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A sustainable waste-to-protein system to maximise waste resource utilisation for developing food- and feed-grade protein solutions†

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A waste-to-protein system that integrates a range of waste-to-protein upgrading technologies has the potential to converge innovations on zero-waste and protein security to ensure a sustainable protein future. We present a global overview of food-safe and feed-safe waste resource potential and technologies to sort and transform such waste streams with compositional quality characteristics into food-grade or feed-grade protein. The identified streams are rich in carbon and nutrients and absent of pathogens and hazardous contaminants, including food waste streams, lignocellulosic waste from agricultural residues and forestry, and contaminant-free waste from the food and drink industry. A wide range of chemical, physical, and biological treatments can be applied to extract nutrients and convert waste-carbon to fermentable sugars or other platform chemicals for subsequent conversion to protein. Our quantitative analyses suggest that the waste-to-protein system has the potential to maximise recovery of various low-value resources and catalyse the transformative solutions toward a sustainable protein future. However, novel protein regulation processes remain expensive and resource intensive in many countries, with protracted timelines for approval. This poses a significant barrier to market expansion, despite accelerated research and development in waste-to-protein technologies and novel protein sources. Thus, the waste-to-protein system is an important initiative to promote metabolic health across lifespans and tackle the global hunger crisis.

Introduction

Despite continuous efforts to achieve the goal of 'zero hunger' Sustainable Development Goals (SDG), the global undernourished population is projected to increase from 688 million to 841 million by 2030.[1] A major contributor to this forecast is the occurrence of war and disruptive political situations, and failure to distribute economically accessible food to the poorest societies on our planet. In addition, increasing strains on food security are exacerbated by the unsustainable reliance

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§Equivalent contributions.

on finite natural capital resources such as land and water, that are required for traditional farming techniques. Animal-sourced protein is a highly resource-intensive and nutritionally inefficient method of food production based on nitrogen utilisation yet constitutes 18% of the current global protein supply.[2–4] Indeed, the projected increase in demand for meat protein (to almost double by 2050) poses significant environmental concerns, particularly in relation to land and water availability and greenhouse gas emissions.[5–8] The Covid-19 pandemic has threatened global food supply chains at multiple levels, causing interruptions to the planting, harvesting, and transportation of crops.[9–11] Such interruptions exacerbate the issue of food security with the worst post-pandemic scenario estimated to produce 909 million people with undernutrition by 2030,[12–14] highlighting the need for a secure yet sustainable food production system.

Rising food waste presents as an abundant resource for alternative protein solutions.[15–18] It is estimated that one-third of food produced globally is underutilised for reasons related to logistics of supply and demand. This trend is evident in both developed regions with overnutrition and less developed countries with increasing rates of undernutrition, and is equivalent to 1.3 billion tonnes of wasted food which provides sufficient resources to feed 2 billion people worldwide.[15] Globally, considerable amounts of carbon-containing and nutrient-rich waste are generated from the food and drink sector. For instance, in the UK, 1.5 million tonnes of waste is created from the production of meat, dairy, fruits, vegetables, starch products, beverages, brewing by-products, and other food products.[19,20]

This review focuses on the contaminant-free organic component of three broad waste streams that can be converted to food-grade or animal feed-grade protein through sustainable protein production technologies. We consider (i) food waste streams present in organic fraction of municipal solid waste (OFMSW); (ii) lignocellulosic waste, which is defined here as the lignocellulosic agricultural residues from crop cultivation (*e.g.* straw) as well as forestry waste (*e.g.* wood chips); and (iii) food industry waste in the form of organic gas, liquid, and solid streams generated from processing and manufacturing within the food and drink sector. These waste streams offer considerable potential for resource recovery and protein production due to the high concentrations of nutrients, degradable organic compounds and the absence of pathogens, toxic metals, and other hazardous contaminants.

Non-organic wastes have been investigated for a 'power to protein' approach;[21,22] however, here we explore a range of sustainable technologies to extract or convert nutrients and organic compounds present in contaminant-free waste to produce food- or feed-grade protein. Utilisation of microbial biotechnologies such as fermentation can achieve yields of approximately 40% cell biomass from dry waste matter.[23] At least 80 species have been reported to produce microbial protein, but a better understanding of the microbes involved and their potential for protein recovery from waste is needed.[24] Higher organisms such as insects can also be used

as bio-converters within a waste-to-protein system. These higher organisms typically attain a maximum upgrading efficiency of only 10% but can also yield biomass components of significant functional value. Additionally, biochemical and physical treatments can be used to recover extra nutrients from waste streams, upgrade waste-to-protein systems, or convert waste-carbon to fermentable sugars and other platform chemicals for subsequent conversion to protein. Despite the advances in individual technologies, critical gaps remain in the development of innovative systems that integrate these technologies for optimised protein recovery from diverse waste streams.

We define a 'waste-to-protein system' as a collection of pathways using process technologies to recover food-grade and/or feed-grade protein from contamination-free organic waste resources. Accordingly, 'waste-to-protein' refers to the proteins derived or produced from non-contaminated food-safe or feed-safe organic materials exhibiting compositional quality suitable for valuable upgrading. Food-grade and feed-grade proteins have differing requirements with regards to feedstock quality (food-safe *vs.* animal feed-safe, respectively), and must comply with hygienic quality and safety standards set by regulators which vary significantly by country.[25]
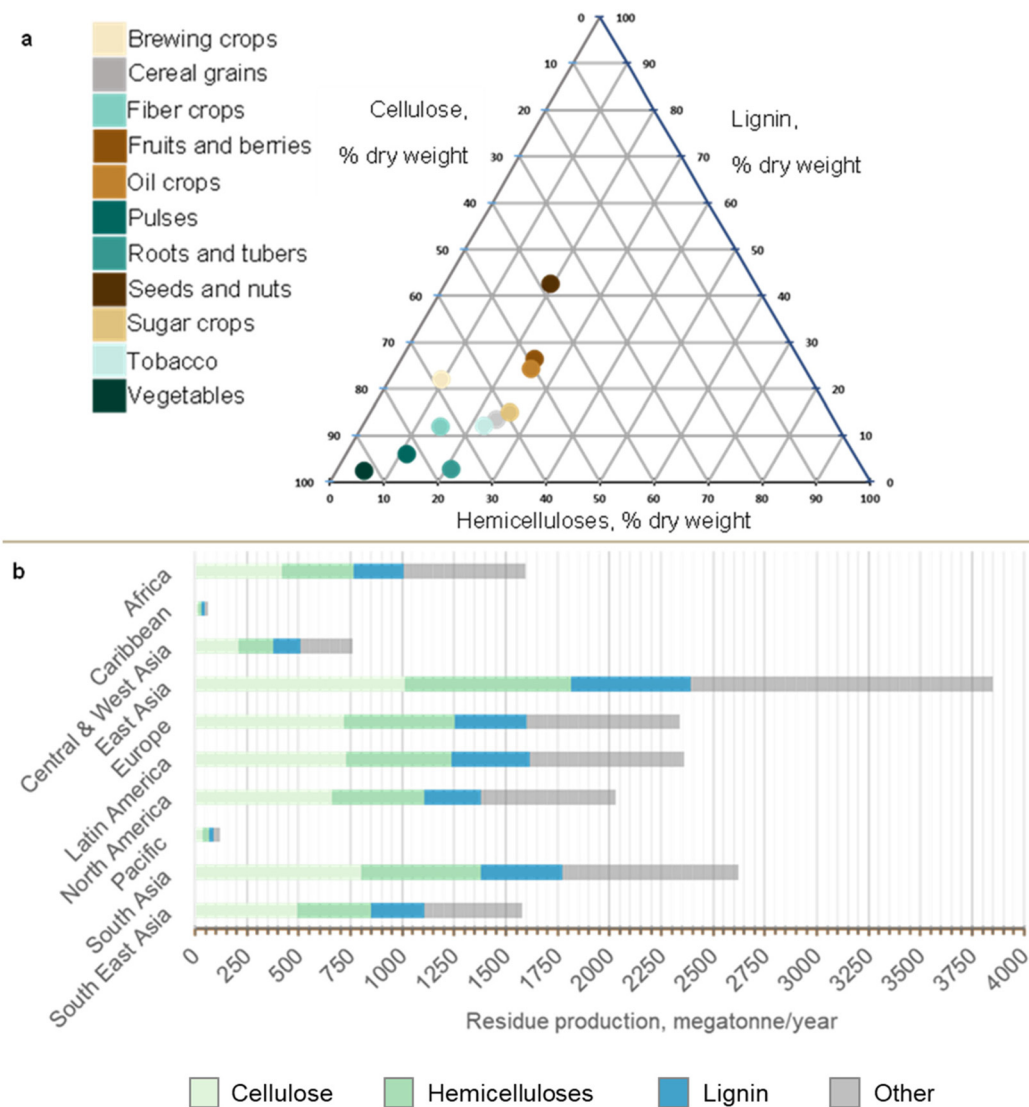
The primary aim of this article is to provide an overview of the strategies and pathways with the potential to transform globally abundant contaminant-free waste into a sustainable 'waste-to-protein system' to achieve global protein security and contribute to a circular-economy aspiration.[26,27] To achieve this, we critically evaluate the viability of food-safe and feed-safe waste streams as 'waste-to-protein' resources, with an emphasis on their abundance and biochemical composition. We then appraise the technologies available for waste-to-protein conversion, focusing on three promising, evidence-based pathways: biochemical and physical treatment, microbial protein, and insects as bio-converters. Finally, we propose a sustainable 'waste-to-protein' system that maximises waste resource utilisation for the development of food-grade and feed-grade protein solutions to promote global food security and ameliorate the hunger pandemic.

Waste-to-protein sources

Feed-grade organic fraction of municipal solid waste

Annual global household waste generation is equivalent to 2.01 billion tonnes of municipal solid waste (MSW). The organic fraction of municipal solid waste (OFMSW) accounts for around 40% of global MSW generated each year, presenting as an abundant source of feed-grade organic waste for a waste-to-protein system.[28,29] It is an overly abundant resource for high-income countries, and a valuable nutrient resource for low-income countries due to its macronutrient profile.[30] Fig. 1 illustrates the rate of MSW generation by country, as well as the regional composition. Rates of generation range from 4.94 kg per capita per day (Antigua and Barbuda) to 0.14 kg

Fig. 1 Global production of Municipal Solid Waste (MSW). (a) Average MSW generation (kg per capita per day) was calculated for each country using data from literature[15,30,38,39] where MSW generation was plotted according to a colour gradient scale ranging from low (minimum 0.14 kg per capita per day) to high (maximum 4.93 kg per capita per day). (b) Regional OFMSW composition and average lipid, carbohydrate and protein contents (g per capita per day) were calculated from previously reported values.[15,40] Detailed data can be found in ESI-1 and ESI Table ST1.†

per capita per day (Nepal). While higher quantities of MSW are produced by high-income countries (Fig. 1a), low-income countries tend to generate a larger organic fraction (food and garden waste) compared to high-income nations (Fig. 1b). On average, 184 g of OFMSW is generated per capita per day with crude protein content ranging from 4.35 g per capita per day (South Asia) to 31 g per capita per day (Caribbean). MSW is projected to increase by 70% in developing countries, and a marked increase in MSW generation has been observed in areas with rapid urbanisation.[15,31] Developing regions such as Africa and South East Asia also account for 91.8% of world-wide undernourishment, highlighting the urgent need to explore new protein solutions, *e.g.* waste-to-protein, to meet increasing nutrient and protein demands in these areas.[1]

Safety of feed-grade organic fraction of municipal solid waste

Crops may accumulate antibiotic resistant genes (ARGs) from organic fertiliser (*e.g.* manure) applied to the soil, potentially contaminating sources of OFMSW.[32,33] Furthermore, OFMSW sourced from mixed domestic waste may be further contaminated due to direct contact with other ARG- and pathogen-rich wastes.[32] Pre-treatment of OFMSW prior to protein valorisation is therefore imperative to mitigate health effects posed by such contaminants. Ozonation is commonly used to treat waste-water containing ARGs and has been applied to solid wastes in previous works.[34–37] However, it requires tightly controlled conditions that are highly dependent on solid waste feedstock properties, such as pH, water content, particle size.[35]
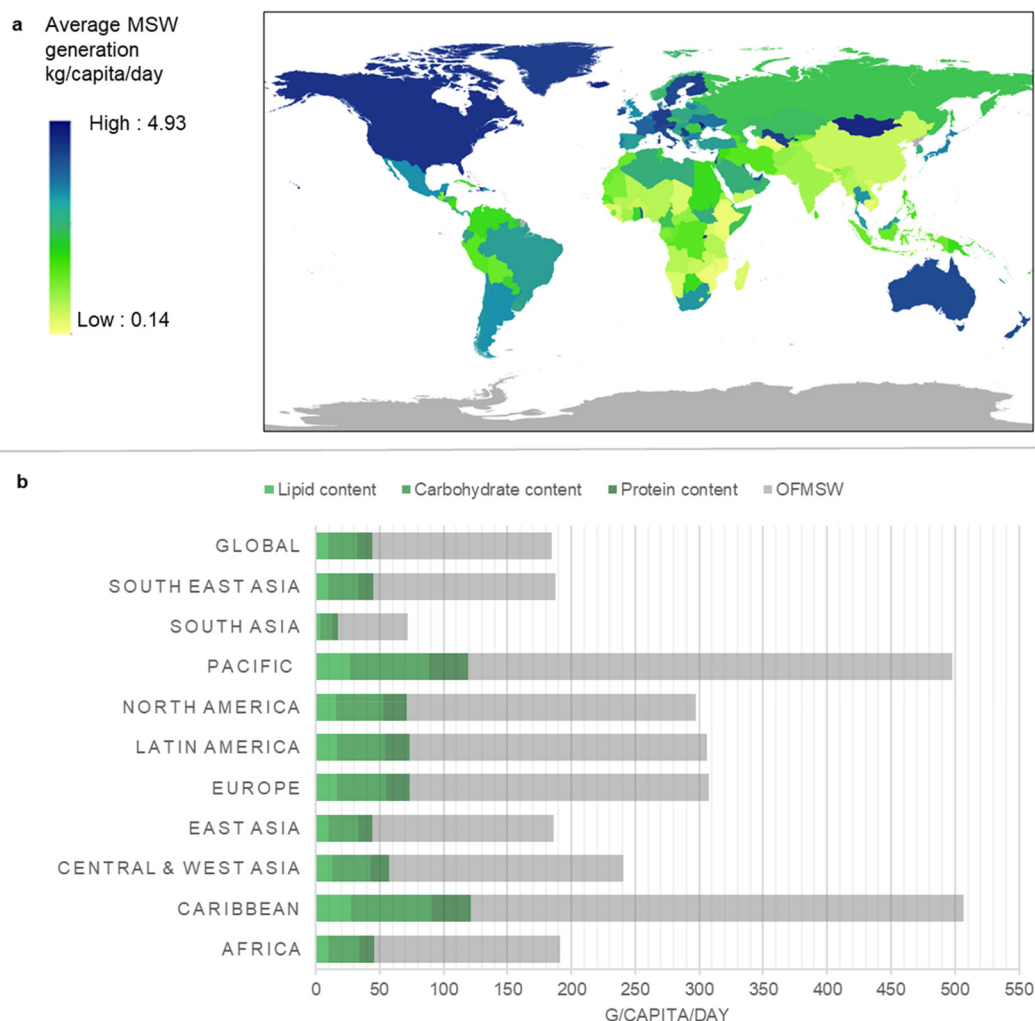
Furthermore, the impact on protein quality resulting from ozonation pre-treatment of OFMSW requires further investigation to assess the potential for integration into a waste-to-protein valorisation process system. On the other hand, thermal treatments (*e.g.* microwaves[41]) and high-pressure processing technologies[42,43] have been reported to destroy pathogens through disruption of cell wall structure, while simultaneously increasing protein and sugar solubility.[44] However, ARG reduction potential of such technologies is less understood.

Lignocellulosic waste

Agricultural residues. Lignocellulosic waste from agriculture is a globally distributed, carbon-rich, non-contaminated and food-safe resource presenting as a potential candidate for the recovery of nutritionally valuable protein.[11] Although different countries and regions exhibit varying production rates of agricultural crops, all countries generate lignocellulosic waste in the form of agricultural residues.[45,46] In this review, we define agricultural crops as terrestrial plants cultivated on a large scale including cereal grains, fruits, vegetables, oil crops, and sugar crops. We assessed the potential carbon and nutritional values of food-grade lignocellulosic wastes from agriculture sector by examining the biochemical composition of non-edible parts of crops, *i.e.* agricultural residues (Fig. 2). Crude protein content often constitutes less than 8% of agricultural residues. However, sustainable technologies could be deployed to convert the lignocellulosic component to protein. For example, microbial strains capable of metabolising lignocellulosic feedstock could be used to produce food-grade or feed-grade protein.

Fig. 2a presents the lignocellulosic contents of the main agricultural product residues, ranging from 34% to 60% for

Fig. 2 Biochemical analysis of agricultural lignocellulosic residues. Agricultural products were categorised as: brewing crops; cereal grains; fibre crops; fodder; fruits and berries; oil crops; pulses; roots and tubers; seeds and nuts; sugar crops; tobacco; and vegetables. (a) Biochemical composition of lignocellulosic component of agricultural product residues based on the Phyllis database.[47] Values are given as a % of dry weight. (b) Regional lignocellulosic production rate and its biochemical composition as part of the total agricultural residue production. Residue production was estimated by applying residue production ratios to production values for 2018 for each region.[47,48] Detailed data can be found in ESI-2 and ESI Table ST2.†

lignin, 15% to 43% for cellulose and 17% to 36% for hemicelluloses. We focus on cellulose, hemicelluloses and lignin but acknowledge that other cell wall components (*e.g.* pectins) and intracellular components (*e.g.* oligosaccharides and starch) warrant future exploratory research.

Geographical variations in climate and soil conditions contribute to regional differences in production rate and biochemical composition of agricultural residues. East Asia is the largest global producer of lignocellulosic agricultural residues (2389 megatonnes per year), which constitutes approximately 62% of the total residue production. In comparison, the Caribbean agricultural sector generates only 44 megatonnes per year of lignocellulosic residues, constituting 68% of its total residue production. Overall, total residue production is higher in South and Southeast Asia. However, other regions including both high-income and low-income countries also show abundant agricultural residue production, highlighting global potential for lignocellulosic conversion of crop residues to protein (Fig. 2b).

Forestry residue. Forestry residue is another lignocellulosic waste source.[45,49,50] Global forest resources amount to 600 066 megatonnes per year and comprise of above- and below-ground biomass, plus 67 000 megatonnes per year of deadwood. The global distribution and analyses of forestry biomass and corresponding residue biomass can be found in published databases.[51] Residues generated by forest management, harvesting and processing (particularly in regions with active forestry industries such as Canada and parts of Latin America, and from areas employing tree-cutting for wildfire prevention) could provide substantial lignocellulosic feedstock for a waste-to-protein process system.[52,53] The fact that upgrading of lignocellulosic content from forestry residues to human food or animal feed has not taken any dimensions of scale relates to aspects of logistics and particularly cost competitiveness of the products. Furthermore, protein derivation from forestry waste for human consumption is particularly problematic, as forestry land can have significant contamination *e.g.* those used for phytoremediation.

Safety of lignocellulosic waste

Based on safety concerns, pre-treatment may be required to remove harmful toxins prior to protein or carbohydrate extraction processes. Technologies such as pressurised liquid extraction (PLE), supercritical fluid extraction (SFE) and subcritical water extraction have shown promising effectiveness at reducing the content of organic pesticides and heavy metals contained in lignocellulosic waste streams.[54–58] However, they are rarely employed for industrial processing due to their high cost.[57,58] Furthermore, due to the high purity requirements required for human and animal consumption, intense research efforts are required to optimise pre-treatment to achieve maximum contaminant reduction while mitigating deleterious chemical alterations of feedstock compounds, which can significantly reduce downstream efficiency and yield of protein extraction and bioconversion processes.[56] Combined contaminant remediation and protein value-

upgrading is a promising approach that integrates process stages through multi-objective bioconversion. For example, one strategy is the use of fungal strains capable of simultaneously degrading pollutants/contaminants while assimilating lignocellulose into biomass through subsequent saccharification and fermentation (SSF).[59–63] However, the efficacy of this approach is highly dependent on the feedstock composition, process conditions and the strain type employed. Furthermore, due to the high content of chemically stable lignin, a co-culture containing a lignin-degrading species, such as white rot fungi species, may be required to maximise feedstock extraction efficiency and reduce downstream separation burden, at the expense of increased bioremediation process complexity, due to difference in optimal growth conditions of microbial strains.

Food and drink industry waste

Quantifying food industry waste production is challenging, due to its complex nature and enormous scale. We have therefore selected quantifiable waste streams of two industries (shrimp fishing, and brewer's spent grain) as examples to show the potential of food industry waste within a waste-to-protein system.

Shrimp waste

The shrimp fishing industry is a good target for waste-to-protein resource recovery, being well-established in Africa and South East Asia and generating 6–8 megatonnes per year of protein-richorganic waste (40% protein) during the processing phase.[64] Shrimp waste also contains chitin, which constitutes 20–30% of its biomass. Chitin can be converted to water-soluble chitosan, a value-added polysaccharide with a range of functional properties and industrial applications (*e.g.* drug delivery, food thickening and stabilising).[65,66] Combined recovery of protein and value-added polysaccharides such as chitosan has the potential to improve the economics and sustainability of waste-to-protein system processes.

Brewer's spent grain

The most abundant by-product generated by the brewing industry is brewer's spent grain (BSG), which offers great potential for protein recovery due to its protein and carbon-rich chemical composition.[67] The major component of BSG tissues are the cell walls consisting primarily of non-starch polysaccharides (NSP), some of which are lignified.[68] The NSP include cellulose and non-cellulosic polysaccharides ('hemicelluloses'), particularly arabinoxylans which constitute 25–52% of BSG composition. BSG also has high protein contents, comprising 15–31% of its composition.[69,70] Research efforts have focussed on existing chemical processes (*e.g.* solvent pre-treatment followed by enzymatic hydrolysis) to fractionate the protein components and convert NSP to fermentable sugars for microbial protein production.[71,72] However, optimised routes to integration of BSG into the conventional feed and food supply chains using novel processing methods remains as an outstanding research gap.

Safety of food and drink industry waste

Despite the relative lack of chemical contaminants (for example heavy metals), waste streams from the food and drink sector are highly susceptible to contamination through growth of potentially pathogenic microbes.[73] Employment of controlled pasteurisation at sufficiently high temperatures before processing is therefore used to prevent contamination of downstream products. Integration of continuous toxicological and pathogen testing of feedstock pre- and post-processing should also be employed to assure food/feed safety and for adequate quality control.[73] However, research and development of novel pasteurisation technologies such as high-pressure processing is required, as current high temperature processes have been shown to impact sensory and functional properties of valorised protein.[43,74]

Sustainable protein production technologies

Promising technologies presenting sustainable methods of protein recovery include: (i) biochemical, chemical, and physical treatments, (ii) bioconverters (microbial protein and insects).

Biochemical, chemical and physical treatments

A wide range of biochemical, chemical, or physical treatments can be applied to contaminant-free organic waste streams to extract valuable proteins, produce protein hydrolysates with favourable functionality, palatability and reduced allergenicity, or to transform carbohydrates to sugars as feedstock for bio-conversion technologies.[75,76]

Protein extraction and purification technologies

Membrane filtration (*e.g.* ultrafiltration, reverse osmosis) and precipitation (*e.g.* isoelectric precipitation, salting out, organic solvent methods) and adsorption technologies offer great advantages as cost-effective techniques for continuous protein extraction from waste feedstock. The advantages and drawbacks of these technologies with regards to process operation and product safety/nutrition are summarised in Table 1.

Membrane filtration. Membrane filtration has been well-established as a physical treatment to mitigate nutrient concentration and carbon oxygen demand (COD) of industrial effluents, as in the dairy industry to recover value-added caseins and whey proteins from wastewater.[77] Such methods have demonstrated high efficiency, for example Das *et al.* (2015) were able to achieve 90% protein recovery from whey waste using combined ultrafiltration and nanofiltration.[78] Filtration methods are also low in energy consumption and protein denaturation but are challenged by performance issues such as membrane fouling caused by particle deposition and coagulation of charged proteins at the membrane surface. This issue has been observed in various studies, including tuna and dairy wastewater processing, as well as commercially, for example during production trials of flavour enhancer Mycoscent (Quorn), a concentrate containing glutamate and ribonucleotides from mycoprotein wastewater.[79,80]

Precipitation. A variety of methods exist to precipitate proteins from solution, including isoelectric precipitation, salting out, and organic solvent methods. Typically, precipitation is a rapid, easily scalable process that can be operated at low temperatures, enabling high throughput, low heat duty and recovery of proteins without denaturation effects. Taskila *et al.* (2017) investigated the use of low-temperature evaporation followed by ethanol precipitation to recover value-added proteins from potato fruit juice. Implementation at pilot scale demonstrated a 50% recovery of proteins from industrial starch waste streams.[81] Xu *et al.* (2019) studied epigallo-catechin-3-gallate (a polyphenol derived from green tea) as a precipitating agent for protein valorisation from soy whey wastewater, achieving a high recovery of 60.7% with a protein purity of 69.51%.[82]

Adsorption. Adsorption technologies have been explored primarily to extract valuable enzymes from waste, as detailed in a review by Shahid *et al.* (2021). Typically, various structural forms of mesoporous silica with modified surface properties are employed for targeted protein valorisation and are capable of operating at low temperatures. However, residence time, adsorption capacity and operating pH vary significantly as a function of adsorbent, substrate, and target protein of study.[79]

Despite promising results of new filtration, precipitation, and adsorption technologies, further studies are required to determine recovery performance and protein structure alterations when targeting proteins of high nutritional value from a wider range of waste streams. Research efforts focused on adsorbent/membrane regeneration and precipitant recovery and recycle capacity are also essential to ensure sustainability and economic viability of extraction.

Assisted extraction technologies. A variety of technologies can be used in hybrid with extraction and hydrolysis technologies to improve process efficiency and environmental impact, while improving the functional properties of product. These include hydrodynamic cavitation extraction (HCE), microwave assisted extraction (MAE), pulsed electric fields (PEF) and ultrasound assisted extraction (UAE), the working mechanisms of which have been reviewed in-depth in other works.[83-107] Key advantages and drawbacks of these technologies with regards to process operation and product safety/nutrition are summarised in Table 2.

Hydrolysis technologies. Hydrolysis technologies can be employed to produce both protein and carbohydrate hydrolysate from waste streams. Hydrolysis agents include acid, alkali, organic solvents, subcritical water, and enzymes. The advantages and drawbacks of these technologies with regards to process operation and product safety/nutrition are summarised in Table 3, and a critical evaluation of enzymatic hydrolysis is given in the following section.

Enzymatic hydrolysis. In contrast with chemical, solvent, and subcritical water hydrolysis, enzymatic hydrolysis is effective at low temperatures, pressures, and mild pH, reducing reactor capital cost, heat duty and preservation of amino acid profile and other nutrients.[87,108-110] Furthermore, favour-

Table 1 Protein extraction and purification technologies: advantages and drawbacks of process efficiency/operation and product safety/nutrition (MW = molecular weight; COD = chemical oxygen demand; OPEX = operating expenditure; CAPEX = capital expenditure)

Technology	Extraction and purification technologies		Ref.
Membrane filtration	**Process advantages**	**Process drawbacks**	99, 104, 110, 115 and 191–195
	Can be integrated with simple pre-treatment processes (centrifugation, pre-filtration, dissolved air flotation) to reduce fouling by waste particles containing fat, starch, and high MW proteins	Protein agglomeration on membrane surface leads to concentration polarisation and pore blocking, which causes severe membrane fouling	
	High yield of non-denatured proteins due to low operating temperatures	High OPEX/CAPEX for membrane regeneration/ replacement	
	Low energy consumption		
	Membrane unit configurations such as rotating disk membranes can reduce fouling by increasing shear rate	Permeate may contain high COD due to presence of residual waste and chemicals used in pre-treatment steps, requiring further downstream processing before discharge	
	Cascading membrane systems of varying pore size can increase protein yield and water recovery while reducing COD of effluent	Throughput levels capped by flooding and loading limits	
	Modular and flexible usage, highly scalable for industrial processing with small physical footprint	Use of harsh chemicals may be required to regenerate fouled membrane	
	Backflushing and rinsing of the membrane during or after operation can decrease fouling	Potentially large solvent inventory for cleaning purposes	
	Safety/nutritional advantages	**Safety/nutritional drawbacks**	
	Enhanced functional/nutritional properties of extracted proteins compared to precipitation	High MW proteins associated with allergenicity and digestibility issues (post-extraction hydrolysis may be required)	
	Denaturation of high MW proteins may occur which can improve digestibility and reduce allergenicity		
Precipitation (organic solvent, pH-shift, salting-out)	**Process advantages**	**Process drawbacks**	95, 104, 108, 110, 196 and 197
	Increased efficiency when integrated with membrane filtration	Low overall protein yield, sensitive to impurities in feed	
	Isolate can be processed downstream (e.g., enzymatic hydrolysis) to produce shorter peptides with higher solubility and improved functionality	pH shift requires controlled addition of harsh alkali/acid chemicals	
		Chemical/salt addition may introduce further impurities, intensifying the downstream purification load	
	Long-established technology in bioprocessing industry	High environmental impact when using organic solvents	
	Relatively simple, inexpensive and highly scalable process (especially salting-out)	Intense centrifugation is often required downstream to remove chemicals and impurities, increasing energy costs	
	Mild operating temperature (but must be controlled carefully for sensitive proteins)	Operating at extreme pH may result in functionality loss of many proteins in waste stream	
	Safety/nutritional advantages	**Safety/nutritional drawbacks**	
	Products often used as emulsifiers, stabilisers, and foaming agents and as fortifiers to enhance the nutritional value of food products due to favourable functionality	High MW proteins associated with allergenicity and digestibility issues (post-extraction hydrolysis may be required)	
	Precipitating agents or flocculants used to increase efficiency are food safe	Use of acid-alkali impacts functionality and amino acid content of proteins due to denaturation effects	
Adsorption	**Process advantages**	**Process drawbacks**	99, 198 and 199
	Mesoporous silica structure can be modified to include functional groups to extract specific proteins	Unmodified silica adsorbent is electronically neutral and has lower affinity for charged proteins, resulting in leaching of proteins	
	Low operating temperature	Increased CAPEX/OPEX due to adsorbent replacement/regeneration	
	Adsorbent can be used to immobilise enzymes which hydrolyse incoming feed (e.g., to hydrolyse carbohydrates and lipids in waste stream)	Enzyme leaching from surface can occur for poorly selected adsorbent (enzyme regeneration can also be an issue)	
	Effective for targeted extraction of bioactive proteins	Difficult and expensive to modify silica adsorbent	
	Many adsorbents are low-cost (e.g. silica)	Extraction efficiency and selectivity is highly dependent on process conditions and adsorbent surface structure	
	Safety/nutritional advantages	**Safety/nutritional drawbacks**	
	Products often used as emulsifiers, stabilisers, and foaming agents and as fortifiers to enhance the nutritional value of food products due to favourable functionality	Relatively little available research on the mechanisms of protein–surface interactions and effect on protein structure (especially for complex waste feedstock)	

Table 2 Assisted extraction technologies: advantages and drawbacks of process efficiency/operation and product safety/nutrition (OPEX = operating expenditure; CAPEX = capital expenditure)

Technology	Assisted extraction technologies		Ref.
Hydrodynamic cavitation extraction (HCE)	**Process advantages** Good scalability for continuous processing compared with UAE Lowers CAPEX, OPEX and production time Higher efficiency compared with UAE Recent scale-up studies have demonstrated improved economics at pilot/industrial scale compared to lab scale Can be used in conjunction with other extraction techniques (*e.g.*, enzymatic hydrolysis, solvent extraction) to significantly increase yield Potential use as one-step valorisation process, reducing downstream processing burden Relatively low environmental impact due to reduced energy consumption **Safety/nutritional advantages** Enhances nutritional quality, solubility, and digestibility of product	**Process drawbacks** Relatively little available research (denaturation effects and efficiency at industrial scale are largely unknown) Process efficiency is highly dependent on interaction between reactor configuration, operational parameters, and feedstock properties **Safety/nutritional drawbacks** Evaluation of potential toxic by-product generation is yet to be fully evaluated	90, 94, 96, 100 and 107
Microwave assisted extraction (MAE)	**Process advantages** Can be used in conjunction with other extraction techniques (*e.g.*, enzymatic hydrolysis, solvent extraction) to significantly increase yield Relatively simple and inexpensive compared to SFE Reduces energy consumption and environmental impact of process Shorter extraction time compared to UAE Can reduce solvent/chemical requirement of extraction process **Safety/nutritional advantages** Reported to assist pathogen expulsion during thermal pre-treatment of waste by disrupting cell wall structure Enhances nutritional quality, solubility, and digestibility of product	**Process drawbacks** In conjunction with solvent extraction, organic/inorganic solvents are preferred to water due to lower relative electrical permittivity Relatively difficult to operate compared to UAE High equipment CAPEX Denaturation an issue when operating at high power and prolonged time periods **Safety/nutritional drawbacks** Relatively little research to understand the full effects of MAE on food/feed products and their safety	83, 84, 88, 90, 91, 93, 98, 103 and 105
Pulsed electric fields (PEF)	**Process advantages** Modular and flexible technology is highly scalable for continuous processing Significantly increases protein yield and functionality when used in combination with other technologies (*e.g.*, enzymatic, acid/alkali hydrolysis) Enhances product purity reducing downstream processing load Non-thermal and can reduce solvent/chemical requirement of extraction process) Reduces energy consumption, duration, and environmental impact of process **Safety/nutritional advantages** Demonstrated to preserves nutritional value, flavour, texture, and colour of product, reduces allergenicity, and can enhance product functionality Can destroy pathogens in dairy wastewater	**Process drawbacks** Can cause air bubble entrapment in the treatment chamber, lowering efficiency Further research is required to fully understand the molecular mechanisms of the process Higher OPEX when implemented for complex waste streams is expected to increase product cost Implementation at industrial scale has been limited Process parameters must be optimised to achieve best efficiency and yield increase at industrial scale (contributing to complexity) **Safety/nutritional drawbacks** Food/feed safety assurance requires further investigation of protein functionality alteration when waste exposed to PEF	85, 86, 90, 99, 101 and 106
Ultrasound assisted extraction (UAE)	**Process advantages** Reduces overall energy consumption and environmental impact of process Water can be used as solvent with greater efficiency than MAE (and lowers required organic solvent if used) Simple applicability of bath-type reactor configuration	**Process drawbacks** Probe-type reactor configuration improves efficiency, but is not simple to implement Pilot studies indicate very poor scaling with diminished increase in protein yield compared to lab-scale Attenuation of ultrasound waves is an issue leading to operational losses	89, 90, 92, 95, 97, 102 and 104

Table 2 *(Contd.)*

Technology	Assisted extraction technologies		Ref.
	Can be used in conjunction with other extraction techniques (*e.g.*, enzymatic hydrolysis, solvent extraction) to significantly increase yield	Efficiency is highly dependent on reactor configuration, power intensity, duration, and is specific to substrate factors	
	Shortens extraction time	High energy consumption	
	Safety/nutritional advantages	**Safety/nutritional drawbacks**	
	Favourable amino acid profile for well-optimised processes	Protracted sonication at high power can result in severe protein denaturation and loss of functionality/solubility	
	Favourable protein functionality for well-optimised processes	Amino acid and nutritional profile can be diminished for poorly optimised processes	

able functional characteristics and improvements in safety and palatability for consumption (*e.g.*, reduced allergenicity of smaller peptides, better digestibility, colour, and texture) have been observed in hydrolysates.[87,111–113] However, enzymes remain expensive due to their production complexity and are highly sensitive to operating conditions (which must be tightly controlled) due to low stability, presenting a significant economic and complexity barrier to commercial implementation.[87,99,114]

Furthermore, enzymes generally have high substrate specificity, requiring careful consideration of enzyme choice for a given waste stream or potential use of a "cocktail" solution incorporating various enzyme types to broaden specificity, increasing process cost and complexity further.[90] Enzymatic hydrolysis using proteases can be applied to waste feedstock directly to recover protein, however this can cause issues of enzyme inhibition by impurities in the feedstock. One way to address this is to apply enzymatic hydrolysis to high purity isolate recovered from upstream protein extraction processes.[87,110,113,115]

Carbohydrase enzymes can also be employed to produce sugar hydrolysate from organic waste containing complex carbohydrates by breaking down cell wall components (*e.g.* lignocellulosic cell walls) into constituent monomers, in addition to releasing dissolved proteins and reducing sugars from the intracellular matrix.[116] Research into enzymatic hydrolysis of lignocellulosic waste has been detailed in previous reviews.[2,114,117,118] In brief, according to Modenbach and Nokes (2013) cellulases and xylanases are the most commonly adopted enzymes to degrade cellulose and xylan, respectively.[117] The degradation mechanisms of these enzymes on their corresponding carbohydrate substrates are discussed by Aditiya *et al.*, (2016). In addition to the common sugars (*e.g.* sucrose, glucose, fructose, galactose, mannose, ribose, xylose, and arabinose), which occur in nature in the free form, or as monomers of oligosaccharides and polysaccharides, other rare monosaccharides and sugar alcohols (*e.g.* xylitol, mannitol, erythritol as sugar substitutes) can also be produced by enzyme-catalysed reactions.[119] The wide range of platform chemicals, in particular the fermentable sugars, provide substrates to produce microbial protein or alternative protein sources. The capacity of microbial protein produced from such resources to replace conventional protein from animal husban-

dry was estimated by Pikaar and colleagues.[23] The authors calculated that in terms of amino acid requirements, up to 10–19% of current global feed crops (occupying 6% of global arable area and equivalent to the entire current cropland of China) could be replaced by microbial protein, freeing up arable land area for other important agricultural practices.

However, with regards to lignocellulosic waste in particular, pre-treatments are required to fractionate complex carbohydrates from the biomass to increase substrate degradability and downstream process performance. Fractionation pre-treatment technologies include chemical (*e.g.* alkali, acid, ionic liquid), thermal (*e.g.* steam), biological (*e.g.* ligninolytic microbes) and physical (*e.g.* extrusion) methods, individually or in combination. Extensive research has focused on pre-treatment technologies, as detailed in several reviews.[120–125] In short, these reviews conclude that the chemical processes successfully render effective fractionation but introduce design challenges such as solvent recycling and the need for reactor anti-corrosion steps. Physical and thermal routes may lead to cost-effective, solvent-free but energy-intensive solutions. Despite the advantages of low-energy demand and effective lignin depolymerisation, biological routes might be challenged by low reaction rate and inhibitor generation issues. Furthermore, food-safe methods of pre-treatment that are capable of high efficiency fractionation requires further research and development to improve economic viability and food/feed safety assurances for the downstream production chain.

The variety of technology options available offers great potential for novel protein solutions capable of transforming global food production as we know it. For example, Indonesia primarily relies on imported feed-protein such as soybean meal, fish meal and meat bone meal from America and Brazil, exposing the country to feed shortages in the event of global supply chain disruptions.[126] Recognising this, Indonesian researchers have focused on protein recovery from local palm and coconut oil waste using microbial enzymes.[127] Transitioning to local waste-to-protein solutions has the potential to significantly improve protein security and sustainability, while reducing the cost of meeting regional and national nutritional demands.

Bioconverter technologies. Bioconverter technologies refer specifically to the use of value-upgrading organisms

Table 3 Hydrolysis technologies: advantages and drawbacks of process efficiency/operation and product safety/nutrition (OPEX = operating expenditure; CAPEX = capital expenditure)

Technology	Hydrolysis technologies		Ref.
Acid/alkali hydrolysis	**Process advantages** Inexpensive compared to enzymatic hydrolysis Can be integrated with assisted extraction techniques for increased yield Proteins highly soluble under these conditions **Safety/nutritional advantages** Harsh temperatures and pH sterilise the feedstock	**Process drawbacks** Unpleasant flavour of product Harsh chemical conditions and high operating temperatures Relatively low yield **Safety/nutritional drawbacks** Acid: Destruction of tryptophan, asparagine, and glutamine (partial destruction of methionine and cysteine). Alkali: Destruction of majority of amino acids but tryptophan is retained	108–110 and 200
Enzymatic hydrolysis	**Process advantages** Mild operating temperature and pH Harsh chemicals replaced by biological catalysts (enzymes) Available research is relatively extensive Alcalase has broad substrate specificity (can achieve high yields for variety of waste feedstocks) Hydrolysate has improved rheological (texture) and taste profile Low environmental impact Specificity of enzymes minimises undesirable side-reactions and enables control of the degree of hydrolysis Carbohydrases can be employed to hydrolyse pre-treated lignocellulosic biomass into platform sugars for microbial protein fermentation Genetic engineering can be used to broaden enzyme specificity and increase efficiency **Safety/nutritional advantages** Protein hydrolysate has higher solubility and smaller peptides with improved functionality and reduced allergenicity compared with whole protein extraction No destruction of amino acids (protein quality retained) Food-grade enzymes available and commonly employed (*e.g.*, alcalase)	**Process drawbacks** Longer operational time compared to acid-alkali hydrolysis due to low temperature operation Acid/alkali is added to maintain optimum pH Difficult to operate at industrial scale due to tight multiparameter control requirements and sensitivity of enzymes Impurities in the feedstock such as phytochemicals in food waste can act as enzyme inhibitors, reducing efficiency Hydrolysate can retain impurities from feedstock (polluted downstream effluent requires purification) High OPEX (requires the use of expensive enzymes) Substrate specificity of individual enzymes – "enzyme cocktail" may be required to efficiently hydrolyse the waste feedstock containing complex array of proteins and other compounds Pre-treatment of lignocellulosic biomass required to fractionate complex carbohydrates Genetic engineering to broaden enzyme specificity is restricted in many countries. **Safety/nutritional drawbacks** Heat inactivation step may impact physiochemical properties of hydrolysate	90, 99, 109–113 and 115
Subcritical water hydrolysis	**Process advantages** Expensive enzymes not required Addition of acid, alkali and organic solvents not required (but addition of sodium bicarbonate, NaOH and acetone modifiers has been shown to increase yield) Concentrated CO_2 and O_2 gas to pressurise the atmosphere can increase amino acid yield and decrease reaction time **Safety/nutritional advantages** Hydrolysates have demonstrated improved functionality and compared to enzymatic hydrolysis in some studies Amino acid profile is usually not significantly impacted (process condition dependent)	**Process drawbacks** High temperatures and pressures required compared to enzymatic hydrolysis If required, reducing temperature to preserve protein quality will lead to increased reaction times Presence of other compounds in the feed may impact process yields (may be necessary to remove them through pre-treatment) High CAPEX due to expensive equipment Moisture extraction (*e.g.*, evaporation) is required downstream to obtain purified protein **Safety/nutritional drawbacks** Health and nutritional effect of modifications to the side chains and the amino acid profile have not been evaluated Addition of O_2 gas can decrease functionality of hydrolysate due to amino acid alterations	42, 99, 108 and 201–213

through metabolism of waste protein, nutrients, and waste-derived sugars into biomass, namely microbes and insects. The advantages and drawbacks of these technologies with regards to process operation and product safety/nutrition are summarised in Table 4 and 5.

Table 4 Bioconverter technologies (microbial protein): advantages and drawbacks of process efficiency/operation and product safety/nutrition (OPEX = operating expenditure; CAPEX = capital expenditure; R&D = research and development)

Technology	Bioconverter technologies		Ref.
Microbial protein	**Process advantages** Intracellular proteases eliminate the requirement of costly cell-free enzymes as in enzymatic hydrolysis	**Process drawbacks** Nutrient assimilation efficiency and selectivity is highly dependent on microbial strain Wastewater stream may require further processing to reduce nutrient content to acceptable limits	99, 174 and 214–232
	Rapid (exponential) growth rates – high productivity compared to traditional protein sources	High R&D cost for microbial strain screening, improvement, and process design/scale-up Genetic engineering of microbial strains is restricted in many countries	
	Microbial co-culture can increase conversion efficiency of complex waste resources, autotrophic bacteria as potential carbon capture solution	Sterility of cultivation broth is required and is difficult to achieve	
	Relatively large availability of research and expertise	Difficulty of scale-up for continuous industrial processes High CAPEX of process equipment Shear stress from agitation and aeration can negatively impact growth efficiency of microbes and product texture	
	Reduced environmental impact due to lower water/energy consumption and no arable land requirement compared to traditional farming	Strain evolution occurring over lengthy production times can result in dominant strain with undesirable phenotype (*e.g.*, lower protein content, poor texture, protein functionality). Considerable downtime between batches (required even for continuous processes to reduce contamination risk and ensure product quality)	
	Growth is season/climate-independent so can be operated year-round	Requires tight control of many process parameters for maximum efficiency, increasing operation complexity High cost of product relative to traditional food/feed protein resources	
	Microorganisms are relatively easy to genetically modify	Batch variability of feedstock impacts process performance Inhibitory and unfermentable compounds in feedstock can negatively impact process performance (requires upstream purification)	
	Safety/nutritional advantages High protein content compared to many traditional sources of protein	**Safety/nutritional drawbacks** Novel feed/food protein and regulatory approval process can be time and resource intensive (use of waste feedstock may further complicate this) Low consumer acceptance as food source in some global regions	
	Long history of use as human food protein source in many global regions	Requires downstream processing to remove intracellular compounds unsafe for consumption (particularly nucleic acid, which causes severe gastrointestinal and other health problems) Strains may produce toxic compounds under certain conditions (*e.g.*, mycotoxins)	
	Favourable nutrient profiles including vitamins, minerals and no cholesterol compared to traditional sources of protein	Allergic reactions to consumption have been reported for several microbial strains Long feeding trials required to assess toxicological and carcinogenic potential of product	
	Animal feed replacement has demonstrated favourable digestibility and prolonged survival of animals due to probiotic contents (of yeast)	Composition and quality are highly dependent on feedstock content. Can be difficult to ensure safety and consistent nutritional profile due to batch variability in waste feedstock Some microbes (especially bacteria) have poor palatability and colour, making them unsuitable for human food purposes	
	Cell walls contribute to significant fibre content, thus potentially improving gut function and metabolism	High risk of contamination during production and processing Feedstock must be/derived from food/feed-safe resource to avoid introduction of toxic contaminants	

Microbial protein

Microbial protein technology utilises yeast, fungal, bacterial, or algal strains capable of converting sources of carbon, nitrogen, and oxygen into protein-rich biomass fit for human consumption or animal feeding. Approximately 80 different microbial strains have been reported to enable the production of food-grade or feed-grade protein (Fig. 3).

Table 5 Bioconverter technologies (insects): advantages and drawbacks of process efficiency/operation and product safety/nutrition

Technology	Bioconverter technologies		Ref.
Insects	**Process advantages**	**Process drawbacks**	162–164, 166–169, 233 and 234
	Can be further processed downstream (*e.g.*, enzymatic hydrolysis/precipitation) to produce protein hydrolysate/isolate with favourable functionality	Processing techniques are currently poorly optimised and regulated at scale	
	Low energy consumption and environmental impact	Processing methods (*e.g.*, boiling, drying, freezing) can reduce lipid and protein yield and quality	
	Can be formed using 3D printing to improve texture & appearance		
	Insects are fast-growing (high productivity)	Efficiency is sensitive to pH, oxygen, light, and temperature conditions	
	Low temperatures required during cultivation	Sanitary environment is difficult to achieve during cultivation, processing, storage	
	Safety/nutritional advantages	**Safety/nutritional drawbacks**	
	Demonstrated to increase protein content, improve amino acid, nutrient profile and digestibility when insect powder used as a food additive	Use as food additive can negatively affect the colour and palatability of the product	
	No significant difference in allergenicity of insects and traditional food sources	Spore-generating bacteria species can survive microbiocidal processing, increasing risk of potential food-borne diseases	
		May accumulate harmful chemicals such as persistent organic pollutants (POPs) from feedstock if not food-safe	
	Chitin and/or chitosan contribute to high fibre content (has been reported to reduce cholesterol and improve gastrointestinal function)	Insect biomass has a high fat content (boiling process may be necessary)	
	Long history of use as human food protein source in many global regions	Flame retardants and plasticisers used in processing may accumulate in insect biomass	
	Microbiocidal processing step (*e.g.*, boiling) can be used to eliminate pathogens	Cooking stage can damage nutrient profile and reduce protein content	
	Low moisture content can improve texture when used as food additive	Toxicological and carcinogenic identification is difficult due to high biodiversity of insect species	
	Insect powders have demonstrated favourable mineral, fatty acids, and vitamin profiles	Microbial contamination of insect communities is significant (novel high-pressure microbiocidal techniques should be explored to eliminate spore-forming bacteria while retaining functional and sensory properties)	
	Favourable gel-forming ability, emulsion capacity, and water/oil absorption ability	Allergic reactions in humans have been reported due to high chitin, and uric acid content of insects	
	Has demonstrated decreased leaching of nutrients from animal feed when used as additive	Very low consumer acceptance as food source, especially in developed regions	

Microalgae and bacteria. Microalgae and bacteria represent the most protein-rich sources, within the range of 60–70 wt% and 50–80 wt%, respectively, whereas fungi/yeasts contain approximately 30–50 wt% protein, followed by protists at 10–20 wt%.[128] The high protein content positions bacteria as a desirable candidate for microbial protein conversion. However, reported palatability issues are yet to be addressed, posing a challenge to the successful commercialisation of bacterial protein as a food product.[129,130]
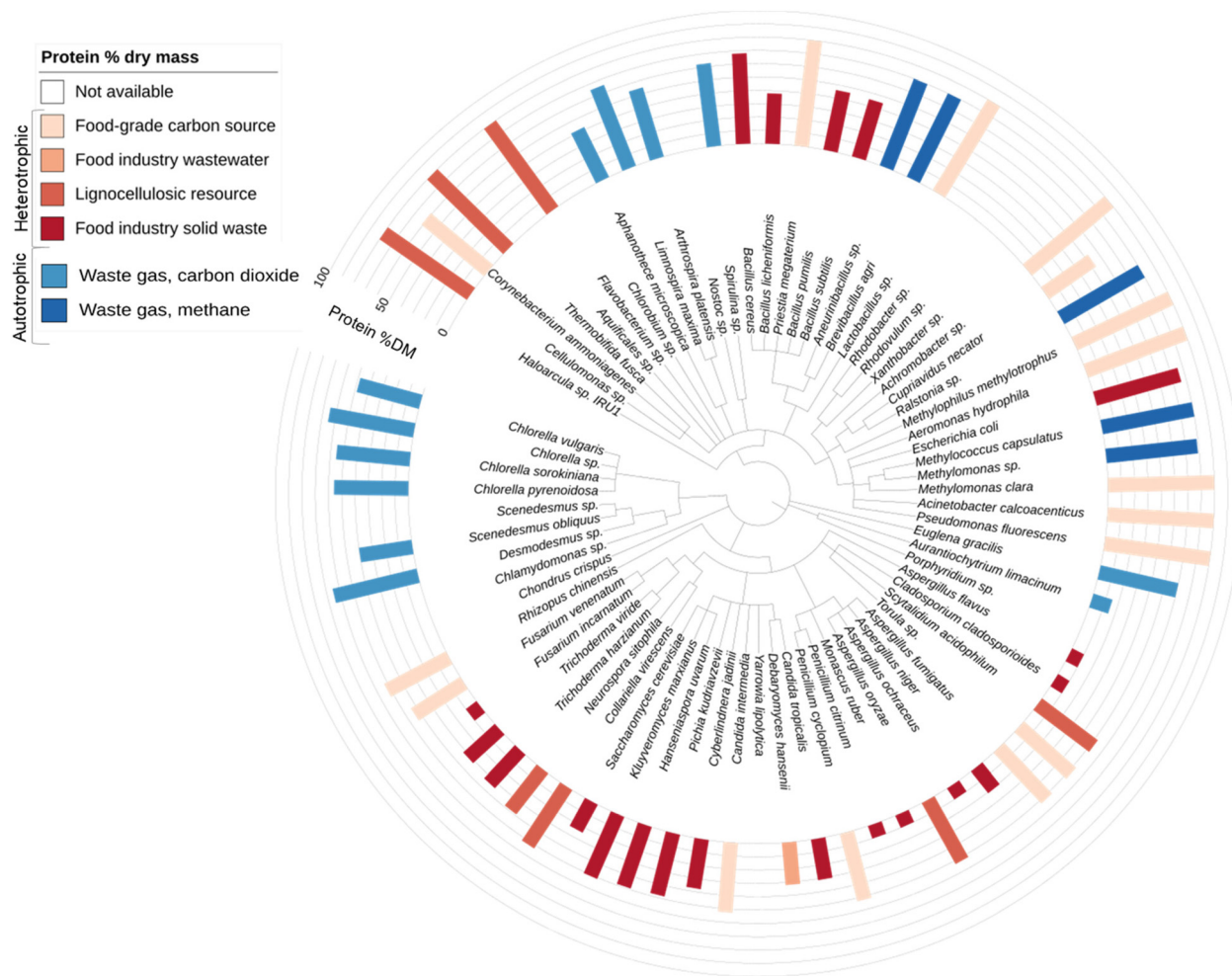
Fungi. Fungi (including yeast) have a longstanding history of use in the production of microbial protein food products, some of which are now mass-produced and widely distributed *e.g.* tempeh. Oncom, a traditional food closely related to tempeh and consumed mainly in West Java, Indonesia, is produced by fermenting *Rhizopus oligosporus* and *Neurospora sitophila*. Interestingly, waste by-products from food production such as soy pulp, peanut press cake and cassava tailings are typically employed as substrates for the fermentation process. Despite serving as a historical waste-to-protein proof of concept, a high quality, mass-produced oncom product has

not yet been realised, and very few research efforts have been made to this end.[131]

Industrial production of microbial protein. As early as the 1970s, a variety of high-quality upgrade products that are rich in microbial protein were established on farm and industrial scales, *e.g.* volatile fatty acids from Candida yeast[133,134] and methanol to Pruteen.[135] Despite relative ease of operation and access to a large body of expertise built by long-established fermentation industries, established supply chains (*e.g.* soybean-based protein) held an economically competitive edge, stifling many early businesses.

Mycoprotein has become one of the most successful food-grade microbial proteins and was originally produced in response to concerns regarding the insufficiency of meat as a sustainable and healthy protein source. It has been commercialised since 1985 as Quorn™[136] and is currently sold in 17 countries, predominantly in Europe but also in developing nations such as the Philippines, and is the largest microbial protein meat alternative (by sales) with over 6 billion meals supplied globally in 2020.[137] Quorn™ mycoprotein is pro-

Fig. 3 Taxonomic tree of reported microbial protein producing species. Species are sorted according to the National Centre for Biotechnology Information (NCBI) taxonomy database.[132] Species are grouped by domain: Archaea, Eukaryota or Bacteria. Reported protein contents (% dry mass) are indicated by bar chart ranging from 10% to 80% dry mass (ESI Table ST3†). Where multiple protein values have been reported an average was calculated. Food-grade carbon source refers to pure food-grade soluble compounds such as glucose, lactose and maltose. Detailed data can be found in the ESI-3 and ESI Table ST3.†

duced *via* fermentation of fungus species *Fusarium venenatum A3/5* utilising glucose as feedstock, with the addition of oxygen, nitrogen, vitamins, and minerals.[138] Mycoprotein has a moderate protein content (45% of biomass) and contains all essential amino acids (44% of total protein).[139] Additionally, it offers positive health attributes compared with animal protein, such as a favourable fatty acid profile and high fibre content.[140]

These properties make Quorn™ mycoprotein well-suited to regions with high prevalence rates of obesity-related diseases such as North America and Europe.[1,141] A series of recent studies in human physiology by Monteyne *et al.* (2020) have examined the capacity for mycoprotein to regulate skeletal muscle protein metabolism in young and older adults, with encouraging results.[142]

Other industrial pioneers have utilised microbial protein technologies to produce protein for human consumption, as well as for animal and aquaculture feed purposes. Notable

feed-grade protein products that have been commercialised include All-G Rich® (Alltech), UniProtein® (Unibio) and Feedkind® (Calysta).[143–145] Fungal species *Neurospora sitophila* also has a longstanding history of involvement in food production.[146] White Dog Labs, Inc. (New Castle, Delaware) actively produces microbial protein for animal feed but has not disclosed strain information. Moreover, the carbon transformation company Kiverdi, Inc. (Pleasanton, California) recently introduced 'Air Protein™', which converts CO_2 to food-grade protein by microbial fermentation; however, no detailed information has been disclosed on the hydrogenotrophic microorganisms used.[147] Solar Foods (Helsinki, Finland) also produces food-grade microbial protein (Solein®) *via* CO_2 fermentation at pilot scale and has recently been awarded €35 million in funding. Avecom (Ghent, Belgium) aims to integrate their microbial protein technology with existing food processing businesses as a waste recovery solution, allowing them to produce proteins for food or feed purposes.

Furthermore, Avecom's 'Power-to-Protein' research partnership has been investigating renewable hydrogen and atmospheric CO_2 as drivers for autotrophic and mixotrophic upgrading of nitrogen from waste to produce feed protein.[120] However, issues of poor hydrogen mass transfer are still being addressed to ensure adequate rates of production. Phototrophic bacteria are also being explored to produce human food and animal feed from secondary resources.

Research and development of microbial protein. Many microorganisms are still at the research and development stage. Microbial protein production that utilises lignocellulosic waste resources have generated increasing research attention. Two potential technology solutions have been reported, namely *Fusarium venenatum A3/5* fed on glucose and xylose derived from lignocellulosic biomass[11] and cellulose-consuming strains such as *Aspergillus niger*, *Neurospore sitaphila*, and *Trichoderma viridae.*[121,122] Recently, SylPro® Arbiom has gained attention for scaling up trials of protein production based on the conversion of lignocellulosic forestry waste by yeast species for aquaculture feed.[123,128] Producing novel food ingredients with desirable techno-functional and sensory qualities for use in the food and drink industry remains a formidable challenge,[124] and the development of microbial protein ingredients is no exception.[120] Currently, the preferred strategy is to focus on the nutritional value (amino acid composition) of microbial proteins and then search for smart combinations with other food ingredients to provide properties such as taste, texture and structure to the final food, such is the case with current mycoprotein products.[125]

Regulation and safety of microbial protein. Although there is a large list of potential upgraders, the legislator formulates strict requirements regarding which organisms are accepted as human food. In the European Union, applications for novel food status require preparation of detailed technical dossiers as evidence for the safety of products. When added to the considerable costs and complexity involved in the application procedure, this creates an significant barrier to the development and commercialisation of novel foods[148] in the EU and in countries adopting a similarly 'cautionary' regulatory model. With regards to the safety of microbial protein, it is imperative that toxicological testing is performed continuously for the detection of secondary metabolites (*e.g.* mycotoxins) as a food/feed safety assurance protocol.[149] Furthermore, food processing operation must be performed under controlled conditions to mitigate the risk of contamination of the microbial culture, which could lead to the introduction of pathogens. Although this can prove difficult to achieve for industrial scale processing, good operator training, oversight and development of sound operation and testing procedures should be employed to address this issue. Furthermore, allergenicity and digestibility issues have been reported for several microbial strains, for example, cases of allergic reactions have been reported as a result of mycoprotein consumption.[150] Therefore, extensive animal feeding trials are required prior to distribution to fully characterise the potential health risks posed by consumption of a particular microbial strain.

Insects

Contamination-free biowaste provides a theoretical feed stream for insects to act as waste-to-protein bio-converters. High conversion rates for *Orthoptera* sp. (1.7 kg feed : 1 kg live-weight)[65] and *Hermentia illucens*, *i.e.* black soldier fly larvae (1.95–13.42% carbon and 5.4–18.93% nitrogen recycling) have been reported.[151] The cultivated insects can be harvested and converted into human food through relatively simple processing methods. For example, caterpillars and mealworms are prepared by scalding, drying and cooking (*i.e.* roasting or boiling), and insect protein bars are prepared by milling and processing (*i.e.* baking).[20,152] According to recent estimates, one billion of the world's population are estimated to rely on insects as a primary protein source, particularly in African, South American and South East Asian countries.[153] Insect-based foods are seeing increasing global acceptance in recent years, with the combined insect market of the US, Belgium, France, UK, the Netherlands, China, Thailand, Vietnam, Brazil and Mexico, predicted to increase from £25 million in 2015 to £398 million in 2023.[154]
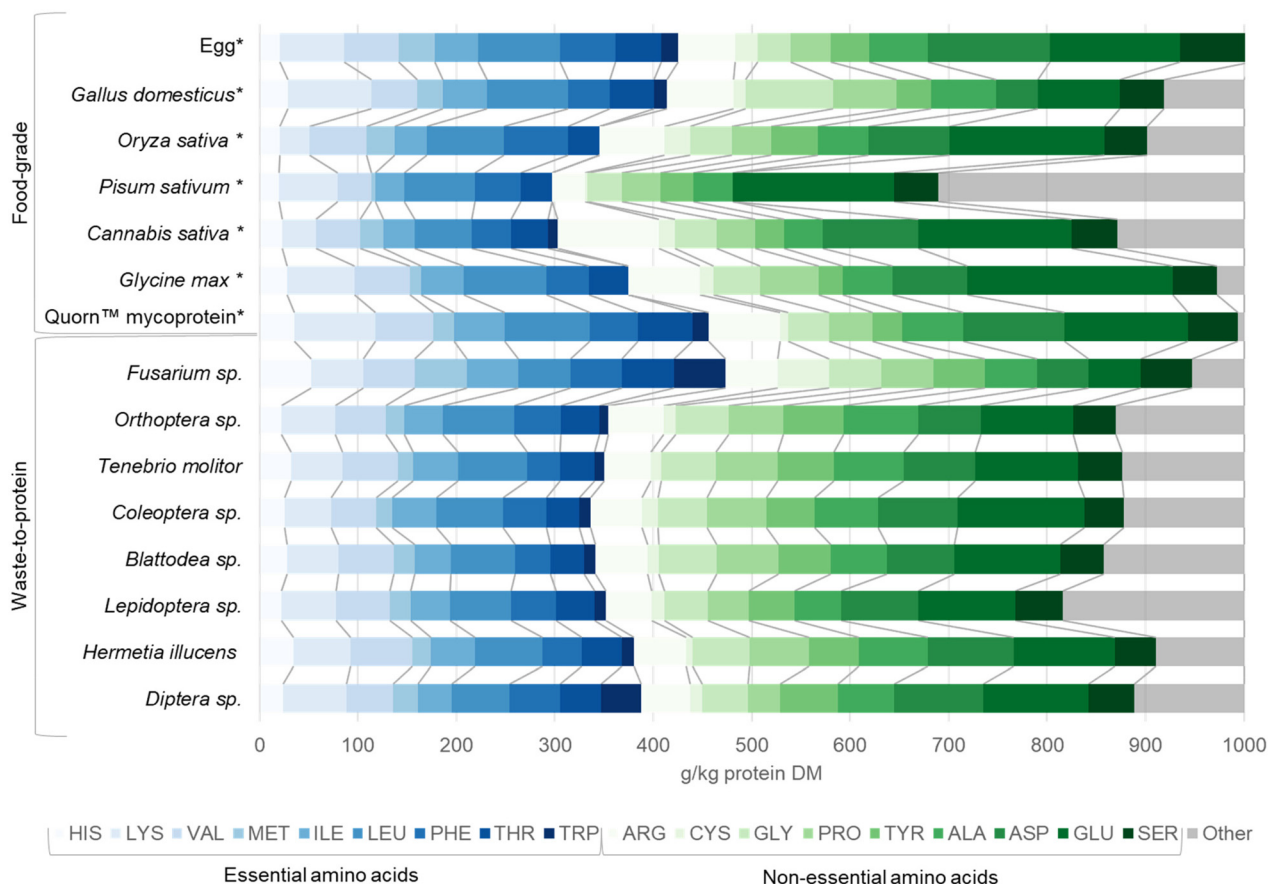
Insect protein nutritional value analysis

Most insects are rich in protein and other nutrients such as iron and vitamin A.[155] Oibiopka *et al.*, (2018) found that the protein content of a diet consisting of *Orthoptera*, *Lepidoptera* and *Blattodea* fed to rats exhibits a 12–20% higher biological value compared to the standard protein casein.[156] Moreover, *in vitro* digestion experiments evaluating mineral bioavailability indicated that *Orthoptera* sp. and *Tenebrio molitor* contain significantly higher chemically available calcium, magnesium, manganese, and zinc than sirloin beef.[157]

Fig. 4 shows the amino acid profiles of different food-grade benchmark animal-based, plant-based, and microbial proteins, as well as waste-to-protein insect and microbial protein sources. Compared to food-grade benchmark protein sources, waste-to-protein insect and microbial sources are richer in the essential amino acids.[158] Waste-to-protein *Fusarium* spp. demonstrated the highest total essential amino acid contents of all protein sources, followed by food-grade egg and Quorn™ mycoprotein products, while *Diptera* sp. (including *Hermetia illucens*) protein exhibited a similar profile of essential amino acids to egg. Amongst insect proteins, *Diptera* sp. (including *Hermetia illucens*) and *Coleoptera* sp. (including *Tenebrio molitor*) appear to have the highest total amino acid contents (Fig. 4). However, the nutritional quality of edible insect protein could diminish during digestion due to low content of the limiting essential amino acids, tryptophan and lysine.[65] Previous research also reported that methionine and cysteine were limiting amino acids in *Blattodea* sp., whereas isoleucine was limiting in some *Orthoptera* sp.[159]

Accounting for the time taken for insects to reach maturity, *Hermetia illucens* and *Tenebrio molitor* larvae may be considered favourable new protein sources for rapid technology scale-up due to their relatively short lifecycles (ESI-6†).

Fig. 4 Amino acid profile of various microbial and insect protein sources. Egg albumin is included as a standard for comparison. Eighteen amino acids are included: Histidine (HIS), Lysine (LYS), Methionine (MET), Isoleucine (ILE), Leucine (LEU), Phenylalanine (PHE), Threonine (THR), Tryptophan (TRP), Arginine (ARG), Cysteine (CYS), Glycine (GLY), Proline (PRO), Tyrosine (TYR), Alanine (ALA), Aspartic acid (ASP), Glutamic acid (GLU) and Serine (SER). We were unable to obtain values for asparagine and glutamine. Amino acid profiles are displayed for waste-to-protein protein sources including: *Fusarium* sp. (mycoprotein), *Orthoptera* sp. (crickets, grasshoppers, locusts), *Tenebrio molitor* (mealworm) *Coleoptera* sp. (beetles), *Blattodea* sp. (cockroaches, termites), *Lepidoptera* sp. (butterflies, moths), *Hermetia illucens* (black soldier fly larvae) and *Diptera* sp. Bench mark food-grade* protein sources were provided for comparison including *Gallus domesticus* (chicken), *Oryza sativa* (Asian rice), *Pisum sativum* (pea), *Cannabis sativa* (hempseed), *Glycine max* (soya), and Quorn™ mycoprotein. Essential amino acid profiles are shown in blue, non-essential amino acids are shown in green on a g kg^{-1} protein dry mass basis. 'Other' is presented in grey and represents missing values or errors due to methodology limitations reported in original literature. Detailed data can be found in ESI-4 and ESI Table ST4.†

Depending on the grade of organic waste used as substrate, insect farming technologies provide a source of protein for human consumption or animal feed purposes. As efficient waste-to-protein bio-converters, insects achieve high conversion efficiency to turn low-grade waste into protein sources. For example, 100 g of *Hermetia illucens* prepupae fed on food waste produced 80–85 g of pressed cake with a high protein content of 53.1%.[160] There is a growing number of institutions and programmes dedicated to researching insect farming as a means to address increasing global feed demands, including the International Centre of Insect Physiology and Ecology, the Sanergy project in Kenya, the Entofood partnership with Veolia in Malaysia, and Innovafeed in France (ESI Table ST6.4†). Introducing insects such as *Hermetia illucens* as protein feed substitutes for livestock and aquaculture could bring significant socio-economic benefits such as job creation and circular economy opportunities.

Safety of insect protein

With regards to safety and quality assurance of insects for food and feed purposes, a major complicating issue is with regards to the high content of insect-borne microbes, that if not treated effectively, can be a source of food/feed-borne disease.[161] Commonly employed microbiocidal techniques such as commercial and domestic cooking (*e.g.* boiling) are effective as destroying the microbiome post-harvesting, and subsequent drying and refrigeration can be employed to maintain sterility.[162] However, due to the large biodiversity of insects and consequently large variability in microbiome composition, safety assurance can become more complicated. For example, spore-forming bacterial species such as *Bacillus* sp. and *Clostridia* can effectively survive traditional microbiocidal techniques.[163–165] It is therefore imperative that pathogen testing is performed pre- and post-processing as part of a food

and feed safety assurance protocol, yet this remains a challenging endeavour.[166] The application of new microbiocidal techniques such as high-pressure processing should be investigated at scale, which have demonstrated greater effectiveness at eliminating spore-forming bacteria while mitigating negative impacts on functional and sensory properties of insects that occur during high temperature processing.[43] Another safety concern is allergenicity and digestibility of consumed insects. Post-processing using protein extraction and hydrolysis techniques may be required to reduce risks of adverse reactions to compounds found in whole insects such as high chitin and uric acid content.[90,109,149,167] As insect processing is relatively poorly optimised for efficiency and safety, greater research focus is required to improve the efficacy of commercialisation of insect protein globally, and to improve consumer and regulatory acceptance rates.[166,168–171]
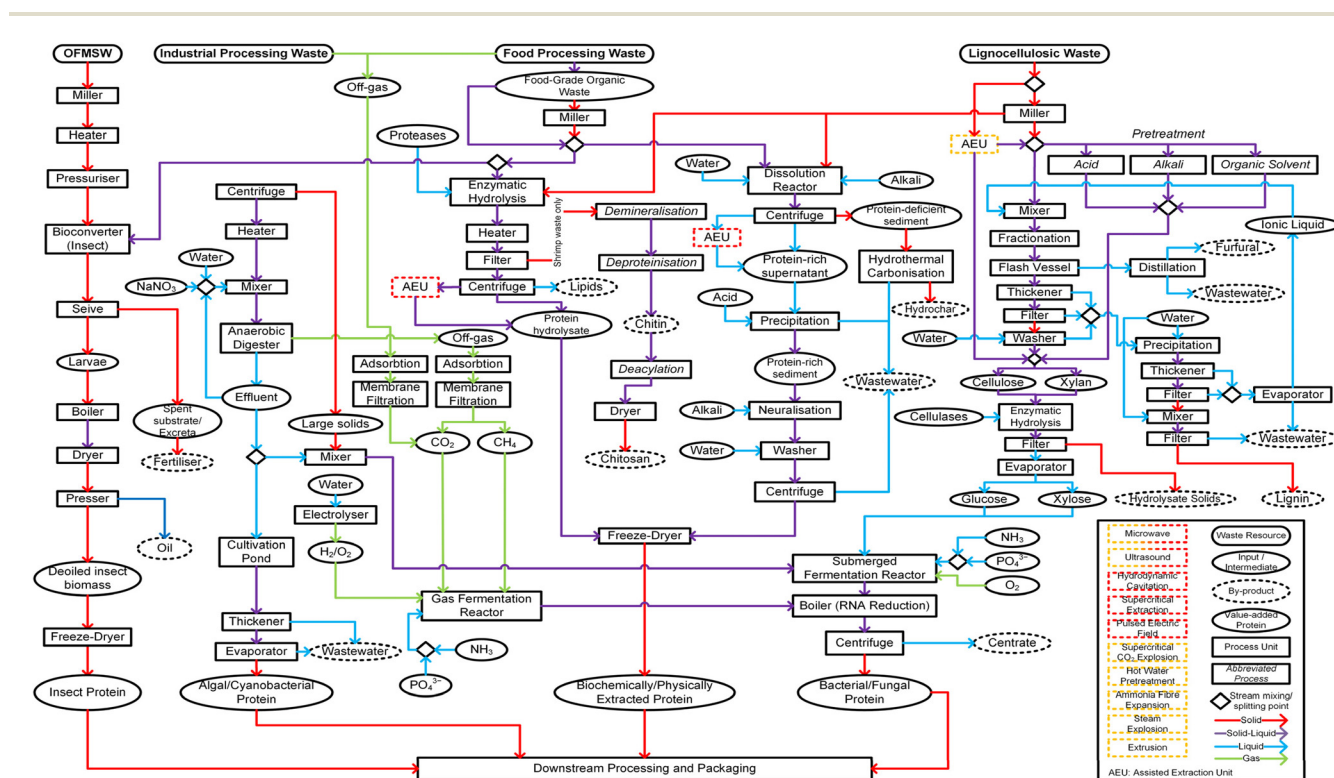
Waste-to-protein system

A waste-to-protein system has the potential to converge waste-recovery and protein security towards a resource-circular protein future. To date, waste-to-protein technologies have been safely developed and scaled-up including the food-waste derived insect protein as animal feed (*e.g.* Entofood and Livalta technologies) and waste-gas to microbial protein as aquafeed (*e.g.* Deep Branch gas fermentation technology). Under the waste-to-protein vision, we propose to synergistically integrate biotechnologies to maximise the recovery of food or feed-grade protein from contaminant-free organic waste while systematically considering regional characteristics on a global scale. This initiative would consider waste resource abundance and composition as well as existing industries and waste recovery infrastructure. Specifically, there is a need to develop and introduce efficient logistical approaches of supply and demand in cooperation with regulators and feed/food safety authorities.
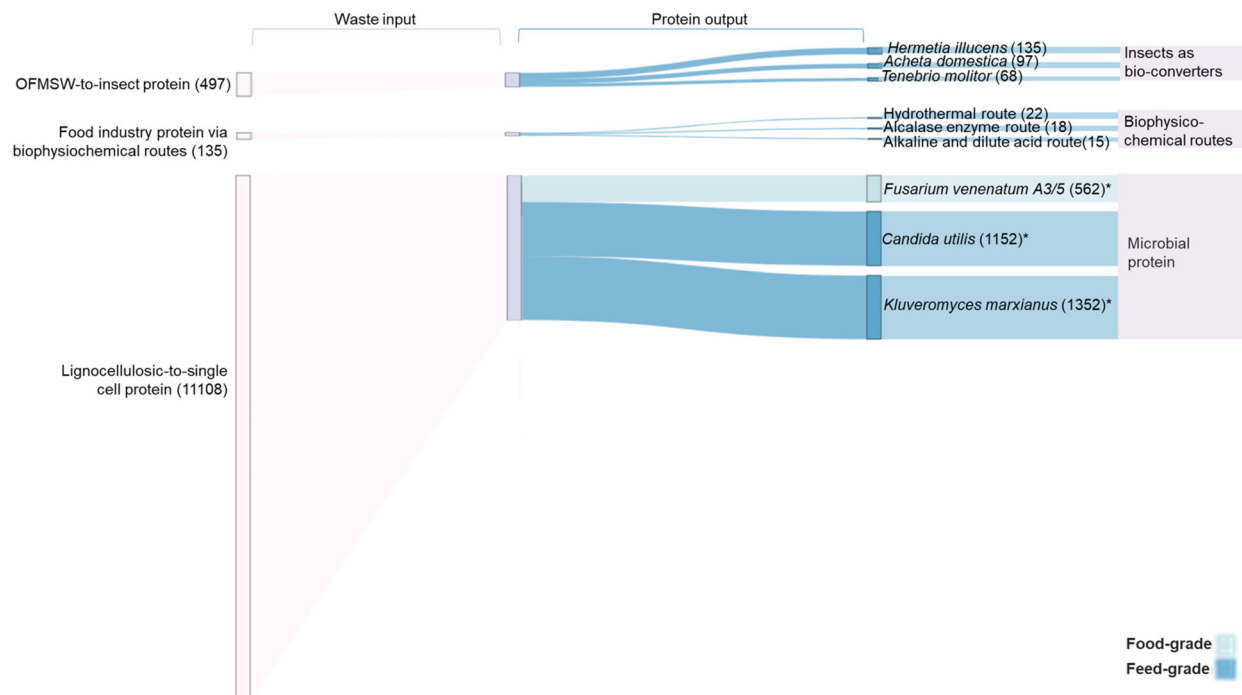
Bioprocess analysis

Fig. 5 displays a range of chemical, physical, and biological processes that can be applied to extract protein and nutrients directly from waste, or to convert waste-carbon to sugars or other platform chemicals for subsequent protein production. As presented in Fig. 6, considerable amounts of food/feed grade waste are generated globally every year, including feed-grade OFMSW, lignocellulosic waste from agriculture and forestry sectors, and waste streams from the food and drink industry. Our estimated protein recovery potential was based

Fig. 5 Waste-to-protein system. Process flow diagram demonstrating potential pathways for protein valorisation from organic fraction of municipal solid waste (OFMSW); industrial processing waste; food processing waste; and lignocellulosic waste to obtain value-added protein. Nodes: rectangle (rounded) = waste resource; oval (thin border) = input/intermediate; oval (dashed border) = by-product; oval (thick border) = value-added protein; rectangle = process unit; rectangle (italicised font) = abbreviated process; diamond = stream mixing/splitting point. Stream arrows: red = solid phase; purple = solid–liquid mixture; blue = liquid phase; green = gas phase. Assisted extraction unit (AEU) (red-dashed) refers to any of the following: microwave; ultrasound; supercritical extraction; pulsed electric field. AEU (orange-dashed) refers to any of the following: microwave; ultrasound; supercritical CO$_2$ explosion; hot water pre-treatment; ammonia fibre expansion; steam explosion; extrusion.[18,70,90,166,235–245]

Fig. 6 Quantitative mass balance for a theoretical waste-to-protein system. Input waste streams are shown on the left: OFMSW-to-insect protein, agricultural lignocellulosic-to-microbial protein, and food industry (including fishing, aquaculture and brewery industry) protein *via* biophysicochemical routes. The protein outputs are shown on the right. *Candida Utilis* and *Kluveromyces marxianus* are capable of utilising hexose and pentose sugars. Values given are for glucose utilisation only. Inclusion of pentose sugars increases conversion outputs to an upper range of 893 megatonne per year, 1831 megatonne per year and 2149 megatonne per year for *Fusarium venenatum A3/5*, *Candida utlilis*, and *Kluveromyces marxianus*, respectively. All values in brackets are given in megatonne per year. Detailed data can be found in ESI-5 and ESI Table ST5.†

on conversion rates (ESI Table ST5.3†) of different technologies reported to be food- or feed-grade. With highly efficient insect bio-converters, it is estimated that 68 to 135 megatonnes per year of insect proteins could be recovered from carbon-rich OFMSW waste, depending on the insect species employed. Microbial protein technologies represent an effective lignocellulosic carbon-to-protein conversion pathway, offering protein recovery in the range of 562 megatonnes per year using food grade *F. venenatum*, or up to 1352 megatonnes per year using feed-grade *K. marxianus* species. The estimated protein recovery potential from global food and drink industry waste (135 megatonnes per year) ranges between 15 to 22 megatonnes per year. However, our estimated recovery value focuses on *F. venenatum* due to its history as an widely-accepted food source.[136] This pathway offers a potential 562 megatonnes per year recovery of food protein from the 11 108 megatonnes per year cellulosic waste content produced by global agricultural and forestry sectors, supplying 72 g per capita per year (197 g per capita per day) waste-derived protein to meet the average adult daily protein recommendation (50 g per 70 kg).[172] However, as these estimates were based on conversion rates derived from literature data, further characterisation of region-specific waste composition and exploratory research on resource recovery potential at scale are essential to provide evidence for informed decision-making.

Geographical analysis

It should be noted that both waste compositions (Fig. 1 and 2) and existing waste recovery systems differ significantly across countries. Developed and urbanised regions tend to produce higher quantities of MSW with a lower organic component than low-income countries and offer established centralised waste collection and treatment infrastructure. Thus, centralised waste-to-protein systems represent great potential for increased efficiency.[15] In less developed countries, there are still large amounts of untapped waste resources including OFMSW, agricultural and forestry lignocellulosic waste, and food and drink industry waste that represent unexploited future potential for a waste-to-protein system.[30] The more sporadic distribution of organic waste and lack of sustainable waste-recovery systems positions decentralised waste-to-protein solutions as the most suitable approach for such countries. Examples include those in recent studies focused on *Hermetia illucens* as bio-converters of food processing waste, and microbial protein routes developed by Deep Branch for aquafeed production from decentralised waste gas streams.[173,174] The significant global variations discussed above call for a systems approach to synergistically integrate centralised and decentralised technologies and optimise waste-to-protein solutions, which consider the spatial distribution of regional waste and existing industries and infrastructures.

Consumer perception analysis and safety

Perceptions of a 'waste-to-protein' concept vary significantly by country and also warrant consideration. African and South East Asian countries appear to be good candidates for expansion of technologies that utilise insects as bio-converters due to their relatively strong cultural acceptance of insects as food.[175] Microbial fermentation is already well-established in Europe and North America, with Quorn™ being a popular and mainstream food product in both regions. These regions would therefore be a good target for expanding microbial protein technologies. It is essential that upgraded 'waste-to-protein' products are regarded as high-quality and safe by consumers globally. As such, conversion and upgrading must proceed within the conditions set out by the feed/food chain alliance and must comply with hygiene quality and safety standards set by regulators[176,177]

Regulatory analysis

New protein sources have been highlighted as novel food, which need to meet general food safety requirements stipulated in national or regional food safety regulations.[178] Global approaches to the regulation of novel food vary significantly. In the EU, Canada, Singapore, and India, evidence of 'history of safe use' (HOSU) is considered globally, whereas in China, Australia/New Zealand (AU/NZ) and Brazil, the scope of HOSU is restricted to native consumption.[179–183] AU/NZ and Canada are exceptional in that there is no rigid cut-off date defined for HOSU, giving their respective regulatory authorities an extra degree of freedom in determining novel status.[179] In these countries, if a protein for food purposes is deemed novel by the responsible authoritative body, a risk assessment is then undertaken considering evidence submitted in the form of a dossier by the manufacturer.[179–183] Pre-submission consultations can help to identify missing information and errors in the dossier to avoid 'clock-stop' delays in the risk assessment stage. Food Standards Australia/New Zealand, Singapore Food Association and Health Canada have established organisations specifically for this purpose.[179,180] In the US, novel status is commonly self-determined by the manufacturer in accordance with generally recognised as safe (GRAS) standards, through convening of an expert panel to review publicly available scientific data on the HOSU of their product.[179] Alternatively, a food additive petition can be submitted to the Food and Drug Administration. However, data from in-house testing pertaining to safety of the product is required in this case, incurring similar issues of high cost and extended timelines from submission-to-market as in countries adopting an EU-style model.[179,184] Further details on global novel food/protein regulations and notification processes can be found in ESI-7.†

Recent regulatory advances on waste-to-protein for animal feed purposes in the EU includes Regulation (EU) 2021/1372, an amendment that allows the use of insect-processed proteins as feed.[185] Subsequently, Regulation (EU) 2021/1925 was implemented to authorise the use of *Bombyx mori* (silkworm) processed animal proteins in animal feed, the eighth insect

species to be approved.[186] It should be noted that regulatory discrepencies between feed and food safety assurance criteria for novel proteins exist. For example, the Singapore Food Agency requires that substances used to feed insects are "properly handled and traceable" to ensure the safety of insect-derived animal feed, but does not require that microbiocidal post-harvest treatment is performed to destroy pathogens, which is required when harvesting insects for human food purposes.[187] Furthermore, EU regulations allow the use of processed animal protein derived from eight species of insects for aquaculture, poultry, and pig feed purposes.[188,189] However, substrates used for the cultivation of insects for human food purposes are restricted to those of vegetal origin, restricting the scope of waste-to-protein system with respect to the types of waste that can be valorised for a particular region and usage purpose.[186,189,190]

Future research and technology development

Insect proteins and microbial proteins offer environmental advantages over conventional animal-source or plant-sourced proteins, in particular on climate change mitigation and arable land use reduction (ESI-8 and ESI Table ST7†). However, novel protein research and technologies are still at the infant stage in contrast to conventional protein sources, which operate at higher technological readiness levels (TRL) 7–9. Thus, future research into waste-to-protein scale-up potential, particularly with regards to process integration and optimisation, is necessary to enable novel waste-to-protein products to become economically competitive.

Safety/nutritional assurance and process efficacy at each stage of the waste-to-protein system remains a complex and significant barrier to implementation. There are significant knowledge gaps regarding the efficacy of novel waste-to-protein technologies, particularly insects/microbial protein as bioconverters, subcritical water hydrolysis, and assisted extraction techniques (Table 1–4). Due to large compositional variety of waste feedstocks (and species of bioconverters), the effects of these processing methods on protein structures and subsequent functionality are not comprehensively understood. Hence, greater research efforts are required to formalise the underlying mechanisms of protein extraction for novel technologies. Additionally, feeding trials and rigorous testing should be performed for protein valorised from a wide range of waste feedstocks to assess the allergenicity, digestibility and toxicity potentials.

Furthermore, knowledge of the regional regulations regarding feed-safe and food-safe novel proteins should be a major consideration when defining key design specifications for industrial production, such as feedstock type and composition, protein valorisation technology flowsheet and operating conditions, as well as product functionality and purity. On the other hand, despite promising process efficiencies, protein yields and functionalities at lab-scale demonstrated by novel technologies, future work should also focus on pilot and/or industrial studies to evaluate scale-up effects on the process performance and product quality. Utilising this information to

perform technoeconomic analyses and process optimisation is key to maximising the value of waste-derived protein while minimising environmental impacts and comporting with regional safety assurance standards.

Nevertheless, new protein sources have the potential to contribute towards food systems that operate within scientifically defined targets for sustainability, both at local and Earth system scales, *i.e.* planetary boundaries.[246]

Overall, it is not only conversion efficiency and nutritional quality of proteins recovered from waste that are of importance, but also the processability, scalability and acceptability of a waste-to-protein system that are highly relevant to future work. Thus, future research and technology development should focus on the waste resources and protein solutions that (i) offer food- or feed-grade nutrition values; (ii) are easily processed and harvested, and thereby able to fit into existing food supply chains; (iii) consider perception, safety and acceptability to the consumers and regulators; and (iv) advance the understanding of waste-to-protein technology performance, including process optimisation at scale, techno-economic viability, and environmental sustainability.

Conclusions and future work

Animal-sourced proteins are not only carbon-intensive and resource-demanding, but also vulnerable to pandemic effects (*e.g.* Covid-19) due to long-production cycles (except for chicken, ESI-6†) and animals being susceptible to infection. These factors, combined with increasing protein demands and the persistent global hunger pandemic, highlight the complex challenges of ensuring protein security for human health within environmental boundaries. In this quantitative analysis, we have proposed a waste-to-protein upgrading system. By synergistically integrating waste-to-protein technologies, this system has the potential to solve a significant component of the global challenge of a planet degrading food system and converge innovations on zero-waste and protein security towards a sustainable protein future. Our study emphasises the importance of upstream quality preservation by assuring contaminant-free organic waste streams and systems analysis to estimate the waste-to-protein potential involving chemical, physical, and biological conversion pathways. We quantified global waste streams, which are rich in carbon and nutrients and absent of pathogens and hazardous substances. These streams present a global annual resource potential of 497 megatonnes of OFMSW, 135 megatonnes of by-products from the brewing and shrimp fishing industries and 11 108 megatonnes of lignocellulosic agricultural and forestry waste. This is equivalent to 9386 megatonnes of holocellulosic contents, which can be converted to fermentable sugars amounting to 2503 megatonnes of glucose, or 3980 megatonnes of glucose and xylose.

Over 80 microbial species have been discovered to enable efficient waste recovery of microbial protein with preferable amino acid profiles that are characteristic of proteins of high biological value. A concerted effort to broaden the range of micro-organisms is warranted, either independently or in combination with microbiomes or designed cultures that can be regarded as safe for upgrading secondary resources to safe animal feed and foods. Insects as bio-converters offer efficient mechanisms to convert different grades of waste to food or feed proteins, which are generally rich in protein, vitamins, and minerals such as iron, calcium, manganese and zinc compared with other animal-sourced proteins.

Despite advances in individual technologies, critical gaps remain in the development of innovative systems which will enable 'plug-and-play' solutions, synergistic technology integration, and optimisation of the protein recovery from diverse waste streams. Although we demonstrate that waste-to-protein system has the potential to recover waste and catalyse novel protein solutions, scientific targets that define healthy and sustainable protein production remain absent. Integrated assessment and optimisation of waste-to-protein value chains that consider scientifically quantified planetary boundaries[246] represent a future research frontier to further understand the implications of a waste-to-protein transition for water, land, biodiversity, carbon, nitrogen and phosphorus (5 of the 9 planetary boundaries). Notably, evidence-informed regulatory response timelines are considerably lagging behind the accelerated food and feed technology innovations including novel proteins. For waste-to-protein, many aspects remain unknown, such as the quality of low-value waste streams, nutritional values and health effects. Such regulatory barriers hinder the development of waste-to-protein technologies.

Future research to enable deep scanning of the fast-paced protein innovation landscape and develop a system for rapid regulatory response is needed. A sustainable protein system can only be achieved by multi-sector, multi-level actions that include a substantial global shift towards reduction in food loss and waste, and deployment of innovative protein technologies. Under the international policy framework, human health and environmental sustainability are included in most of the United Nations Sustainable Development Goals (SDGs).[247] Integrated analyses of different future diet and protein scenarios and their impacts on humans (SDGs 1 and 2) and on planetary boundaries (SDGs 6, 13, 14, 15, on water, climate, ocean, and biodiversity) are necessary to inform future policy and technology development. A crucial element is the linkage of the waste-to-protein supply chains, environment footprint and the overall regulatory measures in relation to the sustainability and safety of upgrade-protein to help ameliorate the persistent and ongoing hunger pandemic and to protect the planet.

Author contributions

M.G., G.K., A.K. and H.F.R. designed the study. E.P., M.B., Y.H. and M.G. performed research and data analyses. E.P., W.V., P. E., M.B. and M.G. drafted the manuscript sections. J.R., P.S., O.W., C.H., J.H. and M.B. contributed significantly to the

paper revision. A.L. contributed to the data visualisation. All authors contributed to the final paper revision and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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