

The biotechnological potential of the *Chloroflexota* phylum

André Freches,^{1,2} Joana Costa Fradinho^{1,2}

AUTHOR AFFILIATIONS See affiliation list on p. 16.

ABSTRACT In the next decades, the increasing material and energetic demand to support population growth and higher standards of living will amplify the current pressures on ecosystems and will call for greater investments in infrastructures and modern technologies. A valid approach to overcome such future challenges is the employment of sustainable bio-based technologies that explore the metabolic richness of microorganisms. Collectively, the metabolic capabilities of *Chloroflexota*, spanning aerobic and anaerobic conditions, thermophilic adaptability, anoxygenic photosynthesis, and utilization of toxic compounds as electron acceptors, underscore the phylum's resilience and ecological significance. These diverse metabolic strategies, driven by the interplay between temperature, oxygen availability, and energy metabolism, exemplify the complex adaptations that enabled *Chloroflexota* to colonize a wide range of ecological niches. In demonstrating the metabolic richness of the *Chloroflexota* phylum, specific members exemplify the diverse capabilities of these microorganisms: *Chloroflexus aurantiacus* showcases adaptability through its thermophilic and phototrophic growth, whereas members of the *Anaerolineae* class are known for their role in the degradation of complex organic compounds, contributing significantly to the carbon cycle in anaerobic environments, highlighting the phylum's potential for biotechnological exploitation in varying environmental conditions. In this context, the metabolic diversity of *Chloroflexota* must be considered a promising asset for a large range of applications. Currently, this bacterial phylum is organized into eight classes possessing different metabolic strategies to survive and thrive in a wide variety of extreme environments. This review correlates the ecological role of *Chloroflexota* in such environments with the potential application of their metabolisms in biotechnological approaches.

KEYWORDS filamentous anoxygenic phototroph, photoautotrophic bacteria, extremophiles, biotechnological applications, decontamination technologies, value-added substances production

The utilization of microorganisms in biotechnological processes has been widely reported, and the search for new metabolisms can lead to the development of several innovative technologies focused on environmental decontamination, wastewater treatment, and production of energy and value-added substances (1–4). In all these applications, the adaptability and versatility of microorganisms make them valuable tools for addressing environmental and industrial challenges. In every microbial phylum, we can find interesting metabolisms, and in the specific case of the *Chloroflexota* phylum, it encompasses a wide spectrum of metabolic diversity, with some organisms exhibiting remarkable traits such as a bicycle-like mechanism for inorganic carbon fixation, others harnessing the power of halogens, and others performing denitrification, although often being involved in the cycling of several elements (5). This diversity, which arises from the natural adaptation of *Chloroflexota* to harsh environmental conditions, contains a unique array of metabolic processes and distinctive features that can be explored for biotechnological purposes.

Editor Haruyuki Atomi, Kyoto University, Kyoto, Japan

Address correspondence to Joana Costa Fradinho, j.fradinho@fct.unl.pt.

The authors declare no conflict of interest.

See the funding table on p. 16.

Published 6 May 2024

Copyright © 2024 Freches and Fradinho. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

The *Chloroflexota* bacteria phylum, formerly known as green non-sulfur bacteria (GNSB), comprises extensively diverse microorganisms that can be found in several environments, both terrestrial and aquatic. This phylum nomenclature derives from the species *Chloroflexus aurantiacus*, first isolated and described by Pierson and Castenholtz (6) as a filamentous anoxygenic phototroph (FAP), a term currently used specifically for phototrophic members of the *Chloroflexota*. The constant discoveries of new microbial organisms and advances in phylogenetic analysis are leading to a continual redefinition and reorganization of this phylum. Currently, the *Chloroflexota* phylum is divided into eight classes: *Anaerolineae*, *Ardenticatenia*, *Caldilineae*, *Chloroflexia*, *Dehalococcoidia*, *Ktedonobacteria*, *Tepidiformia*, and *Thermoflexia* (Fig. 1). Interestingly, the entire phylum only has 18 families, with many of the classes owing their name to one single genus characteristic of the class.

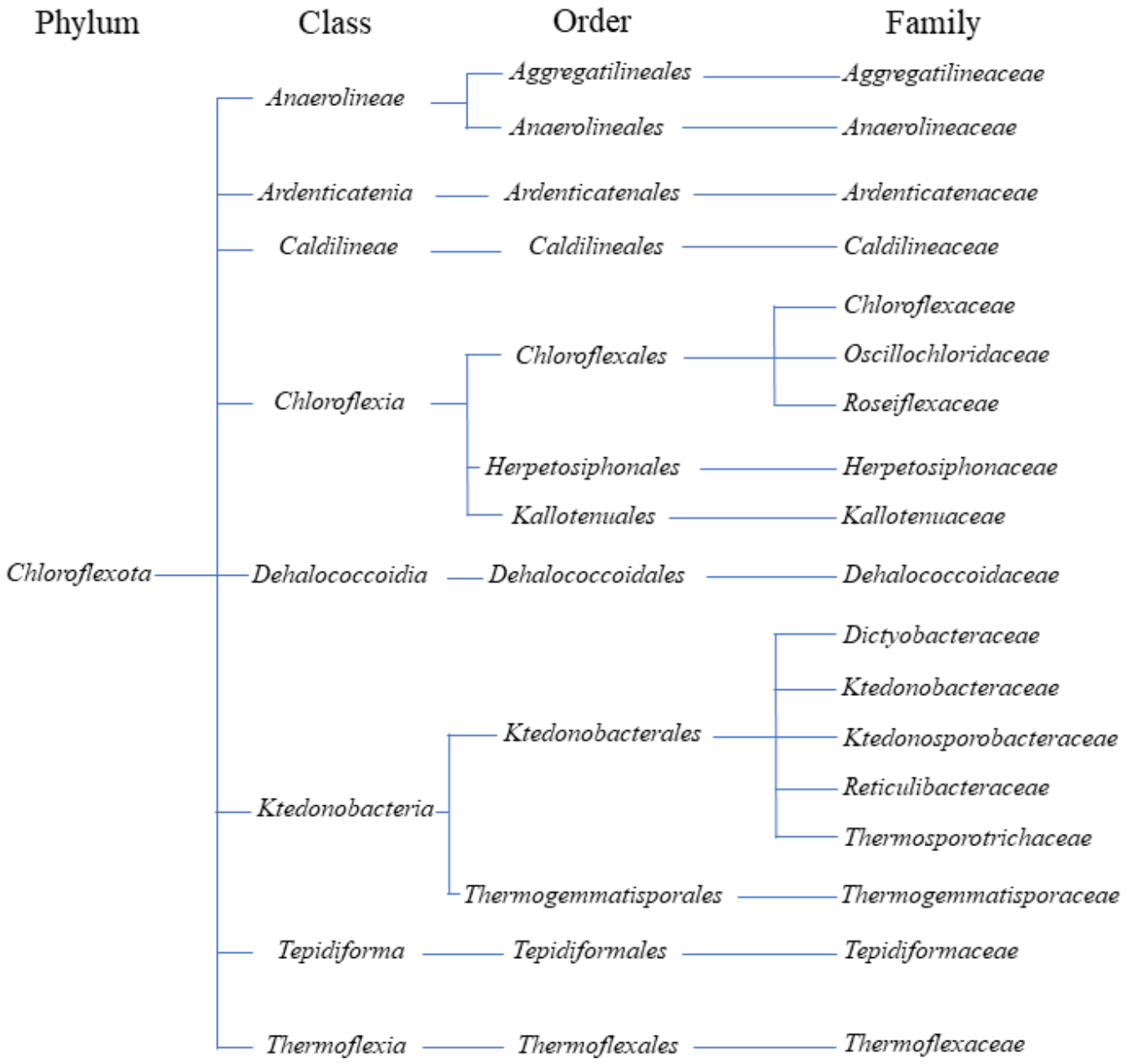


FIG 1 The *Chloroflexota* phylum is divided into eight classes of bacteria, each with its specific orders and families (<https://lpsn.dsmz.de/phylum/chloroflexota>, accessed 10 February 2024).

The uniqueness of *Chloroflexota* members is reviewed in this manuscript, analyzing its metabolic features and placing a great focus on the much-needed overview of the biotechnological potential of this bacterial phylum.

CHLOROFLEXOTA CLASSES AND CHARACTERISTICS

The *Chloroflexota* phylum is characterized according to the metabolism, phylogeny, cell shape, motility, ability to form multicellular aggregates, and spore formation capacity. There are several similar traits (morphology, environment, and growth conditions) between the constituents of this bacterial phylum, as shown in Fig. 2.

The class *Anaerolineae* is divided into two orders, *Aggregatilineales* and *Anaerolineales*, each containing a single family. Its representatives are strictly anaerobic chemoorganotrophic organisms with a filamentous morphology. Organisms from this class have been isolated from diverse environments including anaerobic digesters, hot springs, and sub-seafloor sediments (7, 8).

The class *Ardenticatenia* was proposed based on a single species, *Ardenticatena maritima*, which was isolated from a costal hydrothermal field, and is a facultative aerobe that can reduce ferric ion and nitrate under anaerobic conditions. This chemoorganotrophic species forms thin multicellular filaments and can grow at temperatures of up to 75°C (9).

The class *Caldilineae* contains a single described order, *Caldilineales*, and family, *Caldilineaceae*, which consists of two genera, *Caldilinea* and *Litorilinea*. Species belonging to these genera are aerobic, facultative aerobic, or anaerobic chemoorganotrophic filamentous organisms found in hot springs and hot aquifers (7, 10, 11).

The class *Chloroflexia* contains the first known members of this phylum that possess phototrophic and/or chemoheterotrophic growth under mesophilic or moderately thermophilic conditions, presenting a filamentous growth morphology. Moreover, the phototrophic members of this class, usually referred to as FAP bacteria, belong to the *Chloroflexales* order, which can be divided into three families: *Chloroflexaceae*

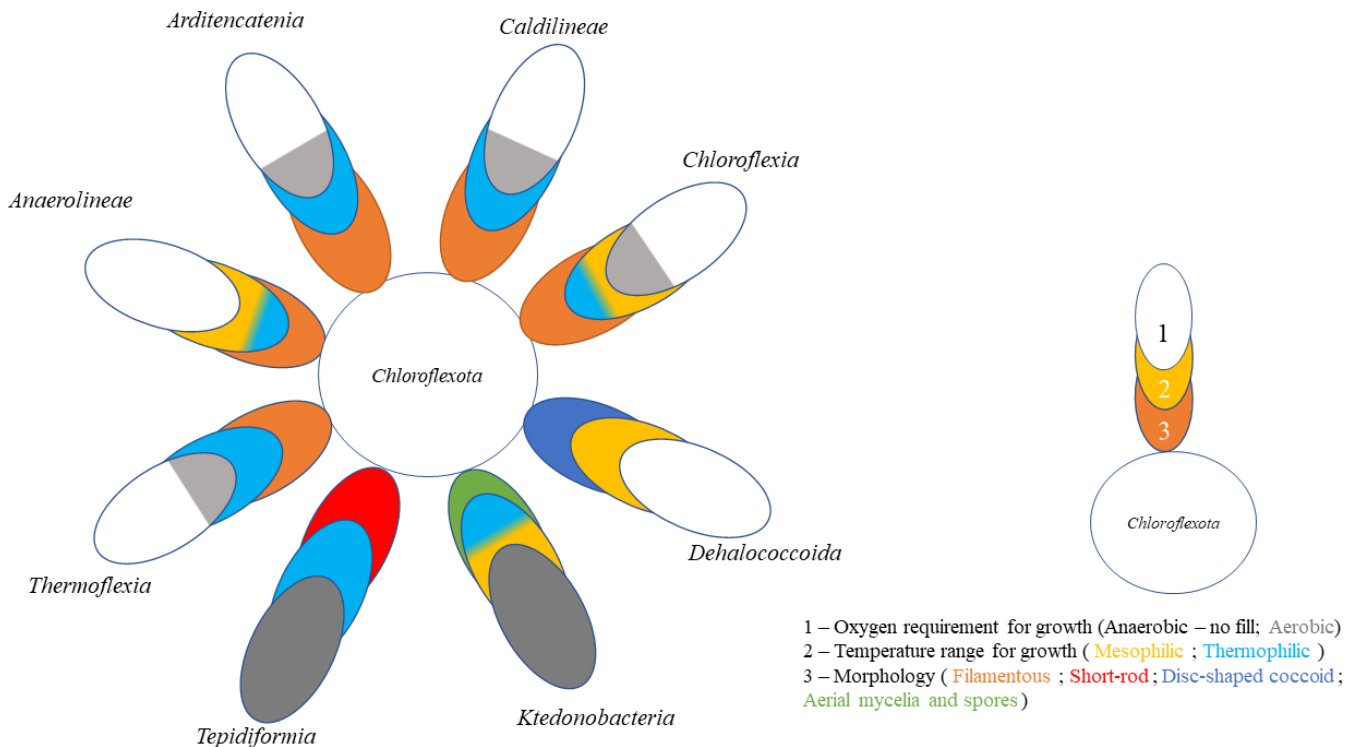


FIG 2 The *Chloroflexota* phylum organized in terms of predominant morphology, oxygen requirements, and temperature range for growth (mesophiles: 20°C–45°C; thermophiles: 45°C–80°C).

(photoheterotrophic but also with photoautotrophic abilities), *Roseiflexaceae*, and *Oscillochloridaceae* (both predominantly photoheterotrophic). The first two families are composed of thermophilic FAP bacteria isolated from terrestrial hot springs, whereas the latter contains photoheterotrophic mesophilic freshwater species (12, 13). Although phototrophy in *Chloroflexota* isolates is limited to *Chloroflexia* class, metagenomic studies indicate the existence of potentially phototrophic members also in other classes, which could be attributed to horizontal gene transfer of sequences for reaction center and bacteriochlorophyll synthesis proteins (14). The *Chloroflexia* class has two additional orders, *Herpetosiphonales* and *Kallotenuales*, containing non-photosynthetic species that rely on heterotrophic metabolism for growth (12, 15).

The class *Dehalococcoidia* is characterized by the disc-shaped coccoid form of its members, instead of the filamentous morphology attributed to other classes within the *Chloroflexota* phylum. All isolates from this class can grow in chemoorganotrophic conditions and perform dehalogenation of chlorinated and brominated alkanes under strict anaerobic conditions, which grants them great importance in the bioremediation field (16).

The class *Ktedonobacteria* contains heterotrophic bacteria capable of growing under microaerophilic conditions. They have been isolated from soil samples with the peculiarity of forming aerial mycelia and spores. This class is divided into two orders, *Ktedonobacterales* and *Thermogemmatisporales*. The order *Ktedonobacterales* contains all the mesophilic and some thermophilic representatives of this class, whereas the *Thermogemmatisporales* order encompasses only thermophilic species (17–22).

The class *Tepidiformia* is composed of one order, *Tepidiformales*, and one family, *Tepidiformaceae*, containing moderately thermophilic bacteria with a regular short rod morphology, being able to grow hetero- or auto-trophically in aerobic conditions (23).

The class *Thermoflexia*, proposed based on its type species *Thermoflexus hugenholtzii*, englobes filamentous thermophilic chemoheterotrophic microaerobes (optimally growing at 1% (vol/vol) O₂ with an upper limit of 8% O₂) being also facultatively anaerobic (24).

CHLOROFLEXOTA METABOLISMS AND ENVIRONMENTS

There are several metabolisms present within the *Chloroflexota* phylum, such as anoxygenic phototrophy, obligate anaerobic heterotrophy, organohalide respiration, and facultative or aerobic heterotrophy. For this reason, bacteria from the *Chloroflexota* phylum can be found in several environments (natural or industrial) with different properties and singularities (25–29). Their metabolic versatility allowed the adaptation of *Chloroflexota* to microbial mats, soils, aquatic environments, and other extreme environments as shown in Fig. 3.

Anoxygenic phototrophy

The first metabolism to be described in this phylum was anoxygenic phototrophic growth (6), in which the filamentous phototrophs use light energy to generate chemical energy in the form of ATP. The isolated members from the *Chloroflexota* phylum exhibiting photoautotrophy are exclusively contained in the *Chloroflexia* class; however, most organisms belonging to this class demonstrated the ability to grow photoheterotrophically assimilating organic carbon compounds (30–33), or chemotrophically under aerobic dark conditions. *Chloroflexia* is commonly found in phototrophic microbial mat communities, specifically in neutral and alkaline spring waters with temperatures between 40°C and 70°C (6, 34–36).

To perform their main metabolism, the phototrophic bacteria rely on light-harvesting organelles known as chlorosomes, oval structures attached to the inner surface of the cytoplasmic membrane, consisting of paracrystalline aggregates of bacteriochlorophylls that are surrounded by a galactolipid non-unit membrane (37). Regarding the antenna pigments, there are some slight differences within the order *Chloroflexales*: for example, members of the *Chloroflexus* and *Oscillochloris* genera possess bacteriochlorophyll *c*,

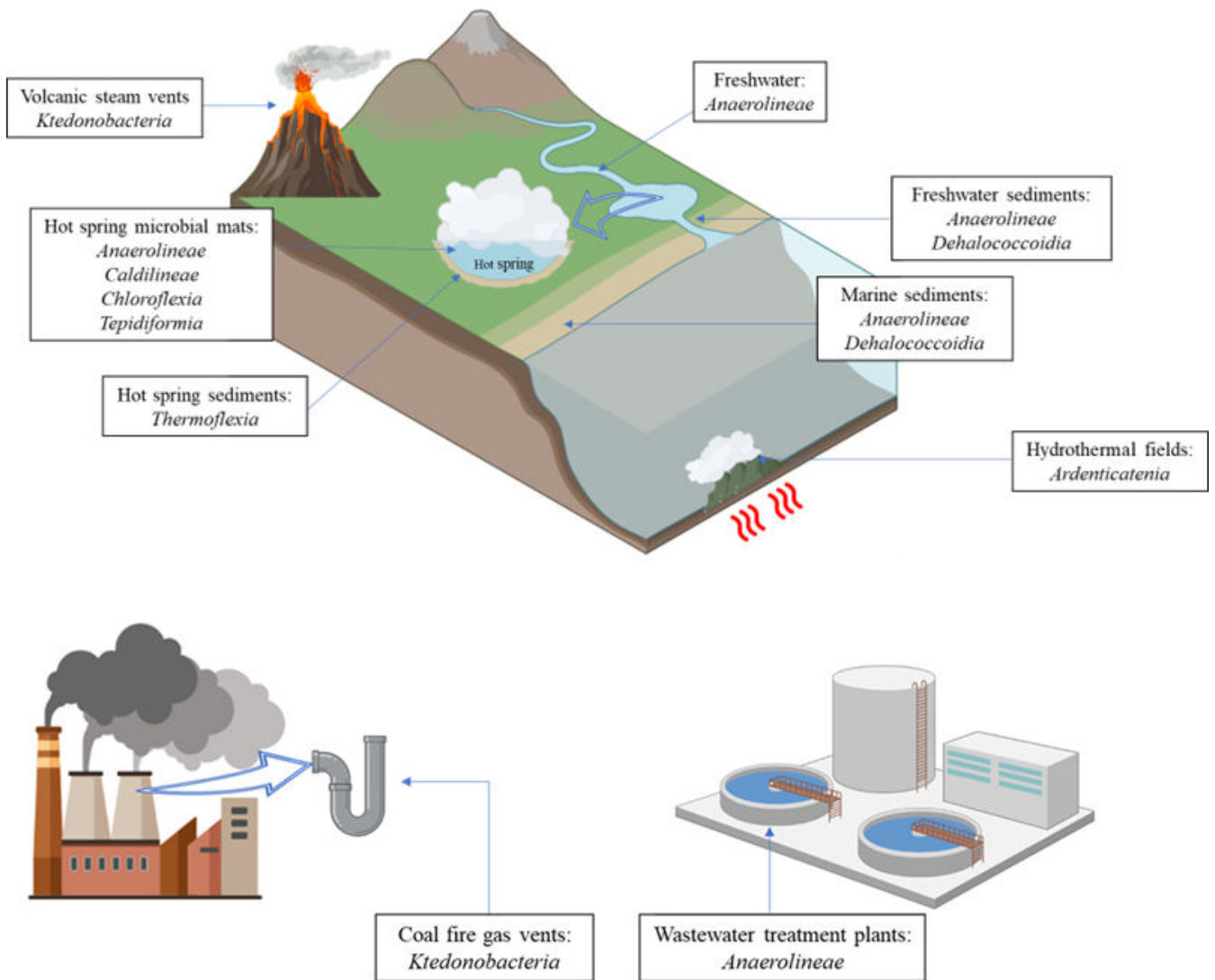


FIG 3 Natural and industrialized environments where *Chloroflexota* can be found. The pictures were designed with templates available at <https://www.bioren-der.com/>.

whereas *Chloronema* is rich in bacteriochlorophylls *c* and *d* (31, 32). The presence of bacteriochlorophylls *c* or *d* gives cells their green color, and carotenoids (β - and γ -carotene, and derivatives) are also present as part of the light-harvesting cellular apparatus. *Roseiflexus castenholzii* and *Heliobacterium oregonensis* (from the family *Roseiflexaceae*) contain solely bacteriochlorophyll *a*, which makes them chlorosome-free. These cells usually present a red/orange color because of their oxo- γ -carotene and glucoside-rich nature. Moreover, the carotenoid β -carotene (which is present in *Chloroflexus*, *Oscillochloris*, and *Chloronema*) is missing in the red-colored genera *Roseiflexus* and *Heliobacterium* (38–40).

In terms of inorganic carbon fixation, *C. aurantiacus* can use the 3-hydroxypropionate bi-cycle, in which bicarbonate fixation is preceded by the carboxylation of acetyl-CoA and propionyl-CoA yielding pyruvate as the net product; glyoxylate, an intermediate of the bi-cycle, can also be assimilated into cell material (41–44). Energy-wise, inorganic carbon fixation via the 3-hydroxypropionate bi-cycle requires 7 ATP, 5 NAD(P)H, and 3 HCO_3^- to produce one pyruvate. Comparatively, alternative carbon fixation pathways found in other phototrophs can produce pyruvate through the reverse tricarboxylic cycle, which requires, equivalently, 3 CO_2 , 2 ATP, and 5 NAD(P)H, whereas the Calvin-Benson-Bassham cycle inputs 3 CO_2 , 7 ATP, and 5 NAD(P)H to produce one pyruvate

molecule (45, 46). However, the 3-hydroxypropionate bi-cycle allows the consumption of bicarbonate, which can provide a competitive advantage to *Chloroflexus* in alkaline environments or under low carbon dioxide availability (47). To support inorganic carbon fixation, different compounds can be used as electron sources, such as sulfide, thiosulfate, small organic molecules, or molecular hydrogen (45, 48–50).

Given the ability to use a wide range of electron donors, members of the *Chloroflexia* class (specifically members of the *Chloroflexales* order) have also been observed in marine and hypersaline microbial mats (51, 52), which points to the adaptability of such organisms to different environments. Indeed, the presence of *Chloroflexales* members has also been reported in extreme soil environments such as sediments from the Arctic (53). Furthermore, *Chloroflexia* has been isolated from volcanic vents (54), and *Chloroflexus islandicus* has been isolated from a geyser in Iceland (30). Ecologically, *Chloroflexia* serves as both primary and secondary producers (55–57), performing mixotrophic growth using CO₂ and simple organic molecules as carbon sources.

Anaerobic heterotrophy

Strict anaerobic chemoheterotrophic growth has been reported in the classes *Anaerolineae* and *Dehalococcoidia*. The strict anaerobic metabolisms of *Anaerolineae* are characterized by a fermentative metabolism, with some isolates performing sugar fermentation to produce acetate, lactate, succinate, propionate, and hydrogen and this class has shown to be present in methanogenic sludge systems (58), wastewaters with recalcitrant compounds (59), and sugar-fed microbial fuel cells systems (60, 61). Additionally, members of the *Anaerolineae* class have been reported to contribute to the transformation of cellulose and hemi-cellulose to smaller carbon molecules such as lactate, formate, and acetate, even in adverse conditions, such as uranium-rich sediments, which was possible, given the presence of genes related to uranium tolerance (62). Members of the *Anaerolineae* classes can also be found in marine environments contributing to the re-cycling of dissolved organic matter and degrading carbohydrates (8, 63, 64).

Bacteria from the *Dehalococcoidia* class can perform anaerobic organohalide respiration, being repeatedly found in marine sediments at different worldwide locations, often with high relative abundances (65, 66). In fact, Krzmarzick et al. (29) investigated the role of *Dehalococcoidia* on chlorine cycle by establishing a correlation between their concentration and the concentration of organochlorine compounds, stating the pivotal role of these bacteria in the biogeochemical chlorine cycle (29). Additionally, a sulfur-oxidizing/reducing ability was reported by different authors, which could imply a role of *Dehalococcoidia* in the sulfur cycle of marine shallow surfaces (67).

Overall, members of the *Anaerolineae* and *Dehalococcoidia* contribute to the fermentation of sugars and fixation of carbon dioxide, participating in carbon cycling and constituting around 5%–25% of the bacterial communities detected in freshwater sediments from lakes and rivers (68, 69).

Facultative or aerobic heterotrophy

Aerobic chemoheterotrophic metabolism can be found in the *Ardenticatenia*, *Ktedonobacteria*, *Tepidiformia*, and *Thermoflexia* classes. *A. maritima* (sole species of the *Ardenticatenia* class) is an aerobe that can also use ferric iron as an electron acceptor and tolerates high NaCl concentrations and temperatures, which can explain its abundance in iron-rich coastal hydrothermal fields (9). Members of *Ktedonobacteria* can grow in mesophilic or thermophilic conditions and have been reported to be dominant in coal-fire gas vents at 58°C, able to oxidize hydrogen and carbon monoxide for its metabolism (70). Additionally, *Ktedonobacteria* members have been found in steam vents from volcanoes (54). *Thermoflexia* members have been reported to have optimal growth at 72.5°C–75°C in microaerophilic conditions (1% vol/vol of O₂), conditions usually found in hot springs sediments, where these bacteria may be found (24).

Within the aerobic organisms of the *Chloroflexota*, *Tepidiformia* class members, here represented by its single species *Tepidiforma bonchosmolovskayae*, are aerobic bacteria that can grow chemoorganoheterotrophically using different carbohydrates or volatile fatty acids and chemolithoautotrophically using FeCO_3 as the electron donor, being usually found in hot springs (23).

Chloroflexota members exhibit several metabolisms, and despite being taxonomically divided into only eight classes, these bacteria can be found in extremely diverse environments adapted to different conditions, playing a role in the cycle of several elements such as carbon, sulfur, and halogens (71). The adaptability of *Chloroflexota* to different organic matter inputs allows their survival in adverse environments, a feature undoubtedly important for their application in the biotechnological industry.

BIOTECHNOLOGICAL RELEVANCE

The metabolic versatility of *Chloroflexota* and its natural occurrence in different habitats make this a very interesting group of bacteria to be used in several biotechnological applications, which can range from the production of chemical compounds to the degradation of contaminants, among many others (Fig. 4).

In fact, *Chloroflexota* bacteria can be found as sole contributors to certain applications or as an integrative of mixed microbial culture-based solutions. Regardless, this review aims to discuss *Chloroflexota* role in each technology, providing insights about the advantages of the utilization of these bacteria and prospecting the unexplored metabolisms that can be applied to the development of new biotechnological approaches.

Production of enzymes and energy

Energy production is fundamental for human activities, powering essentially every aspect of daily life. Transportation, communications, and industrial production are paramount examples of the current energy demands. Complementarily, enzymes are widely applied in various industries (food and beverages, nutrition, textiles, cleaning products, and health and drugs sector) (72–74), ensuring product quality and stability while increasing production efficiency. The development of new approaches to produce enzymes and energy is imperative to decrease the environmental footprint of these activities, namely through the reduction of waste generation as well as water, energy, and raw materials demand.

Within *Chloroflexota*, the genus *Chloroflexus* has been a great source of enzymes. Shin et al. cloned and expressed in *Escherichia coli* the homodimer enzyme α -L-rhamnosidase (200 kDa MW) from *C. aurantiacus* and purified it as a soluble enzyme to use in the transformation of rutin (the bioflavonoid vitamin P) into isoquercitrin (flavonoid) (75). The obtained product has several important properties acting as an antioxidant, anti-inflammatory, anti-carcinogenic, antidiabetic, and anti-allergic agent (76), with the results displaying a natural ability from *C. aurantiacus* to effectively produce isoquercitrin. In fact, the purified α -L-rhamnosidase displayed the highest substrate-specific activity when compared with other isoquercitrin-producing enzymes. Moreover, isoquercitrin productivity from *C. aurantiacus*-derived α -L-rhamnosidase was almost two times higher than commercial α -L-rhamnosidase. Interestingly, this enzyme is also widely used in the industrial field for debittering citrus fruit juices, enhancement of wine aromas, and drug precursor production (77).

C. aurantiacus metabolic versatility was also investigated in the search for thermophilic alcohol dehydrogenases (78). These enzymes catalyze the regio- and stereo-selective reduction in aldehydes or ketones to primary or secondary alcohols, a process applied in several industrial-scale processes (79). Loderer et al. expressed this enzyme gene from *C. aurantiacus* in *E. coli* and reported its optimal temperature activity (70°C) and possible substrates, a relevant result given the industries' demand for a larger diversity of well-characterized enzymes (78). The expressed enzyme showed high tolerance to ethylenediamine tetraacetic acid (EDTA), compared with other alcohol dehydrogenases, reflecting a stronger binding of the catalytic zinc ion, attributed to a

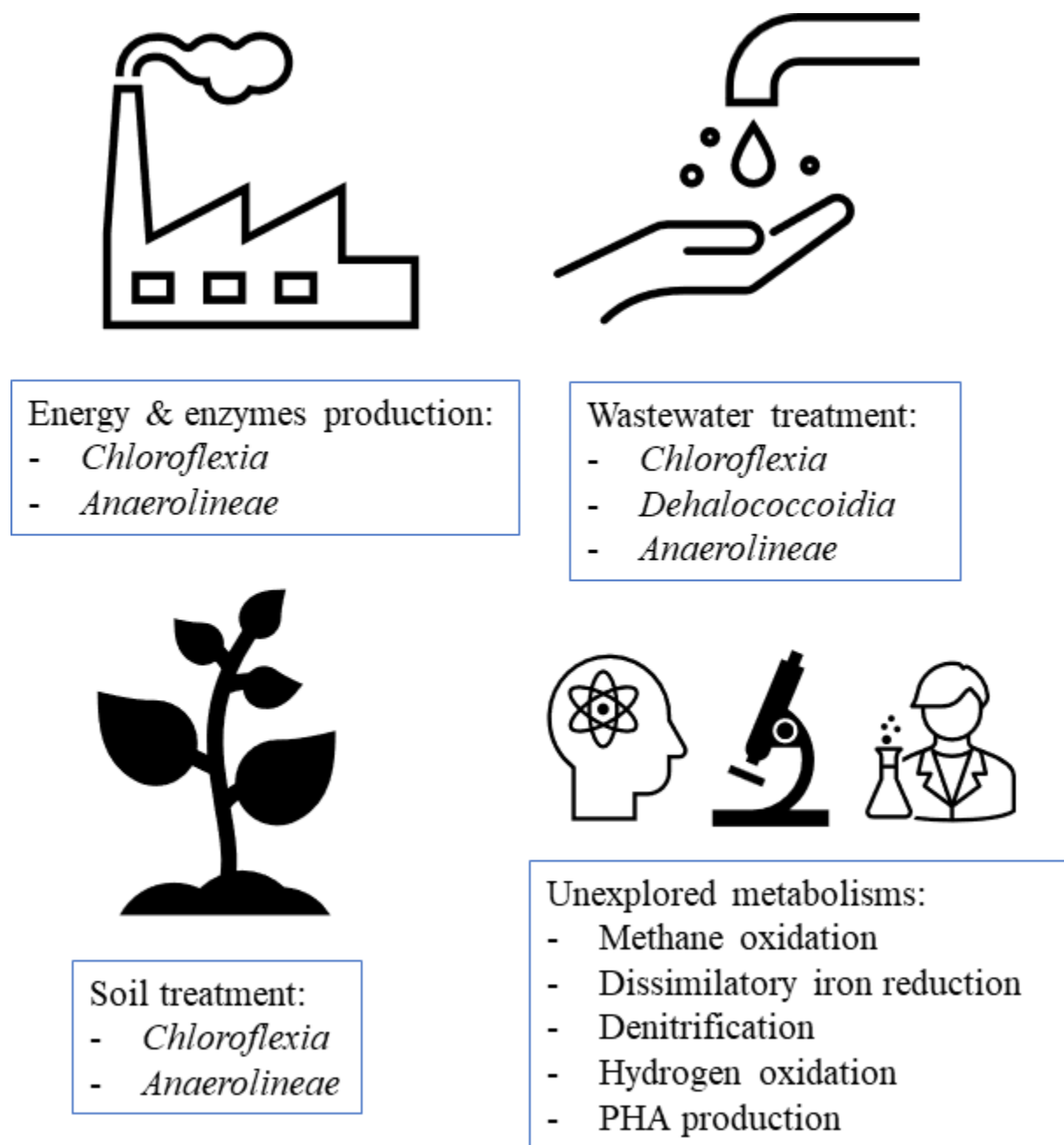


FIG 4 Applications of *Chloroflexota* members in different biotechnological areas.

more robust enzyme fold derived from a thermophilic host. Overall, the unique properties of the *Chloroflexota* enzyme (high-temperature activity, substrate versatility, and high tolerance to EDTA) broaden the enzyme's applicability in various industrial processes, such as in the synthesis of fine chemicals, pharmaceuticals, or biofuels, benefiting from high-temperature stability, substrate flexibility, and robustness in the presence of chelating agents.

Chloroflexia bacteria are indeed a unique reservoir of new biocatalytic activities and the production of ene-reductase enzymes by *Chloroflexus aggregans* was investigated (80). These enzymes catalyze the asymmetric hydrogenation of alkenes and have collected great interest from academia and industries. Robescu et al. reported that the ene-reductases produced by *C. aggregans* were robust biocatalysts with high

thermostability, presenting acceptable solvent tolerance and a wide range of optimal pH, which can be important for bigger-scale applications of this enzyme.

Chloroflexi bacterium, unclassified *Chloroflexota*, has been used to obtain ω -transaminases (ω -TA) highly reactive to aromatic amino donors/receptors at pH 8.5, 40°C, and showing affinity to cyclic substrates such as 1-Boc-3-piperidone (81). The enzymatic properties of this *Chloroflexota*-derived enzyme displayed good thermal stability, organic solvent tolerance, and broad substrate specificity. The ω -TA enzymes produce chiral amines with applications in the medical and fine chemical industries, such as the oral antihyperglycemic drug, sitagliptin. Therefore, the results of this study showed that *Chloroflexota* is a valuable source of catalysts for the asymmetric synthesis of these chiral amines from the corresponding aldehydes or ketones.

There are several examples of enzyme production with *Chloroflexota* bacteria, such as α -L-rhamnosidase, alcohol dehydrogenase, ene-reductase, and ω -transaminases. However, the reported studies have been using strains from the *Chloroflexia* class, which means that given the metabolic diversity presented by *Chloroflexota* members, there is still a tremendous amount of unexplored potential for enzyme production within this bacterial phylum, and further understanding of metabolic pathways and enzyme functions could help overcome this bottleneck.

Another relevant application is the utilization of *Chloroflexota* metabolic pathways to produce energy precursors, namely hydrogen or biogas. Both can be used as renewable sources of energy (82), and the development of new production approaches can contribute to the implementation of completely circular and bio-based solutions.

Production of hydrogen by *Chloroflexota* has been studied through the utilization of catalytic systems based on whole cells or organelles of photosynthetic bacteria specialized in the conversion of light energy into H₂ (83). For this reason, Gogotov et al. studied the hydrogenases of several photosynthetic bacteria (including *C. aurantiacus*), proving the involvement of Ni (from the enzyme Ni-Fe active center) on the activation of molecular hydrogen and reporting a high denaturing factor resistance from these enzymes, which can be attributed to their thermophilic origin. Moreover, hydrogenase from *C. aurantiacus* was able to reversibly activate H₂ at a high rate at more anaerobic conditions, in contrast with other studied hydrogenases that exhibited low activity under similar conditions. The ability to activate hydrogen efficiently at low redox potentials could be an evolutionary adaptation of *C. aurantiacus* to its natural habitat. In fact, hot springs often present anaerobic and reduced environments, and the microbial life in these locations has evolved mechanisms to optimally harness available resources, like hydrogen, for survival and growth. This feature can also be linked to potential biotechnological applications. For instance, enzymes that are active at low redox potentials and high temperatures could be advantageous in industrial processes involving hydrogen gas, such as in biohydrogen production or in enzymatic fuel cells.

Hydrogen production in photosynthetic microbial mats has been further expanded by biogeochemical and molecular studies reporting H₂ production mainly under dark and anoxic conditions (84). The authors stated the importance of incorporating carbon during sunlight availability for dark production of H₂ and concluded about the inexistence of competition between nitrogen fixation and H₂ production. *Chloroflexales* participated in this process by being involved in the carbon capture and producing reduction equivalents for the dark production of H₂, which states the importance of *Chloroflexota* bacteria in a mixed microbial culture approach specialized in coupling carbon capture with energy production (84).

Recent research reported the presence of *Anaerolineae* in the upgrading of antibiotic fermentation residue (AFR) to biogas (85). The study showed that the addition of Fe₃O₄ acted as a biostimulator for *Anaerolineae* activity, which enhanced methane production by as high as 48%. The contribution of *Anaerolineae* for the valorization of this protein-rich biosolid is another proof of the potential role of *Chloroflexota* bacteria in residue management strategies and energy production.

Biodegradation technologies

Environmentally, microorganisms are the most important agents for the breakdown of organic pollutants or biodegradation, given their ability to use different harmful substances as carbon and energy sources (86). There are several examples of *Chloroflexota* microbial organisms' contribution to biodegradation and decontamination technologies.

In the case of phenol-polluted environments, *Chloroflexota* can be used for their bioremediation. Phenolic compound pollution can be associated with wastewater discharges from several industries (87), with its removal being considered a priority by several countries and entities. Huang et al. assessed and stated the optimal conditions for phenol degradation (1.5 M of NaCl and 350 mg/L of phenol) using a mixed bacterial culture containing *Chloroflexus sp.* from a saline environment (88). Moreover, the authors studied the metabolic pathways related to phenol biodegradation and reported the importance of phenol hydroxylase, catechol 1,2-dioxygenase, and catechol 2,3-dioxygenase for this process. Furthermore, the dependence of this bacteria on ectoine and hydroxyectoine presence was established, contributing to improving bioremediation strategies in phenol-contaminated saline environments. Sánchez-González et al. also stated the involvement of *Chloroflexales* in the degradation of phenol, highlighting the extremophile's participation on the degradation process and the microbial community strategies to survive under severe environmental conditions (89). Overall, *Chloroflexales* bacteria can be considered effective phenolic degraders, and given their ability to thrive in anaerobic conditions, their utilization might be advantageous when treating certain types of industrial effluents. Moreover, their ability to establish synergies with other species can lead to more efficient degradation processes compared with microbes working in isolation. In comparison, genera like *Pseudomonas*, *Acinetobacter*, and *Sphingomonas* are more versatile and efficient in aerobic conditions and are widely used in industrial bioremediation processes (90, 91). Fungi offer a different mechanism through extracellular enzymatic degradation and are particularly effective against more complex pollutants (92). However, *Chloroflexales* might have a unique niche in phenol degradation, especially in anaerobic and extreme environments.

Zhang et al. studied the interactions of bacterial populations along sediment pollution gradients in shallow eutrophic lakes (93). The authors reported that *Chloroflexales* were among the dominant taxa at severe pollution concentrations, possibly contributing to photosynthesis and pollutant degradation, which further demonstrates the adaptability of *Chloroflexota* bacteria to adverse conditions and their importance in bioremediation technologies.

The organohalide respiration of *Dehalococcoides* presents great biotechnological interest in bioremediation applications, and Zanaroli et al. described the dechlorination capacity of members of this class, which was able to dechlorinate more than 75% of polychlorinated biphenyls (PCBs) in just 30 weeks (94). This extensive removal of pollutants is remarkable, given that PCBs are persistent organic pollutants and, due to bioaccumulation, are responsible for negative health effects on humans (95). In addition to being the first dechlorinator identified in marine sediments, the displayed dechlorination activity and specificity were more comprehensive than other bacteria described in the literature. Moreover, the activities of dechlorination took place under biogeochemical circumstances that closely mirror those found naturally in marine environments. This aspect is particularly important when considering the development of customized approaches for encouraging the *in situ* dechlorination of aged PCBs.

Padilla-Crespo et al. studied the environmental distribution of the genetic sequence encoding for the reductive dehalogenase, which catalyzes the dichloro elimination of 1,2-dichloropropane (a carcinogenic compound formerly used as industrial solvent) to propene (96). The authors reported gene sequences from different continents sharing high sequence identities (>98%), indicating that this enzyme is highly conserved or was recently disseminated. Moreover, *Dehalococcoides mccartyi*, from the *Dehalococcoidia* class, appeared to be the major microbial contributor for this bioremediation process.

Organohalide-respiring *Dehalococcoidia* has been described, and its importance in bioremediation technologies has been stated before (16, 97). In fact, *Dehalococcoidia* presence has also been observed in industrialized estuaries sediments after extreme weather conditions (98). In this study, after Hurricane Harvey in 2017, the presence of several xenobiotic and polychlorinated compounds degrading microorganisms was correlated with sediment properties and contaminant concentrations of the estuary water.

Chloroflexota can also contribute to the biodegradation of polycyclic aromatic hydrocarbons (PAHs) and mutagenic/carcinogenic toxic compounds produced by incomplete combustion of fossil fuels. Specifically, in constructed wetlands, the low dissolved oxygen concentrations decrease the activity of PAHs-degrading microorganisms, such as *Pseudomonas aeruginosa* and *Pseudomonas putida*, which require aerobic conditions to perform aromatic hydrocarbon degradation (99). Therefore, the development of anaerobic approaches is important to enhance PAH removal for polluted sites with low available oxygen. Hence, Lu et al. reported the important role of *Anaerolineae* bacterium in the degradation of PAHs in an iron-enhanced anaerobic process, in which the metal presence functioned as an electron conduit to promote interspecies electron transfer between iron-reducing bacteria and *Anaerolineae* (100).

Wastewater treatment technologies

The consistently high abundance of *Chloroflexota* bacteria in wastewater treatment systems illustrates their ecological role in nutrient transformation processes. These bacteria are often identified in nutrient (phosphorous and nitrogen) removal systems acting as anaerobic chemoorganotrophs with sugar fermentation abilities, being present in floccular biomass and/or in bulking-related representatives (101, 102). The understanding of *Chloroflexota* distribution and physiology is determinant to establish correlations between their ecology and operational issues in full-scale plants. Table 1 summarizes processes for wastewater treatment processes involving *Chloroflexota* bacteria.

As can be observed, these bacteria are present in several treatment processes, contributing to pollutant removal in a wide range of wastewater types, often as dissolved organic matter decomposers.

Specifically, *Chloroflexota* members have been detected in membrane bioreactors acting as soluble microbial product decomposers (103), this feature being previously demonstrated by the work of Miura et al., who correlated the carbohydrates degradation

TABLE 1 Wastewater treatment processes where the involvement of *Chloroflexota* bacteria was reported

Water type	Pollutant removal	Process type	Bacteria	Reference
Synthetic wastewater	97% of COD 97% of nitrogen	Submerged membrane bioreactors	<i>Chloroflexia</i> , <i>Anaerolineae</i>	(103)
Municipal wastewater	90% of DOC 92% of phosphorus 38% of nitrogen	Submerged membrane bioreactors	Unspecified <i>Chloroflexota</i>	(104)
Mainstream wastewater	90% of nitrogen	Anammox at low temperature	<i>Chloroflexales</i>	(105)
Aniline wastewater	80% of aniline 100% of nitrogen	Electro-enhanced sequencing batch reactor	<i>Anaerolineae</i>	(106)
Domestic saline wastewater	93% of COD	Membrane-aerated biofilm reactor	<i>Anaerolineae</i>	(107)
Saline wastewater	51.8% of COD	Constructed wetlands	<i>Anaerolineae</i> , <i>Dehalococcoides</i>	(108)
Thermal hydrolysis and anaerobic digestion wastewater	12% of COD 0.58 ± 0.06 g N/(L·d)	Partial nitrification-anammox	<i>Anaerolineae</i>	(109)
p-Fluoronitrobenzene (p-FNB) wastewater	100% of p-FNB	Bioelectrochemical degradation	<i>Anaerolineae</i>	(110)
Quinoline wastewater	83.5% of quinoline	Anaerobic degradation	<i>Anaerolineae</i>	(111)
Effluents of wastewater treatment plants	83% of nitrogen 43% of COD	Constructed wetlands	<i>Anaerolineae</i> Other <i>Chloroflexota</i>	(112)

ability of the system with the concentration of *Chloroflexota* and stated an imperative *Chloroflexota* cell concentration above 10% to avoid membrane fouling (104), suggesting the ecological significance of *Chloroflexota* members in the reduction of membrane fouling in membrane bioreactors.

Chloroflexota bacteria were also found to be important in specific treatments of mainstream wastewaters. Lv et al. reported an abundance of *Chloroflexales* genera in flocculent sludge establishing symbiotic microbial interactions with anaerobic ammonia-oxidizing bacteria, which favored the anammox process in mainstream wastewaters at low temperatures, maintaining a nitrogen removal efficiency above 90% (105). Therefore, the coexistence of anaerobic ammonia-oxidizing bacteria and *Chloroflexales* appears to be an effective solution to overcome the challenges of anammox processes at low temperatures.

Other reports displayed the importance of *Anaerolineae* in the effectiveness of aniline and nitrogen removal (106), treatment of saline wastewater (107, 108), treatment of waste streams rich in ammonium and low on organic compounds (109), biodegradation of p-fluoronitrobenzene (110), complete anaerobic mineralization of quinoline (111), and denitrification processes (112).

Soil treatment technologies

Chloroflexota bacteria have been reported in numerous approaches for soil treatment, contributing as producers of biodegradable organic matter and nutrients, and degrading recalcitrant molecules. They have been found in metal-contaminated soils of abandoned mines, and their concentration varied with the application of different phytostabilization techniques (113). In this study, among other reported bacteria, *Chloroflexota* organisms belonged to the *Chloroflexales* order and could perform anaerobic photoheterotrophy and, in dark aerobic conditions, chemoheterotrophy. Moreover, *Chloroflexota* members can present heavy metal resistance (114), which could imply their involvement in the decontamination of soils, especially in the reduction of heavy metal bioavailability and in the production of biodegradable organic matter.

Studies on the improvement of soil fertility also reported the relevance of *Chloroflexota*. Huang et al. successfully applied biochar to alleviate salt stress in a rice plantation field, to prevent crop production inhibition (115). The study reported the abundance of *Chloroflexota* (*Anaerolineaceae* family members) in the untreated soil, which decreased after the treatment with biochar mainly due to changes in the soil pH, suggesting that the regulation of the bacterial community is a key factor in achieving a satisfactory soil decontamination. Despite decreasing in concentration, *Chloroflexota* bacteria were still important in the transformation of inorganic carbon into organic matter and production of nutrients such as phosphorous and nitrogen, implying a central role in the symbiotic relationships established between soil bacteria, fungi, and plants. Indeed, in a study by Chen et al., *Chloroflexota* was demonstrated to be beneficial for soil treatment, acting as organic matter and nutrient producers and contributing to the decrease in N-loss bacterial activities. The authors studied a maize rhizosphere for 3 years and concluded that the presence of bacteria from the unclassified group *Chloroflexia* KD4-96 in soils contributed not only to plant growth but also to grain production (116).

Chloroflexota has also been demonstrated to participate in composting processes, specifically in the treatment of textile wastes (117). In this study, the authors mixed several textile waste concentrations with paper waste for composting and reported the presence of *Chloroflexota* bacteria participating in recalcitrant molecular degradation for mixtures containing 40%–60% of textile wastes.

Anaerolineae carbon fixation metabolism, via Arnon-Buchanan cycle (118), was also demonstrated to be important during the reduction of contaminants bioavailability by natural processes in anthropized freshwater sediments with high phosphorous concentrations and alkaline pH (119). The carbon fixation activity of *Anaerolineae* in surface sediments creates a flux of carbon that aids in the degradation of xenobiotic compounds by bacteria residing in the deeper, non-surface sediments. This dynamic

illustrates a noteworthy symbiotic relationship, where the metabolic processes of surface-dwelling *Anaerolineae* enhance the capacity of deeper sediment bacteria to recover contaminated sites.

Hereabove, a variety of *Chloroflexota* metabolisms (carbon fixation, nutrient production, switch between anaerobic photoheterotrophy, and, in dark aerobic conditions, chemoheterotrophy) were highlighted, being mostly found in microbial communities for the treatment of soils and sediments. Their wide presence can be attributed to their resistance to adverse environmental conditions, and their role as primary producers may potentiate the activity of other microorganisms, establishing symbiotic relationships in these applications. However, it can be observed that most of the described applications that make use of *Chloroflexota* mainly report bacteria from the *Chloroflexia*, *Anaerolineae*, and *Dehalococcoides* classes (Table 2). Therefore, the discovery of new metabolic pathways and functions can act as a driving force for the development of biobased technologies containing *Chloroflexota* bacteria as the main players or as important contributors in mixed microbial systems.

PROSPECTIVE BIOTECHNOLOGICAL APPLICATIONS

Chloroflexota metabolic diversity continues to expand as scientific studies report previously unknown microbial processes. For instance, (120) described a new candidate of the *Chloroflexota* phylum (*Candidatus Chlorolinea photoalkanotrophicum*) with the ability to both perform phototrophy and oxidize methane and/or other small alkanes (120), which not only extends the known metabolic diversity of *Chloroflexota* but also offers exciting possibilities for biotechnological applications, especially in the areas of environmental remediation (biodegradation of alkanes), renewable energy (conversion of methane into biofuels), and carbon cycle management (carbon sequestration or transformation). In fact, the metagenomic-assembled genome of this species showed the ability of this species to perform several metabolisms, namely phototrophy, aerobic respiration, reduction of nitrites, carbon monoxide oxidation, and oxidation of carbon from methane and/or propane, and potentially fixate carbon using the pathway composed of hybridized components of the serine cycle and the 3-hydroxypropionate bi-cycle. These findings contribute to demonstrating the evolution and incorporation of new pathways into *Chloroflexota* promoted by horizontal gene transfer occurrences in natural habitats (120, 121).

The work of Kawaichi et al. described, for the first time, another interesting metabolism for a representative of the *Chloroflexota* phylum that could be biotechnologically viable (9). In this study, an isolate (belonging to the *Ardenticatenia* class) from a hydrothermal field with high iron concentrations was shown to perform dissimilatory

TABLE 2 Reported applications for bacteria belonging to the *Chloroflexota* phylum^a

Bacterial class	Application	Metabolism	Reference
<i>Chloroflexia</i>	H ₂ production	Anaerobic photoheterotrophic growth	(84)
<i>Anaerolineae</i>	Biogas production	Carbohydrate hydrolysis and proteolysis	(85)
<i>Chloroflexia</i>	Phenolic compound removal	Meta-cleavage pathway	(88, 89)
<i>Dehalococcoides</i>	PCB treatment	Dehalogenation	(94, 96)
<i>Anaerolineae</i>	Biodegradation of PAHs	Anaerobic heterotrophic growth	(100)
<i>Anaerolineae</i>	Avoidance of membrane fouling in SMBR	Anaerobic heterotrophic growth	(103, 104)
<i>Chloroflexia</i>			
<i>Anaerolineae</i>	N-removal, biodegradation of pollutants	Anaerobic (photo)-heterotrophic growth	(105, 106, 110)
<i>Chloroflexia</i>			
<i>Chloroflexia</i>	Reduction of heavy metal availability and production of biodegradable organic matter	Anaerobic photoheterotrophic and chemoheterotrophic growth	(114)
<i>Anaerolineae</i>	Soil treatment	Anaerobic photoautotrophic and heterotrophic growth	(115, 116)
<i>Chloroflexia</i>			
<i>Anaerolineae</i>	Reduction of contaminant bioavailability	Carbon fixation (Arnon-Buchanan cycle)	(119)

^aPCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons; SMBR, submerged membrane bioreactor.

iron reduction. Presenting a versatile metabolism with the ability to grow using oxygen, ferric iron, and nitrate as electron acceptors, this isolate also grows at different temperatures (30°C–75°C) and salt concentrations (0%–6% NaCl). This metabolic trait can play an important role in the bioremediation of subsurface environments contaminated with organic or metal contaminants.

Regarding the discovery of interesting metabolic pathways for *Chloroflexota* bacteria, there are also studies reporting the existence of newly found aerobic respiration and partial denitrification for *Anaerolineae* (122). In fact, despite most described *Anaerolineae* being classified as strict anaerobes, *Levilinea saccharolytica* displayed a branched aerobic respiration pathway, containing a NADH dehydrogenase, a succinate dehydrogenase, a heme-copper oxygen reductase, and a bd oxidase. Moreover, two nitrite reduction pathways were reported containing different nitrite reductases, able to reduce nitrite into nitric oxide or into ammonia. The presence of previously unknown pathways in *Anaerolineae* species suggests a wider physiological diversity than previously recognized for this *Chloroflexota* class, offering new opportunities for biotechnological applications: the aerobic and denitrification capabilities of these bacteria could play a role in converting nitrite into less toxic forms, contributing to the health of the soil microbiome, improving soil quality, and potentially enhancing crop growth. Moreover, the presence of enzymes like NADH dehydrogenase, succinate dehydrogenase, and oxygen reductases indicates their potential role in more efficient breakdown of organic matter in wastewater, leading to more effective and possibly faster treatment methods.

A. maritima, from the *Ardenticatenia* class, also presents a wide range of physiologies, which include aerobic respiration (containing enzymes from complex I, II, III, and three oxygen reductases), iron reduction, and a complete denitrification pathway composed of nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (123). These findings could enhance the role of *Chloroflexota* bacteria in nitrogen removal technologies in wastewater treatment plants and, to a broader extent, the coupling of nitrogen, sulfur, and carbon cycles to be used in multipurpose bioreactors, which is of utmost technological relevance (124).

Hydrothermal systems, including terrestrial hot springs, contain diverse geochemical conditions that promote the discovery of novel metabolisms. Among the isolated bacteria from the different redox environments existing in an intertidal, anoxic, iron-, and hydrogen-rich hot spring that mixes with the oxygenated atmosphere and sulfate-rich seawater, there was a *Chloroflexota* member that not only presented the ability to fixate carbon, via Calvin cycle, but also had genes encoding for a hydrogenase, suggesting a lithoautotrophic capacity to oxidize hydrogen (125). This ability could be important in the utilization of these bacteria in waste-free biotechnological processes to directly convert electrical energy and inorganic substances into amino acids and other biologically active substances, contributing to sustainable bioproduction, where the goal is to minimize waste and maximize efficiency. Overall, the thermophilic nature of these *Chloroflexota* bacteria, thriving in hydrothermal systems, suggests that they possess enzymes and proteins that are stable and active at high temperatures. This thermophilic property can be particularly advantageous in industrial processes that operate at elevated temperatures, providing more robust and efficient systems, as in the production of bioenergy or in biocatalysis processes.

Chloroflexota from the *Ktenodobacteria* class (*Thermogemmatispora* sp. T81) has been demonstrated to persist using sub-atmospheric levels of H₂ and CO (126). The authors reported that group 1 h [NiFe]-hydrogenases and type I carbon monoxide dehydrogenases were encoded in most of the studied reference genomes within the *Ktedonobacteriales*. Additionally, a meta-transcriptome study revealed that homologs of the group 1 f[NiFe]-hydrogenase of *Roseiflexus* species are highly expressed in geothermal microbial mats at night (55), possibly indicating atmospheric H₂ oxidation within the photosynthetic *Chloroflexota* strains. These findings could also indicate a possible application of *Chloroflexota* bacteria in the treatment of syngas and industrial off-gas streams, often rich in hydrogen and carbon monoxide. In fact, the ability of

C. aurantiacus to use hydrogen or sulfide for photoautotrophic growth (127, 128), combined with its capacity for polyhydroxyalkanoates (PHA) accumulation (129) and pigment production (130), could be explored as a circular process focused on carbon dioxide mitigation coupled with the production of value-added substances. PHA are natural polyesters with thermoplastic properties that are internally accumulated by bacteria as carbon and energy reserves, being considered interesting candidates for substituting traditional plastics (131). Recently, purple phototrophic bacteria have been described as suitable PHA-accumulating organisms (132), and its upscale challenges have been reviewed (133). There are several examples of phototrophic production of PHA using purple bacteria or cyanobacteria (134–138), and these bacteria have been reviewed as suitable phototrophic factories to couple resource recovery with the production of value-added substances (139). However, the PHA production capacity of phototrophic organisms of the *Chloroflexota* phylum remains mostly unexplored. In fact, phototrophy has been demonstrated to be present in seven bacterial phyla, and the development of omics methodologies can contribute to valuable metabolic, ecological, and physiological insights regarding photosynthesis and carbon fixation (5). A potential *Chloroflexota* member to develop such studies is *C. aurantiacus*, a phototrophic species that can use the 3-hydroxypropionate bi-cycle pathway for autotrophic carbon fixation, in which some intermediates of the pathway (such as acetyl-coA and propionyl-coA) are precursors for PHA accumulation (140). Studying PHA production in *Chloroflexota*, specifically *C. aurantiacus*, is crucial due to its unique metabolic pathways which may offer more efficient or varied PHA synthesis compared with well-studied purple bacteria. The photoautotrophic growth capabilities of *C. aurantiacus*, using hydrogen or sulfide, presents opportunities for sustainable, energy-efficient bioplastic production. In fact, exploring *Chloroflexota*'s PHA production can broaden the understanding of bioplastic synthesis and applications, complementing and extending the current knowledge derived from studies on purple bacteria. Furthermore, *Chloroflexota* potential to use CO₂ fixation toward PHA production could contribute to new carbon capture technologies focused on bioplastic production, hence advancing new *Chloroflexota*-based bioprocesses for photoautotrophic biodegradable polymers production.

CONCLUSION

The *Chloroflexota* phylum encompasses several biotechnologically interesting bacteria that can be frequently found in extreme environments and naturally adapted to unfavorable conditions due to the unique characteristics of its members. The resilience of these organisms can be attributed to their metabolic diversity, responding to site-specific requirements. In fact, *Chloroflexota* is present in several biotechnological approaches, including water treatment, pollutant biodegradation, and energy production. Due to the metabolic diversity and adaptability of the members belonging to this bacterial phylum, several interesting *Chloroflexota* enzymes have been isolated and studied, often reported as thermostable and highly efficient. However, the available literature reveals that several *Chloroflexota* microorganisms remain unexplored, which means that several bacterial functions and possibly interesting metabolisms are being overlooked. Therefore, further research is required to unlock the full potential of these microorganisms, especially within their photoautotrophic members, which could be useful for the development of CO₂-negative technologies. The understanding of *Chloroflexota* role in natural cycles, under specific conditions, is fundamental for the development of new technologies based on these microorganisms.

ACKNOWLEDGMENTS

This work was supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. A.

Freches also acknowledges the financial support of FCT - Fundação para a Ciência e a Tecnologia through the doctoral grant SFRH/BD/145555/2019.

AUTHOR AFFILIATIONS

¹Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal

²Department of Chemistry, UCIBIO - Applied Molecular Biosciences Unit, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

AUTHOR ORCIDs

Joana Costa Fradinho  <http://orcid.org/0000-0001-7192-5367>

FUNDING

Funder	Grant(s)	Author(s)
MEC Fundação para a Ciência e a Tecnologia (FCT)	UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020	André Freches Joana Costa Fradinho
MEC Fundação para a Ciência e a Tecnologia (FCT)	SFRH/BD/145555/2019	André Freches

REFERENCES

- Kuleshova T, Rao A, Bhadra S, Garlapati VK, Sharma S, Kaushik A, Goswami P, Sreekirshnan TR, Sevda S. 2022. Plant microbial fuel cells as an innovative, versatile agro-technology for green energy generation combined with wastewater treatment and food production. *Bio Bioener* 167:106629. <https://doi.org/10.1016/j.biombioe.2022.106629>
- Sodhi KK, Mishra LC, Singh CK, Kumar M. 2022. Perspective on the heavy metal pollution and recent remediation strategies. *Curr Res Microb Sci* 3:100166. <https://doi.org/10.1016/j.crmicr.2022.100166>
- George DM, Vincent AS, Mackey HR. 2020. An overview of anoxygenic phototrophic bacteria and their applications in environmental biotechnology for sustainable resource recovery. *Biotechnol Rep (Amst)* 28:e00563. <https://doi.org/10.1016/j.btre.2020.e00563>
- Yaashikaa PR, Senthil Kumar P, Varjani SJ, Saravanan A. 2019. A review on photochemical, biochemical and electrochemical transformation of CO₂ into value-added products. *J CO₂ Util* 33:131–147. <https://doi.org/10.1016/j.jcou.2019.05.017>
- Thiel V, Tank M, Bryant DA. 2018. Diversity of chlorophototrophic bacteria revealed in the omics era. *Annu Rev Plant Biol* 69:21–49. <https://doi.org/10.1146/annurev-arplant-042817-040500>
- Pierson BK, Castenholz RW. 1974. A Phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. *Arch Microbiol* 100:5–24. <https://doi.org/10.1007/BF00446302>
- Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, Kamagata Y. 2006. *Anaerolinea thermolimos* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum *Chloroflexi*. *Int J Syst Evol Microbiol* 56:1331–1340. <https://doi.org/10.1099/ijs.0.64169-0>
- Nakahara N, Nobu MK, Takaki Y, Miyazaki M, Tasumi E, Sakai S, Ogawara M, Yoshida N, Tamaki H, Yamanaka Y, Katayama A, Yamaguchi T, Takai K, Imachi H. 2019. *Aggregatilinea Lenta* gen. nov., sp. nov., a slow-growing, facultatively anaerobic bacterium isolated from subseafloor sediment, and proposal of the new order *Aggregatilineales* ord. nov. within the class *Anaerolineae* of the phylum *Chloroflexi*. *Int J Syst Evol Microbiol* 69:1185–1194. <https://doi.org/10.1099/ijsem.0.003291>
- Kawaichi S, Ito N, Kamikawa R, Sugawara T, Yoshida T, Sako Y. 2013. *Ardenticatena maritima* gen. nov., sp. nov., a ferric iron- and nitrate-reducing bacterium of the phylum *Chloroflexi* isolated from an iron-rich coastal hydrothermal field, and description of *Ardenticatena* classis nov. *Int J Syst Evol Microbiol* 63:2992–3002. <https://doi.org/10.1099/ijs.0.046532-0>
- Kale V, Björnsdóttir SH, Friðjónsson ÓH, Pétursdóttir SK, Ómarsdóttir S, Hreggviðsson GÓ. 2013. *Litorilinea aerophila* gen. nov., sp. nov., an aerobic member of the class *Caldilineae*, phylum *Chloroflexi*, isolated from an Intertidal hot spring. *Int J Syst Evol Microbiol* 63:1149–1154. <https://doi.org/10.1099/ijs.0.044115-0>
- Sekiguchi Y, Yamada T, Hanada S, Ohashi A, Harada H, Kamagata Y. 2003. *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain bacteria at the subphylum level. *Int J Syst Evol Microbiol* 53:1843–1851. <https://doi.org/10.1099/ijs.0.02699-0>
- Gupta RS, Chander P, George S. 2013. Phylogenetic framework and molecular signatures for the class *Chloroflexi* and its different clades; proposal for division of the class *Chloroflexi* class. nov. into the suborder *Chloroflexineae* subord. nov., consisting of the emended family *Oscillochloridaceae* and the family *Chloroflexaceae* fam. nov., and the suborder *Roseiflexineae* subord. nov., containing the family *Roseiflexaceae* fam. nov. *Antonie Van Leeuwenhoek* 103:99–119. <https://doi.org/10.1007/s10482-012-9790-3>
- Keppen OI, Tourova TP, Kuznetsov BB, Ivanovsky RN, Gorlenko VM. 2000. Proposal of *Oscillochloridaceae* fam. nov. on the basis of a phylogenetic analysis of the filamentous anoxygenic phototrophic bacteria, and emended description of *Oscillochloris* and *Oscillochloris trichoides* in comparison with further new isolates. *Int J Syst Evol Microbiol* 50 Pt 4:1529–1537. <https://doi.org/10.1099/00207713-50-4-1529>
- Ward LM, Hemp J, Shih PM, McGlynn SE, Fischer WW. 2018. Evolution of phototrophy in the *Chloroflexi* phylum driven by horizontal gene transfer. *Front Microbiol* 9:260. <https://doi.org/10.3389/fmicb.2018.00260>
- Cole JK, Gieler BA, Heisler DL, Palisoc MM, Williams AJ, Dohnalkova AC, Ming H, Yu TT, Dodsworth JA, Li W-J, Hedlund BP. 2013. *Kallotenue papyrolyticum* gen. nov., sp. nov., a cellulolytic and filamentous thermophile that represents a novel lineage (*Kallotenuales* ord. nov., *Kallotenuaceae* fam. nov.) within the class *Chloroflexia*. *Int J Syst Evol Microbiol* 63:4675–4682. <https://doi.org/10.1099/ijs.0.053348-0>
- Löffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT, Müller JA, Fullerton H, Zinder SH, Spormann AM. 2013. *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation,

- belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and family *Dehalococcoidaceae* fam. nov., within the phylum *Chloroflexi*. Int J Syst Evol Microbiol 63:625–635. <https://doi.org/10.1099/ijs.0.034926-0>
17. Wang C-M, Zheng Y, Sakai Y, Toyoda A, Minakuchi Y, Abe K, Yokota A, Yabe S. 2019. *Tengunoibacter tsumagoiensis* gen. nov., sp. nov., *Dictyobacter kobayashii* sp. nov., *Dictyobacter alpinus* sp. nov., and description of *Dictyobacteraceae* fam. nov. within the order *Ktedonobacterales* isolated from Tengu-no-mugimeshi, a soil-like granular mass of micro-organisms, and emended descriptions of the genera *Ktedonobacter* and *Dictyobacter*. Int J Syst Evol Microbiol 69:1910–1918. <https://doi.org/10.1099/ijs.0.003396>
 18. Cavalletti L, Monciardini P, Bamonte R, Schumann P, Rohde M, Sosio M, Donadio S. 2006. New lineage of filamentous, spore-forming, Gram-positive bacteria from soil. Appl Environ Microbiol 72:4360–4369. <https://doi.org/10.1128/AEM.00132-06>
 19. Yan B, Guo X, Liu M, Huang Y. 2020. *Ktedonosporobacter rubrisoli* gen. nov., sp. nov., a novel representative of the class *Ktedonobacteria*, isolated from red soil, and proposal of *Ktedonosporobacteraceae* fam. nov. Int J Syst Evol Microbiol 70:1015–1025. <https://doi.org/10.1099/ijs.0.003864>
 20. Yabe S, Zheng Y, Wang C, Sakai Y, Abe K, Yokota A, Donadio S, Cavalletti L, Monciardini P. 2021. *Reticulibacter mediterranei* gen. nov., sp. nov., within the new family *Reticulibacteraceae* fam. nov., and *Ktedonospora formicarum* gen. nov., sp. nov., *Ktedonobacter robiniae* sp. nov., *Dictyobacter formicarum* sp. nov. and *Dictyobacter arantisoli* sp. nov., belonging to the class *Ktedonobacteria*. Int J Syst Evol Microbiol 71:1–24. <https://doi.org/10.1099/ijs.0.004883>
 21. Yabe S, Aiba Y, Sakai Y, Hazaka M, Yokota A. 2010. *Thermosporothrix hazakensis* gen. nov., sp. nov., isolated from compost, description of *Thermosporotrichaceae* fam. nov. within the class *Ktedonobacteria* cavalletti et al. 2007 and emended description of the class *Ktedonobacteria*. Int J Syst Evol Microbiol 60:1794–1801. <https://doi.org/10.1099/ijs.0.018069-0>
 22. Yabe S, Aiba Y, Sakai Y, Hazaka M, Yokota A. 2011. *Thermogemmatisspora onikobensis* gen. nov., sp. nov. and *Thermogemmatisspora foliorum* sp. nov., isolated from fallen leaves on geothermal soils, and description of *Thermogemmatissporaceae* fam. nov. and *Thermogemmatissporales* ord. nov. within the class *Ktedonobacteria*. Int J Syst Evol Microbiol 61:903–910. <https://doi.org/10.1099/ijs.0.024877-0>
 23. Kochetkova TV, Zayulina KS, Zhigarkov VS, Minaev NV, Chichkov BN, Novikov AA, Toshchakov SV, Elcheninov AG, Kublanov IV. 2020. *Tepidiforma bonchosmolovskayae* gen. nov., sp. nov., a moderately thermophilic *Chloroflexi* bacterium from a Chukotka hot spring (Arctic, Russia), representing a novel class, tepidiformia, which includes the previously uncultivated lineage olb14. Int J Syst Evol Microbiol 70:1192–1202. <https://doi.org/10.1099/ijs.0.003902>
 24. Dodsworth JA, Gevorkian J, Despujols F, Cole JK, Murugapiran SK, Ming H, Li W-J, Zhang G, Dohnalkova A, Hedlund BP. 2014. *Thermoflexus hugenholtzii* gen. nov., sp. nov., a thermophilic, microaerophilic, filamentous bacterium representing a novel class in the *Chloroflexi*, *thermoflexia* classis nov., and description of *Thermoflexaceae* fam. nov. and *Thermoflexales* ord. nov. Int J Syst Evol Microbiol 64:2119–2127. <https://doi.org/10.1099/ijs.0.055855-0>
 25. Mehrshad M, Salcher MM, Okazaki Y, Nakano SI, Šimek K, Andrei AS, Ghai R. 2018. Hidden in plain sight - highly abundant and diverse planktonic freshwater *Chloroflexi*. Microbiome 6:176. <https://doi.org/10.1186/s40168-018-0563-8>
 26. Yamada T, Sekiguchi Y, Imachi H, Kamagata Y, Ohashi A, Harada H. 2005. Diversity, localization, and physiological properties of filamentous microbes belonging to *Chloroflexi* subphylum I in mesophilic and thermophilic methanogenic sludge granules. Appl Environ Microbiol 71:7493–7503. <https://doi.org/10.1128/AEM.71.11.7493-7503.2005>
 27. Hanada S. 2003. Filamentous anoxygenic phototrophs in hot springs. Microb Environ 18:51–61. <https://doi.org/10.1264/jsme2.18.51>
 28. Gorlenko VM, Bryantseva IA, Samylnina OS, Ashikhmin AA, Sinetova MA, Kostrikina NA, Kozyaeva VV. 2020. Filamentous anoxygenic phototrophic bacteria in microbial communities of the Kulunda Steppe soda lakes (Altai Krai, Russia). Microbiology 89:697–707. <https://doi.org/10.1134/S0026261720060053>
 29. Krzmarzick MJ, Crary BB, Harding JJ, Oyerinde OO, Leri AC, Myneni SCB, Novak PJ. 2012. Natural niche for organohalide-respiring *Chloroflexi*. Appl Environ Microbiol 78:393–401. <https://doi.org/10.1128/AEM.06510-11>
 30. Gaisin VA, Kalashnikov AM, Grouzdev DS, Sukhacheva MV, Kuznetsov BB, Gorlenko VM. 2017. *Chloroflexus islandicus* sp. nov., a thermophilic filamentous anoxygenic phototrophic bacterium from a geyser. Int J Syst Evol Microbiol 67:1381–1386. <https://doi.org/10.1099/ijs.0.001820>
 31. Hanada S, Takaichi S, Matsuura K, Nakamura K. 2002. *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. Int J Syst Evol Microbiol 52:187–193. <https://doi.org/10.1099/00207713-52-1-187>
 32. Gorlenko VM, Bryantseva IA, Kalashnikov AM, Gaisin VA, Sukhacheva MV, Grouzdev DS, Kuznetsov BB. 2014. "Candidatus *Chloroploca asiatica*" gen. nov., sp. nov., a new mesophilic filamentous anoxygenic phototrophic bacterium. Microbiology 83:838–848. <https://doi.org/10.1134/S0026261714060083>
 33. Gaisin VA, Burganskaya EI, Grouzdev DS, Ashikhmin AA, Kostrikina NA, Bryantseva IA, Kozyaeva VV, Gorlenko VM. 2019. *Candidatus viridilinea mediisalina*, a novel phototrophic *Chloroflexi* bacterium from a Siberian soda Lake. FEMS Microbiol Lett 366:fnz043. <https://doi.org/10.1093/femsle/fnz043>
 34. Gaisin VA, Burganskaya EI, Grouzdev DS, Osipova NS, Ashikhmin AA, Sinetova MA, Krutkina MS, Bryantseva IA, Sukhacheva MV, Kochetkova TV, Kozyaeva VV, Kalashnikov AM, Gorlenko VM. 2019. *Candidatus oscillochloris fontis*: a novel mesophilic phototrophic *Chloroflexota* bacterium belonging to the ubiquitous *Oscillochloris* genus. FEMS Microbiol Lett 366:fnz097. <https://doi.org/10.1093/femsle/fnz097>
 35. Bennett AC, Murugapiran SK, Hamilton TL. 2020. Temperature impacts community structure and function of phototrophic *Chloroflexi* and cyanobacteria in two alkaline hot springs in Yellowstone National Park. Environ Microbiol Rep 12:503–513. <https://doi.org/10.1111/1758-2229.12863>
 36. Gaisin VA, Grouzdev DS, Namsaraev ZB, Sukhacheva MV, Gorlenko VM, Kuznetsov BB. 2016. Biogeography of thermophilic phototrophic bacteria belonging to *Roseiflexus* genus. FEMS Microbiol Ecol 92:fw012. <https://doi.org/10.1093/femsec/fw012>
 37. Sprague SG, Staehelin LA, DiBartolomeis MJ, Fuller RC. 1981. Isolation and development of chlorosomes in the green bacterium *Chloroflexus aurantiacus*. J Bacteriol 147:1021–1031. <https://doi.org/10.1128/jb.147.3.1021-1031.1981>
 38. Frigaard N-U, Gomez A, Chew M, Maresca JA, Bryant DA. 2006. Bacteriochlorophyll biosynthesis in green bacteria, p 201–221. In Grimm B, Porra RJ, Rudiger W, Scheer H (ed), Chlorophylls and bacteriochlorophylls: biochemistry, biophysics, functions and applications. Springer.
 39. Bryant DA, Liu Z, Li T, Zhao F, Costas AMG, Klatt CG, Ward DM, Frigaard N-U, Overmann J. 2012. Comparative and functional genomics of anoxygenic green bacteria from the taxa *Chlorobi*, *Chloroflexi*, and *Acidobacteria*, p 47–102. In Burnap RI, Vermaas WFJ (ed), Functional genomics and evolution of photosynthetic systems, advances in photosynthesis and respiration. Springer.
 40. Takaichi S, Maoka T, Yamada M, Matsuura K, Haikawa Y, Hanada S. 2001. Absence of carotenoids and presence of a tertiary methoxy group in a carotenoid from a thermophilic filamentous photosynthetic bacterium *Roseiflexus castenholzii*. Plant Cell Physiol 42:1355–1362. <https://doi.org/10.1093/pcp/pce172>
 41. Garritano AN, Song W, Thomas T. 2022. Carbon fixation pathways across the bacterial and archaeal tree of life. PNAS Nexus 1:pgac226. <https://doi.org/10.1093/pnasnexus/pgac226>
 42. Sirevag R, Castenholz R. 1979. Aspects of carbon metabolism in *Chloroflexus*. Arch Microbiol 120:151–153. <https://doi.org/10.1007/BF00409101>
 43. van der Meer MT, Schouten S, de Leeuw JW, Ward DM. 2000. Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. Environ Microbiol 2:428–435. <https://doi.org/10.1046/j.1462-2920.2000.00124.x>
 44. Liang B, Zhao Y, Yang J. 2020. Recent advances in developing artificial autotrophic microorganism for reinforcing CO₂ fixation. Front Microbiol 11:592631. <https://doi.org/10.3389/fmicb.2020.592631>

45. Zarzycki J, Fuchs G. 2011. Coassimilation of organic substrates via the autotrophic 3-hydroxypropionate bi-cycle in *Chloroflexus aurantiacus*. *Appl Environ Microbiol* 77:6181–6188. <https://doi.org/10.1128/AEM.00705-11>
46. Hügler M, Sievert SM. 2011. Beyond the calvin cycle: autotrophic carbon fixation in the ocean. *Annu Rev Mar Sci* 3:261–289. <https://doi.org/10.1146/annurev-marine-120709-142712>
47. Zarzycki J, Brecht V, Müller M, Fuchs G, Bryant DA. 2009. Identifying the missing steps of the autotrophic 3-hydroxypropionate CO₂ fixation cycle in *Chloroflexus aurantiacus*. *Proc Natl Acad Sci U S A* 106:21317–21322. <https://doi.org/10.1073/pnas.0908356106>
48. Overmann J. 2008. Green nonsulfur bacteria, p 1–10. In *Encyclopedia of life sciences*. Wiley.
49. Frigaard NU, Dahl C. 2008. Sulfur metabolism in phototrophic sulfur bacteria. *Adv Microb Physiol* 54:103–200.
50. Camacho A. 2009. Sulfur bacteria, p 261–278. In *Protists, bacteria and fungi: planktonic and attached*. Elsevier.
51. Klappenbach JA, Pierson BK. 2004. “Phylogenetic and physiological characterization of a filamentous anoxygenic photoautotrophic bacterium “*Candidatus chlorothrix halophila*” gen. nov., sp. nov., recovered from hypersaline microbial mats”. *Arch Microbiol* 181:17–25. <https://doi.org/10.1007/s00203-003-0615-7>
52. Burganskaya EI, Bryantseva IA, Krutkina MS, Grouzdev DS, Gorlenko VM. 2019. Bacterial communities of the microbial mats of chokrak sulfide springs. *Arch Microbiol* 201:795–805. <https://doi.org/10.1007/s00203-019-01648-6>
53. Gaisin VA, Grouzdev DS, Krutkina MS, Ashikhmin AA, Sinetova MA, Osipova NS, Koziavaeva VV, Gorlenko VM. 2020. *Candidatus Oscillochloris kuznetsovii* a novel mesophilic filamentous anoxygenic phototrophic chloroflexales bacterium from arctic coastal environments. *FEMS Microbiol Lett* 367:fnaa158. <https://doi.org/10.1093/femsle/fnaa158>
54. Brito EMS, Romero-Núñez VM, Caretta CA, Bertin P, Valerdi-Negreros JC, Guyoneaud R, Goñi-Urriza M. 2019. The bacterial diversity on steam vents from Parícutin and Sapichu volcanoes. *Extremophiles* 23:249–263. <https://doi.org/10.1007/s00792-019-01078-8>
55. Klatt CG, Liu Z, Ludwig M, Kühl M, Jensen SI, Bryant DA, Ward DM. 2013. Temporal metatranscriptomic patterning in phototrophic *Chloroflexi* inhabiting a microbial mat in a geothermal spring. *ISME J* 7:1775–1789. <https://doi.org/10.1038/ismej.2013.52>
56. Kawai S, Martinez JN, Lichtenberg M, Trampe E, Kühl M, Tank M, Haruta S, Nishihara A, Hanada S, Thiel V. 2021. *In-situ* metatranscriptomic analyses reveal the metabolic flexibility of the thermophilic anoxygenic photosynthetic bacterium *Chloroflexus aggregans* in a hot spring cyanobacteria-dominated microbial mat. *Microorganisms* 9:1–22. <https://doi.org/10.3390/microorganisms9030652>
57. van der Meer MTJ, Schouten S, Sinnighe Damsté JS, de Leeuw JW, Ward DM. 2003. Compound-specific isotopic fractionation patterns suggest different carbon metabolisms among chloroflexus-like bacteria in hot-spring microbial mats. *Appl Environ Microbiol* 69:6000–6006. <https://doi.org/10.1128/AEM.69.10.6000-6006.2003>
58. Sun L, Toyonaga M, Ohashi A, Matsuura N, Tourlousse DM, Meng XY, Tamaki H, Hanada S, Cruz R, Yamaguchi T, Sekiguchi Y. 2016. Isolation and characterization of *Flexilinea flocculi* gen. nov., sp. nov., a filamentous, anaerobic bacterium belonging to the class anaerolineae in the phylum *Chloroflexi*. *Int J Syst Evol Microbiol* 66:988–996. <https://doi.org/10.1099/ijsem.0.000822>
59. Cao S, Yan W, Yu L, Zhang L, Lay W, Zhou Y. 2021. Challenges of THP-AD centrate treatment using partial nitrification-anammox (PN/A) – inhibition, biomass washout, low alkalinity, recalcitrant and more. *Water Res* 203:117555. <https://doi.org/10.1016/j.watres.2021.117555>
60. Park Y, Cho H, Yu J, Min B, Kim HS, Kim BG, Lee T. 2017. Response of microbial community structure to pre-acclimation strategies in microbial fuel cells for domestic wastewater treatment. *Bioresour Technol* 233:176–183. <https://doi.org/10.1016/j.biortech.2017.02.101>
61. Zhang B, Tian C, Liu Y, Hao L, Liu Y, Feng C, Liu Y, Wang Z. 2015. Simultaneous microbial and electrochemical reductions of vanadium (V) with bioelectricity generation in microbial fuel cells. *Bioresour Technol* 179:91–97. <https://doi.org/10.1016/j.biortech.2014.12.010>
62. Sutcliffe B, Chariton AA, Harford AJ, Hose GC, Stephenson S, Greenfield P, Midgley DJ, Paulsen IT. 2018. Insights from the Genomes of microbes thriving in uranium-enriched sediments. *Microb Ecol* 75:970–984. <https://doi.org/10.1007/s00248-017-1102-z>
63. Bayer K, Jahn MT, Slaby BM, Moitinho-Silva L, Hentschel U. 2018. Marine sponges as *Chloroflexi* hot spots: genomic insights and high-resolution visualization of an abundant and diverse symbiotic clade. *mSystems* 3:117555. <https://doi.org/10.1128/mSystems.00150-18>
64. Wang Q, Liu W, Yuan X, Wang R, Liu L, Li H, Zhao C, Kong Q. 2022. Characteristics of bacterial and archaeal communities in microbial-enhanced constructed wetlands under NaCl stress. *Clean Soil Air Wat* 50:2100152. <https://doi.org/10.1002/clen.202100152>
65. Yang Y, Zhang Y, Cápiro NL, Yan J. 2020. Genomic characteristics distinguish geographically distributed *Dehalococcoidia*. *Front Microbiol* 11:546063. <https://doi.org/10.3389/fmicb.2020.546063>
66. Vuillemin A, Kerrigan Z, D’Hondt S, Orsi WD. 2020. Exploring the abundance, metabolic potential and gene expression of subseafloor *Chloroflexi* in million-year-old oxic and anoxic abyssal clay. *FEMS Microbiol Ecol* 96:faa223. <https://doi.org/10.1093/femsec/faa223>
67. Wasmund K, Cooper M, Schreiber L, Lloyd KG, Baker BJ, Petersen DG, Jørgensen BB, Stepanauskas R, Reinhardt R, Schramm A, Loy A, Adrian L. 2016. Single-cell genome and group-specific dsrAB sequencing implicate marine members of the class *Dehalococcoidia* (phylum *Chloroflexi*) in sulfur cycling. *mBio* 7:e00266-16. <https://doi.org/10.1128/mBio.00266-16>
68. Biderre-Petit C, Dugat-Bony E, Mege M, Parisot N, Adrian L, Moné A, Denonfoux J, Peyretailade E, Debroas D, Boucher D, Peyret P. 2016. Distribution of *Dehalococcoidia* in the anaerobic deep water of a remote meromictic crater lake and detection of *Dehalococcoidia*-derived reductive dehalogenase homologous genes. *PLoS One* 11:e0145558. <https://doi.org/10.1371/journal.pone.0145558>
69. Gich F, Garcia-Gil J, Overmann J. 2001. Previously unknown and phylogenetically diverse members of the green nonsulfur bacteria are indigenous to freshwater lakes. *Archives Microb* 177:1–10. <https://doi.org/10.1007/s00203-001-0354-6>
70. Kadnikov VV, Mardanov AV, Beletsky AV, Grigoriev MA, Karnachuk OV, Ravin NV. 2021. Thermophilic *Chloroflexi* dominate in the microbial community associated with coal-fire gas vents in the Kuznetsk coal Basin, Russia. *Microorganisms* 9:948. <https://doi.org/10.3390/microorganisms9050948>
71. Liu R, Wei X, Song W, Wang L, Cao J, Wu J, Thomas T, Jin T, Wang Z, Wei W, Wei Y, Zhai H, Yao C, Shen Z, Du J, Fang J. 2022. Novel *Chloroflexi* Genomes from the deepest ocean reveal metabolic strategies for the adaptation to deep-sea habitats. *Microbiome* 10:75. <https://doi.org/10.1186/s40168-022-01263-6>
72. Raveendran S, Parameswaran B, Ummalyma SB, Abraham A, Mathew AK, Madhavan A, Rebello S, Pandey A. 2018. Applications of microbial enzymes in food industry. *Food Technol Biotechnol* 56:16–30. <https://doi.org/10.17113/ftb.56.01.18.5491>
73. Meghwanshi GK, Kaur N, Verma S, Dabi NK, Vashishtha A, Charan PD, Purohit P, Bhandari HS, Bhojak N, Kumar R. 2020. Enzymes for pharmaceutical and therapeutic applications. *Biotechnol Appl Biochem* 67:586–601. <https://doi.org/10.1002/bab.1919>
74. Singh R, Kumar M, Mittal A, Mehta PK. 2016. Microbial enzymes: industrial progress in 21st century. *3 Biotech* 6:174. <https://doi.org/10.1007/s13205-016-0485-8>
75. Shin K-C, Seo M-J, Oh D-K, Choi M-N, Kim D-W, Kim Y-S, Park C-S. 2019. Cloning and characterization of a -L-rhamnosidase from *Chloroflexus aurantiacus* and its application in the production of isoquercitrin from rutin. *Biotechnol Lett* 41:419–426. <https://doi.org/10.1007/s10529-019-02648-8>
76. Makino T, Kanemaru M, Okuyama S, Shimizu R, Tanaka H, Mizukami H. 2013. Anti-allergic effects of enzymatically modified Isoquercitrin (α -oligoglucosyl quercetin 3-O-glucoside), quercetin 3-O-glucoside, α -Oligoglucosyl rutin, and quercetin, when administered orally to mice. *J Nat Med* 67:881–886. <https://doi.org/10.1007/s11418-013-0760-5>
77. Vila-Real H, Alfaia AJ, Bronze MR, Calado ART, Ribeiro MHL. 2011. Enzymatic synthesis of the flavone glucosides, prunin and isoquercetin, and the aglycones, naringenin and quercetin, with selective α -L-rhamnosidase and β -D-glucosidase activities of naringinase. *Enzyme Res* 2011:692618. <https://doi.org/10.4061/2011/692618>
78. Loderer C, Wagner D, Morgenstern F, Spieß A, Ansorge-Schumacher MB. 2018. Discovery of a novel thermostable Zn²⁺-dependent alcohol

- dehydrogenase from *Chloroflexus aurantiacus* through conserved domains mining. *J Appl Microbiol* 124:480–490. <https://doi.org/10.1111/jam.13664>
79. Huisman GW, Liang J, Krebber A. 2010. Practical chiral alcohol manufacture using ketoreductases. *Curr Opin Chem Biol* 14:122–129. <https://doi.org/10.1016/j.cbpa.2009.12.003>
 80. Robescu MS, Niero M, Loprete G, Cendron L, Bergantino E. 2021. A new thermophilic ene-reductase from the filamentous anoxygenic phototrophic bacterium *Chloroflexus aggregans*. *Microorganisms* 9:953. <https://doi.org/10.3390/microorganisms9050953>
 81. Wang C, Tang K, Dai Y, Jia H, Li Y, Gao Z, Wu B. 2021. Identification, characterization, and site-specific mutagenesis of a thermostable *w*-transaminase from *Chloroflexi* bacterium. *ACS Omega* 6:17058–17070. <https://doi.org/10.1021/acsomega.1c02164>
 82. Torzillo G, Scoma A, Faraloni C, Giannelli L. 2015. Advances in the biotechnology of hydrogen production with the microalga *Chlamydomonas reinhardtii*. *Crit Rev Biotechnol* 35:485–496. <https://doi.org/10.3109/07388551.2014.900734>
 83. Gogotov IN, Zorin NA, Serebriakova LT. 1991. Hydrogen production by model systems including hydrogenases from phototrophic bacteria. *Intern J Hydrogen Ene* 16:393–396. [https://doi.org/10.1016/0360-3199\(91\)90137-8](https://doi.org/10.1016/0360-3199(91)90137-8)
 84. Burow LC, Woebken D, Debout BM, McMurdie PJ, Singer SW, Pett-Ridge J, Prufert-Bebout L, Spormann AM, Weber PK, Hoehler TM. 2012. Hydrogen production in photosynthetic microbial mats in the Elkhorn slough estuary, Monterey Bay. *ISME J* 6:863–874. <https://doi.org/10.1038/ismej.2011.142>
 85. Cai C, Li L, Hua Y, Liu H, Dai X. 2021. Ferroferric oxide promotes metabolism in anaerolineae other than microbial syntrophy in anaerobic methanogenesis of antibiotic fermentation residue. *Sci Total Environ* 758:143601. <https://doi.org/10.1016/j.scitotenv.2020.143601>
 86. Bala S, Garg D, Thirumalesh BV, Sharma M, Sridhar K, Inbaraj BS, Tripathi M. 2022. Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. *Toxics* 10:484. <https://doi.org/10.3390/toxics10080484>
 87. Arutchelvan V, Kanakasabai V, Elangovan R, Nagarajan S, Muralikrishnan V. 2006. Kinetics of high strength phenol degradation using *Bacillus brevis*. *J Hazard Mater* 129:216–222. <https://doi.org/10.1016/j.jhazmat.2005.08.040>
 88. Huang Z, Wang P, Li H, Lin K, Lu Z, Guo X, Liu Y. 2014. Community analysis and metabolic pathway of halophilic bacteria for phenol degradation in saline environment. *Intern Bio Biod* 94:115–120. <https://doi.org/10.1016/j.ibiod.2014.07.003>
 89. Sánchez-González M, Álvarez-Urbe H, Rivera-Solís R, González-Burgos A, Escalante-Réndiz D, Rojas-Herrera R. 2018. Analysis of a phenol-adapted microbial community: degradation capacity, taxonomy and metabolic description. *J Appl Microbiol* 126:771–779.
 90. Li Q, Huang Y, Wen D, Fu R, Feng L. 2020. Application of alkyl polyglycosides for enhanced bioremediation of petroleum hydrocarbon-contaminated soil using *Sphingomonas changbaiensis* and *Pseudomonas stutzeri*. *Sci Total Environ* 719:137456. <https://doi.org/10.1016/j.scitotenv.2020.137456>
 91. Méndez V, Fuentes S, Morgante V, Hernández M, González M, Moore E, Seeger M. 2017. Novel hydrocarbonoclastic metal-tolerant *Acinetobacter* and *Pseudomonas* strains from Aconcagua river oil-polluted soil. *J Soil Sci Plant Nutr* 17:1074–1087. <https://doi.org/10.4067/S0718-95162017000400017>
 92. Silva A, Delerue-Matos C, Figueiredo SA, Freitas OM. 2019. The use of algae and fungi for removal of pharmaceuticals by bioremediation and Biosorption processes: a review. *Water* 11:1555. <https://doi.org/10.3390/w11081555>
 93. Zhang H, Yang L, Li Y, Wang C, Zhang W, Wang L, Niu L. 2022. Pollution gradients shape the co-occurrence networks and interactions of sedimentary bacterial communities in Taihu lake, a shallow eutrophic lake. *J Environ Man* 305:114380. <https://doi.org/10.1016/j.jenvman.2021.114380>
 94. Zanolli G, Balloi A, Negroni A, Borruso L, Daffonchio D, Fava F. 2012. A *Chloroflexi* bacterium dechlorinates polychlorinated biphenyls in marine sediments under *in situ*-like biogeochemical conditions. *J Hazard Mater* 209–210:449–457. <https://doi.org/10.1016/j.jhazmat.2012.01.042>
 95. Carpenter DO. 2006. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. *Rev Environ Health* 21:1–23. <https://doi.org/10.1515/revhe.2006.21.1.1>
 96. Padilla-Crespo E, Yan J, Swift C, Wagner DD, Chourey K, Hettich RL, Ritalahti KM, Löffler FE. 2014. Identification and environmental distribution of *dcpA*, which encodes the reductive dehalogenase catalyzing the dichloroelimination of 1,2-dichloropropane to propene in organohalide-respiring *Chloroflexi*. *Appl Environ Microbiol* 80:808–818. <https://doi.org/10.1128/AEM.02927-13>
 97. Yan J, Rash BA, Rainey FA, Moe WM. 2009. “Detection and quantification of *Dehalogenimonas* and “*Dehalococcoides*” populations via PCR-based protocols targeting 16S rRNA genes”. *Appl Environ Microbiol* 75:7560–7564. <https://doi.org/10.1128/AEM.01938-09>
 98. Govindarajan A, Crum M, Adolacion J, Kiaghadi A, Acuña-Gonzalez E, Rifai HS, Willson RC. 2022. Sediment and their bacterial communities in an industrialized estuary after hurricane harvey. *Mar Pollut Bull* 175:113359. <https://doi.org/10.1016/j.marpolbul.2022.113359>
 99. Sakshi Haritash AK. 2020. A comprehensive review of metabolic and genomic aspects of PAH-degradation. *Arch Microbiol* 202:2033–2058. <https://doi.org/10.1007/s00203-020-01929-5>
 100. Lu J, Guo Z, Pan Y, Li M, Chen X, He M, Wu H, Zhang J. 2022. Simultaneously enhanced removal of PAHs and nitrogen driven by Fe²⁺/Fe³⁺ Cycle in constructed Wetland through automatic tidal operation. *Water Res* 215:118232. <https://doi.org/10.1016/j.watres.2022.118232>
 101. Kiersztyn B, Chróst R, Miłobędzka A. 2023. Homogenisation and dilution in metabolic evaluation of activated sludge rich in *Chloroflexi*. *Int J Environ Sci Technol* 20:3295–3308. <https://doi.org/10.1007/s13762-022-04191-y>
 102. Speirs LBM, Rice DTF, Petrovski S, Seviour RJ. 2019. The phylogeny, biodiversity, and ecology of the *Chloroflexi* in activated sludge. *Front Microbiol* 10:2015. <https://doi.org/10.3389/fmicb.2019.02015>
 103. Xiao X, You S, Guo H, Ma F, Zhang J, Zhang R, Bao X. 2021. Mycelial pellets for alleviation of membrane fouling in membrane bioreactor. *J Membrane Sci* 635:119545. <https://doi.org/10.1016/j.memsci.2021.119545>
 104. Miura Y, Watanabe Y, Okabe S. 2007. Significance of *Chloroflexi* in performance of submerged membrane bioreactors (MBR) treating municipal wastewater. *Environ Sci Technol* 41:7787–7794. <https://doi.org/10.1021/es071263x>
 105. Lv Y, Pan J, Huo T, Li J, Liu S. 2020. Enhance the treatment of low strength wastewater at low temperature with the coexistence system of Anaerobic and heterotrophic bacteria: performance and bacterial community. *Sci Total Environ* 714:136799. <https://doi.org/10.1016/j.scitotenv.2020.136799>
 106. Feng J, Zhang Q, Tan B, Li M, Peng H, He J, Zhang Y, Su J. 2022. Microbial community and metabolic characteristics evaluation in start-up stage of electro-enhanced SBR for aniline wastewater treatment. *J Water Pro Eng* 45:102489. <https://doi.org/10.1016/j.jwpe.2021.102489>
 107. Tian H, Liu J, Feng T, Li H, Wu X, Li B. 2017. Assessing the performance and microbial structure of biofilms adhering on aerated membranes for domestic saline sewage treatment. *RSC Adv* 7:27198–27205. <https://doi.org/10.1039/C7RA03755D>
 108. Wang Q, Cao Z, Liu Q, Zhang J, Hu Y, Zhang J, Xu W, Kong Q, Yuan X, Chen QF. 2019. Enhancement of COD removal in constructed wetlands treating saline wastewater: intertidal Wetland sediment as a novel inoculation. *J Environ Manage* 249:109398. <https://doi.org/10.1016/j.jenvman.2019.109398>
 109. Wang S, Yu H, Su Q, Zuo J. 2021. Exploring the role of heterotrophs in partial nitrification-anammox process treating thermal hydrolysis process - anaerobic digestion reject water. *Bio Tech* 341:125762. <https://doi.org/10.1016/j.biortech.2021.125762>
 110. Feng H, Wang Y, Zhang X, Shen D, Li N, Chen W, Huang B, Liang Y, Zhou Y. 2017. Degradation of p-fluoronitrobenzene in biological and bioelectrochemical systems: differences in kinetics, pathways, and microbial community evolutions. *Chem Eng J* 314:232–239. <https://doi.org/10.1016/j.cej.2016.12.097>
 111. Wang Y, Tian H, Huang F, Long W, Zhang Q, Wang J, Zhu Y, Wu X, Chen G, Zhao L, Bakken LR, Frostegård Å, Zhang X. 2017. Time-resolved analysis of a denitrifying bacterial community revealed a core microbiome responsible for the anaerobic degradation of quinoline. *Sci Rep* 7:14778. <https://doi.org/10.1038/s41598-017-15122-0>
 112. Zhang Y, Ji Z, Pei Y. 2021. Nutrient removal and microbial community structure in an artificial-natural coupled wetland system. *Process Saf*

- Environmental Prot 147:1160–1170. <https://doi.org/10.1016/j.psep.2021.01.036>
113. Garaiyurrebaso O, Garbisu C, Blanco F, Lanzén A, Martín I, Epelde L, Becerril JM, Jechalke S, Smalla K, Grohmann E, Alkorta I. 2017. Long-term effects of aided phytostabilisation on microbial communities of metal-contaminated mine soil. *FEMS Microbiol Ecol* 93:fw252. <https://doi.org/10.1093/femsec/fw252>
 114. Rastogi G, Barua S, Sani RK, Peyton BM. 2011. Investigation of microbial populations in the extremely metal-contaminated Coeur d'Alene river sediments. *Microb Ecol* 62:1–13. <https://doi.org/10.1007/s00248-011-9810-2>
 115. Huang J, Zhu C, Kong Y, Cao X, Zhu L, Zhang Y, Ning Y, Tian W, Zhang H, Yu Y, Zhang J. 2022. Biochar application alleviated rice salt stress via modifying soil properties and regulating soil bacterial abundance and community structure. *Agronomy* 12:409. <https://doi.org/10.3390/agronomy12020409>
 116. Chen L, Li K, Shang J, Wu Y, Chen T, Wanyan Y, Wang E, Tian C, Chen W, Chen W, Mi G, Sui X. 2021. Plant growth-promoting bacteria improve maize growth through reshaping the rhizobacterial community in low-nitrogen and low-phosphorus soil. *Biol Fertil Soils* 57:1075–1088. <https://doi.org/10.1007/s00374-021-01598-6>
 117. Bijada S, Merzouki M, Dëmčenko T, Vasiliauskiene D, Marčiulaitienė E, Vasarevičius S, Urbonavičius J. 2022. The effect of feedstock concentration on the microbial community dynamics during textile waste composting. *Front Ecol Evol* 10:813488. <https://doi.org/10.3389/fevo.2022.813488>
 118. Banda DM, Pereira JH, Liu AK, Orr DJ, Hammel M, He C, Parry MAJ, Carmo-Silva E, Adams PD, Banfield JF, Shih PM. 2020. Novel bacterial clade reveals origin of form I Rubisco. *Nat. Plants* 6:1158–1166. <https://doi.org/10.1038/s41477-020-00762-4>
 119. Madueño L, Starevich VA, Agnello AC, Coppotelli BM, Laprida C, Vidal NC, Di Marco P, Oneto ME, Del Panno MT, Morelli IS. 2021. Assessment of biological contribution to natural recovery of anthropized freshwater sediments from Argentina: autochthonous microbiome structure and functional prediction. *Front Microbiol* 12:601705. <https://doi.org/10.3389/fmicb.2021.601705>
 120. Ward LM, Shih PM, Hemp J, Kakegawa T, Fischer WW, McGlynn SE. 2019. Genomic evidence for phototrophic oxidation of small alkanes in a member of the *Chloroflexi* Phylum. *bioRxiv*. <https://doi.org/10.1101/531582>
 121. Ward LM, Shih PM, Hemp J, Kakegawa T, Fischer WW, McGlynn SE. 2019. Phototrophic methane oxidation in a member of the *Chloroflexi* phylum. *bioRxiv*. 531582.
 122. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Levilinea saccharolytica* KIBI-1, a member of the *Chloroflexi* class *Anaerolineae*. *Genome Announc* 3:24–25. <https://doi.org/10.1128/genomeA.01357-15>
 123. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Ardenticatena maritima* 110S, a thermophilic nitrate- and iron-reducing member of the *Chloroflexi* class *Ardenticatena*. *Genome Announc* 3:110–111. <https://doi.org/10.1128/genomeA.01347-15>
 124. González-Blanco G, Casas-Reyes A, Velasco-Garduño O, Ruiz-Gómez ML, Aguirre-Garrido JF, Beristain-Cardoso R. 2021. simultaneous nitrification/denitrification and desulfurization of wastewater polluted with ammonium, COD and sulfide: effectiveness of a new up-flow vertical hybrid reactor. *3 Biotech* 11:123. <https://doi.org/10.1007/s13205-021-02671-5>
 125. Ward LM, Idei A, Nakagawa M, Ueno Y, Fischer WW, McGlynn SE. 2019. Geochemical and metagenomic characterization of jinata onsen, a proterozoic-analog hot spring, reveals novel microbial diversity including iron-tolerant phototrophs and thermophilic lithotrophs. *Microbes Environ* 34:278–292. <https://doi.org/10.1264/jsme2.ME19017>
 126. Islam ZF, Cordero PRF, Feng J, Chen Y-J, Bay SK, Jirapanjawan T, Gleadow RM, Carere CR, Stott MB, Chiri E, Greening C. 2019. Two *Chloroflexi* classes independently evolved the ability to persist on atmospheric hydrogen and carbon monoxide. *ISME J* 13:1801–1813. <https://doi.org/10.1038/s41396-019-0393-0>
 127. Kanno N, Haruta S, Hanada S. 2019. Sulfide-dependent photoautotrophy in the filamentous anoxygenic phototrophic bacterium, *Chloroflexus aggregans*. *Microbes Environ* 34:304–309. <https://doi.org/10.1264/jsme2.ME19008>
 128. Kawai S, Nishihara A, Matsuura K, Haruta S. 2019. Hydrogen-dependent autotrophic growth in phototrophic and chemolithotrophic cultures of thermophilic bacteria, *Chloroflexus aggregans* and *Chloroflexus aurantiacus*, isolated from Nakabusa hot springs. *FEMS Microbiol Lett* 366:fnz122. <https://doi.org/10.1093/femle/fnz122>
 129. Rahman A, Susmi TF, Yasmin F, Karim ME, Hossain MU. 2020. Functional annotation of an ecologically important protein from *Chloroflexus aurantiacus* involved in polyhydroxyalkanoates (PHA) biosynthetic pathway. *SN Appl Sci* 2:1810. <https://doi.org/10.1007/s42452-020-03598-x>
 130. Izaki K, Haruta S. 2020. Aerobic production of bacteriochlorophylls in the filamentous anoxygenic photosynthetic bacterium, *Chloroflexus aurantiacus* in the light. *Microbes Environ* 35:ME20015. <https://doi.org/10.1264/jsme2.ME20015>
 131. Almeida J.R., Serrano E, Fernandez M, Fradinho JC, Oehmen A, Reis MAM. 2021. Polyhydroxyalkanoates production from fermented domestic wastewater using phototrophic mixed cultures. *Wat Res* 197:117101. <https://doi.org/10.1016/j.watres.2021.117101>
 132. Almeida Juliana R., Fradinho JC, Carvalho G, Oehmen A, Reis MAM. 2022. Dynamics of microbial communities in phototrophic polyhydroxyalkanoate accumulating cultures. *Microorganisms* 10:351. <https://doi.org/10.3390/microorganisms10020351>
 133. Fradinho J, Allegue LD, Ventura M, Meler JA, Reis MAM, Puyol D. 2021. Up-scale challenges on biopolymer production from waste streams by purple phototrophic bacteria mixed cultures: a critical review. *Bioresour Technol* 327:124820. <https://doi.org/10.1016/j.biortech.2021.124820>
 134. Afreen R, Tyagi S, Singh GP, Singh M. 2021. Challenges and perspectives of polyhydroxyalkanoate production from microalgae/cyanobacteria and bacteria as microbial factories: an assessment of hybrid biological system. *Front Bioeng Biotechnol* 9:624885. <https://doi.org/10.3389/fbioe.2021.624885>
 135. Ángeles R, Arnaiz E, Gutiérrez J, Muñoz R, Lebrero R. 2021. Biogas-based production of glycogen by *Nostoc muscorum*: assessing the potential of transforming CO₂ into value added products. *Chemosphere* 275:129885. <https://doi.org/10.1016/j.chemosphere.2021.129885>
 136. Higuchi-Takeuchi M, Morisaki K, Toyooka K, Numata K. 2016. Synthesis of high-molecular-weight polyhydroxyalkanoates by marine photosynthetic purple bacteria. *PLoS One* 11:e0160981. <https://doi.org/10.1371/journal.pone.0160981>
 137. Higuchi-Takeuchi M, Morisaki K, Numata K. 2016. A screening method for the isolation of polyhydroxyalkanoate-producing purple non-sulfur photosynthetic bacteria from natural seawater. *Front Microbiol* 7:1509. <https://doi.org/10.3389/fmicb.2016.01509>
 138. Arias DM, Fradinho JC, Uggetti E, García J, Oehmen A, Reis MAM. 2018. Polymer accumulation in mixed cyanobacterial cultures selected under the feast and famine strategy. *Algal Res* 33:99–108. <https://doi.org/10.1016/j.algal.2018.04.027>
 139. Fradinho JC, Carvalho VCF, Reis MAM. 2021. New Phototrophic factories for resource recovery, p 413–438. In Moura JJG, Moura I, Maia LB (ed), *Enzymes for solving humankind's problems: Natural and artificial systems in health, agriculture, environment and energy*. Springer.
 140. Shih PM, Ward LM, Fischer WW. 2017. Evolution of the 3-hydroxypropionate bicycle and recent transfer of anoxygenic photosynthesis into the *Chloroflexi*. *Proc Natl Acad Sci U S A* 114:10749–10754. <https://doi.org/10.1073/pnas.1710798114>