?
	1000	?
	2000	?
SUM	50	?
	100	?
	400	?
	650	?
	1000	?
	3000	?

While some weight are known, most have to approximated by interpolation/extrapolation.

Data from Table 30 is plotted to receive a potency function to calculate the weight of unknown volumes:

$$m_{bag}[kg] = 0.0406 \cdot V_{bag}^{0.6256}[L]$$

Exemple for 25 L:

$$m_{25L} = 0.0406 \cdot 25[L]^{0.6256} \approx 0.30[kg]$$

Data from Table 31 is plotted to receive a linear function to calculate the weight of unknown volumes:

$$m_{bag} = 0.0022 \cdot V_{bag}[L] + 0.1014$$

Exemple for 1000 L:

$$m_{1000L} = 0.0022 \cdot 1000[L] + 0.1014 \approx 2.3[kg]$$

Data from Table 32 is plotted to receive a linear function to calculate the weight of unknown volumes:

$$m_{bag} = 0.0057 \cdot V_{bag}[L] + 6.4016$$

Exemplary for 500 L:

$$m_{500L} = 0.0057 \cdot 500[L] + 6.4016 \approx 9.3[kg]$$

Type	Volume	Known mass [kg]	Approximated by	Approximated mass [kg]
2D bag	1		Data from Table 30	0.0406
	10	0.185		
	20	0.2506		
	25		Data from Table 30	0.3041
3D bag	50	0.18		
	100	0.36		
	200		Data from Table 31	0.5414
	250		Data from Table 31	0.6514
	500	1.208		
	650		Data from Table 31	1.5314
	1000		Data from Table 31	2.3014
	1500		Data from Table 31	3.4014
WAVE	25		Data from Table 30	0.3041
	50		Data from Table 30	0.4692
	100		Data from Table 30	0.7240
SUB	200	6.5		
	500		Data from Table 32	9.2516
	1000	14.0		
	2000	17.0		
SUM	50		Data from Table 32	6.6866
	100		Data from Table 32	6.9716
	400		Data from Table 32	8.6816
	650		Data from Table 32	10.1066
	1000		Data from Table 32	12.1016
	3000		Data from Table 32	23.5016

The finalized table:

Table 35 Finalized table with known weights (grey) and calculated weights (white).

Type	Volume	Mass [kg]	Quantity	Total weight for one batch [kg]
2D bag	1	0.0406	1	0.0406
	10	0.185	0	0.0000
	20	0.2506	2	0.5012
	25	0.3041	0	0.0000
3D bag	50	0.18	1	0.1800
	100	0.36	2	0.7200
	200	0.5414	3	1.6242
	250	0.6514	1	0.6514
	500	1.208	1	1.2080
	650	1.5314	0	0.0000
	1000	2.3014	53	121.9000
	1500	3.4014	2	0.4000
WAVE	25	0.3041	0	0.0000
	50	0.4692	0	0.0000
	100	0.7240	1	0.7240
SUB	200	6.5	0	0.0000
	500	9.2516	0	0.0000
	1000	14.0	0	0.0000
	2000	17.0	1	17.0000
SUM	50	6.6866	0	0.0000
	100	6.9716	0	0.0000
	400	8.6816	0	0.0000
	650	10.1066	2	20.2132
	1000	12.1016	8	96.8128
	3000	23.5016	1	23.5016
Sum				285.4770

The calculated values are just approximated values for the lack of provided data. With known weight the carbon emissions for the production and incineration of single-use bags is calculated by multiplying the mass with emission factors for plastic film extrusion and incineration of polyethylene.

10.13 Emission factor of PE/PP – preliminary calculations

Polyethylene (PE)

$$C_2H_4 + 3O_2 \rightharpoonup 2CO_2 + 2H_2O$$

Molar ratio is 1:2, one mole polyethylene to two moles carbon dioxide

The molar mass of polyethylene is 28.05 kg/kmol, so one kg of polyethylene contains $0.\overline{0357}$ kilo mol:

$$1kg \cdot \frac{1[kg]}{28.05[kmol]} = 0.\overline{0357}[kmol]$$

The molar ratio is 1:2, meaning that for every mole of polyethylene (C_2H_4) two moles of carbon dioxide (CO_2) are emitted:

$$n_{CO2} = 2 \cdot 0.\overline{0357}[kmol] = 0.\overline{0714}[kmol]$$

The molar mass of carbon dioxide (CO2) is 44.91 kg/kmol, meaning that for every kg of polyethylene that is burned, 3.2069 kg CO_2 are emitted:

$$m_{CO2} = 0.\overline{0714}[kmol] \cdot 44.91[\frac{kg}{kmol}] \approx 3.2069[kg]$$

The emission factor for polyethylene incineration is 3.2069[kg_{CO2}/kg_{PE}].

Polypropylene (PP)

$$C_3H_6 + 4.5O_2 \rightharpoonup 3CO_2 + 3H_2O$$

Molar ratio is 1:3, one mole polyethylene to three moles carbon dioxide

The molar mass of polyethylene is 42.08 kg/kmol, so one kg of polyethylene contains 0.0238 kilo mol:

$$1kg \cdot \frac{1[kg]}{42.085[kmol]} = 0.0238[kmol]$$

The molar ratio is 1:3, meaning that for every mole of polyethylene (C_2H_4) two moles of carbon dioxide (CO_2) are emitted:

$$n_{CO2} = 3 \cdot 0.0238[kmol] = 0.0714[kmol]$$

The molar mass of carbon dioxide (CO_2) is 44.91 kg/kmol, meaning that for every kg of polyethylene that is burned, 3.2069 kg CO_2 are emitted:

$$m_{CO2} = 0.0714[kmol] \cdot 44.91[\frac{kg}{kmol}] \approx 3.2066[kg]$$

The emission factor for incineration of polypropylene is 3.2066[kg_{CO2}/kg_{PP}].

10.14 Cargo transport emission – preliminary calculations

Cargo transport emissions cover the transport of steel tanks from the production site to the SST facility, the transport of bag support structures from production site to the SUT production facility as well as the transport of buffer man the buffer generation plant to the SUT facility. Calculations are based on transport via trucks on roads or via trains on rails. The mass of each shipment is calculated to perform a conversion into CO_2 equivalents via emission factors.

Buffer

To ship 7526.37 m^3 (or 7526.37 t with an assumed density of 1000 kg/m^3) via trucks, for a distance of 600 km, 631.31 t_{CO2} are emitted:

$$m_{CO2,buffer} = 7526.3[t] \cdot 139.8 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 631306044[g_{CO2}]$$

$$m_{CO2,buffer} = 631306044[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 631.31[t_{CO2}]$$

For shipment via train 70.45 t_{CO2} are emitted:

$$m_{CO2,buffer} = 7526.3[t] \cdot 15.6 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 70446168[g_{CO2}]$$

$$m_{CO2,buffer} = 70446168[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 70.45[t_{CO2}]$$

Steel tanks

To transport steel tanks with a weight of 1.41 t for a distance of 600 km via trucks, 0.12 t_{CO2} are emitted:

$$m_{CO2,SST\ tanks} = 1.41[t] \cdot 139.8 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 118270.8[g_{CO2}]$$

$$m_{CO2,SST\ tanks} = 118270.8[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 0.12[t_{CO2}]$$

For shipment via train 0.0132 t_{CO2} are emitted:

$$m_{CO2,SST\ tanks} = 1.41[t] \cdot 15.6 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 13197.6[g_{CO2}]$$

$$m_{CO2,SST\ tanks} = 13197.6[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 0.0132[t_{CO2}]$$

Bag support structures

Number and type of single-use support structures are known shows a compiled list with according weights:

Table 36 Data on mass on single-use support structures.

Flowchart label	Type	Volume [L]	Mass [kg]	Reference
0.9	SUM	500	432	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
1	SUB	2000	942.1	Thermo Fisher - Bioprocessing solutions to address your unique challenges (catalogue) [98]
2	SUM	3000	1730	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
3	SUM	2000	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
4	SUM	1000	516	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
5	SUM	1000	516	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
6	break bag	1000	163.3	Personal communication with supplier
7	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
8	SUM	450	432	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer[97]
9	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]

10	break bag	1000	163.3	Personal communication
11	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
12	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
13	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
14	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
15	SUM	650	503	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
16	SUM	400	432	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer [97]
17	SUM	400	432	Thermo Fisher - DATA SHEET imPULSE Single-Use Mixer[97]
Σ			**9782.7**	

To transport bag support structures with a weight of 9.7827t for a distance of 600 km via trucks, 0.82 t_{CO2} are emitted:

$$m_{CO2,bag\ support} = 9.7827[t] \cdot 139.8 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 820572.876[g_{CO2}]$$

$$m_{CO2,bag\ support} = 820572.876[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 0.82[t_{CO2}]$$

For shipment via train 0.09 t_{CO2} are emitted:

$$m_{CO2,bag\ support} = 9.7827[t] \cdot 15.6 \cdot 600[km] \left[\frac{g_{CO2}}{t_{cargo} \cdot km}\right] = 91566.072[g_{CO2}]$$

$$m_{CO2,bag\ support} = 91566.072[g_{CO2}] \cdot \frac{1[kg]}{1000[g]} \cdot \frac{1[t]}{1000[kg]} = 0.09[t_{CO2}]$$

10.15 Tank diameter/height – preliminary calculations

Optimized cylinder surface

(I) $V_c = \pi \cdot r^2 \cdot h_c$

(II) $\rightarrow h_c = \frac{V}{\pi \cdot r^2}$

(III) $S_c = 2 \cdot \pi \cdot r \cdot h + 2 \cdot \pi \cdot r^2$

Insert II in III:

$$S_c = 2 \cdot \pi \cdot r \cdot \frac{V_c}{\pi \cdot r^2} + 2 \cdot \pi \cdot r^2 = \frac{2V_c}{r} + 2 \cdot \pi \cdot r^2 = 2 \cdot V_c \cdot \frac{1}{r} + 2 \cdot \pi \cdot r^2$$

First derivative of the surface:

$$\dot{S}_c(r) = 4\pi r - 2V\frac{1}{r^2}$$

Second derivative of the surface:

$$\ddot{S}_c(r) = 4\pi + 2V_c\frac{2}{r^3} + 2\pi r^2 = 4\pi + \frac{4V_c}{r^3}$$

Set first derivative to zero:

$$\dot{S}_c(r) = 0$$

Rearrange to r:

$$0 = 4\pi r - 2V_c\frac{1}{r^2} \quad \left| + \frac{2V_c}{r^2}\right.$$

$$\frac{2V_c}{r^2} = 4\pi r \quad \left| \cdot r^2\right.$$

$$2V_c = 4\pi r^3 \quad \left| \cdot \frac{1}{4\pi}\right.$$

$$r^3 = \frac{2V_c}{4\pi} \quad \left|\sqrt[3]{}\right.$$

$$r = \sqrt[3]{\frac{2V_c}{4\pi}} = \sqrt[3]{\frac{V_c}{2\pi}}$$

Since $V_c > 0$ and > 0 , the term of the second derivative is positive, meaning that a minimum has been found

$$\ddot{S}_c(r) = \ddot{S}_c\left(\sqrt[3]{\frac{2V_c}{4\pi}}\right) > 0 \checkmark$$

There the optimized height can be calculated:

$$h_{tank,optimized} = \frac{V_c}{\pi r^2} = \frac{V_c}{\pi \cdot (\sqrt[3]{\frac{V_c}{2\pi}})^2} = \frac{V_c}{\pi \cdot \sqrt[3]{\frac{V_c^2}{2^2 \cdot \pi^2}}} \sqrt[3]{\frac{V_c^3}{\pi^2 \cdot \frac{V_c^2}{4 \cdot \pi^2}}} = \sqrt[3]{\frac{V_c^3 \cdot 4 \cdot \pi^2}{\pi^3 \cdot V_C^2}}$$

$$= \sqrt[3]{\frac{4 \cdot V_c}{\pi}}$$

The optimized radius can be calculated with the following formula:

$$r_{tank,optimized} = \sqrt[3]{\frac{V_c}{2 \cdot \pi}}$$

The optimized diameter is essential calculated with the same formula as the optimized height:

$$d_{tank,optimized} = 2 \cdot r_{tank\ optimized} = 2 \cdot \sqrt[3]{\frac{V_c}{2 \cdot \pi}} = \sqrt[3]{\frac{2^3 \cdot V_c}{2 \cdot \pi}} = \sqrt[3]{\frac{4 \cdot V_c}{\pi}}$$

10.16 Typical CIP cycles and spray ball models

Typical CIP cycle as stated Nicholas Jeffry and Elliot Sutton (Suncombe Ltd; www.suncombe.com):

Typical CIP Programme

Step	Operation	Cleaning Agent	Temp. (ºC)	Time (Min.)	Usage
1	Pre-Rinse	Water	20 – 30	2 – 5	To drain
2	Alkali Clean	2% Caustic	70 – 90	5 – 30	Re-circulated
3	Inter-rinse	Water	20 – 30	1 – 5	To drain
4	Acid clean	1% Phosphoric	50 – 70	3 – 15	Re-circulated
5	Inter-rinse	Water	20 – 30	4 – 10	To drain
6	Sterilant	Peracetic Acid	20 – 30	3 – 15	Re-circulated
7	Final Rinse	Water	20 – 30	4 – 10	To drain

Typical CIP cycle as stated by Dale A. Seiberling on page 77 in his book “Clean-In-Place for Biopharmaceutical processes”:

TABLE 1 Typical Recirculating Chemical Wash Program

Phase(s)	Function	Water usage (L)	Time (min)
CIP program initiation	Confirms utilities, CIP boundary, permissives	NA	5
Rinse	Flush circuit of all free-rinsing soil	300	1.5
Intermediate drain	Drains return side, CIP supply side remains charged with water	NA	2
Chemical wash	Establish circuit recirculation, feed chemical A, confirm conductivity and temperature, wash for required duration	130	10
Gas blow and intermediate drain	Clears CIP supply of chemical A, drain circuit for effective minimum volume rinse	NA	2
Rinse	Flush circuit of spent chemical A	300	2
Intermediate drain	Drains return side, CIP supply side remains charged with water	NA	1
Chemical wash	Establish circuit recirculation, feed chemical B, confirm conductivity and wash temperature, wash for required duration	130	10
Gas blow and intermediate drain	Clears CIP supply of chemical B, drain circuit for effective minimum volume rinse	NA	2
Rinse	Flush circuit of spent chemical B	300	2
Intermediate drain	Drains return side, CIP supply side remains charged with water	NA	1
Final rinse with high quality water	Flush with high-quality water to defined end point, removing soil and chemical	700	5
Gas blow and intermediate drain	Clears CIP supply of water, drain rinse	NA	2
Final drain	Gravity drain of CIP boundary low points	NA	5
Program complete	Releases clean CIP boundary	Total volume 2860	Total time 64

Spray angle	Ordering number Type	E Ø [mm]	V̇ [l/min] p [bar] (p_{max} = 5 bar)					Dimensions approx. [mm]						Max. tank diameter [m]
			0.5	1	2	3	at 40 psi [US gal./ min]	Ø D	Height H	Con-nection B	Slip-on	C	A	
360°	**591.M11.17.00**	0.8	7	10	14	17	4	20	32.5	8.2	DN8	2.2	9.0	2.0
	591.X11.17.00	1.2	25	35	49	61	15	24	37.5	12.2	DN10	2.2	9.0	2.2
	591.Y11.17.00	1.6	49	70	99	121	31	30	42	18.2	DN15	2.2	9.0	2.5
	591.A21.17.00	2.0	91	128	181	222	56	40	53	22.2	DN20	2.5	9.0	3.5
	591.B31.17.00	2.1	130	183	259	318	80	64	90	28.2	DN25	2.8	18.0	5.2
	591.B51.17.00	3.0	206	292	412	505	128	64	90	28.2	DN25	2.8	18.0	5.4
180°	**591.A23.17.00**	2.0	74	105	148	182	46	40	53	22.2	DN20	2.5	9.0	2.5
	591.B53.17.00	3.0	146	207	292	358	91	64	90	28.2	DN25	2.8	18.0	4.6
180°	**591.B32.17.00**	2.1	103	145	205	251	64	64	90	28.2	DN25	2.8	18.0	5.2
	591.D42.17.00	2.2	230	325	460	563	142	90	122	52.3	DN50	3.3	25.0	5.5

E = Narrowest free cross-section
Female thread and more slip-on sizes on request

The maximum tank diameter shown above applies for the recommended operating pressure and is indicative only. The cleaning result is also affected by the type of soiling.

Figure 72 Static spray ball model from Lechler GmbH [91, S. 24]

1

Spray angle	Ordering no. Type	Connection 3/4 BSPP female	3/4" Slip-on	1" Slip-on	1" Tri-Clamp	E Ø [mm]	V̇ [l/min] p [bar] (p_{max} = 6 bar) 1	2	3	at 40 psi [US gal./ min]	Max. tank diameter [m]
270°	**569.055.1Y**	**AL**	**TF07**	**TF10**	**10**	3.6	36	48	62	15	1.8
	569.135.1Y	**AL**	**TF07**	**TF10**	**10**	4.8	52	71	87	22	2.1
	569.195.1Y	**AL**	**TF07**	**TF10**	**10**	5.6	69	97	119	30	2.6
270°	**569.056.1Y**	**AL**	**TF07**	**TF10**	**10**	3.6	36	48	62	15	1.8
	569.106.1Y	**AL**	**TF07**	**TF10**	**10**	4.8	41	58	71	18	2.1
	569.196.1Y	**AL**	**TF07**	**TF10**	**10**	5.6	69	97	119	30	2.6
360°	**569.059.1Y**	**AL**	**TF07**	**TF10**	**10**	3.2	36	48	62	15	1.8
	569.139.1Y	**AL**	**TF07**	**TF10**	**10**	3.6	52	71	87	22	2.1
	569.199.1Y	**AL**	**TF07**	**TF10**	**10**	4.8	69	97	119	30	2.6
	569.279.1Y	**AL**	**TF07**	**TF10**	**10**	7.1	103	145	178	45	3.0

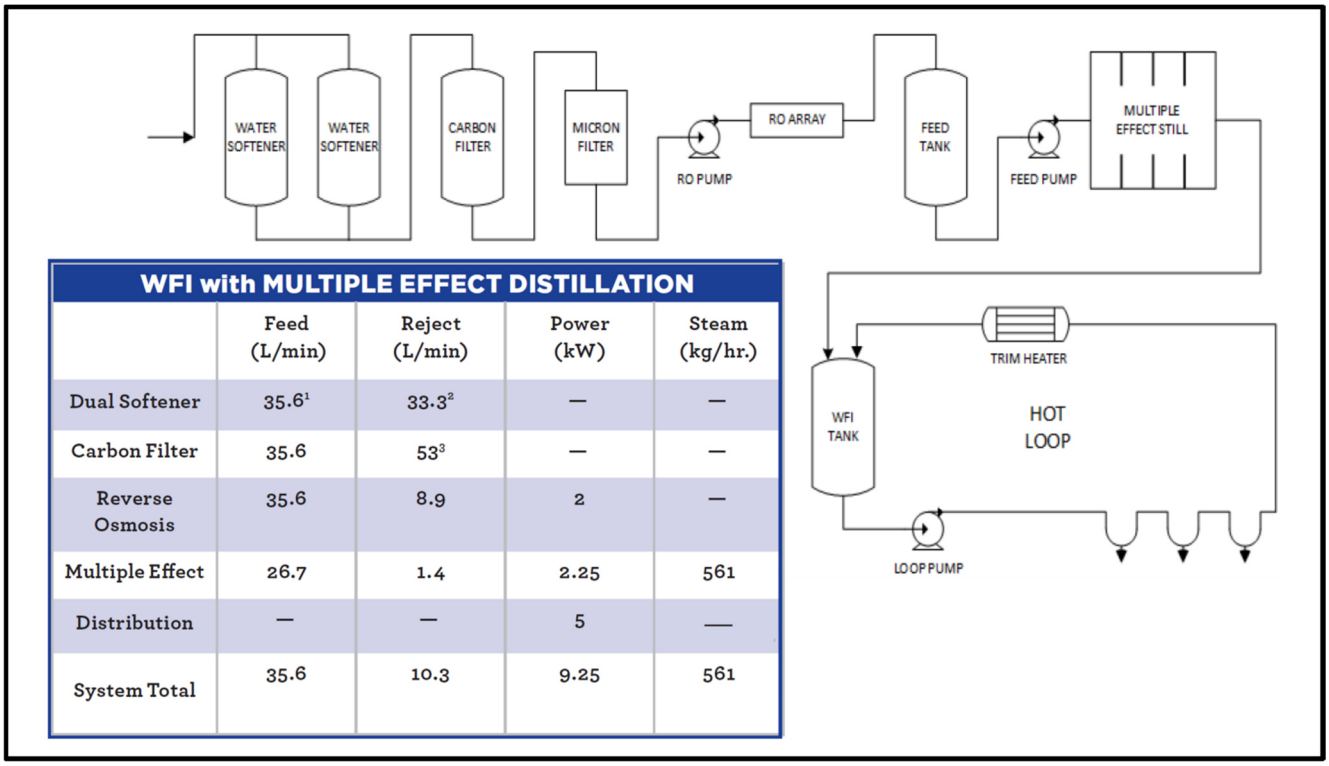
E = narrowest free cross-section · NPT on request

The maximum tank diameter shown above applies for the recommended operating pressure and is indicative only. The cleaning result is also affected by the type of soiling.

Figure 73 Rotating spray ball model “Whirly”from Lechler GmbH [92, S. 52].

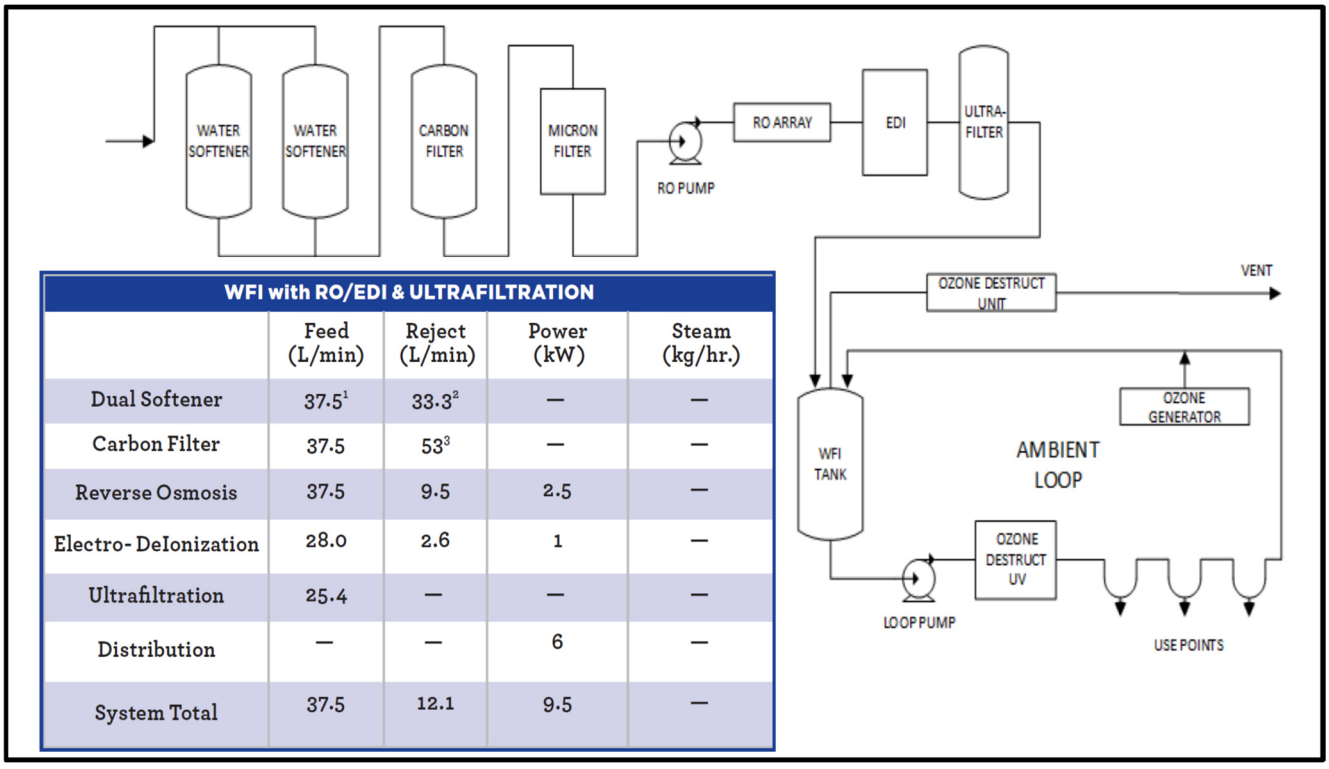
10.17 Exemplary WFI generation systems

The commercially available WFI generation systems by Meco, that are presented below produce 1500 L_{WFI}/h [99]. All systems presented are adapted from a brochure by Meco [99, S. 1-16].



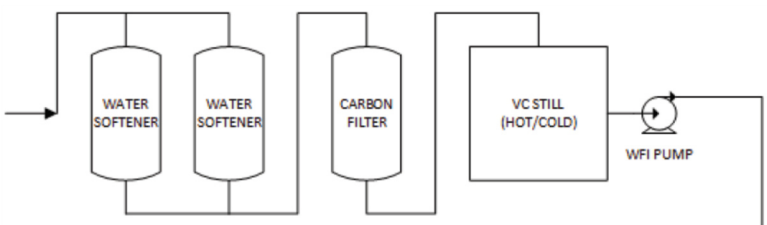
WFI with MULTIPLE EFFECT DISTILLATION

	Feed (L/min)	Reject (L/min)	Power (kW)	Steam (kg/hr.)
Dual Softener	35.6[1]	33.3[2]	—	—
Carbon Filter	35.6	53[3]	—	—
Reverse Osmosis	35.6	8.9	2	—
Multiple Effect	26.7	1.4	2.25	561
Distribution	—	—	5	—
System Total	35.6	10.3	9.25	561



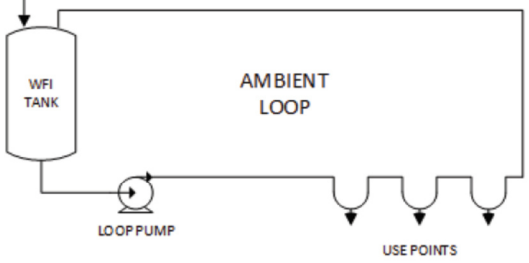
WFI with RO/EDI & ULTRAFILTRATION

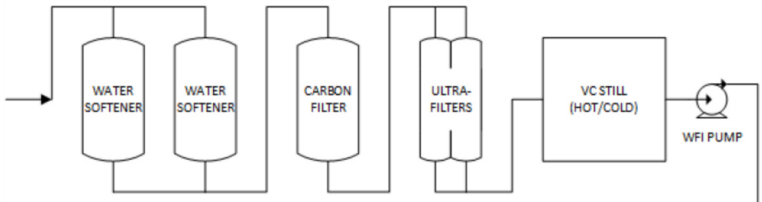
	Feed (L/min)	Reject (L/min)	Power (kW)	Steam (kg/hr.)
Dual Softener	37.5[1]	33.3[2]	—	—
Carbon Filter	37.5	53[3]	—	—
Reverse Osmosis	37.5	9.5	2.5	—
Electro- DeIonization	28.0	2.6	1	—
Ultrafiltration	25.4	—	—	—
Distribution	—	—	6	—
System Total	37.5	12.1	9.5	—



WFI with CARBON FILTRATION, WATER SOFTENING AND VAPOR COMPRESSION

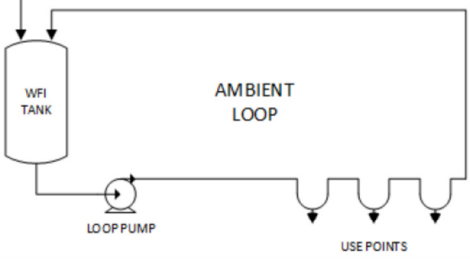
	Feed (L/min)	Reject (L/min)	Power (kW)	Steam (kg/hr.)
Dual Softener	31.4[1]	33.3[2]	—	—
Carbon Filter	31.4	53[3]	—	—
Vapor Compression	29.9	4.5	18.6	55
Distribution	—	—	5	—
System Total	31.4	6.0	25.6	



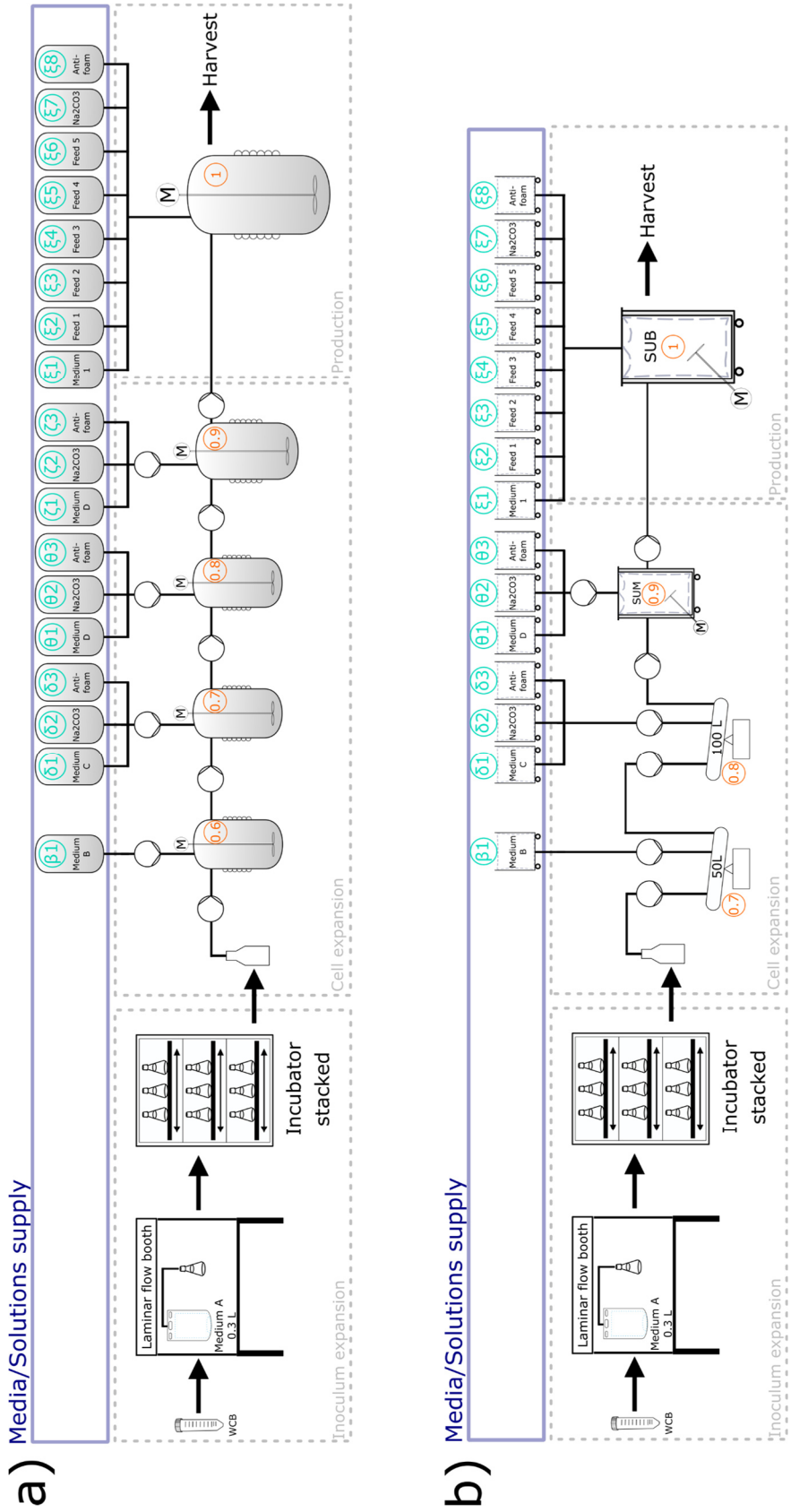


WFI with ULTRAFILTRATION AND VAPOR COMPRESSION

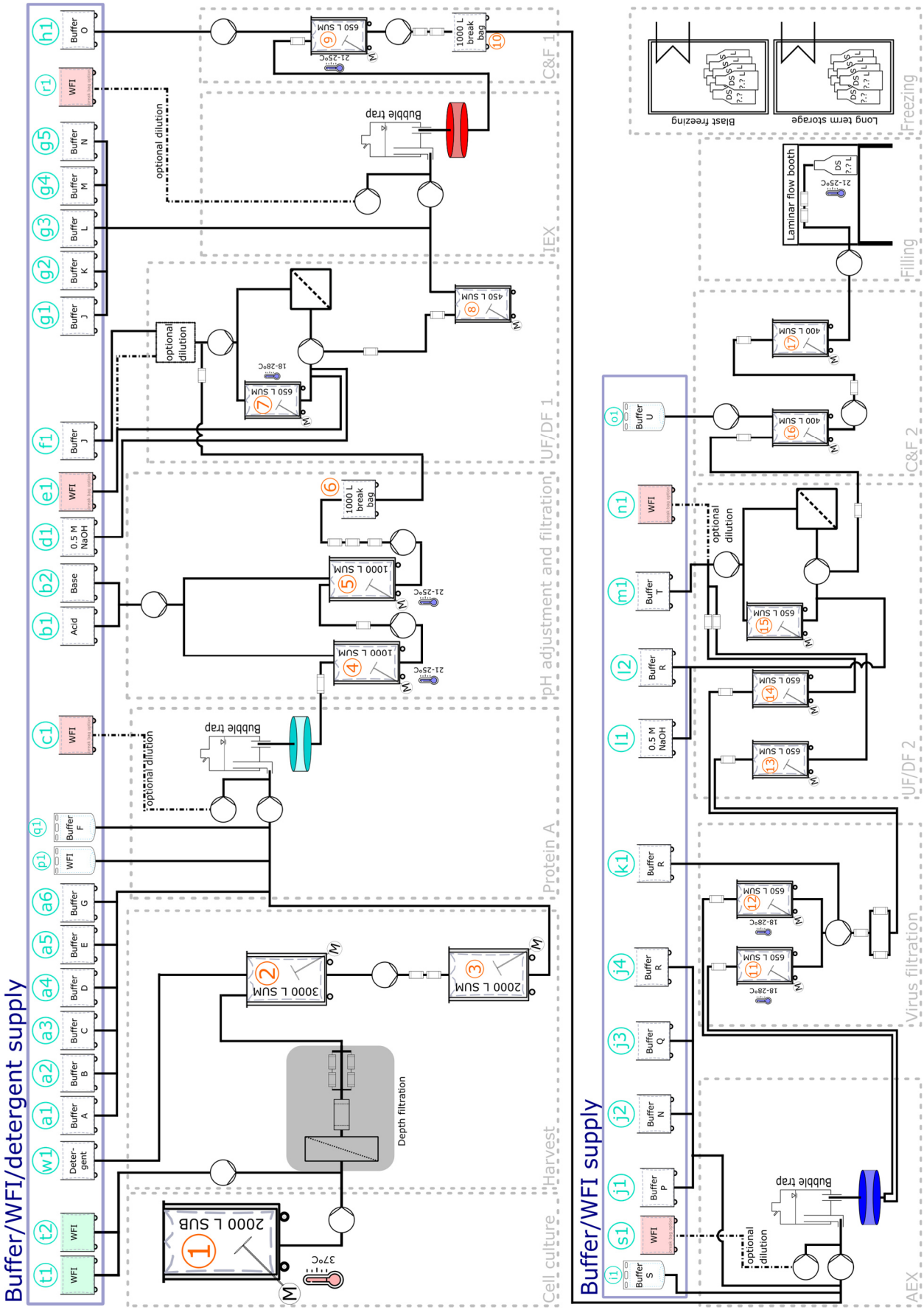
	Feed (L/min)	Reject (L/min)	Power (kW)	Steam (kg/hr.)
Dual Softener	31.4[1]	33.3[2]	—	—
Carbon Filter	31.4	53[3]	—	—
Ultrafiltration	29.9	1.5	2	—
Vapor Compression	29.9	4.5	18.6	55
Distribution	—	—	5	—
System Total	31.4	6.0	25.6	55



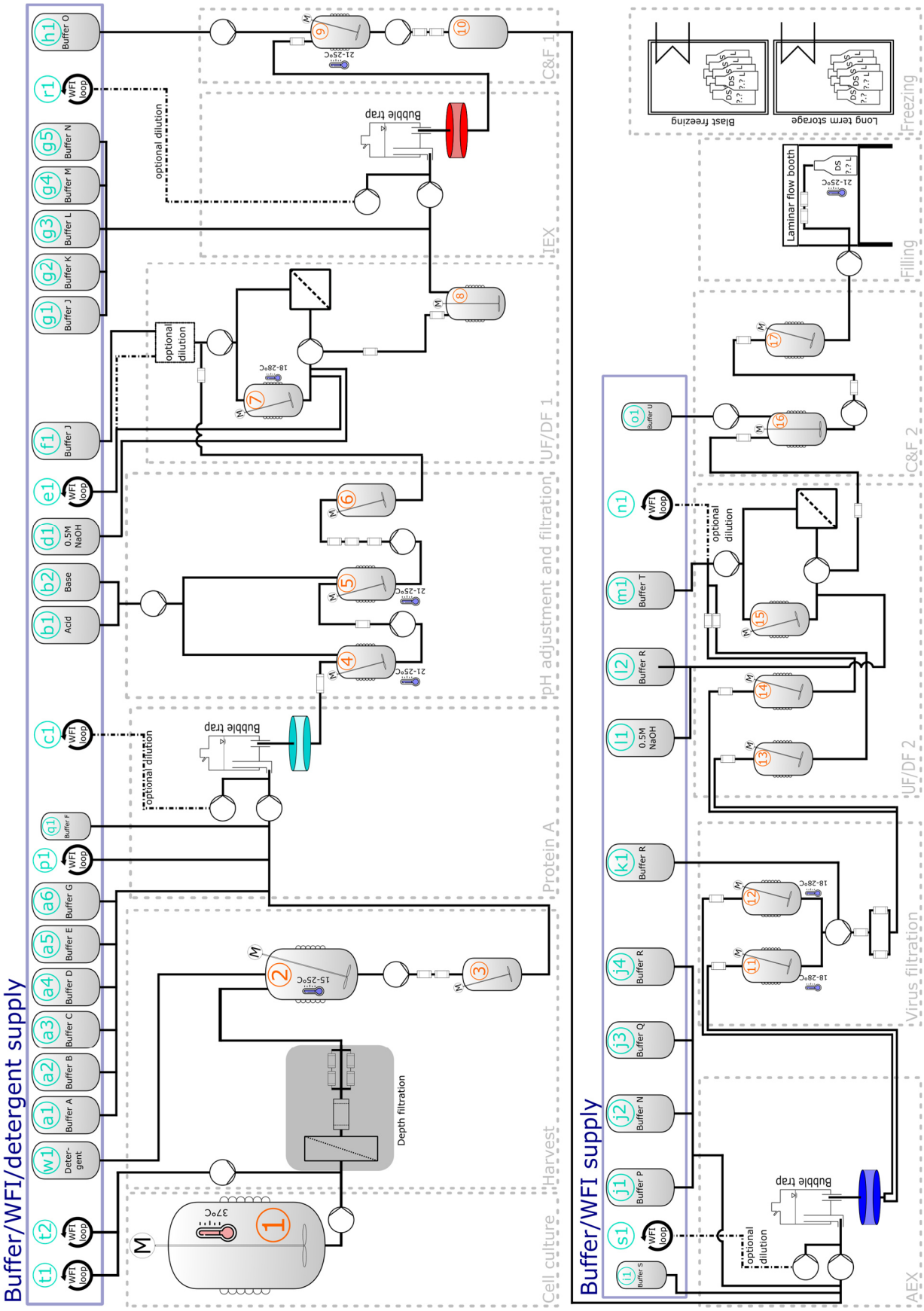
10.18 USP flow chart for the SST and SUT facility



10.19 Downstream process flow chart for the SUT facility



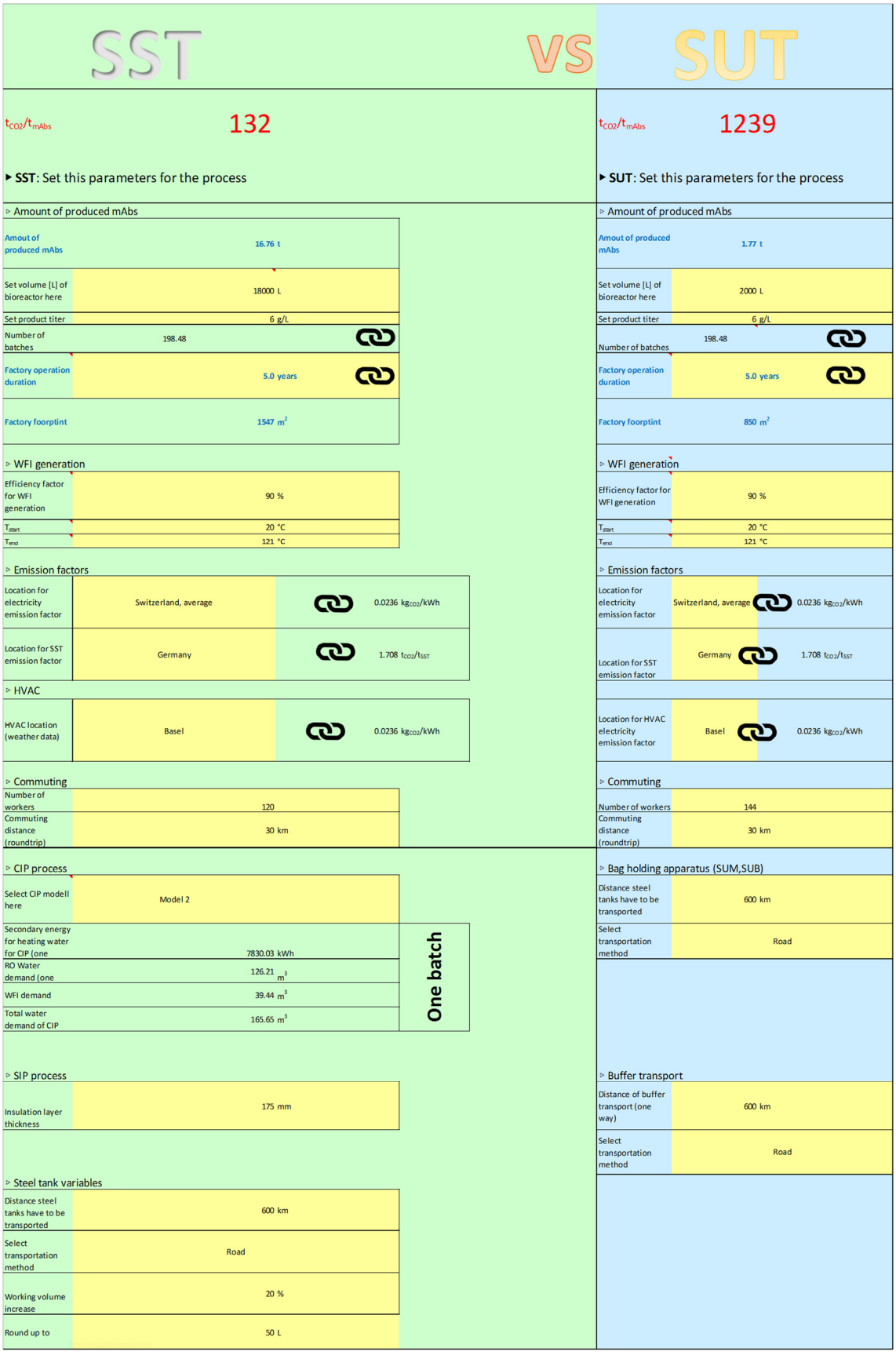
10.20 Downstream process flow chart for the SST facility



10.21 HVAC fan data

Modelle 400V 3~				Umdrehung	Leistung	Luftmenge	Schalldruck		
Ring	Kurz	Lang	Durchm.	U/min	kW	m³/h	dB(A)	m3/h/kW	Model No.
		DHCT	25-4T	1320	0.09	1000.00	50	11111	1
		DHCT	31-4T	1320	0.09	1550.00	52	17222	2
DHCH		DHCT	35-4T	1320	0.09	3100.00	59	34444	3
DHCH		DHCT	40-4T-0,33	1350	0.25	5150.00	64	20600	4
DHCH		DHCT	45-4T-0,5	1370	0.37	7100.00	68	19189	5
		DHCT	50-4T-0,75	1380	0.55	10400.00	70	18909	6
DHCH	DHFT	DHCT	56-4T-0,75	1380	0.55	11050.00	72	20091	7
DHCH	DHFT	DHCT	56-4T-1	1410	0.75	12950.00	73	17267	8
DHCH	DHFT	DHCT	56-4T-1,5	1400	1.10	14000.00	74	12727	9
DHCH	DHFT	DHCT	56-4T-2	1430	1.50	15300.00	75	10200	10
DHCH	DHFT	DHCT	56-6T-0,33	900	0.25	8500.00	61	34000	11
DHCH	DHFT	DHCT	56-6T-0,5	900	0.37	9300.00	61	25135	12
DHCH	DHFT	DHCT	56-6T-0,75	900	0.55	10000.00	62	18182	13
DHCH	DHFT	DHCT	63-4T-1	1410	0.75	14150.00	73	18867	14
DHCH	DHFT	DHCT	63-4T-1,5	1400	1.10	17000.00	74	15455	15
DHCH	DHFT	DHCT	63-4T-2	1430	1.50	18900.00	75	12600	16
DHCH	DHFT	DHCT	63-4T-3	1445	2.20	22100.00	76	10045	17
DHCH	DHFT	DHCT	63-4T-4	1445	3.00	25400.00	77	8467	18
DHCH	DHFT	DHCT	63-6T-0,5	900	0.37	12150.00	64	32838	19
DHCH	DHFT	DHCT	63-6T-0,75	900	0.55	12750.00	65	23182	20
DHCH	DHFT	DHCT	71-4T-1,5	1400	1.10	19750.00	78	17955	21
DHCH	DHFT	DHCT	71-4T-2	1430	1.50	21100.00	79	14067	22
DHCH	DHFT	DHCT	71-4T-3	1445	2.20	23950.00	81	10886	23
DHCH	DHFT	DHCT	71-4T-4	1445	3.00	29400.00	82	9800	24
DHCH	DHFT	DHCT	71-6T-0,75	900	0.55	15150.00	67	27545	25
DHCH	DHFT	DHCT	71-6T-1	945	0.75	17250.00	68	23000	26
DHCH	DHFT	DHCT	71-6T-1,5	945	1.10	20950.00	69	19045	27
DHCH	DHFT	DHCT	80-4T-3	1445	2.20	28000.00	82	12727	28
DHCH	DHFT	DHCT	80-4T-4	1445	3.00	32700.00	83	10900	29
DHCH	DHFT	DHCT	80-4T-5,5	1440	4.00	37200.00	84	9300	30
DHCH	DHFT	DHCT	80-6T-1	945	0.75	20600.00	71	27467	31
DHCH	DHFT	DHCT	80-6T-1,5	945	1.10	24250.00	72	22045	32
DHCH	DHFT	DHCT	80-6T-2	955	1.50	28000.00	73	18667	33
DHCH	DHFT	DHCT	80-6T-3	955	2.20	32500.00	74	14773	34
DHCH	DHFT	DHCT	90-4T-4	1445	3.00	37750.00	87	12583	35
DHCH	DHFT	DHCT	90-4T-5,5	1440	4.00	41850.00	89	10463	36
DHCH	DHFT	DHCT	90-4T-7,5	1440	5.50	47000.00	91	8545	37
DHCH	DHFT	DHCT	90-4T-10	1455	7.50	53000.00	92	7067	38
DHCH	DHFT	DHCT	90-6T-3	955	2.20	35000.00	78	15909	39
DHCH	DHFT	DHCT	90-6T-4	960	3.00	40000.00	79	13333	40
DHCH	DHFT	DHCT	100-4T-7,5	1440	5.50	52500.00	92	9545	41
DHCH	DHFT	DHCT	100-4T-10	1455	7.50	58500.00	93	7800	42
DHCH	DHFT	DHCT	100-4T-15	1460	11.00	68000.00	94	6182	43
DHCH	DHFT	DHCT	100-4T-20	1460	15.00	71850.00	95	4790	44
DHCH	DHFT	DHCT	100-6T-3	955	2.20	40500.00	82	18409	45
DHCH	DHFT	DHCT	100-6T-4	960	3.00	46950.00	83	15650	46

10.22 Exemplary input (case study one)



SST		VS	SUT	
t_{CO2}/t_{mAbs}	132		t_{CO2}/t_{mAbs}	1239
▸ **SST**: Set this parameters for the process			▸ **SUT**: Set this parameters for the process	
▹ Amount of produced mAbs			▹ Amount of produced mAbs	
Amout of produced mAbs	16.76 t		Amout of produced mAbs	1.77 t
Set volume [L] of bioreactor here	18000 L		Set volume [L] of bioreactor here	2000 L
Set product titer	6 g/L		Set product titer	6 g/L
Number of batches	198.48		Number of batches	198.48
Factory operation duration	5.0 years		Factory operation duration	5.0 years
Factory foorptint	1547 m^2		Factory foorptint	850 m^2
▹ WFI generation			▹ WFI generation	
Efficiency factor for WFI generation	90 %		Efficiency factor for WFI generation	90 %
T_{start}	20 °C		T_{start}	20 °C
T_{end}	121 °C		T_{end}	121 °C
▹ Emission factors			▹ Emission factors	
Location for electricity emission factor	Switzerland, average	0.0236 kg_{CO2}/kWh	Location for electricity emission factor	Switzerland, average 0.0236 kg_{CO2}/kWh
Location for SST emission factor	Germany	1.708 t_{CO2}/t_{SST}	Location for SST emission factor	Germany 1.708 t_{CO2}/t_{SST}
▹ HVAC				
HVAC location (weather data)	Basel	0.0236 kg_{CO2}/kWh	Location for HVAC electricity emission factor	Basel 0.0236 kg_{CO2}/kWh
▹ Commuting			▹ Commuting	
Number of workers	120		Number of workers	144
Commuting distance (roundtrip)	30 km		Commuting distance (roundtrip)	30 km
▹ CIP process			▹ Bag holding apparatus (SUM,SUB)	
Select CIP modell here	Model 2		Distance steel tanks have to be transported	600 km
Secondary energy for heating water for CIP (one	7830.03 kWh	One batch	Select transportation method	Road
RO Water demand (one	126.21 m^3			
WFI demand	39.44 m^3			
Total water demand of CIP	165.65 m^3			
▹ SIP process			▹ Buffer transport	
Insulation layer thickness	175 mm		Distance of buffer transport (one way)	600 km
			Select transportation method	Road
▹ Steel tank variables				
Distance steel tanks have to be transported	600 km			
Select transportation method	Road			
Working volume increase	20 %			
Round up to	50 L			

10.23 Exemplary output data (case study one)