Embedding Safe-by-Design in circular bioeconomy workflows

From feedstock to product and value-added waste

November 2023

Commissioner

Dutch Ministry of Infrastructure and Water Management (IenW)

Project period

1st October 2022 to 30th November 2023

Authors

Alexandra Müller, MSc

Fathya Hekmatyar

Nikhil Sairam

Dr. Christos Batianis

Dr. Zoë Robaey

Dr. Enrique Asin-Garcia

Prof. Dr. ing. Vitor Martins dos Santos

Research institution

Wageningen University & Research

Bioprocess Engineering chair group

Biomanufacturing & Digital Twins division

Table of Contents

I Summary

The pursuit of a circular economy involves a heightened focus on carbon efficiency and the reduction of virgin fossil feedstocks. Biomass waste, often overlooked, emerges as a promising unconventional resource for value addition. However, challenges persist, especially with less defined feedstocks, concerning usability, feasibility, and safety. Industrial biotechnology utilises microorganisms and enzymes as tools, while synthetic biology, incorporating engineering principles, takes a lead role in transforming non-food agricultural and other feedstocks into high-value products. Recognising and addressing the inherent challenges and knowledge gaps in incorporating alternative biowaste feedstocks for the synthesis of high-value products is crucial for enhancing the efficiency of the circular bioeconomy.

This study involves a comprehensive focus on three key aspects: usability, feasibility, and safety in circular bioeconomy. Firstly, it aims to identify the critical steps, decisions, and risks that significantly influence the safety and circularity of bioprocess value chains. Secondly, the research endeavours to assess the feasibility of employing industrial waste as feedstock for synthetic biology, specifically in the generation of high-value compounds. Lastly, the study seeks to propose preliminary Safe-by-Design principles to be implemented throughout circular bioproduction value chains. By addressing these key aspects, the research aims to contribute to a comprehensive understanding and enhancement of safety and circularity in the context of bioprocess value chains.

Employing a comprehensive methodology, this study incorporates a thorough literature review, conducts stakeholder interviews, and showcases experimentation involving an engineered *Pseudomonas putida* strain for the transformation of Soda Lignin, a by-product from the pulp and paper industry, into curcuminoids, a high value compound.

This approach, incorporating a mix of methodologies, offers in-depth insights into our three principal goals. Initially, our focus was on identifying the pivotal steps, choices, and risks that impact the safety and circularity of bioprocess value chains. This entailed examining waste as feedstock, demanding a comprehensive evaluation of the entire value chain and related chains. Factors such as economic feasibility, quality, reliable supply, regulation, and public perception come into play when selecting a feedstock. Choosing the right biocatalyst is of utmost importance, involving considerations of economic efficiency comparable to the oil and gas sector, while also addressing safety and security issues linked to genetically engineered microorganisms. Dealing with novel fermentation processes requires the optimisation of technical efficiency, resource efficiency, and the utilisation of feedstocks. Additionally, evaluating the final product is essential to ensure that biotechnologies used in the process do not compromise the integrity of

the end product. This includes a rigorous assessment of safety, recyclability, degradability, adherence to local regulatory requirements, and integration into local production systems. Lastly, waste management circles back to the initial step, prompting considerations of the economic viability of waste produced, advocating for clear distinctions between wastes and by-products, and sharing responsibility for end-of-life scenarios. Addressing all five of these critical steps in coordination is crucial before implementing a new process. Only by tackling these steps simultaneously can the safe implementation of these new technologies in industrial biotechnology be ensured.

Moreover, this research delves into the assessment of the feasibility of utilising industrial waste as feedstock for synthetic biology to produce high-value compounds. As a case study, we use Soda Lignin as a "waste" feedstock for the bioproduction of curcuminoids. By assimilating monomers released by the fractionation of lignin into the curcuminoid biosynthetic pathway, our Pseudomonas putida curcumin-producing strain was able to enhance its production credentials. Furthermore, the study reveals variations in waste characteristics and highlights challenges associated with regulatory labels on waste. These challenges significantly impact the economic feasibility of selecting specific waste for a given process. Further research is warranted to explore case-specific considerations and foster shared learning on the utilisation of waste.

Early-stage Safe-by-Design principles for an entire circular bioproduction value chain involve considerations such as viewing feedstock holistically, optimising biocatalyst integration in both economic and socio-technical systems, and sharing responsibility for product lifecycle sustainability through the use of diverse tools and expertise.

Overall, our findings reveal various dilemmas and challenges across the circular bioprocess value chain, emphasising the complexity of the issue. Notably, challenges related to the terminology and classification of waste, (pre)treatment of waste, public perception, gaps in research, and technical and economic issues were more pronounced than concerns related to the use of genetically modified organisms or potential interference from residues in agricultural or industrial side-streams. Finally, we provided insights and recommendations to guide the incorporation of Safe-by-Design principles into a circular bioeconomy workflow, acknowledging the multifaceted nature of these challenges. the pursuit of a circular economy, an increased focus on carbon efficiency and the minimisation of virgin fossil feedstocks is crucial. To reach this goal, utilising biomass waste for added value offers promising avenues. Industrial biotechnology, which leverages microorganisms and enzymes, is at the forefront of converting biomass, *i.e.*, non-food agricultural and other feedstocks into high-value products. One game-changer for this sector is synthetic biology, which applies engineering principles to design and construct artificial biological systems for diverse applications, fostering innovation and customised solutions.

I Openbare samenvatting

Het streven naar een circulaire economie impliceert een grotere focus op de vermindering van nieuwe fossiele grondstoffen. Biomassa afval, dat vaak over het hoofd wordt gezien, komt naar voren als een veelbelovend, onconventioneel alternatief. Echter, vooral bij deze minder gedefinieerde grondstoffen blijven er uitdagingen bestaan op het gebied van bruikbaarheid, haalbaarheid en veiligheid. Industriële biotechnologie maakt gebruik van micro-organismen en enzymen, terwijl synthetische biologie technische principes integreert en daarmee een leidende rol speelt bij het omzetten van niet eetbare landbouw- en andere grondstoffen in hoogwaardige producten. Het herkennen en aanpakken van deze inherente uitdagingen en kennistekorten bij het integreren van alternatieve bioafvalgrondstoffen is van cruciaal belang voor het verbeteren van de efficiëntie van de circulaire bio-economie.

Deze studie omvat een uitgebreide focus op drie belangrijke aspecten: bruikbaarheid, haalbaarheid en veiligheid in de circulaire bio-economie. Het eerste doel is het identificeren van de kritische stappen, beslissingen en risico's die de veiligheid en circulariteit van de waardeketens van bioprocessen aanzienlijk beïnvloeden. Als tweede doel probeert het onderzoek de haalbaarheid te beoordelen van het gebruik van industrieel afval als grondstof voor synthetische biologie, met name bij het genereren van hoogwaardige producten. Ten slotte probeert de studie voorlopige Safe-by-Design-principes voor te stellen die in de gehele waardeketen van de circulaire bioproductie kunnen worden geïmplementeerd. Door deze belangrijke aspecten aan te pakken, wil het onderzoek bijdragen aan een alomvattend begrip en verbetering van veiligheid en circulariteit in de context van bioproceswaardeketens.

Deze studie maakt gebruik van een alomvattende methodologie, omvat een grondig literatuuronderzoek, voert interviews uit met belanghebbenden en demonstreert experimenten met een ontwikkelde Pseudomonas putida-stam voor de transformatie van Soda Lignine, een bijproduct uit de pulp- en papierindustrie, in curcuminoïden.

Deze aanpak, die een mix van methodologieën omvat, biedt diepgaande inzichten in onze drie hoofddoelen. In eerste instantie lag onze focus op het identificeren van de cruciale stappen, keuzes en risico's die van invloed zijn op de veiligheid en circulariteit van de waardeketens van bioprocessen. Dit bracht met zich mee het onderzoeken van het afval als grondstof, wat een alomvattende evaluatie van de gehele waardeketen en aanverwante ketens vereiste. Factoren zoals economische haalbaarheid, kwaliteit, betrouwbare levering, regelgeving en publieke perceptie spelen een rol bij het selecteren van een grondstof.

Het kiezen van de juiste biokatalysator is van het allergrootste belang, waarbij rekening wordt gehouden met economische overwegingen in efficiëntie die vergelijkbaar zijn met die van de olie- en gassector. Tegelijkertijd, veiligheids- en beveiligingskwesties worden aangepakt die in verband staan met het houden met genetisch gemanipuleerde micro-organismen.

Nieuwe fermentatieprocessen vereist de optimalisatie van de technische efficiëntie, de hulpbronnenefficiëntie en het gebruik van grondstoffen. Bovendien is het evalueren van het eindproduct essentieel om ervoor te zorgen dat de biotechnologieën die in het proces worden gebruikt de integriteit van het eindproduct niet in gevaar brengen. Dit omvat een rigoureuze beoordeling van de veiligheid, recycleerbaarheid, afbreekbaarheid, naleving van lokale wettelijke vereisten en integratie in lokale productiesystemen. Ten slotte keert het afvalbeheer terug naar de eerste stap, waarbij overwegingen over de economische levensvatbaarheid van het geproduceerde afval worden overwogen, waarbij wordt gepleit voor een duidelijk onderscheid tussen afval en bijproducten, en het delen van de verantwoordelijkheid voor scenario's aan het einde van de levensduur. Het aanpakken van al deze vijf stappen is van cruciaal belang voordat een nieuw proces kan worden geïmplementeerd. Alleen door deze stappen gelijktijdig aan te pakken kan de veilige implementatie van nieuwe technologieën in de industriële biotechnologie worden gewaarborgd.

Bovendien gaat dit onderzoek dieper in op de beoordeling van de haalbaarheid van het gebruik van industrieel afval als grondstof voor synthetische biologie om hoogwaardige verbindingen te produceren. Als case study gebruiken we Soda Lignine als “afval” grondstof voor de bioproductie van curcuminoïden. Door de monomeren die vrijkomen bij de fractionering van lignine te assimileren kon onze Pseudomonas putida-stam de productie van curcuminoïden verbeteren. Bovendien brengt de studie variaties in afvalkenmerken aan het licht en worden de uitdagingen benadrukt die geassocieerd zijn met wettelijke etiketten op afval. Deze uitdagingen hebben een aanzienlijke invloed op de economische haalbaarheid van het selecteren van specifiek afval voor een bepaald proces. Verder onderzoek is nodig om specifieke overwegingen te onderzoeken en het gebruik van afval te bevorderen.

De Safe-by-Design-principes in een vroeg stadium voor een volledige waardeketen voor circulaire bioproductie omvatten overwegingen zoals het holistisch bekijken van grondstoffen, het optimaliseren van de integratie van biokatalysatoren in zowel economische als sociaal-technische systemen, en het delen van de verantwoordelijkheid voor de duurzaamheid van de productlevenscyclus door het gebruik van diverse instrumenten en expertise.

Onze bevindingen brengen verschillende dilemma’s en uitdagingen aan het licht in de waardeketen van circulaire bioprocessen, waarbij de complexiteit van het probleem wordt benadrukt. Met name de

uitdagingen in verband met de terminologie en classificatie van afval, de (voor)behandeling van afval, de publieke perceptie, kennistekorten in het onderzoek en technische en economische kwesties waren groter dan de zorgen in verband met het gebruik van genetisch gemodificeerde organismen of mogelijke interferentie door residuen in de productie van afval. agrarische of industriële zijstromen. Ten slotte hebben we inzichten en aanbevelingen gegeven om de integratie van Safe-by-Design-principes in een circulaire bio-economie workflow te begeleiden, waarbij we de veelzijdige aard van deze uitdagingen erkennen.

II Introduction

II.1 Relevance of Safe-by-Design for circular bioeconomic workflows

In order to transition towards a circular economy, challenges include increased carbon efficiency, divesting from virgin fossil feedstocks, and developing an integrative approach between industrial production and end-of-life waste management.[1,2] A key role to this transition plays the industrial biotechnology sector, which harnesses microorganisms and enzymes to convert agricultural or industrial feedstocks into valuable products.[3,4] In this context, synthetic biology approaches have the potential to revolutionise industrial biotechnology. By implementing high-throughput genome engineering and model-driven designs, synthetic biology provides pathways for process developments previously deemed impossible, non-scalable, or economically non-viable.[5–9]

In the recent history of industrial biotechnology, microbial production predominantly relied on defined, costly, and often unsustainable feedstocks like glucose.[10] Such practices, grounded in a linear economy, necessitate change. The spotlight is now on harnessing synthetic biology to engineer microbes capable of utilising non-conventional feedstocks, predominantly waste side-streams from agriculture and industry (second-generation feedstocks and beyond). These abundant yet under-exploited resources, when transformed into high-value products, herald a dual benefit: environmental pollution mitigation and waste reduction.[1,11,12] This seamlessly aligns with the concept of circularity, converting waste into valuable products in line with circular economy principles. Circular practices in industrial biotechnology involve continuous recycling and repurposing of materials, establishing a closed-loop system that minimises waste and optimises resource utilization. This paradigm shift towards circularity underscores a commitment towards sustainability, fostering a more resilient and environmentally friendly bioproduction landscape.

However, the journey is not devoid of challenges. The utilisation of these variable-quality feedstocks presents safety concerns. Specifically, how do feedstock fluctuations and contaminants impact the reproducibility and safety of microbial cell factory processes? Ensuring product safety, avoiding unwanted toxic by-products, and determining the optimal method for biomass disposal or reuse post-cultivation are all paramount.[13–15]

Amid this complexity, the “Safe-by-Design” (SbD) principle stands out as a fundamental concept. SbD is a proactive approach that seamlessly integrates safety and risk management considerations into the design and development phase of new products and processes. This integration encompasses the entire chain and involves stakeholders, incorporating their perspectives on Responsible Research and Innovation (RRI)

aspects. The overarching goal of this pre-emptive framework is to prevent harmful consequences in the future. Recognising the interconnectedness of safety and sustainability, especially against the backdrop of climate change, has evolved SbD into "Safe-and-Sustainable-by-Design" (SSbD) in some contexts.[16–21] While some sectors have embraced these ideals, there remains a pressing need for a more widespread acknowledgment and seamless integration of "sustainability" as a core and inherent quality, thereby reinforcing the crucial role of environmental responsibility in the design and innovation process. While we acknowledge this interconnectedness of the two aspects, and even though sustainability will be a recurrent topic throughout, this report was envisioned and has been elaborated from an initial SbD standpoint.

The ambition of the EU's Green Deal, aspiring for a zero-pollution environment and a circular economy, aligns seamlessly with the SbD and SSbD approaches.[22,23] Alongside, the Dutch government is committed to realising a fully circular bioeconomy by 2050.[24] While technical nuances, like the choice of host organisms or energy sources, can be addressed through collaborative efforts between scientists and industry stakeholders, broader issues require a more diverse dialogue involving ethicists, ecologists, and policymakers. For instance, the origin of renewable feedstocks and their societal implications necessitate holistic discussions. Creating awareness for SbD principles remains a great challenge. Prior studies have indicated a marked lack of responsibility and care among stakeholders, attributes crucial for the success of the SbD strategy.[16,25–28] While tools and models have emerged in recent times to embed SbD principles from product conceptualisation to manufacturing[29–31], a comprehensive model addressing a variety of value chains in industrial biotechnology processes, including end of life scenarios remains to be formulated.

In addressing the prevailing challenges of integrating safety into research, the existing frameworks have often fallen short of providing tangible, experimentally validated methodologies in the context of biotechnological innovations and processes. There remains a gap between the conceptual underpinnings of SbD principles and their concrete implementation across an entire bioeconomic value chain. With this study we endeavour to bridge this gap by linking the SbD framework to a hands-on experimental showcase, examining the major steps phase of a process value chain.

II.2 Bridging theory and practice: SbD in an experimental contexts

In an effort to bridge the theoretical SbD principles with experimental applications, our study delves into a comprehensive exploration evaluating the applicability of the SbD framework[17] within a lab-scale experimental context. Going beyond theoretical considerations, our focus actively engages with the practical implementation of SbD choices, assessing their effectiveness in a real-world application scenario. To achieve this, we leverage one of our research lines within the Biomanufacturing and Digital Twins division of the Bioprocess Engineering chair group at Wageningen University & Research. As an academic

research group in biomanufacturing, we focus on developing bioproduction strategies and more sustainable bioprocesses, which serve as suitable subjects for SbD analysis. The chosen research line illustrates a bioeconomic workflow that spans from feedstock to product and value-added waste, providing an ideal case for bridging SbD theory with day-to-day biotechnology practices. The term bioeconomic workflow is often used in the context of bioprocessing and industrial biotechnology, where the goal is to optimize both biological and economic aspects of the production process. In this report, we refrain from investigating and discussing purely economic considerations, but still refer to our case study as bioeconomic workflow. This is because it encompasses the entire process from the selection of raw materials (such as feedstocks) to the final production of valuable products, and because it works as a lab-scale example of biotechnological efforts towards the bioeconomy.

Using this case study, our emphasis is on elucidating critical stages, inherent risks, decision-making paradigms, and challenges involved in integrating safety and circularity across the bioeconomic workflow, from feedstock sourcing to product end-of-life (Figure 1).

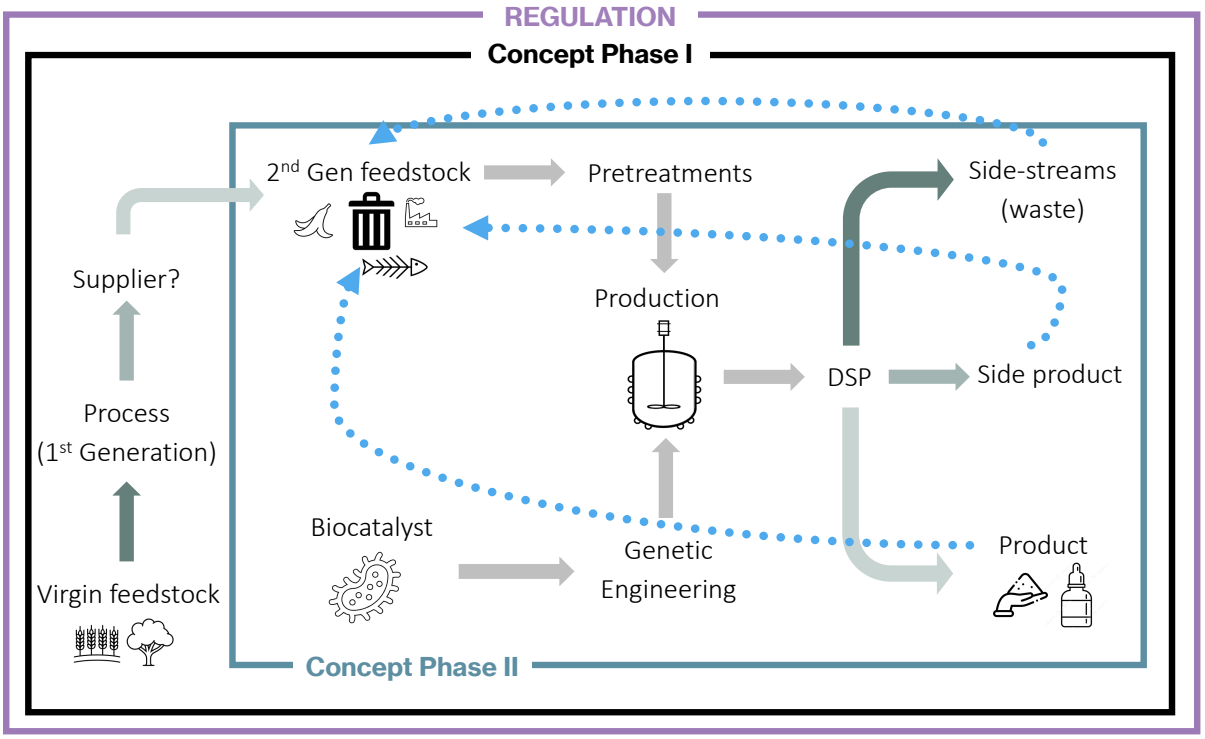


Figure 1. Simplified scheme of a (circular) bioprocess workflow. Depiction of the various bioprocess stages, where choices need to be made and risks identified, especially regarding waste valorisation, reusability/ recyclability or side-streams, end-of-life scenarios of (by-) products. On top of the entire process are the regulatory confinements that need to be established for new innovations and followed by the respective industries that want to apply these.

The trajectory begins with the selection of plant-based sustainable second-generation feedstocks, particularly from agricultural and industrial waste streams. We explore various feedstock pre-treatment

options and identify a suitable biocatalyst. The discussion includes synthetic biology engineering methodologies to facilitate the production of high-value added products from waste-stream derived feedstock. A fermentation process is followed by downstream processing (DSP), where we critically examine DSP treatments, solvents used, and the resulting wastes or side-streams. To achieve a truly circular process, the recycling or valorisation of most side-streams is essential, which may involve serving as feedstock for another bioproduction process, finding material applications, or producing a by-product that can be purified for other purposes. This approach also incorporates the safe disposal or re-use of bacterial waste. The workflow concludes with the synthesis of a high-value aromatic compound. While we underscore the integration and application of SbD principles in laboratory workflows, it is essential to acknowledge the study's limitations. Notably, our investigation did not delve into the safety nor into the sustainability aspects encompassing social and economic dimensions. While, these facets are integral to a comprehensive SbD approach, which is holistic in nature, this research provides valuable insights into the challenges that can be addressed in a research-lab setting. This focus on practice helps situating what decisions are possible for scientists in the bigger picture of SbD.

II.3 Research question and aims of the study

This study aims to bridge the gap between theoretical ambitions and the practical reality of incorporating Safe-by-Design values throughout a circular bioproduction process.

Central research question

> What are the essential challenges that must be addressed to successfully integrate Safe-by-Design into an actual circular bioeconomic workflow using a synthetic biology approach for valorising industrial wastes to a value-added product?

Specific aims of the study

1. Make an inventory of the **critical steps**, **choices** and **risks** that affect safety and circularity of the various steps in a bioprocess value chain.

2. Investigate the **feasibility** and **usability** of (industrial) waste as feedstock for synthetic biology applications to produce high-value added compounds.

3. Formulate **recommendations** (lessons learned) on how to embed Safe-and-Sustainable-by-Design principles in an early stage and throughout an entire circular bioproduction value chain.

A note on the research approach of this report

In this section, three streams of data collection are presented: literature review, interviews and experimental showcase. The streams complement each other in that the literature review gives us a state of the art, that not all practitioners may be aware of. In turn, literature reviews may miss important tacit knowledge on SbD that are then gathered throughout the interviews. The sources of this information come from different specific practices within industrial biotechnology. These are then contrasted with a real-life experimental showcase, as a site of reflection for SbD in the practice of a bioprocessing university research laboratory. In section III, the methods for each stream are described.

Subsequently, results are presented in section IV as five main critical steps along with a reflection on their implications. As Figure 1 above demonstrates, there are many more steps than these five where decisions for SbD are relevant. As a result of this research, we selected five critical steps not only where impact can be made at the level of practice of a research laboratory, but also where sufficient literature and attention in interviews was given to these issues. This focus is the result of an iterative process considering the different types of data collected, as well as group discussions reflecting on the data.

Finally, there are some salient overarching issues where we find better coordination and communication would benefit implementing SbD that we present in section V.

III Methodology

III.1 Literature review

This literature review intended to address the main research question of this report by identifying and making an inventory of critical steps, choices and risks in the production workflow of plant-derived aromatic compounds through microbial conversion from the viewpoint of product and process safety.

The universal procedure of (i) formulating the problem, (ii) searching the literature (used databases: google, google scholar, Science Direct), (iii) screening for inclusion, (iv) assessing the quality, (iv) extracting the data and (v) analysing the data was followed, to present a combination of a narrative and developmental review for this interdisciplinary study.[32] First, the procedure was followed on the general topics and terms of Safe-by-Design/ Safe-and-Sustainable-by-Design, circular economy, bioeconomy, bioprocess value chains, and second, on the steps of a bioprocess workflow. Next, we expanded by going further to the more specific parts of the experimental showcase that dealt with the valorisation of Soda Lignin to curcuminoids using a genetically engineering *Pseudomonas putida* strain. Problem formulations linked to the sub-topics and keywords used for the literature study can be found in Annex III (Literature study details). The literature study was carried out between 20-09-2022 and 12-10-2023.

III.2 Stakeholder interviews

To complement our literature review and its objectives, we resorted on models for reflexive research by engaging stakeholders involved in various phases of the process. In this approach, stakeholders critically examine and reflect on their own role, assumptions, biases, and the influence of their presence on the research process. Their intervention helped us identify and make an inventory of critical steps, choices and risks that affect safety of the processes, study the disposal of waste and the safety aspects of reuse of the waste after bioreactor operation, and explore different feedstocks and their fate as well as that of the biocatalysts. These interviews were also instrumental for the formulation of recommendations included at the end of this report.

III.2.1 Recruitment of interview participants

Prospective participants were identified based on their roles as direct stakeholders in industrial biotechnology and the circular bioeconomy, spanning three key categories: academia (A), industry professionals (I), and regulatory authorities (R).[33] These categories of stakeholders are selected because they have a direct influence in implementing SbD. More stakeholders that are typically qualified as indirect

stakeholder are relevant to SbD but are beyond the scope of this study. An effort was made to find participants from the industrial sector, with biotech firms being sourced through online searches, recommendations from Bioprocess Engineering colleagues at Wageningen University & Research (WUR), and referrals from the Ministry of Infrastructure and Water Management (IenW) and the Dutch National Institute for Public Health and the Environment (RIVM) representatives. Additionally, the professional platform LinkedIn, was instrumental in identifying potential interviewees. During the interview sessions, current participants were also asked to recommend other potential candidates, using a snowballing technique to widen our reach. Of the 30 individuals approached (A=3, A/I=3, I=19, R=5) via email with a detailed project description and a consent form, ten (N=10) responded positively. Between 06-06-2023 and 17-10-2023, these interviews were facilitated online using the Microsoft Teams (MS Teams) platform. An overview of the study's respondents is presented in table 1. Our approach based on reflexive research involves self-awareness and acknowledgement of the interviewees' subjectivity and the limitations of the study derived from the size of the list of participants. More than a comprehensive analysis of the landscape, our stakeholder interviews aim to enhance the rigor and transparency of the literature research by considering the authors' position in shaping the study.

Table 1. Overview of interview participants. All companies are part of the food and fragrance (F&F) production sector, and two are as well producing biomedical or biochemical compounds. Only I5 uses GMMs in their process.

Sectors/Categories	**Area of expertise**	**Participant codes**
Academia/Industry	Biopolymer research	A/I1
Academia/Industry	Sustainable chemistry & technology	A/I2
Academia/Industry	Bio-based chemistry & technology	A/I3
Industry	Biotechnology innovation/Bacterial cell factories	I1
Industry	Bioprocess engineering	I2
Industry	Sustainable bio-based and chemistry innovation and technology	I3
Industry	Mixed culture fermentation for plant oil production	I4
Industry	Bio-based F&F production	I5
Regulator	Microbiology/ Food safety	R1
Regulator	Safety of substances and products	R2

During the period of this study, the project was discussed with several colleagues from academia who have shown great interest in the project, as well as in its transdisciplinary nature, incorporating natural, social, and health sciences into a humanities context, transcending traditional boundaries. During the discussions with senior colleagues (assistant and full professors) in the fields of biorefinery and synthetic biology/ metabolic engineering, interesting points were raised, of which some are included in the report findings and are marked as an external reference with E1 to E5.

III.2.2 Interview structure

Participants were invited for a one-hour semi-structured interview to delve into discussions about safety and SbD implementation in the biomanufacturing process, industrial biotechnology waste management, and circularity. A predefined set of questions (Annex IV) served as the foundation for these discussions, though interviewers had the flexibility to pursue follow-up questions or delve into areas pertinent to each participant's experiences. While the MS Teams programme was utilised for both recording and transcription, manual reviews were conducted to ensure the accuracy of transcriptions. To foster an environment of trust and honesty, participants' identities and their respective transcripts were anonymised.

Each interview began with an inquiry about the participant's background and their familiarity with safety measures within biotech manufacturing or research sectors. They were also questioned about their specific roles within bioeconomic workflows. The interview questions were framed around two primary themes related to the SbD approach: safety measures in the biomanufacturing process (spanning upstream, processing, and downstream), and safety evaluations regarding the approach of circular waste management and recycling of elements derived from a previous fermentation. The questions tailored for each participant were grounded in their expertise and their role in integrating SbD practices that underpin a circular bioeconomy. Participants first outlined key safety protocols relevant to their roles, and subsequent questions further probed these areas. Later, they shared their views on harmonising safety with sustainability of the bioprocess, particularly in waste management and promoting circularity.

III.2.3 Analysis interviews

The analysis of interview results followed a systematic approach. We began by categorising the interview questions (Annex V) and arranging them into primary tables in Microsoft Excel. These tables served as predefined categories, corresponding to critical steps of a value chain, streamlining the organisation of participants' feedback. These tables were then enriched with additional participant details, such as their specific sectors and unique identification codes (Table 1). Following this, the transcribed interviews were methodically sorted in Microsoft Excel, aligning responses with their corresponding categories. This methodology allowed us to classify interview data to the topics of interest, promoting coherent data management. We subsequently delved into reviewing pivotal responses, insightful remarks, and standout statements, specifically inductively relating them to key categories identified through desk research. Analysis of these statements led to the formulation of recommendations and insights designed to bolster the integration of SbD within circular bioeconomy workflows.

III.3 Experimental case study

To bridge the experimental and theoretical grounds of SbD implementation in biotechnology, we undertook a laboratory exploration focused on the production of an aromatic compound as a building block for industrial applications, using agricultural waste as feedstock. The specific showcase involved Soda Lignin, an industrial by-product from the pulp and paper sector, as our chosen waste material. This waste material stands out as one of the most prolific yet underutilized across several industries, particularly generated from the pulping process in the paper industry using the Soda-Anthraquinone process. To convert Soda Lignin into high-value products, we employed an in-house engineered *Pseudomonas putida* strain, thoughtfully chosen for its biosafety level-1 (BSL-1) status. The *P. putida* strain was kindly provided by our colleague María Martín Pascual, a PhD candidate within the Biomanufacturing and Digital Twins division of the Bioprocess Engineering chair group of Wageningen University & Research. The Soda Lignin was, in turn, generously supplied by Dr. Richard Gosselink from Wageningen Food & Biobased Research, BBP Biorefinery & Sustainable Value Chains. Its generation was therefore not considered as part of this research.

The rationale for selecting this showcase extends beyond its successful ongoing status in our research laboratory. It aligns seamlessly with the objectives and scheme of our workflow, which involves second-generation feedstock, microbial cell factories, and the production of aromatic target products along with the presence of by-products. This approach provided an insightful preliminary evaluation of the intricacies associated with incorporating SbD principles and aspects gathered in the literature study and the stakeholder interviews in a lab-scale bioprocess.

The strategic choices in waste stream selection, strain engineering, and process conditions were deliberate and are critical aspects that warrant attention. The use of Soda Lignin not only serves as a representative example of an abundant waste material but also addresses the dual challenge of waste reduction and the generation of high-value products. The chosen *P. putida* strain, being a BSL-1 organism, adds an extra layer of value, aligning with safety considerations in bioprocessing. Our focus on the production of aromatic molecules such as bisdemethoxycurcumin, demethoxycurcumin, and curcumin is significant given their therapeutic, dietary, and cosmetic applications. As the name suggests, we use this strain for showcase purposes. It is beyond the scope of the study to investigate the social and economic contexts of curcuma production. Like a lot of research done in university laboratories, the focus is on discovering possibilities. This report does not investigate the ethical justifiability of producing curcuma. The research team, however, is collaborating with an ethics of technology research project on this matter led by one of the co-authors of this report.

Looking ahead, as we move from lab-scale experiments to larger-scale endeavours, we foresee potential challenges and considerations. The successful conversion of Soda Lignin in a controlled laboratory setting prompts questions about the scalability of this process, potential variations in waste composition, and the broader applicability of SbD principles in an industrial context. These are key issues that could potentially be addressed in a follow-up study, as we transition from the insights gained at the lab-scale to the complexities of large-scale experiments.

In conclusion, this showcase not only exemplifies the practical application of SbD principles but also sets the stage for a more comprehensive understanding of the challenges and opportunities associated with scaling up sustainable bioprocesses. The chosen case study serves as a valuable starting point for further exploration and integration of SbD principles in the broader landscape of industrial biotechnology. While this study does not extensively explore the sustainability and socioeconomic aspects of bioproduction, it highlights, connects and puts into perspective relevant insights from literature and stakeholder discussions. At this point, it is worth mentioning that each biomanufacturing process is unique, and no broad guidelines can be derived from a single case study; however, critical steps can be identified in every value chain with the potential of being generally extrapolated to other cases.

▪ Showcase: Soda Lignin to curcuminoids by *P. putida*

Solubilising and depolymerisation of Soda Lignin

Lignin, an intricate aromatic polymer, must be depolymerised into accessible monomers for effective microbial valorisation of lignin-derived wastes.[11,34,35] *P. putida* has demonstrated the capacity to enzymatically degrade lignin polymers autonomously, harnessing them for growth, making it an ideal choice for lignin valorisation.[36,37] Additionally, our engineered *P. putida* strain can utilize aromatic molecules such as tyrosine, ferulic acid, coumaric acid, and caffeic acid for curcuminoid synthesis (María Martín Pascual, 2024, Manuscript in preparation). These aromatic compounds become available through further depolymerisation of the complex lignin structures.[11] Different depolymerisation strategies were considered and assessed. Given the resource and time constraints for this study, we adapted a mild-alkali extraction method to solubilise lignin, with prospect to fractionise the lignin for higher monomer availability to be used by *P. putida* for product synthesis.[38–40]

Assessment of usability of Soda Lignin as microbial feedstock

A series of experiments were carried out to analyse the composition and usability of the solubilised Soda Lignin for microbial product synthesis. Initially, the monomer content of the feedstock was assessed using high-performance liquid chromatography (HPLC), that allows to quantify molecules in solution by detection of certain wavelengths linked to the molecules chemical structure. This was followed by growth and

production experiments where the solubilised Soda Lignin was supplemented to the engineered producer strain. Subsequent HPLC analysis followed to quantify lignin-derived monomer utilisation and curcuminoid production.

IV Results, lessons learned and implications

This report examines the details of establishing a circular bioeconomic workflow, encompassing critical decisions at every phase. Based on a thorough examination of literature, policies, and guidelines, coupled with interactions with stakeholders and a parallel lab-scale experiment which covered some of the most crucial process phases, this research delivered a number of outcomes, including the interconnected nature of safety and sustainability. Safety, traditionally viewed as primarily focused on human protection, has expanded to include a broader definition that extends to environmental, social, and economic dimensions.

- **How to read this report section**

This results section will predominantly feature tables elucidating challenges at critical steps: (I) feedstock selection and pre-treatment choice, (II) biocatalyst selection, (III) fermentation process and downstream processing, (IV) product assessment and (V) waste management. These stages ultimately represent the critical steps in which major safety (and sustainability) decisions must be addressed. However, the most pressing challenges due to their novelty in research and only recent advancements and consideration, are the (less-defined) waste feedstock, their required pre-treatment and the needed optimisation of downstream processing (including side-stream separation, solvent recovery, etc.).

The results are presented as a combination of the literature study and the conducted interviews (referenced as codes, see Table 1) in text and tabular form. The critical choices, risks and/or challenges are summarised in tables in each section of a critical step in the biomanufacturing workflow. Interesting findings from the desk study are highlighted in framed textboxes. Results specifically related to the experimental case study are highlighted in the blue 'SHOWCASE' boxes and under VI.6 Lessons learned from the experimental showcase.

Lessons learned and implications (aim three) regarding the outcomes of the critical bioprocess steps are highlighted at the end of each subsection, with aims one (identifying critical steps, choices, and risks that affect embedding SbD principles) and two (investigating the feasibility and usability of waste valorisation approaches for biomanufacturing) of this project in mind.

IV.1 Sustainable feedstock selection and pre-treatment issues

The circular bioeconomy seeks to reduce waste and pollution.[41,42] Research into sustainable feedstocks focuses on exploring unconventional renewable materials, emphasising safety in feedstock selection, especially concerning environmental impact, product safety, and waste management.[43–46] Sustainable

feedstocks include gaseous emissions like CO_2 and solid organic materials such as plant residues.[45,46] Recently, microbial biomass has emerged as an innovative feedstock option.[47,48] Using these materials in industry can shift away from fossil-based chemicals, promoting a low-carbon economy, reducing resource depletion, and aligning with next-generation biotechnology goals of affordability, reduced freshwater use, and energy conservation.[46,49,50]

Safety evaluations of using intricate agricultural or industrial by-products as carbon sources are notably limited in scientific literature, driven by two primary reasons: (i) The ambiguous composition of these side-streams necessitates extensive chemical analyses and optimisation of often strenuous chemical treatments to prepare them for bioproduction. For some industrial companies, whose business identity does not evolve around a sustainability image, analysing and subsequently separating their waste side-streams for future reusability for a circular bioeconomy purpose has not been a top priority until now. Nonetheless, the trend is there to reuse and recycle their own side-streams (I1-I4); (ii) Researchers have primarily pursued the adaptability of these ambiguous feedstocks, especially low-energy molecules like CO_2, for fermentation. Their objective has been to demonstrate the potential of these materials to replace fossil-based production lines (proof-of-concept), relegating safety considerations for the time being (E2). For instance, in the case of lignin: pure lignin-derived monomers were initially added directly to the fermentation mixture (research by María Martín Pascual, manuscript in preparation, and Linger et al. (2014)[11]), instead of using an industrially derived lignin side-stream.

While safety concerns originally pinpointed on, for example, pesticide residues in the agricultural derived feedstocks or living contaminants, these seemed to be of less or even no concern due to the extensive washing and sterilising steps prior to biorefinery or fermentation processes (E1, I2, I4, I5). While these practices could raise, in turn, sustainability concerns related to the use of water and energy, the safety concerns have shifted towards the potentially aggressive solvents required for the pre-treatments and their accumulation as well as safe disposal. Hence, decisions on less hazardous solvents need to be based on further research to find more solutions, or to find the least aggressive methods and optimise those for less solvent usage (E1,A/I2,I3).

The principal safety apprehensions relate to the possible creation of toxic by-products during the biocatalytic process. Due to the variable nature of waste components, making precise predictions is a challenge (I3). It is essential to meticulously assess waste to discern viable carbon sources, chemical hazards and potential microbial hazards. Emerging computational tools (as *e.g.*, Life-Cycle-Assessment (LCA) software), unfortunately, cater mainly to specific pure carbon sources.[51,52] With most by-products currently used as fertilisers or animal feed, or still being placed into landfills or being incinerated for energy production, there is a pressing need for more research into leveraging these complex streams for

fermentative bioproduction of both higher-value and bulk products.[53–58] Table 2 lists choices of potential sustainable feedstocks with their safety concerns that would need to be addressed to sustainably valorise them.

Table 2. Choices and process risk concerns of various feedstocks. Listed are materials that were identified as sustainable, originating from agricultural or industrial side-streams, or declared as wastes and their main safety concerns.

Feedstock categories	Safety concerns & comments	Reference
Gaseous Emission		
• Syngas (CO_2, CO, H_2) • Prior electrochemical conversion of CO_2 to formate and methanol	Formation of toxic substances in the waste gas possible (*e.g.*, CO) Usage for bioproduction for industrial scale still unfeasible	59–63
Organic Materials		
• Plant biomass side-streams		
○ Agricultural	Harsh pre-treatments for lignin breakdown	64–66
○ Forestry	Harsh pre-treatments for lignin breakdown	67–69
○ Industry	Harsh pre-treatments	14,70,71
○ Food wastes	Presence of other organisms	54,55
• Material of animal origin		
○ Manure	Presence of other organisms	72,73
○ Food wastes	Presence of other organisms	55,57
• Microbial biomass	Possibility of transferring genetic material	74
• Sewage sludge	Presence of other organisms	75
• Municipal solid waste	Presence of other organisms Potential presence of heavy metals	,57,76

When selecting an appropriate feedstock for bioconversion, it is crucial to incorporate a thorough risk assessment. However, the primary components of such assessments typically focus exclusively on environmental safety.[77,78] The various risk categories to be analysed thoroughly when designing a circular bioeconomic workflow are outlined in Table 3. These categories can be clustered into distinct themes that highlight crucial considerations for ensuring safety, sustainability, and responsible decision-making throughout the biomanufacturing process. The themes are the following: (i) Feedstock characteristics (availability and stability); (ii) Resource depletion and environmental impact (non-renewable resource depletion, water withdrawals and consumption); (iii) Climate and atmospheric impact (climate change, ozone depletion); (iv) Ecosystem health (acidification, photochemical oxidation, eutrophication, ecotoxicity); (v) Land use and biodiversity (natural land transformation and occupation, biodiversity loss); (vi) Human health and safety (human health impacts); (vii) Risks to the microbial cell factories (microbial pathogens, microbial contaminants). Each category within these clusters contributes to a comprehensive understanding of the potential risks associated with different feedstock choices, enabling a more informed and responsible decision-making process in biomanufacturing. It goes without saying, that for a complete approach we would not only need to assess the industrial or agricultural side-stream for these categories,

but also the preliminary process from which these streams are originating from (*e.g.*, for our showcase, the safety and sustainability assessment of the pulp and paper industry processes and their possible use of virgin feedstocks). Additionally, it is important to note that socio-economic factors are intentionally excluded from this part of the study and therefore Table 3.

Table 3. Risk categories (environmental and economic) for feedstock choice. Relevant factors for SbD assessment for circular industrial biomanufacturing processes.[17,44,79-83]

Categories	**Details**
Feedstock availability	Dry weight of residual biomass flow as a resource, typically justified in metric tons per year.
Feedstock stability	Quality and quantity of the feedstock, steadiness in composition and distribution, and predictability over the long term.
Non-renewable resource depletion	Utilisation of non-renewable resources (fossil fuels, metals, minerals) that cannot be replenished within a relevant human timescale.
Water withdrawals & consumption	Water consumption and potential environmental impacts, such as warmer temperatures or water contamination contributing to hostile aquatic environments.
Climate change	Greenhouse gas emissions (CO_2, N_2O, CH_4) contributing to climate change by trapping infrared radiation, leading to extreme weather and sea-level rise.
Ozone depletion	Emission of chlorofluorocarbons (CFC) causing ozone layer depletion, resulting in hazards like crop damage, plankton impact, and risks to human skin and eyes.
Acidification	Elevated water and soil acidity levels impacting the ecosystem, potentially causing the death of local organisms, attributed to nitrogen oxide (NO_x) and sulfur dioxide (SO_2) presence.
Photochemical oxidation	NO_x and other volatile organic compounds contributing to photochemical oxidation reactions, forming ground-level ozone and potential smog development.
Eutrophication	Nutrient influx (nitrogen and phosphorus compounds) into water ecosystems leading to excessive aquatic vegetation growth, changes in aroma, toxic chemical generation, and aquatic animal death due to reduced dissolved oxygen (DO).
Ecotoxicity	A broad category capturing toxic effects of substances affecting the ecosystem.
Natural land transformation and/or occupation	Consequences of inappropriate land use practices, including soil erosion, diminished land availability, decreased soil fertility, habitat degradation, and fragmentation.
Biodiversity loss	Species decline and extinction driven by factors like ecotoxicity and land transformation.
Human health impacts	Various pollutants affecting human health, such as benzene (carcinogenic), lead, and methanol (neurotoxic), along with compounds like NO_x, SO_2, and particulate matter contributing to respiratory illnesses and premature death. In addition, biological entities like pathogens can also affect human health.

Microbial pathogens	Possibility of microbial pathogens in the feedstock material.
Microbial contaminants	Possibility of chemical or microbial contaminants in the feedstock material.

For each factor relevant to SbD, several check points and guidelines with criteria that the feedstock should meet are available to prevent and minimise these, either during the design phase of a new process or in the early lab-scale and pilot-scale stages.[17,69,78,82,84] For example, ensuring that spent microbial biomass is devoid of harmful components for further valorisation is paramount, particularly for food or feed applications. This assurance involves a range of techniques such as toxicological assessments which test the biomass for safety.[85] Heat treatments can deactivate microbial toxins, while chemical agents can neutralise harmful compounds.[86,87] Extraction and purification processes, using solvents and methods like filtration, remove potential contaminants.[88] Genetically modifying strains and controlling fermentation can reduce unwanted metabolites.[89] Advanced tools like HPLC and GC-MS identify and measure potential contaminants.[90] Regular microbiological tests ensure no pathogens are present, while sensory evaluations assess the biomass's quality and safety for consumption.[91,92] While these measures follow general regulatory guidelines, the chosen methods should be tailored to the specific intended use of the biomass and the specific risks of each feedstock and process.

SHOWCASE

Why using lignin as a sustainable carbon source for bioproduction is a great choice – and a challenge

Lignocellulosic biomass, primarily consisting of cellulose, hemicellulose, and lignin, offers a renewable alternative to fossil-based feedstocks without compromising global food security. While sugar-based feedstocks from plants like sugar beet and sugarcane currently dominate, there is a shift of focus from these first-generation sugars, which compete for human and animal consumption and possess environmental drawbacks, to second-generation sources like lignocellulosic biomass. Lignocellulosic biomass, especially lignin, an aromatic biopolymer, has a vast untapped potential. Making up 15-40% of the dry weight of lignocellulosic biomass, lignin is the most abundant aromatic biopolymer on Earth, with billions of metric tons produced annually, especially as a by-product from the pulp and paper industry.

Furthermore, agricultural and forestry residuals offer vast reserves of lignin, bolstering the promise of a circular bioeconomy. Although lignin is a prime candidate for sourcing fine chemicals and other valuable compounds, most of it is currently undervalued, with the bulk being used for low-value energy generation. A few challenges, like lignin's heterogeneous structure and its solubility issues, remain. Overcoming these usually requires harsh pre-treatment methods to optimise lignocellulose structures for further processing.[12,64,93–97] However, the shift towards leveraging lignin aligns with the broader goal of creating a green, sustainable, and safe feedstock source for various bioprocesses.

SHOWCASE

Technical lignins

Lignin extraction from lignocellulosic biomass requires diverse pre-treatment techniques such as Kraft, Lignosulfonates, and Steam Explosion. These techniques employ chemical, mechanical, or thermal methods, leading to lignins with varying properties. The extracted lignin's application scope depends on the pre-treatment method, with processes modifying lignocellulose structure for better accessibility to chemical or enzymatic agents. Lignins that underwent one of these processes are referred to as technical lignins and are mainly left as side-streams to be used for low-value processes (*e.g.*, combustion for heat generation).

Bio-based feedstock like lignin could potentially have a lesser environmental footprint compared to petrochemical feedstock, but a thorough underpinning encompassing tecno-economic analysis and life cycle assessment should be undertaken to shed more light in this comparison. For example, lignin production still contributes to GHG emissions, acidification, and other environmental issues. In the same vein, environmental impact and risks will depend on the nature of the chemicals used for pre-treating the feedstock (*e.g.*, SVHC classified or not). Lignin from pre-treatments can be categorised into sulphur-containing and sulphur-free groups. Kraft and sulphite lignin are sulphur-containing, while solvent pulping yields sulphur-free lignin. Sulphur-free lignin is environmentally preferable and versatile, free from odour, and suitable for multiple applications.[96,98–102]

Choosing the right pre-treatment for Soda Lignin: Mild-alkali extraction

In the experimental showcase, we used Soda Lignin, specifically Protobind 1000, as the feedstock, exemplifying a lignin product derived from these methods. Although Soda Lignin is often viewed as less desirable due to its high recalcitrance, it is a sulphur-free lignin. To fractionate the Soda Lignin into monomers for bioconversion, we opted for a mild alkali extraction method. This choice was motivated by its sulphur-free nature, which prevents lignin condensation, enhances material solubility, and eliminates the need for harsh acid treatments, making the down-stream processing and solvent recovery less hazardous. This approach also presents cost advantages when considering future upscaling.[103-108]

Since the biomass used in this study is made of wheat straw and Sarkanda grass, it differs from woody materials. Specifically, its unique properties make it easier and require less energy to break down. Therefore, a gentler alkali treatment, known as mild-alkali extraction, was chosen to separate the components of this biomass. This method is particularly effective for such plant residues and ensures that the lignin, a key component, remains in a form that is easy to dissolve and remove.[39,109] In practical terms, in our tests, the highest solubility of lignin was achieved in the mild-alkali method compared to the organosolv-acetone method.

From an SbD perspective, mild-alkali treatment is preferable because it is less aggressive than methods used for woody biomass. This means it might require fewer chemicals, less energy, and be less disruptive to the biomass's natural structure. Such a gentler approach aligns with sustainability principles by potentially reducing environmental impacts and conserving resources. Moreover, by avoiding the harsh conditions some other methods might require, it can also ensure a safer working environment for researchers and technicians.[110,111]

- **Implications on sustainable feedstock choice**

The project's central focus was to determine the viability and safety of using industrial or agricultural waste streams as novel bioprocess feedstocks in terms of technical feasibility, and safe alternativity. One primary concern was the potential for harmful residues, such as pesticides. However, our interviews with industry stakeholders dispelled this due to rigorous quality checks and thorough pre-treatment of waste-derived feedstocks before fermentation. There remains a question of who sets required limits for safety, and who should carry this burden of proof, which echoes much of the literature on SbD on distribution of responsibilities in such processes.[112]

A notable challenge highlighted by stakeholders was the nomenclature obstacles arising when a side-stream is labelled "waste". This nomenclature can be restrictive in valorising side-streams and requires coordination and communication between stakeholders, what has been coined as an alignment problem.[112] The project also emphasised the need for further research to make bio-based production from side-streams as economically competitive as fossil-based counterparts. Waste stream dependence and related stability of supply adds layers of complexity when considering scaling up waste-based production.

Public perception regarding waste-derived products was a concern, particularly in the Flavour and Fragrance sector (I5), demanding high-quality products, *i.e.*, stable and reliable products. This challenge can be at least technically surmounted with refined processes and advanced filtration methods. The nature of waste, whether liquid or solid, also influences the necessity and intensity of pre-treatment. While liquid waste like fruit remnants may not need pre-treatment, solid wastes like lignin demand extensive procedures. Despite lignin's abundance and industrial potential, which is the focus of this study, some waste streams may be economically unsuitable for large-scale production due to their size or complexity. This echoes lessons 3, 5 and 6 from the previous section, which become in this context particularly relevant.

In summary, to foster sustainable biomanufacturing using waste feedstocks, key areas of focus include regulatory adjustments, technological advancements, and in-depth feedstock understanding. Ensuring quality and safety with variable feedstocks hinges on stringent monitoring and control. These implications should be considered alongside the other implications for the other critical steps. Addressing them as a discrete step does not give the full picture.

- **Lessons learned on using wastes as feedstock**

1. Terminology and classification issues regarding "wastes" complicate their use as feedstock from a regulatory point of view. Regulatory hurdles hinder the establishment of circular bioeconomies, necessitating a re-evaluation of waste classification and use regulations.

2. Potential harmful residues in the feedstock affecting product quality remain a possibility. However, they are less of a safety concern than affecting the quality of delicate products, such as for the flavours and fragrances industry (taste, odour, colour).
3. More research on different kinds of side-streams to be used as feedstocks is needed to boost valorisation of these for a bio-based economy.
4. Public perception of products made from waste can be a concern in the industry.
5. (Economic) competition with highly efficient and optimised fossil-based processes remains a main challenge.
6. Dependency on fluctuating waste supply can be a risk to utilise this as main resource for an entire production workflow.
7. Quality control and monitoring are crucial for safety when using less-defined food feedstocks.
8. Clarification is needed on who is responsible for providing analysis and assessment of the value-added less defined side-streams.
9. The challenges of solid-waste utilisation require harsher pre-treatments and solvent optimisation.
10. Abundant resources like lignin may require extensive pre-treatment but offer long-term benefits.

IV.2 Choosing the right biocatalyst

There has been a great effort over the past decades to assess the safety of using microbes for production purposes, and while there are uncertainties over the usability of genetically modified microorganisms in the open environment,[19,113–115], their use in enclosed biotechnological industrial settings is regarded as safe by the scientific and industrial community (I1-I5). Nonetheless, proper handling, biocontainment, and classification of any microorganism, engineered or not, should be assessed thoroughly within the design stage of any new biomanufacturing process. The main risk categories to be evaluated when choosing a suitable biocatalyst are listed in Table 4. When considering these, host-organisms should be selected in order to avoid, or greatly minimise these risks.

Numerous guidelines governing the utilisation of genetically modified microorganisms (GMMs) within product manufacturing are outlined in a variety of regulations and directives. Regulations of GMMs employment linked to products are described in section IV.4 Product evaluation. Moreover, further details into the regulatory aspects concerning the handling of microbial residues are stated in section IV.5 Waste management. Based on the analysis of the interview results, it was observed that several interviewees showed apprehension regarding the public's perception of the products generated by GMMs and supported the establishment of regulatory systems about this issue (A/I2, R1, R2). According to one participant, the concerns about the potential hazards associated with genetically modified organisms

Table 4. Risk categories related to safety choices when selecting a host microorganism selection.[17-19,118]

(GMO) primarily originated from people with limited scientific knowledge about them (A/I2). Another participant confirmed the assertion that the public generally lacks comprehension of the role of GMMs in industrial processes and displays an apathy towards the subject matter (R2). These statements underline the literature findings where efforts are being described to eliminate the negative connotation towards synthetic biology and discussions about GMOs.[116–118] This statement also highlights that there are more than communication and education efforts involved in perception of GMMs. In contrast, one interviewee stated that the existing regulations are deemed adequate for managing role and potential hazards related to GMM utilisation in industries. Thus, another main goal of an approach such as the SbD concept, should be to ensure higher safety for products in order to increase public trust (I1).

Categories	**Details**
Pathogenicity and toxicity	Causing illness to other organisms, such as allergic reactions of the novel food derived from GMMs.
Possibilities of spread of modified microorganisms to the environment	Persistence, invasiveness, and unintended effect of non-target organisms → changing the balance in ecosystem Gene pool contamination → changing the existing genetic make-up of population that affect biodiversity
Horizontal gene transfer	The ability to pass on genetic material between individuals. This can affect biodiversity and have unintended consequences, like anti-microbial resistance.
Genetic safeguard availability	Availability of existing genetic safeguards to prevent unintended host microbes spreading into the environment (biological isolation) and genetic material transfers between organisms (genetic isolation), like recoding, auxotrophy, etc.

SHOWCASE

A versatile and safe choice for the bioindustry: Pseudomonas putida

The soil bacterium *P. putida* boasts a remarkable stress tolerance, high adaptability, and the capacity to bioremediate toxic compounds.[119-121] The resistance of this microorganism to toxins and its capacity to thrive on a wide range of substrates enhance its potential as a promising candidate for industrial applications in biofuel and biochemical production from less-defined feedstocks as industrial side streams.[122] *P. putida* KT2440 has become a key player in industrial biotechnology after the discovery of the full sequencing of its genome. It is considered safe for use in food additives, although it does not hold the "Generally Regarded as Safe" or GRAS status. Instead, it is classified as host-vector system safety level 1 (HV1), meaning that it is safe to work with in certain facilities without extra precautions. This safety is ensured by the absence of factors that cause illness.[123] Ultimately, *P. putida* is a preferred choice to be used in (contained) fermentation processes.

P. putida strain KT2440, renowned for its well-studied physiological and metabolic traits, as well as the possibility for domestication, offers promise for lignin valorisation.[37,120] In the experimental part showcasing curcumin production, we employed an engineered *P. putida* KT2440 to produce curcumin from lignin. An in-house *P. putida* strain that was genetically engineered to utilise lignin-derived monomers (such as ferulic acid, caffeic acid, L-tyrosine) for curcuminoid production, was used for showcasing lignin valorisation. Genetic modifications encompassed the deletion of several native genes, gene mutations and overexpression, and the introduction of heterologous genes. These genetic modifications target the Shikimate, the aromatic amino acid, and the curcumin degradation pathways (María Martín Pascual, 2024, Manuscript in preparation).

- **Implications on biocatalyst choice**

Utilising biocatalysts, whether engineered or natural, for sustainable bioproduction presents a set of challenges that need thorough assessment. However, after discussions with all interview participants and colleagues, the primary concerns were less about safety and more about the technical challenges that need to be addressed first. Firstly, the competitiveness of bio-based production against established oil and gas industries poses significant feasibility questions. These industries have matured processes and vast infrastructure that biotechnological approaches need to match or surpass.

Secondly, the public's perception of GMOs weighs heavily on the success of biocatalytic methods.[125–128] Asveld et al. (2019) highlight five themes that must be addressed and even more so when engaging with

the public on industrial biotechnology innovations: sustainability, naturalness, innovation trajectories, risk management and economic justice.[129]

Introducing SbD principles can be pivotal in this context. Adopting SbD ensures that safety concerns related to GMMs are addressed from the outset of process development. A detailed examination of SbD could be beneficial in bolstering public confidence and in elaborating the responsibility of the different parts, including researchers and developers.

Selecting the right microorganisms for bioconversion is crucial. The ideal candidate would specialise in processing targeted aromatic substrates, be genetically controllable, and be resilient to the stresses of industrial bioreactors. *Pseudomonas* and *Rhodococci*, for instance, are prominent choices for lignin valorisation. Uncertainties about safety when choosing engineered biocatalyst need to be put into context: Is the fermentation happening in an enclosed, monitored, sterile environment? If yes, which is the case in most designed processes when utilising monocultures of GMMs, then the safety risks for workers and the environment are not higher or different than using non-engineered microbes.

General safety categories that should be assessed in the design phase when choosing a suitable biocatalyst, apart from its technical requirements, were presented in Table 4. The showcase study on *P. putida* underscored its minimal adverse effects, suggesting a low inherent risk, setting a precedent for future investigations.

At present, no insurmountable safety concerns hinder the bio-based economy's complete operation to utilise engineered or non-engineered host microbes, provided proper design assessments of the fermentation process are conducted beforehand.

- **Lessons learned on using and choosing biocatalysts**

1. Feasibility challenges arise due to competition with the oil and gas sector.
2. Public perception, heavily influenced by the internet and social media, can both support and impede the adoption of biotechnologies.
3. SbD principles have the potential to bolster trust and mitigate risks.
4. Choosing the right microorganisms is pivotal for effective bioconversion.
5. Regulatory frameworks offer guidance on how to choose a safe biocatalyst but need backing by comprehensive research data on risk assessment and feasibility.
6. Proper safety protocols are paramount, and preliminary studies show promise for certain organisms like *P. putida*.

7. Proactive safety evaluations may enable the growth of a sustainable bio-based economy.
8. Using non-GMOs simplifies their repurposing (*e.g.*, as protein source in animal feed) from a regulatory point of view.

IV.3 Safety of a production process: from fermentation to downstream processing

Biomanufacturing processes in the European Union are stringently regulated to ensure both environmental and human safety. The European Commission's guidelines on Good Manufacturing Practice (GMP) play a crucial role in standardising and ensuring the quality of biological products. Over the years, these regulatory bodies have thoroughly employed a comprehensive set of safety precautions, streamlining them based on evolving scientific evidence and technological advancements. As it stands, the current regulatory framework is deemed comprehensive, and there appears to be no immediate need for further regulation, ensuring that both producers and consumers benefit from the highest standards of safety and quality.[113,125–127]

The biomanufacturing process, particularly during fermentation and downstream processing, raises several safety concerns that require meticulous attention to ensure both worker safety and product quality. The safety concerns are presented in detail in Table 5 below. During the fermentation phase, the potential for microbial contamination is ever-present. Contaminating microorganisms can outcompete the production strain, leading to reduced yields and potentially generating toxic by-products that can compromise the safety of the final product.[128,130] Over-pressurisation in fermentation vessels is another concern; if pressure builds up without an appropriate release mechanism, it can lead to equipment rupture and possible injury.[131] In addition, the accidental release of volatile compounds during fermentation can pose both health risks to workers and fire hazards.[132]

Downstream processing, which includes steps like filtration, purification, and concentration, also has its set of challenges. The use of chemicals and solvents for product recovery can pose inhalation, skin contact, and flammability risks. However, the conditions in chemical industry are much harsher since microorganisms are grown under “moderate” conditions (temperature, pH, solvent usage).[133,134] There is also a risk of producing aerosols during processes like centrifugation and filtration, which might lead to respiratory concerns if inhaled. Inefficient separation processes might not effectively remove all toxins or contaminants, compromising product safety.[135,136]

Table 5. Risk categories of bioreactor processes which are highly monitored and regulated for large-scale production processes.

Category	Description	Reference
Contamination risk	Possible entry of foreign microbes compromising product purity.	130,141–143
Bioreactor overpressure	Build-up of gases or foaming.	144
Inadequate sterilisation	Insufficient sterilisation affecting product quality.	143,145,146
Handling of hazardous chemicals	Harmful chemicals being leaked, spilled, or improperly handled.	147
Biological aerosol release	Respiratory health risks.	132,148
Release of VOCs (volatile organic compounds)	Environmental and health risks.	132
Waste disposal	Environmental contamination.	149
Undesired by-products	Risks from toxic or reactive by-products.	150
Cross-contamination	Cross-contamination risk affecting product quality.	130,142,151,152
Operator exposure	Exposure to harmful chemicals, bioactive compounds, and pathogens.	

Equipment integrity is fundamental in both phases. Any equipment malfunction or breakdown can lead to leaks, spills, or uncontrolled reactions, which might expose workers to hazardous chemicals or result in environmental contamination.[125,137] Additionally, the high temperatures used in certain downstream processes can cause burns if there is direct contact.[138]

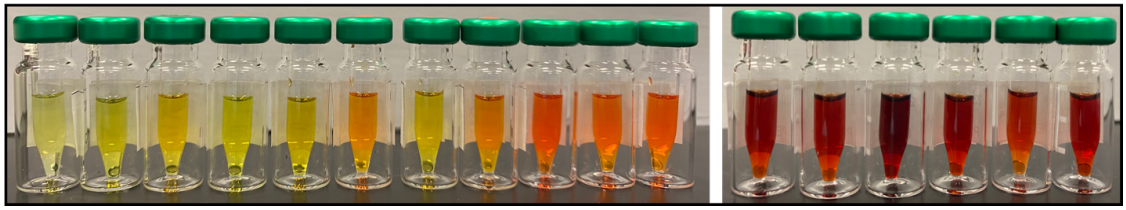
In summary, while biomanufacturing processes, especially fermentation and downstream processing, hold immense promise in various sectors, they come with several safety concerns that need stringent monitoring and control measures to protect both workers and the end product's consumers. The rules and guidelines, however, are well established, since fermentation with bioreactors in the context of modern biotechnology have been used since several decades (early 20th century).[139,140]

SHOWCASE

Curcuminoid production from lignin monomers

Successful lab-scale production experiments with Soda Lignin were conducted in 25 ml flasks, following significant challenges in finding a suitable pre-treatment to solubilise the Soda Lignin and release monomer structures. However, within this project's timeline, bioreactor experiments could not be executed. In contrast, collaborators from our group and in the US performed up-scaling bioreactor experiments using the *P. putida* curcuminoid production strain. A major difference there was, nonetheless, that instead of side-stream derived lignin, pure lignin-derived monomers like ferulic acid and coumaric acid, were used for the initial proof-of-concept experiments.

In those experiments, different *P. putida* strains were used to produce curcuminoids from lignin in bioreactors of 500 ml using fermentation volumes of 0250 ml per bioreactor. Different ratios of glucose and aromatic monomers (ferulic acid and coumaric acid) were employed as carbon sources: 1:0.5, 1: 0.25, 1:0.125, 1:0.0625, 1:0.03125. The reason to test different ratios was the fact that high concentrations of these aromatics can become toxic and decrease titers, yields and productivities. This can result in different concentrations of curcuminoids as can be seen in the following image, in which colour can be used as a proxy of product concentration for the purpose of this report.



Coming back to the experiments performed in 25 ml flasks, our *P. putida* strain cultivated in minimal mineral medium supplemented with 30 mM glucose and 3% alkali lignin produced 128 µM of curcumin and 160 µM total curcuminoids. In comparison, the *P. putida* curcumin-producing strain cultivated in minimal mineral medium supplemented with 30 mM glucose and 6% alkali lignin produced 123 µM of curcumin and 153 µM total curcuminoids.

Compared with *P. putida* cultivated without lignin, the productions were reported to be 3.72 (with 3% lignin) and 3.5 fold (with 6% lignin) higher. Curcumin titers were 10-fold higher in the cases of medium supplemented with lignin.

- **Implications on fermentation and down-stream processes**

Using biocatalysts, whether engineered or naturally occurring, in the pursuit of safe, circular, and sustainable bioproduction comes with its unique set of challenges. Safety and risk challenges linked to biocatalysts are well-studied. Fermentation, a cornerstone of this field, has been in practice for hundreds of years, tracing back to the early days of brewing and dairy production. The industry is subject to stringent regulations, particularly concerning hygiene. For products intended for direct consumption, such as food, feed, and cosmetics, a plethora of quality control assessments and continuous process monitoring are mandated.

Currently, open mixed fermentation processes, which do not involve GMOs, dominate the industry. Not using GMOs is less restrictive . An interviewee from the fermentation industry, which employs open mixed culture fermentation, highlighted the robustness of their system (I2). The system can handle varied waste streams, negating the necessity for a highly pure, homogenous carbon source.

When considering the potential adoption of GMOs, one must take into account tightly controlled environments that ensure minimal risk of generating unwanted by-products. This is largely because of engineering endeavours that aimed at ensuring the efficient use of the provided substrate by the biocatalysts.[153,154] Furthermore, when employing a biocatalyst like *P. putida*, capable of using a plethora of substrates (some potentially unknown), there might be an increased risk of by-product formation or reduced primary product yield.[155,156] But this risk is limited if unknown waste streams are subjected to preliminary testing before scaling up. The broader safety and risk considerations for industrial plants should be integrated within the SbD framework, ensuring holistic process assessment.

Furthermore, the pivotal challenges lie in optimising energy and chemical consumption, especially during pre-treatment, downstream processing, filtration, and extraction, even though these aspects are related to sustainability assessment and not necessarily to safety (E3). Industries, in turn, should focus on refining extraction methodologies and enhancing recycling potential, for instance, by reusing solvents (E3-E5).

- **Lessons learned on novel fermentation processes**

1. Safety and risk challenges with biocatalysts are well-understood.
2. The industry's longevity ensures experience with processes like fermentation.
3. Strict regulations guarantee quality and safety, especially for consumable products.
4. Open mixed fermentation processes are prevalent and versatile.

5. Engineering certain control measures aimed at biocontainment or cell self-lysis in GMOs minimise risks related to biological contamination.
6. Thorough preliminary assessment of feedstocks is essential.
7. Researching the composition of different types of waste can yield crucial insights and valorisation benefits.
8. Integrating an SbD framework may ensure comprehensive process scrutiny.
9. Optimising energy and chemical use, particularly in pre-treatment and downstream processes, is crucial.
10. The industry should intensify efforts to refine downstream-processing and recycle, emphasising resource efficiency.

IV.4 Product evaluation

Product safety and quality are at the forefront of considerations when it comes to bioprocesses. This is not only a concern for industries but crucial for ensuring consumer health and environmental protection (R2). In the broader context, the European Union has instituted key regulations. Relevant regulatory organisations are described in the information box "From waste to food, feed & cosmetics" which take care of evaluating an entire production process including the starting material.

When shifting focus to food and feed products, the ISO 22000:2018 is the gold standard for the food safety management system. These standard mandates rigorous measures to ascertain the safety of ingredients and processes throughout the production journey. It integrates key components like interactive communication, system administration, prerequisite programmes (PRPs), and the globally recognised hazards analysis and critical control points (HACCP) to mitigate food safety risks. Incorporating both HACCP and good manufacturing practices (GMP) has been highlighted as pivotal for upholding safety in expansive food systems.[126,157–159] Moreover, in managing the entire production process, quality control is vital, overseeing product quality at all stages and ensuring regular monitoring of equipment and facilities. The generic quality management system, ISO 9001:2015, complements these efforts, establishing consistent safety and acceptability standards for food-related products and services (R1).[157,160]

To provide a framework for food and feed products derived from GMMs, the European Food Safety Authority (EFSA) outlines specific risk assessment guidelines. Given the extent of GMM-derived products, EFSA classifies them into four distinct groups, with the information required for process modification approvals contingent on the specific product category.[161]

In summary, product safety and quality are overall highly regulated, monitored and assessed in pre-market tests. Two interviewees suggest that a thorough and long-term environmental safety analysis, following proper action to minimise harmful impact is still lacking in well-established industries. However, this is not for new bio-based processes, since these are being included upfront (I1, I2).

Evolva's vanillin production case (see highlight box next page) emphasises rather the challenges that persist in public reception of novel bio-based production to substitute unsustainable agricultural practices, and the socio-economic challenges that need to be addressed when obtaining products will not depend on production and harvesting in developing countries.[129]

Evolva's Vanillin

A controversial example that highlights the challenges of public perception and socio-economic issues

In 2011, Evolva, a Swiss company, pioneered a synthetic version of vanillin using engineered yeast. Contrary to the traditionally harvested vanilla from the vanilla orchid, which forms less than 1% of total vanillin consumption, Evolva's innovation promised superior cost-effectiveness. They positioned their variant as a sustainable and natural alternative with a quality edge over other synthetics. However, this assertion faced scrutiny from environmental groups who questioned its genuine naturalness and sustainability, given its derivation from highly engineered organisms. Additionally, there were apprehensions about the potential negative repercussions on the livelihoods of traditional vanilla farmers, who practice sustainable farming harmoniously with nature.[128]

While biotechnological advancements have the potential to revolutionise industries, they can also introduce complex challenges, particularly in the realms of societal and ethical dimensions. Such challenges, though not directly harmful to individual health, might influence broader aspects of human life, including economic stability and self-sufficiency.[161]

The Evolva scenario underscores the importance of a comprehensive perspective when assessing biotechnological innovations in food. It is essential to weigh not only the safety and ecological benefits but also the broader socioeconomic, political, and ethical ramifications that shape the trajectory of such novel products.

FROM WASTE TO FOOD, FEED & COSMETICS

Utilising waste for the bio-based production of food, feed and cosmetic supplements is encouraged by many countries.[22,162-164] However, its permissibility is contingent upon several regulatory considerations. Especially those concerning safety and quality standards that need to be addressed are an immense hurdle for companies and start-ups to start researching and valorising waste utilisation as feedstock (I2).

In the Netherlands, the use of waste for the bio-based production of food, feed and cosmetic supplements is subject to specific national regulations, which also align with overarching European Union directives and regulations.

For food applications

The Netherlands adheres to the EU's General Food Law Regulation (EC) No 178/2002, which sets out general principles for food safety. Waste-derived ingredients intended for food must be safe and should not deceive the consumer.[165]

Dutch national regulations on food safety are overseen by the Netherlands Food and Consumer Product Safety Authority (NVWA). They ensure that food products, including those derived from waste, are safe for consumption.[166]

Specifically, for waste-derived animal by-products, the EU's Regulation (EC) No 1069/2009 sets health rules concerning animal by-products not intended for human consumption. It classifies animal by-products into different categories based on the risk, and each category has specific allowed uses.[167]

For feed applications

The Dutch Commodities Act (Warenwet) sets requirements for both food and feed products. Waste-derived feed must adhere to these national standards.[168] In line with EU regulations, the Netherlands follows Regulation (EC) No 767/2009 on the placing on the market and use of feed. This regulation stipulates that feed, whether derived from waste or not, must be safe and should not have a direct adverse effect on the environment.[169]

FROM WASTE TO FOOD, FEED & COSMETICS

For cosmetic supplements

The Netherlands implements the EU's Cosmetic Regulation (EC) No 1223/2009 and Biocidal Products Regulation (BPR, Regulation (EU) 528/2012), which stipulates that cosmetic products must be safe for human health. Ingredients derived from waste streams are allowed in cosmetics as long as they meet the necessary safety criteria, and the manufacturing process ensures their purity and quality. The NVWA also oversees the safety of cosmetic products in the Netherlands, ensuring they comply with both national and EU regulations.[170]

Waste Management in the Netherlands

The Netherlands has a proactive stance on waste management and the circular economy. The Dutch Waste Management Act promotes recycling and upcycling of waste, which includes the transformation of waste into new products, such as bio-based food, feed and cosmetics. However, any product derived from waste and intended for consumption or topical application must undergo rigorous safety assessments.[171,172]

In summary, in the Netherlands, while waste can be used as a raw material to produce food, feed and cosmetic supplements, it must meet stringent safety and quality standards. The regulatory framework ensures that any potential risks associated with such products are effectively managed, guaranteeing the safety of consumers and the environment.

SHOWCASE

Curcumin

Curcumin is the primary bioactive substance found in the spice turmeric, which is derived from the root of the plant *Curcuma longa*. It has been consumed for centuries in Asian countries as a part of the daily diet and has also been extensively studied for its antioxidant, anti-inflammatory, anticancer, and various other therapeutic properties.[173-176] Moreover, curcumin is strongly associated with textiles because of its intrinsic bactericidal capabilities, making it a valuable natural dye for fabrics.[177] Despite its natural origin, curcumin may be produced involving non-natural procedures, including chemical synthesis (although its application in food areas is prohibited) and biotechnological processes employing GMMs such as engineered *E. coli* and *P. putida*.[178]

In general, curcumin is considered safe when consumed in the amounts commonly found in foods. The U.S. Food and Drug Administration (FDA) has granted curcumin Generally Recognized As Safe (GRAS) status as a food additive.[179,180] However, like any substance, curcumin might pose risks in certain situations:

- Dosage: High doses or long-term use of curcumin may cause gastrointestinal problems.[181]
- Interactions with Drugs: Curcumin might interact with certain medications, like blood thinners, which can increase bleeding risk. Always consult with a healthcare professional if considering curcumin supplements while on other medications.[182,183]
- Bioavailability: Curcumin has low bioavailability, meaning it is hard for the body to absorb when taken orally. Many supplements contain formulations like curcumin with piperine (black pepper extract) to improve absorption, but this can also increase the potential for drug interactions.[184-186]
- Allergic Reactions: Some people might be allergic to curcumin or turmeric.[187]
- Other Health Conditions: Those with gallbladder disease, kidney stones, or certain other conditions might be advised to avoid high doses of curcumin.[187]

In summary, while curcumin is generally safe for most people when consumed in culinary amounts, high doses or supplements might pose risks for some individuals.

SHOWCASE

Curcumin by P. putida

The curcumin produced from engineered *P. putida* is categorised as a type 1 product, given that both GMM and the introduced genes are not involved in the final product composition. Nevertheless, EFSA still requires some principal information for products falling under this category. For example, they require details about genetic modification, a comprehensive outline of the production process, and a thorough characterisation of the GMM.[160] Paoletti et al. (2008) reached the conclusion that there is a critical necessity for global harmonisation and standardisation of regulations concerning GMOs and GMMs in the context of risk analysis and assessment. This urgency is particularly pronounced in specific areas such as experimental design, data requirements, and data evaluation. It is essential that the production process aligns with the guidelines established by FAO/WHO and the EFSA.[188,189]

Since the curcumin product is derived from genetically modified *P. putida*, it falls outside the scope of the requirements outlined in the novel food law Regulation (EU) 2015/2283 (2005).[190] The subject matter refers to the regulatory framework of genetically modified food and feed as outlined in Regulation (EC) No 1829/2003 (2003).[191] The standing committee of the Food Chain and Animal Health (SCFCAH) conducted an evaluation on 24 September 2004 regarding the classification of products produced through the fermentation of GMMs in compliance with Regulation (EC) No 1829/2003. SCFCAH (2004) has determined that food products derived from GMMs are not subject to the Regulation (EC) No 1829/2003.[192] The only requirements are that the fermentation process must be contained and that the GMM should not be detectable in the final product. Given that the procedure fulfils both criteria, the curcumin produced from the genetically modified *P. putida* falls beyond Regulation (EC) No 1829/2003. When marketing this product, it is not required to be designated as a genetically modified product.

- **Implications on microbially produced compounds**

In the journey towards sustainable and circular bioproduction, microbial products offer an innovative approach. While microbes are fundamentally known to produce biodegradable compounds, their vast biochemical diversity allows them to generate a spectrum of molecules. This ranges from natural, short-lived substances to more persistent ones like specific polysaccharides, toxins, lipids, waxes, and even polyesters.[194–198] The advent of metabolic engineering further widens this spectrum, enabling the synthesis of increasingly synthetic and durable products. The nature of the product being produced determines the intricacies of its assessment. The paramount need, therefore, is to carry out a meticulous evaluation to ascertain the safety and sustainability of these products. Beyond just being non-toxic, the ideal end-

products should either be biodegradable or recyclable. This caution stems from lessons learned from past crises, such as the Dutch PFAS debacle of 2019 and the overarching global challenge of plastic waste management.[199,200]

From a regulatory perspective, the European Union provides a robust framework. Initially designed with an emphasis on safeguarding workers and consumers, these regulations have evolved, particularly with the introduction of the 17 Sustainable Development Goals (SDGs).[201] They now encapsulate broader considerations, including environmental protection and global societal impacts.

The safety profiles of compounds which have been integral to our food and flavour sectors for centuries, as the showcase of curcuminoids, regardless of their source – be it traditional plants or innovative microbes – remain unchanged in themselves.

In this report we do not address the social and economic implications of such products. This is not to say that they are not significant and present. As mentioned earlier in the report, the research team engages in these reflections via other on-going collaborations.

Another important social dimension is the public perception of microbial production, especially when it involves GMOs. While there is a case to be made about the alleged "naturalness" of microbial production when compared against the polluting fossil-based industries that use crude oil, gas and coal, which pose evident health and safety risks when handled improperly, the unease surrounding GMOs remains tangible.[202–204]

- **Lessons learned on product assessment**

1. Case-specific nature: product assessments depend on the specific end-product.
2. Microbes can produce biodegradable compounds, but they can also produce persistent substances. The advancement of the field is expanding towards synthetic products so it is important to have the degradability of those into account in the first place.
3. Proper evaluation is important: products must be safe, recyclable, or degradable to avoid crises like the PFAS event.[199,200]
4. Rigorous regulations: EU regulations stress safety, now extending to environmental and global societal impacts due to the 17 SDGs.
5. Holistic social safety assessments: localised production could disrupt traditional cultivators and food cultures in certain countries.

6. Public perception: despite potential advantages, public perception remains sceptical of GMOs and microbial production.
7. Certification concerns: while GMP+ ensures standards, "non-GMO" labels might be counterproductive.

IV.5 Waste management

This section mainly circles back to section IV.1 where sustainable non-conventional feedstock choices and their safety, sustainability and regulatory challenges are discussed, closing the loop as it is envisioned for a circular bioeconomy. A pragmatic aim should be to minimise and optimise waste/ side-stream valorisation as much as possible.[205]

This section presents findings on regulation, tools, and challenges to waste management from literature review and interviews. Regulation is essential for both fermentation and downstream processing because they generate a myriad of waste products, including biomass residues, spent media, volatile compounds, and other by-products, which need careful handling. Proper waste management is critical not only for environmental sustainability but also to ensure public and ecological safety.[206,207] Adhering to European Union regulations is essential for companies operating within the EU (Table 6).

Furthermore, interviews point to potential objections from the public and business actors regarding safety issues like toxic components in residual feedstocks and microbial activities call for strict compliance to regulation (A/I2). Due to the regulation burden, using waste streams can become a challenge in view of economic feasibility, premium on price of waste derived products, and the uncertainty in the supply chain (I1).

Table 6. EU regulatory frameworks either directly or indirectly relating to waste management.

Regulation	**Description**
Directive 2008/98/EC (2018) on waste[208]	This Directive aims to safeguard the environment and human health by minimising negative effects from waste generation and management.
Directive 2010/75/EU (2011) on industrial emissions (integrated pollution prevention and control)[209]	This Directive establishes guidelines for preventing and controlling pollution caused by industrial activities. Its objective is to minimise emissions into air, water, and land, as well as to reduce waste generation, with the ultimate goal of achieving a high standard of environmental conservation.
Council Directive 91/271/EEC (2014) concerning urban wastewater treatment[210]	This Directive focuses on managing urban and industrial wastewater collection, treatment, and discharge to protect the environment from any negative impacts caused by these actions.
Directive 2001/18/EC (2021) on the deliberate release into the	The purpose of this Directive is to accomplish lawful coherence among Member States and to protect the environment and human

environment of genetically modified microorganisms[211]	health in two scenarios: the intentional release of GMOs into the environment without employing tailored safeguards and placing GMOs or products containing GMOs on the market.
Regulation (EU) 2020/741 (2020) on minimum requirements for water reuse[212]	This regulation specifies the minimum criteria of water quality monitoring, as well as regulations on risk management, in relation to the secure utilisation of reused water within the integrated water management framework.
Council Directive 1999/31/EC (2018) on the landfill of waste[213]	The primary objective of this Directive is to minimise adverse impacts on the environment, including surface water, groundwater, soil, and air pollution, by implementing strict operational and technical waste and landfills throughout their entire life cycle.

Besides regulation, there are several tools available to help dealing with wastes. To ensure the long-term ecological and economic sustainability of biotechnological approaches, life cycle assessment (LCA) and techno-economic analyses (TEA) can be utilised to enhance output efficiency and reduce waste.[206,214] Additionally, these tools assist in measuring the environmental impact.[205] An innovative tool highlighted by an interviewee from the regulatory sector is the Safe and Sustainable Material Loops (SSML) approach, developed by the RIVM.[215,216] This tool leverages multi-criteria decision analysis to holistically evaluate a substance, especially concerning its safety and circularity based on its application. Comprehensive criteria, such as energy consumption, water, and land usage, are integral to this assessment (R1). Another interviewee highlighted the potential value of incorporating socioeconomic analysis to gauge broader public perceptions of bio-industrial processes (R2). A notable point of contention was the depth required for assessments like LCA. Rather than delving into intricate details of LCAs, which can be time-intensive, it may be more pragmatic to prioritise and define the most pertinent criteria for evaluation (A/I2, R1, R2).

Besides the main product and possible valuable by-products, the remaining part of a fermentation process is encompassed by side-streams, which are usually referred to as bioprocess waste streams (Table 7). The nature and quantity of these components can vary based on the specific bioprocess, the organism used, and the process conditions.[47,217,218] Proper management and potential valorisation of these side-products and side-streams are crucial for the economic and environmental sustainability of bioprocesses. Here the laboratory scale of our experimental showcase did not add further data, and analysis should be done on a case-by-case basis since actual fraction distribution values can be influenced by the factor mentioned above. However, the volume of the side-streams is seldom negligible as this fraction includes elements as the medium's water, gases, and often the biomass itself. Considering the water of the media as an example, the implementation of wastewater recycling represents a substantial investment for companies due to the need for significant infrastructure. Establishing an efficient wastewater recycling system involves the deployment of advanced technologies and the construction of tailored facilities, contributing to the overall

cost of the process. Table 8 consolidates the primary challenges identified from the interview sessions. These challenges require a holistic and thorough approach within the companies, and with further stakeholders who would be involved in the further valorisation of the side-streams. A guideline could serve as a valuable starting point to address these issues.

Table 7. Primary components of a bioprocess can be grouped as main product, side-product and side-streams (I1-I5). [47,218,219]

Components	Description
I. Main product	The primary desired output of the bioprocess. It could be a biochemical compound (like ethanol in fermentation) or a biological entity (like a specific protein or biomass).
II. Side-Products (By-products)	Compounds that are formed alongside the main product. These might be the result of secondary metabolic pathways or degradation of substrates. Examples include glycerol in yeast fermentation or acetate in bacterial fermentation.
III. Side-Streams	These are elements or compounds that are not directly part of the main bioprocess pathway but are crucial for its functioning or result from it.
a. Water	Often used as a solvent in many bioprocesses. Post-process, water can be present in the waste stream and might need purification or treatment before release or reuse.
b. Carbon Dioxide (CO_2)	A common by-product in many fermentation processes, such as ethanol production.
c. Oxygen and Nitrogen	Often used for aeration in aerobic fermentations or to maintain an inert atmosphere in certain reactors.
d. Heat	Bioprocesses are exothermic or endothermic, meaning they can produce or consume heat. Proper temperature regulation is essential for optimal functioning.
e. Salts and Minerals	Resulting from nutrient feeds and can be part of the effluent.
f. Unconsumed Nutrients	Substrates or nutrients that are not fully consumed by the microbial culture can be part of the waste stream.
g. Cells/Biomass	Post-production, the microbial or cell biomass used in the process needs to be separated and dealt with. In some processes, this biomass is the main product, but in others, it is a by-product.
h. Other Wastes	Metabolic waste products, residues from substrate breakdown, or any compounds that are not part of the desired products.

Table 8. Main challenges and risks in waste management from bioprocesses (I1-I5).

Category	Description
Terminology	
Waste, side-stream, residue, by-product, etc.	• Bias in what is declared as waste and what not, depends on the producer of the side-stream. • As soon as material is declared as waste, it has according to legislation, no further valorisation prospect. • Using waste side-streams as feedstock possess further an immense regulation and assessment hurdle for anyone attempting to valorise it further, which makes it less attractive to industry and researchers.
Safety aspects	
Toxic residues	Waste may contain compounds harmful to the environment or human health. Proper detoxification is essential.
Biological hazards	Risks associated with potential pathogenic contaminants (*e.g.*, antibiotics used in the fermentation process) or GMOs that may adversely affect local ecosystems if not handled correctly.
Sustainability aspects	
Resource loss	Failure to recover valuable compounds from waste streams leads to economic loss and greater environmental burden.
Intensive energy consumption	Some waste treatment processes, like incineration, require significant energy, contributing to a larger carbon footprint.
Water pollution	If untreated or improperly treated waste reaches water bodies, it can lead to eutrophication and other ecological imbalances.
Solid waste	Accumulation of non-degradable solid residues can contribute to land pollution.
Air emissions	Processes like incineration can lead to emissions of greenhouse gases or other pollutants if not properly managed.
Regulatory non-compliance	Failure to adhere to local, national, or international waste management regulations can result in legal repercussions and damage to the company's reputation.

▪ Implications waste management

Waste management, particularly in the bioeconomy sector, presents a multitude of challenges, as observed from a series of interviews and project discussions. A primary concern emerging from these conversations is the pressing debate surrounding the very terminology of “waste”. Many stakeholders argue for the re-evaluation and potential replacement of this term, especially when discussing value-added side-streams. There is a tangible risk in labelling potentially reusable or recyclable materials as “waste”, which could create regulatory barriers to their subsequent reuse.

Then there is the intricate matter of by-products, substances generated during production that are neither the desired output nor end-of-life waste. These have inherent value and further utilisation potential. The

indiscriminate use of the term "waste" in scientific publications, even when referring to direct by-products or alternative feedstocks, has led to confusion. This ambiguity, particularly within the circular economy framework, necessitates a clear distinction between product, by-product, and (value-added) side-stream and waste. Spent biomass, rich in proteins, fibres, and amino acids, is one such area where companies are actively seeking end-of-life solutions. Possible outcomes were mentioned as either supplementing the fermentation media with these nutrients, hence bringing this side-stream back to use in the production process, or as a well-suited supplement for animal feed. While the repurposing of spent microbial biomass as nutrient supplements is a viable strategy, stringent measures must be in place to ensure the inactivity or sterilisation of the cells. Safely repurposing microbial biomass requires thorough processes to deactivate or eliminate any viable microorganisms.

Nevertheless, among these considerations, a pivotal question remains: Who is responsible for evaluating these complex, mixed-material feedstocks? An interviewee (I2) from the industrial sector claimed responsibility for the waste their plant produces, emphasising their role in assessing and potentially marketing the by-products. Undoubtedly, the producers of the waste have the most comprehensive understanding of its contents. This insight, combined with financial motivations like repurposing or selling waste, could shape the future of waste management in this sector (I1-I5). However, the matter of responsibility appeared ambiguous to most interviewees. Beyond just assessing the feedstocks, the challenge of correctly segregating them for diverse applications persists. Here, we observe a knowledge gap with regards to what happens with these by-products and how this can, in turn, inform what characteristics we need from these by-products.

- **Lessons learned on waste management**

1. The redefinition of "waste" is crucial to navigate and address regulatory hurdles more effectively.
2. Distinguishing and separating "waste" from "side-product" is vital after a production process, as the two terms carry different implications for utilisation and regulatory compliance.
3. While companies take effort into emphasising circularity in their processes, their commitment to sustainability can be overshadowed by the economic viability of their operations.
4. Recycling usually requires substantial additional infrastructure, making it a significant investment for companies.
5. Spent microbial biomass can be safely repurposed as nutrient supplements when the cells are ensured to be inactive or sterilised.
6. Distinguishing (and technical separation) of final bioprocess waste from side-products is vital, as the two terms carry different implications for utilisation and regulatory compliance.

7. Exploring end-of-life applications for by-products, especially materials like spent biomass, is becoming an increasingly important aspect of sustainability.
8. Determining who bears the responsibility for evaluating and managing waste remains unclear for some of the involved parties, which might have implications for both business and regulatory environments.

IV.6 Implications on the experimental showcase

▪ The main limiting steps in designing the experimental showcase: time & resources

Designing an experimental study to explore a fermentation production process tailored to a specific research objective is a multifaceted endeavour. Its complexity and direction are intricately tied to the nature of the research question it aims to address. In our project, we set out to investigate two key aspects: (i) identifying the critical decision-making safety factors within a biomanufacturing workflow, and (ii) evaluating the viability and practicality of using agricultural or industrial waste as carbon sources in the biocatalytic production of high-value compounds.

Given our one-year project timeline, practical considerations were paramount. We opted to use a biocatalyst developed in-house, bypassing the extensive time and resources typically required for creating a new biocatalyst. This decision also sidestepped potential complications associated with external collaborations, such as the need for additional protocol adjustments when integrating a production strain from another group. Our approach faced two other primary constraints: finding and securing an appropriate feedstock that could be safe and sustainably sourced from industrial or agricultural by-products.

This project navigated various decision points throughout the bioprocess workflow, which we will delve into in greater detail in the following sub-section.

▪ Decision points during the experimental set-up

As previously stated, the specific focus of our study imposed initial constraints on how we addressed the research questions and objectives, which were primarily aimed at exploring the integration of SbD principles within a circular bioeconomy framework. Although we acknowledge the limitations of a lab-scale experiment in fully capturing the complexities of real-world scenarios, this study afforded us an opportunity to re-evaluate our conventional approaches to academic research.

Guided by the framework outlined in Figure 2, our decision-making process was informed by critical stages identified during our preliminary desk study. These decisions not only validated some findings and hypotheses from the earlier desk study and interviews but also highlighted areas we had overlooked or where alternative approaches could have offered deeper insights.

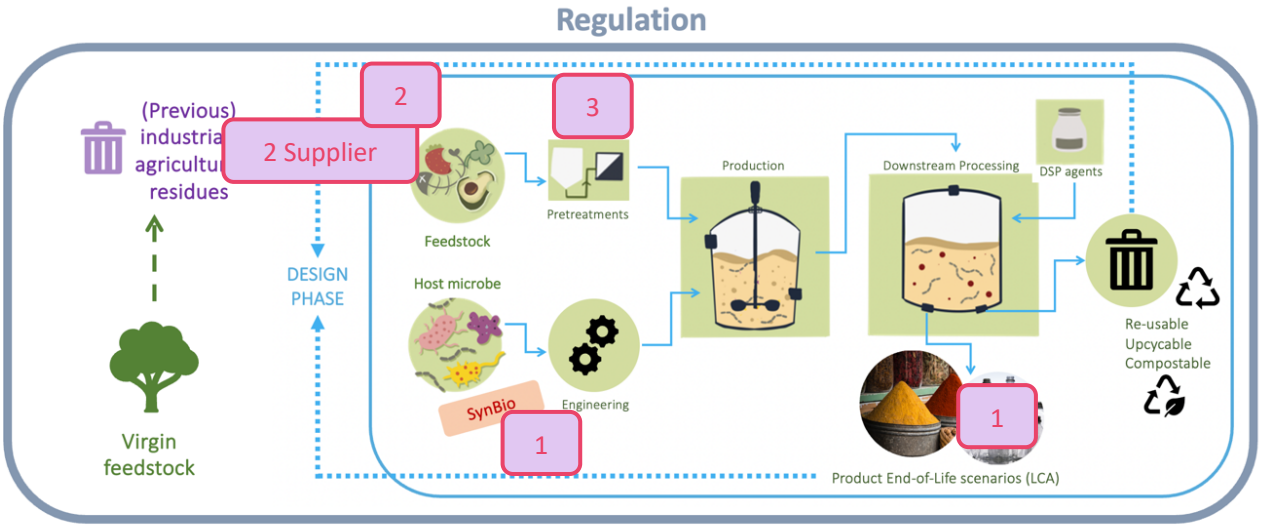


Figure 2. Decisions points during the experimental showcase study. Highlighted with the pink boxes (1 to 3) are the decision points along the bioeconomy workflow in the order we approached the project and which were touched upon. The main challenges were here perceived the most feasible and usable biotechnological production experiment that would give answers on the feasibility and usability of wastes/side-streams as feedstocks and on how to incorporate SbD principles in each step.

1. **Biocatalyst & Product**

   It is important to note that, given sufficient time, interest and resources, a more conventional approach to biomanufacturing would involve selecting the target product first, or, in the case of valorising waste streams, choosing the waste component to analyse its molecular makeup. This would help identify if there is another organism capable of efficiently breaking down and utilising these molecules.

   In the Biomanufacturing & Digital Twins group at Wageningen University, we have developed significant expertise and established a research trajectory focused on utilising and enhancing the soil bacterium *P. putida* (for more details, refer to the showcase box in section IV: Choosing the Right Biocatalyst). Given that this microbe is HV1-certified and we have in-house strains engineered for the production of various compounds, we found it most practical to leverage *P. putida* in our proof-of-principle research for integrating SbD principles into our workflow. Additionally, *P. putida*'s remarkable capacity to utilize diverse carbon sources for growth and production meant that we could feasibly replace glucose with an industrial by-product as our primary feedstock.

Since we were working with an in-house strain already known to convert lignin-derived monomers into food-grade products, the decision-making process was straightforward regarding the product. However, upon deeper examination of regulatory aspects and the potential social impact of displacing manual labour (a consideration when producing compounds like curcuminoids), we recognised that this might not be the most suitable case study. Curcumin, being a natural food ingredient, generally presents low safety and environmental sustainability concerns in moderate amounts. However, considering social and public perception, a product like polyhydroxyalkanoates (PHA), a naturally produced polyester, might have been a more appropriate choice due to its lower social impact and fewer regulatory barriers.

2. Feedstock/ Supplier

In practical scenarios, the choice of valorising industrial side-streams naturally comes before other decisions. In our case, we leveraged our network and expertise to identify a suitable industrial by-product for our study. Our criteria focused on finding a side-stream that was both abundantly available and underutilised for high-value chemical production, and which could be metabolised by *P. putida*.

Based on our experience with the in-house curcuminoid production strain, we knew that *P. putida* could synthesise curcuminoids from lignin-derived monomers such as tyrosine, ferulic acid, coumaric acid, and others. A critical factor in our selection process was the availability, timely delivery, and cost-effectiveness of acquiring the necessary lignin. Ultimately, we sourced the Soda Lignin not directly from a pulp and paper industry but from the BBP Biorefinery & Sustainable Value Chains research group of Wageningen Research. We received therefore a chemical information sheet with the main attributes of the Soda Lignin and its purity. Upon further readings we knew what kind of chemical transformations/ pre-treatments were performed beforehand to receive the Soda Lignin. Interestingly, this lignin originated from a pulp and paper industry in India. While ideally, bio-based industries should aim for more localised value chains, especially from an environmental perspective, the sourcing of Soda Lignin from India may contribute to social sustainability for the workers and the local industry there. By purchasing the by-product, it is plausible to assume that Wageningen Research indirectly supports the local economy and workforce in India.

3. Pre-treatment

This stage of the process offered us the most freedom in making safe and sustainable choices. However, having acquired Soda Lignin, we faced the necessity of employing chemicals to break down and solubilise it, making it accessible for *P. putida*. This requirement is common to most forms of

lignin, but Soda Lignin presents particular challenges, which underscores the importance of finding viable methods to valorise it (see showcase box in section IV.1 Sustainable feedstock selection & pre-treatment issues).

In devising our pre-treatment strategies, we prioritised methods that were well-established, utilised less toxic or irritating chemicals, and were relatively simple to implement, keeping in mind the laboratory equipment available at our facilities. Ultimately, we opted for a mild-alkali pre-treatment. Had there been more time and expertise available, we would have explored further optimisation to reduce chemical use, streamline the process, or discover other more sustainable alternatives.

▪ Embedding SbD in research

In the context of embedding SbD principles into our research, we have collected valuable insights into the intrinsic complexity of the decisions that need to be made. This complexity became evident even without reaching the stages of up-scaling, fermentation waste and side-stream separation and analysis. This project highlighted the necessity for a multidisciplinary approach when designing a biomanufacturing research project. It underlined the importance of effective communication, knowledge transfer, and the resolution of conflicts of interest when engaging with external stakeholders and experts.

The RIVM guide by Hogervorst et al. (2023) informing about safe, sustainable and circular designs for industrial biotechnology applications gave us a first understanding of the regulatory frameworks we would need to comply to in a “real-life-scenario”, and a primary idea about the critical stages where choices need to be made and assessments are necessary (Table 1). Indicators for safety, sustainability and circularity in an industrial biotechnology process’, RIVM guide).[17] It became clear that time and resources are major constraints in this realm, not only in terms of material resources but also in the availability of professional expertise. For example, there is a scarcity of professionals in the wider research community with the necessary skills and knowledge to perform assessments as TEA and LCA which should become apparent in the context of SbD goals.

Personal motivation among young researchers or the academic network also emerged as a critical factor: From a scientist's perspective, research often depends heavily on the expertise available within the group or institution. This reliance can inadvertently limit project designs, particularly in decisions like selecting a host organism, as we tend to rely on existing infrastructure, tools, and expertise. In this regard, it is crucial to further encourage and support researchers to challenge themselves to think creatively and step beyond the conventional boundaries.

Furthermore, it is worth noting that research projects are typically not designed to have SbD as the starting point. Generally, the starting point is a problem, such as the unsustainable and resource-intensive procedures involved in curcumin extraction from plant, which often involves cheap labour and extensive transportation across the globe, underscoring the need for more sustainable alternatives.

V Concluding remarks

The various streams of research in this project point to an inherent tension: while we seem to know a lot about safety issues, interviews reveal that compliance is the name of the game. This is because the pursuit of a safe and sustainable design is not yet profitable in these new processes. For this, tools can become incentivised, and regulatory definitions can create better incentives. This paradigm shift requires commitment from multiple stakeholders to ensure that sustainable, circular processes become as (economically) viable as traditional linear, fossil-based approaches.

Advancements in synthetic biology and metabolic engineering offer the potential to replace fossil fuels, utilise diverse feedstock sources and give hope that a shift towards a safe and circular bioeconomy future is possible. However, although microorganisms such as bacteria, fungi, and viruses have been utilised by humans for millennia, and fossil-based workflows are generally less safe and sustainable, safety and sustainability of bioengineered biocatalysts need to be further addressed in public debates.

▪ Terminology

Terminological challenges often accompany new technologies. Misunderstandings arise from outdated or inaccurate definitions, leading to regulatory issues for safety. In this research we encounter this challenge when it comes to defining wastes, ambiguity of the term natural, and polysemic nature of values of safety and sustainability.

The term “waste” generated significant debate in this study. Although organisations like the EU and UN advocate using waste-derived side-streams, regulatory frameworks lag, imposing stringent rules on companies looking to repurpose waste as feedstock. This has implications for process and product safety as one does not want to expose workers to potentially harmful waste products, and by the same token, want to make sure that toxicity of a side-stream feedstock will not make it to a final product. A recent news item shows how traces of amphetamine are found in manure and this is a problem when we think of circular processes. Having good definitions for what counts as waste for circular processes can help avoid unsafe organic materials from entering bioprocesses.[221]

Similar ambiguity exists around terms like “bio-based” or “natural”. For instance, it is worth questioning whether products like vanillin or curcumin, produced by engineered microorganisms, can be termed as “natural”. This is ambivalent because many products we already consume as a result of agricultural production, from vegetables to dairy, have been modified by humans to a point where they scarcely resemble their wild counterparts. This is relevant for safety in a different way: industry will not want to

invest in bio-based products that might be perceived as unsafe by consumers because of the use of bioengineering. Here, having clear communication on what is modified and how the modified strains actually do not reach the consumer is extremely important for issues of safety.

In this study, a notable third example centred on the challenge of distinguishing between safety and sustainability, and ultimately the definition of both terms affords several interpretations. This is a risk with polysemic concepts. In our research we find an interesting dynamic, where while identifying safety challenges in bioeconomy workflows, we found that the interviews often veered back towards sustainability gaps. Interestingly, it appears that safety now inherently includes environmental safety, and hence a sustainability factor, at least in European perspectives. This reveals the intertwined nature of both safety and sustainability.

- **Assessment tools**

Assessment tools, including not only Life Cycle Analysis (LCA) and Techno-Economic Analysis (TEA) but also Social Life Cycle Analysis (SLCA), play a pivotal role in the SbD approach, offering holistic insights into safety, and also into sustainability in the extended SSbD dimension. The relatively novel SSML approach was highlighted by a single interviewee, emphasising the dominance of established frameworks like GMP(+). Rather than developing new industry assessment tools, platforms enabling stakeholder collaboration can be more impactful. These would facilitate discussions around side-stream management, leading to improved pre-treatment protocols.

- **Limitations of the study**

The concept of Safe-by-Design (SbD) heralds a paradigm shift in the development and implementation of technologies and processes, advocating for the intrinsic embedding of safety from the outset. This forward-thinking framework insists on a holistic incorporation of environmental, economic, and social dimensions, ensuring that products and processes are designed to minimise risks to both people and the planet.
In pursuit of this comprehensive approach, it is imperative to engage a broader range of stakeholders than the ones contributing to this study report. For example, including international participants or depending on where the industrial or agricultural wastes are originating, the stakeholders of these companies and industries would give a more inclusive idea also of their challenges and possibilities to contribute to a comprehensive conceptualisation of an SbD framework. Such inclusivity is pivotal for harmonising divergent safety perspectives globally and from different market perspectives. Additionally, representatives of waste management companies would have been a big asset to include, since waste separation and treatment are an oft-overlooked facet. This phase of the process demands a rigorous assessment strategy, ensuring that post-consumer impacts align with the SbD principles.

The setting-up and prioritisation of stakeholder collaboration in form of a communication platform for example, over the introduction of new guidelines or assessment tools, is a further idea that sprung from this study, especially from the interviews. These platforms could serve as a room for critical discussions on valorisation, sector-specific needs, and innovative solutions for side-stream management.

Regarding assessment tools, LCA and TEA are a future perspective of the experimental showcase study, and should in any aspect be a mandatory incorporation for researchers from, at least, pilot scale level and above. Prediction tools such as the ex-Ante method, can aid with assessing novel processes already in early design stage, allowing for the assessment of potential impacts before they occur, hence before a high amount of experimental data is generated, aiming to secure a sustainable future by design rather than by retrofit.

Additionally, addressing the socio-economic aspects is non-negotiable, and this is where the incorporation of Social Life Cycle Assessment (SLCA) comes into play. SLCA extends the sustainability evaluation to include the social impacts of products throughout their lifecycle, ensuring that human labour, community impacts, and societal well-being are factored into the design process.

Further, a more comprehensive experimental setup, one that mandates collaboration with a diverse array of partners, thereby pooling resources and expertise would have led to more useful outcomes and technical insights on feedstock pre-treatments, fermentation and downstream processes and the end-of-life scenarios for the product and side-streams to evaluate the SbD concept effectively.

In essence, the limitations of the study highlight the need for a more connected, anticipatory, and socially inclusive approach to SbD. By engaging a comprehensive network of stakeholders and leveraging ex-ante methods such as LCA and TEA, the pursuit of safety, but also of sustainability, can be more than a checklist — it can be an inherent characteristic of innovation.

To conclude this report, we revisit our main aims.

i) Identify the critical steps, choices, and risks influencing the safety and circularity of bioprocess value chains.

1. Considering waste as feedstock: this critical step requires thinking about the entire value chain and the other related value chains. Issues relating to economic feasibility, quality, reliable supply, regulation and public perception need to come into consideration when selecting a feedstock.

2. Choosing the right biocatalyst: this critical step requires thinking about the comparability in economic efficiency to the oil and gas sector, together with issues of safety and security that usually come with the use of genetically modified microorganisms.
3. Dealing with novel fermentation processes: this critical step requires thinking about the technical efficiency of a process, how to optimise the process, resource efficiency, and use of feedstocks.
4. Assessing a final product: this critical step requires making sure that the biotechnologies used in the process are not present in the final product, as well as rigorous assessment on safety, recyclability, or degradability, while meeting local regulatory requirements, global and regional certification requirements, and integrating in local systems of production.
5. Managing waste: this critical step brings us back to the first step. It requires us to think of the economic viability of the waste produced, advocate for clear distinctions between wastes and by-products, and sharing responsibility for end-of-life scenarios.

These five critical steps should be addressed before rolling out a process and in coordination with each other. Only addressing all five steps at the same time can insure a safe roll out of these new technologies for industrial biotechnology.

(ii) examine the viability of industrial waste as feedstock for synthetic biology to generate high-value compounds.

The embeddement of an experimental case study within this project allowed us to consider the safety aspects in the design stages of a real case in line with SbD principles. This proof of concept elucidated the effect of lignocellulosic waste in the enhancement of curcumin production using an engineered *Pseudomonas putida* equipped with the curcumin biosynthesis pathway.

In this context, we learnt that not all waste is the same. A main finding of this research is that there are issues with regulatory labels on waste. These, in turn, affect greatly the economic viability of the waste chosen for a given process. Case-by-case consideration and shared learning on using waste is a topic for further research.

(iii) propose early-stage Safe-by-Design principles for an entire circular bioproduction value chain.

From our research, we suggest three design principles. These principles should still be validated with other projects for their usability and practicability.

- Consider the feedstock from a holistic perspective: the feedstock is not only the starting point but also the end point.

- Optimise biocatalyst integration: integration relates not only to integration in an economic system, but also a socio-technical system where value like safety and security are paramount.
- Share responsibility for Product Lifecycle Sustainability: this relates to the use of several types of tools and expertise that help get the full picture.

VI Recommendations and messages to the ministry

VI. 1 Recommendations

From these concluding remarks, we formulate a set of recommendations:

- **Recommendation 1:** Implement tools and regulatory definitions that incentivise the pursuit of safe and sustainable designs. Relevant for stakeholders in emerging industries transitioning from traditional linear, fossil-based approaches to sustainable, circular processes.
- **Recommendation 2:** Demonstrate the safety and sustainability aspects of bioengineered biocatalysts and communicate about these to the public. Relevant for researchers, policymakers, and industry leaders in synthetic biology and metabolic engineering.
- **Recommendation 3:** Develop clear definitions for terms such as "waste" and "bio-based" in order to establish guidelines for the safe utilisation of waste-derived side-streams, balancing safety and product quality considerations. Relevant for researchers, policymakers, and industry leaders in synthetic biology and metabolic engineering.
- **Recommendation 4:** Prioritise the use of holistic assessment tools like Life Cycle Analysis (LCA), Techno-Economic Analysis (TEA), and Social Life Cycle Analysis (SLCA). Relevant for researchers, policymakers, and industry leaders in synthetic biology and metabolic engineering.

VI.2 Interviewees' messages to the ministry

This section comprises answers from our interviewees, when asked at the end of the session if they would like us to transmit a message to the ministry, or what topics would need further discussion and improvements. Context information or questions, added words or deleted parts between thoughts/ sentences (that would reveal the interviewees background) are placed in brackets. The answers are given as unedited as possible from the transcripts.

Interviewee 1

"I think the ministries and the authorities, they will play an important role in advertising the safety regulations and of course scientists could help them develop these kinds of regulations.
I think it's only important to have other stakeholders involved in that discussion. So maybe it should be a kind of joint effort between scientists and company representatives to get into discussion with the authorities to set up a good set of regulations and measures. And also, how to maintain that for the future because safety is for all of us and a very important topic."

Interviewee 2

[on topics that should be further improved and implemented in an SSbD approach:]

"*HACCP: Hazard analysis and critical control points and GMP for the safety part. And for the sustainability part, I would say energy or carbon dioxide production, and in combination with the circularity, water use, and land use. These are the big issues dealing with sustainability."*

Interviewee 3

"Be brave and do what needs to be done and not what all kinds of parties with special interests tell you. Look at the science. Make decisions and come up with a good framework that everybody knows what the possibilities and the freedoms are and what their responsibilities are."

Interviewee 4

"I think the work you are doing could be good, for example, if there is something on paper, like a good discussion with industry how to approach questions. Because all industry, as in the chemical industry, is really struggling, also, on how can we do SbD. […] I think your biggest struggle would be the level of abstraction you have to go to. Because you cannot go into detail of everything because it takes too much time, you will not find the data anyway, but still, you want to make some predictions on the basis of limited information. And I think if you succeed in this to give insight into the process with limited data and which tools you can use, this would be your perfect showcase to discuss with industry, this would be a way you can pick up SbD for example."

[In your opinion, is it the most important to involve all the industry stakeholders in the whole discussion and to take them along and to provide a more detailed guideline for them than for anyone else regulators/policy makers, (academic) scientists?:]

"Yes, because the way SbD is positioned now, it's industry that should do the work. So, if you can think of [a guideline/ material] they can use, I would very much encouraged to include industries somewhere, right? So maybe, this can be a workshop for industry […] just to get the message across."

Interviewee 5

[on the meaningfulness of an SbD guideline:]

"I guess in industry quite some parts of the industry, in the engineering part, the Safety-by-Design is more or less an intern integral part of it [already]. So, if you then look at the overall scheme in the circularity, I'm not sure. Because it goes over so many chains. And so, so many parties involved, and I guess it is quite difficult to pinpoint if you have to do this, because of that."

[Would you say this is happening already or is there room for improvement from industry, academia and with the ministries?]

"That's a tricky one to answer to be honest. I mean, there's always room for improvement and communication is always a weak part anywhere. And so there could be - but then because it's the difficult what you might end up with is a set of guidelines or rules that you have to comply with which are just not it. That's also not what you want. So that's why I'm a bit careful with [an answer/opinion]. So what we definitely do not want is more regulation. And say, maybe, fine tuning of existing one, is okay."

Interviewee 6

[on the root of the problem]

"For biogas, [for example], if the government wouldn't give them subsidies, it would not be feasible in many cases. And that's the other dilemma that we are facing, is that we have to compete with the oil industry. And the oil industry, of course, has a gigantic economic scale. It has optimised all their processes, they found outlets for all the by-products they might make. So that's completely optimised there. So they are so good at what they do, making products out of oil, that it is very difficult to compete with them, of course and you have to really find the one process where you can get enough added value and then also convince customers that what you're doing is circular and that it has extra local economical value and it's a big struggle that you have to compete with companies that have been completely optimised and at the same time they of course are often not paying for the environmental destruction they're causing and that's artificially lowering their prices.

Yeah, maybe something interesting as well is that you're not allowed to use waste to make a lot of products. Waste to food is not allowed - but it is allowed to put products that are based on petrol in food, in medicine, in everything. And if you look at petrol or [the crude material] what they take out of the ground, the oil directly, it's really dirty stuff, right? You don't want to eat it. You don't want to, so that's also considered safe… So if that is considered safe, why can't we do it out of waste products. We always try to make that comparison because if you eat the oil coming out of the ground directly, you'll just die."

[message to the ministry]

"We all want to go to a circular economy and we want to do that in a safe way. But then it should be also allowed when you have a safe product, you can apply a process and [bring] a safe product [on the market]. So, legislations should be adapted to enable that and we should be very strict on the processes that are being applied there.

The process of reviewing what companies are actually going to do, to turn a waste product into a useful product that should be very carefully monitored by the government or related agency, but we should allow it with careful consideration."

[Would an SbD guideline be helpful?]

"I think it would be indeed very helpful. I think what would be really good if such a guideline would be formalised, for example, in an ISO norm or by SER [Social and economic council of the Netherlands]. In the end, it should be referred to in legislation, so I think a guideline would be a first step telling companies "OK,

this is a way to do it" and then at one point you would have to formalise it. […] I think that there's a couple steps to be taken and I think a guideline is very good first step and then it should be formalised to a norm and then taken up into European regulation."

Interviewee 7 *"We need a flexible legislative framework for all these organic streams, […] at a conference we're talking about using side streams from agriculture to make all kinds of new products, but if preferred streams are by law considered waste, then nobody will touch it.*
We need to be flexible in how we define waste and side streams coming from renewable sources, so more flexible than we've been so far, because once something is that designated waste, this value drops because you can no longer turn it into food and then the value drops dramatically. […]
So, you're taking one side stream, you're extracting or you're changing it, you're making it into a high value product, but you'll end up with the majority of what you started with [organic material].
And it's usually mainly water and so being able to treat those watery streams is usually important for the bioeconomy. So if we take that watery stream now and we would just put it into in the municipal water treatment, it would cost us a lot of money. So that's why we are forced to look for other outlets, as I said. [For example it can be sold] as animal feed and some of it is being turned into bio gas again.
But this is the challenge of this business, you take a biological product, you extract something, you make something useful, and you have a lot of watery waste leftover again, which you then have to use. This is something for you to realize as a PhD student. If you look at your circular story, I missed the water. Where were the water side streams? So, if you talk about Safe-by-Design, maybe this is one of the things you need to add to this. If you make such a design, where is the water going to go from your feedstock that is A, and [B] you need to start thinking about it before you do anything else. We didn't think about it and then later on we realise, oh […], we have all this wastewater, we have to do something with it."

Interviewee 8

"Especially the comparison one [comparing various processes/ products] I find very much important and do not expect a one size fit all number. I know that this is [what they would like to have] but this is not going to happen. It's intrinsic to this whole thing. You will not get a from one size fits all answer unfortunately."
[on the meaningfulness of an SbD guideline:]
"Good question, I would say it would be already useful to have it at this time, but it should not hamper to start because you [think you might] know what comes out. So here I'm a bit [doubtful]. I think it's useful to have it in the beginning, but it should not be already the decisive to not do something.
Well, you don't know what comes out of the research, and that is the underlying problem I have a bit with all these things with life cycle and environmental [assessments/ predictions] and now Safe-by-Design that I'm afraid that it will hamper a lot of research. […]

I think it would be useful – well, it depends on what the word guideline means. I think again I would say I would use it as a comparison of processes and then make a decision of which process I want to do. I'm not sure if it should have forced things on people because these guidelines they will change every time if there is new research, a new catalyst or a new conversion process found. […] So it needs to be used flexibly. You cannot make legislation based on this. I guess at least not on the absolute numbers."

Interviewee 9

"Well, the first message - I will keep it simple. Because there are so many regulations, both locally and European, but most companies will also look at the US. The US has its own set of rules.
There are a lot of rules already around novel foods around food in general, and I would say treat these products that are upcycled from the side stream or waste stream, treat them as a food product and make sure that they have the same food safety around it as a normal food would have. Don't over-complicate it by making a difference. It's still a food product and it should be safe as regular food and some products might need a big dose shape to show that it's new and it's not harmful if it's really a new product.
If something similar then just show that it is similar and it has the same safety proposition as the regular product. I would say don't treat it too much as a different thing and don't consider waste as waste, but call it the side stream and make sure that it's just treated properly."
"[…] I mean if we don't produce for the Dutch market, we produce for the global market. So, everything we do, we evaluate by the Dutch regulation or by the EFSA or the European regulation, but also by the FDA regulation because we want to enter the US market as well. So, we already have like three sets of rules that we want and need to attend to, to make sure that we are approved for the [different] markets.
So no, we don't consider moving to the US as such because it's so hard here, but the reality is that in general, the US market is a bit easier to enter because you have more your own responsibility rather than the government that has to approve you [with a] yes or no. […] So don't over-complicate it by getting another set of rules."

Interviewee 10

"It's good to think about safety but also think about safety around new examples [as in occurrences that indeed happened] and not about things that could happen, or might happen, at least in my perception. [There should not be] an economic penalty on it because as I said there are big difficulties in the biotech industry to make a sustainable life, economically. I appreciate efforts to make things better and we should for sure investigate it, I think, but, to put a regulation on it – I would be cautious with that, from a competitive perspective, I would say."

VII References

1. Mishra K, Siwal SS, Nayaka SC, Guan Z, Thakur VK. Waste-to-chemicals: Green solutions for bioeconomy markets. *Sci Total Environ*. 2023;887:164006. doi:10.1016/j.scitotenv.2023.164006

2. Neves SA, Marques AC. Drivers and barriers in the transition from a linear economy to a circular economy. *J Clean Prod*. 2022;341:130865. doi:10.1016/j.jclepro.2022.130865

3. Salmenperä H, Pitkänen K, Kautto P, Saikku L. Critical factors for enhancing the circular economy in waste management. *J Clean Prod*. 2021;280:124339. doi:10.1016/j.jclepro.2020.124339

4. Ewing TA, Nouse N, Van Lint M, Van Haveren J, Hugenholtz J, Van Es DS. Fermentation for the production of biobased chemicals in a circular economy: a perspective for the period 2022–2050. *Green Chem*. 2022;24(17):6373-6405. doi:10.1039/D1GC04758B

5. Lorenzo V de, Prather KL, Chen GQ, et al. The power of synthetic biology for bioproduction, remediation and pollution control: The UN's Sustainable Development Goals will inevitably require the application of molecular biology and biotechnology on a global scale. *EMBO Rep*. Published online March 2018:e45658. doi:10.15252/embr.201745658

6. El Karoui M, Hoyos-Flight M, Fletcher L. Future Trends in Synthetic Biology—A Report. *Front Bioeng Biotechnol*. 2019;7:175. doi:10.3389/fbioe.2019.00175

7. Nielsen J, Tillegreen CB, Petranovic D. Innovation trends in industrial biotechnology. *Trends Biotechnol*. 2022;40(10):1160-1172. doi:10.1016/j.tibtech.2022.03.007

8. DeLisi C, Patrinos A, MacCracken M, et al. The Role of Synthetic Biology in Atmospheric Greenhouse Gas Reduction: Prospects and Challenges. *BioDesign Res*. 2020;2020:1016207. doi:10.34133/2020/1016207

9. Yaashikaa PR, Kumar PS, Saravanan A, Varjani S, Ramamurthy R. Bioconversion of municipal solid waste into bio-based products: A review on valorisation and sustainable approach for circular bioeconomy. *Sci Total Environ*. 2020;748:141312. doi:10.1016/j.scitotenv.2020.141312

10. Kumar M, Sun Y, Rathour R, Pandey A, Thakur IS, Tsang DCW. Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. *Sci Total Environ*. 2020;716:137116. doi:10.1016/j.scitotenv.2020.137116

11. Linger JG, Vardon DR, Guarnieri MT, et al. Lignin valorization through integrated biological funneling and chemical catalysis. *Proc Natl Acad Sci*. 2014;111(33):12013-12018. doi:10.1073/pnas.1410657111

12. Sethupathy S, Murillo Morales G, Gao L, et al. Lignin valorization: Status, challenges and opportunities. *Bioresour Technol*. 2022;347:126696. doi:10.1016/j.biortech.2022.126696

13. Dey T, Bhattacharjee T, Nag P, Ritika, Ghati A, Kuila A. Valorization of agro-waste into value added products for sustainable development. *Bioresour Technol Rep*. 2021;16:100834. doi:10.1016/j.biteb.2021.100834

14. Son J, Lim SH, Kim YJ, et al. Customized valorization of waste streams by Pseudomonas putida: State-of-the-art, challenges, and future trends. *Bioresour Technol*. 2023;371:128607. doi:10.1016/j.biortech.2023.128607

15. Dharma Patria R, Rehman S, Vuppaladadiyam AK, et al. Bioconversion of food and lignocellulosic wastes employing sugar platform: A review of enzymatic hydrolysis and kinetics. *Bioresour Technol*. 2022;352:127083. doi:10.1016/j.biortech.2022.127083

16. van de Poel I, Robaey Z. Safe-by-Design: from Safety to Responsibility. *NanoEthics*. 2017;11(3):297-306. doi:10.1007/s11569-017-0301-x

17. Hogervorst P, Heens F, Schuijff M. Safe, sustainable and circular design in industrial biotechnology: Guidance for researchers and developers. Published online February 2023. https://www.rivm.nl/documenten/safe-

sustainable-and-circular-design-in-industrial-biotechnologyguidance-for-researchers

18. Robaey Z. Dealing with risks of biotechnology: understanding the potential of Safe-by-Design. Published online 2018. doi:10.13140/RG.2.2.13725.97769

19. Asin-Garcia E, Kallergi A, Landeweerd L, Martins dos Santos VAP. Genetic Safeguards for Safety-by-design: So Close Yet So Far. *Trends Biotechnol*. 2020;38(12):1308-1312. doi:10.1016/j.tibtech.2020.04.005

20. European Commission. Joint Research Centre. *Safe and Sustainable by Design Chemicals and Materials :Framework for the Definition of Criteria and Evaluation Procedure for Chemicals and Materials.* Publications Office; 2022. Accessed November 23, 2023. https://data.europa.eu/doi/10.2760/404991

21. Packroff R, Marx R. Safe and Sustainable by Design—An Interdisciplinary Challenge for Future-Proof Chemistry. *ChemPlusChem*. 2022;87(3):e202100534. doi:10.1002/cplu.202100534

22. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. *The European Green Deal*.; 2019. Accessed December 12, 2021. https://ec.europa.eu/info/sites/info/files/european-green-deal-communication_en.pdf

23. A European Green Deal. Published July 14, 2021. Accessed February 10, 2023. https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en

24. Beekman M, Bakker JC, Bodar CWM, et al. Coping with substances of concern in a circular economy. Published online 2020. doi:10.21945/RIVM-2020-0049

25. Nyholm S. Responsibility Gaps, Value Alignment, and Meaningful Human Control over Artificial Intelligence. In: *Risk and Responsibility in Context*. 1st ed. Routledge; 2023:191-213. doi:10.4324/9781003276029-14

26. Santoni De Sio F, Mecacci G. Four Responsibility Gaps with Artificial Intelligence: Why they Matter and How to Address them. *Philos Technol*. 2021;34(4):1057-1084. doi:10.1007/s13347-021-00450-x

27. Baas L, Metselaar S, Klaassen P. Circles of Care for Safety: A Care Ethics Approach to Safe-by-Design. *NanoEthics*. 2022;16(2):167-179. doi:10.1007/s11569-022-00419-w

28. Soeteman-Hernández LG, Bekker C, Groenewold M, et al. Perspective on how regulators can keep pace with innovation: Outcomes of a European Regulatory Preparedness Workshop on nanomaterials and nano-enabled products. *NanoImpact*. 2019;14:100166. doi:10.1016/j.impact.2019.100166

29. Cucurachi S, Van Der Giesen C, Guinée J. Ex-ante LCA of Emerging Technologies. *Procedia CIRP*. 2018;69:463-468. doi:10.1016/j.procir.2017.11.005

30. Saling P. Assessing Industrial Biotechnology Products with LCA and Eco-Efficiency. In: Fröhling M, Hiete M, eds. *Sustainability and Life Cycle Assessment in Industrial Biotechnology*. Vol 173. Advances in Biochemical Engineering/Biotechnology. Springer International Publishing; 2019:325-357. doi:10.1007/10_2019_102

31. Schuurbiers D. *Modellen Voor Een Reflexieve Component in Technisch Onderzoek Gericht Op Safe-by-Design*. Ministerie van Infrastructuur en Waterstaat (IenW); 2019. https://www.safe-by-design-nl.nl/documenten/over+safe-by-design+documenten/daan+schuurbiers++modellen+voor+een+reflexieve+co/handlerdownloadfiles.ashx?idnv=1877630

32. Templier M, Paré G. A Framework for Guiding and Evaluating Literature Reviews. *Commun Assoc Inf Syst*. 2015;37. doi:10.17705/1CAIS.03706

33. Asin-Garcia E, Robaey Z, Kampers LFC, Martins dos Santos VAP. Exploring the Impact of Tensions in Stakeholder Norms on Designing for Value Change: The Case of Biosafety in Industrial Biotechnology. *Sci Eng Ethics*. 2023;29(2):9. doi:10.1007/s11948-023-00432-6

34. Chen Z, Wan C. Biological valorization strategies for converting lignin into fuels and chemicals. *Renew Sustain Energy Rev*. 2017;73:610-621. doi:10.1016/j.rser.2017.01.166

35. Bugg TDH, Williamson JJ, Alberti F. Microbial hosts for metabolic engineering of lignin bioconversion to renewable chemicals. *Renew Sustain Energy Rev*. 2021;152:111674. doi:10.1016/j.rser.2021.111674

36. Azubuike CC, Allemann MN, Michener JK. Microbial assimilation of lignin-derived aromatic compounds and conversion to value-added products. *Curr Opin Microbiol*. 2022;65:64-72. doi:10.1016/j.mib.2021.10.014

37. Lee S, Sohn JH, Bae JH, Kim SC, Sung BH. Current Status of Pseudomonas putida Engineering for Lignin Valorization. *Biotechnol Bioprocess Eng*. 2020;25(6):862-871. doi:10.1007/s12257-020-0029-2

38. Kim JS, Lee YY, Kim TH. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour Technol*. 2016;199:42-48. doi:10.1016/j.biortech.2015.08.085

39. Oriez V, Peydecastaing J, Pontalier PY. Lignocellulosic Biomass Mild Alkaline Fractionation and Resulting Extract Purification Processes: Conditions, Yields, and Purities. *Clean Technol*. 2020;2(1):91-115. doi:10.3390/cleantechnol2010007

40. Liu H, Pang B, Zhao Y, Lu J, Han Y, Wang H. Comparative study of two different alkali-mechanical pretreatments of corn stover for bioethanol production. *Fuel*. 2018;221:21-27. doi:10.1016/j.fuel.2018.02.088

41. Clark JH, Farmer TJ, Herrero-Davila L, Sherwood J. Circular economy design considerations for research and process development in the chemical sciences. *Green Chem*. 2016;18(14):3914-3934. doi:10.1039/C6GC00501B

42. Sherwood J. The significance of biomass in a circular economy. *Bioresour Technol*. 2020;300:122755. doi:10.1016/j.biortech.2020.122755

43. Lucia LA. Lignocellulosic biomass: A potential feedstock to replace petroleum. *BioResources*. 2008;3(4):981-982.

44. Venkatesh A, Posen ID, MacLean HL, Chu PL, Griffin WM, Saville BA. Environmental Aspects of Biotechnology. In: Fröhling M, Hiete M, eds. *Sustainability and Life Cycle Assessment in Industrial Biotechnology*. Vol 173. Advances in Biochemical Engineering/Biotechnology. Springer International Publishing; 2019:77-119. doi:10.1007/10_2019_98

45. Coma M, Martinez-Hernandez E, Abeln F, et al. Organic waste as a sustainable feedstock for platform chemicals. *Faraday Discuss*. 2017;202:175-195. doi:10.1039/C7FD00070G

46. Dahiya S, Katakojwala R, Ramakrishna S, Mohan SV. Biobased Products and Life Cycle Assessment in the Context of Circular Economy and Sustainability. *Mater Circ Econ*. 2020;2(1):7. doi:10.1007/s42824-020-00007-x

47. Stikane A, Dace E, Stalidzans E. Closing the loop in bioproduction: Spent microbial biomass as a resource within circular bioeconomy. *New Biotechnol*. 2022;70:109-115. doi:10.1016/j.nbt.2022.06.001

48. Stikane A, Baumanis MR, Muiznieks R, Stalidzans E. Impact of Waste as a Substrate on Biomass Formation, and Optimization of Spent Microbial Biomass Re-Use by Sustainable Metabolic Engineering. *Fermentation*. 2023;9(6):531. doi:10.3390/fermentation9060531

49. Mekonnen TH, Misra M, Mohanty AK. Processing, performance, and applications of plant and animal protein-based blends and their biocomposites. In: *Biocomposites*. Elsevier; 2015:201-235. doi:10.1016/B978-1-78242-373-7.00017-2

50. Yu L, Wu F, Chen G. Next-Generation Industrial Biotechnology-Transforming the Current Industrial Biotechnology into Competitive Processes. *Biotechnol J*. 2019;14(9):1800437. doi:10.1002/biot.201800437

51. Scott WT, Benito-Vaquerizo S, Zimmerman J, et al. *A Structured Evaluation of Genome-Scale Constraint-Based Modeling Tools for Microbial Consortia*. Systems Biology; 2023. doi:10.1101/2023.02.08.527721

52. Li J, Suvarna M, Li L, et al. A review of computational modeling techniques for wet waste valorization: Research trends and future perspectives. *J Clean Prod*. 2022;367:133025. doi:10.1016/j.jclepro.2022.133025

53. Anand U, Reddy B, Singh VK, et al. Potential Environmental and Human Health Risks Caused by Antibiotic-Resistant Bacteria (ARB), Antibiotic

Resistance Genes (ARGs) and Emerging Contaminants (ECs) from Municipal Solid Waste (MSW) Landfill. *Antibiotics*. 2021;10(4):374. doi:10.3390/antibiotics10040374

54. Yaashikaa PR, Kumar PS, Nhung TC, et al. A review on landfill system for municipal solid wastes: Insight into leachate, gas emissions, environmental and economic analysis. *Chemosphere*. 2022;309:136627. doi:10.1016/j.chemosphere.2022.136627

55. National Research Council (U.S.), ed. *Waste Incineration & Public Health*. National Academy Press; 2000.

56. Shunda Lin, Jiang X, Zhao Y, Yan J. Disposal technology and new progress for dioxins and heavy metals in fly ash from municipal solid waste incineration: A critical review. *Environ Pollut*. 2022;311:119878. doi:10.1016/j.envpol.2022.119878

57. Samoraj M, Mironiuk M, Izydorczyk G, et al. The challenges and perspectives for anaerobic digestion of animal waste and fertilizer application of the digestate. *Chemosphere*. 2022;295:133799. doi:10.1016/j.chemosphere.2022.133799

58. Hoornweg D, Bhada-Tata P. What a Waste : A Global Review of Solid Waste Management. Published March 2012. Accessed October 15, 2023. http://hdl.handle.net/10986/17388

59. Bruinsma L, Wenk S, Claassens NJ, Martins Dos Santos VAP. Paving the way for synthetic C1 - Metabolism in Pseudomonas putida through the reductive glycine pathway. *Metab Eng*. 2023;76:215-224. doi:10.1016/j.ymben.2023.02.004

60. Pacheco M, Moura P, Silva C. A Systematic Review of Syngas Bioconversion to Value-Added Products from 2012 to 2022. *Energies*. 2023;16(7):3241. doi:10.3390/en16073241

61. Lee J, Park HJ, Moon M, Lee JS, Min K. Recent progress and challenges in microbial polyhydroxybutyrate (PHB) production from CO2 as a sustainable feedstock: A state-of-the-art review. *Bioresour Technol*. 2021;339:125616. doi:10.1016/j.biortech.2021.125616

62. Claassens NJ. Reductive Glycine Pathway: A Versatile Route for One-Carbon Biotech. *Trends Biotechnol*. 2021;39(4):327-329. doi:10.1016/j.tibtech.2021.02.005

63. Kim S, Lindner SN, Aslan S, et al. Growth of E. coli on formate and methanol via the reductive glycine pathway. *Nat Chem Biol*. 2020;16(5):538-545. doi:10.1038/s41589-020-0473-5

64. Beckham GT, Johnson CW, Karp EM, Salvachúa D, Vardon DR. Opportunities and challenges in biological lignin valorization. *Curr Opin Biotechnol*. 2016;42:40-53. doi:10.1016/j.copbio.2016.02.030

65. Saini JK, Saini R, Tewari L. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*. 2015;5(4):337-353. doi:10.1007/s13205-014-0246-5

66. Guo XM, Trably E, Latrille E, Carrère H, Steyer JP. Hydrogen production from agricultural waste by dark fermentation: A review. *Int J Hydrog Energy*. 2010;35(19):10660-10673. doi:10.1016/j.ijhydene.2010.03.008

67. Hossain N, Haji Zaini J, Mahlia TMI. A Review of Bioethanol Production from Plant-based Waste Biomass by Yeast Fermentation. *Int J Technol*. 2017;8(1):5. doi:10.14716/ijtech.v8i1.3948

68. Cheng F, Brewer CE. Conversion of protein-rich lignocellulosic wastes to bio-energy: Review and recommendations for hydrolysis + fermentation and anaerobic digestion. *Renew Sustain Energy Rev*. 2021;146:111167. doi:10.1016/j.rser.2021.111167

69. Handler RM, Shonnard DR, Griffing EM, Lai A, Palou-Rivera I. Life Cycle Assessments of Ethanol Production via Gas Fermentation: Anticipated Greenhouse Gas Emissions for Cellulosic and Waste Gas Feedstocks. *Ind Eng Chem Res*. 2016;55(12):3253-3261. doi:10.1021/acs.iecr.5b03215

70. Koutinas AA, Vlysidis A, Pleissner D, et al. Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. *Chem Soc Rev*. 2014;43(8):2587. doi:10.1039/c3cs60293a

71. Mahmoud A, Hamza RA, Elbeshbishy E. Enhancement of denitrification efficiency using municipal and industrial waste fermentation

liquids as external carbon sources. *Sci Total Environ*. 2022;816:151578. doi:10.1016/j.scitotenv.2021.151578

72. Donner M, Verniquet A, Broeze J, Kayser K, De Vries H. Critical success and risk factors for circular business models valorising agricultural waste and by-products. *Resour Conserv Recycl*. 2021;165:105236. doi:10.1016/j.resconrec.2020.105236

73. Bhatnagar N, Ryan D, Murphy R, Enright AM. A comprehensive review of green policy, anaerobic digestion of animal manure and chicken litter feedstock potential – Global and Irish perspective. *Renew Sustain Energy Rev*. 2022;154:111884. doi:10.1016/j.rser.2021.111884

74. Wei L, Liang S, Coats ER, McDonald AG. Valorization of residual bacterial biomass waste after polyhydroxyalkanoate isolation by hydrothermal treatment. *Bioresour Technol*. 2015;198:739-745. doi:10.1016/j.biortech.2015.09.086

75. Battista F, Frison N, Pavan P, et al. Food wastes and sewage sludge as feedstock for an urban biorefinery producing biofuels and added-value bioproducts. *J Chem Technol Biotechnol*. 2020;95(2):328-338. doi:10.1002/jctb.6096

76. Molina-Peñate E, Artola A, Sánchez A. Organic municipal waste as feedstock for biorefineries: bioconversion technologies integration and challenges. *Rev Environ Sci Biotechnol*. 2022;21(1):247-267. doi:10.1007/s11157-021-09605-w

77. Vyas S, Prajapati P, Shah AV, Varjani S. Municipal solid waste management: Dynamics, risk assessment, ecological influence, advancements, constraints and perspectives. *Sci Total Environ*. 2022;814:152802. doi:10.1016/j.scitotenv.2021.152802

78. Chen C, Reniers G. Risk Assessment of Processes and Products in Industrial Biotechnology. In: Fröhling M, Hiete M, eds. *Sustainability and Life Cycle Assessment in Industrial Biotechnology*. Vol 173. Advances in Biochemical Engineering/Biotechnology. Springer International Publishing; 2018:255-279. doi:10.1007/10_2018_74

79. Elbersen W, Schultze-Jena A, Van Berkum S, Dengerink J, Naranjo-Barrantes M, Obeng E. *Identifying and Implementing Circular Applications of Agri-Residues : A Circular Evaluation Framework for Assessing Impacts and Circularity of Different Agri-Residue Applications*. Wageningen Food & Biobased Research; 2022. doi:10.18174/563389

80. Quik JTK, Mesman M, van der Grinten E. Assessing sustainability of residual biomass applications: Finding the optimal solution for a circular economy. Published online 2016. https://www.rivm.nl/bibliotheek/rapporten/2016-0135.pdf

81. Robaey Z, Asveld L, Sinha KM, Wubben E, Osseweijer P. Identifying practices of inclusive biobased value chains: Lessons from corn stover in Iowa, sugar cane in Jamaica, and sugar beet in the Netherlands. *Clean Circ Bioeconomy*. 2022;3:100032. doi:10.1016/j.clcb.2022.100032

82. Broeren MLM, Zijp MC, Waaijers-van Der Loop SL, et al. Environmental assessment of bio-based chemicals in early-stage development: a review of methods and indicators. *Biofuels Bioprod Biorefining*. 2017;11(4):701-718. doi:10.1002/bbb.1772

83. Lindner JP, Beck T, Bos U, Albrecht S. Assessing Land Use and Biodiversity Impacts of Industrial Biotechnology. In: Fröhling M, Hiete M, eds. *Sustainability and Life Cycle Assessment in Industrial Biotechnology*. Vol 173. Advances in Biochemical Engineering/Biotechnology. Springer International Publishing; 2019:233-254. doi:10.1007/10_2019_114

84. FBR BP Biorefinery & Sustainable Value Chains, LEI International Policy, CDI advisory, et al. *Identifying and Implementing Circular Applications of Agri-Residues : A Circular Evaluation Framework for Assessing Impacts and Circularity of Different Agri-Residue Applications*. Wageningen Food & Biobased Research; 2022. doi:10.18174/563389

85. Van Egmond HP. Natural toxins: risks, regulations and the analytical situation in Europe. *Anal Bioanal Chem*. 2004;378(5):1152-1160. doi:10.1007/s00216-003-2373-4

86. Mañas P, Pagán R. Microbial inactivation by new technologies of food preservation. *J Appl Microbiol*. 2005;98(6):1387-1399. doi:10.1111/j.1365-2672.2005.02561.x

87. Pankaj SK, Bueno-Ferrer C, Misra NN, et al. Applications of cold plasma technology in food packaging. *Trends Food Sci Technol*. 2014;35(1):5-17. doi:10.1016/j.tifs.2013.10.009

88. Dufossé L, Galaup P, Yaron A, et al. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends Food Sci Technol*. 2005;16(9):389-406. doi:10.1016/j.tifs.2005.02.006

89. Nielsen J, Keasling JD. Engineering Cellular Metabolism. *Cell*. 2016;164(6):1185-1197. doi:10.1016/j.cell.2016.02.004

90. Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: A review. *Anal Chim Acta*. 2009;632(2):168-180. doi:10.1016/j.aca.2008.11.010

91. Jay JM, Loessner MJ, Golden DA. *Modern Food Microbiology*. 7th ed. Springer; 2005.

92. Lawless HT, Heymann H. *Sensory Evaluation of Food: Principles and Practices*. Springer New York; 2010. doi:10.1007/978-1-4419-6488-5

93. Gosselink RJA, Van Dam JEG, De Jong E, et al. Fractionation, analysis, and PCA modeling of properties of four technical lignins for prediction of their application potential in binders. *Holzforschung*. 2010;64(2). doi:10.1515/hf.2010.023

94. Savitskaya T, Rezkinov I, Grinshpan D. Rheological Behavior of Lignin Based Dispersions Intended for Composite Fuel Production. Published online 2016. doi:10.3933/APPLRHEOL-26-63476

95. Zoghlami A, Paës G. Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. *Front Chem*. 2019;7:874. doi:10.3389/fchem.2019.00874

96. Bugg TD, Rahmanpour R. Enzymatic conversion of lignin into renewable chemicals. *Curr Opin Chem Biol*. 2015;29:10-17. doi:10.1016/j.cbpa.2015.06.009

97. Matsushita Y. Conversion of technical lignins to functional materials with retained polymeric properties. *J Wood Sci*. 2015;61(3):230-250. doi:10.1007/s10086-015-1470-2

98. Hodásová Ľ, Jablonsky M, Skulcova AB, Haz A. Lignin, potential products and their market value. *Wood Res*. 2015;60(6):973-986.

99. Mathews SL, Pawlak J, Grunden AM. Bacterial biodegradation and bioconversion of industrial lignocellulosic streams. *Appl Microbiol Biotechnol*. 2015;99(7):2939-2954. doi:10.1007/s00253-015-6471-y

100. Moretti C, Corona B, Hoefnagels R, Vural-Gürsel I, Gosselink R, Junginger M. Review of life cycle assessments of lignin and derived products: Lessons learned. *Sci Total Environ*. 2021;770:144656. doi:10.1016/j.scitotenv.2020.144656

101. Kucharska K, Rybarczyk P, Hołowacz I, Łukajtis R, Glinka M, Kamiński M. Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes. *Molecules*. 2018;23(11):2937. doi:10.3390/molecules23112937

102. Skals PB, Krabek A, Nielsen PH, Wenzel H. Environmental assessment of enzyme assisted processing in pulp and paper industry. *Int J Life Cycle Assess*. 2008;13(2):124-132. doi:10.1065/lca2007.11.366

103. Bajwa DS, Pourhashem G, Ullah AH, Bajwa SG. A concise review of current lignin production, applications, products and their environmental impact. *Ind Crops Prod*. 2019;139:111526. doi:10.1016/j.indcrop.2019.111526

104. Cabral Almada C, Kazachenko A, Fongarland P, Da Silva Perez D, Kuznetsov BN, Djakovitch L. Oxidative depolymerization of lignins for producing aromatics: variation of botanical origin and extraction methods. *Biomass Convers Biorefinery*. 2022;12(9):3795-3808. doi:10.1007/s13399-020-00897-6

105. Patil V, Adhikari S, Cross P, Jahromi H. Progress in the solvent depolymerization of lignin. *Renew Sustain Energy Rev*. 2020;133:110359. doi:10.1016/j.rser.2020.110359

106. Kouris PD, Van Osch DJGP, Cremers GJW, Boot MD, Hensen EJM. Mild thermolytic solvolysis of technical lignins in polar organic solvents to a crude lignin oil. *Sustain Energy Fuels*. 2020;4(12):6212-6226. doi:10.1039/D0SE01016B

107. Sebhat W, El-Roz A, Crepet A, et al. Comparative study of solvolysis of technical lignins in flow reactor. *Biomass Convers Biorefinery*. 2020;10(2):351-366. doi:10.1007/s13399-019-00435-z

108. Alfaro A, Perez A, Garcia JC, Lopez F, Zamudio MA, Rodriguez A. Ethanol and soda pulping of Tagasaste wood: Neural fuzzy modeling. *Cellul Chem Technol*. 2009;43(7):295.

109. Łucejko JJ, De Lamotte A, Andriulo F, et al. Evaluation of Soda Lignin from Wheat Straw/Sarkanda Grass as a Potential Future Consolidant for Archaeological Wood. *Forests*. 2021;12(7):911. doi:10.3390/f12070911

110. Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour Technol*. 2010;101(13):4851-4861. doi:10.1016/j.biortech.2009.11.093

111. Verma N, Kumar V, Bansal MC. Valorization of Waste Biomass in Fermentative Production of Cellulases: A Review. *Waste Biomass Valorization*. 2021;12(2):613-640. doi:10.1007/s12649-020-01048-8

112. Horst M van der, Robaey ZH, García EA, Santos VAPM dos. An exploratory study on embedding Safe-and-Sustainable-by-Design (SSbD) in Circular Bioeconomy Research. Published online March 15, 2023. Accessed November 22, 2023. https://research.wur.nl/en/publications/an-exploratory-study-on-embedding-safe-and-sustainable-by-design-

113. Sundaram LS, Ajioka JW, Molloy JC. Synthetic biology regulation in Europe: containment, release and beyond. *Synth Biol*. 2023;8(1):ysad009. doi:10.1093/synbio/ysad009

114. Kumar NM, Muthukumaran C, Sharmila G, Gurunathan B. Genetically Modified Organisms and Its Impact on the Enhancement of Bioremediation. In: Varjani SJ, Agarwal AK, Gnansounou E, Gurunathan B, eds. *Bioremediation: Applications for Environmental Protection and Management*. Energy, Environment, and Sustainability. Springer Singapore; 2018:53-76. doi:10.1007/978-981-10-7485-1_4

115. Hewett JP, Wolfe AK, Bergmann RA, Stelling SC, Davis KL. Human Health and Environmental Risks Posed by Synthetic Biology R&D for Energy Applications: A Literature Analysis. *Appl Biosaf*. 2016;21(4):177-184. doi:10.1177/1535676016672377

116. Krink N, Löchner AC, Cooper H, Beisel CL, Di Ventura B. Synthetic biology landscape and community in Germany. *Biotechnol Notes*. 2022;3:8-14. doi:10.1016/j.biotno.2021.12.001

117. Bouchaut B, Asveld L. Safe-by-Design: Stakeholders' Perceptions and Expectations of How to Deal with Uncertain Risks of Emerging Biotechnologies in the Netherlands. *Risk Anal*. 2020;40(8):1632-1644. doi:10.1111/risa.13501

118. Ancillotti M, Rerimassie V, Seitz SB, Steurer W. An Update of Public Perceptions of Synthetic Biology: Still Undecided? *NanoEthics*. 2016;10(3):309-325. doi:10.1007/s11569-016-0256-3

119. RIVM. Safety framework Biotechnology: Basic knowledge with links to in-dept information. Published online 2021. https://biotechnologie.rivm.nl/documenten/safety-framework-biotechnologybasic-knowledge-with-links-to-in-dept-information

120. Tokic M, Hatzimanikatis V, Miskovic L. Large-scale kinetic metabolic models of Pseudomonas putida KT2440 for consistent design of metabolic engineering strategies. *Biotechnol Biofuels*. 2020;13(1):33. doi:10.1186/s13068-020-1665-7

121. Kampers LF, Volkers RJ, Martins dos Santos VA. Pseudomonas putida KT 2440 is HV 1 certified, not GRAS. *Microb Biotechnol*. 2019;12(5):845-848.

122. Nikel PI, De Lorenzo V. Pseudomonas putida as a functional chassis for industrial biocatalysis: From native biochemistry to trans-metabolism. *Metab Eng*. 2018;50:142-155. doi:10.1016/j.ymben.2018.05.005

123. Weimer A, Kohlstedt M, Volke DC, Nikel PI, Wittmann C. Industrial biotechnology of Pseudomonas putida: advances and prospects. *Appl Microbiol Biotechnol*. 2020;104(18):7745-7766. doi:10.1007/s00253-020-10811-9

124. Lammens EM, Nikel PI, Lavigne R. Exploring the synthetic biology potential of

bacteriophages for engineering non-model bacteria. *Nat Commun*. 2020;11(1):5294. doi:10.1038/s41467-020-19124-x

125. Beeckman DSA, Rüdelsheim P. Biosafety and Biosecurity in Containment: A Regulatory Overview. *Front Bioeng Biotechnol*. 2020;8:650. doi:10.3389/fbioe.2020.00650

126. GMP+ International. GMP+ International. Accessed November 26, 2023. https://www.gmpplus.org/en/

127. EMA. European Medicines Agency. European Medicines Agency. Accessed November 26, 2023. https://www.ema.europa.eu/en

128. Shaw AJ, Lam FH, Hamilton M, et al. Metabolic engineering of microbial competitive advantage for industrial fermentation processes. *Science*. 2016;353(6299):583-586. doi:10.1126/science.aaf6159

129. Asveld L, Osseweijer P, Posada JA. Societal and Ethical Issues in Industrial Biotechnology. In: Fröhling M, Hiete M, eds. *Sustainability and Life Cycle Assessment in Industrial Biotechnology*. Vol 173. Advances in Biochemical Engineering/Biotechnology. Springer International Publishing; 2019:121-141. doi:10.1007/10_2019_100

130. Brexó RP, Sant'Ana AS. Impact and significance of microbial contamination during fermentation for bioethanol production. *Renew Sustain Energy Rev*. 2017;73:423-434. doi:10.1016/j.rser.2017.01.151

131. Hopkins D, St. Amand M, Prior J. Bioreactor Automation. In: Baltz RH, Davies JE, Demain AL, et al., eds. *Manual of Industrial Microbiology and Biotechnology*. ASM Press; 2014:719-730. doi:10.1128/9781555816827.ch51

132. Ma J, Li L. VOC emitted by biopharmaceutical industries: Source profiles, health risks, and secondary pollution. *J Environ Sci*. 2024;135:570-584. doi:10.1016/j.jes.2022.10.022

133. Singh RS, Kaur N, Singh D, Bajaj BK, Kennedy JF. Downstream processing and structural confirmation of pullulan - A comprehensive review. *Int J Biol Macromol*. 2022;208:553-564. doi:10.1016/j.ijbiomac.2022.03.163

134. Cottam A. Risk assessment and control in biotechnology. In: Institution of Chemical Engineers; 1991:341.

135. Zydney AL. New developments in membranes for bioprocessing – A review. *J Membr Sci*. 2021;620:118804. doi:10.1016/j.memsci.2020.118804

136. Bennett AM, Benbough JE, Hambleton P. Biosafety in Downstream Processing. In: Pyle DL, ed. *Separations for Biotechnology 2*. Springer Netherlands; 1990:592-600. doi:10.1007/978-94-009-0783-6_63

137. Sebesta J, Xiong W, Guarnieri MT, Yu J. Biocontainment of Genetically Engineered Algae. *Front Plant Sci*. 2022;13:839446. doi:10.3389/fpls.2022.839446

138. Liu S. Combustion, reactive hazard, and bioprocess safety. In: *Bioprocess Engineering*. Elsevier; 2020:719-772. doi:10.1016/B978-0-12-821012-3.00016-6

139. Abraham EP, Chain E, Fletcher CM, et al. Further observations on penicillin. 1941. *Eur J Clin Pharmacol*. 1992;42(1):3-9.

140. Stanbury PF, Whitaker A, Hall SJ. Design of a fermenter. In: *Principles of Fermentation Technology*. Elsevier; 2017:401-485. doi:10.1016/B978-0-08-099953-1.00007-7

141. Göğüş N, Hakgüder Taze B, DemiR H, Tari C, Ünlütürk S, Lahore MF. Evaluation of orange peel, an industrial waste, for the production of Aspergillus sojae polygalacturonase considering both morphology and rheology effects. *Turk J Biol*. 2014;38:537-548. doi:10.3906/biy-1308-47

142. Boehm J, Bushnell B. Contamination Prevention: How Single-Use Systems Can Ensure a Safe, Clean, and Efficient Bioprocess Environment. *Pharm Technol*. 2008;32(6). Accessed November 26, 2023. https://www.pharmtech.com/view/contamination-prevention-how-single-use-systems-can-ensure-safe-clean-and-efficient-bioprocess-envir

143. Junker B, Lester M, Leporati J, et al. Sustainable reduction of bioreactor contamination in an industrial fermentation pilot plant. *J Biosci Bioeng*. 2006;102(4):251-268. doi:10.1263/jbb.102.251

144. Routledge SJ. BEYOND DE-FOAMING: THE EFFECTS OF ANTIFOAMS ON BIOPROCESS PRODUCTIVITY. *Comput Struct Biotechnol J*. 2012;3(4):e201210001. doi:10.5936/csbj.201210014

145. Durand A. Bioreactor designs for solid state fermentation. *Biochem Eng J*. 2003;13(2-3):113-125. doi:10.1016/S1369-703X(02)00124-9

146. Berovic M. Sterilisation in biotechnology. In: *Biotechnology Annual Review*. Vol 11. Elsevier; 2005:257-279. doi:10.1016/S1387-2656(05)11008-4

147. Peron M, Arena S, Paltrinieri N, Sgarbossa F, Boustras G. Risk assessment for handling hazardous substances within the European industry: Available methodologies and research streams. *Risk Anal*. 2023;43(7):1434-1462. doi:10.1111/risa.14010

148. Srikanth P, Sudharsanam S, Steinberg R. Bio-aerosols in indoor environment: Composition, health effects and analysis. *Indian J Med Microbiol*. 2008;26(4):302. doi:10.4103/0255-0857.43555

149. Yin FW, Guo DS, Ren LJ, Ji XJ, Huang H. Development of a method for the valorization of fermentation wastewater and algal-residue extract in docosahexaenoic acid production by Schizochytrium sp. *Bioresour Technol*. 2018;266:482-487. doi:10.1016/j.biortech.2018.06.109

150. Teng TS, Chin YL, Chai KF, Chen WN. Fermentation for future food systems: Precision fermentation can complement the scope and applications of traditional fermentation. *EMBO Rep*. 2021;22(5):e52680. doi:10.15252/embr.202152680

151. Kim S, Han JH, Chua B, Pak JJ. A pH Sensing Pipette for Cross-Contamination Prevention in Industrial Fermentation. *IEEE Trans Ind Electron*. 2022;69(7):7461-7469. doi:10.1109/TIE.2021.3099251

152. Scholz J, Suppmann S. A re-usable wave bioreactor for protein production in insect cells. *MethodsX*. 2016;3:497-501. doi:10.1016/j.mex.2016.08.001

153. Noh MH, Cha S, Kim M, Jung GY. Recent Advances in Microbial Cell Growth Regulation Strategies for Metabolic Engineering. *Biotechnol Bioprocess Eng*. 2020;25(6):810-828. doi:10.1007/s12257-019-0511-x

154. Batianis C, van Rosmalen RP, Major M, et al. A tunable metabolic valve for precise growth control and increased product formation in Pseudomonas putida. *Metab Eng*. 2023;75:47-57. doi:10.1016/j.ymben.2022.10.002

155. Ma H, Yang J, Jia Y, Wang Q, Ma X, Sonomoto K. Alleviation of harmful effect in stillage reflux in food waste ethanol fermentation based on metabolic and side-product accumulation regulation. *Bioresour Technol*. 2016;218:463-468. doi:10.1016/j.biortech.2016.06.123

156. Mohanty SS, Koul Y, Varjani S, et al. A critical review on various feedstocks as sustainable substrates for biosurfactants production: a way towards cleaner production. *Microb Cell Factories*. 2021;20(1):120. doi:10.1186/s12934-021-01613-3

157. ISO. ISO 22000:2018, Second Edition: Food safety management systems - Requirements for any organization in the food chain. Published online 2018.

158. Chen H, Liou B, Hsu K, Chen C, Chuang P. Implementation of food safety management systems that meets ISO 22000:2018 and HACCP: A case study of capsule biotechnology products of chaga mushroom. *J Food Sci*. 2021;86(1):40-54. doi:10.1111/1750-3841.15553

159. 14:00-17:00. ISO 22000:2018. ISO. Published June 5, 2023. Accessed November 26, 2023. https://www.iso.org/standard/65464.html

160. 14:00-17:00. ISO 9001:2015. ISO. Published September 1, 2021. Accessed November 26, 2023. https://www.iso.org/standard/62085.html

161. EFSA. Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. *EFSA J*. 2011;(2011;9(6):2193). doi:10.2903/j.efsa.2011.2193

162. Regulation (EC) No 178/2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements

of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Published online July 1, 2022. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002R0178-20220701

163. web K. Home - NVWA-English. Published September 22, 2023. Accessed November 26, 2023. https://english.nvwa.nl/

164. Regulation (EC) No 1069/2009. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Published online October 21, 2009. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:300:0001:0033:en:PDF

165. Kassim FO, Thomas CLP, Afolabi OOD. Integrated conversion technologies for sustainable agri-food waste valorization: A critical review. *Biomass Bioenergy*. 2022;156:106314. doi:10.1016/j.biombioe.2021.106314

166. Bottausci S, Midence R, Serrano-Bernardo F, Bonoli A. Organic Waste Management and Circular Bioeconomy: A Literature Review Comparison between Latin America and the European Union. *Sustainability*. 2022;14(3):1661. doi:10.3390/su14031661

167. Kumar Sarangi P, Subudhi S, Bhatia L, et al. Utilization of agricultural waste biomass and recycling toward circular bioeconomy. *Environ Sci Pollut Res*. 2022;30(4):8526-8539. doi:10.1007/s11356-022-20669-1

168. Regulation (EC) No 1223/2009. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Published online November 30, 2009. https://health.ec.europa.eu/system/files/2016-11/cosmetic_1223_2009_regulation_en_0.pdf

169. National Waste Management Plan. Rijkswaterstaat Environment. Accessed November 26, 2023. https://rwsenvironment.eu/subjects/from-waste-resources/national-activities/national-waste/

170. Zaken M van A. Environmental Management Act - Environment - Government.nl. Published December 13, 2011. Accessed November 26, 2023. https://www.government.nl/topics/environment/roles-and-responsibilities-of-central-government/environmental-management-act

171. Bouchaut B, De Vriend H, Asveld L. Uncertainties and uncertain risks of emerging biotechnology applications: A social learning workshop for stakeholder communication. *Front Bioeng Biotechnol*. 2022;10:946526. doi:10.3389/fbioe.2022.946526

172. Commodities Act. business.gov.nl. Accessed November 26, 2023. https://business.gov.nl/regulation/commodities-act/

173. Regulation (EC) No 767/2009. Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. Published online July 13, 2009. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:229:0001:0028:EN:PDF

174. Kim EJ, Cha MN, Kim BG, Ahn JH. Production of Curcuminoids in Engineered Escherichia coli. *J Microbiol Biotechnol*. 2017;27(5):975-982. doi:10.4014/jmb.1701.01030

175. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. *Eur J Cancer*. 2005;41(13):1955-1968. doi:10.1016/j.ejca.2005.05.009

176. Stankovic I. CURCUMIN: Chemical and Technical Assessment (CTA). Published online 2004. https://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/61/Curcumin.pdf

177. Urošević M, Nikolić L, Gajić I, Nikolić V, Dinić A, Miljković V. Curcumin: Biological Activities and Modern Pharmaceutical Forms. *Antibiotics*. 2022;11(2):135. doi:10.3390/antibiotics11020135

178. Han S, Yang Y. Antimicrobial activity of wool fabric treated with curcumin. *Dyes Pigments*. 2005;64(2):157-161. doi:10.1016/j.dyepig.2004.05.008

179. Incha MR, Thompson MG, Blake-Hedges JM, et al. Leveraging host metabolism for bisdemethoxycurcumin production in Pseudomonas putida. *Metab Eng Commun*. 2020;10:e00119. doi:10.1016/j.mec.2019.e00119

180. Hassanizadeh S, Shojaei M, Bagherniya M, Orekhov AN, Sahebkar A. Effect of nano-curcumin on various diseases: A comprehensive review of clinical trials. *BioFactors*. 2023;49(3):512-533. doi:10.1002/biof.1932

181. Ryu V, Chuesiang P, Corradini MG, et al. Synergistic photoinactivation of Escherichia coli and Listeria innocua by curcumin and lauric arginate ethyl ester micelles. *LWT*. 2023;173:114317. doi:10.1016/j.lwt.2022.114317

182. Lao CD, Ruffin MT, Normolle D, et al. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med*. 2006;6(1):10. doi:10.1186/1472-6882-6-10

183. Hewlings SJ, Kalman DS. Curcumin: A Review of Its' Effects on Human Health. *Foods*. 2017;6(10):92. doi:10.3390/foods6100092

184. Burkina V, Zamaratskaia G, Rasmussen MK. Curcumin and quercetin modify warfarin-induced regulation of porcine CYP1A2 and CYP3A expression and activity *in vitro*. *Xenobiotica*. 2022;52(5):435-441. doi:10.1080/00498254.2022.2089932

185. Prasad S, Tyagi AK, Aggarwal BB. Recent Developments in Delivery, Bioavailability, Absorption and Metabolism of Curcumin: the Golden Pigment from Golden Spice. *Cancer Res Treat*. 2014;46(1):2-18. doi:10.4143/crt.2014.46.1.2

186. Servida S, Panzeri E, Tomaino L, et al. Overview of Curcumin and Piperine Effects on Glucose Metabolism: The Case of an Insulinoma Patient's Loss of Consciousness. *Int J Mol Sci*. 2023;24(7):6621. doi:10.3390/ijms24076621

187. Heidari H, Bagherniya M, Majeed M, Sathyapalan T, Jamialahmadi T, Sahebkar A. Curcumin-piperine co-supplementation and human health: A comprehensive review of preclinical and clinical studies. *Phytother Res*. 2023;37(4):1462-1487. doi:10.1002/ptr.7737

188. Niranjana Sri S, Thiagarajan R, Manikandan R, Arumugam M. Curcumin-Based Food Supplements: Challenges and Future Prospects. In: *Nonvitamin and Nonmineral Nutritional Supplements*. Elsevier; 2019:119-128. doi:10.1016/B978-0-12-812491-8.00016-3

189. Paoletti C, Flamm E, Yan W, et al. GMO risk assessment around the world: Some examples. *Trends Food Sci Technol*. 2008;19:S70-S78. doi:10.1016/j.tifs.2008.07.007

190. Joint FAO/WHO Codex Alimentarius Commission. *Foods Derived from Modern Biotechnology - Second Edition (2nd Ed.)*. WHO/FAO; 2009. https://www.fao.org/sustainable-food-value-chains/library/details/en/c/265868/

191. Regulation (EU) 2015/2283. Regulation (EU) 2015/2283 of The European Parliament and The Council of 25 November 2015 on novel foods. Published online December 11, 2005. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283

192. Regulation (EC) No 1829/2003. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Published online October 18, 2003. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1829&qid=1692420063731

193. SCFCAH. *Standing Committe on The Food Chain and Animal Health: Section on Geneteically Modified Food and Feed and Environmental Risk - Summary Record of the 3rd Meeting - 24 September 2004*.; 2004.

194. Daffé M, Draper P. The Envelope Layers of Mycobacteria with Reference to their Pathogenicity. In: Poole RK, ed. *Advances in Microbial Physiology*. Vol 39. Academic Press; 1997:131-203. doi:10.1016/S0065-2911(08)60016-8

195. Paerl HW, Otten TG. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microb Ecol*. 2013;65(4):995-1010. doi:10.1007/s00248-012-0159-y

196. Bai X, Nakatsu CH, Bhunia AK. Bacterial Biofilms and Their Implications in Pathogenesis and Food Safety. *Foods*. 2021;10(9):2117. doi:10.3390/foods10092117

197. Hwangbo M, Chu KH. Recent advances in production and extraction of bacterial lipids for biofuel production. *Sci Total Environ*. 2020;734:139420. doi:10.1016/j.scitotenv.2020.139420

198. Choi SY, Cho IJ, Lee Y, Kim Y, Kim K, Lee SY. Microbial Polyhydroxyalkanoates and Nonnatural Polyesters. *Adv Mater*. 2020;32(35):1907138. doi:10.1002/adma.201907138

199. Nitrogen and PFAS suddenly big societal issues in the Netherlands | RIVM. Accessed October 18, 2023. https://www.rivm.nl/en/newsletter/content/2020/issue1/nitrogen-pfas-in-NL

200. Dickman RA, Aga DS. A review of recent studies on toxicity, sequestration, and degradation of per- and polyfluoroalkyl substances (PFAS). *J Hazard Mater*. 2022;436:129120. doi:10.1016/j.jhazmat.2022.129120

201. THE 17 GOALS | Sustainable Development. Accessed October 18, 2023. https://sdgs.un.org/goals

202. Voigt B. Genome Editing: Why go beyond Precaution? The (Non-) Place for 'Irrational'Public Opinions in EU Risk Regulation. In: Nomos Verlagsgesellschaft mbH & Co. KG; 2019:123-134.

203. Pechlaner G. Biotechnology activism is dead; long live biotechnology activism! The lure and legacy of market-based food movement strategies. *Agric Hum Values*. Published online September 5, 2023. doi:10.1007/s10460-023-10501-y

204. Jha S, Thakur ND, Singh AA. Regulatory Framework for GMOs-Interplay Between Regulatory Policies and Socio-Ethical Concern. In: *Policy Issues in Genetically Modified Crops*. Elsevier; 2021:401-419. doi:10.1016/B978-0-12-820780-2.00018-2

205. Usmani Z, Sharma M, Awasthi AK, et al. Bioprocessing of waste biomass for sustainable product development and minimizing environmental impact. *Bioresour Technol*. 2021;322:124548. doi:10.1016/j.biortech.2020.124548

206. Wang X, Li C, Lam CH, et al. Emerging waste valorisation techniques to moderate the hazardous impacts, and their path towards sustainability. *J Hazard Mater*. 2022;423:127023. doi:10.1016/j.jhazmat.2021.127023

207. Pongrácz E, Pohjola VJ. Re-defining waste, the concept of ownership and the role of waste management. *Resour Conserv Recycl*. 2004;40(2):141-153. doi:10.1016/S0921-3449(03)00057-0

208. Directive 2008/98/EC. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. Published online May 7, 2018. https://eur-lex.europa.eu/eli/dir/2008/98/oj/eng

209. Directive 2010/75/EU. Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control). Published online June 1, 2011. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32010L0075

210. Council Directive 91/271/EEC. Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). Published online January 1, 2014. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A01991L0271-20140101

211. Directive 2001/18/EC. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. Published online March 27, 2021. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32001L0018

212. Regulation (EU) 2020/741. Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse. Published online May 25, 2020. https://eur-lex.europa.eu/eli/reg/2020/741/oj

213. Council Directive 1999/31/EC. Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste. Published online July 4, 2018. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A01999L0031-20180704&qid=1690203968989

214. Escobar N, Laibach N. Sustainability check for bio-based technologies: A review of process-based and life cycle approaches. *Renew Sustain Energy Rev*. 2021;135:110213. doi:10.1016/j.rser.2020.110213

215. Quik JTK, Lijzen JPA, Spijker J. Creating safe and sustainable material loops in a circular economy : Proposal for a tiered modular framework to assess options for material recycling. Published online 2018. https://www.rivm.nl/bibliotheek/rapporten/2018-0173.pdf

216. Traas TP, Zweers P, Quik JTK, Waaijers SL, Lijzen JPA. *Towards Sustainable Recycling : A Method for Comparing the Safety and Sustainability of the Processing of Residual Flows*. Rijksinstituut voor Volksgezondheid en Milieu RIVM; 2021. Accessed October 17, 2023. https://rivm.openrepository.com/handle/10029/624783

217. Ampelli C, Perathoner S, Centi G. CO2 utilization: an enabling element to move to a resource- and energy-efficient chemical and fuel production. *Philos Trans R Soc Math Phys Eng Sci*. 2015;373(2037):20140177. doi:10.1098/rsta.2014.0177

218. Doran PM. *Bioprocess Engineering Principles*. Elsevier; 1995.

219. McAloone TC, Bey N. *Environmental Improvement through Product Development: A Guide*. Danish Environmental Protection Agency; 2009.

220. Cooper RG. The Stage-Gate Idea to Launch System. In: *Wiley International Encyclopedia of Marketing*. John Wiley & Sons, Ltd; 2010. doi:10.1002/9781444316568.wiem05014

221. Kuiper M. 'Wilde theorieën' over hoe sporen van amfetamine bij 23 mestfabrieken terechtkwamen. *NRC*. https://www.nrc.nl/nieuws/2023/11/01/wilde-theorieen-over-hoe-sporen-van-amfetamine-bij-23-mestfabrieken-terechtkwamen-a4179393. Published November 1, 2023. Accessed November 28, 2023.

Annex I: Reflection on the SSbD guideline by RIVM[17]

The RIVM guideline provides a comprehensive overview and introductory guidance on addressing safety and sustainability in industrial biotechnology. It effectively marks a step towards embracing a circular economy, enhancing understanding of sustainability and circularity concepts. The guideline successfully highlights the critical steps and challenges in bioprocess design and offers a detailed depiction of the current regulatory framework, complete with useful resources for further reading.

However, our feedback indicates a lack of awareness about the guide's existence, suggesting a need for more proactive promotion. This gap in awareness was echoed by several interviewees. Regarding the tools listed in the guideline, aside from Tool 4 (Life Cycle Assessment - LCA) and Tool 8 (Information on legal frameworks and chemical hazards), most interviewees were unaware of their existence. These tools, however, are valuable for student learning and reflection.

Recommendations

1. Early Integration of LCA: Introduce Life Cycle Assessment (LCA) at earlier stages and explore the potential of predictive tools such as the ex-Ante method. Include guidance on evaluating and substituting solvents for safer alternatives. (*e.g.*, at least process design stage, if not already in concept stage).
2. Social LCA (S-LCA): Incorporate Social Life Cycle Assessment to address global socio-economic questions comprehensively (*e.g.*, in concept stage)
3. Stakeholder Involvement: Provide a more concrete guide on stakeholder involvement, potentially through the creation of platforms or innovation communities.
4. Incorporate a Stage-Gate Model: This would provide a structured approach to project management and decision-making.[220]
5. DTU Guideline Reference: Consider referencing the guideline by McAloone et al. (2009) from DTU (Environmental improvement through product development – a guide) for its step-by-step approach to environmental improvement in product processes.[219]
6. Certifications and Directives: Include information on relevant certifications (*e.g.*, GMP+, ISO standards) and additional EU directives applicable to the biotech industry.
7. Practical Examples: Use case studies or examples to clarify the guideline's application and relevance.
8. Waste Management Focus: Place greater emphasis on waste recovery and separation, highlighting the importance of feedstock choices.

9. Terminology Section: A dedicated section explaining key terms (Safety, Sustainability, Product/By-product/Side-stream/Waste, End-of-Life/Recycling, “Cradle to Cradle” or “Cradle to Grave” principles) would enhance understanding.
10. Design Stage Impact: Emphasize that 80% of a product’s sustainability is determined at the design stage, highlighting the responsibility of stakeholders to collaborate effectively.
11. Comment on Figure 2: The current depiction of a circular bioeconomy workflow in Figure 2 is lacking. It misses crucial steps and thoughts about end-of-life scenarios for products and wastes, as well as the valorisation of waste and side-streams.

By addressing these areas, the RIVM guideline could significantly enhance its utility and effectiveness in guiding safety and sustainability improvements in the biotech industry.

Annex II: Reflection on prior SSbD embedding study

The preliminary exploratory study by Maaike van der Horst et al. (2023)[112] , commissioned by the Ministry of Infrastructure and Water Management (IenW), laid the groundwork for integrating Safe-and-Sustainable-by-Design (SSbD) principles in circular bio-economy research. Building upon this foundation, our current study supports these findings and delves deeper in two significant ways.

Firstly, it accentuates the critical need to integrate sustainability into the early design stages of novel biotechnological processes. While SSbD principles have been established in nanotechnology and the chemical industry, their application in circular biotechnology workflows is still evolving.

In the previous study integrating the Design-Build-Test-Learn (DBTL) strategy, established in synthetic biology and strain engineering, with the SSbD related "Who?", "How?", "What?", and "When?" (WHWW) questions, was suggested. This integration can map the principles and questions of the DBTL cycle and WHWW questions to the key steps identified in our study, which align with a circular bioeconomy workflow. Applying the DBTL approach in this context is particularly relevant for designing entirely new bioprocesses, especially those utilising unconventional feedstocks to revise and optimise the process designs. Moreover, we see the potential for this strategy as a means to revaluate and optimise established processes, incorporating new knowledge over time.

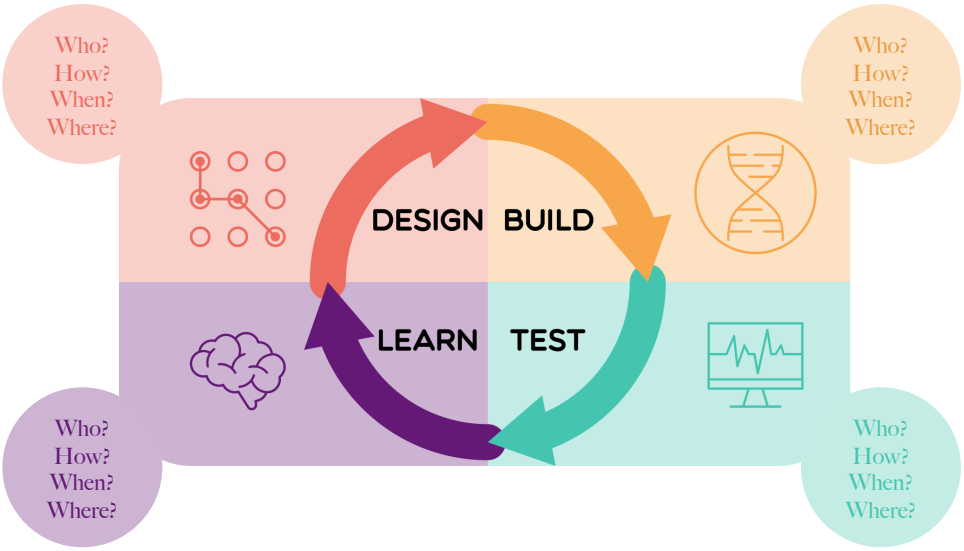


Figure 3. DBTL cycle schematic highlighting that the WHWW questions need to be addressed for each step. Figure adapted from the 2022 iGEM Wageningen Team wiki: https://2022.igem.wiki/wageningen-ur/engineering.

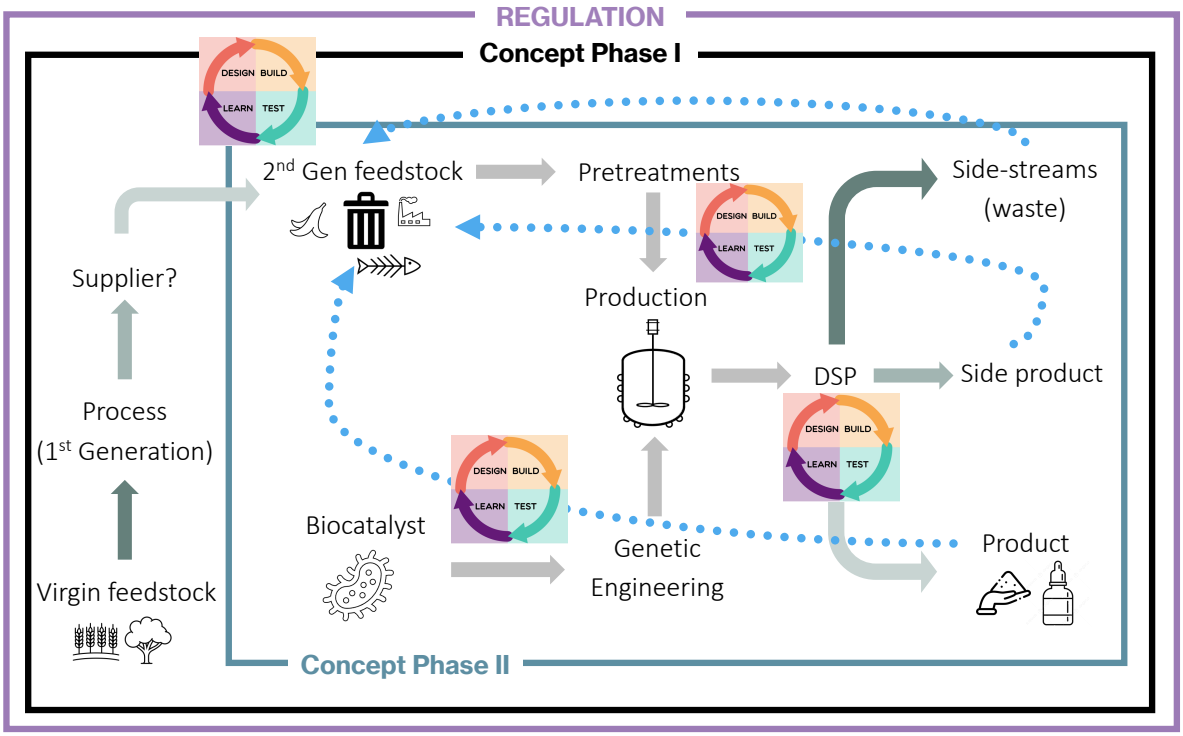


Figure 4. Value chain approach including the DBTL cycle framework at various critical steps where new technologies will be needed to achieve a circular bioeconomy.

Secondly, addressing the previous report's call for concrete SSbD guidelines for practitioners, our report reviews various existing guidelines, including the newly published guide by RIVM. These guidelines, along with additional recommendations highlighted in the previous section (see Annex I), emphasise the continuous necessity to revisit and refine the "Who?", "How?", "What?", and "When?" (WHWW) questions. These are vital for making informed decisions and evaluations in the design and analysis of new bioprocesses. Developing a comprehensive and practical guideline for the diverse and case-specific nature of circular bioprocesses is a long-term endeavour requiring the commitment of multiple stakeholders.

Given the complexity of this task, we also recommend the formation of innovation communities. These communities would proactively engage stakeholders over extended periods, fostering ongoing discussions and collaborations throughout various stages of design and upscaling in the value chain. This approach is vital for advancing towards a truly circular bioeconomy.

Annex III: Literature study details

The literature study for the project "Embedding Safe-by-Design in bioeconomy workflows: From feedstock to product and valorizable waste" was carried out between 20-09-2022 and 12-10-2023. The table below (Annex III) shows the (sub)topics that needed to be studied to understand the complex matters of the research question, and to acquire knowledge about the current research status to give a profound opinion and recommendations on how to embed Safe-by-Design in circular bioprocesses. The respective keywords used in the online databases (google, google scholar, Science Direct) are listed accordingly.

Table Annex III. Overview of topics and keywords used for the literature study on how to embed Safe-by-Design in bioeconomy workflows.

Topic	Search (knowledge) goal	Keywords
General		
Safe-by-Design/ Safe-and-Sustainable-by-Design	Status on S(S)bD definition, implementation & guidelines, stakeholder identification & role	"Safe by Design responsibility", "Safe by Design biotechnology", "Safe by Design biotechnology guidelines", "stakeholder involvement in Safe-by-Design" " 'safe innovation approach' AND 'regulatory preparedness' "
Circular economy/ Bioeconomy	Definition, status of research & achievements, identifying challenges	"critical factors in circular economy", "bioeconomy challenges", "bioeconomy prospects", "sustainability in industrial biotechnology"
Bioprocess value chain	Identifying workflow steps, understanding complexity, risks biotechnological bioprocesses	"risks in biotechnology", "challenges industrial biotechnology", "bioprocess steps", "bioprocess value chain", "bioprocess workflow", "sustainable bioprocess"
Bioprocess steps		
Sustainable feedstock	Identify sustainable feedstocks, feedstocks in the context of circular bioeconomy and biotechnology	"biomass in circular economy", "sustainable feedstock for chemicals", "CO2 as sustainable feedstock", "CO2 reuse and capture", "Syngas bioconversion", "organic waste feedstock", "microbial conversion CO2", "agri food waste fermentation", "first second generation sugar AND review", "'sugar feedstocks' AND 'life cycle assessment' ", "animal manure feedstock", " 'spent microbial biomass' AND 'circular economy' ", "forestry waste

		further use", "green chemicals challenge", "environmental assessment of sustainable feedstocks"
Host microbe	Industrial biocatalyst, safety risks & concerns, current SbD implementation for biocatalyst choice, risk assessment	"microbial biotechnology", "role of microbes biotechnology", "industrial biotechnology AND sustainability", "biosafety tool", "safety frame work biotechnology", "GMO debate in Dutch society", "genetic safeguards", "Safe-by-Design AND xenobiology", "GMO Risk assessment" , ""risk assessment" AND "genetically modified microorganisms"", "EU directive release GMO"
Fermentation	Safety challenges & risks during production process	"cross-contamination fermentation", "challenges microbial fermentation", "technical challenges fermentation", "risks in fermentation"
Downstream process	Safety challenges & risks during downstream process	"challenges downstream processing", "safety challenges downstream processing", "safety risks downstream processing"
Product	(Biotechnological) product specific regulations regarding safety & sustainability	"food management systems global regulation", "food safety management systems implementation AND ISO22000:2018", "genetically engineered microorganisms AND food production", "regulation food additives", "EC regulation genetically modified food", "fermentation product risk", "GMO product risk"
Waste management (WM)/ Valorisation/ End-of-life scenarios	waste side-streams, regulation, possibilities/current implementation of WM in biotechnology/ fermentation, link between proper WM and circularity/safety/sustainability, possibilities of valorisation of fermentation waste, current regulation	"bioreactor waste management", "fermentation waste disposal", "waste definition", "EC regulation waste", "waste biomass AND sustainable", ""waste valorisation" AND "circular economy"", ""waste biomass" AND sustainable", "waste management minimization in food processing", "EU directive industrial emissions", "EU directive wastewater treatment", "EU directive landfill", "safety considerations in waste management", "waste

		incineration", "conversion culture media waste", "bacterial culture waste reuse", "ecological advantage solvent recovery", "bacterial culture waste reuse", "spent cell culture medium re-use", "EC regulation solvents food ingredients", "solvent recovery and reuse", "EC regulation REACH", "water reuse and recycling", "water reuse in industrial food processing", "EC regulation water reuse", "wastewater in food industry", "wastewater treatment and disposal"
Experimental showcase		
(Soda) Lignin	Status of valorising lignin (microbial and non-microbial), sustainability of lignin, utilization of lignin as carbon source by *P. putida*, Soda Lignin attributes, pretreatments for valorization	"enzymatic conversion of lignin", "lignin valorisation", "conversions of technical lignin", "lignin potential products", "environmental impact of lignin", "pre-treatment of lignocellulosic materials", "life cycle assessment of lignin", "life cycle assessment of lignin AND aromatics", "environmental impact of pulp and paper", "environmental assessment AND pulp and paper", "degradation of lignin", "opportunities and challenges of lignin utilization", "microbial degradation of lignin", "lignin valorisation pretreatment"
Curcuminoids	Compound classification & safety, (microbial) production ways	"curcumin characteristics", "microbial curcumin production", "curcumin technical assessment", "curcuminoids production engineered bacteria", "curcumin production pseudomonas putida", "extraction curcuminoids process design"
Pseudomonas putida	Safety classification, suitability as safe and sustainable biocatalyst, suitability as industrial host microorganism	"Pseudomonas putida capabilities", "Pseudomonas KT2440 host characteristics", "Pseudomonas putida AND industrial biotechnology", "Pseudomonas putida AND metabolic engineering", "Pseudomonas putida certification", "Pseudomonas putida AND Safe-by-Design", "Pseudomonas putida AND ecotoxicity"

Culture valorisation	Valorization/recycling of flask experiment culture media, reuse of GMMs	“bacterial culture waste reuse”, “spent cell culture medium re-use”

Annex IV: Interview guide

Semi-structured interview guide with open questions

Note: R = Regulator; O = Others

Colours: Dark grey = topics; Light grey = mandatory questions; White = keywords and follow-up questions (if needed)

Table Annex IV. Interview guide: Questions formulated prior to the semi-structured interviews.

Stakeholder: Academia	Stakeholder: Industrial sector	Stakeholder: Society (regulator (R) and others (O))
Introduction		
• Introduction of interviewers, framework of the project (theoretical & experimental part, SbD concept, focus of the study, aspects of safety concerns in a biotechnological process, mention main questions we are trying to answer…) • Interview structure, recording, consensus • Could you briefly introduce yourself, your (professional) background and current work, please?	• Introduction of interviewers, framework of the project (theoretical & experimental part, SbD concept, focus of the study, aspects of safety concerns in a biotechnological process, mention main questions we are trying to answer …) • Interview structure, recording, consensus • Could you briefly introduce yourself, your (professional) background and current work, please?	• Introduction of interviewers, framework of the project (theoretical & experimental part, SbD concept, focus of the study, aspects of safety concerns in a biotechnological process, mention main questions we are trying to answer …) • Interview structure, recording, consensus • Could you briefly introduce yourself, your (professional) background and current work, please?
Safety/ SbD implementation in the biomanufacturing process		
Could you tell us in which part of a bioproduction process your work focus lies on? (Showing diagram from upstream to downstream process) In your opinion, what are the most critical steps here regarding safety rules and concerns?	Could you tell us in which part of a bioproduction process your work focus lies on? (Showing diagram from upstream to downstream process) In your opinion, what are the most critical steps here regarding safety rules and concerns?	Would you say regulation of safety aspects in biotechnological processes play a crucial role in your daily work? In your opinion, in which steps of a biotechnological processes do you see the relevance of safety considerations/ risks the most?
Keywords: Research topic; detail of their roles (follow-up questions depend on the interviewee's answers)	**Keywords:** Upstream and/or downstream process, waste/side stream (follow-up questions depend on the interviewee's answers)	**Keywords:** Connection (yes/no); detail of their roles (follow-up questions depend on the interviewee's answers)

Stakeholder: Academia	Stakeholder: Industrial sector	Stakeholder: Society (regulator (R) and others (O))
Follow-up questions: • In which way would you say is safety playing a role when choosing the three main building blocks of a bioconversion process: the feedstock, host-organism, and product? To which extend would you say are critical aspects and choices regarding the material (feedstock, biocatalyst, product) taken into account in the design phase of a project? • Could you evaluate the choices that are being made for the feedstock (or host organism), especially in regard to safety and quality? • In your experience, what are the main critical steps in the manufacturing (bioconversion/fermentation) process? • Would you suggest that life cycle analysis (LCA) and techno economic analysis (TEA) are always necessary to be performed for a bioeconomic process, if yes, in which stage would you recommend focusing on these? If the person is more related to the upstream process: • What would you say are your main concerns when talking about using non-conventional feedstocks from agricultural or industrial side-streams for bioconversion processes? • Is your team taking safety considerations for the follow-up fermentation and downstream processes into account? Are these evaluated in a joined matter together with other stakeholders beforehand? • Would you say there are specific steps that need	Follow-up questions: • In which way would you say is safety playing a role when choosing the three main building blocks of a bioconversion process: the feedstock, host-organism, and product? To which extend would you say are critical aspects and choices regarding the material (feedstock, biocatalyst, product) taken into account in the design phase of a project? • Could you evaluate the choices that are being made for the feedstock (or host organism), especially in regard to safety and quality? • In your experience, what are the main critical steps in the manufacturing (bioconversion/fermentation) process? • Would you suggest that life cycle analysis (LCA) and trace element analysis (TEA) are always necessary to be performed for a bioeconomic process, if yes, in which stage would you recommend focusing on these? • In your opinion, what would you say the main differences are regarding needed safety considerations between academic research and industrial production? If the person is more related to the upstream process: • What would you say are your main concerns when talking about using non-conventional feedstocks from agricultural or industrial side-streams for bioconversion processes? • Is your team taking safety considerations for the follow-up fermentation and downstream processes into account? Are these evaluated	Follow-up questions: • Could you guide us through the process and critical steps of your work, when working on new safety regulations/ implementations/ recommendations for companies and research? • Where would you see the most critical choices and steps, and safety risks to be taken in a bioproduction process? Especially when focusing on the decisions of feedstock, biocatalyst and the product (focus on food additives/products)? • When using a less well-defined feedstock, such as agricultural or industrial waste, as potential bioconversion feedstock, seems contradicting in the first place with thorough safety measurements, what are in your experience the main questions that need to be addressed when choosing these feedstocks and their treatment for the near future? • Are you including the public's opinion in your work when it comes down to using or designing processes that include GMOs or usage of wastes as feedstocks? What relevance might it have? • Could you describe the differences regarding safety risks and considerations when it comes to food products in particular (than from chemical building blocks for other applications)? • Are there particular assessments/ analysis for using various feedstocks and for the re-use of the biocatalyst, fermentation broth and side-streams that

Stakeholder: Academia	Stakeholder: Industrial sector	Stakeholder: Society (regulator (R) and others (O))
further investigation or improvement regarding safety concerns? Or were there recent new guidelines being implemented in your work/ team? • Are safety and sustainability considerations ultimately intertwined in your work? • Do you have the feeling we missed to touch upon an aspect when talking about critical choices and risks that affect safety in your work? If person is more related to the downstream process: • What are your thoughts on safety when we focus on the biocatalyst, the product and the fermentation broth? • Are there measurements taken in your group for assessing the possible reuse of the broth and catalyst? Are there concerns of safety occurring in this process, or are there specific criteria and guidelines? • What are your thoughts on GMO product safety evaluation/ assurance? What are the critical choices in your opinion? • Do you have the feeling we missed to touch upon an aspect when talking about critical choices and risks that affect safety in your work?	in a joined matter together with other stakeholders beforehand? • Would you say there are specific steps that need further investigation or improvement regarding safety concerns? Or were there recent new guidelines being implemented in your work/ team? • Are safety and sustainability considerations ultimately intertwined in your work? • Do you have the feeling we missed to touch upon an aspect when talking about critical choices and risks that affect safety in your work? If person is more related to the downstream process: • What are your thoughts on safety when we focus on the biocatalyst, the product and the fermentation broth? • Are there measurements taken in your group for assessing the possible reuse of the broth and catalyst? Are there concerns of safety occurring in this process, or are there specific criteria and guidelines? • What are your thoughts on GMO product safety evaluation/ assurance? What are the critical choices in your opinion? • Do you have the feeling we missed to touch upon an aspect when talking about critical choices and risks that affect safety in your work?	reduce the risks of harmful side-products or other safety risks?

Waste management/ Circularity		
From your experience in academic research, would you say that safety of the waste management programme is considered appropriately, or do you see room for improvements? When thinking about circularity, and hence	From your experience in industrial production processes, would you say that safety of the waste management is considered appropriately, or do you see room for improvements?	What would you suggest as the ideal handling of industrial biotechnology waste? What are your thoughts on circularity when it comes to the production/biomanufacturing

Stakeholder: Academia	Stakeholder: Industrial sector	Stakeholder: Society (regulator (R) and others (O))
diminishing the term 'waste', what are in your opinion the main challenges?	When thinking about circularity, and hence diminishing the term 'waste', what are in your opinion the main challenges? What are the main dilemmas when facing recycling of agricultural/industrial waste?	process and reusing side-streams?
Keywords: waste stream process; waste reuse; recycle; waste disposal; GMO waste; circularity	**Keywords:** waste stream process; waste reuse; recycle; waste disposal; GMO waste; circularity	**Keywords:** waste reuse; recycle; waste disposal; circularity
Follow-up questions: • Is your team, or collaborators, performing LCA or TEA on the waste/ side-streams that are produced? • Are you considering/ analysing various end-of-life scenarios for the biocatalyst, product and the remaining fermentation broth? • Where there processes in which, e.g. the agriculturally used chemicals for the main crop, caused undesired interference with the fermentation process? (Or industrial chemicals from pulp/paper industry) If yes, did your team encounter toxic/ undesired side-product formation? • Is it possible for you to formulate a few general recommendations regarding safe (and sustainable) waste management in the context of circularity?	Follow-up questions: • Is your team, or collaborators, performing LCA or TEA on the waste/ side-streams that are produced? • Are you considering/ analysing various end-of-life scenarios for the biocatalyst, product and the remaining fermentation broth? • Were there processes in which, e.g. the agriculturally used chemicals for the main crop, caused undesired interference with the fermentation process? (Or industrial chemicals from pulp/paper industry) If yes, did your team encounter toxic/ undesired side-product formation? • Is it possible for you to formulate a few general recommendations regarding safe (and sustainable) waste management in the context of circularity?	Follow-up questions: • Could you evaluate on how the different regulatory frameworks are applied and assessed (checked) in terms of waste management in the different industrial biotechnology sectors? • Could you name methods/ measurements (as LCA, TEA) for waste reuse/recycling? • Are you considering/ analysing various end-of-life scenarios for the biocatalyst, product and the remaining fermentation broth? • Is it possible for you to formulate a few general recommendations regarding safe (and sustainable) waste management in the context of circularity?

Conclusion and recommendations		
• After discussing the role of safety in your work thoroughly in the past hour, do you believe that a framework as the safe-by-design concept with involvement of various stakeholders throughout the whole process? Do you believe a guideline would accelerate/ support the application and acceptance	• After discussing the role of safety in your work thoroughly in the past hour, do you believe that a framework as the safe-by-design concept with involvement of various stakeholders throughout the whole process? Do you believe a guideline would accelerate/ support the application and acceptance	• After discussing the role of safety in your work in the past hour, do you believe that a framework as the safe-by-design concept with involvement of various stakeholders throughout the whole process is the right way forward? Do you believe a guideline would accelerate/ support the application and

Stakeholder: Academia	Stakeholder: Industrial sector	Stakeholder: Society (regulator (R) and others (O))
of biomanufacturing processes? • Do you have some recommendations to implement SbD in current bioeconomy industrial workflows? and in future ones? • Is there something you would like to say or add that has not been discussed? • Would you like to include a particular message for the ministry/policy makers regarding your work concerning the context of this project?	of biomanufacturing processes? • What kind of guideline would you invision now that might be useful? • *Since your product is directly linked to the consumer/ the general public, would you say there is the need to include the public from early on in the discussion of safety and sustainability of the product?* • Do you have some recommendations to implement SbD in current bioeconomy industrial workflows? and in future ones? • Is there something you would like to say or add that has not been discussed? • Would you like to include a particular message for the ministry/policy makers regarding your work concerning the context of this project?	acceptance of biomanufacturing processes? • would you say there is the need to include the public from early on in the discussion of safety and sustainability of the product? Whose involvement do you miss on a daily/monthly/ yearly basis? • Do you have some recommendations to implement SbD in current bioeconomy industrial workflows? and in future ones? • Is there something you would like to say or add that has not been discussed? • Would you like to include a particular message for the ministry/policy makers regarding your work concerning the context of this project?
Keywords: SbD implementation, problems, recommendation	**Keywords:** SbD implementation, problems, recommendation	**Keywords:** SbD implementation, problems, recommendation
Closing		
With this, we would like to thank you for your time and fruitful participation, your experience, and suggestions. Your contribution was very much appreciated and helpful. We will keep in touch to send you the final report about our study if you wish to receive it.	Ask for further recommendations of colleagues/ partners who might be interesting to talk to in the context of incorporating safety aspects in biotechnological processes. With this, we would like to thank you for your time and fruitful participation, your experience, and suggestions. Your contribution was very much appreciated and helpful. We will keep in touch to send you the final report about our study if you wish to receive it.	Recommendations to talk to other people from the industry? Especially Waste management? With this, we would like to thank you for your time and fruitful participation, your experience, and suggestions. Your contribution was very much appreciated and helpful. We will keep in touch to send you the final report about our study if you wish to receive it.