Chapter 12

Photosynthesis and Hydrogen Production in Purple Non Sulfur Bacteria: Fundamental and Applied Aspects

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Summary

Light-dependent hydrogen production by purple non sulfur bacteria (PNSB) has been studied for several decades. However the exact route that energy takes from the moment a photon is absorbed to the formation of a molecule of hydrogen is quite complex. The aim of this

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chapter is to review the researches carried out on the metabolic processes related to hydrogen production in PNSB, in particular stressing the issues related with the efficiency in the conversion of the energy deriving from the light in the energy-rich H_2 molecule produced. The metabolic processes that bring form the light capturing to hydrogen production are described, with the relative bottlenecks and hurdles.

The information currently available on the light distribution in various kind of photobioreactors are also reviewed, mainly focusing on the photosynthetic efficiency and on the efficiency in substrate conversion to H_2 obtained in laboratory and outdoor experiments.

From these data, it comes out how many different cellular processes can interact and affect photosynthetic efficiency and how complex is the route that brings from light energy to hydrogen energy.

I. Introduction

In 1949, Howard Gest and Martin Kamen first observed light dependent hydrogen production by the purple non sulfur bacterium (PNSB) *Rhodospirillum rubrum* (Gest and Kamen 1949). The dependence of H_2 production on nitrogenase activity was also observed, opening the way for research in this field. Since 1949, the metabolic routes that bring to the production of a molecule of hydrogen gas by PNSB have been thoroughly investigated and elucidated.

The process will be analyzed step by step in this chapter, stressing the complexity of the different metabolic pathways that interact with and influence the production of hydrogen.

II. The H_2 Production Process in Purple Bacteria

PNSB produce hydrogen via nitrogenase (see also Bothe et al., Chap. 6 of this volume). Although the major role of nitrogenase is to fix molecular nitrogen to ammonia, giving molecular hydrogen as a by-product (Eq. 12.1), the enzyme can also work in absence of molecular nitrogen and give hydrogen as the sole product (Eq. 12.2).

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \quad (12.1)$$

$$8H^+ + 8e^- + 16ATP \rightarrow 4H_2 + 16ADP + 16P_i \quad (12.2)$$

Nitrogenase is a two-protein complex consisting of a dinitrogenase containing Fe and Mo as cofactors and having a molecular weight of 250 kDa, and of a dinitrogenase reductase (containing Fe) of about 70 kDa. Mo-nitrogenase is the most common and the most efficient nitrogenase for converting N_2 to NH_3 (12.1), but other two isozymes have been described (Eady 1996) that contain Fe or V as cofactors and *Rhodopseudomonas palustris* is the only purple bacterium that encodes all three of them (Larimer et al. 2004). Those alternative nitrogenases are less efficient in reducing N_2 and more efficient in producing H_2 in nitrogen fixing conditions; however, they are produced only in case of lack of Mo and presence of Fe or V (Oda et al. 2005).

PNSB synthesize large amounts of nitrogenase (up to 2 % of cellular protein), not only because of its crucial role in cellular metabolism, but also because the enzyme is known as a slow catalyst (its turnover time per electron is ~5 s^{-1}), so a larger amount of enzyme provides a larger amount of nitrogen fixed. Although the mechanism by which nitrogenase reduces its substrates has been deeply

Abbreviations: BChl – Bacteriochlorophyll; Cyt bc_1 – Cytochrome bc_1 complex; Cyt c_2 – Cytochrome c_2; Fd – Ferredoxin; LH – Light harvesting; PE – Photosynthetic efficiency; PHB – Poly-β-hydroxybutyrate; PNSB – Purple non sulfur bacterium/bacteria; PSU – Photosynthetic unit; RC – Reaction center; SC – Substrate conversion

studied during the last decades -the first comprehensive scheme was proposed by Thorneley and Lowe in 1985- it proves to be very complex so that it still has not been completely clarified (Seefeldt et al. 2009).

H_2 production via nitrogenase has a specific activity one order of magnitude lower than Ni–Fe hydrogenase typical of oxygenic photosynthetic organisms (e.g. 1.3 mmol mg protein^{-1} min^{-1} for Mo-nitrogenase). Even so, in vivo H_2 production rates by nitrogenase-utilizing PNSB are comparable with those by hydrogenase-utilizing oxygenic phototrophs (Harwood 2008).

The H_2 produced during N_2-fixation by nitrogenase can be reused by uptake-hydrogenases (Vignais and Billoud 2007). High uptake-hydrogenase activities have been observed in cells possessing an active nitrogenase; the hydrogen produced by the nitrogenase stimulated the activity of hydrogenase in growing cells even though the synthesis of hydrogenase is not closely linked to the synthesis of nitrogenase (Colbeau et al. 1980).

Uptake-hydrogenases usually need to be genetically deleted to accumulate larger amounts of H_2. However, a few H_2-producing hydrogenases in PNSB have been described. *Rs. rubrum* and *Rp. palustris* BisB18 have a Ni–Fe hydrogenase that couples H_2 production to CO or formate oxidation (Fox et al. 1996; Oda et al. 2008).

A. Electron Transport to Nitrogenase

Ferredoxins are small proteins that function as electron carriers and take part in various metabolic processes, such as photosynthesis, nitrogen fixation, steroid hydroxylation, and degradation of aromatic compounds (Bruschi and Guerlesquin 1988). *Rhodobacter capsulatus* has been shown to synthesize six soluble ferredoxins which can be divided into two groups according to the number of [2Fe-2S] clusters present: FdI, FdII, and FdIII constitute the group of dicluster ferredoxins; FdIV, FdV, and FdVI contain a single [2Fe-2S] cluster. The genes encoding FdI, FdIII, FdIV, and FdV were localized within three *nif*-regulated operons (Moreno-Vivian et al. 1989; Schatt et al. 1989; Grabau et al. 1991; Willison et al. 1993), indicating that these ferredoxins participate in nitrogen fixation. FdI has been shown to serve as the physiological electron donor to nitrogenase (Jouanneau et al. 1995; Naud et al. 1996).

Göbel (1978) calculated that 1.5 photons are needed to synthesize 1 ATP molecule at 860 nm. Moreover, Miyake (1998) calculated that at 860 nm 11 photons are required for the production of a single molecule of H_2. Considering reaction (12.2), 4 ATP molecules are consumed for every H_2 molecule produced (i.e. 6 photons at 860 nm, according to Göbel 1978); the remaining 5 photons are most probably needed for the ferredoxin mediated (ATP consuming) electron transfer to nitrogenase.

B. The Role of ATP in Nitrogenase Activity

As it has been above described, nitrogenase catalyses a very expensive reaction in terms of ATP consumption to synthesize hydrogen (12.2), being the latter an endothermic reaction requiring external energy to overcome the positive free energy barrier ($G_0 = 75$ kJ). The balance is of 4 ATP molecules and 2 electrons consumed per molecule of H_2 produced. Besides these 4 ATP molecules, an additional number of ATP molecules is needed, through an energy requiring process, for reducing the ferredoxins that act as electron donors to nitrogenase (see Sect. II.A).

In detail, it has been schematically reported that 2 molecules of ATP are needed for every electron transfer from the Fe-protein nitrogenase subunit to the Mo-Fe protein subunit (in Mo-nitrogenase). However, experimental results demonstrated that the actual ratio of ATP hydrolyzed per couple of protons reduced is ~4.5 (Eady 1996).

Furthermore, it looks like the energy gained from the hydrolysis of those ATP molecules is used not only to overcome the thermodynamic barrier for N_2 reduction, but also has a role in the kinetic mechanism, not yet completely understood (Rees and Howard 2000).

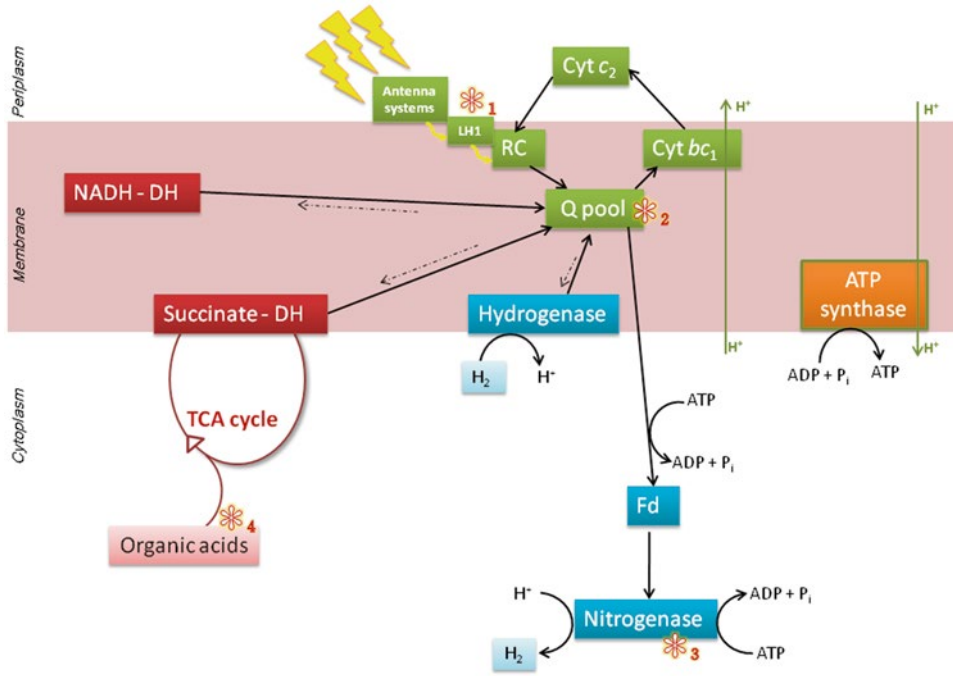

Fig. 12.1. Main processes related to hydrogen production, under photoheterotrophic growth in non-nitrogen fixing conditions: anoxygenic photosynthesis (in *green*), ATP synthesis (in *orange*), TCA cycle (in *red*), hydrogenase and nitrogenase activities (in *blue*). The straight *black arrows* indicate the electron flow. The *dotted lines* indicate minor electron flow. The lightning symbols indicate light excitation. The waving *arrows* indicate light energy transfers. The straight green lines indicate proton translocation. Abbreviations: *Cyt* bc_1, cytochrome bc_1 complex; *Cyt* c_2, cytochrome c_2, *Fd* ferredoxin, *RC*, Reaction center, *Succinate – DH* succinate dehydrogenase; *NADH-DH* NADH dehydrogenase. Numbered 1–4* signs will be discussed in Sect. VI of this chapter (Scheme modified from Adessi and De Philippis 2012, with kind permission from Springer Science+Business Media BV).

During photofermentation, cells are in photoheterotrophic conditions, i.e. the ATP is formed via anoxygenic photosynthesis, while the reducing power is derived by the catabolism of organic substrates, as it is schematically shown in Fig. 12.1.

The main energy carrier of a cell, ATP, is thus only synthesized by photosynthesis and many molecules are consumed while nitrogenase is active; this makes the parameters that refer to light and photosynthesis of crucial importance for the nitrogenase-mediated hydrogen production process in PNSB.

For this reason in recent years a large number of researches was aimed at optimizing illumination protocols in order the parameters capable of increasing photosynthetic efficiency (see Sect. IV).

III. Anoxygenic Photosynthesis

Generally speaking, in the reaction center of a photosynthetic organism, the excitation energy of photons is used to move one electron from one chemical compound (donor) towards another compound (acceptor). Thus, the reaction center is where the separation of charges actually occurs: the excitation energy is then stored into an energy-rich chemical bond.

The specificity of purple bacteria is given by their ability to form their energy carrier (ATP) in absence of oxygen by using sunlight as a source of energy (Imhoff 1995). Indeed, in anoxygenic photosynthesis, a special pair of bacteriochlorophylls, $[BChl]_2$, are both the primary electron donors and the final electron acceptors, as this photosynthetic

process is operationally defined as cyclic (Fig. 12.1).

A photon is absorbed by the light harvesting (LH) complexes that funnel the excitation towards bacteriochlorophylls in the reaction center (RC) and charge separation occurs. This energy is used for the release of an electron which reduces the quinone into a semiquinone. Once the quinone is doubly reduced (i.e. after a second photon is captured) it picks up protons from the cytoplasmic space and translocates them through the membrane to reach the cytochrome bc_1 complex: here electrons are channeled to the cytochrome c_2 (Cyt c_2) while protons are released in the periplasmic space. Cyt c_2 is then able to reduce the oxidized primary electron donors in the RC, i.e. $[Bchl]_2$, thus closing the cycle. The protons accumulated in the periplasmic space form an electrochemical gradient ($\Delta\mu H^+$) which is used by the ATP-synthase to generate ATP.

The photo-electron cycle can be opened by the "Δp-driven reversed electron transport" (indicated by the dotted black arrows in Fig. 12.1), i.e. by the action of both the NADH dehydrogenase working in the "reversed" way to reduce NAD^+ to NADH, and the succinate dehydrogenase also working "backwards" reducing fumarate to succinate (Klamt et al. 2008). As those two enzymes are able to catalyze both the forward and reverse reactions, the force that drives the direction is the presence or absence of the products; in particular the reversed reactions are ways to get rid of the possible excess of the reduced quinol. The reversed NADH dehydrogenase reaction is also the way to refurnish the cell with NADH reducing equivalents.

A. The Photosynthetic Unit (PSU)

The absorption spectrum of purple bacteria (Fig. 12.2) is very wide as covers the two ends of the visible spectrum, and even a little wider. The 'three fingered' absorption bands between 450 and 550 nm are due to carotenoids. Bacteriochlorophylls (BChls) show two characteristic absorption bands: the Soret band in the near UV region, around

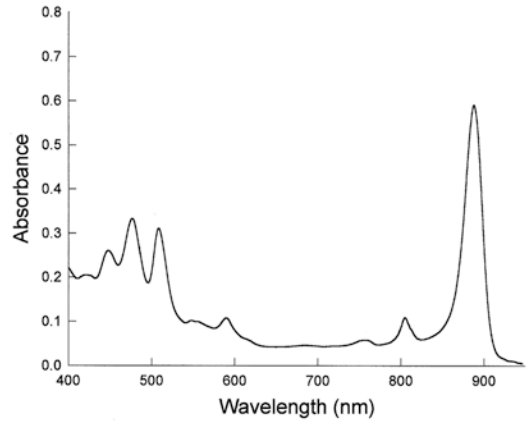

Fig. 12.2. Absorption spectrum of *Rb. sphaeroides* taken as an example for purple bacteria absorption spectrum. Peak wavelengths slightly vary among species.

390 nm (not shown in Fig. 12.2), and a band called Q band in the visible region of the spectrum. This band can usually be decomposed in two distinct bands called Q_x andQ_y according to their predominant polarization. The absorption peaks at 800, 850 and 880 nm in the near infrared region are due to the Q_y shift. The peaks at 850 and 880 often merge in one larger peak, having the maximum at an intermediate wavelength in between, but shoulders can be observed occasionally. The peak at 590 is due to the Q_x shift of BChl *a* (Hoff and Deisenhofer 1997; Blankenship 2002; Frank and Polívka 2008; Loach and Parker-Loach 2008; Robert 2008).

The pigment-protein complexes in the bacterial PSU are responsible for the absorption of light energy and its conversion to electronic excitation that drives the primary charge separation process. All the pigment-protein complexes bind both BChls and carotenoids; with a typical number of 10 LH-2 s, 1 LH-1 and 1 RC the PSU contains approximately 300 BChls. The observed stoichiometric ratio BChl:carotenoids of 3:2 implies the presence of 200 carotenoids (Hu et al. 2002). Out of all these pigments, only very few BChls in the RC directly take part in photochemical reactions; most BChls serve as light-harvesting antennae capturing the sunlight and channeling electronic excitation towards the RC. A wealth of evidence has accumu-

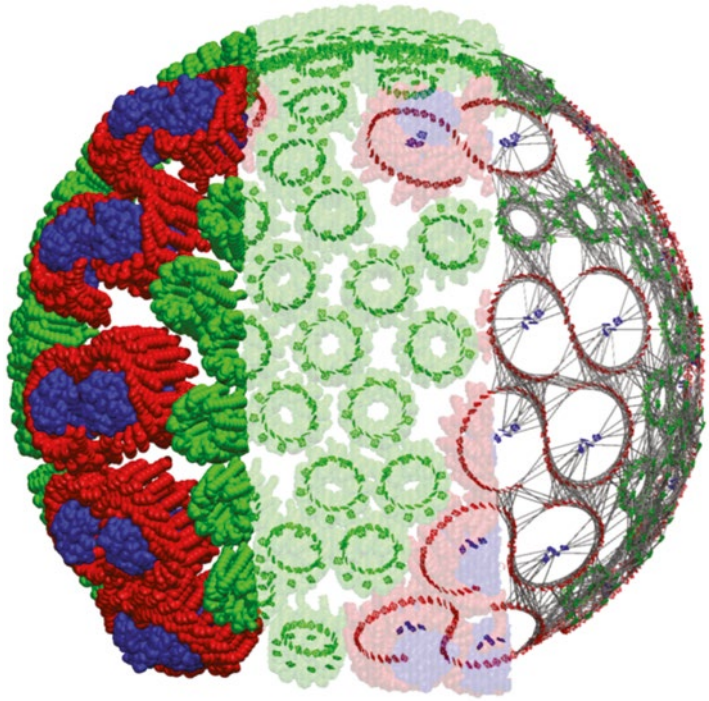

Fig. 12.3. Organization of the photosynthetic unit (PSU) from the supramolecular organization (*left hand side*) to individual bacteriochlorophylls and the electronic couplings (*right hand side*). Protein complexes and the BChls they respectively embed are colored as follows: LH2 in *green*, LH1 in *red* and RC in *blue*. On the *left* side, proteic subunits are represented with tubes; in the *middle* the proteic complexes are transparent to make the BChls visible; on the *right* side the strong electronic couplings of BChls are shown (Figure from Şener and Schulten 2008, with kind permission from Springer Science+Business Media BV).

lated now which proves that the organization of PSUs, to surround an RC with aggregates of chlorophylls and associated carotenoids, is universal in photosynthetic bacteria, higher plants and other photosynthetic organisms (Cogdell et al. 1996; Fromme 1996; Hankamer et al. 1997; Hu and Schulten 1997).

An overall scheme of the photosynthetic units is given in Fig. 12.3, where are represented the protein complexes, differently colored for LH2, LH1 and RCs, the pigment organization and the electronic couplings.

1. Carotenoids

An amount of about 50 distinct carotenoids have been described for purple anaerobic bacteria, and most of them have structures that significantly differ from those found in other photosynthetic or non-photosynthetic organisms.

The function of carotenoids in photosynthetic organisms, and in purple anaerobic bacteria as well, is both to harvest light energy and to protect the cell from photo-induced stress; also structural roles cannot be excluded (Takaichi 2008).

The pathways for carotenoid-genesis in purple bacteria have been classified in two distinct main pathways that contain many variations on their inside: the spirilloxantin pathway (that contain normal spirilloxantin, unusual spirilloxantin, sphaeroidene and carotenal pathways) and the okenone pathway (comprising okenone and *R.g.*-keto carotenoids pathways).

The structure of a carotenoid molecule affects its ability to transfer energy to BChls: *Rb. sphaeroides* containing spheroidene showed a 80–100 % energy transfer efficiency; *Rp. acidophila* containing rhodopin glucoside showed a 35–70 % efficiency; even a

lower efficiency has been detected (~30 %) for *Rs. rubrum* LH1 containing spirilloxantin (Frank and Polívka 2008).

2. Light Harvesting (LH) Complexes

LH complexes are able to harvest a great portion of photons hitting the cellular membrane and then to transfer the energy to the "open" (i.e. ready to accept photons) reaction center. LH1 and LH2 differ in structure and in the role covered: LH1 is intimately associated to the RC (for that reason is often referred to as the "core" or "primary" complex) and is found in all purple photosynthetic bacteria. LH2 is present in most species, but not all of them; this complex is distal from the RC (and is also referred to as the "secondary" complex) and channels the excitation to the RC only trough LH1 complexes. This is the reason why the LH2 complexes present absorption bands at higher energy levels (i.e. lower wavelengths) than those of LH1 (Gabrielsen et al. 2008).

Both antenna complexes are composed of repetitions of an elementary unit formed by α and β hydrophobic apoproteins (Zuber and Brunhisolz 1991). Those αβ subunits bind two BChl molecules and one or two carotenoids molecules (Cogdell et al. 2006); the subunits are organized in rings of different dimensions for the two kinds of complexes and the rings themselves can form different structures (Sheuring et al. 2004, 2005). "Basic" LH1 complexes are composed of at least 16 αβ subunits forming a ring that surrounds the RC (Karrash et al. 1995; Walz and Ghosh 1997; Walz et al. 1998; Jamieson et al. 2002); however, the structure varies significantly among bacterial species. In some cases LH1 complexes also contain an additional polypeptide named PufX (Cogdell et al. 1996).

LH2 is structured as circles as well, but composed of a different number of subunits compared to LH1; differences in the number of subunits and in their organization have been observed among different species. Generally speaking LH2 are smaller cycles than LH1 (Robert 2008).

These structural differences between LH1 and LH2, and the different environment and organization of the BChls they contain, give to the two complexes a difference in the absorption bands (Gabrielsen et al. 2008). In particular, LH2 contains B800 and B850 BChls, while LH1 contains B880 BChls (the numbers following the letter B indicate the wavelength corresponding to the Q_y transition of BChl; for details see Frank and Polívka 2008).

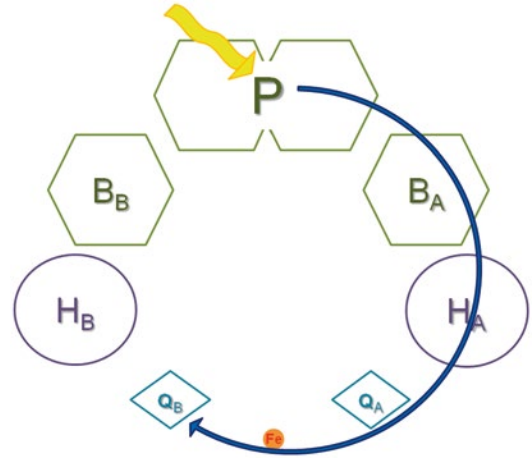

Fig. 12.4. Schematic representation of the reaction center (RC) of purple bacteria. RC is an integral membrane protein complex composed of L, M and H subunits. The H subunit contributes to the complex stability, while L and M compose the core of the RC and embed nine cofactors, symmetrically disposed in an A and a B branch: 2 BChl *a* molecules that form a dimer (P or $[BChl]_2$) connecting the two branches, 2 monomeric bacteriochlorophyll *a* molecules (B_A and B_B), 2 bacteriopheophytin *a* molecules (H_A and H_B), 2 ubiquinone molecules (Q_A and Q_B), and an iron (Fe) atom. The *round arrow* shows the electron path. The *waving arrow* shows excitation energy.

3. The Reaction Center (RC)

The reaction center (RC) of purple bacteria is an integral membrane protein complex composed of L, M and H subunits. The H subunit contributes to the complex stability and is involved in proton binding and transfer, while L and M compose the core of the RC and embed 9 cofactors, symmetrically disposed in an A and a B branch as shown in Fig. 12.4: 2 BChl *a* molecules that form a dimer (P or $[BChl]_2$) connecting the two branches, 2 monomeric bacteriochlorophyll *a* molecules (B_A and B_B), 2 bacteriopheophy-

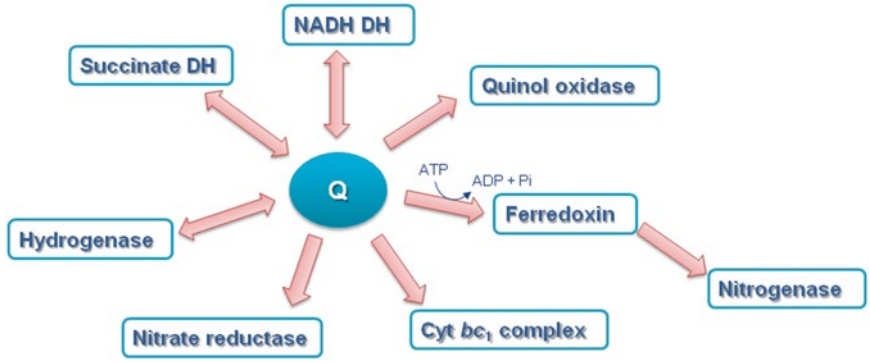

Fig. 12.5. The quinone pool functions. *Q* quinones, *Cyt* bc_1 complex: cytochrome bc_1 complex, *NADH DH* NADH dehydrogenase, *Succinate DH* succinate dehydrogenase.

tin *a* molecules (H_A and H_B), 2 ubiquinone molecules (Q_A and Q_B), and an iron (Fe) atom. Some authors suggested also the presence of a carotenoid molecule located next to the B_B monomer. Bacteriochlorophylls and bacteriopheophytins can be substituted by some other molecules of the same class (Williams and Allen 2008).

When a photon is conveyed to the P dimer an electron is raised to a higher energy level; the excited dimer P* transfers the electron, selectively through the A branch, to B_A (Fig. 12.4). Then, the electron is transferred to H_A; the overall transfer from P* to H_A takes from 3–5 ps, depending on bacterial species. Then the electron is transferred from H_A^- to Q_A and finally to Q_B, reducing it to QH_2. The oxidized P^+ is reduced back to P by an electron donated by Cyt c_2 (Fig. 12.1).

B. The Role of Quinones

Purple bacteria export reducing equivalents in pair from the RC, and the molecule migrating from the RC to the other membrane proteins is the reduced quinol (QH_2).

Two electrons are needed to reduce the quinone to quinol, so, as the RC transfers electrons one by one, two quinones (Q_A and Q_B) are needed and act in a cycle in order to accumulate two reducing equivalents; after two RC turnovers a QH_2 is released from the Q_B site (Okamura and Feher 1992, 1995; Shinkarev and Wraight 1993; Okamura et al. 2000; Paddock et al. 2003; Wraight 2004, 2005). During this cycle, protons are captured from the cytoplasm through the acceptor quinone cycle, and then released in the periplasm by the cytochrome bc_1 complex to form the proton gradient needed for ATP synthesis.

QH_2 is enough lipophilic to freely move throughout the membrane and to transport its reducing power to different membrane-bound enzymes. Indeed, quinones hold a crucial role for the energetic processes in the cell, as it is schematically shown in Fig. 12.5. They not only take part in photosynthesis, as it has been above reported, but they also function as electron carriers for all of the redox processes taking place in the membrane (Adessi and De Philippis 2013); for the sake of brevity not all redox processes are treated in this chapter, but specific references are given for further information.

During photosynthesis, they acquire electrons from the RC and reach the cytochrome bc_1 complex to donate them; they also can accept or donate electrons from/to NADH dehydrogenase and succinate dehydrogenase, as described earlier.

During respiration, they receive electrons from NADH-dehydrogenase and succinate dehydrogenase and carry the electrons to both the quinol oxidase and the Cyt bc_1 complex (Zannoni 1995; Zannoni et al. 2008).

During denitrification, they carry the electrons to nitrite reductase (Shapleigh 2008).

In anaerobic conditions and under H_2 containing atmosphere, quinones can accept electrons from hydrogenase (either donate them, but rarely and depending on the abundance of substrates and products of the hydrogenase catalyzed reaction).

Again, in anaerobic conditions electrons can also be transferred to ferredoxins, with the expense of a molecule of ATP, that will then carry the reducing power to nitrogenase in order to fix nitrogen and/or reduce protons to hydrogen.

Moreover, they are not only involved in many essential redox reactions, but also are the mediators of the integrative cell-redox state signal to the RegA/RegB regulon (Swem et al. 2006) which regulates the major processes taking place in purple non sulfur bacteria: photosynthesis, respiration, nitrogen and carbon fixation (Elsen et al. 2004).

IV. Photosynthetic Efficiency (PE)

Generally speaking, the photosynthetic efficiency (PE) is defined as the energy stored as biomass produced per unit of light energy absorbed (Gadhamshetty et al. 2011). It is also called light conversion efficiency. The calculation of the light energy absorbed can be based either on the full solar irradiance or on the photosynthetically active radiation (PAR) range. As a measure for efficiency, when working with photosynthetic organisms, biomass yield (calculated as protein content or dry weight) on light energy is often used. However, in the case of hydrogen production, the product of the process is energy in the form of H_2 and the limiting factor is light; therefore, in this specific case, expressing the efficiency on the basis of the hydrogen-related energy produced per unit of light energy absorbed is more accurate (Eq. 12.3).

$$\mathrm{PE} = \frac{\text{Free energy of the total amount of } H_2}{\text{Total energy of the light incident on the culture}} \% \tag{12.3}$$

It has to be stressed that in this calculation only the energy input of light is considered, and not the energetic contribution of organic substrates consumed by the cell during hydrogen production (Hallenbeck and Benemann 2002).

The efficiency by which the light energy can be transformed into hydrogen gas energy is dependent not only on the part of the energy that is absorbed by the antenna systems of the organism studied but also on the energy loss during the several steps of excitation and electron transfers that follow. In addition to that, PE is a measure derived from the amount of hydrogen produced, thus all cellular processes that deviate from the routes that bring to hydrogen production negatively affect PE. To summarize, hydrogen production depends both on photoparameters such as quality and quantity of light and on biological factors such as pigment composition, quantum requirements and the metabolism of the different PNSB strains. Therefore, qualitative and quantitative understanding of each of those factors is important to optimize PE. Moreover, only the 65.8 % of the spectrum is actually part of the PAR for purple bacteria; the energy associated to one mole of photons depends on the wavelengths inside this 65.8 %, so PE should be calculated for each wavelength. However, quantum yields are only known for 522 and 860 nm wavelenghts (Miyake 1998), and lead to wavelength specific PEs of 8.4 % and 19 % respectively. From these data it was calculated that the theoretical maximum PE for PNSB, based on the natural sunlight spectrum, is at least 10 % (Akkerman et al. 2002).

PE is a crucial factor for optimum hydrogen production and it is the most important aspect to be taken into account designing a photobioreactor (Akkerman et al. 2002; Gadhamshetty et al. 2011). Indeed, high surface to volume ratios are necessary to capture sufficient light (Dasgupta et al. 2010); this means that operating at a scarce PE implicates the need to occupy a large surface area with the photobioreactor for producing satisfactory amounts of hydrogen. Consequently, photobioreactors that optimize

light distribution and capturing by cells are required for efficient hydrogen production processes.

Moving from theory to practice, low PE has always constituted a very hard obstacle to overcome, not only using natural sunlight but also using artificial irradiation. Indeed, up to 2010, high photosynthetic efficiencies have been reached using such low light intensities that the production rates were not enough to be considered interesting for an H_2 production process. Barbosa et al. (2001) observed that higher light intensities may decrease PE, but usually increase hydrogen productivity. Miyake and Kawamura (1987) reported light conversion efficiencies of 7.9 and 6.2 %: they illuminated the cultures with a xenon lamp at 50 W m^{-2} and by a solar simulator at 75 Wm^{-2}, respectively. Those light intensities allowed to reach a relevant value of PE, but are too low to reach gas evolution rates that can be considered interesting for production processes. However, recently, impressive high PEs have been obtained (Tian et al. 2010; Wang et al. 2013) associated to high hydrogen production rates, by paying attention to the quality of the incident light. These data will be discussed thoroughly in this paragraph, Sect. IV.B.

A. Light Intensity

In *Rb. capsulatus* it has been observed that light strongly stimulates not only the activity but also the amount of nitrogenase synthesized (up to 25 % of all soluble proteins); as a consequence, a larger amount of hydrogen under high light conditions was observed as well (Jouanneau et al. 1985; Vignais et al. 1985). The photophosphorylation capacity is also slightly greater in cells grown under high light-intensity than in cells grown under low-light intensities (Steinborn and Oelze 1989). Therefore, Kars and Gündüz (2010) proposed that high ATP production rates under well-illuminated conditions result in higher hydrogen production activity.

Uyar et al. (2007) indicated, for *Rb. sphaeroides*, a minimum light intensity of 270 W m^{-2} to obtain high hydrogen production rates, this value being equivalent to 4,000 lx and 1,370 μmol(photons) m^{-2} s^{-1}.

However, it must be underlined that the intensity of light impinging on the photobioreactor, which is the parameter usually reported in the literature, is not equivalent to the intensity of light actually faced by the single cells. Indeed, due to the self-shading of cells, a phenomenon always present in the cultivation systems used for growing phototrophic microorganisms (Vonshak and Richmond 1985; Tredici 1999), the amount of light reaching the single cell may be hundreds of times lower than the light reaching the photobioreactor, or even zero (Gadhamshetty et al. 2011). This well known phenomenon depends on the concentration of the cells, the light path in the photobioreactor and the amount of light absorbed by the single cell. This amount, on its side, depends on cell diameter, pigment composition and concentration, antenna size.

From these considerations, it is quite evident that it is a problematic task to evaluate the exact amount of light that is absorbed by a cell, thus making the photosynthetic efficiency a parameter that relies on a few approximations.

B. Light Quality and Sources

Purple bacteria are able to absorb light at the very extremities of the visible spectrum (Fig. 12.2) with one main absorption band at 300–500 nm and the other above 800 nm in the near infrared region.

When using artificial light, the most frequently used light sources are incandescent lamps. Among them, tungsten lamps have an emission spectrum that covers the whole absorption spectrum of PNSB (Adessi and De Philippis 2012). Particularly important is the high near infrared emission, where is located the absorption maximum of bacteriochlorophylls. Halogen lamps are quite frequently used as well.

As incandescent lamps are energy-expensive light sources, an interesting alternative is offered by Light Emitting Diodes (LEDs). Kawagoshi et al. (2010) prefigured a

reduction of energy cost by 98 % using LEDs instead of tungsten lamps. In a cost effective scaled-up system, though, the best solution would appear the use of natural solar light.

Table 12.1 shows the results deriving from different kinds of photobioreactors illuminated by different light sources: incandescent lamps, LEDs and solar light. Summarizing the experimental results reported in Table 12.1, PE is <10 % in the experiments carried out using incandescent lamps, it stands around 1 % when using solar light but reaches very high values when using LEDs.

Apparently, specific LED illumination granted the highest ever obtained PE values. Those data were obtained using immobilized systems: glass beads biofilm (Tian et al. 2010) or a mix of PVA, carrageenan and alginate (Wang et al. 2013). These outstanding efficiencies, respectively 56 and 82 % (see Table 12.1), were to a great extent due to the use of LEDs illuminating at a selected specific wavelength (590 nm). This specific excitation allowed the cells to utilize a large part of the incident energy, as it was at the exact wavelength that could be absorbed by the culture.

590 nm as an emission wavelength was the best among other wavelength specific LEDs (470, 520, 590, 620 nm), tested by Zhang et al. (2010); comparing those emission wavelengths with the PNSB absorption spectrum (Fig. 12.2) it emerges that 590 nm is very close to one of the absorption peaks of bacteriochlorophylls. Also long-wavelength emitting LEDs have been used for hydrogen production processes with PNSB (Kawagoshi et al. 2010); those LEDs provided an emission spectrum having a maximum at 850 nm and were used for illuminating a halotolerant *Rhodobacter* sp., but poor hydrogen production was obtained. However, the scarce hydrogen production was not due to the light source, but most probably to sub- optimal growth conditions.

The use of natural sunlight opens a series of more complex issues, not all related to light itself. Indeed, most sunlight illuminated photobioreactors are outdoor large-scale systems, whose management complicates the processes comparing it with a lab-scale process (Chen et al. 2011). However, focusing exclusively on light-related issues, not all of the solar spectrum is part of the PAR for PNSB, as mentioned earlier. Moreover, Miyake et al. (1999) pointed out how the intrinsic variability of solar light makes the rates vary along with light intensity during the day, giving an endemic inconstancy to the process. Furthermore, they observed a probable photoinhibition under the highest irradiation of the day (0.9 kW m^{-2}).

Generally speaking, when H_2 production is carried out outdoors using solar irradiation, PE is around 1 %. The highest PE reported with a solar photobioreactor containing purple bacteria (1.4 %) was obtained with a flat plate reactor provided with light shading bands (Wakayama and Miyake 2002). The special feature of the system used by Wakayama and Miyake (2002) was the reduction of the total amount of light irradiation reaching the cells through the use of shading bands.

Photoinhibition in outdoors purple bacteria culturing systems has not been studied thoroughly yet, as a few studies on PNSB pay a specific attention to it. Miyake et al. (1999) observed a delay of 2–4 h of the maximum H_2 production rate after the maximum irradiation at noon. At the same regard, Adessi et al. (2012a) tried to avoid photoinhibition cutting sunlight intensity by 50 % using a light-shield. Hydrogen production rates were not negatively affected by this light intensity decrease; on the contrary, the maximum rate (27.2 ml l^{-1} h^{-1}), reached 2 h after noon, was comparable to artificial lab-conditions results. However, this delay in achieving the maximum production rate compared to the peak of irradiation might still indicate photoinhibition by the highest irradiation of the day.

Although the photoinhibition might affect hydrogen production during the peak irradiation of the day, Adessi et al. (2012a) observed a good physiological long-term fitting of a *Rp. palustris* culture, as confirmed by the BChl *a* fluorescence analysis. Twenty-one days of exposure to solar irradiation was

Table 12.1. Light sources and intensities and types of photobioreactors (PBR) with the corresponding hydrogen production rates (HPR), photosynthetic efficiencies (PE) and substrate conversions (SC).

Light source and intensity	Type of PBR	Organism	HPR (ml l^{-1} h^{-1})	PE (%)	SC (%)	Ref.
Traditional lamps						
Tungsten lamp 200 W m^{-2}	Tubular, vertical	*Rb. sphaeroides*	20.0	1.1[a]	n.a.	Eroglu et al. (1998)
Tungsten lamp 10.25 W m^{-2}	Flat, rocking	*Rb. sphaeroides*	11.0	3.31	45	Gilbert et al. (2011)
Tungsten lamp 500 W m^{-2}	Double-layer	*Rb. sphaeroides* (wild type + reduced pigment mutant)	3640[b]	2.18	n.a.	Kondo et al. (2002a, b)
Tungsten lamps 500 lx	Fermentor type	*Rs. rubrum*	107.5 [c]	8.67	n.a.	Ismail et al. (2008)
Halogen lamp	Flat, floating type	*Rp. palustris*	10.0–12.0	0.3	n.a.	Otzuki et al. (1998)
Halogen lamp	Induced diffuse light	*Rb. sphaeroides*	7577[b]	6.12	61	El-Shishtawy et al. (1997)
				Peak value: 9.23		
Luminine tubular light 15 W m^{-2}	Annular triple jacketed	*Rb. sphaeroides*	6.5	3.7	75	Basak and Das (2009)
Halogen +Tungsten lamps 95 W m^{-2} (with optical fibers)	Immobilized, clay	*Rp. palustris*	43.8	2.34	90.8	Chen and Chang (2006)
Incandescent lamp 150 W m^{-2}	Immobilized, activated carbon fibers	*Rp. faecalis*	32.9	0.96	77.0	Xie et al. (2012)
Metal–halide lamp 12 W m^{-2} (with optical fibers)	Biofilm, rough surface	*Rp. palustris*	39.2 [c]	9.3	75	Guo et al. (2011)

LED lights							
LED (590 nm) 6.75 W m^{-2}	Biofilm, glass beads	*Rp. palustris*	38.9		56	1.7	Tian et al. (2010)
LED (590 nm) 6.75 W m^{-2}	Biofilm, groove-type	*Rp. palustris*	86.46[b,c]		3.8	6.25	Zhang et al. (2010)
LED (590 nm) 6,000 lx	Immobilized, PVA + carrageenan + alginate	*Rp. palustris*	58.4		82.3	62.3	Wang et al. (2013)
LED (590 nm)	Flat, 30° inclined	*Rp. palustris*	26.9		8.9	2.1	Liao et al. (2010)
Solar light							
Sulight (+ initial artificial light)	Tubular, near horizontal	*Rb. capsulatus*	Avg:6.9[a]	Max: 16.6 [a]	1.0	16	Boran et al. (2010)
Sulight (+ initial artificial light)	Tubular, near horizontal	*Rb. capsulatus hup*- mutant	Avg:4.5[a]	Max: 8.9[a]	0.2	12	Boran et al. (2012)
Sunlight (shielded)	Tubular, horizontal	*Rp. palustris*	Avg:10.7	Max: 27.2	Avg 0.63 Max 0.92	10.3	Adessi et al. (2012a)
Sunlight	Flat, 30° inclined	*Rb. sphaeroides*	10.0		n.a.	76.7	Eroglu et al. (2008)
Sunlight	Flat with light shading bands	*Rb. sphaeroides*	0.87[b]		1.4	n.a.	Wakayama and Miyake (2002)
Sunlight + tungsten lamp (with optical fibers)	Fermentor type	*Rp. palustris*	22.7		n.a.	62.3	Chen et al. (2008)

n.a not available

[a]Calculated by Akkerman et al. (2002)

[b]Volume expressed as ml m^{-2} h^{-1}

[c]Value in mmoles of H_2 converted to ml of H_2 by 22.4 multiplying factor

not affecting the functionality of the photosystem, and it was not degraded by the excess of solar radiation. This indicates a very high capability of purple bacteria to acclimate to conditions that would potentially be a source of stress, as an excessive light irradiance. Structural and functional homology between the purple bacterial RC and the RC of the photosystem II (PSII) of oxygenic photosynthesis allows the use of variable bacteriochlorophyll (Bchl *a*) fluorescence to investigate the energy transfer and electron transport within the photosynthetic apparatus. There is evidence that confirms the applicability of chlorophyll fluorescence analysis (usually used for oxygenic photosynthetic organisms) to photosynthetic bacteria (Koblízek et al. 2005; Maróti 2008; Asztalos et al. 2010; Adessi et al. 2012a).

V. Substrate to Hydrogen Conversion (SC)

As it has been described earlier (see Fig. 12.1), under photoheterotrophic growth conditions the ATP is formed via anoxygenic photosynthesis, while the reducing power is derived by the catabolism of organic substrates. Thus, the efficiency of the carbon related metabolic processes has a role in determining the amount of electrons accumulated as quinols and then, if that is the case, transferred to nitrogenase through ATP-dependent ferredoxin reduction. Therefore, even going back to the role that quinones have in the cell (Fig. 12.5), it looks clear how hydrogen production in purple bacteria is related to many metabolic processes that deal with ATP generation (photosynthesis), carbon metabolism and nitrogen fixation. Usually, all the processes involved in energy generation, as photosynthesis and H_2 oxidation, and energy consumption, as N_2 and CO_2 fixation, are globally regulated by the two component system RegB-RegA (Elsen et al. 2000).

The preferred substrates for hydrogen production are the low-molecular weight organic acids that can easily enter the TCA cycle, which is very active during anaerobic photosynthetic growth.

Carbon metabolism in purple non-sulfur bacteria has been schematically compiled by Koku et al. (2002). That scheme describes the metabolism of *Rb. sphaeroides*, but not all species and genera follow the same scheme: for example *Rp. palustris* does not possess the Entner-Doudoroff pathway (Larimer et al. 2004). A well documented description of C metabolism in PNSB in relation with hydrogen production is reported in Chap. 7 of this volume (McKinlay).

An important parameter to evaluate the yield of a hydrogen production process is the substrate conversion efficiency, calculated as the ratio between the moles of hydrogen produced and the moles theoretically obtainable if all the substrate consumed was converted to CO_2 and H_2. Thus, considering the most common organic acids utilized in photofermentation processes (Barbosa et al. 2001), the conversion yields can be calculated from the following reactions:

$$\text{Lactate}: \ C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2 \quad (12.4)$$

$$\text{Acetate}: C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (12.5)$$

$$\text{Malate}: \ C_4H_6O_5 + 3H_2O \rightarrow 6H_2 + 4CO_2 \quad (12.6)$$

It has to be stressed that these reactions are theoretical, because they are neither considering the utilization of the substrate for the growth neither limiting factors occurring in a culture. On the basis of these reactions, the gas expected should be composed of a 66.7 % of H_2 and a 33.3 % of CO_2 when growing on lactate and on acetate; a 60 % of H_2 and a 40 % of CO_2 when growing on malate. Actually, the gas phase above the culture is much richer in H_2 then in CO_2, due to a partial solubilization of CO_2 in the culture medium and also to a partial fixation to CO_2 for anabolic reactions (McKinlay, Chap. 7 of this volume).

The presence of Calvin cycle in carbon metabolism has been described by Joshi and Tabita (1996). They demonstrated that the

absence of the reductive pentose phosphate CO_2 fixation pathway enhances the synthesis of nitrogenase also in presence of ammonium ions, as the reduction of CO_2, in photoheterotrophy, is another way to dissipate the excessive reducing power deriving from organic carbon compounds. McKinlay and Harwood (2010, 2011) followed the exact pathway of CO_2 molecules, getting to the conclusion that CO_2 fixation actually deprives the cells from a part of the electrons that would either be useful for hydrogen production, but that it is not true that a more reduced substrate generates a larger quantity of electrons available for H_2 production.

A 100 % substrate conversion efficiency has been reported by Sasikala et al. (1990), but in a limited culture volume (2 ml); conversion efficiencies (reported in Table 12.1) mainly range between 60 % and 90 %.

The substrate conversion efficiency is strongly affected by the C/N ratio in the culture. Indeed, a high C/N ratio in the culture media usually leads to higher hydrogen production compared with low C/N ratio, where a higher cell growth occurs (Kapdan and Kargi 2006; Redwood et al. 2009; Keskin et al. 2011). In the latter case, the conversion efficiency decreases due to the consumption of the organic acids for cell growth instead that for hydrogen production. This problem becomes a very relevant matter when wastewaters or liquors deriving from other fermentation processes are utilized for the production of H_2 by means of photofermentation (Eroglu et al., Chap. 11 of this volume).

As regarding the processes that compete with the conversion of the carbon substrate to hydrogen, PNSB are also capable of producing poly-β-hydroxybutyrate (PHB) a valuable by-product which is a biodegradable thermoplastic having industrial and medical interest (De Philippis et al. 1992; Sasikala and Ramana 1995; Reddy et al. 2003; Franchi et al. 2004). PHB synthesis not only consumes the carbon substrate itself (acetate molecules are needed as building blocks for PHB), but also the reducing power, as PHB synthesis utilizes NADH. Both those aspects are in competition with hydrogen production during photofermentation (De Philippis et al. 1992; Hustede et al. 1993; Vincenzini et al. 1997; Koku et al. 2003; Franchi et al. 2004).

Other factors affecting substrate conversion are the mixing and the availability of the substrate for the cells in terms of exchanging surface between cells and medium. In particular, immobilized or biofilm reactors show a higher conversion efficiencies for that reason (Table 12.1).

VI. Process Bottlenecks – Conclusions

In this chapter, an overview of the process leading from light energy to the production of a molecule of hydrogen was given. From this description, it appears evident that this process encounters many hurdles and deviations from the desired route.

In Fig. 12.1, the most critical steps, that can be bottlenecks of the process are indicated by star signs:

$*_1$ Energy transfer through the photosynthetic unit;
$*_2$ Reducing power deviation;
$*_3$ Nitrogenase activity;
$*_4$ Substrate deviation.

Recently, Harwood (2008) and Kars and Gündüz (2010) reviewed the research on mutagenesis of strains in order to overcome the hurdles described. Below are listed the cultivation strategies that can be adopted for each of the previous points.

$*_1$ Strategies for improving the energy transfer through the photosynthetic unit:

Reduced pigment strains: reducing pigment content by genetic manipulations is aimed at reducing the self-shading effect of the cells. As a consequence, the light penetration inside the bioreactor increases, causing an increase in H_2 production (Vasilyeva et al. 1999; Kondo et al. 2002a, b).

Protection from photoinhibition: light shading bands (Wakayama and Miyake 2002) or light shields (Adessi et al. 2012a) prevent the cells to be burnt by the excess of sunlight.

Use of specific light sources: the use of specific-wavelength LEDs brought to higher PEs (Tian et al. 2010; Wang et al. 2013).

*$_2$ Strategies for preventing the of deviation reducing power:

Hydrogenase inhibition: since uptake-hydrogenase decreases the efficiency of H_2 production, it was targeted to be eliminated in many PNSB either by antibiotic resistance gene insertion into the *hup* genes or by deletion of *hup* genes (Kern et al. 1994; Ooshima et al. 1998; Franchi et al. 2004; Kim et al. 2006; Öztürk et al. 2006; Kars et al. 2008; Kars et al. 2009); other deviations are avoided by supplementing the cultures with reduced carbon substrates in order not to let the cells run out of reducing equivalents.

CBB defective strains: PNSB fix CO_2 via the Calvin Benson Bassham (CBB) cycle; the overall pathway consumes both NADPH and ATP to synthesize cell material; carbon dioxide fixation defective strains have been constructed in order to save electrons for hydrogen production (Joshi and Tabita 1996; Qian and Tabita 1996; McKinlay and Harwood 2010, 2011).

*$_3$ Strategies for enhancing nitrogenase activity:

Enhanced electron flow to nitrogenase: the *rnf* operon has been identified as related to the electron transport to nitrogenase. Overexpression of this operon led to higher nitrogenase activity (Jeong and Jouanneau 2000).

Enhanced nitrogenase activity: the limiting step for nitrogenase mediated catalysis is difficult to overcome due to the complexity of the mechanism. However, a possible strategy could be acting on the substrate selectivity of nitrogenase in presence of dinitrogen (Harwood 2008), as it has already been proved to be successful in *Azotobacter vinelandii* (Barney et al. 2004).

Deregulation of nitrogenase: nitrogenase is strongly inhibited by the presence of fixed nitrogen in the cell (Rey et al. 2007; Heiniger et al. 2012); deregulating nitrogenase appears to have a stabilizing effect on hydrogen production even on ammonia containing substrates (Adessi et al. 2012b).

*$_4$ Strategies for avoiding substrate deviation:

PHB route inactivation: polyhydroxyalkanoic acids biosynthesis constitutes a way for disposing of the excess of reducing power in the cell; it competes with hydrogen production and consumes acetate. Deletion of the PHB synthesizing route resulted in enhanced hydrogen production (Hustede et al. 1993); following studies reported an enhanced hydrogen production only when PHB deficiency was coupled with uptake hydrogenase inactivation (Franchi et al. 2004; Kim et al. 2006).

It looks clear how complex is the itinerary that brings from light to hydrogen and how many different routes have been taken to optimize the process. A "supermutant" bringing all the mutations above reported is not a solution that the authors consider feasible, due to the consequent fragility of an organism that would bring all those kind of genetic alterations. A fragile organism, even if the hydrogen production route might be stabilized in its metabolism, would not be a good choice for an up-scaled process in particular if operating on complex substrates derived from other fermentation or degradative processes. On the contrary the authors believe that a limited number of genetic manipulations carefully combined with the more appropriate culture conditions and photobioreactor geometry should be designed for each specific process in order to obtain the maximum hydrogen production without affecting process stability.

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References

Adessi A, De Philippis R (2012) Hydrogen production: photofermentation. In: Hallenbeck PC (ed) Microbial technologies in advanced biofuels production. Springer Science+Business Media, New York, pp 53–75

Adessi A, De Philippis R (2013) Purple bacteria: electron acceptors and donors. In: Lennarz WJ, Lane MD (eds) The encyclopedia of biological chemistry, vol 3. Academic, Waltham, pp 693–699

Adessi A, Torzillo G, Baccetti E, De Philippis R (2012a) Sustained outdoor H_2 production with *Rhodopseudomonas palustris* cultures in a 50 L tubular photobioreactor. Int J Hydrog Energy 37:8840–8849

Adessi A, McKinlay JB, Harwood CS, De Philippis R (2012b) A *Rhodopseudomonas palustris nifA** mutant produces H_2 from NH_4^+-containing vegetable wastes. Int J Hydrog Energy 37:15893–15900

Akkerman I, Janssen M, Rocha J, Wijffels RH (2002) Photobiological hydrogen production: photochemical efficiency and bioreactor design. Int J Hydrog Energy 27:1195–1208

Asztalos E, Italiano F, Milano F, Maróti P, Trotta M (2010) Early detection of mercury contamination by fluorescence induction of photosynthetic bacteria. Photochem Photobiol Sci 9:1218–1223

Barbosa MJ, Rocha JMS, Tramper J, Wijffels RH (2001) Acetate as a carbon source for hydrogen production by photosynthetic bacteria. J Biotechnol 85:25–33

Barney BM, Igarashi RY, Dos Santos PC, Dean DR, Seefeldt LC (2004) Substrate interaction at an Iron-Sulfur face of the FeMo-cofactor during nitrogenase catalysis. J Biol Chem 279:53621–53624

Basak N, Das D (2009) Photofermentative hydrogen production using purple non-sulfur bacteria *Rhodobacter sphaeroides* O.U.001 in an annular photobioreactor: a case study. Biomass Bioenergy 33:911–919

Blankenship RE (2002) Molecular mechanisms of photosynthesis. Blackwell Science Ltd, Oxford

Boran E, Özgür E, Van der Burg J, Yücel M, Gündüz U, Eroğlu I (2010) Biological hydrogen production by *Rhodobacter capsulatus* in solar tubular photo bioreactor. J Clean Prod 18:S29–S35

Boran E, Özgür E, Yücel M, Gündüz U, Eroğlu I (2012) Biohydrogen production by *Rhodobacter capsulatus* Hup- mutant in pilot solar tubular photobioreactor. Int J Hydrog Energy 37:16437–16445

Bruschi M, Guerlesquin F (1988) Structure, function and evolution of bacterial ferredoxins. FEMS Microbiol Rev 54:155–176

Chen CY, Chang JS (2006) Enhancing phototrophic hydrogen production by solid-carrier arrested fermentation and internal optical-fiber illumination. Process Biochem 41:2041–2049

Chen CY, Saratale GD, Lee CM, Chen PC, Chang JS (2008) Phototrophic hydrogen production in photobioreactors coupled with solar energy excited optical fibers. Int J Hydrog Energy 33:6686–6695

Chen CY, Liu CH, Lo YC, Chang JS (2011) Perspectives on cultivation strategies and photobioreactor designs for photo-fermentative hydrogen production. Bioresour Technol 102:8484–8492

Cogdell R, Fyfe P, Barrett S, Prince S, Freer A, Isaacs N, McGlynn P, Hunter C (1996) The purple bacterial photosynthetic unit. Photosynth Res 48:55–63

Cogdell RJ, Southall J, Gardiner AT, Law CJ, Gall A, Roszak AW, Isaacs NW (2006) How purple photosynthetic bacteria harvest solar energy. CR Chim 9:201–206

Colbeau A, Kelley BC, Vignais PM (1980) Hydrogenase activity in *Rhodopseudomonas capsulata*: relationship with nitrogenase activity. J Bacteriol 144:141–148

Dasgupta CN, Gilbert JJ, Lindblad P, Heidorn T, Borgvang SA, Skjanes K, Das D (2010) Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. Int J Hydrog Energy 35:10218–10238

De Philippis R, Ena A, Guastini M, Sili C, Vincenzini M (1992) Factors affecting poly-β-hydroxybutyrate accumulation in cyanobacteria and in purple non-sulfur bacteria. FEMS Microbiol Rev 103:187–194

Eady RR (1996) Structure-function relationships of alternative nitrogenases. Chem Rev 96:3013–3030

Elsen S, Dischert W, Colbeau A, Bauer CE (2000) Expression of uptake hydrogenase and molybdenum nitrogenase in *Rhodobacter capsulatus* is coregulated by the RegB-RegA two-component regulatory system. J Biotechnol 182:2831–2837

Elsen S, Swem LR, Swem DL, Bauer CE (2004) RegB/RegA, a highly conserved redox-responding global two-component regulatory system. Microbiol Mol Biol Rev 68:263–279

El-Shishtawy RMA, Kawasaki S, Morimoto M (1997) Biological H_2 production using a novel light-induced and diffused photoreactor. Biotechnol Tech 11:403–407

Eroglu I, Aslan K, Gunduz U, Yucel M, Turker L (1998) Continuous hydrogen production by Rhodobacter sphaeroides O.U.001. In: Zaborsky OR (ed) Biohydrogen. Plenum Press, London, pp 143–151

Eroğlu I, Tabanoğlu A, Gündüz U, Eroğlu E, Yücel M (2008) Hydrogen production by *Rhodobacter sphaeroides* O.U.001 in a flat plate solar bioreactor. Int J Hydrog Energy 33:531–541

Fox JD, He Y, Shelver D, Roberts GP, Ludden PW (1996) Characterization of the region encoding the CO-induced hydrogenase of *Rhodospirillum rubrum*. J Bacteriol 178:6200–6208

Franchi E, Tosi C, Scolla G, Penna DG, Rodriguez F, Pedroni MP (2004) Metabolically engineered *Rhodobacter sphaeroides* RV strains for improved biohydrogen photoproduction combined with disposal of food wastes. Mar Biotechnol 6:552–565

Frank HA, Polívka T (2008) Energy transfer from carotenoids to bacteriochlorophylls. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer Science+Business Media, Dordrecht, pp 213–230

Fromme P (1996) Structure and function of photosystem I. Curr Opin Struct Biol 6:473–484

Gabrielsen M, Gardiner AT, Cogdell RJ (2008) Peripheral complexes of purple bacteria. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer Science+Business Media, Dordrecht, pp 135–153

Gadhamshetty V, Sukumaran A, Nirmalakhandan N (2011) Photoparameters in photofermentative biohydrogen production. Environ Sci Technol 41:1–51

Gest H, Kamen MD (1949) Photoproduction of molecular hydrogen by *Rhodospirillum rubrum*. Science 109:558–559

Gilbert JJ, Ray S, Das D (2011) Hydrogen production using *Rhodobacter sphaeroides* (O.U.001) in a flat panel rocking photobioreactor. Int J Hydrog Energy 36:3434–3441

Göbel F (1978) Quantum efficiencies of growth. In: Clayton RK, Sistrom WR (eds) Photosynthetic bacteria. Plenum Press, New York, pp 907–925

Grabau C, Schatt E, Jouanneau Y, Vignais PM (1991) A new [2Fe-2S] ferredoxin from *Rhodobacter capsulatus*. Coexpression with a 2[4Fe-4S] ferredoxin in *Escherichia coli*. J Biol Chem 266:3294–3299

Guo CL, Zhu X, Liao Q, Wang YZ, Chen R, Lee DJ (2011) Enhancement of photo-hydrogen production in a biofilm photobioreactor using optical fiber with additional rough surface. Bioresour Technol 102:8507–8513

Hallenbeck PC, Benemann JR (2002) Biological hydrogen production; fundamentals and limiting processes. Int J Hydrog Energy 27:1185–1193

Hankamer B, Barber J, Boekema EJ (1997) Structure and membrane organization of photosystem II in green plants. Annu Rev Plant Physiol 48:641–671

Harwood CS (2008) Nitrogenase-catalyzed hydrogen production by purple nonsulfur photosynthetic bacteria. In: Demain AL, Wall JD, Harwood CS (eds) Bioenergy. Springer Science+Business Media, Dordrecht, pp 259–271

Heiniger EK, Oda Y, Samanta SK, Harwood CS (2012) How posttranslational modification of nitrogenase is circumvented in *Rhodopseudomonas palustris* strains that produce hydrogen gas constitutively. Appl Environ Microbiol 78:1023–1032

Hoff AJ, Deisenhofer J (1997) Photophysics of photosynthesis. Structure and spectroscopy of reaction centers of purple bacteria. Phys Rep 287:1–247

Hu X, Schulten K (1997) How nature harvests sunlight. Phys Today 50:28–34

Hu X, Ritz T, Damjanović A, Autenrieth F, Shulten K (2002) Photosynthetic apparatus of purple bacteria. Q Rev Biophys 35:1–62

Hustede E, Steinbüchel A, Schlegel HG (1993) Relationship between the photoproduction of hydrogen and the accumulation of PHB in non-sulfur purple bacteria. Appl Microbiol Biotechnol 39:87–93

Imhoff JF (1995) The anoxygenic phototrophic purple bacteria. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 631–637

Ismail KSK, Najafpour G, Younesi H, Mohamed AR, Kamaruddin AH (2008) Biological hydrogen production from CO: bioreactor performance. Biochem Eng J 39:468–477

Jamieson SJ, Wang P, Qian P, Kirkland JY, Conroy MJ, Hunter CN, Bullough PA (2002) Projection structures of the photosynthetic reaction centre-antenna complex from *Rhodospirillum rubrum* at 8.5 Å resolution. EMBO 21:3927–3935

Jeong HS, Jouanneau Y (2000) Enhanced nitrogenase activity in strains of *Rhodobacter capsulatus* that overexpress the *rnf* genes. J Bacteriol 182:1208–1214

Joshi HM, Tabita FR (1996) A global two component signal transduction system that integrates the control of photosynthesis, carbon dioxide assimilation, and nitrogen fixation. Proc Natl Acad Sci U S A 93:14515–14520

Jouanneau Y, Wong B, Vignais PM (1985) Stimulation by light of nitrogenase synthesis in cells of *Rhodopseudomonas capsulata* growing in N-limited continuous cultures. BBA-Bioenerg 808:149–155

Jouanneau Y, Meyer C, Naud I, Klipp W (1995) Characterization of an *fdxN* mutant of *Rhodobacter capsulatus* indicates that ferredoxin I serves as electron donor to nitrogenase. Biochim Biophys Acta 1232:33–42

Kapdan IK, Kargi F (2006) Bio-hydrogen production from waste material. Enzyme Microb Technol 38:569–582

Karrash S, Bullough PA, Ghosh R (1995) The 8.5 Å projection map of the light-harvesting complex I from *Rhodospirillum rubrum* reveals a ring composed of 16 subunits. EMBO J 14:631–638

Kars G, Gündüz U (2010) Towards a super H_2 producer: improvements in photofermentative biohydrogen production by genetic manipulations. Int J Hydrog Energy 35:6646–6656

Kars G, Gündüz U, Rakhely G, Yücel M, Eroğlu I, Kovacs LK (2008) Improved hydrogen production by hydrogenase deficient mutant strain of *Rhodobacter sphaeroides* O.U.001. Int J Hydrog Energy 33:3056–3060

Kars G, Gündüz U, Yücel M, Rakhely G, Kovacs K, Eroğlu I (2009) Evaluation of hydrogen production by *Rhodobacter sphaeroides* O.U.001 and its *hup*SL deficient mutant using acetate and malate as carbon sources. Int J Hydrog Energy 34:2184–2190

Kawagoshi Y, Oki Y, Nakano I, Fujimoto A, Takahashi H (2010) Biohydrogen production by isolated halotolerant photosynthetic bacteria using long-wavelength light-emitting diode (LW-LED). Int J Hydrog Energy 35:13365–13369

Kern M, Klipp W, Klemme HJ (1994) Increased nitrogenase dependent H_2 photoproduction by *hup* mutants of *Rhodospirillum rubrum*. Appl Environ Microbiol 60:1768–1774

Keskin T, Abo-Hashesh M, Hallenbeck PC (2011) Photofermentative hydrogen production from wastes. Bioresour Technol 102:8557–8568

Kim MS, Baek JS, Lee JK (2006) Comparison of H_2 accumulation by *Rhodobacter sphaeroides* KD131 and its uptake hydrogenase and PHB synthase deficient mutant. Int J Hydrog Energy 31:121–127

Klamt S, Grammel H, Straube R, Ghosh R, Gilles ED (2008) Modeling the electron transport chain of purple non-sulfur bacteria. Mol Syst Biol 4:156–174

Koblízek M, Shih JD, Breitbart SI, Ratcliffe EC, Kolber ZS, Hunter CN, Niederman RA (2005) Sequential assembly of photosynthetic units in *Rhodobacter sphaeroides* as revealed by fast repetition rate analysis of variable bacteriochlorophyll a fluorescence. Biochim Biophys Acta 1706:220–231

Koku H, Eroğlu I, Gündüz U, Yücel M, Türker L (2002) Aspects of metabolism of hydrogen production by *Rhodobacter sphaeroides*. Int J Hydrog Energy 27:1315–1329

Koku H, Eroğlu I, Gündüz U, Yücel M, Türker L (2003) Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001. Int J Hydrog Energy 28:381–388

Kondo T, Arakawa M, Hirai T, Wakayama T, Hara M, Miyake J (2002a) Enhancement of hydrogen production by a photosynthetic bacterium mutant with reduced pigment. J Biosci Bioeng 93:145–150

Kondo T, Arakawa M, Wakayama T, Miyake J (2002b) Hydrogen production by combining two types of photosynthetic bacteria with different characteristics. Int J Hydrog Energy 27:1303–1308

Larimer FW, Chain P, Hauser L, Lamerdin J, Malfatti S, Do L, Land ML, Pelletier DA, Beatty JT, Lang AS, Tabita FR, Gibson JL, Hanson TE, Bobst C, Torres y Torres JL, Peres C, Harrison FH, Gibson J, Harwood CS (2004) Complete genome sequence of the metabolic versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nat Biotechnol 22:55–61

Liao Q, Wamg YJ, Wang YZ, Zhu X, Tian X, Li J (2010) Formation and hydrogen production of photosynthetic bacterial biofilm under various illumination conditions. Bioresour Technol 101:5315–5324

Loach PA, Parker-Loach PS (2008) Structure-function relationships in bacterial light-harvesting complexes investigated by reconstitution techniques. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer Science+Business Media, Dordrecht, pp 181–198

Maróti P (2008) Kinetics and yields of bacteriochlorophyll fluorescence: redox and conformation changes in reaction center of *Rhodobacter sphaeroides*. Eur Biophys J 37:1175–1184

McKinlay JB, Harwood CS (2010) Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proc Natl Acad Sci U S A 107:11669–11675

McKinlay JB, Harwood CS (2011) Calvin cycle flux, pathway constraints, and substrate oxidation state together determine the H_2 biofuel yield in photoheterotrophic bacteria. mBio 2:e00323-10

Miyake J (1998) The science of biohydrogen. In: Zaborsky OR (ed) Biohydrogen. Plenum Press, London, pp 7–18

Miyake J, Kawamura S (1987) Efficiency of light energy conversion to hydrogen by the photosynthetic bacterium *Rhodobacter sphaeroides*. Int J Hydrog Energy 12:147–149

Miyake J, Wakayama T, Schnackenberg J, Arai T, Asada Y (1999) Simulation of the daily sunlight illumination pattern for bacterial photo-hydrogen production. J Biosci Bioeng 88:659–663

Moreno-Vivian C, Hennecke S, Pühler S, Klipp W (1989) Open reading frame 5 (ORF5), encoding a ferredoxin-like protein, and *nif*Q are cotranscribed with *nif*E, *nif*N, *nif*X, and ORF4 in *Rhodobacter capsulatus*. J Bacteriol 171:2591–2598

Naud I, Meyer C, David L, Breton J, Gaillard J, Jouanneau Y (1996) Identification of residues of *Rhodobacter capsulatus* ferredoxin I important for its interaction with nitrogenase. Eur J Biochem 237:399–405

Oda Y, Samanta SK, Rey FE, Wu L, Liu X, Yan T, Zhou J, Harwood CS (2005) Functional genomic analysis of three nitrogenase isozymes in the photosynthetic bacterium *Rhodopseudomonas palustris*. J Bacteriol 187:7784–7794

Oda Y, Larimer FW, Chain PS, Malfatti S, Shin MV, Vergez LM, Hauser L, Land ML, Braatsch S, Beatty JT, Pelletier DA, Schaefer AL, Harwood CS (2008) Multiple genome sequences reveal adaptations of a phototrophic bacterium to sediment microenvironments. Proc Natl Acad Sci U S A 105:18543–18548

Okamura MY, Feher G (1992) Proton transfer in reaction centers from photosynthetic bacteria. Annu Rev Biochem 61:861–896

Okamura MY, Feher G (1995) Proton-coupled electron transfer reactions of QB in reaction centers from photosynthetic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria, vol 2, Advances in photosynthesis and respiration. Springer Science+Business Media, Dordrecht, pp 577–593

Okamura MY, Paddock ML, Graige MS, Feher G (2000) Proton and electron transfer in bacterial reaction centers. Biochim Biophys Acta 1458:148–163

Ooshima H, Takakuwa S, Katsuda T, Okuda M, Shirasawa T, Azuma M, Kato J (1998) Production of hydrogen by a hydrogenase deficient mutant of *Rhodobacter capsulatus*. J Ferment Bioeng 85:470–475

Otzuki T, Uchiyama S, Fujiki K, Fukunaga S (1998) Hydrogen production by a floating-type photobioreactor. In: Zaborsky OR (ed) Biohydrogen. Plenum Press, London, pp 369–374

Öztürk Y, Yücel M, Daldal F, Mandacı S, Gündüz U, Türker L, Eroğlu I (2006) Hydrogen production by using *Rhodobacter capsulatus* mutants with genetically modified electron transfer chains. Int J Hydrog Energy 31:1545–1552

Paddock ML, Feher G, Okamura MY (2003) Proton transfer pathways and mechanism in bacterial reaction centers. FEBS Lett 555:45–50

Qian Y, Tabita FR (1996) A global signal transduction system regulates aerobic and anaerobic CO2 fixation in *Rhodobacter sphaeroides*. J Bacteriol 178:12–18

Reddy CSK, Ghai R, Rashmi V, Kalia C (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87:137–146

Redwood MD, Paterson-Beedle M, Macaskie LE (2009) Integrating dark and light bio-hydrogen production strategies: towards the hydrogen economy. Rev Environ Sci Biotechnol 8:149–185

Rees DC, Howard JB (2000) Nitrogenase: standing at the crossroads. Curr Opin Chem Biol 4:559–566

Rey FE, Heiniger EK, Harwood CS (2007) Redirection of metabolism for biological hydrogen production. Appl Environ Microbiol 73:1665–1671

Robert B (2008) Spectroscopic properties of antenna complexes from purple bacteria. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer, Dordrecht, pp 199–212

Sasikala C, Ramana CV (1995) Biotechnological potentials of anoxygenic phototrophic bacteria: II. Biopolyesters, biopesticide, biofuel and biofertilizer. Adv Appl Microbiol 41:227–278

Sasikala K, Ramana CV, Raghuveer Rao P, Subrahmanyam M (1990) Effect of gas phase on the photoproduction of hydrogen and substrate conversion efficiency in the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001. Int J Hydrog Energy 15:795–797

Schatt E, Jouanneau Y, Vignais PM (1989) Molecular cloning and sequence analysis of the structural gene of ferredoxin I from the photosynthetic bacterium *Rhodobacter capsulatus*. J Bacteriol 171:6218–6226

Seefeldt LC, Hoffman BM, Dean DR (2009) Mechanism of Mo-dependent nitrogenase. Annu Rev Biochem 78:701–722

Sener MK, Schulten K (2008) From atomic-level structure to supramolecular organization in the photosynthetic unit of purple bacteria. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer, Dordrecht, pp 275–294

Shapleigh JP (2008) Dissimilatory and assimilatory nitrate reduction in the purple photosynthetic bacteria. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28,

Advances in photosynthesis and respiration. Springer, Dordrecht/London, pp 623–642

Sheuring S, Rigaud JL, Sturgis JN (2004) Variable LH2 stoichiometry and core clustering in native membranes of *Rhodospirillum photometricum*. EMBO J 23:4127–4413

Sheuring S, Lévy D, Rigaud JL (2005) Watching the components of photosynthetic bacterial membranes and their in situ organization by atomic force microscopy. Biochim Biophys Acta 1712:109–127

Shinkarev VP, Wraight CA (1993) Electron and proton transfer in the acceptor quinone complex of reaction centers of phototrophic bacteria. In: Deisenhofer J, Norris JR (eds) The photosynthetic reaction center, vol 1. Academic, San Diego, pp 193–255

Steinborn B, Oelze J (1989) Nitrogenase and photosynthetic activities of chemostat cultures of *Rhodobacter capsulatus* 37b4 grown under different illuminations. Arch Microbiol 152:100–104

Swem LR, Gong X, Yu CA, Bauer CE (2006) Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. J Biol Chem 281:6768–6775

Takaichi S (2008) Distribution and biosynthesis of carotenoids. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer, Dordrecht/London, pp 97–117

Tian X, Liao Q, Zhu X, Wang Y, Zhang P, Li H, Wang H (2010) Characteristics of a biofilm photobioreactor as applied to photo-hydrogen production. Bioresour Technol 101:977–983

Tredici MR (1999) Bioreactors, photo. In: Flickinger MC, Drew SW (eds) Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation, vol 1. Wiley, New York, pp 395–419

Uyar B, Eroğlu I, Yücel M, Gündüz U, Türker L (2007) Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors. Int J Hydrog Energy 32:4670–4677

Vasilyeva L, Miyake M, Khatipov E, Wakayama T, Sekine M, Hara M, Nakada E, Asada Y, Miyake J (1999) Enhanced hydrogen production by a mutant of *Rhodobacter sphaeroides* having an altered light-harvesting system. J Biosci Bioeng 87:619–624

Vignais PM, Billoud B (2007) Occurrence, classification, and biological function of hydrogenases: an overview. Chem Rev 107:4206–4272

Vignais PM, Colbeau A, Willison JC, Jouanneau Y (1985) Hydrogenase, nitrogenase, and hydrogen metabolism in the photosynthetic bacteria. Adv Microb Physiol 26:155–234

Vincenzini M, Marchini A, Ena A, De Philippis R (1997) H_2 and poly-β-hydroxybutyrate, two alternative chemicals from purple non sulfur bacteria. Biotechnol Lett 19:759–762

Vonshak A, Richmond A (1985) Problems in developing the biotechonology of algal biomass production. Plant Soil 89:129–135

Wakayama T, Miyake J (2002) Light shade bands for the improvement of solar hydrogen production efficiency by *Rhodobacter sphaeroides* RV. Int J Hydrog Energy 27:1495–1500

Walz T, Ghosh R (1997) Two-dimensional crystallization of the light-harvesting I-reaction center photounit from *Rhodospirillum rubrum*. J Mol Biol 256:107–111

Walz T, Jamieson SJ, Bowers CM, Bullogh PA, Hunter CN (1998) Projection structures of three photosynthetic complexes from *Rhodobacter sphaeroides*: LH2 at 6 Å, LH1 and RC-LH1 at 25 Å. J Mol Biol 282:833–845

Wang YZ, Liao Q, Zhu X, Chen R, Guo CL, Zhou J (2013) Bioconversion characteristics of *R. palustris* CQK 01 entrapped in a photobioreactor for hydrogen production. Bioresour Technol 135:331–338

Williams JC, Allen JP (2008) Directed modification of reaction centers from purple bacteria. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer, Dordrecht/London, pp 337–353

Willison JC, Pierrard J, Hübner P (1993) Sequence and transcript analysis of the nitrogenase structural gene operon *nif*HDK of *Rhodobacter capsulatus*: evidence of an intramolecular processing of *nif*HDK mRNA. Gene 133:39–46

Wraight CA (2004) Proton and electron transfer in the acceptor quinone complex of bacterial photosynthetic reaction centers. Front Biosci 9:309–327

Wraight CA (2005) Intraprotein proton transfer – Concept and realities from the bacterial photosynthetic reaction center. In: Wilkström M (ed) Biophysical and structural aspects of bioenergetics. Royal Society of Chemistry, Cambridge, pp 273–313

Xie GJ, Liu BF, Ding J, Xing DF, Ren HY, Guo WG, Ren NQ (2012) Enhanced photo-H_2 production by *Rhodopseudomonas faecalis* RLD-53 immobilization on activated carbon fibers. Biomass Bioenergy 44:122–129

Zannoni D (1995) Aerobic and anaerobic electron transport chains in anoxygenic phototrophic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria, vol 2,

Advances in photosynthesis and respiration. Springer Science+Business Media, Dordrecht, pp 949–971

Zannoni D, Schoepp-Cothenet B, Hosler J (2008) Respiration and respiratory complexes. In: Hunter CD, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria, vol 28, Advances in photosynthesis and respiration. Springer, Springer Science+Business Media, Dordrecht, pp 537–561

Zhang C, Zhu X, Liao Q, Wang Y, Li J, Ding Y, Wang H (2010) Performance of a groove-type photobioreactor for hydrogen production by immobilized photosynthetic bacteria. Int J Hydrog Energy 35:5284–5292

Zuber H, Brunhisolz RA (1991) Structure and function of antenna polypeptides and chlorophyll-protein complexes: principles and variability. In: Sheer H (ed) Chlorophylls. CRC Press, Boca Raton, pp 627–703