enviromed

Next generation toolbox for greener pharmaceuticals design and manufacturing towards reduced environmental impact

D2.1 - Report Green Pharmaceutical Manufacturing Process Requirements

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Executive Summary

The deliverable D2.1 produced from TU Wien is the output from T2.1 and T2.2. D2.1 is the first of three deliverables due for WP2. It lays a foundation for the requirements for the more sustainable production of the pharmaceuticals in question, and the future tasks and WPs set for the ENVIROMED project.

The following pharmaceuticals were chosen with discussion from the end users (partners NOVO Nordisk and Pfizer) for their fate in wastewater/sewage treatment, their negative environmental impact, and their widespread and significant production and consumption: insulin, diclofenac (DCF), ibuprofen (IBF) and metformin. Additionally, the compounds carbamazepine, metoprolol, benzotriazole, and hydrochlorothiazide were later suggested as the analytes pose problematic environmental issues, and are included for wastewater monitoring in the proposal for a revised Urban Wastewater Treatment Directive.

The four initially selected compounds listed above will be investigated in terms of green pharmaceutical production (Task 2.1). The full list of compounds (8 compounds) given above will be part of the experimentation for the sensor development. The four latter analytes (in addition to DCF) were also chosen due to their detection limits at the wastewater treatment plants (WWTPs).

Deliverable D2.1 outlines the following topics, as defined by the project proposal:

- the state-of-the-art production and manufacturing processes for the eight pharmaceuticals listed above
- an overview of requirements for sustainable pharmaceutical manufacturing (SPM) as well as an attempt to improve the sustainability of the eight compounds described in this deliverable
- fate and amount of the eight pharmaceuticals in wastewater/sewage treatment plants
- review on the potential of digitalization methods and its effects on Green Pharmaceutical Manufacturing (GPM)- digital twin technologies from single process analytical technologies (PAT) measurements.

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List of Acronyms

Table 1: Acronyms

Term	Definition
AI	Artificial Intelligence
AOP	Activated Oxidation Process
APC	Advanced Process Control
API	Active Pharmaceutical Ingredient
BHC	Boots Hoechst-Celanese
BNR	Biological Nutrient Removal
BTA	Benzotriazole
CANP	2-chloro-N-2, 6-dichlorophenyl-N-phenylacetamide
CAS	Conventional Activated Sludge
CBM	Continuous BioManufacturing
CBZ	Carbamazepine
CFD	Computational Fluid Dynamics
CIP	Clean-In-Place
CMA	Critical Material Attribute
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
DCF	Diclofenac
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DoE	Design of Experiments
DT	Digital Twin
E-Factor	Environmental Factor
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
FIM	Fisher Information Matrix
GMP	Good Manufacturing Practice
GPM	Green Pharmaceutical Manufacturing
HCTZ	Hydrochlorothiazide
HF	Hydrofluoric Acid
H_2O_2	Hydrogen Peroxide
IBF	Ibuprofen
KPI	Key Performance Indicator

LCA	Life-Cycle Assessment
LCI	Life-Cycle Impact
MBC	Model-Based Control
MBR	Membrane Bioreactor
MIMO	Multiple Input, Multiple Output
MPC	Model Predictive Control
NaOH	Sodium Hydroxide
NORMAN	Network of Reference Laboratories, Research Centres and Related Organisations for Monitoring of Emerging Environmental Substances
NSAID	Non-Steroidal Anti-Inflammatory Drug
OFAT	One-Factor-at-A-Time
PAT	Process Analytical Technologies
PAV	Proven Acceptable Ranges
PID	Proportional-Integral-Derivative
PMI	Process Mass Intensity
QbD	Quality by Design
SIP	Sterilize-In-Place
SPM	Sustainable Pharmaceutical Manufacturing
STPs	Sewage Treatment Plants
STY	Space-Time-Yield
TPQP	Target Product Quality Profile
UV	Ultraviolet
WARIEN	WAter Related Impact of ENergy
WHO	World Health Organisation
WWTPs	Wastewater Treatment Plants

1 Introduction

Deliverable D2.1 is the first of three deliverables in WP2 that discusses the output of T2.1 and T2.2. WP2 establishes the specifications and requirements for future WPs encompassed within this project. Tasks T2.1 and T2.2 deal with two different aspects of the project, both integral to the further tasks and work packages.

The first task in WP2 (T2.1) deals with the specification of the requirements for a GPM process, in terms of waste minimization, resource efficiency maximization, and environmental impact reduction. For these goals, a review of state-of-the-art SPM technologies has been carried out (for the eight chosen pharmaceuticals). Focus has been applied to methods in which these goals can be accomplished- namely through process simulation to derive material balance schemes for the selected production routes, and life-cycle assessment (LCA) with a particular focus on reduction of chemicals, solvents, cleaning agents, water supply and energy consumption.

The second task in WP2 (T2.2) deals with the review of digitalization methods for environmental impact reduction. Different ways can be utilized to fulfil the goal of T2.2, such as Continuous BioManufacturing (CBM), waste effluents minimization during cleaning, and media and buffer feeds' optimization. To accomplish this task, a review of not only the state-of-the-art but also potential future digitalization methods on the above-stated three aspects has been carried out. Here, some desirable techniques include:

- predictive potentials of digital twins from single PAT measurements
- optimal digital twin-based experimental design as well as enhanced data analytics to achieve process understanding and optimize operations
- digital twin-based model predictive control of CBM for enhanced scalability and higher productivity.

This document is structured as follows:

- *Chapter 1 – Introduction*: The deliverable is introduced and a short summary is provided about the contents and work done within the scope of this deliverable.
- *Chapter 2 – Requirements of Green Manufacturing Processes*: An overview of the requirements for sustainable pharmaceutical manufacturing is provided and a few important green metrics are examined. Additionally, the state-of-the-art production of eight pharmaceutical compounds and their fate, toxicity and amount in wastewater and sewage treatment plants is researched.
- *Chapter 3 – Review of Applicable Digitalisation Methods for Environmental Impact Reduction*: An overview of the applicable digitalisation methods for energy, resource and water consumption is provided. Additionally, the application of digital twins and their predictive potential is explored. Process control and its potential to streamline a process towards sustainability is also discussed.
- *Chapter 4 – Conclusion*: This chapter concludes the deliverable, providing a summary of the literature research as well as an outlook for future work.

2 Requirements of Green Manufacturing Processes

2.1 Requirements for Sustainable Pharmaceutical Manufacturing (SPM)

To be able to fulfil the future WPs and the goals of the broader project, it is necessary to specify precisely the requirements for the sustainable manufacture of the pharmaceuticals chosen and with which metrics and techniques the manufacture can not only be analysed and tested against other processes/pharmaceuticals but also to reduce the environmental impact and effectively sense and remove them in wastewater/sewage treatment plants.

The aim of reducing the environmental impact of the active pharmaceutical ingredient (API) and the development of the API itself is imperative for the pharmaceutical industry's future. Many APIs are designed to produce a therapeutic effect in the body before they are metabolized by the human body. This property of the pharmaceuticals means that functional groups are incorporated into the chemical structure that do not biodegrade readily and thus persists even after treatment in wastewater/sewage treatment plants (Peake et al., 2016).

Another problem to be addressed is the generation of waste and by-products in the pharmaceutical industry. Drug and pharmaceutical manufacture create the most significant amount of waste and by-products compared to other chemical industry sectors. This is due to the fact that products of high purity are required from a medical and regulatory standpoint, as compared to the different sectors (Cue and Zhang, 2009).

Table 2: Chemical Industry Sector Comparison by E-factor (Cue and Zhang, 2009; Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018)

Industry Sector	Product Tonnage	kg waste/kg product (E-Factor)
Oil refining	10^6-10^8	~0,1
Bulk chemicals	10^4-10^6	<1-5
Fine chemicals	10^2-10^4	5-50
Pharmaceuticals	10-10^3	25-100

A second pressing issue that needs to be addressed is the massive utilization of solvents in pharmaceutical production. According to an LCA study performed on a typical API from GlaxoSmithKline (Jiménez-González et al., 2004, p.), solvent utilization accounts for 50-80% of energy use, total life cycle mass, greenhouse gases, and photochemical ozone creation potential. Assuming that the solvent utilization is similar for many other typically produced APIs, this aspect of pharmaceutical manufacture requires optimization, innovation, and implementation of sustainable technologies that reduce solvent usage. There has been considerable research on the type and amount of solvent necessary for pharmaceutical manufacture and the more sustainable replacements of such crucial solvents (Dunn, Wells, and Williams, 2010).

A high solvent utilization occurs due to the high demands for purity and undesired product contamination. Solvent usage is extensive during the development phase of a product, where only a few batches are manufactured per step. The amount of cleaning solvents needed to avoid contamination in the manufacturing process drives particular metrics up, as the reactors are typically rinsed with polar solvents (e.g., acetone) followed by water until total carbon (TC) levels or substance-specific cleaning values are met (Becker, Manske and Randl, 2022).

2.1.1 Green Metrics and Their Utilization in SPM

To quantify the 'greenness' or the sustainability of SPM processes (or processes in general), various metrics/assessments are necessary. It is crucial for these metrics to be simple to use and apply, clearly defined, and be an integral part of the decision-making of the project and manufacturing process.

Green metrics have been developed mainly in the last few decades. While the earlier metrics mainly dealt with waste, green metrics have evolved into measuring the efficiency and utilization of a process's most important aspects, raw materials, reagents, and outputs. The elements of green chemistry and green engineering principles that are taken into account to measure the sustainability of a process include (Jimenez-Gonzalez and Lund, 2022):

- Resource efficiency
- Environment, health, and safety
- Life-cycle assessment considerations (Jimenez-Gonzalez and Lund, 2022)

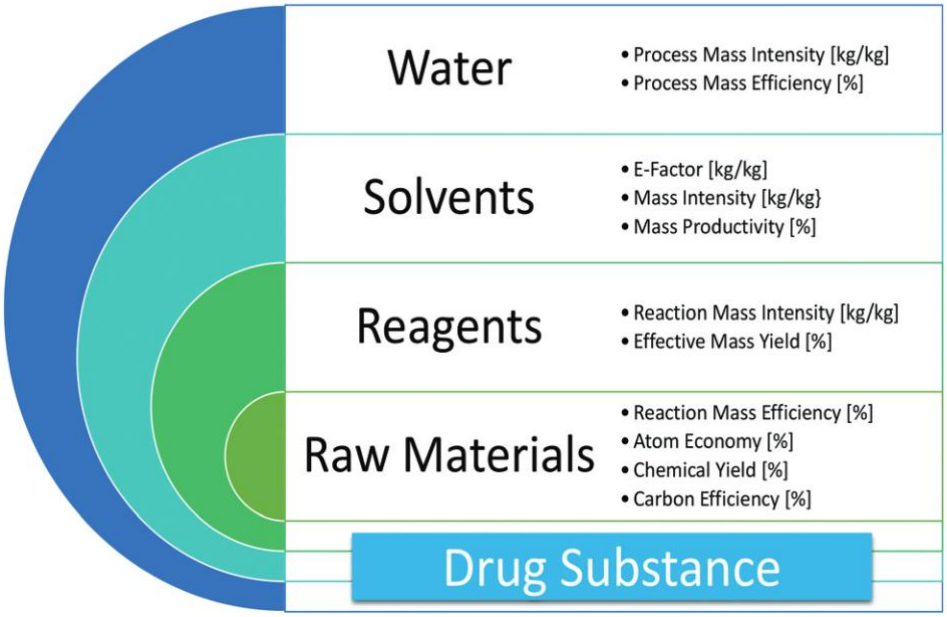


Figure 1: 'Green' mass metrics relationships for pharmaceutical production (Roschangar, A. Sheldon and H. Senanayake, 2015; GreenChemUOFT, 2017)

As shown in Figure 1, many different metrics have been developed to measure the 'greenness' of a process. Different metrics consider various aspects of a process, whether it be the amount of water, solvents, or reagents used or energy consumption, or greenhouse gas emissions. However, these are not the only metrics used to measure and compare API and pharmaceutical production. Table 6 lists the different metrics that measure the amount of each material utilized in the production of critical pharmaceuticals and provides brief definitions for the metrics listed in Figure 1. This deliverable provides a brief overview of the most relevant metrics utilized in the pharmaceutical sector over the subsequent few subunits.

The two metrics widely utilized in the pharmaceutical and biochemical sector are the process mass intensity (PMI) and the environmental factor (E-Factor) (Rose et al., 2022). It is proposed, however, that for the future WPs, other sustainable metrics be considered not only in the development of greener-by-design compounds but also in the development steps of digitalization, CFD, and process simulation for CBM and optimization to SPM technologies.

2.1.1.1 Process Mass Intensity (PMI)

As described in Table 6, PMI is a mass-based metric defined as the ratio of the total input mass to a unit product mass. In other words, PMI describes the total mass of materials used to produce a unit mass of a product. All materials required for the product manufacture are included- reactants, reagents, solvents, catalysts, and water. Ideally, when no waste is produced and all materials are utilized thoroughly, the PMI equals 1. The PMI was selected by the American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable (ACS GCI PR) as the critical green metric to benchmark the sustainability of fine chemical and pharmaceutical industries (Jimenez-Gonzalez et al., 2011; Roschangar, A. Sheldon and H. Senanayake, 2015; Jimenez-Gonzalez and Lund, 2022). This was chosen due to several different reasons:

- To place a more prominent focus not only on waste minimization but also on the maximization of efficiency, value, and further innovation toward sustainable manufacture
- The PMI metric is one of the few mass-based metrics that closely resembles the more time-consuming but informative and conclusive life-cycle assessment (LCA) approach (Jimenez-Gonzalez et al., 2011; Jimenez-Gonzalez and Lund, 2022)
- A better indicator of the general sustainability of a process as compared to other mass-based metrics; however, it must be stated that the PMI is not a perfect metric, and that a more holistic approach and a clearer and larger picture of the impact of a product and its manufacture on the environment, health, and safety can only be achieved with an LCA

Lastly, it is of interest to this project and WP to identify the critical materials utilized in producing the chosen pharmaceutical compounds, where the innovation towards SPM can be implemented, and where it is most necessary. The PMI metric is critical for this aspect. The emphasis on the more commonly available inputs and their utilized masses indicates where more efficient and greener practices in the supply chain can be applied. Figure 2 depicts the results of the PMI benchmarking carried out by the ACS GCI PR in 2008. It is evident that solvent utilization is the highest contributor to the PMI and, thus, to the environmental life cycle impacts associated with API production (Jimenez-Gonzalez et al., 2011).

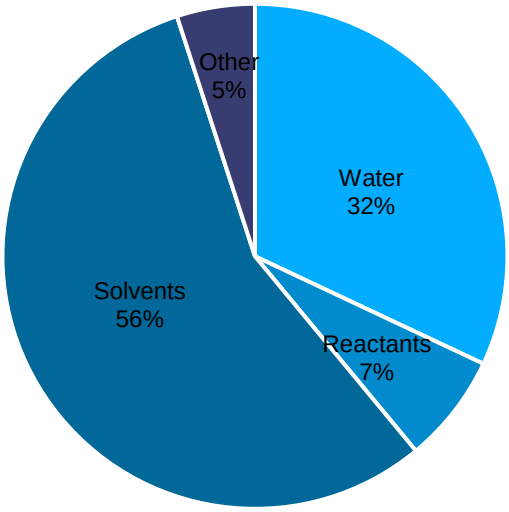


Figure 2: Composition by mass of materials required for API production, adapted from (Jimenez-Gonzalez et al., 2011)

2.1.1.2 Environmental Factor (E-Factor)

The E-factor is one of the oldest green mass-based metrics utilized to measure the sustainability of a product and its manufacture. The E-factor is defined as the mass of waste produced in the process per unit mass of the product. It is evident from the definition that the higher the E-factor, the higher the amount of waste produced along the product's manufacture. Ideally, the E-factor should be zero, and lower E-factors correspond to lower manufacturing costs of the products. The lesser input of process materials directly represents this phenomenon, as do the reduced hazardous and toxic waste disposal costs, increased capacity usage, and reduced energy demand and consumption of processes with lower E-factors, compared to alternatives with high E-factors (Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022).

As can be seen in Table 6, the E-factor can be standardized to provide two different E-factors for different purposes and times of the process and development. The simple E-factor (sEF) does not take solvents and water into account, whereas the complete E-factor (cEF) accounts for all process materials (including solvents and water). However, it does not take any recycling into account and lends itself more to the analysis of total waste stream analysis. On the other hand, the sEF is more appropriate for early process route development and determination. The real and commercial E-factor lies between the sEF and cEF and can only be determined when data for solvent losses are available (Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018).

Table 2 shows the high E-factors typical of the pharmaceutical industry, partly due to the abovementioned reasons. However, another reason for high E-factors is the high molecular complexity and the large number of chemical steps necessary for their manufacture. Other metrics, such as the Green Aspiration Level (GAL), have been developed to account for this unique property. This concept considers the complexity of such processes to enable the comparison of the E-factor of a particular process to the industry norm (Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018).

It also should be noted that mass metrics like the PMI and the E-factor have their downfalls and should be used with the correct context and background. Initially, both mass metrics excluded water as the resulting substantial values would make difficult comparisons. They both have evolved to include water as water scarcity, and the increasing importance of water in biological systems, have become a critical issue that needs to be addressed. PMI or the E-factor are both easy to calculate and simple to use. However, both do not consider the process route's complexity. On the contrary, other mass-based metrics like the reaction mass efficiency (Table 6) include atom economy, yield, and stoichiometry of the process and are also easy to calculate and use but exclude the utilization of solvents and other materials (Jimenez-Gonzalez and Lund, 2022).

2.1.2 Life-Cycle Assessment (LCA)

The literature research for this deliverable shows that a practical gap exists between green engineering and chemistry metrics and LCA in the pharmaceutical field. A literature review of the LCAs performed in the pharmaceutical sector, to assess the sustainability and management of pharmaceutical production, discovered that only ca. 30 LCAs of pharmaceutical products, processes, or pre-cursors were performed in or after 2000. Additionally, the LCAs performed do not have standardized system boundaries, are carried out on many incomparable APIs and reagents, and/or are conducted with a focus on specific life stages, instead of the full life cycle of pharmaceuticals, such as, on the packaging necessary for the pharmaceutical product (Emara et al., 2018).

Figure 3 provides an overview of the LCA process, the system boundary, and the inputs and outputs of a production/process. The LCA approach has been standardized and consists of four main steps. Such an approach is usually a comprehensive 'cradle-to-grave' approach which involves:

- Definition of goal and scope of the assessment
- Compilation of a life-cycle inventory (LCI)
- Assessment of the environmental impact from the data compiled from the LCI
- Interpretation of the overall results of the assessment from data obtained from the LCI and environmental impact assessment (Peake et al., 2016)

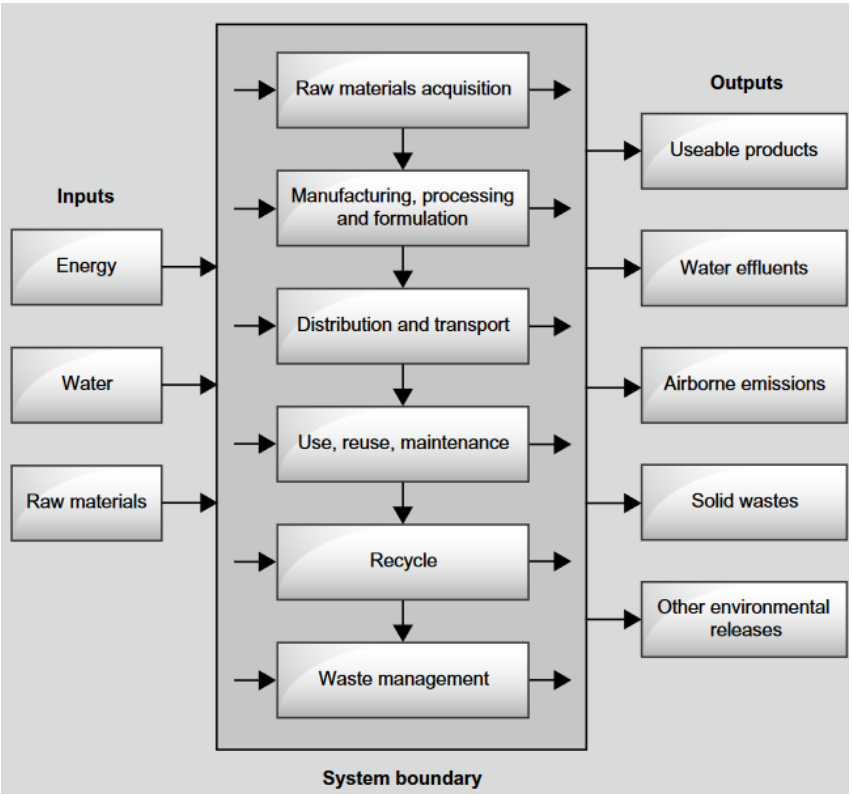


Figure 3: An LCA overview (Peake et al., 2016)

Specific aspects must be addressed for an LCA to be performed appropriately and for a thorough environmental impact assessment. The approach to the LCA needs to be specified based on the relevant data and databases available to the project partners. The choice of the type of LCA needs to be addressed- 'cradle-to-grave,' 'cradle-to-gate,' or a 'gate-to-gate' LCA. The product and the scope of the production process need to be addressed, as well as the system boundaries of the process. The material inputs, outputs, and flows are to be quantified, and their environmental impact to be assessed.

The type of LCA, the LCI as well as the environmental impact assessments tie into the green sustainability metrics, as the mass and energy balances of the chosen product/process have to be quantified and simulated so that the ultimate goal of this project can be reached- to optimize current state-of-the-art pharmaceutical technologies and products with the help of digitalization, process simulation, and LCA into sustainable pharmaceutical manufacturing (SPM).

2.2 Currently Applied State-of-the-Art SPM Technologies

One of the tasks reported on in D2.1 is the review of currently utilized state-of-the-art technologies for the sustainable manufacture of the four chosen materials. A further aim of D2.1 was to highlight further techniques to characterize and analyse the sustainable, waste-minimizing, and resource-maximizing manufacture of the four chosen pharmaceuticals.

To develop well-built and functioning monitoring sensors in wastewater and waste streams, knowledge about the compound to be measured, their amount present, and the possible further metabolisms/reactions that the compound undergoes is crucial. It is also essential to know how these pharmaceutical compounds are manufactured so that waste and wastewater streams can be quantified. This knowledge also serves as the diving board for possible further optimization toward sustainable pharmaceutical manufacture (SPM). Understanding the fate and amount of the specific APIs and the processes behind the pharmaceutical compounds and their metabolites towards their 'grave' (end-of-life in an LCA, for example, Figure 4) is also helpful for a more efficient digitalization, process simulation, and LCA of the process routes.

Comprehensively understanding the fate and amount of the compounds and their metabolites in wastewater/sewage play a prominent role in creating sensitive and appropriate sensors for monitoring waste and wastewater effluents, a significant aim of the ENVIROMED project as well.

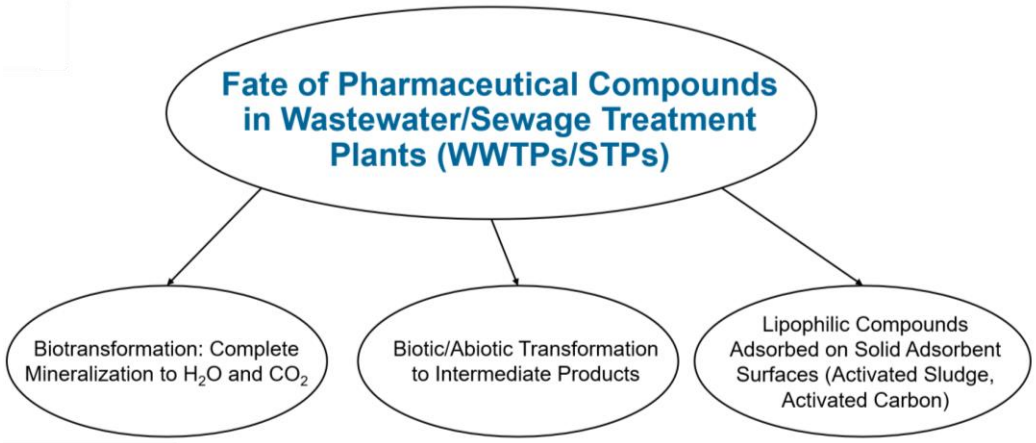


Figure 4: Fate of pharmaceutical compounds in wastewater/sewage treatment plants (WWTPs/STPs), adapted from (Ram et al., 2020)

Many SPM technologies have been developed over the years, keeping in line with the development of many green metrics and more widespread use of LCA methodology. A large number of such technologies have seen a reduction in PMI and E-factor with the simultaneous utilization of water and the minimization of solvent usage. Additionally, the utilization of innovative technologies such as process intensification and continuous manufacturing has also played a significant role in reducing PMI values (Becker, Manske and Randl, 2022).

The key to the development and widespread implementation of continuous pharmaceutical manufacturing is the extensive comprehension of the characteristic reactions that occur in the process. This can be achieved through kinetic data acquisition- a combination of inline process analytical technologies (PAT) and modelling technologies (Becker, Manske and Randl, 2022), which is a large part of the review in WP2 (discussed in T2.2) and the tasks in WP3. Continuous manufacturing and processing have many benefits to it. Unlike batch processing (a typical manufacturing technique in the pharmaceutical industry), it is currently somewhat limited by

the chemistry and physics of the relevant reactions, as processes with higher complexities require more adaptation and planning (Jiménez-González et al., 2011). Plenty of motivations exist to transition from batch processing to continuous processing (Figure 5):

- Economics: lower production costs due to reduced inventory, footprint, waste, emissions, and energy consumption
- Quality: continuous steady-state operation can lead to improved product quality and consistency; batch-to-batch variability can be overcome due to PAT integration and real-time release of the product in continuous processing
- Safety: smaller reactor volumes and holdup volumes of potentially hazardous reagents or solvents leads to enhanced process safety
- Environment: potential for solvent reduction leading to reduced PMI; potential for simplification of operations, compared to batch processes being inherently wasteful with frequent nonvalue-added operations; solvent usage and emissions' reduction due to the lower frequency of cleaning compared to cleaning in batch operation (Jiménez-González et al., 2011; Baumann et al., 2020; Kavara et al., 2020)

Figure 5 compares the batch and continuous processing of 4-D-Erythronolactone at the lab scale and the pilot plant level. The PMI for a continuous process is higher than the batch process due to the higher number of reagents and the higher dilution factor of the reagents. However, continuous processing proves to be the most sustainable approach since the cumulative MI is smaller than that of the batch processing (Lee, Khoo and Tan, 2016). While 4-D-Erythronolactone is not the focus of this project, the comparison between batch and continuous processing can be extrapolated for the larger pharmaceutical sector and the compounds in question in this project.

Green-by-design chemicals, solvents, and materials feed into the life-cycle environmental approach, ultimately leading to a greener pharmaceutical product production line. While this aspect has its tenets firmly in the foundations of green chemistry (Rogers and Jensen, 2019; Mishra et al., 2021; Martínez, Cortés and Miranda, 2022), it aims at more sustainable upstream processing of a pharmaceutical product. However, this aspect must work and go hand-in-hand with continuous processing (applied to both upstream and downstream processing (Kavara et al., 2020)) to lead to a truly sustainable pharmaceutical manufacturing process (Table 3). An example of such a continuous operation with a high degree of flexibility and automation/digitalization has been proposed by Pfizer and Boehringer Ingelheim (Vogel, 2022). Additionally, plenty of industrially implemented continuous processing examples/applications have been documented, which would be a great jumping point for the ENVIROMED project (Kavara et al., 2020).

Another aspect that would benefit from CBM and integration of PAT, digital twin models, and real-time sensing, is the crucial cleaning step. Strict regulations and highly rigorous conditions are set in place to clean the equipment prior to manufacturing to prevent undesired contamination of products. This critical step is also a leading factor in the widespread batch processing in the pharmaceutical industry (i.e., to allow equipment cleaning between consecutive batches).

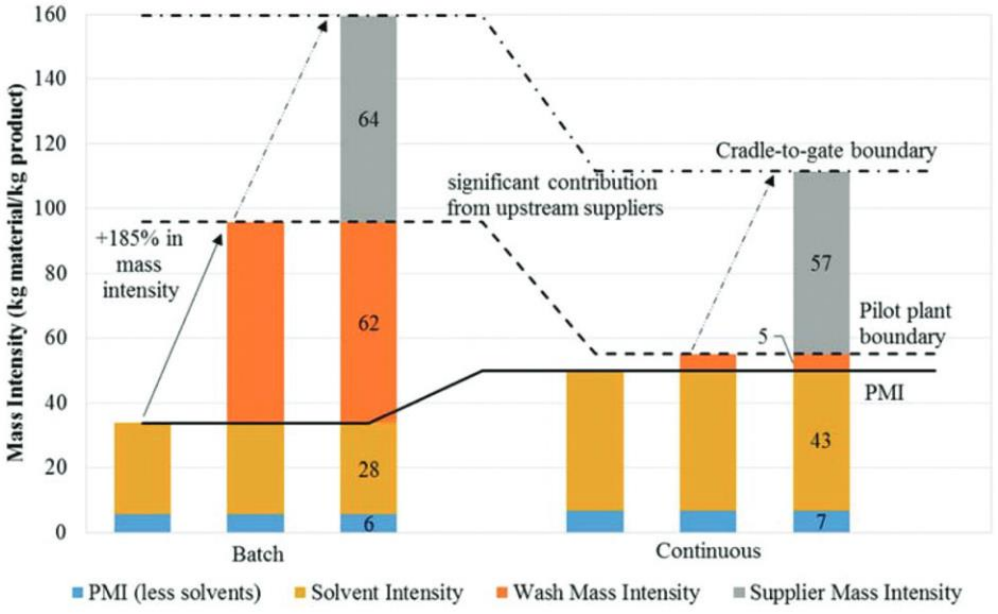


Figure 5: Use of Green Chemistry metrics to analyse batch and continuous processing systems for 4-D-Erythronolactone; MIs presented are calculated using base case values. The bolded line depicts PMI, the dotted line the MI at pilot plant level and the dash-dotted line refers to the cradle-to-gate (CtG) boundary and depicts the cumulative MI (Lee, Khoo and Tan, 2016).

Apart from continuous processing, PAT and real-time, online and inline sensing integration, and solvent use reduction, plenty of aspects of techniques and technologies can be utilized to result in SPM. Rose et al. (2022) discusses the green-by-design reagents and solvents used in the production process. This is another aspect of the ENVIROMED project as well.

Table 3: Environmental and economic considerations and their relation to CBM, adapted from (Rogers and Jensen, 2019; Mishra et al., 2021)

	Thinking environmental	Thinking continuous	Thinking economic
Atom economy	Minimal by-product formation Reduced environmental burden	More extensive toolbox of reactions due to increased safety and process intensification	More from less, incorporate the total value of materials Reduced cost
Solvent reduction	Less solvent required, less solvent waste Reduced environmental burden	Reduced solvent volumes through the elimination of large reactors	Reduced capacity requirements, less energy required Reduced cost
Reagent optimization	Catalytic, low stoichiometry, recyclable Reduced environmental burden	Increased process understanding and performance	Higher efficiency, higher selectivity Reduced cost

Convergence	Reduced environmental burden Related to improved process efficiency	Fewer potential intermediate and/or product isolations	Higher efficiency, fewer operations Reduced cost
Energy reduction	Reduced environmental burden Related to power generation, transport, and use	Smaller energy requirements to run continuous platforms	Higher efficiency, shorter processes, milder conditions Reduced cost
***In-situ* analysis**	Reduced potential for exposure or release to the environment	Significant utilization of PAT for CBM to ensure product quality and reduce burden for final product testing	Real-time data increases throughput and efficiency, with fewer reworks Higher efficiency, fewer operations Reduced cost
Safety	Non-hazardous materials and processes Reduced risk of exposure, release, explosions, and fires	Small volumes of hazardous materials being processed at any given time, increased control over process parameters	Worker safety and reduced downtime Reduced special control measures Reduced cost

Lastly, the eight selected pharmaceutical compounds will be briefly discussed. State-of-the-art manufacture and current innovations from a literature review of SPM for each compound will be examined. Finally, a closer look is given to their metabolites and transformation products, their fate in WWTPs/STPs, and their environmental effect.

2.2.1 Diclofenac (DCF) Production and Fate in Wastewater/Sewage Treatment Plants

DCF is widely utilized for the treatment and management of acute and chronic pain associated with inflammatory conditions, e.g., different forms of arthritis and spondylitis, cataract condition, eye pain, etc. DCF is a non-steroidal anti-inflammatory drug (NSAID), as seen in

Table 4, belonging to the family of phenylacetic acids (Alfaro and Davis, 2022). Diclofenac is a highly administered drug with an estimated annual consumption of over 100 tons globally, coupled with a constant release through body excretions into the environment (Yu et al., 2013).

The medication DCF is usually sold as diclofenac sodium and is currently produced in a one-pot batch process. Different mechanisms and synthetic routes have been developed since DCF was first synthesized in 1965. Currently, the synthesis utilizing 2-chloro-N-(2,6-dichlorophenyl)-N-phenyl acetamide (CANP) as an advanced intermediate via the Smiles rearrangement/amide hydrolysis remains the most attractive and reliable synthesis so far developed. Performing this synthesis in a one-pot batch process carries some unrealized potential for optimisation and a more sustainable operation. The amide hydrolysis of the phenoxy acetamide during the Smiles rearrangement is undesired and inevitable. Additionally, the use of the toxic chloroacetyl chloride in this synthesis as a reagent also leads to undesired side products that possibly hinder the production of CANP (Wang et al., 2022).

Thus, operating the process in a continuous mode might be able to make the process more sustainable. Utilizing chloroacetic acid and phenylamine, the synthesis can be carried out in a two-step flow synthesis with two continuous flow reactors. The synthesis can be broken down into six elementary steps with esterification/Smiles rearrangement to produce diclofenac sodium. This is just one example of continuous manufacturing proposed to improve the yield, efficiency, and sustainability of the entire process, as seen in Figure 6. This continuous flow process results in a total yield of 63% and 99% purity and a total residence time of less than 3,5 h, which is admittedly less than the batch process (Wang et al., 2022). A literature review for D2.1 only yielded one publication that highlighted a proposal of continuous flow for DCF sodium production. The lack of such research only serves to highlight the need for research, development, and innovation in turning the traditional batch process into more efficient and less wasteful continuous flow operations.

Like most other pharmaceutical compounds, diclofenac is biologically active and thus should, in theory, be readily biodegradable. However, like most NSAIDs, DCF is poorly biodegradable and has low elimination rates during biological wastewater treatment. DCF is biologically persistent and is toxic to the environment, habitats, flora and fauna and many different animal species. Due to its wide availability and administration, the growing persistence and occurrence in the environment has raised concern over the last decade. Based on multiple studies (Zhang, Geißen and Gal, 2008; Brozinski et al., 2013; Yu et al., 2013; Vieno and Sillanpää, 2014; Dasenaki and Thomaidis, 2015; Bottoni and Caroli, 2018) carried out on the occurrence and removal efficiency of DCF in WWTPs effluents and surface water, DCF was detected in the µg/L range. Even in such amounts, DCF and its metabolites have caused adverse environmental effects such as, most notably, the decline of the vulture population in Pakistan. DCF is known to harmfully affect several aquatic and avian organisms as well as bacterial communities, even in small quantities. DCF was classified to the EU watch list of pharmaceuticals in the Water Framework Directive (2000/60/EC) in 2015 but was later removed in 2018 (Vieno and Sillanpää, 2014). However, DCF, along with many other analytes in this deliverable are proposed to be part of a revised EU Urban Wastewater Treatment Directive (2022).

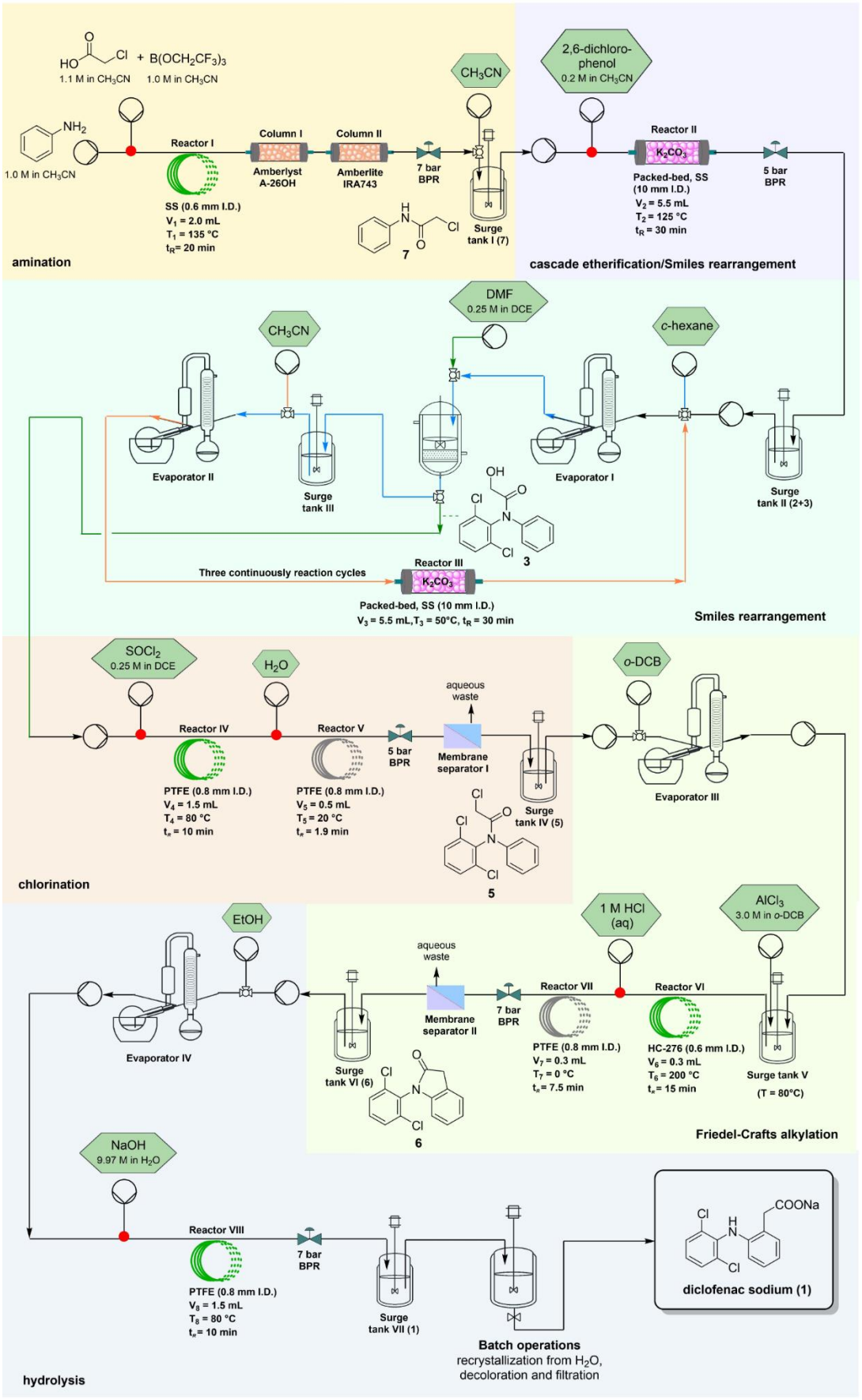


Figure 6: Six-step continuous flow synthesis of DCF sodium (Wang et al., 2022)

Multiple reviews and studies show that removing DCF in WWTPs/STPs is inefficient. Additionally, the removal efficiency of DCF is inconsistent among many different publications and reports (Zhang, Geißen and Gal, 2008; Yu et al., 2013; Vieno and Sillanpää, 2014; Dasenaki and Thomaidis, 2015; Bottoni and Caroli, 2018). Modern treatments for DCF in WWTPs that have shown some form of success at removing DCF and its metabolites are sludge and sewage treatments like conventional activated sludge (CAS) and biological nutrient removal (BNR). However, all common treatments and processes are unable to eliminate DCF and its' metabolites completely. Some optimization ideas have been reviewed in literature to effectively and efficiently remove DCF from the wastewater influents (Zhang, Geißen and Gal, 2008; Vieno and Sillanpää, 2014):

- Longer hydraulic retention times
- Membrane bioreactor processes
- Bioaugmentation: addition of cultured microorganisms to degrade DCF
- Other pre- or post-treatments, e.g., chemical oxidation, photo-oxidation, etc.

Due to its poor biodegradability, low removal efficiencies in WWTPS/STPs, the molecule's size (for imprinting experiments and sensor development), and its toxic effects in the environment, DCF was chosen as the target analyte for the ENVIROMED project.

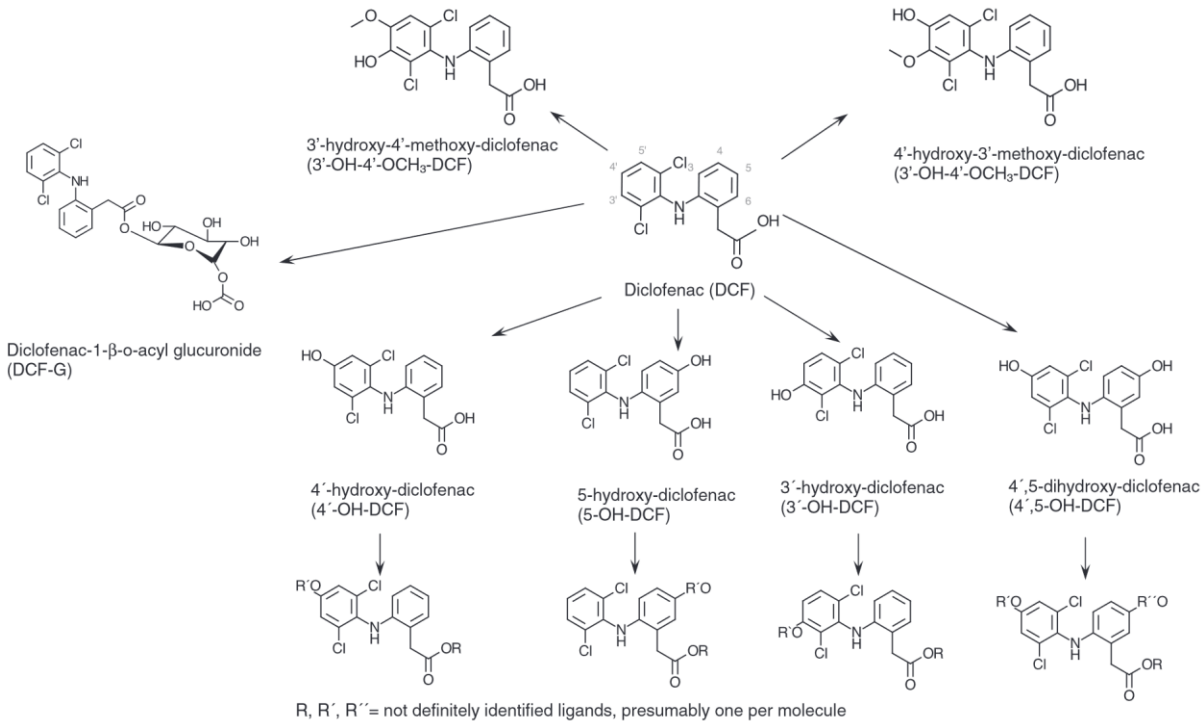
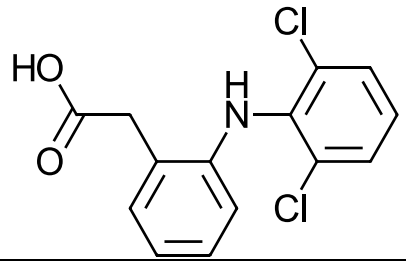
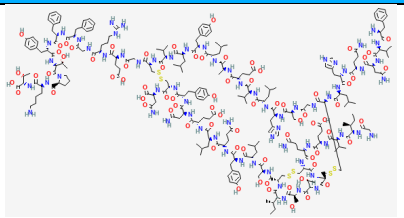
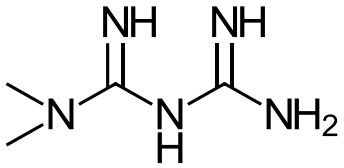
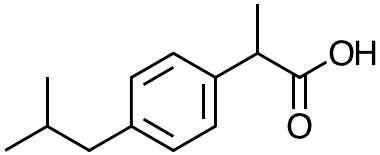


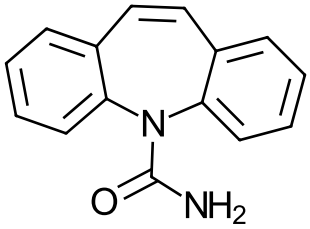
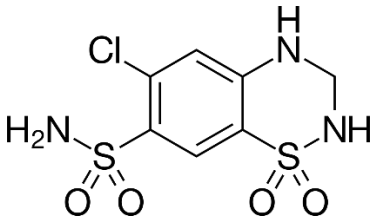
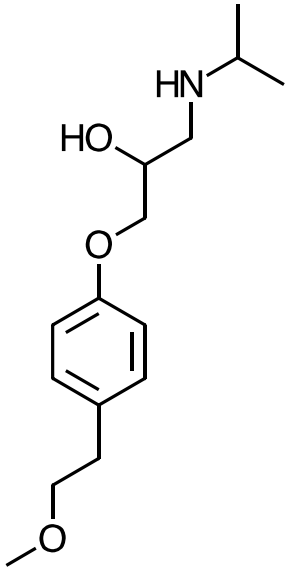
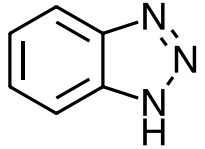
Figure 7: Common metabolic pathways of diclofenac in the human body with many metabolites being present in body excretions that ultimately end up in WWTPs influents (Vieno and Sillanpää, 2014)

Table 4: An overview of the pharmaceutical compounds in this deliverable I (Lougheed et al., 1981; Rabkin, Ryan and Duckworth, 1984; Dunn and Peters, 1995; Zhang, Geißen and Gal, 2008; Vieno and Sillanpää, 2014; Briones, Sarmah and Padhye, 2016; Alfaro and Davis, 2022; Balakrishnan et al., 2022; PubChem, 2022a; 2022b; Kennedy, n.d.)

	Diclofenac (DCF)	Insulin	Metformin	Ibuprofen (IBF)
Structure				
Molecular weight [g/mol]	296,16	5805	129,167	206,28
Formula	$C_{14}H_{10}Cl_2NO_2$	$C_{257}H_{383}N_{65}O_{77}S_6$	$C_4H_{11}N_5$	$C_{13}H_{18}O_2$
Usage	Analgesic, anti-inflammatory	Antihyperglycemic	Antidiabetic	Analgesic, anti-inflammatory
Water solubility [mg/L] at 25 °C	23,73	Poor solubility	$3*10^5$	21
Elimination half-life [hr]	2	2,3 - 4,3	4 - 8,7	1,8 - 2,2
Excretion	65% of oral dosage excreted in urine	Small amounts (a definite number not found)	70% excreted unchanged in urine	15% of oral dosage excreted unchanged

Metabolites	5-OH-DCF 4'-OH-DCF 3'-OH-DCF 4'-5-diOH-DCF 4'-OH-5-Cl-DCF 3'-OH-4'-CH_3O-DCF	Oligopeptides Amino acids	Guanylurea	(β)-2-40-(2-hydroxy-2-methylpropyl)-phenylpropionic acid (β)-2-20-(2-carboxypropyl)-phenylpropionic acid Conjugated ibuprofen Carboxyibuprofen Hydroxyibuprofen Carboxyhydratopic acid
Dosage	75 - 150 mg daily	Depending on the type of diabetes, based on a strict formula	Up to 2000 mg daily	200 - 1200 mg daily

Table 5: An overview of the pharmaceutical compounds in this deliverable II (Regårdh and Johnsson, 1980, p.1; Vardanyan and Hruby, 2006a; 2006b; Zhang, Geißen and Gal, 2008; Suma, Natesh and Madhavan, 2011; Tolou-Ghamari et al., 2013; International Agency for Research on Cancer, 2015; Kasonga et al., 2021; Morris and Dunham, 2023; PubChem, 2023d; 2023c; 2023a; 2023b)

	Carbamazepine (CBZ)	Hydrochlorothiazide (HCTZ)	Metoprolol	Benzotriazole (BTA)
Structure				
Molecular weight [g/mol]	236,27	297,74	267,36	119,12
Formula	$C_{15}H_{12}N_2O$	$C_7H_8ClN_3O_4S_2$	$C_{15}H_{25}NO_3$	$C_6H_5N_3$
Usage	Antiepileptic, anticonvulsant	Diuretic and antihypertension agent	β-adrenoblocker used to treat angina and severe myocardial infarction	Drug precursor; antimicrobial and antiprotozoal
Water solubility [mg/L] at 25 °C	17,7	722	402	$1{,}98*10^4$

Elimination half-life [hr]	25 – 65	5,6 - 15	3 - 4	-
Excretion	72% of oral dose excreted in urine, 28% in faeces	Mostly excreted unchanged in urine	5% excreted as unchanged drug	-
Metabolites	CBZ-epoxide CBZ-diol CBZ-acridan 2-OH-CBZ 3-OH-CBZ Iminostilbene 10, 11-dihydro-10-hydroxy CBZ CBZ-2, 3-quinone Iminoquinone	2-amino-4-chloro-1, 3-benzenedisulfonamide Chlorothiazide	H104/83 H117/04 O-demethyl-metoprolol α-hydroxymetoprolol	-
Dosage	Usually 800 – 1200 mg daily	12,5 – 25 mg daily	50 – 200 mg daily	-

2.2.2 Insulin Production and Fate in Wastewater/Sewage Treatment Plants

As seen in Table 4, insulin is a large molecule in the human body. Human insulin is a peptide hormone produced by beta cells in the pancreas. It regulates the metabolism of carbohydrates and fats by promoting glucose absorption from the blood into various cells in the body (Weiss, Steiner and Philipson, 2000; Nandy and Srivastava, 2018; PubChem, 2022a). Insulin discovery and production has a long history and belongs to the WHO Model List of Essential Medicines (World Health Organisation, 2022).

The medication insulin (sold under many different brand names) is produced using recombinant DNA technology- where the human insulin gene is inserted into *Escherichia coli* bacteria or *Saccharomyces cerevisiae*. These organisms are then placed in a bioreactor, where they then produce insulin. Insulin is harvested and purified in downstream processes and finally made available for use (Weiss, Steiner and Philipson, 2000; PubChem, 2022a). This process is, again, a typical batch process. While the review shows that some research and effort have gone into changing the batch process into a continuous one, it is currently in the development phase in lab-scale production.

Considering the composition of the Consortium in this research project, insulin would be of particular interest to investigate, as it is produced in large quantities by one of the involved partners, and it is therefore readily available to investigate in terms of manufacturing process know-how, as well as in terms of molecule availability for experimental testing. Furthermore, optimisations in the manufacturing process derived from the results of ENVIROMED could be directly incorporated in the production facilities of the involved partners, thus resulting in fast and effective up-take of the project's results in relevant global production scales.

Although the insulin molecule itself is quite large (making experimenting and sensor development more challenging than for smaller molecules), there has been evidence (Kasonga et al., 2021) that insulin and other metabolites can disrupt endocrinal systems in aquatic species and human beings if not adequately removed from the wastewater influents. Unfortunately, more information could not be found despite research into insulin manufacture and its ecotoxicity effects. This strongly supports the argument for setting insulin and other materials required for its production under closer observation.

2.2.3 Metformin Production and Fate in Wastewater/Sewage Treatment Plants

Another suggested target analyte (as little information was found on insulin regarding the relevant aspects for this deliverable) was the pharmaceutical compound metformin. Metformin belongs to the biguanide class of antidiabetics and is a member of the model essential medicines for hypoglycemia list created by the World Health Organization (WHO) (2022). This is because metformin is widely utilized for various treatments, from cancer suppression, polycystic ovarian syndrome, and weight loss to COVID-19 treatments. It is typically orally administered with a maximum daily dose of 2 g, and the majority is not metabolized in the body (Balakrishnan et al., 2022). It is excreted chiefly unaltered and undergoes partial biological degradation into guanylurea (Figure 8) in WWTPs/STPs (Scheurer et al., 2012; Jacob et al., 2019; Balakrishnan et al., 2022).

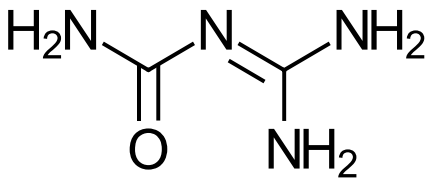


Figure 8: Guanylurea: most common metabolite of metformin, adapted from (Scheurer et al., 2012)

WWTPs have employed several treatments/processes to remove metformin and guanylurea, as shown in Figure 9. Metformin and guanylurea removal showed low removal efficiencies using coagulation or flocculation methods, whereas chlorination and advanced oxidation processes (AOPs) exhibited higher degrees of removal. Other treatment methods, like bioremediation, have become more common in WWTPs/STPs and have shown great promise in metformin and guanylurea removal (Balakrishnan et al., 2022).

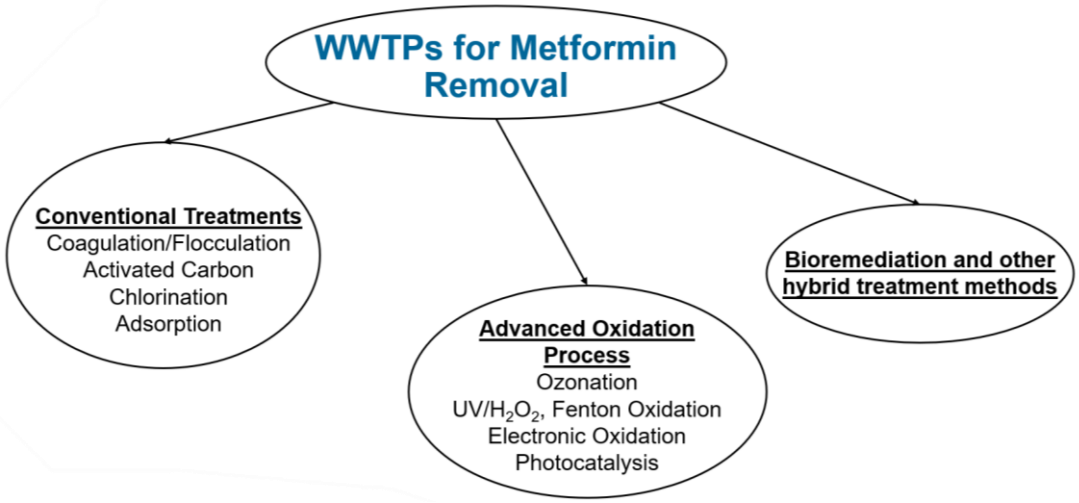


Figure 9: Treatment for metformin removal in wastewater, adapted from (Scheurer et al., 2012; Balakrishnan et al., 2022)

While the long-term effects of metformin and guanylurea on the environment and living organisms are not completely clear, it is evident that more significant concentrations over more extended periods of time may disrupt endocrinal systems in aquatic organisms (Scheurer et al., 2012; Jacob et al., 2019). Metformin and its metabolite guanylurea can be found in large amounts in wastewater influents. While they are somewhat efficiently removed, large quantities are still present in wastewater effluents, and observation, optimization, and experimentation for this analyte are still warranted.

2.2.4 Ibuprofen (IBF) Production and Fate in Wastewater/Sewage Treatment Plants

Ibuprofen is the third most popular, highly prescribed, over-the-counter medication globally and is excreted from the body into the environment. Ibuprofen was listed among the potential target analytes for ENVIROMED due to its nature- being part of the NSAIDs and belonging to a group of medications that has the potential to be easily overdosed due to its ready availability. For this reason, IBF is an attractive choice to sense and monitor in WWTPs/STPs.

The manufacturing process for IBF has undergone significant progress toward more efficient and sustainable production. IBF was discovered by the Boots Pure Drug Company, which was then developed through six steps with stoichiometric amounts of reagents, low atom efficiency, and significant amounts of inorganic salt formation. This process was utilized for large-scale

production of IBF until a new alternative (developed by the Boots Hoechst-Celanese BHC company) was proposed (Figure 10). This alternative entailed merely three catalytic steps (Sheldon, 2010):

- The first step involves the dual use of anhydrous HF as catalyst and solvent in a Friedel-Crafts acylation- HF recovered and recycled with >99,9% efficiency
- Hydrogenation- first catalytic step- 100% atom efficient
- Carbonylation- second catalytic step- also 100% efficient

IBF manufacture in this way requires no other solvent, simplifying product recovery and minimizing emissions. This process was commercialized in 1992 and is an excellent example of the prevalent problem of large volumes of waste associated with traditional stoichiometric use in the pharmaceutical industry (Sheldon, 2010).

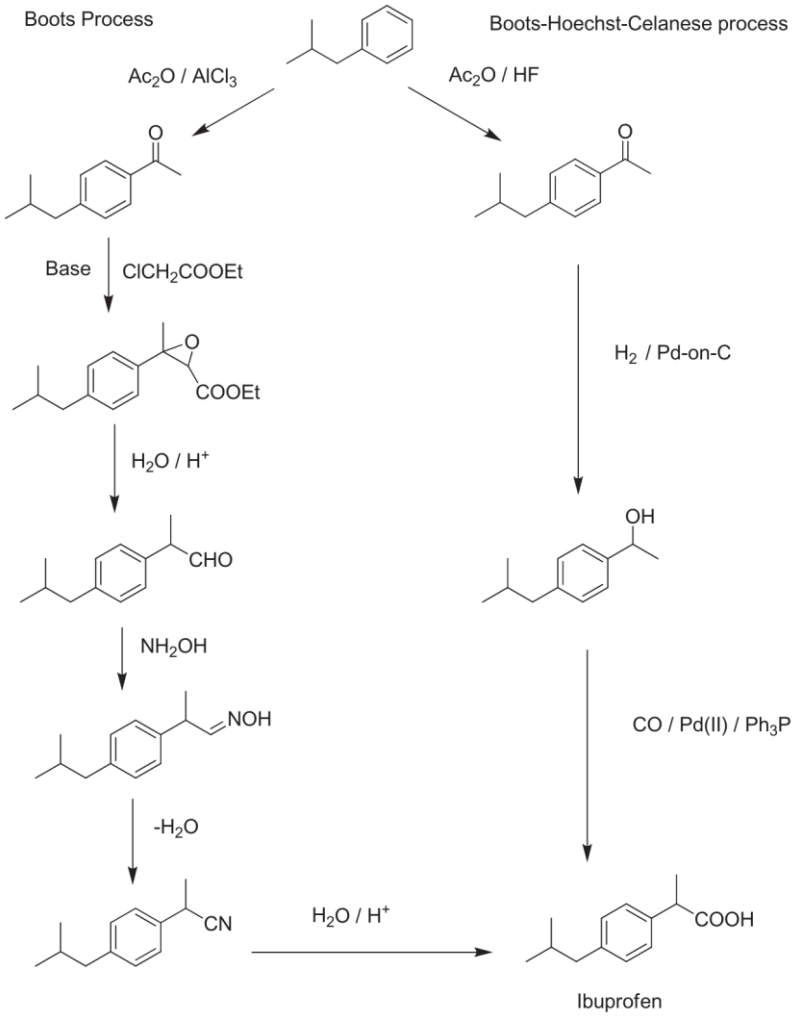


Figure 10: Two processes for ibuprofen (Sheldon, 2010)

According to studies (Brozinski et al., 2013; Chopra and Kumar, 2020), IBF has been found in the WWTPs effluents and later in the plasma of fish exposed to treated wastewater. It has been shown that IBF negatively affects aquatic organisms and ecosystems. Unlike DCF, however, IBF has been shown to be more effectively removed. Removal efficiencies range from 60-99%, and IBF concentrations in wastewater effluents seem to be lower than DCF, and thus IBF proves to be less of an environmental issue than DCF (Figure 11). However, IBF has toxic

metabolites, which are further hydrolysed in the environment and WWTPs. Therefore, it is worth taking a closer look at within the scope of this project.

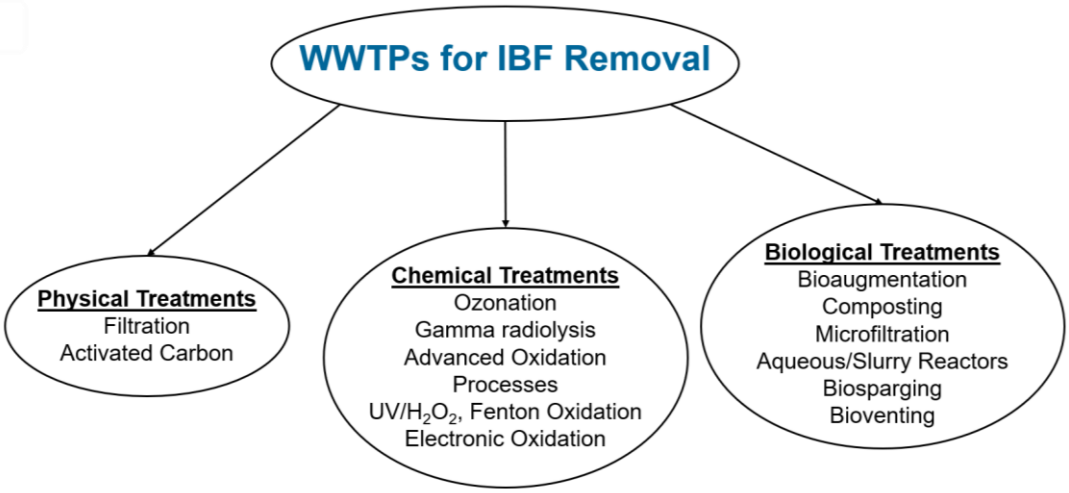


Figure 11: Treatment for IBF removal in wastewater (Chopra and Kumar, 2020)

2.2.5 Carbamazepine (CBZ) Production and Fate in Wastewater/Sewage Treatment Plants

Carbamazepine (CBZ) is, like diclofenac, a widely prescribed and used pharmaceutical drug, with an estimated annual consumption totalling 1014 tons and a continuously rising trend (Zhang, Geißen and Gal, 2008). CBZ serves as a critical antiepileptic drug and is prescribed as an anticonvulsant and a mood-stabilizing agent in treatment. CBZ is processed in the liver and upon its transformation there, it blocks voltage-dependent sodium channels, thus positively acting on the central nervous system (Yan et al., 2021).

CBZ is undoubtedly an important pharmaceutical compound, with widespread usage, and a significant potential to improve the quality of life. However, exposure of aquatic species to CBZ has been proven to be of concern. Yan et al. (2021) studied the effects of different concentrations of CBZ (concentrations which are environmentally relevant and typically found as micropollutants) on Chinese minnows (*Gobiocypris rarus*) and discovered that continuous CBZ exposure led to increased DNA damage in the liver. CBZ fundamentally led to further mitochondrial apoptosis. Additionally, APIs such as CBZ and hydrochlorothiazide tend to be subject to bioaccumulation in many marine species. The negative effects on the flora and fauna and on the environment of many such APIs needs to be subject to further testing and investigation, as much of the research carried out has been on a lab-scale level (Biel-Maeso et al., 2018).

CBZ, 5H-dibenz[b, f]azepine-5-carboxamide, was first discovered by chemist Walter Schindler in Switzerland in 1953 and has been used to treat epilepsy since the 1960s (Tolou-Ghamari et al., 2013). The traditional synthesis of CBZ occurs by the reaction of 5H-dibenz[b, f]azepine and phosgene, forming the intermediate product 5-chlorcarboxy-5H-dibenz-[b, f]azepine. This subsequently reacts with ammonia to produce CBZ. Alternatively, another synthesis method is utilised with reacting 5H-dibenz[b, f]azepine with potassium cyanate (Vardanyan and Hruby, 2006a).

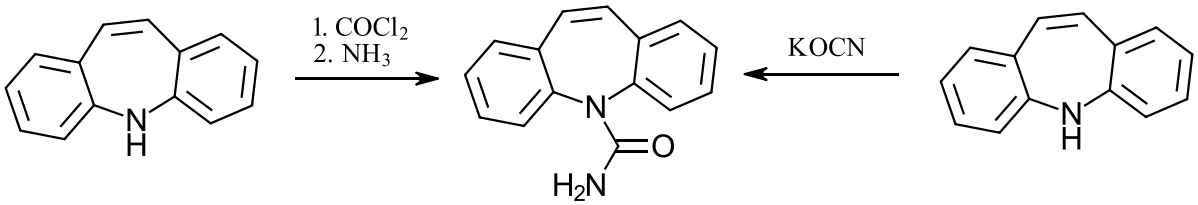


Figure 12: CBZ synthesis, adapted from (Vardanyan and Hruby, 2006a)

Unfortunately, unlike IBF, very little information was found in literature about which alternative is more energy and resource efficient. Additionally, little information was found about the state-of-the-art continuous manufacturing of CBZ. As has been mentioned before, many APIs are still produced using a batch/fed-batch process, despite of the advantages and endorsement by regulatory boards and authorities of a continuous mode of operation. Within the course of this deliverable, only one paper (Wang et al., 2012) was found that investigated the utilization of electrospray technology to produce solid dosage forms of CBZ. It is evident that more studies are sorely needed to develop efficient and sustainable processes, combined with excellent process understanding to modernize such classic batch-run processes.

Lastly, a literature review was carried out on the occurrence and fate of CBZ in WWTP influents and effluents as well treatment techniques employed in WWTP/STPs to remove CBZ before the effluents enter the environment. Due to its occurrence in the environment and its fate in WWTP/STPs, CBZ is also a target analyte proposed to be a part of the EU Proposal for a revised urban wastewater treatment directive (2022). CBZ is one of the most common and frequently found API in river areas, and thus used as a marker for contamination in water and wastewater. Due to its presence in drinking and groundwater, as well as its persistence in the environment, CBZ has been added into many watchlists- namely the NORMAN List of Emerging Substances, and has been categorised as an Endocrine Disrupting Chemical by the US Environmental Protection Agency as well (Feijoo, Kamali and Dewil, 2023).

Many studies and reviews (Leclercq et al., 2009; Meyer et al., 2016; Biel-Maeso et al., 2018; Kasonga et al., 2021; Feijoo, Kamali and Dewil, 2023) have shown that CBZ and its metabolites persist in coastal and river waters, as it is hard to biodegrade. CBZ concentrations in WWTP/STP influents and effluents range widely, as described in literature (depending on where the study and/or review was performed). Unfortunately, it is still a challenge to completely remove CBZ from the flow streams, as WWTP/STP effluents show a presence of CBZ (Miao, Yang and Metcalfe, 2005; Zhang, Geißen and Gal, 2008; Leclercq et al., 2009; Biel-Maeso et al., 2018).

There are multiple ways in which CBZ in wastewater streams is treated and removed, as can be seen in Figure 13. The classical wastewater treatment using CAS or MBR are not very efficient at removing CBZ and its metabolites, and the average removal efficiencies in WWTPs are in a range of 21-40% (Zhang, Geißen and Gal, 2008). However, AOP processes have been utilised to efficiently remove CBZ and its metabolites in WWTP/STPs. This technology has been gaining more popularity and application over the last few decades (Feijoo, Kamali and Dewil, 2023). Due to its versatility and different techniques, AOPs are quite attractive, with different types of AOPs achieving up to a 100% removal from wastewater. It has to be noted, however, that removal efficiencies depend on the type of process, CBZ and other analytes' concentrations, and the operating conditions as dismal efficiencies are also routinely reported in literature (Feijoo, Kamali and Dewil, 2023).

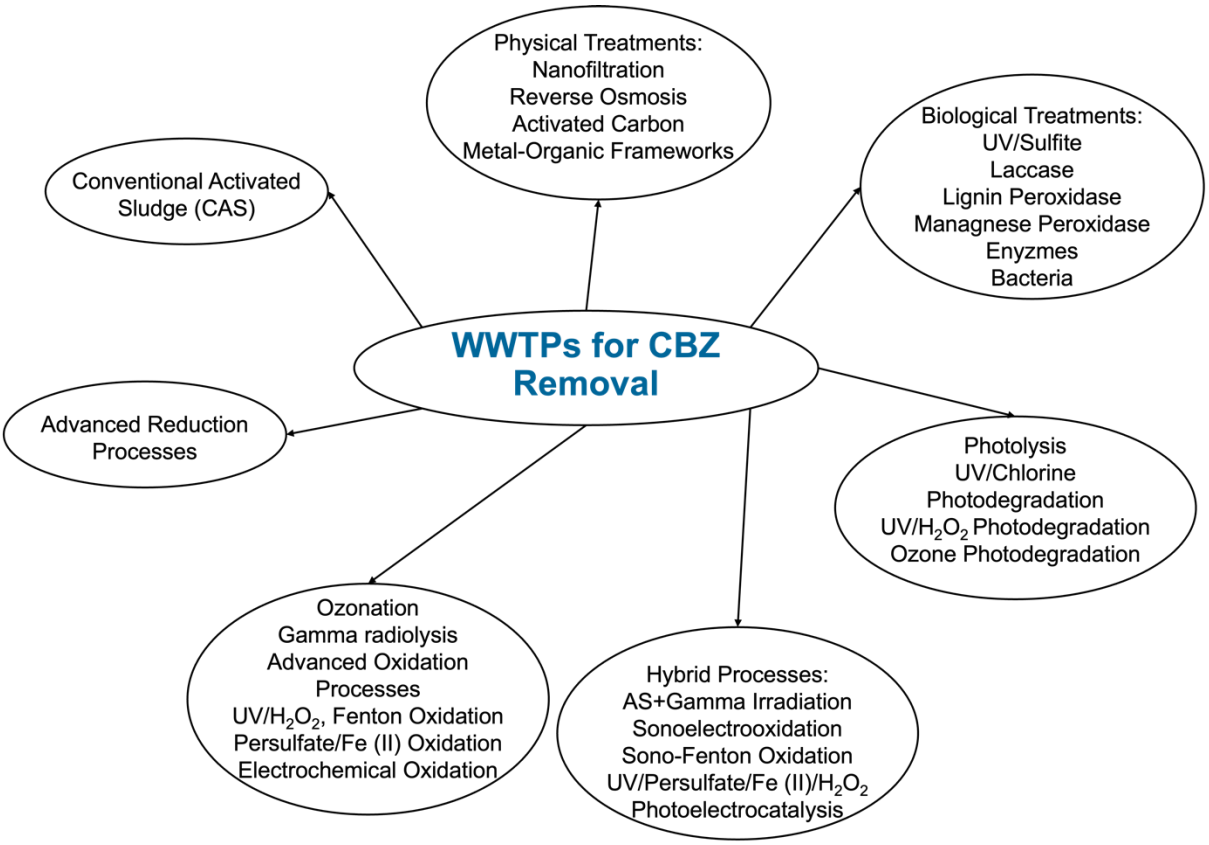


Figure 13: Treatment for CBZ removal in wastewater; adapted from (Miao, Yang and Metcalfe, 2005; Leclercq et al., 2009; Feijoo, Kamali and Dewil, 2023)

Depending on the immediate environment of the WWTP/STP, and the geographical conditions around, the environmental risk of CBZ and many of the other analytes can be lower or higher than the impacts expected or predicted, based solely on the detected concentrations (Biel-Maeso et al., 2018). CBZ, in this case, has been shown to be of medium environmental risk in WWTP influents and effluents and of very low risk in the bay and gulf region (where samples were taken). Keeping this in mind, more monitoring campaigns in different geographical regions are necessary to provide a clearer picture and more comprehensive information of how analytes like CBZ affect the environment.

2.2.6 Hydrochlorothiazide (HCTZ) Production and Fate in Wastewater/Sewage Treatment Plants

Hydrochlorothiazide is usually utilised medicinally on its own or in combination with other antihypertensive medications to treat hypertension and oedema. It belongs to the class of thiazide diuretics prescribed for the control of elevated blood pressure (International Agency for Research on Cancer, 2015, 2023a).

Hydrochlorothiazide is usually produced and found in a white crystalline powder form. Within the scope of this literature research, multiple production methods were found. Many of these production methods have been patented and the details of the production can be found online (Deo et al., 2009; International Agency for Research on Cancer, 2015; Xuezhi, Wang and Mei Mei, 2016). Multiple different synthesis pathways exist and have been patented. However, it seems that all the patents found in the course of this research were run in a batch process, or in a small lab-scale level, especially research and patents on the purification of

hydrochlorothiazide (Deo et al., 2009). Since the 1980's, hydrochlorothiazide was produced one of two ways, as seen in Figure 14 (International Agency for Research on Cancer, 2015):

i. Non-aqueous reaction between 5-chloro-2, 4-disulfamylaniline and *para*formaldehyde

ii. Reaction between 6-chloro-7-sulfamyl-2H-1, 2, 4-benzothiadiazine-1, 1-dioxide with formaldehyde in aqueous alkaline solution

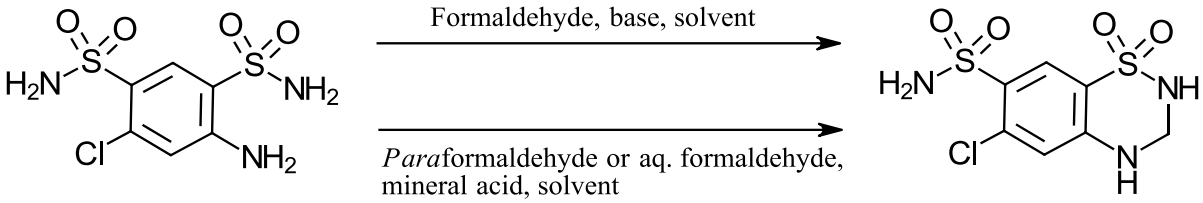


Figure 14: Alternate production methods for producing hydrochlorothiazide; adapted from (Deo et al., 2009; International Agency for Research on Cancer, 2015)

An improved production was invented by Deo et al. (2009), where 5-chloro-2, 4-disulfamylaniline is reacted with formaldehyde in the presence of a solvent without any acid or base. Any number of solvents can be utilised for the process – alcohols, acetates, nitriles, ethers, chlorinated solvents, polar aprotic solvents and other solvents such as water, carbon disulphide and other mixtures, whereas the formaldehyde can be used in the form of paraformaldehyde, trioxane or as an acetal. This process leads to a purity of 99,16% (compared to 98,88% using sulphuric acid) and a higher yield (92% from 86%). Such an improvement is resource efficient as no extra acid or base was used with simultaneous improvement in purity and yield. Further purification steps were performed to purify HCTZ to 99,5% by adding aqueous ammonia, a base solution, activated charcoal, a mineral acid and finally isolating pure HCTZ.

HCTZ, like CBZ and DCF, was found to persist in water bodies and in aquatic and marine species (Biel-Maeso et al., 2018). For example, HCTZ was quantified in the lagoon water all year round, with an increase of HCTZ concentrations in the summer due to increased tourism near a specific coastal lagoon in south-east Spain (Moreno-González et al., 2015). The real effect of HCTZ on many species has yet to be researched thoroughly. However, research shows that long-term exposure to HCTZ can lead to the development of keratinocyte carcinoma in patients (Adalsteinsson et al., 2021).

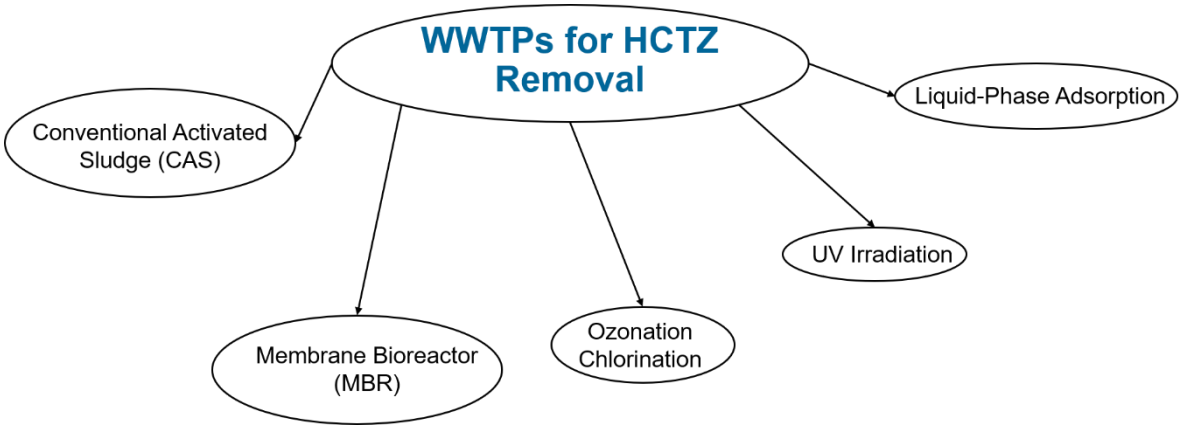


Figure 15: Treatment for HCTZ removal in wastewater; adapted from (Rhoden et al., 2021)

As HCTZ persists in the marine environment and can be found in the marine sediments, it is safe to assume that HCTZ does not get completely treated and removed in WWTP/STPs. HCTZ is also a target analyte proposed to be a part of the EU Proposal for a revised urban wastewater treatment directive (2022). HCTZ concentration in wastewater influents varied largely, depending on the region and the type of waste streams the WWTP/STP treats. HCTZ is usually only partially removed through conventional technologies (CAS, MBR etc) – with reported removal efficiencies of 56 – 85% (Radjenovic, Petrovic and Barceló, 2007), and further exploration and investigation is necessary (Figure 15). Optimization of such techniques, and additional to monitoring campaigns are needed to understand the environmental impact of such technology.

2.2.7 Metoprolol Production and Fate in Wastewater/Sewage Treatment Plants

Metoprolol is another analyte chosen for this deliverable and is also proposed to be a part of the EU Proposal for a revised urban wastewater treatment directive (2022). Metoprolol is a cardioselective β_1-blocker in therapeutic doses. It is utilised medicinally to treat angina, hypertension and aids in treatment of heart problems (Vardanyan and Hruby, 2006b; Morris and Dunham, 2023). The drug metoprolol was first selected for research and testing for its selective β_1-blocking abilities in the 1960s. After successful testing and human studies, metoprolol was registered for marketing in 1975 (Regårdh and Johnsson, 1980).

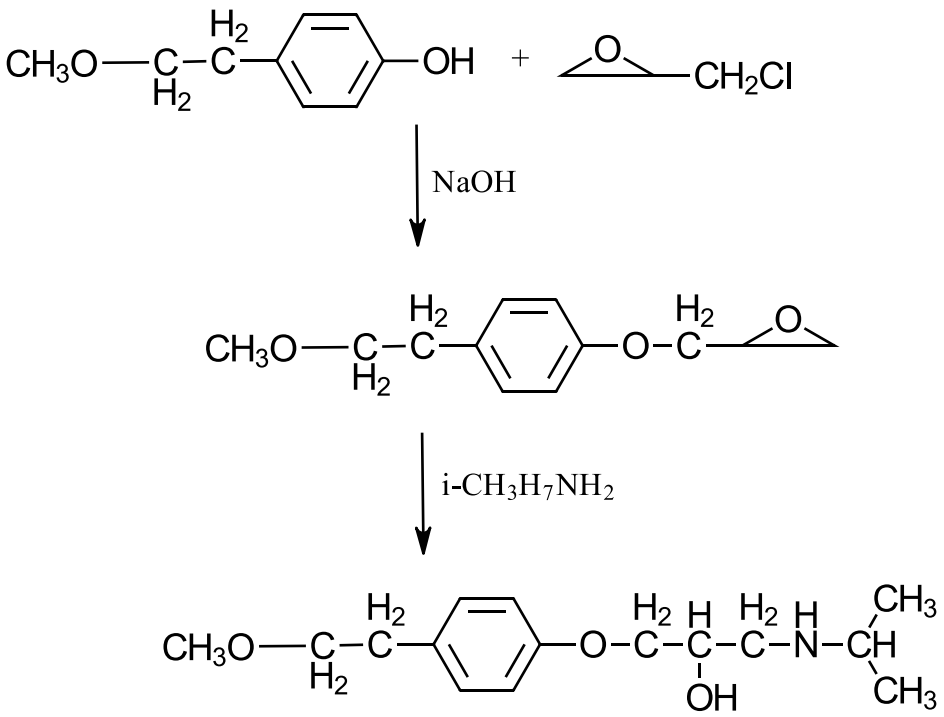


Figure 16: Synthesis pathway of metoprolol, adapted from (Vardanyan and Hruby, 2006b)

Similarly to HCTZ, there are multiple patents (Palmér and Sidenqvist, 2001; Mehra et al., 2005) that can be found online for metoprolol production. Historically, metoprolol, 1-(iso-propylamino)-3-[4'(2-methoxyethyl)phenoxy]-2-propanol, is synthesized by the reaction of 4-(2-methoxyethyl)phenol with epichlorhydride in a basic environment (NaOH). The subsequent intermediate 1, 2-epoxy-3-[4'(2-methoxyethyl)phenoxy]propane is isolated and reacted with iso-propylamine that leads to the epoxide ring opening. This leads to the production of metoprolol, as shown in Figure 16 (Vardanyan and Hruby, 2006b). From Figure 17, the patented production of metoprolol can be seen. The method requires only water as a solvent at

temperatures ranging from 50 – 70 °C with distillation steps to produce metoprolol (Palmér and Sidenqvist, 2001). Other patents (Mehra et al., 2005) follow similar processes.

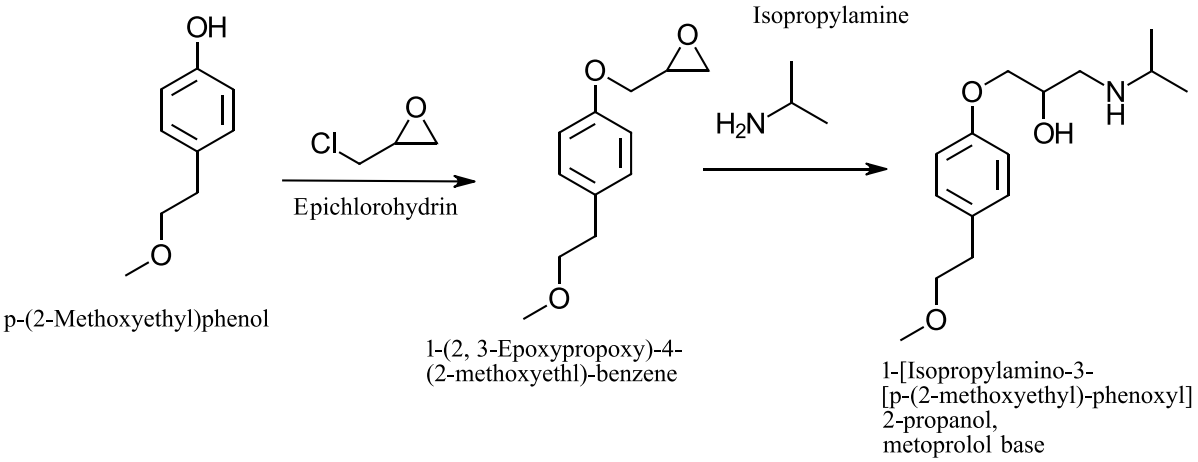


Figure 17: Metoprolol manufacturing process from patent (Palmér and Sidenqvist, 2001)

Metoprolol persists in wastewater effluents at relatively large concentrations. Metoprolol was also found in river and surface waters (Meyer et al., 2016). Little information was found about the effects of metoprolol on the environment, marine and freshwater aquatic life. However, different technologies have been utilised to treat wastewater with metoprolol in them, as shown in Figure 18.

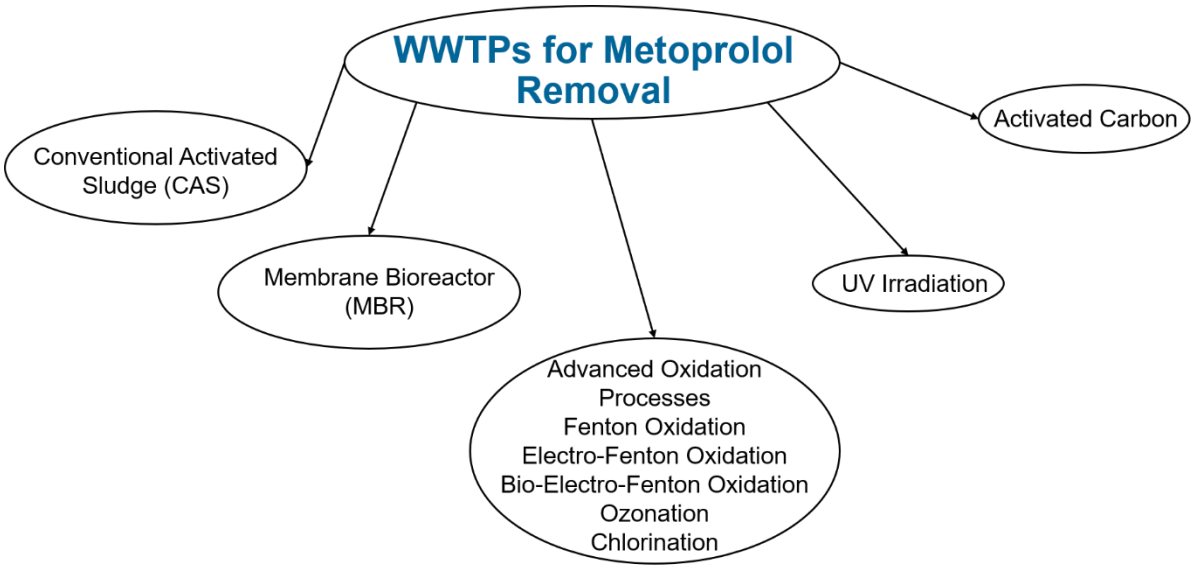


Figure 18: Treatment for metoprolol removal in wastewater; adapted from (Yang et al., 2021; Pedrosa et al., 2022)

However, it is clearly evident that the environmental impact of metoprolol needs to be further researched. Effective monitoring campaigns need to be performed to be able to quantify the effects of metoprolol. Additionally, while some steps have been taken to improve the sustainability of the manufacturing process, more research is necessary to transition to continuous mode of operation.

2.2.8 Benzotriazole (BTA) Production and Fate in Wastewater/Sewage Treatment Plants

Unlike the other API analytes described in this deliverable, BTA is considered to be a precursor and a synthetic auxiliary that can be easily utilised to produce antifungal drugs. Benzotriazole is relatively stable, non-toxic, odourless and inexpensive. Due in part to some of these qualities, BTA is widely utilised as a precursor. It can be used to prepare amines and a number of different derivatives that serve different purposes. BTA itself acts as an antimicrobial and antiprotozoal and shows potential to be applied for difficult-to-treat infections due to antibiotics resistance (Katritzky and Rogovoy, 2003; Briguglio et al., 2015).

Regarding the manufacturing process of BTA, older patents (Long, 1971; Chan and Hunter, 1981) were found within the scope of this deliverable. Two different methods were outlined in the patents found. However, both patents produced BTA for non-medicinal and pharmaceutical purposes. Hence, the purities of BTA made with these processes might not fulfil the strict criteria that the pharmaceutical industry faces. In lieu of reported production processes for pharmaceutical-grade BTA, the retrieved patented processes will be presented here. Long (1971) patented the manufacturing process shown in Figure 19. However, this process requires a considerable amount of water to wash the crude BTA and thus requires subsequent evaporation, distillation and condensation. Chan and Hunter (1981) patented a similar production process of BTA with differing temperatures and operating conditions. However, both patents show examples of production that are batch-run and exhibit the same need for a transition to continuous mode of operation.

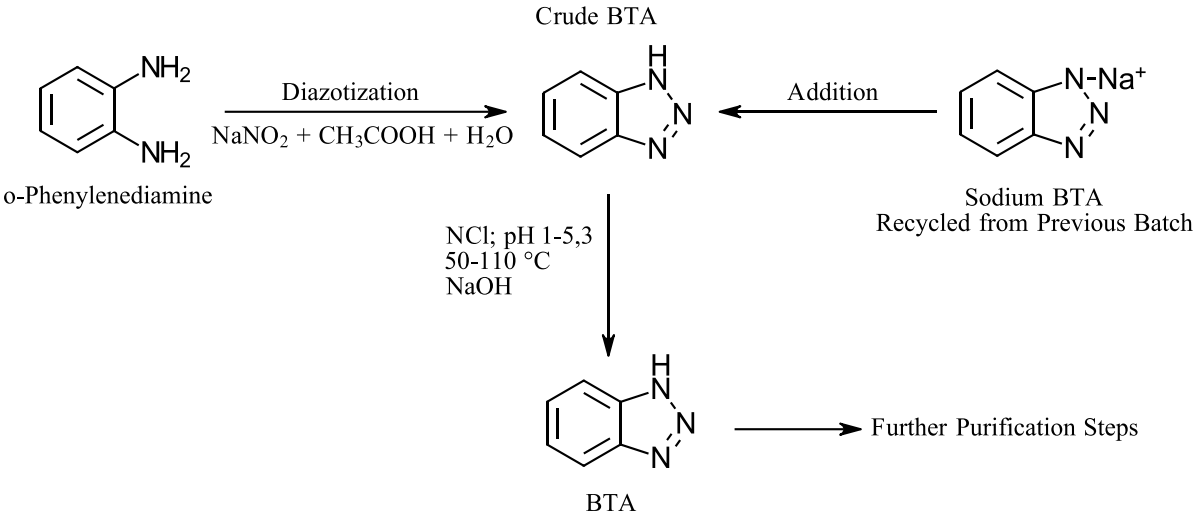


Figure 19: BTA manufacturing process; adapted from (Long, 1971)

The environmental fate and impact of BTA and its derivatives have been researched, as BTA has been shown to persist in the environment. However, the specific contribution of the pharmaceutical sector to these reported impacts is not clear, as BTA finds its application in other industries as well – as anti-freezes, automotive coolants, hydraulic brake fluids etc. and can find its way through means other than the WWTP/STPs highlighted in this deliverable. BTA has been shown to be toxic to marine and freshwater aquatic species, flora and fauna, and can induce toxic effects in higher plants at environmentally relevant concentrations. Similar to metformin, BTA can have an endocrine-disrupting effect on organisms (Durjava et al., 2013; Shi et al., 2019; Im et al., 2023). BTA and its derivatives can be found in wastewater influents and effluents and these analytes have been shown to have low removal efficiencies in WWTPs. However, research has been carried out to optimise existing technology and to develop new technology to improve removal efficiencies in WWTPs (Figure 20).

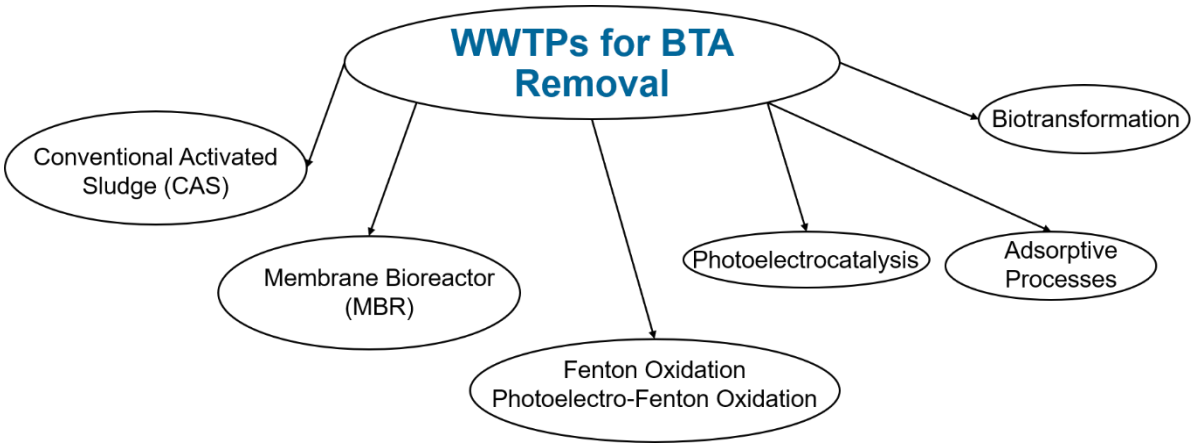


Figure 20: Treatment for BTA removal in wastewater; adapted from (Wu et al., 2013; Ye et al., 2018; Kowalska et al., 2019)

Conclusively, it can be seen that further research is needed to transition the mode of operation and manufacture of BTA and its derivatives towards continuous mode. Additionally, more research is necessary to improve removal efficiencies in WWTPs. BTA poses a harder literature research as it is unclear how much of the BTA waste originates from the pharmaceutical industry, as BTA and its derivatives have many functions across the board. Nevertheless, from the perspective of WWTPs and their operators, more research, effort and work are required to effectively remove BTA from the wastewater.

3 Review of Applicable Digitalisation Methods for Environmental Impact Reduction

3.1 Introduction

The potential of digitalization methods towards Green Pharmaceutical Manufacturing (GPM) is presented and evaluated in the following section. The application of digitalization methods can result in i) minimization of waste effluents during cleaning, ii) optimization of the need of media and buffer feeds, iii) advantageous process modes such as Continuous Bio-Manufacturing (CBM). The pharmaceutical industry is a strictly regulated field that has not used the full potential of digitalization yet. The realization of industry 4.0 means overcoming regulatory, technical and logistical challenges and implementing advanced manufacturing technologies (Arden et al., 2021). Digitalization is the key for industry 4.0 since it enables full connectivity of devices, sensors, instruments, whole units and human operators in a facility. Using the full potential of digitalization methods also requires the acceptance of users by providing user-friendly software and tools, the integration of those methods in existing processes (brownfield scenarios) and structured workflows for setting up digital twins and models (Kroll et al., 2017). Digitalization itself is an interdisciplinary mission involving advanced sensors for in-/at quality testing, real-time manufacturing environments for continuous production chains, advanced process control for minimizing failure and waste, using Artificial Intelligence (AI) ensuring optimal and adaptive process conditions. While digitalization and methods of industry 4.0 enable more sustainable manufacturing, scientific publications are mainly covering concepts & theories, key technologies, shop floor – equipment and human – machine interactions. Sustainability aspects are addressed in just 18% of the publications about industry 4.0 (Kamble, Gunasekaran and Gawankar, 2018). Within the ENVIROMED project, the next steps towards the vision of greener and smarter manufacturing are followed by linking digital methods along the whole lifecycle of a product as well as the process chain (Figure 21).

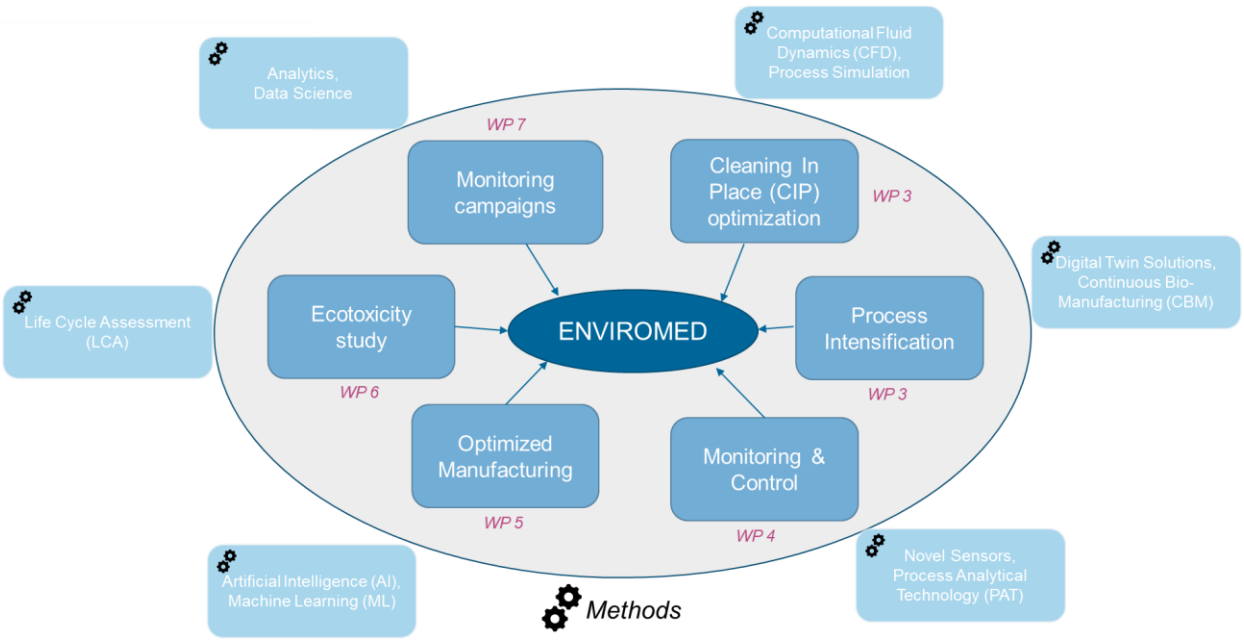


Figure 21: Digital methods for greener manufacturing.

3.2 Predictive Potentials of Digital Twins for Continuous BioManufacturing (CBM)

3.2.1 Continuous Manufacturing for Greener Pharmaceutical Production

The term process intensification describes the concepts of novel equipment designs (e.g., membrane reactors), continuous processing (e.g., flow processing) or process integration (e.g., heat integration) (Boodhoo and Harvey, 2013). Flow processing is referring to continuous material and energy streams and very promising for greener processes, since a more efficient utilization of raw materials and energy resources is achievable. Continuous manufacturing is an established mode of operation in chemical industry (Barenji et al., 2019). However, in pharmaceutical industry conventional fed-batch processes are still dominating. While other sectors have done a successful transformation towards continuous manufacturing, the pharmaceutical industry is a strictly regulated sector with high standards for manufacturing. High standards were defined by regulatory authorities (FDA, EMA) to guarantee efficacy and safety to patients (EMA, 2018). The standards of pharmaceutical production are summarized in the good manufacturing practice (GMP) guidelines. Product testing to fulfil GMP requirements is mainly done batch-wise by testing intermediates or end-product quality (Hole, Hole and McFalone-Shaw, 2021). The transition towards CBM demands a full integration of upstream and downstream unit operations into continuous mode which is a challenging and ongoing procedure (Hong et al., 2018). The upstream process is carried out in continuous bioreactors, in Chemostats or perfusion reactors. Therefore, the concept of cascaded processing has proven to enable continuous upstream operation. Cascaded processing is set up with a series of continuous stirred tank reactors. The higher the number of reactors in the cascade, the more the setup mimics a tubular reactor. Cascaded processing enables the spatial separation of process phases where each cascade is a chemostat providing the favoured conditions. Kittler et al. present a workflow to establish cascaded processing with *E. coli* BL21(DE3) having the process phases of biomass cultivation and protein expression separated (Kittler et al., 2021). It has been proven that the key performance indicators (KPIs) space time yield (STY [mg/L/h]) and specific productivity (q_P (mg/g/h)) were increased significantly in comparison to conventional fed-batch processes. Jungbauer highlights the importance of upstream and downstream units integration and gives suggestions for technical realization of continuous downstream equipment (Jungbauer, 2013). Downstream processing encompasses all unit operations from cell harvesting, cell lysis, refolding, capture, purification, polishing to the final pure product. While the required equipment for all continuous downstream processing steps is equivalent to batch processing, the efficiency can be increased by reduced washing and CIP (clean-in-place) / sterilize-in-place (SIP) cycles and less buffer consumption. The reduction of cleaning-related downtimes leads to savings in cleaning media, less human interventions and lower potential of contamination. The benefit of CBM is summarized by a higher plant efficiency and an overall smaller footprint, thus increasing process sustainability. Nevertheless, the process stability in continuous operation is critical (Kopp et al., 2019a). Growth of biomass might be inhibited in time invariant conditions, long term effects on the culture are unknown and genetic instabilities due to selection pressure are problematic.

Data availability for holistic process evaluation along the path from raw materials supply to final product release is a key challenge in pharmaceutical manufacturing (Figure 22). Becker et al. emphasize that green metrics for process evaluation were already evolving in the 1990s, but their application towards greener process and product design suffers from shortcomings in holistic data acquisition (Becker, Manske and Randl, 2022). Additional challenges for holistic process evaluation are the current trends towards rapid drug development, lean production and the increasing demand of process and product flexibility. Holistic approaches, e.g., life cycle

assessment (LCA), are considered to provide the most accurate translation of process data to green metrics. Their complexity might be a limitation in terms of the lack of complete process data.

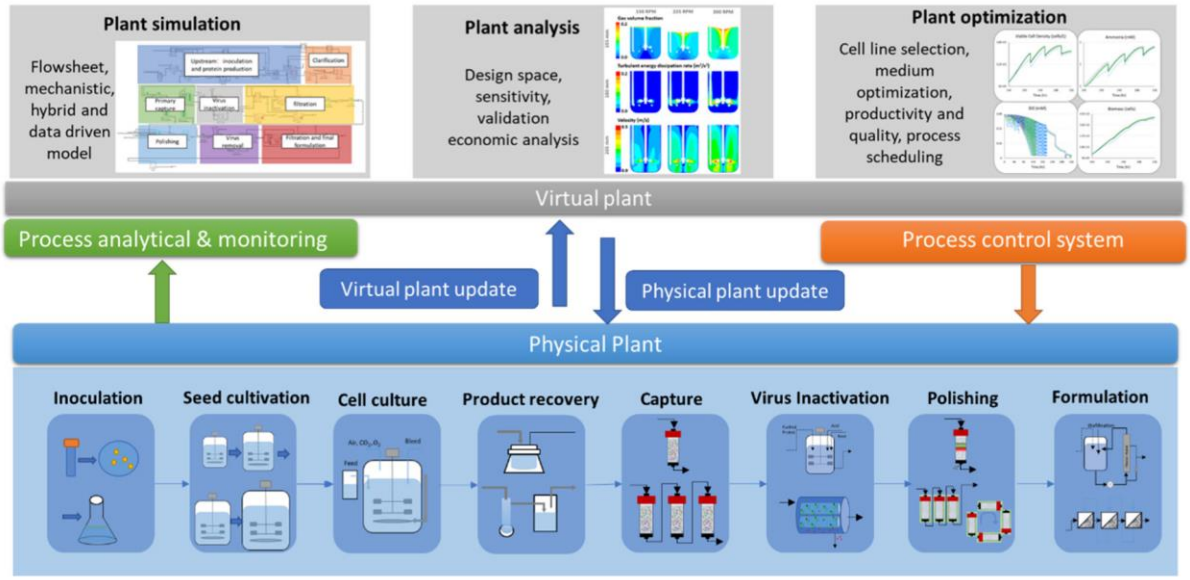


Figure 22: Physical plant interacts with virtual plant (digital twin) (Chen et al., 2020)

3.2.2 The Digital Twin in a Continuous Manufacturing Environment

The traditional batch-wise testing with significant time delays between sampling and analysing the sample is not applicable for a continuous manufacturing line. An immediate result indicating whether the process is operated within the quality limits is mandatory for CBM. The induced delay of at-line/offline measurements leads to a time-delay in control and quality checks (Kopp et al., 2019b). Proper PAT is a bottleneck in both upstream and downstream to enable stable, controlled processes and assure product quality. The digital twin (DT) is seen as a solution to merge the knowledge gained from PAT with the needs of CBM. The regulatory authorities don't require batch operations, but quality control and assurance of defined batches within the production line. Transferred to continuous operation, engineers are facing the challenge of defining batches within the continuous production units (Jungbauer, 2013). The DT is considered to build a bridge for selected online PAT while it is utilized as a soft sensor to observe KPIs indirectly. By observation of indirect (or secondary) online measurements, information about the critical process parameters is obtained in real-time and the time delay of quality checks is avoided. The soft sensor is a state observer in control theory context and predicts input-output relations in a finite time horizon (Golabgir et al., 2015). The input-output relation is expressed by a certain model. Hereby, data-driven, mechanistic models or hybrid models describe the mathematical relation between secondary measurements and the actual KPIs. While data-driven models are not as laborious and don't take the inner structure of the system into account, mechanistic models enable the integration of prior knowledge and their parameters have physiological meaning (Solle et al., 2017). The application of data-driven, hybrid or mechanistic models depend on the availability of process data and the requirements of the bioprocess development use case.

However, the implementation of soft sensors in industry is facing some challenges. Golabgir et al. point out that the successful implementation of soft sensors relies on the set of available secondary measurements and their combination with respect to inherent uncertainty. The approach of observability analysis might help to find the required number and combination of measurements for capturing the system behaviour.

The implementation of a digital twin in a CBM environment is done in a stepwise approach (Figure 23). I) The DT is set up with available knowledge and offline data, II) its predictive potential is used to monitor selected PAT measurements (open-loop), III) a control strategy is derived for assuring product quality with varying raw material attributes and under consideration of the observability (closed-loop), IV) the DT is utilized to predict optimal process conditions in each unit operation, to reduce waste by detecting early failure in production, and to minimize the environmental footprint expressed by green metrics (PMI, WARIEN, etc.). The vision of the DT in a CBM environment is not only covering the whole process from raw materials to final products, but also use its potential along the supply chain (Herwig, Pörtner and Möller, 2021). As a first step the DT supports business planning by forecasting the demand and providing KPIs in real time. The second step is the prescriptive ability of the DT in the context of self-driving supply chains. The DT detects demands and places automatically orders in the pharmaceutical production. This vision would be a huge extension of the well-known lean production concept to a smart and lean manufacturing environment (Kamble, Gunasekaran and Gawankar, 2018).

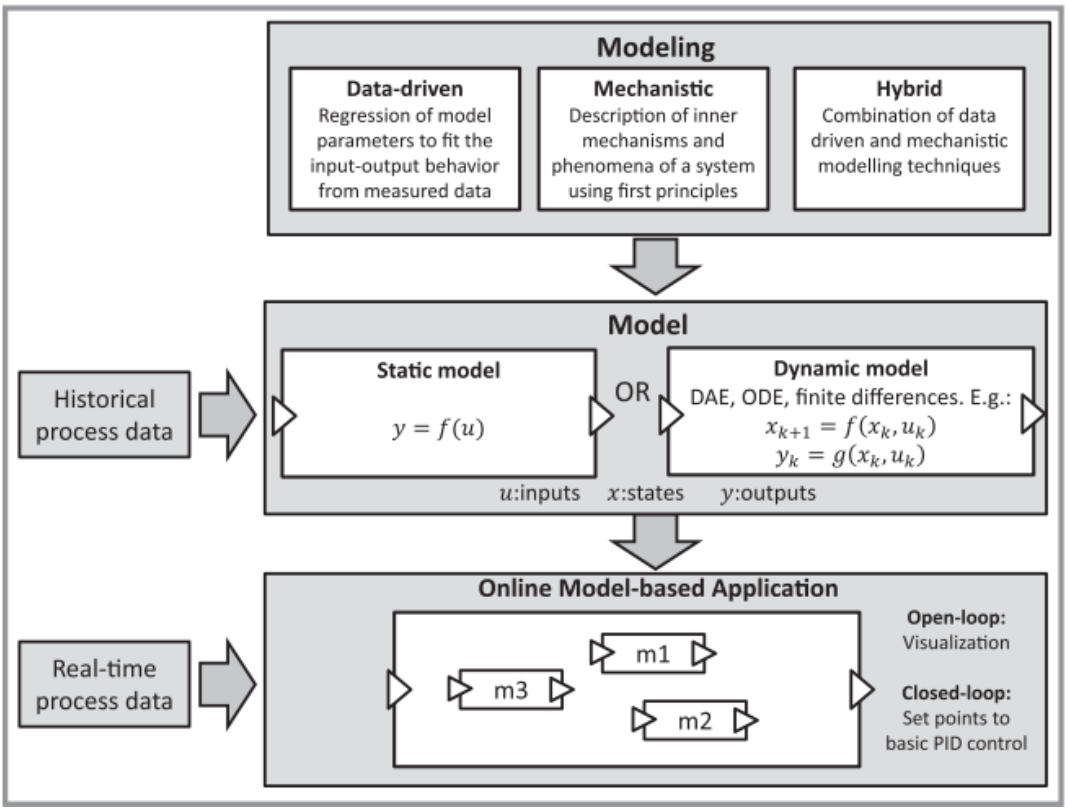


Figure 23: Modeling methodologies and their applications (Solle et al., 2017)

3.3 Digital Twins and Optimal Experimental Design

3.3.1 A Quality-oriented Approach Towards Bioprocess Understanding

Biopharmaceutical development is a time and resources consuming process (Abt et al., 2018). Many steps need to be taken from the initial laboratory experimental trial to the FDA or EMA approved industrial process at large scales. Process understanding is the basis for many bioprocess development tasks like strain characterization, media optimization, process optimization or scale up / scale down. A hurdle in bioprocess development is the way to gain process understanding in an efficient manner and consequently reduce time to market,

production failures, waste and costs (Grangeia et al., 2020). Following the systematic Quality-by-Design (QbD) approach, quality should be built into the process design during development (Figure 24) (ICH, 2017). The QbD approach emphasizes the need of process and product understanding to assure quality. Hereby, quality is defined in the target product profile concerning safety and efficacy of the pharmaceutical product for the patient. The critical quality attributes (CQAs) are derived by the general target product quality profile (TPQP). The CQAs are physical, chemical, biological or microbiological properties that need to be in a specific range, distribution or limit (Lionberger et al., 2008). CQAs are defined after a unit operation step or at the end of the entire process. To assure product quality represented in the CQAs, risk assessment is applied to identify critical process parameters (CPPs) and critical raw material attributes (CMAs) (von Stosch et al., 2020). A certain number of experiments has to be done to explore the relations of process inputs (CPPs, CMAs) and outputs (CQAs). The proven relation between CPPs, CMAs and CQAs is usually expressed by mathematical models and considered as process understanding (Abt et al., 2018). For quality assurance the CQAs have to be maintained in narrow ranges, which are set as proven acceptable ranges (PAR). The multidimensional space (design space) within the PAR depending on CPPs and CMAs can be constructed (von Stosch et al., 2020).

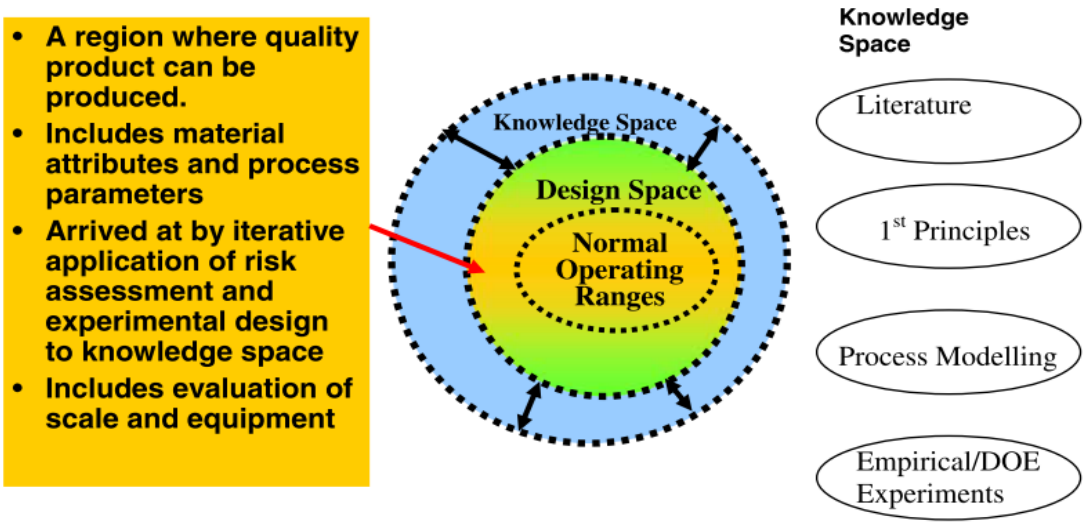


Figure 24: The design space extends the normal operation ranges within the knowledge space while assuring product quality. (Lepore and Spavins, 2008)

3.3.2 Comparison of DoE Strategies for Gaining Process Knowledge

Experiments are carried out to test a hypothesis, find favourable process conditions for optimal yield, determine the impact of CPPs and CMAs on CQAs including robustness analysis and in general to increase process understanding (Abt et al., 2018; Oberleitner et al., 2022). Traditional approaches are following trial-and-error learning, one-factor-at-a-time methods or statistical design of experiments (DoE), ranked from naive to more structured approaches (Herwig, Pörtner and Möller, 2021). Statistical DoE plans are aiming for high information content while being simple and fast applicable (Lee, 2019). Statistical DoE is a commonly used method for screening and optimization purposes. Screening designs are exploring the experimental region of interest in an early process development stage. Hereby, full factorial 2^3 or fractional factorial $2^{4,1}$ designs are to be considered. The screening runs are conducted to uncover the most influential factors and outline appropriate design regions. After experimental screening, the process response on target variables (e.g., final product concentration) is investigated to detect optimal process conditions. Box-Behnken and Central Composite designs are suitable for response surface modelling (Abu-Absi et al., 2010). Underlying the

statistical DoE approaches is the fact that they do not take into account the internal structure of the process, but rather prescribe a rigid design to explore the experimental region. Usually, the static planning of experiments leads to a higher number of required experiments to assure a certain level of process understanding.

In contrast, optimal experimental designs and model-based designs are highly promising since they are incorporating the existing knowledge expressed by mathematical models (Puente-Massaguer et al., 2019). Optimal DoE is aiming to increase the information content of the design matrix, which is the fisher information matrix (FIM) (Oberleitner et al., 2022). An example of optimal DoE is the D-optimality criterion which is targeting to maximize the determinant of the FIM. Optimal DoE is highly flexible and handles distorted or non-convex design regions well. While statistical DoE plans specify a certain number of runs, optimal plans are flexible according to available resources. Even existing runs can be incorporated leading to a reduced experimental effort.

Model-assisted and model-based design of experiments are techniques utilizing an *a priori* known bioprocess model (Figure 25) (Abt et al., 2018). Those approaches are based on mechanistic knowledge being derived by preliminary experiments. The mechanistic knowledge might be reflected by yield coefficients, limiting substrates or reaction kinetic rates. Due to the strong physiological context of the approach, fewer experiments are required to characterize the process and even extrapolation capabilities might be inherent (Lee, 2019). Nevertheless, mechanistic models are considered to be laborious and have higher development effort since they are relying on expert knowledge (Sokolov et al., 2021). Kroll et al. identified the lack of user-friendly tools as well as consistent workflows for setting up mechanistic models as a bottleneck in biopharma (Kroll et al., 2017). An alternative for model-based DoE, if no process model is available, might be the application of Bayesian optimization for experimental design. Instead of a known process model, a surrogate model (Gaussian process model) is sequentially trained with data. Only a few publications were found regarding experimental planning based on the Bayesian approach (Greenhill et al., 2020; Narayanan et al., 2021).

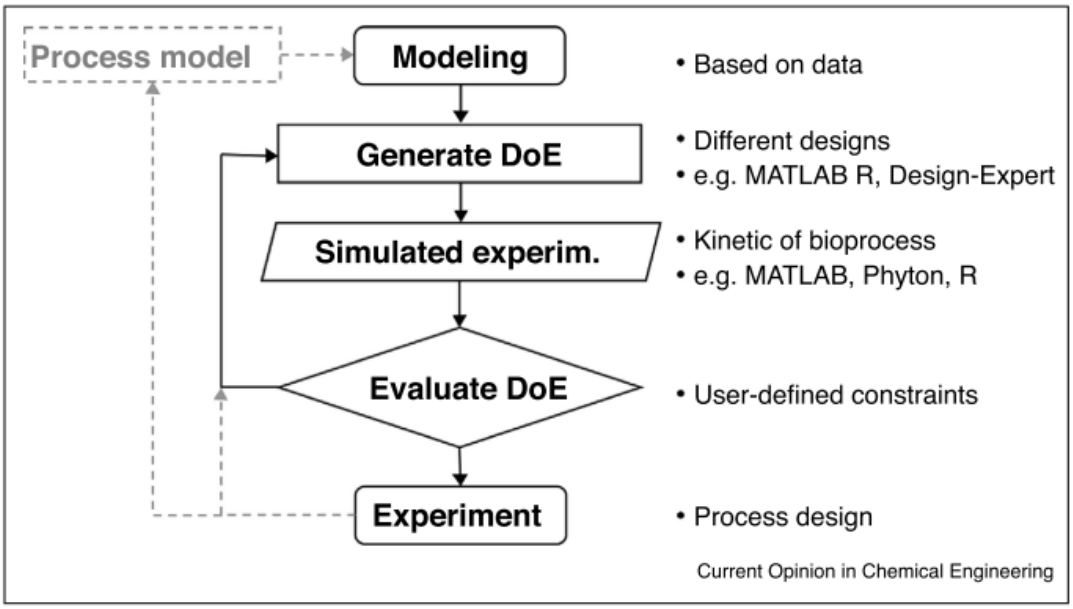


Figure 25: Model-assisted design of experiments with several iterations between modeling and experiments proposed by Abt et al. (2018)

The strategy to run experiments can be serial, parallel or hybrid. All experiments can be realized at once in a parallel setup. With modern upstream cultivation systems (e.g., Ambr250©), high throughput is realized and process characterization is accelerated (Xu et al., 2017). The classical way is running one experiment after another according to the DoE plan (serial approach). De Luca et al. propose a hybrid framework where mini-batches of experiments are carried out (De Luca et al., 2023). After each series of runs the experimental results are evaluated, the process model is updated and new experiments planned. The benefit of this highly flexible strategy is that a trade-off between process exploitation (finding optimal conditions) and process exploration (gaining new information) is implemented leading to more robust process models and feasible optimal regions.

3.3.3 Greener Process Development enabled by Model-assisted DoE

The digital twin is the knowledge representation of advanced bioprocesses. The enriched knowledge should be acquired in the way to (i) gain the highest possible amount of information from a set of experimental runs and (ii) minimize waste related to experimental effort created by a high number of characterization experiments (Möller et al., 2019). By including mechanistic knowledge in experimental planning, a process model can be utilized to plan the next experiments iteratively in a more efficient manner. Thus, a significant number of experimental runs and resources can be saved. Furthermore, the experimental data is supposed to contain a higher information content, which enables more accurate process models. Summarized, model-assisted DoE strategies are supporting greener manufacturing in terms of decreased experimental effort, and more reliable process models for further tasks (e.g., media optimization, monitoring, model predictive control) (Herwig, Pörtner and Möller, 2021).

3.4 Advanced Process Control based on Digital Twins

3.4.1 Control under the Quality-by Design Paradigm

Biopharmaceutical processes are running under varying input parameters while targeting to match the quality gates within a specific range (Sommeregger et al., 2017). Although it is attempted to keep CQAs in the targeted range, failures are occurring due to lack of CQA monitoring techniques in real-time. Process control is the ability to find process inputs for achieving the desired outcome (Kroll et al., 2017). The desired outcomes are in terms of the QbD approach robust CQAs which lead to reduced failure-related costs and resources (Figure 26). The aforementioned goals of the QbD paradigm are consistent with the pursuit of sustainability in the pharmaceutical industry and will be reflected in green metrics beneficially. To achieve the desired outcomes, different control strategies are applicable (i - iv). Rathore et al. (2021) gave a concise review about bioprocess control strategies (Rathore et al., 2021).

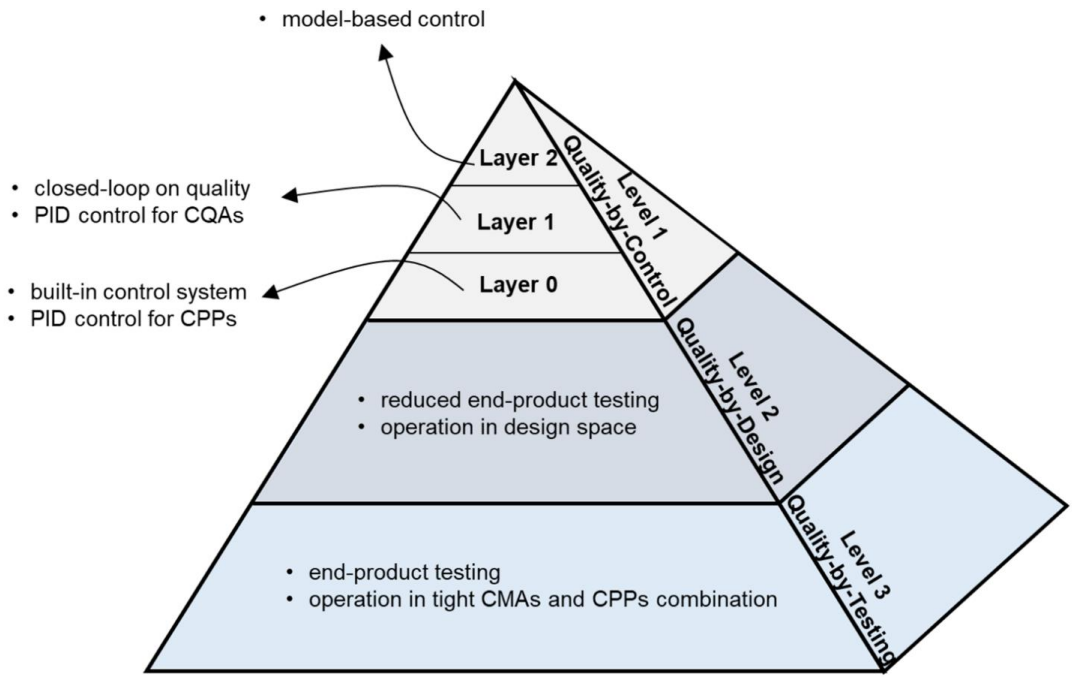


Figure 26: Levels of control strategies for pharmaceutical manufacturing (Destro and Barolo, 2022)

3.5 Control Strategies for Bioprocesses

A common method in industry is the open loop (feedforward) control of the process at predefined setpoints (i). The open loop strategy instructs the system, regardless of the system's current state. In bioprocesses, open loop control is applied to feeding strategies where predefined exponential feeding rates are provided. The system response is not taken into account, although the feeding profile relies on system knowledge by assuming unlimited exponential cell growth (Aehle et al., 2012). Although open loop PID control is simple and cheap, there are several drawbacks when it comes to the robust control of complex bioprocesses. The disadvantage of the open loop strategy is the lack of integration of process feedback. Possible process limitations, random events and resulting deviations are not considered. Especially, if the pre-assumed behaviour of the process is deviating significantly, a huge risk of failure exists in the manufacturing line showing the need for plant-wide control architectures (Aehle et al., 2012; Hong et al., 2018).

Widely used in industry is the closed loop (feedback) PI or PID control (ii). PID feedback control is beneficial for controlling process parameters with high frequency online/inline monitoring, while the underlying system response is well-known. The controlled variables are directly measured with a high frequency e.g., dissolved oxygen (DO) or pH measurements in the reactor. The manipulated variables are changed by a simple, model free PID controller in order to maintain the setpoints of the controlled variables. For example, the DO is controlled by adjusting stirrer speed or aeration rate. Critical for the successful implementation of PID control is the tuning procedure of the controller parameters in order to get a fast response with sufficient accuracy, to avoid overshooting and instable oscillating behaviour (Kager et al., 2020). In addition, the risk of saturating the PID controller must be handled by anti-windup solutions. The disadvantages of PID control are the limited capability to react towards significant disturbances in the process due to the non-linearity of bioprocesses, the lack of deviation detection before their occurrence, the non-smoothness of control actions resulting in unsatisfactory reproducibility between charges, the risk of losing the preferred path of the

process due to time delays in offline measurements and limited options for optimization tasks (Aehle et al., 2012).

It is often the case that the controlled variables are not directly measurable. Consequently, a combined approach of a model, which describes the controlled variable via indirect measurements, with a PID controller is convenient. This approach is referred to as model-assisted, model-supported or Model-Based (PID) Control (MBC) (iii). The model is in the control theory context a state observer, which predicts the states by taking online data as inputs and incorporating sparse, noisy data (Lee and Majda, 2016). The statistical, empirical or mechanistic model is based on historical data and/or knowledge. The derived model with the underlying reaction kinetics can be utilized to monitor critical, non-accessible parameters, maintain metabolic rates at certain levels and operate at proven, robust conditions (Kager et al., n.d.). However, in this approach the model is only translating the measurements for the PID controller without anticipating future process behaviour. The shortcomings of model-based PID control are the limited ability to cope with non-linear processes and handling varying input parameters. Furthermore, the static setpoint control of measurable process parameters might be not ideal for many processes. Preferably, specific metabolic rates are controlled at certain setpoints (Ulonska, Kager and Herwig, 2018). Kager et. al. (2020) demonstrated the applicability of MBC to control the biomass specific substrate uptake rates $q_{S,i}$ in a *Penicillium chrysogenum* fed-batch process by combining an inverted process model with a particle filter for state estimation. In total, three different control strategies (PID, MBC, MPC) were compared in order to provide suitable feeding rates for the process. MPC showed the best results in terms of reducing unwanted side reactions and substrate accumulation while yielding the highest product concentration (Kager et al., 2020).

Model predictive control (MPC) is an advanced control strategy for highly non-linear bioprocesses (iv). Prerequisite for MPC is the availability of a reliable process model and the careful choice of a set of measurements (Sommeregger et al., 2017). MPC enables multiple input, multiple output (MIMO) systems, which means the controller handles several control variable inputs, calculating an optimal control action in a finite control horizon for all manipulated variables (Figure 27) (Schwenzer et al., 2021). The benefit is that interactions of the inputs on the manipulated variable are taken into account. Furthermore, MPC is able to perform optimization tasks on constrained problems. While hard constrains are related to process or equipment limitations, soft constrains are targeting to penalize unfavoured conditions (e.g., secondary substrate accumulation, low product formation) or harsh control actions (e.g., changing feed rates) (Kager et al., 2020).

Despite the beforementioned benefits of MPC, there are some shortcomings addressed by (Ulonska, Kager and Herwig, 2018; Rathore et al., 2021). MPC is highly dependent on the model accuracy, since inaccurate models might mislead the process from the right track. The model should be able to capture disturbances of the system in an appropriate way. Mechanistic models are more laborious than data-driven models, but require much less data points for calibration. Critical for the successful implementation of MPC is the ability to apply the control actions in a sufficient time span. Particularly, if the MPC algorithm is optimizing the control actions at each time step, high computational demands are raised.

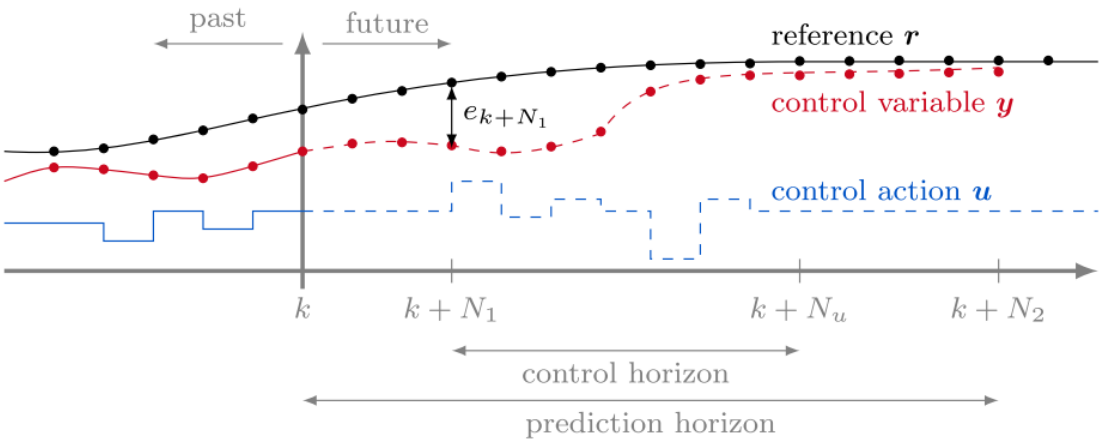


Figure 27: MPC principle: the trajectories of the control variable (y) are predicted in a finite time horizon, the underlying model is used to optimize the control action (u). (Schwenzer et al., 2021)

MPC seems to be the most promising strategy for controlling continuous operated bioprocesses due to the advantages in real-time optimization of control inputs and product variability reduction. With respect to greener pharmaceutical manufacturing, the application of MPC strategies can reduce waste related to failures in product quality and reduce media consumption by optimal forecasting of feed addition. In the authors opinion, the broad application of MPC suffers from high computational efforts during the real time optimization as well as the not fully uncovered potential of soft sensors as process models. Nevertheless, to meet the overall *ENVIROMED* project goal enabling more sustainable manufacturing, advanced control algorithms based on robust process knowledge provide the highest potential (Figure 28).

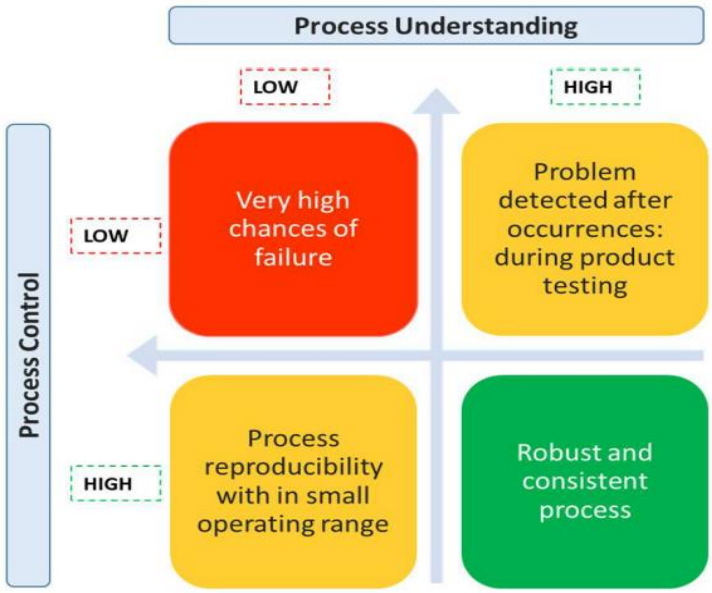


Figure 28: Robust process control is based on process understanding (Rathore et al., 2021)

4 Conclusion

D2.1 aimed to report on the requirements for SPM and the different metrics that can be utilized to measure the sustainability of a (pharmaceutical) process. The deliverable D2.1 is a report, which defines requirements for greener pharmaceutical manufacturing processes. The process requirements are translated in task T2.1 into a set of green metrics. The green metrics are quantifying the degree of sustainability of a process and allow comparison of different processes. In Task 2.2 the digitalization methods for greener pharmaceutical production are introduced. State-of the-art technologies are compared to novel approaches.

Task 2.1 of this deliverable outlines the state-of-the-art production of the eight analytes in the project's scope. These analytes were chosen for their harmful effects on the environment and the challenging treatment in WWTPs/STPs. The benefits of integrating green metrics in the design of pharmaceutical plants and existing production plants were also observed.

A review of the most essential and relevant green metrics- PMI and the E-Factor- was thoroughly examined. This yielded many interesting aspects of sustainability that still need to be achieved within the pharmaceutical industry. A brief overview of LCA strategies and approaches was provided as well. Such metrics and mindset are imperative in identifying the problem areas and opportunities for optimization toward sustainability.

Lastly, an examination of the eight chosen molecules, their production, occurrence and fate in WWTPs/STPs was carried out. While some molecules have already seen significant advantages in switching to a continuous mode of operation, other pharmaceutical compounds have been and still are being produced in a batch/fed-batch mode. These great examples illustrate the necessity for further research and present opportunities for further optimization and sustainability.

All of the compounds do not get entirely treated in WWTPs/STPs and end up in the environment. Their effects have been studied in research, and it is imperative for the project and the project partners to comprehend the treatment of these compounds and their metabolites, as they play a prominent role in the harmful effects on the environment.

The first subchapter within T2.2 highlights the importance of continuous (flow) processing. By increased space-time-yields and achieving higher productivities, classical fed-batch operation is outperformed. Thus, plant efficiency, resource utilization and energy consumption are enhanced. Since continuous processes have higher complexity to be maintained in a robust mode of operation, advanced monitoring techniques and control algorithms are required. The way to gain robust process understanding is drafted in the next subchapter. By using model-based approaches for experimental design the number of experiments can be significantly reduced, and resources can be saved. Furthermore, the increased information content in the data will decrease the model uncertainty. Having more reliable process models is a prerequisite for all model-based tools described in this report. Different control strategies for bioprocesses are highlighted and the benefits of a predictive control approach that allows the incorporation of process knowledge and performs optimized control actions are emphasized. The implementation of advanced process control is supposed to reduce failures and waste in production and provides a broader flexibility, consequently it makes production more sustainable. Plant-wide implemented advanced control architectures are still rarely seen and are a field with huge potential for greener manufacturing.

Within the ENVIROMED project, the aim of is to benchmark the suggested new methods and demonstrate the positive impact of the application of green metrics on the overall sustainability of the production of pharmaceutical analytes.

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ANNEX A

Table 6: Process green metrics; E: energy; m: mass; mw: molecular weight; WFI: water for injection; PW: purified water; CS: clean steam; CSC: clean steam cold; CEQ: CO_2 equivalent; WC: water cold

Metric	Calculation	Definition and Comments	References
Atom economy (AE) [%]	$\mathrm{AE} = \frac{mw_{product}}{\sum mw_{reactants}} * 100$	Atomic percent of reactants incorporated into the product Minimal by-product formation Reduced environmental burden and cost	(Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022)
Reaction mass efficiency (RME) [%]	$\mathrm{RME} = \frac{m_{product}}{\sum m_{reactants}} * 100$	Ratio of mass of reactants incorporated into product's mass	(Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022)
Carbon efficiency (CE) [%]	$\mathrm{CE} = \frac{m_{carbon\ in\ product}}{\sum m_{carbon\ in\ raw\ materials}} * 100$		(Sheldon, 2018)
Mass intensity/Process mass intensity (MI/PMI)	$\mathrm{PMI} = \frac{\sum m_{inputs}}{m_{product}}$	Total mass of inputs per unit mass of product PMI includes process water, and MI typically doesn't	(Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022)
Environmental factor (E-Factor)	$\mathrm{E-Factor} = \frac{m_{waste}}{m_{product}}$ $\mathrm{cEF} = \frac{\sum(m_{raw\ materials} + m_{reagents} + m_{solvents} + m_{water}) - m_{prod}}{m_{product}}$ $\mathrm{sEF} = \frac{\sum(m_{raw\ materials} + m_{reagents}) - m_{product}}{m_{product}}$	Mass of waste produced per 1 kg of product Simple E-factor (sEF): for early development phase process route activities Complete E-factor (cEF): accounts for all process materials	(Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022)
Mass efficiency/Mass productivity (ME/MP)	$\mathrm{ME} = \frac{m_{product}}{\sum m_{inputs}} = \frac{1}{\mathrm{PMI}}$	Mass ratio of inputs incorporated into the product	(Sheldon, 2018; Jimenez-Gonzalez and Lund, 2022)

Energy intensity (EI)	$\mathrm{EI} = \frac{\sum E_{inputs}}{m_{product}}$	Total energy input per unit mass of product	(Jimenez-Gonzalez and Lund, 2022)
Effective mass yield (EMY)	$\frac{m_{product}}{\sum m_{non-benign\ inputs}}$	Ratio of product's mass to nonbenign inputs	(Jimenez-Gonzalez and Lund, 2022)
Solvent intensity (SI)	$\mathrm{SI} = \frac{\sum m_{solvents}}{m_{product}}$	Ratio of solvent mass to product mass Solvent and solvent waste reduction Reduced environmental burden and cost	(Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018)
Wastewater intensity (WWI)	$\mathrm{WWI} = \frac{\sum m_{process\ water}}{m_{product}}$		(Roschangar, A. Sheldon and H. Senanayake, 2015; Sheldon, 2018)
Renewables Intensity (RI)	$\mathrm{RI} = \frac{\sum m_{renewably\ derived\ materials}}{m_{product}}$	Ratio of renewable materials mass to product mass Determination of the renewability of reagents used	(Henderson, Constable and Jiménez-González, 2010; Jiménez-González, Constable and Ponder, 2012)
Renewables Index (RI) [%]	$\mathrm{RI} = \frac{\text{Carbon from renewable materials [kg]}}{\text{Total cradle mass Carbon MI}} + \frac{\text{Carbon from renewable energy [kg]}}{\text{Total energy mass Carbon MI}}$	Ratio of carbon from renewable sources to total carbon Determination of the extent to which renewable materials are used throughout the life cycle Accounting for energy used to produce a reagent	(Jiménez-González, Constable and Ponder, 2012)

		Scored from 1-10, with 10 being the highest renewability fraction	
Water-related impact of energy (WARIEN)	$\mathrm{WARIEN} = (\mathrm{PMI_{WFI}} + \mathrm{PMI_{PW}}) * \mathrm{CEQ_{WC}} + \mathrm{PMI_{CS}} * \mathrm{CEQ_{CSC}}$ $\mathrm{PMI_W} = \frac{\text{Total water used in process [kg]}}{\text{API [kg]}} = \mathrm{PMI_{PW}} + \mathrm{PMI_{CS}} + \mathrm{PMI_{WFI}}$	Directly correlates the amount of CO_2 emitted per kg biopharmaceutical, including membrane- and distillation-based methods for clean water production	(Cataldo et al., 2020)
Fast Life Cycle Assessment of Synthetic Chemistry (FLASC)	-		(Curzons et al., 2007; Sheldon, 2018)
Sustainability Metrics:		Units	
Net mass of materials used		kg	
Energy consumed		MJ	
GHG equivalents		kg CO_2 equivalents	
Oil & natural gas depletion for materials' production		kg	
Acidification potential (AP)		kg SO_2 equivalents	
Eutrophication potential (EuP)		kg PO_4^{3-} equivalents	
Photochemical ozone creation potential (POCP)		kg ethylene equivalents	