

Microbes and the Next Nitrogen Revolution

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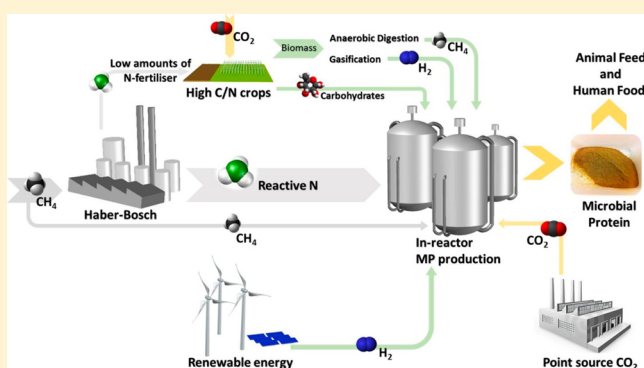
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ABSTRACT: The Haber Bosch process is among the greatest inventions of the 20th century. It provided agriculture with reactive nitrogen and ultimately mankind with nourishment for a population of 7 billion people. However, the present agricultural practice of growing crops for animal production and human food constitutes a major threat to the sustainability of the planet in terms of reactive nitrogen pollution. In view of the shortage of directly feasible and cost-effective measures to avoid these planetary nitrogen burdens and the necessity to remediate this problem, we foresee the absolute need for and expect a revolution in the use of microbes as a source of protein. Bypassing land-based agriculture through direct use of Haber Bosch produced nitrogen for reactor-based production of microbial protein can be an inspiring concept for the production of high quality animal feed and even straightforward supply of proteinaceous products for human food, without significant nitrogen losses to the environment and without the need for genetic engineering to safeguard feed and food supply for the generations to come.



■ THE HABER BOSCH PROCESS: AN INVENTION THAT CHANGED THE WORLD

Thomas Malthus (1766–1834) stated that the increasing human population would suffer hunger because of the limited capacity of the earth to produce food crops.¹ This would particularly be caused by the lack of reactive nitrogen, the critical factor in agro-production. About a century later, in 1908, Fritz Haber patented the Haber Bosch process, so-called “synthesis of ammonia from its elements”, which led to the production of synthetic fertilizers and stepped up agricultural production intensively.² This has allowed the human population to grow to 7 billion. At present, per unit of biological fixed nitrogen, more than 3 units of Haber Bosch derived nitrogen enter the plant based production chain of protein and calories.³ This represents around 80% of the total Haber Bosch produced nitrogen and requires about 1% of the total annual world energy expenditure.² While the Haber Bosch process itself is optimized and is reaching near thermodynamic process efficiencies,⁴ the subsequent use of the Haber Bosch nitrogen in agriculture suffers from many losses. These include, leaching, runoff and volatilization, which result in high inefficiencies and a set of indirect detrimental environmental impacts. This so-called nitrogen cascade effect^{3,5,6} particularly relates to the production of plants destined to feed livestock, with a large fraction of croplands being devoted to produce protein-rich animal feed like soybean and cereals.⁷ As shown in Figure 1,

if 100 units of reactive nitrogen are used in the agro-production system, only 4–14% end up as consumable protein,^{8,9} illustrating the inefficiency of conventional agriculture based protein production chains and the need for a more sustainable and efficient path. Almost half of the reactive nitrogen is dissipated when used to fertilize fields, causing serious environmental damage and affecting human health at a global scale (because of, for example, water and air pollution).^{10–14} Moreover, these externalities also come with serious economic repercussions for society at large. Indeed, recent studies highlight the enormous scale of the externalities of reactive nitrogen pollution (see Table 1), on the order of the estimated benefits the fertilizer industry constitutes for the agriculture.

In recent years a lot of research has been conducted to address the problems associated with the use of reactive nitrogen in agriculture. Yet so far, proposed solutions favor optimization of existing agricultural practices, rather than advancing agriculture-free approaches. The production of microbial proteins as a disruptive technology has not been described as yet. Here we provide an overview of the need for and expectation of a coming revolution in securing nitrogen for food and feed using microbes as the feedstock rather than traditional agricultural production routes.

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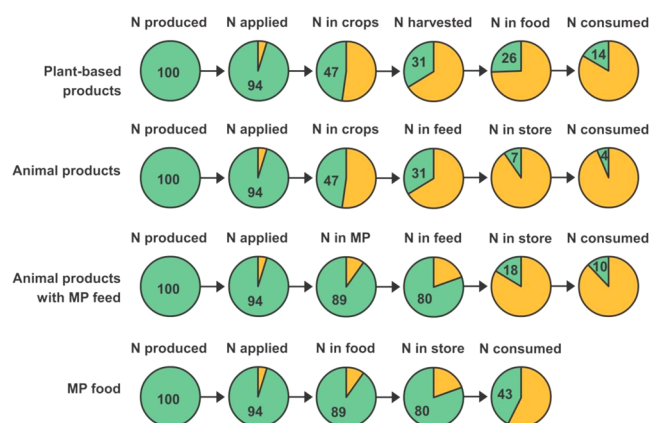


Figure 1. Amount of Haber Bosch nitrogen required and its fate during the different protein production routes. The orange and green fractions represent the amount of reactive nitrogen lost or retained, respectively, within the protein supply chain (expressed in percentage of total input, i.e., 100%). The vegetarian- and animal-product-based protein supply routes are adapted from ref 52. A nitrogen uptake efficiency in the microbial protein production step of 90% was assumed; all other losses were based on Galloway and Cowling.⁵²

THE NITROGEN CYCLE TO COME

The world's population is expected to grow to 9–10 billion by 2050.¹⁵ Moreover the demand for high-quality protein will increase as more people converge on more developed countries consumption patterns due to increase in wealth.^{16,17} Estimates by the United Nations Food and Agriculture Organization (FAO) suggest a further 50% increase in N fertilizer demand by 2050, while at the same time recent studies foresee nitrogen losses to the environment of up to 70%.^{11,18} Available mitigation

measures to reduce nitrogen pollution include reduced food waste, lower consumption of animal products, better live-stock feeding, more efficient fertilization and improved manure use.^{19,20} However, a model estimate³ showed that even under ambitious mitigation efforts that combine all above measures, nitrogen losses still amount to some 94 million tons per year, well above the critical thresholds for greenhouse gases, air pollution and water pollution (see Figure 2).³ Under these conditions, it is uncertain whether environmental sustainability thresholds can be reached^{3,21} and that the nitrogen cycle can return within the planetary boundaries.^{13,17,18,22,23} Moreover, implementing these ambitious measures, which include, for example, a doubling of nitrogen use efficiency on fields or halving the consumption of animal products, could be very challenging in political, technological and economic terms.³ In addition to conventional nitrogen mitigation measures,¹⁹ it is therefore paramount to think “outside the box” for new innovations to effectively decrease nitrogen pollution.

MICROBIAL PROTEIN PRODUCTION IS A VERY EFFICIENT WAY TO PRODUCE VALUABLE PROTEIN

An alternative to plant and animal protein is the aerobic production of microbial proteins (MP) by bacteria, fungi, yeast, and algae.²⁴ Typically, this mode of production involves the supply of nitrogen, an electron donor, a carbon source (can be the donor) and an electron acceptor (e.g., oxygen) to a reactor system enabling highly efficient production and harvesting of the protein. The concept of MP is not new. In fact, methanol based production of bacterial MP was achieved at industrial scale in the 1970s,²⁵ but a combination of low prices for soy and fishmeal, increased oil prices, the underdeveloped state of the fermentation technology and limited focus on nitrogen

Table 1. Recent Studies Highlighting the Significance of Environmental Costs of Reactive Nitrogen Pollution

region	ref	costs of reactive nitrogen pollution
China	54	This study estimates that agriculture accounted for 95% of the NH_3 and 51% of the N_2O in China in the year 2008. In the same study, it was also estimated that the total atmospheric emissions of reactive nitrogen causing related health damage ranged US\$19–62 billion per year. Of this number, agricultural-induced emissions accounted for more than 50% of the costs (in 2008 US dollars).
EU	55	This study revealed that the costs of agricultural-induced reactive nitrogen losses exceed the economic benefit because of increased primary crop production by a factor of 4. Overall, the annual costs associated with agricultural reactive nitrogen losses was estimated to range between €35 and 230 billion per year (equal to ~38–251 billion in 2008 US dollars).
USA	56	This study is the first assessing the cost associated with reactive nitrogen losses to the biosphere from human activities in the United States. The study revealed that the total potential environmental and health economic impact of reactive nitrogen losses from anthropogenic nitrogen summed up to an average of US\$210 (\$81–\$441) billion per year in the beginning of the 21st century. Of this, ~75% of the estimated costs were associated with agricultural induces losses. Costs are in 2008 US dollars or as reported otherwise in the manuscript.
World	19	In this report, conducted by the European Nitrogen Assessment, a costing procedure based on the European situation was implemented aiming at calculating the global cost of nitrogen pollution. Taking into account that the global costs would be approximately a factor 3-fold of the European situation, resulting in an overall estimated costs associated with reactive nitrogen losses ranging between 200 and 2000 billion US dollars annually (in 2008 US dollars).
USA	57	In this study, the Air Pollution Emission Experiments and Policy (APEEP) model (an integrated assessment model) was used to determine the economic impact of air pollution by means of air quality modeling, exposure, dose–response and valuation for a large range of point sources, based on data of more than 10 000 sources measured by the United States EPA. Costs for NH_3 and NO_x emissions are estimated at \$900 (\$100–\$59 400) and 250 (\$20–\$1780) per ton NH_3 and NO_x , respectively. No information is given regarding year of reference.
USA	58	This study aimed to determine the environmental and health externalities associated with the production of different agricultural crops such as corn and switch grass for the production of ethanol. While the purpose and crops used are different, the externalities are directly assessed based on the emissions of NH_3 and NO_x . Estimated costs (in 2008 US dollars) for NH_3 and NO_x emissions were \$3.03 (\$1.25–\$4.80) and 14.6 (\$2.0–\$27.27) per kg NH_3 and NO_x , respectively.
USA and EU	59	In this study, the findings of several previous studies ^{60–62} on the externalities of reactive nitrogen emissions in terms of health, ecosystems/coastal systems, crop decline and climate change were summarized. Costs were estimated at €3.1–€30 kg NH_3 -N (to air), €13–€43 kg NO_x -N (to air), €5–€54 kg N _r (to water) and €2–18 kg N_2O -N (to air) which equals to \$3.4–\$33 kg NH_3 -N, \$14–\$47 kg NO_x -N, \$5.5–\$59 kg N _r and \$2.2–\$120 kg N_2O -N when expressed in US dollars. Note that emission data for the year 2008 was used, where the damage costs were derived from studies between 1995 and 2005 and were not corrected for inflation.

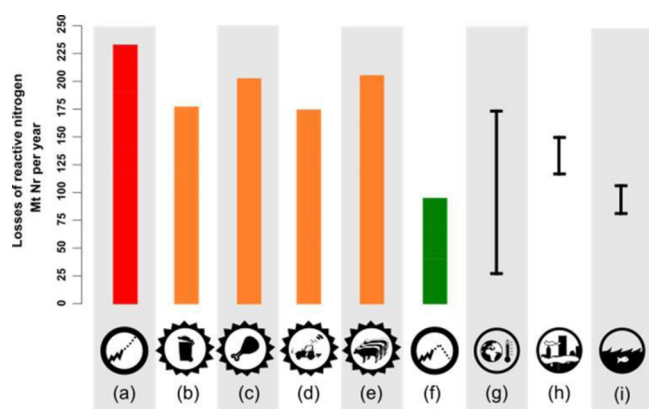


Figure 2. Currently available mitigation measures may not have the capacity to decrease nitrogen losses sufficiently.³ The scenarios described in the figure are (a) a middle-of-the road scenario of the shared socio-economic pathways,⁵³ (b) reduction to a maximum of 20% in household waste with increased waste recycling, (c) animal consumption reduced to 50% of western diets, (d) improved fertilization, (e) improved livestock management, and (f) with all mitigation measures in combination. Panels g–i describe a range for critical thresholds for reactive nitrogen related greenhouse gases, air pollution, and water pollution, respectively. Please refer to Bodirsky et al. (2014)³ for a detailed description of all the mitigation methods, simulated reactive nitrogen flows, and critical thresholds.

efficiency resulted in the discontinuation of it as a production process. The increases in soybean and fishmeal prices in recent years,²⁶ together with the enormous progress made in industrial fermentation technologies, justifies revisiting the potential of MP as a source of usable nutritive protein. Additionally, the recent findings and increased awareness of the enormous environmental costs of nitrogen pollution (Table 1) make it imperative to rectify this planetary boundary.

A fundamental difference between MP and plant based production of protein is the fact that leaching, runoff and volatilization of nitrogen can be completely avoided. MP production can take place in fully controlled, enclosed and automated bioreactors similar to those widely used in the fermentation processes by the food industry for the production of, for example, beer and yoghurt. As microbes can convert Haber Bosch nitrogen into cellular protein with an unmatched efficiency of close to 100%^{24,27} in such reactor systems, the overall nitrogen efficiency of the total feed/food chain would become substantially higher than conventional protein supply routes. As illustrated in Figure 1, with microbial protein based systems 10–43% ends up as consumable protein, compared to only 4–14% for agricultural based protein production.

■ BACTERIA CAN USE A BROAD RANGE OF CARBON SOURCES

Compared to fungi, algae, and yeast, bacteria have the advantage of not only growing rapidly on organic substrates like carbohydrates, starch, and cellulose but also on gases, such as methane, hydrogen and syngas (i.e., a mixture of CO + H₂) (Figure 3).^{28–30} When bacteria are supplied with one of these substrates they can produce highly concentrated cellular protein up to 75 wt % of the dry microbial biomass²⁴ at achievable protein production rates of 2–4 kg per m³ reactor volume per hour.²⁵ The latter protein production rates, using naturally available microorganisms, are several orders of magnitude higher than plant based protein production (i.e., up to

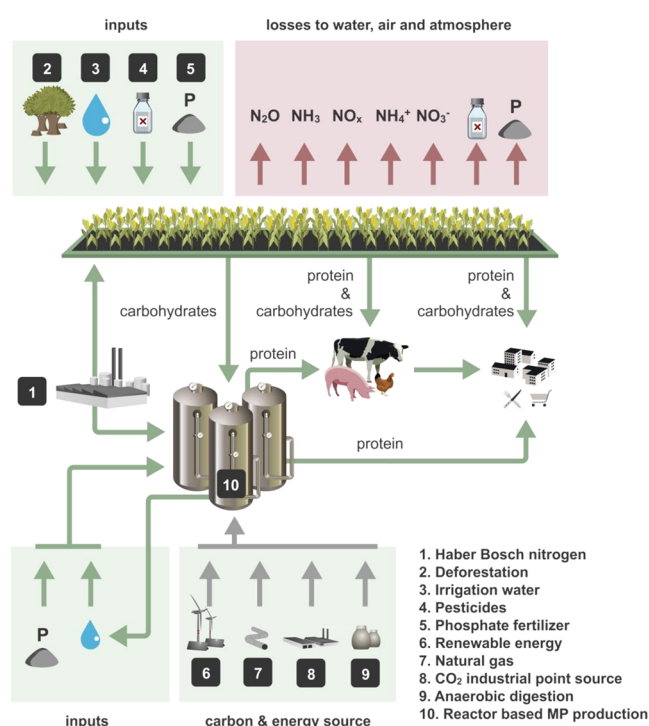


Figure 3. Potential technological pathways for industrial-reactor-based production of MP using different feedstocks with their respective inputs and environmental losses for livestock production and human consumption. Direct upgrading of Haber Bosch generated reactive nitrogen into microbial proteins using hydrogen, methane, or carbohydrates as energy source for microbial growth. Green and gray arrows represent inputs, whereas red arrows represent losses to water, atmosphere, and soil.

3120 tons/hectare per year of microbial protein⁹ versus 3–8 ton dry matter per ha per year for conventional soy production). The fact that bacteria can use hydrogen (in combination with carbon dioxide), methane gas or syngas as their energy source opens up the unique opportunity to completely short-cut agriculture-based feed and food production, enabling virtually land free production of MP which concomitantly is less dependent on weather conditions. The process can be placed on marginal land, but also in urban areas. This decreases the pressure on agriculture in general and on high carbon and biodiversity ecosystems in particular.³¹ The production of MP using hydrogen, generated through water electrolysis powered by wind or photovoltaic energy would require an additional inorganic carbon source. CO₂ from industrial point sources, such as flue gases from power stations could serve as a carbon source,³² and would offer the valuable opportunity for large CO₂ emitting industries to substantially reduce their carbon footprint via substitution.

■ BACTERIAL PROTEIN PRODUCTION HAS MANY ADDITIONAL ENVIRONMENTAL BENEFITS

Land Use Change, Greenhouse Gas Emissions, and Biodiversity Loss. Agriculture currently constitutes a dominant form of land use globally. According to the FAO, about 5 billion hectares (approximately 38% of the land surface) is currently used for agriculture, either as cropland (12%) or pasture (26%).³³ It is important to realize that agriculture uses the most fertile and suitable land available. A large fraction of remaining terrestrial land on our planet is deemed unsuitable

for agriculture and represents deserts, mountains, and tundra.³⁴ MP production would help to decrease future pressures on fertile land to the benefit of natural ecosystems, thereby, positively affecting biodiversity and CO₂ emissions from land use change.³¹ Of particular importance are biodiversity hotspots, such as the Amazon, where a large fraction of the deforestation is related to the expansion of soybean fields.³⁵

Phosphorus Pollution. Agricultural loss of phosphorus, another essential nutrient for crops applied as fertilizer in large amounts (i.e., ~20 Mton P in 2012),³³ through erosion and leaching, is also considered a major environmental burden, already falling outside its safe planetary boundary.¹³ Similar to nitrogen, phosphorus losses are completely avoided during the reactor-based MP production process. Note that P losses still can occur in the overall protein supply chain (see Figure 1).

Fresh Water Withdrawals. Agriculture requires major inputs of fresh water, with ~70% of the global fresh water withdrawals used for irrigation.³⁶ Reactor-based MP production requires very limited amounts of water (i.e., ~5 m³/ton MP²⁷ versus 2364 m³/ton for soy³⁷), which can be further reduced if water is recycled or recycled water is used.

Pesticides. MP production does not require the use of chemicals to control weeds or insect pests. Contamination of surface and groundwater with agricultural pesticides is a global environmental and health concern.^{38,39}

■ OPPORTUNITIES AND CHALLENGES

Technological Implementation. Large-scale production of MP is connected to certain challenges in terms of growing the cultures, processing the cells to product and assuring the quality of the final product. The obstacles should be, relative to comparable production processes dealing with microbial fermentations, in all respects achievable. The first key challenge is the development of microbial cultures, either as pure culture, as combination of pure cultures or as microbiome. In the latter two approaches, cooperating microorganisms that complement each other to fully use low-value forms of carbon (e.g., CO, CO₂, carbohydrates, methane derived from anaerobic digestion and oils) are present. Such mixed cooperative cultures, which by natural selection evolve to highly efficient biocatalysts are currently named microbiomes.^{40,41} A two-pronged approach can be followed for nonpure culture systems. Microbiomes have been successfully enriched from natural samples to achieve high protein content.²⁸ One can also use defined cultures to make a mixed inoculum and let it gradually evolve to improved cooperation whereby a predefined ratio between the cell types can be aimed at.

The second challenge is to scale these reactor microbiomes up to a relevant scale and maintain stability as well as guarantee microbial and chemical safety of the resulting product. From the microbiomes, novel organisms can be obtained, complementing the already existing pure cultures enabling protein production. Working with pure and mixtures of pure cultures results in some new challenges, but may lead to a high level of product quality control and facilitated consumer acceptance. On the basis of the substrate, different feeds and foods could be tailored toward a diverse end-use while maximizing usage of substrate. The central element is to come to assemblages of beneficial organisms which at high rates produce in a stable way proteinaceous metabolites. It should be noted that the latter at no point can have negative nutritional attributes, such as raising allergic reactions. On the contrary, they may have certain special properties, such as being rich in high value

amino acids or being capable of inducing a stimulation of the immuno-system.⁴²

The third challenge (and opportunity) is then to deal with these single-cell microbial biomass rich in protein and transform it to a commodity that has functionalities (e.g., specific taste and odor, the capacity to coagulate and provide structure) that would allow MP to compete in the context of food and feed quality with widely used milk, egg, or meat proteins.

Establishing a Large-Scale Demand for MP. The main challenge for establishing MP as a major sector in the feed/food industry is that it must be accepted by the public as an appropriate alternative to conventional agriculture-based protein. This involves a transition of the existing protein production industries. The protein market is in the order of several hundreds of million tons annually,²⁷ so in the first instance the production of MP would still rely on Haber Bosch nitrogen.

The direct supply route of MP to human food would be from an efficiency standpoint most beneficial. Even small replacement rates of conventional protein concentrate like soy and cereals could lead to a substantial change in the food supply chain, in particular if it would substitute for animal-based products. A particular issue here can be the regulatory hurdles about food safety and quality assurance that need to be overcome for MP to be fully accepted as a protein supplement. Yet, using carbohydrates to produce fungal protein is already an established concept in human consumption under the name of Quorn,⁴³ presently produced at some 25 000 ton per year and growing. The main challenge lies in creating a product of competitive texture, nutritional attributes, and taste, which will require applied consumer research involving partners from the food industry. Using MP as feed for animals is comparatively easier, as in the European Union (EU), it is officially recognized and approved as commercial feed for all types of livestock.⁴⁴ Moreover, the feed industry is already used to integrating various brewery and distillery yeast-containing products.

Cost Competitiveness of MP. The production cost of MP will depend mostly on the natural gas price and energy costs, which determine the price for Haber Bosch, as well as for substrates like methane or hydrogen. Considering the current prices of natural gas, energy (e.g., for hydrogen production through electrolysis), and carbohydrates on the one hand,⁴⁵ and the rising prices of conventional protein (i.e., in particular those of fish meal) on the other hand, this allows MP to be produced at competitive costs. Indeed, methane-based MP, as well as Quorn, are effectively entering the market economy.^{43,46} Similarly important to the MP production costs will be the effective pricing of environmental pollution of agricultural production. MP can reach its full market potential only if the strongly reduced external costs of the food supply chain are reflected in market prices. This requires a shifting of the current policy paradigm away from supporting industrial output-oriented agriculture toward a resource efficient nitrogen economy with a low environmental footprint.

Toward Protein Self-Sufficiency. Having an amino acid composition resembling that of fishmeal, MP will be mainly eligible to replace the protein portion (e.g., soybean meal) within the feed basket, while the potential to replace other feed components (e.g., calories and fibers) is limited. The protein demand of intensified agricultural systems, such as pork or poultry production, is, however, so large that Europe or China (as well as Japan, the Middle East, Korea, and Mexico) cannot settle this demand domestically but have to import large quantities of protein animal feed from Latin and North America.³³

For example, in 2012, the EU produced 96% and 99%, respectively, of the total meat and dairy products available to the European market. However, up to 64% of the total protein-rich feed additives used to support livestock production are currently provided by soybean meal, with domestic EU production only able to cover 3% of the internal demand of soy.⁴⁷ This highlights the endemic production and dependence of EU on soy imports, resulting in exposure to price volatility and to risk of scarcity of soybean on the global market. The fact that MP can be locally produced at industrial scale holds the potential for protein-importers, such as Europe and China, to become protein self-sufficient and independent of soy imports.

■ CONCLUDING REMARKS

The supply of quality protein is of crucial importance for the overall health and wellbeing of the global population. To find a long-term solution to the environmental burdens of our protein-supply chain, we ultimately need to reshape the nitrogen cycle back to its state prior to the Haber Bosch revolution when significant losses of reactive nitrogen into the biosphere did not exist. Contemporary agriculture alone, hindered by its inefficient use of reactive nitrogen, will not achieve this. We therefore foresee a revolution in the use of Haber Bosch nitrogen that allows the radical restructuring of agricultural practices and holds the potential to completely bypass agriculture by directly upgrading Haber Bosch nitrogen into microbial protein for use in animal feed or human food. The new platform does not generate issues in terms of ethics in relation to production animals, genetically modified organisms, and has low water and land footprints. Other routes to protein production should be explored simultaneously to assist in alleviating the environmental burdens. Examples are the use of improved biological nitrogen fixation,^{48,49} engineering nitrogen-fixing cereals (although these types of GMOs would likely face substantial challenges in terms of public acceptance), and developing N fixation by electrochemical processes not involving pressures and temperatures in the Haber Bosch process⁵⁰ or even by hybrid nitrogenases.⁵¹ Moreover, some microbes could even use atmospheric nitrogen directly. However, it is questionable that nitrogen fixation would lead to high productivity in terms of cell growth; in the context of MP we are talking about assimilation, not dissimilatory production of a metabolite.

Despite the technical challenges and research needs outlined above, first and foremost the widespread use and acceptability of MP will be dependent on careful and correct marketing to receive overall acceptability from regulators and consumers. Triggering a constructive public dialogue on the mutual benefits for the consumer and the environment as well of this new protein supply route will be essential to safeguard feed and food supply for the generations to come in a sustainable way.

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