LIFE CYCLE ASSESSMENT

BIO-MANUFACTURING AT SCALE

ARSENALE BIOYARDS

As the first European venture capital fund, Planet A relies on its own scientific team to assess the environmental and climate impact of an innovation. Prior to an investment, a life cycle assessment, like this one, is conducted and integral part of the investment decision. All assessments as well as the methodology are published for maximum transparency.

Terminology, units and abbreviations

EC	European Commission
EF	Emission factor
EU	European Union
FAO	United Nations Food and Agriculture Organisation
Functional unit	Quantified performance of a product system for use as a reference unit
g	Gram
GHG	Greenhouse gas
IEA	International Energy Agency
kg	Kilogram
kg CO_2-eq.	Kilogram carbon dioxide equivalent
LCA	Life Cycle Assessment
MJ	Megajoule
Rate	Speed at which the desired product is being synthesized by the microorganisms over a certain period. Unit measure g/h
Titer	The concentration or level of the target product (e.g., a specific protein) within the fermentation broth or culture medium. Unit measure g/L.
Yield	Amount of the desired product (e.g., a specific protein) produced by the microorganisms in a bioprocess in relation to the initial substrate. Unit measure in g/g or %.
Productivity	Amount of product made per unit of time (takes into consideration fermentation titer and length. Unit measure g/L/h.

About Arsenale BioYards

Arsenale BioYards offers an end-to-end hardware / software platform to make bio-manufacturing economically viable. They accelerate product development and reduce the cost of manufacturing by enabling design and development in industrial conditions and reduce unit economics by increasing the yield, titer, rate, productivity, and scaling capacity of precision fermentation at industrial scale.

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Summary

The current use of petrochemical-based materials results in severe negative impacts on the environment with petrochemicals and its derivatives accounting for 14% of the global oil and 8% of global gas demand. The chemical industry is one of the largest energy consumers in the industrial sector, responsible for almost 7% of total energy consumption and 1.5 Gt direct CO_2 emissions/ year (IEA 2018). Economically competitive bio-manufactured materials are needed at scale across all industries to support decarbonisation and develop the bioeconomy. Furthermore, bio-manufactured proteins can also help reduce dependency on emission intensive animal husbandry industry which has massive negative effects on the environment: It accounts for 14% of anthropogenic greenhouse gas (GHG) emissions, utilises 30% of ice-free land, and consumes 33% of global freshwater resources (UNFCC 2021). In contrast, bio-manufactured proteins, such as proteins contained in or derived from plants, fungi, bacteria, or insects offer a more sustainable protein supply.

Arsenale BioYards develops end-to-end hardware-software integrated systems for precision fermentation to enable cost effective and sustainable bio-manufacturing at scale.

Our analysis using two exemplary products made by precision fermentation shows that:

- Changing the protein source from a conventional meat protein to an alternative bio-manufactured protein, myoglobin results in:
    - net GHG savings ranging from 21 to 296 kg CO_2-eq. per kg of protein,
    - a reduction in land demand between 42 and 854 m^2a per kg of protein, and
    - net water savings ranging from 8 to 56 m^3 of water saved per kg protein.

- Switching from cow milk-based whey protein to bio-manufactured whey protein products can save 88 kg CO_2-eq. per kg of protein and offers up to 98% GHG emission reduction when compared with diverse whey protein-based products.

Overall, Arsenale BioYards’ innovation will support the transition to a more sustainable bioeconomy by accelerating the transition from petrochemical-based products to bio-based products.

About this study

This study is divided into two parts. **Part I** provides insights into the market for bio-manufactured products, the sustainability potential across various sectors, major challenges and how Arsenale BioYards addresses them. In **Part II**, two exemplary Life Cycle Assessments (LCAs) are presented for alternative proteins and bio-manufactured whey protein as these are two likely product types that Arsenale BioYard could help scale faster. Lastly, a **summary** combines the key findings to provide an overview on the environmental implications of Arsenale BioYards' innovation.

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1. Part I - Sustainability potential of bio-manufacturing

Bio-manufacturing is defined as the use of biological systems to produce goods and services at a commercial scale (NIST 2022). A 2020 McKinsey report titled “The Bio Revolution” points out that as much as 60% of the physical inputs to the global economy could be produced biologically - about one-third of these inputs are biological materials (wood or animals bred for food) and two-thirds are non-biological products (plastics, fuels etc.). Similarly, nearly all key petrochemical groups can be derived from renewable resources like biomass (IEA 2018). Bio-manufacturing enables the bioeconomy. According to FAO, bioeconomy is "the production, use and conservation of biological resources, including related knowledge, science, technology, and innovation to provide information, products, processes and services to all economic sectors with the aim of moving towards a sustainable economy" (Thaís Linhares-Juvenal and Pierre Bouillon 2018).

The European bioeconomy generates a turnover estimated at around 2,4 trillion euros (2019 figures) (nova-Institute for Ecology and Innovation 2022). The European Union's (EU) commitment to shifting from a fossil-based economy to a sustainable and circular economy is articulated in the "Bioeconomy Strategy" from 2012 and updated in 2018. It ties into the "European Green Deal", which aspires to make the EU climate-neutral by 2050. Figure 1 (see below) depicts the relationship between the green economy, bioeconomy, bio-based economy, and circular economy.

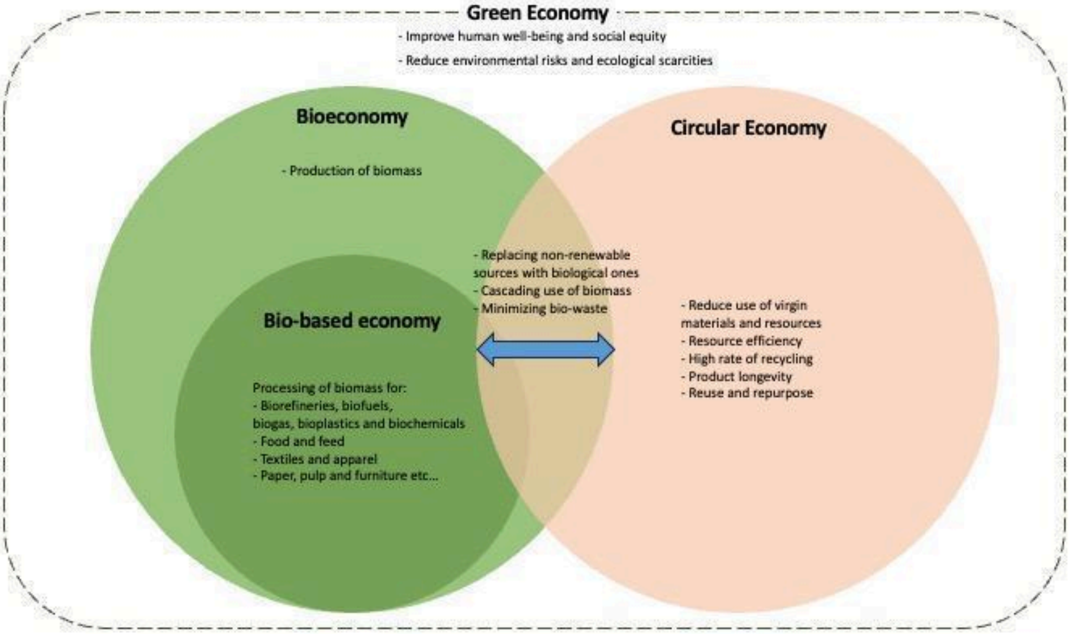


Figure 1 *Overview of opportunities in industrial biotechnology (adapted from Kardung et al. 2021).*

1.1. Conventional materials and bio-manufactured substitutes

The following section discusses the role of petrochemicals, protein sources, and their bio-manufactured alternatives as well as their effect on the environment.

1.1.1 Environmental impact of conventional materials

Petrochemicals are the invisible fabric of modern society. They are used in most industrial products such as plastics, synthetic rubber, solvents, fertilisers, pharmaceuticals, additives, adhesives, explosives etc. They are also used in many consumer goods such as cars, building materials, paints, clothing, packaging etc. (R.J. Clews 2016) and even in 'non-intuitive' products such as food colourings, food preservatives, cosmetics, detergents, medicines, crayons etc. (CPV 2023).

Petrochemicals and its derivatives account for 14% of the global oil and 8% of global gas demand and the chemical industry - being one of the largest energy consumers in the industrial sector (equal to the steel and cement sectors combined) - was responsible for about 7% of total energy consumption and 1.5 Gt direct CO_2 emissions (IEA 2018). IEA 2018 further suggests that it is one of the biggest "blind spots" in terms of energy demand despite the chemical industry having one of the highest foreseeable growth trajectories.

In addition, the end-of-life emissions of the chemical sector, which are usually accounted for in downstream sectors (e.g., ammonia used in the agriculture sector), are estimated to be equivalent to the direct emissions, i.e., in reality, the total emissions from chemicals are close to twice the emissions of the chemical industry (Huang et al. 2021). Apart from high energy demand and GHG emissions, the manufacturing, use and end-of-life of petrochemicals and their derivatives also have severe negative impacts on freshwater and biodiversity (Sharma et al. 2017).

Another key area contributing to global emissions, energy consumption, and natural ecosystems loss — underscoring the interconnectedness of industrial activities and environmental sustainability is the food production industry. Specially, our current **protein sources** have a major environmental footprint. For instance, livestock contributes significantly to global environmental issues, accounting for 14% of anthropogenic greenhouse gas (GHG) emissions, utilising 30% of ice-free land, and consuming 33% of global freshwater resources (UNFCC 2021), and it has many negative effects on biodiversity, water pollution as well as land use change. More broadly, agriculture accounts for a high share of anthropogenic GHG emissions, ranging from 24 to 37% of total anthropogenic GHG emissions if all impacts of meat production are included (Xu et al. 2021).

1.1.2 Environmental impact of bio-manufactured substitutes and industrial biotechnology

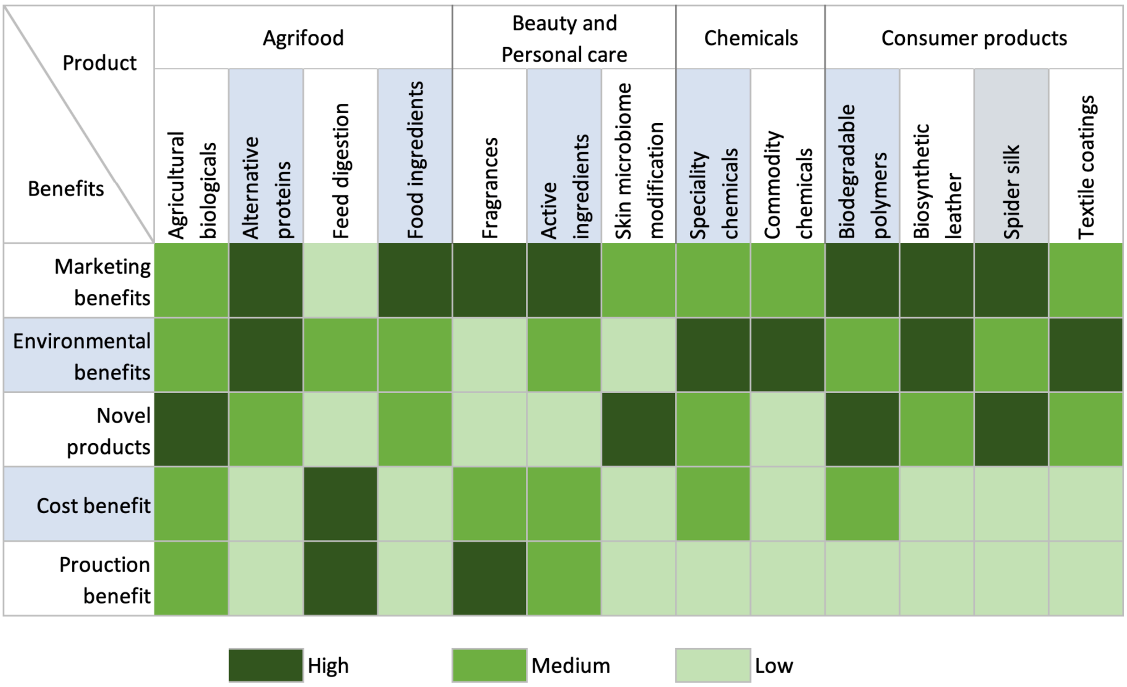
Scientific literature suggests that replacing oil and natural gas feedstocks with biomass-based sources can lead to 88% GHG emission reduction under most conservative estimates (i.e. 25% conversion and high separation energy) and up to 94% GHG emission reduction under most optimistic estimates (i.e. 75% conversion and easy separation) (Huang et al. 2021). The 'Rainbow Code of Biotechnology (Paweł Kafraski, 2012), assigns different color codes to biotechnological applications based on their use cases in various domain, reflecting the versatility and potential of biotechnology across various sectors.

Industrial biotechnology processes enable sustainable large-scale industrial production of products and ingredients such as biomass, chemicals, proteins, small molecules (including volatiles), medicines and biologics etc. The processes can produce high yields of specific products with low energy use and minimal waste generation (EuropaBio and ESAB 2005). It uses enzymes and / or microorganisms to manufacture products in sectors such as food & beverages, cosmetics, chemistry, feed, paper and pulp, textiles, and energy. For example, it could provide new chances to the chemical industry by allowing easy access to building blocks and materials that were only accessible before via complicated and expensive processes or not at all. The use of renewable raw materials as alternative feedstock will reduce consumption of fossil resources and energy consumption (EuropaBio and ESAB 2005).

More specifically, **alternative proteins** offer promising solutions to combat the environmental challenges posed by traditional animal agriculture. Precision fermentation, a groundbreaking technology, can revolutionise the production of animal-sourced foods like red meat and whey by creating identical proteins using microorganisms, reducing GHG emissions, land use change, and freshwater impacts. This aligns with the growing global awareness of the need to mitigate climate

change, as over 30% of current alternative protein consumers prioritise climate impact alongside animal welfare and health considerations (Grasso et al. 2019; Ibrahimi Jarchlo and King 2022).
Recent trends indicate a surging interest in alternative proteins. Sales have increased by 38% in recent years (Jahn, Furchheim, and Strässner 2021), driven primarily by "Flexitarians" who occasionally incorporate plant-based meat alternatives into their diets. Globally, 36% of consumers intend to consume more alternative proteins (Joseph et al. 2020), with projections suggesting they could comprise up to 22% of global protein consumption by 2035, leading to a decline in animal protein consumption. Investments in fermentation-based animal-cell meat have risen by 150% to $1.7 billion in 2021, and the European substitute protein market is projected to reach 3.5 billion by 2027, enhancing taste, texture, and affordability (Morach et al. 2022).

Figure 2 gives a high-level overview of the benefits of industrial biotechnology across different sectors (non exhaustive, excluding pharmaceuticals).



Product / Benefits	Agrifood				Beauty and Personal care			Chemicals		Consumer products			
	Agricultural biologicals	Alternative proteins	Feed digestion	Food ingredients	Fragrances	Active ingredients	Skin microbiome modification	Speciality chemicals	Commodity chemicals	Biodegradable polymers	Biosynthetic leather	Spider silk	Textile coatings
Marketing benefits	▣	■	□	■	■	■	▣	▣	▣	■	■	■	▣
Environmental benefits	▣	■	▣	▣	□	▣	□	■	■	▣	■	▣	■
Novel products	■	▣	□	▣	□	□	■	▣	□	■	▣	■	▣
Cost benefit	▣	□	■	□	▣	▣	□	▣	□	▣	□	□	□
Prouction benefit	▣	□	■	□	■	▣	□	□	□	□	□	□	□

■ High ▣ Medium □ Low

Figure 2 *Overview of opportunities in industrial biotechnology (adapted from Nielsen et al. 2022)*

Below is a non-exhaustive list and impact potential of industries (TAB 2016):

<u>Chemical industry</u>

Biochemicals have an immense potential for enzymes (fine and speciality chemicals) where high specificity, selectivity, and activity under moderate reaction conditions offer comparative advantages compared to 'conventional synthetic chemistry'. Biochemicals have the potential to create large-scale impact by replacing fossil-based bulk and platform chemicals with both direct (current drawback: price parity) and functionally substitutable (e.g. bioplastics, spider silk protein etc.) products. The chemical industry in Germany has set the goal of using 50% more renewable raw materials for its processes by 2030 as compared to 2015 (Verband der chemischen Industrie e.V. 2015).

<u>Personal care, detergents, and cleaning products</u>

For personal care, biochemicals have potential applications in hair colour, sunscreen & after-sun products, peelings and dental care products. In detergents and cleaning agents, biochemical enzymes with specific functions lead to increased flexibility in the formulation of detergents, higher tolerance of the enzymes to the washing conditions and occupational and consumer safety.

Food and Beverage industry

Biotechnology plays an important role in food and beverage production. Food and beverages have been produced using fermentation methods for thousands of years. The initially traditional and manual processes have been enhanced with modern scientific methods and are being further developed and optimised for industrial scale production.

Figure 3 provides an overview of the use of fermentation products in different applications.

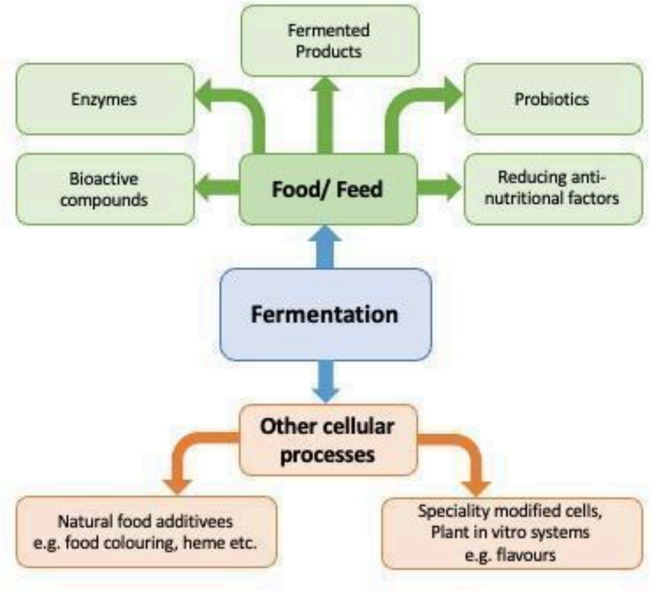


Figure 3 *Fermentation enabled food valorisation potential (adapted from Boukid et al. 2023)*

Textile industry

Wet-chemical processes traditionally used to process the fibres offer the potential to supplement or replace chemical processes with biotechnical ones. Biomolecules immobilised on the textile matrix is another opportunity to open new doors to develop biomedical textiles, textile-based biosensors, environmental remediation, functional textiles etc.

Leather industry

Leather production involves more than a dozen wet-chemical process steps where enzymes are used. In addition to increasing the efficiency of the enzymes, the biotechnical process can also help with enzymatic dehairing, extraction of recyclable material from residues of leather production and leather recycling (recovery of chromium from chromium-tanned leather, conversion of used leather into valuable materials etc.).

Pulp and paper industry

Biotechnical processes can replace the use of petrochemicals along the entire value chain of paper and pulp production. Biochemicals can enable enzymatic removal of bark, production of fibrous materials (biopulping), production of ‘dissolving pulp’, bleaching of fibrous materials (bio-bleaching), enzymatic fibre modifications, pitch removal, control of slime formation in paper manufacture, removal of sticky inks (de-inking) and control of sticky contaminants.

Mining and metal industry

Biochemicals have the potential to enable and transform many processes in these industries like 'biomining' which refers to technical processes that use the metabolic performance of certain microorganisms to extract metals from ores or residues, 'bioleaching' which refers to metal-dissolving bioprocesses and 'bio-oxidation' which is the release of the metal by dissolving the surrounding mineral.

Environmental biotechnology

Biotechnological processes have been used in aftercare environmental technology for decades, i.e. in waste-water treatment in sewage treatment plants, organic waste treatment, in exhaust air purification plants, in the remediation of contaminated soil, bodies of water and groundwater as well as sediments. Innovation in biotechnical processes and biochemicals opens new avenues such as increasing the performance of established processes, minimising the production of biomass as an (undesirable but unavoidable) by-product of the actual degradation and purification processes and further reducing the area and bioreactor volume requirements of the processes and developing advanced biosensors.

Automotive industry

Many bio-manufactured products are relevant for the automotive industry like bio-plastics, bio-lubricants, biobased paints, rubbers, bio-adhesives, and biofuels. Biotechnical processes and bio-manufactured products can play a significant role in changing to more climate-friendly raw materials and by increasing the efficiency of motor vehicles (e.g. through lightweight construction). Biotechnical developments in fuel cells in another avenue to challenge drivetrain options like lithium-ion batteries.

In a modern car, around 20% are made of plastic (by weight of the components); this is about 100 to 150 kg/car and in 2013 world production of passenger cars by German automobile manufacturers was 14.1 million cars. The automotive industry could thus be a major potential customer for bio-manufactured plastics, resulting in significant GHG emission savings.

Biopharmaceuticals, another promising use case, initially drove biomanufacturing, laying the groundwork for research that facilitated advancements in other biotechnology domains, including industrial biotechnology.

Biopharmaceuticals are drugs that are produced using living cells or organisms, such as bacteria, yeast, or mammalian cells. Biotechnical processes and biochemicals are a boon for the industry as they enable the development of biosimilars (biopharmaceutical equivalent of generic drugs) and biobetters (advanced versions of existing biopharmaceutical drugs). These drugs have revolutionised the treatment of many diseases, including cancer, autoimmune disorders, and genetic diseases (Malgorzata Kesik-Brodacka 2017).

Biopharmaceuticals contain 'recombinant proteins' as their active pharmaceutical ingredient which can be produced using different microbial expression systems, including yeast and fungi. These systems have several advantages over traditional methods of biopharmaceutical production, including the ability to produce proteins with proper folding and post-translational modifications, which are essential for the efficacy of many biopharmaceuticals. It has the potential to reduce the cost and complexity of pharmaceutical production, while also addressing key challenges by improving the quality and consistency of these drugs (Malgorzata Kesik-Brodacka 2017).

The sheer breadth of application of bio-manufactured products signifies that bioeconomy can be regarded as a key driver of the transition towards a sustainable and climate-neutral economy. However, there are many challenges that must be addressed in order to fully realise the potential of this technology, including regulatory hurdles and the need for more research and development to enable cost effective scale up and commercialization. These challenges and Arsenale BioYards' contribution are discussed in the next section.

1.2. Challenges and Arsenale BioYards' contribution

The following section outlines the biggest challenges (opportunities) in the bio-manufacturing space and gives a brief overview of Arsenale BioYards' potential to address these challenges.

1.2.1 Challenges in the bio-manufacturing (precision fermentation) space

Review of literature sources like Banks et al. 2022; Carter et al. 2023; Katy Askew 2022; Nielsen et al. 2022 and Yu et al. 2019 helped identify several challenges in bio-manufacturing. Apart from consumer acceptance and regulatory approvals the primary challenges/ barriers to enabling bioeconomy are:

Technical challenges and equipment innovation: Scaling up precision fermentation involves addressing technical challenges related to the fragility of cells used in the process. Production parameters can vary and even fluctuate significantly when scaling up from lab scale to industrial scale equipment as biology is context dependent and often with limited predictability. Achieving commercial-scale production often necessitates significant innovation in equipment and processes.

Collaboration and De-risking: Collaborating with other experts, including those from different industries, can help de-risk the scaling process. Learning from cross-industry experiences and outsourcing certain parts of development and production can accelerate innovation and reduce the likelihood of costly mistakes. A notable example of this is the biofuel industry's online process monitoring and control technology for ethanol production plants which was derived from the online monitoring processes used in refineries in the petrochemical sector (Glen Austin et al., 2015).

End-Product Considerations: Scaling requires careful consideration of the end-product and downstream processing. Ensuring that the final product aligns with market needs and can be efficiently processed at scale is crucial for successful expansion.

Digital Technologies: Reliable collection of experimental data and the subsequent use of digital tools, such as computational modelling and software development, can help mitigate risks by allowing companies to simulate and optimise feedstock options, substrate behaviour and production processes before physical implementation, reducing trial-and-error efforts. It also enables efficient product development at lower cost.

Price Parity: Economies of scale are seen as a solution to reduce unit costs and make bio-manufactured products more competitive with conventional products. The key factor in reaching this goal is maximising throughput and productivity, measured in grams per litre per day, which requires advanced technology and innovation. Exploring other by-product or industrial waste substrates can also help lower the cost of goods manufactured.

1.2.2 How does Arsenale BioYards address these challenges

Arsenale BioYards with their precision fermentation at scale technology using yeast such as *Saccharomyces cerevisae* and *Pichia pastoris* at first and exploring other hosts such as fungi or bacteria afterwards, allows for the production of numerous products, including but not limited to proteins, chemicals or biologics across various industries. This makes Arsenale BioYards a key enabling technology player in unlocking biomanufacturing.

Arsenale BioYards' technology addresses several challenges mentioned above and has multiple benefits including time- and cost-efficient scaling, access to production capacity, and achievement of economic viability:

Navigating across scales: Small scale bioreactors can be used to explore a broader option space of conditions which could be encountered at large scale. With these trials in the small scale bioreactors, combined with a **"**design at scale" based modelling approach, the economic viability of the strain and bioprocess will be better understood and anticipated in the large scale bioreactors, allowing further strain and process improvements.

Efficient bioprocess: Arsenale BioYards employs a two phase production process (fed-batch) through multiple industrial scale bioreactors in its BioYards. Biomass is built in the first phase ("batch" phase), and substrate is added to the bioreactor in increments throughout the rest of the process ("fed-batch" phase). This method provides a higher conversion rate, diminishes substrate and end product inhibition, reduces viscosity of the culture broth, reduces water loss by evaporation, etc.. The use of multiple smaller reactors reduces the risk and consequence of failures, and it allows for a faster adoption and modification of process parameters.

Efficient scaling: The large scale bioreactors are designed for container transport, standardisation and scale out of equipment with the help of a modular setup mechanism based on the required product. This helps expand production capacity faster and produce bio-manufactured products in the desired scalable quantities and in a distributed network of BioYards.

Leveraging data: Live sensing and actuation algorithms help faster and efficient design of host organisms and production of products. Over the course of time, the collected data will support development of new feedstocks, substrates, bioprocesses, equipment and products and act as a backbone of sustainable bioeconomy.

2. Part II - Exemplary LCAs

This section provides exemplary life cycle assessments (LCA) of two products: alternative proteins and whey protein which can be bio-manufactured using Arsenale BioYards' technology. It also describes the usefulness of LCA in the context of bio-manufacturing to enable product and industry selection for maximising environmental benefit.

2.1. Alternative proteins and myoglobin

Livestock rearing has many negative effects beyond GHG emissions, impacting biodiversity, water use and pollution, as well as land use and land use changes, as outlined in Section 1.1.1. In contrast, alternative proteins, such as proteins contained in or derived from yeasts, fungi, bacteria, and insects offer a more sustainable protein supply. It is estimated that there could be a +100% increase in exclusive or near-exclusive consumers of plant-based proteins if the main barriers like cost, taste, texture, and nutritional value are resolved (Morach et al. 2022).

Fermentation proteins, such as the ones developed by Planet A`s portfolio company Paleo, can significantly improve taste and colour while reducing the ingredients of meat alternatives, such as iron supplements etc. Paleo's myoglobin (and many other fermentation proteins) can be produced efficiently at scale using Arsenale BioYards' technology. Thus, this section presents an LCA assessing the environmental benefits of Paleo's bio-manufactured product.

2.1.1. System description

The aim of this LCA study is to assess the potential systemic changes in environmental impacts of a switch in diets from conventional animal-based proteins (beef, mutton, lamb, pig and poultry) to bio-manufactured fermentation proteins. A consequential LCA approach was applied to assess the impact on the environment. The approach evaluates marginal changes within the overall economy because of a change in the market structure (e.g. entry of Paleo's bio-manufactured myoglobin), production modalities, demands as well as political, consumer or any other decision affecting the former aspects (Ekvall et al. 2016). To account for marginal changes, marginal data is used wherever possible, e.g. marginal suppliers are identified and the change in their production output is considered (in contrast to using market averages).

Paleo's myoglobin addresses several barriers regarding the market uptake of alternative protein products and will likely lead to an increase in demand for alternative protein products and a decrease in the demand for conventional meat products. On a systemic level, this change comprises an increase in all production processes related to Paleo's myoglobin production, additional production processes to produce alternative protein products, and less demand for conventional meat (Figure 4). Additionally, the demand for glycerol, used in the fermentation medium of Paleo, might trigger additional market responses which are explained in our detailed LCA report on Paleo along with the product level environmental impact assessment of Paleo's myoglobin production and sensitivity analysis for displacement effects and parameter variation.

Functional unit and indicators

The LCA comprises three main elements: The impact of Paleo's myoglobin production, the impact of protein supply and the systemic change in environmental impacts resulting from a change in protein consumption. The functional unit is **one kg of protein** (Paleo's myoglobin, other conventional animal proteins and alternative proteins).

The system is assessed using the indicators **climate change** (Intergovernmental Panel on Climate Change (IPCC) 2014) and several indicators related to resource use, **cumulative fossil energy demand** - CED$_f$ (Verein Deutscher Ingenieure (VDI) (ed.) 2012), **land demand** and **water demand** - WSI (Pfister, Koehler, and Hellweg 2009).

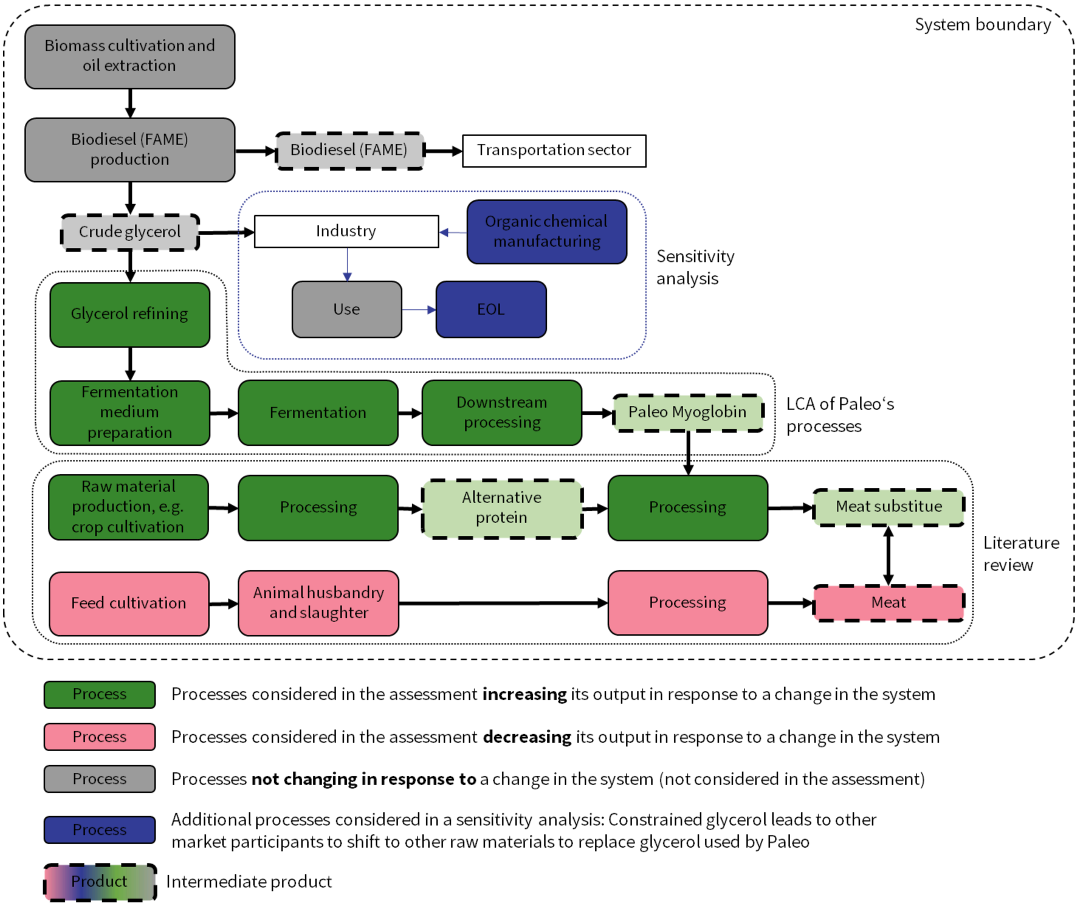


Figure 4 *Depiction of system boundaries. The grey process steps do not change in response to a shift from meat-based proteins to alternative protein sources. Green processes will increase their output, while red processes reduce their output. Additional processes (blue) are considered in a sensitivity analysis explained in our detailed LCA report on Paleo.*

2.1.2. Paleo's myoglobin production

Paleo's production process consists of the following elements considered in the LCA:

- Medium preparation: The fermentation medium used in the process contains multiple ingredients. The complete list of ingredients is known to Planet A. A cut-off was applied to exclude all nutrients contained in trace quantities. All ingredients of at least 0.1% concentration (1g per L) were considered in the assessment.
- Inoculum production: A seed train is used to produce inoculum containing the microorganisms to start the fermentation process. The inoculum medium, energy requirements as well as technical parameters, such as cell density of inoculation medium, nutrient demand etc. were considered in the assessment. The medium used is a buffered glycerol-complex medium (BMGY).
- Fermentation: Most important parameters, such as fermentation time, myoglobin titer, nutrient demand, technical specifications as well as the demand for energy and auxiliary materials were considered in the assessment. This step, as well as inoculum preparation, can be supported and further improved by employing Arsenale BioYards's precision fermentation at scale technology.

- Downstream processing: In several downstream processing steps, myoglobin is purified, concentrated and preserved by either drying or freezing. Downstream processing includes biomass removal, concentration and purification by successive microfiltration and ultrafiltration steps.
- Additional activities: Equipment sanitation and transportation of the product is included. A transport of 500 km by truck is assumed.
- Infrastructure and its operation: The assessment includes infrastructure that needs to be installed and its operation. The facility was modelled by using a proxy (ethanol production facility contained in the ecoinvent database scaled according to the size of Paleo’s plant). The operation of warehouses, offices and refrigerated storage facilities requires energy. The energy demand for buildings is included in the assessment.

2.1.3. Impact assessment of conventional and alternative protein sources

This assessment comprehensively evaluates the environmental implications of conventional meat production and alternative protein production and draws upon a broad spectrum of peer-reviewed studies and reports, utilising the latest available data and analyses as outlined in our detailed LCA report on Paleo. The goal is to offer a thorough understanding of the potential environmental implications associated with various protein production approaches. This approach was chosen over an assessment of individual production systems to cover the widest range of production modalities and data sources possible. Beyond environmental considerations, the digestibility of proteins was also delved into, as it is a critical factor in assessing their nutritional value. Two widely recognized methods, the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score (DIASS), enable comparisons of protein nutritional quality, considering amino acid composition and digestibility. For a compilation of PDCAAS and DIASS values for the protein sources in this study and our sensitivity analysis for adjusted protein quantities based on PDCAAS and DIASS see our LCA report on Paleo.

2.1.4. Environmental impact of different protein sources

The comparison of literature values of different types of animal- and plant-based proteins shows that plant-based proteins perform much better than animal proteins in terms of environmental impact (Table 1). Novel protein sources, such as lab grown meat, mycelium, insects and microbial proteins are also included in the assessment. In almost all cases they perform better than animal proteins.

Lab grown meat shows a comparably wide range of results indicating a worse performance than other non–conventional animal protein sources. This is most likely because most studies are an ex-ante assessment of future production systems. Since many technical difficulties associated with lab grown meat are yet to be solved, most assessments rely on assumptions as explained in our LCA report on Paleo.

Table 1 *Comparison of GHG emissions, fossil energy demand, land use and water use of different protein sources. All values per kg protein (dry mass). The impact of Paleo's myoglobin is listed, too. The ranges presented refer to all scenarios discussed in section 2.2.3.1. Additionally, values for the base case scenario are provided. Note: The final ready-to-eat product will contain between 0.1% and 1% Paleo myoglobin. Abbr.: Max - maximum, Min - minimum, N - number of values included in the assessment. Data derived from all sources stated in Table 1 of our [LCA report on Paleo](). *

	Climate change (kg CO_2-eq.)				**Fossil energy demand (MJ)**				**Land use (m2a)**				**Water use (m^3)**			
	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max
Bovine Meat (beef herd)	724	302.71	188.42	1350.00	0				724	854.41	353.11	4564.19	724	3.71	01.03	956.85
Bovine Meat (dairy herd)	490	172.95	75.63	287.13	0				490	131.41	62.16	538.86	490	13.24	213.65	1085.21
Lamb & Mutton	757	202.95	118.44	300.65	0				757	636.73	239.13	3621.44	757	2.30	1.29	2974.90
Pig Meat	116	65.33	42.71	147.03	0				116	83.07	45.67	210.69	116	11.19	0.32	941.47
Poultry Meat	326	43.42	22.81	120.21	0				326	63.57	37.30	117.78	326	2.14	0.12	381.32
Tofu	354	16.13	8.81	45.44	0				354	21.31	9.81	36.69	354	0.04	0.08	196.77
Lab grown	25	17.79	7.26	2814.29	17	186.84	9.70	2828.57	17	11.53	2.42	157.14	21	1.32	0.00	4.44
Microbial proteins	5	1.57	0.81	11.56	2	16.97	9.70	24.24	5	0.04	0.00	0.47	5	9.70	01.01	24.24
Mycelium	5	19.16	7.90	55.50	2	310.49	20.29	600.70	5	3.84	0.35	7.90	3	22.32	0.74	714.24
Insects	4	12.32	5.23	21.04	4	156.54	11.13	320.00	4	15.01	4.44	18.07	0			
Plant based	27	5.19	1.90	21.76	25	61.70	11.12	397.00	27	16.36	1.14	55.00	0			
Paleo myoglobin	12	38.55	18.23	98.61	12	596.46	278.53	1534.89	12	5.89	3.55	13.87	12	4.28	2.79	
				Drying: 41.11 Freezing: 24.39				Drying: 639.99 Freezing: 373.42				Drying: 5.78 Freezing: 4.57				Drying: 3.99 Freezing: 3.60

2.1.5. Impact of a change in protein supply

Final ready-to-eat products contain between 0.1 and 1% Paleo myoglobin. Changing the protein source from a conventional meat protein to an alternative results in **net GHG savings ranging from 21 to 296 kg CO_2-eq. per kg of protein** (Table 2). Values in Table 2 are provided per kg protein as the protein content is the major nutritional decision criterion to consume meat or any alternatives. The overall influence of Paleos myoglobin is negligible in comparison with the net reductions in GHG emissions that can be achieved by switching from conventional meat proteins to any other of the alternatives depicted here. These values only include direct impacts of protein supply. **If additional carbon sequestration by restoration of natural vegetation is considered that could take place on land no longer needed for livestock or feed production, an estimated 809 Gt CO_2 are removed from the atmosphere over a period of 100 years** (Poore and Nemecek 2018). This equates to all anthropogenic GHG emissions emitted between 1996 and 2021 or an annual average net removal equal to 22% of global GHG emissions emitted in 2022 (own calculation based on (Ritchie and Roser 2022; World Economic Forum 2022). A 50% reduction in the consumption of animal products translates into a net removal of 551 Gt CO_2 over a period of 100 years (Poore and Nemecek 2018). These high savings in GHG emissions and potential net removal of CO_2 by a restoration of natural vegetation arises from the high land demand for livestock and animal based products.

Table 2 *Net change in GHG arising from a shift from one protein source to another in kg CO$_2$-eq. per kg of protein. Calculations based on median GHG emissions of different products. Alternative protein sources (*) contain myoglobin. Each product contains Paleo's myoglobin (1% of its fresh mass). The value of myoglobin is the base case scenario incl. drying of myoglobin. Green shaded values depict net reductions in GHG emissions. Red shaded values depict a net increase in emissions. Abbr.: bh - beef herd; dh - dairy herd.*

	Shift from…										
Shift to…	Bovine (bh)	Bovine (dh)	Lamb & Mutton	Pig	Poultry	Tofu*	Lab	Microbial*	Mycelium*	Insects*	Plant based*
Bovine (bh)	0	130	100	237	259	284	285	301	280	289	296
Bovine (dh)	-130	0	-30	108	130	155	155	171	151	159	167
Lamb & Mutton	-100	30	0	138	160	185	185	201	181	189	197
Pig	-237	-108	-138	0	22	47	48	63	43	51	59
Poultry	-259	-130	-160	-22	0	25	26	41	21	29	37
Tofu*	-284	-155	-185	-47	-25	0	1	16	-4	4	12
Lab	-285	-155	-185	-48	-26	-1	0	16	-4	4	12
Microbial*	-301	-171	-201	-63	-41	-16	-16	0	-20	-12	-4
Mycelium*	-280	-151	-181	-43	-21	4	4	20	0	8	16
Insects*	-289	-159	-189	-51	-29	-4	-4	12	-8	0	8
Plant based*	-296	-167	-197	-59	-37	-12	-12	4	-16	-8	0

* Additional impact of Paleo's myoglobin included (Table A3)-

Switching from any type of conventional meat to alternative protein sources reduces the land demand between 42 and 854 m^{2}a per kg of protein (Table 3). These high net savings arise from the fact that meat is a highly inefficient source of proteins in terms of land-use as livestock occupies 77% of agricultural land while only supplying 37% of the global protein supply (Ritchie and Roser 2017; Shepon et al. 2016). The weighted average efficiency of protein-to-protein conversion of meat consumed in the United States is 8%. It should be noted that not all land is suitable for crop cultivation, nor is it always desirable from an environmental point of view to switch from pasture to cropland (refer to Part III of our detailed LCA report on Paleo). However, 81% of protein originating from cultivated feed crops depicted in Figure 5 would be suitable for human consumption. Considering the low efficiency of protein-to-protein conversion of animals, these crops could provide 12.5 times more protein to humans than by feeding these proteins to animals. Even if the lower protein digestibility of plant-based proteins is considered, these crops fed to animals could provide around 6 times more protein to humans (or reduce the land demand for these feed crops by a factor of 6).

__Table 3__ Net change in land use from a shift from one protein source to another in m^2 per kg of protein. Calculations based on median land use of different products. Alternative protein sources (*) contain myoglobin. Each product contains Paleo's myoglobin (1% of its fresh mass). The value of myoglobin is the base case scenario incl. drying of myoglobin. Green shaded values depict net reductions in GHG emissions. Red shaded values depict a net increase in emissions. Abbr.: bh - beef herd; dh - dairy herd.

	Shift from…										
Shift to…	Bovine (bh)	Bovine (dh)	Lamb & Mutton	Pig	Poultry	Tofu*	Lab	Microbial*	Mycelium*	Insects*	Plant based*
Bovine (bh)	0	723	218	771	791	833	843	854	850	839	838
Bovine (dh)	-723	0	-505	48	68	110	120	131	127	116	115
Lamb & Mutton	-218	505	0	554	573	615	625	637	632	621	620
Pig	-771	-48	-554	0	19	61	72	83	79	68	67
Poultry	-791	-68	-573	-19	0	42	52	63	59	48	47
Tofu*	-833	-110	-615	-61	-42	0	10	22	17	6	5
Lab	-843	-120	-625	-72	-52	-10	0	11	7	-4	-5
Microbial*	-854	-131	-637	-83	-63	-22	-11	0	-4	-15	-16
Mycelium*	-850	-127	-632	-79	-59	-17	-7	4	0	-11	-12
Insects*	-839	-116	-621	-68	-48	-6	4	15	11	0	-1
Plant based*	-838	-115	-620	-67	-47	-5	5	16	12	1	0

* Additional impact of Paleo's myoglobin included (Table A3)-

The literature used to compile data did not include values for water demand of plant-based proteins. Mekonnen and Hoekstra 2010 show that average vegetables have a substantially lower water footprint than beef, lamb, pig, and chicken meat. The data of Mekonnen and Hoekstra (2010) shows **net water savings ranging from 8 to 56 m^3 of water saved per kg protein by switching from meat to vegetables.** In conclusion, shifting from a meat-based diet to other alternatives has a significant positive impact on the environment.

2.2. Whey protein

White biotechnology uses enzymes and microorganisms to produce bio-products from renewable sources. These bio-products can be used in various industries, such as food, pharmaceuticals, agriculture, chemicals etc. (see section 2.1.2.). One example is bio-manufactured protein which can be used as a substitute for animal-derived protein, such as whey protein, which is a by-product of cheese making and is widely used as a dietary supplement and food ingredient. Emissions from milk range between 1.5 to 7 kg CO_2-eq./ kg fat and protein corrected milk, which results in emissions ranging from 46 to 212 kg CO_2-eq./kg protein (Gerber et al. 2010a). These emissions are a result of various factors, including the methane produced by cows, the energy used in farming and processing, and the transportation of the product.

The bio-manufactured whey protein production process has a lower environmental impact than traditional animal-based protein production methods and has the potential to be more efficient and scalable than traditional methods. Some of the challenges associated with bio-manufactured whey protein are high COGS, lack of necessary production capabilities and processing capacity (McKinsey 2019) which can be addressed with Arsenale BioYards' technology. Thus, this section presents an LCA highlighting the environmental impact of bio-manufactured whey protein and its scaling with Arsenale BioYards' technology.

2.2.1. System description

The aim of this study is to assess the change in environmental impact of switching from conventional whey protein to bio-manufactured whey protein and the effect of its scaling using Arsenale BioYards' technology. The approach follows a consequential LCA approach seeking to assess the environmental impact i.e. the savings in CO_2-eq. as a consequence of shifting from conventional to bio-manufactured whey protein (Ekvall et al. 2016).

Bio-manufactured whey protein provides possibilities to develop more sustainable (lower carbon footprint) animal-free dairy products (Carter et al. 2023). Its manufacturing at scale using Arsenale BioYards is likely to promote an increase in demand for alternative protein products and a decrease in the demand for animal-based whey products. On a systemic level, this change leads to an increase in all production processes related to bio-manufacturing of whey protein along with an increase in downstream production processes related to production of animal-free whey protein-based products like protein powder, ice cream, chocolates etc. (Carter et al. 2023).

Functional unit and assessed indicators

The LCA comprises two main elements: The GHG reduction potential by replacing whey protein from diverse sources with bio-manufactured whey protein and the cumulative impact of replacing milk-based whey protein with bio-manufactured protein using Arsenale BioYards' technology. The functional unit to assess both aspects is **one kilogram whey protein** (bio-manufactured, milk based and other sources).

The system is assessed using the indicators **climate change** (Intergovernmental Panel on Climate Change (IPCC) 2014) and several indicators related to resource use, **cumulative fossil energy demand** - CED_f (Verein Deutscher Ingenieure (VDI) (ed.) 2012), **land demand** and **water demand** - WSI (Pfister, Koehler, and Hellweg 2009).

Temporal score

The assessment evaluates the net benefit of manufacturing whey protein using Arsenale BioYards' technology till 2035.

2.2.2. Environmental impact of different sources of whey protein and their replacement

Whey is derived from the cheesemaking process by adding enzymes to separate the curds from the liquid whey. The liquid whey is pasteurised, and the protein is concentrated and isolated. The two main methods to achieve this are membrane filtration and ion exchange technology (Agropur 2023). The amount of whey is determined by the amount of protein content of a milk source. Thus, the emissions of whey protein are a factor of the emission of the respective milk and its protein content. Table 4 shows the GHG emissions from different types of milk and its protein content.

Table 4 *GHG emission from diverse sources of whey protein along with its protein content [References - A: (Beatriz Queiroz Silva and Sergiy Smetana 2022), B: (U.S. Department of Agriculture 2019c), C: (U.S. Department of Agriculture 2019a), D: (U.S. Department of Agriculture 2019b), E:(U.S. Department of Agriculture 2021)]*

Source	Minimum	Median	Maximum	Unit	Protein Content	Reference
Cow Milk	1.5	2.7	7.0	kg CO_2-eq./kg milk	3.3%	A, B
Oat milk	0.021	0.4605	0.9	kg CO_2-eq./kg milk	1.25%	A, C
Almond milk	0.3	2.075	3.85	kg CO_2-eq./kg milk	0.4%	A, D
Rice milk	0.83	2.05	3.27	kg CO_2-eq./kg milk	0.28%	A, E
Soy milk	0.03	0.505	0.98	kg CO_2-eq./kg milk	3.55%	A, F
Bio-manufactured protein	2.985	8.1	21.783	kg CO_2-eq./kg protein	100%	Confidential MB protein data

Based on the information provided in table 4, the GHG emission reduction potential was calculated for replacing diverse milk products with bio-manufactured whey protein. The results show a minimum of 13% savings potential for almond and rice milk and up to 98% savings for soy milk. Further, manufacturing vegan cheese using bio-manufactured whey protein reduces GHG emission by a minimum of 34% for vegan feta and up to 68% GHG emissions for vegan mature cheddar cheese. Table 5 shows the emission savings potential for different milk sources and types of cheeses.

Table 5 *GHG emission savings potential by replacing milk-based products with bio-manufactured whey protein-based products*

Product	Minimum Saving	Maximum Saving
Oat milk+	76%	95%
Almond milk+	13%	74%
Rice milk+	13%	44%
Soy milk+	81%	98%
Soy milk+	57%	66%
Vegan Mozzarella+	59%	67%
Vegan Cheddar+	63%	68%
Vegan Mature Cheddar+	53%	55%
Vegan Feta+	34%	43%

2.2.3. Impact of scaling whey protein production

In the short to mid-term producing bio-manufactured whey proteins alone is unlikely to affect the overall demand for milk because milk is used to produce many other products, e.g. most cheeses are made from the casein fraction. Precision fermentation based bio-manufactured whey proteins may shift the production focus from higher-value whey protein (isolates) to lower-value whey protein (concentrates) within the whey processing industry. This whey protein concentrate can substitute other proteins, e.g. plant-based proteins, such as soy. However, in the long term, milk can be fully substituted by a range of products, e.g. microbial casein and whey protein, plant fats, etc. Results suggest that substituting cow milk-based whey protein with bio-manufactured whey protein offers potential savings of 88 kg CO_2-eq./ kg protein. As shown in figure 5, it leads to cumulative savings of 9.33 Mt CO_2-eq. (assuming 100 operational BioYards with 300 tons each production at a specific titer and rate) by 2035 if milk is fully displaced.

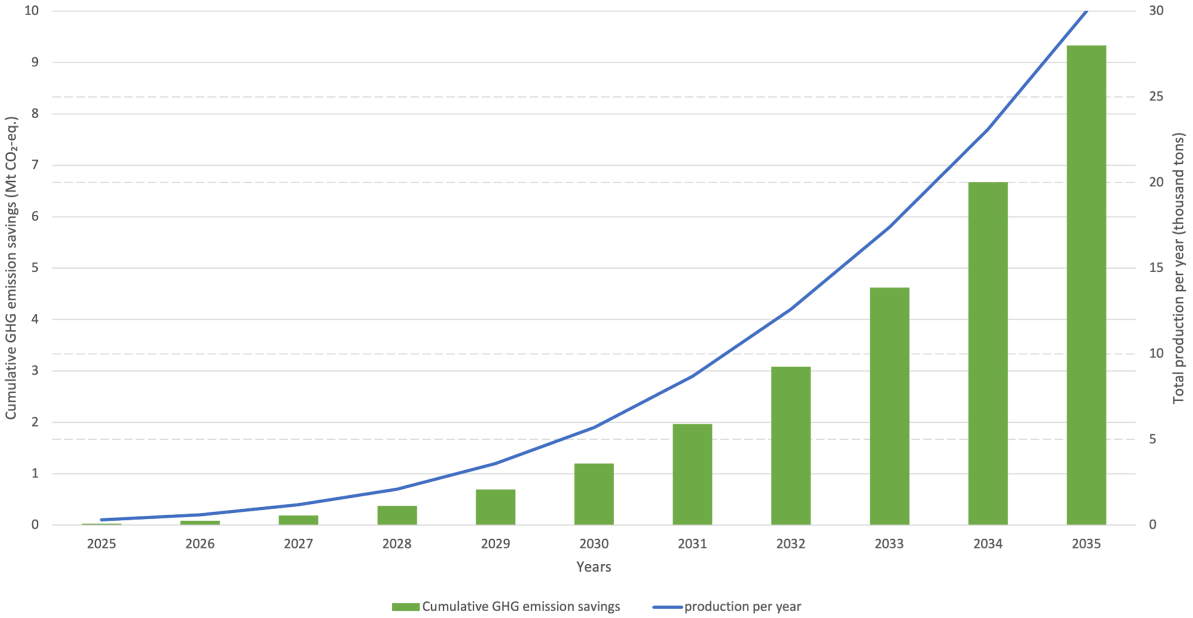


Figure 5 *GHG emission reduction potential by substituting cow milk based whey protein with bio-manufactured whey protein.*

Further, substituting cow milk-based whey protein with bio-manufactured whey protein also saves around 18,390 litres of blue water and 600m2 land use per kg of bio-manufactured whey protein. However, it leads to a slight increase in cumulative fossil energy demand of about 28 MJ/ kg of bio-manufactured whey protein due to an increase in industrial production steps.

2.3. Using LCAs to support decision-making for bio-manufactured products

These exemplary LCAs help to understand the positive environmental impact of bio-manufactured proteins, especially across the sensitive water and land use categories, along with climate change. It also helps to identify the different levers to improve the sustainability of the product. Below, we present a brief overview of the identified environmental hotspots across various categories from the alternative protein exemplary LCA presented above (for a detailed description, refer to our LCA report on Paleo):

2.3.1. Climate change

Paleo's myoglobin when dried shows downstream processing as the largest contributing factor to GHG emissions, accounting for 47% and 34% of GHG emissions in the base and upside case, respectively. The high impact of product drying reveals that reducing the moisture to be removed from the product stream for drying, a reduction in energy consumption of the drying step, as well as the GHG intensity of drying present the biggest levers to reduce overall GHG emission reductions of Paleo's operations. Instead of drying using natural gas, drying could be accomplished using biogenic energy sources, such as biogas.

The second and third largest contribution arises from ingredients of the fermentation medium other than glycerol and glycerol, ranging from 21 to 39% and 13 to 22% of total GHG emissions in case of the ingredients other than glycerol and glycerol, respectively. All other processes, activities and materials only contribute to a minor share of GHG emissions. Aside from drying, most GHG emissions are emitted upstream by processes providing materials needed by Paleo. A decrease in supply chain GHG emissions (scope 3) will result in a substantial reduction in GHG emissions associated with Paleo's myoglobin production.

2.3.2. Fossil energy demand

The assessment of the fossil energy demand mirrors findings of the climate change impacts: the largest demand of fossil energy arises from drying, followed by the production of ingredients of the fermentation medium as well as refined glycerol. This indicates again a high potential for reducing the demand for fossil fuels and associated GHG emissions.

2.3.3. Land use

The land-use indicator in production of Paleo's myoglobin is dominated by the land use for the ingredients of the fermentation medium. Diving deeper into the ingredients of the fermentation medium and looking for alternatives can further reduce the land use for this alternative protein.

2.3.4. Water demand

The water demand indicator is dominated by ingredients of the fermentation medium, accounting for 60 to 63% of the total water demand. All other processes and activities contribute only a minor share to the total water demand. Thus indicates the potential of significantly reducing the water demand by altering the ingredients of the fermentation medium.

In both the exemplary LCAs presented in this study, the changes in the supply chain and wider market structures have been excluded from the LCA, but it might be very relevant when switching from conventional materials to bio-manufactured products as some materials like whey protein are an integral part of a multi-product output system.

For Arsenale BioYards, the LCAs underline the significance of scaling bio-manufacturing at a cost-competitive level to create a massive environmental impact in innumerable sectors. It also proves that LCAs are a well-suited tool for Arsenale BioYards to support their decision-making in the selection of industries and products to provide bio-manufactured substitutes.

3. Conclusion

Our assessment of markets for bio-manufacturing shows that there are numerous challenges like technological, industrial, economical etc. preventing wider adoption of bio-manufactured products. **Arsenale BioYards addresses several of these challenges such as efficient product and bioprocess development, access capacity for sustainable manufacturing at scale, achieving price parity etc. that has the potential to accelerate the development of a bioeconomy** and substitute conventional petrochemical products in industrial sectors, animal-based products in the food sector, as well as cheaper and novel active pharmaceutical ingredients. Such a transition in industrial raw materials and consumer products can have major positive impacts on the environment and human health. The current petrochemical and livestock industries combined with a growing population and consumerism results in high GHG emissions, a high demand for land and water, increased pollution in water bodies and negative biodiversity impact.

The most effective way to counteract these negative impacts while maintaining a high quality of life is to to switch to bio-manufactured substitute products. Our analysis using two exemplary products shows that:

- Changing the protein source from a conventional meat protein to an alternative bio-manufactured protein results in:
    - net GHG savings ranging from 21 to 296 kg CO_2-eq. per kg of protein,
    - a reduction in land demand between 42 and 854 m^2a per kg of protein, and
    - net water savings ranging from 8 to 56 m^3 of water saved per kg protein.

- Switching from cow milk-based whey protein to bio-manufactured whey protein products can save 88 kg CO_2-eq. Per kg of protein and offers up to 98% emission reduction when compared with diverse whey protein-based products.

These results demonstrate that Arsenale BioYards can have a significant positive impact by enabling a shift from conventional materials to bio-manufactured substitutes as they address major challenges to the development and adoption of bio-manufactured products. Arsenale BioYards' unique approach is not just targeting a specific product or industry but rather makes them a 'game-changing' enabling technology necessary to transition from a petrochemical-based economy to a bio-based economy.

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