

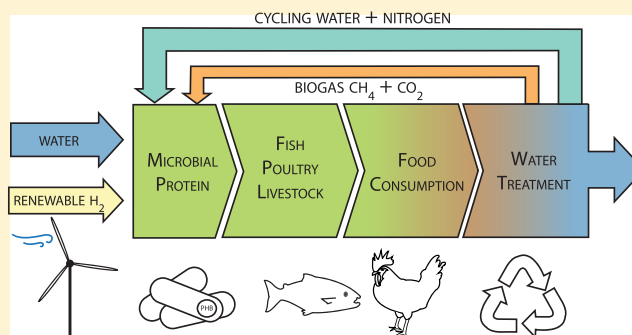
Engineering the Dark Food Chain

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S Supporting Information

ABSTRACT: Meeting global food needs in the face of climate change and resource limitation requires innovative approaches to food production. Here, we explore incorporation of new dark food chains into human food systems, drawing inspiration from natural ecosystems, the history of single cell protein, and opportunities for new food production through wastewater treatment, microbial protein production, and aquaculture. The envisioned dark food chains rely upon chemoautotrophy in lieu of photosynthesis, with primary production based upon assimilation of CH_4 and CO_2 by methane- and hydrogen-oxidizing bacteria. The stoichiometry, kinetics, and thermodynamics of these bacteria are evaluated, and opportunities for recycling of carbon, nitrogen, and water are explored. Because these processes do not require light delivery, high volumetric productivities are possible; because they are exothermic, heat is available for downstream protein processing; because the feedstock gases are cheap, existing pipeline infrastructure could facilitate low-cost energy-efficient delivery in urban environments. Potential life-cycle benefits include: a protein alternative to fishmeal; partial decoupling of animal feed from human food; climate change mitigation due to decreased land use for agriculture; efficient local cycling of carbon and nutrients that offsets the need for energy-intensive fertilizers; and production of high value products, such as the prebiotic polyhydroxybutyrate.



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I. INTRODUCTION: THE GROWING NEED FOR NEW SUSTAINABLE FOOD PRODUCTION

Humanity depends on natural and engineered food chains for its existence. These chains in turn depend upon the availability of water, nutrients and light. The need for light limits food production to locations with large surface area to capture sunlight or where energy-intensive artificial lighting can be provided. Substantial land requirements in turn result in widespread deforestation, inefficient application of synthetic fertilizers and increased distance from farm to table (or trough)—all factors that contribute to increased greenhouse gas emissions and global climate change. With the global population projected to exceed 11 billion by 2100,¹ and 800 million people currently undernourished,² meeting worldwide food needs while reducing climate change impact requires innovative approaches to food production.

In this review, we explore dark food chains, microbially based food production systems that do not rely on light. First, we review natural environments in which dark food chains contribute to the overall carbon biomass of the ecosystem. We then review historic efforts to produce human food or animal feed in the absence of light. Finally, we discuss how dark food chains can be incorporated into human food systems, their engineering, and future implementation. We focus on largely untapped chemoautotrophic food chains where the ultimate sources of carbon are low-cost and renewable feedstocks with potential benefits for climate change mitigation (CO_2 , CH_4 , H_2). This list of dark food chain substrates is not meant to be exclusive. Future innovations will likely result in dark food chains based upon renewable

production of substrates that support heterotrophic growth, such as acetate generated by microbial bioelectrosynthesis³ or ethanol, generated by fermentation of syngas (CO and H_2)⁴ or by degradation of cellulosic waste streams.⁵

Engineering and intensifying dark food chains is attractive for several reasons. Because such chains are independent of light, high volumetric delivery of chemical energy is possible, enabling smaller land and water footprints. Because they are based upon consumption of CO_2 and CH_4 —the end products of methane fermentation—they can enable efficient carbon recycling through anaerobic biodegradation of food wastes and animal wastes back into CO_2 and CH_4 , the original feedstocks. Because they largely provide food for aquaculture and livestock, they can enable decoupling of animal feed production from food for human consumption (Box 1).

II. NATURAL DARK FOOD CHAINS AS MODEL SYSTEMS

In natural environments, dark food chains have evolved in the absence of light, harnessing energy from reduced compounds in lieu of photosynthesis (Figure 1). Three dark environments are especially noteworthy: oceans, caves, and lakes. Within oceans

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Box 1. Benefits of Dark Food Chains

- Generates in a protein alternative for animal feed that does not compete with human protein feedstocks and can offset demand for fishmeal in farmed aquatic species.
- Enables high volumetric delivery of chemical energy and dense production of protein; well-suited for urban environments, decreasing transport distances to market and pressure for deforested land needed to cultivate animal feed.
- Supports production of microbial protein that could avoid use of over 100 million hectares of cropland, avoiding emissions of 40 Gt of CO₂ equivalents.¹⁰⁹
- Enables efficient local recycling of nutrients, specifically carbon and nitrogen, decreasing land requirements and offsetting the need for energy-intensive fertilizers.
- Enables sustained production of 1 ton of microbial protein from approximately 1 m³ of freshwater, 140 times less than the water requirement for protein alternative soybean.⁴⁶
- Facilitates low cost feed production when coupled to the use of cheap gaseous substrates that can be delivered at low energy cost using existing pipeline infrastructure.
- Enables beneficial use of greenhouse gases CO₂ and CH₄ providing life-cycle benefits for climate change mitigation.
- Enables production and beneficial use of polyhydroxybutyrate, a polymer with documented positive health benefits for aquatic animals, potentially increasing yields for aquaculture without risks associated with antibiotic use.

and caves, dark food chains extract energy from reduced compounds of geological origin; in lakes, dark food chains coexist with light food chains with the result that higher trophic level animals (e.g., fish) contain organic carbon derived from both photosynthesis and chemoautotrophy. These dark food chains can potentially provide baseline values for carbon use efficiency and productivity and can serve as models for intensified human food production in engineering systems. They can also inspire innovation based upon biomimicry.

2.1. Marine Dark Food Chains. In marine environments, dark food chains form in the presence of deep-sea hydrothermal vents and cold seeps (Figure 1A). Hydrothermal vents form in deep sea spreading zones (the one known exception to this is the Lost City Hydrothermal field), where volcanic gases and fluids are released,⁶ with temperatures reaching 405 °C. Lower-temperature flows are released from deep-sea fissures and porous surfaces of volcanic structures. These flows mix with cold seawater to create warm water (usually less than 40 °C). Such fluids are enriched with electron donors, including H₂S (3–110 mmol/kg), H₂ (0.1–50 mmol/kg), CH₄ (0.05–4.5 mmol/kg),⁷ which serve as primary substrates for diverse ecosystems that include grazers, deposit feeders, and predators.⁸ Symbiotic associations are established between chemoautotrophic bacteria that oxidize H₂S, CH₄ and H₂ bacteria and marine animals (e.g., tube worms, mollusks, clams, mussels, snails, limpets and worms).⁹ This impressive diversity is sustained by methane and carbon dioxide as the sole sources of carbon.¹⁰ Upwelling of reduced compounds also occurs in cold seeps along active and passive continental margins, giving rise to dark food chains based upon metabolism of methane and petroleum hydrocarbons.¹¹ Additionally, recent reports indicate the importance of inorganic carbon fixation in the abyssal seafloor, where the rate of bacterial

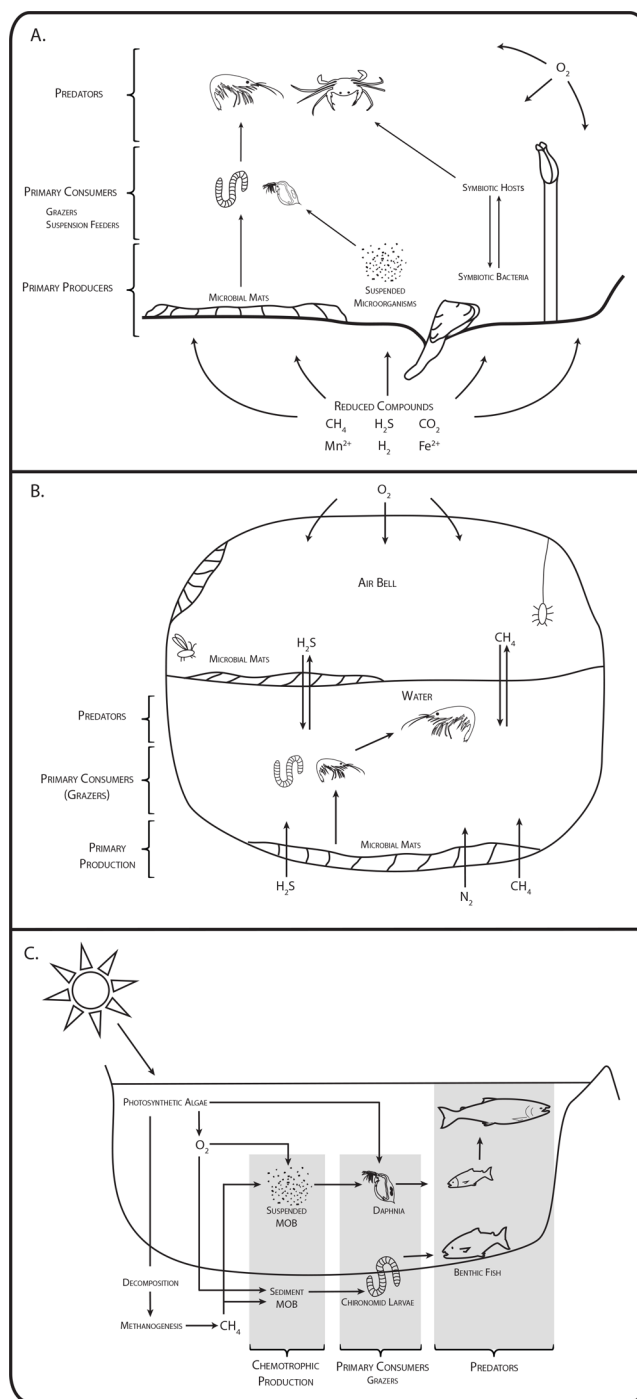


Figure 1. Dark food chains in the following natural environments: A. Deep-sea B. Caves C. Lakes.

CO₂ assimilation was observed to be as high as that of algal photosynthesis. Importantly, the mechanism of this carbon sequestration has yet to be defined, underscoring the importance of further research on marine dark food chains.¹²

2.2. Cave Dark Food Chains. Dark food chains in caves (Figure 1B) host communities and symbiotic associations that appear less evolved than those of deep-sea environments.^{13,14} Cave environments contain fewer species: 40–50 compared to hundreds in the deep sea,¹⁴ and the documented symbioses are far more limited.¹³ Nevertheless, species in caves are highly specialized and demonstrate the capacity of dark food chains to self-assemble when nutrients are available (Figure 1b).

The Movile Cave in Romania and the Frasassi Cave System in Italy are well-studied cave systems containing established complex food chains. The atmosphere of the Movile Cave is enriched with H₂S, CO₂ and CH₄.^{15,16} Microbial mats oxidize CH₄, H₂S and NH₃ for primary production supporting an ecosystem of grazers and carnivorous arthropods (e.g., cave scorpions, millipedes, spiders).^{15,17} The Frasassi Cave is similarly enriched with reduced compounds, including H₂S,¹⁸ CH₄ and Mn²⁺.¹⁹ The sulfidic sections of the cave contain rich chemoautotrophic microbial communities, including higher trophic levels such as mollusks, crustaceans (aquatic and insects) and arachnids.¹⁸

2.3. Lake Dark Food Chains. Unlike deep-sea and cave environments, the dominant form of anaerobic metabolism in freshwater ecosystems is CH₄ production (Figure 1C).²⁰ Methane produced in anaerobic lake sediment diffuses to aerobic sediment or water where it is consumed by methane oxidizing bacteria (MOB).²⁰ MOB can account for up to 40% of total bacterial biomass in some lakes²¹ and play a key role in preventing the release of methane into the atmosphere.²²

Methane-derived biomass enters the lake food chain when MOB are consumed by grazing organisms such as chironomid larvae^{20,23–25} and *Daphnia*,²⁶ and these organisms are in turn consumed by fish. In fact, stable isotope analysis of $\delta^{13}\text{C}$ indicates methane-derived carbon reaches the fish level in several lakes.^{23,27,28} Fish may also play a “top down” role in the regulation of lake methane emissions. Increasing fish predation of *Daphnia* decreases *Daphnia* levels, relieving MOB predation, and increasing MOB abundance—ultimately reducing methane emissions from the lake.²²

III. HISTORY OF DARK FOOD CHAINS

Humanity has continuously sought to identify and exploit new food resources. Over 10,000 years ago, domestication of plants and livestock supported permanent human settlements.²⁹ As early as 2000 years ago, Chinese, Indian, and Roman civilizations engaged in early forms of aquaculture.³⁰ From the early 20th century onward, researchers have sought to incorporate microbial biomass into human diets and animal feed. Although technologies initially developed to produce microbial protein were not commercially viable due to high feedstock costs and process inefficiencies, the experience gained with large-scale bioreactor technology laid the groundwork for 21st-century efforts.

3.1. Microbial Protein in the Early 20th Century.

Production of microbial biomass for use as feedstock (later known as single cell protein, SCP) is strongly tied with historic patterns of food scarcity and costs of growth substrates. As early as World War I, researchers in Germany and England evaluated processes for production of microbial protein. In Germany, yeast grown on molasses was marketed for human consumption, but large-scale production was not achieved, likely due to wartime sugar shortages.³¹ In England, microbial biosolids from production of acetone and butanol was used as a diet supplement for pigs, improving growth compared to the standard diet.³¹ During World War II, researchers in England continued to study microbial protein, focusing on yeast. Studies tested yeast as a feed component for pigs³² and researchers advocated yeast as a new protein source, suggesting its use in spaghetti, dumplings, curries, pies, and gravies.³¹ Feedstocks considered for large-scale yeast production (e.g., molasses, maize, waste citrus fruit) relied upon extraction of resources from regions under British imperial rule.³¹ After World War II, the research focus shifted from yeast to bacteria—allowing consideration of a broader range of feedstocks and cultivation conditions suitable for large-scale production.

3.2. Late 20th Century Single Cell Protein. In the 1960s, concerns about rapid human population growth prompted a surge of interest in the use of microbial protein from yeast, bacteria and algae. In 1966, MIT professor Carroll Wilson³³ recommended replacement of nonstandard terms, such as “petroprotein,” in favor of “single cell protein”.³⁴ In the following year, MIT hosted the First International Conference on Single Cell Protein.³⁵ During this period, researchers promoted SCP for human consumption, including feeding of children in low-income households,³⁶ astronauts in space systems,³⁷ and for animal feed.

A challenge with SCP production was identification of feedstocks that could support rapid and dense microbial growth. Most research focused on growth of heterotrophic bacteria or yeast capable of using fossil carbon-derived feedstocks (methanol, ethanol, gas—oil, and paraffins) or industrial wastes (molasses, food wastes, sulfite waste, whey, and cellulosic waste).³⁸ Separation of such feedstocks from the SCP products proved to be a significant challenge,³⁹ contributing to high costs for infrastructure, equipment, and large energy inputs.⁴⁰ The situation was exacerbated by the 1973 oil crisis, as costs increased for both hydrocarbon-based feedstocks and fuel needed to produce SCP, making the process economically uncompetitive with soybean and fishmeal alternatives.⁴¹

In the late 20th century, British engineers with Imperial Chemical Industries (ICI) used natural gas methane as feedstock for production of SCP product marketed as “Pruteen.” Concerned about the slow growth rates and inefficiencies of MOB, ICI engineers chose to chemically convert methane into methanol (a decision described at the time “as a Eureka situation”).⁴² Methanol was then used to support growth of methylotrophic bacteria.⁴² The Pruteen plant, opened in 1980, was the world’s largest fermenter (50 m high) and produced 50 000 tons of SCP per year for use in animal feed.⁴² Due to high costs of infrastructure (£100 million) and continuous operation, Pruteen was not economically viable.⁴² By 1984, the plant was running below capacity, and ICI switched to other products.⁴³ By 1985, the goal of using SCP to feed the world was viewed as largely unsuccessful due to the high costs of fossil fuel feedstock, energy, and infrastructure.⁴¹ Key lessons of the 20th century were the need for cheap feedstocks where the cost of feedstock is ideally independent of the cost of food and energy, and where the feedstock is readily separable from the SCP product.

3.3. 21st-Century Dark Food Chains and the Rise of Aquaculture.

Twenty-first century opportunities and challenges have spurred renewed interest in sustainable food supplies including microbial SCP.^{44–47} Drivers include climate change, limited natural resources, deforestation, increased urbanization, global poverty and decreased cost of methane as a SCP feedstock. Demand has been further increased by overharvesting of ocean fisheries, a boom in aquaculture⁴⁷ and research indicating that SCP and associated coproducts can stimulate the innate immune system of animals^{48,49} and, for some products, decrease gut inflammation.^{50,51}

Aquaculture is a major source of protein for human consumption and has experienced rapid growth over the last several decades. From 1990–2010, the annual growth rate was 7.8%, a rate far exceeding the annual growth rate of the global human population (0.5%) and also exceeding annual growth rates in production of grain (1.4%), dairy (1.4%), pork (2.2%), and poultry (4.6%).⁵² Fish play an increasingly important role in human diets, comprising more than one-third of the total animal protein supply for 30 countries; 22 of which are low-income and food deficient.⁵³ Increasing fish supply has the potential to

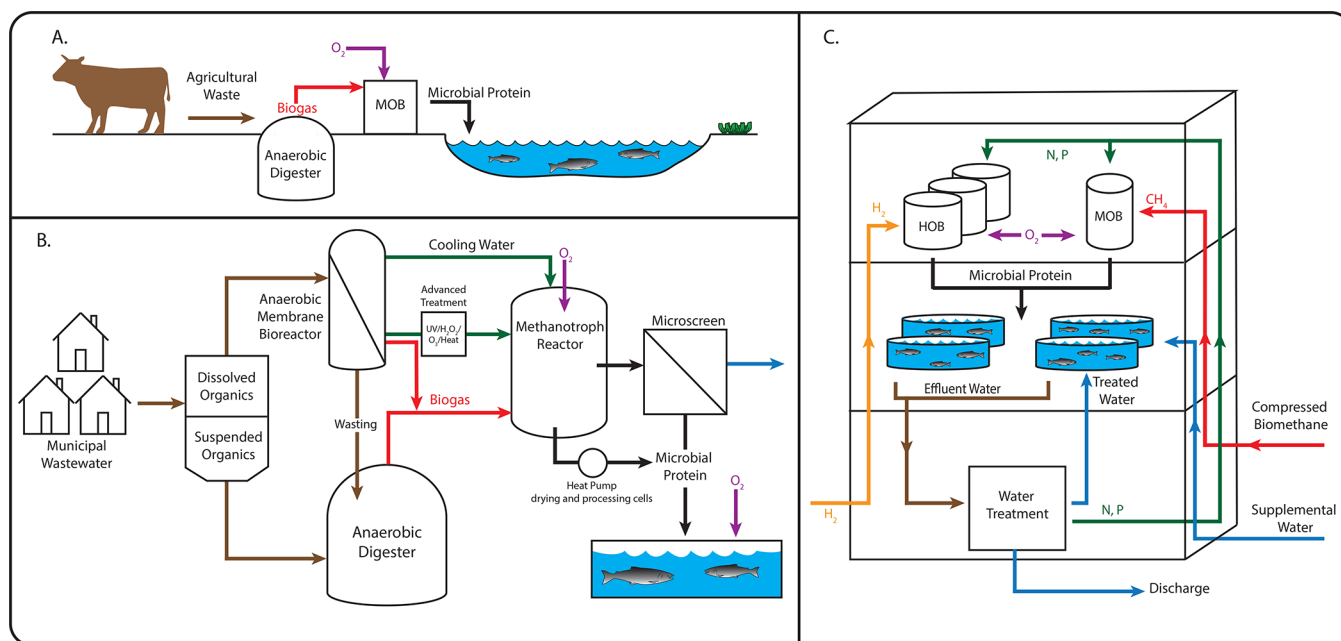


Figure 2. Engineered dark food chains in different economic environments. A. Low-income setting B. Municipal wastewater: incorporating dark food chains into existing wastewater infrastructure. C. Urban dark food chains connected to compressed biomethane and hydrogen.

improve human health by increasing the nutrient density of diets and by providing sources of Vitamins A, B, and D, calcium, phosphorus, iodine, iron, and zinc, in addition to protein.⁵⁴

While the growth of aquaculture offers many advantages as a food supply, current practices have resulted in severe environmental damage.⁵⁵ Of particular concern is the high demand for fishmeal, a source of protein for farmed aquatic species obtained by harvesting of forage fish or low trophic level (LTL) fish from the ocean. LTL fish fill an essential role in the marine ecosystem: transferring primary production from plankton to larger fish, mammals, and birds.⁵⁶ At present, however, the majority of the world's fisheries are fully exploited, overexploited, or slowly recovering from overexploitation.⁵⁷ Thus, if aquaculture is to serve as a sustainable human food supply, the aquaculture sector must develop new, environmentally friendly sources of feed. Microbial protein is a promising substitute. The commercial sector has recognized this opportunity, and several companies have developed intellectual property related to microbial protein animal feed products as a replacement for fishmeal.⁴⁵

IV. ENGINEERING THE DARK FOOD CHAIN

4.1. Methane and Hydrogen as SCP Feedstocks. As noted previously, cheap and abundant feedstocks are essential for SCP production, with a cost that is ideally independent of the cost of food and energy, and where the feedstock is readily separable from SCP. Methane is a promising on both accounts and commercial production is now feasible and undergoing field-scale implementation. Hydrogen is likewise promising though over a longer planning horizon, as renewable production becomes widely available.

Biogas methane is generated by anaerobic digestion of domestic and agricultural organic waste streams (Figure 2A and B). This sustainably sourced methane is thus readily available in many locations, and compressed biomethane can be transported via natural gas pipelines. Methane is a potent greenhouse gas, and its capture for use as a substrate can potentially mitigate emissions. Use of biogas generated by anaerobic

digestion of organic wastes can sustain food systems through a cradle-to-cradle cycle in which biogas derived from anaerobic digestion of animal and food wastes becomes feedstock for methanotrophs which then become feed for animal protein. Because agricultural and human wastes are treated anaerobically in both low- and high-income settings, biogas-based dark food chains are possible across a variety of economic contexts. Globally, 58.7 billion Nm³ of biogas were produced in 2014, an increase from 13.2 billion Nm³ in 2000—a trend that is expected to continue.⁵⁸ In low-income settings, anaerobic digesters are often promoted as a source of methane fuel for clean cookstoves (Figure 2A), but the economic incentives needed to fully maintain digesters and completely capture biogas are lacking, resulting in damaged and dysfunctional digesters.⁵⁹ In high income settings, biogas is also readily available but is often flared rather than used beneficially. In the United States, for example, approximately, 48% of wastewater is treated using anaerobic digestion technology, but less than 10% of these facilities use the biogas produced.⁶⁰ In both low- and high-income settings, microbial protein produced from methane by methane-oxidizing bacteria (MOB) could incentivize biogas capture by providing an affordable means of generating a high-value product.

While current infrastructure does not support hydrogen production, future dark food chains can be expected to increasingly rely on renewably generated hydrogen (Figure 2c).⁴⁴ Hydrogen-oxidizing bacteria (HOB)-based SCP is not recommended for human feed⁶¹ but laboratory tests have demonstrated its potential as animal feed,^{62–64} and HOB-generated SCP is the subject of increased scientific interest due to their rapid growth rates and lower oxygen demand.⁴⁴

4.2. Stoichiometry and Kinetics of MOB and HOB. Large-scale production of microbial protein requires engineering understanding of the stoichiometry and nutrient requirements for microbial growth of MOB and HOB. Methane is typically conceptualized as a high energy substrate, with eight free electrons that are released during oxidation to CO₂, but aerobic microbial transformation requires an energy-intensive

initial attack on the methane molecule using methane mono-oxygenase (MMO). Oxygen is consumed at a ratio of 1–2 mol per mole of methane, and four reducing electron equivalents are lost: two for oxidation of methane to methanol and an additional two as NAD(P)H.⁶⁵ Thermodynamic models that enable yield estimates have accounted for energy losses in different ways: VanBriesen and collaborators^{65–67} developed a model that accounts for the specific number of electrons invested in mono-oxygenase reactions; McCarty⁶⁸ adopted an approach based on the number of oxygenase reactions; Rostkowski and collaborators used a different approach, meeting the MMO oxygen demand by setting aside one mole of oxygen for each mole of methane oxidized and assuming loss of reducing equivalents required to convert methane into formaldehyde.⁶⁹ New evidence indicates that methanotrophic bacteria can uptake significant levels of CO₂, a finding not yet incorporated into stoichiometric models.⁷⁰ For PHB-producing methanotroph *Methylosinus tricosporium* OB3b, CO₂-derived carbon makes up over 60% of cell biomass, assimilated through the serine cycle.⁷⁰ Incorporating this level of CO₂ likely requires a complex regulatory system of carboxylases that warrants further investigation. Further research on metabolic regulation of MOB may provide insights into conditions that optimize carbon assimilation from mixtures of CO₂/CH₄ mixtures such as biogas.

Table 1 summarizes typical stoichiometric and kinetic parameters for both MOB and HOB. For methanotrophs,

Table 1. Stoichiometric and Kinetic Parameters for Methane-Oxidizing Bacteria and Hydrogen-Oxidizing Bacteria

	feedstock requirement ^a (g substrate/g vss)	oxygen requirement (g O ₂ /g vss)	heat production(kJ/g vss)	maximum specific growth rate (day ⁻¹)
methane-oxidizing bacteria	1.4	4.2	55	4.0 ⁶⁹
hydrogen-oxidizing bacteria	0.45	2.1	43.5	10.1 ⁴⁴

^aFeedstock requirement = (maximum yield)⁻¹, where assumed MOB yield is 0.72 g vss/g CH₄⁶⁵ and HOB yield is 2.24g vss/g H₂.⁷⁸ See Box 2 for calculations of oxygen requirement and heat production. Maximum specific growth rate is reported average for 30 °C from Rostkowski et al. (2013).⁶⁹

a wide range of maximum specific growth rates have been reported, with specific growth rates ranging from 0.03 to 0.3 h⁻¹, corresponding to doubling times ranging from 2 to 20 h.^{71–75} Reported methane and oxygen half saturation coefficients are low, in the micromolar range,^{76,77} suggesting that methanotrophs achieve their maximum growth rates at very low levels of dissolved methane and oxygen. HOB have generally higher growth rates and lower oxygen requirements, and both the hydrogen and oxygen requirements can be met by electrolysis of water using renewable energy.

Trace metal requirements of HOB and MOB must be satisfied to enable balanced growth. HOB requirements include Ni and Fe for hydrogenase,^{79,80} and MOB requirements include Cu, Fe, Mo, and Zn.⁸¹ For MOB, recent research indicates the importance of rare earth elements, such as cerium.^{82,83} These reports point to the importance of lanthanides in the leakage of methanol from MOB. Specifically, there are two forms of methanol-dehydrogenase, XoxF, a lanthanide-dependent form that rapidly converts methanol to formaldehyde, and MxaF,

a calcium-dependent form that oxidizes methanol more slowly. The ecological consequence of this difference is accumulation and leakage of methanol into the environment when the calcium-dependent form is active, stimulating downstream growth of methylotrophic bacteria.⁸⁴ Thus, trace metal regulation can result in changes in community structure, changing the composition of microbial protein products.

Stoichiometry and kinetics of MOB and HOB growth inform considerations for engineering reactor design. Box 2 illustrates an example of design calculations for a MOB chemostat. In this example, S^o_{eff} represents the effective influent concentration achieved through operation at high pressure or use of substrate vectors to enable enhanced delivery and mass transfer of sparingly soluble substrates.

4.3. Thermodynamics of Chemoautotrophy. In order to scale chemoautotrophic dark food chains for a meaningful impact on food production and greenhouse gas mitigation, researchers must address the unique problems presented by the thermodynamics of gas feedstocks. By harnessing energy through chemoautotrophy, dark food chains rely upon conversion of high entropy substrate gases into lower entropy biomass, a decrease in the entropy of the products (biomass, PHB) relative to the gaseous reactants.⁸⁵ This entropy decrease must be offset by heat release to enable a net negative change in free energy.^{85–89} In other words, chemoautotrophic metabolism must be highly exothermic in order to compensate for the decrease in entropy that occurs when carbon is sequestered as biomass. Figure 3 illustrates the relationships for MOB and HOB in comparison with other microorganisms. For both MOB and HOB, heat release results in ~70% loss of energy available for combustion in the initial substrate (calculations in Box 2).

Exothermic growth impacts bioreactor design and selection of organism type. Cooling is required in high productivity bioreactors. In one technoeconomic analysis of methanotrophic production of PHB,⁹⁰ cooling costs dominated. Delivery of cooling water at 30 °C was sufficient to cool thermophilic methanotrophic cultures, but for operation under mesophilic conditions, more expensive coolants were needed. This underscores the importance of coolant availability, as costs of microbial protein production thus depends on siting of the production facility. Co-location of facilities at or near a wastewater treatment plant may enable cost-effective cooling with recycled water in addition to providing a source of biogas as feedstock (Figure 2b).

4.4. Substrate Solubility and Opportunities for Biomimicry. Importantly, methane and hydrogen are sparingly soluble in aqueous media, with solubilities that are orders of magnitude less than other feedstocks such as glucose (SI Figure S2). To improve performance of engineered systems and maximize specific growth rates, high rates of mass transfer are needed, as well as provisions to avoid explosion hazard.⁹² This may be the single largest obstacle to commercial production using flammable gases. A disadvantage of operation at higher temperature is the decreased solubility of CH₄, O₂, and H₂, but an advantage is the higher specific growth rates (approximately doubling for every 10 degree increase in temperature up to an upper limit for the species).⁹³ Such trade-offs require careful assessment.

Commercial ventures have implemented innovative reactor design to achieve high rates of mass transfer for methanotrophic production. Unibio A/S in Denmark developed a U-loop bioreactor with a recirculation loop, allowing for high rates of gas recirculation. Residence time for gases are less than 1 min in this system, compared to several hours for the liquid phase in which microbial growth occurs, and mass transfer coefficients reach

Box 2. Example Calculations for Well-Mixed SCP Bioreactors

Variables:

V = Reactor volume [L³]
 Q^o = Flow rate [L³c⁻¹]
 S_{eff} = Effective influent substrate concentration [ML⁻³]
 S = Substrate concentration in reactor and effluent [ML⁻³]
 X = Biomass concentration in reactor and effluent [ML⁻³]
 θ = $\frac{V}{Q}$ [c⁻¹]
 R_S = Volumetric rate of electron donor consumption [Mr⁻¹L⁻³]
 P_X = Volumetric biomass production rate [Mr⁻¹L⁻³]
 Y_X = Biomass yield
 R_O = Volumetric oxygen consumption rate [Mr⁻¹L⁻³]
 γ = COD/Weight Ratio
 $Y_{CH_4} = \frac{4 \text{ g COD}}{8 \text{ g CH}_4}$
 $Y_{H_2} = \frac{8 \text{ g COD}}{8 \text{ g CH}_4}$
 R_N = Volumetric nitrogen consumption rate [Mr⁻¹L⁻³]
 Δ_cH_{Donor} = Heat released by electron donor combustion *
 Δ_cH_X = Heat stored in cells produced
 Δ_cH_{MET} = Metabolic Heat

*Heat released by electron donor combustion represents the theoretical heat released by oxidation of all electron donor consumed by the microorganisms

Volumetric rate of Substrate Consumption
 $R_S = \frac{(S^o - S)}{\theta}$

Volumetric rate of Cell Production
 $P_X = Y_X R_S$

Volumetric rate of Oxygen Consumption (assuming N source is ammonia)
 $R_O = R_S \gamma - 1.42 P_X$

Volumetric Rate of Nitrogen Consumption (for cells with stoichiometry C₅H₇O₂N)
 $R_N = 0.12 P_X$

Heat released per gram of cells produced:
 $\Delta_c H_{Donor} = \Delta_c H_X + \Delta_c H_{MET}$
 $\Delta_c H_X = \text{Enthalpy of combustion of cell biomass [Energy-Mc}^{-1}]$
 $= -460,29 \text{ kJ/C-mole of biomass } C^{85}$
 $= -14.3 \text{ kJ/g COD}$
 (calculated assuming: biomass is C₅H₇O₂N and 1.42 g COD/g vss)
 $\Delta_c H_{MET} = \Delta_c H_{CH_4} - \Delta_c H_X * Y * 1.42 \frac{OD}{g \text{ vss}}$

Methanotrophic Bacteria

$Y = 0.72 \text{ g vss/g CH}_4$
 $CH_4(g) + 2O_2(g) = CO_2(g) + 2H_2O(g) + 802.3 \text{ kJ Heat}$
 $\Delta_c H_{CH_4} = -55 \text{ kJ/g CH}_4$
 $\Delta_c H_{MET} = -55 \text{ kJ/g CH}_4 - \left(-14.3 \frac{\text{kJ}}{\text{g COD}} * 0.73 \frac{\text{g vss}}{\text{g CH}_4} * 1.42 \frac{\text{OD}}{\text{g vss}} \right)$
 $\Delta_c H_{MET} = -40 \text{ kJ/g CH}_4 = -643 \text{ kJ/mol CH}_4$

Heat released per gram of vss = $-40 \text{ kJ/g CH}_4 * Y^{-1} = -55 \text{ kJ/g vss}$
 Energy Loss = $\frac{\Delta_c H_{MET}}{\Delta_c H_{CH_4}} = 73\% \text{ heat loss}$
 Or, only 27% of energy in methane is captured

$CH_4 + 1.48O_2 + 0.10NH_3 = 0.10C_5H_7O_2N + 0.48CO_2 + 1.79H_2O + 643 \text{ kJ Heat}$
 $10CH_4 + 14.8O_2 + NH_3 = C_5H_7O_2N + 4.8CO_2 + 17.9H_2O + 6,430 \text{ kJ Heat}$

Hydrogenotrophic Bacteria

$Y = 2.24 \text{ g vss/g H}_2$
 $H_2 + \frac{1}{2}O_2 = H_2O + 286 \text{ kJ Heat}$
 $\Delta_c H_{H_2} = -143 \text{ kJ/g H}_2$
 $\Delta_c H_{MET} = -143 \text{ kJ/g H}_2 - \left(-14.3 \frac{\text{kJ}}{\text{g COD}} * 2.24 \frac{\text{g vss}}{\text{g H}_2} * 1.42 \frac{\text{OD}}{\text{g vss}} \right)$
 $\Delta_c H_{MET} = -97.5 \text{ kJ/g H}_2 = -195 \text{ kJ/mol H}_2$

Heat released per gram of vss = $-97.5 \text{ kJ/g H}_2 * Y^{-1} = -43.5 \text{ kJ/g vss}$
 Energy Loss = $\frac{\Delta_c H_{MET}}{\Delta_c H_{H_2}} = 68\% \text{ heat loss}$
 Or, only 32% of energy in hydrogen is captured

$H_2 + 0.2CO_2 + 0.3O_2 + 0.04NH_3 = 0.04C_5H_7O_2N + 0.92H_2O + 195 \text{ kJ Heat}$
 $25H_2 + 5CO_2 + 7.5O_2 + NH_3 = C_5H_7O_2N + 23H_2O + 4,875 \text{ kJ Heat}$

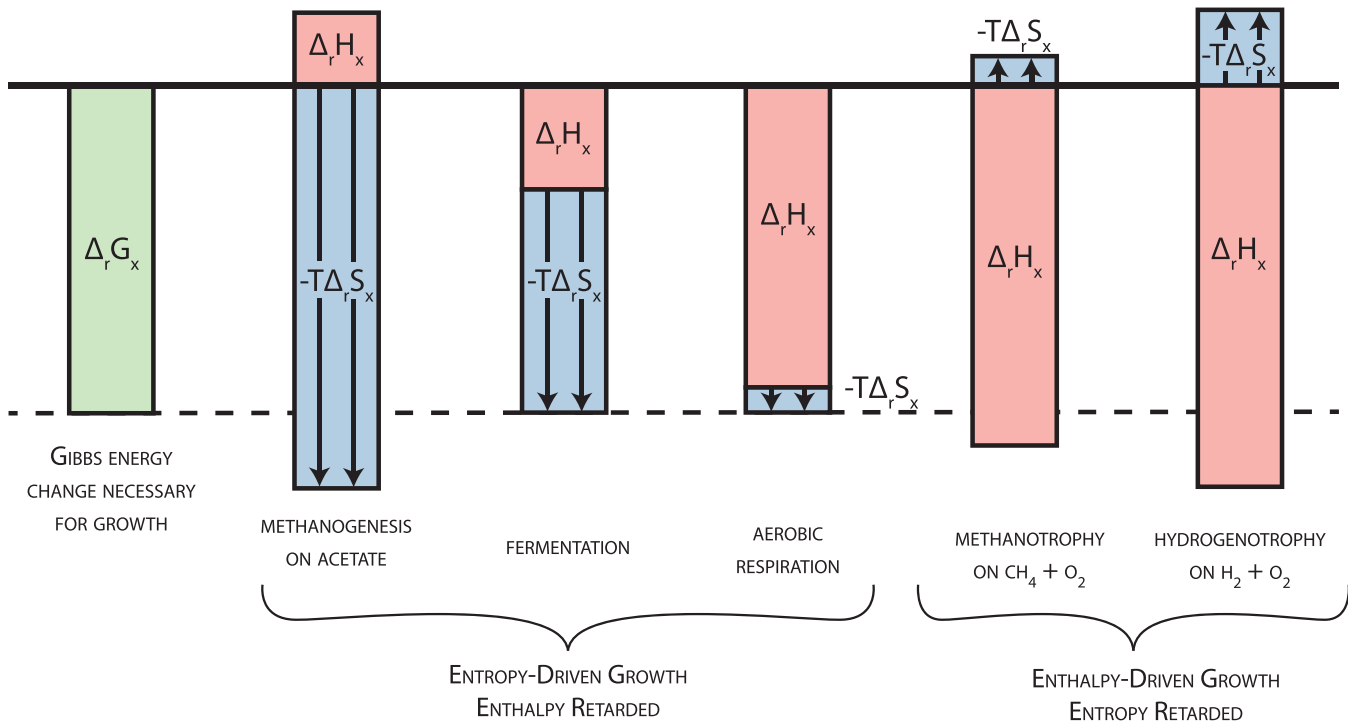


Figure 3. Thermodynamics of chemoautotrophic growth, modified from von Stockar et al. (2006).⁹¹ Microbial growth is only possible when Δ_rG_x is negative, so 0 < Δ_rH_x - TΔ_rS_x. For chemoautotrophs, the gas reactants have higher entropy than the products, thus Δ_rS_x is negative and -TΔ_rS_x. The microbial reaction will only proceed spontaneously from reactants to products when Δ_rH_x < -TΔ_rS_x. A highly negative change in enthalpy, Δ_rH_x, is required. See Supporting Information (SI) for calculation details.

3000 h⁻¹.⁹⁴ Mango Materials in California utilizes a proprietary gas delivery system in a deep tank bioreactor which operates at atmospheric pressure. The system delivers two gases separately while maintaining mixtures outside the flammable range.⁹⁵

For design of engineered systems that efficiently deliver sparingly soluble gases while dissipating heat, natural dark food chains can provide inspiration as they have evolved to overcome these limitations and support high levels of productivity. Giant

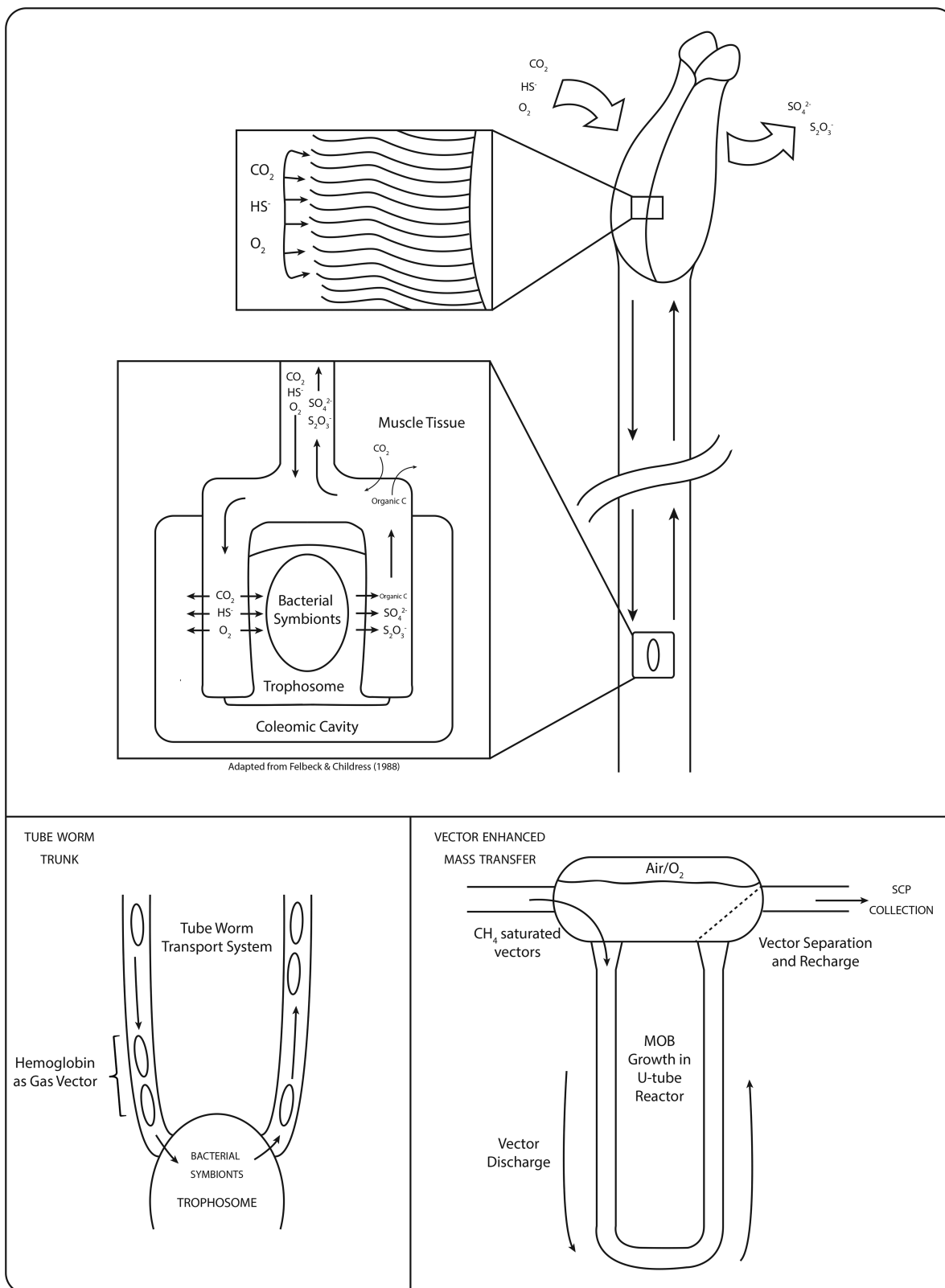


Figure 4. Tube worm, illustrating whole tube worm and transfer of gases from plume and transport to trophosome (top). Bottom left: transport of gaseous substrate bound to hemoglobin; Bottom right: engineered use of substrate vectors (biopolymers, surfactants, oils, etc.) for enhanced transport of sparingly soluble gases (biomimicry).

tube worms have evolved a highly specialized method of delivering gases to their bacterial symbionts (Figure 4). These organisms maximize surface area for mass and heat transfer in their plume and trophosome, the vascular organ that houses

intracellular symbionts. Gases bind to hemoglobin and are transported from the plume to the trophosome.⁹⁶ The trophosome is in turn surrounded by a coelomic cavity, where gaseous substrates are stored in case of shortage.⁹⁶ An engineering analog

might involve use of solid vectors or liquids (such as emulsions or oil droplets) that have high solubility for the gases to be delivered (Figure 4).^{97–99} Alternative approaches might incorporate hollow fiber membranes that enable formation of small gas bubbles with high surface area for efficient gas delivery and ionic strength solutions that stabilize small bubbles, preventing coalescence and formation of larger bubbles.¹⁰⁰

4.5. Energy and Space Requirements for Production of SCP and Aquaculture Protein. Table 2 summarizes

Table 2. Energy Demand (MJ/kg N-protein) for Methanotrophic and Hydrogenotrophic-Derived Protein Synthesis

	energy for microbial protein production ^a	energy for feed to shrimp (FCR = 1.7) ⁴⁷	energy for feed to salmon (FCR = 1.3) ⁴⁷
methane-oxidizing bacteria	361 ¹⁰²	679	606
hydrogen-oxidizing bacteria	452 ¹⁰²	850	758

^aEnergy for production of 1 kg of edible protein are from Matassa et al. (2015).¹⁰² This value is based on the thermodynamic assessment of Heijnen et al. (1992a),¹⁰³ Heijnen et al. (1992b),¹⁰⁴ and Heijnen et al. (2002).¹⁰⁵ The input energy for MOB and HOB is a theoretical thermodynamic minimum equal to the $-T\Delta_r S_x$ term illustrated in Figure 3. Energy for production of shrimp and salmon assumes: (1) Feed conversion ratio (FCR) is the same for conventional feed as for microbial protein feed and (2) Feed is 100% microbial protein. Shrimp are assumed to be 70% protein;¹⁰⁶ salmon are assumed to be 60% protein;¹⁰⁷ protein is assumed to be 16% nitrogen;¹⁰⁸ empirical formula for microbial biomass is assumed to be $C_5H_7O_2N$.

estimated energy requirements for nitrogen assimilation in MOB and HOB microbial protein and subsequent conversion into shrimp or salmon protein can be estimated using the method of Matassa et al. (2015).⁴⁴ The lower values for MOB reflects the lower assimilatory energy requirement of MOB illustrated in Figure 3. For comparison, chicken, turkey, and beef have estimated energy requirements of 400, 1000, and 4000 MJ/kg N-protein.¹⁰¹

Use of waste-derived biogas- CH_4 and renewable H_2 can potentially save land for agriculture. In one analysis, use of microbial protein production over the 2005–2050 period can offset demand for 109 million hectares of cropland. Such a change could avoid emissions of 40 Gt of CO_2 equivalents over the same time period.¹⁰⁹ In dark bacterial cultures, high volumetric productivities can be achieved with both MOB and HOB: researchers have reported upper values of around 100 g/L-day^{78,110} compared to 0.2–0.4 g/L-day for light-limited algal cultures.¹¹¹

Table 3 summarizes estimated protein productivity values for MOB. These values can be many orders of magnitude greater than values reported for conventional agriculture (soy beans shown for comparison), indicating dramatically reduced space requirements and making SCP production particularly attractive for space-constrained environments, such as cities. Use of MOB also suggests possible opportunities for N_2 fixation analogous to the use of legumes in conventional agriculture. As shown in Table 3, use of N_2 in air as the sole source of nitrogen for Type II methanotrophs enabled a protein productivity (unoptimized) similar to that of soybeans.¹¹²

4.6. Microbial SCP with Value Added Products. Aquaculture is subject to high mortality risks due to bacterial and viral pathogens,¹¹⁶ but recent discoveries indicate that

Table 3. Estimated Protein Productivity for Methanotrophic Bioreactor Systems and Soybeans^a

product	growth conditions	protein productivity (kg protein/m ³ -day)
MOB microbial protein	air and methane fed fluidized bed reactor (N_2 as N-source)	0.00052 ¹¹²
	batch serum bottle incubation (ammonia N source)	2.8 ⁶⁹
	high pressure (6 atm) Chemostat	60.5 ¹¹⁰
soybean	typical yield in midwest U.S. (1995–2012)	0.00054 ¹¹⁵

^aAssumed protein content is 60% for MOB¹¹³ (a conservative assumption) and 35% for soybean.¹¹⁴ Soybean production determined using the yield per area with the assumption that 1 m of vertical space is required (height of plant plus air space).

microbial products coproduced with SCP can improve health outcomes for aquatic animals. These findings increase the value of microbial biomass as a feedstock and the economic viability of dark food chains. By replacing fishmeal, microbial biomass can potentially increase the sustainability of aquaculture while simultaneously increasing yields and profitability.

Polyhydroxyalkanoates (PHAs) are a broad class of ubiquitous naturally occurring biopolymers stored as granules within many microorganisms under conditions of unbalanced growth,¹¹⁷ that is, when carbon/electron donor is present in excess, but an essential nutrient or electron acceptor needed for cell division (N, P, or O_2) is lacking.¹¹² In the absence of added cosubstrates, methanotrophs (Type I and some Type II)¹¹⁸ and hydrogen-utilizing bacteria produce granules that only contain poly-3-hydroxybutyrate (PHB).¹¹⁹ If cosubstrates are added during the PHA accumulation phase, the granules can contain copolymers, such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate).^{119–123}

Over the past decade, many studies have established that PHB can be a valuable aquaculture feed supplement—improving health outcomes for brine shrimp,¹²⁴ prawn,¹²⁵ crab¹²⁶ and fish.¹²⁷ Many microorganisms produce PHB, including MOB¹²⁸ and HOB.⁴⁴ A typical PHB yield for Type II MOB is 0.5 g PHB/g methane (equal to 0.12 g PHB/g CH_4 as oxygen demand)¹¹⁰ and 0.12 g PHB/g H_2 as oxygen demand.⁴⁴ MOB production of PHB occurs after cells have become limited for a critical nutrient, with time requirements ranging from 12 to 24 h for MOB;¹¹⁰ HOB production times are similar.¹¹⁹

PHB improves health outcomes of aquatic animals under normal growth conditions and when subject to pathogenic and environmental stress (Table 4). One of the most well studied phenomena is the ability of PHB to improve health outcomes for a variety of animals challenged with bacterial pathogens,^{48,49,125,129–136} making it a promising alternative to antibiotics. Both PHB and its hydroxybutyrate monomer suppress pathogenic Vibrios, completely inhibiting *Vibrio* growth when the monomer is present at 100 mM.¹³⁰ At concentrations achievable in the intestinal tract of *Artemia* (24 mM), hydroxybutyrate can decrease production of pathogenic *Vibrio* virulence factors.¹³⁷ PHB also acts as an immunostimulant, with its inclusion in diet resulting in increased activity of the innate immune system.⁴⁸ Increased antibody response was observed in tilapia⁴⁹ and synthesis of Heat Shock Protein 70, a signal of immune response, was observed in brine shrimp.⁴⁸ Recent research indicates that ingestion of PHB may improve survival of *P. monodon* against White Spot Syndrome Virus,¹³⁸ supporting the hypothesis that PHB activates the innate immune system, providing nonspecific protection against an array of pathogens.

Table 4. Effects of PHB Supplemented Diet on Aquatic Organisms

animal fed PHB	scientific name	life stage	unchallenged survival	effect on growth	type of challenge	survival in challenge test	references
brine shrimp	<i>Artemia franciscana</i>	Nauplii			<i>Vibrio campbellii</i> and <i>Vibrio harveyi</i>	increased	48,124, 130,144
freshwater prawn	<i>Macrobrachium rosenbergii</i>	larvae	increased	increased	<i>Vibrio harveyi</i>	increased	131,133
Asian tiger shrimp	<i>Penaeus monodon</i>	post larvae	increased	no significant effect	lethal ammonia dose	increased	125,134
		adult			low level ammonia + <i>Vibrio campbellii</i>	increased	
					<i>Vibrio campbellii</i>	increased	138
Pacific white shrimp	<i>Litopenaeus vannamei</i>	post larvae	increased	increased	salinity + <i>V. anguillarum</i>	increased	145
Chinese mitten crab	<i>Eriocheir sinensis</i>	larvae	increased	increased	<i>Vibrio anguillarum</i>	increased	135
Siberian sturgeon	<i>Acipenser baerii</i>	larvae	no significant effect	decreased	salinity and ammonia	decreased	140
		fingerling	increased	increased			139
Mozambique tilapia	<i>Oreochromis mossambicus</i>	adult			<i>Aeromonas hydrophila</i>	increased	49
Nile tilapia	<i>Oreochromis niloticus</i>	juvenile	no significant change	trend of increased weight gain, not significant			136
		larvae			<i>Edwardsiella ictaluri</i>	increased	136
Rainbow trout	<i>Oncorhynchus mykiss</i>	fry	no significant effect	decreased weight week 1–2, increased weight week 5–6	<i>Yersinia ruckeri</i>	increased	129
European sea bass	<i>Dicentrarchus labrax</i>	Yok-sac larvae	increased	trend toward increased weight	<i>Vibrio anguillarum</i>	no significant effect	146
		juvenile	no significant effect	increased			127
red drum (channel bass)	<i>Sciaenops ocellatus</i>	juvenile	no significant effect	decreased			147
blue mussel	<i>Mytilus edulis</i>	larvae	increased	no significant effect			148

Experimental evidence also suggests that aquatic animals derive energy from PHB consumption, potentially further contributing to improved health outcomes.^{126,127,130}

PHB particle size, delivery method, and proper dosage affect the efficacy of PHB ingestion. A decrease in size of crystalline PHB particles decreases optimal dose concentration by an order of magnitude.⁴⁸ Several studies underscore the importance of optimal PHB dosage, a value that appears to be both species-specific and life stage-specific. Both Siberian Sturgeon and Rainbow trout experienced adverse effects on growth when fed PHB at younger life stages, but positive outcomes at slightly more advanced stages of growth.^{129,139,140} While optimal dosing is necessary, the overall picture is that PHB can have very positive impacts. Importantly, health benefits for aquaculture are observed when PHB is delivered packaged within a bacterial cell.^{124,136,141} This is significant because feeding of PHB inside harvested dead cells or its provision as live feed avoids the need for chemical- and energy-intensive PHB extraction, and enhances the feasibility of controlled use of PHB in conjunction with SCP production.

Preliminary research indicates that other substances in microbial biomass besides PHB can have beneficial impacts as animal feed. Ingestion of feed containing the Type I methanotroph *Methylococcus capsulatus* reduces gut enteropathy in salmon^{50,51} and mice.¹⁴² These methanotrophs do not accumulate PHB, suggesting a health benefit based upon a different mechanism.¹²⁸ Other microbial products that improve animal feed quality include phospholipids that improve human cholesterol levels and vitamin B12.¹⁴³

V. SYSTEM INTEGRATION: CYCLING OF WATER, CARBON, AND NUTRIENTS

5.1. Food Chains, Carbon Use Efficiency, And Protein Quality. Useful metrics for assessment of dark food chains are microbial biomass yields within pure culture bioreactors (g cell biomass/g substrate), carbon use efficiencies (g biomass production per g substrate for mixed cultures and communities),¹⁴⁹ and feed conversion ratios (kg feed/kg animal biomass).⁴⁷ Ecologists have observed that C-use efficiency (CUE, carbon assimilated in grazer/carbon in autotroph consumed × 100%) in ecosystems is determined by the elemental ratio of autotrophic biomass (i.e., C:N and C:P). In turn, CUE increases when C:N and C:P ratios approximate those of the grazers or consumers.¹⁵⁰ Because PHB and other storage products (e.g., glycogen) lack N and P, manipulation of carbon storage within cells presents a possible avenue for optimization of CUE.

Carbon use efficiency within engineered food chains is affected by the number of trophic levels. The simplest scenario consists of a two-level chain in which microbial protein is used for direct human consumption. FDA-approved products such as Quorn, Vegemite, and Marmite (all fungi) have achieved commercial success in such a food chain.⁴⁵ Use of bacterial microbial protein in a two-level food chain would require treatment to reduce RNA content to levels acceptable for human consumption—in fact, high nucleic acid content is a primary reason why microbial protein from bacteria is considered preferable when used as a feed for animals with short lifespans.^{45,151,152} Thus, given practical and cultural considerations, the most

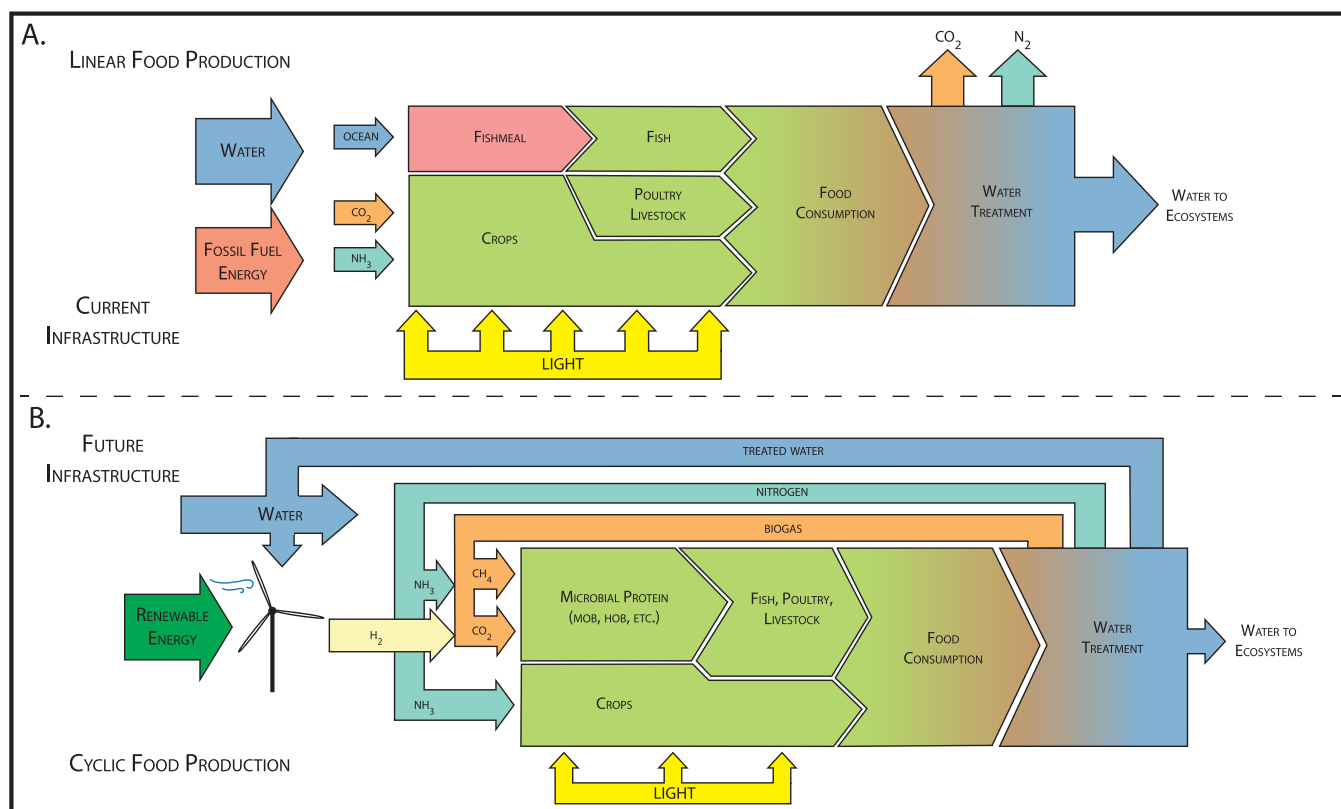


Figure 5. A. Schematic representation of current infrastructure with linear food production. B. Future infrastructure incorporating microbial protein production, taking advantage of resource recycling enabled by wastewater treatment.

probable food chain would consist of three levels: microbial protein as a feed for animals, which are in turn consumed by humans. Research has explored use of microbial protein for livestock (pigs, beef, and dairy cattle) and poultry (broiler chickens and laying hens).^{109,113,153} Additional trophic levels are also possible: microbial protein could serve as feed for *Daphnia*, for example, which can then serve as live feed for aquaculture. However, each additional level reduces carbon use efficiency of the overall process, resulting in increased CO₂ production.¹⁴⁹

5.2. Integrated Resource Recovery Systems. Engineered dark food chains create interesting opportunities for recycling of water, carbon and nitrogen (Figures 2 and 5). Treatment and reuse of water for a SCP growth and for aquaculture offsets the demand for imported water. Similarly, recycling of biogas carbon and nitrogen can offset requirements for imported carbon and nitrogen. At present, recirculation aquaculture practice includes technologies for water purification via solids removal, nitrogen removal via biofilters, re-aeration and disinfection.⁵⁵ Configurations designed for resource recovery can be envisioned for SCP production.

Carbon reuse can be enabled by dark food chains because CH₄ and CO₂ are at once feedstocks for microbial protein and products of wastewater treatment. Methanogens generate biogas from waste; methanotrophs consume the biogas to produce SCP; SCP is used to produce animal protein; and the loop is closed when the animal protein is consumed and converted again into waste.

Nitrogen recovery and reuse are likewise enabled by dark food chains because ammonium and nitrate are at once feedstocks for microbial protein production and products of wastewater treatment. Through recycle loops, nitrogen cycles that incorporate dark food chains can avoid loss of nitrogen through nitrogen

recovery and its upcycling into animal protein (Figures 2c and 5). Recovery of nutrients may be achieved in settings where domestic wastewater is treated, an alternative that has been successfully tested with urine streams in low-income settings.¹⁵⁴ Further benefits are incurred by avoiding energy-intensive fixation of N₂ to NH₃ (37–45 MJ/kg N as NH₃) via the Haber-Basch process.¹⁰² The minimum energy required for production of 1 kg of edible animal protein grown on microbial protein ranges from 606 to 850 MJ/kg-N protein (Table 2). Caution must of course be taken to avoid accumulation of toxic levels of ammonia within recirculation systems.

Heat is another resource that can potentially be harvested within combined microbial protein-aquaculture systems. As discussed in Section 4.3, microbial chemoautotrophy is accompanied by high rates of heat production. Heat pumps can be used to harness this energy. Heat can also be used at various disinfection stages in water treatment (for Advanced Treatment in Figure 2c), or for treatment of microbial protein. Specifically, heat can be used for both drying of cells and for reduction of total RNA content via activation of endogenous RNA degrading ribonucleases,⁴⁵ an important consideration if SCP is to be used for human consumption (as discussed in Section 5.1).

VI. CONCLUSIONS AND RESEARCH GAPS

Engineered dark food chains have the potential to provide many benefits as a supply of microbial protein. Such food chains have the potential to decrease reliance upon fishmeal and enable high volumetric rates of productivity, thereby reducing land requirements and associated greenhouse gas emissions. Additionally, valuable coproducts, such as PHB, can be produced, enabling increased survival of aquaculture animals and reduced use of

antibiotics. However, life cycle assessment and technoeconomic analyses of such food chains are needed for successful integration of technologies within different cultural and environmental settings.

Research is needed to improve the quality of feedstocks, optimize low-energy bioreactor designs, and integrate systems to enable reuse of water, carbon, and nitrogen. Renewable hydrogen offers significant advantages: it is clean and can potentially be deployed anywhere hydrogen can be generated.⁴⁴ Biogas is ubiquitous and promising, but of variable quality, and methane leaks are a serious concern that must be prevented. Depending upon the source of biogas, contaminants such as hydrogen sulfide and siloxanes may need to be removed before use as a feedstock.

For both hydrogen- and biogas-based bioreactors, methods are needed to safely deliver sparingly soluble gases in aerobic fermentations. Renewable bioelectrosynthesis technologies now in development could enable low-cost production of soluble substrates from CO₂, such as acetic acid, formate, and methanol.^{3,155–157} Such feedstocks could support heterotrophic dark food chains that avoid the challenges associated with delivery of sparingly soluble gases.

Increased fundamental knowledge of dark food chains in natural ecosystems can facilitate new engineering applications. For example, understanding of carbon sequestration mechanisms in the deep ocean may reveal currently unknown microbial metabolic processes that can motivate development of new systems.¹² Currently, dark food chains are researched in many different academic fields, and increasing connectivity across these disciplines can enable more rapid and improved development of sustainable food systems of the future. Ref 144.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b04038.

Dark food chain generic schematic, solubility of substrate gases, thermodynamic calculations (PDF)

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The authors declare no competing financial interest.

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■ REFERENCES

(1) World Population Prospects: The 2017 Revision Key Findings and Advance Tables, Working Paper No. ESA/P/WP/248; United Nations, 2017.

(2) United Nations. Goal 2: End Hunger, achieve food security and improved nutrition and promote sustainable agriculture <https://www.un.org/sustainabledevelopment/hunger/> (accessed April 10, 2018).

(3) Deutzmann, J. S.; Spormann, A. M. Enhanced Microbial Electrosynthesis by Using Defined Co-Cultures. *ISME J.* **2017**, *11* (3), 704–714.

(4) Abubakar, H. N.; Veiga, M. C.; Kennes, C. Carbon Monoxide Fermentation to Ethanol by *Clostridium Autoethanogenum* in a Bioreactor with No Accumulation of Acetic Acid. *Bioresour. Technol.* **2015**, *186*, 122–127.

(5) Kozubal, M. A.; Macur, R. E.; Inskip, W. P. Acidophilic *Fusarium Oxysporum* Strains, Methods of Their Production and Methods of Their Use. U.S. Patent 9796989 B2, 2017.

(6) Kelley, D. S.; Baross, J. A.; Delaney, J. R. Volcanoes, Fluids, and Life at Mid-Ocean Ridge Spreading Centers. *Annu. Rev. Earth Planet. Sci.* **2002**, *30* (1), 385–491.

(7) Martin, W.; Baross, J.; Kelley, D.; Russell, M. J. Hydrothermal Vents and the Origin of Life. *Nat. Rev. Microbiol.* **2008**, *6* (11), 805–814.

(8) Van Dover, C. L. *The Ecology of Deep-Sea Hydrothermal Vents*; Princeton University Press: Princeton, NJ, 2000.

(9) Petersen, J. M.; Zielinski, F. U.; Pape, T.; Seifert, R.; Moraru, C.; Amann, R.; Hourdez, S.; Girguis, P. R.; Wankel, S. D.; Barbe, V.; Pelletier, E.; Fink, D.; Borowski, C.; Bach, W.; Dubilier, N. Hydrogen Is an Energy Source for Hydrothermal Vent Symbioses. *Nature* **2011**, *476* (7359), 176–180.

(10) Dubilier, N.; Bergin, C.; Lott, C. Symbiotic Diversity in Marine Animals: The Art of Harnessing Chemosynthesis. *Nat. Rev. Microbiol.* **2008**, *6* (10), 725–740.

(11) Levin, L. A. Ecology of Cold Seep Sediments: Interactions of Fauna with Flow, Chemistry and Microbes. *Oceanogr. Mar. Biol. Annu. Rev.* **2005**, *43*, 1–46.

(12) Sweetman, A. K.; Smith, C. R.; Shulze, C. N.; Maillot, B.; Lindh, M.; Church, M. J.; Meyer, K. S.; van Oevelen, D.; Stratmann, T.; Gooday, A. J. Key Role of Bacteria in the Short-Term Cycling of Carbon at the Abyssal Seafloor in a Low Particulate Organic Carbon Flux Region of the Eastern Pacific Ocean. *Limnol. Oceanogr.* **2018**, 1–20.

(13) Dattagupta, S.; Schaperdoth, I.; Montanari, A.; Mariani, S.; Kita, N.; Valley, J. W.; MacAlady, J. L. A Novel Symbiosis between Chemoautotrophic Bacteria and a Freshwater Cave Amphipod. *ISME J.* **2009**, *3* (8), 935–943.

(14) Forti, P.; Galdenzi, S.; Sarbu, S. M. The Hypogenic Caves: A Powerful Tool for the Study of Seeps and Their Environmental Effects. *Cont. Shelf Res.* **2002**, *22* (16), 2373–2386.

(15) Sarbu, S. M.; Kane, T. C.; Kinkle, B. K. A Chemoautotrophically Based Cave Ecosystem. *Science* **1996**, *272* (5270), 1953–1955.

(16) Kumaresan, D.; Wischer, D.; Stephenson, J.; Hillebrand-Voiculescu, A.; Murrell, J. C. Microbiology of Movile Cave—A Chemolithoautotrophic Ecosystem. *Geomicrobiol. J.* **2014**, *31* (3), 186–193.

(17) Chen, Y.; Wu, L.; Boden, R.; Hillebrand, A.; Kumaresan, D.; Moussard, H.; Baciu, M.; Lu, Y.; Colin Murrell, J. Life without Light: Microbial Diversity and Evidence of Sulfur- and Ammonium-Based Chemolithotrophy in Movile Cave. *ISME J.* **2009**, *3* (9), 1093–1104.

(18) Sarbu, S. M.; Galdenzi, S.; Menichetti, M.; Gentile, G. *Geology and Biology of the Frasassi Caves in Central Italy: An Ecological Multi-Disciplinary Study of Hydrogenetic Underground Karst System*; Wilkens, H., Culver, D. C., Humphreys, W. F., Eds.; 2014.

(19) Macalady, J. L.; Dattagupta, S.; Schaperdoth, I.; Jones, D. S.; Druschel, G. K.; Eastman, D. Niche Differentiation among Sulfur-Oxidizing Bacterial Populations in Cave Waters. *ISME J.* **2008**, *2* (6), 590–601.

(20) Jones, R. I.; Grey, J. Biogenic Methane in Freshwater Food Webs. *Freshwater Biol.* **2011**, *56*, 213–229.

(21) Sundh, I.; Bastviken, D.; Tranvik, L. J. Abundance, Activity, and Community Structure of Pelagic Methane-Oxidizing Bacteria in Temperate Lakes. *Appl. Environ. Microbiol.* **2005**, *71* (11), 6746–6752.

- (22) Devlin, S. P.; Saarenheimo, J.; Syväranta, J.; Jones, R. I. Top Consumer Abundance Influences Lake Methane Efflux. *Nat. Commun.* **2015**, *6*. DOI: 10.1038/ncomms9787
- (23) Ravinet, M.; Syväranta, J.; Jones, R. I.; Grey, J. A Trophic Pathway from Biogenic Methane Supports Fish Biomass in a Temperate Lake Ecosystem. *Oikos* **2010**, *119* (2), 409–416.
- (24) Agasild, H.; Zingel, P.; Tuvikene, L.; Tuvikene, A.; Timm, H.; Feldmann, T.; Salujõe, J.; Toming, K.; Jones, R. I.; Nõges, T. Biogenic Methane Contributes to the Food Web of a Large, Shallow Lake. *Freshwater Biol.* **2014**, *59* (2), 272–285.
- (25) Kiyashko, S. I.; Imbs, A. B.; Narita, T.; Svetashev, V. I.; Wada, E. Fatty Acid Composition of Aquatic Insect Larvae *Stictochironomus pictulus* (Diptera: Chironomidae): Evidence of Feeding upon Methanotrophic Bacteria. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2004**, *139* (4), 705–711.
- (26) Taipale, S.; Kankaala, P.; Jones, R. I. Contributions of Different Organic Carbon Sources to *Daphnia* in the Pelagic Foodweb of a Small Polyhumic Lake: Results from Mesocosm DI 13C-Additions. *Ecosystems* **2007**, *10* (5), 757–772.
- (27) Agasild, H.; Zingel, P.; Tuvikene, L.; Tuvikene, A.; Timm, H.; Feldmann, T.; Salujõe, J.; Toming, K.; Jones, R. I.; Nõges, T. Biogenic Methane Contributes to the Food Web of a Large, Shallow Lake. *Freshwater Biol.* **2014**, *59* (2), 272–285.
- (28) Sanseverino, A. M.; Bastviken, D.; Sundh, I.; Pickova, J.; Enrich-Prast, A. Methane Carbon Supports Aquatic Food Webs to the Fish Level. *PLoS One* **2012**, *7* (8).e42723
- (29) Diamond, J. Evolution, Consequences and Future of Plant and Animal Domestication. *Nature* **2002**, *418* (6898), 700–707.
- (30) Nash, C. E. *The History of Aquaculture*; Blackwell Publishing Ltd.: Singapore, 2011.
- (31) Thaysen, A. C. Food Yeast: Its Nutritive Value and Its Production from Empire Sources. *R. Soc. Encour. Arts, Manuf. Commer.* **1945**, *93* (4693), 353–364.
- (32) Braude, R.; Foot, A. S. War-Time Rations for Pigs: Report of Experiments with Mangolds and Biscuit Waste, Fodder Yeast, Urea and Dried Skim Milk. *J. Agric. Sci.* **1942**, *32* (1), 70–84.
- (33) Scrimshaw, N. S. Introduction. In *Single Cell Protein*; Mateles, R. I., Tannenbaum, S. R., Eds.; The MIT Press: Cambridge, MA, 1968; pp 3–7.
- (34) Scrimshaw, N. S. Single-Cell Protein for Human Consumption—An Overview. In *Single Cell Protein II*; Tannenbaum, S. R., Wang, D. I., Eds.; The MIT Press: Cambridge, MA, 1975; pp 24–45.
- (35) Mateles, R. I.; Tannenbaum, S. R. Preface. In *Single Cell Protein*; Mateles, R. I., Tannenbaum, S. R., Eds.; The MIT Press: Cambridge, MA, 1968; pp v–vi.
- (36) Wilson, C. L. Concluding Remarks. In *Single Cell Protein*; Mateles, R. I., Tannenbaum, S. R., Eds.; Cambridge, MA, 1968; pp 457–461.
- (37) Foster, J. F.; Litchfield, J. H. A Continuous Culture Apparatus for the Microbial Utilization of Hydrogen Produced by Electrolysis of Water in Closed-Cycle Space Systems. *Biotechnol. Bioeng.* **1964**, *6* (4), 441–456.
- (38) Goldberg, I. Organisms and Substrates. In *Single Cell Protein*; Aiba, S., Fan, L. T., Fiechter, A., Schügerl, K., Eds.; Springer-Verlag: Berlin, 1985; pp 11–66.
- (39) Goldberg, I. Fermentation Processes for Microbial SCP Production. In *Single Cell Protein*; Aiba, S., Fan, L. T., Fiechter, A., Schügerl, K., Eds.; Springer-Verlag: Berlin, 1985; pp 67–128.
- (40) Labuza, T. P. Cell Collection: Recovery and Drying for SCP Manufacture. In *Single Cell Protein II*; Tannenbaum, S. R., Want, D. I. C., Eds.; The MIT Press: Cambridge, MA, 1975; pp 69–104.
- (41) Goldberg, I. Concluding Remarks and Epilogue. In *Single Cell Protein*; Aiba, S., Fan, L. T., Fiechter, A., Schügerl, K., Eds.; Springer-Verlag: Berlin, 1985; pp 153–160.
- (42) Anthony, C. John Rodney Quayle: 18 November 1926 – 2006. *Biogr. Mem. Fellows R. Soc.* **2015**, *61* (February 2006), 331–349.
- (43) Erlichman, J. Biotech Fungus for Our Daily Bread. *Guardian.* **1984**, 16.
- (44) Matassa, S.; Boon, N.; Verstraete, W. Resource Recovery from Used Water: The Manufacturing Abilities of Hydrogen-Oxidizing Bacteria. *Water Res.* **2015**, *68*, 467–478.
- (45) Ritala, A.; Häkkinen, S. T.; Toivari, M.; Wiebe, M. G. Single Cell Protein – State-of-the-Art, Industrial Landscape and Patents 2001–2016. *Front. Microbiol.* **2017**, *8*, 1–18.
- (46) Matassa, S.; Boon, N.; Pikaar, I.; Verstraete, W. Microbial Protein: Future Sustainable Food Supply Route with Low Environmental Footprint. *Microb. Biotechnol.* **2016**, *9* (5), S68–S75.
- (47) Naylor, R. L.; Hardy, R. W.; Bureau, D. P.; Chiu, A.; Elliot, M.; Farrell, A. P.; Forster, I.; Gatlin, D. M.; Goldberg, R. J.; Hua, K.; Nichols, P. D. Feeding Aquaculture in an Era of Finite Resources. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (42), 15103–15110.
- (48) Baruah, K.; Huy, T. T.; Norouzitallab, P.; Niu, Y.; Gupta, S. K.; De Schryver, P.; Bossier, P. Probing the Protective Mechanism of Poly- β -Hydroxybutyrate against Vibriosis by Using Gnotobiotic *Artemia franciscana* and *Vibrio campbellii* as Host-Pathogen Model. *Sci. Rep.* **2015**, *5*, 9427.
- (49) Suguna, P.; Binuramesh, C.; Abirami, P.; Saranya, V.; Poornima, K.; Rajeswari, V.; Shenbagarathai, R. Immunostimulation by Poly- β -Hydroxybutyrate-Hydroxyvalerate (PHB-HV) from *Bacillus thuringiensis* in *Oreochromis mossambicus*. *Fish Shellfish Immunol.* **2014**, *36* (1), 90–97.
- (50) Romarheim, O. H.; Øverland, M.; Mydland, L. T.; Skrede, A.; Landsverk, T. Bacteria Grown on Natural Gas Prevent Soybean Meal-Induced Enteritis in Atlantic Salmon. *J. Nutr.* **2011**, *141* (1), 124–130.
- (51) Romarheim, O. H.; Landsverk, T.; Mydland, L. T.; Skrede, A.; Øverland, M. Cell Wall Fractions from *Methylococcus capsulatus* Prevent Soybean Meal-Induced Enteritis in Atlantic Salmon (*Salmo salar*). *Aquaculture* **2013**, *402–403* (S75), 13–18.
- (52) Troell, M.; Naylor, R. L.; Metian, M.; Beveridge, M.; Tyedmers, P. H.; Folke, C.; Arrow, K. J.; Barrett, S.; Crépin, A.-S.; Ehrlich, P. R.; Gren, Å.; Kautsky, N.; Levin, S. A.; Nyborg, K.; Österblom, H.; Polasky, S.; Scheffer, M.; Walker, B. H.; Xepapadeas, T.; de Zeeuw, A. Does Aquaculture Add Resilience to the Global Food System? *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (37), 13257–13263.
- (53) Béné, C.; Arthur, R.; Norbury, H.; Allison, E. H.; Beveridge, M.; Bush, S.; Campling, L.; Leschen, W.; Little, D.; Squires, D.; Thilsted, S. H.; Troell, M.; Williams, M. Contribution of Fisheries and Aquaculture to Food Security and Poverty Reduction: Assessing the Current Evidence. *World Dev.* **2016**, *79*, 177–196.
- (54) Kawarazuka, N.; Béné, C. Linking Small-Scale Fisheries and Aquaculture to Household Nutritional Security: An Overview. *Food Secur.* **2010**, *2* (4), 343–357.
- (55) Klinger, D.; Naylor, R. Searching for Solutions in Aquaculture: Charting a Sustainable Course. *Annu. Rev. Environ. Resour.* **2012**, *37* (1), 247–276.
- (56) Smith, A. D. M.; Brown, C. J.; Bulman, C. M.; Fulton, E. A.; Johnson, P.; Kaplan, I. C.; Lozano-Montes, H.; Mackinson, S.; Marzloff, M.; Shannon, L. J.; Shin, Y.; Tam, J. Impacts of Fishing Low-Trophic Level Species on Marine Ecosystems. *Science* **2011**, *333* (August), 1147–1150.
- (57) Alder, J.; Campbell, B.; Karpouzi, V.; Kaschner, K.; Pauly, D. Forage Fish: From Ecosystems to Markets. *Annu. Rev. Environ. Resour.* **2008**, *33* (1), 153–166.
- (58) Kumamuru, B. *WBA Global Bioenergy Statistics*. World Bioenergy Association, 2017.
- (59) Bond, T.; Templeton, M. R. History and Future of Domestic Biogas Plants in the Developing World. *Energy Sustainable Dev.* **2011**, *15* (4), 347–354.
- (60) Shen, Y.; Linville, J. L.; Urgun-Demirtas, M.; Mintz, M. M.; Snyder, S. W. An Overview of Biogas Production and Utilization at Full-Scale Wastewater Treatment Plants (WWTPs) in the United States: Challenges and Opportunities towards Energy-Neutral WWTPs. *Renewable Sustainable Energy Rev.* **2015**, *50*, 346–362.
- (61) Waslien, C. I.; Calloway, D. H.; Margen, S. Human Intolerance to Bacteria as Food. *Nature* **1969**, *221*, 84–85.
- (62) Calloway, D. H.; Kumar, A. M. Protein Quality of the Bacterium *Hydrogenomonas eutropha*. *Appl. Microbiol.* **1969**, *17* (1), 176–178.

- (63) Repaske, R.; Mayer, R. Dense Autotrophic Cultures of *Alcaligenes Eutrophus*. *Appl. Environ. Microbiol.* **1976**, *32* (4), 592–597.
- (64) Schlegel, H. G.; Lafferty, R. M. Novel Energy and Carbon Sources A. The Production of Biomass from Hydrogen and Carbon Dioxide. In *Advances in Biochemical Engineering*; Springer: Berlin, Heidelberg, 1971; Vol. 1, pp 143–168.
- (65) Xiao, J.; VanBriesen, J. M. Expanded Thermodynamic True Yield Prediction Model: Adjustments and Limitations. *Biodegradation* **2008**, *19* (1), 99–127.
- (66) Vanbriesen, J. M. Thermodynamic Yield Predictions for Biodegradation through Oxygenase Activation Reactions. *Biodegradation* **2001**, *12*, 265–281.
- (67) Yuan, Z.; Vanbriesen, J. M. Yield Prediction and Stoichiometry of Multi-Step Biodegradation Reactions Involving Oxygenation. *Biotechnol. Bioeng.* **2002**, *80* (1), 100–113.
- (68) McCarty, P. L. Thermodynamic Electron Equivalents Model for Bacterial Yield Prediction: Modifications and Comparative Evaluations. *Biotechnol. Bioeng.* **2007**, *97* (2), 377–388.
- (69) Rostkowski, K. H.; Pfluger, A. R.; Criddle, C. S. Stoichiometry and Kinetics of the PHB-Producing Type II Methanotrophs *Methylosinus Trichosporium* OB3b and *Methylocystis Parvus* OB3b. *Bioresour. Technol.* **2013**, *132*, 71–77.
- (70) Yang, S.; Matsen, J. B.; Konopka, M.; Green-Saxena, A.; Clubb, J.; Sadilek, M.; Orphan, V. J.; Beck, D.; Kalyuzhnaya, M. G. Global Molecular Analyses of Methane Metabolism in *Trichosporium* OB3b. Part II. Metabolomics and ¹³C-Labeling Study. *Front. Microbiol.* **2013**, *4* (April), 1–13.
- (71) Broholm, K.; Christensen, T. H.; Jensen, B. K. Modelling TCE Degradation by a Mixed Culture of Methane-Oxidizing Bacteria. *Water Res.* **1992**, *26* (9), 1177–1185.
- (72) Heijnen, J. J.; Roels, J. A. A Macroscopic Model Describing Yield and Maintenance Relationships in Aerobic Fermentation Processes. *Biotechnol. Bioeng.* **1981**, *23*, 739–763.
- (73) Oldenhuis, R.; Oedzes, J. Y.; Van der Waarde, J. J.; Janssen, D. B. Kinetics of Chlorinated Hydrocarbon Degradation by *Methylosinus Trichosporium* OB3b and Toxicity of Trichloroethylene. *Appl. Environ. Microbiol.* **1991**, *57* (1), 7–14.
- (74) Anderson, J. E.; McCarty, P. L. Model for Treatment of Trichloroethylene by Methanotrophic Biofilms. *J. Environ. Eng.* **1994**, *120* (2), 379–400.
- (75) Ferenci, T.; Ström, T.; Quayle, J. R. Oxidation of Carbon Monoxide and Methane by *Pseudomonas Methanica*. *J. Gen. Microbiol.* **1975**, *91*, 79–91.
- (76) Anderson, J. E.; McCarty, P. L. Effect of Three Chlorinated Ethenes on Growth Rates for a Methanotrophic Mixed Culture. *Environ. Sci. Technol.* **1996**, *30* (12), 3517–3524.
- (77) van Bodegom, P.; Stams, F.; Mollema, L.; Boeke, S.; Leffelaar, P. Methane Oxidation and the Competition for Oxygen in the Rice Rhizosphere. *Appl. Environ. Microbiol.* **2001**, *67* (Aug), 3586–3597.
- (78) Ishizaki, A.; Tanaka, K. Batch Culture of *Alcaligenes Eutrophus* ATCC 17697T Using Recycled Gas Closed Circuit Culture System. *J. Ferment. Bioeng.* **1990**, *69* (3), 170–174.
- (79) Adam, N.; Perner, M. Microbially Mediated Hydrogen Cycling in Deep-Sea Hydrothermal Vents. *Front. Microbiol.* **2018**, *9* (November). DOI: 10.3389/fmicb.2018.02873
- (80) Hausinger, R. P. Nickel Utilization by Microorganisms. *Microbiol. Rev.* **1987**, *51* (1), 22–42.
- (81) Glass, J. B.; Orphan, V. J. Trace Metal Requirements for Microbial Enzymes Involved in the Production and Consumption of Methane and Nitrous Oxide. *Front. Microbiol.* **2012**, *3* (FEB), 1–20.
- (82) Gu, W.; Haque, M. F. U.; DiSpirito, A. A.; Semrau, J. D. Uptake and Effect of Rare Earth Elements on Gene Expression in *Methylosinus Trichosporium* OB3b. *FEMS Microbiol. Lett.* **2016**, *363* (13), 1–6.
- (83) Gu, W.; Semrau, J. D. Copper and Cerium-Regulated Gene Expression in *Methylosinus Trichosporium* OB3b. *Appl. Microbiol. Biotechnol.* **2017**, *101* (23–24), 8499–8516.
- (84) Krause, S. M. B.; Johnson, T.; Samadhi Karunaratne, Y.; Fu, Y.; Beck, D. A. C.; Chistoserdova, L.; Lidstrom, M. E. Lanthanide-Dependent Cross-Feeding of Methane-Derived Carbon Is Linked by Microbial Community Interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (2), 358–363.
- (85) Von Stockar, U.; Liu, J. Does Microbial Growth Always Feed on Negative Entropy Thermodynamic Analysis of Microbial Growth. *Biochim. Biophys. Acta, Bioenerg.* **1999**, *1412*, 191–211.
- (86) Von Stockar, U. Biothermodynamics of Live Cells: A Tool for Biotechnology and Biochemical Engineering. *J. Non-Equilib. Thermodyn.* **2010**, *35* (4), 415–475.
- (87) Von Stockar, U.; Marison, I.; Janssen, M.; Patiño, R. Calorimetry and Thermodynamic Aspects of Heterotrophic, Mixotrophic, and Phototrophic Growth. *J. Therm. Anal. Calorim.* **2011**, *104*, 45–52.
- (88) Liu, J. S.; Vojinović, V.; Patiño, R.; Maskow, T.; von Stockar, U. A Comparison of Various Gibbs Energy Dissipation Correlations for Predicting Microbial Growth Yields. *Thermochim. Acta* **2007**, *458* (1–2), 38–46.
- (89) von Stocker, U. Biothermodynamics of Live Cells: Energy Dissipation and Heat Generation in Cellular Cultures. In *Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering*; von Stockar, U., Ed.; Taylor & Francis Group, 2013; pp 475–534.
- (90) Levett, L.; Birkett, G.; Davies, N.; Bell, A.; Langford, A.; Laycock, B.; Lant, P.; Pratt, S. Techno-Economic Assessment of Poly-3-Hydroxybutyrate (PHB) Production from Methane - The Case for Thermophilic Bioprocessing. *J. Environ. Chem. Eng.* **2016**, *4*, 3724–3733.
- (91) Von Stockar, U.; Maskow, T.; Liu, J.; Marison, I. W.; Patiño, R. Thermodynamics of Microbial Growth and Metabolism: An Analysis of the Current Situation. *J. Biotechnol.* **2006**, *121*, 517–533.
- (92) Matheson. Lower and Upper Explosive Limits for Flammable Gases and Vapors (LEL/UEL) [https://www.mathesongas.com/pdfs/products/Lower-\(LEL\)-&-Upper-\(UEL\)-Explosive-Limits-.pdf](https://www.mathesongas.com/pdfs/products/Lower-(LEL)-&-Upper-(UEL)-Explosive-Limits-.pdf) (accessed July 22, 2018).
- (93) Rittman, B.; McCarty, P. L. *Environmental Biotechnology: Principles & Applications*; McGraw Hill Education, 2013.
- (94) Petersen, L. A. H.; Villadsen, J.; Jørgensen, S. B.; Gernaey, K. V. Mixing and Mass Transfer in a Pilot Scale U-Loop Bioreactor. *Biotechnol. Bioeng.* **2017**, *114* (2), 344–354.
- (95) Morse, M. *Mango Materials*; Personal Communication: Redwood City, CA, 2018.
- (96) Felbeck, H.; Childress, J. J. *Riftia Pachyptila: A Highly Integrated Symbiosis*. In *Hydrothermalism, Biology and Ecology Symposium*; Oceanologica Acta: Paris, 1988; pp 131–138.
- (97) Lee, J.; Kim, K.; Chang, I. S.; Kim, M.-G.; Ha, K.-S.; Lee, E. Y.; Lee, J.; Kim, C. Enhanced Mass Transfer Rate of Methane in Aqueous Phase via Methyl-Functionalized SBA-15. *J. Mol. Liq.* **2016**, *215*, 154–160.
- (98) Lee, S.-Y.; Mo, K.-S.; Choi, J.-H.; Hur, N. H.; Kim, Y.-K.; Oh, B.-K.; Lee, J. Enhancement of CH₄-Water Mass Transfer Using Methyl-Modified Mesoporous Silica Nanoparticles. *Korean J. Chem. Eng.* **2015**, *32* (9), 1744–1748.
- (99) Zhang, S.; Wang, D.; Fan, P. P.; Sun, L. P. Enhancement of Gas-to-Liquid Oxygen Transfer in the Presence of Fine Solid Particles for Air-Exposed Multiphase System. *Chem. Eng. Res. Des.* **2015**, *100*, 434–443.
- (100) Myung, J.; Kim, M.; Pan, M.; Criddle, C. S.; Tang, S. K. Y. Low Energy Emulsion-Based Fermentation Enabling Accelerated Methane Mass Transfer and Growth of Poly(3-Hydroxybutyrate)-Accumulating Methanotrophs. *Bioresour. Technol.* **2016**, *207*, 302–307.
- (101) Pimentel, D.; Pimentel, M. Sustainability of Meat-Based and Plant-Based Diets and the Environment. *Am. J. Clin. Nutr.* **2003**, *78* (3 SUPPL), 660–663.
- (102) Matassa, S.; Batstone, D. J.; Huelsen, T.; Schnoor, J. L.; Verstraete, W. Can Direct Conversion of Used Nitrogen to New Feed and Protein Help Feed the World? *Environ. Sci. Technol.* **2015**, *49*, 5247–5254.
- (103) Heijnen, J. J.; van Loosdrecht, M. C. M.; Tijhuis, L. A Black Box Mathematical Model to Calculate Auto-and Heterotrophic Biomass Yields Based on Gibb Energy Dissipation. *Biotechnol. Bioeng.* **1992**, *40*, 1139–1154.

- (104) Heijnen, J. J.; Dijken, J. P. In Search of a Thermodynamic Description of Biomass Yields for the Chemotrophic Growth of Microorganisms. *Biotechnol. Bioeng.* **1992**, *39*, 833–852.
- (105) Heijnen, J. J. Bioenergetics of Microbial Growth. In *Encyclopedia of Bioprocess Technology*; Heijnen, J. J., Kleerebezem, R., Eds.; John Wiley & Sons Inc.: Delft, 2002; pp 267–291.
- (106) Cheng, Z. J.; Hardy, R. W. Protein and Lipid Sources Affect Cholesterol Concentrations of Juvenile Pacific White Shrimp, *Litopenaeus Vannamei* (Boone). *J. Anim. Sci.* **2004**, *82* (4), 1136–1145.
- (107) Opstvedt, J.; Aksnes, A.; Hope, B.; Pike, I. H. Efficiency of Feed Utilization in Atlantic Salmon (*Salmo Salar* L.) Fed Diets with Increasing Substitution of Fish Meal with Vegetable Proteins. *Aquaculture* **2003**, *221* (1–4), 365–379.
- (108) Perrett, D. From “protein” to the Beginnings of Clinical Proteomics. *Proteomics: Clin. Appl.* **2007**, *1* (8), 720–738.
- (109) Pikaar, I.; Matassa, S.; Bodirsky, B. L.; Weindl, I.; Humpenöder, F.; Rabaey, K.; Boon, N.; Bruschi, M.; Yuan, Z.; van Zanten, H.; Herrero, M.; Verstraete, W.; Popp, A. Decoupling Livestock from Land Use through Industrial Feed Production Pathways. *Environ. Sci. Technol.* **2018**, *52*, 7351–7359.
- (110) Wendlandt, K. D.; Jechorek, M.; Helm, J.; Stottmeister, U. Producing Poly-3-Hydroxybutyrate with a High Molecular Mass from Methane. *J. Biotechnol.* **2001**, *86* (2), 127–133.
- (111) Ho, S. H.; Chen, C. Y.; Chang, J. S. Effect of Light Intensity and Nitrogen Starvation on CO₂ Fixation and Lipid/Carbohydrate Production of an Indigenous Microalga *Scenedesmus Obliquus* CNW-N. *Bioresour. Technol.* **2012**, *113*, 244–252.
- (112) Pfluger, A. R.; Wu, W.-M.; Pieja, A. J.; Wan, J.; Rostkowski, K. H.; Criddle, C. S. Selection of Type I and Type II Methanotrophic Proteobacteria in a Fluidized Bed Reactor under Non-Sterile Conditions. *Bioresour. Technol.* **2011**, *102*, 9919–9926.
- (113) Øverland, M.; Tauson, A.-H.; Shearer, K.; Skrede, A. Evaluation of Methane-Utilising Bacteria Products as Feed Ingredients for Monogastric Animals. *Arch. Anim. Nutr.* **2010**, *64* (3), 171–189.
- (114) Wolf, R. B.; Cavins, J. F.; Kleiman, R.; Black, L. T. Effect of Temperature on Soybean Seed Constituents: Oil, Protein, Moisture, Fatty Acids, Amino Acids and Sugars. *J. Am. Oil Chem. Soc.* **1982**, *59* (5), 230–232.
- (115) Lobell, D. B.; Roberts, M. J.; Schlenker, W.; Braun, N.; Little, B. B.; Rejesus, R. M.; Hammer, G. L. Greater Sensitivity to Drought Accompanies Maize Yield Increase in the U.S. Midwest. *Science* **2014**, *344* (May), 516–520.
- (116) Defoirdt, T.; Boon, N.; Sorgeloos, P.; Verstraete, W.; Bossier, P. Alternatives to Antibiotics to Control Bacterial Infections: Luminescent Vibriosis in Aquaculture as an Example. *Trends Biotechnol.* **2007**, *25* (10), 472–479.
- (117) Jendrossek, D.; Handrick, R. Microbial Degradation of Polyhydroxyalkanoates. *Annu. Rev. Microbiol.* **2002**, *56* (1), 403–432.
- (118) Heyer, J.; Berger, U.; Hardt, M.; Dunfield, P. F. *Methylohalobium Crimeensis* Gen. Nov., Sp. Nov., a Moderately Halophilic, Methanotrophic Bacterium Isolated from Hypersaline Lakes of Crimea. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 1817–1826.
- (119) Volova, T. G.; Kiselev, E. G.; Shishatskaya, E. I.; Zhila, N. O.; Boyandin, A. N.; Syrvacheva, D. A.; Vinogradova, O. N.; Kalacheva, G. S.; Vasiliev, A. D.; Peterson, I. V. Cell Growth and Accumulation of Polyhydroxyalkanoates from CO₂ and H₂ of a Hydrogen-Oxidizing Bacterium, *Cupriavidus Eutrophus* B-10646. *Bioresour. Technol.* **2013**, *146*, 215–222.
- (120) Myung, J.; Flanagan, J. C. A.; Waymouth, R. M.; Criddle, C. S. Methane or Methanol-Oxidation Dependent Synthesis of Poly (3-Hydroxybutyrate- Co -3-Hydroxyvalerate) by Obligate Type II Methanotrophs. *Process Biochem.* **2016**, *51* (5), 561–567.
- (121) Myung, J.; Flanagan, J. C. A.; Waymouth, R. M.; Criddle, C. S. Expanding the Range of Polyhydroxyalkanoates Synthesized by Methanotrophic Bacteria through the Utilization of Omega-Hydroxyalkanoate Co-Substrates. *AMB Express* **2017**, *7* (1), 118–128.
- (122) Park, I.; Jho, E. H.; Nam, K. Optimization of Carbon Dioxide and Valeric Acid Utilization for Polyhydroxyalkanoates Synthesis by *Cupriavidus Necator*. *J. Polym. Environ.* **2014**, *22* (2), 244–251.
- (123) Ghysels, S.; Mozumder, M. S. I.; De Wever, H.; Volcke, E. I. P.; Garcia-Gonzalez, L. Targeted Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) Bioplastic Production from Carbon Dioxide. *Bioresour. Technol.* **2018**, *249* (October 2017), 858–868.
- (124) Halet, D.; Defoirdt, T.; Van Damme, P.; Vervaeren, H.; Forrez, I.; Van De Wiele, T.; Boon, N.; Sorgeloos, P.; Bossier, P.; Verstraete, W. Poly-Beta-Hydroxybutyrate-Accumulating Bacteria Protect Gnotobiotic *Artemia Franciscana* from Pathogenic *Vibrio Campbellii*. *FEMS Microbiol. Ecol.* **2007**, *60*, 363–369.
- (125) Laranja, J. L. Q.; Ludevese-Pascual, G. L.; Amar, E. C.; Sorgeloos, P.; Bossier, P.; De Schryver, P. Poly-β-Hydroxybutyrate (PHB) Accumulating *Bacillus* Spp. Improve the Survival, Growth and Robustness of *Penaeus Monodon* (Fabricius, 1798) Postlarvae. *Vet. Microbiol.* **2014**, *173* (3–4), 310–317.
- (126) Sui, L.; Liu, Y.; Sun, H.; Wille, M.; Bossier, P.; De Schryver, P. The Effect of Poly-β-Hydroxybutyrate on the Performance of Chinese Mitten Crab (*Eriocheir Sinensis* Milne-Edwards) Zoea Larvae. *Aquacult. Res.* **2014**, *45* (3), 558–565.
- (127) De Schryver, P.; Sinha, A. K.; Kunwar, P. S.; Baruah, K.; Verstraete, W.; Boon, N.; De Boeck, G.; Bossier, P. Poly-Beta-Hydroxybutyrate (PHB) Increases Growth Performance and Intestinal Bacterial Range-Weighted Richness in Juvenile European Sea Bass, *Dicentrarchus Labrax*. *Appl. Microbiol. Biotechnol.* **2010**, *86* (5), 1535–1541.
- (128) Pieja, A. J.; Rostkowski, K. H.; Criddle, C. S. Distribution and Selection of Poly-3-Hydroxybutyrate Production Capacity in Methanotrophic Proteobacteria. *Microb. Ecol.* **2011**, *62* (3), 564–573.
- (129) Najdegerami, E. H.; Bakhshi, F.; Tokmechi, A.; Shiri Harzevili, A.; Sorgeloos, P.; Bossier, P. Dietary Effects of Poly-Beta-Hydroxybutyrate on the Growth Performance, Digestive Enzyme Activity, Body Composition, Mineral Uptake and Bacterial Challenge of Rainbow Trout Fry (*Oncorhynchus Mykiss*). *Aquacult. Nutr.* **2017**, *23*, 246–254.
- (130) Defoirdt, T.; Halet, D.; Vervaeren, H.; Boon, N.; Van de Wiele, T.; Sorgeloos, P.; Bossier, P.; Verstraete, W. The Bacterial Storage Compound Poly-Beta-Hydroxybutyrate Protects *Artemia Franciscana* from Pathogenic *Vibrio Campbellii*. *Environ. Microbiol.* **2007**, *9* (2), 445–452.
- (131) Nhan, D. T.; Wille, M.; De Schryver, P.; Defoirdt, T.; Bossier, P.; Sorgeloos, P. The Effect of Poly β-Hydroxybutyrate on Larviculture of the Giant Freshwater Prawn *Macrobrachium Rosenbergi*. *Aquaculture* **2010**, *302* (1–2), 76–81.
- (132) Laranja, J. L. Q.; De Schryver, P.; Ludevese-Pascual, G. L.; Amar, E. C.; Aerts, M.; Vandamme, P.; Bossier, P. High Amorphous Poly-Beta-Hydroxybutyrate (PHB) Content in a Probiotic *Bacillus* Strain Displays Better Protective Effects in *Vibrio*-Challenged Gnotobiotic *Artemia*. *Aquaculture* **2018**, *487* (January), 15–21.
- (133) Thai, T. Q.; Wille, M.; Garcia-Gonzalez, L.; Sorgeloos, P.; Bossier, P.; De Schryver, P. Poly-β-Hydroxybutyrate Content and Dose of the Bacterial Carrier for *Artemia* Enrichment Determine the Performance of Giant Freshwater Prawn Larvae. *Appl. Microbiol. Biotechnol.* **2014**, *98* (11), 5205–5215.
- (134) Ludevese-Pascual, G.; Laranja, J. L. Q.; Amar, E. C.; Sorgeloos, P.; Bossier, P.; De Schryver, P. Poly-Beta-Hydroxybutyrate-Enriched *Artemia* Spp. for Giant Tiger Prawn *Penaeus Monodon* Larviculture. *Aquacult. Nutr.* **2017**, *23* (2), 422–429.
- (135) Sui, L.; Cai, J.; Sun, H.; Wille, M.; Bossier, P. Effect of Poly-β-Hydroxybutyrate on Chinese Mitten Crab, *Eriocheir Sinensis*, Larvae Challenged with Pathogenic *Vibrio Anguillarum*. *J. Fish Dis.* **2012**, *35* (5), 359–364.
- (136) Situmorang, M. L.; De Schryver, P.; Dierckens, K.; Bossier, P. Effect of Poly-β-Hydroxybutyrate on Growth and Disease Resistance of Nile Tilapia *Oreochromis Niloticus* Juveniles. *Vet. Microbiol.* **2016**, *182*, 44–49.
- (137) Defoirdt, T.; Mai Anh, N. T.; De Schryver, P. Virulence-Inhibitory Activity of the Degradation Product 3-Hydroxybutyrate

Explains the Protective Effect of Poly- β -Hydroxybutyrate against the Major Aquaculture Pathogen *Vibrio Campbellii*. *Sci. Rep.* **2018**, *8*, 1–9.

(138) Monica, M.; Priyanka, T.; Akshaya, M.; Rajeswari, V.; Sivakumar, L.; Somasundaram, S. T.; Shenbhagarathai, R. The Efficacy of Poly- β -Hydroxy Butyrate (PHB)/Biosurfactant Derived from *Staphylococcus Hominis* against White Spot Syndrome Virus (WSSV) in *Penaeus Monodon*. *Fish Shellfish Immunol.* **2017**, *71* (October), 399–410.

(139) Najdegerami, E. H.; Tran, T. N.; Defoirdt, T.; Marzorati, M.; Sorgeloos, P.; Boon, N.; Bossier, P. Effects of Poly- β -Hydroxybutyrate (PHB) on Siberian Sturgeon (*Acipenser Baerii*) Fingerlings Performance and Its Gastrointestinal Tract Microbial Community. *FEMS Microbiol. Ecol.* **2012**, *79* (1), 25–33.

(140) Najdegerami, E. H.; Baruah, K.; Shiri, A.; Rekecki, A.; Van den Broeck, W.; Sorgeloos, P.; Boon, N.; Bossier, P.; De Schryver, P. Siberian Sturgeon (*Acipenser Baerii*) Larvae Fed Artemia Nauplii Enriched with Poly- β -Hydroxybutyrate (PHB): Effect on Growth Performance, Body Composition, Digestive Enzymes, Gut Microbial Community, Gut Histology and Stress Tests. *Aquacult. Res.* **2015**, *46* (4), 801–812.

(141) Laranja, J. L. Q.; De Schryver, P.; Ludevese-Pascual, G. L.; Amar, E. C.; Aerts, M.; Vandamme, P.; Bossier, P. High Amorphous Poly-Beta-Hydroxybutyrate (PHB) Content in a Probiotic *Bacillus* Strain Displays Better Protective Effects in *Vibrio*-Challenged Gnotobiotic *Artemia*. *Aquaculture* **2018**, *487* (April 2017), 15–21.

(142) Kleiveland, C. R.; Hult, L. T. O.; Spetalen, S.; Kaldhusdal, M.; Christofferesen, T. E.; Bengtsson, O.; Romarheim, O. H.; Jacobsen, M.; Leaa, T. The Noncommensal Bacterium *Methylococcus Capsulatus* (Bath) Ameliorates Dextran Sulfate (Sodium Salt)-Induced Ulcerative Colitis by Influencing Mechanisms Essential for Maintenance of the Colonic Barrier Function. *Appl. Environ. Microbiol.* **2013**, *79* (1), 48–57.

(143) Strong, P. J.; Xie, S.; Clarke, W. P. Methane as a Resource: Can the Methanotrophs Add Value? *Environ. Sci. Technol.* **2015**, *49* (7), 4001–4018.

(144) Van Cam, D. T.; Van Hao, N.; Dierckens, K.; Defoirdt, T.; Boon, N.; Sorgeloos, P.; Bossier, P. Novel Approach of Using Homoserine Lactone-Degrading and Poly-Beta-Hydroxybutyrate-Accumulating Bacteria to Protect *Artemia* from the Pathogenic Effects of *Vibrio Harveyi*. *Aquaculture* **2009**, *291* (1–2), 23–30.

(145) Gao, M.; Du, D.; Bo, Z.; Sui, L. Poly- β -Hydroxybutyrate (PHB)-Accumulating *Halomonas* Improves the Survival, Growth, Robustness and Modifies the Gut Microbial Composition of *Litopenaeus Vannamei* Postlarvae. *Aquaculture* **2019**, *500* (May 2018), 607–612.

(146) Franke, A.; Roth, O.; De Schryver, P.; Bayer, T.; Garcia-Gonzalez, L.; Kü, S.; Bossier, P.; Miest, J. J.; Clemmesen, C. Poly- β -Hydroxybutyrate Administration during Early Life: Effects on Performance, Immunity and Microbial Community of European Sea Bass Yolk-Sac Larvae. *Sci. Rep.* **2017**, *7* (15022), 1–11.

(147) Rodriguez, M. G. M.; Pohlenz, C.; Gatlin, D. M., III. Supplementation of Organic Acids and Algae Extracts in the Diet of Red Drum *Sciaenops Ocellatus*: Immunological Impacts. *Aquacult. Res.* **2017**, *48*, 1778–1786.

(148) Van Hung, N.; De Schryver, P.; Tam, T. T.; Garcia-Gonzalez, L.; Bossier, P.; Nevejan, N. Application of Poly- β -Hydroxybutyrate (PHB) in Mussel Larviculture. *Aquaculture* **2015**, *446* (May), 318–324.

(149) Geyer, K. M.; Kyker-Snowman, E.; Grandy, A. S.; Frey, S. D. Microbial Carbon Use Efficiency: Accounting for Population, Community, and Ecosystem-Scale Controls over the Fate of Metabolized Organic Matter. *Biogeochemistry* **2016**, *127* (2–3), 173–188.

(150) Hessen, D. O.; Ågren, G. I.; Anderson, T. R.; Elser, J. J.; Peter, C.; Hessen, D. a G. O.; Ågren, G. I.; De Ruiter, P. C. Carbon Sequestration in Ecosystems: The Role of Stoichiometry. *Ecology* **2004**, *85* (5), 1179–1192.

(151) Kuhad, R. C.; Singh, A.; Tripathi, K. K.; Saxena, R. K.; Eriksson, K.-E. L. Microorganisms as an Alternative Source of Protein. *Nutr. Rev.* **1997**, *55* (3), 65–75.

(152) Miller, S. A. Nutritional Factors in Single Cell Protein. In *Single Cell Protein*; Mateles, R. I., Tannenbaum, S. R., Eds.; The MIT Press: Cambridge, MA, 1968; pp 79–89.

(153) Waldroup, P. W.; Payne, J. R. Feeding Value of Methanol-Derived Single Cell Protein for Broiler Chicks. *Poult. Sci.* **1974**, *53* (3), 1039–1042.

(154) Tarpeh, W. A.; Wald, I.; Omollo, M. O.; Egan, T.; Nelson, K. L. Evaluating Ion Exchange for Nitrogen Recovery from Source-Separated Urine in Nairobi, Kenya. *Dev. Eng.* **2018**, *3* (July), 188–195.

(155) Gildemyn, S.; Verbeeck, K.; Slabbinck, R.; Andersen, S. J.; PrévotEAU, A.; Rabaey, K. Integrated Production, Extraction, and Concentration of Acetic Acid from CO₂ through Microbial Electrosynthesis. *Environ. Sci. Technol. Lett.* **2015**, *2* (11), 325–328.

(156) Lienemann, M.; Deutzmann, J. S.; Milton, R. D.; Sahin, M.; Spormann, A. M. Mediator-Free Enzymatic Electrosynthesis of Formate by the *Methanococcus Maripaludis* Heterodisulfide Reductase Supercomplex. *Bioresour. Technol.* **2018**, *254* (November 2017), 278–283.

(157) Rabaey, K.; Rozendal, R. A. Microbial Electrosynthesis - Revisiting the Electrical Route for Microbial Production. *Nat. Rev. Microbiol.* **2010**, *8* (10), 706–716.