Trends in Biotechnology

CellPress OPEN ACCESS

Opinion

Food production from air: gas precision fermentation with hydrogen-oxidising bacteria

Margarita Bernal-Cabas [1,7,8], Karan Kumar [2,8,9], Owen Terpstra [1,7,8], Samira van den Bogaard [2,8,9], Aziz B. Ammar[2,8,9], Susanna Mäkinen [3,8,10], Lukas Herwig[4,8,11], Marta de Almeida[4,8,11], Petri Tervasmäki [3,8,10,*], Lars M. Blank [2,8,9], Tobias B. Alter [2,8,9,*], and Sonja Billerbeck [1,5,6,7,8,*]

The breach of six planetary boundaries highlights the need for sustainable food production. Aerobic hydrogen-oxidising bacteria (HOBs) convert atmospheric CO_2 and green hydrogen (H_2) into biomass via gas fermentation, a process already used for food-grade single-cell protein production. This approach enables a supply chain independent of agriculture, requiring minimal land and water, with potential for carbon-neutral production and carbon capture. To expand beyond single-cell protein, HOBs must be engineered into cell factories for precision fermentation. Advances in synthetic biology, metabolic engineering, computational modelling, and bioreactor design have accelerated the development of scalable bioprocesses providing a blueprint for gas-based fermentation. We present a path forward using secreted recombinant milk protein as a case study, highlighting key challenges and opportunities.

Highlights

Hydrogen-oxidising bacteria (HOBs) convert CO_2, hydrogen (H_2), and potentially nitrogen (N_2) into biomass via gas fermentation, promising to decouple food production from agriculture.

Industrial-scale gas fermentation has been shown to be feasible across a variety of microbial species and gases.

The development of engineered microbial strains for the precision fermentation of novel foods gains momentum due to maturing synthetic biology tools, pressing environmental concerns, and a consequential shift in consumer preferences.

Combining industrial-scale gas fermentation capabilities with state-of-the-art precision fermentation technologies enables the development of gas precision fermentation processes.

Product development and commercialisation would greatly benefit from focused generation of HOB tools and improved gas fermentation capacities in academia and industry.

Feasibility of industrial-scale gas fermentation suggests an alternative food chain decoupled from agriculture

The global population is projected to exceed 10.3 billion by 2080 [1], while six out of nine **planetary boundaries** (see Glossary) have already been breached [2]. Consequently, ensuring sufficient food supply for the world population while addressing environmental challenges has become critically important. Central to this challenge is meeting the rising demand for protein for human consumption. The global demand for protein is expected to grow by 40% by 2050 compared with present production levels [3].

Currently, almost all globally consumed protein is produced by traditional agriculture. This includes growing crops for plant-based protein supply and growing feed for livestock (Box 1). Traditional agriculture contributes 37% of global greenhouse gas emissions and places significant burden on the environment as vast amounts of **arable land** and fresh water are used [4,5].

Several companies and research consortia are already pioneering **industrial-scale fermentation** with hydrogen-oxidising bacteria (HOBs) for food production (Table 1). To mitigate this looming crisis, microbial fermentations using bacteria, yeast, or fungi have emerged as a more sustainable way for food production, especially protein (Box 1) [6]. Quorn® is an exemplary microbial protein product derived from the fungus *Fusarium venenatum*. It has been on the market for 40 years, indicating the economic feasibility of microbial protein substitutes [6]. The viability of the concept of producing foods via fermentation is further substantiated by the fact that microbial fermentations have delivered real-world products outside the food space for decades, such as

[1]Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, 9747 AG, The Netherlands
[2]Institute of Applied Microbiology – iAMB, Aachen Biology and Biotechnology – ABBt, RWTH Aachen University, Worringerweg 1, Aachen 52074, Germany
[3]Solar Foods Oyj, Härkälenkki 11, 01730 Vantaa, Finland

© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Box 1. Production schemes for alternative protein and their current reliance on arable land

Due to the growing demand for protein and due to planetary health challenges, protein sources have been diversified from a primarily livestock-based food chain to include three main additional categories of alternative protein:[iv] plant-based, microbe-derived, and cultured meat and seafood. Each alternative protein production scheme is designed – once fully optimised – to cause less burden on planetary health, when compared with conventionally produced protein. This box places precision gas fermentation into the context of other alternative protein production schemes and points out differences in terms of requirement for agriculture and thus the use of arable land.

Plant-based alternative protein is produced by directly processing high-protein plant material such as soybean, nuts, peas, or wheat. Products like tofu and tempeh have been consumed for centuries and plant-based products have the highest current market share within the alternative protein space with over 88% in 2023 [23]. The process relies on farming crops by agriculture, and hence plant-based alternative protein products require arable land.

Cultivated meat involves growing animal cells in fermenters to produce meat without raising animals. Animal cells require glucose or other highly reduced sugars as feedstock, which are currently derived from crops such as sugar cane or sugar beet. These crops are farmed and thus require arable land. Despite the development of such non-pharma-grade substrates, the cost of cell culture media for growing animal cells depicts the major bottleneck in the advancement of cultivated meat.

Fermentation-derived microbial protein involves growing microorganisms such as filamentous fungi, yeast, bacteria, and algae in large quantities in fermenters to produce protein-rich biomass also known as single-cell protein (biomass fermentation). Alternatively, microorganisms can be engineered to produce desired proteins or other functional ingredients (precision fermentation). Microbial protein has the second largest market share within the alternative protein space with over 8% [23].

Depending on the nutritional capabilities of the microbial cells, fermentation-based processes can use 1G to 3G feedstocks. For example, Mycoprotein like Quorn® is derived from fungal mycelium [78] cultured on 1G (sugar) and 2G (agricultural waste) feedstocks. Microbially derived dairy or egg replacements produced by precision fermentation of engineered yeast or bacteria currently also rely on 1G (sugar) and 2G (agricultural waste) feedstocks, which are derived from farming [4]. By contrast, Algae protein – the protein derived from the biomass fermentation of protein-rich microalgae such as *Spirulina* spp. and *Chlorella* spp. [79] – can be produced from the 3G feedstock CO_2 using light as the energy source. This is as algae are photosynthetic organisms. Thus, like precision gas fermentation, Algae protein does not rely on farming and arable land.

Given the complexity of each process, and the complexity of our food system, the different processes can be seen as complementary rather than competitive. Up-to-date information on each process and products is provided by the Good Food Institute.[iv]

[4]FGen AG, a subsidiary of Ginkgo Bioworks Holding Inc. (USA), Hochbergerstrasse 60C, 4057 Basel, Switzerland
[5]Department of Bioengineering, Imperial College London, South Kensington Campus, London, SW72AZ, UK
[6]Bezos Centre for Sustainable Protein, Imperial College London, London SW72AZ, UK
[7]www.billerbecklab.com
[8]www.hydrocow.eu
[9]www.iamb.rwth-aachen.de
[10]www.solarfoods.com
[11]www.ginkgo.bio

*Correspondence:
petri.tervasmaki@solarfoods.com (P. Tervasmäki),
tobias.alter@rwth-aachen.de (T.B. Alter), and
s.k.billerbeck@rug.nl (S. Billerbeck).

the bioproduction of insulin in bacteria and yeast [7,8] and the production of bioethanol [9], which exemplify two large-scale markets, currently valued at multi-million euros. Countless other food and non-food-related products – amino acids, enzymes for the food industry, antibiotics, vitamins, flavours, fats, antibodies, and material precursors, amongst others – have been produced via natural and metabolically engineered **microbial cell factories**, both in academia and industry [10].

The potential of these microbial fermentations to outperform or at least become on par with agriculture-based food production in terms of water use, land use, and greenhouse gas emissions strongly depends on the feedstock. Feedstocks have been classified into different categories based on the origin of their fermentable carbon source and named by their historic use [11,12]. Of note, the nitrogen source is historically not considered in the feedstock classification but has attracted increased attention in sustainability assessments [12]: first generation (1G) feedstocks refer to carbon in simple sugars (i.e., glucose) from food crops like sugarcane and corn. 1G feedstocks depend on agriculture, thus providing little sustainability advantage and even creating a direct competition with agricultural food production. Second generation (2G) feedstocks consist of carbon sources from lignocellulosic biomass like agricultural waste and forestry bioproducts and offer improved sustainability and circularity but arable land is still required [13–15].

Third generation (3G) or next generation feedstocks use carbon from non-arable sources such as seaweed biomass and include C_1 gases. By design, these feedstocks do not compete with food crops and do not depend on arable land. Gas-based 3G feedstocks include CO_2, methane (CH_4), and **syngas** [carbon monoxide (CO)/H_2 mix], amongst others. In combination with a

suitable energy source to generate reduction power (e.g., sunlight, hydrogen, electricity), these gases can be used by a variety of natural and engineered microorganisms to create biomass [16–18]. 3G feedstocks align exceptionally well with circular principles and can lead to net-zero or even carbon-negative processes [19,20].

Currently, most **industrial-scale microbial fermentations** for food and non-food applications rely on 1G and 2G feedstocks. Over the last century, historical and economic factors have driven key innovation in fermentation technology relying on 1G and 2G feedstock, ultimately enabling broad commercial scale-up [6,16]. In comparison, solving challenges intrinsic to the scaling of 3G gas-based microbial fermentations has gained less attention: mass transfer remains inefficient due to the poor solubility of gases, there are still safety challenges related to explosive gas mixtures (e.g., H_2/O_2 mixtures) [3,20,21], and the intrinsic slow C_1 assimilation into biomass by chemoautotrophic organisms results in slow growth rates [22].

However, supported by pressing sustainable development goals, and shifting consumer behaviour towards a positive attitude to alternative (microbial) proteins [23], several companies pioneered into the commercial gas fermentation space, demonstrating scalability across various gases and product types [17,20,24]. For example, the New Zealand-founded/US-based company LanzaTech has pioneered anaerobic syngas fermentations of non-food-related molecules at an industrial scale using the acetogenic bacteria *Clostridium autoethanogenum*, amongst others [25]. Bio-based ethanol and soon a wider range of products like acetone and isopropanol can directly substitute their petroleum-derived counterparts, serving as fuels and material precursors [19,26–28]. Further, the US-based company Calysta scaled aerobic CH_4-based fermentations to 400 000-liter scale for producing **single-cell protein** – a term used for the product of biomass fermentations where the edible and dried biomass from natural strains is the final product – commercialised as aquaculture feed.[i]

Most importantly for our focus on food production, in 2024, the Finnish company Solar Foods scaled up aerobic CO_2 and H_2-based gas fermentation to produce Solein®, a food-grade single-cell protein at 20 000-liter scale[ii] using the **hydrogen-oxidising bacterium (HOB)** *Xanthobacter sp.* SoF1, a new species isolated from the Baltic Sea [29]. Solein® has obtained self-affirmed Generally Recognized As Safe (GRAS) status in the USA, and novel food approval in Singapore.[iii]

As new 1G and 2G novel foods might reach the market soon, consumer acceptance will likely increase and help solve regulatory challenges, which in turn will help the market entry for gas precision fermentation products. While reaching consumer acceptance is still in its infancy, more studies become readily available that carefully evaluate consumer resistance and fears towards microbial foods, paving the way to address these concerns in the future [30,31].

A diverse agriculture-free food chain requires gas precision fermentation capabilities

This industrial-scale capacity for food-grade single-cell protein production for human consumption marks a significant step towards an agriculture-decoupled alternative food chain. However, to enable a resilient supply chain capable of delivering a variety of delicious and healthy foods, product diversification beyond single-cell protein is essential.

In the 1G and 2G feedstock space, precision fermentation technologies – a speciality term for microbial cell factories producing food ingredients – already deliver diversified microbial food products such as flavours, fats, vitamins, colorants, and proteins, projected to substitute for their agriculturally derived counterparts [6]. Numerous academic laboratories and many start-ups

Glossary

Accessory genes: a collection of genes found only in a subset of strains of a specific species.
Adaptive laboratory evolution (ALE): involves culturing microorganisms under controlled conditions and selecting for beneficial mutations that allow them to adapt to specific environments or improve desired traits.
Alternative protein: food products that replace animal proteins, including meat, egg, dairy, seafood, and eggs.
Arable land: land suitable for growing crops.
Autotrophic: organisms capable of synthesising their own organic compounds from inorganic carbon sources (such as CO_2), using either light or chemical energy.
Computational fluid dynamics (CFD) simulation: computer-aided numerical analysis for the simulation of fluid flows and their interaction with defined surfaces.
Core genes: the set of genes shared by all members of a given species or group of organisms.
Enzyme-constrained models: constraint-based metabolic models with included linear relationships between reaction rates and enzyme concentration required to sustain a metabolic flux.
Flux balance analysis: a computational technique used to analyse the flow of metabolites through a metabolic network, allowing the prediction of cellular behaviour to help optimise bioengineering strategies.
Genome-scale metabolic model: a computational representation of an organism's metabolic network, used to simulate and analyse cellular functions, metabolic pathways, and bioengineering strategies.
Green hydrogen: hydrogen produced through a renewable energy route, such as electrolysis powered by renewable energy, making it a sustainable alternative to fossil fuel-based hydrogen.
Heterotrophic: organisms that utilise organic carbon sources for growth and energy production.
High-throughput screening: a method for rapidly screening large populations of microbial strains expressing a product of interest.

explore proof-of-concepts and commercialisation [6]. Companies are particularly focused on dairy and egg protein production [4] as there is both a market and sustainability case: current milk protein consumption accounts for 12% of global protein consumption [32], while their production requires a disproportionate amount of arable land and consumes vast amounts of freshwater [33].

As of today, gas-based precision fermentation has not been extensively explored. However, critical technologies for both gas fermentation and cell engineering are getting mature enough to de-risk academic and industrial research and development towards carbon net-zero food production processes.

Specifically, decades of research in synthetic biology have yielded a robust foundation for engineering organisms and this knowledge can be adopted to successfully engineer non-model organisms that ferment gases, exemplified by LanzaTech's development of non-model acetogens into cell factories for fuels and protein based on synthetic biology principles [19,34].

As such, merging now-ready industrial-scale gas fermentation capabilities with state-of-the-art synthetic biology capacities creates momentum to develop gas precision fermentation processes for novel food ingredients. These could include scents, flavours, and vitamins, among others, whose production has been demonstrated in Gram-negatives before [35,36] and could be expanded to precision fermentation processes using engineered **hydrogen-oxidising bacteria (HOBs)**.

Here, we outline the development of a gas-based precision fermentation process using secreted milk protein from engineered HOBs, outlining reasons for choosing this example, substantiating feasibility and highlighting critical challenges and mitigation paths.

Engineering hydrogen-oxidising bacteria for gas precision fermentation of secreted milk protein

Milk (dairy) protein production is already explored within several start-ups in the 1G/2G precision fermentation space [4] and milk proteins have already been expressed in Gram-negative bacteria (Box 2). As such, there is knowledge on viable business cases and downstream processing. Although critical challenges need to be addressed, air-based production could yield a major sustainability advantage.

Opportunities

HOBs can grow on air: aerobic HOBs (also known as Knallgas bacteria) are an evolutionary diverse group of mostly Gram-negatives that assimilate CO_2 via the Calvin-Benson-Bassham cycle (Figure 1) and derive energy through soluble and membrane-bound hydrogenases that oxidise H_2 with O_2 as the final electron acceptor. This allows cultivating them in a bioreactor with a controlled supply of H_2, O_2, and CO_2. Of note, anaerobic HOBs exist [37] but will not be discussed here due to the technical challenges in anaerobic cultivations.

Protein derived from HOBs has been studied as a viable dietary protein replacement since the 1960s [38] and available life cycle analyses confirm that the environmental impact of protein production from HOBs is lower than for any of the plant- or animal-based reference products evaluated, especially when renewable energies are used for electrolysis of H_2O to hydrogen [39]. In addition, some HOB species are capable of fixing gaseous nitrogen via nitrogenases [37,40]. Although nitrogen fixation has not yet been widely explored, this capability in theory makes HOBs ideal candidates for fully **autotrophic** food production from gases available in 'air' alone: CO_2, N_2, and O_2.

Hydrogen-oxidising bacteria (HOBs): also known as Knallgas bacteria, a group of bacteria that utilise hydrogen gas as an energy source through oxidation (i.e., electron donor), typically using oxygen as the final electron acceptor.
Industrial-scale fermentation: large-scale production of bio-based products aiming for commercial viability.
Microbial cell factories: genetically engineered microorganisms designed to produce bio-based chemicals, proteins, or pharmaceuticals.
Overflow metabolism: a metabolic strategy where cells produce fermentative byproducts like acetate, lactate, or ethanol to regenerate reduction equivalents (RE), even when oxygen is available, and the more efficient oxidative phosphorylation could be used for RE recycling and energy generation.
Pangenome: a bacterial pangenome represents the complete genetic repertoire of a bacterial species or clade (or any user-defined group of genomes), encompassing all genes found across this group.
Planetary boundaries: a framework that describes the limits to the impacts of human activities on the Earth system. Beyond these limits, the environment may not be able to continue to self-regulate. The nine evaluated limits are change in climate, change in biosphere integrity, change in biogeochemical fluxes, ocean acidification, change in land use, change in freshwater fluxes, ozone depletion, atmospheric aerosols, and introduction of novel entities.
Single-cell protein: protein-rich biomass produced by microbial fermentation, used as an alternative protein source for food or feed.
Syngas: syngas, or synthesis gas, is a mixture of hydrogen (H_2) and carbon monoxide (CO) produced through the gasification of carbonaceous materials like biomass, coal, or waste, and is used as a fuel or to produce chemicals.
Technology readiness levels (TRLs): a measurement system used to assess the maturity of a technology, ranging from basic research to proven system operation.

Table 1. List of companies and research consortia actively developing food production platforms based on HOBs[a,b]

Name	Location	Creation	Product	Business model/funder	Organism	Link
Company						
UCDI (Utilization of Carbon Dioxide Institute Co., Ltd)	Japan	1970s	Protein, fuels, plastics, and others	B2B	N/A	v
Avecom	Belgium	1995	SCP for human and animal consumption	B2B	N/A	vi
LanzaTech	USA	2005	SCP for human consumption	B2B	*Cupriavidus necator*	vii
Unibio	Denmark	2011	UniProtein® SCP for aquaculture feed	B2B	N/A	viii
Solar Foods	Finland	2017	Solein®, SCP for human consumption	B2B	*Xanthobacter sp.*	ix
Novonutrients	USA	2017	SCP for human and animal consumption	B2B	N/A	x
Air Protein	USA	2019	SCP for human consumption	B2C	*Cupriavidus necator*	xi
AerBio	The Netherlands	2024	Proton™, SCP for animal consumption	B2B	N/A	xii
Joules	New Zealand	2021	SCP	B2B	N/A	xiii
Econutri	Austria	2021	SCP for human and animal consumption	B2B	*Cupriavidus necator*	xiv
Research consortia						
Development of innovative biomanufacturing technologies using CO_2 and H_2 as feedstocks for hydrogen-oxidising bacteria.	Japan	2023	Chemical products/alternative protein	Sojitz Corporation Central Research Institute of Electric Power Industry (CRIEPI) Green Earth Institute Co., Ltd. DIC Corporation Toray Industries, Inc. Daicel Corporation	N/A	xv
HYDROCOW – hydrogen-oxidising bacteria engineered to valorise CO_2 for whey protein production.	Europe	2023	Dairy protein	European Innovation Council	*Xanthobacter sp.*	xvi
HERO – protein using hydrogen-oxidising bacteria to convert CO_2 to protein.	Europe	2024	SCP and food proteins	Nordic Energy Research	*Rhodococcus opacus*	xvii
UNICO2RN	Europe	2025	Alternative protein	Circular Bio-based Europe Joint Undertaking (CBE-JU)	N/A	N/A

[a]Abbreviations: B2B, business to business; B2C, business to costumer; N/A, not available; SCP, single-cell protein.
[b]The list has been assembled based on an extensive online search but might be incomplete.

HOBs are genetically tractable: *Cupriavidus necator*, the most extensively studied HOB, has a wide range of genetic, genomic, and metabolic modelling tools (recently reviewed [41,42]) available and has been used to create academic proof-of-concept cell factories [42]. Other HOB species, especially those belonging to the *Xanthobacter* family, have available cloning toolkits, plasmid-based gene-expression systems [43], deletion tools [44], and genome sequences that are available [45]. Moreover, aerobic HOBs are **heterotrophic** and can be grown, maintained, and engineered with a variety of organic carbon sources [45], facilitating their use in standard microbiology laboratories. While further optimisation is needed to establish HOBs as robust cell factories, many species already show strong potential for genetic engineering and industrial biotechnology applications.

Product secretion avoids costly downstream processing: life cycle analysis and techno-economic analysis show that downstream processing has a huge impact on cost and sustainability [3]. Protein secretion inherently separates the target protein from biomass, which simplifies downstream processing towards a pure protein product.

A major challenge towards commercialisation

Product yield needs to be high enough for prototyping and commercialisation. Today, the entire **alternative protein** sector is facing significant challenges to cost-effectively scale-up production. This is critical to meet market needs in terms of volume, but also to offer competitive pricing [23]. A further challenge for microbial protein is achieving suitable nutritional and sensory profiles (colour, smell, and texture) that match their conventional counterparts [23].

This food processing challenge requires that food technologists have access to enough product early on during product development, to develop downstream processes and product formulations. Thus, sufficient yield at moderate scales is critical. Encouragingly, titres of milk protein obtained by microbial fermentation have developed a thousandfold over the past 30 years from

Box 2. State of the art: secretion of milk proteins from Gram-negative bacteria

Precision fermentation of milk proteins: cow milk is composed of a large set of post-translationally modified proteins (Figure I), each contributing nutritional, health-beneficial, and food textual value [4,80].

In precision fermentation, single milk proteins are either intracellularly expressed or secreted, with secretion requiring simpler downstream processing. The highest levels of β-lactoglobulin have been achieved in the eukaryotic fungus *Trichoderma reesei* [46].

Challenges in secreting milk proteins from Gram-negatives: secreting proteins in Gram-negative bacteria across the inner and outer membrane involves various sophisticated secretion systems. The general secretory (Sec) pathway, twin-arginine (Tat) pathway, type I, II, III, and V secretion systems in *Escherichia coli* have been widely used for heterologous secretion of proteins with reported yields of at least 6 g/l [60,81,82]. However, these systems are highly substrate-specific, requiring the correct protein folding in the periplasm or extracellular space, and have been the focus of intensive engineering efforts. Moreover, suitable signal peptides that guide a protein to the targeted secretion machinery need to be identified, as they can vary across species and substrates [83]. Proteins can either be transported across both membranes in a single step, for example, by type I and III secretion systems [82]. The type I secretion system has been used multiple times to transport heterologous protein, albeit with product concentration in the 100 mg/l range [84]. Alternatively, proteins can cross the membrane in a two-step transport. First, proteins cross the inner membrane from the cytosol to the periplasm either by the Sec or Tat pathway. Next, proteins are transported to the outer membrane by the type II or V secretion system [82]. In some cases, proteins can leak into the extracellular space after chemical or genetic outer-membrane destabilisation.

A second challenge lies in the post-translational modification of milk proteins, which is essential to their functional properties but difficult to engineer in prokaryotic systems [85]. Caseins contain 1–13 phosphorylation sites, with κ-casein containing 0–6 glycosylation sites [86] and 2 disulfide bonds [87]. α-lactalbumin contains four disulfide bonds and one phosphorylation site [88,89]. β-lactoglobulin only contains two disulfide bonds, making it the most promising for prokaryotic synthesis [85].

How to achieve milk protein secretion: the first step to achieve high secretion is to obtain high levels of intracellular expression. All major bovine milk proteins have been successfully expressed intracellularly in *E. coli*, with titres reported at 1 g/l and 10 g/l for αs1-casein and β-casein, respectively, however, without post-translational modifications [90,91]. To date there is only a single report of milk protein secretion from *E. coli* (αs1-casein) [92]. However, lessons can be drawn from work on high-level secretion of other eukaryotic proteins such as Vh anti-TNFR1 domain antibody (6 g/l), hirudin (3.1 g/l), [49] and anti-lysozyme Fab fragment (1.5 g/l) [49]. These examples used Sec periplasmic signal peptides followed by outer-membrane destabilisation and leakage. This strategy is particularly promising for the secretion of β-lactoglobulin, since disulfide bridges are formed in the periplasm. Sec periplasmic signal peptides and membrane leakage have also been used to secrete α-amylase at 236 mg/l in *Cupriavidus necator* under autotrophic growth. This shows that this strategy is compatible with HOBs [93]. As the Sec pathway is conserved across bacteria, this secretion strategy is likely applicable to other HOBs as well [94].

Alternatively, one-step secretion from the cytosol to the periplasm via Type I systems is another option. While Type I secretion systems are not conserved across species, known Type I secretion system/secretion peptide pairs have been heterologously expressed multiple times cross-species. However, Type I systems do not support disulfide bond formation, which might limit their utility for milk proteins with complex folding requirements [95–97].

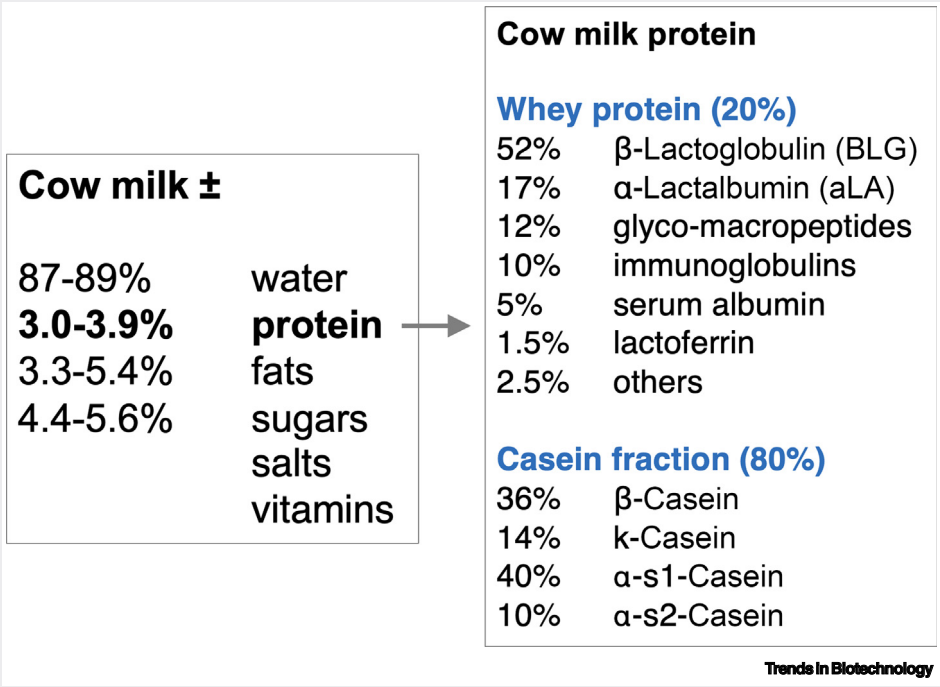


Figure I. Composition of cow milk [4].

0.001 g/l in 1990 to 1 g/l [4] – for comparison, bovine milk contains up to approximately 3 g/l beta-lactoglobulin (BLG). The reported current maximum titres for secretion of a milk protein of 1 g/l of BLG has been achieved in a eukaryotic host, the fungus *Trichoderma reesei* [46].

Secretion of authentic eukaryotic proteins from bacteria faces several challenges due to differences in eukaryotic and prokaryotic secretion machinery, folding, and post-translational modifications (Box 2) [47]. Nevertheless, secretion of some eukaryotic proteins showed comparable titres: for example, two antibody fragments (Vh anti-TNFR1 domain antibody and anti-lysozyme Fab fragment) have been secreted at 6 g/l [48] and 1.5 g/l [49], respectively and the blood anticoagulant hirudin has been secreted at 3.1 g/l [49], all by *Escherichia coli,* thus indicating that secretion of eukaryotic proteins by prokaryotes is possible.

Required research and innovation

Using synthetic biology's design-build-test-learn (DBTL) cycle for creating Gram-negative cell factories is a mature, well-proven strategy for achieving specific engineering goals, especially when enhanced by laboratory evolution and machine learning (a roadmap has been published [10]). However, critical research and innovation are needed to adapt the DBTL cycle to HOBs.

First, improved understanding of HOB physiology, metabolism, and stress responses is essential for rational cell factory design. For effective cell factory design, it is important to understand and predict an organism's behaviour under specific growth conditions [10]. While the model HOB *C. necator* has been partially studied and metabolically modelled to some extent [42]. significant gaps remain, especially for less studied HOB species such as *Xanthobacter* spp. Key insights into stress adaptation, carbon flux distribution, **overflow metabolism**, and redox balancing are still lacking, limiting our ability to fully engineer these organisms as efficient production hosts [50].

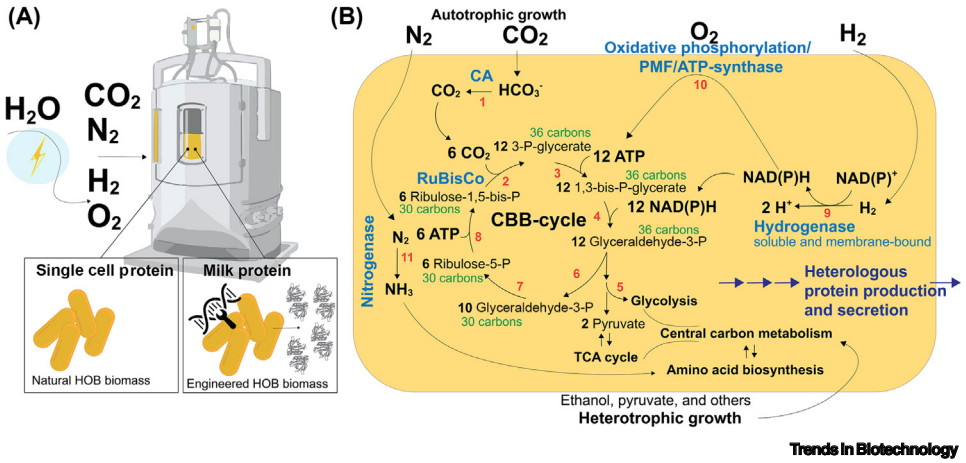


Figure 1. Food production by gas fermentation. (A) Overview of the production of food from carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2). Hydrogen is derived from water electrolysis. (B) Simplified overview of hydrogen-oxidising bacterium (HOB) metabolism showing a limited number of key steps (red) and key enzymes (blue) for autotrophic growth: CO_2 is fixed via the Calvin-Benson-Bassham (CBB) cycle, here exemplifying how six molecules of CO_2 can be fixed into two molecules of pyruvate (C_3 sugar), a central branching point towards central carbon metabolism and amino acid biosynthesis. 1. Carboanhydrase (CA) converts bicarbonate ($HCO3^-$) to gaseous CO_2, the substrate for ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo). 2. RuBisCo performs the central carbon fixing step by carboxylating the C_5 compound ribulose-1,5-bisphosphate to generate two C_3 compounds (3-phospho-glycerate). 3,4. Input of energy (ATP) and reducing power (NADPH) converts 3-phospho-glycerate to glyceraldehyde-3-phospate, a key glycolytic intermediate. 5,6. Part of the generated glyceraldehyde-3-phospate is used for biosynthesis, part for recycling the CO_2 acceptor ribulose-1,5-bisphosphate. 7. Various sugar re-arrangements lead to the creation of six C_5 compounds (ribulose-5-phosphate) per 10 C_3 compounds (glyceraldehyde-3-phosphate). 8. Energy input (ATP) allows recycling the CO_2 acceptor ribulose-1,5-bisphosphate. Hydrogen serves as an electron donor to reduce $NAD(P)^+$ to $NAD(P)H+H^+$, by a set of soluble and membrane-bound hydrogenases. ATP is generated via oxidative phosphorylation, using the concept of an electron transport chain with $NADPH+H^+$ as an electron donor. The resulting proton motif force (PMF) is used by ATP synthase to phosphorylate ADP to ATP. 11. N_2 is fixed (reduced) to ammonia (NH_3) by nitrogenases; NH_3 is then used for nitrogen assimilation, connecting central carbon metabolism with amino acid biosynthesis. Heterotrophic growth happens on various carbon feedstocks, depending on species. Abbreviation: TCA, tricarboxylic acid.

Affordable access to next generation sequencing and mass spectrometry has enabled a widespread generation of genomic, transcriptomic, and proteomics data. Systems biologists increasingly use this data to start unravelling complex biological processes across organisms and to predict biological outcomes during DBTL cycling [51]. However, these research investments are still missing for HOBs. We still need to understand how HOBs cope with the extra stress of making heterologous proteins, how results from heterotrophic cultures translate to autotrophic growth, how hydrogenase and nitrogen-fixation pathways shape redox balance, which secretion machinery works best, and which genes and regulatory tweaks are truly essential for high-yield protein production.

Second, effective genetic tools are needed to create cell factories that reach high levels of secreted heterologous proteins. Decades of synthetic biology have delivered a wealth of plasmid toolkits and clustered regularly interspaced short palindromic repeats (CRISPR)-based genome editing kits [52,53] for Gram-negatives that can be ordered off the shelf from plasmid repositories. However, it still takes time to test and adjust their performance in a new organism, especially as HOBs show slow growth rates, which significantly slows strain and process development. Automation and parallelisation in, for example, biofoundries [54] in combination with cell-free prototyping [55,56] could have a big impact on speeding up tool development. Once effective tools are developed these can be used to engineer protein-secreting HOB cell factories. High secretion yield depends on effective biomass accumulation over time, high intracellular protein expression, and

eventually successful secretion across both membranes to the extracellular space (Box 2). Several challenges need to be addressed to achieve this in HOBs. First, allocating resources to protein expression and secretion during autotrophic growth needs to be improved: autotrophic growth is highly energy consuming, and it is highly likely that HOBs do not actively secrete proteins (or very few) during this growth state. However, little research has been done on that question. While low endogenous protein secretion during autotrophic cultivation is an advantage for recovery of clean heterologous protein, strong metabolic engineering will likely be required to reallocate resources into heterologous protein expression and secretion. Metabolic models with incorporated resource allocation constraints (Box 3) and burden aware design principles [57] could guide this process, combined with available or newly developed strong promoters, to achieve high expression levels [43,58]. Stress response and chaperone analysis could guide the way to achieve expression of correctly folded protein [59,60]. Second, effective secretion to the extracellular space needs to be achieved: while protein secretion is well understood in other Gram-negatives (Box 2), very little is known about protein secretion in HOBs. Research is required to understand which secretion systems are functional in different HOB species and which secretion signal/secretion system pairs are promiscuous enough to allow high levels of translocation of recombinant proteins. Here, proteomics of the periplasm and the extracellular space, combined with predictive tools such as Signal P6.0 [61] or DeepSecc [62], could be used to identify signal peptides. Third, slow growth rate and hence slow biomass formation over time needs to be improved. This could be achieved by reducing

Box 3. Computational frameworks to assess and improve protein production in non-model microorganisms

A core element to a metabolic engineering success is a deep understanding of a species' metabolism and cellular processes. A systems-level view on metabolic processes is particularly valuable for recombinant protein production, where cellular resources must be efficiently directed toward synthesis of the target protein without compromising growth or viability. **Genome-scale metabolic models** are powerful computational frameworks for mapping the entire metabolic repertoire of an organism, bridging genomic information and biochemical reactions to predict how cells utilise substrates under different genetic or environmental constraints. Central to genome-scale models is the concept of **flux balance analysis** [98], in which steady-state metabolic flux distributions are optimised (e.g., for biomass formation), revealing potential bottlenecks or alternative pathways for biotechnological targets. Thus, genome-scale models have become instrumental for strain design in model and non-model microbes [99,100]. Taking heterologous protein expression as an example, flux balance analysis is able to reveal pathway-level flux shifts and metabolic bottlenecks that arise when the metabolic profile of recombinant proteins differs from that of the host's biomass. Yet, complex metabolic behaviours – like overflow metabolism or excessive byproduct formation – can remain elusive without additional mechanistic detail on cellular resource constraints.

Resource allocation models address this need by coupling metabolic reactions to essential cellular components – enzymes, ribosomes, energy, and more – thereby providing a resource-aware lens on metabolic function. Within resource allocation models, **enzyme-constrained models** [101] and specifically protein allocation models [102] incorporate enzyme turnover numbers and quantitative descriptions of coarse-grained protein sectors, such as translational protein, allowing simulation of cost–benefit trade-offs as cells allocate finite resources toward growth, maintenance, or the production of a desired product. Thus, protein allocation models enable the prediction of the metabolic burden accompanied phenotypic consequences caused by a diversion of native homeostatic protein towards heterologous protein expression. While uncovering the merits and advantages of heterologous protein secretion is an obvious application example, translation of the insights gained by resource allocation model simulations into strain and process designs for improved recombinant protein production has yet to be demonstrated.

Complementing both genome-scale models and resource allocation models with **pangenome** analysis broadens the scope from single-strain genomics to species-wide comparisons, capturing gene presence–absence variation across multiple isolates or closely related species [103]. By identifying **core genes** shared by all strains and **accessory genes** present only in subsets, pangenome analysis reveals untapped genetic diversity that can be harnessed to expand the metabolic toolkit for strain engineering [104]. When integrated with genome-scale metabolic models and resource allocation models, the insights from the pangenome analysis enable researchers to incorporate novel genes or pathways into computational models, predict their metabolic impact, and prioritise promising targets for high-throughput gene library construction [105]. Promising application examples include uncovering and transfer of protein secretion machinery across strains, as well as the identification of beneficial gene variants, for example, engineered hydrogenases [106] to optimise hydrogen fixation.

and streamlining the enzymatic repertoire using metabolic model-informed rational design or evolutionary adaptation (Box 3). **Adaptive laboratory evolution (ALE)** has proven effective at enhancing growth in other autotrophically growing organisms such as the acetogen *C. autoethanogenum* [63] and thus be a likely successful strategy. Close attention must be paid to ensure that strain selection occurs under conditions that closely mimic the final bioprocess (Box 4). This helps prevent unintended optimisation. Therefore, a rigorous scale-up strategy is essential to bridge the gap between lab-scale and industrial-scale conditions. By integrating the strategies, it becomes possible to generate libraries of robust strains that can be screened to identify candidates for a successful bioprocess.

Third, prototyping strategies are required for testing designs under relevant process conditions. Screening libraries of engineered mutant strains for desired yield requires testing under relevant process conditions. Several **high-throughput screening** capacities for protein-secreting cell factories have been developed and could be adopted to screen HOBs (Box 4). However, relevant gas fermentation conditions are difficult to implement during screening. Although gas fermentation is gaining traction in industry, safe and user-friendly small-scale gas fermentation capacities are limited as they cannot be bought off the shelf at an affordable cost, limiting broader adoption in academic settings and early-stage development settings.

Box 4. HOB screening campaigns to identify the best-performing food protein secretors from large libraries under process conditions

At the start of a HOB engineering campaign, low throughput microtiter plate-based screening methods, combined with fluorescence or colorimetric assays, can be used to identify promising candidates that secrete the food protein of interest. As development continues, higher-throughput screening methods are likely needed to evaluate genetic variants generated via random or combinatorial libraries to identify top performers that achieve target titres under industrially relevant conditions. A variety of screening platforms now exist to support such multi-dimensional search efforts [107]. However, identifying the best-performing food protein secretors during industrial production requires key screening features:

Compartmentalisation: for secretion-based screening, it is essential to compartmentalise each library member to link the secretion phenotype to the corresponding genotype. Microtiter plate assays support such compartmentalisation of library members in single wells and can it be scaled down to the microliter level by using commercially available 384- or 1536-well plates. Higher throughput can be reached by compartmentalising single cells in water-in-oil droplets (picolitre volume) [107], or encapsulating single cells in nanometre-scale alginate beads (nanolitre volume) [83,108]. This allows pooling up to millions of individual compartments in a small volume during screening. Other compartmentalisation strategies involve using the periplasmic space as a compartment [109], given it is the compartment where proteins secreted via the Sec and Tat machinery accumulate (Box 2).

Screening under process conditions: an effective screening campaign should follow the 'You get what you screen for' rule, meaning that an applied screen should closely reflect a desired result. In the context of HOB engineering, this implies (performing) selection under autotrophic (hydrogen-oxidising) conditions. Successful upscaling begins with effective downscaling by replicating critical process parameters such as gas exchange, nutrient limitations, pressure, and mixing at a microscale to ensure that screening results are translatable to industrial conditions. Thus, the pooled screening systems based on water-in-oil or alginate compartmentalisation could in theory be suitable for this challenge as pooled compartments can be incubated in large-scale gas fermenters during the screen. It is important, however, to think about the gas permeability of the compartment, and it remains to be tested if hydrogel-based 3D polymers or hydrophobic lipid-surrounded water-in-oil droplets perform better.

Read-outs for native food proteins: while in food-related processes, tagged protein expression is useful during early-stage screening to accelerate strain development, they pose regulatory and physicochemical limitations in the final product as regulatory requirements might demand unmodified products and tags might change the sensual or textual properties of a protein [110,111]. Commonly used protein screening tags, such as fluorescent protein tags or enzymatic tags, can negatively impact secretion levels and results obtained with tags do not necessarily correlate to the behaviour of untagged proteins. Therefore, alternative methods are required that avoid the use of tags. One such approach might consist of employing very small peptide tags or native labelling methods. Promising tools include co-culturing of whole-cell biosensors that can detect short peptide tags [112] or short tags that bind a fluorescent small molecule that can be added to the screen [113]. Additionally, native labelling could be achieved by using fluorescence-based derivatisation.

In the meantime, alternative strategies can be followed: given HOBs grow heterotrophically and most synthetic biology labs have capacity for small-scale heterotrophic cultivation (e.g., microtiter plates), metabolic modelling enhanced by multi-omics data (transcriptomics, proteomics, and metabolomics) from autotrophic and heterotrophic growth could help in identifying a heterotrophic condition under which the intracellular flux distribution resembles that of autotrophic growth as close as possible. In parallel, phenotypic differences in the performance of a specific genetic design under heterotrophic versus autotrophic conditions could be systematically analysed to develop predictive algorithms. Such tools could enable the prediction of design performance under gas fermentation based on data obtained from heterotrophic screenings.

Another option is using cell-free systems prepared from autotrophically grown HOBs for prototyping, as they can serve as proxies to autotrophic metabolic states. Cell-free prototyping has accelerated the design of cell factories for scaled syngas fermentations [55]. While such systems are well suited for evaluating high expression levels and metabolic activity, they are currently less suitable for assessing protein secretion [64].

Finally, while the engineering of HOBs lays the foundation for viable production processes, bioreactors and fermentation processes must be properly designed to facilitate optimal conditions for maximising production yields. As with any bioprocess, obtaining such optimal conditions at industrial scale presents a major challenge for gas precision fermentation. In large-scale fermentations, microorganisms are exposed to heterogeneous, dynamic environmental changes, known as lifelines, due to unavoidable spatial gradients of dissolved substrate concentrations at large bioreactor scale. These environmental fluctuations can lead to reduced microbial performance at industrial scale [65,66]. Uncovering and quantifying such lifelines in gas fermentation bioreactors using **computational fluid dynamics (CFD) simulations** and scale-down bioreactors is the subject of ongoing research [67,68] and will facilitate the optimisation of bioreactor designs and help in specifically improving the robustness of HOBs.

Additionally, bioreactor design and optimisation of gas fermentations with HOBs must address the inherently low solubility of gaseous substrates, particularly hydrogen, in water. Insufficient hydrogen transfer limits growth, requiring strategies to enhance gas-liquid mass transfer rates. One approach involves increasing the partial pressure of gases. This can be achieved by increasing the hydrostatic pressure in bioreactors [21], thereby improving gas solubility and boosting biomass and protein yields. Moreover, aerobic HOBs require hydrogen and oxygen, which can be highly explosive, requiring rigorous safety protocols and carefully controlled process conditions.

In summary, efficient gas fermentation processes demand specialised bioreactor designs [69]. One example in this regard is external-loop gas-lift reactors [68], which employ upward gas flow to promote mixing, reduce concentration gradients, and increase mass transfer rates to some extent. Comparative analysis of reactor technologies based on process-specific parameters is available for aerobic heterotrophic bioprocesses [70] and anaerobic syngas fermentations [69,71], but not yet for HOB-based processes. Balancing process efficiency, capital costs, and operational safety remains a central challenge in scaling up protein production using HOBs. Proper research, engineering, standardisation, and cost reduction in this field could minimise the hurdles for implementing gas fermentations technologies, not only at industrial scale, but also for laboratory settings.

Alternative routes to gas precision fermentation of food proteins

Given these challenges, exploring available parallel strategies for scaling 3G gas precision fermentation seems critical to deliver on the promise of a carbon-zero or carbon-negative food

chain. While HOBs have naturally evolved to use gaseous substrates, engineering model microorganisms (e.g., *E. coli* and several yeasts) to fix CO_2 [17,72–74] and other C compounds [75] through synthetic metabolisms offers an attractive alternative. This approach leverages the advantage of extensive genetic and system biology knowledge while combining an autotrophic lifestyle with robust protein production traits. However, this undertaking comes with its own challenges [76] as engineered model organisms will eventually face similar hurdles regarding prototyping under autotrophic conditions or achieving high protein yields at commercial scale.

An alternative concept involves a consolidated bioprocess, where HOBs create biomass – as already achieved at industrial scale – which is then used as a feedstock for precision fermentation using well-established bacterial and yeast cell factories. Beyond microbial and bioprocessing innovations, advances in *de novo* protein design [77] could be leveraged to create food proteins that mimic functional properties of animal-derived proteins while being simpler to express in microbial cell factories. However, consumer acceptance and regulatory challenges may hamper the introduction of such novel foods into the market.

Concluding remarks

HOBs and their engineered counterparts offer a carbon-negative, sustainable path towards protein production that circumvents the limitations of traditional agriculture. While edible single-cell protein is already on the market, we explore how the food supply chain could be diversified through gas precision fermentation with HOBs.

Precision fermentation has already yielded economically viable products, and it is increasingly viewed as a novel platform to produce nutritional and sensorially enhanced foods [6]. A next step is thus to merge recent advances in scaling gas fermentation capacities with knowledge in precision fermentation to work towards an agriculturally decoupled food system.

While we focus on outlining a specific case study and associated technical challenges, realising gas precision fermentation as a viable industry sector will require that multiple actors come together to develop a roadmap able to lift scientific innovation to high **technology readiness levels (TRLs)** (see Outstanding questions). Later we outline critical points that a roadmap should include.

First, continue integrating key digital capabilities. Specifically, artificial intelligence (AI) tools will play a key role in accelerating the DBTL cycle, and if employed early for HOB development, they might significantly reduce process development timelines. Applications range from metabolic modelling and multi-omics integration to real-time processes control, digital twin development, bioprocess optimisation, and the use of AI for genetic design optimisation and predictive upscaling, all of which can enable a faster and more robust upscaling. When combined with strategic developments in small-scale autotrophic testbeds, AI tools will likely significantly reduce cost and risk during development and increase the success rate of industrial deployment.

Second, beyond expanding capabilities for low TRLs, processes need to be scaled to industrial levels, integrated with upstream feedstock supply and downstream processing technologies, and designed for circularity in terms of waste and heat recovery. Collaboration between industry and academia funded by public–private partnerships will be critical as academic environments lack access to industrial-scale production facilities and sector-specific insights, while industry often cannot pursue the high-risk, high-reward research needed for transformative innovation. Access to critical infrastructure such as pilot plants and open innovation platforms will enable pre-commercial scale experimentation to help lower market entry barriers for start-ups. Scale-up will

Outstanding questions

Can food proteins be secreted by HOBs?

Can protein design help with creating food proteins that mimic functional properties of animal-derived proteins but are simpler to express in microbial cell factories?

Will HOBs be established as model organisms in academia and industry?

Can we effectively downscale process conditions to eventually upscale engineered processes?

Can bottlenecks in reactor design and reactor availability for safe and user-friendly academic use but also for broad scaling in the industrial sector be overcome?

Can HOB-based gas precision fermentation perform at scale and reach commercially relevant yields?

Will consumers and regulators be open to novel gas-fermented foods and which ethical considerations would arise?

also allow the production of enough material to integrate expertise in downstream processing and formulation, ultimately enabling the delivery of tasty foods. In terms of feedstock supply, key technologies and actors in the **green hydrogen** production and carbon (CO_2) capture sector need to be involved. Eventually life cycle analysis will need to be employed to model energy, water, and land use to benchmark each process against conventional agriculture, but also other alternative protein production schemes (Box 1).

Finally, early engagement with regulators on novel foods and biosafety policies will be critical to understand and address market barriers. Lean and effective regulatory processes are key for safe, but timely, introduction of products. Techno-economic analyses should assess feasibility, identify business models and early-adopter markets, and target commercially viable products. We envision that establishing robust supply chains for novel foods will spur growth of precision gas fermentation enterprises, supporting a full transition to sustainable food production.

Acknowledgments

This work was funded by the European Innovation Council (EIC) Pathfinder project HYDROCOW, Grant agreement ID: 101115118. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the European Innovation Council. Neither the European Union nor the granting authority can be held responsible for them.

Declaration of interests

All authors are part of the HYDROCOW consortium, developing precision fermentation technology for HOBs. Solar Foods Oyj is a company producing single-cell protein by hydrogen-oxidising bacteria, and P.T. and S.M. are employees of Solar Foods.

Resources

[i]https://calysta.com

[ii]https://investors.solarfoods.com/release/f84c2804-c99d-431d-bb34-22ec1079c274

[iii]https://solarfoods.com/solein/

[iv]https://gfi.org/defining-alternative-protein/

[v]https://www.co2.co.jp/en/top/

[vi]https://avecom.be/

[vii]https://lanzatech.com/

[viii]https://www.unibio.dk/

[ix]https://solarfoods.com/

[x]https://www.novonutrients.com/

[xi]https://www.airprotein.com/

[xii]https://aer.bio/

[xiii]https://www.jooules.com/

[xiv]https://econutri.com/

[xv]https://www.sojitz.com/news/news_file/file/230804e.pdf

[xvi]https://www.hydrocow.eu/

[xvii]https://www.nordforsk.org/projects/energy-efficient-and-resilient-food-production-system-using-hydrogen-oxidizing-bacteria

References

1. Lam, D. (2025) The next 2 billion: can the world support 10 billion people? *Popul. Dev. Rev.* 51, 63–102
2. Richardson, K. *et al.* (2023) Earth beyond six of nine planetary boundaries. *Sci. Adv.* 9, eadh2458
3. Ye, L. *et al.* (2024) Single-cell protein: overcoming technological and biological challenges towards improved industrialization. *Curr. Opin. Biotechnol.* 88, 103171
4. Nielsen, M.B. *et al.* (2024) The next food revolution is here: recombinant microbial production of milk and egg proteins by precision fermentation. *Annu. Rev. Food Sci. Technol.* 15, 173–187
5. Xu, X. *et al.* (2021) Global greenhouse gas emissions from animal-based foods are twice those of plant-based foods. *Nat. Food* 2, 724–732
6. Graham, A.E. and Ledesma-Amaro, R. (2023) The microbial food revolution. *Nat. Commun.* 14, 2231
7. Baeshen, N.A. *et al.* (2014) Cell factories for insulin production. *Microb. Cell Factories* 13, 141

8. Walsh, G. (2005) Therapeutic insulins and their large-scale manufacture. *Appl. Microbiol. Biotechnol.* 67, 151–159
9. Jain, S. and Kumar, S. (2024) A comprehensive review of bioethanol production from diverse feedstocks: current advancements and economic perspectives. *Energy* 296, 131130
10. Song, Y. and Prather, K.L.J. (2024) Strategies in engineering sustainable biochemical synthesis through microbial systems. *Curr. Opin. Chem. Biol.* 81, 102493
11. Liu, Z. *et al.* (2020) Third-generation biorefineries as the means to produce fuels and chemicals from CO2. *Nat. Catal.* 3, 274–288
12. Grossmann, L. (2024) Sustainable media feedstocks for cellular agriculture. *Biotechnol. Adv.* 73, 108367
13. Mustafa, A. *et al.* (2024) Converting lignocellulosic biomass into valuable end products for decentralized energy solutions: a comprehensive overview. *Sustain. Energy Technol. Assess.* 72, 104065
14. Wang, K. *et al.* (2024) The transition from 2G to 3G-feedstocks enabled efficient production of fuels and chemicals. *Green Energy Environ.* 9, 1759–1770
15. Ayodele, B.V. *et al.* (2020) An overview of integration opportunities for sustainable bioethanol production from first- and second-generation sugar-based feedstocks. *J. Clean. Prod.* 245, 118857
16. Choi, K.R. *et al.* (2024) From sustainable feedstocks to microbial foods. *Nat. Microbiol.* 9, 1167–1175
17. Jiang, W. *et al.* (2021) Metabolic engineering strategies to enable microbial utilization of C1 feedstocks. *Nat. Chem. Biol.* 17, 845–855
18. Dürre, P. and Eikmanns, B.J. (2015) C_1-carbon sources for chemical and fuel production by microbial gas fermentation. *Curr. Opin. Biotechnol.* 35, 63–72
19. Liew, F.E. *et al.* (2022) Carbon-negative production of acetone and isopropanol by gas fermentation at industrial pilot scale. *Nat. Biotechnol.* 40, 335–344
20. Dürre, P. (2016) Gas fermentation – a biotechnological solution for today's challenges. *Microb. Biotechnol.* 10, 14
21. Van Hecke, W. *et al.* (2019) Effects of moderately elevated pressure on gas fermentation processes. *Bioresour. Technol.* 293, 122129
22. Bar-Even, A. *et al.* (2012) Thermodynamic constraints shape the structure of carbon fixation pathways. *Biochim. Biophys. Acta BBA Bioenerg.* 1817, 1646–1659
23. Malila, Y. *et al.* (2024) Current challenges of alternative proteins as future foods. *Npj Sci. Food* 8, 53
24. Heijstra, B.D. *et al.* (2017) Gas fermentation: cellular engineering possibilities and scale up. *Microb. Cell Factories* 16, 60
25. Pavan, M. *et al.* (2022) Advances in systems metabolic engineering of autotrophic carbon oxide-fixing biocatalysts towards a circular economy. *Metab. Eng.* 71, 117–141
26. Li, Y.P. *et al.* (2024) Recent advances and challenges in single cell protein (SCP) technologies for food and feed production. *Npj Sci. Food* 8, 66
27. Liew, F. *et al.* (2016) Gas fermentation—a flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. *Front. Microbiol.* 7, 694
28. Watson, E. (2024) Gas fermentation co LanzaTech enters food arena: 'We've developed a path to mass producing protein from CO2'. *AgFunderNews* [Online]. Available:. https://agfundernews.com/gas-fermentation-co-lanzatech-enters-food-arena-weve-developed-a-path-to-mass-producing-protein-from-co2, Accessed date: 3 May 2025
29. Holmström, S. and Pitkänen, J.-P. (2023) Strains and processes for single cell protein or biomass production, EP3816293B1
30. Kühl, S. *et al.* (2024) Beyond the cow: consumer perceptions and information impact on acceptance of precision fermentation-produced cheese in Germany. *Future Foods* 10, 100411
31. Agency, F.S. (2025) A rapid evidence review on consumer responses to precision fermentation. *FSA Res. Evid.* 1–55. https://doi.org/10.46756/001c.136898
32. Smith, N.W. *et al.* (2022) Modeling the contribution of milk to global nutrition. *Front. Nutr.* 8, 716100
33. Behm, K. *et al.* (2022) Comparison of carbon footprint and water scarcity footprint of milk protein produced by cellular agriculture and the dairy industry. *Int. J. Life Cycle Assess.* 27, 1017–1034
34. Simpson, S. *et al.* (2020) Gas fermentation for the production of protein or feed, US10856560B2
35. Dunstan, M.S. *et al.* (2020) Engineering *Escherichia coli* towards de novo production of gatekeeper (2S)-flavanones: naringenin, pinocembrin, eriodictyol and homoeriodictyol. *Synth. Biol.* 5, ysaa012
36. Carroll, A.L. *et al.* (2016) Microbial production of scent and flavor compounds. *Curr. Opin. Biotechnol.* 37, 8–15
37. Angenent, S.C. *et al.* (2022) Hydrogen oxidizing bacteria as novel protein source for human consumption: an overview. *Open Microbiol. J.* 16, 1–14 e187428582207270
38. Waslien, C.I. *et al.* (1969) Human intolerance to bacteria as food. *Nature* 221, 84–85
39. Järviö, N. *et al.* (2021) An attributional life cycle assessment of microbial protein production: a case study on using hydrogen-oxidizing bacteria. *Sci. Total Environ.* 776, 145764
40. Li, R. *et al.* (2025) Hydrogen-oxidizing bacteria: a promising contributor to environmental sustainability and resource recycling. *Green Carbon* https://doi.org/10.1016/j.greenca.2025.02.004
41. Morlino, M.S. *et al.* (2023) *Cupriavidus necator* as a platform for polyhydroxyalkanoate production: an overview of strains, metabolism, and modeling approaches. *Biotechnol. Adv.* 69, 108264
42. Weldon, M. and Euler, C. (2025) Physiology-informed use of *Cupriavidus necator* in biomanufacturing: a review of advances and challenges. *Microb. Cell Factories* 24, 30
43. Soltysiak, M.P.M. *et al.* (2025) XanthoMoClo—a robust modular cloning genetic toolkit for the genera *Xanthobacter* and *Roseixanthobacter*. *ACS Synth. Biol.* 14, 1173–1190
44. Jämsä, T. *et al.* (2023) Inactivation of poly(3-hydroxybutyrate) (PHB) biosynthesis in 'Knallgas' bacterium *Xanthobacter* sp. SoF1. *AMB Express* 13, 75
45. Soltysiak, M.P.M. *et al.* (2024) Expansion and revision of the genus *Xanthobacter* and proposal of *Roseixanthobacter* gen. nov. *bioRxiv* 2024.07.11.603134
46. Aro, N. *et al.* (2023) Production of bovine beta-lactoglobulin and hen egg ovalbumin by *Trichoderma reesei* using precision fermentation technology and testing of their techno-functional properties. *Food Res. Int. Ott. Ont* 163, 112131
47. Rettenbacher, L.A. *et al.* (2022) Microbial protein cell factories fight back? *Trends Biotechnol.* 40, 576–590
48. Voulgaris, I. *et al.* (2015) Enhancing the selective extracellular location of a recombinant *E. coli* domain antibody by management of fermentation conditions. *Appl. Microbiol. Biotechnol.* 99, 8441–8453
49. Dassler, T. *et al.* (2010) Process for the production of proteins by fermentation, EP1903105B1
50. Montaño López, J. *et al.* (2022) Physiological limitations and opportunities in microbial metabolic engineering. *Nat. Rev. Microbiol.* 20, 35–48
51. Gurdo, N. *et al.* (2023) Automating the design-build-test-learn cycle towards next-generation bacterial cell factories. *New Biotechnol.* 74, 1–15
52. Krink, N. *et al.* (2024) A Hitchhiker's guide to CRISPR editing tools in bacteria. *EMBO Rep.* 25, 1694–1699
53. Bird, J.E. *et al.* (2022) A user's guide to Golden Gate cloning methods and standards. *ACS Synth. Biol.* 11, 3551–3563
54. Hillson, N. *et al.* (2019) Building a global alliance of biofoundries. *Nat. Commun.* 10, 2040
55. Vögeli, B. *et al.* (2022) Cell-free prototyping enables implementation of optimized reverse β-oxidation pathways in heterotrophic and autotrophic bacteria. *Nat. Commun.* 13, 3058
56. Jeung, K. *et al.* (2025) Cell-free systems: a synthetic biology tool for rapid prototyping in metabolic engineering. *Biotechnol. Adv.* 79, 108522
57. Grob, A. *et al.* (2021) Experimental tools to reduce the burden of bacterial synthetic biology. *Curr. Opin. Syst. Biol.* 28, 100393
58. Blázquez, B. *et al.* (2023) Golden Standard: a complete standard, portable, and interoperative MoClo tool for model and non-model proteobacteria. *Nucleic Acids Res.* 51, e98
59. Eicholt, L.A. *et al.* (2022) Heterologous expression of naturally evolved putative de novo proteins with chaperones. *Protein Sci. Publ. Protein Soc.* 31, e4371
60. Kleiner-Grote, G.R.M. *et al.* (2018) Secretion of recombinant proteins from *E. coli*. *Eng. Life Sci.* 18, 532–550
61. Teufel, F. *et al.* (2022) SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nat. Biotechnol.* 40, 1023–1025

62. Zhang, Y. *et al.* (2023) DeepSecE: a deep-learning-based framework for multiclass prediction of secreted proteins in Gram-negative bacteria. *Research* 6, 0258
63. Ingelman, H. *et al.* (2024) Autotrophic adaptive laboratory evolution of the acetogen *Clostridium autoethanogenum* delivers the gas-fermenting strain LAbrini with superior growth, products, and robustness. *New Biotechnol.* 83, 1–15
64. Yue, K. *et al.* (2023) Advancing synthetic biology through cell-free protein synthesis. *Comput. Struct. Biotechnol. J.* 21, 2899
65. Mu, X. and Zhang, F. (2023) Diverse mechanisms of bioproduction heterogeneity in fermentation and their control strategies. *J. Ind. Microbiol. Biotechnol.* 50, kuad033
66. Nadal-Rey, G. *et al.* (2021) Understanding gradients in industrial bioreactors. *Biotechnol. Adv.* 46, 107660
67. Puiman, L. *et al.* (2023) Downscaling industrial-scale syngas fermentation to simulate frequent and irregular dissolved gas concentration shocks. *Bioengineering* 10, 518
68. Puiman, L. *et al.* (2024) High-resolution computation predicts that low dissolved CO concentrations and CO gradients promote ethanol production at industrial-scale gas fermentation. *Biochem. Eng. J.* 207, 109330
69. Puiman, L. *et al.* (2025) Dos and don'ts for scaling up gas fermentations. *Curr. Opin. Biotechnol.* 93, 103294
70. Humbird, D. *et al.* (2017) Aeration costs in stirred-tank and bubble column bioreactors. *Biochem. Eng. J.* 127, 161–166
71. Bokelmann, C. *et al.* (2025) Pros and cons of airlift and bubble column bioreactors: how internals improve performance. *Biochem. Eng. J.* 213, 109539
72. Antonovsky, N. *et al.* (2016) Sugar synthesis from CO2 in *Escherichia coli*. *Cell* 166, 115–125
73. Satanowski, A. *et al.* (2025) Design and implementation of aerobic and ambient CO2-reduction as an entry-point for enhanced carbon fixation. *Nat. Commun.* 16, 3134
74. Luo, S. *et al.* (2023) Construction and modular implementation of the THETA cycle for synthetic CO2 fixation. *Nat. Catal.* 6, 1228–1240
75. Orsi, E. *et al.* (2023) Synergistic investigation of natural and synthetic C1-trophic microorganisms to foster a circular carbon economy. *Nat. Commun.* 14, 6673
76. Puiggené, Ò. *et al.* (2025) Seven critical challenges in synthetic one-carbon assimilation and their potential solutions. *FEMS Microbiol. Rev.* 49, fuaf011
77. Kortemme, T. (2024) De novo protein design—from new structures to programmable functions. *Cell* 187, 526–544
78. Linder, T. (2024) What next for mycoprotein? *Curr. Opin. Food Sci.* 58, 101199
79. Espinosa-Ramírez, J. *et al.* (2023) Algae as a potential source of protein meat alternatives. *Front. Nutr.* 10, 1254300
80. Deng, M. *et al.* (2023) Cell factory-based milk protein biomanufacturing: advances and perspectives. *Int. J. Biol. Macromol.* 244, 125335
81. Hui, X. *et al.* (2021) Computational prediction of secreted proteins in Gram-negative bacteria. *Comput. Struct. Biotechnol. J.* 19, 1806–1828
82. Burdette, L.A. *et al.* (2018) Developing Gram-negative bacteria for the secretion of heterologous proteins. *Microb. Cell Factories* 17, 196
83. Grasso, S. *et al.* (2023) Signal peptide efficiency: from high-throughput data to prediction and explanation. *ACS Synth. Biol.* 12, 390–404
84. Pourhassan, N., .Z. *et al.* (2021) Biotechnological applications of type 1 secretion systems. *Biotechnol. Adv.* 53, 107864
85. Hettinga, K. and Bijl, E. (2022) Can recombinant milk proteins replace those produced by animals? *Curr. Opin. Biotechnol.* 75, 102690
86. Bijl, E. *et al.* (2020) Chapter 5 - Posttranslational modifications of caseins. In *Milk Proteins* (Third Edition) (Boland, M. and Singh, H., eds), pp. 173–211, Academic Press
87. Holland, J.W. *et al.* (2008) Analysis of disulphide linkages in bovine kappa-casein oligomers using two-dimensional electrophoresis. *Electrophoresis* 29, 2402–2410
88. Vanaman, T.C. *et al.* (1970) The disulfide bonds of bovine alpha-lactalbumin. *J. Biol. Chem.* 245, 4583–4590
89. Thesbjerg, M.N. *et al.* (2022) Differences in post-translational modifications of proteins in milk from early and mid-lactation dairy cows as studied using total ion chromatograms from LC-ESI/MS. *Int. Dairy J.* 130, 105262
90. Wang, Y. *et al.* (2021) Bioconversion of lignocellulosic 'waste' to high-value food proteins: recombinant production of bovine and human αS1-casein based on wheat straw lignocellulose. *GCB Bioenergy* 13, 640–655
91. Gangnard, S. *et al.* (2007) Modifications of the charges at the N-terminus of bovine β-casein: consequences on its structure and its micellisation. *Food Hydrocoll.* 21, 180–190
92. Goda, S.K. *et al.* (2000) Recombinant expression analysis of natural and synthetic bovine alpha-casein in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 54, 671–676
93. Tang, R. *et al.* (2025) Production of α-amylase from gluconate and carbon dioxide by protein synthesis and secretion optimization in *Cupriavidus necator* H16. *Bioresour. Technol.* 416, 131744
94. Cranford-Smith, T. and Huber, D. (2018) The way is the goal: how SecA transports proteins across the cytoplasmic membrane in bacteria. *FEMS Microbiol. Lett.* 365, fny093
95. Ryu, J. *et al.* (2015) A vector system for ABC transporter-mediated secretion and purification of recombinant proteins in *Pseudomonas* species. *Appl. Environ. Microbiol.* 81, 1744–1753
96. Angkawidjaja, C. *et al.* (2006) Extracellular secretion of *Escherichia coli* alkaline phosphatase with a C-terminal tag by type I secretion system: purification and biochemical characterization. *Protein Eng. Des. Sel.* 19, 337–343
97. Palacios, J.L. *et al.* (2001) Subset of hybrid eukaryotic proteins is exported by the type I secretion system of *Erwinia chrysanthemi*. *J. Bacteriol.* 183, 1346–1358
98. Orth, J.D. *et al.* (2010) What is flux balance analysis? *Nat. Biotechnol.* 28, 245–248
99. Zielinski, D.C. *et al.* (2020) The expanding computational toolbox for engineering microbial phenotypes at the genome scale. *Microorganisms* 8, 2050
100. Kim, G.B. *et al.* (2025) Comprehensive evaluation of the capacities of microbial cell factories. *Nat. Commun.* 16, 2869
101. Sánchez, B.J. *et al.* (2017) Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints. *Mol. Syst. Biol.* 13, 935
102. Alter, T.B. *et al.* (2021) Proteome regulation patterns determine *Escherichia coli* wild-type and mutant phenotypes. *mSystems* 6. https://doi.org/10.1128/msystems.00625-20
103. Matthews, C.A. *et al.* (2024) A gentle introduction to pangenomics. *Brief. Bioinform.* 25, bbae588
104. Kumar, K. *et al.* (2024) Genomic insights into clostridia in bioenergy production: comparison of metabolic capabilities and evolutionary relationships. *Biotechnol. Bioeng.* 121, 1297–1312
105. Gu, C. *et al.* (2019) Current status and applications of genome-scale metabolic models. *Genome Biol.* 20, 121
106. Patra, A.K. *et al.* (2022) A regulatory hydrogenase gene cluster observed in the thioautotrophic symbiont of *Bathymodiolus* mussel in the East Pacific Rise. *Sci. Rep.* 12, 22232
107. Bozkurt, E.U. *et al.* (2025) Accelerating enzyme discovery and engineering with high-throughput screening. *Nat. Prod. Rep.* https://doi.org/10.1039/D4NP00031E
108. Meyer, A. *et al.* (2015) Optimization of a whole-cell biocatalyst by employing genetically encoded product sensors inside nanolitre reactors. *Nat. Chem.* 7, 673–678
109. Osgerby, A. and Overton, T.W. (2023) Approaches for high-throughput quantification of periplasmic recombinant proteins. *New Biotechnol.* 77, 149–160
110. Regulation (EU) 2015/2283 on novel foods. *OJ L 327*, 11 Dec 2015, 1–22 [Online]. Available: https://eur-lex.europa.eu/eli/reg/2015/2283/oj/eng [Accessed: 03-May-2025]
111. *Guidance for Industry: Q6B—Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*, FDA Issued August 18, 1999; Content reviewed as current as of August 28, 2025
112. Matragrano, J. *et al.* (2023) A multiplex GPCR-mediated peptide tagging system for a growing yeast synthetic biology toolbox. *Helv. Chim. Acta* 106, e202300094
113. Adams, S.R. and Tsien, R.Y. (2008) Preparation of the membrane-permeant biarsenicals, FlAsH-EDT2 and ReAsH-EDT2 for fluorescent labeling of tetracysteine-tagged proteins. *Nat. Protoc.* 3, 1527–1534