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# COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

## Item 8.3 of the Provisional Agenda

## Twentieth Regular Session

## Rome, 24–28 March 2025

# DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF FERMENTATION-ASSOCIATED MICROORGANISMS WITHIN THE AGRIFOOD SYSTEM

## NOTE BY THE SECRETARIAT

1. The Commission on Genetic Resources for Food and Agriculture (Commission), at its Seventeenth Regular Session, adopted its Work Plan for the Sustainable Use and Conservation of Micro-organism and Invertebrate Genetic Resources for Food and Agriculture (Work Plan).[1] The Work Plan addresses microorganisms and invertebrates as functional groups[2] and foresees that the two functional groups considered by the Commission at its Twentieth Regular Session will be (i) edible fungi and invertebrates used as dietary components of food/feed and (ii) microorganisms used in food processing and agro-industrial processes.[3]

2. In response to the Work Plan, FAO commissioned the preparation of a study on the sustainable use and conservation of microorganisms used in food processing and agro-industrial processes. As some of the agro-industrial processes covered in Background Study Paper 64, notably those related to nutrient cycling, biological control and biostimulation, have been addressed in other background study papers recently prepared under the Work Plan, the current study focuses on the use of microorganisms in food processing and in the processing of agro-industrial materials into non-food value-added products.

3 A draft version of study was made available to the Intergovernmental Technical Working Group on Micoorganism and Invertebrate Genetic Resources for Food and Agriculture at its First Session. The Working Group took note of the study and provided comments in writing. A revised draft is contained in this document.

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[1] CGRFA-17/19/Report, *Appendix E*.
[2] CGRFA-17/19/Report, *Appendix E*, paragraphs 8–14.
[3] CGRFA-17/19/Report, *Appendix E*, paragraph 14.

# SUSTAINABLE USE AND CONSERVATION OF FERMENTATION-ASSOCIATED MICROORGANISMS WITHIN THE AGRIFOOD SYSTEM

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This document has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture with a view to facilitating consideration by the Commission of the sustainable use and conservation of edible fungi and invertebrates used as dietary components of food/feed, at its Twentieth Regular Session. The content of this document is entirely the responsibility of the authors, and does not necessarily represent the views of the FAO or its Members.

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## Executive summary

The global agrifood system faces significant challenges related to both human and planetary health. It is responsible for 21–37 percent of anthropogenic greenhouse gas emissions[4] and is the primary driver of biodiversity loss. In addition to intensive use of resources, inefficiencies lead to more than 30 percent of the food produced globally being wasted.[5] At the same time, healthy diets are unaffordable to an estimated 2.8 billion people.[6] With a growing population, climate-related disruptions and a decline in the availability of agricultural land, meeting increasing demand will require improvements to the sustainability and resilience of the system.

Innovations in the use of fermentation-associated microorganisms in food processing and non-food agro-industrial processes offer technological solutions that could help the agrifood sector reduce environmental damage, adapt to a changing climate, promote a circular bioeconomy and ensure access to safe and nutritious foods.

Food-processing applications can be broadly grouped into the following categories: production of fermented foods (FFs) through traditional fermentation; production of fermentation-derived foods (FDFs) through biomass fermentation; and production of FDFs through precision fermentation. Production of non-food value-added products, including biofuels, pharmaceuticals and a variety of materials and chemicals (e.g. plastics, fabrics, solvents and clothing fibres), from agro-industrial by-products and waste is also made possible through precision fermentation.

Traditional fermentation, a technology that pre-dates agriculture, involves the transformation of a plant or animal substrate through the activity of microorganisms (bacteria or fungi). It can improve food preservation, food safety, nutritional value and flavour, and remains integral to many cultures worldwide. An estimated one-third of the human diet globally is composed of fermented foods. The fermented milk drink kefir, for example, has been evaluated in clinical, animal and *in vitro* studies for its health-promoting benefits and its potential as an intervention strategy for diet-related non-communicable diseases via its effect on the gut microbiome.[7] Promising results have been identified in both *in vitro* and animal studies, although small sample sizes, methodological variation and differences in kefir types limit the extent to which generalizations can be drawn from clinical studies[8][9][10]. The drink has been included as a recommended dairy item in some national dietary guidelines.[11]

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[4] Rosenzweig, C., Mbow, C., Barioni, L.G., Benton, T.G., Herrero, M., Krishnapillai, M., Liwenga, E.T. *et al.* 2020. Climate change responses benefit from a global food system approach. *Nature Food*, 1(2): 94–97. https://doi.org/10.1038/s43016-020-0031-z

[5] FAO. 2019. *The State of Food and Agriculture 2019: moving forward on food loss and waste reduction*. Rome. https://doi.org/10.18356/32f21f8c-en

[6] FAO, IFAD, UNICEF, WFP & WHO. 2024. *The State of Food Security and Nutrition in the World 2024 – Financing to end hunger, food insecurity and malnutrition in all its forms*. Rome. https://doi.org/10.4060/cd1254en

[7] Bourrie, B.C.T., Willing, B.P. & Cotter, P.D. 2016. The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology*, 7. https://doi.org/10.3389/fmicb.2016.00647

[8] Bessa, M.K., Bessa, G.R. & Bonamigo, R.R. 2024. Kefir as a therapeutic agent in clinical research: a scoping review. *Nutrition Research Reviews*, 37(1): 79–95. https://doi.org/10.1017/S0954422423000070

[9] Culpepper, T. 2022. The Effects of Kefir and Kefir Components on Immune and Metabolic Physiology in Pre-Clinical Studies: A Narrative Review. *Cureus*, 14(8): e27768. https://doi.org/10.7759/cureus.27768

[10] Rosa, D.D., Dias, M.M.S., Grześkowiak, Ł.M., Reis, S.A., Conceição, L.L. & Peluzio, M.D.C.G. 2017. Milk kefir : nutritional, microbiological and health benefits. *Nutrition Research Reviews*, 30(1): 82–96. https://doi.org/10.1017/S0954422416000275

[11] For example: USDA & HHS (United States Department of Agriculture & United States Department of Health and Human Services). 2020. *Dietary guidelines for Americans, 2020-2050.* 9th Edition. Washington DC.https://www.dietaryguidelines.gov/sites/default/files/2020-12/Dietary_Guidelines_for_Americans_2020-2025.pdf

Biomass fermentation leverages microbial biomass as a direct source of protein and other nutrients, whereas precision fermentation utilizes “microbial cell factories” to manufacture compounds such as important micronutrients.

Key advantages of FF and FDF production include shorter timelines (because of the speed at which microorganisms grow), reduced land and water use, potential to valorize waste and by-products from agrifood systems, and greater stability of supply and flexibility in the site of production because of reduced dependence on climatic and weather conditions. For example, the production of dairy protein β-lactoglobulin from a fungal cell factory eliminates the need to use pastureland to meet demand for whey-focused ingredients, although the carbon footprint of the process is greatly affected by the energy source used.[12] The use of photovoltaics to power microbial protein production using atmospheric carbon dioxide ($CO_2$) has been found to have the potential to provide a protein yield that is ten times as high and a caloric yield that is twice as high as those of any staple crop.[13] For non-food applications, precision fermentation provides an alternative to petrochemical-based systems.

Underpinning any fermentation process is the microbial diversity behind it. The diversity of the world’s more than 5 000 different types of fermented food reflects this bacterial and fungal diversity. Fermentation-associated microbial communities are products of traditional knowledge, as their members and structure are linked to the chosen substrate and the design of the fermentation process, which select for the microbial strains that define the qualities of the final food product.

Although insights into potential losses of fermentation-associated microbial biodiversity are limited, the disappearance of traditional practices and the knowledge associated with them represents a loss of microbial communities and the functionalities that may be associated with them. Globalization and industrialization are being accompanied by increasing use of starter cultures composed of one to three well-characterized domesticated microbial strains as opposed to the complex microbial communities associated with traditional practices. Further characterization of the latter communities can enable the identification of novel microbial species with properties that can potentially contribute to the production of nutritional and flavour compounds and to the design of more sustainable bioprocesses. Biomass and precision fermentation platforms make use of unique non-model microorganisms to achieve high yields and efficient resource use. The term non-model microorganism refers to species that have not been heavily studied and are not used as a standard organism in scientific research (e.g. *Escherichia coli* for bacteria, *Saccharomyces cerevisiae* for yeast).

*Status and trends in the production of fermented and fermentation-derived foods*

At present, production of FFs takes place at several scales, from the household level, where fermentation serves as a low-cost technique for preserving raw materials such as milk, to large industrial operations designed to meet increasing consumer demand. To enable standardization of products, improve control over microbial communities and lower the risk of contamination, extensive efforts are being put into starter-culture design, including identification of key strains in natural communities, use of non-conventional strains and assembly of semi-synthetic communities with genetically engineered or evolved members. Traditional fermentation is also being explored as a means of overcoming bottlenecks in the development of plant-based alternative proteins, including those related to sensory properties (e.g. taste, texture and smell), bioavailability of micronutrients, allergenicity and prevalence of antinutritional compounds.

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[12] Nielsen, M.B., Meyer, A.S. & Arnau, J. 2024. The next food revolution is here: recombinant microbial production of milk and egg proteins by precision fermentation. *Annual Review of Food Science and Technology*, 15(1): annurev-food-072023-034256. https://doi.org/10.1146/annurev-food-072023-034256; Behm, K., Nappa, M., Aro, N., Welman, A., Ledgard, S., Suomalainen, M. & Hill, J. 2022. Comparison of carbon footprint and water scarcity footprint of milk protein produced by cellular agriculture and the dairy industry. *The International Journal of Life Cycle Assessment*, 27(8): 1017–1034. https://doi.org/10.1007/s11367-022-02087-0

[13] Leger, D., Matassa, S., Noor, E., Shepon, A., Milo, R. & Bar-Even, A. 2021. Photovoltaic-driven microbial protein production can use land and sunlight more efficiently than conventional crops. *Proceedings of the National Academy of Sciences*, 118(26): e2015025118. https://doi.org/10.1073/pnas.2015025118

Because of concerns about the environmental impact of animal-derived proteins, a key target for FDF production is the supply of alternative proteins. Biomass fermentation circumvents many of the constraints associated with livestock production while providing products that have high protein quality, low energy density and low total and saturated fats. Given the diversity of edible microorganisms, microbial protein can be produced from a variety of feedstocks, ranging from inedible plant biomass and agricultural waste to direct $CO_2$ capture. Currently, however, microbial proteins for human consumption generally rely on sugars. The use of a bioreactor (a large vertical vessel) enables protein to be produced with a small land-use footprint and provides flexibility in terms of where this can be done, as the primary needs are access to a feedstock, water, air and electricity. In the case of precision fermentation-based FDFs, a variety of animal proteins (e.g. egg and dairy proteins) produced using microorganisms are already on the market, in addition to various micronutrients, food ingredients and additives that are too complex, costly and/or environmentally damaging to manufacture via conventional means.

Innovation in biomass and precision fermentation is striving to use engineering biology[14] tools and/or bioprocess design[15] to address challenges related to economic feasibility, sustainability and consumer acceptance. This is exemplified by carbon-capture-based production systems, which unlock the possibility of decoupling food production from land, biophysical conditions and photosynthesis. Engineering biology approaches utilized at different biological scales, from the protein-level to microbial communities, are not only being used to expand the range of products and compounds that can be feasibly produced by microorganisms but also enable this to be done through the valorization of by-products and waste from agrifood systems, for example lignocellulosic biomass.

*Challenges and opportunities for increased FF and FDF use*

Despite the potential contributions of FFs to food security, their inclusion in policy and intervention strategies remains limited. Major constraints include limited clinical evidence for their health benefits, and difficulties with the standardization of products and with the implementation of safe practices that minimize the risk of food-borne diseases in household-scale and informal production. Overcoming these constraints will require further studies on the metabolic factors underpinning the health benefits of FFs, and the development of processes that allow standardization and improved safety in artisanal set-ups.

With regard to technical barriers to expanding the use of FDFs, there is a pressing need to increase global fermentation capacity, especially in resource-limited regions. The lack of facilities for scaling up bioprocesses is a key factor limiting the transfer of technologies from the laboratory into industrial use. Both the construction of new commercial manufacturing facilities and the retrofitting of existing ones for new technological processes are capital intensive and require significant investments. The lack of demo-scale plants makes it difficult to ensure that designed bioprocesses perform at scale and can compete in terms of cost. Economic barriers, energy-requirements and feedstock selection are also critical in determining the feasibility, competitiveness and sustainability of bioprocesses. The choice of feedstock, which supplies the essential nutrients carbon and nitrogen, substantially effects the land- and water-use efficiency, carbon footprint and circularity of the process. Although at present most bioprocesses rely on sugar derived from high-yielding sugar crops, for some food and feed applications the use of agricultural by-products, food-processing wastewater and direct carbon capture have been proven at scale.

Further development of the sector will require legislative action and technical innovation. Fundamentally, however, realization of the potential of fermentation technologies to improve the

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[14] Engineering biology consists of the design and construction of biological systems (e.g. proteins, metabolisms, whole cells and microbial communities) via the application of engineering principles to biology.

[15] Bioprocess design deals with the techniques involved in the manufacturing of products using living cells or their components. This encompasses preparation, production and purification steps.

capacity, resilience and sustainability of the agrifood system hinges on consumers' trust in products, manufacturers and regulatory bodies.

### *Non-food product biomanufacturing from agro-industrial materials*

Precision fermentation involving the use of one or more natural or engineered microorganisms has been extensively explored as a means of producing non-food products, including biofuels, platform and bulk chemicals with extensive applications (e.g. organic acids such as lactic and succinic acid, amines and short-chain diols), biomaterials, bio-based solvents and pharmaceuticals. Fermentation technologies have the potential to play a role in the shift towards a circular economy in the chemical industry, which currently relies heavily on non-renewable fossil fuels. The use of agro-industrial by-products and wastes as feedstocks for such bioprocesses has been investigated as a way of making manufacturing more sustainable and economically viable. Examples include non-edible lignocellulosic biomass residues (e.g. stalks, straws and bagasse), non-food crops (e.g. silvergrass), forestry residues, fruit waste (e.g. peels) and food-processing wastewater.

Biomanufacturing fuelled by agro-industrial materials has reached commercial scale for some applications and is being piloted in biorefinery plants around the world. However, the cost of production still exceeds that of petrochemical alternatives, posing a challenge to the expansion of this approach. Innovation in integrated biorefinery models, where a single processing facility converts biomass into power, heat and value-added products, holds promise as a means of improving efficiency and lowering environmental impacts.

### *Conservation of fermentation-associated microorganisms*

Conservation and characterization of microbial diversity are pivotal to its sustainable use in the further development of a bio-based economy. The long-term storage and stable preservation of microorganisms, whether isolated from nature or engineered, are possible through culture collections. These can be large public repositories accessible in accordance with international agreements, or research collections in academic institutions or industrial research laboratories. The Culture Collections Information Worldwide[16] repository currently records 856 culture collections worldwide, based in 80 countries and storing more than 4 million microorganisms. However, in many parts of the world shortages of funding (especially for meeting high long-term operating costs), infrastructure and expertise restrict opportunities to identify, characterize and store isolates relevant to fermentation applications. Technical challenges surrounding the preservation of microbial communities as a whole are another major obstacle.

### *Policy landscape*

The policy landscape for fermentation-associated microorganisms encompasses frameworks regulating the use and sale of FFs and FDFs, including those related to genetically engineered microorganisms as they pertain to FDFs, frameworks related to conservation and frameworks related to ownership of genetic resources.

As products of traditional knowledge with great potential for commercialization, fermentation-associated microorganisms must be protected via access and benefit-sharing frameworks to ensure equitable use. The Convention on Biological Diversity and the Nagoya Protocol strive to achieve this. However, unintended consequences of their application include constraints to the expansion of culture collections and microbial biological resource centres (mBRCs) because of the complex bureaucratic bilateral negotiations required for sampling and collaborative characterization efforts. This has an impact on both academic researchers and biotechnological companies, potentially hindering the use of promising microbial strains. Questions remain about the ownership status of microorganisms prevalent around the world and not closely linked to a particular geographical location. Moreover, in the case of

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[16] https://ccinfo.wdcm.org

microbial cell factories designed using genetic sequences from multiple sources and with lengthy value chains there are ambiguities as to how benefit-sharing should be pursued.

To promote *in situ* conservation and help realize the bioeconomic opportunities associated with the sustainable use of genetic resources by Indigenous Peoples and local communities, capacity building is needed, for example in the construction of culture collections.

Harmonizing international standards would allow regulatory processes to be streamlined, facilitating market entry for FFs and FDFs. Current frameworks are often fragmented and out of step with industry developments. In the case of labelling in particular, this can have marked effects on consumer acceptance.

Policy surrounding the equitable integration of novel technologies and products into the agrifood system is another pressing issue. Scaling-up of FDF products has the potential to disrupt current food production methods, causing concern about impacts on food sovereignty, rural communities and landscapes, the labour force involved in livestock production, and the industries that supply the agricultural sector and depend on non-food animal-derived products, such as leather. Responsible innovation and a just transition require the development of effective policy and/or legal frameworks. This would greatly benefit from multistakeholder engagement and involvement.

Global and regional research and industrial networks relevant to FFs and FDFs and to the conservation of related microorganisms are emerging worldwide. The following is a non-exhaustive list of significant players among these: the International Scientific Association for Probiotics and Prebiotics,[17] the Good Food Institute,[18] Precision Fermentation Alliance,[19] Food Fermentation Europe,[20] the International Dairy Federation,[21] the Lactic Acid Bacteria Industrial Platform,[22] Future Food Asia,[23] European Food & Fermentation Cultures Association,[24] the UK Research and Innovation Microbial Food Hub, the Bezos Centre for Sustainable Protein, the COST (European Cooperation in Science and Technology) Action PIMENTO (Promoting Innovation of ferMENTed fOods),[25] DOMINO (a European network on fermented foods),[26] the Swedish South Asian Network for Fermented Foods,[27] World Federation for Culture Collections,[28] Global Biological Resource Centre Network,[29] Global Microbiome Conservancy[30] and the Microbiota Vault.[31]

*Recommendations*

The following recommendations are intended to guide efforts to address current challenges in the conservation and sustainable use of fermentation-associated microorganisms in the agrifood system.

- Frameworks for the long-term financial support of culture collections and mBRCs need to be developed. One avenue could be to involve the private sector, given its dependence on such collections. Another would be funding via international organizations for centralized

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[17] https://isappscience.org
[18] https://gfi.org
[19] https://www.pfalliance.org
[20] https://www.foodfermentation.eu
[21] https://fil-idf.org
[22] https://labip.com
[23] https://futurefoodasia.com
[24] https://effca.org
[25] https://www.cost.eu/actions/CA20128
[26] https://www.domino-euproject.eu/about/m4sf
[27] https://fermented-foods.net.in
[28] https://wfcc.info
[29] https://www.bionity.com/en/associations/69535/global-biological-resource-centre-network-gbrcn.html
[30] https://microbiomeconservancy.org
[31] https://www.microbiotavault.org

collections, with clear and simple pathways for accessing resources in line with international agreements.

- In parts of the world where such capacity is currently weak or non-existent, there is a need to build the infrastructure and skills needed to preserve and characterize the microbial communities behind local fermented foods and enable the development of starter cultures. Similar efforts are needed to support the characterization and preservation of microbial strains from various environmental niches that may have potential for use in the production of platform chemicals and high-value compounds from non-food agro-industrial materials.
- Better documenting traditional knowledge related to fermented foods would help promote food security, the preservation of cultural heritage and the identification of novel microbial strains with industrial potential. Where traditional practices are in decline, opportunities to revitalize them need to be explored and where relevant supported.
- Issues related to the ownership of microbial strains and intellectual property require attention. Equitable use and benefit-sharing are pivotal issues, and the rights of Indigenous Peoples and local communities over their microbial genetic resources need to be safeguarded through appropriate frameworks. To enable the identification and preservation of fermentation-associated microorganisms in places where the equipment and infrastructure required are not yet available, there is a need to develop and implement frameworks that promote fair international research collaborations, which can be hindered by unclear guidelines and lengthy bureaucratic procedures. A multilateral approach could be a potential solution. Especially in the light of developments in engineering biology applications, there is a need to determine how to deal with digital sequence information related to fermentation-associated microorganisms.
- Although FDFs and wider applications of precision fermentation hold great promise as means of enhancing the sustainability and resilience of agrifood systems, the low rate of successful commercialization of products and bioprocesses has been a persistent challenge. Engineering biology has the potential to facilitate commercialization by improving yields, both for food/feed and for non-food products. Economic viability can be increased by incentivizing research and development of FFs and FDFs based on engineering biology and by improving regulation. Globally, there is insufficient commercial fermentation capacity to meet projected demand for FDFs. Current manufacturing capacity does not meet needs in terms of scale, technical capability or geographical distribution. Support for efforts to increase fermentation capacity and improve infrastructure is needed.
- Harmonization of definitions related to fermentation technologies such as precision fermentation could be an initial step towards harmonized policies governing their use, including those related to labelling, an issue that has a substantial effect on consumer understanding of novel food products and their acceptance.
- Integrating fermentation into agricultural practice as a means of valorizing by-products and waste *in situ* is another promising avenue. This would promote circularity in production systems and help to shape how novel food-processing and production technologies affect the food industry. For example, it could help address concerns over the impacts of FDF production on food sovereignty. Exploring opportunities and frameworks for partnerships between agricultural and FF/FDF producers could further support these objectives.
- Further efforts are needed to promote and research the contributions of fermented foods to healthy diets. There is also a need for more effective science communication and awareness raising on fermentation technologies and FF and FDF products. Better understanding of the components of FFs that confer health benefits, along with appropriate quality control and certification procedures, can help ensure consumers are well informed about the health and nutritional value of the wide array of products entering the market. New evidence emerging from microbiome science may provide the basis for further refinement of food-based dietary guidelines to cover the consumption of a range of FFs.
- To effectively realize the potential of non-food product biomanufacturing from agro-industrial materials, especially as an alternative to petrochemical-based production, concerted efforts need to be made to direct resources, including both public and private investments, towards the compounds that hold the greatest potential for economically feasible and sustainable

production. This requires effective communication between industry and academia. Improving technology transfer terms at universities and public institutions could also help to lower barriers to commercialization.

# 1. Introduction

Continued growth of the global population, the rise of climate-related disruptions and a decrease in the availability of agricultural land and other natural resources constitute a compounding set of challenges to current and future food security (Kompas, Che and Grafton, 2024). Enhancing the sustainability and resilience of global agrifood systems and their ability to provide "sufficient, appropriate and accessible food to all" in the face of disruptions is thus a priority for the international community (Nguyen, 2014). Innovations in food processing and production can bring us closer to meeting this goal while minimizing adverse impacts on the environment, the economy and society. Fermentation technologies – encompassing traditional fermentation, biomass fermentation and precision fermentation – may be pivotal.

This study delves into these technologies, elucidating their mechanisms, trends in their innovation and their possible contributions to food security in the context of the above-mentioned global challenges and the need to develop a circular bioeconomy. It aims to inform the work of the Commission on Genetic Resources for Food and Agriculture (Commission) on the genetic resources of microorganisms used in food processing and agro-industrial processes. In line with the stipulations of the Commission's Work Plan on Microorganism and Invertebrate Genetic Resources it "builds on" Commission Background Study Papers No. 64 (Chatzipavlidis, *et al.*, 2013) and No. 65 (Alexandraki *et al.*, 2013), which respectively addressed microorganisms used in agro-industrial processes and in food processing.

Some of the subject matter of Background Study Papers Nos 64 and 65 has been covered in recent background study papers prepared for the Commission in the context of the Work Plan, specifically those on biological control agents and biostimulants (Buitenhuis *et al.*, 2023) and on the roles of soil biodiversity in bioremediation and nutrient cycling (Csorba *et al.*, 2024). The present study therefore focuses on the use of fermentation technologies and the associated microbial diversity in fermentation-derived food production (microbial-based food), food processing and the valorization of agro-industrial materials (including non-food crops, agricultural and food-processing by-products and waste streams) in the production of non-food products such as fuels and platform chemicals. It aims to provide an overview of recent advances in the use of microorganisms in these contexts and particularly of the significant advances that have been made over the last decade in precision fermentation technologies and applications. It also covers the state of conservation of relevant microbial diversity and the policy landscape related to the equitable, safe and sustainable use of this diversity.

### Defining fermentation technologies

#### Fermented food – traditional fermentation

Traditional fermentation is an age-old process that utilizes naturally occurring microorganisms such as bacteria, yeasts and moulds to convert organic substrates into desired food products. The International Scientific Association for Probiotics and Prebiotics defines fermented foods (FFs) as "foods made through desired microbial growth and enzymatic conversions of food components" (Marco *et al.*, 2021). This method has been employed for millennia to produce a variety of foods and beverages, including bread, yoghurt, cheese, beer and fermented vegetables, fruits, roots, meat and fish (Cuamatzin-García *et al.*, 2022). Several local foods cherished around the world are produced through traditional fermentation, well-known examples including kefir, kimchi, tempeh, dahi, natto, miso, sauerkraut and sourdough bread (Ramos *et al.*, 2023). The process can not only enhance food preservation and safety but also improve nutritional value and flavour. Traditional fermentation remains integral to many cultures and diets, providing a natural and accessible means of food production and preservation that contributes to healthy diets and to gastronomy.

#### Fermented-derived food

Fermented-derived food (FDF) products are made with microbial biomass or ingredients produced using microorganisms. The processes behind these are, respectively, biomass fermentation and precision fermentation. FDF production often involves using both processes to achieve a final product that meets target nutritional and sensory properties.

### Biomass fermentation

Biomass fermentation consists in the production of large quantities of microbial biomass that can be used whole as a food product or further processed to serve as food ingredients such as proteins, lipids or other nutrients. In this process, microorganisms such as fungi, algae or bacteria are cultivated on various feedstocks to produce biomass rich in protein, vitamins and minerals. The resulting biomass can be harvested and processed into food or feed products (Graham and Ledesma-Amaro, 2023). The technology offers a sustainable alternative to conventional animal agriculture as it requires less use of limited resources, particularly land and water (Matassa *et al.*, 2016). In addition, it can be implemented in controlled environments, reducing dependence on arable land and mitigating risks associated with climate variability (Linder, 2023a). Biomass fermentation is primarily being explored as a means of manufacturing alternative protein products, with those already on the market including Quorn.[32] Commonly used terms in the literature associated with this production process are mycoprotein (protein derived from the biomass of fungi), microbial protein (protein derived from the biomass of microorganisms), single-cell protein (whole cells as a protein source), microbial oils (lipids derived from the biomass of microorganisms) and single-cell oil (whole cells as a lipid source).

### Precision fermentation

While the term “precision fermentation” does not have an internationally accepted definition, it often refers to the use of microorganisms in fermentation as “microbial cell-factories” for the manufacturing of specific compounds. In generic terms, the production process involves supplying cells with a feedstock, which is consumed to produce the compound of interest, which in turn is separated from the cells and purified (Nielsen, Meyer and Arnau, 2024). The microorganisms used may naturally produce the compound or may be engineered to do so utilizing synthetic biology tools, including new genetic techniques and evolutionary engineering approaches. Although the term “precision fermentation” may be relatively new, the process itself has been used since the 1980s to produce a wide range of food ingredients and additives, ranging from vitamins to dairy proteins, without the environmental burden of traditional agriculture (Lawrence, 1988). Efficient bioprocess design can allow microbial cell factories to be used to valorize food waste and agricultural by-products, making them a pivotal technology in the development of a circular bioeconomy (Salazar-López *et al.*, 2022).

#### Non-food product biomanufacturing from agro-industrial materials

Precision fermentation has been extensively explored as a means of producing non-food products such as biofuels, platform chemicals, high value-added chemicals, pharmaceuticals and materials (e.g. plastics, clothing fibres and leather alternatives) (Nielsen, Tillegreen and Petranovic, 2022). Fermentation technologies are being explored as a means of using biomass as a source of carbon in the chemical industry, helping to defossilize the sector and contributing to a broader shift towards a circular economy (The Royal Society, 2024). To reduce environmental impacts, agro-industrial by-products and wastes are being investigated as feedstock for such bioprocesses. Examples include non-edible lignocellulosic biomass residues (e.g. stalks, straws and bagasse), non-food crops (e.g. silvergrass), forestry residues, fruit waste (e.g. peels) and food-processing wastewater (Ubando, Felix and Chen, 2020). Some bioprocesses can also use $CO_2$ and $CO_2$-derived feedstocks such as methane, methanol or formate (Dürre and Eikmanns, 2015). Table 1.1. provides some of examples of how particular fermentation-based products or processes are contributing to particular objectives in the agrifood sector.

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[32] https://www.quorn.co.uk

**Table 1.1 Selected examples of fermented food (FF), fermentation derived food (FDF) and non-food product bioproduction applications and impacts**

| **Objective** | **Example product or process** | **Potential environmental, social and economic impact** | **References** |
|---|---|---|---|
| **Reduction of food spoilage/wastage** | Mabisi (FF):<br>a traditional Zambian dairy product made through spontaneous fermentation | • About 50 percent of raw milk produced annually goes to waste because of inadequate infrastructure and underdeveloped value chains. Fermentation can be used to process this raw substrate into a nutritious and palatable food product.<br>• Production of mabisi can provide an additional source of income for farmers.<br>• 70–90 percent of the Zambian population is lactose intolerant. Fermentation converts the lactose present in raw milk into lactic acid and other compounds. Mabisi can therefore be more readily consumed than fresh milk.<br>• Consumption of mabisi can facilitate adequate intakes of fat, protein, and various minerals and B vitamins.<br>• Processing of fermented foods requires little capital investment and relies on traditional knowledge. Support for upscaling, centring mostly on local women producers, would have beneficial effects on livelihoods and on the accessibility and availability of nutritious locally embedded food products. | Materia *et al.*, 2021; Moonga *et al.*, 2022; Chileshe *et al.*, 2020a b |
| **Valorization of agricultural waste for feed production** | Microbial-based fish feed ingredient from soybean-processing wastewater (FDF) | • Seafood is a key source of dietary protein. It is estimated that aquaculture production needs to increase by 57.2 percent by 2050 to meet increasing demand.<br>• Reducing the environmental impact of aquaculture production includes finding sustainable feed sources that are high in protein.<br>• The use of food-processing wastewater in microbial protein production addresses both the challenge of providing sustainable feed and the environmental challenge associated with wastewater treatment and disposal.<br>• The production strategy fits within a circular bioeconomy framework, as FDF production is achieved via the recovery of resources from a waste stream rich in nutrients such as carbon, nitrogen and phosphorus.<br>• A microbial community approach aimed at valorizing soybean-processing wastewater for the production of microbial-based fish feed resulted in a maximum protein content of 50 percent and the removal of 75 percent of soluble total nitrogen.<br>• A 24-day feeding trial found that the FDF could replace 50 percent of the fishmeal used in Asian seabass (*Lates calcarifer*) aquaculture without affecting growth or survival.<br>• The above study did not include a life-cycle assessment. However, a life-cycle assessment of similar microbial protein production from food-processing wastewater compared its human health, environmental and resource-use impacts to those of soybean meal (a common aquaculture feed ingredient). Soybean meal was found to have up to a 52 percent higher impact on human health and up to an 87 percent higher impact on ecosystems than the microbial protein. However, the microbial protein was estimated to have an 8–88 percent higher level of resource exploitation as a result of its high energy requirement. The use of renewable or waste energy sources could mitigate this. | Vethathiri *et al.*, 2023; Santillan *et al.*, 2024; Spiller *et al.*, 2020) |
| **Valorization of food waste for** | Polyhydroxyalkanoates (PHAs, used to produce | • It is estimated that a third of all food produced globally is lost or wasted annually.<br>• A circular economy model requires efforts to process and valorize nutrient-rich resources. This also addresses challenges in the | Gontard *et al.*, 2018 |

| | | | |
|---|---|---|---|
| **production of chemicals** | bioplastic) and biogas (FDF) produced using mixed microbial culture grown on food waste | disposal of food waste, which can cause contamination of air, soil and groundwater, and require significant resources for proper treatment.<br>• Fermentation valorizes food waste by producing high-value compounds relevant to industries beyond food and agriculture. The technology used is often termed a “biorefinery”.<br>• The creation of a new value chain may open new job and market opportunities.<br>• A pilot-scale study based in Italy developed an urban biorefinery technology with a technical readiness level of five (technology development and demonstration). The location selected allowed efficient collection of municipal solid waste (food and biological sludge).<br>• The study used mixed microbial cultures to convert the waste into high added-value compounds (PHAs) and biogas. PHAs are used to develop bioplastic with a range of applications, thus replacing conventional petrochemical-derived plastic. An overall yield of 7.6 percent biopolymer mass on the initial volatile solids was estimated. In terms of economic evaluation, revenue from the system was estimated to be 23 percent higher than from co-digesting organic waste. A social acceptance survey found that shopping or bin bags were the items produced through waste valorization that consumers were most willing to purchase.<br>• For a biorefinery system to compete with the prices of petrochemical-derived products is still a considerable challenge. | |
| **Food production without pastureland** | Dairy protein (β-lactoglobulin) production using engineered fungal cell factories (FDF) | • In 2020, an estimated 746 million tonnes of bovine milk were produced. According to one estimate, 35 percent of this supply is directed towards supplementary ingredients; this is equivalent to 261 million tonnes.<br>• Dairy farming demands significant land resources, with a 2017 study reporting that it occupies 7 percent of the world’s land, 85 percent of which is used for pasture. The demand for land for pasture and feed production is a key driver of deforestation and biodiversity loss.<br>• Precision fermentation of β-lactoglobulin (BLG) aims to provide an alternative source for the growing global whey-focused supplementary ingredients market.<br>• Precision fermentation eliminates the need for pastureland. Production can take place in any geographical location with access to nutrients, water, air, electricity and skilled labour, without affecting quality. However, choice of location affects both the environmental impacts and cost of the process.<br>• A study modelling the replacement of animal products with FDF, including dairy and egg proteins, found that a shift to FDFs from 2020 to 2050 would result in 83 percent less land use. Because of a lack of data, it is not known whether this land could be reclaimed for other purposes such as rewilding.<br>• According to a life-cycle analysis evaluating carbon footprint and water use, BLG production via precision fermentation has a similar magnitude of environmental impact to extracted dairy protein unless renewable energy and sustainable feedstock sources are used to fuel the process.<br>• A titre of 1 g/litre of BLG has been achieved using a *Trichoderma reesei* production host. To ensure this production method is economically feasible and capable of meeting at least 10 percent of demand, it is estimated that titre must be improved to 50 g/litre. This represents a technical challenge that can be tackled through engineering-biology techniques and bioprocess engineering. | El Wali *et al.*, 2024; Hill, 2017; Nielsen, Meyer and Arnau, 2024; Aro *et al.*, 2023; Behm *et al.*, 2022 |

| | | | |
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| | | • The waste fungal biomass from precision fermentation can be valorized as animal feed.<br>• BLG produced from microbial cell factories is bio-identical to that obtained from animals – it contains the same essential amino acids. However, nutritional impact depends on how BLG is used as an ingredient. The final food product (e.g. a bovine milk alternative) may not feature other bioavailable and nutritious food components contained in the food it is intended to replace. | |
| **Climate-resilient food production** | Microbial protein based on hydrogen-oxidizing bacteria biomass (FDF)<br>Carbon-capture based biomass fermentation system. | • Depending on the metabolism of the microorganism used, microbial protein production can be decoupled from land and photosynthesis. This can be done when the required feedstock is not an agriculturally derived material but assimilated $CO_2$ from the atmosphere.<br>• Sugars and agricultural by-products both depend on arable land, and their supply is sensitive to all the factors that can affect plant productivity, including climate, pathogens and soil quality. Carbon-capture-based systems are thus expected to be more resilient than those relying on conventional feedstocks.<br>• A study that explored the use of photovoltaics to power microbial protein production using atmospheric $CO_2$ found that the process has the potential of reaching a protein yield that is more than ten-fold higher than any staple crop and a caloric yield that is twice as high. Specifically, the microbial protein system was estimated to be capable of providing 15 tonnes of protein per year from 1 hectare. In comparison, microbial protein produced using sugar beet resulted in 2.7 tonnes per hectare per year, and a soybean field resulted in 1.1 tonnes per hectare per year. This exemplifies the higher land-use potential of the system. However, the same study estimated a production cost of USD 4–5 per kg of feed protein, which is almost twice the market price of fishmeal and four to five times that of soybean meal.<br>• Another study, which used empirical data for microbial protein production from hydrogen-oxidizing bacteria, found that because of its high energy demand the approach has a global warming potential that is equivalent to or higher than soy protein. Thus its sustainability is heavily dependent on access to renewable energy. | Leger *et al.*, 2021; Linder, 2023a; Järviö *et al.*, 2021 |
| **Nutrition improvement, health promotion and disease prevention** | Milk kefir (FF)<br>A fermented milk product made using kefir grains or starter culture. | • Numerous animal and clinical studies have associated the consumption of kefir with health benefits, although findings vary with the method of production and the resulting microbial composition of the product.<br>• Health-promoting benefits identified include antifungal and antimicrobial properties, tumour suppression and prevention, gastrointestinal immunity and cholesterol assimilation.<br>• Nutritional value is dependent on the milk and the production method used, but kefir generally has lower lactose content and higher vitamin content, protein digestibility and levels of essential amino acids.<br>• Kefir has also been identified as a source of, and means of delivering, probiotics, live microorganisms that when consumed in adequate amounts confer a health benefit. Kefir's role in modulating the gut microbiota and potential as a dietary intervention for diet-related non-communicable diseases is being explored.<br>• The United States of America's dietary guidelines for 2020–2050 feature kefir as a recommended dairy item. | USDA & HHS, 2020; Rosa *et al.*, 2017; Bourrie, Willing and Cotter, 2016 |

*Sources*: See the references in the table.

**The role of fermentation technologies in sustainable agrifood systems**

Addressing climate change

Fermentation technologies offer substantial environmental benefits by reducing the land-use footprint and potentially the carbon footprint associated with food production, particularly in the case of animal-derived products (El Wali *et al.*, 2024; Shahid *et al.*, 2024). The livestock industry has been estimated to be one of the largest producers of greenhouse-gas (GHG) emissions (FAO, 2023a). It is also responsible for the use of more than two-thirds of agricultural land and one-third of the world's total land (Ritchie and Roser, 2019), making it one of the leading causes of forest clearance (Díaz *et al.*, 2019). Production of animal food products is also the greatest agricultural cause of water pollution (Heinke *et al.*, 2020).

FDFs are an alternative protein source that can be produced using significantly less land and water (Humpenöder *et al.*, 2022). Collectively, biomass and precision fermentation align with global efforts to combat climate change by promoting low-impact food production methods. FFs and FDFs can also help the food system adapt to a changing climate (Teng *et al.*, 2021).

Enhancing food security and nutrition

Fermentation technologies play a crucial role in enhancing food security by providing diverse, nutritious and accessible food options. Traditional fermentation techniques enhance nutrition, preserve food and extend shelf-life, thus reducing waste (Siddiqui *et al.*, 2023). Biomass fermentation can produce protein-rich foods rapidly, and depending on the bioprocess designed, can do so with minimal inputs and at any geographical location with access to electricity, water, air and feedstock (Linder, 2019). Precision fermentation, with its ability to manufacture essential nutrients and ingredients, ensures stability in their supply, irrespective of agricultural constraints (Knychala *et al.*, 2024). This includes important nutritional elements such as human milk oligosaccharides, a prebiotic in human breast milk (Walsh *et al.*, 2020).

Promoting a circular bioeconomy

Transition towards a circular bioeconomy may hold the key to sustainable development (Muscat *et al.*, 2021). Biomass and precision fermentation technologies contribute significantly to this paradigm by utilizing agricultural by-products and food waste as feedstocks, thereby closing the resource loop. Traditional fermentation often uses locally available ingredients and enables valorization of raw materials for which storage capacity is unavailable (e.g. dairy and fruits), thus supporting local economies and reducing waste (Materia *et al.*, 2021).

## 2. Fermented foods: traditional fermentation

### 2.1 State of traditional fermentation applications in the food system

Traditional fermentation, as defined in Section I, is an ancient technology primarily developed and used as a method of preserving perishable food materials, including fruits, vegetables, meat and dairy products, thus ensuring their availability during periods of food scarcity (Ross, Morgan and Hill, 2002). Today, traditional fermentation contributes to two key objectives in food systems: improving nutrition and preventing food loss. Four main distinguishing features of FFs underpin these contributions:

1) **Increased shelf-life and preservation of raw food materials**
   Multiple mechanisms allow the end-product of a well-conducted traditional fermentation process to have greater microbiological safety than the raw food material started with. The metabolites produced by fermentation-associated microbes, such as lactic and acetic acid, create an acidic environment that inhibits the growth of spoilage and/or pathogenic microorganisms (Adesulu-Dahunsi, Dahunsi and Ajayeoba, 2022). Members of the FF microbiome can exhibit biopreservative actions, producing compounds that are directly inhibitory to foodborne pathogens (Zapaśnik, Sokołowska and Bryła, 2022). Bacteriocin, which

is produced by lactic acid bacteria (LAB), one of the types of microorganisms most frequently used in the production of fermented foods, has been reported to have broad-spectrum effects on both spoilage and pathogenic microorganisms (Naghmouchi *et al.*, 2020; Vieco-Saiz *et al.*, 2019).

2) **Increased nutritional value**
Fermentation-associated microbes improve the nutritional value of FFs through the following primary avenues:

   a) *Increased bioavailability of nutrients*. Microbial action can improve the bioavailability of food components in the human digestive tract. Mechanisms include the enzymatic hydrolysis of macromolecules such as polysaccharides, lipids and proteins (Gänzle, 2014) as well as improved uptake of minerals such as iron, calcium, zinc and phosphorus as a result of the degradation of phytic acid, which inhibits uptake (Gänzle, 2020; Gibson, Raboy and King, 2018; Shevade *et al.*, 2019). Other compounds known to interfere with the digestibility and uptake of nutrients, such as tannins and trypsin and amylase inhibitors, have been found to be reduced or eliminated via fermentation (Osman, 2004).
   b) *Degradation of undesirable elements*. Enzymatic activities present in the fermentation process have shown potential for degradation and detoxification of toxins, such as aflatoxin, a substance found in foods contaminated with *Aspergillus flavus*, potentially offering an inexpensive control measure (Verheecke, Liboz and Mathieu, 2016; Adebiyi *et al.*, 2019). As microbial-based biodegradation of mycotoxins is still an emerging approach, studies have predominantly been at laboratory scale, and further research is needed to identify high-performance microbial strains and protocols (Vanhoutte, Audenaert and De Gelder, 2016). Fermentation has also been found to reduce the allergenicity of raw food materials, such as peanut, soy and milk, through the degradation of allergen proteins (Pi *et al.*, 2022).
   c) *Production of nutritionally beneficial compounds*. By-products of fermentation include polyunsaturated fatty acids, conjugated linoleic acids, antioxidant compounds, minerals and essential vitamins, such as thiamine, folic acid and riboflavin (Mukherjee *et al.*, 2024).

Several recent review articles provide thorough analyses of the health-promoting and nutritional benefits of FFs (Melini *et al.*, 2019; Diez-Ozaeta and Astiazaran, 2022; Mukherjee *et al.*, 2024).

3) **Improved organoleptic properties of the raw food material**
Fermentation transforms foods’ organoleptic properties, including their flavour, texture and aroma. These sensory attributes are pivotal to the popularity and commercial viability of FFs. The varied metabolism of fermentation-associated microorganisms results in the production and accumulation of flavour compounds that impart unique and rich tastes to the final food products. For example, propionic acid bacteria used in the production of Swiss cheese are responsible for the $CO_2$ that gives the cheese its airy and spongy texture as well as its slightly sour taste (Siddiqui *et al.*, 2023).

4) **Increased health benefits through promotion of the gut microbiota**
The diversity of the microbial strains (some probiotic), metabolites and other bioactive components present in FFs is thought to provide considerable health benefits to the consumer, especially via effects on the gut microbiota. A healthier gut microbiota can prevent the proliferation of some pathogenic microbes, boost the immune system and support the treatment of dysbiosis (unbalanced or dysfunctional gut microbiota) and gastrointestinal diseases (Okoniewski *et al.*, 2023). Recent studies examining the gut–brain axis have also shed light on the potential roles of fermented foods in promoting better neural and mental health (Balasubramanian *et al.*, 2024).

FFs are cemented in the diets and cultures of populations across the world, contributing to food security, improved nutrition and the food sovereignty of many developing countries (Quave and Pieroni, 2014). It is estimated that fermented foods and beverages make-up a third of the human diet (Marco *et al.*, 2021). Interest in FFs has been on the rise, as reflected in the increasing number of both traditional and novel fermented products being released by the food industry. The global fermented food and beverage market, including dairy and bakery products and alcoholic and non-alcoholic drinks, was valued at USD 1.04 trillion in 2017 (Shiferaw Terefe, 2022) and USD 1.83 trillion in 2023 (Mordor Intelligence, 2024). It is expected to register a compound annual growth rate (CAGR) of 6.70 percent over the next five years (Mordor Intelligence, 2024).

The perception of FFs as wholesome, healthy and natural products, accompanied by growing availability of evidence supporting such claims, has been identified as a factor driving renewed consumer interest in them (Taylor *et al.*, 2020). This is reflected in the popularity of products such as kefir and kombucha that have spread beyond their places of origin. The global kefir market tripled between 2011 and 2016, reaching USD 1.23 billion in 2019. In the case of kombucha, global market size grew from USD 0.5 billion to USD 1.84 billion in 2019, and is forecast to reach USD 10.45 billion by 2027 (Shiferaw Terefe, 2022).

The last decade has seen a focus on innovation in plant-based FFs, the global market for which is expected to grow to USD 422.26 million by 2026, with a CAGR of 5 percent (Boukid *et al.*, 2023). The popularity of traditional FFs has paved the way for the development of novel designer FFs, which use microbial strains assembled in starter cultures to develop unique products. Although growing demand has led to the growth of large industrial fermentation operations, traditional fermentation is still widely used at the household level. With advances in microbiome science, the more complex fermentations associated with traditional FFs are starting to be used in industrial settings. The availability of fermentation substrate, climate, cultural traditions and local knowledge systems are key determinants of the scale at which FFs are produced, whether that is within households, in community production or at commercial industrial scale (Gänzle, 2022).

## 2.2 Trends in traditional fermentation applications in the agrifood system

### 2.2.1 Microbial diversity

Across the world, there are more than 5 000 different varieties of fermented food, reflecting local knowledge, cultural influences, geographical and climatic differences, and microbial heterogeneity. The diversity of FFs represents the diversity of fermenting microorganisms. These can be derived either from the autochthonous microbiota of raw animal and plant substrates or from starter cultures (defined or undefined) added to the substrate at the beginning of, or during, the fermentation process. In 2022, an inventory of food cultures compiled by the International Dairy Federation, included more than 226 bacterial and 95 fungal species, encompassing three bacterial phyla (Actinomycetota, Bacillota and Pseudomonata), represented by 17 families, and three fungal phyla, represented by 13 families (International Dairy Federation, 2022). The web-based Omics Database of Fermentative Microbes (ODFM), which provides freely accessible "-omics" information (e.g. genome, metagenome, metataxonome, metatranscriptome and metabolome) on microorganisms associated with fermented foods (Whon *et al.*, 2021), featured entries for 145 species and 206 strains as of May 2024. The composition of these communities has been found to be strongly associated with the raw substrate and the processing method used (e.g. spontaneous fermentation, back-slopping or starter culture) as opposed to geography (Landis *et al.*, 2021). Beyond the individual strains, the structure of the microbial community is fundamental to the function and the product of the fermentation process (Tu *et al.*, 2024).

Advances in high-throughput sequencing have made it possible to interrogate the broader microbial ecologies of artisanal fermented foods, including to catalogue the taxa present. Multi-omics tools (e.g. metatranscriptomics and metabolomics) further enable studies on the functional roles of member strains. Predictive computational tools, such as metagenome-scale metabolic modelling, are also being developed to analyse which enzymes, and ultimately metabolites, may be produced by the microbiome. These developments are pivotal to efforts to address knowledge gaps on the potential health benefits of

FFs and how they arise as well as on how FFs influence the microbial diversity of the human gut microbiome. Commercial interest in this is also high, as characterization efforts can lead to insights into how to modulate starter cultures for ideal flavour profiles and to the identification of novel strains that have probiotic potential or favourable characteristics for use in biotechnological applications (Walsh *et al.*, 2023).

### 2.2.1 Innovations in use and technology

#### Emerging processing technologies

Non-thermal processing technologies, such as ultrasound, high pressure (HPP) and pulsed electric field (PEF), are being explored as means of enhancing the safety and efficiency of FFs and altering their sensory and nutritional properties. Applications of these tools span every stage of production, from treatment of the feedstock to processing of the food product post-fermentation. Though initial investigations have been promising, use of these technologies is still limited to the laboratory scale, and further research will be required before they can be integrated into standard FF production processes (Shiferaw Terefe and Augustin, 2020).

#### Microbial culture design

Industrial production of FFs generally relies on the use of defined starter cultures to obtain standardized products, control the microbial community during fermentation and lower the risk of contamination. Knowledge garnered using “-omics” technologies is being used in the selection of strains found in traditional fermented foods to develop novel designed starter cultures (Gänzle *et al.*, 2023). Increasingly, non-conventional organisms are being explored for potential use in culture design, given their unique metabolisms and their influence on organoleptic properties (Gänzle *et al.*, 2023). Combinations of *Saccharomyces cerevisae* with non-conventional yeasts for wine and beer production are commercially available (Capece *et al.*, 2018). Machine learning and artificial intelligence (AI) enable the analysis of large sets of “-omics” data, providing predictive insights into the performance of microbial communities in novel FFs (Chelliah *et al.*, 2022; Galimberti *et al.*, 2021).

Genetic strategies for selection are also broadening the possibilities of starter culture development. In the case of yeasts, de novo laboratory hybrids achieved through breeding between well-known organisms and wild isolates allow expansion of the functional diversity in starter cultures (Turgeon *et al.*, 2021). This translates into the introduction of beneficial traits that improve the efficiency of the fermentation process and innovation in the sensory (e.g. flavour profile) complexity of the novel FF product. Multistrain cultures or synthetic communities composed of more than two or three strains are the norm in industry today and are seen as the future of food starters (Gänzle *et al.*, 2023; Navarrete-Bolaños and Serrato-Joya, 2023). Genetic engineering and synthetic biology tools are also being explored for use in the development of single microbial strains and microbial communities. For example, use of the genome-editing tool CRISPR/Cas9 to develop a strain that would combine increased ester and glycerol production in wine has generated promising results (van Wyk *et al.*, 2020). Other examples include the use of synthetic biology tools to exploit metabolic traits that are key to cocoa and coffee quality (Da Silva Vale *et al.*, 2023).

#### Enhancing plant-based food products

The growth of the global population, urbanization, changing dietary habits and climate-related impacts on the agrifood system are expected to put increasing pressure on animal-based food supply chains, which account for the bulk of GHG emissions associated with the agrifood system. There is, therefore, a need for more sustainable alternative protein sources that offer equivalent, if not superior, nutritional value and the sensory attributes needed for consumer acceptance. FFs such as tempeh are examples of traditional plant-based food products used as meat substitutes. Although well-established in East Asia, consumer acceptance of these products has been lower in Western countries, although this is changing (Szenderák, Fróna and Rákos, 2022). Plant-based alternative proteins (PBAP), defined as plant proteins that are structured and formulated to mimic the flavour, appearance, texture and nutritional profile of meat, have therefore emerged (FAO, 2022).

Although several commercial products are on the market, including via fast-food outlets and industry giants, bottlenecks remain. While efforts are being made to improve the nutritional content and stability of PBAPs through the use of plant-protein blends, fortification and preservatives, concerns remain about their safety, low digestibility, high allergenicity, high costs and nutrient deficiencies (Elhalis *et al.*, 2023). Fermentation using tailor-made starter cultures to improve the properties of PBAPs is garnering attention as a highly effective processing method that has potential to address these concerns (Abbaspour, 2024; Pua *et al.*, 2022).

In particular, even though plant-based foods are intrinsically rich in minerals such as iron and zinc, these have lower bio-accessibility and bio-availability than in animal-based products because of barriers such as the presence of antinutrients and the structure of the food matrix. This a significant issue if PBAPs are being considered as direct alternatives to meat, as iron deficiency remains the world's most common micronutrient deficiency, affecting over 30 percent of the global population (Kumar *et al.*, 2022). It is also believed to be the only such deficiency that is significantly prevalent in industrialized countries – disproportionately affecting women, especially during pregnancy. Zinc deficiency is another global health concern and had a global average prevalence rate of 17.3 percent in the 2003–2007 period, largely in developing countries (Wessells and Brown, 2012). Fermentation using LAB can help improve the bioavailability of minerals in PBAPs by degrading phytic acid and polyphenol content (antinutrients limiting uptake) as well as by producing organic acids that form soluble and absorbable ligands with the minerals (Rousseau *et al.*, 2020).

LAB fermentation has also been found to decrease soy immunoreactivity, highlighting the possibility of developing nutritious hypoallergenic soy products. Maung *et al.* (2020) found textured vegetable proteins (consisting of soy protein, corn starch and wheat gluten) to have improved chewiness, hardness and layered structure after fermentation using *Bacillus subtilis.* Similarly, the inclusion of 10 percent fermented soy products in alternative protein formulations has been found to improve texture and flavour (Razavizadeh *et al.*, 2022).

## 2.3 Challenges and opportunities for development

### 2.3.1 Knowledge gaps: an obstacle to expanded use of traditional fermentation as an intervention for food insecurity

Given their nutritional profile, potential to reduce food waste, cultural significance and low production cost, FFs could provide the basis for effective intervention strategies aimed at improving food security and nutrition, especially in communities at risk of malnutrition and hunger (Obafemi *et al.*, 2022; Misci *et al.*, 2021; Narzary, Wahengbam and Shemesh, 2023). However, their inclusion in such strategies has been limited because of reasons such as insufficient understanding or lack of robust evidence of the microbial metabolic factors underpinning health-promoting effects, especially when it comes to non-LAB FFs (Materia *et al.*, 2021). Moreover, further research is needed on how traditional FF production processes can be utilized to provide guaranteed nutritional profiles. A starting point would be further research on the isolation, identification and utilization of all the microorganisms, both culturable and non-culturable, present in the microbial communities behind FFs, following the example of initiatives such as the Puratos Sourdough Library, the Global Microbiome Conservancy and the Microbiota Vault Project (Nithya *et al.*, 2023). Insight from -omics technologies combined with AI algorithms that can link sequence and function would enable not only better taxonomic classification but also better understanding of which species are responsible for which desired effects. Genome sequencing can further lead to the selection of target genes that can be used in metabolic engineering strategies, either to improve properties such as the production of post-biotics (e.g. vitamins, bacteriocins and enzymes) or to delete genes responsible for potential allergens (Owusu-Kwarteng *et al.*, 2022). In addition, although there are significant *in vitro* data suggesting that traditional FFs provide health benefits, more extensive clinical trials to substantiate these claims, especially with regard to their effect on the gut microbiome, are needed (Gille *et al.*, 2018).

#### 2.3.2 Safety risks with artisanal fermentation

Production of traditional FFs around the world often remains artisanal, especially at the household and small- and medium-enterprise levels, which are the commonest sources of FFs in rural communities, where their roles in preventing malnutrition have the greatest impact (Skowron *et al.*, 2022). Although traditional fermentation provides excellent food safety and preservation when well-conducted, lack of control over fermentation procedure, substrates, storage and sanitation throughout the process creates the risk that spoilage and/or pathogenic microorganisms may proliferate. The hygiene quality of the ingredients, water and utensils used, as well as the practices of the producer, can affect the composition of the microbial community. This is a source of concern, as certain fungal and bacterial genera are associated with safety risks such as the production of spores, toxins and large amounts of biological amines (Ramos *et al.*, 2023).

Unsuccessful fermentation and post-processing contamination can not only reduce the nutritional quality of the final FF but also lead to outbreaks of food poisoning (Anal *et al.*, 2020). Further understanding of which strains give grounds for concern and how to prevent their proliferation can enable the design of intervention strategies that maintain the authenticity and health benefits of the FFs while preventing potential negative effects (Chin *et al.*, 2024). This is crucial for their inclusion in public policy. Advances in best-practices for manufacturing, capacity building and strong food control systems can enable producers to ensure their products are food-safe.

#### 2.3.3 Legislative limitations to introducing fermented foods into markets

Current regulatory frameworks for FFs, especially non-dairy products, are insufficiently mature to manage the growing diversity of the products on the market, thus limiting their commercialization, despite their potential benefits. Many FFs, particularly artisanal and functional FFs, lack relevant standards and regulations. Legislative efforts have been found to be largely reactive rather than proactive (Metras *et al.*, 2021; Laulund *et al.*, 2017; Gänzle *et al.*, 2023). Regulatory frameworks for assessing whether products may be authorized to enter markets have also been found by researchers to lack harmonization at international, national and regional levels (Mukherjee *et al.*, 2022). Where standards are concerned, only a few fermented food products feature in the Codex Alimentarius[33] (Marco *et al.*, 2021), a limitation that makes harmonization more difficult. Legislation is thus out of step with developments in the private sector, where consumer interest in FFs has led to growing innovation and development of novel products. For instance, differences between the regulations in place in different states within Australia and the fact that FFs fall under several overlapping legal instruments in Brazil and South Africa have cause confusion among manufacturers and hindered the development of the sector in the respective countries (Mukherjee *et al.*, 2022). Consolidating standards and specifications in legislation and standard codes, as recently done in India and the Republic of Korea, could improve harmonization across national structures (Mukherjee *et al.*, 2022).

## 3. Fermentation-derived foods

### 3.1 Biomass fermentation

#### 3.1.1 State of biomass fermentation applications in the agrifood system

Biomass fermentation has been employed throughout history, from the edible cyanobacteria used by the Aztecs and by the Kanembu people in Chad, who still cultivate it to this day (Carcea *et al.*, 2015), to the spent brewer's yeast used by the Marmite Food Company to make its namesake product, first produced in 1902 (Nyyssölä *et al.*, 2022). Interest in microbial protein surged in the mid-twentieth century amid persistent famines plaguing the developing world. At the time, the technological

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[33] The Codex Alimentarius is a collection of international standards, guidelines and codes of practice to ensure safe and fair practices in the food trade. The Codex standards are recognized in the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures as the international reference point for food safety.

innovations of the Green Revolution, including increased agrochemical usage that has had long-lasting impacts on human health and the environment (Linder, 2023a), superseded microbial foods.

Use of microbial protein as feed, rather than direct human consumption, was explored in the 1970s and 1980s, with successful scale-up of products such as Pruteen and Pekilo. However, these were not able to compete with low-cost protein feed such as soy because of increased energy costs and the development of high-yielding crops (Matassa *et al.*, 2016). The only successful product from this wave of interest in microbial protein is Quorn, a meat-imitation product derived from the mycelium of the soil fungus *Fusarium venenatum*, first released onto the market in the United Kingdom in 1985. In addition to maintaining popularity in the United Kingdom, Quorn products are now available in 11 other countries (Finnigan *et al.*, 2019).

Over the past decade, interest in microbial proteins as sustainable alternatives to animal-derived products has again surged (Section I). This is because, as noted above, livestock production remains one of the major contributors to GHG emissions within the food system, which as a whole is responsible for one-third of global anthropogenic emissions (Crippa *et al.*, 2021). The land- and water-use efficiency of livestock production is another source of great concern, as it uses 80 percent of agricultural land (Wirsenius, Azar and Berndes, 2010) and 41 percent of agricultural water (Heinke *et al.*, 2020). With meat consumption projected to rise because of both population growth and changes in dietary habits, alternatives to conventional animal-derived products are eagerly being sought.

Biomass fermentation circumvents many of the constraints commonly associated with the production of protein, particularly land shortages and unfavourable climactic conditions (Malila *et al.*, 2024). The process involves the growth of an edible microorganism within a bioreactor. Once the desired amount of biomass has been accumulated, it is processed into a product that can be consumed. Key to the process is the selection of the edible microorganism and a feedstock that is appropriate for the organism and has an acceptable cost and environmental impact. Given the rapid growth rate of microorganisms and their high protein content (30–80 percent w/w of dry biomass depending on the strain), they produce protein much more efficiently than any farm animal or plant (Bajić *et al.*, 2022). In terms of land protein productivity (kg of protein per hectare per year), microbial protein from bacteria performs around 600 times better than animals and about 90 times better than soybean. Similarly, the use of energy (MJ per kg of protein) for spirulina production is more than five times lower than for meat (beef, pork and poultry) production (Ciani *et al.*, 2021). There are, however, significant challenges to cost-effective scale-up of the approach (Malila *et al.*, 2024).

Bioreactors, large metal cultivation vessels, typically in the form of a vertical cylinder, allow protein to be produced with a small land-use footprint and in any location, as long as feedstock, water, air, electricity and the necessary skilled labour can be accessed. The precise control that it allows over cultivation also means the process is not vulnerable to changing climatic conditions.

Given the diversity of microorganisms explored (Section III.1.2.1), feedstocks can range from inedible plant biomass and agricultural waste to direct $CO_2$ capture. At present, however, products sold for human consumption generally rely on sugar as a feedstock, and as such are not entirely decoupled from the need for arable land or from climatic shocks affecting crop production. Nonetheless, a recent study found that the replacement of 20 percent of per capita ruminant meat consumption with sugar-based microbial protein at a global scale by 2050 would cut annual deforestation and related $CO_2$ emissions roughly in half by limiting expansion of global pasture area, while also lowering methane emissions (Humpenöder *et al.*, 2022).

In addition to their reduced environmental impact, the nutritional profile of microbial foods and proteins also makes them an attractive alternative. They have low energy density and low total and saturated fats, are classed as high in fibre and provide the nine main essential amino acids (Li *et al.*, 2023). Their protein quality, measured using the protein digestibility-corrected amino acid score (PDCAAS), is equivalent to that of soy protein (PDCAAS of 91 percent) and beef (92 percent), if not comparable to optimal sources such as eggs and cow’s milk (100 percent). In the case of Quorn’s *F. venenatum* product and Nature Fynd's *Fusarium* Fy microbial protein, the figures are 96–97 percent and 91 percent, respectively (Linder, 2019).

Some microorganisms are excellent at accumulating lipids, which can comprise up to 50–70 percent of their total biomass and can act as a source of "single-cell oils" for use in food and feed supplements given their high content of beneficial polyunsaturated fatty acids, including omega-3 fatty acids (Patel *et al.*, 2020). Beyond proteins and lipids, microbial biomass can also supply key micronutrients. It is high in phosphorus, zinc, manganese and many vitamins of the B family (e.g. folic acid, biotin, riboflavin and thiamine). Depending on the edible microorganism chosen, products can also be rich in vitamins C and E and precursors of vitamin A and vitamin B12 (Bratosin, Darjan and Vodnar, 2021).

One concern is the high purine content in crude microbial biomass caused by high intracellular levels of RNA, a by-product of rapid growth. Humans are unable to fully metabolize purines, which can result in hazardous levels of uric acid, potentially leading to health issues such as gout and kidney stones. Crude microbial biomass is therefore processed using heat or alkaline treatments to reduce RNA content, although potential residues mean microbial protein products must be consumed with caution by individuals predisposed to conditions such as gout (Cardoso Alves *et al.*, 2023). Nevertheless, the safety of the edible microorganisms on the market has been well studied, especially in the case of fungi, which are capable of producing toxic compounds (mycotoxins) (Sharif *et al.*, 2021). Microorganisms considered safe for human consumption receive regulatory approval for given applications by the relevant regulatory authorities. In the United States of America, microorganisms used for the production of microbial protein products have "Generally Regarded As Safe" (GRAS) status from the Food and Drug Administration (FDA), either on the basis of historical use or following applications for premarket review and approval by the FDA (Gänzle *et al.*, 2023; FDA, 2018a). In the European Union, the European Food Safety Authority (EFSA) maintains a list of microorganisms with Qualified Presumption of Safety (QPS) status. EFSA assesses the safety of microorganisms in products for which it receives applications for market authorization, including in the case of novel foods, the category within which FDFs generally fall (EFSA, 2025).

Interest in microbial protein products is growing in the private sector, and this is reflected in the increasing number of patents being sought and granted for innovations in process and product, with nearly 178 patents awarded in the 2021 to 2023 period, and 417 and 319 patent applications in 2022 and 2023, respectively (Battle, Mackenzie *et al.*, 2023). Although exact market-size projections vary depending on the research firm, most sources indicate a CAGR of 6–7 percent for the microbial protein (or mycoprotein) sector and a forecast value of USD 1 billion by 2030. The total capital invested in fermentation (including both FF and FDF) in the last decade (2013–2023) amounts to USD 4.1 billion. As of 2023, according to the Good Food Institute, the number of biomass fermentation companies has grown to 80, with several forming partnerships with multinational companies such as Nestlé, Kraft Heinz, Cargill and Tyson, for funding, infrastructure and distribution access (Battle, Mackenzie *et al.*, 2023).

### 3.1.2 Trends in biomass fermentation applications in the food system

#### 3.1.2.1 Microbial diversity

Microbial protein producers span all three domains of life: Eukarya, Bacteria and Archaea. One approach to classifying them is based on their feedstock use, with two main functional categories: heterotrophic and autotrophic microorganisms.

**Heterotrophic microorganisms**

Heterotrophs are microorganisms that utilize simple organic carbon that has already been synthesized, or "fixed", from $CO_2$. Filamentous fungi and yeast species are by far the most popular choices for microbial protein production because of their nutritional profile, sensory attributes and ability to utilize a wide range of complex feedstocks (Koukoumaki *et al.*, 2024). Examples include *Fusarium, Aspergillus*, *Candida, Saccharomyces*, *Kluyveromyces*, *Meyerozyma*, *Nectaromyces*, *Yarrowia,* *Rhodotorula* and *Trichoderma* spp. Filamentous fungi are especially attractive for the development of meat alternatives given how well their mycelium can mimic the texture of meat.

Heterotrophs can be further categorized based on the type of fixed carbon they utilize. These may be compounds accessible in waste streams or via carbon-capture systems. An example of a commercialized product that utilizes sidestreams from other industries is Pekilo from Enifer Bio (Enifer Bio, 2025). The process, which uses the fungus *Paecilomyces variotii*, was initially developed in the 1970s to valorize spent sulphite liquor from forestry in Finland but was adapted to utilize other, similar, industrial side-streams as the pulp industry evolved and ceased producing liquor (Voutilainen, Pihlajaniemi and Parviainen, 2021). Such examples demonstrate the capacity of biomass fermentation to valorize waste and support the development of a circular bioeconomy.

**Autotrophic microorganisms**

Autotrophs can assimilate $CO_2$ directly as a source of the carbon necessary for growth. This is possible either through photosynthesis (photoautotrophs) or chemosynthesis (chemoautotrophs), the latter enabling the use of a range of compounds as energy sources, including hydrogen sulphide gas, ferrous iron and ammonia. This ability lends itself to the design of a biomass fermentation process that is fully independent of crop production and can be used in any geographical location. This concept is explored further in Section III.1.2.2, which discusses the example of hydrogen-oxidizing chemoautotrophic microorganisms that can be used in a carbon-capture system. Companies producing food and/or feed via chemoautotrophic growth include Solar Foods, Novonutrients, Deep Branch, Arkeon and Air Protein (Pander *et al.*, 2020).

Commonly used phototrophs include cyanobacteria and eukaryotic microalgae (e.g. *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus* and *Schizochytrium)*, although these are mainly used for feed production, as their high light intensity requirement means companies opt to reduce capital costs by using open culture systems (e.g. open ponds), which are more vulnerable to contamination (Sobhi *et al.*, 2023). Recent studies have explored the potential of purple phototrophic bacteria species (e.g. *Rhodospirillum*, *Rhodopseudonomonas and Rhodobacter*) to produce microbial protein for feed applications using industrial or agricultural wastewater (Delamare-Deboutteville *et al.*, 2019).

Beyond the choice of feedstock, factors influencing the selection of one microorganism over another include protein content, growth rate, neutral taste, harvestability, digestibility, lack of toxicity and infrastructure requirements. Further exploration of the microbial diversity present in both food-associated environments and extreme environments can enable the identification of microbial strains that permit the development of novel, scalable, low-cost, efficient and sustainable production processes. Indeed, the discovery of *Fusarium. venenatum A3/5* strain, now synonymous with microbial protein thanks to Quorn, involved an initial screening process of more than 3 000 fungi collected from soil samples from all over the world and evaluated on the criteria of fast growth in culture, filamentous growth, ability to assimilate inorganic nitrogen, lack of pigments, odours and toxins, and protein content of more than 45 percent of total biomass (Tong *et al.*, 2022).

### 3.1.2.2 Innovations in use and technology

**Advances in genetic engineering tools and workflows**

Although products on the market already showcase the versatility and commercial promise of biomass fermentation, there are some bottlenecks to their wider use. Microbial-protein production remains cost intensive and has low production efficiency because of a variety of factors, including costs associated with feedstock and its utilization efficiency, as well as low protein yield, biomass accumulation and capacity to produce nutritional compounds. Most products rely on a few isolated strains that have inherent limitations in their industrial capacity, metabolism and structure. This can limit the range of potential products in terms of nutritional profile and sensorial attributes, as well as affecting the cost, scalability and sustainability of the production process. Generally, the carbon and nitrogen sources needed by the microorganism are determining factors for both cost and environmental impact, measured as carbon footprint. One strategy for overcoming these challenges is to use strain engineering.

Synthetic biology, which entails the engineering of biological systems, has given rise to the development of standardized and modular toolkits for the genetic engineering of a wide variety of microorganisms, composed of well-characterized biological parts necessary for protein expression. The use of such toolkits enables researchers to make precise and predictable modifications within shorter research and development (R&D) cycles (Baldwin *et al.*, 2016).

One trend in the optimization of microbial protein production is the development of such toolkits for (and the application of synthetic biology approaches to) historically consumed, safe, edible microorganisms (Lv *et al.*, 2021). One recent study saw the development of a CRISPR-Cas9-based toolkit for *Aspergillus oryzae*, an edible fungus with a long history of human consumption (Maini Rekdal *et al.*, 2024). The toolkit enables the introduction of genes at specific well-studied, neutral locations in the genome, under the control of promoter elements that allow strong expression of genes and thus high production of the encoded protein, independent of the composition of the feedstock. The researchers utilized the novel tool to overproduce ergothioneine, a potent antioxidant, and heme, a key protein imparting both flavour and colour, thus tailoring the nutritional value and sensory appeal of the fungal biomass for alternative-meat applications.

Metabolic engineering, i.e. the rewiring of an organism's native metabolic pathways or the design of new networks, can also be used to tailor microbial biomass used for feed to fit the nutritional needs of the target animal, thus improving the nutritional profile of the final animal product and the growth and health of the animal (Balagurunathan *et al.*, 2022; Wang, Wu and Yin, 2022). An example of a successful genetically modified microbial protein product approved for commercial use is KnipBio Meal,[34] a fishmeal replacement with elevated carotenoid content for use in aquaculture.

Although some edible microorganisms are capable of using complex, low-cost and renewable feedstocks, such as pretreated lignocellulosic biomass, this is not a preferred nutrient source and can alter the microorganisms' metabolism, impacting protein content, growth and the production of secondary compounds of interest (Stoffel *et al.*, 2019). Synthetic biology tools have been utilized in laboratory settings to overcome this challenge. In yeast, well-characterized native sugar-uptake, carbon and glycerol transporters have been engineered – leading to improved growth (Nijland and Driessen, 2019; Rodriguez *et al.*, 2016).

Another approach is to use adaptive laboratory evolution or chemical mutagenesis. Both techniques involve evolutionary engineering in which artificial conditions are designed to imitate and accelerate a microorganism's natural evolution (Wang *et al.*, 2023). The result is the selection of a novel strain better suited to particular environmental conditions, for example with increased tolerance to potential inhibitors in culture media (Hirasawa and Maeda, 2022). The accumulated mutations are ones that could have occurred naturally, thus qualifying the strains as not being genetically modified organisms (GMOs) under certain regulatory frameworks (Lee and Kim, 2020). Another promising avenue is the development of engineering tools for microorganisms capable of utilizing $CO_2$ as a feedstock in order to target bottlenecks in their use for microbial-protein production (Pan *et al.*, 2021). A recent study improved the production rate of *Cupriavidus necator* in a bioelectrochemical system 2.7-fold by engineering the organism to display superoxide dismutase enzymes on its surface, thus providing a novel strategy for the production of a carbon-neutral protein source (Chen *et al.*, 2023).

Advances in automation and high-throughput equipment accessible in biofoundries across the world, combined with genetic engineering tools, have the potential to contribute to the next generation of candidate microorganisms for microbial-protein production, expanding the range beyond that present in natural biodiversity.

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[34] https://www.knipbio.com

### Development of mixed or co-cultures

Biomass accumulation of edible microorganisms for microbial-protein production can be achieved through the synergistic growth of more than one microorganism in mixed or co-cultures. Depending on the bioreactor set-up, the use of more than one microorganism can allow production to be divided into two steps, each leveraging the unique metabolism of the chosen microorganism: first, low-cost intermediate feedstocks such as C5/C6 sugars, methane and ethanol are produced by a microorganism capable of assimilating crude feedstocks. These are then supplied to an edible microorganism for the second step, which entails the production of microbial biomass. This can allow valorization of food wastes (e.g. dairy or fruit wastes) (Myint *et al.*, 2020; Zhou *et al.*, 2019) and agricultural and industrial sidestreams, without compromising on the quality and safety of the final food product. Mixed cultures can also enable the use of renewable sources of nitrogen, which is otherwise supplied in the form of inorganic nitrogen obtained through the highly polluting Haber-Bosch process. Hu *et al.* (2020) report the use of a mixed culture of nitrogen-fixing hydrogenotrophic bacteria to produce microbial biomass through the fermentation of gas containing $H_2$, $O_2$, $CO_2$ and $N_2$ (Hu *et al.*, 2020). Mixed cultures or microbial communities can also enable the assimilation of ammonia as a nitrogen source from wastewater (Hu, Vandamme and Boon, 2022).

### Carbon-capture-based processes

From a global agrifood system perspective, one notable advantage of biomass fermentation is its potential to serve as a food source that is not ultimately constrained by photosynthetic capacity. This would unlock production that is independent of both climate and arable land, and resilient to the vulnerabilities of photosynthesis-dependent production, such as those associated with pests and pathogens. Decoupling production from biophysical conditions widens the range of possible geographical locations where it can happen, creating opportunities for increased food sovereignty and tailored interventions targeting food insecurity. Overall, this would serve as a means not only of adapting food production to a changing climate but also of reducing the impact of food production on the climate by being a carbon-neutral process.

One significant area of interest is therefore the “carbon capture, conversion and cultivation” processes whereby microbial protein is produced either via purely chemical conversion methods or by biocatalytic processes, also known as gas fermentation, where $CO_2$ is reduced to simple organic carbon substrates through anaerobic microbial fermentation. In the latter case, the produced substrates, for example methanol, formate, methane and carbon monoxide, are collectively known as C1 substrates. Significant research efforts are being invested in the study of both native and synthetic C1 assimilation pathways (Jiang *et al.*, 2021). Use of formate and methanol has received particular attention, as they are completely miscible and do not have problems with low water solubility, thus potentially supporting higher microbial productivities (Choi, Jung and Lee, 2024).

Aerobic hydrogen-oxidizing chemoautotrophic microorganisms, such as *Cupriavidus necator* and *Rhodococcus opacus*, can be cultured for microbial protein applications using a gas fermenter where $CO_2$ is supplied alongside $H_2$ and molecular oxygen ($O_2$) obtained via electrolysis using electricity generated through renewables such as solar, wind or hydropower (Woern and Grossmann, 2023; Pander *et al.*, 2020; Bachleitner, Ata and Mattanovich, 2023). One study estimated that such a system could lead to a ten-fold reduction in land requirement per unit of protein weight compared to that of soybean, and even greater land savings with the use of wind power (Sillman *et al.*, 2020). Another study, which looked at the use of photovoltaics to power a process such as the one outlined above or an electrochemical process generating methanol for use by heterotrophs, found that it would be possible to obtain roughly a ten-fold increase in protein production and a two-fold increase in caloric yield per unit of land relative to those achievable with conventional staple crops (Leger *et al.*, 2021).

### Bioprocess design

The primary two fermentation processes are solid-state and submerged. Submerged fermentation involves microbial cells being suspended in a liquid nutrient medium. Most fermentation facilities are designed for this bioprocess, with the main types of bioreactors being continuous stirred tank, bubble column, fluidized bed and trickle bed. These may be unsuitable for biomass fermentation applications because of the importance of conserving structure. Instead, such applications often rely on solid-state fermentation, where microbes are inoculated onto a moistened feedstock (Majumder *et al.*, 2024). There is significant room for improvement in the design of bioreactors, given the need to improve scale, cost and sustainability in the context of food applications (Banks *et al.*, 2022). An example of successful innovation is the air-lift design[35] pioneered by Quorn to reduce energy consumption in the cultivation of filamentous fungi (Moore, Robson and Trinci, 2020). Several start-ups, including Unibio and Calysta, are currently developing novel bioprocess designs that utilize methanotrophs cultivated on gas feedstocks (But *et al.*, 2024).

## 3.2 Precision fermentation

### 3.2.1 State of precision fermentation applications for food production

Precision fermentation has been recognized as an emerging trend in the fourth industrial revolution of the food industry. As a manufacturing method, industrial fermentation dates back to the 1910s (Ewing *et al.*, 2022), and it has been used for decades in the production of high-value recombinant proteins, such as rennet for cheese (Singh, Singh and Sachan, 2019). The programmability of biology enabled by advances in genetic engineering tools has unlocked the potential of this technology to address bottlenecks across the food system. In particular, precision fermentation allows:

1) production of essential nutrients, food ingredients and additives (e.g. bioactive, texturizing, flavouring, aroma, colourant and preservative compounds) that are too complex, costly and/or environmentally damaging to manufacture via conventional means (Sun, Xin and Alper, 2021);
2) production of a variety of proteins naturally found in animal-derived food products (dairy and egg protein) and non-food products (e.g. leather) for which alternative sources are required if livestock production is to be curbed (Knychala *et al.*, 2024); and
3) valorization of food waste, a USD 400 billion scale problem given that an estimated 14 percent of food is wasted between harvest and retail globally (FAO, 2021).

The process of developing a microbial cell factory generally begins with the selection of a host microorganism and the study of the biosynthetic pathway of the desired product, for example a protein or a vitamin. The pathway is then reconstructed in the microbial host through the introduction of genes found in the relevant plant or animal species (Nielsen, Meyer and Arnau, 2024). In the context of food ingredients and proteins, costs of production must be substantially lower than those for pharmaceuticals because of the lower profit margins; the use of this approach is therefore usually contingent on high yield and high production efficiency (Eastham and Leman, 2024). Rapid development of metabolic engineering techniques and their intersection with advances in computational tools and high-throughput equipment have enabled the implementation of more efficient strategies for strain engineering (Abbate *et al.*, 2023). Once established, engineered strains are cultivated in a bioreactor, supplied with the necessary feedstock and kept under conditions adjusted to favour the production of the compound of interest. The compound is then separated from the biomass and recovered through downstream processing that makes it ready to be used as a food-safe ingredient.

To date, precision fermentation has been used in the production of macronutrients (proteins, carbohydrates and lipids), vitamins and a wide array of nutraceuticals and additives (including terpenes and flavonoids with flavouring, colourant and preservative properties). A key example is the production of riboflavin (vitamin B2), where precision fermentation has replaced chemical synthesis since the 1990s. Further examples of microbially produced food additives that have both received regulatory approval and can be produced at industrially relevant titres are xanthan (a stabilizer), L-glutamate (a

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[35] Air-lift bioreactors are designed for large-scale aerobic cultures where the mixing of the culture broth is done by the inserted gas via an airlift pump.

flavouring), erythritol (a sweetener), 2’-fucosyllactose (a sweetener) and a-galactosidase (an enzyme used in food processing) (Sun, Xin and Alper, 2021; Seo *et al.*, 2022).

In 2020, the global market for the use of synthetic biology in food and beverages, agriculture and consumer products was USD 0.45 billion, USD 0.39 billion and USD 0.35 billion, respectively. The market for food and beverages specifically is forecast to grow at a CAGR of 51.3 percent, reaching an estimated value of USD 5.7 billion by 2026 (Augustin *et al.*, 2023). Innovation in the private sector is being driven both by start-ups and small to mid-sized companies and by large industrial chemical companies, such as DSM, BASF and DuPont (Augustin *et al.*, 2023).

In terms of environmental impact, production of specific animal proteins via precision fermentation has been modelled to be more efficient in the use of land and water and to have a potentially lower carbon footprint, depending on the feedstock and energy source used. According to one study on the replacement of animal products, including dairy and egg proteins, with FDFs, a shift to FDFs over the period from 2020 to 2050 would reduce agricultural land requirements by 83 percent by the latter year (El Wali *et al.*, 2024). Noteworthy achievements in this space, representing the three key areas of focus of the industry (dairy, egg and meat analogue proteins) have been:

- production of β-lactoglobulin (milk protein) using engineered *Trichoderma reesei* fungus (FDA, 2020);
- synthesis of egg-white protein using engineered *Komagatella phaffi* (FDA, 2021); and
- production of soy leghaemoglobin (which imparts meat colouring and flavour) using engineered *K. phaffi*, (EFSA Panel on Food Additives and Flavourings *et al.*, 2024).

Notably, both for plant-based and for microorganism-based alternative proteins, precision fermentation holds the key to nutrition, taste, texture and cost parity with animal-derived products (Boukid *et al.*, 2023). This is critical both for consumer acceptance, which remains the fundamental bottleneck in this field, and for the realization of the sustainability and circularity potentials of these products. One example is the development of precision fermentation platforms for alternative fats and oils, which has already attracted several start-ups. Products targeted include plant-derived oils such as palm oil, which is extensively used in the food industry and in the production of plant-based meat alternatives (Zahari *et al.*, 2022) but is highly environmentally damaging to produce; one recent study estimated that palm plantation expansion could be responsible for 0.44–0.74 percent of global GHG emissions (Cooper *et al.*, 2020).

Beyond the environmental benefits, this technology could also serve as an essential means of maintaining stable and sufficient supplies of vital nutritional elements such as human milk oligosaccharides, unique prebiotic components of human breast milk (Walsh *et al.*, 2020).

The possibility of engineering microorganisms to efficiently utilize food waste (e.g. bread and fruit waste) and agricultural by-products (e.g. rice bran, wheat straw and corn cobs) could also create opportunities to biomanufacture industrial bulk chemicals, fuels and high value-added compounds (Cardoso Alves *et al.*, 2023; Javourez, O’Donohue and Hamelin, 2021). This valorization of waste has the potential to increase the economic feasibility of alternatives to petrochemical-based chemical synthesis, while also reducing environmental impacts relative to those of using sugar-based feedstocks (Spiller *et al.*, 2020; Ullah *et al.*, 2022). This is pivotal, as the value of the global market for biomanufacturing is projected to reach USD 30.3 billion by 2027 (Liao, Ma and Tang, 2022). Overall, precision fermentation has promise in the development both of a more sustainable agrifood system and of a wider bio-based circular economy.

### 3.1.2 Trends in precision fermentation applications in the food system

#### 3.1.2.1 Microbial diversity

Precision fermentation processes can make use of the metabolism of wild-type microorganisms isolated from nature, engineered microorganisms featuring heterologous DNA (DNA derived from a different organism) or engineered microorganisms featuring modifications that could have occurred naturally. Microorganisms that are considered food-grade, are on the QPS list in the European Union and/or are recipients of GRAS status in the United States of America are preferred for food applications. Thus, strain engineering often utilizes benign bacteria (such as *Bacillus* spp.), yeasts (such as *Saccharomyces cerevisiae*, *Komagataella phaffii*, *Yarrowia lipolytica* and *Kluyveromyces* spp.) or filamentous fungi (such as *Trichoderma* spp., notably the popular *T. reesei* strains) (Augustin *et al.*, 2023).

The selection and engineering of an industrial host to develop a production platform is recognized as major bottleneck in the commercialization of bioprocesses. There is tremendous untapped potential within natural microbial biodiversity to provide efficient microbial cell factories for novel and safe fermented foods with minimal strain engineering required (Ramírez Rojas, Swidah and Schindler, 2022). In particular, native stress-tolerant phenotypes of non-conventional microbes are highly attractive for bioproduction platforms. Growth at high temperature, high salt and solvent concentrations and low pH can provide cost savings by reducing the energy required for product separation, bioreactor cooling and the maintenance of sterile conditions (Thorwall *et al.*, 2020). These features are often associated with the ability to use low-cost sugars and water resources. Non-conventional hosts are needed because these phenotypes have thus far proven difficult to introduce into model hosts such as *Saccharomyces cerevisiae*. New stress-tolerant organisms may be identified through bioprospecting in extreme environments, by searching existing physical collections and through bioinformatic analysis of sequenced genomes (Overmann and Smith, 2017; Boundy-Mills *et al.*, 2016; Lin *et al.*, 2023). Once they have been identified, such strains must be extensively tested to ensure their safety for use in food applications, even though the final product is extracted and separated from any biomass.

### 3.1.2.2 Innovations in use and technology

### Advances in engineering biology at different scales

The construction of efficient microbial cell factories involves multiple iterations of strain engineering. In recent decades, leaps forward in DNA sequencing and synthesizing technologies have enabled significant reductions in the cost and time required for the “build” stage, in particular. However, even in the case of optimizing single pathways of 5 to 12 steps, the traditional trial-and-error approach requires significant development times. The manufacturing of the immediate precursor of the antimalarial artemisinin took the highly trained expert team at Amyris an estimated 150 person-years (Kwok, 2010). Similarly, Dupont spent an estimated 575 person-years to produce propanediol (Hodgman and Jewett, 2012). The ability to accurately predict the performance of a genetic design, and thus engineer a biological system to a given specification such as titre, would revolutionize development timelines. Innovations in genetic engineering tools, as well as advances in bioinformatics, AI and automated workflows, are bringing this closer to becoming a reality (Lawson *et al.*, 2021). Advances are being made at levels of biological complexity ranging from the single protein level to the whole cell level (see the following subsections for short overviews). Strain engineers employ these tools synergistically to get closer to maximum theoretical yield and design strains that are robust enough to perform at the bioreactor scale (Han *et al.*, 2023).

### Enzyme level

Production of non-native compounds requires the expression of proteins originating from a different species in the chosen microbial host. This first step is facilitated by computational tools used to ensure that the sequence introduced leads to the expression of a functional protein (Fu *et al.*, 2020). Based on the original biological function, enzymes may not be efficient enough for the titre needed for an industrial bioprocess. Thus, protein engineering can be essential for precision fermentation. A wide variety of computational tools, leveraging recent advances in AI and machine learning, are available for efficient protein engineering, enabling both improvements in well-known enzymes and discovery of the functionalities of less-characterized ones, a crucial step in the reconstruction of poorly understood biosynthetic pathways (Sequeiros-Borja, Surpeta and Brezovsky, 2021). The intersection of advanced computational tools and high-throughput experimental methods has also made it possible to design and construct de novo enzymes, also known as “artificial” or “new to nature” (Lovelock *et al.*, 2022). Fusion proteins built upon existing protein domains are one example, and allow better carbon flux, lower levels of by-products, compartmentalization of a pathway in subcellular organelles or cell surface display of enzymes (Li *et al.*, 2020).

Genetic module level

Expression of a protein requires the use of certain genetic elements, generally a promoter,[36] a ribosome-binding-site[37] in the case of bacteria, a gene, a terminator[38] and transcription factors,[39] which together form what is called a genetic module. These elements control when and how strongly a protein is expressed. Promoters are essential in tuning the flux of a pathway and ensuring that the right amount of each enzyme is produced, i.e. enough to maximize production but not so much as to be a burden to the cell, affecting its growth and the overall yield of a bioprocess. Researchers have used rational methods and extensive experimentation to improve upon native promoters already present in the genome of microorganisms as well as to design synthetic ones involving, for example, the fusion of different promoter parts (Jin *et al.*, 2019). With the availability of extensive and high-quality datasets for model organisms, such as *Escherichia coli*, machine and deep learning tools are now being used (LaFleur, Hossain and Salis, 2022).

Transcription factors are another key engineering target. These proteins can act as biosensors, changing the flux in metabolic pathways in response to an internal or external metabolite. This phenomenon can be exploited for “dynamic metabolic engineering”, an approach in which a synthetic genetic circuit is implemented to enable the self-regulation of metabolism to optimize bioproduction (Hartline *et al.*, 2021). This can be a powerful way of creating more robust cells that can maintain production even if unfavourable environmental niches form in the bioreactor (Jiang *et al.*, 2020).

Pathway level

Traditionally, introducing a new biosynthetic pathway into a microorganism involves arduous trial and error, as there may be multiple bottlenecks limiting the availability of precursors and cofactors from the native metabolism. Computational pathway design can enable data-driven identification of possible metabolic routes from native precursor to the desired compound, ranking of these routes and enzyme assignment based on information available in databases (Sveshnikova, MohammadiPeyhani and Hatzimanikatis, 2022). This can lead to more resource-efficient pathways that do not compete with essential native metabolic pathways, accelerating the development of successful microbial cell factories (Wang *et al.*, 2017). One major limiting factor is the availability of enough high-quality data (Kitano *et al.*, 2023), and thus these approaches are limited to a few model microorganisms that may not be ideal for food applications. Additionally, computational tools are enabling retrosynthetic pathway discovery[40] (Lin, Warden-Rothman and Voigt, 2019).

Genome level

Multiple genome-editing tools are now available that can successfully perform genome-wide manipulations, such as the insertion, deletion or alteration of genes, in model systems and a growing number of hosts. Termed new genetic techniques, these include zinc finger nucleases, transcription activator-life effector nucleases, multiplex-automated genome engineering and CRISPR-Cas tools (Gaj

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[36] Promoters are regions of DNA where transcription begins. This is the process whereby an RNA copy is made of a DNA sequence. This can then be translated into a protein. Depending on the sequence, promoters can have different strengths, meaning they can result in more or less protein being produced.

[37] As the name suggests, the ribosome binding site regulates the binding of a ribosome to an RNA copy of DNA. Ribosomes are small structures within the cell that are responsible for protein synthesis from RNA.

[38] Terminators are genetic sequences that cause transcription to stop.

[39] Transcription factors are proteins that bind to DNA and regulate gene expression by promoting or suppressing transcription.

[40] A technique that works backwards from the target compound to identify enzymatic transformations that would be required to build it from the metabolites made by the cell.

*et al.*, 2016). The latter is one of the most widely used thanks to its adaptability, specificity and robustness. CRISPR stands for clustered regularly interspaced short palindromic repeat DNA sequences. CRISPR-Cas gene-editing tools allow precise cutting of DNA using an RNA guide, and harness natural DNA repair mechanisms to introduce desired modifications. CRISPR editing allows microorganisms to be engineering without traces of non-native DNA, which could allow them to be classified as non-GMOs under future regulatory frameworks (Hanlon and Sewalt, 2021).

Cutting-edge genome-level engineering efforts include the refactoring of genetic codes to create synthetic ones that deviate from the near-universal genetic code defining the correspondence of nucleotide sequence to amino acid sequence (DNA to protein) (Ostrov *et al.*, 2020). The relevance of this to microbial cell factories is that it can enable the design of bacterial systems that feature genetic firewalls that isolate synthetic organisms from natural ones, thus safeguarding the synthetic cells from viruses and ensuring the containment of synthetic genetic information (Zürcher *et al.*, 2022; Nyerges *et al.*, 2023). Bacteriophages, viruses that affect bacteria, are a persistent challenge in industrial biotechnology, with contamination leading to fermentation failure and low-quality and inconsistent end-products, and thus to considerable economic loss. Another genome-level approach used to tackle this challenge has been the introduction of defence modules into the genome and mutation of components essential for the life cycle of the virus (Zou *et al.*, 2022).

Flux level

Genome-scale metabolic models and constraint-based modelling such as flux balance analysis are widely used in metabolic engineering to estimate metabolic flux, which gives a more dynamic view of what is happening within the cell, informing engineering and fermentation optimization strategies (Gong *et al.*, 2024). Recently, flux balance analysis has been improved by integrating machine learning, which enables the inclusion of kinetics and regulatory events in the model, allowing more representative predictions (Sahu *et al.*, 2021).

Cell level

For the production of compounds that are toxic to the host or difficult to release out of the cell, adaptive laboratory evolution has been extensively used to improve tolerance, substrate use and growth under specific conditions. Adaptive laboratory evolution uses natural selection to obtain a population of cells that are better adapted to a defined laboratory environment featuring a particular set of conditions (Wang *et al.*, 2023). The mutations accumulated are thus all naturally occurring. Another approach at the cell level is to change the shape, size or structure of cells in a way that benefits production. In *Yarrowia lipolytica*, β-carotene production was increased by 139 percent after the deletion of two genes related to cell shape (Liu *et al.*, 2021). Modifications to cell shape and the thickness of cell walls can also help improve the efficiency of downstream processing – the final product recovery step, the success of which defines the yield and purity of the product. In the case of oligosaccharides, downstream processing can account for 50–70 percent of total manufacturing cost (Kruschitz and Nidetzky, 2020). Innovation is needed in how to optimize strategies from the perspectives of both strain engineering and analytical and material science.

As building strains has become easier, one bottleneck that has emerged is the ability to generate and analyse data related to their performance. Ideally, every strain would be tested at manufacturing scale, but this is prohibitively expensive (Abbate *et al.*, 2023). New analytical technologies generalized to many molecule classes show promise as a means of addressing this bottleneck. Attention needs to be paid to small-culture conditions that have predictive ability for performance at manufacturing scale, with high data quality required as relative improvements get smaller closer to maximum theoretical yield (Leavell, Singh and Kaufmann-Malaga, 2020). Development of computational techniques for analysis and making predictions using the resulting data is equally important (Lawson *et al.*, 2021).

Community level

As outlined in Section III.1.2.2, an emerging approach in the field of biomass fermentation is the use of microbial communities. This is relevant to precision fermentation too, where the use of more than one microbial strain is being explored as a way of increasing production via division of labour, improving the use of alternative feedstocks and increasing robustness to environmental challenges by exploiting the diverse innate metabolic capabilities of different microorganisms (McCarty and Ledesma-Amaro, 2019).

**Valorization of waste**

Increasing food demand, coupled with the scarcity of natural resources and the growing burden of waste disposal, has renewed interest in the remediation and valorization of waste. Precision fermentation is attractive for this compared to purely chemical technologies because of the ability of microorganisms to metabolize a wide variety of compounds and reroute the carbon towards a single value-added product under mild conditions. In the context of the agrifood system, this allows the valorization of food waste, agricultural by-products and inedible biomass, and provides a way to reduce the cost of the culture media needed for precision fermentation production systems, improving both their sustainability and their economic viability (Nadar *et al.*, 2024).

Agricultural by-products, including protein hydrolysates (e.g. soy meal, maize distiller's dried grain with solubles [DDGS], canola meal, brewer's spent grain, maize gluten meal and tomato pomace) and lignocellulosic sugars (e.g. maize stover, soy straw, rice hulls, sugarcane trash/bagasse and barley straw/husks) have been explored for use in the production of alternative proteins, bulk chemicals and biofuels (Ewing *et al.*, 2022). Host microorganisms are either native isolates from waste streams or metabolically engineered hosts using enzymes from such isolates to assimilate the sugars present in sidestreams. Lignocellulosic biomass in particular has been identified as a potent resource with carbon-neutral potential; about 181.5 billion tonnes are produced per year (Singh *et al.*, 2022a). Recent reviews have covered advances both in pretreatments and in the genetic engineering of microorganisms to convert the substance into products (Ashokkumar *et al.*, 2022). Food waste represents another resource, including carbohydrate-rich wastes (such as fruit peels and whey from dairy) and lipid-rich wastes (such as waste cooking oil, fatty acid distillates and waste animal fat) (Lad, Coleman and Alper, 2021).

Limiting factors for the scaling-up of such production platforms are the logistics and costs of transporting agricultural by-products and food waste to fermentation facilities, the pretreatments needed, the stability of the waste or sidestreams, and the consistency of their composition (Ewing *et al.*, 2022). One solution is the construction of fermentation facilities close to sources of agricultural residues or food manufacturing plants (Augustin *et al.*, 2023). Overall, the use of microorganisms to valorize food and agricultural waste holds promise as a pillar of a bio-based circular economy.

## 3.3 Challenges and opportunities in the development of FDFs

### 3.3.1 Food safety and nutritional impact

As outlined in Section III.1.1, microbial proteins have desirable nutritional properties, although the amount and type of nutrients present depend on the profile of the microorganism, the specific strain used (Sakarika *et al.*, 2023) and the methods used for harvesting, drying and processing (Hashempour-Baltork *et al.*, 2020). However, the nucleic acid content can be elevated and potentially harmful, and food-processing techniques have to be used to mitigate this.

Studies exploring nutritional impact, potential health benefits of FDF consumption and their efficacy as meat substitutes have so far been limited in number, small in size and involved short study periods. Short-term clinical trials and dietary studies have found a range of products to be safe for humans and to be associated with health benefits (Cherta-Murillo and Frost, 2022; Derbyshire, 2022). A systematic review and meta-analysis of nine randomized control trials investigating the effect of mycoprotein intake on selected biomarkers of human health identified potentially important effects on blood lipids, particularly a reduction of low-density cholesterol, a risk factor for cardiovascular disease (Shahid *et al.*, 2023). However, the authors of the review highlighted the magnitude of the evidence base as a limitation to the statistical significance of their results, pointing to the need for definitive larger-scale trials that could provide the data necessary to define the effects of mycoprotein consumption on a wider range of outcomes over a longer period. Concerns about potentially severe allergies remain (Food Forum *et al.*, 2022; Williams, 2021). Although data are limited, fungi-based microbial protein products have been found to cause adverse reactions in individuals with a history of mould allergies (Hoff *et al.*, 2003; Katona, 2002; Jacobson and DePorter, 2018). Production of mycotoxins is also a concern, and this needs to be evaluated as novel strains and feedstocks are tested (Upcraft *et al.*, 2021).

In the case of microalgal protein, the use of open-air systems creates the risk of contamination with heavy metals, other toxins and pathogenic bacteria. Hadi and Brightwell (2021) provide an extensive review of this topic. Regulatory bodies ultimately make the decision on whether a microbial protein product is safe for human consumption or for use as animal feed. In the case of precision fermentation (as opposed to biomass fermentation), downstream processing that purifies out the final product means that the approval process can be more streamlined.

The final product formulation of biomass fermentation-based products is another consideration in their assessment as safe and nutritious foods. The widely used NOVA food classification system categorizes foods into four groups based on the use of food processing and food additives. One of these, NOVA group 4, termed ultra-processed foods (UPF), includes many ready-to-consume and ready-to-heat food items, such as packaged snacks, carbonated drinks, sausages, burgers and other reconstituted meat products. According to NOVA, microbial protein products fall under the UPF category, as these foods generally cannot be produced at home given the food processing steps required and often make use of food additives to improve sensorial attributes, including colour, flavour and texture. A 2019 FAO report examined peer-reviewed literature on the effects of UPFs on both diet quality and health, finding a consistent association between UPF intake and both dietary profiles prone to non-communicable diseases and increased risk of such diseases (Monteiro *et al.*, 2019). It is important to consider that the UPF category is broad and that different foods within this category can have significantly different effects on health (Fang *et al.*, 2024). In December 2023, the Lancet published a multinational prospective cohort study examining the relationship between UPF consumption and the risk of multimorbidity, defined as the co-occurrence of two or more chronic diseases (cancer, cardiovascular disease and type 2 diabetes) in an individual (Cordova *et al.*, 2023). The study created nine mutually exclusive UPF subgroups and examined the associations between total and subgroup intake of UPFs and the risk of multimorbidity. Among the subgroups, higher intake of artificially and sugar-sweetened beverages, and animal-based products were associated with higher risk of multimorbidity, whereas an inverse association was found with ultra-processed breads and cereals, and none with plant-based alternatives (including dairy alternatives and meat substitutes). The differential relationship of subgroups of UPFs suggests that a more nuanced subgroup analysis of microbial protein is necessary, especially given its potential to replace ultra-processed meats.

In the case of proteins produced via microbial cell factories, the goal is to provide a drop-in replacement for their animal-derived counterparts. However, because of impacts that processing, formulation and/or post-translational modifications may have on techno-functional properties (including foaming capacity, solubility, emulsifying capacity and gelation), this does not always happen (Eastham and Leman, 2024). Bioprocess conditions and strain engineering must be optimized to ensure that the functionality of the protein is preserved (Hettinga and Bijl, 2022). Post-translational modifications affecting product integrity and/or protein function featured in discussions about potential product-hazards associated with food ingredients derived from precision fermentation at a recent scientific colloquium of the European Food Safety Authority (EFSA *et al.*, 2024). The issue was also mentioned in relation to the assessment of acceptable levels of identity between food ingredients derived from precision fermentation and their native counterparts. It is, thus, an area requiring attention in future developments related to the selection of host microorganisms, strain engineering and bioprocess conditions.

On the horizon are discussions about the potential use of microorganisms that have great potential for industrial applications but also have known pathogenicity, often restricted to a small immunocompromised subset of the population (Dupuis *et al.*, 2023). There are examples of known opportunistic pathogens that are widely used in the food and beverage industry given their low virulence and the availability of effective antifungal treatments; *Saccharomyces cerevisiae* is one example (Pérez-Torrado and Querol, 2016).

### 3.3.2 Artificial intelligence

The intersection of AI and synthetic biology, the broader field enabling the development of precision fermentation platforms, holds great promise as a means of overcoming technical barriers (Lawson *et al.*, 2021). An example of this is AlphaFold, which has addressed a problem previously deemed unsolvable, the near-perfect prediction of protein structure and folding from sequence alone (Jumper *et al.*, 2021). This monumental breakthrough has transformed the landscape of what is possible with precision fermentation, opening doors to the design of more efficient, high-yielding and even new-to-nature pathways (Cho *et al.*, 2022). The recent developments in generative AI, such as GPT-4, already show promise as tools with predictive potential enabling more accurate and effective design of microbial cell factories, bypassing longer, costlier and more labour-intensive laboratory-based trial and error approaches. One recent study demonstrated the potential of leveraging natural language processing tools to accelerate the extraction of published information related to microbial performance under complex strain engineering and bioreactor conditions, thus enabling biomanufacturing predictions (Xiao *et al.*, 2023). The pipeline successfully extracted knowledge from 176 publications on two oleaginous yeasts (*Yarrowia lipolytica* and *Rhodosporidium toruloides*), and analysis enabled by machine learning allowed researchers to predict *Y. lipolytica* fermentation titres with good accuracy (Xiao *et al.*, 2023).

Coupling of AI with automated workflows enabled by specialized high-throughput equipment is sure to accelerate strain engineering, especially in terms of enabling data-driven techniques that more accurately capture the complexity of cellular process in strain design (Liao, Ma and Tang, 2022). In particular, there is potential to take advantage of advances in AI to increase the success rate in the development of strains that can achieve industrially relevant yields at the bioreactor scale, thus overcoming a major bottleneck to the use of precision fermentation platforms and FDFs. However, data remain a limiting factor in the development of such applications. In the case of GRAS microbial strains, a subset of microorganisms often featuring non-model species, multi-omics data are particularly lacking.

Although AI can demonstrably solve grand challenges in biology, there are significant risks surrounding its use. As exemplified by the AlphaFold case, the application of AI brings serious dual-use concerns. Within the scientific community, this challenge is being dealt with by promoting and fostering a responsible mindset at a global scale. However this is not a standardized approach, and several influential factors are affecting the success of the strategy in different parts of the world (Undheim, 2024). One major concern is that the convergence of large language models (GPT-4) and AI-based biological design tools raises the potential for harm, with the former lowering the barrier to laboratory-based work for non-experts and the latter increasing the capabilities of sophisticated actors. Agile governance at the national level is needed, as is the establishment of an appropriate set of internationally recognized biosafety standards and potential guardrails for the use of AI in this field, for example technical AI safeguards, stronger DNA synthesis screening (ensuring bad actors cannot order DNA sequences that can be used for dangerous or nefarious applications) or even restrictions on access to tools, (Undheim, 2024).

### 3.3.3 Technical barriers

#### 3.3.3.1 Infrastructure

Globally, there is insufficient commercial fermentation capacity to meet forecast demand for FDFs. As one measure, the number of fermentation-derived alternative protein product companies, including both biomass and precision fermentation, grew from 7 to 136 between 2013 and 2022, with the average global investment tripling (Carter *et al.*, 2023). Current manufacturing capacity does not meet needs in terms of scale, technical capability or geographic distribution. According to a recent report from the Good Food Institute (GFI) (Leman *et al.*, 2023), global fermentation-derived product manufacturing capacity is approximately 16 million litres. This is split between 48 known producers (business-to-consumer or business-to-business companies) and 41 food-exclusive contract manufacturing organizations (CMOs). If dedicated to alternative protein products exclusively, this volumetric capacity should translate into 0.4–2.8 million tonnes of food product. In terms of accessibility, this production capacity is concentrated in Europe (47 percent) and the United States of America (34 percent), with a lack of food-grade facilities in Africa, Latin America and the Asia–Pacific region (Leman *et al.*, 2023).

The two means of increasing capacity are:

1) greenfield construction – building new purpose-built facilities for fermentation and downstream processing. This is associated with high capital cost and long timelines; and
2) brownfield retrofit – retrofitting of existing fermentation facilities that were often designed for significantly different bioprocesses. This generally requires new purpose-built downstream processing facilities. The approach can significantly reduce up-front capital expenditure but is nonetheless capital intensive.

One obstacle impeding investment in bioreactor infrastructure is investor uncertainty about consumer adoption of FDFs (Linder, 2023a).

#### 3.3.3.2 Microbial robustness

A major bottleneck to the translation of a precision fermentation platform is the scale-up of the bioprocess from the laboratory to commercial scale. Knowledge of how microorganisms adapt to the bioreactor environment in terms of the interplay between ecology and evolution is limited. Without experimental tests at different scales, it is difficult to predict how capable microorganisms are of continuing to produce the target product in the bioreactor environment, where they face both predictable and stochastic disturbances (Becker *et al.*, 2023). Even with mixing, the distribution of nutrients and oxygen within the vessel will be unequal, creating different niches where cells will experience different stressors, including product toxicity, low pH, high temperature and oxygen limitation. These perturbances can significantly affect productivity and titre, as cells that naturally accumulate mutations may thrive and out-compete producing cells. This is of particular concern in the case of bioproduction platforms with narrow profit margins, where minimal yield losses may affect the economic viability of the process (Olsson *et al.*, 2022).

Microbial robustness is a term used to describe the ability of a microorganism to maintain production performance in the face of perturbations (Trivellin, Olsson and Rugbjerg, 2022). Recent reviews have outlined advances in metabolic engineering strategies that can enable the design of robust cell factories (Wehrs *et al.*, 2019; Xu *et al.*, 2024; Jiang *et al.*, 2020; Mohedano, Konzock and Chen, 2022). Another approach is bioprospecting to identify novel microbial strains that possess native tolerance to a broader range of temperatures, pH and potentially end products (Vuong, Chong and Kaur, 2022). Bioprocess design can also be instrumental in improving robustness.

#### 3.3.3.3 Accessibility

As outlined in Section III.2.2.1, one of the main factors limiting FDF production around the world is a lack of access to bioreactor capacity. However, there are significant constraints at every stage from upstream (strain development) and midstream (fermentation) to downstream (recovery and purification). For upstream development, access to microbial strains that are not readily available in culture collections in the same institution or country can be a significant challenge. Similarly, although DNA synthesis is now more affordable than ever, access to these services, as well as to DNA sequencing, varies widely across the world. This creates substantial bottlenecks and delays in the R&D process. The equipment, infrastructure and expertise needed to commercialize a bioprocess require significant and long-term investments.

The 35 publicly funded biofoundries that are part of the Global Biofoundry Alliance[41] facilitate access to cutting-edge workflows that can be pivotal for start-ups, small and medium-sized enterprises and researchers alike. However, excluding China, only 2 of the 35 are located in the Global South (Mexico and India) (Global Biofoundry Alliance, 2024). This discrepancy is reflected in the lack of geographical diversity in innovation, with North America, the United Kingdom, the rest of Europe and China leading the field. An analysis of the spread of research papers referencing synthetic biology as of February 2023 found that only 1 128 out of 24 000 came from low- and middle-income countries (Dasgupta *et al.*, 2023). The agrifood system challenges that FDFs may help to address are global, but effective solutions need to be tailored to the local context. Enabling local autonomy in research in this field can foster equitable innovation and allow advantage to be taken of geographical diversity and local knowledge bases.

### 3.3.4 Achieving sustainable bioprocesses

#### 3.3.4.1 Economic barriers

A persistent challenge for FDFs, but also for wider industrial biotechnology, is the low rate of successful commercialization, with less than 1 percent of innovation derived from academia reaching industrial practice (De Lorenzo and Couto, 2019). This is because numerous ventures fail as R&D on technologies progresses across technical readiness levels (TRLs) from lab-scale success to pilot-scale and beyond (TRLs 1 to 9). This phenomenon is referred to as the Valley of Death. There are many factors associated with this pattern of failure. One of the major bottlenecks, as noted above, is scale-up (Kampers *et al.*, 2022). The development of a successful lab-scale proof-of-concept in academia covers TRLs 1 to 3, while industry generally covers TRLs 8 and 9. The path from TRL 4 to TRL 7 requires equipment and investment that may be beyond the means of academic funding but too risky for industry to commit to. One way of potentially bridging this gap is to develop partnerships between academia and industry. Start-ups established to operate between TRLs 4 to 7 are another option. These can spin out of academia and if successful are often acquired by larger companies or investment groups (Meramo, Fantke and Sukumara, 2022). Nevertheless, the lack of investment and access to bioreactor capacity at the right scale hampers the development of fermentation technology and limits its ability to compete with livestock or petrochemical-derived production, for example by limiting accessibility to economies of scale that can lower costs and allow competitive pricing. Early understanding of factors that may lead to failure can be enabled by using techno-economic assessment (TEA) and life-cycle assessment (LCA) tools. TEA allows the technical and economic viability of a production process to be assessed, while LCA gives insight into environmental sustainability (Meramo, Fantke and Sukumara, 2022).

#### 3.3.4.2 Resource use

**Source of carbon in feedstocks**

The use of pure sugars, as is the norm in industry, is both economically and environmentally expensive. Land, water and fertilizer use associated with the cultivation of sugar-yielding crops, including maize and sugar cane, result in a significant environmental footprint that limits the sustainability of the overall bioprocess as measured by LCA (Ögmundarson *et al.*, 2020). With a growing global population, increasing demands for sugar-yielding crops as food may add further pressure: competition with other food production supply chains would be undesirable and potentially detrimental to food security (Linder, 2023b). The availability of sugar feedstock is also a point of concern given that – like the availability of any crop product – it is vulnerable to plant disease, extreme weather events and other shocks to the food system. A recent report from the Stockholm Environment Institute predicted that global maize and sugarcane yields may decrease by 27 percent and 58 percent, respectively (Adams *et al.*, 2021). This places into question the economic viability of FDFs as competitors with conventional animal products as well as the degree to which they may contribute to improving the resilience of the agrifood system (Zhang *et al.*, 2022). From both a food security and a circular bioeconomy perspective, further research into carbon capture-based processes and waste valorization opportunities need to be

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[41] https://www.biofoundries.org/members

prioritized. Policy and infrastructure changes that facilitate transition to alternative feedstocks should also be promoted.

**Source of nitrogen in feedstocks**

Compared to conventional agricultural methods of food production, the use of bioreactors to produce FDFs allows precise control over the input of nitrogen into the production system and its flow out. This allows the environmentally damaging consequences of nitrogen leakage, such as eutrophication and $N_2O$ emissions, to be prevented (Linder, 2023b). However, unlike crop plants and livestock, microorganisms used in industrial bioprocesses require refined forms of nitrogen substrate such as ammonia, nitrate, urea, amino acids or protein hydrolysates from soy. In industry there is a preference for inorganic ammonium salts, ultimately derived from ammonia (Noroozi and Jarboe, 2023). Manufacturing of ammonia via the Haber-Bosch process accounts for roughly 1 percent of global $CO_2$ emissions and utilizes around 1 percent of global energy production (Capdevila-Cortada, 2019). Reducing reliance on this process is thus an area of great research interest. The design of FDFs should aim to reduce dependence on industrial nitrogen fixation over biological nitrogen fixation. Both use of nitrogen-rich waste streams (Shirvani *et al.*, 2023) and engineering of nitrogen-fixing bacteria (Yoshidome *et al.*, 2024) are garnering increasing interest.

**Energy requirement**

In both TEA and LCA analysis, the energy required to run a production facility is a key factor affecting cost and sustainability. Without the use of renewable sources of electricity, bioproduction processes run the risk of being similarly or more environmentally damaging than conventional agriculture, especially with respect to GHG emissions (Behm *et al.*, 2022; Sillman *et al.*, 2020). It is not guaranteed that improved land- and water-use efficiency can offset this for every bioprocess. This is demonstrated by LCA analysis highlighting the need for renewable energy sources to ensure the sustainability of carbon capture-based processes in particular (Järviö *et al.*, 2021). Dependence on the green-energy sector, which in turn requires critical materials, such as chromium, nickel, zinc, silicon and gallium, means that a transition to microbial protein would increase demand for these materials (El Wali *et al.*, 2024).

#### 3.3.4.2 Waste management

To fully realize the potential of FDFs to support a circular bioeconomy, the waste generated from the bioprocess needs to be managed efficiently. One organic residue arising from precision fermentation is spent microbial biomass, 50 million tonnes of which were generated from biomanufacturing in 2013 (Stikane, Dace and Stalidzans, 2022). Regardless of the type of microorganism from which the biomass is derived, such waste is typically rich in protein and cell-wall materials (Guedes *et al.*, 2019). Because of the nature of industrial bioprocesses, this waste stream is quite stable in terms of its composition and the quantity produced (Stikane *et al.*, 2023). To date, research efforts have primarily focused on methods for valorizing one type of spent biomass – brewer’s yeast. Mapping projected amounts and types of spent biomass can help identify opportunities to make use of them, ranging from repurposing as fertilizer to use as animal feed or even as biosorbent for reducing the prevalence of heavy metals in the environment (Halter *et al.*, 2020; Chwastowski *et al.*, 2023). Further use of spent biomass can improve the environmental impact of a bioprocess (Behm *et al.*, 2022).

## 4. Non-food product biomanufacturing from agro-industrial materials

### 4.1 Current landscape

The use of microorganisms and precision fermentation technology can improve the sustainability of industries beyond food and agriculture. Modern society is dependent on carbon-based chemicals for products ranging from pharmaceuticals and plastics to textiles and cleaning products (The Royal Society, 2024). The vast majority of these are produced using fossil fuels, a non-renewable feedstock with grave environmental impacts (Tickner, Geiser and Baima, 2021). Demand for embedded carbon in chemicals is expected to reach over 1.1 gigatonnes by 2050, double the current approximately

550 million tonnes of embedded carbon present in feedstocks for chemicals and derived materials (Kähler, Porc and Carus, 2023). Only 8 percent of the latter has been found to be bio-based, with an estimated 88 percent being fossil based (Kähler, Porc and Carus, 2023). The possibility of converting biomass into carbon-based chemicals holds potential for the defossilization of the chemical industry and the development of a circular bioeconomy.

### Overview of agro-industrial materials utilized

Agro-industrial materials are renewable resources that can be utilized as a more sustainable alternative in the manufacturing of chemicals and/or in energy production. These materials can range from non-food crops (e.g. switchgrass and miscanthus) to by-products and waste biomass from agricultural and food-processing activities. A non-exhaustive list includes straw (rice, barley, wheat, sugarcane), rice husk, wheat stover, corn cobs, maize stover, sugarcane bagasse, bran (maize, rice, soybean and wheat), forestry residues (e.g. wood branches, wood sawdust, fruit bunch), horticultural residue, marine biomass, waste vegetable oil, animal fat, vegetable skins, fruit waste, and municipal food and garden waste. Biomass feedstocks are complex, consisting of a range of molecules including carbohydrates, oils, fats, proteins and lignin (Philippini *et al.*, 2020; Calvo-Flores and Martin-Martinez, 2022; Singh *et al.*, 2022b; Ubando, Felix and Chen, 2020).

Conversion technologies, including fermentation, are used to transform these molecules into valuable bio-based chemicals. To ensure microorganisms can effectively access these molecules, biomass feedstocks must first be fractioned or processed into biomass platforms, such as sugar, oil, lignin, $CO_2$ or syngas (Sparks, 2024). Commercial-scale bioprocesses are well-established for the use of sugars, namely glucose and sucrose obtained from crops such as sugar cane, sugar beet, maize, cassava and sorghum (Ewing *et al.*, 2022). However, concerns over cost, sustainability and competition with food applications have increased focus on the use of agro-industrial by-products, waste and non-food plant biomass (Arias, Feijoo and Moreira, 2023). Such feedstocks tend to be more heterogenous, and pretreatment steps are required in order to obtain the fraction of biomass necessary for a particular process. There are a number of different processing technologies, and the nature of these steps can affect the environmental impact, cost and quality of the final product (Awogbemi and Kallon, 2022; Gallego-García *et al.*, 2023).

In particular, significant research efforts have been directed towards the valorization of lignocellulosic biomass residues such as maize stover, barley straw, sugarcane bagasse, wheat straw and coconut husk (Rajesh Banu *et al.*, 2021). This is because lignocellulosic biomass is the most abundant biomass source on Earth (Dahmen *et al.*, 2019) ,with an annual global production of about 181.5 billion tonnes (Singh *et al.*, 2022b). Only 3 percent of this is efficiently utilized and incorporated into the circular bioeconomy, showcasing the untapped potential (Li *et al.*, 2024).

The source of lignocellulosic biomass selected for a bioprocess depends on the local landscape. For example, sugarcane bagasse residues dominate in Brazil, maize stover in the United States of America, cassava skin in sub-Saharan Africa, olive mill solid waste in the Mediterranean region and cocoa pods in Indonesia (Mujtaba *et al.*, 2023).

Given the wide range of sources, there is a lot of variability in the composition of this type of biomass, and thus there is no standard method for valorizing it. Its major components are cellulose (40–60 percent), hemicellulose (20–40 percent) and lignin (10–30 percent) (Sharma, Xu and Qin, 2019). Cellulose and hemicellulose can be broken down through a process called saccharification into simple monomeric sugars that can be used for fermentation in the production of chemicals and biofuels (Jahangeer *et al.*, 2024). However, a major challenge to this end is the recalcitrance of lignocellulosic biomass, in other words the inherent resistance of plants to being broken down and releasing their sugars (McCann and Carpita, 2015). Energy-intensive and costly pretreatment steps are needed to overcome this problem (Lorenci Woiciechowski *et al.*, 2020). Approaches include simultaneous saccharification and fermentation, separate hydrolysis and fermentation, and consolidated bioprocessing Li *et al.*, 2024).

Each of these approaches has its advantages and disadvantages in terms of technoeconomic feasibility and environmental footprint. One study looking at the bioproduction of the platform chemical D-glucaric acid from lignocellulose evaluated all three approaches (Li *et al.*, 2021). The findings indicated that consolidated bioprocessing using a synthetic microbial community of *T. reesei* and *S. cerevisiae* was the most promising in terms of high titre and yield. In this system, the filamentous fungus *T. reesei* produced enzymes that break down cellulose, while *S. cerevisiae* utilized the resulting sugars to produce the target product. This example showcases how microbial diversity and engineering biology tools could play a pivotal role in realizing the potential of lignocellulosic biomass valorization (Gaur *et al.*, 2024). Recent reviews have extensively mapped the current global status of lignocellulose use, including future challenges and potential ways forward (Singh *et al.*, 2022b; Usmani *et al.*, 2021).

Fermentation-based conversion of agro-industrial materials into chemicals and fuels remains technologically and economically challenging. The conversion efficiency, meaning the amount of waste turned into product, varies significantly, as some biomass may turn into by-products, be lost as $CO_2$ or end up as waste (Vom Berg *et al.*, 2023). This depends on the specific feedstock used, the technology (both the processing of the biomass and the design of the bioprocess) and the nature of the product. Higher conversion efficiency means more product from the same volume of feedstock, which equates to major benefits in terms of land use, environmental impact and economic feasibility (Sparks, 2024). Indeed, fermentation-based technologies may not be the most appropriate for the valorization of every type of agro-industrial material, extensive research has been dedicated to exploring thermochemical techniques as well as combinations of different conversion technologies (Jha *et al.*, 2022; Ewing *et al.*, 2022).

### Overview of non-food products manufactured using microorganisms

Precision fermentation has been explored as a means of producing a myriad of non-food products. These can broadly be grouped into biofuels and energy-related products (Barragán-Ocaña *et al.*, 2023; Shahid *et al.*, 2021), platform chemicals (Ewing *et al.*, 2022;  de Jong *et al.*, 2020), high value-added chemicals (Gargalo *et al.*, 2022) and materials (Talan *et al.*, 2022). For the chemical industry, fermentation technologies can be used to produce both drop-in and novel bio-based products (Serra *et al.*, 2022).

Drop-in chemicals are those that are structurally identical to existing fossil-based ones. Adipic acid, a compound used in the production of nylon and other materials, is one example of a drop-in chemical that can be produced more sustainably via fermentation (Skoog *et al.*, 2018). Fossil-based adipic acid is derived from benzene in a process that involves the emission of nitrous oxides, and production of nitric and adipic acid is projected to become the third largest source of nitrous oxide emissions (He, Han and Qin, 2024). The fermentation process creates no such emissions.

Novel bio-based chemicals, on the other hand, have properties that are similar to fossil-based chemicals but do not have the same chemistry. In the context of materials, novel properties can improve the sustainability of products over their entire life cycles. Biodegradable polymers have attracted attention in the production of plastics (Rosenboom, Langer and Traverso, 2022), synthetic rubbers (Boon, Teo and Ang, 2022), textiles (Schiros *et al.*, 2021) and polymers in liquid formulations (Kelly, 2023). A well-established example is polylactic acid (PLA), a commercially available bio-based and biodegradable plastic whose production process involves fermentation to produce lactic acid (De Albuquerque *et al.*, 2021). At laboratory scale, studies have explored the use of renewable resources and waste streams for lactic acid production, including fermentation of food waste and spent mushroom using *Enterococcus mundtii* (Ma *et al.*, 2021), fermentation of corn cob hemicellulose hydrolysate using *Lactobacillus brevis* and *Lactobacillus plantarum* (Zhang and Vadlani, 2015), and fermentation of pretreated rice straw using *Lacotbacillus plantarum SKL-22* (Yadav, Nain and Khare, 2021).

An additional advantage of novel bio-based chemicals is their potential to replace particularly toxic or hazardous organic chemicals, such as plastic additives, certain solvents and formaldehyde resins (Sparks, 2024). Ethyl lactate is an alternative to the solvent dimethylformamide, a liver toxin found in paints, gums, food additives, cosmetics and industrial cleaners (Taipabu *et al.*, 2023). Its synthesis from biomass leverages LAB to produce lactic acid. A laboratory-scale study has also explored the synergistic fermentation of LAB and yeast for ethyl lactate production from biomass (Li *et al.*, 2019).

Recent reviews (Ewing *et al.*, 2022; Calvo-Flores and Martin-Martinez, 2022) provide an overview of the scale, commercial success, technological readiness, sustainability and adoption of agro-industrial materials in the production of non-food products using biological processes, with key examples including bioethanol, lactic acid, 1,3-propanediol, xylitol, succinic acid, microbial oil, polyhydroxyalkanoates (PHA polymers), poly-gamma-glutamic acid (γ-PGA), itaconic acid and 1,4-butanediol.

### Biorefineries

The International Energy Association (IAE) defines the biorefinery concept as an upstream, midstream and downstream processing facility for the conversion of biomass into a spectrum of marketable products, such as fuels, energy, materials and chemicals, in an economical, socially acceptable and environmentally sustainable manner (Annevelink *et al.*, 2022). A biorefinery may use physical, chemical or biological methods, with fermentation technologies classified as an effective biochemical process. The integrated biorefinery model aims to optimize the use of biomass feedstocks, reduce energy costs, reduce environmental impacts and improve profitability via the production of multiple products from a given feedstock (Kumar and Verma, 2020). According to a 2022 report from the IAE, there are 42 countries running pilot to commercial scale biorefinery plants, including Australia, Austria, China, Denmark, Germany, Ireland, Italy, the Kingdom of the Netherlands, the Republic of Korea, Sweden and the United States of America (Annevelink *et al.*, 2022). A case study is presented in Box 1.

**Box 1. The Bazancourt-Pomacle Biorefinery**

The Bazancourt–Pomacle Biorefinery in France (Allais, Lescieux-Katir and Chauvet, 2021) was first developed as a sugar refinery but is now an integrated biorefinery that converts 4 million tonnes of biomass (mostly sugar beet and wheat, but also alfalfa and woody material) per year into chemicals, fuels, and food and feed ingredients, leveraging a range of technologies, including fermentation processes. The use of biomass from agricultural activities in the surrounding region benefits local producers. The development of the site was made possible by the close involvement of two world-scale agro-industrial cooperative groups, Vivescia[42] and Cristal Union,[43] which has led to the mobilization of nearly EUR 1 billion of investment.

As of 2021, the plant was responsible for 1 200 employees and 800 indirect jobs. The co-location of multiple companies at the site enables both economic and environmental optimization, as they supply each other with intermediates, energy and services, reducing waste and the energy and water consumption of the plant as a whole. The plant also hosts a multidisciplinary research academic centre, with the infrastructure and partnerships in place to innovate and scale novel technologies up to technical readiness level (TRL) 9. More recently, the centre has collaborated with experts in synthetic biology to produce non-endocrine disruptive sustainable UV filters from forestry, wheat and mustard by-products through the use of fermentation and green chemical processes (Magee, 2020).

Source: See the references within the box.

### Environmental impact

In terms of GHG emissions, the use of biomass feedstocks as an alternative to fossil fuels can improve sustainability, as the carbon released during manufacturing and at the end of the life of the product is biogenic, i.e. has been captured from the atmosphere through the growth of biomass (Sazdovski *et al.*, 2024). However, this does not necessarily translate into a net-zero or carbon-neutral process, as the

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[42] https://www.vivescia.com/en

[43] https://www.cristal-union.fr/en

transport of feedstock, use of certain reagents and energy requirements of bioreactors result in additional GHG emissions (Culaba *et al.*, 2023). Comparative assessments rely on LCA analysis, although differences in data availability, assumptions made and methodologies used mean that estimations of potential GHG savings may diverge significantly for the same target product (Zuiderveen *et al.*, 2023; Gaffey, Collins and Styles, 2024). Overall, however, improved life-cycle GHG emissions can be achieved for bioprocesses through the use of low-emission feedstocks (e.g. agricultural residues or food-processing waste, although pretreatments required for these can significantly add to emissions) (Moutousidi and Kookos, 2021), replacing petrochemical products with bio-based alternatives that have lower life-cycle emissions (e.g. via biodegradability) and maximizing energy and biomass-conversion efficiency (Sparks, 2024).

Beyond reducing GHG emissions, one of the key advantages of waste valorization through a biorefinery model is that it mitigates the environmental impact of traditional waste-disposal methods (Prado-Acebo *et al.*, 2024). Agrifood waste accumulation in landfills and disposal via incineration have significant carbon footprints and can reduce air quality and pollute groundwater (Capanoglu, Nemli and Tomas-Barberan, 2022). Problems associated with agrifood waste disposal particularly affect developing countries, where safe disposal methods may be inaccessible (Freitas *et al.*, 2021).

### 4.2 Challenges and opportunities for future development

The commercialization of biorefineries faces four main roadblocks:

- **Technical maturity**: Various valorization techniques are still at the laboratory or pilot scale. Difficulties remain with optimizing the processing and pretreatment of biomass to reduce cost and energy requirements, improving downstream processing and product recovery, ensuring robust bioproduction performance at scale, and improving biomass conversion efficiency by valorizing all biomass components, improving tolerance of inhibitors present in the feedstock, reducing carbon loss through by-product formation and optimizing fermentation conditions (Prado-Acebo *et al.*, 2024; Singh *et al.*, 2022b).

- **Biomass availability**: The amount of biomass available is not sufficient to fully replace all fossil fuel-derived products. According to a 2018 IEA report, to shift to fully bio-based routes for all primary chemical production, approximately half of the world’s sustainable biomass would be required (IEA, 2018). Biomass is a limited resource, with myriad applications, ranging from chemicals and fuels to food and feed. Priority uses of biomass need to be identified in order to ensure that resources are directed to the sectors and products with the highest economic feasibility and the greatest potential impact in terms of climate change mitigation (Louw *et al.*, 2023a). At a global scale, this sort of evaluation is going to require improved standardization of how TEAs and LCAs are conducted (Mahendrasinh Kosamia *et al.*, 2022).

  In addition to the volume of biomass available, feedstock complexity, acquisition and logistics pose significant challenges (Nunes, Causer and Ciolkosz, 2020). Compared to petroleum, biomass is expensive to transport and may be widely distributed, as well as being seasonal in some cases. Biorefineries that are flexible to a range of feedstock sources, for example lignocellulosic biomass from food processing as well as forestry waste, are better positioned to face challenges related to biomass availability and prices when demand for particular feedstock sources becomes competitive in the context of the transition towards a bio-based circular economy (Garcia-Ochoa *et al.*, 2021).

- **Investment requirements**: The capital investment required for building new infrastructure is large and high-risk (Makepa and Chihobo, 2024). Biomass availability issues limit the size of biorefineries, unlike their petroleum refinery counterparts (Sparks, 2024). The result is a higher capital expenditure requirement per tonne of product, which increases costs. The operating cost of biorefineries is also high (Mossberg *et al.*, 2018). Integrated biorefinery strategies aim to mitigate this by providing production pathways for more than one product, thus improving profitability and resource use.

A study that used an integrated biorefinery model based on valorization of waste cooking oil to evaluate profitability and sustainability under different process integration scenarios (Ullah *et al.* (2022) found that combining biodiesel and bio-succinic acid production increased net present value by 7 percent and reduce energy requirement, waste and $CO_2$ emissions by 22 percent, 80 percent and 20 percent, respectively. However, inability to compete with the prices of products from the well-established petrochemical industry, especially in the absence of supportive policies, has made commercialization extremely challenging (Zetterholm *et al.*, 2020). Restrictions on economies of scale, uncertainty over future demand and lack of cost-competitiveness, in addition to the need for new infrastructure, make biorefineries high-risk investments (Giwa, Akbari and Kumar, 2023).

- **Scale-up challenges**: As outlined throughout this study, unlike chemical processes, the scale-up of biological processes driven by living microorganisms is complex. Metabolic reactions and process stoichiometry are not straightforward. Parameters optimized at laboratory scale generally do not demonstrate a similar level of efficiency at pilot-scale. Even when technical challenges to achieving commercial production are overcome, as has been the case with bioethanol, 1,3-propanediol, succinic acid, xylitol and PHA, high production costs and low production capacity place ventures at risk (Millinger *et al.*, 2022). When gasoline prices fall, biorefinery units either remain idle or close down, as has been the case for some succinic acid production units (Li and Mupondwa, 2021). Bioethanol production from lignocellulosic sources at an industrial scale is not cost competitive but is made viable through government incentives, such as the renewable identification number credits in the United States of America, and subsidies (Stewart *et al.*, 2023; Makepa and Chihobo, 2024).

  One reported constraint to the scaling up and operation of biorefineries is the lack of an appropriately skilled workforce (Sparks, 2024). Studies in Europe have found that the establishment of biorefineries in rural locations, which is the most convenient option in terms of access to biomass, has the potential to provide employment opportunities in the surrounding areas through direct employment at the facility and by maintaining jobs in farming and service industries (Zhu *et al.*, 2024; Zhu, Vrachioli and Sauer, 2023). Exploration of the societal impact of biorefineries in local contexts could help inform policy decisions.

# 5. State of conservation of microorganisms relevant to fermentation

## 5.1 State of biodiversity loss and conservation

The scale of microbial diversity is immense, one statistic showcasing this is that there are more prokaryotic cells, about $10^{30}$ considering bacterial and archaeal microorganisms alone, than stars estimated to exist in the observable universe (Vuong, Chong and Kaur, 2022). Although only a sliver of this diversity is relevant to FFs or FDFs, the incredible volume of species and strains represents a challenge for conservation efforts. In addition to this, there are no straightforward automatic or remote monitoring systems for accounting for changes in global microbial diversity (Shaffer *et al.*, 2022). Instead, such efforts are reliant on individual and large-scale collaborative characterization studies that provide sequence data, which can lead to insights into microbiome composition and functionality and provide the foundation for comparative studies (Louw *et al.*, 2023b).

Awareness of how climate change and agricultural practices are affecting microbial diversity is growing. The Microbiologists' Warning Consensus Statement, published in 2019, details concerns about how changes in temperature, precipitation and another climate-related factors may affect the abundance, diversity and function of microorganisms, especially fungi, in agricultural systems

(Cavicchioli *et al.*, 2019). Given that traditional fermentation arises from the spontaneous fermentation of microorganisms present on the surface of substrates, it can be theorized that the loss of soil microbial diversity may affect the diversity of microbial strains present in fermentation-associated microbial communities (Louw *et al.*, 2023b). However, this requires investigation.

The loss of cultural practices and traditional foods represents a loss of local knowledge and relevant starter cultures and the functionalities that may be associated with them (Asogwa, Okoye and Oni, 2017). Indeed, the major threats to fermentation-associated microbial diversity are those affecting the practice and valorization of traditional knowledge, including globalization and industrialization, which have contributed to the trend towards an increased use of commercial starter cultures and loss of traditional ferments (Asogwa, Okoye and Oni, 2017; Rest, 2021; Sõukand *et al.*, 2015).

Key to conserving these genetic resources is the documentation of traditional knowledge and long-term preservation of microbial communities. Conservation through *ex situ* methods, such as storing strains and starter cultures in culture collections or mBRCs, can play a supporting role in efforts to conserve traditional knowledge, for example by ensuring that these materials are preserved and can be accessed by future generations (Vijayan *et al.*, 2022; Boundy-Mills *et al.*, 2016; De Guidi *et al.*, 2023). However, it has been recognized that *ex situ* methods must be developed carefully via engagement with Indigenous Peoples and local communities because of the risks of exploitative bioprospecting and biopiracy. Capacity building to ensure the infrastructure necessary to preserve microorganisms is available *in situ* would empower countries to manage, maintain and sustainably use their microbial genetic resources (Ryan *et al.*, 2019).

Initiatives aimed at the identification and conservation of microbial communities behind traditional FFs before these are lost, include the Puratos Sourdough Library,[44] Global Microbiome Conservancy[45] and the Microbiota Vault Project[46] as well as the work of individual research groups such as the Heirloom Microbes Project.[47] The latter is an interesting case study of effective engagement with Indigenous Peoples and local communities, and benefit-sharing between foreign research groups and local stakeholders via training opportunities, co-authorship with local scientists on research papers and knowledge-sharing.

Where engineered or domesticated microorganisms are concerned, maintenance depends on the discretion of the responsible academic research group/institution or private company. The loss of a group leader in an academic institution may lead to the destruction of a culture collection (Boundy-Mills *et al.*, 2020). Internal protocols surrounding the maintenance of unique wild isolates or engineered strains owned by private companies are more obscure and presumably vary with the size and management of the organization and the availability of funds. However, companies may be required to deposit strains associated with patent applications in culture collections (WIPO, 2025).

With the rise of high-throughput strain construction strategies and evolutionary engineering techniques, microbial biodiversity is being expanded through the development of new-to-nature strains with unique functionalities and adaptations (Hirasawa and Maeda, 2022). The rich genetic diversity generated from such experiments can be utilized in the investigation of fundamental biological phenomena or in interventions aimed at obtaining functionalities or behaviours unrelated to the original objectives of the experiment (Sandberg *et al.*, 2019). Although there are no clear systematic efforts to preserve evolved microbial strains, recognition of the value of experimental evolution information led to the establishment of the ALEDb database (Phaneuf *et al.*, 2019). As of 21 August 2024, this hosts data from over 92 different publications, in particular 703 647 unique mutations from 11 858 isolates, making it the largest collection of microbial evolution mutation data in the world. A widely used

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[44] https://sourdoughlibrary.puratos.com/en

[45] https://microbiomeconservancy.org

[46] https://www.microbiotavault.org

[47] https://christinawarinner.com/research-2/research-h/

publicly accessible collection for the preservation and accessibility of genetic parts referenced in publications, including those related to precision fermentation, is Addgene.[48]

## 5.2 Conservation strategies

### 5.2.1 Culture collections

Culture collections play a fundamental role in the long-term storage and stable preservation of microorganisms. They can be large public repositories that are accessible to the research community in accordance with international agreements, including the Nagoya Protocol, or research collections stored in academic institutions or industrial research laboratories that are not publicly accessible (Boundy-Mills *et al.*, 2020). Depending on the scope of the collection, it may be considered a genetic stock centre preserving thousands of genetic variants of the same species or a biodiversity collection with numerous species isolated from natural environments. Beyond the microbial sample itself, the resources available from these collections can include replicable parts of the microorganisms (e.g. genomes and plasmids), data associated with samples, and information such as heredity and function (McCluskey, 2013).

The concept of a Biological Resource Centre (BRC) was established by the Organisation for Economic Cooperation and Development (OECD), and refers to collections that can provide the above-described resources and operate according to the best practices of the OECD (Ozerskaya, 2008). For microbial domain BRCs (mBRCs), these include validation of the taxonomy of the preserved materials and quality control to guarantee their viability, purity and authenticity (Smith, McCluskey and Stackebrandt, 2014). Crucially, to ensure long-term storage and prevent irreparable loss, use of at least two different preservation methods is required, including one specifically long-term preservation technique such as cryopreservation or freeze-drying (also termed lyophilization) (Becker *et al.*, 2019). mBRCs also maintain strains deposited in association with patents, thus securing intellectual property. They must be designated for this purpose by the World Intellectual Property Organization based on the Budapest Treaty (WIPO, 2025). The Microbial Resource Research Infrastructure (MIRRI) brings together more than 50 mBRCs from ten European countries and aims to provide sophisticated services that facilitate the valorization of microbial resources by researchers and industries, including for the production of healthier food and feed (MIRRI-ERIC, 2020).

The Korean Collection for Type Cultures, the BRC of the Korea Research Institute of Bioscience and Biotechnology, is an example of a BRC that, in addition to acquisition, preservation and distribution of biological resources, conducts R&D, including a programme for identifying and building a bank of secondary metabolites produced by bacteria with potential applications in fields such as the production of pigments, vitamins and herbicides (KRIBB, 2022).

Information about culture collections registered with the World Federation for Culture Collections (WFCC) is available online (http://www.wfcc.info), with catalogues available from the World Data Centre for Microorganisms, which includes Culture Collections Information Worldwide (CCINFO), a directory of all registered culture collections. As August 2024, CCINFO holds information on 861 culture collections based in 80 countries and regions containing more than 4 million microorganisms (CCINFO, 2024). Affiliate collections of the WFCC are under the long-term care of an institution and cannot be dependent on a single individual. Information on the properties of strains held by collections may not have been updated since the time they were deposited and thus may not reflect contemporary food quality and safety requirements or changes that the strains may have incurred over time during storage.

The United States of America and Japan are recognized as strong hubs for microbial resource preservation and utilization, and have large holdings. European networks also have a long history of microbial research, which is reflected in the size of their holdings, for example those maintained by the members of the European Culture Collections' Organisation (ECCO). In these countries, the tendency is to have fewer centres with large holdings, suggesting a focus on centralized publicly accessible

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[48] https://www.addgene.org/mission

culture collections or certified mBRCs (Wu *et al.*, 2017). In contrast, countries such as Brazil and Thailand that have high native biodiversity have many collections that specialize in particular regional characteristics or have a particular research focus, with a smaller average number of strains per collection (Wu *et al.*, 2017; Glienke *et al.*, 2024). Access to funds, infrastructure and expertise are key influences on the size and maintenance of collections, with sophisticated mBRCs having high operating costs (Smith, McCluskey and Stackebrandt, 2014).

Every culture collection maintains microorganisms isolated from microbiomes. However, these are usually only the culturable components preserved in an axenic state, i.e. isolated from any other microorganism. A few culture collections, for example the German DSMZ collection, act as biobanks, which host deposits of microbiome samples. Biobanks serve as archive repositories, storing samples without assuring the viability or stability of all microbial constituents (Ryan *et al.*, 2021). This is relevant to fermentation applications, as advances in microbiome preservation techniques (Prakash, Nimonkar and Desai, 2020) can provide a means of safeguarding the microbial communities of traditional FFs.

Initiatives aimed at building capacity for the preservation of food-associated microorganisms not only serve to safeguard culturally significant foods but can also enable effective valorization of local knowledge and microbial heritage via new economic opportunities. One example is the GreenGrowth project (Mengu and Jespersen, 2019), which saw the establishment of biobanks (-80 °C freezers with green power backup supply) in Benin, Burkina Faso and Ghana for the long-term preservation of microorganisms seen as potential starter cultures. Microorganisms were isolated from local fermented foods and beverages, characterized for nutritional and technological properties, and assessed for green food processing through trials conducted by small to medium enterprises and local women's cooperatives.

Culture collections and mBRCs also have potential for bioprospecting initiatives. Characterization of current holdings and improving access to uncharted microbial diversity can lead to the identification of biotechnologically relevant strains (de los Santos-Villalobos *et al.*, 2021). Such efforts can be integrated into the business model of mBRCs and used to promote private investment in the long-term preservation of microbial diversity (Overmann and Smith, 2017).

### 5.2.2 Microbiome vs. pure-culture preservation

Methods of preserving microorganisms *ex situ* have been explored for decades, and include preservation on gelatine discs, storage in sterile soil, mineral oil or silica gel, spray-drying, liquid-drying, desiccation, freeze-drying and cryopreservation (De Vero *et al.*, 2019). The last two are considered the most reliable methods for long-term maintenance of a wide range of microbial species, as reflected in OECD best-practice guidelines and international standards (Smith, Fritze and Stackebrandt, 2013). Specific regimes must be developed for different strains, including the selection of cryoprotectants and cooling rates. Strains can be cryopreserved at -80 °C in the freezer and -196 °C in liquid nitrogen. Analysis of the timelines for which regimes are successful in maintaining the viability and integrity of samples is necessary, as this can vary from strain to strain and protocol to protocol (Vekeman and Heylen, 2017; Janssens *et al.*, 2010). Advances relevant to the preservation of strains that are not readily culturable include the addition of signalling and growth-promoting molecules from helper strains to the media, the use of diffusion bioreactors that allow the passage of necessary elements from the natural environment into the inoculated chamber, and other cultivation techniques, including the use of transwell plates,[49] optical tweezers and laser microdissection (Chaudhary, Khulan and Kim, 2019; Vartoukian, Palmer and Wade, 2010).

The importance of human-, plant- and food-associated microbiomes in safeguarding human and planetary health has fuelled efforts to preserve microbial communities in addition to single culturable elements (Paramithiotis and Dimopoulou, 2023). However, there are significant technical challenges to achieving this. Microbial communities are dynamic, i.e. change in composition over time. This raises

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[49] A type of small plastic cell culture device.

questions concerning what components are vital for preservation and at what point in time should a "snapshot" of the community be saved (Ryan *et al.*, 2021). Developing a process for storage that can preserve the viability of a community is incredibly complex, as unintentional alteration at any stage may affect its functionality and integrity (Kerckhof *et al.*, 2014). Cryopreservation, the current gold standard, may not be appropriate for every community, as only freeze-tolerant cells will survive. A recent review (Prakash, Nimonkar and Desai, 2020) provides an overview of research work dedicated to tackling this challenge.

## 5.3 Unmet needs in conservation

Unmet needs related to the conservation of microbial diversity include the following:

- *Adequate microbial diversity assessment*. Despite technological innovation and conservation efforts, only 1–10 percent of estimated microbial diversity has been cultured, meaning the functionality, abundance and ecological relevance of the remaining 90–99 percent remain largely unknown (Vitorino and Bessa, 2018; Bernard *et al.*, 2018; Bodelier, 2011). This not only limits the investigation, use and safeguarding of valuable microbial strains but clouds understanding of trends in the loss of microbial diversity. Novel strategies could be used to monitor and investigate the microbial communities present in artisanally produced fermented foods (Louw *et al.*, 2023b).

- *Study of long-term viability of preserved strains*. Validation of preservation protocols is needed to assess possible time limits to the viability, purity, identity and stability of preserved strains. Extensive characterization of cultures pre-preservation and at successive intervals, including morphological, physiological, metabolomic and genomic analysis, will be instrumental in this (De Vero *et al.*, 2019).

- *Innovation in preservation strategies*. As outlined above, to enable use of microbial diversity as a resource for sustainable development, advances in preservation techniques for non-culturable microorganisms and microbial communities are required. Low-cost, robust techniques can also help make the preservation of microorganisms more accessible (Al-Bedak, Sayed and Hassan, 2019).

- *Prevention of orphan culture collections*. A common cause of the loss of private culture collections is the retirement of the researchers responsible for founding or maintaining them. Common strategies are to transfer the collection to another researcher, distribute it among alumni of the research laboratory or continue to maintain it *in situ*. If a plan is not put in place, such orphaned collections are often lost, quietly destroyed or dispersed. Policies for preventing such losses are needed (Boundy-Mills *et al.*, 2020).

- *Funding*. A major challenge for culture collections and mBRCs alike is ensuring the funding needed to maintain their functions and services. In the vast majority of cases, funds from the distribution of strains do not cover operating costs. Thus, there is significant reliance on government funding and institutional support. The evaluation of alternative business plans and potential sources of income, taking into consideration the stakeholders served by their work, will be vital to the sustainability, stability and expansion of culture collections (Overmann and Smith, 2017).

## 5.4 Global and regional networks and groups

Global and regional research and industrial networks relevant to FFs and FDFs and to the conservation of related microorganisms are emerging worldwide. The following is a non-exhaustive list of significant players among these: the International Scientific Association for Probiotics and Prebiotics,[50] the Good Food Institute,[51] Precision Fermentation Alliance,[52] Food Fermentation Europe,[53] the International Dairy Federation,[54] the Lactic Acid Bacteria Industrial Platform,[55] Future Food Asia,[56] European Food & Fermentation Cultures Association,[57] the UK Research and Innovation Microbial Food Hub, the Bezos Centre for Sustainable Protein, the COST (European Cooperation in Science and Technology) Action PIMENTO (Promoting Innovation of ferMENTed fOods),[58] DOMINO (a European network on fermented foods),[59] the Swedish South Asian Network for Fermented Foods,[60] the World Federation for Culture Collections,[61] the Global Biological Resource Centre Network,[62] the Global Microbiome Conservancy[63] and the Microbiota Vault.[64]

## 6 Policy landscape surrounding fermentation-associated microorganisms

### 6.1 Policy landscape governing the use of microorganisms of relevance to fermentation applications within the food system

#### 6.1.1. Current policy landscape

**Defining terminology**

Genetically engineered microorganism (GEM): refers specifically to microorganisms (i.e. bacteria or fungi, including yeasts) that have been modified using *in vitro* molecular biology techniques. This does not include techniques that allow for the accumulation of modifications that could have occurred naturally (Hanlon and Sewalt, 2021). For example, chemical mutagenesis and interspecies crossing can be used to modify the genetic makeup of a microorganism (National Research Council of the United States, 2004), yet these techniques do not typically fall under the regulatory definitions of genetically engineered (or genetically modified in the European Union). Examples of GEMs used in the food industry include those designed for precision fermentation-based processes, in particular for the production of food enzymes.

Genetically modified organism (GMO): used interchangeably with GEM, but by definition can encompass other organisms in addition to microorganisms, for examples animals or plants (US EPA, 2024).

Genetically modified microorganism (GMM): defined according to Directive 2009/41/EC of the European Parliament and Council as “a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination” (EU, 2009).

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[50] https://isappscience.org
[51] https://gfi.org
[52] https://www.pfalliance.org
[53] https://www.foodfermentation.eu
[54] https://fil-idf.org
[55] https://labip.com
[56] https://futurefoodasia.com
[57] https://effca.org
[58] https://www.cost.eu/actions/CA20128
[59] https://www.domino-euproject.eu/about/m4sf
[60] https://fermented-foods.net.in
[61] https://wfcc.info
[62] https://www.bionity.com/en/associations/69535/global-biological-resource-centre-network-gbrcn.html
[63] https://microbiomeconservancy.org
[64] https://www.microbiotavault.org

New genomic technique (NGT): defined as a technique that is capable of changing the genetic material of an organism and has emerged or has been developed after the adoption of Directive 2001/18/EC in the EU (EU Commission, 2021; EU, 2001).

## Global landscape

Driven by societal and industrial trends there are continuous efforts to invest in and integrate new technologies within the agrifood sector. Regulation of fermented food products, and more specifically those derived from engineered microorganisms, is at different stages of development in different parts of the world. The use of engineered microorganisms for biomass or precision fermentation is tightly regulated in certain contexts (e.g. in the European Union), faces stewardship-style governance in others (e.g. in the United States of America) and is under development, with discussions ongoing, in some countries (e.g. Brazil, China and India).

### European Union

In the European Union, the European Commission and EFSA are responsible for authorizing the placement of novel foods onto the market, with the latter conducting safety assessments (EFSA, 2024a). The legal frameworks relevant to the introduction of FFs originating from countries outside the European Union and FDFs are the General Food Law (which constitutes the core of European Union food legislation) (EU, 2002), the Food Information Regulation (EU, 2011), the Novel Food Regulation (EU, 2015), the Genetically Modified Food and Feed Regulation (EU, 2003) and the Common Organization of the Markets Regulation (Nègre, 2023).

The Novel Food Regulation encompasses foods not used for human consumption to a significant degree in the European Union before 15 May 1997 (EU, 2015). This includes newly developed foods with innovative formulations, foods produced using new technologies and production processes, and traditional foods eaten outside of the European Union. Thus, most FFs and FDFs fall under this category and require explicit permission to be marketed after authorization and inclusion in a European Union list of novel foods. Quorn products were introduced into the European Union market before the Novel Food Regulation (Wiebe, 2004).

In the case of precision fermentation, there is no legal definition for the technology. For bioidentical food additives, processing aids and enzymes produced through fermentation, the regulatory pathway that has to be followed is contingent on whether the food product is considered to be made “from” GMMs or “with” GMMs (Hanlon and Sewalt, 2021). Although, typically, precision fermentation would be a process where food production occurs “‘with” GMMs, as the GMMs are completely removed during the production process, in reality the presence of any recombinant DNA in the final product would classify the entire process as being “from” GMMs, meaning the more stringent Genetically Modified Food and Feed Regulation must be followed (Ronchetti, Springer and Purnhagen, 2024). Notably, there is a zero-tolerance policy on the presence of recombinant DNA, making the possibility of adhering to the “with” classification, and thus the Novel Food Regulation (as is the case with precision fermentation-derived products involving no GMMs), both technologically and economically unfeasible (FSA, 2021).

Researchers have proposed establishing a minimum threshold approach instead, although there are several challenges to implementing this, mainly surrounding the analytical issues involved in developing a standardized and consistent solution, including the absence of standardized recombinant DNA detection methods, the extremely high sensitivity of detection methods and the lack of an established threshold for distinguishing between “real” and “fortuitous” presence of recombinant DNA (Lensch *et al.*, 2022). According to guidance from the EFSA GMO Panel, the risk assessment of GMMs in food or feed products involves the characterization of the GMM (based on the parental organism, the donor of the genetic material used, the genetic modifications made and the traits of the final strain), the possible effects the modifications made may have on the safety of the product, and the product’s composition, nutritional value, potential toxicity/allergenicity and impact on the environment (EFSA

Panel on Genetically Modified Organisms, 2010). The outcome of the risk assessment determines whether any safety issues should be raised.

The introduction of the qualified presumption of safety (QPS) status from EFSA has been one development that has enabled shorter and simpler approval processes. This status is granted to microbial strains that are well known and have been thoroughly evaluated for various aspects of safety. The EFSA Panel of Biological Hazards is responsible for granting a biological agent safety status. Inspection covers the taxonomic identity of the microorganisms, the related body of knowledge and potential safety concerns (EFSA, 2024b). The approved list is updated every three years and features bacteria, protists/algae, viruses and yeast. FFs or FDFs using microbial strains with QPS status are not automatically approved for market but are exempt from a full safety assessment, which requires the generation of safety data, a process involving significant costs and time.

In the case of GMMs, the QPS approach can be extended to engineered production strains as long as the species of the parental/recipient strain has QPS status and the genetic modification does not raise safety concerns (Herman *et al.*, 2018). In an EFSA assessment published in December 2023, QPS status was extended to an engineered *Yarrowia lipolytica* strain for the production of steviol glycosides (food additives), predominantly Rebaudioside M, with the concluding opinion being that there was no safety concern with regard to the use of this precision fermentation process for its intended purposes (EFSA Panel on Food Additives and Flavourings *et al.*, 2023).

In the case of premarket authorization under the Genetically Modified Food and Feed Regulation, after EFSA has received a valid application from a food business operator, it has up to six months to issue a scientific opinion regarding the safety of the product. If more data are required by the agency, the clock can be stopped until the required information is provided. After the EFSA opinion is published, a 30-day public consultation phase begins, part of a three-month window given to the European Commission to decide whether to grant approval to the product, with assistance from the Standing Committee on the Food Chain and Animal Health (FSA, 2021). However, it has been reported that these timelines are often not adhered to, with delays, causing uncertainties for applicants, often start-ups for whom funding is a challenge (EFSA *et al.*, 2024).

It is of note that under the European Union Food Law "placing on the market" involves a range of activities beyond simply sale (FSA, 2024). This means that tastings require premarket approval, making it very difficult for companies to gather consumer acceptance data (Verzijden, 2023). Testing and validation are steps that provide essential evidence of a product's safety, quality and efficacy, without which it is difficult to instil confidence in consumers or investors.

One issue involved in obtaining preauthorization is that of labelling. European Union food labelling law is intended to prevent consumer deception. Thus, there are stringent requirements surrounding the use of labels with health and nutrition claims, such as "vegan" or "organic", and clean labels[65] (Lähteenmäki-Uutela *et al.*, 2021). According to the Genetically Modified Food and Feed Regulation, products that are produced from or contain ingredients produced from GMOs must be labelled clearly as "genetically modified" or "produced from genetically modified (name of the ingredient)" (EU, 2003). Difficulties in understanding the classification of their product and compliance with labelling regulations mean that food business operators struggle with planning future steps, and this affects investor confidence (Wesseler *et al.*, 2023). There are challenges particularly with precision fermentation products, which may feature proteins that are bioidentical to those found in milk or eggs even though they have been produced without these products and without using animals. Further clarification for this category of FDFs would enable the use of labels that potentially attract consumer

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[65] Clean labels are simple labels, not featuring any codes or difficult names, intended to indicate to consumers that a food product is minimally processed and does not feature any artificial food additives. Examples include "natural" and "without artificial preservatives".

interest based on environmental benefits while ensuring that the contents of the food product can be understood from the perspective of allergenicity.

United States of America

The regulatory framework for FFs and FDFs in the United States of America differs greatly from that in the European Union. The United States of America assesses food products primarily on the basis of the qualities of the final product, rather than on the process. This is one of the key tenets of the Coordinated Framework for Regulation of Biotechnology (CF), established in 1986 to simplify oversight of biotechnology for which multiple agencies are relevant, as is the case with GEMs (USDA, FDA and EPA, 2015). The last update of the CF was in 2017 and clarifies the roles and areas of focus of different agencies, including the FDA, the United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA); it also reaffirms core tenets such as the need for regulations to be based on science and the sufficiency of existing statutes as the basis for the review of biotechnology products (USDA, FDA and EPA, 2017).

Although the United States of America does not have a specific definition for novel foods, most food business operators aim to obtain GRAS determination if they intend to introduce any innovative food products produced by or consisting of microorganisms, engineered or not, onto the market. This status can be obtained via two pathways: either the substance was used in food before 1958, and thus GRAS status can be granted on the basis of common use experience, or the status must be established through a scientific procedure (Shams *et al.*, 2024). In the case of engineered FDFs, the FDA Center for Food Safety and Applied Nutrition (CFSAN) is responsible for determining whether the product is safe or can be classed as GRAS.

Food business operators can also self-affirm GRAS determination independently of the FDA. They must provide data and convene a panel of experts to agree upon the safety status of the substance, for example a precision fermentation-derived ingredient. If the outcome is positive, the product can be marketed without the FDA’s prior approval. The FDA may at a later stage challenge the GRAS status of such substances or products after they are on the market. To avoid this, businesses can also voluntarily notify the FDA of their obtained GRAS claim, after which the agency can express an opinion on whether the reported data are sufficient to allow the safety status to be assigned (Gaynor, 2018). If satisfied, a “no questions” letter is issued.

In 2018, a “no questions” letter was published for precision fermentation-derived soy leghaemoglobin ingredient, which is used as a colourant in alternative protein products (FDA, 2018b). In 2020, a company developing precision fermentation-derived whey protein (β-lactoglobulin) was issued a “no questions” letter for its intended conditions of use (FDA, 2020). Since then, GRAS status has also been given for other precision fermentation-derived dairy proteins (FDA, 2022). In September 2021, another company obtained a “no questions” letter for its precision fermentation-based egg protein aimed for use as an ingredient in food and beverage products (FDA, 2021). Compared to the European Union framework, this process has several advantages for food manufacturers, mainly in terms of time and cost. The ability to enter the market after an independent GRAS determination provides a considerable time advantage and reduces the risk of bottlenecks caused by the saturation of an agency’s capacity to process applications.

In terms of labelling, instead of using GMO or non-GMO, the United States of America uses the term “bioengineered”. The USDA Agricultural Marketing Service (AMS) implements the National Bioengineered Food Disclosure Standard, which requires food manufacturers, importers and, in certain cases, retailers to disclose whether retailed food is bioengineered or uses bioengineered food ingredients (USDA, 2022). Bioengineered foods are defined as those that contain detectable genetic material that has been modified through recombinant DNA techniques, with modifications that could not have occurred naturally or been obtained through conventional breeding (Department of Agriculture and Agricultural Marketing Service, 2018). Different states within the country have additional policy on labelling, banning the use of terms such as “burger” or “sausage” that may be deemed to deceive consumers, and compelling the use of language such as “lab-grown” or “lab-created” (Granoff, 2024).

Countries where regulation is under development

In recent years, several countries have recognized the value of FDFs to their food security and bioeconomy, paving the way for the development of related regulatory frameworks. In 2022, the Food Safety and Standards Authority of India (FSSAI) updated its regulatory frameworks for the premarket approval of alternative proteins (Saha *et al.*, 2023). In the same year, it granted premarket approval to a company’s dairy alternative precision fermentation-derived and mycoprotein products (Perfect Day, 2022). In 2023, it did this for an algal protein powder produced utilizing biomass fermentation (GFI, 2024).

In China’s Fourteenth Five Year Plan, covering the years 2021 to 2025, alternative proteins, including egg analogues and recombinant proteins, were identified as areas of interest (BitsxBites, 2023). In November 2023, FAO and the China National Center for Food Safety Risk Assessment (CFSA) held a stakeholder roundtable meeting at which the latest developments in cell-based food and precision fermentation were discussed with the aim of collecting insights from developers and producers to inform food-safety considerations and improve risk assessments by competent authorities (FAO, 2023b).

In December 2023, the Brazilian Health Regulatory Agency (ANVISA) published the Resolution of the Board of Directors (RDC 839/2023), providing a regulatory framework for novel foods and ingredients, including those derived from precision fermentation (Souto Correa Advogados, 2023).

### 6.1.2 Trends in the development of regulatory frameworks

As highlighted in Section V.1, interest in FFs and FDFs has risen globally, resulting in efforts to establish regulatory frameworks that can allow innovative food products to enter the market while safeguarding consumer safety. Landmark approvals by Singapore and the United States of America have paved the way for FDFs in particular (GFI, 2023). Driven by the goal of enhancing food security via sustainable production of 30 percent of Singapore’s nutritional needs by 2030, the Singaporean Food Authority (SFA) developed the Novel Food Regulatory Framework in 2019, which has led to the country becoming a hub of novel food innovation (Singapore Food Agency, 2021). Notably, Singapore was the first country to allow the gas-fermentation based microbial protein Solein from Solar Foods onto market, a decision made in 2022 (Mäkinen, 2023).

Advocacy for changes in regulations related to food innovation is occurring in the European Union. In March 2023, a consortium of leaders in the precision-fermentation sector established Food Fermentation Europe (FFE, 2023). The group advocates for a risk-based regulatory approach that may allow a more predictable and efficient market approval process, and for an increase in the resources available to the EFSA. Globally, the Precision Fermentation Alliance is a trade organization established in February 2023 that similarly champions the implementation of such procedures for FDF products, with one of the three main goals being to “develop market access and the ability to operate and market products effectively by engaging with regulators” (Onego Bio, 2023).

### 6.1.3 Challenges associated with current regulatory frameworks and unmet needs

#### Consumer acceptance

As innovation drives advances in FF and FDF development and technical challenges are overcome, the fundamental bottleneck in the translation of this technology into an effective tool to improve the capacity, resilience and sustainability of the food system is consumer acceptance. Although fermentation is potentially pivotal as a means of reducing the negative externalities associated with protein production while ensuring that demand from the growing global population is met, its adoption and success hinge on consumers' trust in products, manufacturers and regulatory bodies (Onwezen *et al.*, 2021).

The way consumers evaluate new technologies such as precision fermentation affects the acceptance of food products and, ultimately, their success on the market. Many factors, including perceived benefits, technology neophobia and the framing of novel products, shape consumer attitudes (Szenderák, Fróna and Rákos, 2022). Regulation can play a significant role in this regard. Engagement between policymakers, industry and consumers can promote transparency and hence trust. Labelling, in particular, is frequently cited as a determinant of how consumers perceive novel foods (Edenbrandt and Lagerkvist, 2021). This is especially challenging for FDFs. While FFs may be embraced because of their perceived naturalness and cultural significance, the novelty, technological application and perceived lack of naturalness of processed foods means that FDFs are often viewed with negatively (National Academies of Sciences *et al.*, 2023). Clarity in labelling and definitions, for example with regard to what is deemed "organic", "natural", "GMO" and "non-GMO", and a sound scientific basis for decisions on this are crucial.

Beyond the perception of the safety and nutritional qualities of FFs and FDFs, the socioeconomic issues surrounding them are another source of mistrust. There are concerns about the impact alternative meat products, including FDFs, will have on livestock farmers, the potential for consolidation of food production under large corporations, and how relative price may affect inequality (IPES-Food, 2022). Policy related to the equitable integration of novel technologies and products into the food system is a pressing issue. A recent study of perceptions of cultured meat, including alternative proteins, in the United Kingdom farming sector revealed the complexity of how farmers may engage with the FDF sector, with an understanding of the potential opportunities associated with co-production accompanied by major concerns about the actors and business models currently driving the field and about how possible concentration of power within the food system could affect the development of rural communities and their access to land and safe and affordable food products (Manning *et al.*, 2023). Responsible innovation and a just transition require the development of effective policy and/or legal frameworks, which would greatly benefit from multistakeholder engagement and involvement.

With regard to equity in the development of the sector, the World Intellectual Property Organization approved a historic treaty on intellectual property, genetic resources and associated traditional knowledge on 24 May 2024 (WIPO, 2024). Essentially, where a claimed invention in a patent application is based on genetic resources, applicants will be required to disclose the source or country of origin of these genetic resources. Similarly, if it is based on traditional knowledge associated with genetic resources, applicants will be required by each contracting party to the treaty to disclose the Indigenous Peoples or local community who provided this knowledge.

**Need for definitions, clarity in classification and harmonization**

Although regulatory approaches differ from country to country, one common challenge food business operators face in obtaining premarket approval is a lack of clarity with regard to the classification of their food products. This affects the regulatory pathway that needs to be followed and the risk assessments that need to be undertaken, and overall causes significant time delays (FSA, 2021). A legal definition for precision fermentation in the European Union, for example, could possibly help to address these issues. An initial effort to enable a regulatory classification of GEMs beyond whether or not they are GMOs and thus to aid in the streamlining of the regulatory process has been made by ASTM International,[66] which developed an international voluntary consensus standard for classifying industrial microbes based on genotype, biosafety, use and available sequence information (Standard 3214-19) (Hanlon and Sewalt, 2021). Notably, the genotype classification field features four classes: a) microorganisms without intentional modification; b) microorganisms that were deliberately subject to genetic alteration, however, none that resulted in the introduction of non-native DNA; c)

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[66] https://www.astm.org

microorganisms modified with non-native DNA; and d) microorganisms engineered to produce chemical substances that are new to nature.

Lack of harmonization at both national and international levels has also been a hindrance. Developing international food-safety standards and aligning national regulations to them allow regulatory divergence and unnecessary trade barriers to be avoided. International standards are developed by the Codex Alimentarius Commission and are recognized by the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (Mukherjee *et al.*, 2022). Harmonization is also beneficial to consumer trust and to innovation in the food industry. Given the rapid development of FDF technologies and their applications, one aspect worth considering in the development of international standards is their adaptability to future advances.

## 6.2 Access and benefit-sharing frameworks

Fermentation-associated microorganisms have been receiving increasing attention on account of their potential roles in nutrition, food security and the preservation of cultural heritage and their commercialization potential as microbial protein and/or microbial cell factories. Access to the genetic resources of such organisms and associated traditional knowledge for research and development, as well as the sharing of resulting benefits, is therefore an issue of high relevance to various stakeholders ranging from the Indigenous Peoples and the local communities that have enabled the growth and domestication of the genetic resources of interest through traditional practices, to consumers, producers, private industry and academic institutions. Given the patentability of microorganisms in all major jurisdictions, there are concerns about the potential for resources once considered common heritage to be subject to exclusion and privatization, paralleling concerns in the plant genetic resources sector (Peschard and Randeria, 2020). The Convention on Biological Diversity and the Nagoya Protocol are global legal frameworks intended to promote access to genetic resources, including microorganism genetic resources, and the equitable sharing of benefits arising from their use (Secretariat of the Convention on Biodiversity, 2011a). This section provides a snapshot of discussions surrounding how fermentation-associated microorganisms fit into access and benefit-sharing frameworks and how this may affect different stakeholders.

### 6.2.1 Fermentation-associated microorganisms and traditional knowledge

Traditional knowledge is defined as “the knowledge, innovations and practices of indigenous and local communities related to genetic resources … developed through the experiences of communities over centuries, adapted to local needs, cultures and environments and passed down from generation to generation” (Secretariat of the Convention on Biodiversity, 2011b). The fermentation process behind traditional foods fits within this definition. The structure and members of fermentation-associated microbial communities have been found to be closely linked to the designed fermentation process and the selected substrate (Phiri *et al.*, 2020). Practitioners in this case act as selection agents for microbial strains that are pivotal in imbuing the final food product with its sensory and nutritional properties (Sõukand *et al.*, 2015; Ojeda-Linares, Solís-García and Casas, 2022). For example, a study of traditional dairying in Mongolia, part of the Heirloom Microbes project, uncovered the role of the specific fermentation vessels in promoting the growth of certain microbial species that define the final product (Gevin, 2020; Reichhardt and Abrahms-Kavunenko, 2022). Spontaneous fermentation in wine production has been found to be influenced by the microbiome associated with the grapes used, which thus contributes to the regional distinctiveness of the final product (Liu *et al.*, 2020; Bokulich *et al.*, 2016; Wei *et al.*, 2022). As such, in certain instances, fermentation-associated microorganisms and microbial communities fit within the Nagoya Protocol’s description of products of traditional knowledge and have particular territorial origins (Golan, 2022).

However, in many scenarios it can be difficult to determine the origin of microorganisms. For example, LAB present in foods are ready to be used and reproduced. It is common to culture these microorganisms from products such as yoghurt and cheese, improve on them and use them to produce a new product. Even without intentional modifications, genetic information can change through mutations. Because such products have moved across countries over time, with the line between providers and users of genetic resources becoming blurred, it may be impossible to trace the origin of the respective genetic resources back to a single country (Flach *et al.*, 2019; Parlindungan *et al.*, 2021).

Moreover, the biogeography of organisms such as plants and animals differs greatly from that of microorganisms. Some studies characterizing microbial communities in fermented foods sampled in different continents have found the composition of these communities to be correlated to substrate rather than geographical location (Wolfe *et al.*, 2014; Gänzle, 2022). A comprehensive study of how the microbial diversity of sourdough starters varies across and between continents (North America, Europe and Australasia) that involved collaboration with a community-scientist network of bread bakers found that geographical location did not determine sourdough microbial composition (Landis *et al.*, 2021). One explanation is the widespread movement of starters, as well as flour (a major source of microorganisms) through starter sharing and commercial distribution. Microorganisms are considered to be related to microniches, which can be similar in different regions of the world. As the Nagoya Protocol covers access to and use of genetic resources presumed to have developed because of specific features of geographical sites within national jurisdictions, there are questions concerning how well this framework is able to account for the ecology of microorganisms.

### 6.2.2 Impact of frameworks on conservation efforts

Microbial biodiversity conservation efforts are reliant on both individual and large-scale collaborative characterization studies that provide sequence data that can lead to insights into microbiome composition and functionality and provide the foundation for comparative studies (Louw *et al.*, 2023b). Sampling and characterization of microbiomes in biodiversity-rich countries have been challenging as a result of the unintended consequences of the Nagoya Protocol (Salem and Kaltenpoth, 2023). Variability in national policies, challenges in obtaining the information needed to perform due diligence and long waiting times can deter research efforts and leave genetic resources unidentified and underutilized (Flach *et al.*, 2019).

One of the three main goals of the Convention on Biological Diversity is the sustainable use of the components of biological diversity. The use of a particular microorganism at different scales, whether for academic research or for household or industrial production, does not deplete the resource (Sara *et al.*, 2022). A microorganism of interest does not need to be harvested from the environment once isolated. It can be stored long term and grown on nutrients as and when needed. Thus, the use of microorganisms is not a direct threat to their conservation or the ability of future generations to benefit from their use, rather it can promote their preservation and characterization. The major threats to fermentation-associated microbial diversity are those affecting the practice and valorization of traditional knowledge (Asogwa, Okoye and Oni, 2017; Rest, 2021; Sõukand *et al.*, 2015). There is a need for legal frameworks that facilitate collaborative conservation efforts that respect the spirit of the Nagoya Protocol without creating bottlenecks and allow the implementation of both *ex situ* and *in situ* methods, for example sampling and storage of strains and starter cultures in culture collections or mBRCs as well as efforts to conserve traditional knowledge through documentation, knowledge-sharing and capacity building.

### 6.2.3 Impact of frameworks on research and innovation

Since the implementation of the Nagoya Protocol, there have been discussions about how certain access and benefit-sharing regimes can create obstacles to collaboration, research and development, negatively affecting the potential sustainable use of genetic resources and the sharing of benefits (Prathapan *et al.*, 2018; Sara *et al.*, 2022). This affects both FFs and FDFs.

In the case of FDFs, one major concern is how the inclusion of digital sequence information in bilateral access and benefit-sharing measures may affect the work of both academia and private industry (Scholz *et al.*, 2023). Precision fermentation often relies on engineered microbial cell factories developed using synthetic biology techniques. This methodology sees the development of multi-origin constructs in which a combination of genetic sequences of different origins, potentially with different origin claims, is used to produce a single cell factory (Pascual *et al.*, 2021). Within the field of synthetic biology, standardization of the parts needed to express genes and build genetic circuits means that open-access databases of both material and immaterial parts are now available (McLaughlin *et al.*, 2018). These databases are fundamental to research in this field. Legal barriers to using the multiple parts needed for the development of engineered microorganisms are likely to slow development and hamper commercialization (Rourke, 2022).

There are also concerns from private industry about the complexity of commercial value chains for FDFs and the stage at which benefit-sharing is expected to occur (Michiels *et al.*, 2021). A key application of FDFs is in the production of ingredients that can be used in food and feed – potentially leading to an array of different commercial products. The stage at which benefit-sharing takes place can affect the magnitude of benefits shared (Halewood *et al.*, 2023). Complexity in terms of claims of origin surrounding the cell factories involved in FDF production and in terms of responsibility for benefit-sharing throughout the value chain can make implementation of bilateral ABS measures difficult.

### 6.2.4 Impact of frameworks on Indigenous Peoples and local communities

As FFs and FDFs are being explored for their potential roles in efforts to meet many of the challenges affecting the agrifood system, interest in the microorganisms and microbial communities present in traditional foods is increasing (Ramírez Rojas, Swidah and Schindler, 2022; Mendoza Salazar *et al.*, 2022). The rapid development of technological tools in the field of engineering biology has changed the ways in which parts and unique information pertaining to fermentation-associated microorganisms are utilized to make commercial products, which increasingly rely solely on digital sequence information (Akpoviri, Baharum and Zainol, 2023; Delgado, 2024). There are concerns about how robust access and benefit-sharing frameworks are in ensuring that the rights of Indigenous Peoples and local communities over these resources are preserved and that benefits are appropriately shared (Ambler *et al.*, 2021).

Traditional fermented foods play a vital role in local food systems (Ghosh, Meyer-Rochow and Jung, 2023). However, there is a decline in the use of low-cost fermentation-based processing methods because of factors such as urbanization, globalization, the labour intensiveness of the techniques, the amount of time required to perform them, and a lack of documentation and/or sharing of them (Vijayan *et al.*, 2022; Asogwa, Okoye and Oni, 2017). As research on FFs and the associated microbial communities grows, the study of traditional fermented foods can potentially be leveraged to revitalize traditional food-processing methods – with the possibility of improvements to the selection of microorganisms and to the standardization and safety of products. Partnerships and capacity-building efforts within the framework of access and benefit-sharing could be valuable in this context.

It is worth evaluating how the use of fermentation-associated microorganisms may impact the Indigenous Peoples and local communities from which genetic resources are obtained. As discussed above, different applications of fermentation technologies may have different levels of environmental impact, which may have downstream effects on vulnerable populations (Järviö *et al.*, 2021; Behm *et al.*, 2022; Sillman *et al.*, 2020; Spiller *et al.*, 2020; Humpenöder *et al.*, 2022). Although highly efficient in terms of land use, an FDF production system may have a high carbon footprint because of its high energy consumption. At the same time, the process may be an effective form of waste treatment, producing value-added products while limiting the leakage of contaminants into groundwater. The sustainability of FDF production, and precision fermentation in general, can be complex from an environmental perspective.

There are discussions about how different applications of fermentation-associated microorganisms may affect Indigenous Peoples and local communities economically and socially, and whether monetary and non-monetary benefit-sharing may outweigh the risks. Concerns have been raised over how the replacement of protein from livestock with FDFs may affect food sovereignty in the long term (La Via Campesina, 2022). The technology itself has the potential to be adopted by small-scale producers and act as an additional income source by valorizing wastes. However, market developments suggest that this is an industry that will feature increasing power concentrated in centralized production systems and large firms (IPES-Food, 2022). Additionally, the labour force required for FDF production would need skills that do not significantly overlap with those needed for current methods of producing animal-derived products. Although highly likely still to be dependent on agricultural inputs, FDF production at scale has the potential to disrupt rural communities as livestock production is reduced (Stephens *et al.*, 2018). How these communities and labour forces can be supported may be an important consideration in the context of benefit-sharing.

## 7. Recommendations

The following recommendations are intended to address current challenges in the conservation and sustainable use of fermentation-associated microorganisms in the agrifood sector.

- Frameworks for the provision of long-term financial support for culture collections and mBRCs need to be developed. One avenue would be to involve the private sector in the maintenance and funding of mBRCs given the sector's need for such facilities. Another would be funding via international organizations for centralized collections, with clear and simple pathways for accessing resources in accordance with international agreements. The presence of such collections and the human, financial and technical resources needed for the maintenance and periodic evaluation of the properties of strains, are key to the identification of powerful strains for both food and non-food applications.

- To improve accessibility to microorganisms and promote their valorization, capacity building is required in regions of the world with no history of investment in the necessary infrastructure and expertise, including investment in the equipment needed to preserve microbial samples, for example -80 °C freezers. Such efforts can promote the characterization of microbial communities behind local fermented foods, enabling the development of starter cultures and thus the realization of potential entrepreneurial opportunities. Beyond the food microbiome, such infrastructure can be used to preserve and characterize microbial strains from different environmental niches, which may have potential for use in the production of platform chemicals and high value-added compounds from agro-industrial materials.

- Documentation of traditional knowledge related to fermentation techniques and traditional FFs, as well as the revitalization of these practices, could benefit efforts to improve nutrition and food security, preserve cultural heritage and realize the economic potential of microbial strains with industrial relevance.

- Attention needs to be given to the issue of the ownership of microbial strains and intellectual property related to microorganisms. Equitable use and benefit-sharing are pivotal, and the rights of Indigenous Peoples and local communities over their microbial genetic resources must be safeguarded through frameworks appropriate to the nature and prevalence of microorganisms. To enable the identification and preservation of fermentation-associated microorganisms in places where the equipment and infrastructure required are not yet available, there is a need to design and implement frameworks that promote fair international research collaborations. Such collaborations can be hindered by unclear guidelines and lengthy bureaucratic procedures. A multilateral approach could be a potential solution, with a clear determination of how digital sequence information is to be classified, especially in the light of engineering-biology applications.

- Although FDFs and wider applications of precision fermentation hold great promise in enhancing agrifood system sustainability and resilience, a persistent challenge has been the low rate of successful commercialization of products and bioprocesses. Engineering biology has the potential to favour commercialization by improving yields, both for food and feed and for other products. Incentivizing research on FFs and FDFs and their development and regulation based on engineering biology can improve their economic viability. Globally, there is insufficient commercial fermentation capacity to meet forecast demand for FDF production. Current manufacturing capacity does not meet needs in terms of scale, technical capability or geographic distribution. Efforts to increase fermentation capacity and address shortages of infrastructure require support.

- Harmonization of definitions related to fermentation technologies such as precision fermentation could represent an initial step towards harmonized policies governing their use, including labelling. The latter has a substantial impact on consumer understanding of novel food products and their acceptance.

- Integration of fermentation into agricultural practice as a means of valorizing by-products and waste *in situ* is another promising avenue. In the context of farming, this would promote circular production systems and help shape how novel food processing and production technologies affect the food industry. For example, it could help address concerns over how FDF production may affect food sovereignty. Exploration of opportunities and frameworks for partnerships between agricultural and FF/FDF producers could further support this.

- Efforts are needed to promote and further research the roles of FFs as components of healthy diets, along with effective science communication and awareness raising on fermentation technologies, FFs and FDFs. Better understanding of the components of FFs that confer health benefits, along with appropriate quality control and certification procedures, would help ensure that consumers are well informed about the health and nutritional value of the wide array of products entering the market. New evidence emerging from microbiome science may provide the basis for further refinement of food-based dietary guidelines to cover the consumption of a range of FFs.

- To take full advantage of the potential of non-food product biomanufacturing from agro-industrial materials, especially as an alternative to petrochemical-based production, concerted efforts are needed to direct resources, including both public and private investments, towards compounds that hold the greatest potential to be economically feasible and sustainable to produce. Effective communication between industry and academia is needed to realize this. In addition, improvement in technology transfer terms at universities and public institutions could lower barriers to commercialization.

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