Adv Biochem Eng Biotechnol (2016) 156: 139–154
DOI: 10.1007/10_2015_5006

Published online: 24 February 2016

Biotechnology of Anoxygenic Phototrophic Bacteria

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Abstract Anoxygenic phototrophic bacteria are a diverse collection of organisms that are defined by their ability to grow using energy from light without evolving oxygen. The dominant groups are purple sulfur bacteria, purple nonsulfur bacteria, green sulfur bacteria, and green and red filamentous anoxygenic phototrophic bacteria. They represent several bacterial phyla but they all have bacteriochlorophylls and carotenoids and photochemical reaction centers which generate ATP and cellular reductants used for CO_2 fixation. They typically have an anaerobic lifestyle in the light, although some grow aerobically in the dark. Some of them oxidize inorganic sulfur compounds for light-dependent CO_2 fixation; this ability can be exploited for photobiological removal of hydrogen sulfide from wastewater and biogas. The anoxygenic phototrophic bacteria also perform bioremediation of recalcitrant dyes, pesticides, and heavy metals under anaerobic conditions. Finally, these organisms may be useful for overexpression of membrane proteins and photobiological production of H_2 and other valuable compounds.

Keywords Biogas, Bioremediation, Carotenoids, Green sulfur bacteria, Hydrogen sulfide, Membrane proteins, Photosynthetic bacteria, Purple bacteria

Contents

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Abbreviations

BChl	Bacteriochlorophyll
E_0'	Standard reduction potential at pH 7 and 25 °C
EPS	Extracellular polymeric substances
FAP	Filamentous anoxygenic phototrophs
GSB	Green sulfur bacteria
PBR	Photobioreactor
PNSB	Purple nonsulfur bacteria
PSB	Purple sulfur bacteria

1 Introduction

Phototrophic organisms are defined by their ability to convert light energy into chemical energy in forms useful for growth and other metabolic processes [1–3]. Cyanobacteria and microalgae are the only phototrophic microorganisms that evolve O_2 as a result of this process. This is because they have an enzyme complex capable of oxidizing water to oxygen ($H_2O \rightarrow 2H^+ + ½O_2 + 2e^-$) and donating the electrons to a type II photochemical reaction center. These organisms are therefore oxygenic (i.e., oxygen-evolving) phototrophs. Phototrophs that do not oxidize water may, for example, oxidize hydrogen sulfide ($H_2S \rightarrow 2H^+ + S + 2e^-$) and donate the electrons to the photochemical reaction center in the organism. These organisms are therefore anoxygenic (i.e., not oxygen-evolving) phototrophs. In either case, these electron transfer reactions generate a transmembrane proton motive force used for ATP generation and other physiological purposes. The reductants generated by the reaction center are also used for biosynthetic purposes and cyclic electron transfer around the reaction centers to generate even more proton motive force.

The vast majority of anoxygenic phototrophic bacteria grow phototrophically only under anaerobic conditions [4]. Some may be capable of chemotrophic growth under aerobic conditions in the dark (typical of purple bacteria), but others are obligate anaerobic and are killed by O_2 (typical of green sulfur bacteria; GSB). Anoxygenic phototrophic bacteria rarely form visibly large accumulations in natural environments because of their anaerobic lifestyle, but occasionally various

purple bacteria may bloom and color ponds, coastal areas, and wastewater reservoirs red, purple, or reddish-brown.

Anoxygenic phototrophic bacteria may not be as extensively used for biotechnological applications as chemotrophic microorganisms. However, given their diversity and the rising interest in biological solutions to societal challenges, more applications should be explored. For example, the extensive intracellular membrane systems in purple bacteria may have practical applications (Sect. 8). Another underexplored area is the potential for production of proteins and metabolites that are O_2 sensitive. In the following, a survey is made of biotechnological applications of the anoxygenic phototrophic bacteria. The bioremediating properties of oxygenic and anoxygenic photosynthetic bacteria were recently reviewed [5].

1.1 Phototrophy and Photosynthesis

There are only two principal sources of energy for living organisms: energy from light (phototrophy) or energy from chemical compounds (chemotrophy) [3]. Photosynthesis usually refers specifically to photoautotrophy, i.e., growth based on CO_2 fixation where the required energy is derived from light. Thus, all photosynthetic organisms are phototrophic but not all phototrophic organisms are photosynthetic. The principal cellular component that allows phototrophy is the photochemical reaction center [1]. This is a large enzyme complex that is always membrane-bound and has the ability to convert excitation energy obtained from light into chemical energy by creating a light-induced charge separation. The chemical energy is used for generation of strong reductants and ATP that are used in CO_2 fixation, biosynthetic pathways, and other cellular activities (Fig. 1). There are only two types of photochemical reaction centers: type I and type II. Type I (also called iron-sulfur type) has a relatively low redox potential and reduces soluble, cytoplasmic ferredoxins with E_0' values between −0.5 and −0.6 V. Type II (also called quinone-type) has a relatively high redox potential and reduces membrane-bound isoprenoid quinones with E_0' values between +0.1 and −0.1 V. The oxygenic phototrophs (cyanobacteria, algae, and plants) have both types of reaction centers (known as photosystems I and II) whereas the anoxygenic phototrophic bacteria have only either type I or type II. Light-harvesting pigment-protein antenna complexes are associated with the reaction centers to increase the amount of light energy harvested by the cells [1, 2]. The excitation energy in the antenna pigments is channeled to the photochemical reaction center in the order of picoseconds. There is a large diversity of evolutionary unrelated light-harvesting antenna complexes, but common to most of them is the presence of chlorophylls or bacteriochlorophylls and carotenoids. Most of the pigmentation in phototrophic bacteria (>99%) is found in these light-harvesting antenna complexes.

In principle, phototrophy can also be supported by alternative enzymes: the rhodopsin-like proteins [2, 3]. These membrane-bound enzymes absorb light energy and, as a result, transfer protons across the membrane, thereby conserving the light energy as a transmembrane electrochemical proton gradient. Rhodopsins

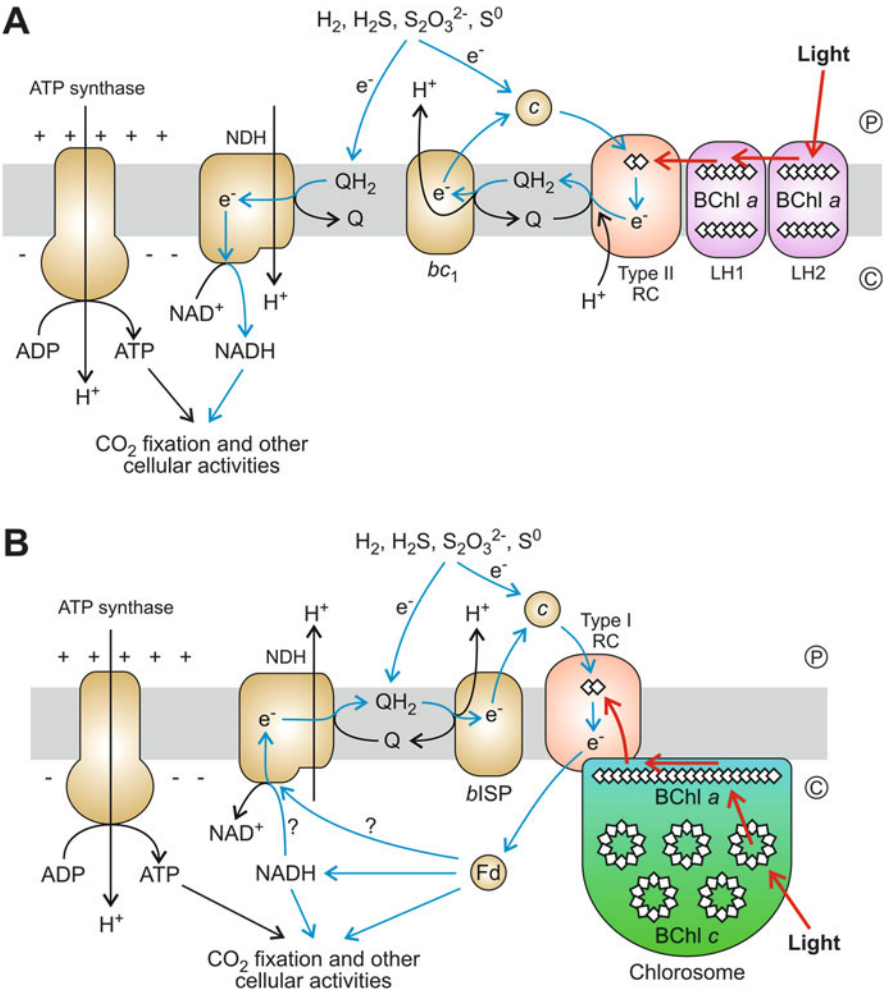

Fig. 1 Simplified models of the photosynthetic machinery in (**a**) purple bacteria and (**b**) green sulfur bacteria (GSB) during photoautotrophic growth on inorganic sulfur compounds. Light is harvested by the peripheral antennae (LH1, LH2, or chlorosomes) and the excitation energy is transferred to the reaction center (RC). Light-driven cyclic electron transfer occurs when electrons are continuously transferred between the reaction center and the membrane-bound cytochrome bc_1/*b*ISP in a light-dependent manner. This electron transfer generates a proton motive force, which fuels other physiological processes such as ATP synthesis. NADH is generated by reverse electron flow in purple bacteria under photoautotrophic growth conditions. If suitable organic substrates are available for photoheterotrophic growth in purple bacteria, NADH for cellular activities is instead obtained by oxidation of these organic substrates and the photochemical system may function exclusively in a cyclic mode to produce ATP. For details on oxidation of sulfur compounds (H_2S, $S_2O_3^{2-}$, and S^0) see Fig. 2. Pathways that carry electrons for cyclic electron transfer, CO_2 fixation, and other cellular activities are shown with *blue arrows*. Transfer of light and excitation energy is shown with *red arrows*. bc_1 cytochrome bc_1 complex, *BChl* bacteriochlorophyll, *b*ISP cytochrome *b* iron-sulfur protein, *C* (*circled*) cytoplasm, *Fd* ferredoxin, *NDH* NADH:quinone oxidoreductase, *P* (*circled*) periplasm, *Q* isoprenoid quinone (oxidized), *RC* photochemical reaction center. Modified from [1, 2]

do not transfer electrons and are not associated with large light-harvesting antennae as are photochemical reactions centers. Rhodopsins are well-known components of halophilic archaea where they support a phototrophic lifestyle under anaerobic conditions. Rhodopsin-like enzymes have recently been found in numerous very different marine microbes (archaea, bacteria, and eukaryotes) but the exact contribution to cell physiology is not always clear in these organisms. Phototrophy based on rhodopsins is not considered further here.

1.2 Types of Anoxygenic Phototrophic Bacteria

Purple bacteria belong to the phylum Proteobacteria and are characterized by having type II photochemical reaction centers and bacteriochlorophyll (BChl) *a* or BChl *b* as the major pigment [4, 6]. The light-harvesting (LH) antennae known as LH1 and LH2 are located in the cytoplasmic and intracytoplasmic membranes. As a group, the purple bacteria are very physiologically versatile and may grow photoautotrophically, photoheterotrophically, or chemotrophically in the dark by fermentation or by aerobic or anaerobic respiration. A subgroup of the purple bacteria, the purple sulfur bacteria (PSB), is characterized by a predominant photoautotrophic lifestyle where inorganic sulfur compounds such as sulfide serve as electron donors for photosynthesis. The other major subgroup of purple bacteria, known collectively as purple nonsulfur bacteria (PNSB), is characterized by a predominantly photoheterotrophic lifestyle where many organic compounds can be assimilated and these organisms are not as tolerant to sulfide as the PSB. PSB are Gammaproteobacteria whereas PNSB are Alfaproteobacteria and Betaproteobacteria.

GSB belong to the phylum Chlorobi and are characterized by having BChl *c*, *d*, or *e* organized into large light-harvesting organelles known as chlorosomes [4]. These organisms contain type I reaction centers and typically oxidize sulfur compounds for CO_2 fixation. Compared to the purple bacteria, the GSB have much more restricted growth requirements: they are strict anaerobic, obligate phototrophic, and obligate autotrophic.

Filamentous anoxygenic phototrophic (FAP) bacteria belong to the phylum Chloroflexi and are characterized by having BChl *a* organized into type II reaction centers and light-harvesting antennae similar to those found in purple bacteria [4]. There are two kinds of FAPs: the red FAPs have BChl *a* as the sole chlorophyll-like pigment, whereas the green FAPs, in addition to BChl *a*, also have BChl *c* or *d* organized in chlorosome structures similar to those found in GSB. The green FAPs are also known as green nonsulfur bacteria.

The only other groups of anoxygenic phototrophic bacteria known are the Heliobacteria, Chloracidobacteria, and the recently discovered Gemmamonatiales [7]. There are no known archaea that contain photochemical reaction centers.

Cyanobacteria are oxygenic phototrophs [8]. However, a few cyanobacteria are capable of anoxygenic photosynthesis and an anaerobic phototrophic lifestyle

where the organisms consume hydrogen sulfide as electron donor for photosynthesis, very similar to the phototrophic GSB and PSB described above. Microalgae are also oxygenic phototrophs but are not known to grow phototrophically under anaerobic conditions. However, this does not exclude the possibility that some microalgae have light-independent lifestyles under anaerobic conditions or in the dark.

1.3 Occurrence

In general, anoxygenic phototrophic bacteria thrive where anaerobic conditions intersect with light [4]. In natural environments these conditions occur in lakes and sediments, often in narrow bands limited by the extent of light penetration. If sulfide is present, typically either PSB or GSB or both accumulate. Purple bacteria in general are physiologically versatile and thrive in many man-made polluted environments such as wastewater lagoons where light coincides with anaerobic or microaerobic conditions and high loads of organic compounds.

1.4 Sulfur Metabolism and Sulfide Tolerance

Reduced inorganic sulfur compounds are abundant in anaerobic environments because of the degradation of sulfur-containing organic materials and H_2S production by sulfate-reducing bacteria. These compounds are oxidized by various chemotrophic bacteria (often collectively denoted "colorless sulfur bacteria") under aerobic conditions or by phototrophic bacteria under anaerobic conditions. The metabolism of sulfur compound oxidation in phototrophic bacteria is complex and not fully elucidated (Fig. 2) [9]. Sulfide and thiosulfate are often incompletely oxidized to elemental sulfur if the sulfide and thiosulfate are supplied in excess. This elemental sulfur is deposited outside or inside the cells as sulfur globules with diameters ranging from very small up to 2 μm. Intracellular sulfur globules are found in PSB of the family *Chromatiaceae* and extracellular sulfur globules are found in GSB, PNSB, and most PSB of the family *Ectothiorhodospiraceae*. Upon depletion of sulfide and other electron sources, the sulfur globules are oxidized completely to sulfate.

Although sulfide is metabolized by most, if not all, phototrophic bacteria, the levels of tolerance vary significantly [9]. Sulfide concentrations above 15–30 mg/L H_2S (0.5–1 mM) tend to inhibit the PNSB and favor the PSB and GSB. The highest sulfide concentrations tolerated by most PSB and GSB are about 100–150 mg/L H_2S (about 3–5 mM), where the GSB typically are the most tolerant. In extreme cases, up to 375 mg/L H_2S (11 mM) is tolerated.

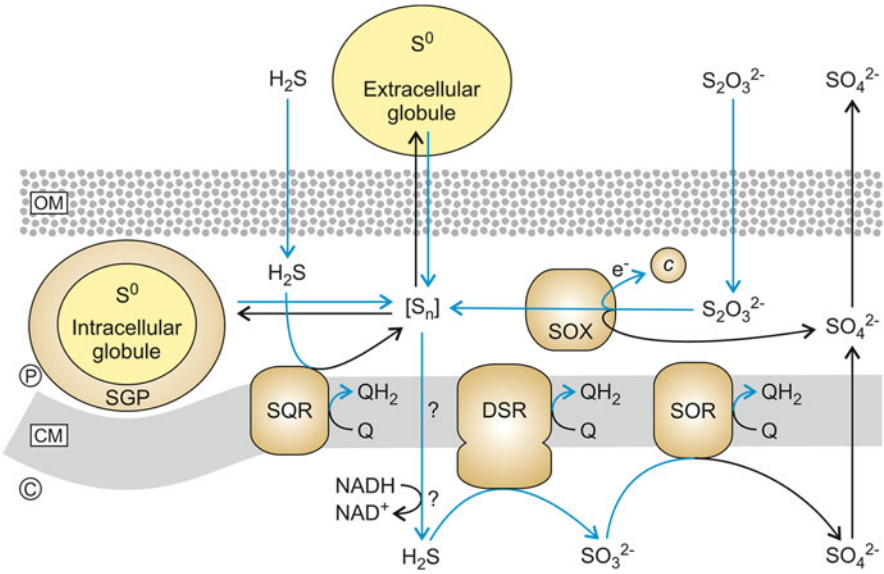

Fig. 2 Simplified model of the oxidative sulfur metabolism in phototrophic sulfur bacteria. Sulfur globules are shown in *yellow*. Intracellular sulfur globules in purple sulfur bacteria (PSB) are located in the periplasm and are associated with sulfur globule proteins (SGP). Pathways that carry electrons towards cellular electron carriers (isoprenoid quinones and cytochromes *c*) are shown in *blue*. *C* (*circled*) cytoplasm, *CM* (*boxed*) cytoplasmic membrane, *DSR* dissimilative sulfite reductase, *OM* (*boxed*) outer membrane, *P* (*circled*) periplasm, *Q* isoprenoid quinone (oxidized), *SGP* sulfur globule protein, [S_n] oligosulfide pool, *SOR* sulfite oxidoreductase (several types), *SOX* sulfur compound oxidizing system, *SQR* sulfide:quinone oxidoreductase. Modified from [9]

2 Removal of Hydrogen Sulfide from Wastewater Streams

Sulfide is a malodorous, corrosive, and toxic compound often present in liquid waste streams from domestic and industrial sources. The source is typically organically bound sulfur, which is liberated as sulfide or sulfate upon degradation, and inorganic sulfur (mostly sulfate), which is reduced to sulfide under anaerobic conditions by ubiquitous sulfate-reducing bacteria. Anoxygenic phototrophic bacteria offer an environmentally friendly biological approach to remove this sulfide [10]. Partial oxidation of H_2S to elemental sulfur (S^0) rather than complete oxidation to sulfate (SO_4^{2-}) is desirable for a number of reasons. (1) Elemental sulfur can be physically removed from the waste stream by sedimentation of the sulfur granules. This alleviates downstream problems with sulfate (such as reduction to sulfide by sulfate-reducing bacteria). In addition, this biogenic elemental sulfur has commercial value as fertilizer and chemical feedstock. (2) Oxidation of sulfide to elemental sulfur generates less H^+ than oxidation of sulfide to sulfate. This minimizes corrosion and the requirement for pH control. (3) Conversion to elemental sulfur requires less light than conversion to sulfate; this maximizes the sulfide removal per light input. For these reasons, conditions in the photobioreactor

(PBR) have to be carefully controlled (e.g., flow rate, light intensity) to avoid complete sulfide oxidation and to maximize the output of elemental sulfur. Alternatively, naturally occurring or genetically engineered strains of phototrophic sulfur bacteria could be used which are deficient in sulfate formation and only oxidize sulfide to elemental sulfur, regardless of the sulfide load [11].

For sulfide removal from waste streams, GSB have certain advantages over other anoxygenic phototrophic bacteria [10]. (1) GSB deposit elemental sulfur extracellularly, which enables recovery of the sulfur by sedimentation (most PSB accumulate the elemental sulfur intracellularly). (2) GSB oxidize more sulfide per light input than purple bacteria, which is important for practical design and economic reasons. (3) GSB have higher tolerance for sulfide and higher affinity for sulfide uptake than purple bacteria. (4) Sulfide uptake in purple bacteria is diminished when organic nutrients are available. For these reasons, if a defined microbial culture is to be used for photobiological sulfide removal, a GSB culture may be beneficial. In one such study, a pilot-scale fixed-film continuous-flow PBR (21 mL) with the GSB *Chlorobium limicola* strain DSM 257 was designed and successfully optimized for sulfide removal [12]. At loading rates of 111–286 mg/L/h sulfide, about 100% of the influent sulfide was consumed and 92–95% recovered as elemental sulfur [12].

In another pilot-scale study in Brazil, up-flow anaerobic PBRs were tested with a continuous flow of domestic wastewater containing 1–6 mg/L sulfide [13]. These systems relied on naturally developing microbial communities under the influence of natural light. Sulfide removal efficiencies reached 90% and the effluent contained less than 0.5 mg/L sulfide, which is below the national sulfide discharge standard in Brazil of 1 mg/L. Most of the sulfide was oxidized to elemental sulfur (S^0) although some sulfate (SO_4^{2-}) was also formed. The sulfide was oxidized by a mixed microbial community which was shown by molecular techniques to contain GSB, PSB of the *Chromatiaceae* lineage, and green FAPs of the *Chloronema* lineage. Sulfide removal by chemotrophic denitrification was unlikely to occur because the influent and effluent wastewater contained very little nitrate and nitrite, and microbes capable of preforming this process were not identified in the microbial community.

3 Removal of Hydrogen Sulfide from Gas Streams

Hydrogen sulfide is present in small amounts in biogas (typically 0.1–2%) and off-gases from wastewater treatment plants, for example. Besides being toxic and malodorous, H_2S in biogas causes corrosion and poisoning of the equipment using the biogas. Therefore, a number of chemical technologies are currently used to remove H_2S from biogas. A commercialized approach using chemotrophic sulfide-oxidizing bacteria in a fixed-film bioreactor under controlled oxygen conditions is also available [14]. Fixed-film or suspended-growth PBRs with anoxygenic phototrophic bacteria could be interesting alternatives for cost-effective H_2S

removal because of their ability to operate for long periods of time without requiring a biomass separation step and their ability to operate under high and variable sulfide loadings [14]. Illumination and design of the PBR is a key concern for practical application because light is rapidly attenuated in dense cell suspensions [15]. Studies suggest that illumination using light-emitting diodes (LED) appears to be a very cost-effective approach if the LED emission wavelength is matched to the pigment absorption by the cells [16].

A variety of reactor designs have been investigated which allow the influent gas to exchange H_2S with an aqueous medium containing suspended or immobilized sulfide-oxidizing bacteria. In one study, a continuous-stirred PBR with *Chlorobium thiosulfatophilum* was used to remove sulfide from a synthetic gas stream containing 2.5% H_2S [17]. At its optimum operation, the 1.25-L PBR consumed 118 mg/h H_2S with negligible formation of sulfate corresponding to a conversion rate of sulfide to elemental sulfur per PBR volume of about 100 mg/h/L H_2S. Another study with a larger PBR working volume (11.9 L) used a flat-panel gas-lift PBR design with a PBR thickness of 10 cm and LED illumination [16]. In this system, the sulfide conversion to elemental sulfur was only about 20 mg/h/L H_2S but the energy expenditure for illumination per amount of sulfide removed was the lowest reported in the literature because of the use of LED for illumination.

4 Degradation of Recalcitrant Dyes and Pesticides

Azo dyes are the most important group of synthetic colorants and are generally recalcitrant to biodegradation because of their xenobiotic nature. Degradation of azo dyes in wastewater streams is most efficient under anaerobic conditions because many anaerobic bacteria apparently have a broad-range ability to reduce the azo bond [18]. Azo dyes are decolorized by many PNSB and this degradation is dependent on the enzyme azoreductase [19, 20]. Pure culture studies with different isolates of *Rhodopseudomonas palustris* have shown that this organism efficiently decolorizes various azo dyes in concentrations of around 1 g/L dye [20, 21]. In these studies, decolorization and at least partial degradation of azo dyes occurred only under anaerobic conditions and in the light. In another study, different PNSB strains isolated from various water sources (*Rhodobacter adriaticus*, *Rhodobacter blasticus*, *Rhodobacter capsulatus*, *Rhodovulum strictum*, and *R. palustris*) decolorized up to 96% of the tested azo dyes after only 2 days of illuminated and anaerobic incubation [22].

To circumvent problems associated with wastewater treatment using suspended cultures of bacteria, Wang and colleagues constructed a PBR to treat azo dye-contaminated wastewater [23]. The naturally developed biofilm contained anoxygenic phototrophic bacteria related to the *Rhodopseudomonas*, *Rhodomicrobium*, and *Chlorobium* lineages. This PBR allowed the removal of most of the organic load and up to 90% of the azo dyes. Over a 30-day run, the predominant phototrophic bacteria in the biofilm changed from purple bacteria to a

mixture of purple and GSB, and finally to mainly GSB. During this period the decolorization of azo dye increased from 60% to 90%, suggesting that GSB may also be capable of efficiently decolorizing azo dyes.

Purple bacteria belonging to the *Rhodospirillum* and *Rhodopseudomonas* genera have also been investigated for use in anaerobic biodegradation of halogenated aromatic pollutants such as 3-chlorobenzoate [5, 24].

5 Removal of Toxic Metals and Radioisotopes

Microorganisms – dead or alive, free or immobilized – can be used to remove toxic metals and radioisotopes from the environment [25]. Among anoxygenic phototrophic bacteria, this ability has especially been studied in PNSB [5].

The PNSB *Rhodobacter sphaeroides* bioaccumulates heavy metals including cadmium (Cd), nickel (Ni), and lead (Pb) [26–28], and metals with radioactive isotopes, cesium (Cs) and strontium (Sr) [29]. In one study, *R. sphaeroides* strain S accumulated Cd^{2+} with a higher efficiency under aerobic-dark conditions (93%) than under anaerobic-light conditions (50%) over 7 days at an initial Cd^{2+} concentration of 5 mg/L [27]. However, the ability to bioaccumulate metal ions is dependent on the strain of the organism under investigation. *R. sphaeroides* strain SSI is a spontaneous mutant of *R. sphaeroides* strain S with an increased ability to produce extracellular polymeric substances (EPS) on the cell surface [29]. The SSI strain has been shown to remove efficiently toxic metals including cadmium, uranium (U), cobalt (Co), mercury (Hg), and chromium (Cr). The removal of these metals has been attributed to the high amount of EPS produced on the surface of strain SSI because the strong negative charges of EPS efficiently adsorb many different kinds of metal ions. In one experimental setup with immobilized cells of strain SSI and 5 mg/L of each Cs^{+} and Sr^{2+}, about 100% of the cesium and 50% of the strontium were removed in 3 days. Other strains of *R. sphaeroides* and other PNSB have also been shown to remove efficiently (>90%) copper (Cu^{2+}), zinc (Zn^{2+}), and Cd^{2+} from contaminated shrimp pond water [30].

Zinc consumption by live *R. capsulatus* strain B10 cells has been reported at levels of 164 mg Zn^{2+} per gram of cell dry weight [31]. This is among the highest zinc biosorption capacities reported for any microorganism and this suggests that *R. capsulatus* could be useful for zinc bioremediation. The unusually high zinc biosorption capacity was again mainly attributed to the physicochemical properties of the EPS on the cell surface.

6 Photobiological Production of Hydrogen

Hydrogen (H_2) has potential as an environmentally friendly fuel. Most, if not all, oxygenic as well as anoxygenic phototrophic microorganisms have the capacity to produce H_2 but the physiological mechanisms for H_2 production vary among these organisms [32]. Here, only H_2 production in anoxygenic phototrophic bacteria is considered.

Although H_2 production from phototrophic bacteria may not be economically viable by itself, H_2 could be a valuable byproduct from other application of these organisms such as wastewater or biogas cleanup [33]. Photobiological H_2 formation is catalyzed either by a hydrogenase ($2H^+ + 2e^- \leftrightarrows H_2$) or by nitrogenase as a byproduct from N_2 fixation ($8H^+ + N_2 + 8e^- \rightarrow H_2 + 2NH_3$). Most bacteria have the ability to re-uptake the produced H_2 so this ability may have to be eliminated if the H_2 production is to be useful.

In *Rhodospirillum rubrum* and other PNSB, photobiological H_2 production is primarily caused by nitrogenase and therefore H_2 production is induced under nitrogen limitation. Interestingly, if N_2 is completely removed (for example, by using an inert gas), nitrogenase produces exclusively H_2 and thus the H_2 productivity is increased. In a nitrogen-limited batch culture of *R. rubrum*, a continuous production of 0.48 L/day H_2 per gram cell dry weight was observed with lactate as electron donor [34]. *R. rubrum* and other purple bacteria also catalyze light-dependent H_2 production from CO with a net reaction corresponding to the water-gas shift reaction ($CO + H_2O \rightarrow H_2 + CO_2$) [35].

GSB also exhibit light- and nitrogenase-dependent H_2 production. In these bacteria, inorganic sulfur compounds (sulfide, sulfur, and thiosulfate) are electron donors for photosynthesis and H_2 production. Photobiological H_2 production from organic compounds using these bacteria was demonstrated in a syntrophic co-culture of the GSB *Chlorobium vibrioforme* and the acetate-oxidizing, sulfur-reducing bacterium *Desulfuromonas acetoxidans* [36]. In this co-culture, the chemotrophic partner oxidized acetate and reduced sulfur to H_2S, and the phototrophic partner oxidized H_2S back to sulfur and produced H_2 using nitrogenase. In this co-culture the productivity was 1.3 L/day H_2 per gram cell dry weight with acetate as electron donor.

An alternative approach to photobiological H_2 production in GSB might be engineered. In principle, the redox potentials of the type I photochemical reaction center (E_0' approx. −0.6 V) and the soluble ferredoxins (E_0' approx. −0.5 V) in GSB are low enough to allow reduction of H^+ to H_2 (E_0' −0.42 V). These strong cellular reductants are required for CO_2 fixation by the reductive TCA cycle found in GSB. However, this reducing power might also be used for photobiological H_2 production if a suitable hydrogenase could be inserted in these organisms that would accept electrons from the indigenous strong reductants. Then H_2 production would be independent of N_2 and nitrogenase.

7 Biosynthesis of Carotenoids and Other Terpenoids

Terpenoids (or isoprenoids) are compounds derived from one or more isoprene C_5 units and constitute a very large range of natural compounds. A prominent group is the carotenoids (C_{40} compounds) found in all phototrophic organisms and some chemotrophic bacteria, archaea, and fungi. Carotenoids are yellow, orange, and red pigments with broad applications in the food, feed, nutraceutical, cosmetic, and pharmaceutical industries because of their vibrant colors and health-promoting activities [37]. Most commercialized carotenoids, such as beta-carotene and astaxanthin, are produced in microalgae although genetically modified *Escherichia coli* and yeasts that produce commercially valuable carotenoids are also available [38]. Anoxygenic phototrophic bacteria naturally produce a range of carotenoids (such as okenone and lycopene derivatives) which may have commercial interest [37, 39] (Fig. 3).

The most important role of carotenoids in phototrophic organisms is in protection from light [40]. The detrimental effects of light are much more serious under aerobic conditions than under anaerobic conditions because excitation of (bacterio) chlorophylls under aerobic conditions causes formation of reactive oxygen species (ROS). Mechanistically this occurs by reaction of excited triplet-state chlorophyll (^{3}Chl*) with ground-state molecular oxygen (O_2), which results in formation of singlet oxygen ($^1O_2$). Singlet oxygen is extremely reactive and detrimental to the cell. Carotenoids quench ^{3}Chl* and thereby prevent formation of singlet oxygen. This means carotenoids cannot be completely removed from phototrophic organisms growing under aerobic conditions. However, phototrophic bacteria growing under anaerobic conditions do not have this requirement and therefore carotenoids are not essential for anoxygenic phototrophic bacteria. This in turn means the carotenoids of these bacteria in principle are freely available for biosynthetic engineering.

Genetic engineering has been used to synthesize the commercially valuable carotenoid lycopene in the purple non-sulfur bacterium *R. rubrum* [41]. Here, the indigenous carotenoid biosynthetic pathway was interrupted by targeted gene inactivation and as a result the cells accumulated lycopene as the sole carotenoid in a content of 2 mg/g cell dry weight. Although this is not high compared to the yield obtained in genetically engineered *E. coli* (33 mg/g cell dry weight lycopene as the sole carotenoid [42]), additional engineering of *R. rubrum* could surely increase the yield. Using a similar approach, lycopene and zeta-carotene have also been produced as the sole carotenoid species in GSB by genetic manipulation of *Chlorobaculum tepidum* [43]. Because carotenoids are not required in anaerobic phototrophic bacteria, the flux of isoprene precursors to carotenoid biosynthesis in these organisms could be redirected to any isoprenoid compound such as valuable plant-type terpenoids [44].

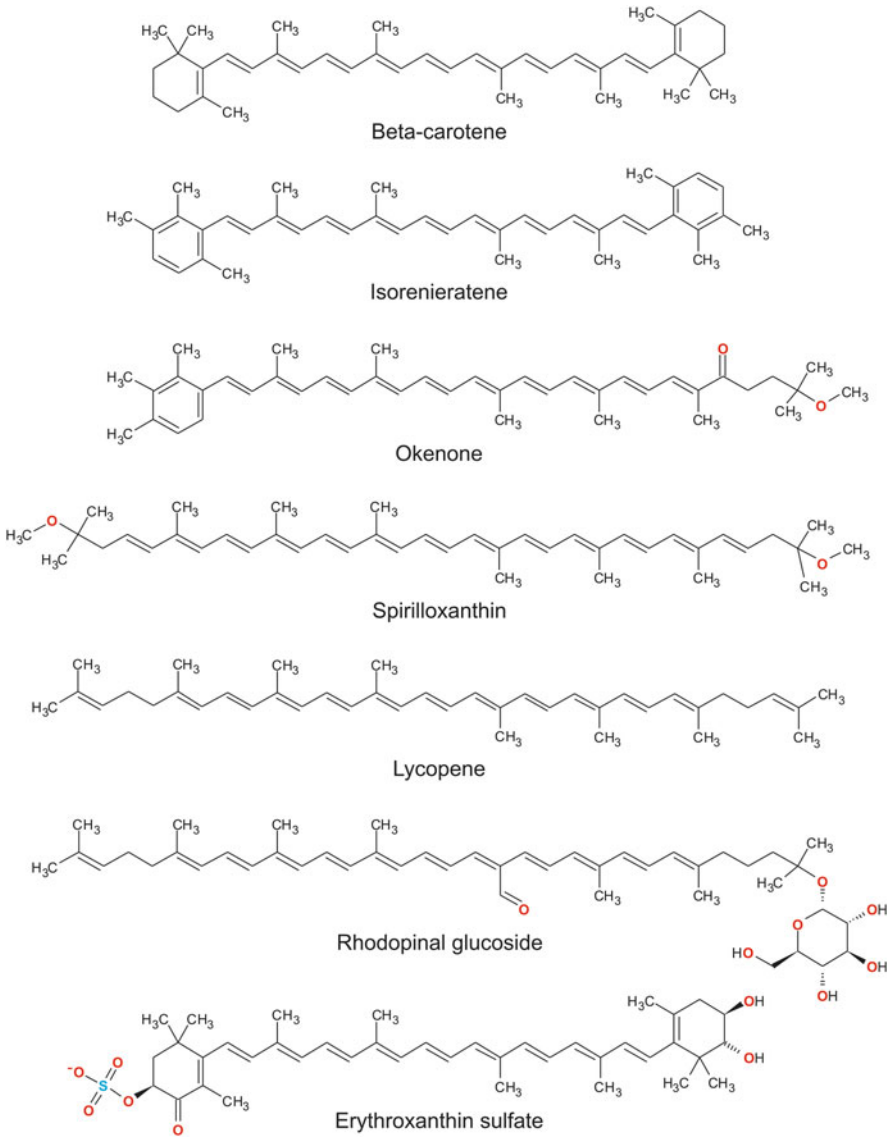

Fig. 3 Examples of carotenoids found in anoxygenic phototrophic bacteria. From [39] and Carotenoid Database (http://carotenoiddb.jp/)

8 Production of Functional Membrane Proteins

To study the structure and function of proteins, it is useful to overexpress the proteins in a foreign host organism to obtain amounts sufficient for experimentation. However, membrane proteins pose a challenge because they often denature in

the absence of a suitable membrane environment. For example, heterologous membrane proteins that are overexpressed in *E. coli* in a functional form are usually found in much lower titers than heterologous soluble proteins and tend to form inclusion bodies. To overcome this problem, the PNSB *Rba sphaeroides* has been developed as a host for overexpression of functional membrane proteins [45]. This system takes advantage of the large content of intracellular membranes in purple bacteria which hold the membrane-bound antennae and enzymes of the photochemical machinery. In *Rba. sphaeroides* these internal membranes form intracytoplasmic membrane (ICM) vesicles that sequester newly synthesized foreign proteins and enable easy purification following cell lysis. Using this system, challenging membrane proteins in a functional form have recently been prepared and examined: human aquaporin 9 (hAQP9), human tight junction protein occludin (Occ), *Rba. sphaeroides* cellulose synthase enzyme complex (BcsAB), and *Rba. capsulatus* cytochrome c_y [46].

References

1. Blankenship RE (2008) Molecular mechanisms of photosynthesis. Blackwell Science, Oxford, UK
2. Hohmann-Marriott MF, Blankenship RE (2011) Evolution of photosynthesis. Annu Rev Plant Biol 62:515–548
3. Bryant DA, Frigaard N-U (2006) Prokaryotic photosynthesis and phototrophy illuminated. Trends Microbiol 14(11):488–496
4. Blankenship RE, Madigan MT, Bauer CE (1995) Anoxygenic photosynthetic bacteria. Springer, The Netherlands
5. Idi A et al (2015) Photosynthetic bacteria: an eco-friendly and cheap tool for bioremediation. Rev Environ Sci Biotechnol 14(2):271–285
6. Hunter CN et al (2009) The purple phototrophic bacteria, Advances in photosynthesis and respiration. Springer, London
7. Zeng Y et al (2014) Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum *Gemmatimonadetes*. Proc Natl Acad Sci U S A 111(21):7795–7800
8. Cohen Y, Gurevitz M (2006) The cyanobacteria—ecology, physiology and molecular genetics. Prokaryotes 4:1074–1098
9. Frigaard N-U, Dahl C (2009) Sulfur metabolism in phototrophic sulfur bacteria. Adv Microb Physiol 54:103–200
10. Hurse TJ, Kappler U, Keller J (2008) Using anoxygenic photosynthetic bacteria for the removal of sulfide from wastewater. In: Sulfur metabolism in phototrophic organisms. Springer, Dordrecht, pp 437–460
11. Holkenbrink C et al (2011) Sulfur globule oxidation in green sulfur bacteria is dependent on the dissimilatory sulfite reductase system. Microbiology 157(Pt 4):1229–1239
12. Henshaw PF, Zhu W (2001) Biological conversion of hydrogen sulphide to elemental sulphur in a fixed-film continuous flow photo-reactor. Water Res 35(15):3605–3610
13. Garcia GP et al (2015) Biological sulphide removal from anaerobically treated domestic sewage: reactor performance and microbial community dynamics. Environ Technol 36 (17):2177–2189
14. Syed M et al (2006) Removal of hydrogen sulfide from gas streams using biological processes – a review. Can Biosyst Eng 48:2.1–2.14

15. An JY, Kim BW (2000) Biological desulfurization in an optical-fiber photobioreactor using an automatic sunlight collection system. J Biotechnol 80(1):35–44
16. Kim YJ, Kim BW, Chang HN (1996) Desulfurization in a plate-type gas-lift photobioreactor using light emitting diodes. Korean J Chem Eng 13(6):606–611
17. Basu R, Clausen EC, Gaddy JL (1996) Biological conversion of hydrogen sulfide into elemental sulfur. Environ Prog 15(4):234–238
18. Pandey A, Singh P, Iyengar L (2007) Bacterial decolorization and degradation of azo dyes. Int Biodeter Biodegr 59(2):73–84
19. Bin Y et al (2004) Expression and characteristics of the gene encoding azoreductase from *Rhodobacter sphaeroides* AS1.1737. FEMS Microbiol Lett 236(1):129–136
20. Liu G-f et al (2006) Bacterial decolorization of azo dyes by *Rhodopseudomonas palustris*. World J Microbiol Biotechnol 22(10):1069–1074
21. Wang X et al (2008) Biodecolorization and partial mineralization of Reactive Black 5 by a strain of *Rhodopseudomonas palustris*. J Environ Sci (China) 20(10):1218–1225
22. Kim TTH et al (2003) Decolorization of azo dyes by purple non-sulfur bacteria. In: Annual Report of FY 2002, The Core University Program between Japan Society for the Promotion of Science (JSPS) and National Centre for Natural Science and Technology (NCST). pp 112–118
23. Wang X et al (2015) Formation characteristics of an anoxygenic photosynthetic bacterial biofilm in a photorotating biological contactor for azo dye wastewater treatment. J Chem Technol Biotechnol 90(1):176–184
24. Mutharasaiah K, Govindareddy V, Karigar C (2010) Photobiodegradation of halogenated aromatic pollutants. Adv Biosci Biotechnol 01(03):238–240
25. Dixit R et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212
26. Seki H, Suzuki A, Mitsueda S-I (1998) Biosorption of heavy metal ions on *Rhodobacter sphaeroides* and *Alcaligenes eutrophus* H16. J Colloid Interface Sci 197(2):185–190
27. Watanabe M et al (2003) Biosorption of cadmium ions using a photosynthetic bacterium, *Rhodobacter sphaeroides* S and a marine photosynthetic bacterium, *Rhodovulum* sp. and their biosorption kinetics. J Biosci Bioeng 95(4):374–378
28. Buccolieri A et al (2006) Testing the photosynthetic bacterium *Rhodobacter sphaeroides* as heavy metal removal tool. Ann Chim 96(3–4):195–203
29. Sasaki K et al (2013) Simultaneous removal of cesium and strontium using a photosynthetic bacterium, *Rhodobacter sphaeroides* SSI immobilized on porous ceramic made from waste glass. Adv Biosci Biotechnol 04(01):6–13
30. Panwichian S et al (2011) Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from contaminated shrimp ponds. Electron J Biotechnol 14(4). http://dx.doi.org/10.2225/vol14-issue4-fulltext-2
31. Magnin JP, Gondrexon N, Willison JC (2014) Zinc biosorption by the purple non-sulfur bacterium *Rhodobacter capsulatus*. Can J Microbiol 60(12):829–837
32. Sakurai H et al (2013) Photobiological hydrogen production: bioenergetics and challenges for its practical application. J Photochem Photobiol C 17:1–25
33. Lazaro CZ, Varesche MBA, Silva EL (2015) Sequential fermentative and phototrophic system for hydrogen production: an approach for Brazilian alcohol distillery wastewater. Int J Hydrogen Energy 40(31):9642–9655
34. Zürrer H, Bachofen R (1979) Hydrogen production by the photosynthetic bacterium *Rhodospirillum rubrum*. Appl Environ Microbiol 37(5):789–793
35. Najafpour GD, Younesi H (2007) Bioconversion of synthesis gas to hydrogen using a light-dependent photosynthetic bacterium, *Rhodospirillum rubrum*. World J Microbiol Biotechnol 23(2):275–284
36. Warthmann R, Cypionka H, Pfennig N (1992) Photoproduction of H2 from acetate by syntrophic cocultures of green sulfur bacteria and sulfur-reducing bacteria. Arch Microbiol 157(4):343–348

37. Kirti K et al (2014) Colorful world of microbes: carotenoids and their applications. Adv Biol 2014:1–13
38. Sun Z et al (2014) Microalgae as the production platform for carotenoids. Recent advances in microalgal biotechnology. OMICS Group eBooks, Foster City, CA, USA, pp 1–17
39. Takaichi S (1999) Carotenoids and carotenogenesis in anoxygenic photosynthetic bacteria. In: The photochemistry of carotenoids. Kluwer, Dordrecht, pp 39–69
40. Fraser NJ, Hashimoto H, Cogdell RJ (2001) Carotenoids and bacterial photosynthesis: the story so far. Photosynth Res 70(3):249–256
41. Wang GS et al (2012) High-level production of the industrial product lycopene by the photosynthetic bacterium *Rhodospirillum rubrum*. Appl Environ Microbiol 78(20):7205–7215
42. Chen YY et al (2013) Chromosomal evolution of *Escherichia coli* for the efficient production of lycopene. BMC Biotechnol 13:6
43. Frigaard NU et al (2004) Genetic manipulation of carotenoid biosynthesis in the green sulfur bacterium *Chlorobium tepidum*. J Bacteriol 186(16):5210–5220
44. Englund E et al (2015) Metabolic engineering of *Synechocystis* sp. PCC 6803 for production of the plant diterpenoid manoyl oxide. ACS Synth Biol 4(12):1270–1278
45. Laible PD, Mielke DL, Hanson DK (2009) Foreign gene expression in photosynthetic bacteria. In: The purple phototrophic bacteria. Springer, Dordrecht, pp 839–860
46. Erbakan M et al (2015) Advancing *Rhodobacter sphaeroides* as a platform for expression of functional membrane proteins. Protein Expr Purif 115:109–117